

1966  
ANNUAL REPORT

of

HOP INVESTIGATIONS  
(CRe5, OAES 36)

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## INTRODUCTION

S. N. Brooks

This 1966 annual report of the investigations carried out by the regional hop project headquartered at Corvallis, Oregon, includes data collected and summarized during the period March 1, 1966 to February 28, 1967. It includes data in some cases which were collected by personnel at the Irrigated Agriculture Research and Extension Center at Prosser, Washington. All data are reported under one of four main lines of study or line projects. Detailed discussions and summarizing data are presented for each experiment or phase as a separate section within a line project report. Additional data or notes which are important enough to be included as a matter of permanent record are appended to the report.

Some of the line projects are conducted cooperatively by investigators located at Oregon State University. In these cases, it is necessary that a line project report be prepared by more than one person. Where this has occurred an attempt has been made to give each project leader full credit for his contribution to the report.

The work summarized in this report is supported by public and private funds. Cooperative research is carried out by Crops Research Division, ARS, USDA; Oregon Agricultural Experiment Station; and United States Brewers Foundation through the Agricultural Research Foundation under Memorandum of Understanding. In addition certain phases of the federal breeding program are cooperative with the agricultural experiment stations in California, Idaho and Washington also under Memorandum of Understanding. This report does not summarize work done at any of the institutions which does not involve direct cooperation of federal personnel.

The immediate staff of the hop research project during the preceding twelve months consisted of the following persons. This list is made up of regularly employed personnel who were associated with the cooperative State-Federal hop research program and thus contributed directly to the work reported herein. Personnel doing independent research at Oregon State University and field assistants hired for intermittent or seasonal jobs on the cooperative program are not included.

Mrs. J. M. Barnes, Secretary, USDA,  
Dr. S. N. Brooks, Research Agronomist, USDA,  
Mr. John Burnett, Student helper, intermittent, OSU,  
Mrs. Doris Cox, Student helper, 4 mo., OSU,  
Mr. Wm. Eisenbart, Student helper, 3 mo., OSU,  
Mr. Charles Florence, Laborer, 2 mo., OSU,  
Dr. Alfred Haunold, Research Plant Geneticist, USDA,  
Dr. C. E. Horner, Plant Pathologist, USDA and OSU,  
Mr. S. T. Likens, Research Chemist, USDA,  
Mrs. Betty McCoy, Lab. Tech., OSU,  
Mr. Robert McKittrick, Laborer, intermittent, 9 mo., OSU,  
Mr. Craig Mills, Laborer, 3 mo., OSU,  
Miss G. B. Nickerson, Chemist, OSU,  
Mrs. Pat. Sturdevant, Laborer, intermittent, OSU,



Mr. B. R. Swanson, Biology Aide, OSU,  
 Mr. John Waldron, Laborer, part-time, 3 mo., OSU,  
 Mr. Terry Wheeler, Laborer, part-time, 2 mo., OSU,  
 Mr. Jeffrey Williams, Laborer, 2 mo., OSU,  
 Mr. H. Zehsazian, Graduate Assistant, OSU,  
 Mr. C. E. Zimmermann, Plant Physiologist, USDA,

There have been only two short papers published by project personnel during the past 12 months. These are:

Haunold, A. 1966. A modified Venetian turpentine mounting technique for chromosome spreading and karyotype analysis. WSCS Crop Sci. Abstr. p. 13. (mimeo.) June.

Zimmermann, C. E. 1967. Treating hop string with copper naphthenate. Ore. Agric. Expt. Sta. Misc. Publ. (mimeo.) Feb.

According to the Oregon Crop and Livestock Reporting Service (27 Dec. 1966), U. S. production of hops was 55,418,000 pounds, down 1 percent from 1965 but 20 percent above average. Washington and Oregon each produced more hops, but Idaho and California produced less than last year. Total acreage was down slightly, mostly because California had nearly 20 percent less acreage than in 1965. Yields were above average in Washington, Oregon, and Idaho, but slightly below average in California.

In Idaho, cool weather early in the season was a factor in holding yields below last year's level. Damage from mildew and wind was less than usual. Washington yields were above both last year and average. The growing season was good, but wind caused damage at the start of harvest. Wind whip and dehydration reduced yields in many yards. Wind caused several yards to go down and this resulted in some unharvested acreage. Although California's season started off with good yield prospects, high temperatures in August sharply curtailed production. Oregon hops were set back by cool spring weather, but subsequent weather was favorable.

Oregon hop growers produced 7,150,000 pounds of hops in 1966, 7 percent above 1965 and 31 percent above the 1960-64 average, according to the Oregon Crop and Livestock Reporting Service. Yield per acre, at 1,430 pounds, was down 1 percent from 1965, but 3 percent above the 1960-64 average. All varieties yielded well except Fuggle which did not develop as well as was expected. With the increased production and a slightly higher price per pound, the total value of the crop was 9 percent above 1965 and the highest since 1952.

According to the Consumer and Marketing Service (29 Dec. 1966) the 1966 world hops crop was estimated at 205.0 million pounds or only marginally below the record 1964 total of 205.6 million pounds and slightly above the 203.4 million pound 1965 harvest. The 1960-64 average production was 183.4 million pounds. The 1966 crop was grown on 176,900 acres -- up slightly from the 175,700 acres grown in 1965. The 1966 average yield per acre at 1,159 pounds was almost identical to the 1,158 pounds produced in 1965, but was slightly below the 1,182 averaged during the record 1964 harvest.

The United States, as usual, was the world's leading hops producer with 55.4 million pounds.

While acreage rose 3% in Western Europe, production dropped 5 percent from the 1965 level. Both the United Kingdom and West Germany, the world's second and third largest producers, had smaller crops than in recent years. This more than offset increases in most of the rest of the region.

Eastern Europe, on the other hand, generally had a good crop with an increase in production of 9 percent from a 1 percent smaller acreage. The average yield for the region at 755 pounds, was well above the 690 pound 1965 average and somewhat above normal, but was still well below that of other major production areas.

Table 1. Hops: Acreage, yield, production, season average price received by growers, and value, Average 1960-64; annual 1965-66.

State	Acreage harvested			Yield per acre			Production			Price per pound		Value	
	Average	1965	1966	Average	1965	1966	Average	1965	1966	1965	1966	1965	1966
	1960-64			1960-64			1960-64			-Cents-		-1,000 dollars	
	-Acres-			-Pounds-			-1,000 pounds-						
Idaho	3,580	3,900	4,000	1,746	1,950	1,810	6,205	7,605	7,240	46.0	49.0	3,498	3,548
Washington	17,700	21,100	20,700	1,570	1,710	1,790	27,833	36,081 <sup>1/</sup>	37,053	44.7	45.0	16,128	16,674
Oregon	3,920	4,600	5,000	1,392	1,450	1,430	5,447	6,670	7,150	48.0	49.0	3,202	3,504
California	4,140	3,100	2,500	1,605	1,840	1,590	6,607	5,704	3,975	54.5	54.0	3,109	2,146
United States	29,340	32,700	32,200	1,569	1,714	1,721	46,092	56,060	55,418	46.3	46.7	25,937	25,872

<sup>1/</sup> Production includes 113,000 pounds lost in kiln.





CR5-1 (OAES FC:36) BREEDING AND EVALUATING  
NEW AND IMPROVED VARIETIES OF HOPS

S. N. Brooks, C. E. Horner, and Alfred Haunold

The work done under this line project consists of development of improved varieties of hops, studies of techniques of breeding or evaluating genetic lines, basic studies of inheritance or inherent variation in the plant itself, and studies on the botany of hops. The report is divided into three sections:

- (1) That phase dealing with crossing and initial selection of seedlings,
- (2) Preliminary and advanced evaluation of selections for field performance, and
- (3) That phase of this project dealing with botanical and genetic studies.

The work done under this line project is varied with different personnel involved in different aspects of the research. Therefore, the initials of the investigator follow the section or subsection headings to identify the contributions of each to the line project report.

Exchange of Germ Plasm (SNB)

There was considerable activity in exchange of germ plasm in 1966 and early 1967 (Table 1). Eight genotypes were received into our program. Alliance and Progress are two new Verticillium wilt resistant lines developed in the Wye/East Malling program in England. Both are more vigorous but later maturing than Fuggle, have smaller cones, shatter less, and are symptomless carriers of mosaic virus. The three varieties from New Zealand are new Phytophthora resistant ones developed from "Californian" x unknown parentage. Little information is available on the two varieties from Australia except that they are recently developed.

Table 1. Exchange of germ plasm in 1966 and early 1967.

<u>Accession Number</u>	<u>Name</u>	<u>P.I.number</u>	<u>Destination or Source</u>
<u>Received</u>			
I 66/52	Pride of Ringwood	314046	A.S.Nash, Melbourne, Australia
I 66/53	Ringwood Special	314047	A.S.Nash, Melbourne, Australia
I 66/54	Calicross	314968	R.H.J.Roborgh, Riwaka, New Zealand
I 66/55	First choice	314969	R.H.J.Roborgh, Riwaka, New Zealand
I 66/56	Smoothcone	314970	R.H.J.Roborgh, Riwaka, New Zealand
I 66/50	Alliance(W/E 1778)	312510	R.A.Neve, Wye, England
I 66/51	Progress(W/E 1008)	312511	R.A.Neve, Wye, England
I 67/51	King		Mel King, Grants Pass, Oregon
<u>Sent</u>			
	Yakima Cluster (65/102), E-2 (65/103), and Brewers Gold (19/01)	Oct. '66	R.H.J.Roborgh, Riwaka, New Zealand
	Fuggle (19/209) and Late Cluster (19/208)	Mar. '67	C.L.Younkman, Ohio State Farm Science Review, Columbus, Ohio.
	Late Cluster (19/208)	May '66	O.C.Ruelke, University of Florida, Gainesville, Florida.
	Late Cluster (19/208), Fuggle (19/209) and Brewers Gold (19/01)	Mar. '67	R.M.Indra, Colombo, Ceylon
	Late Cluster (19/208), Early Cluster (59/08), Fuggle (19/209) and Brewers Gold (19/01)	Mar. '67	M.W.Hardas, New Delhi, India
	Late Cluster (19/208), Fuggle (19/209), and Brewers Gold (19/01)	Mar. '67	F.R.Palacio, Cuernavaca, Mexico
	Late Cluster (19/208) Fuggle (19/209), and Early Cluster (59/08)	Mar. '67	L.Zub, Pulawy, Poland



Table 1. cont.

<u>Accession Number</u>	<u>Name</u>	<u>P.I.number</u>	<u>Destination or Source</u>
	<u>Sent</u>		
Late Cluster (19/208), Early Cluster (59/08), Ariz.1-3 (60/14), N.M.2-2 (60/18), N.M.2-3 (60/19M), Colo.1-1 (60/23M), Colo.2-2 (60/27), and Ariz.1-2 (60/13M)		Mar. '67	D.V.Ter-Avanesyan, Leningrad, USSR

1966 Selections (SNB)

Because the screening program on the seedlings resulting from the 1963 crosses was not successful in isolating downy mildew resistant genotypes, an additional year's observation in the field was made necessary. As a result, no clones were selected for advancement to the observation block. Instead, 101 clones were taken on the basis of freedom from disease in the 1964 nursery and planted into a second 1964 nursery in the new yard (Table 2).

Several crosses appeared to be outstanding in the degree of resistance to downy mildew exhibited by the various seedlings. However, additional observation will be needed in 1967 to be sure of disease reaction of these seedlings.

Single-hill plants and multiple-hill plots of about 200 genotypes were grown at Prosser, Washington by C. E. Nelson. He considered this material (1962 crosses) the best we had ever sent him, supporting our own conclusions last year that the 1963 selections were the best ever at Corvallis. Based on observations made at Prosser in 1966, the following material (Table 3) appears to warrant additional study. Data from most of the lines grown at Prosser in 1966 are appended to this report. Arrangements are being made to submit brewers' inspection samples from Prosser in 1967.

Table 2. 1964 Seedling Nursery (transplanted 1966)

<u>Cross</u>	<u>Pedigree</u>	<u>Row</u>	<u>No. plants</u>
63004	1/2 Fu; 1/8 EKG; 1/16 EG; 1/32 KG; 9/32 X	30	5
63005	1/2 Fu; 1/4 Str; 1/8 LC; 1/8 X		5
63023	3/16 Fu; 1/8 LG; 1/8 EKG; 1/16 EG; 1/32 KG; 15/32 X		<u>4</u>
63010	1/2 Ha; 1/4 Str; 1/8 LC; 1/8 X	31	5
63006	1/2 Fu; 1/4 EG; 1/4 X		5
63022	1/4 Str; 3/16 Fu; 1/8 LG; 1/8 LC; 5/16 X		<u>4</u>

At Corvallis, screening tests, under insectory conditions, were repeated in 1966 on those genotypes which appeared most promising in 1965 for reduced fecundity of spider mites and aphids. No clones appeared to resist both pests, but several clones were considerably better than Fuggle for either spider-mites or aphids. Although there were some inconsistencies between the two years, the results are still encouraging. A portion of the data is given in table 4.

Table 4. Reduced fecundity of insect pests (compared with Fuggle) in 1965 and 1966.

Clone	% reduced fecundity		Clone	% reduced fecundity	
	1965	1966		1965	1966
<u>For aphids:</u>			<u>For spider mites:</u>		
BB 118-2	54	47	BB 517-5	71	59
BB 420-2	74	66	BB 205	72	19
35-S	80	67	BB 221-2	80	34
56-S	54	80	OB 835	59	42
94-S	68	70	Colo. 4-1	78	27

Aphid and spider-mite populations were followed on the 300 genotypes in the field. Due to poor field infestations, under natural conditions, the data obtained were inadequate for complete or conclusive findings.

A complete report on the Corvallis work will be forthcoming as a graduate thesis prepared by Mr. Mayberry.

At Prosser Dr. Cone made periodic counts of mites and aphids on several genotypes. The selections exhibited marked differences in degree of resistance to attack of two-spotted spider mites. Although mite populations were generally low throughout the Yakima Valley in 1966, numbers were sufficient to establish tendencies in reaction.

Aphid populations were large, and some selections suffered partial or complete defoliation. There seemed to be no correlation between occurrence of mites and aphids in spite of large amounts of honeydew which, in some cases, would have been expected to discourage mite build-up.

In Experiment A (see last year's report) selections 20(11-48), 23(11-42), and 7(11-1) showed greatest promise for mite resistance, but only 23 and 7 could be considered for both mite and aphid resistance. Selection 6(64-10) showed greatest susceptibility to both pests. Selection 8(10-3) has shown intermediate resistance to spider mite in both 1965 and 1966. Results for the rest are not consistent between years, except that several genotypes have shown marked susceptibility to spider mites both years.

In Experiment B in 1966 selections 46(68-7), 1(67-16), 6(69-9), 55(51-30), 8(8-33), 13(52-28), and 4(69-49) showed greatest promise for mite resistance. Low numbers of aphids were found on all of these except 6, 13, and 4. Only selection 16(52-19) has appeared to be resistant to mites 2 years in a

row. Selection 44(65-30) has had a high mite population both years.

In general, the mite population at Prosser developed very slowly during the 1966 growing season. The probable cause for the lack of mite numbers early in the season was cool night temperatures. Population development in late July 1966 was corresponded with late June of 1965. Aphid numbers, on the other hand, were favored by the cooler temperatures. Where not controlled with insecticides, aphids remained abundant throughout the season.

#### Reaction of 1965 Crosses to Downy Mildew (CEH).

Twenty six crosses made in 1965 yielded 5231 seedlings which were tested in 1966 for resistance to downy mildew. Seeds were planted in the greenhouse in March and April, 1966. Seedlings were allowed to grow and mature until August when top growth was removed. Seedling crowns were inoculated by injecting a mixture of zoospores and sporangia into the phloem area of the upper crown and by spraying the crown buds with spores. Favorable conditions for infection and disease development were maintained in the greenhouse. Seedling crowns were dug and washed about 70 days after inoculation. Evaluation for infection was made by slicing into the injection wound with a razor blade then determining visually if infection had spread into the crown. Many seedling crowns were completely rotted by mildew infection.

The seedling represented four different groups of crosses and open pollinated source as follows:

- Group I -- Backcrosses
- Group II -- Crosses for earliness
- Group III -- High alpha acid crosses
- Group IV -- Crosses to obtain genetic information
- Group V -- Open pollinated seed sources.

Table 5 shows the number of plants in each cross, the downy mildew reaction of seedlings and the number of plants saved for further evaluation.

#### Discussion:

Seedlings resistant to downy mildew crown infection were obtained from all crosses producing sufficient numbers of seedlings for evaluation. Cross number 17, a backcross with Hallertau (mildew susceptible) as the recurring parent, was outstanding in producing resistant seedlings.



Table 5. Crosses evaluated for downy mildew resistance in 1966.

<u>Cross No.</u>	<u>Total plants</u>	<u>Downy mildew</u>	<u>% DM</u>	<u>No. plants kept</u>
I <u>Back Crosses</u> (10)				
8	1	0	0	1
10	1	0	0	0
13	392	164	41.8	21
14	2	1	50.0	0
15	6	5	83.3	0
16	360	170	47.2	30
17	400	79	19.7	60
19	23	5	21.7	12
20	2	2	100.0	0
38	255	109	42.7	39
Totals	1442	535	37.7% inf.	163
II <u>Earliness cross</u> (1)				
4	9	3	33.3	3
III <u>High alpha</u> (2)				
12	227	94	41.4	28
24	11	5	45.4	3
Totals	238	99	41.6% inf.	31
IV <u>Genetic info.</u> (4)				
2	12	6	50.0	6
3	57	17	29.8	30
26	10	7	70.0	3
27	70	39	55.7	23
Totals	149	69	46.3% inf.	62
V <u>Open pollinated</u>				
29	552	255	46.2	7
30	651	418	64.2	15
31	86	39	45.3	19
32	603	406	67.3	28
33	488	289	59.2	23
34	7	5	71.4	1
35	362	241	66.6	19
36	584	385	65.9	13
37	60	33	55.0	3
Totals	3393	2071	61.0% inf.	128
Grand total	5231	2777	53.1% inf.	387

Crosses Made in 1966 (SNB).

Seventy-six crosses and open pollinated seed lots produced seed in 1966 (Table 6). These were made in six groups. Two groups were made up of material which showed some promise of aphid and/or spider mite resistance in 1965. Another group was rather conventional in that the parental material has shown some resistance to downy mildew through the years. An attempt was made in one group to get segregation for early maturity. A fifth group was made up of crosses for which at least one parent possesses high  $\alpha$ -acid content. In some cases certain crosses fit the categories of more than one group. Various crosses were made to furnish progenies for genetic studies.

Table 6.

Crosses made in 1966

Cross No. <u>1</u> /	<u>2</u> / Purpose	Parentage	
601	AR	113-2 (C52/18)	x 221-2 (C51/114M)
602	AR	113-2 "	x 318-1,2 (19/43M)
603	AR	113-2 "	x 419-1,2 (19/37M)
604	AR	113-2 "	x 517-5 (C54/66M)
605	AR	114-3 (C52/20)	x 221-1 (C51/114M)
606	AR	114-3 "	x 221-2 (C51/101M)
607	DR	122 (19/208)	x 219-4 (C51/61M)
608	DR	122 "	x 123-S (19/182M)
609	DR	122 "	x 317-1,2 (19/41M)
610	DR	122 "	x 421-1,2 (19/40M)
611	DR	122 "	x OP
612	AR	205 (19/04)	x 221-1 (C51/114M)
613	AR	205 "	x 318-1,2 (19/43M)
614	AR	205 "	x 517-5 (C54/66M)
615	AR	205 "	x OP
616	$\alpha$ -DR	311 (19/01)	x 6339-9 (19/209 x I 60/26M)
617	$\alpha$	311 "	x 63/13M (19/01 x I58/15M)
618	$\alpha$	311 "	x 63/23M (19/01 <sup>2</sup> x 19/62M)
619	$\alpha$ (BC)	311 "	x 63/25M (19/01 x 19/40M)
620	$\alpha$	311 "	x Ariz. 1-2 (I60/13M)
621	$\alpha$ -AMR	313 (64/100)	x 221-1 (C51/114M)
622	$\alpha$ -AMR	313 "	x 318-1,2 (19/43M)
623	$\alpha$ -AMR	313 "	x 419-1,2 (19/37M)
624	$\alpha$ -AMR	313 "	x 517-5 (C54/66M)
625	$\alpha$	313 "	x OP

Table 6 cont.

Cross No. <u>1</u> /	<u>2</u> / Purpose		Parentage
626	AR	420-2 (19/38) <u>3</u> /	x 221-2 (C51/101M)
627	AR	420-2 "	x 419-1,2 (19/37M)
628	AR	420-2 "	x 517-5 (C54/66M)
629	AR	420-2 "	x OP
630	AR	519-5 (C52/43) <u>4</u> /	x OP
631	DR	522 (59/08)	x 121-2 (19/62M)
632	DR	522 "	x 219-4 (C51/61M)
633	DR	522 "	x 521-4,5 (19/10M)
634	DR	522 "	x Ut 524-2 (I58/06M)
635	DR	522 "	x OP
636	AR	FOB 215 (I62/02)	x 221-1 (C51/114M)
637	AR	FOB 215 "	x 318-1,2 (19/43M)
638	AR	FOB 215 "	x 419-1,2 (19/37M)
639	AR	FOB 215 "	x 517-5 (C54/66M)
640	AR	FOB 215 "	x OP
641	AR	35-S (19/124)	x 221-2 (C51/114M)
642	AR	35-S "	x OP
643	AR	94-S (19/164)	x 221-1 (C51/114M)
644	AR	94-S "	x 221-2 (C51/101M)
645	AR	94-S "	x OP
646	AR	131-S (19/185)	x OP
647	MR	148-S (19/200)	x 221-1 (C51/114M)
648	MR	148-S "	x 318-1,2 (19/43M)
649	MR	148-S "	x 419-1,2 (19/37M)
650	MR	148-S "	x 517-5 (C54/66M)

Table 6 cont.

Cross No. <u>1</u> /	<u>2</u> / Purpose		Parentage
651	α	129-I (19/115H)	x OP
652	MR	Fu H (48/01)	x 221-1 (C51/114M)
653	MR	Fu H "	x 221-2 (C51/101M)
654	MR	Fu H "	x 419-1,2 (19/37M)
655	MR	Fu H "	x 517-5 (C54/66M)
656	MR	Fu H "	x OP
657	α	6347-42 (C63/20)	x 6344-36 (C63/13M)
658	α	6347-42 "	x 6347-32 (C63/23M)
659	α	6347-42 "	x 6348-15 (C63/25M)
660	α	6347-42 "	x Ariz. 1-2 (I60/13M)
661	α	6348-21 (C63/10)	x 6344-36 (C63/13M)
662	α	6348-21 "	x 6347-32 (C63/23M)
663	α	6348-21 "	x 6348-15 (C63/25M)
664	α	6348-21 "	x Ariz. 1-2 (I60/13M)
665	Gen	135-I (19/151)	x 317-1,2 (19/41M)
666	Gen	222 (19/209)	x 110-S (19/173M)
667	Gen	128-I (19/113)	x OP
668	Gen	OB 826 (56/08)	x OP
669	α	Goschie Bu Yard	x OP
670	αE	311 (19/01)	x 106-S (19/170M)
671	α	311 "	x 123-S (19/182M)
672	DR	311 "	x OB 79 (I64/103M)
673	Gen	311 "	x 63/23 M (A/01 <sup>2</sup> x 19/62M)
674	Gen	311 "	x Fu-1-1 (reversed Fu)
675	Gen	311 "	x Fu-2-4 (reversed Fu)

Table 6 cont.

Cross No. <u>1/</u>	Purpose <u>2/</u>	Parentage
676	Gen 311	(19/01) x 63/22M (19/01 x I58/15M)

- 1/ Crosses 616, 617, 618, 619, 620, 657, 658, 659, 660, 661, 662, 663, and 664 were planted directly into the field.
- 2/ AR = aphid resistance; DR = disease resistance; MR = spider mite resistance;  $\alpha$  = alpha-acid content; Gen = genetic studies; E = earliness.
- 3/ Originally listed as male 19/38M.
- 4/ Originally listed as male C52/43M -- reversed previously in SA.

## EVALUATION

## Objectives:

1. To provide preliminary quality evaluation of new selections and make observations on vigor and disease reaction.
2. To make preliminary field evaluation of new selections in replicated variety trials.
3. To provide advanced field and quality evaluation of experimental varieties.
4. To increase planting stock of promising experimental varieties for ultimate distribution.

Preliminary Quality Evaluation

Quality and agronomic data and observations were obtained from 32 genotypes. Most of this material was composed of introductions received from foreign countries in 1961 or 1962, selections taken in 1963 from the 1961 crosses, or older clones in the breeding block that have never been examined thoroughly.

The overall effort was somewhat disappointing. Most of the foreign introductions appear to lack adaptation to Oregon, particularly the Russian and Polish material is weak. This material will be maintained until we find out if desirable genes are available in some of the stocks. In addition, only two out of the eight 1962 selections harvested will be maintained; and only one of these shows any promise as a potential variety. As a result, only five lines were submitted for physical quality evaluation in 1966. Data are given in table 7. Results of physical evaluation are given in table 8.

Table 7. 1966 Summary of Brewer Inspection Sample Evaluation

Accession No.	Location	Picking date	%mc	At 8% moisture content				Yield Bales/A.	Drying time
				mg DM cone	ml oil 100 gm	% $\alpha_S$	% $\beta_S$		
I61/08	FOB 201	8/26	9.8	188	1.12	4.9	4.0	2	5.5
I61/09	" 202	8/23	10.0	208	1.02	4.4	3.3	2	4.5
I61/10	" 203	9/8	5.8	---	0.98	5.2	3.4	1	---
I61/11	" 204	8/26	10.6	226	1.03	6.5	4.6	2	5.2
I61/12	" 205	8/30	(25.3)	342	1.53	4.5	4.1	<1	---
I61/13	" 206	9/8	6.7	---	1.11	5.1	3.8	1	---
I61/14	" 207	---	---	---	---	---	---	-	---
I61/15	" 208	9/2	6.8	---	0.89	5.2	3.3	<1	---
I61/16	" 209	8/26	10.6	153	1.07	3.8	3.5	1	5.2
I61/19	" 210	8/23	10.3	148	0.55	5.3	2.3	4	4.5
I61/17	" 211	9/2	---	---	---	---	---	<1	---
I61/18	" 212	9/8	6.6	---	1.28	5.0	4.3	1	---
I61/20	" 213	8/26	9.8	---	1.23	5.9	2.4	6	5.0
I62/51	" 214	8/26	10.8	174	0.55	4.8	2.1	1	5.5
I62/52	" 215	---	---	---	---	---	---	-	---
I62/53	" 216	8/26	10.7	208	1.18	3.8	1.2	3	6.0
I64/106	" 219	---	---	---	---	---	---	-	---
I64/107	" 220	9/2	7.6	---	2.73	8.6	3.6	-	---
C50/54	BB 111-2	9/2	6.85	---	1.06	3.1	2.8	7	---
C19/94	BB 111-3	9/12	10.25	---	---	4.0	3.6	7	6.6
C19/20	BB 307	9/12	8.45	---	1.30	6.1	3.0	7	6.6
C19/80	BB 316-3	8/30	9.10	---	1.82	9.1	7.2	7	4.0
?	BB 508-2	8/26	10.8	---	1.03	5.9	2.4	11	6.0
C62/05	OB 805	8/26	7.63	---	1.66	9.0	4.5	7	5.0
C62/04	OB 806	8/30	8.85	---	0.55	4.8	1.7	6	3.7
C62/12	OB 808	9/12	10.00	---	0.77	3.3	2.3	6	6.6
C62/09	OB 809	9/2	9.65	---	0.97	5.3	4.0	4	5.9
C62/11	OB 811	8/30	8.85	---	0.71	6.3	3.4	3	4.8
C62/03	OB 835	9/16	10.05	---	0.41	5.3	4.0	7	7.0
C62/08	OB 839	9/12	8.95	---	1.41	4.9	6.5	7	6.6
C62/06	OB 840	9/12	8.70	---	1.41	4.4	4.7	6	6.6
C19/120	25-S	9/2	6.70	151	1.97	8.2	3.6	<1	---

Table 7 cont.

Access. No.	Pickability			Dry Shatter	Aroma	General Evaluation	Disposition		General Comments
	Cone	Vine	Overall				1966	1967	
I61/08	2	2	Fair	--	--	--		keep	
I61/09	2	2	Poor	--	--	--		keep	
I61/10	3-4	3-4	Poor	--	--	--		keep	Mite damage
I61/11	2	2	Fair	--	--	--		keep	
I61/12	2	2	Avg.	--	--	--		keep	
I61/13	3	3	Fair	--	--	--		keep	
I61/14	-	-	--	--	--	--		keep	
I61/15	3	3	Poor	--	--	--		keep	Hills 2 & 3 virus
I61/16	2	2	Fair	--	--	--		keep	
I61/19	2-3	2	Avg.	4	mild-med. sharp, heavy under.	Fair	USBA	Re-exam.	Golding
I61/17	2-3	2-3	V. poor	--	--	--		keep	
I61/18	3-4	3	Poor	--	--	--		keep	
I61/20	2	2	Avg.	5	mild-dull grassy	Fair	USBA	Re-exam.	Sav. Golding
I62/51	2-3	3	Avg.	--	--	--		keep	Defender
I62/52	-	-	--	--	--	--		keep	Density-green oil
I62/53	3-4	3-4	Poor	--	--	--		keep	Janus-low $\alpha$ -acid
I64/106	-	-	--	--	--	--		keep	Green oil
I64/107	2	2	Avg.	1	mild-pleasant str. estery	Good	Comm. variety		N. Brewer, Green oil
C50/54	3-4	3-4	Poor	--	--	--	Reject	Discard	Low $\alpha$ -good vigor
C19/94	2	3	Good	--	--	--	Reject	Discard	Low $\alpha$ flower, good cone & vigor
C19/20	2-3	1-2	Good	4	Pl. pepper, ester med-sharp	Fair	Reject	Discard	Hills 2, 4 & 5 virus
C19/80	2	2	Good	2	floral, spicy str. penetr.	Fair	Reject	Discard	Sev. cone DM
?	2	2	Good	2-3	mild off, spicy sl. rancid	Good	USBA	Re-exam.	Early
C62/05	3	2	Avg.	4	W.A. strong sharp, spicy	Fair	USBA	Re-exam.	Sl. cone DM
C62/04	5	4-5	V. poor	5	mild-off grassy	Poor	Reject	Discard	
C62/12	4-5	4	Poor	3	mild-off rancid	Poor	Reject	Discard	Low $\alpha$ -acid
C62/09	2	2-3	Good	1	mild-pleasant perfumey (rancid)	Poor	Reject	Discard	
C62/11	3	2-3	Poor	3-4	sweet-floral mild off	Poor	Reject	Discard	
C62/03	3	3-4	Poor	3	S-W.A. sweet floral	Poor	Reject	Discard	
C62/08	1-2	1-2	Poor	1	mild-pleas.	Fair	Reject	Re-exam.	Low seed content
C62/06	4	3	Poor	3	mild-off	Poor	Reject	Discard	
C19/120	2	2-3	Good	--	--	--		keep	



Table 8. USBA evaluation of 1966 experimental hop lines.

	<u>Appearance</u>	<u>Aroma</u>	<u>Analysis</u>	<u>Desirability</u>	<u>Potential</u>
19/209 (Fuggle) (5.9% $\alpha$ ; 2.4% $\beta$ ; 1.03 ml oil) (S)					
AS/PD	4	3	3	4	Ltd.
PWS	4	7	3	10	Ltd.
RTC	2	5	3	5	Ltd.
JBS	3	6	3	12	Ltd.
AGW	3	6	3	8	Ltd.
FJH	3	8	3	12	Unl.
RGW	1.5	5	3	4	None
RH	3	7	3	13	Ltd.
JBB	4	8	3	12	Unl.
LSG	5	5	3	7	Ltd.
61/20 (Savinja Golding) (5.9% $\alpha$ ; 2.4% $\beta$ ; 1.23 ml oil) (S)					
AS/PD	2	6	3	7	Ltd.
PWS	3	9	3	12	Unl.
RTC	0	3	3	2	Ltd.
JBS	4	9	3	12	Ltd.
AGW	3	6	3	8	Ltd.
FJH	4	8	3	12	Unl.
RGW	2	4	3	4	Ltd.
RH	3	7	3	13	Ltd.
JBB	2	6	3	8	Ltd.
LSG	5	7	3	1	--
61/19 (Yugoslav Golding) (5.3% $\alpha$ ; 2.3% $\beta$ ; 0.5 ml oil) (S)					
AS/PD	2	6	2	7	Ltd.
PWS	5	8	2	12	Unl.
RTC	1	4	2	2	None
JBS	4	10	2	12	Unl.
AGW	3	7	2	9	Ltd.
FJH	4	7	2	10	Ltd.
RGW	1	6	2	3	None
RH	3.5	8	2	13.5	Unl.
JBB	3	8	2	11	Unl.
LSG (evaluation form not found)					

Table 8 cont.

	<u>Appearance</u>	<u>Aroma</u>	<u>Analysis</u>	<u>Desirability</u>	<u>Potential</u>
BB 508-2 (5.9% $\alpha$ ; 2.4% $\beta$ ; 1.03 ml oil) (S)					
AS/PD	3	7	3	9	Ltd.
PWS	4	3	3	3	None
RTC	2	0	3	0	Ltd.
JBS	2	2	3	-	None
AGW	3	3	3	6	None
FJH	4	9	3	13	Unl.
RGW	3	5	3	5	Ltd.
RH	3	7	3	13	Ltd.
JBB	3	4	3	7	None
LSG	5	5	3	7	--

62/05 (OB 805) (9.0%  $\alpha$ ; 4.5%  $\beta$ ; 1.70 ml oil) (S)

AS/PD	3	10	9	15	Unl
PWS	4	7	9	11	Ltd.
RTC	3	9	9	12	Ltd.
JBS	1	0	9	0	--
AGW	3.5	8	9	10	Ltd.
FJH	1	2	9	3	None
RGW	3	0	9	0	None
RH	3	0	9	12	None
JBB	3	7	9	10	Ltd.
LSG	4	5	9	-	Ltd.

A preliminary yield trial of 15 entries was planted in the spring of 1966 in the new yard. The first data from this trial will be available in 1967. Plots are seven hills in size and there are five replications. Additional plants of each clone were established also for miscellaneous testing during the trial period. Following is a list of the experimental lines and varieties to be tested:

57/11 (OB 813)	19/137 (50-S)	48/209 (Fuggle H)
56/12 (OB 830)	56/08 (OB 826)	65/101 (Talisman)
56/13 (OB 831)	58/112 (OB 835)	19/01 (Brewers Gold)
19/20 (BB 307)	65/103 (E-2)	I 61/20 (Sav. Golding)
19/110 (15-S)	65/102 (Yakima Cluster)	I 61/19 (Yug. Golding)

Advanced Evaluation (SNB)

Small observation plots of Fuggle H and three advanced experimental lines were planted in several growers yards in 1966 (Table 9). Plantings were made under memorandum of understanding in cooperation with the Marion County Agricultural Extension Agent. The purpose of these plantings was to furnish observational notes on 2-year-old plants in 1967, since regular off-station tests will be in their baby year in 1967.

Table 9. Observation plots established off-station in Oregon in 1966.

Grower	Address	Number of hills of:			
		Fu H	OB 826	OB 835	OB 831
Krebs Brothers	Jefferson	12			
Robert Merten	St. Paul	7			
Dick Kirk	St. Paul			24	
Father Dominic	St. Benedict	24	24		24
Ralph Case	St. Paul	24			
Frank Fobert	Hubbard	6			
Carl Weathers	Salem	6	12	12	12
Robert Coleman & Sons	Gervais	24	12		12
Pokorny Brothers	Woodburn	13			
Stauffer Brothers <u>1/</u>	Hubbard			12	

1/ Stauffer Brothers have an older planting of Fuggle H

One inspection trip was made to most of the plots in July. Most of the plants were very small and will furnish no worthwhile observational data until 1967. The planting at Weathers grew relatively well and some hops were produced. All three experimental lines compared favorably with Bullion and Brewers Gold planted at the same time in the same site.

Larger two-acre commercial blocks are being planned for the three advanced selections 56/08, 58/112, and 56/13. One clone will be grown under contract by each of three selected growers in Oregon. Approximately 1500 one-year-old crowns of each of these three selections are available for planting.

Planting stock and management of the off-station trials will be controlled as strictly as possible. Hops produced from these trials will become the property of the United States Brewers Association. It is hoped that brewing trials will be made in 1968 and 1969.

Clonal Increase (SNB)

Approximately 2000 cuttings each of 56/08, 58/112, and 56/13 were planted to furnish rooted crowns for off-station trials in 1967. In addition, 275 rooted crowns of Fuggle H were produced to provide nuclear stocks in a certified root program on this variety. Rooted crowns of 56/12 (OB 830), Yakima

Cluster, Bullion, 57/11 (OB 813), 19/110 (15-S) and 19/137 (50-S) were produced to provide nuclear stocks for certified programs or to provide planting stocks sufficient to establish future yield trials.

Increase plots (4 hills) of most of the 1963 selections, and other material, were grown in the Smith Yard. Larger plantings of E-2, LC, YC (L-1), BG, Ta, Bu, Ha, Swiss, Fuggle H, and the three advanced selections were grown also. In addition to providing increase material, these plots will furnish hop samples under seedless conditions for comparison with seeded samples.

## BREEDING BEHAVIOR, GENETICS, AND BOTANY.

Genetic and Cytogenetic Investigations (AH)Polyploidy Breeding with Fuggle:

Major emphasis this year was placed on developing a tetraploid Fuggle from colchicine-treated material described in the 1965 report. Shoots from colchicine-treated material were rooted in a sand bed and rootings checked for ploidy level. Four cycles of selection (from softwood cuttings) were screened in 1966 and several clones at this point look very promising. It appears that a pure tetraploid Fuggle clone may be obtained during the coming year after at least one more screening cycle. Should a pure clone become available early in the year it will be crossed to a Fuggle-like male plant in order to produce a triploid.

Collection of Genotypes for Genetic Studies:

Several seedlings from the 1965 crosses which showed certain phenotypic traits were included in a genetic block in the new hop yard to be used for future genetic studies. Other genotypes in this block came from the 1959-63 nurseries in the old hop yard. Initially the material will be kept for observation purposes only and crosses to study the inheritance of certain traits will be made in the future.

Spider-mite Resistance Study:

Segregating progenies from three crosses were grown in a greenhouse soil bed under natural spider mite infestation. Plants were rated visually based on the amount of leaf damage. Some genotypes in this test look very promising and they were selected for further studies.

Meiosis in hops:

Microsporocytes which had been in cold storage for several years were analyzed and generally no difference was found in meiotic activity between the 8 AM and 11 AM samplings. The time of sampling, however, was inadequate and a sampling trial for meiotic activity in the male hop is planned for the next summer.

CR5-2 (OAES Bot.:36) HOP DISEASES  
THEIR ETIOLOGY, EPIPHYTOLOGY AND CONTROL.

C. E. Horner

Verticillium Wilt

Testing hops for resistance

Testing for Verticillium wilt resistance was expanded in 1966. A wilt nursery yard was constructed and planted to 23 varieties and selections listed below. These plants were grown from rooted cuttings. Both the soil and the roots were inoculated at planting time which varied from June 25 to July 28. Six to twelve plants of each variety were inoculated with each of five different strains of Verticillium. Three of the Verticillium strains were from hops and one each was from mint and potatoes. These are representative of the Verticillium strains present in Oregon and Washington. The experiment is so arranged that the Verticillium strains are physically separated and several plants of each hop variety are exposed to each fungus strain.

Varieties and Selections Assayed for Verticillium Resistance

Fuggle H	15-S
Fuggle (common)	25-S
Early Cluster	50-S
Late Cluster	OB-831
Bullion	BB-119
Brewers Gold	BB-120
Savinja Golding	BB-123-S
Density ) (Wilt	BB-217
Defender) resistant	BB-219
Janus ) from England)	BB-307
E-2(Skotland)	BB-317
	135-I

The planting was made too late to obtain good field symptoms of wilt. To get data on infection, stem sections were removed from the base of the stem, surface disinfected, chopped in a blender with sterile water, and assayed on special media to determine the number of Verticillium propagules. These results are shown in the following table.

The results suggest that isolate #148 obtained from dying Bullion hops near Woodburn, Oregon is the most pathogenic. This fits the field observations. No definite conclusions can be made from the meager preliminary data. We have, however, developed a wilt nursery that will be satisfactory for screening hops for wilt resistance. The nursery will again be inoculated with the same strains of Verticillium, plantings will be made in early spring, and sufficient plant materials have been propagated to conduct proper trials in 1967.

Results of Stem Assay for Verticillium Infection

<u>Verticillium isolate</u>	<u>Hop variety</u>	<u>Planting date</u>	<u>No. plants assayed</u>	<u>No. plants infected</u>	<u>Verticillium /gm of stem</u>
No. 146 Fuggle strain)	B. Gold	June 25	12	2	6200 800
	135-I	July 26	8	1	800
No. 148 Bullion strain)	Fuggle	June 25	12	1	8600
	Fuggle H	June 25	12	3	7000 4000 800
	E-2	July 5	12	3	14400 4000 3800
	Bullion	June 25	12	2	10000 7400
	BB-307	July 5	8	2	11000 2400
	135-I	July 26	6	1	5000
	S.Golding	July 26	6	1	12600
No. 138 Fuggle DM strain)	BB-119	July 26	3	1	2200
No. 95 Mint strain)	Bullion	June 25	12	1	11200
	Fug. H	June 25	10	1	1000
	E. Clus.	June 25	8	1	200
	BB-307	June 25	12	1	400
	Janus	July 26	6	1	200
	OB-831	July 26	6	1	1400
No. 119 Potato strain)	E. Clus.	June 25	10	1	400
	E-2	June 25	12	1	3800
	Density	June 25	12	1	400

Another test was made to determine if satisfactory screening for wilt resistance could be conducted by pot tests and to find the desirable soil inoculum levels to use in such tests. Rooted cuttings of Fuggle, Brewers Gold, Late Cluster and Janus varieties were inoculated with strains 138, 146, and 148 of Verticillium at levels of 500,000, 100,000, 20,000 and 0 (control) spores per gram of potting soil. The test involved 5 plants in each variety - strain - inoculum level combination (240 total plants). We obtained no results at all from this test and conclude that this method is unsatisfactory for testing wilt resistance in hops, even though it works well for testing mint, tomatoes, and other crops.

Prevalence and importance of Verticillium wilt in commercial hop plantings.

Through the Marion County Agents' office a questionnaire was sent to Oregon hop growers asking them to list the location, size, and age of any hop yards they wanted surveyed for diseases, principally Verticillium wilt. Eighteen growers responded, representing almost 2000 acres. We surveyed 24 yards totaling 588 acres.

Summary of Verticillium Survey

<u>Variety</u>	<u>No. yards surveyed</u>	<u>No. yards with Verticillium</u>	<u>Range of infection %</u>
Fuggle	9	8	7 - 56
Bullion	12	3	1 - 15
Brewers Gold	3	0	--

The results are based on recovery of the Verticillium fungus in laboratory assays of stem sections collected at regular intervals throughout each yard. The number of Fuggle plantings with some Verticillium wilt is very high. Combined results of our 1965 and 1966 survey show 15 of 17 Fuggle plantings infected, some with nearly 100% infection. Based on observations made regularly since 1957, we expect these infected plantings to show gradual decline in productivity.

Virus Diseases

Heat Treatment and Meristem Tip Culture:

Heat treatment alone and combined with meristem tip culture has been used to obtain virus-free clones of many vegetatively propagated plants, i.e. strawberries, stone fruits, chrysanthemums and others. Several experiments were performed to determine if these methods could be used to obtain virus-free clones of Fuggle hops.

A. Hot water rhizome soak.

Rhizomes of Fuggle were numbered and cut into two pieces, both having buds. One piece of each rhizome was labeled for treatment and the other as control.

The following treatments were made to each of 5 rhizomes in a laboratory hot water bath: 50°C. for 5, 8, and 10 min.; 52°C. for 5, 8, and 10 min. The treated plants and their untreated counterparts were then planted in the greenhouse. All plants emerged and grew and both treated and untreated plants showed similar virus-like symptoms.



### B. Dry heat treatment.

One hundred rhizomes of Fuggle were planted in gallon cans and placed immediately in heat chambers, 50 plants at 36°C. and 50 at 38°C. These temperatures were maintained for two weeks as plants emerged. Most plants emerged and grew at 36°C.; at 38°C. many plants did not emerge and those that did showed severe leaf distortion. All plants were removed to a greenhouse bench and observed. Most plants held at 38°C. emerged later and formed shoots. Of plants treated at 36°C., 48 of 50 survived; at 38°C., 35 of 50 survived. These plants were grown for 3 months and compared to their untreated counterparts for virus symptoms. Virus-like symptoms appeared in all plants, both treated and untreated.

### C. Meristem tip culture.

Plants surviving the 36 and 38°C. heat treatment were used as a source of meristem tips. Vegetative shoots about 10 cm. long were removed and brought to the laboratory. The leaves were aseptically removed from the shoot tip to expose the meristem. Meristem tips 0.2 to 1.1 mm. were excised and placed in several growth media which had been used successfully with other plants by various investigators.

Ten to fifteen meristem tips were placed on each of the following nutrient media in an attempt to obtain tiny rooted plants free from viruses.

1. Neergard's medium (Stone, O. M. *Annals Appl. Biol.* 52: 199-209. 1963)
2. Modified Neergard's (Hollings, M. *Ann. Rev. Phytopath.* 3: 367-396. 1965)
3. Neergard's plus coconut milk.
4. Neergard's with various amounts of NAA (1-10 ppm)
5. Various media used successfully for the growth of tobacco cells and tissue cultures.

The published procedures were followed exactly, but all meristem tips failed to form roots.

The results of these preliminary tests have been disappointing. Probably the most useful result has been the determination that 38°C. for 2 weeks approaches the lethal temperature-time for hops. This would be the treatment most likely to inactivate virus and still yield live plants for further testing.

Selections of outstanding Fuggle plants collected from growers' yards in 1965 were planted in observation plots along with high yielding Fuggle clones previously selected by Keller and Brooks. These have not yet been tested for freedom from virus infection. They could, however, form the nucleus of superior stock if it proves unfeasible to obtain known virus-free stock by other methods.

CR 5-4 (OAES AC 36) AGRONOMIC AND PHYSIOLOGICAL INVESTIGATIONS  
TO IMPROVE YIELD AND QUALITY OF HOPS.

C. E. Zimmermann

The two major objectives under this line project are (a) to develop cultural and management practices for growing commercial hops, and (b) to provide an understanding of the physiological processes associated with yield and quality of hops.

General problems associated with hop management have been minimized by technical knowledge and mechanization. Plant spacing, trellis heights and varieties have been stabilized during the last few years. Harvesting and processing is completely mechanized, though some improvements or changes occur from year to year. A mechanized stringing machine will be available for commercial use in 1968. Hops have been mechanically pruned for several years and recently a field loading device was developed which reduced the field harvesting crew by 50%. Increased mechanization is responsible for decreasing labor requirements during spring operations and harvesting.

Streptomycin and dinitro chemicals have reduced labor cost for the control of downy mildew and the stripping and suckering operation. Many growers use dinitro to eliminate the excessive vine growth after training as well as the sucker growth present later in the year.

Today, the problems associated with hop culture are related to the market situation whereby, the net return per acre is declining. The hop grower wants a higher price per pound, a lower cost of production and/or a higher production. There is a need for chemical weed control, for nutritional studies, for additional pesticides and for basic information of the environmental requirements for maximum production and quality.

In 1966 project studies included the following lines of work:

- (1) AC 36-3 Cooperative work with breeding and insecticide studies.
- (2) AC 36-5 (OHC) Studies of quality and production in seeded and seedless hops.
- (3) AC 36-6 (Merck) Study of endogenous gibberellins in hops.
- (4) AC 36-8 (USBA 20) Investigations into causes of cone breakage in hops.
- (5) AC 36-9 (OHC) Chemical treatment of paper hop string for rot resistance.
- (6) FC 36-9 (OHC) The use of herbicides on hops.

## AC 36-5 STUDY OF QUALITY AND PRODUCTION IN SEEDED AND SEEDLESS HOPS.

C. E. Zimmermann and S. T. Likens

Objectives:

1. To determine the effect of controlled pollination on yield and quality of seeded hops.
2. To evaluate different male hops with controlled pollinations.

Nature and extent of previous work:

There are conflicting opinions concerning the quality of seedless and seeded hops. A report by Williams <sup>1/</sup> states "There appears to be no significant difference in the percent of total soft resins, or preservative value of seeded, semi-seeded, and seedless hops." In 1964 the Wye College research Group <sup>2/</sup> used Fuggle with 14% and 2.5% seed and showed seedless had 20% higher  $\alpha$ -acid content than seeded. They also reported that mature seedless had twice as much oil as seeded. Wye reported in 1963 that the  $\alpha$ -acid in seedless Northern Brewer and Bullion was slightly higher than seeded.

The brewing industry recognizes a difference in quality and is willing to pay a premium for seedlessness, although this frequently fails to offset a corresponding production loss. It is well-known that a seeded hop adds the weight of the seed plus additional weight from fertilization-stimulated strigs and bracteoles. This may easily total 20 to 30%.

All data available in the literature recognizes only percentage values for  $\alpha$ -acid which reflects something about the lupulin and about the remainder of the cone. If the total amount of  $\alpha$ -acid is the same in seeded and seedless cones, the percentage would be less in seeded due to the larger mass or cone size.

Our studies in 1965 showed that the seeded Fuggle (18% seed) had 25% less  $\alpha$ -acid than seedless, but the seeded cone was 25% larger (see 1965 AR, p. 53-55). Therefore, the same quantity of  $\alpha$ -acid in seeded and seedless hops was expressed as being less percentage-wise in the heavier seeded hop. Seeded hops which resulted from controlled pollination were twice the size of commercially seeded hops, but had the same  $\alpha$ -acid percentage. Hence, the control seeded hop cones had twice the  $\alpha$ -acid content.

Procedure:

Brewers Gold and Fuggle were grown seeded, seedless, and control seeded. Production, pickability and quality were evaluated for both varieties. Control seeded hops were obtained with several different male hops. Practical application of controlled pollination was attempted on a commercial scale in Oregon.

Results:

Table 1. Production and quality of control seeded, seeded and seedless Brewers Gold and Fuggle hops.

<u>Fuggle</u>	<u>Cone Wt (mg)</u>		<u>% Seed</u>		<u>% <math>\alpha</math>-acid(DWB)</u>		<u><math>\mu</math>g <math>\alpha</math>/cone</u>	
	<u>1965</u>	<u>1966</u>	<u>1965</u>	<u>1966</u>	<u>1965</u>	<u>1966</u>	<u>1965</u>	<u>1966</u>
Controlled OP	229	185	21.2	12.9	4.88	5.51	11.2	10.2
Field OP	130	188	17.6	16.9	4.95	6.71	6.4	12.6
Seedless	96	108	0	0	6.38	7.21	6.1	7.8
<u>Brewers Gold</u>								
Controlled OP	257	300	17.5	18.7	-	13.33	-	40.0
Field OP	200	181	15.2	14.4	-	13.19	-	23.9
Seedless	133	164	0	0	-	13.98	-	22.9

Data summarized in Table 1 include results obtained in 1965-66. Field pollination (uncontrolled) increased Fuggle cone size 30% and 70% during the two years, but approximately 17% of this increase was contributed by seeds. Brewers Gold cones were similarly increased during 1965, but less during 1966. Controlled pollination doubled the seedless cone size of both hop varieties.

The  $\alpha$ -acid percentage was higher in seedless hops, but the quantity of  $\alpha$ -acid was greater in seed hops.

Pollen used in 1965 for controlled fertilization was obtained from males that were shedding pollen on the particular day required, without regard as to when they started shedding or to amount of pollen. In 1966 males were selected on the basis of vigor, mildew tolerance and flowering compatibility with Fuggle and Brewers Gold.

Table 2. Selected male genotypes for pollinating Brewers Gold and Fuggle hops.

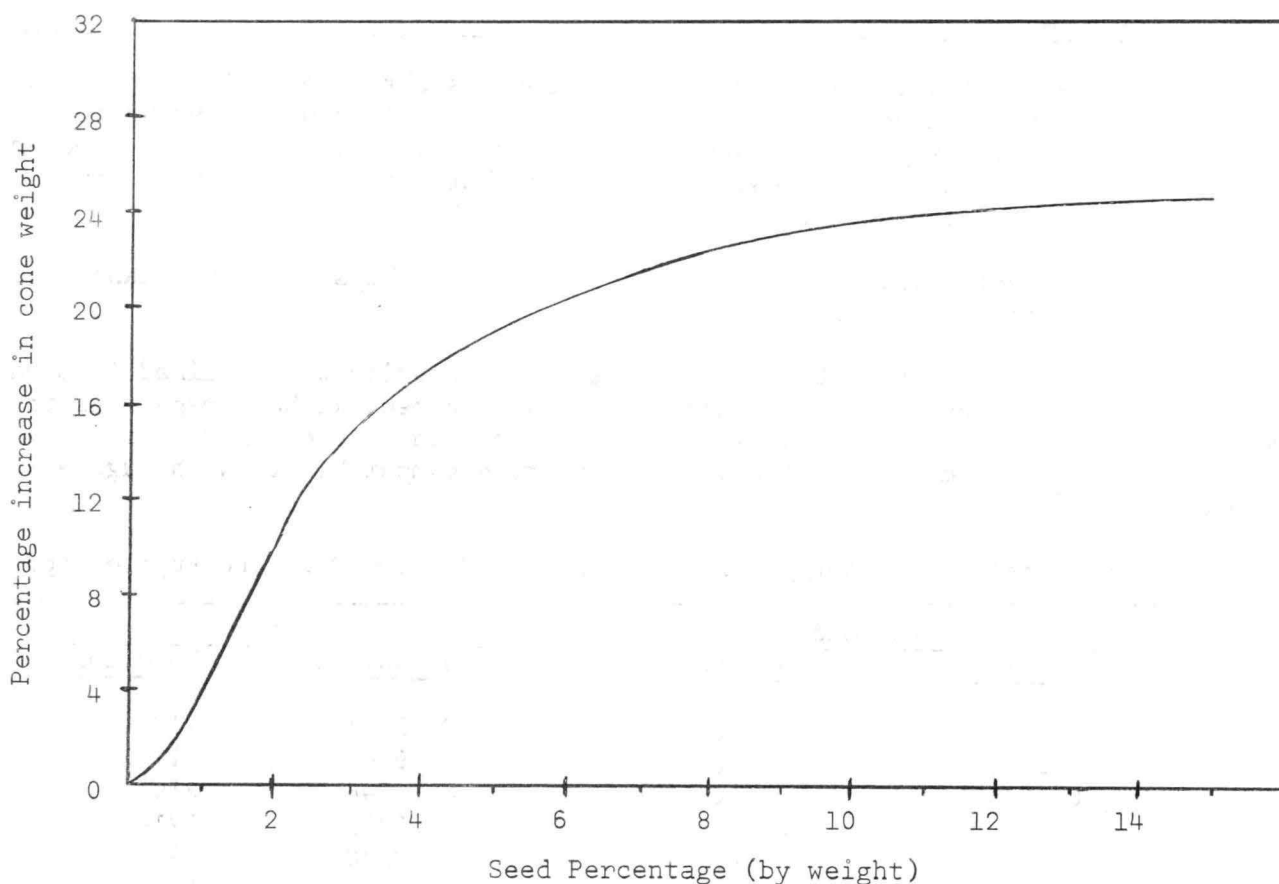
<u>Brewers Gold</u>			<u>Fuggle</u>	
<u>Acc. No.</u>	<u>Location</u>		<u>Acc. No.</u>	<u>Location</u>
I 19/05	118-4,5		C 51/61	219-4
C 19/57	119-4,5		C 19/41	317-2
C 19/50	217-1,2		C 19/46	319-4,5
C 19/43	318-1,2		C 58/11	321-4,5
C 19/47	320-1,2		C 19/40	421-1
C 52/40	518-2		C 52/45	521-2
C 19/10	521-4			

Discussion:

Hop production is based on the cone set and cone size. The number of cones per vine is apparently controlled by the genotypic-environmental interaction. The same controlling mechanism may function in determining the size of seedless

cones. Observations and data indicate considerable variation in cone size exists under seeded conditions, depending on the time and amount of fertilization. Seeded cone weight is comprised of the seed weight and the weight of the seedless cone, plus the growth stimulation associated with fertilization. We are concerned with the variation in growth stimulation of cones with the same seed content and cones with different seed contents. Seeded cones, containing 10% seed, should display an increased weight greater than seedless of the 10% seed contribution and an additional 20% growth stimulation of the strig and cone bracteoles. The problem exists with seeded cone weights which only reflect an increase due to seeds. Figure 1 is a proposed relationship of seed content and cone growth stimulation associated with fertilization.

Figure 1. Relationship of increased cone weight and seed content.



The values from figure 1 show a 2% seeded hop with a 10% yield increase and a 5% seeded hop with a 20% increase. This synergistic effect due to fertilization is not realized by the commercial grower. Data obtained from commercial yards follow the proposed yield curve at the lower levels of seededness, but fail at the high seed levels. Yields obtained with 12 to 14% seed are the same or lower than those at 4 to 6% seed. This apparent discrepancy is also noted with our

open-pollinated hops grown on the experimental yard. The variation is probably a result of the time interval required to fertilize the cones on a given plant. The maximum growth stimulation from fertilization is realized with a simultaneous pollination of the florets within an inflorescence. Year to year variation occurs with differences in flowering compatibility of the male and female which develop as a result of management practices and environmental conditions. Hop growers do not select males on any basis other than availability, therefore, males within a yard flower before or after females are receptive, males lack vigor and pollen-shedding potential, and others may shed some pollen over a long period of time. The growers problem is also complicated with the lack of uniformity in flowering of females due primarily to a mixture of genotypes within the Fuggle variety.

The ideal male for maximum seeded production should have the following characteristics:

1. Shed pollen 2 to 3 weeks after the initial burr stage of the female. This would occur about July 20 with Fuggle hops.
2. A maximum pollen shedding should occur initially. Therefore, male should be vigorous and possess good flower development.
3. Tolerant to downy mildew and verticillium wilt disease..

Male genotypes listed in table 2 were selected on the basis of their desirable characteristics. Genotypes listed under Fuggle are early males which could be used on other early varieties such as Hallertau and possibly Bullion. The males listed for Brewers Gold could be used on varieties such as Cluster and Talisman. Selected male varieties will be increased during 1967 for limited off-station planting this fall as a replacement of presently grown males.

Therefore, it appears that with compatible males and resultant controlled pollination, a two-fold yield increase above seedless yield is possible. Presently this yield increase is accomplished with hops containing 15% seed by weight. Commercial interest is confined to seedless or semi-seeded hops which are lower yielding. It may be possible to realize a yield increase with pollen stimulation and a low seed set by an abortion of the fertilized ovule or other incompatibility.

Alpha-acid percentage was noted to be higher in seedless hops as reported by English workers, but the amount of  $\alpha$ -acid per cone was higher in seeded hops (Table 1). This difference is often overlooked from the quality standpoint, in that brewers prefer seedless hops. Future emphasis on quality may stress the need for greater  $\alpha$ -acid production per acre.

If one assumes that seeded hops continue to be commercially accepted, it is of interest to suggest the commercial application of pollen to hop yards by mechanical means. Artificial pollination would have the advantage of providing: (a) controlled fertilization to gain maximum growth stimulation, (b) a control of specified seed set, (c) an increased yield by growing 2 to 10 additional females per acre, (d) a control of downy mildew, in that males often provide the inoculum source for field infection. In 1966 we pollinated a 35 acre commercial hop yard from an airplane with moderate success. The grower's yard was not uniform, therefore, flowering was variable at the time of pollination. The small number

of males present in the yard were not vigorous and probably were ineffective in contributing to the 8 percent seed set obtained at harvest.

AC 36-3 COOPERATIVE RESIDUE STUDY WITH PHOSPHATE INSECTICIDES.

Fuggle hops were treated with four different phosphate insecticides to obtain residue samples for L. C. Terriere, Agricultural Chemistry Dept. at Oregon State University. The following table is a list of chemicals and application rates used in the residue study.

<u>Chemical</u>	<u>lbs. active material/acre</u>	<u>Concentration</u>
1. Di Syston	1.25	10% Granular
2. "	2.50	"
3. "	5.00	"
4. Thimet	1.25	"
5. "	2.50	"
6. "	5.00	"
7. Dimethoate	.25	2 lbs/gal EC
8. "	.50	"
9. Phosphamidon	2.00	8 lbs/gal EC
10. "	4.00	"
11. Check	0	0

Granular Di Syston and Thimet was applied at the base of each hop hill, whereas Phosphamidon and Dimethoate was applied with a mechanical sprayer. Each treatment was applied to 20 hop hills (1/32 acre) on July 1, 1966 and plots were harvested with a mechanical picker on August 25. A six-pound green cone sample was obtained from each treatment and stored at  $-10^{\circ}\text{F}$ . A duplicate sample was dried at  $130^{\circ}\text{F}$ , baled the following day, and stored at  $38^{\circ}\text{F}$ .

Dr. L. C. Terriere's laboratory used the samples to develop an analytical method for determining phosphate residues in hops and to determine residue levels which were traceable to the pesticide application.

## AC 36-6 STUDY OF ENDOGENOUS GIBBERELLINS IN HOPS

Objectives:

See Annual Report, 1963, p. 59.

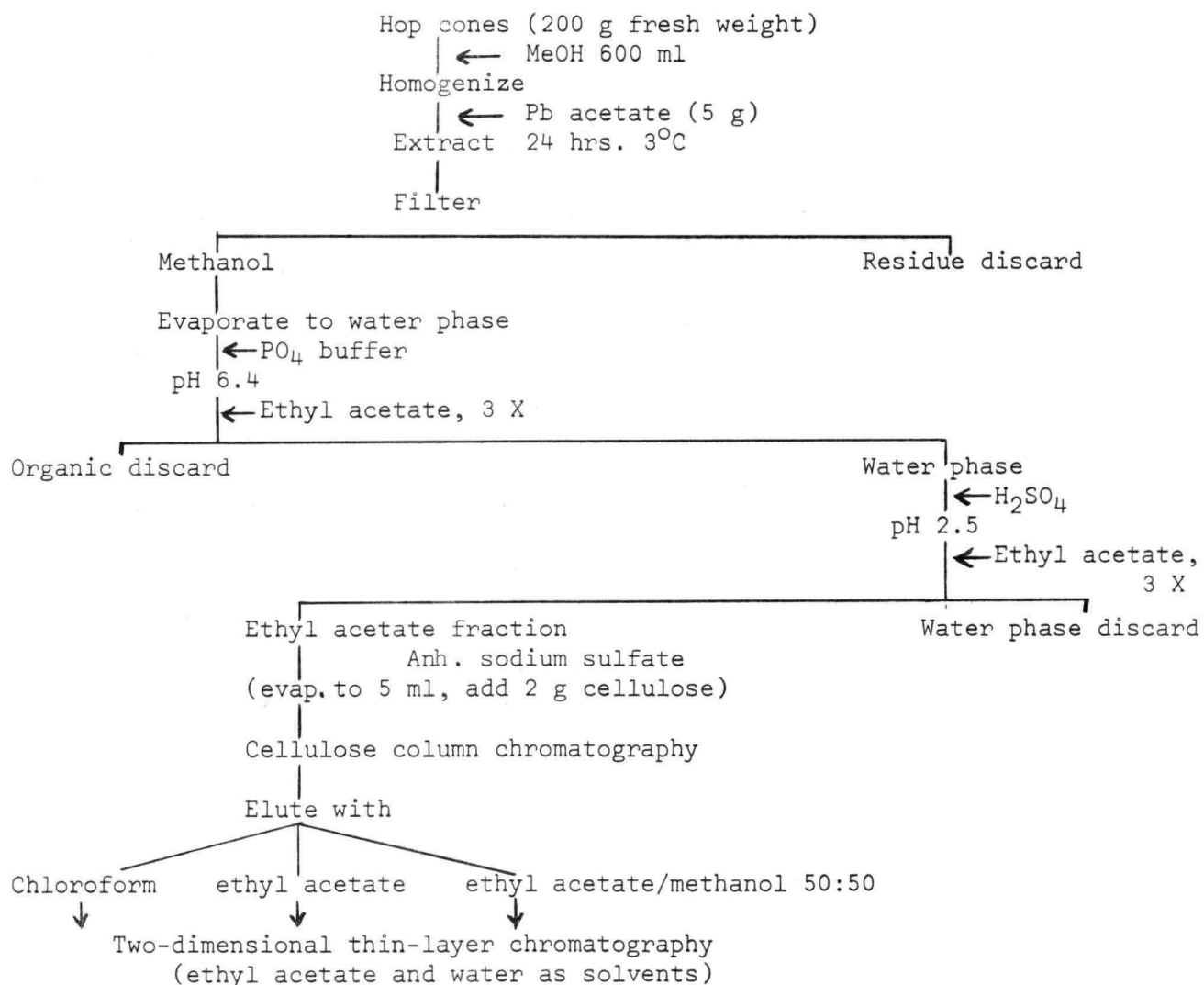
Reasons for undertaking study:

See Annual Report, 1963, p. 59.

Procedure:

The method of extraction and separation of gibberellin-like substances from hop cones is shown in a flow diagram (fig. 1).

Figure 1. Extraction and Separation of Gibberellin-like Substances.





Silica gel scraped from the thin-layer plate, after the two-way development, was eluted with methanol. Acetone and ethyl acetate (dry or saturated) are poor solvents for partitioning on silica gel. The evaporated fraction was activated in an 85%  $H_2SO_4$  -- 0.1% stannous chloride solution in preparation for fluorometric determination. A 50%  $H_2SO_4$  -- MeOH solution, commonly used for activation, caused an interference and lower sensitivity in the qualitative determination of gibberellins in hop fractions.

#### Results and discussion:

Seeded Fuggle cones sprayed with a 100 ppm solution of  $GA_3$  at 21, 14, and 7 days before harvest were extracted for endogenous  $GA_3$ . Untreated hops contained 2.4  $\mu g$  endogenous  $GA_3$  per 100 grams (green weight) on cones. Cones from hops treated 21 days before harvest had the same endogenous level as untreated cones. The endogenous  $GA_3$  level of cones treated 14 and 7 days prior to harvest was 3 times greater than the untreated cones.

An application of 10 ppm  $GA_3$  to seeded Fuggle, when vines were 10 feet in length, resulted in a  $GA_3$  residue level which was greater than the level from hops sprayed with 100 ppm  $GA_3$  21 days prior to harvest. The 10 ppm treatment was applied 75 days before harvest and resulted in a residue of 0.012 ppm in green hop cones. This residue could reflect an increase in endogenous  $GA_3$ , through an increased synthesis, rather than a residue which was traceable to the exogenous application.

An application of 50, 100, and 200 ppm of kinin stimulated seedless cone growth similar to that treated with 100 ppm  $GA_3$ . Chemical treatments were applied to cones shortly after the bracts were visible. Harvested cone weights were 20% greater than untreated seedless cones. The increased weight resulted from an increase in bracteole and strig size.

Treated cones had enlarged bracteoles, similar to those on seeded cones, and on openness which resembled seeded cones. The physical similarity to seeded cones may be an indication of the growth hormone stimulation associated with fertilization. This change in cone morphology could be used to alter the undesirable physical features of cones borne by hop genotypes which are otherwise beneficial.

## AC 36-8 INVESTIGATIONS RELATIVE TO HOP CONE PICKABILITY

C. E. Zimmermann

Objectives:

To evaluate cone morphology and features associated with cone shatter.

Procedure:

Cone shatter studies have been conducted during the last five years to obtain data on objective methods of evaluating shatter, on associated factors, and on cultural techniques. Two facts were obtained from this study (a) shatter resistance must be present initially in the hop variety, and (b) shatter resistant varieties only retain their advantage with good management.

Consequently, our breeding material and subsequent progenies are evaluated as to their pickability properties. This program will assure us of developing good picking varieties, but still retain the problem of cone breakage at the commercial level associated with drying, handling, and baling.

Discussion:

The following is a summary of the hop shatter program.

The hop cone being an inflorescence borne on the female hop plant is a unique agricultural commodity utilized by the brewing industry. The majority of raw agricultural products involved in commerce from crops are seeds, fruit, or plant products. Intact floral structures, such as the hop cone, are rarely used directly by industry without some type of processing. Hop cones are harvested, dried and packaged by growers for direct use by brewers.

The chemical components extracted from hops during the brewing process are primarily located in the miniature glands attached to the bract-like petals of the cone. Therefore, the cone itself should be considered as a package or container for a commercial product.

## Cone Composition of Seeded and Seedless Hops.

<u>Hop Class</u>	<u>Bract wt.(mg.)</u>	<u>Bracteole wt.(mg.)</u>	<u>Strig wt.(mg.)</u>	<u>Seed wt.(mg.)</u>	<u>Cone wt.(mg.)</u>
<u>Seedless</u>					
Fuggle	32.6	54.7	8.6	0	96
Brewers Gold	53.2	69.2	10.6	0	133
<u>Seeded:</u>					
Fuggle	35.1	58.5	13.0	23.4	130
Brewers Gold	56.0	92.0	22.0	30.0	200
Late Cluster	75.0	128.0	38.0	93.0	374

The four main components of the cone are bracts, bracteoles, strigs, and seeds. Each node on the cone strig has four bracteoles with each having one ovary and two bracts. The table lists the separate weights of the four main components of the seeded and seedless hop cone. Lupulin glands, as a component, are not listed separately, but included as an integral part of the bracts, bracteoles and seeds. A majority of the lupulin glands are located on the outer epidermal layer of bracteoles, a few are located on bracts, and an equal quantity can be found on the perianth cover enclosing the seed. Approximately 15 to 25% of the cone consists of lupulin glands, depending on the hop variety.

Increased cone weight associated with fertilization is a result of seed weight, bracteole and strig stimulation. Brewers Gold cones have about twice as many bracteoles as Fuggle, therefore, a greater increase in seeded cone weight is noted with Brewers Gold.

Hop vines grow to a length of approximately 20-25 feet and based on weight, are composed of 25-30% cones, 25-35% leaves, 25-30% vine and 15-20% lateral branches. An acre of hops produces approximately 12 to 20 ton of green vegetative material from which a grower can harvest and recover about one ton of dried hops. Though harvesting is by mechanical means, the process is compounded by the fact that 75% of the hop cones and leaves are located on the upper 1/3 of the strung vine plus top growth and the bulk of the cones are located proximally on the lateral branches.

Though the cone-like floral structure of hops is in nature fragile, the harvesting and processing by mechanical means is considered to be efficient under our mass-production operations.

Hop shatter or cone breakage has been a controversial problem during the last 25 years or since the advent of mechanical harvesters.

The affirmative point of view has been supported by the brewers with a focus on loss of quality. Hop growers are of the opinion that cone breakage (shatter) is a consequence of our present day production-line processing. The loss of hop quality associated with shatter was published 15 years ago and noted that broken cones contain 1/3 less  $\alpha$ -acid than whole cones. During this period commercial hops contained approximately 10% leaf and stem and 70% whole cones. Recently, growers have installed complicated recleaning machinery to reduce the extraneous material content in hops to less than 1%. Consequently, broken cones are also eliminated during the same operation.

Cone breakage in the past was directly associated with the picking operation, whereas today most of the shatter takes place after the cones are removed from the vine. Modern harvest equipment will return 90% whole green cones, but the grower will realize a 10% yield loss from cones left on the vine, discarded broken cones, and petals. Hand-picking and mechanical harvest have a comparable loss of green hops, but the leaf and stem content is greater in hand-picked hops. Cone loss during harvest is comparable with both hand and machine picking, but machines are capable of removing additional shatter and broken cones during the recleaning operation.

Today the majority of cone shatter is associated with Fuggle, Brewers Gold and Bullion hops grown under seeded conditions in the Willamette Valley of

Oregon. These 3 varieties when grown seedless are resistant to shatter, but normally yields are reduced approximately 30% when seedless. Stimulated growth associated with fertilization or seed set is reflected by an increase in bracteole size, strig size and seed content. A cone weight increase due to seed content accounts for about 50% of the total increase in weight whereas the remainder is due to an increase in bracteole size and strig elongation. The grower's loss in shatter is from the removal of bracteoles which bear the seed and also contain most of the lupulin glands. Varieties, such as Brewers Gold and Bullion or seedless hops tolerant to shatter, have a dense cone and the bracts overlay the bracteole. The bracts of Brewers Gold are wider and longer than the bracteoles, hence provide additional compactness to the cone, whereas the seedless Fuggle cone has a larger bracteole than Brewers Gold and when seeded the bracteole is greatly elongated.

#### Economic loss at grower level:

##### 1. Broken cones and petals and lupulin loss during picking.

Generally very few cones remain attached to the hop vine after machine picking due to intense picking force of present day efficient machines. The average force required to pick a single cone varies from one pound for Fuggle to 1 1/2 pounds for English and Cluster varieties. The picking force required increases from the base of the vine to the apex and also from the proximal to distal end of laterals. Therefore, the force requirement of cones on a single vine may vary two-fold. Since vines are picked free of all cones, the machines are so adjusted to pick the most securely attached cones. This task is accomplished at the expense of cone breakage and shatter of those cones which require a high plucking force and eventually detaching the laterals which bear unpicked cones. Broken cones, loose petals, lupulin, leaves and laterals become trash which must be separated from the commercial hop.

There appears to be a direct correlation between cone plucking force and lateral breakage, in that English varieties have less lateral breakage than Fuggle. This observation is confounded in varieties or hops grown with a dense top growth, which is an entanglement, and hence subject to excessive breakage when machine picked.

#### Possible considerations to reduce picking loss.

##### (a) Means to lower cone plucking force.

Seeded cones require an additional 20 to 50% greater plucking force than seedless. Seedless cones are easier to detach and have small bracteoles necessary for compactness and shatter tolerance.

A lower pluckability force was obtained with varieties grown on a 16 ft. trellis as compared with an 18 or 20 ft. height. This relationship probably indicates the association of shade or dense growth and succulent growth. Since low trellis height is associated with dense top growth, it is imperative to have an optimum trellis height and spacing which will be economical.

Succulent cones or turgidity result in low pluckability. This condition can be obtained with trellis height as discussed previously, with high humidity and/or low temperature during harvest, with early harvest, with adequate soil moisture near harvest, with adequate nitrogen nutrition and minor element balance, and a good cultural management to produce vigorous high yielding hops. Vines should be picked as soon as possible after cutting in the field and protected to prevent drying. It may be possible to prevent excessive shatter from over-ripe hops or dried out vines by applying a water mist just prior to picking, but this practice is only necessary because of high pluckability. One must remember that turgidity necessary to reduce pluckability will also lower the shatter resistance of cones.

Several hormones have been applied to hops at various stages of growth, but only gibberellic acid applied at the vegetative stage of growth was beneficial in reducing the plucking force by 20-30%. The hormone benefit was probably associated with the increased growth of the hop whereby shading and succulent growth were indirectly related.

#### (b) Adjustment of harvest machinery.

Broken cones, loose petals and lateral breakage is related to the plucking force of the cone, providing the striking force of the picking machine does not exceed the maximum requirement. The striking requirement of a machine will vary with variety and also during the harvest of a given variety, due to maturation, seed content and other factors which influence pluckability. Adjustment of the mechanical picker, such as speed, picking finger number, finger width, finger shape, and clearance between fingers, may be necessary during the picking season to reduce unnecessary yield loss.

#### 2. Cone breakage and lupulin loss associated with drying and baling.

Cone breakage associated with harvest is usually discarded with other extraneous material from whole cones with the aid of recleaners and dribble belts. Present day picking operations are capable of delivering 90% whole cones to the kiln floor void of loose petals and other material. Therefore, all operations prior to drying involve green shatter which is a direct economic loss to the grower. All operations from the kiln floor to the baler involve dried hops with a minimum loss to the grower. During this period, loose lupulin and sweepings remaining on the kiln and cooling bed are usually included in the hop bale. Therefore, the brewer's concern is for the broken cones and shatter which occurs during the handling and baling of dried hops.

#### Factors associated with dry hop breakage

1. High yielding hops display a wide variation in cone size and growers are required to dry hops to 8% moisture to meet brewer requirement. This low moisture content is obtained at the expense of over drying the small hop cones and by judging the excessive moisture content in the large cones for proper equilibration moisture during the cooling period. Moisture equilibration of cones is accomplished during the 1 to 5 day storage period in cooling bins, but over dried cones still must be handled from the kiln to the storage area.

2. Hop cones are normally removed from the kiln floor with hand scoops and dumped into a cooling bin at a lower level. Hops are piled 10 to 15 feet high in cooling bins prior to the baling operation. Removing cones from the kiln involves considerable cone breakage especially if cones are overdried or still warm from the drying process.

3. Hops held in cooling bins settle during the 1 to 5 day holding period and become compact piles. Cones are then moved from the cooling area to the baler with hand scoops, but occasionally it is necessary to use forks to loosen the piled cones prior to using the scoop. This entire operation will result in additional cone breakage due to excessive handling and enhanced by insufficient cooling or low cone moisture.

4. Prior to baling, cones which are broken during kiln removal, and subsequent removal from cooling bins, are included in the final bale package. The growers' only loss is the lupulin shattered from the cones, and this loss is primarily a reduction in quality and not yield. Hops are compressed into bales at a density of approximately 11 pounds per cubic foot. The density varies considerably without the use of measuring boxes, due to human error in judgement. Since the volume and weight are fixed, any variation in cone weight with similar cone size is not compensated for in the overall compression. Therefore it is possible to compress 30% more seedless cones by number, into a bale, than with seeded hops.

Possible considerations to reduce broken cones and loose petals in dried hops.

(1) Cone breakage during handling and baling by grower.

Dry cone shatter is directly correlated with cone moisture. Over dried hops are associated with the majority of dry shatter, since it is more difficult to replace moisture in cone strigs than in petals. Variation in cone size could be corrected with a size sorting device such as the type utilized by some Europeans. The sorted cones would require separate kiln floors, but could be efficiently dried on a continuous drying belt, such as the type developed in Europe. Breakage which occurs during handling of hops could be minimized by (a) removing dried hops from the kiln floor as a unit, similar to the rotating floors in England, instead of hand scoops, (b) drying hops to 9-11% moisture, instead of 8%, as was the practice 15 years ago, (c) provide adequate cooling bins for moisture equilibration, (d) remove hops from cooling bins, as a unit, with a movable back wall which moves the hops to a baling conveyor, (e) reduce bale pressure to compensate for differences in cone density (f) use a weight-measure box to maintain weight control.

(2) Quality loss realized by brewer.

A reduction in hop quality is related to both green and dry cone shatter, but the brewing industry is primarily concerned with cone shatter present in the hop bale. The brewer's viewpoint is based on the fact that the deterioration of hop quality during storage is enhanced by the presence of broken cones. The 1/3 reduction of  $\alpha$ -acid in broken cones, reported by Bullis in 1956, was probably an evaluation of cone breakage which occurred during harvest which was not discarded after picking. Prior to the installation of modern recleaning machinery, broken cones could not be eliminated from the hop bale; consequently the shatter observed by the brewer was primarily a result of green shatter which



occurred during the harvest operation. Modern harvest facilities are capable of separating and discarding any matter related to shatter. Therefore, today the bale shatter is primarily due to dry shatter which occurs during the handling and baling operation.

Discarding the broken cones and petals during the harvest operation is reflected in a yield loss to the grower, but it also returns a lower quality hop to the brewing industry. Lupulin is removed from hop cones during harvest by the constant motion of picking fingers, conveyors, recleaners and dribble belts. Additional lupulin is lost by the shattering of bracteoles from the cone. The discarded material from recleaners and dribble belts during the harvest of Fuggle contains approximately 2%  $\alpha$ -acid.

Bale shatter, particularly with Oregon grown hop varieties, is a problem which can be altered by the brewing industry more than by hop growers. The standards established by the brewers for Bullion and Brewers Gold hops are for high seed content and delayed harvest, both of which favor a high bale shatter. In addition, the decrease in cone moisture standard, during the last decade, and the standard density for baling high quality hops has lowered hop quality beyond what the grower is capable of producing.

Fuggle bale shatter is under similar influence but is related to additional conditions as well. The brewing industry demands are for more seedless than seeded Fuggle which, in turn, minimizes the bale shatter. Seedless Fuggle (less than 3% seed by weight) are more difficult to produce due to the presence of uncultivated male hops and the increased acreage of other varieties grown seeded. The Fuggle hop is also low yielding and growers realize a 30% production loss with seedless hops. Growers are inclined to produce Fuggle with 5 to 8% seed, instead of seedless, with the present marketing situation, but as a consequence increase bale shatter.

The morphological features of the Fuggle cone make it more susceptible to breakage than other commercial varieties. Shatter slowly increases with seed content to approximately 8% seed after which there is severe shatter and only a small increase in cone weight other than the additional seed weight. Brewers Gold and Bullion have a cone morphology which is tolerant to breakage and the threshold for seed content may be 10 or 12%.

#### Phenotypic characters of hop cones for shatter resistance.

##### 1. Ratio of bract-bracteole length greater than one.

Cluster and English hop varieties have ratios greater than one (approximately 1.25) when grown seeded or seedless, whereas seedless Fuggle cones have bracts and bracteoles of equal length (ratio 1.0) and the seeded cones have a ratio of 0.85. Brewers Gold and Bullion cones have bracts which are twice the width of Fuggle and contributes to the cone compactness. The openness of seeded Fuggle is also related to the strig internode length which is greater than other commercial varieties.

Bract-bracteole comparisons can be readily determined in the field and thereby provide a rapid assessment of genotypes for varietal improvement.

## 2. Descriptive strig configuration

- (a) Short internodes, shaped in the form of a quarter-ellipse or of small angularity.
- (b) Pedicel angle less than  $90^{\circ}$  from strig axis.
- (c) Overlapping bract scars which angle across strig.
- (d) Wide circular bracteole scar.

### Additional Considerations Concerning Cone Shatter

- 1. Influence of lower drying temperatures.
- 2. Effect of kiln-floor depth. Floors layed to a 30-36 inch depth are dried with a wide moisture differential from top to bottom.
- 3. Longer cooling period with deep floors.
- 4. Higher air velocity during drying.
- 5. Relationship of shatter to increased seed content.
- 6. Causes for cone openness after drying.
- 7. Utilization of hop waste.
- 8. Bale pressure based on a standard of volume reduction, instead of weight.



## AC 36-9 CHEMICAL TREATMENT OF PAPER HOP STRING FOR ROT RESISTANCE.

C. E. Zimmermann and Don Miller, Forest Res. Lab. (cooperating)

Objectives:

- (a) Determine rot resistance of paper hop string treated with copper naphthenate at various concentrations.
- (b) Investigate factors associated with decreasing wet strength of paper string treated with "Standard Wood Preservative" (SWP).

Reasons for undertaking study:

See 1965 Annual Report, p. 63.

Nature and extent of previous work:

See 1965 Annual Report, p. 63-64.

Procedure:

Table 1 lists the treatments of paper hop string used in the 1966 trial. Each treatment consisted of 50 strings (215 ft/lb. test) with 2 strings being anchored to a depth of 6 to 10 inches at each hill. The dry treatment consisted of soaking the strings two weeks prior to use in the trial, whereas the wet treatment included strings soaked in the field prior to use. The trial was established on a cool day with intermittent showers.

Table 1. Chemical treatments for rot resistance of paper hop string.

<u>Chemical</u>	<u>Conc.</u>	<u>Solvent</u>	<u>Soaking time(sec)</u>	<u>Type of <sup>2/</sup>treat.</u>	<u>Soil <sup>3/</sup>moisture</u>
1 SWP <sup>1/</sup>	Comm.	Comm.	15	wet	FC
2 "	"	"	30	wet	"
3 "	"	"	15	dry	"
4 "	"	"	30	dry	"
5 "	"	"	15	wet	irrig-I
6 "	"	"	30	wet	"
7 "	"	"	15	dry	"
8 "	"	"	30	dry	"
9 "	"	"	15	wet	irrig-II
10 "	"	"	30	wet	"
11 "	"	"	15	dry	"
12 "	"	"	30	dry	"
13 Cu Naphthenate	2.0%	Diesel	30	wet	FC
14 "	2.0%	"	"	dry	"
15 "	1.0%	"	"	wet	"
16 "	1.0%	"	"	dry	"
17 "	0.5%	"	"	wet	"
18 "	0.5%	"	"	dry	"

Table 1. cont.

<u>Chemical</u>	<u>Conc.</u>	<u>Solvent</u>	<u>Soaking time(sec)</u>	<u>Type of <sup>2/</sup> treat.</u>	<u>Soil <sup>3/</sup> moisture</u>
19 Cu Naphthenate	2.0%	Min.Spirits	30	wet	FC
20 "	2.0%	"	"	dry	"
21 "	1.0%	"	"	wet	"
22 "	1.0%	"	"	dry	"
23 "	0.5%	"	"	wet	"
24 "	0.5%	"	"	dry	"

- 1/ Standard Wood Preservative (SWP) is a commercial formulation which contains 69% aromatic oils, 20% tar acids, 10% petroleum oil and 1% pentachlorophenol. SWP concentration and solvent base was the same for all treatments.
- 2/ Wet treatment indicates that strings were soaked just prior to use. Dry treatment consisted of strings which were soaked in preservative two weeks before use.
- 3/ Trial was established when soil moisture was at field capacity. Irrig-I on treatments 5 through 8 included a one-inch sprinkler irrigation twenty-four hours after the strings were anchored in the soil. Irrig-II on treatments 9 through 12 included an additional one-inch irrigation 72 hours after establishment.

Paper hop strings treated with SWP in previous years displayed an untwisting phenomenon and subsequent reduction in wet strength which appeared to be related to soil moisture or rainfall at the time of anchoring in the soil. The two irrigation levels applied to treatments 5 through 12 simulated rain showers which occur in Oregon during hop stringing.

All treatments received the same cultivation and irrigation during the five-month trial. At the end of this period, each string was inspected and tested for tensile strength.

#### Results and discussion:

Paper strings soaked with the commercial formulation of SWP are resistant to rot when anchored in soil for five months. SWP-treated strings which were broken or had a reduced tensile resulted from untwisting of the string, whereas Cu-treated strings broke as a result of decay or rot.

Soaking time did not influence the breakage of SWP-treated strings nor did the wet and dry treatment alter the durability of the Cu-treatment. Therefore, these two variables were combined with their respective chemical treatments and the results tabulated as an average in Table 2.

Table 2. Rot resistance of paper hop string treated with various chemicals and at different soil moistures

Treatment	Chemical	Soil moisture	Percentage of broken strings at various tensile strengths		
			0 lbs.	<50 lbs.	>50 lbs.
1 & 2	SWP-wet	FC	5	17	78
3 & 4	" dry	FC	6	15	79
5 & 6	" wet	Irrig-I	10	16	74
7 & 8	" dry	Irrig-I	12	16	72
9 & 10	" wet	Irrig-II	12	17	71
11 & 12	" dry	Irrig-II	13	29	58
13 & 14	2% Cu-Diesel	FC	0	4	96
15 & 16	1% Cu-Diesel	FC	11	17	72
17 & 18	0.5% Cu-Diesel	FC	22	29	49
19 & 20	2% Cu-Min.Spt.	FC	0	12	88
21 & 22	1% Cu-Min.Spt.	FC	4	28	68
23 & 24	0.5% Cu-Min.Spt.	FC	27	39	34

Data from the SWP-treatments indicated an increase in broken strings which resulted from rainfall or additional moisture immediately following the stringing operation. Approximately 5% of the strings were broken near the soil level at the end of 5 months when anchored in moist soil and subjected to normal spring rainfall. String breakage doubled when subjected to additional moisture applied by irrigation sprinklers. As stated previously, this breakage is not caused by rot, but resulted from an interaction between SWP and moisture which caused the string to unravel and become a paper ribbon. This action can be eliminated if strings are anchored in dry soil and rainfall is restricted until 2 weeks following the stringing operation. Both of these conditions are rare during the spring operation.

Copper naphthenate dissolved in either diesel oil or mineral spirits to contain 2% copper metal was effective in preventing string rot. Lower concentrations were less effective.

#### Conclusion:

A publication was released during February, 1967 to the hop industry recommending a chemical treatment for hop string. The recommended practice was a 2% copper solution of copper naphthenate and diesel oil for use on paper hop string and a 1% copper solution for coir string. This line project was initiated in 1962 and terminated this year.

Additional questions were raised during 1966 concerning the use of a fire retardant on coir string and the chemical treatment of sisal hop string. Coir string is soaked in water prior to use; therefore a water soluble fire retardant, such as boric acid, is impractical. Fireproofing of coir would require a soak in 8% triphenylphosphate which is uneconomical, considering that the flaming practice can be eliminated with the use of dinitrophenol. Sisal hop twine was introduced in California in 1966 and string rot was

reported. Sisal and coir are comparable in their resistance to rot and it would appear that a 1% copper soak would give the necessary rot resistance when anchored in the soil.

## FC 36-9 THE USE OF HERBICIDES ON HOPS.

C. E. Zimmermann and W. R. Furtick

Objectives:

1. To screen herbicides for controlling shallow rooted weeds in 'baby' hops.
2. To evaluate herbicides for the control of deep rooted weeds, such as bindweed and quackgrass in established hops.

Nature and extent of previous work:

Wye College in England has reported satisfactory weed control with the use of two pounds of Simazine per acre and Paraquat at 1 ounce per gallon sprayed to run-off. Simazine was applied in late March or early April and Paraquat applied to persistent weeds by spot treatment during June. Propagation nurseries at Wye ('baby' hops) are treated with one pound active Simazine per acre as a standard procedure. The chemical is applied as a spray in a band along the rows immediately after planting. In 1965 an English grower reported widespread marginal scorching of hop leaves on 'baby' plants started from rhizomes. They noted the damaged vines arose from buds on the rhizome just below the soil surface. In the meantime, Wye recommends that growers using Simazine should plant their rhizomes with the tops below the soil level, particularly if the rhizomes are short, or if the buds are near the top end.

Wye college has also reported the use of 2 lbs. active Simazine in March and 3.25 lbs. active MCPB in August on 'baby' and established hops. The Simazine effectively reduced the annual weed growth, but leaving perennial weeds as couch grass, creeping thistle, and bindweed. An August application of MCPB effectively controlled these perennial weeds. No adverse effects were observed on hop growth, but there was a distortion of lower laterals due to MCPB.

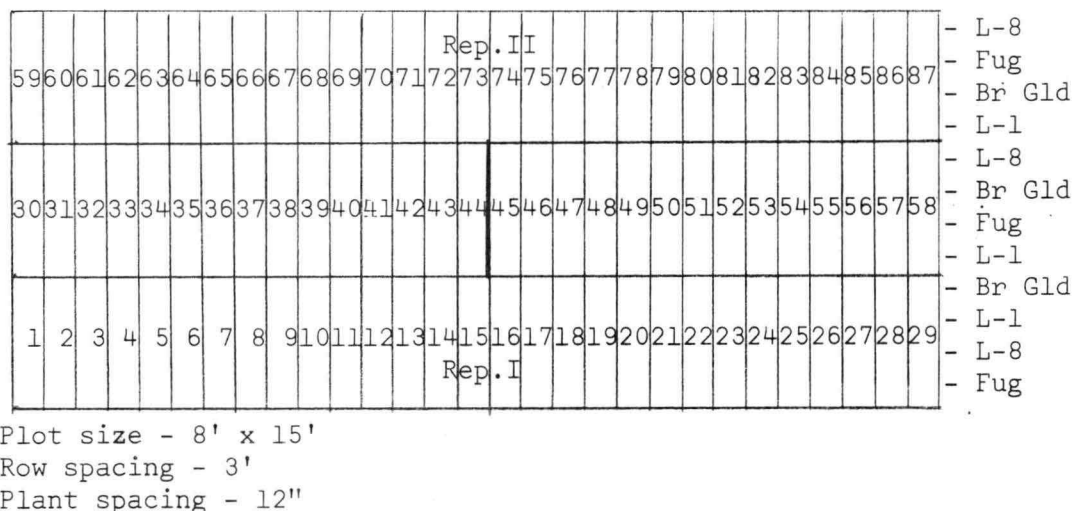
Wye recommends the use of 2 lbs. of Simazine on established hops and 1 lb. on rhizome plantings for annual weed control. Paraquat is used if perennial grasses persist and MCPB is recommended for bindweed control with application during the flower stage.

At OSU we have determined the effectiveness of 2 to 3 lbs. of Simazine for the control of annual weeds on established hops. DNBP which is registered for use on hops as a defoliant is also effective in the control of broad-leaf annuals, but it can not be applied until June or when hops are 8 feet in length. Trials at OSU also indicated that simazine was highly phytotoxic on Fuggle rhizomes planted at a shallow depth.

Procedure:

Fuggle, Brewers Gold, Yakima Cluster and L-8 hop varieties were planted in a herbicide screening trial during mid-December 1966. The experimental area was a split design with two replications. Hop rhizomes were spaced one foot apart in rows planted perpendicular to the spray plots.

Figure 1 Plot diagram for herbicide screening trial on new hop plantings  
Smith Yard, 1965



The pre-plant treatments were applied and incorporated into the soil just prior to planting. Pre-emergence treatments were applied a few days after planting and post-emergence sprays were applied during April, 1967 at which time hop shoots were 2 to 10 inches in length. Table 1 is a listing of treatments, rates, and plot locations for the screening trial.

An additional herbicide trial was initiated on an established hop planting consisting of six hop varieties, which included Fuggle, Brewers Gold, Late Cluster, 128-I, 135-I, and 144-I (Figure 2).

Figure 2 Plot diagram for herbicide trial on established hops, Smith Yard, 1966.

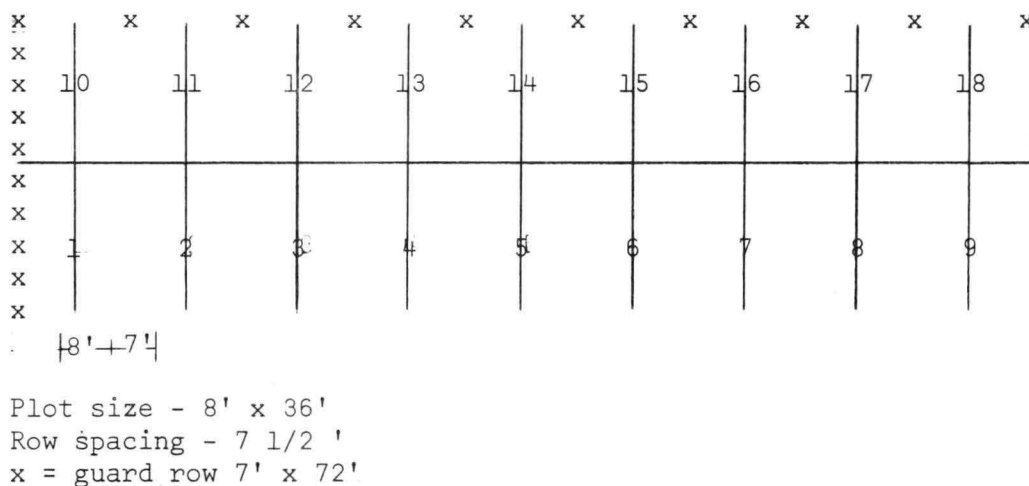


Table 1. Herbicide screening trial on new planting of 4 commercial hop varieties.

No.	Treatment	Lbs. active material/acre	Concen.	Materials for 130 sq. ft.	Reps.	
					I	II
<u>Pre-Emergence</u>						
1	Casaron	2	4%	45.2 gms.	22	56
2	Casaron	4	4%	90.1 gms.	12	70
3	CIPC	4	75%	7.2 gms.	35	48
4	CIPC	8	75%	14.2 gms.	33	51
5	Diuron	2	80%	3.9 gms.	3	52
6	Diuron	4	80%	6.8 gms.	14	75
7	Simazine	2	80%	3.9 gms.	42	60
8	Simazine	4	80%	6.8 gms.	41	83
9	Sinbar	1	80%	2.0 gms	29	46
10	Sinbar	2	80%	3.9 gms.	18	58
11	CP 50144	3	4 lb/A	8.5 mls.	21	85
12	CP 50144	6	4 lb/A	16.5 mls.	37	57
13	Sesone	4	90%	6 gms.	30	49
14	dicamba	4	4 lb/gal.	10.6 mls.	11	80
15	Tordon	2	2 lb/gal.	10.2 mls.	16	78
16	diphenamid	6	50%	16.2 gms.	34	45
17	diphenamid	12	50%	32.4 gms.	39	63
18	RP 11755	4	2.5 lb/gal.	18 mls.	5	74
19	RP 11755	8	2.5 lb/gal.	36 mls.	24	59
20	NIA 11092	2	50%	5.4 gms.	4	79
21	NIA 11092	4	50%	10.8 gms.	17	65
22	CIBA 6313	4	50%	10.8 gms.	26	87
23	CIBA 6313	8	50%	21.6 gms.	27	64
24	OCS 21799	4	4 lb/gal.	11.3 mls.	8	76
25	OCS 21799	8	4 lb/gal.	22.5 mls.	44	71
26	SD 11831	2	80%	3.4 gms.	15	50
27	SD 11831	4	80%	6.8 gms	13	72
<u>Post-Emergence</u>						
28	CIPC +	4	75%	7.2 gms.	40	67
	DNBP	3	3 lb/gal.	10.7 mls.		
29	paraquat	1/2	2 lb/gal.	2.8 mls.	10	53
30	paraquat	1	2 lb/gal.	5.7 mls.	32	81
31	dalapon	5	85%	8 gms.	7	54
32	dalapon	10	85%	16 gms.	1	61
33	2,4-D	1	4 lb/gal.	2.6 mls.	25	68
34	2,4-D	2	4 lb/gal.	5.7 mls	36	77
35	bromoxynil	1/2	2 lb/gal.	2.8 mls.	28	55
36	bromoxynil	1	2 lb/gal.	5.7 mls.	2	84
37	dicamba	1/2	4 lb/gal.	1.3 mls.	9	69
38	dicamba	1	4 lb/gal.	2.6 mls.	20	82

Table 1. cont.

No.	Treatment	Lbs. active material/acre	Concen.	Materials for 130 sq. ft.	Reps.	
					I	II
<u>Pre-Plant (incorporated)</u>						
39	treflan	1/2	4 lb/gal.	1.3 mls.	31	66
40	treflan	3/4	4 lb/gal.	1.8 mls	43	62
41	check	--	--	--	19	73
42	check	--	--	--	23	86
43	check	--	--	--	6	47
44	check	--	--	--	38	

Table 2. Herbicide trial on 6 established hop varieties.

No.	Treatment	Lbs. active material/acre	Concen.	Material for 304 sq. ft.	Reps.	
					I	II
<u>Post-Emergence</u>						
1	Sinbar	2 lb./A	80 wp	7.9 gms.	1	16
2	Diuron	4 lb./A	80 wp	15.9 gms	6	15
3	Simazine	4 lb./A	80 wp	15.9 gms	7	17
4	Diuron - fall	2 lb./A	80 wp	7.9 gms	2	11
	Diuron - spring	2 lb./A	80 wp	7.9 gms		
5	Sinbar - fall	2 lb./A	80 wp	7.9 gms	4	13
	Sinbar - spring	2 lb./A	80 wp	7.9 gms		
6	Simazine - fall	2 lb./A	80 wp	7.9 gms	9	18
	Simazine - spring	2 lb./A	80 wp	7.9 gms		
7	Simazine - fall	2 lb./A	80 wp	7.9 gms	5	10
	Diuron - spring	2 lb./A	80 wp	7.9 gms		
8	Mechanical	-	-	-	3	14
9	Check	-	-	-	8	12



Treatments listed in table 2 include fall applications, applied during late December, and spring applications applied during mid-April. At the time of application, the following weeds were present: annual bluegrass (complete coverage), common chickweed (50% coverage), scattered groundsel, scattered filaree, and sparse annual ryegrass. The plots were planted to wheat during October 1966 as winter cover crop. This cover crop was mowed during April 1967, and the treated plots will remain fallow during the growing season. Check plots will be maintained with normal cultivation during the season.

The herbicide screening trial will provide information on weed control, hop variety-herbicide interaction and other phytotoxic effects. Plots will remain uncultivated during the two-year duration of the trial. Effective treatments will be duplicated the second year.

The herbicide trial on established hops will provide preliminary yield data for an additional trial to be conducted in the fall of 1967.

#### Results and discussion:

Data will be obtained during the 1967 growing season. The two most effective treatments will be included in a cultural trial to establish a chemical practice for grower use.

CRE5-5 (OAES AC:36) CHEMICAL EVALUATIONS  
RELATIVE TO THE EVALUATION OF HOPS.

S. T. Likens

Line project CRE5-5 was reviewed in 1964 (AR p.53) and revised to accommodate four work plans:

AC 36-1 (USBA-8C) Development and Evaluation of Experimental Hop Lines:  
Chemical Investigations.

AC 36-2 Hop Extracts.

AC 36-3 Service Work for Cooperative Agronomic and Breeding Trials.

AC 36-4 Investigation into Analytical Methods and Miscellaneous,

INTRODUCTION

The economic pressure on Brewers to continually cut costs by taking advantage of technological advancement, has resulted in increasing acceptance of high-analysis varieties. With these, the hopping ratio and cost are substantially reduced. For example, brewing with imported Hallertau or Styrian with 5%  $\alpha$ -acid (\$1.00/lb.) costs about 30¢ per barrel, while an American Cluster at 7.5%  $\alpha$ -acid (\$0.50/lb.) costs about 10¢ per barrel and a Brewers Gold with 11%  $\alpha$ -acid (\$0.45/lb.) costs only 6.3¢ per barrel. If a 15%  $\alpha$ -acid hop can be developed, the cost could be reduced an additional 2¢ per barrel.

Since this is a world-wide situation it has been our belief that if the American hop grower is to continue to enjoy a healthy domestic and export market, he must be prepared to compete with high-analysis hops being developed at other hop research centers around the world.

Brewers are also becoming more receptive to the use of hop concentrates which can be used more efficiently than hops. In this way they can further economize on hop usage. There is a possibility that fresh (undried hops) could be extracted and concentrated at the farm level to produce a crude product. This could produce a substantial economy for the grower and the industry as a whole. Although no work along these lines was done this year, we feel it is sufficiently important that the work plan should not be dropped.

Service work for other projects was minimal in 1966, but our lab. facilities remain available for supporting analytical work.

A certain amount of work on analytical methods is necessary in order to maintain and improve day to day laboratory control. In addition to this, however, it is becoming increasingly apparent that analysis (especially for  $\alpha$ -acid content) may soon become an integral part of the marketing system. Brewers are continually increasing their demand for analytical values prior to selecting the lots they wish to buy. If a premium system based on  $\alpha$ -acid content is adopted, each 0.1% error in analysis will represent about \$10.00 per acre, or up to \$1500 per farm unit. Present analytical methods contain experimental errors up to 0.5% with no general attention being paid to improvement. We feel an obligation to apply effort toward improving methods and reducing analytical errors to a minimum.

CR5-5 (AC 36-1) DEVELOPMENT AND EVALUATION OF EXPERIMENTAL HOP LINES:  
CHEMICAL INVESTIGATIONS.

SUMMARY

Parental stock from the breeding block has been evaluated for  $\alpha$ -acid content and oil samples have been collected for permanent identification by their composition profiles (gas chromatographic). Highest quality female and male lines have been selected from the breeding block (9), advanced lines (8) and from nursery material (8). This list is comprised of 25 female lines ranging from 8 to 12%  $\alpha$ -acid. Of all these only C63/20 promises to exceed Bullion or BG in  $\alpha$ -acid content.

Sixteen crosses were attempted combining the highest quality genotypes available. Twelve were successful and have been planted into 3 inch pots to be germinated outdoors. They will be transplanted into the field without screening for downy mildew. The progenies will be searched for highest  $\alpha$ -acid lines.

Data from 1965 BG backcrosses indicated the quality of Brewers Gold is being retained. A larger group of samples was collected in 1966 for evaluation of the BG backcross but analyses are not completed.

A method for obtaining aromagrams from the vapors of crushed lupulin was described. The main advantage of aromagrams over lupulograms (injection of lupulin into the G.C.) is that the resulting recorder charts show the components in the same proportion as they occur during physical evaluation of aroma. Correlations with Brewers inspection samples have not yet been made.

Brewers inspection samples were analyzed as usual.

A manuscript has been accepted by J. Sci. Food and Ag. concerning varietal identification by gas chromatographic analysis of the oil. Another is being prepared. Additional oil samples from foreign hop varieties are being collected for future work.

SO<sub>2</sub> was applied to 3 lots of Fuggle during drying. The rates were 0, 1, and 2 lb. S/100 lb. green hops. Physical evaluation of these by Brewers indicated high rates of sulfur improved the appearance of the cones. No aroma differences could be detected. Presence of S-components could not be detected in aromagrams of lupulin from any of the rates.

Up to 17 S-components were detected in the oils of 5 varieties of hops by use of the Dohrman microcoulometer with a sulfur-titration cell. There are indications that annual differences may be quite high and may account for differences in physical evaluations. High rates of sulfur during drying produce no qualitative changes and there is no evidence of quantitative change. Total elemental sulfur in oil from 5 varieties of hops ranged from 61 ppm to over 700 ppm. This is of significance since Buttery has reported the odor threshold to be less than 1 ppb elemental sulfur from methyl thiohexanoate.

Objectives:

Since the USBA committee has the stated objectives of highest possible  $\alpha$ -acids and oils, the proposal for USBA-8C has set its present objective as:

Locating or assisting in the development of genetic material capable of leading to the production of a variety containing in excess of 15%  $\alpha$ -acid while retaining other desirable properties.

This is to be accomplished by evaluation of each of the steps involved in the introduction of new varieties.

1. Characterization of parental stock to insure that each has a potential for producing lines with acceptable quality.
2. Characterization of segregating populations for the most important quality characteristics in order to increase our understanding of methods of combining genetic material.
3. Evaluation of selections from the nursery in order to obtain early rejection of lines with unacceptable  $\alpha$ -acid content. This provides 3 years' data at the time of the first Brewers' evaluation and saves increasing and maintaining unsatisfactory material.
4. Following through on lines in advanced testing to establish  $\alpha$ -acid and oil potentials as related to maturation and processing.

#### Results and discussion:

##### PARENTAL STOCK

An analytical survey of the East Farm Breeding block was completed in 1965, prior to establishment of the breeding block in the new yard. Histograms of the distribution of  $\alpha$ -acid contents among the genotypes of both the old and new breeding blocks are presented in Figure 1. The new breeding block is an obvious improvement in its proportion of high quality genotypes (from the standpoint of  $\alpha$ -acid contents).

##### SELECTION OF HIGH QUALITY MATERIAL FROM BREEDING BLOCK

Table 1 lists the 14 highest  $\alpha$ -acid lines in the "old breeding block". These selections were based on the general survey of the 101 existing females which was completed in 1965. As a matter of checking, these were re-sampled in 1966. The same 6 lines ranked highest both years, and these would presumably be the most attractive for high  $\alpha$ -acid crosses.

##### SELECTION OF HIGH QUALITY MATERIAL FROM ADVANCED LINES

Table 2 lists the 8 highest  $\alpha$ -lines from advanced lines (1965 and 1966 data). Each of these has sufficient past data to conclude they are unquestionably of good quality.

##### SELECTION OF HIGH QUALITY MATERIAL FROM NURSERY LINES

Ten high analysis lines were selected from the 1963 nursery in 1965 (Table 3). These were re-checked in 1966 (Table 3) and one new selection was added (63N-48-2). Although the 1966 results are somewhat lower, none of these will be removed from the high-quality list since the plot was generally very weak (due to the absence of mildew or insect control).

The highest  $\alpha$ -acids (excepting Bu and BG) are among this group. All except C63/04 (a Hallertau cross) are either the first or second cross in the Brewers Gold back-cross program.

C63/20 has been the highest  $\alpha$ -acid in our program in 1965 and 1966. C63/08 was mixed with C63/09 in 1965 and the results were probably out of line. If C63/08 and C63/04 (the Ha cross) are removed from the high-quality list, the average  $\alpha$ -acid for the remaining 8 lines for the 2 years is 9.5% (at 8% M.C.)

The 2 Wild American crosses, C63/06 and C63/07, have entirely unsatisfactory cone characteristics, but will be kept on the high-quality list until they have been evaluated in the seedless yard.

#### SUMMARY OF HIGH-QUALITY FEMALE LINES AT OSU.

Nine lines from the breeding block representing a spectrum of germ plasm range from 7% to 11%  $\alpha$ -acid with a 1966 average of 8.3%  $\alpha$ -acid (at 8% M.C.) The highest is Bullion and the lowest is early Cluster.

Eight lines from varieties in advanced trials ranging from 7% to 10%  $\alpha$ -acid have averaged 9.0%  $\alpha$ -acid (at 8% M.C.). The highest is OB826 and the lowest is OB831.

Eight outstanding lines from the 1963 nursery range from 8.5%  $\alpha$ -acid to 11.0%  $\alpha$ -acid with a 2-year average of 9.5%  $\alpha$ -acid (at 8% M.C.). These are all results of crosses on Brewers Gold and only one (C63/20) promises to exceed B.G. or Bu in quality.

#### SELECTION OF HIGH QUALITY MALE LINES

The group of high-quality males (lupulin greater than 40%  $\alpha$ -acid) selected in 1965 were re-sampled in 1966. Results of 1966 samples were generally very low. We believe the sampling method employed this year was unsatisfactory. Each of the lines will be examined again in 1967.

FIGURE 1

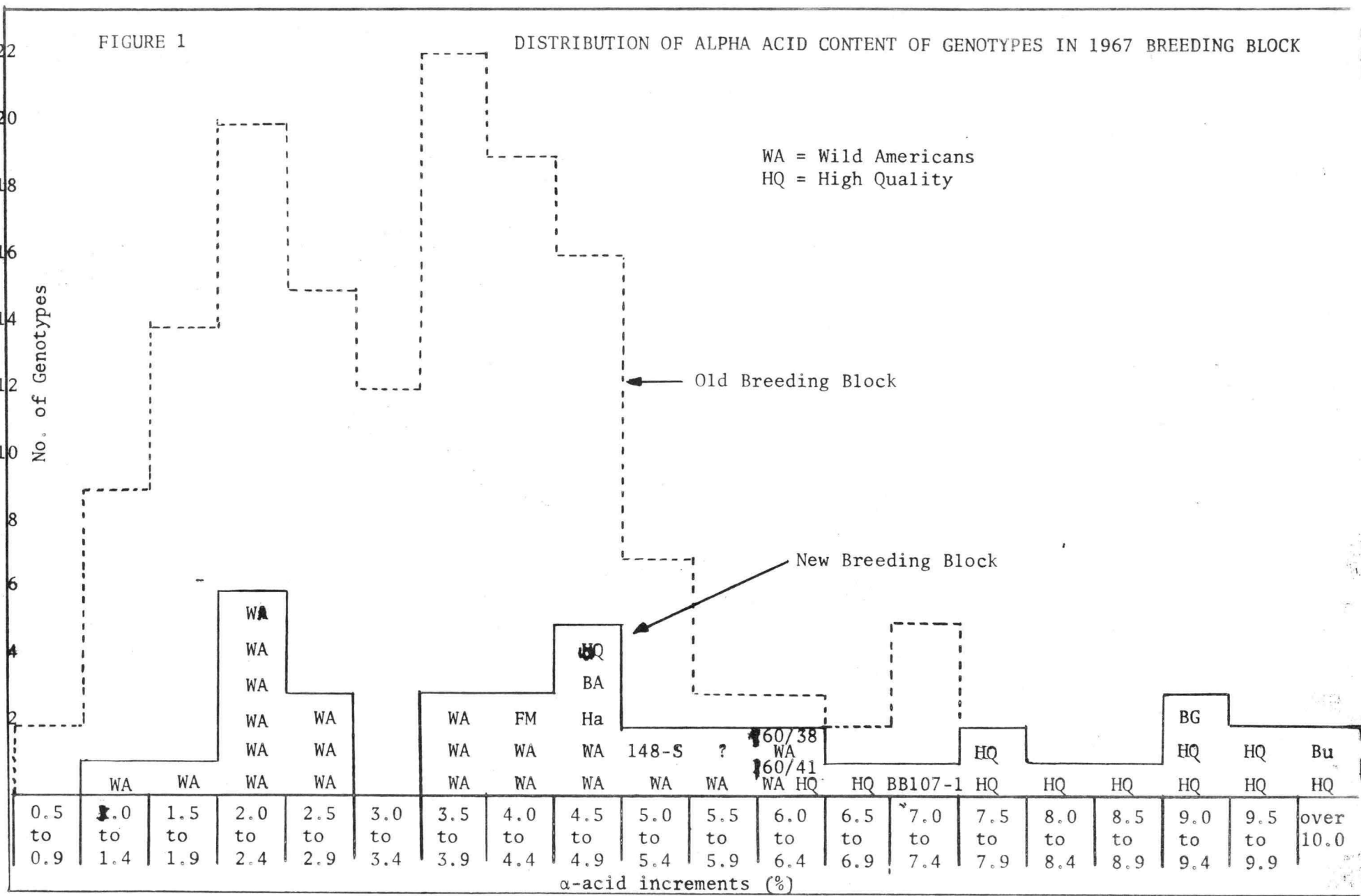
DISTRIBUTION OF ALPHA ACID CONTENT OF GENOTYPES IN 1967 BREEDING BLOCK

WA = Wild Americans  
 HQ = High Quality

No. of Genotypes

← Old Breeding Block

← New Breeding Block



0.5 to 0.9	1.0 to 1.4	1.5 to 1.9	2.0 to 2.4	2.5 to 2.9	3.0 to 3.4	3.5 to 3.9	4.0 to 4.4	4.5 to 4.9	5.0 to 5.4	5.5 to 5.9	6.0 to 6.4	6.5 to 6.9	7.0 to 7.4	7.5 to 7.9	8.0 to 8.4	8.5 to 8.9	9.0 to 9.4	9.5 to 9.9	over 10.0
	WA	WA	WA WA WA WA WA	WA WA		WA WA WA	FM WA WA	BA Ha WA WA	148-S WA	? WA	60/38 WA 60/41 WA HQ	HQ	BB107-1	HQ	HQ	HQ	BG HQ	HQ	Bu HQ

α-acid increments (%)

Table 1. High-quality selections from "old" BREEDING BLOCK (1965 and 1966).

Common Ident.	Access. No.	Year	Harvest Date	Cone wt. (mg)	Oil@ 8%MC	$\alpha$ -acid @8%MC	$\beta$ -acid @8%MC	Remarks
BB313(Bu)		65	--	--	--	--	--	Not established
		66	9/6	259	3.3	11.4	5.1	Good pick
18-S(128-I)C19/113		65	9/2	--	--	6.2*	--	
		66	9/9	179	2.2	10.1	4.6	Sick-sterile-discard
BB311(BG)	C19/01	65	9/1	--	--	7.5*	--	
		66	9/1	119	3.72	9.1	5.6	>11% $\alpha$ Smith yard
92-S	I19/162	65	9/2	--	--	7.3*	--	Sunshine seedl.
		66	8/30	212	0.90	9.0	3.0	Bis'65 8.5% $\alpha$ Easy pick
25-S	C19/120	65	8/28	--	--	7.2*	--	Sunshine seedl.
		66	9/2	151	0.97	8.2	3.0	Bis'65 8.5% $\alpha$ Reject from hi- $\alpha$ v. poor yield
50-S	I19/137	65	8/28	--	--	7.7*	--	Sunshine seedl.
		66	9/7	177	2.15	7.9	3.0	
BB122(LC)	I19/208	65	9/10	--	--	4.6*(?)	--	
		66	9/1	211	0.58	7.7	4.7	Med. pick firm cones
BB201-3	C19/81	65	9/2	--	--	6.2*	--	123-I
		66	9/9	204	0.92	7.5	2.9	
BB526-3	I60/41	65	9/10	--	--	6.4*	--	WA(Mont.)
		66	9/15	220	0.73	7.2	4.6	Poor
BB522(EC)		65	no data					
		66	9/13	167	0.95	6.8	4.4	Over ripe
BB113-2	C52/81	65	9/9	--	--	6.6*		
		66	9/15	187	0.86	4.4	2.3	Reject from hi-anal.
47-S	C19/135	65	9/2	--	--	6.2*	--	105-I
		66	8/30	114	0.47	3.2	3.7	Reject from hi-anal.

\* Conductometric analysis -- can be corrected to spectro by  $\alpha_S = 1.05\alpha_C + 0.32$

Table 2. Highest-analysis females from ADVANCED LINES (1965-66)

Common Identity	Access. No.	% $\alpha$ -acid in cone (8% M.C.)		% $\alpha$ -acid in lupulin (1965 only)	% lupulin in cones (1965 only)	Remarks
		1965	1966			
OB826	C56/08	10.3	8.9*	39	28	Sterile eval. = good
92-S	I19/162	10.2	9.1	--	--	Eval. = fair
OB835	C58/112	10.1	--	52	20	Eval. = good
25-S	C19/120	9.9	8.2	50	20	Sunshine seed. Eval. = good
OB813	C57/11	9.5	--	--	--	Eval. = fair-goo
BB301-2	C50/24	9.3	8.8	--	--	Eval. = fair
OB852	C60/07	8.9	--	--	--	Eval. = fair (discard)
OB831	C56/13	6.8	7.5*	26	33	Incl. because hi.lup. Eval. = good

\* From off-station observ. plots (Carl Weathers, Mission Bottom) 3 Sept.



Table 3. Highest analysis females from 1963 Nursery (1965 and 1966).

Common Ident.	Access. No.	Year	Harvest Date	Cone wt.(mg)	Oil@ 8%MC	$\alpha$ -acid @8%MC	$\beta$ -acid @8%MC	Remarks	
47-42	C63/20	65	9/13	288	3.1	11.2	5.6	BG,BC <sub>1</sub> Very dense	
		66	9/16	254	2.6	11.4	4.8	cone, little shatter	
44-9	C63/06	65	9/10	481	3.6	7.9		BG x WA big, ragged	
		66	9/13	534	2.4	10.7	3.8	cone, poor shatter	
47-40	C63/19	65	9/7	286	2.0	9.6	5.0	BG,BC <sub>1</sub> , good cone	
		66	Vine broke at base-unable to sample/						some shatter
48-1	C63/21	65	9/13	191	2.0	7.7	4.7	BG,BC, small fair	
		66	9/9	--	2.2	9.4	4.9	cone, DM cone	
48-2		65	No data, selected in 66						BG,BC, small good
		66	9/19	203	2.7	9.4	3.9	cone	
44-44	C63/07	65	9/10	311	3.4	9.7	5.2	BG,WA large ragged	
		66	9/9	524	3.2	8.9	4.1	cone	
48-21	C63/10	65	9/10	282	4.1	9.3	4.7	BG,BC	
		66	9/16	308	3.5	8.1	4.2	virus in '66?	
47-35	C63/18	65	9/7	--	3.0	9.7	6.3	BC,BC <sub>1</sub> , very similar	
		66	9/8	171	3.0	8.0	5.6	cone to Bu	
48-8	C63/08	65	9/16	293	3.8	9.0	5.5	BG,BC, mixed with	
		66	9/13	248	3.3	7.1	5.5	48-9 in '65 nice dry cone, excel dry shatt.	
41-34	C63/04	65	9/10	197	2.2	8.1	5.5	Ha,BC	
		66	9/2	273	1.8	6.5	4.8		
42-39	C63/05	65	--		0.8	6.8	--	Excel vig.	
		66	9/13		1.9	6.9	4.4	No G Shatt.	
39-14	C63/02	65	--		1.3	5.8	--	Good vigor	
		66	9/13		1.3	6.5	3.4	No G Shatt.	

## CROSSES MADE SPECIFICALLY FOR ELABORATING HIGH ALPHA-ACID STOCK.

Sixteen crosses were made combining the four best analysis females with the four best analysis males. In addition, one male with apparently high lupulin (visual estimate) was crossed onto B.G.

Female			Males used			
Brewers Gold (Smith)			48-15, 44-36, 47-32, 73-3, 39-9			
47-40 (63 nursery)			" " " "			
47-42 (63 nursery)			" " " "			
48-21 (63 nursery)			" " " "			

Ident.	Accession	Sex	% $\alpha$ -acid in:		Remarks
			(cone) <sup>1/</sup>	lupulin <sup>1/</sup>	
B.G.	I19/01	♀	9, 12	42	Recurrent parent in BG, BC
47-40	C63/19	♀	10, --	43	DM resist. 2nd gen. BG, BC
47-42	C63/20	♀	12, 12	46	Highest $\alpha$ . V. dense cone. 2nd gen. BG, BC
48-21	C63/21	♀	10, 9	?	1st gen. BG, BC. Diff ♂ parent than 47-40, 42
39-9	----	♂	-- --	--	Est. hi lup. DM resist.
48-15	C63/25M	♂	--	47	Hi lup. 1st gen. BG, BC. Diff ♂ parent than 47-40, 42, or 48-1
44-36	C63/13M	♂	--	53	Hi lup. 1st gen BG, BC.
47-32	C63/23M	♂	--	51	2nd gen. BG, BC. Same progeny as 47-40, 47-42
73-3	I60/30M	♂	--	47	W.A. hi lup.

Results of these crosses to date are:

1. Plant 47-40 broke off and all crosses were lost.
2. 3300 seeds from 9 successful crosses were planted and germination appears to be good. See CRE5-1 for details.

## EVALUATION OF BREWERS GOLD BACK-CROSS METHOD.

Last year analysis of many males and 11 females from the Brewers Gold back-cross indicated that the selected females had B.G. quality. This year we will get a much better evaluation of return of quality to the recurrent female (B.G.). A large number of samples have been taken from the progeny of the first and second crosses in the program. The samples are in storage and will be analyzed for  $\alpha$ - and  $\beta$ -acids as time permits.

Oils from 9 of the 11 samples collected in 1965 have been chromatographed (Figures 2, 3):

- 8 have definite methylgeranate peaks similar to B.G.
- 7 are well "balanced" oils similar to B.G.
- 1 contains little (if any) humulene.
- 1 contains excessive selinene.

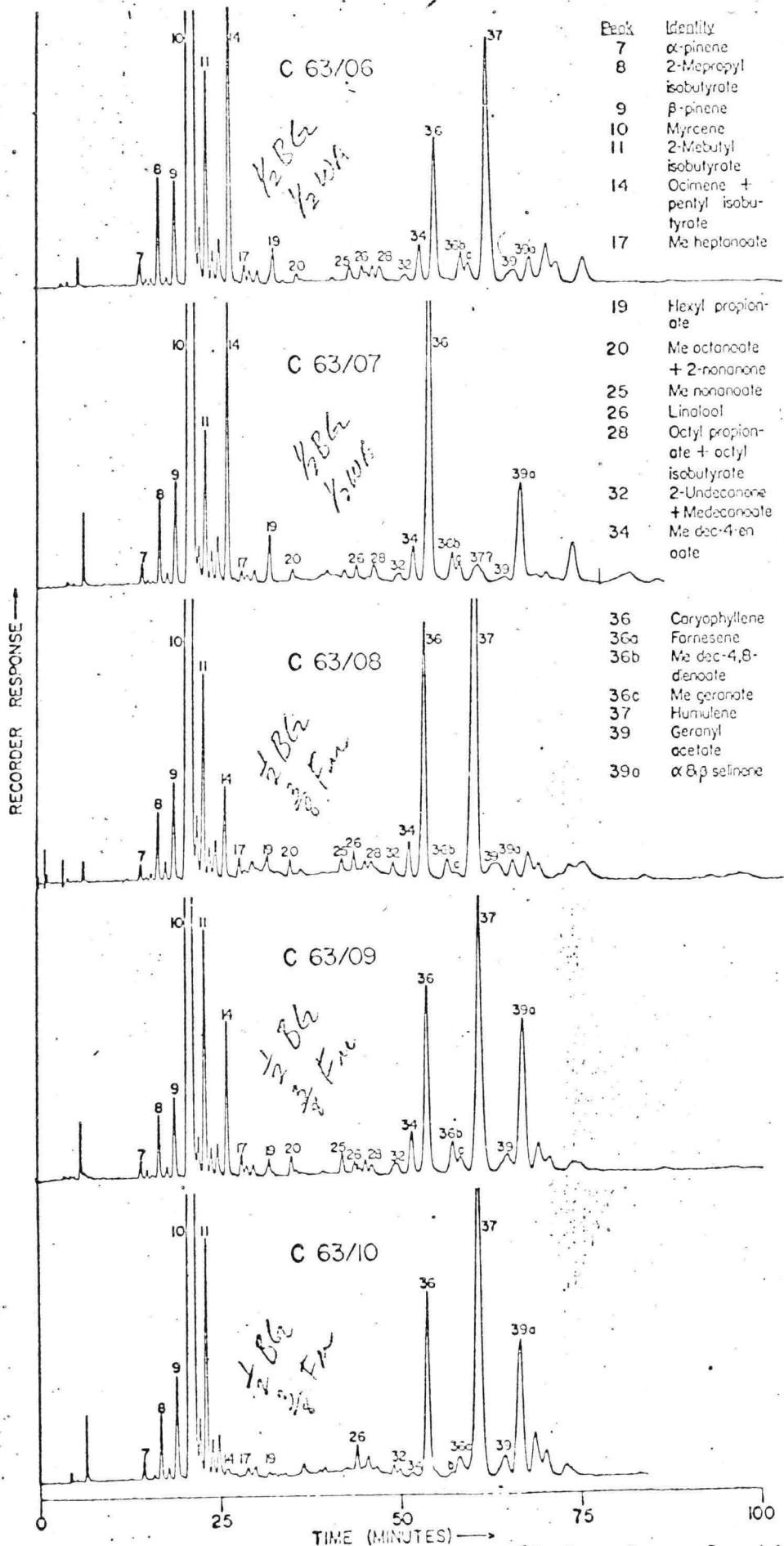


Figure 2. Oil Compositions of 1965 female selections.

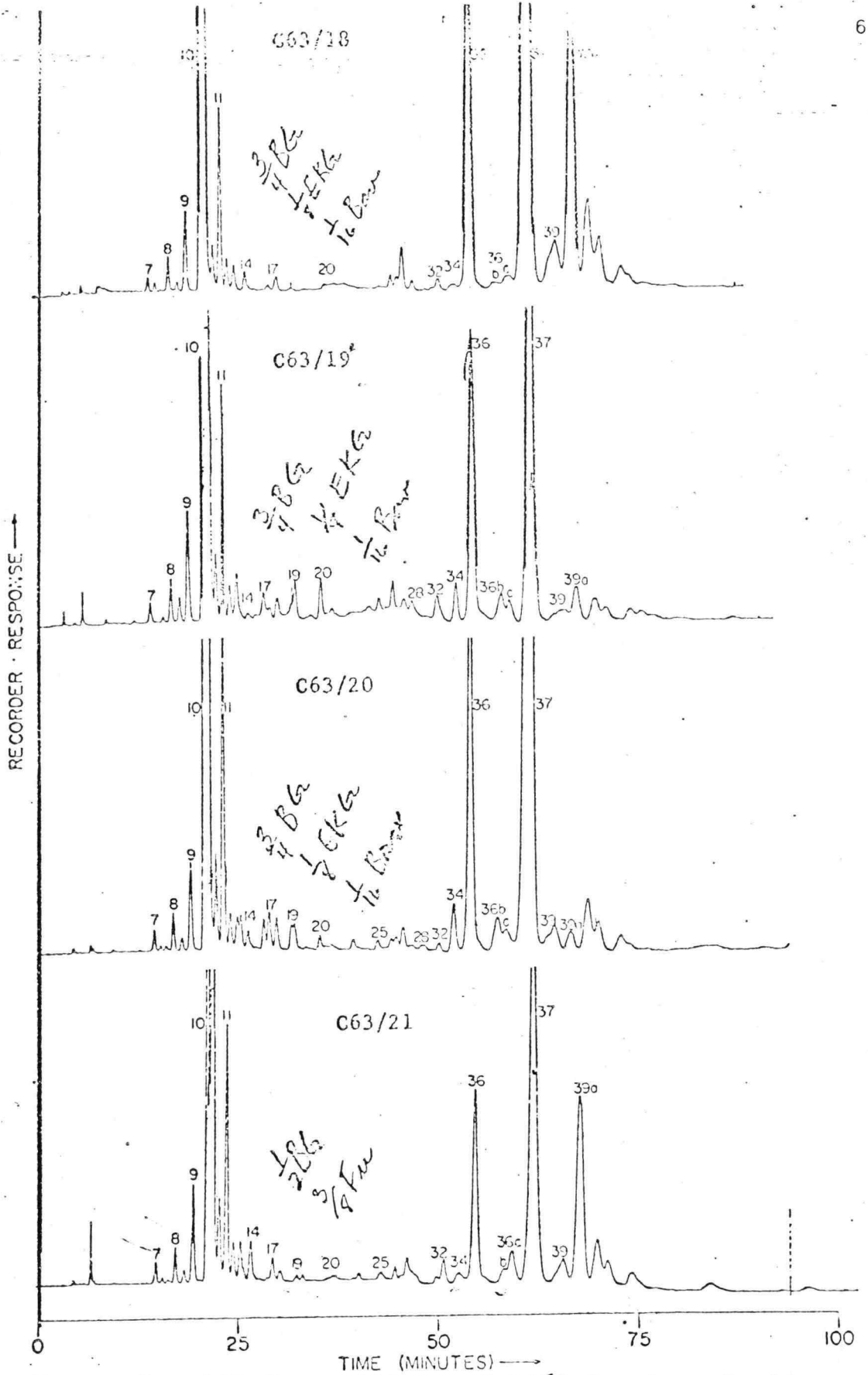


Figure 3. Oil Compositions of 1965 female selections.

The characteristic which separates Brewers Gold and Bullion oils from all others is the methyl geranate content. That 8 out of 9 back-cross progeny have this component indicates that the Brewers Gold back-cross will successfully retain the characteristics of Brewers Gold essential oil.

#### OFF STATION TRIALS

Only 2 samples of the off-station OBSERVATION plots were sampled for quality this year. These were OB826 and OB831 on Carl Weathers' Ranch. The data appear in Table 2.

The observation plots will be in their second year in 1967 and we plan to sample each for quality, etc. to provide preliminary data for the 2 acre plots to be sampled the following year.

#### YIELD TRIAL OF ADVANCING LINES

Dr. Brooks has 8 experimental lines and 7 commercial varieties planted in a replicated (5) yield trial. We plan to take quality samples from 3 replications of these in 1967.

#### VARIETAL IDENTIFICATION BY OIL COMPOSITION ANALYSIS

A 3-part paper on identification of hop varieties by gas chromatographic analysis of their oil compositions has been planned by Dr. Buttery and us. Part one, by S. T. Likens and G. B. Nickerson deals with the consistency of varietal characteristics under a wide range of environmental conditions and thereby establishes the reliability and confidence which can be placed upon a particular G.C. profile. Part two deals with specific varietal properties of oils as determined by capillary-column analyses. Part three is similar to Part two except that the analyses are by packed-column.

Parts one and two have been approved by Earl Stewart and have been submitted to J. Ag. and Food Sci. Data for part three has been completed and a manuscript is being prepared.

Oil samples from foreign varieties are now being collected and will eventually be entered into the identification system.

#### CORRELATION OF SO<sub>2</sub> APPLICATION DURING DRYING AND OCCURRENCE OF S-COMPONENTS IN AROMA OR OIL

At the March 1966 meeting the committee expressed interest in the possibility of undesirable aroma components resulting from the use of sulfur during drying. It was agreed that we would dry 3 lots of hops with 0, 1, and 2 lb. S per 100 lb. fresh hops. The plan was to evaluate these:

1. Physically for aroma with emphasis on S-compounds,
2. Presence of S components in aromagrams,
3. Chromatographically for specific S-components.

Dr. Buttery (WRRL) agreed to take major responsibility for item 3 ... detection and identification of S components in the aroma fraction.

The committee believed that this work should be done on the Cluster variety. However, Cluster hops were available only from commercial operations and the possibility of depreciating the value of \$2,500 worth of hops (one drier load) seemed too risky without some preliminary information. Instead, we used Fuggle, feeling that if SO<sub>2</sub> were absorbed by lupulin and incorporated into the aroma fraction in measurable amounts, that the data should apply as well to Cluster and could be checked later.

The samples were collected and dried according to plan (using SO<sub>2</sub> rather than burning S). The SO<sub>2</sub> was added during the first 60 minutes of drying and the rate adjusted by use of a needle valve and flow meter. About 1 lb. was left loose and several 1 lb. bales were prepared. All samples were put into polyethylene bags and stored at 0°F.

Bale samples were removed from the freezer immediately prior to physical examination by the U. S. Brewers Association on Dec. 12, 1966. Each committee member was asked to evaluate all three S-rates for color and aroma. The results are presented in Table 4.

Table 4. Color Evaluation of Sulfured Fuggle Samples by USBA Dec. 12, 1966.

Evaluator	Check	0.5 lb. S/100 lb.	1.0 lbs. S/100 lb.
L.S.Gimbel	diff. than other	no sig. color	-
F.Haas	may be best	most bleached	brightest color
J.Segal	looks best	-	nice hop
J.Bockelman	neutral	-	fairly good
R.Hansen	-	-	most "washed out" paler
R.Coleman	seems sulfur	-	seems sulfur
P.Stroh	-	normal appearance	more normal
A.Williams	too dark	-	lighter color
C.Horner	variable	-	uniform
G.Nickerson	-	darkest	lightest
R.G.Wright	no comments on color		

Aroma Evaluation of Sulfured Fuggle Samples by USBA Dec. 12, 1966.

L.S.Gimbel	no difference	no difference	no difference
F.Haas	fair flavor, best	not bad but strong	nice flavor
J.Segal	-	best aroma	poorest - not too good.
J.Bockelman	-	best fragrance	poorest
R.Hansen	good, penetrating	good	milder, not as sharp
R.Coleman	no difference	no difference	no difference
P.Stroh	next best	poorest	best
A.Williams	straw-rubber	straw-rubber	weaker, pleasant, best
C.Horner	-	similar to 1.0 lb.	similar to 0.5 lb.
G.Nickerson	nicest	slight straw	-
R.G.Wright	sharp	pleasant	best

Summary of USBA Committee's Remarks on Sulfured Fuggle Samples  
Dec. 12, 1966.

1. The unsulfured and 0.5 lb. S received variable comments on color and 1 lb. S was favored most.
2. The aroma of each S-rate was given "good" to "best" 4 times, indicating there was no clear-cut effect arising from its use.
3. No judge detected aromas of S-compounds.

Since no aroma preferences were noted by committee members it was decided not to run aromagrams.

## SULFUR COMPONENTS IN HOP OIL

A Dohrman microcoulometric detector with a sulfur titration cell was available for a few days so several hop oil samples were analyzed to give preliminary information on the extent of the presence of sulfur components. The samples were selected to provide data on VARIETIES, DRYING, USE OF SULFUR DURING DRYING, MATURITY, and VAPORGRAMS. Since use of the instrument was limited, the data are only indicative and considerable additional work will be required to draw firm conclusions.

Buttery has reported the presence of methyl thiohexanoate and methyl thioheptanoate in hop oil (his report No. 7). Thiohexanoate was reported at 0.2 to 0.6% (2000 to 6000 ppm.). Later (his report No. 8), a mixed peak of methyl thiohexanoate and methyl isooctanoate ranged from 0.1% to 0.3% while methyl thioheptanoate ranged from none to 0.01% of the whole oil.

Our data for methyl thiohexanoate indicates from 25 to 150+ ppm elemental S, or 0.01 to 0.07% of the thio-ester in a sampling of 5 varieties (component no. 11, Table 5, or peak No. 11 Figure 4).

Methyl thioheptanoate was present in much lower amounts ... <0.001 to 0.008% (component No. 13 Table 5, or peak No. 13 figure 4). These data compare favorably with Buttery's observations.

A total of 17 individual S-components were recognized among the 5 varieties. The minimum limit of detection was 2 ppm. elemental sulfur.

Buttery has said the odor threshold for methyl thiohexanoate and methyl thioheptanoate are 1 and 3 ppb respectively, which suggests that any of the 17 sulfur components could easily influence the aroma of hops. Total elemental sulfur for the 5 varieties ranged from 61 ppm for Bullion to 701 ppm for Brewers Gold. This indicates the magnitude of significance of these compounds, that is, combined, they represent from 100,000 to 1,000,000 times the amount necessary for detection.

Varieties:

The five varieties listed in Table 5 were run consecutively with a 1964 sample of Brewers Gold (seedless, Smith Yard) being re-run at the end of the series to insure that analytical reliability was maintained throughout the run.

The Brewers Gold sample was found to contain the greatest number of S-components and in the greatest amount. Fuggle was next and, surprisingly, Bullion contained the least. Early Cluster appeared to be slightly 'milder', from the standpoint of S-components, than late Cluster.

More within-variety variation occurs with S-components than with whole oil as indicated by the Fuggle data in Table 5 (1964 sample) and Table 6 (1966 samples).

A sample of 1966 Brewers Gold from the breeding block on the East Farm showed only methyl thiohexanoate as being present. This suggests that large



annual differences may be found within varieties, and may account for annual evaluation differences.

#### Maturity:

S-components in the oil of Fuggle and Bullion generally increase both in number and in quantity during the usual harvest period (Table 6). Total elemental sulfur increased by approximately a factor of 4 and is probably the first objective measurement of qualitative aroma fraction which accrues during the harvest period.

#### Drying:

Drying appears to increase the concentration of S-components by 20 to 30% (Table 7). This is undoubtedly brought about by a 15 to 30% decrease in the myrcene content during drying.

#### Use of Sulfur dioxide during drying:

SO<sub>2</sub> was applied to hops at the rate of 0.5 lb. S/100 lb. green hops <sup>1/</sup>. S-chromatograms (Figure 5) show no qualitative changes in the sulfur components of the oil. The slight increase in all components was probably due to loss of myrcene during drying.

#### Vaporgrams of lupulin:

Lupulin from 3 rates of applied S during drying of Fuggle (0, 0.5, and 1.0 lb. S/100 lb.) were prepared for vaporgrams (aromagrams) as outlined in AR 1965. No sulfur components were observed in any of the vaporgrams even when the instrument sensitivity was increased 4 times over the oil chromatograms.

#### Summary:

1. Seventeen sulfur containing components were exposed in hop oil. Only 2 have been previously recognized.
2. Within-variety variation may exceed among-variety variation. Annual differences in S components of the oil may contribute to differences in physical evaluations of aroma.
3. Instrument variation is very low.
4. S-components accumulate during maturation.
5. There is a slight increase in the concentration of S-components during drying.
6. There are no qualitative differences in oil from hops which have been dried with high rates of SO<sub>2</sub>.
7. No S-components could be detected in vapor over crushed lupulin from none to high rates of SO<sub>2</sub> during drying.

1/ BBS Experiment, R. Kerr Ranch, 1961.

Table 5. Sulfur Components in the Essential Oil of 5 Commercial Hop Varieties  
(see figure 4)

Component number	Retent. time(min.)	Estimated Element of Sulfur (ppm)				
		BG <sup>2/</sup>	LC <sup>2/</sup>	EC <sup>2/</sup>	Fu <sup>2/</sup>	Bu <sup>2/</sup>
1	6	99+,95+	0	0	40	0
2	9	80,78	8	1	8	0
3	22	10,12	0	0	0	0
4	23	57,56	7	6	4	6
5	26	15,16	0	3	3	0
6-7	27-28	52,54	30	16	22	13
8	32	20,30	0	0	4	0
9	36	25,26	12	6	11	4
10	38	0,0	8	0	0	0
11	39	142+,150+	92	25	144+	36
12	43	15,15	0	0	0	0
13	45	15,21	0	0	16	2
14	49	17,26	14	4	2	0
15	55	25,40	26	15	8	?
16	56	40,60	28	13	5	?
17	72	13,22	21	?	16	0
Total		625+,701+	246	89	283+	61

<sup>1/</sup> Estimated on basis of 10% recovery found for methyl thiohexanoate and methyl thioheptanoate.

<sup>2/</sup> BG, Std reference, Smith 1964; LC, Std reference, 1964; EC, East Farm, 1965; Fu, Std reference, 1964; Bu, Kerr Seeded 27A, 8/25/66.

Table 6. Effect of Maturity on S-components in Hop Oil <sup>1/</sup>

Component No. <sup>2/</sup>	Fuggle (ppm S)		Bullion (ppm S)	
	8/15	8/29	8/15	8/31
1	2	1	6	
2	1			
3				
4				4
5	5	1		
6-7	4	4	7	8
8		3		
9	4	14		7
10	0	0		
11	42	103	10	76
12				
13		28		7
14				
15		14		
16		6		
17		21		
Total	58	195	23	102

<sup>1/</sup> Calculated to ppm elemental sulfur on basis of 10% recovery from GC as noted for methyl thiohexanoate and methyl thioheptanoate (peaks # 11 and 13).

<sup>2/</sup> Component nos. as in table 5 and figure.4.

Table 7. Ppm Elemental Sulfur in Bullion Oil before and after Drying with 0.5 lb. S/100 lb. Green Hops <sup>1/</sup>

Component No.	Fresh (undried)	Dried (Hi S)
4	15	21
6-7	25	30
9	9	12
11	79	104
13	5	6

<sup>1/</sup> See footnotes, table 6

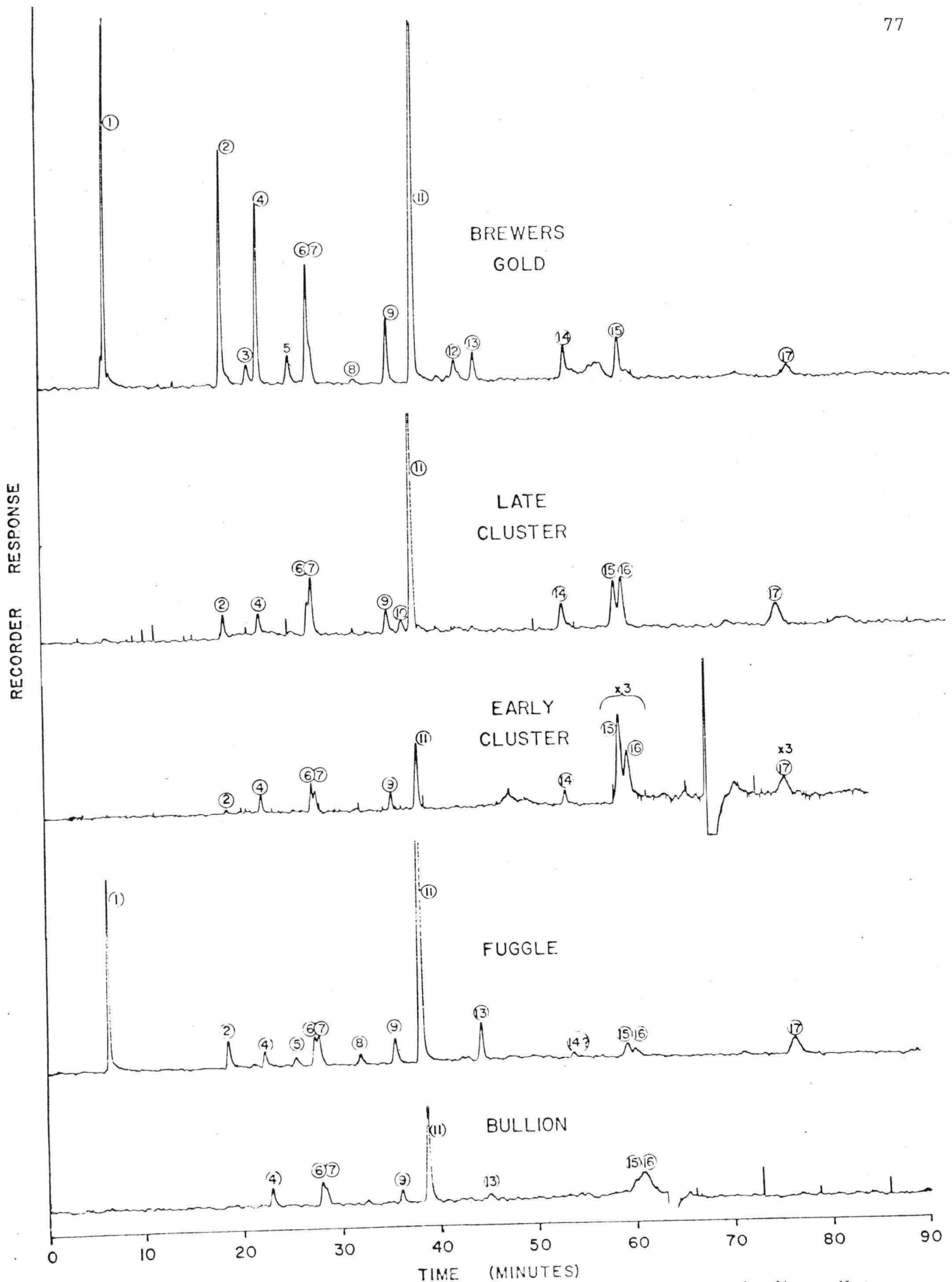


Figure 4. Sulfur components in the essential oil of 5 hop varieties. Note sensitivity is increased 3 times on peaks No.15, 16, and 17 of the Early Cluster. Conditions: 0.5  $\mu$ l sample, 1/8" x 25' aluminum column packed with 10% carbowax 20M. on 60/80 mesh chromosorb P. Programmed 60 $^{\circ}$  to 180 $^{\circ}$ C. @ 20/minute.

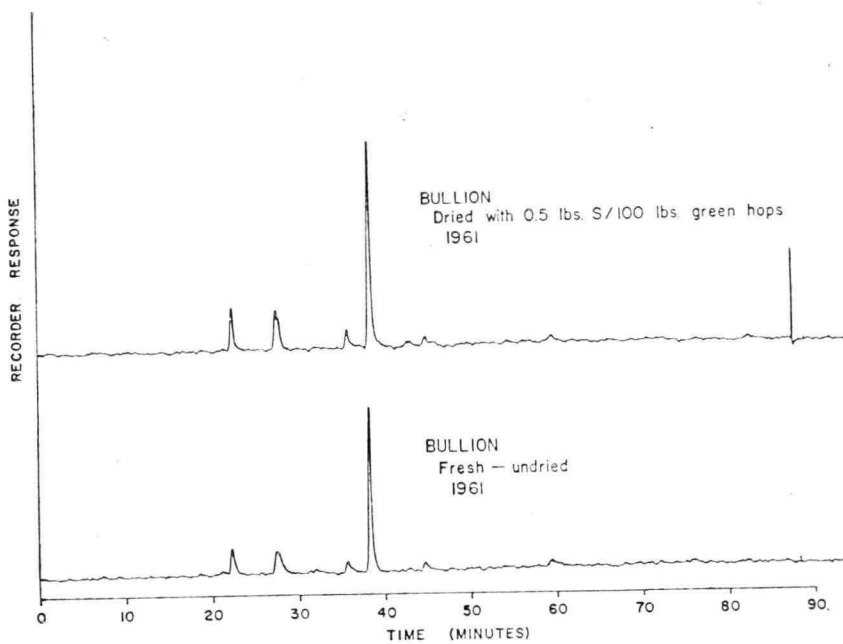


Figure 5. Effect of drying in the presence of  $\text{SO}_2$  on sulfur components in Bullion oil.

## CRe5-5 (OAES AC 36-2) HOP CONCENTRATES.

No new work was initiated on methods of preparation of hop concentrate. All concentrate work involved methods of analysis, most of which was done in collaboration with the Hop Concentrate Analysis Sub-committee of the American Society of Brewing Chemists.

Data concerning the ASBC committee work will not be included in this AR since it is of significance only when considered with data from other participating laboratories. A complete report has been prepared and distributed by the committee.

CRe5-5 (OAES 36-3) COOPERATIVE WORK WITH AGRONOMIC, BREEDING,  
AND DISEASE STUDIES.

Summary:

Five early Cluster selections and 33 "Hallertau-type" selections were analyzed for Dr. R. R. Romanko of the Univ. of Idaho at Parma. The Cluster samples ranged from 7.3% to 10.3%  $\alpha$ -acid and the "Hallertau-type" samples ranged from 3.1% to 5.2%  $\alpha$ -acid.

Results and discussion:

Thirty-eight samples from Dr. Romanko were received in September 1966. These were analyzed for moisture content and for  $\alpha$  and  $\beta$ -acids (Table 1). The samples were too small to obtain oil contents. Selection E.C.3 was outstanding in its  $\alpha$  and  $\beta$ -acid content and sample VII-73 had an exceptionally fine aroma.

Table 1. Analytical data on Idaho selections received from R. R. Romanko, September, 1966.

<u>Identification</u>	<u>%m.c.</u>	<u>at 8% m.c.</u>		<u>Remarks</u>
		<u>% <math>\alpha</math>-acid</u>	<u>% <math>\beta</math>-acid</u>	
EC-1	10.35	7.4,7.2	4.3,4.2	Good early cluster aroma.
EC-2	10.60	6.9,6.9		
EC-3	10.75	10.3,10.1	5.6,5.2	
EC-4	11.65	7.3,7.3	4.1,4.1	
EC-5	10.10	7.8,8.0	4.7,4.3	
I-47	7.85	4.9	3.8	
II-8	7.95	5.3	4.2	
III-7	7.85	4.9	4.2	
III-37	8.05	5.2	4.2	
III-49	7.95	4.3	4.0	
III-59	7.75	4.8	4.1	
III-59A	8.25	5.4	5.0	
III-60	8.10	4.9	4.2	
III-61	7.70	4.3	5.2	
III-69	7.50	4.6	3.9	
IV-20	7.75	4.7	4.2	
IV-28	15.10	5.4	4.8	
VI-35	7.65	4.8	4.0	
VI-53	7.80	4.9	5.0	
VII-26	7.50	4.6	3.9	
VII-28	8.00	3.6	2.8	
VII-54	7.70	4.4	4.2	
VII-73	11.25	4.8	4.1	Nice cones: aroma sweet, sl. spicy and fairly intense.
VII-80	7.90	4.3	4.0	
VIII-62	7.70	4.8	3.9	
VIII-69	7.55	4.7	4.4	
VIII-78	7.50	5.0	4.3	

Table 1 cont.

<u>Identification</u>	<u>% m.c.</u>	<u>at 8% m.c.</u>		<u>Remarks</u>
		<u>% <math>\alpha</math>-acid</u>	<u>% <math>\beta</math>-acid</u>	
IX-43	7.80	4.9	4.7	
IX-59	7.95	4.7	4.2	
IX-62	8.00	3.6	3.5	
IX-63	7.70	4.6	3.7	
IX-65	7.75	4.7	4.0	
IX-66	7.90	4.3	3.5	
IX-68	7.55	4.9	4.2	
IX-69	7.65	5.0	4.3	
IX-71	8.15	4.8	4.2	
IX-72	7.55	4.4	3.9	
IX-75	7.90	3.8	3.1	

Notes: 1. Spectrophotometric method using toluene solvent. 2. All  $\alpha$ - and  $\beta$ -acids calculated to 8% moisture content. 3. Duplicate extractions on early Cluster selections. 4. Single determinations on Hallertau selections. 5. Most Hallertau had a slightly sour aroma and most shattered badly -- individual observations not recorded.



CRe5-5 (AC 36-4) INVESTIGATION INTO ANALYTICAL  
METHODS AND MISCELLANEOUS

SUMMARY

The possibility of using lupulin for indicating maturity was evaluated on the varieties Bullion and Fuggle. It was found that the  $\alpha$ - and  $\beta$ -acid content of lupulin from both varieties was essentially constant throughout the season and would be useless as a maturity guide. The oil content of lupulin from both varieties followed the same accumulation curves as the oil content of cones. Since the oil content of lupulin can be determined in a much shorter time and on a much smaller sample, such an analysis is very appealing for indicating maturity. More detailed information on sample size and source need to be accumulated in 1967.

An appraisal of the conductometric method of  $\alpha$ -acid analysis indicated that it gives lower values on fresh hops than the spectrophotometric method. Higher values are obtained by the conductometric method when applied to aged hops. At some point in the deterioration process, the two methods yield similar results. The relation between the methods provides an indication of the degree of deterioration.

It was found that the addition of pyridine to the titration mixture to the extent of 1% raises the conductometric value by 5 to 10% and is sufficient to bring it into agreement with spectrophotometric values ON FRESH HOPS. As deterioration progresses the two methods diverge ... conductometric being higher. This is considered to be an improvement in the conductometric method.

A few thin layer chromatography separations of hop extracts were made and some of the data are recorded for future reference. The most prominent observation is that decomposition of  $\alpha$  and  $\beta$ -acids on silica gel plates is rapid and both quantitative and qualitative reliability are lost under usual conditions of TLC.

Toluene extracts were evaluated for their storage stability. It was determined that extracts would be sufficiently stable at room temperature for 10 to 15 days to be suitable for distribution in collaborative tests. Glass is preferred to polyethylene. Longer storage requires  $-5^{\circ}\text{F}$  storage where solutions were found to be stable up to 38 days.

## DETERMINATION OF MATURITY BY LUPULIN ANALYSIS.

Samples of Bullion (seeded) and Fuggle (seedless) were collected periodically through the season. These were dried and the cones analyzed for  $\alpha$  and  $\beta$ -acids and for oil content and composition in the usual fashion. In addition lupulin was collected from each sample and analyzed for the same components. The purpose was to determine how the quality of lupulin developed and if it could be used as an index of maturity as is usually done with whole cones.

The experiments were very revealing and the results can be summarized as:

1. Maturity data for  $\alpha$ -acid, oil and cone weight followed normal patterns (Tables 1, 2).
2. Lupulin from both Bullion and Fuggle maintained essentially constant  $\alpha$ -acid content through the entire period (Tables 1, 2).
3. The  $\alpha$ -acid content of lupulin from Bullion was only slightly higher than that from Fuggle (Tables 1, 2).
4. Lupulin from Bullion and Fuggle had equal oil content and followed the same maturity curve. (Figure 1).
5. The oil content of lupulin from both Bullion and Fuggle (as determined by integrater counts of area under GC curves) followed distilled oil curves very well. (Figures 2, 3)
6. Production of lupulin by Fuggle ceased 2 weeks earlier than Bullion ... each "peaking" very near the beginning of the commercial harvest period (mg./lup./cone, Tables 1, 2).
7. Oil accumulation is predominantly a result of myrcene production and verifies earlier results: "Hop Oils, Past and Present." by D. E. Bullis and S. T. Likens (Tables 3, 4).

It was very surprising to find that the  $\alpha$ -acid content of lupulin does not change during the season. This fact obviously rules out  $\alpha$ -acid analysis of lupulin as a device for assessing maturity (Tables 1, 2).

The oil content of lupulin followed the course of maturation very well and it is appealing to speculate on the practical usefulness which might be developed. Since the oil was distilled from 100 to 150 grams of cones but lupulin was taken from only 25 grams, there is a suggestion that lupulin may be much more uniform from cone to cone than was expected. In the event that very small samples were reliable, maturity checks could be made on fresh, undried hops very quickly by simply assaying for myrcene. We plan to determine the uniformity of lupulin during the 1967 season.

The significance of the analytical similarities of lupulin from Bullion and Fuggle is not readily apparent. That each has nearly equal  $\alpha$ -acid content and that Fuggle's lupulin has as much oil as Bullion's ...even accumulating at the same rate and date (Figure 1)... seems to say that the primary difference in quality between the 2 varieties lies in the quantity of lupulin. Differences noted in their brewing behavior would have to result from differences in their cohumulone ratios or (less likely) differences in oil composition.

Table 1. Fuggle Maturity Study (Dry Weight Basis)

Date	mg DM cone	ml oil 100 gm	Cone				Lupulin				% lup. in cone	mg. lup. cone
			$\alpha$ -acid	$\beta$ -acid	total	$\alpha/\beta$	$\alpha$ -acid	$\beta$ -acid	total	$\alpha/\beta$		
8/1	71	0.23	6.31	3.91	10.22	1.62	42.1	25.0	67.1	1.68	15.2	10.8
8/4	--	0.29	6.76	3.96	10.72	1.71	45.2	22.7	67.9	1.99	15.8	--
8/8	--	0.71	7.15	3.77	10.92	1.90	46.3	20.0	66.3	2.31	16.5	--
8/11	--	0.64	7.08	3.43	10.51	2.06	49.0	21.6	70.6	2.27	14.9	--
8/15	114	1.10	7.86	3.46	11.32	2.27	43.2	20.1	63.3	2.15	17.9	20.4
8/18	121	1.55	7.95	3.70	11.65	2.15	53.6	15.6	69.2	3.43	16.8	20.4
8/22	106	2.06	7.68	3.14	10.82	2.44	45.0	18.6	63.6	2.41	17.0	18.0
8/25	96	2.00	7.60	3.52	11.12	2.16	45.2	18.8	64.0	2.40	17.3	16.6
8/29	115	1.82	7.82	3.35	11.17	2.35	39.5	20.3	59.8	1.95	18.7	21.5
9/1	104	2.02	6.89	2.98	9.87	2.31	38.5	18.8	57.3	2.05	17.2	17.9

Table 2. Bullion Maturity Study (Dry Weight Basis)

Date	mg DM/cone		ml oil/100g		Cone				Lupulin				% lup. in cone	mg.lup. cone	
	green	dry	green	dry	% $\alpha$ -acid green	% $\alpha$	% $\beta$	Total	$\alpha/\beta$	% $\alpha$	% $\beta$	Total			$\alpha/\beta$
8/3	--	--	0.26	0.18	2.60	7.90	4.73	12.63	1.67	47.3	23.8	71.1	1.99	17.8	--
8/6	--	--	0.41	0.22	10.00	8.48	4.71	13.19	1.80	48.5	25.5	74.0	1.90	17.8	--
8/9	131	--	0.65	0.42	10.00	10.13	5.40	15.53	1.87	47.1	24.2	71.3	1.95	21.8	23.6
8/12	154	--	0.86	0.62	9.14	9.41	4.95	14.39	1.90	46.5	24.5	71.0	1.90	20.2	30.9
8/15	156	158	1.30	1.15	9.35	11.30	6.05	17.35	1.87	46.9	24.8	71.7	1.79	24.2	38.0
8/18	159	163	1.88	1.54	10.01	11.73	5.55	17.28	2.11	48.7	26.2	75.0	1.86	23.0	36.6
8/21	188	169	1.88	1.71	10.86	10.85	4.25	16.41	2.55	46.3	26.2	72.5	1.77	22.6	42.6
8/25	208	196	2.44	2.44	10.63	11.68	4.95	16.63	2.36	47.3	23.4	71.0	2.02	23.4	45.9
8/28	230	271	3.06	2.67	--	10.93	5.56	16.49	1.97	48.9	22.1	71.0	2.21	23.2	58.1
8/31	226	199	3.21	2.92	--	10.59	4.51	15.10	2.35	41.7	22.6	64.3	1.85	23.5	50.0
9/2	254	216	3.34	3.30	--	11.32	5.57	16.89	2.03						

1/  $\alpha$ -acid, green, was determined conductometrically,  $\alpha$ ,  $\beta$  and total were from UV determinations. All are reported on the basis of 8% moisture content.

Table 3. Fuggle Oil Composition

Date	Steam-distilled			Lupulin			(6400)
	% myr.	% $\beta$ -c	% hum.	% myr.	% $\beta$ -c	% hum.	cts/mg lup.
8/1	16	19	65	11	18	71	74
8/4	16	18	66	33	14	53	95
8/8	44	12	44	60	3	37	169
8/11	53	12	35	72	6	22	107
8/15	57	10	33	72	5	23	282
8/18	75	3	22	75	4	21	295
8/22	69	8	23	72	6	22	456
8/25	73	8	19	81	4	15	503
8/29	74	8	18	81	6	13	464
9/1	83	5	12	81	5	14	526

Table 4 Bullion Oil Composition

Date	Steam Distilled Oil						Lupulin			Cts(at 6400) per mg. lup.
	Dry			Green			% myr.	% $\beta$ -c	% hum.	
	% myr	% $\beta$ -c	% hum.	% myr.	% $\beta$ -c	% hum.	% myr.	% $\beta$ -c	% hum.	
8/3	25	21	54	--	--	--	46	15	39	51
8/6	32	28	40	--	--	--	56	15	29	75
8/9	50	15	35	57	13	30	67	11	22	96
8/12	71	10	19	68	12	20	68	12	20	143
8/15	72	9	19	75	9	16	73	7	20	187
8/18	78	7	15	81	6	13	76	8	16	289
8/21	81	6	13	81	7	12	73	7	20	384
8/25	87	6	7	87	5	8	72	10	18	464
8/28	90	4	6	84	7	9	75	9	16	451
8/31	81	5	14	88	4	8	84	5	11	533

## PICKING DATE VS INTEGRATOR COUNTS

(at 6400 attenuation) PER MG OF LUPULIN

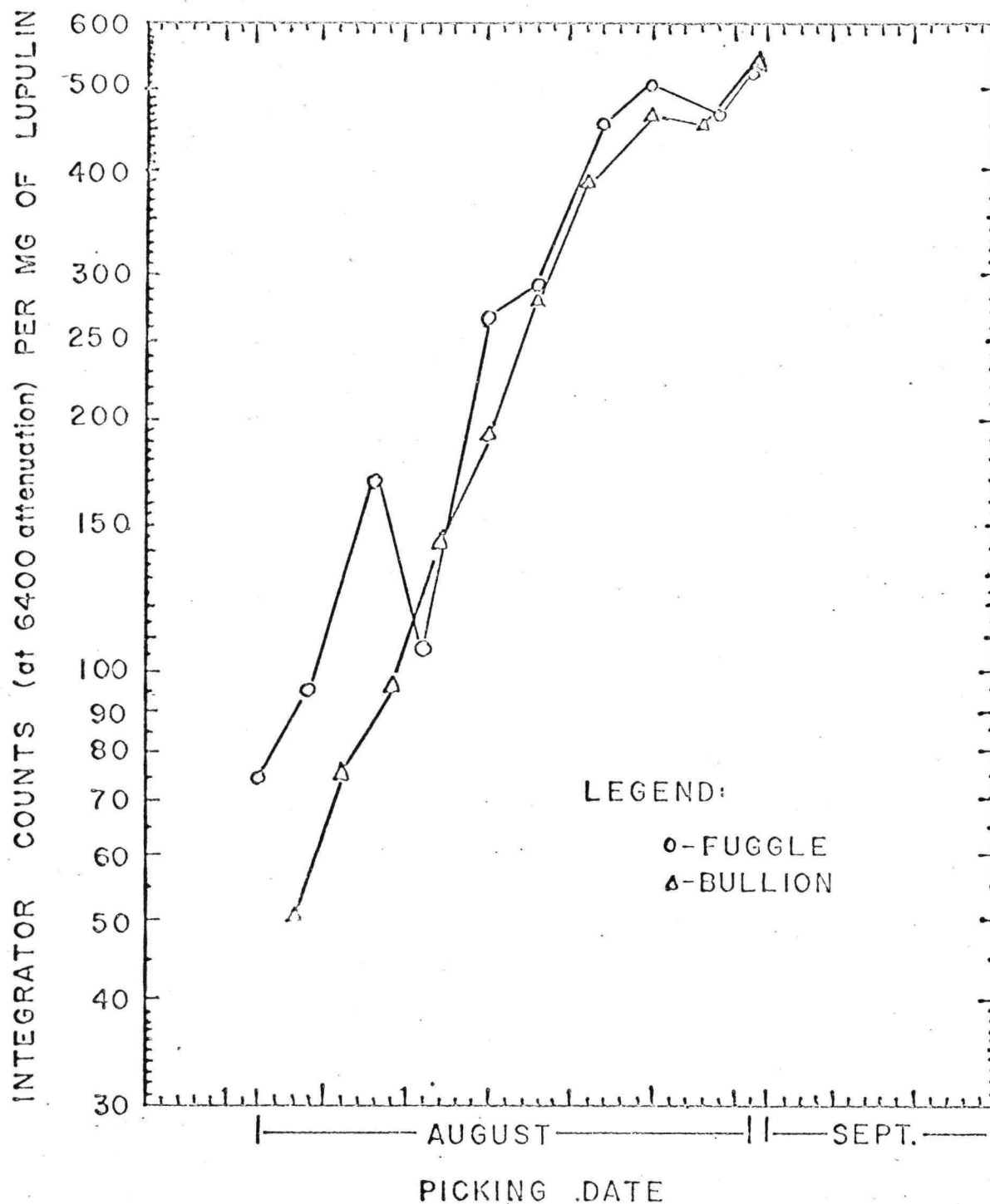


Figure 1. Accumulation of oil in lupulin from Fuggle and Bullion. Determined by solid injection of lupulin and determination of area under resulting G.C. curve (in terms of integrator counts).

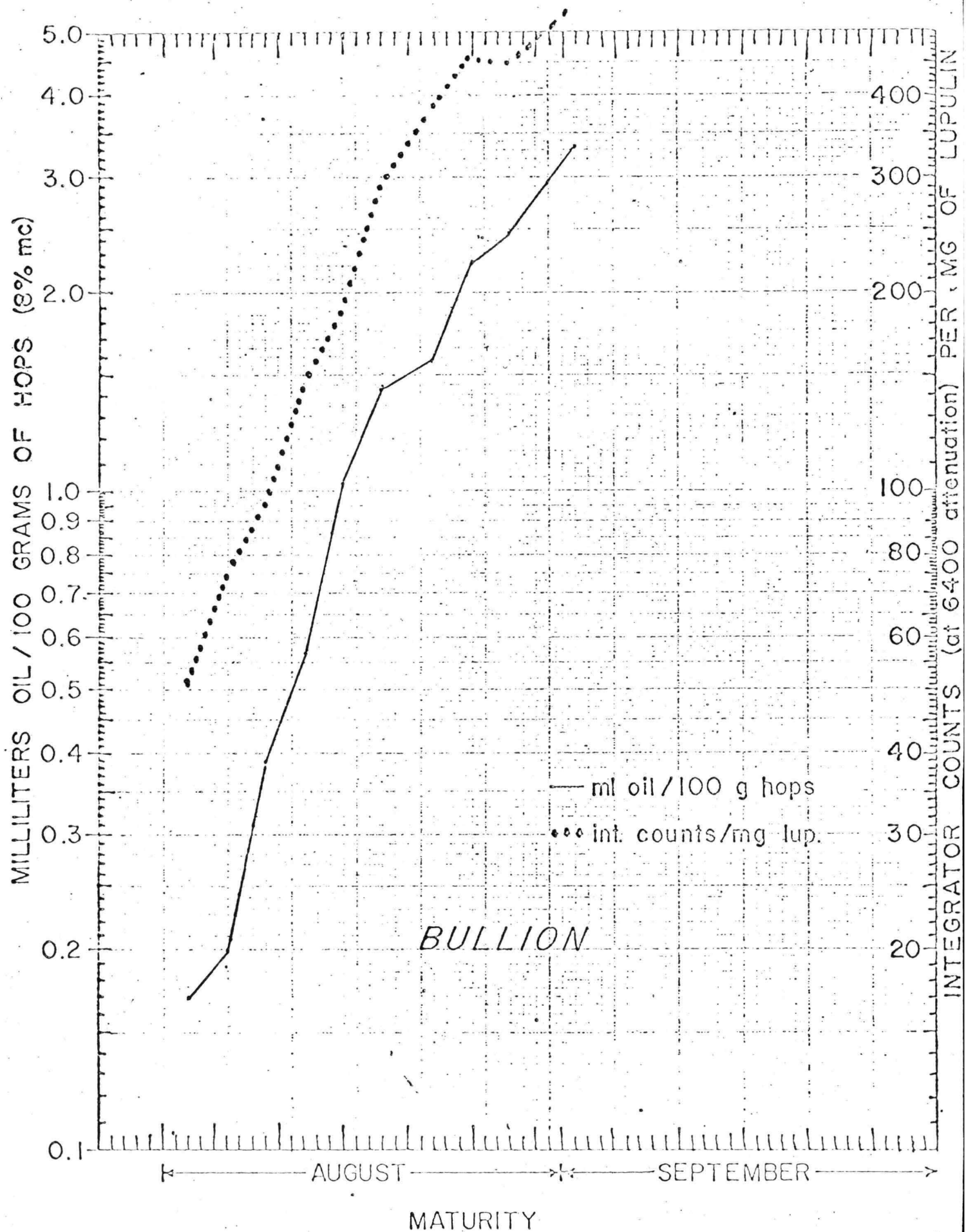


Figure 2. Correlation of accumulation of oil in lupulin and in cones (Bullion).

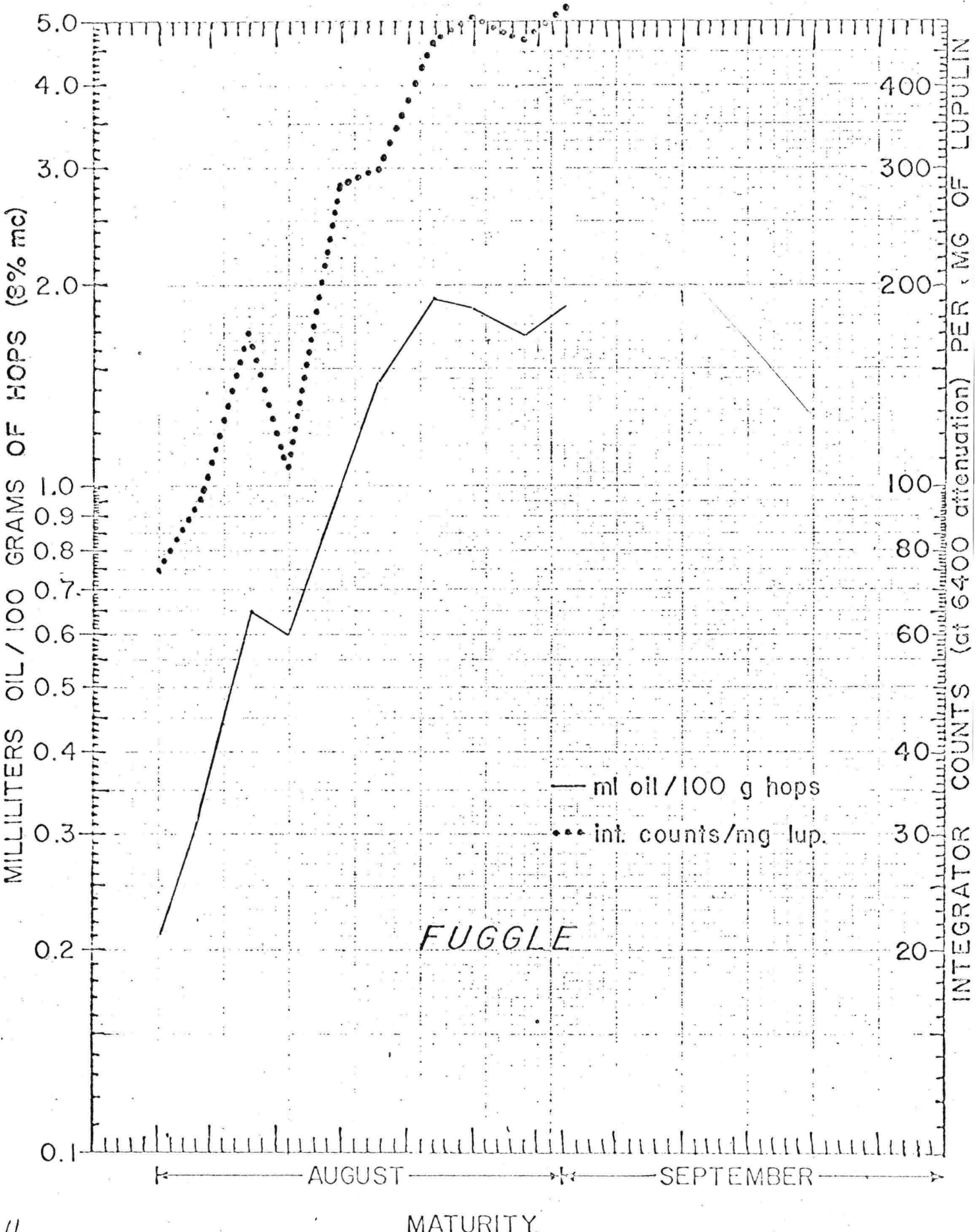


Figure 3. Correlation of accumulation of oil in lupulin and in cones (Fuggle).



## APPRAISAL OF THE CONDUCTOMETRIC METHOD FOR HOP ALPHA ACID ANALYSIS:

Analysis of the 1964, 1965, and 1966 crops by both the spectrophotometric and conductometric methods indicated that the U.V. method consistently yielded higher values. This was contrary to literature reports which claimed either similar results or lower results by U.V. It was decided that an appraisal of the methods and clarification of their relationship would be beneficial to all concerned with hop analysis. Such a study was undertaken and the results have been described before the American Society of Brewing Chemists and will be published in their 1967 Annual Proceedings. Only tables, graphs, and pertinent discussion will be included in this report.

Many compounds (some deterioration products of  $\alpha$ -acids) affect both the conductometric ( $\alpha_c$ ) and U.V. ( $\alpha_s$ ) methods. Table 1 lists these and their effects on each method. It is significant that each affects the 2 methods in opposite fashions. The presence of any tends to increase  $\alpha_c$  and decrease  $\alpha_s$ . It follows that, as deterioration proceeds, the loss of  $\alpha$ -acid would appear to be greater by the U.V. method.

Table 1 also indicates the bittering potential of other components, and it is apparent that although  $\alpha_c$  is not specific, it is a better indicator of bitterness than  $\alpha_s$ . This observation is of consequence from the practical standpoint in determining brewing value.

### Experimental:

A 5 gm. sample of ground hops, or 1 gm. of hop concentrate (weighed to 1 mg.) was extracted on a mechanical shaker for 20 min. with 100 ml. of solvent in a 250 ml extraction bottle stoppered with a neoprene stopper and centrifuged. We usually use toluene as the extraction solvent since it is comparable to benzene and less toxic. A reagent blank was included for spectrophotometric analysis. Hop concentrates were partitioned with 25 ml. of 0.1N HCl during extraction, then centrifuged 5 min. at 2000 rpm. to eliminate emulsions.

The aliquot used for conductometric analysis, usually 15 to 25 ml., plus approximately 150 ml. of methanol, was titrated in 0.25 ml. increments with 4% lead acetate. No water was added to the solution. The lead acetate was standardized frequently with standard acid (0.2286 N  $H_2SO_4$ ).

Another aliquot from the same extract (usually 1 ml.) taken for spectrophotometric analysis was evaporated with air in a warm water bath. The residue was dissolved and made to volume with alkaline methanol. A double dilution was necessary for high  $\alpha$ -acid hops. No  $\alpha$ -acid loss occurred during the evaporation. Table 2 shows the effect of several evaporations in two toluene extracts. The necessity for evaporating in a nitrogen or  $CO_2$  atmosphere seems to have been exaggerated, at least for toluene extracts. Absorption at 275, 325, and 355 m $\mu$  was determined with a Cary Model 11 Recording Spectrophotometer.

### Results and discussion:

As indicated in table 1, disagreement between methods would be expected only after accumulation of deterioration products when  $\alpha_c$  would exceed  $\alpha_s$ .



However, in 1964, 29 samples were analyzed by both methods and  $\alpha_s$  was less than  $\alpha_c$  (Table 3). In 1965, 56 samples were analyzed by both methods with similar results (Table 3). Regression lines for these data are shown in figure 1. In 1966 many samples were first run by the U.V. method and from these a group was selected representing a range of 2% to 12%  $\alpha$ -acid. These were analyzed by both methods in a controlled experiment and  $\alpha_c$  was less than  $\alpha_s$  for the third consecutive year (Table 3), Figure 2).

Several solvents are used for extraction of  $\alpha$ -acids from hops. It was determined that the disagreement between methods was not influenced by the choice of solvent (Table 4). Although chloroform was the most effective extracting solvent, both methods responded equally.

It was found that partitioning extracts with acidified methanol-water raised the value of  $\alpha_s$  while  $\alpha_c$  was unaffected, thus improving the agreement between methods (Table 4). The relationships are summarized in table 5. Successive partitioning, however, failed to improve the situation further. These experiments suggested (1) a water-soluble inhibitor to the reaction between lead and  $\alpha$ -acid which could be entirely removed in a single partitioning, and (2) after its removal, the reaction still failed to go to completion ... possibly due to an accumulation of reaction products, namely acetic acid.

Howard and Martin (J. Inst. Brew. 70: 424 (1964)) investigated methods of analysis and provided data for hops stored 9, 21, and 33 months. If  $\alpha_s/\alpha_c$  is plotted against time, it is possible to extrapolate to 0 months and make a conclusion about the relation between methods on fresh hops. When this is done, the ratio of  $\alpha_s/\alpha_c$  is found to be 1.14 (Figure 3), indicating the two methods would not have agreed and that  $\alpha_s$  would have been 1.14 times as great as  $\alpha_c$ . This is in agreement with equation c, table 3 which would give  $\alpha_s/\alpha_c$  of 1.14 for a hop containing 5%  $\alpha$ -acid.

Aging of hops has an "inverting effect" on the relation of  $\alpha_s$  to  $\alpha_c$ . Two samples of Brewers Gold representing fresh and deteriorated hops were analyzed by 3 methods:  $\alpha_s$ ,  $\alpha_c$  and gravimetric ( $\alpha_g$ ) (Table 6). In fresh hops,  $\alpha_s > \alpha_g > \alpha_c$ , while after deteriorating  $\alpha_g > \alpha_s > \alpha_c$ . In comparison to the gravimetric method, the conductometric method fails to measure all the  $\alpha$ -acid in fresh hops while the U.V. method measures more. The relation is reversed in old hops; the conductometric measures more and the U.V. measures less.

The U.V. spectra of these samples (Figure 4) indicates the accumulation of oxidation products in the  $A_{275}$  region as well as the disappearance of  $A_{325}$  and particularly  $A_{355}$ . The equation for calculating the spectrophotometric  $\alpha$ -acid uses  $A_{275}$  as a background correction. While the  $A_{275}$  corrects for a degree of deterioration, it can overcompensate in badly deteriorated samples such as those in figure 4. That the spectrophotometric method would fail to yield sufficiently high values on an aged sample is as expected from the information in table 1.

#### Conclusions:

1. The initial  $\alpha_s$  in fresh hops will be greater than  $\alpha_c$ , and that the difference can be calculated (Table 3);

2. As the hops age and deteriorate,  $\alpha_s$  and  $\alpha_c$  will become equal, and eventually  $\alpha_c$  will be greater than  $\alpha_s$ ;
3. The fact that partitioning with water-methanol increases  $\alpha_c$  but not  $\alpha_s$  suggests that an inhibitor to the conductometric method is partially responsible for the discrepancies between methods.

Table 1. Compounds, other than  $\alpha$ -acids, that affect the apparent  $\alpha$ -acid content as determined by various methods.

Compound	Bittering potential	Grav. and/or Cond.	Polarimetric	Spectro.	References*
isohumulones	yes	forms complexes with heavy metals - would possibly increase $\alpha_c$	optical rotation iso A $-7.8^\circ$ iso B $+47.6^\circ$ decrease $\alpha_p$	$\lambda_{max}$ 254 $\mu$ shoulder 270 $\mu$ decrease $\alpha_s$	(18,19,38,41)
humulinones	yes, 1/2 of isohumulones	coprecipitates with Pb - increase $\alpha_c$ decrease $\alpha_g$	reduces $\alpha_p$	absorption in UV - decrease $\alpha_s$	(4,18,22,31,44)
humulinic acids	no		no effect	absorption in UV - decrease $\alpha_s$	(10,15,24,33)
$\lambda$ -(hard)resins including $\delta$ -resins	yes intensely bitter	complexes with lead, forms precipitate in some cases - reduce $\alpha_g$ increase $\alpha_c$	no effect	$\lambda_{max}$ 281 $\mu$ decrease $\alpha_s$	(1,9,18,22,26)
hulupones	yes less than isohumulones	forms lead salt soluble in methanol, insoluble in pet. ether. Reduce $\alpha_g$ , increase $\alpha_c$	no effect	$\lambda_{max}$ 270 $\mu$ decrease $\alpha_s$	(8,18,22,27,28,34,35,37)
hulupinic acids	yes	forms insoluble lead salt, increase $\alpha_g$ and $\alpha_c$	no effect	$\lambda_{max}$ 298 $\mu$ decrease $\alpha_s$	(9,38,39)

\*References to be found in "Appraisal of the Conductometric Method of Analysis for Hop Alpha-Acids." Am. Soc. Brew. Chem. Proc., 1967.

Table 2. Effect of air evaporation in the  $\alpha$ -acid content determined by the spectrophotometric method.

<u>Number of evaporations</u>	<u>% <math>\alpha</math>-acid</u>	
	<u>Sample 1</u>	<u>Sample 2</u>
1	12.82	5.71
2	12.76	5.71
3	12.77	5.66

Table 3. Regression Equations derived from 1964, 1965, and 1966 comparisons of  $\alpha_c$  and  $\alpha_s$ .

<u>Equation</u>	<u>Correlation Coefficient</u>	<u>F</u>	<u>Notes</u>
(a) $\alpha_s = 1.085 \alpha_c + 0.02$	$r = 0.953$	265.6*	1964 (29 observations)
(b) $\alpha_s = 1.104 \alpha_c - 0.15$	$r = 0.993$	3631.4*	1965 (56 observations)
(c) $\alpha_s = 1.05 \alpha_c + 0.45$	$r = 0.999$	10793.2**	1966 (33 observations)

\*. Significant at the 5% confidence level.

\*\* Significant at the 1% confidence level.

Table 4. Comparison of  $\alpha_c$  and  $\alpha_s$  with different solvents, and with and without partitioning with acid-methanol. (Avg. of duplicates.)

<u>Solvent</u>	<u>Not Partitioned</u>		<u>Partitioned</u>	
	<u><math>\alpha_c</math></u>	<u><math>\alpha_s</math></u>	<u><math>\alpha_c</math></u>	<u><math>\alpha_s</math></u>
Toluene	11.03	12.25	11.30	12.20
Benzene	11.45	12.42	11.71	12.58
Chloroform	11.67	13.01	12.13	12.96

Table 5. Summary of the effect of different solvents and partitioning with acidified methanol on the apparent  $\alpha$ -acid content of hops.

1. For spectrometric method:  $\text{CHCl}_3 > \text{Benzene} > \text{Toluene}$
2. For conductometric method:  $\text{CHCl}_3 > \text{Benzene} > \text{Toluene}$
3. For spectrometric method:
 
$$\begin{aligned} \text{CHCl}_3 &= \text{CHCl}_3/\text{H}_2\text{O} \\ \text{Benzene} &= \text{Benzene}/\text{H}_2\text{O} \\ \text{Toluene} &= \text{Toluene}/\text{H}_2\text{O} \end{aligned}$$
4. For conductometric method:
 
$$\begin{aligned} \text{CHCl}_3 &< \text{CHCl}_3/\text{H}_2\text{O} \\ \text{Benzene} &< \text{Benzene}/\text{H}_2\text{O} \\ \text{Toluene} &< \text{Toluene}/\text{H}_2\text{O} \end{aligned}$$

Table 6. Comparison of  $\alpha$ -acid content determined by the conductometric, spectrophotometric, and gravimetric methods on fresh and deteriorated hop samples

Sample Condition	Extraction*	$\alpha_s$	$\alpha_c$	$\alpha_g$	$A_{275}/A_{355}$
Fresh	Toluene	11.6	10.8	---	0.26
	TSR	10.7	10.2	10.5	0.29
Old	Toluene	4.8	6.3	---	0.94
	TSR	4.9	6.4	5.3	0.79

\* Toluene = direct analysis of toluene extract, TSR (total soft resins) = analysis of pet. ether solubles partitioned from acid-methanol solution.

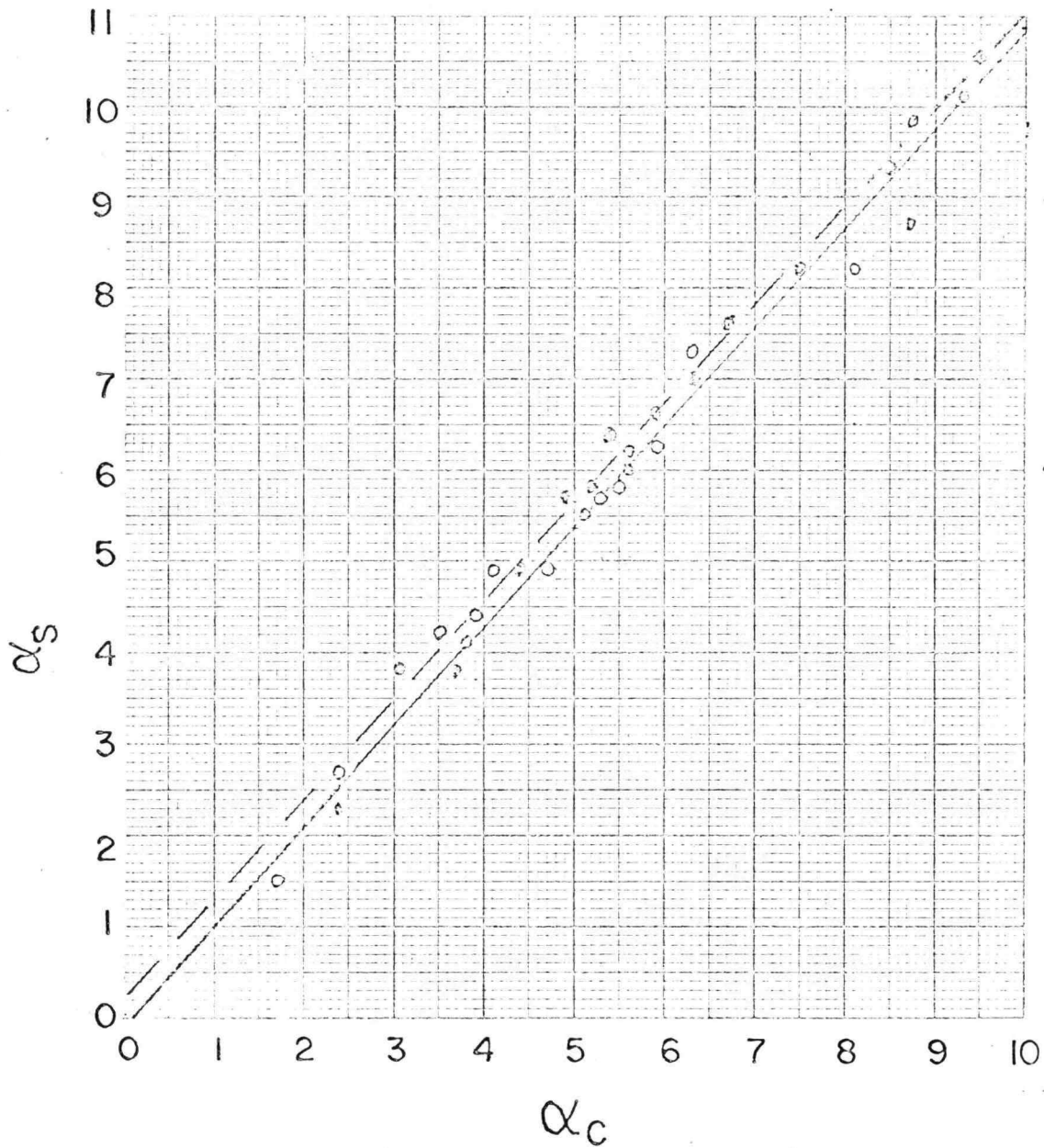


Figure 1. The relationship between  $\alpha_s$  and  $\alpha_c$  for 1964 and 1965 data. The circles and dotted lines represent 1964 data and regression equation; the dots and solid line represent 1965 data and regression equation.

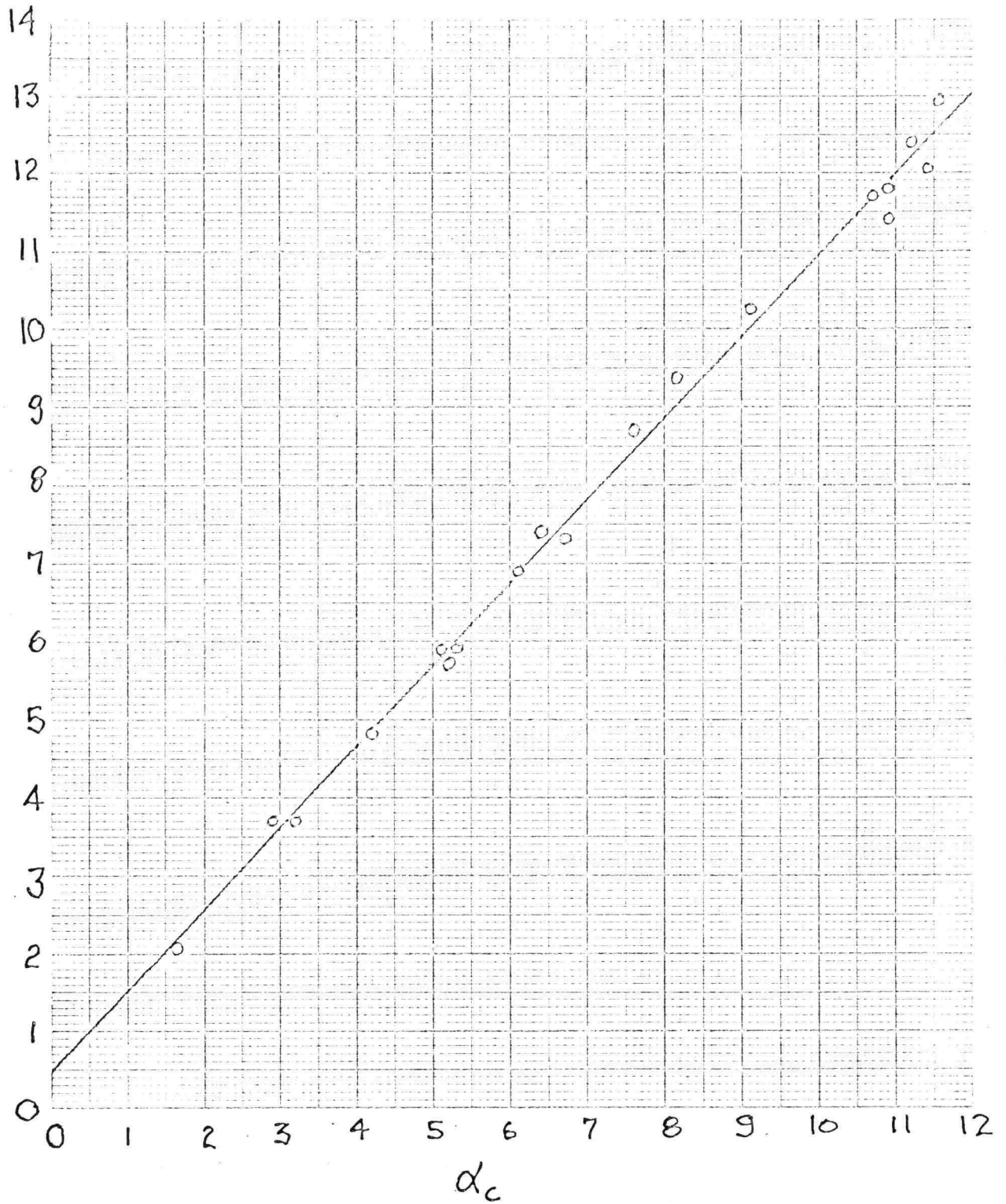


Figure 2. Relationship between  $\alpha_c$  and  $\alpha_3$ , 1966 data.

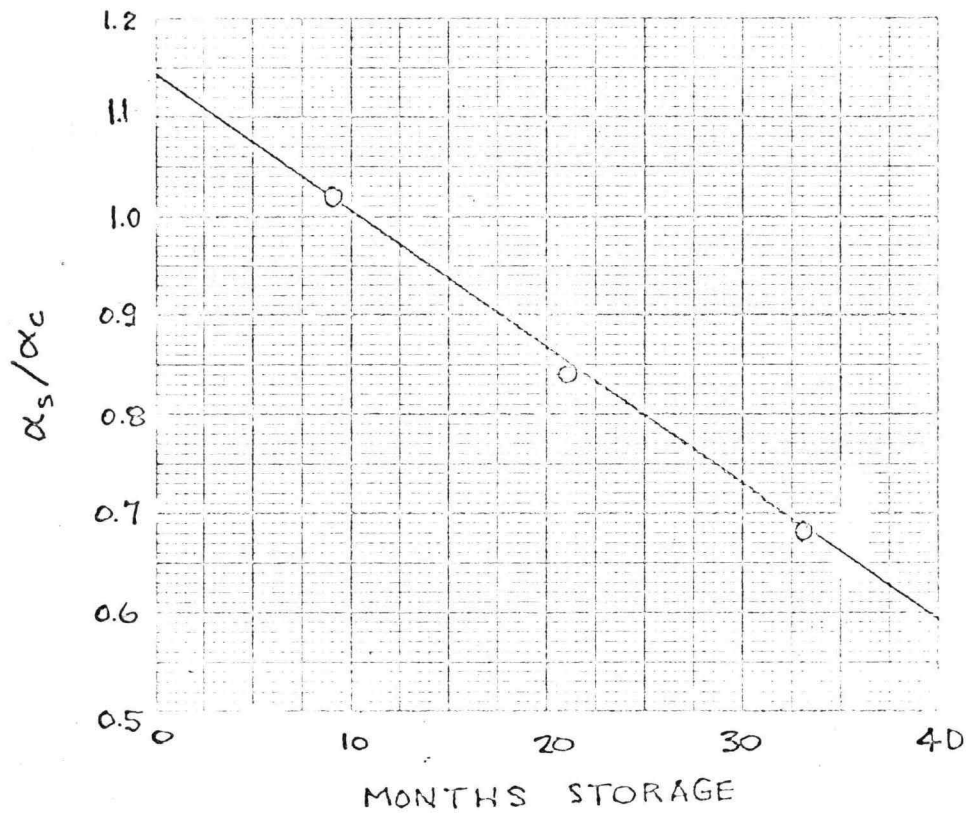


Figure 3. Relationship between the ratio of  $\alpha_s$  to  $\alpha_c$  with age. (Information from p. 70, Howard and Martin (18)). From the data in this paper the intercept at the origin would be 1.12.



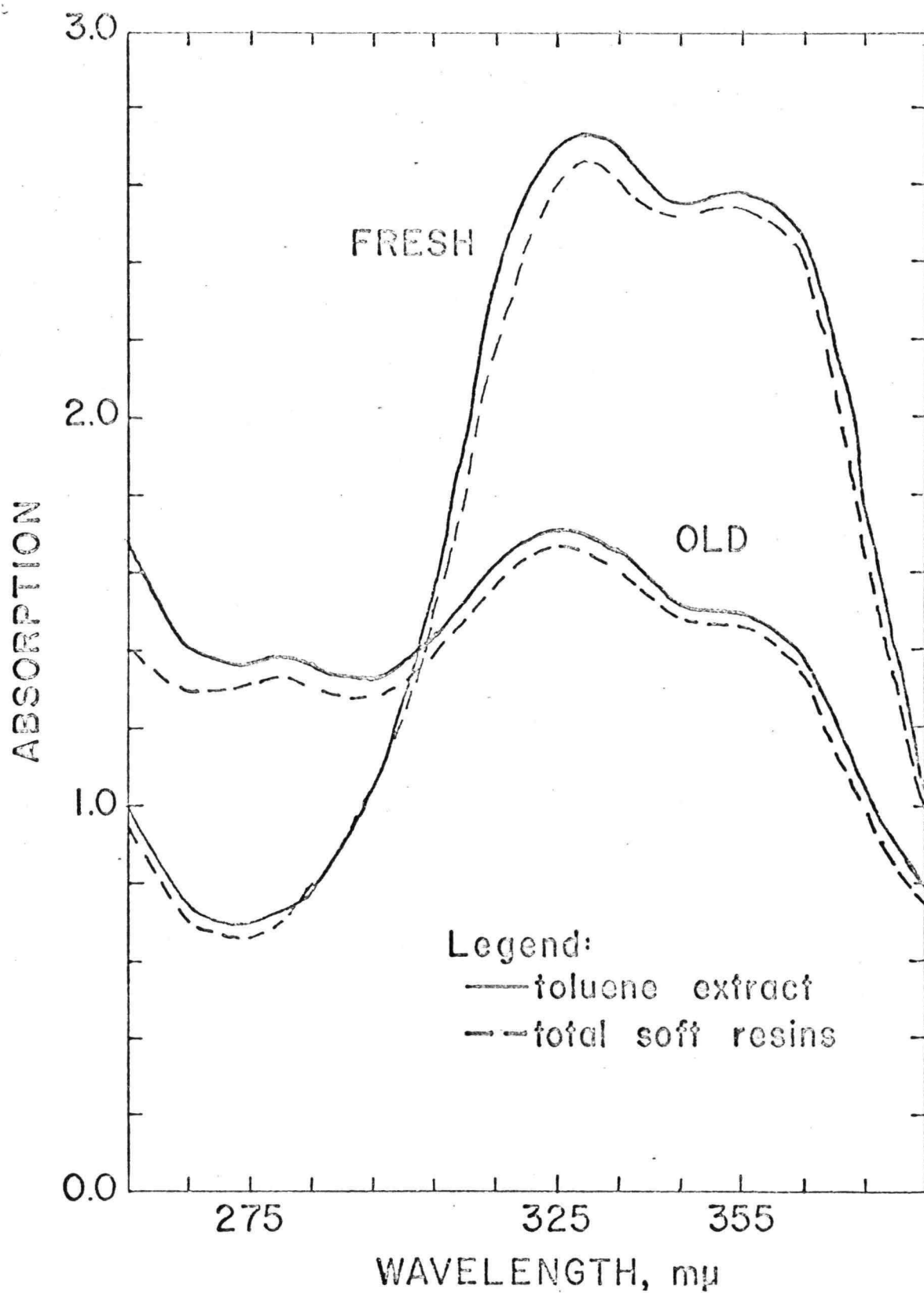


Figure 4. UV spectra of fresh and aged hops in alkaline methanol.

## USE OF PYRIDINE IN THE CONDUCTOMETRIC METHOD FOR ALPHA-ACIDS.

Failure of the conductometric method to measure all the  $\alpha$ -acid present in fresh hops as described under Appraisal of the Conductometric Method (previous section) led to the hypothesis that 2 factors were involved:

1. An inhibitor present in the extract which prevented the completion of the reaction between lead and  $\alpha$ -acid which could be removed by partitioning with acidified water-methanol.
2. Accumulation of Acetic acid during the titration  $(\text{Pb}(\text{Ac})_2 + 2\text{H}\alpha \rightleftharpoons \text{Pb}\alpha + 2\text{HAc})$  prevents the reaction from going to completion.

It was reasoned that, if this were true, removal of acetic acid during the titration would raise the end-point and such a reagent could also react with, and possibly inactivate, an end-point inhibitor. One of the most commonly used organic bases ... pyridine ... was used in preliminary tests and found to possess the required properties:

1. Soluble and undissociated in methanol-toluene system.
2. Does not react with lead acetate and therefore produces a normal blank titration.
3. Reacts with  $\alpha$ -acid to dissociate it and raise the pH of the system.
4. The conductivity of the solution drops during titration, then rises upon addition of excess reagent. This sharpens the end-point.
5. Pyridine has no effect on the end-point of the standardization of lead acetate against sulfuric acid.
6. Most important, it raises the end-point and brings the conductometric and spectrophotometric methods into agreement on fresh hops.

Several experiments were run to test the general applicability of the use of pyridine in the conductometric titration of  $\alpha$ -acids. The total of these are listed in tables 2 through 11 as a matter of record (the published form of this work utilized only 5 of these). A short explanation of each experiment follows:

1. Determination of quantity of pyridine required and demonstration of no influence from excess.
2. Demonstration of independence of pyridine-effect to different solvent systems commonly used for hop extractions.

3. Demonstration of similarity of regression of  $\alpha_{\text{cp}}^{1/}$  on  $\alpha_{\text{c}}^{2/}$  to regression of  $\alpha_{\text{s}}^{3/}$

- 
- 1/  $\alpha_{\text{cp}}$  =  $\alpha$ -acid by conductometric plus pyridine.
  - 2/  $\alpha_{\text{c}}$  =  $\alpha$ -acid by standard conductometric.
  - 3/  $\alpha_{\text{s}}$  =  $\alpha$ -acid by spectrophotometry.

on  $\alpha_c$  to indicate the general equivalence of  $\alpha_{cp}$  to  $\alpha_s$  on fresh hops.

4. Demonstration of the failure of  $\alpha_c$  to agree with  $\alpha_s$  and the ability of pyridine to correct the shortcoming on UNDRIED<sup>C</sup>HOPS (freshest possible).

5. Attempt to demonstrate increase in  $\alpha_c$  by addition of pyridine to bring  $\alpha_{cp}$  into line with  $\alpha_s$  on fresh BG. Also to clarify the pyridine effect on aged hops. "Fresh" sample had apparently undergone some deterioration.

6. Demonstration of pyridine effect on fresh hops (Hi  $\alpha$  BG), partially deteriorated hops (F) and badly deteriorated hops (O).

7. Demonstration of the pyridine-effect on both high and low  $\alpha$ -acid varieties and comparison with low  $\alpha$ -acid resulting from deterioration.

8. Pyridine-effect on partially deteriorated hops using chloroform (to answer question of old hops as related to table 2).

9. Regression of  $\alpha_{cp}$  on  $\alpha_c$  for wide range of  $\alpha$ -acid contents (1.6 to 11.5%).

10. Demonstration of pyridine-effect on hop concentrates.

11. Re-run of 10. Pyridine-effect clear in both, but poor duplication unexplained.

12. Fractionation of hop concentrate to demonstrate pyridine-effect present in both  $\alpha$ -acid and  $\beta$ -fraction ... none in  $\gamma$  resin fraction. Also indicates effects from two fractions are additive.

13. Attempt to apply hypothesis to gravimetric procedure and get higher results in presence of pyridine (Failed ...  $\alpha < \alpha_s = \alpha < \alpha_{cp} = \alpha$ ) (Also tried pptn  $\alpha$ -acid from MeOH-Toluene system. Unable to get complete pptn indicating  $\alpha$  must be pptd from MeOH only as in official procedures). Unable to explain results.

14. Collaborative samples of benzene extracts of fresh and aged B.G. sent to 3 other labs. to test pyridine-effect. Benzene extracts (especially old hops) were unstable and data is unreliable. Insufficient pre-planning. A normal pyridine effect noted in all cases except one.

Table 1. Summary of Varying percent pyridine in the titration mix.

<u>ml pyr.</u>	<u>% pyr.</u>	<u>End point</u>	<u>%<math>\alpha</math> <sup>1/</sup></u>	<u>Angle</u>
0	0	2.82	10.70	125
0.1	0.1	2.95	11.19	117
0.2	0.2	3.08	11.69	107
0.5	0.5	3.15	11.95	104
1.0	1.0	3.28	12.45	107
2.0	2.0	3.29	12.48	104

1% pyridine gives maximum ep and minimum angle.

-----  
 1/ Spectrophotometric = 12.36%  $\alpha$ -acid.

Table 2. Summary of Solvents with pyridine (GBN 12/27/66)

		<u>Spect.</u>	<u>No pyr.</u>	<u>1% pyr.</u>
Fresh BG	Toluene	11.39	10.88	11.40
		<u>11.76</u>	<u>10.72</u>	<u>11.79</u>
		Avg. 11.57	10.80	11.60
Fresh BG	Benzene	11.95	11.10	11.71
		<u>11.80</u>	<u>10.94</u>	<u>11.86</u>
		Avg. 11.87	11.02	11.79
Fresh BG	Chloroform	12.02	11.40	12.16
		<u>11.80</u>	<u>11.02</u>	<u>12.00</u>
		Avg. 11.91	11.21	12.08

Table 3. Summary of Regressions of  $\alpha_s$  and  $\alpha_{cp}$  on  $\alpha_c$ 

<u>Samples</u>	<u>No.</u>	<u>Equation</u>
1st series, solvents, Ext. store (66)	33	$\alpha_s = 1.05 \alpha_c + 0.45$
2nd series (Feb. 10) (1966)	13	$\alpha_s = 1.05 \alpha_c + 0.12$
1965 series (BIS)	38	$\alpha_{cp} = 1.104 \alpha_c - 0.15$
1965 (selections)	18	$\alpha_s = 1.08 \alpha_c + 0.07$
1964	29	$\alpha_s = 1.085 \alpha_c + 0.02$

Table 4. Summary of fresh (undried) Fuggle

	$\alpha_s$	$\alpha_c$	$\alpha_{cp}$
	6.64	5.82	6.58
		<u>5.82</u>	<u>6.67</u>
Avg.	6.64	5.82	6.62

Table 5. Summary of "Fresh and Aged" BG (Benz. extracts STL 1/10/67)

	$\alpha_s$	$\alpha_c$	$\alpha_{cp}$
Fresh	11.19	11.0	11.9
	11.19	10.9	11.8
		11.4	11.9
Aged	4.71	6.60	7.16
	4.71	6.38	7.25
		6.28	7.13
Aged (10 days later)		5.93	7.00

Table 6. Summary of "O" and "F" \* (GBN 1/6/67)

Sample	$\alpha_s$	$\alpha_c$	$\alpha_{cp}$	$\alpha_s$ Initial anal.
O	4.65	6.30	7.17	
	4.94	6.34	7.35	10.2
F	6.75	7.44	7.83	9.1
	7.20	7.70	8.08	
Hi (BG)	11.39	10.88	11.40	11.40
	11.76	10.72	11.79	

O = RT stored BG from RK 66. F = 35° stored Bu from RK 8/28 #65(66)init.  $\alpha_s$  = 9.06

Table 7. Summary of Hi and Low  $\alpha$  hops and aged B.G. (GBN 12/19/66)

	No pyr.	1% pyr.
Hi $\alpha$ (Benz extrn.)	10.75	11.60
	<u>10.68</u>	<u>11.60</u>
Avg.	<u>10.70</u>	<u>11.60</u>
Low $\alpha$ "	5.23	5.86
	<u>5.28</u>	<u>5.62</u>
	<u>5.25</u>	<u>5.75</u>
Aged BG "	7.40	8.34
	<u>7.44</u>	<u>8.41</u>
	<u>7.42</u>	<u>8.37</u>

Table 8. Summary, old hops (12/20,27/66 GBN)

	<u>No pyr.</u>	<u>1% pyr.</u>
Chloroform (BG, RT)	7.56 <u>7.03</u>	7.75 <u>8.11</u>
Avg.	7.30	7.93

Table 9. Comparison of standard conductometric method with conductometric plus pyridine on wide range of  $\alpha$ -acid contents

<u>Ser. No.</u>	<u>Ident.</u>	<u><math>\alpha_c</math></u>	<u><math>\alpha_{cp}</math></u>
48	71-25,26	1.46 1.48	1.63 1.71
60	FOB 202	3.86 3.80	4.20 4.22
85	Fu (s1)	6.36 6.01	6.44 6.67
76	92-S	7.59 7.42	8.16 7.65
54	Bu (RK sd)	9.08 9.23	9.23 10.00
200	BB313 (Bu)	10.21 10.70	10.94 11.30
167	C19/120	10.60	11.40
SS	$\alpha_c$ 128.6415		
SS	$\alpha_{cp}$ 141.4825		
SP	134.6594		

$$\alpha_{cp} = 1.047 \alpha_c + 0.12$$

Table 10. Summary of Pyridine with Extracts (GBN 12/16/66)

	<u><math>\alpha_s</math></u>	<u>No Pyr.</u>	<u>1% Pyr.</u>
ASBC Extract 1 (No HOH sol.)	37.5	37.9	40.1
	<u>38.3</u>	<u>36.9</u>	<u>40.4</u>
Avg.		37.4	40.3
ASBC Extract 2 (hi HOH sol.)	15.7	20.1	21.7
	<u>14.7</u>	<u>20.6</u>	<u>21.5</u>
Avg.		20.3	21.6

Table 11. Summary of ASBC Extracts (STL 12/20/66)

	<u>No Pyr.</u>	<u>1% Pyr.</u>
ASBC Extract 1 (No HOH sol.)	32.0	35.4
	<u>32.6</u>	<u>34.7</u>
Avg.	32.3	35.0
ASBC Extract 2 (hi HOH sol.)	13.9	15.5
	<u>13.2</u>	<u>15.5</u>
Avg.	13.5	15.5

Table 12. Summary of Extract Fractionation (GBN 2/27/67)  
(Pfizer humohop)

<u>Fraction</u>	<u><math>\alpha_s</math></u>	<u><math>\alpha_c</math></u>	<u><math>\alpha_{cp}</math></u>	<u>inc. due to p.</u>	<u><math>\beta_s</math></u>
$\alpha$ from Pba	32.5	28.9	31.5	2.6	-1.1
$\beta$ fraction	2.6	3.7	6.3	2.6	16.6
Hard resins	<u>0.1</u>	-	-	-	<u>-0.1</u>
Sum of recovered	35.2	32.6	37.8	5.2	15.5
Mixed & recovered	35.8	32.7	37.0	4.7	16.10
Original (MeOH Soln.)	40.1	37.6	44.0	6.4	27.6

Table 13. Summary of Gravimetric with pyridine (STL 2/28/67)

	<u>Spect.</u>	<u>Cond.</u>	<u>Grav.(MeOH only)</u>	<u>Grav.(MeOH &amp; Tol.)</u>
No pyridine	12.36	11.16	11.29	9.87
				9.93
				Avg. 9.90
1% pyridine		12.23	10.99	8.83
				9.20
				Avg. 9.02

Table 14. Results of Collaborative Tests on Comparison of Methods

<u>Fresh B.G.</u>				
<u>Laboratory</u>	$\alpha_s$	$\alpha_c$	$\alpha_{cp}$	<u>A275/A355</u>
A	11.2	11.0	11.9	0.34
	11.2	10.9	11.8	0.34
		11.4	11.9	
	<u>11.2</u>	<u>11.1</u>	<u>11.9</u>	<u>0.34</u>
B	11.5	10.9	12.5	0.26
	11.5	10.9	12.5	0.26
	11.5	10.9	12.5	0.26
C <sup>1/</sup>	12.2(10.9)	10.7	10.3	0.33
	12.1(10.9)	--	--	0.32
	12.2(10.9)	10.7	10.3	0.32
D	--	11.8	12.5	
		11.8	--	
		11.8	12.5	
<u>Aged B.G.</u>				
<u>Laboratory</u>				
A	4.7	6.6	7.2	
	4.7	6.4	7.2	
	--	6.3	7.1	
	<u>4.7</u>	<u>6.4</u>	<u>7.2</u>	<u>0.97</u>
B	4.0	5.5	6.4	
	2.3	5.3	6.4	
		5.4	6.4	
				<u>1.5</u>



Table 14 - cont.

<u>Aged B.G.</u>	<u><math>\alpha_s</math></u>	<u><math>\alpha_c</math></u>	<u><math>\alpha_{cp}</math></u>	<u>A275/A355</u>
<u>Laboratory</u>				
C <sup>1/</sup>	--	5.3	6.3	
D	--	6.2	6.5	
	--	6.3	--	

1/ Collaborator C uses a "correction" factor for  $\alpha_s$  and the "corrected" value is given in parentheses. He also uses the Wollmer<sup>s</sup> method of adding water to the titration mix for  $\alpha_c$  and the data are therefore not strictly comparable.

## THIN LAYER CHROMATOGRAPHY

References:

1. Ashurst, P. R., and Whitear, A. L. J. Inst. Brewing 71: 46-51. 1965.
2. Howard, G. A., and Martin, P. A. J. Inst. Brewing 70: 424-439. 1964 (file No. 217)
3. Kuroiwa, Y., and Hashimoto, H. J. Inst. Brewing 67: 347-351. 1961 and 506-510. 1961. (file No. 69)
4. Whitear, A. L. Proc. European Brewery Conv. 1965, Stockholm, pp. 405-415.

Several publications on the separation of hop constituents have mentioned that after TLC separation the spectrophotometric  $\alpha$ -acid agreed with polarimetric  $\alpha$ -acid (1,3,4). Howard and Martin (2) reported on the separation of hop components by TLC with old hops.

During our experiments on the comparison of the conductometric and spectrophotometric methods, we tried TLC as a means of determining what differences could be detected between the extracts of old and fresh hop extracts.

Methods:

Silica gel plates 0.25 mm thick, prepared by Betty McCoy, were developed in several solvent systems. The plates had been dried at 103°C for 30 min. before spotting.

The solvent systems used were:

- A. Chloroform:acetone (80:20)
- B. Chloroform:acetone:formic acid (80:20:1)
- C. Isooctane:isopropanol:formic acid (200:40:1) (2)

Other solvent systems mentioned in published work are:

1. n-Hexane:ethyl acetate (4:1) (3)
2. n-Hexane:ethyl formate:formic acid (12:8:1) (2)
3. n-Hexane:ethyl formate:formic acid (20:6:1) (2)

The spots were detected with UV light and if not recovered, the plates were sprayed with 5%  $H_2SO_4$  in ethanol and heated.

Spotting the plates with a toluene extract worked best since toluene doesn't move most components on silicic acid plates.

Results:

A fresh hop sample (2.5 gm. B.G. in 100 ml. toluene, 12.4%  $\alpha$ ) was plated (approx. 250  $\mu$ l., about 31 mg.  $\alpha$ -acid) and developed in solvent system B. After development there were 4 main components and 2 very faint ones evident under UV light. A hop oil standard (10  $\mu$ l. B.G. oil in 10 ml. acetone, 200  $\mu$ l. spotted) was chromatographed under the same conditions. The spots were scraped off, and ether used to elute them. The ether was evaporated and made to 10 mls with alkaline methanol. The UV spectra was determined with a Cary Recording Spectrophotometer.

From this information it seemed that hop oil wasn't responsible for the other spots on the plate, even though it might be mixed in with the spots.

In order to determine whether degradation on the plates would interfere with attempt to determine differences between old and fresh hops, two experiments were run with different length of time on the plate. In one experiment the spots were not developed but eluted from the plate after varying lengths of time. In the other experiment, the spots were developed after being on the plates for different times. Tables 2 and 3 show the results.

Two different solvent systems were compared and a tentative relationship between them established by their retention times and characteristics before and after spraying and heating. Table 4 shows the information for solvent systems B and C.

Unfortunately the only comparison with TLC of an original toluene extract,  $\beta$ -fraction, and  $\alpha$ -fraction (from Pba) was with solvent system A. (Data Book IV p. 47)

Appearance after 5% H<sub>2</sub>SO<sub>4</sub> in EtOH

<u>R<sub>f</sub></u>	<u>Original</u>	<u><math>\alpha</math>-fraction</u>	<u><math>\beta</math>-fraction</u>
.99	present-light	absent	dark
.98	" "	"	present
.95	bright blue	"	bright blue
.93	present	"	faint
.75	"	"	dark
.38	yellow	"	absent
.21	dark (streak from R <sub>f</sub> 0.18 to 0.38)	"	faint ( $\beta$ )
.18	dark	dark ( $\alpha$ )	faint
.13	absent	absent	light
.00	present	present	faint

Solvent system A does not move either the  $\alpha$  or  $\beta$  very much.

Comments:

The plates have to be developed immediately after spotting if any conclusions are to be made about the differences in old and fresh hops.

Either solvent system B or C is satisfactory. Since Howard and Martin have given tentative retention times (compared to  $\alpha$ -acid) for solvent system C, perhaps this would be best.

Table 1. Thin layer chromatography of a toluene hop extract, and hop oil.  
Solvent system B (Data Book IV p. 42)

Hop extract: <u>R<sub>f</sub></u>	<u>Identity</u>	<u>mg</u> <u><math>\alpha_s</math></u>	<u>mg</u> <u><math>\beta_s</math></u>	<u>A<sub>275</sub>/A<sub>355</sub></u>	<u>UV prop. in alk. MeOH</u>
0.92	? (1)	-0.51	2.13	1.95	little absorption, shoulder at 280 m $\mu$
0.88	$\beta$ -acid (2)	4.70	15.39	0.32	max at 355, shoulder 325, min at 270 m $\mu$
0.81	$\alpha$ -acid (3)	18.38	-0.06	0.50	max at 325, hump at 355, min at 270 m $\mu$
0.65	? (4)	0.15	4.02	0.93	considerable absorption, min at 290 m $\mu$
0.53	?	-----	-----	-----	-----
0.40	?	-----	-----	-----	-----
(1)+(2)+(3)+(4))		19.47	20.01	0.93	
% recovery(total)		63	64		

<u>Hop oil:</u>		<u>Hop extract:</u>	
<u>R<sub>f</sub></u>	<u>Properties under UV light</u>	<u>R<sub>f</sub></u>	<u>Properties under UV light</u>
0.97	red fluorescence	0.92	red fluorescence
0.80	blue fluorescence	0.88	blue fluorescence $\beta$
		0.81	dark $\alpha$
		0.65	dark
		0.53	faint fluorescence
		0.40	faint fluorescence

Table 2. The effect of time on the apparent  $\alpha_s$  and  $\beta_s$  (Data Book V p. 15)

<u>Time in</u> <u>dessicator</u>	<u>Development</u> <u>time</u>	<u>mg</u> $\alpha_s$	<u>mg</u> $\beta_s$	<u>A<sub>275</sub>/A<sub>355</sub></u>	<u>% recovery (<math>\alpha</math>)</u>
*-----	Not spotted-----	6.20	3.08	0.75	100
0	60 min.	5.04	2.83	0.57	81
1 hr.	45 min.	3.15	0.59	0.68	51
2 hr.	45 min.	4.14	0.71	0.71	67
5 hr.	45 min.	3.18	1.83	0.46	51

Table 3. Sample Treatment (Data Book V p. 16)

	Not Spotted	Spotted, removed immediately	Spotted, not developed (Top of plate)	$\alpha$ -acid spot from developed plate
mg $\alpha_s$	11.05	9.86	10.48	8.00
mg $\beta_s$	5.43	4.97	4.25	0.002
A275/A355	0.33	0.44	0.43	0.40
% recovery ( $\alpha$ )	100	89	95	72
% recovery ( $\beta$ )	100	92	78	

Table 4. Comparison of  $R_f$  values and UV characteristics for solvent systems B. and C. (Data Book V p. 11)

$R_f$	C		$R_f$	B		Identity
	Before spray	After Spray		Before spray	After spray	
.88		fawn? bwn-or.				
.83	faint	faint	.75	red	bright yel-or.	
			.71	faint	bright blue(dark)	
			.637			
.76	bright blue	dark	.50	blue	faint	$\beta?$
.72	bright red	dark	.44	orange	dark	$\alpha?$
.69	blue	faint				
.65	yellow	faint	.38	yellow	lt. brown	
.61	brown	faint				
.51	faint (old hops only)	faint	.22	faint (old hops only)	faint	
.34	faint	faint	.18	faint (old hops only)	faint	

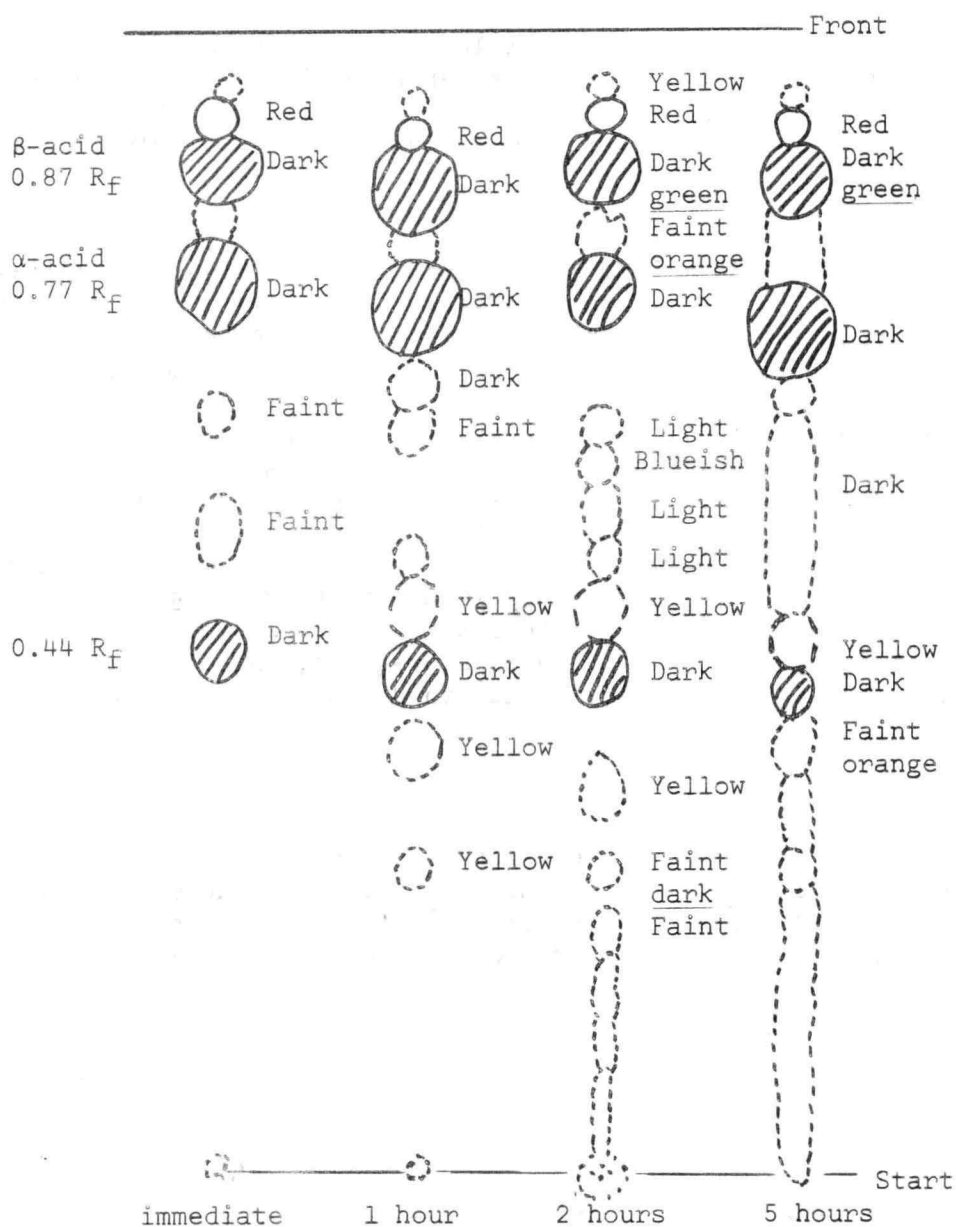


Figure 1. TLC of extracts after varying lengths of time on plate. Solvent system B. Silica-gel plates. About 20  $\mu$ l of toluene extract. (Data Book V p. 15)

STABILITY OF  $\alpha$  AND  $\beta$ - ACIDS IN TOLUENE SOLUTIONS

Stability of hop-acids in toluene solutions were studied in preparation for collaborative studies. If room temperature (R.T.) stability is adequate, collaborative samples can be distributed as extracts and thereby eliminate sub-sampling errors. This permits direct evaluation of the analytical method.

In glass:

2.5 g B.G./100 ml toluene, stored in glass erlenmyer flasks with neoprene stoppers, in freezer and at R.T., in dark. A 1 ml aliquot periodically diluted to 100 ml and  $\alpha$  and  $\beta$ -acid determined spectrophotometrically on Cary. Simultaneously a 25 ml sample taken for standard conductometric analysis.

The data in table 1 indicates good storageability for 8 to 15 days at room temperature by either method. The freezer data indicates good stability for at least a month. However, the apparent rise in  $\alpha$ -acid between the 15th and 38th day at room temperature suggest concentration by solvent loss and an alternate method of evaluation is necessary. In the spectrophotometric method, the ratio of absorbance at 275 m $\mu$  to absorbance at 355 m $\mu$  ( $A_{275}/A_{355}$ ) is an indication of deterioration. When this is plotted against storage time for the two temperatures (figure 1) it is apparent that freezer storage is, indeed, satisfactory; but that at R.T. deterioration begins after the 4th day and becomes unacceptable after 8 to 15 days.

In polyethylene:

A similar test in polyethylene containers suggested that the data from B.G. extracts stored at room temperature become unreliable after about 7 days and at freezer temperatures become unreliable after about 12 days. Fuggle was included in this trial with similar results (data summarized in Book V, p. 6).

Conclusions:

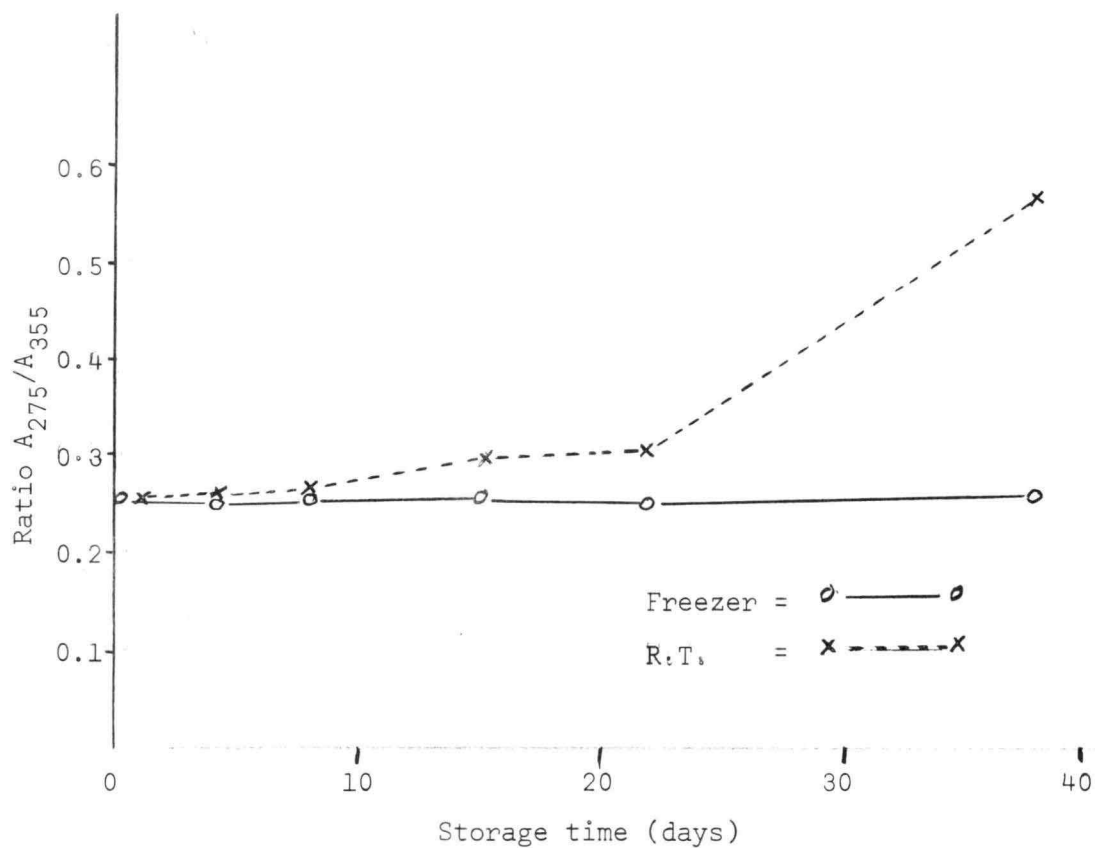
Toluene extracts of hops could be used for collaborative work provided:

1. Glass containers were used,
2. Exposure to room temperature (during mailing and receipt) were restricted to one week,
3. That samples were maintained at  $-5^{\circ}\text{F}$  prior to analysis.

Table 1. Results of 38 day storage test of toluene extracts of Brewers Gold  
(Data Book V p. 1)

Date	No. days	% $\alpha$ -acid			
		Freezer		R.T.	
		$\alpha_S$	$\alpha_C$	$\alpha_S$	$\alpha_C$
10/27	0	12.2	11.4	12.2	11.6
10/28	1	12.7	10.9	12.6	11.4
10/31	4	12.4	11.0	12.4	11.3
11/1	5	--	--	--	12.2 (12.4*)
11/4	8	12.4	11.1 (12.0*)	12.6	11.6 (12.0*)
11/11	15	12.4	--	12.7	--
11/18	22	12.4	--	13.4	--
12/5	38	12.2	10.8 (11.1*)	14.6	--

Figure 1. Evaluation of stability of toluene extracts of B.G. by change in  $A_{275}/A_{355}$  ratio.





A P P E N D I X

## Pollinations made in 1966.

<u>Cross</u>		<u>No. 1/</u>	<u>Pollination</u>			<u>Cross</u>		<u>No. 1/</u>	<u>Pollination</u>		
		<u>Bags</u>	<u>dates</u>					<u>Bags</u>	<u>dates</u>		
BB 313	x 221-1	6	7/26	7/28	8/1	(STL)48-21	x 44-36	1	8/1	8/3	
(26) <u>2/</u>	318-1,2	6	7/26	7/28	8/1		47-32	1	8/1	8/3	
	419-1,2	6	7/26	7/28	8/1		48-15	1	8/1	8/3	
	517-5	6	7/26	7/28	8/1		73-3	1	8/1	8/3	
FOB 215	x 221-1	7	7/27	7/29	8/1	(AH)135-I	x 317-1,2	4	8/2	8/3	
(32)	318-1,2	7	7/27	7/29	8/1						
	419-1,2	7	7/27	7/29	8/1	(AH)Fu	106-S	3	7/18	7/20	
	517-5	7	7/27	7/29	8/1						
BB 205	x 221-1	6	8/2			Late Cluster	x 119-1,2	4	8/4	8/8	
(32)	318-1,2	6	8/2			(32)	121-2	4	8/4	8/8	
	419-1,2	6	8/2				219-4	4	8/4	8/8	
	517-5	6	8/2				421-1,2	4	8/4	8/8	
	221-2	6	8/5				521-4,5	4	8/4	8/8	
							524-2	4	8/4	8/8	
							123-S	4	8/4	8/8	
BB 113-2	x 221-1	5	8/2			Early Cluster	x 119-1,2	4	8/4	8/8	
(27)	318-1,2	5	8/2			(31)	121-2	4	8/4	8/8	
	419-1,2	5	8/2				219-4	4	8/4	8/8	
	517-5	5	8/2				421-1,2	4	8/4	8/8	
	221-2	5	8/5				521-4,5	4	8/4	8/8	
BB 114-3	x 221-1	9	8/2				524-2	4	8/4	8/8	
(18)	221-2	9	8/5				123-S	4	8/4	8/8	
148-S	x 221-1	5	8/3			HL 8-23	x 221-1	4	8/5		
(21)	318-1,2	4	8/3			(19)	221-2	4	8/5		
	419-1,2	5	8/3				419-1,2	4	8/5		
	517-5	5	8/3				517-5	4	8/5		
94-S	x 221-1	5	8/3			420-2	x 221-1	3	8/5		
(14)	221-2	5	8/5				221-2	3	8/5		
35-S	x 221-1	5	8/3				419-1,2	2	8/5		
(21)	221-2	3	8/5				517-5	2	8/5		
(STL) B.G.	x 44-36	2	8/1								
	47-32	2	8/1	8/3							
	48-15	2	8/1	8/3							
	73-3	2	8/1	8/3							
(STL) 47-42	x 44-36	1	8/1								
	47-32	2	8/1	8/3							
	48-15	2	8/1	8/3							
	73-3	2	8/1	8/3							
(STL)47-40	44-36	1	8/1	8/3							
	47-32	1	8/1	8/3							
	48-15	2	8/1	8/3							
	73-3	1	8/1	8/3							

1/ All bags on by 5 July

2/ Numbers in parentheses indicate total number of laterals bagged.

Comments made at harvest time -- 1966 crosses, with estimated amount of viable seed after threshing.

BB 313 x 221-1 200 seeds

1. 1/16 cones -- not developed much, stunted, no indication of seed, dead style still present -- 10 dead cones.
2. 32 cones -- fairly sick looking, 16 dead cones.
3. 49 live cones, 20 dead cones.
4. 41 live cones, 16 dead cones.
5. 49 live cones, 5 dead cones.
6. 30 live cones, 3 dead cones -- small, no seed.

BB 313 x 517-5 20 seeds

1. Some seed, dead style still present, only sharp tipped bracts, 2 dead cones.
  2. 13 live cones, 12 dead cones, cones small.
  3. 21 live cones, 8 dead cones, small, tips of bracts dead.
  4. 23 live cones, 20 dead cones.
  5. 31 live cones, 15 dead cones.
  6. 55 live cones, 6 dead cones.
- 5 and 6 cones looked good seed set.

BB 313 x 318-1,2 150 seeds

1. Some seed stimulation, cone flared open, not closed tight cone. 6 cones -- 2 dead
2. 22 live cones, 1 dead cone, good looking cones, seed set, cone firm although odd looking, not full.
3. 26 live cones, 4 dead cones, same as 2.
4. 10 live cones, 12 dead cones.
5. 27 live cones, 62 dead cones, small cones, no seed.
6. 8 live cones, 2 dead cones, looked good.

BB 313 x 419-1,2 200 seeds

1. 16 cones, 11 dead cones, some seed -- in some places it appears that there has been some stimulation from pollination because round tipped bracts have started to develop, but there is no seed development, aborted seeds?
  2. 10 cones, 22 dead cones.
  3. 15 cones, 20 dead
  4. 23 live cones, 20 dead cones
  5. 31 live cones, 15 dead cones.
  6. 55 live cones, 6 dead cones.
- 5 and 6 looked good

There seems to be no correlation to areas of pollination. What seeds there are, are disbursed over the length of the sidearm.

FOB 215 - check

1. 2 live cones, small, 123 dead cones.
2. 8 live cones, v. small, 0 dead cones.
3. 32 live cones, v. small, 15 dead cones.
4. 37 live cones, v. small, 21 dead cones.

1/ = sidearm number

FOB 215 x 221-1 250 seeds

1. 18 live cones, 5 big, rest small, 50 dead cones.
2. 0 live cones, all cones dead, leaves and stem green, hops and petioles dead.
3. 10 live cones, small, 28 dead cones.
4. 57 live cones, small (5-6 big ones) 52 dead cones. Green hops on proximal and distal end of arm, those in middle were dead.
5. 43 live cones, big cones, 4 dead cones.
6. Entire sidearm dead.
7. 73 live cones, big cones, 21 dead cones.

FOB 215 x 318-1,2 300 seeds

1. 0 live cones, 41 dead cones, all dead, stem and leaves green.
2. 10 live cones, 50 dead cones.
3. 25 live cones, small cones, 30 dead cones. At first node in arm 1 green sidearm, all rest dead.
4. 22 live cones, 30 dead cones.
5. 39 live cones, large cones, 10 dead cones.
6. 26 live cones, large cones, 0 dead cones.
7. 93 live cones, large cones, 41 dead cones.

FOB 215 x 419-1,2 350 seeds

1. 77 live cones, large cones, 48 dead cones.
2. 28 live cones, 128 dead cones.
3. 56 live cones, large cones, 5 dead cones.
4. 33 live cones, small cones, 67 dead cones.  
Some of these that I am listing among dead cones look as though they are at the final stage of burr and their further development has been arrested while they stay green, they don't develop, but in most cases they die at this stage.
5. 63 live cones, large cones, 73 dead cones.
6. 3 live cones, 58 dead cones.
7. 8 live cones, 42 dead cones.

FOB 215 x 517-5 75 seeds

1. 52 live cones, 65 dead cones.
2. 26 live cones, 16 dead cones.
3. 24 live cones, 25 dead cones.
4. Entire sidearm dead, about 30 cones.
5. Entire sidearm dead, about 50 cones.
6. 13 live cones, small cones, 39 dead cones.
7. 27 cones, 47 dead cones.

(STL) 48-21 x 44-36 - 0 cones) Cones looked good, but -- no seeds  
 48-15 - 4 cones) because this ♀ was -- 3 seeds  
 47-32 - 8 cones) bagged late, there are -- 7 seeds  
 73-3 - 8 cones) not many cones. -- 10 seeds  
 47-42 x 48-15 -- sidearm and cones look good -- 31 cones -- 750 seeds  
 47-42 x 47-32 -- " " " " " 16 " -- 50 seeds  
 47-42 x 73-3 -- " " " " " 20 " -- 600 seeds  
 47-42 x 44-36 -- " " " " " 39 " -- 150 seeds  
 47-42 x 48-15 -- " " " " " 52 " -- (included above)  
 47-42 x 73-3 -- " " " " " 23 " -- (included above)

BB 113-2 x 221-1 3 seeds

1. 40 small dead cones, sidearms and leaves still green, cones small, just after burr, either brown or green.
2. 1 cone with seed, rest (20)dead.
3. All cones dead, terminal end of sidearm dead.
4. 3 small non seeded cones, rest (10)dead.

BB 113-2 x 318-1,2 3 seeds

1. 2 green seeded cones, rest (45)dead. Vines and leaves green.
2. All (50)cones dead.
3. All (20)cones dead.
4. All (10) cones dead.
5. All (14) cones dead.

BB 113-2 x 419-1,2 3 seeds

1. 2 seeded cones, 6 dead cones.
2. All (30)cones dead.
3. 1 seeded green cone, rest (50) dead.
4. All (40) cones dead.

BB 113-2 x 517-5 no seeds

1. All (30) cones dead.
2. All (10) cones dead.
3. All (15) cones dead.
4. 1 cone (all the rest (15) dead).

BB 113-2 x 221-2 no seeds

1. 2 cones, rest (45) dead.
2. All cones dead (20).
3. All cones dead (10).
4. 4 cones, rest (50) dead.

BB 114-3 x 221-1 75 seeds

1. Distal end dead. All cones dead (10).
2. All cones dead (75). Stem and leaves healthy, just cones and petioles dead.
3. 11 green cones. Rest (80) dead.
4. All cones dead (50). Terminal 1/2 of sidearm dead.
5. All cones dead (20).
6. 21 green cones, rest dead (40).
7. 63 green cones, rest dead (35).

BB 114-3 x 221-2 150 seeds

1. All (30) cones dead.
2. All (10) cones dead.
3. All (20) cones dead.
4. All (100+) cones dead.
5. 49 green cones (rest dead (50)).
6. 28 green cones, 75 dead cones.
7. All (10) cones dead.
8. All (30) cones dead.
9. 8 green cones, rest dead (30)

- BB 148-S x 221-1            100 seeds
1. All (30) cones dead.
  2. 10 cones, rest dead (30).
  3. All (40) cones dead.
  4. All (40) cones dead.
  5. 2 cones good, rest dead (50) -- sidearm covered with insects.
- BB 148-S x 318-1,2        500 seeds
1. 55 good cones, rest (50) dead.
  2. All cones (30) dead.
  3. All cones (15) dead.
  4. 37 good cones, 50 dead ones -- sidearm covered with aphids and other insects.
- BB 148-S x 419-1,2        500 seeds
1. All cones dead (40).
  2. 53 good cones, 50 dead cones, cones had heavy population of aphids.
  3. All cones dead (40).
  4. All cones dead (20).
  5. 11 good cones, 30 dead ones.
- 148-S x 517-5            750 seeds
1. 44 cones, heavily infested with aphids.
  2. All cones dead (30).
  3. All cones dead (30).
  4. All cones dead (30).
  5. 49 good cones, rest dead (60).
- BB 420-2 x 221-2        75 seeds
1. 20 good cones, 40 dead cones.
  2. 17 good cones, 30 dead cones.
  3. 11 good cones, 30 dead cones.
  4. All (30) cones dead.
- BB 420-2 x 419-1,2        250 seeds
1. 1 good cone, 40 dead cones.
  2. 57 good cones, 30 dead cones.
- BB 420-2 x 517-5        75 seeds
1. 1 good cone, 30 dead cones.
  2. 33 good cones, 10 dead cones.
- BB 205 x 221-1            75 seeds
1. 41 good cones, 30 dead cones.
  2. Entire sidearm dead.
  3. Entire sidearm dead.
  4. All (50) cones dead.
  5. 2 good cones, 30 dead cones.
- BB 205 x 221-2            no seeds
1. All cones on all 4 sidearms dead.

BB 205 x 318-1,2            1 seed  
 1. 2 good cones in 5 sidearms, 150-200 dead cones.

BB 205 x 419-1,2            no seeds  
 1. 3 cones in 3 sidearms, 100-150 dead cones.

BB 205 x 517-5            3 seeds  
 1. 3 cones out of 3 sidearms, 100-150 dead cones.

HL-8-23 x 221-1            no seeds  
 1. Cones (3) small, no indication of seed.  
 2. 2 cones with seed, 3 without.  
 3. 12 cones, 3 with seed.  
 4. 16 cones, 4 with seed.

HL-8-23 x 221-2            6 seeds  
 1. No cones  
 2. 3 cones, no seed.  
 3. 4 cones, no seed.  
 4. No cones.

HL-8-23 x 419-1,2        9 seeds  
 1. 24 cones, 3 with seed.  
 2. 26 cones, 5 with seed.  
 3. No cones.  
 4. No cones.

HL-8-23 x 517-5            1 seed  
 1. No cones.  
 2. No cones.  
 3. 2 cones, no seed.  
 4. 1 cone, no seed.

The sidearms on HL-8-23 were all small, no longer than 6 inches, those outside the bag were longer but no more than 12 to 13 inches. All the sidearms that were under bags remained green, the cones for the most part developed far better than any other genotype. The cones, although smaller than those outside the bag, were about as well developed.

94-S x 221-1            no seeds  
 1. Entire sidearm dead.  
 2. 100+ dead cones, 3 green cones (no seed).  
 3. 100+ dead cones, 5 green cones, seed appear to be aborted.  
 4. 100+ dead cones, 2 green cones.  
 5. 100+ dead cones, 5 green cones (no seed).

94-S x 221-2            1 seed  
 1. Entire sidearm dead.  
 2. 20 cones (aborted seed) 100+ dead cones.  
 3. 150+ dead cones, 3 green cones.  
 4. Entire sidearm dead.

35-S x 221-1 no seeds

1. All cones dead (30).
2. All cones dead (50).
3. All cones dead (50).
4. All cones dead (30).
5. All cones dead (30).

35-S x 221-2 2 seeds

1. All cones dead (20).
2. All cones dead (40).
3. 30 dead cones, 1 live cone with seed.

131-S

There were no crosses on this plant due to the early date of burr. It was burred out considerably on the 30th of June. By the time the males were ready it was too far past burr to pollenate, but overall, this genotype had the best percentage of cone development under the bag. Of the 16 sidearms, taken at harvest, of which 4 were dead, there were approximately 200 to 300 cones of good development. These were picked to obtain some idea of the effectiveness of the bagging procedure and of the bags themselves, to be used as a check. In picking the cones, it was observed that there was a small percentage of seed set.

LC x 123-S 3 seeds

1. Entire vine dead.
2. All cones (30) dead, stem and leaves green.
3. All cones (20) dead, stem and leaves green.
4. 5 good seeded cones, rest (30) dead.

LC x 119-1,2 no seeds

1. All cones dead (20), stem and leaves green.
2. All cones dead (30), stem and leaves green.
3. All cones dead (15), stem and leaves green.

LC x 121-2 no seeds

1. All cones dead (10).
2. All cones dead (30).

LC x 219-4 1 seed

1. 2 good cones with seed, rest dead (40).

LC x 421-1,2 1 seed

1. All cones dead (20).
2. All cones dead (20).
3. 1 good cone, rest dead (30).

LC x 521-4,5 no seeds

1. All cones dead (40).

LC x 524-2 no seeds

1. All cones dead (30).



LC x 317-1,2                    150 seeds

This was a pollination for Dr. Haunold for an ovary development on Late Cluster. 317-1,2 was chosen arbitrarily on the basis that there was an ample supply of pollen.

1. 37 good cones, rest dead (20).

EC x 119-1,2                    no seeds

1. All cones dead (20).

2. All cones dead (20).

3. All cones dead (30).

EC x 121-2                    1 seed

1. All cones dead (30).

2. 4 good cones, rest dead (20).

EC x 123-S                    no seeds

1. All cones dead (20).

2. All cones dead (20).

3. All cones dead (40).

EC x 219-4                    8 seeds

1. All cones dead (40).

2. 2 good cones, rest dead (30).

3. 2 good cones, rest dead (30).

EC x 421-1,2                    no seeds

1. All cones dead (20).

2. All cones dead (15).

3. 1 good cone, rest dead (30).

EC x 521-4,5                    2 seeds

1. All cones dead (10).

2. All cones dead (30).

3. 5 good cones, rest dead (40).

EC x 524-2                    150 seeds

1. All cones dead (20).

2. 8 good cones, rest dead (50).

3. 25 good cones, rest dead (60).

1/ sidearm number.

Ratings of single-hill plants two or more years old at Prosser, Wash. (C.E.Nelson)

Plant vigor and appearance	Cone size and shape					
	Very good		Medium		Fair	
	8	7	6	5	4	3
Very good 8		6249-30			<del>6263-25</del>	25-60 6263-32
7		25-40	6263-23	6259-13	15-14	620-37 6249-29 6249- <del>11</del> 6249-24
6	25-72	25-68 6249-22	15-34	25-71	25-67 35-84	15-1 15-3 25-43 6261-33 6249- <del>10</del>
5		15-33	6262-8	15-11 15-13 25-63 6261-22 6261-25	65-47 6262-9 6249-36 6260-17	15-18 <del>15-19</del> 6262-28 6261-18 6260-12
4					15-12 6259-17	25-62 35-79
Fair 3		25-41				15-5

Those scoring less than 3 in either rating are not shown

Ratings of single-hill plants at Prosser, Wash. Slip roots were planted on April 18, 1966. (C. E. Nelson)

Plant vigor and appearance	Cone size and shape					
	Very good		Medium		Fair	
	8	7	6	5	4	3
Very good 8			6342-37 6338-23	6338-24	6341-40	6337-1 6339-17
7	6340-15	6337-11	6342-8 6346-33 6337-3 6339-7	6388-10 6338-13 6339-19 6344-14	6346-24 6338-29	6347-24 6338-22 6345-15
6	6340-34	6339-13	6339-14 6334-45 6337-13 6337-14	6341-36 6338-3 6339-16	6342-14 6341-2 6341-34 6338-17	6344-46 6346-29 6343-37
5		6338-30		6346-39 6338-5	6338-31 6343-12 6342-23 6342-47	6344-2 6343-14 6341-32 6338-33 6343-39
4		6345-43 6337-16	6344-3 6344-15	6344-41 6343-47 6343-48		6348-30 6348-46 6349-50 6346-8 6344-17
Fair 3						6342-43 6342-49 6343-42 6343-43 6338-14

Plants scoring less than 3 in either rating are not shown.

## Dates of flowering for the April 18, 1966 planting at Prosser, Wash. (C.E.Nelson)

Acc. No.	Date	Acc. No.	Date	Acc. No.	Date	Acc. No.	Date	Acc. No.	Date
6348-2	7-29	6346-51	8-5	6343-48	8-2	6340-15	7-19	6338-26	7-26
6348-9	7-29	6345-15	7-29	6343-49	8-16	6340-34	7-26	6338-30	7-15
6348-21	7-26	6345-43	7-29	6343-50	7-29	6340-52	7-22	6338-31	7-19
6348-30	7-19	6345-44	8-2	6343-52	7-12	6339-7	7-15	6338-33	7-22
6348-40	8-8	6345-46	8-8	6342-2	8-2	6339-13	7-15	6338-41	8-11
6348-46	7-29	6345-47	8-11	6342-3	8-11	6339-14	7-29	6337-1	7-22
6348-53	7-29	6345-50	8-8	6342-8	8-2	6339-16	7-22	6337-3	7-19
6347-3	8-16	6344-2	8-2	6342-14	7-26	6339-28	7-22	6337-8	8-11
6347-7	7-29	6344-5	7-26	6342-15	8-11	6339-44	8-2	6337-11	7-19
6347-10	7-29	6344-14	7-22	6342-16	7-22	6339-45	7-22	6337-13	7-22
6347-22	8-5	6344-15	7-29	6342-23	7-29	6339-46	8-16	6337-14	7-19
6347-24	7-19	6344-17	7-29	6342-34	7-29	6339-47	7-29	6337-16	7-29
6347-29	8-11	6344-18	7-26	6342-38	8-5	6338-3	7-22	6337-20	7-29
6347-36	7-26	6344-26	8-8	6342-40	8-11	6338-5	7-29	6337-23	7-29
6347-45	7-26	6344-39	8-11	6342-42	8-8	6338-8	7-26		
6346-1	8-2	6344-41	7-29	6342-43	7-29	6338-10	7-19		
6346-2	7-29	6344-45	7-19	6342-47	7-22	6338-11	7-29		
6346-3	7-26	6344-46	8-2	6342-49	7-19	6338-13	7-29		
6346-4	7-26	6343-12	7-19	6341-2	7-19	6338-14	7-29		
6346-8	7-19	6343-14	7-19	6341-3	8-16	6338-15	8-11		
6346-24	7-19	6343-37	7-29	6341-31	7-26	6338-17	7-29		
6346-29	8-2	6343-39	7-19	6341-32	7-26	6338-19	7-26		
6346-33	7-29	6343-42	8-2	6341-34	7-19	6338-22	7-29		
6346-39	7-29	6343-43	7-26	6341-36	7-19	6338-23	7-26		
6346-41	7-26	6343-45	8-11	6341-40	7-22	6338-24	7-26		
6346-43	8-16	6343-47	7-22	6341-42	8-11	6338-25	8-2		

Other hills did not grow or males were removed.

Hop selections containing 4 or more per cent alpha acid at Prosser, Wash. (C.E. Nelson)

<u>Acc. No.</u>	<u>% alpha acid</u>	<u>Acc. No.</u>	<u>% alpha acid</u>
<u>Aug. 31, 1966</u>			
3S-102	8.2	6342-47	4.2
3S-112	7.3	6346-24	5.1
4S-143	4.7	6338-22	5.7
6249-24	7.5	6338-23	4.7
5S-168	4.4	6338-24	4.0
6339-16	5.6	6338-19	7.8
6337-11	5.0	6339-7	4.9
6341-34	5.3		
<u>Sept. 15, 1966</u>			
6343-47	7.7	6338-23	4.6
6343-48	8.0	OB826	5.7
6346-29	5.2	6259-13	4.0
6338-22	7.7	3S-90	7.7

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1966 notes on multiple hill plantings at Prosser, Wash. (C.E.Nelson)

10 Hill Plantings May 1, 1966Acc. No.

OB826 Poor vigor, average cones  
 OB831 Fair vigor, average cone quality  
 OB835 Poor vigor, average cone size and quality

5 Hill Plantings (2 yr.)

<u>Acc. No.</u>		<u>Bales per a.*</u>	<u>% alpha acid</u>
68-16	Good vigor, average cones, spray damaged	--	--
68-4	Very good vigor and cone size	8.5	2.37
68-1	Good vigor and cone size, medium set	6.4	6.28
67-43	Fair vigor, fair cones	--	--
66-39	Fair vigor, fair cone, spray damaged		
66-11	Good vigor, very good cones, heavy set	12.4	3.86
66-1	Good vigor, better than average cones, heavy set	12.0	6.44
65-47	Average vigor and cones, wind damaged	--	--
65-41	Average vigor, average cone size	--	--
65-25	Better than average vigor, small tight cones	--	--
13-17	Fair vigor and cones	--	--
10-20	Very good vigor, average cones	7.7	5.87
OB841	Good vigor, poor cone size	--	--

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\* Yield data only on those with adequate stand and sufficient cones for machine harvesting.

## Supplement to Hop Varietal Identification.

C. E. Zimmermann

Strobiles from different hop varieties appear to be similar in several botanical properties, but the apparent differences are quantitative and result in gradient classification.

The number of nodes per strig varies from eight in Fuggle to 12 in the "English" varieties. Each node in all varieties is composed of four florets each with one bracteole and single ovary. Therefore, hop varieties vary in having strobiles composed of 32 to 48 bracteoles and ovaries. The bracteole size is inherent to the variety, but is also influenced by the ovary which is encased by the bracteole fold.

Bracteoles are larger in seeded strobiles than seedless, but this difference in size may vary with variety. Fuggle and Bullion seeded strobiles have bracteoles which are stimulated more than Brewers Gold (Table 1, 2 and 3), and also display a greater increase in length over seedless than Brewers Gold. This same difference, between the 3 varieties, is common with the length of the bracteole fold which encases the ovary.

The ratio of fold length to bracteole length was determined on 7 commercial hop varieties to establish a visual means of identification. In general, ratios were .50 or less on seeded or seedless strobiles, except on seeded Bullion, Early, and Late Cluster. The seeded Bullion has bracteole folds which extend more than half-way as do both Early and Late Cluster. Similar sampling of Early and Late Cluster indicated that Early Cluster had a longer fold than Late Cluster (Table 4), but the bracteole variation within a single cone is reflected in Table 5. This variation in bracteole and fold length is primarily associated with seed content.

Data recorded in Tables 1, 2, 3, and 4 were obtained by sampling two seeded bracteoles from a single node located near the middle of each strobile. The sample was also limited to the two inner bracteoles at a given node. Tables 6 and 7 include the bracteole data from the four bracteoles on the fourth node of each strobile. Though the sampling technique did not consider seededness, the strobiles had a large seed content and each fourth node sampled had at least two seeds. The ratios of 0.74 and 0.68, for Early and Late Cluster respectively, were similar to the ratios obtained by sampling two seeded bracteoles at a given node (Table 4).

Preliminary data would suggest that the sampling of 2 seeded bracteoles from a mid-node on a given hop strobile, is a good estimate of the population. This technique will not distinguish between seedless Fuggle, Bullion, or Brewers Gold (ratios 0.50 or less), but seeded Bullion (0.62 ratio) can be separated from seeded Brewers Gold (0.42). Seeded Early Cluster with a ratio of 0.60 to 0.70 probably cannot be separated from seeded Late Cluster or Bullion, but can be distinguished from Late Cluster and Bullion by its large bracteole size.

Table 1. Bracteole Ratios from Seeded and Seedless Fuggle  
(Paired measurements from 8 cones)

Centimeters			
	<u>Bracteole Length</u>	<u>Fold length</u>	<u>Ratio</u>
<u>Seeded Fuggle</u>			
1	1.83	.80	.44
	1.79	1.10	.61
2	1.86	.91	.49
	1.83	.82	.45
3	1.89	.87	.46
	1.98	1.10	.55
4	1.93	1.11	.57
	1.92	1.07	.56
5	1.96	1.18	.60
	1.84	.91	.49
6	1.64	.62	.38
	1.75	.90	.51
7	1.78	.70	.39
	1.78	1.04	.58
8	1.99	.94	.47
	1.90	.70	.37
		Avg.	.50
<u>Seedless Fuggle</u>			
1	1.50	.80	.53
	1.49	.80	.54
2	1.27	.75	.59
	1.31	.57	.44
3	1.54	.75	.49
	1.43	.63	.44
4	1.54	.74	.48
	1.63	.85	.52
5	1.53	.60	.39
	1.59	.83	.52
6	1.35	.52	.39
	1.38	.65	.47
7	1.42	.69	.49
	1.40	.66	.47
8	1.43	.80	.56
	1.44	.62	.43
		Avg.	.48



Table 2. Bracteole Ratios from Seeded and Seedless Bullion.  
(Paired measurements from 8 cones.)

Centimeters			
	<u>Bracteole Length</u>	<u>Fold Length</u>	<u>Ratio</u>
<u>Seeded Bullion</u>			
1	1.88	.96	.51
	1.85	1.31	.71
2	1.80	1.27	.71
	1.78	1.10	.62
3	1.73	1.20	.69
	1.67	1.26	.75
4	1.68	1.13	.67
	1.83	1.10	.60
5	1.64	.96	.58
	1.64	.89	.54
6	1.85	1.10	.59
	1.78	1.11	.62
7	1.83	.90	.49
	1.84	1.00	.54
8	1.74	1.01	.58
	1.56	1.14	.73
		Avg.	.62
<u>Seedless Bullion</u>			
1	1.48	.54	.36
	1.42	.67	.47
2	1.18	.45	.38
	1.18	.52	.44
3	1.29	.51	.40
	1.39	.60	.43
4	1.48	.76	.51
	1.36	.68	.50
5	1.52	.57	.38
	1.50	.63	.42
6	1.33	.64	.48
	1.37	.66	.48
7	1.36	.35	.26
	1.28	.52	.41
8	1.50	.60	.40
	1.55	.63	.41
		Avg.	.42

Bracteole of medium width.  
Fold generally rises >1/2  
length of bracteole and  
rolls gently into edge of  
bracteole, rather than at  
a sharp angle.

Table 3. Bracteole ratios from seeded and seedless Brewers Gold  
(Paired measurements from 8 cones)

		Centimeters		
		<u>Bracteole Length</u>	<u>Fold Length</u>	<u>Ratio</u>
<u>Seeded Brewers Gold</u>				
1	1.50	.72	.48	
	1.41	.69	.49	
2	1.73	.83	.48	
	1.71	.75	.44	
3	1.62	.61	.38	
	1.51	.69	.46	
4	1.65	.60	.36	
	1.65	.61	.37	
5	1.68	.73	.43	
	1.58	.80	.51	
6	1.59	.62	.39	
	1.45	.66	.45	
7	1.50	.60	.40	
	1.68	.58	.35	
8	1.48	.61	.41	
	1.59	.66	.42	
			Avg.	.42
<u>Seedless Brewers Gold</u>				
1	1.55	.65	.42	
	1.54	.63	.41	
2	1.50	.54	.36	
	1.46	.52	.36	
3	1.35	.55	.41	
	1.41	.71	.50	
4	1.46	.63	.43	
	1.50	.75	.50	
5	1.53	.60	.39	
	1.58	.67	.42	
6	1.50	.68	.45	
	1.50	.59	.39	
7	1.61	.65	.40	
	1.53	.74	.48	
8	1.32	.57	.43	
	1.45	.60	.41	
			Avg.	.42

Table 4. Bracteole Ratios from Seeded Early and Late Cluster.  
(Paired measurements from 8 cones.)

Centimeters			
	<u>Bracteole Length</u>	<u>Fold Length</u>	<u>Ratio</u>
<u>Seeded Late Cluster</u>			
1	1.59	.91	.57
	----	--	--
2	1.65	.83	.50
	1.50	.78	.52
3	1.70	.92	.54
	1.64	.85	.52
4	1.75	.83	.47
	1.75	.96	.55
5	1.77	.86	.49
	1.70	.90	.53
Bracteole wide; fold is generally pressed against bracteole.			
6	1.52	.80	.53
	1.77	.85	.48
7	1.78	.97	.54
	1.57	.72	.46
8	1.59	.82	.52
	1.63	.92	.56
			Avg. .52
<u>Seeded Early Cluster</u>			
1	2.00	1.29	.65
	1.90	1.45	.76
2	2.01	1.38	.69
	2.20	1.75	.80
3	2.32	1.43	.62
	2.09	1.38	.66
4	2.04	Nearly all the way	.98
	2.08	1.40	.67
Bracteole very wide; fold with gradual roll into edge of bracteole.			
5	1.82	1.12	.62
	1.94	1.13	.58
6	2.54	Fold all way to end	.98
	2.42	1.58	.65
7	2.05	1.40	.68
	2.10	1.69	.80
8	2.37	1.77	.75
	2.40	1.68	.70
			Avg. .73

Table 5. Variability of Bracteole Ratios Within One Cone of Seeded Late and Early Cluster. Measurement in Centimeters.

<u>Seeded Late Cluster</u>			<u>Seeded Early Cluster</u>		
<u>Bracteole length</u>	<u>Fold length</u>	<u>Ratio</u>	<u>Bracteole length</u>	<u>Fold length</u>	<u>Ratio</u>
1.10	.65	.59	1.22	.64	.52
1.45	1.00	.69	1.57	.91	.58
1.65	.98	.59	1.07	.60	.56
1.52	.93	.61	2.00	1.40	.70
1.72	1.01	.59	2.00	1.40	.70
1.82	.80	.44	1.55	1.04	.67
1.60	1.02	.64	1.70	1.25	.74
2.12	1.30	.61	1.80	1.45	.81
1.93	1.23	.64	1.90	1.05	.55
1.38	.80	.58	1.85	1.05	.57
1.50	.90	.60	1.69	.95	.56
1.75	1.00	.57	2.14	1.50	.70
1.80	.75	.42	2.18	1.63	.75
1.90	1.15	.61	1.80	1.45	.81
2.00	1.38	.69	1.64	.94	.57
1.92	1.45	.76	1.65	.95	.58
1.80	1.31	.73	1.25	.70	.56
1.68	1.18	.70	1.30	.80	.62
1.78	1.10	.62	1.57	.90	.57
1.39	.80	.58	1.35	.70	.52
1.75	1.25	.71	1.35	.98	.73
1.68	1.15	.69	1.58	.95	.60
1.86	1.23	.66	2.17	1.66	.76
1.32	.90	.68	1.50	1.15	.77
1.00	.80	.80	1.80	1.33	.74
1.25	.70	.56	1.49	1.00	.67
			1.80	1.16	.64
		Avg. .63	2.16	1.74	.81
			1.68	1.20	.71
			1.31	.75	.57
			1.11	.72	.65
			2.13	1.52	.71
			1.80	1.21	.67
			1.42	.92	.65
					Avg. .67

Table 6. Bracteole Ratios from One Middle-node on Each of 8 Seeded Late Cluster Cones. Measurement in centimeters.



<u>Bracteole length</u>			<u>Fold length</u>			<u>Ratio</u>		
1.	1.95	1.29	.66	5.	1.89	1.46	.77	
	2.26	1.72	.76		2.10	1.36	.65	
	2.10	1.40	.67		1.68	.77	.46	
	2.22	1.61	.73		1.43	.91	.64	
2.	2.18	1.65	.76	6.	1.51	.81	.54	
	1.93	1.47	.76		2.10	1.32	.63	
	1.67	1.28	.77		1.91	1.25	.65	
	2.08	1.59	.76		1.86	1.18	.63	
3.	2.27	2.01	.89	7.	1.70	.83	.49	
	2.11	1.58	.75		1.85	1.20	.65	
	1.91	1.33	.70		1.76	1.04	.59	
	1.83	1.15	.63		1.63	1.08	.66	
4.	2.00	1.38	.69	8.	1.96	1.30	.66	
	1.92	1.25	.65		2.01	1.24	.62	
	2.00	1.38	.69		2.08	1.36	.65	
	2.08	1.41	.68		2.10	1.40	.67	
Avg. 0.68								

Table 7. Bracteole Ratios from One Middle-node on Each of 8 Seeded Early Cluster Cones. Measurement in centimeters.

<u>Bracteole length</u>			<u>Fold length</u>			<u>Ratio</u>		
1.	2.10	1.60	.76	5.	2.40	2.00	.83	
	2.10	1.18	.56		2.32	1.83	.79	
	2.20	1.52	.69		2.17	1.60	.74	
	1.93	1.14	.59		2.27	1.66	.73	
2.	2.22	1.62	.73	6.	2.15	1.43	.67	
	2.15	1.45	.67		1.92	1.35	.71	
	2.30	2.04	.89		2.14	1.70	.79	
	2.02	1.62	.80		1.96	1.41	.72	
3.	2.10	1.70	.81	7.	2.12	1.18	.56	
	2.28	2.19	.96		1.79	1.04	.58	
	1.97	1.65	.84		2.25	1.58	.70	
	2.22	1.73	.78		2.07	1.59	.77	
4.	2.30	1.75	.76	8.	2.13	1.54	.72	
	2.19	1.78	.81		2.02	1.83	.91	
	1.70	1.07	.63		2.10	1.76	.84	
	1.76	1.14	.65		1.93	1.48	.77	
Avg. 0.74								

In previous studies with hop strigs certain distinguishing characteristics, as well as some similarities, were noted in some varieties. Strig shape, angularity and thickness, pedicel angularity and pedicel-strig attachment were considered in this study.

Fuggle and Hallertau strigs are easily recognizable by their very open structure. On both varieties, the pedicel extends perpendicular to the main axis of the strig, a characteristic unique to these two varieties. The Fuggle pedicel is longer and thinner than the Hallertau pedicel. The Hallertau strig is slightly more open and thicker when compared with Fuggle. For strigs of comparable size, the measured distance between pedicels on the same side of the main axis is greater for Fuggle than for Hallertau.

The general appearance of Bullion and Brewers Gold is similar. However, close examination of the strig reveals some recognizable differences. The zig-zag pattern of the Bullion strig appears to be composed of segments in the form of a quarter ellipse () alternating right and left of the major axis. The zig-zag pattern of Brewers Gold appears to be composed of nearly linear segments (). When compared, the pedicels of Bullion strigs appear longer than the pedicels of Brewers Gold strigs.

Early and Late Cluster possess strigs similar to Bullion strigs. In Bullion the pedicel attachment appears to be at the apex of the zig-zag pattern, most distant from the main axis, while in Clusters the bract scar is at this apex, with the pedicels attached above it, somewhat closer to the main axis. No significant difference could be observed between Early and Late Cluster.

Talisman exhibits a close packed structure which, combined with its extreme hairiness, gives the strig a massive appearance. The zig-zag pattern of the strig has a similar curvature common in Bullion, Late and Early Cluster. On Talisman also the bract scars occur approximately at the apex, with the pedicel attachments slightly higher and closer to the main axis.

Much variability in strig formation is seen in the extreme portions of the strig, while the central portion appears more consistent. For this reason comparisons and measurements were based on the central half of the strig. Measurements of strig angularity were made primarily from photographs of the sample material. High clarity outlines in the 'photos made this relatively easy and accurate. Strig angularity was determined by placing a transparent protractor over the strig 'photo and measuring the angle between the semi-axes.

Bullion, Late Cluster, Early Cluster and Talisman strigs are characterized by a curved strig internode (figure 3) and a resultant small strig angle (Table 8) as compared with Fuggle, Hallertau and Brewers Gold. This characteristic can be used to separate Bullion from Brewers Gold. Talisman can be distinguished from Clusters by strig pubescence and a vestigial stipular bract located between bract scars.

The following morphological features should be considered in a varietal identification scheme.

- |                   |               |                   |
|-------------------|---------------|-------------------|
| A. Strobile Strig | B. Pedicel    | C. Bracteole      |
| 1. Shape          | 1. Angularity | 1. Fold length    |
| 2. Angularity     | 2. Attachment | 2. Overall length |
| 3. Thickness      |               |                   |

Table 8. Strig Angularity on 7 Commercial Seeded Hop Varieties.  
(Measurements include 4 angles from each of 10 cones.)

Fuggle	150	147	145	150	124	130	141	140	140	130
	148	145	135	140	135	130	140	143	136	140
	140	140	130	120	140	129	138	143	130	135
	140	140	150	160	136	153	142	150	143	135
	<u>145</u>	<u>143</u>	<u>140</u>	<u>143</u>	<u>134</u>	<u>135</u>	<u>140</u>	<u>144</u>	<u>137</u>	<u>135</u>
Avg. 140°										
Hallertau	150	148	145	140	140	150	140	138	140	140
	150	148	140	140	140	150	140	142	140	145
	150	145	145	140	150	150	140	150	137	150
	150	147	140	140	133	145	130	145	140	145
	<u>150</u>	<u>147</u>	<u>142</u>	<u>140</u>	<u>141</u>	<u>149</u>	<u>138</u>	<u>144</u>	<u>139</u>	<u>145</u>
Avg. 144°										
Brewers Gold	140	140	120	150	136	140	150	140	142	140
	142	137	130	140	139	140	150	136	140	135
	140	148	130	138	140	140	140	142	138	140
	140	128	130	145	138	145	136	135	135	140
	<u>140</u>	<u>138</u>	<u>127</u>	<u>143</u>	<u>138</u>	<u>141</u>	<u>144</u>	<u>138</u>	<u>140</u>	<u>139</u>
Avg. 139°										
Bullion	120	130	120	120	125	120	125	130	120	130
	120	130	120	120	122	120	120	120	120	120
	120	123	120	123	120	130	133	125	120	125
	122	130	120	120	120	130	130	125	120	118
	<u>120</u>	<u>128</u>	<u>120</u>	<u>121</u>	<u>122</u>	<u>125</u>	<u>127</u>	<u>125</u>	<u>120</u>	<u>123</u>
Avg. 123°										
Talisman	108	98	120	110	100	100	120	95	115	110
	102	100	110	105	110	100	120	100	95	90
	108	110	110	100	120	108	100	90	110	105
	105	100	105	110	100	110	100	93	120	110
	<u>106</u>	<u>102</u>	<u>111</u>	<u>106</u>	<u>107</u>	<u>105</u>	<u>110</u>	<u>95</u>	<u>110</u>	<u>104</u>
Avg. 106°										
Late Cluster	110	110	120	115	125	100	120	120	125	120
	110	110	115	108	120	110	115	120	115	118
	120	110	120	115	120	105	100	120	110	120
	115	110	105	120	110	110	115	100	120	118
	<u>114</u>	<u>110</u>	<u>115</u>	<u>115</u>	<u>119</u>	<u>109</u>	<u>118</u>	<u>115</u>	<u>117</u>	<u>119</u>
Avg. 115°										
Early Cluster	120	120	120	100	120	110	120	110	120	110
	120	120	130	100	130	100	110	110	110	120
	120	115	120	105	120	90	100	115	120	120
	120	125	125	105	100	120	95	120	115	120
	<u>120</u>	<u>120</u>	<u>124</u>	<u>107</u>	<u>118</u>	<u>105</u>	<u>106</u>	<u>116</u>	<u>116</u>	<u>117</u>
Avg. 115°										

This measures strig angularity between the semi-axes of the strig.



This is the measured angle

BULLION

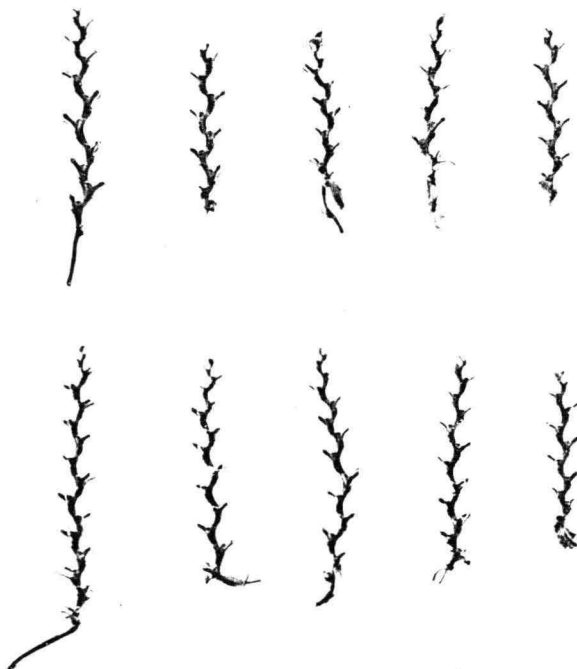
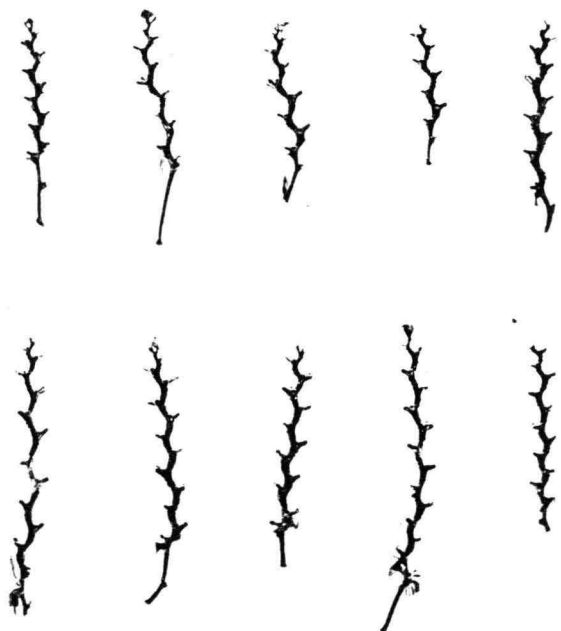


Figure 1. Strobile strigs from seeded Bullion and Brewers Gold. (X1)

BREWERS GOLD





HALLERTAU

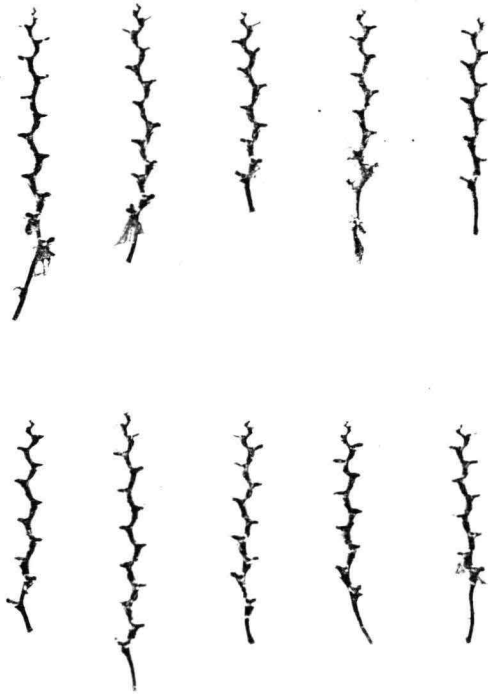
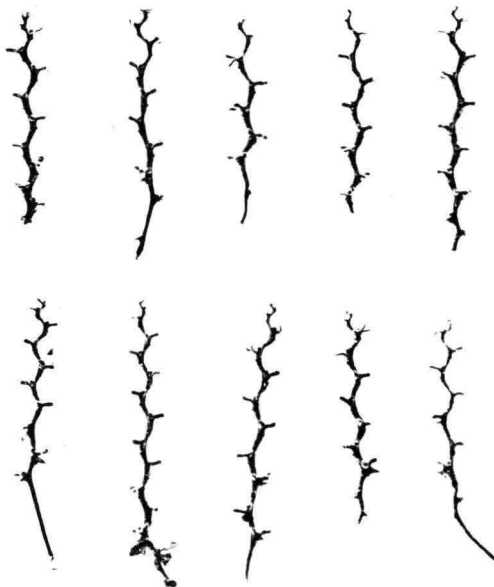
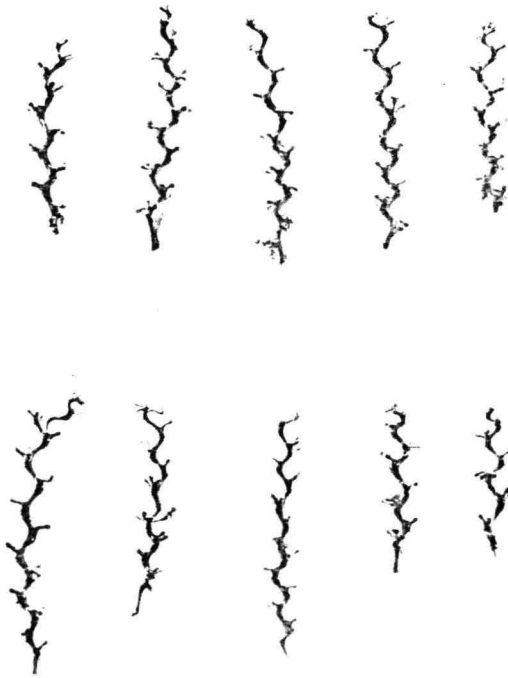


Figure 2. Strobile strigs from seeded Hallertau and Fuggle. (X1)

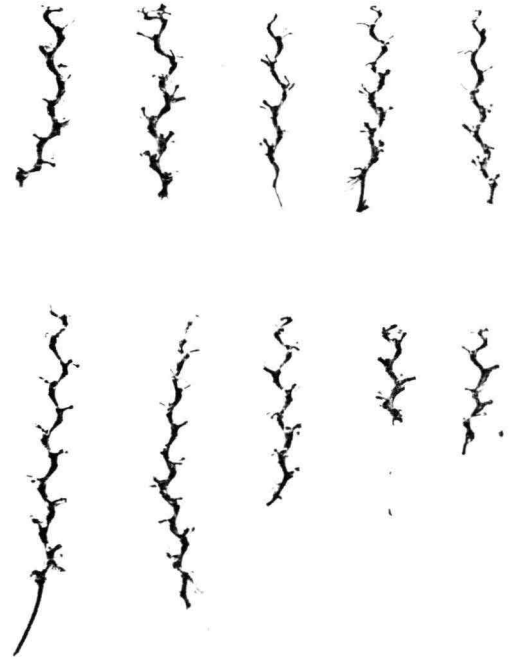
FUGGLE



## LATE CLUSTER



## EARLY CLUSTER



## TALISMAN

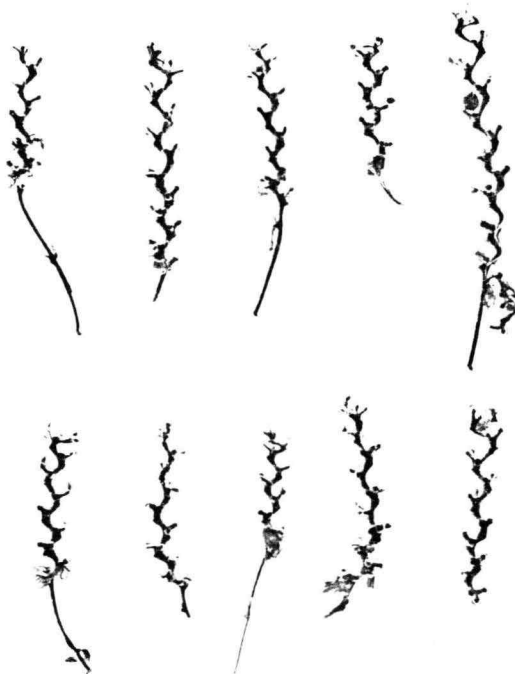


Figure 3. Strobile strigs from seeded Talisman, Late and Early Cluster. (X1)

Seeded

Bu

BG

Fu

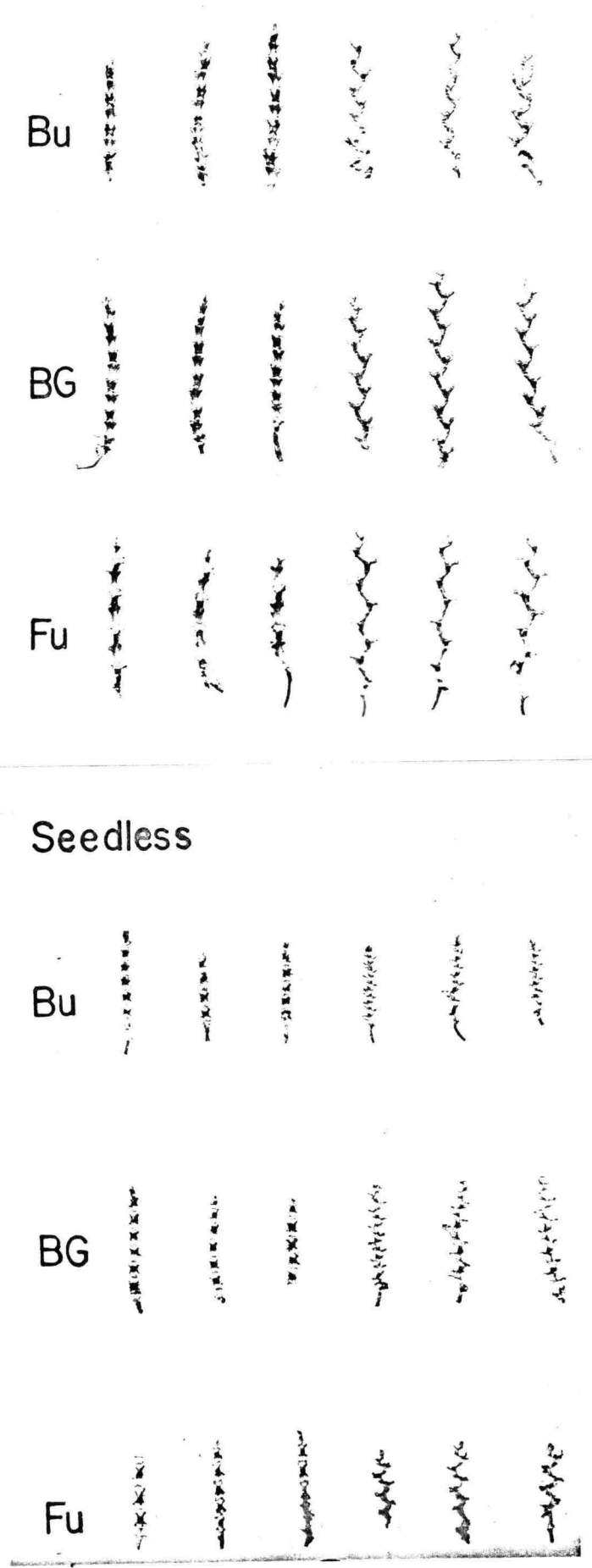
Seedless

Bu

BG

Fu

Figure 4. Comparison of strobile strigs from both seeded and seedless Bullion, Brewers Gold, and Fuggle. Three strigs on edge (left) and three strigs on flat axis.



Seeded

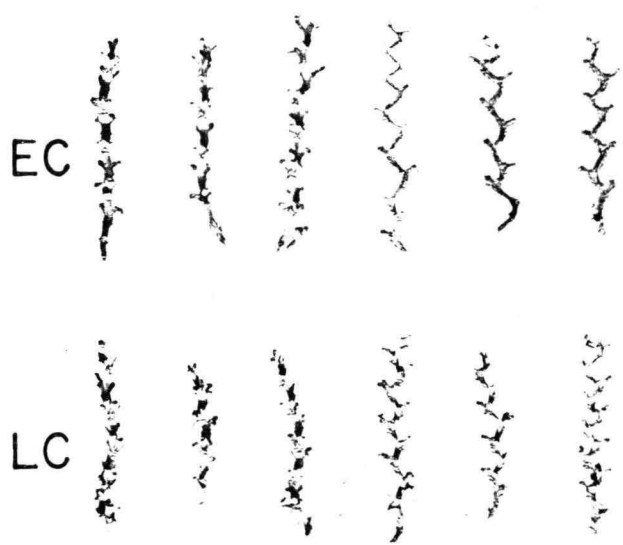
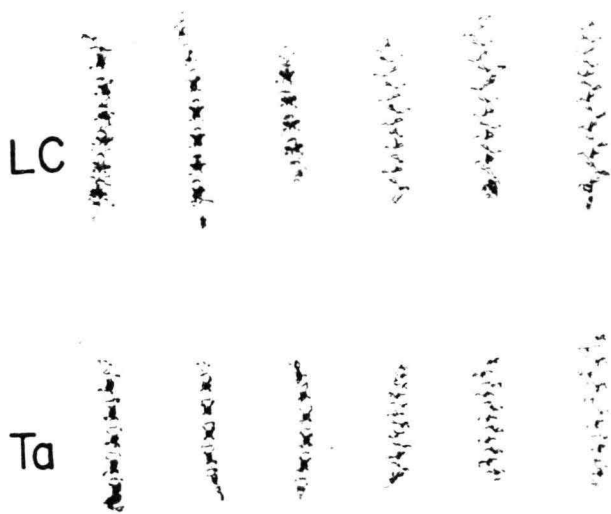


Figure 5. Comparison of strobile strigs from seeded Late Cluster, Early Cluster, and seedless Late Cluster and Talisman.

Seedless



## GENETIC AND CYTOGENETIC INVESTIGATIONS

Alfred Haunold

The following is a detailed description of research work and accomplishments of the past year, which is also summarized in the text of this report on page 26.

Polyploidy breeding with Fuggle:

Major emphasis this year was placed on developing a tetraploid Fuggle from the colchicine treated material described in the 1965 report (page 76). Shoots from colchicine treated material were rooted in the sand bed, and root tips checked for ploidy level, using the previously described Venetian turpentine method. The only difference in the method used today is that roots after Feulgen staining are placed into tap water instead of water plus a few drops of acetic acid. The formula for the "modified Venetian turpentine medium" as reported at the Western Regional Meetings of the American Society of Agronomy at Pullman, Washington (June 28 to 30, 1966) is as follows:

Table 1. Modified Venetian turpentine mounting medium <sup>1/</sup>

Venetian turpentine	50 ml
Phenol (90% liquid)	45 ml
Propionic acid (98%)	35 ml
Acetic acid (glacial)	10 ml
Water, distilled	15 ml

1/ Harleco Brand, manufactured by Hartmen & Leddon Co. Philadelphia, Pa. Not all brands of Venetian turpentine are suitable and considerable variation was found between identically named products from various manufacturers.

In order to be acceptable, Venetian turpentine must be miscible with a limited quantity of water, 45% acetic acid or acetocarmine. A total of 40 shoots from 5 different colchicine treatments (1965 report, Table 6, page 77) was cytologically checked for level of ploidy in the first cycle of selection after colchicine treatment. Many contained tissue of mixed ploidy (Table 2). Three plants from the 1963-64 colchicine program (1965 report Table 5, page 77), namely E-1-2-5, F-1-1 and Fu-6, were identified as tetraploid carriers and were also saved for future screening (Table 3).

The most promising clones listed in Tables 2 and 3 were advanced to the third cycle of selection (Table 4). In all cases, several cuttings from each clone were grown for future screening to guard against occasional loss of cuttings in the mist chamber. Cuttings consisted of at least one node with two leaves and an axillary bud adjacent to each leaf. In order to simplify record keeping, an arbitrary code was adopted which is listed in Table 4. For working convenience, both the material in Tables 2 and 3 was combined into one group.

Table 2. Polyploidy breeding with Fuggle: first cycle of selection after colchicine treatment.

Colchicine concentration	Shoot number	Cytological results <u>1/</u>
0.50% aqueous	1	diploid
	2	"
	3	tetraploid
	4	diploid
	5	"
	6	tetraploid carrier, very good
0.60% aqueous	1	diploid
	2	"
	3	"
	4	"
	5	"
	6	tetraploid carrier, very good
	7	" " " "
	8	diploid
0.75% aqueous	1	tetraploid carrier, good
	2	diploid
	3	tetraploid carrier, good
	4	diploid
0.50% lanolin	1	diploid
	2	"
	3	questionable, 2 tetraploid cells
	4	" 3 " "
	5	diploid
	6	questionable, 2 tetraploid cells
	7	tetraploid carrier, good
	8	discard
	9	questionable, several tetraploid cells
	10	diploid
	11	"
0.60% lanolin	1	"
	2	"
	3	"
	4	tetraploid carrier, very good
	5	diploid
	6	"
	7	"
	8	"
	9	"
	10	"
	11	"

1/ based on 3 cuttings per shoot, 8-20 roottips per cutting.

Table 3. Polyploidy breeding with Fuggle: material from the 1964 colchicine treatments.

Clone	Shoot number	Cytological results <u>1/</u>
D-1(D 1-1)	1	diploid
	2	questionable, 1 tetraploid cell
	3	" " 2 " cells
D-1-2	1	diploid
	2	"
	3	"
	4	questionable, several tetraploid cells
	5	diploid
	6	"
E-1-2-1	1	"
	2	questionable, several tetraploid cells
E-1-2-3	1	diploid
	2	"
E-1-5(E-1-2-5)	1	questionable, several tetraploid cells
	2	tetraploid carrier, very good
E-1-3	1	questionable, several tetraploid cells
F-1-1	1	" " " "
	2	tetraploid carrier, very good
Fu-6	1	" " " "
	2	diploid

1/ based on 3 cuttings per shoot, 8-20 roottips per cutting.

Table 4.- Polyploidy breeding with Fuggle: second cycle of selection after colchicine treatment.

Colchicine treatment	Shoot number	Code	Cytological results
0.5% aqueous	3-1	A-1	diploid
		a-1	"
	3-2	A-2 *	questionable, some tetraploid tissue (A-2-2)
		a-2	diploid
	6-1	B-1 *	tetraploid carrier, very good
		b-1 *	" " " "
	6-2	B-2 *	" " " "
		b-2 *	" " " "
	6-3	B-3	diploid
		b-3	"
0.6% aqueous	6-1	C-1	"
		c-1	"
	6-2	C-2 *	questionable, some tetraploid tissue
		c-2	diploid
	6-3	C-3	"
		c-3	"
	7-1	D-1	diploid
		d-1	"
	7-2	D-2	"
		d-2	"
	7-3	D-3	"
		d-3	"
	7-4	D-4	"
		d-4	"
	7-5	D-5	"
		d-5	"
7-6	D-6	"	
	d-6	"	
0.75% aqueous	1-1	E-1	"
		e-1	"
	1-2	E-2	"
		e-2	"
	1-3	E-3	"
		e-3	"
	3-1	F-1	"
		f-1	"
	3-2	F-2	"
		f-2	"
3-3	F-3	"	
	f-3	"	
0.50% lanolin	7-1	G-1	"
		g-1	"
	7-2	G-2 *	tetraploid carrier
		g-2	diploid
	7-3	G-3	"
		g-3	"
	7-4	G-4	"
		g-4	"



Table 4 - cont.

Colchicine treatment	Shoot number <sup>1/</sup>	Code <sup>2/</sup>	Cytological results
0.50% lanolin	7-5	G-5	diploid
		g-5	"
	7-6	G-6	"
0.60% lanolin	4-1	g-6	"
		H-1	"
		h-1	"
	4-2	H-2 *	tetraploid carrier, very good
		h-2 *	" " " "
	4-3	H-3	diploid
		h-3 *	tetraploid carrier, very good
	4-4	H-4 *	" " " "
Fu-6		h-4	diploid
	1-2	K-2	"
		k-2	"
	1-3	K-3	"
F-1-1		k-3 *	tetraploid carrier
	2-1	L-1	diploid
		l-1	"
E-1-2-5	2-2	L-2 *	questionable, some tetraploid tissue
		l-2	diploid
	2-1	M-1	"
	m-1	"	
2-2	M-2	"	
	m-2	"	
2-3	M-3	"	
	m-3	"	
2-4	M-4	"	
	m-4	"	

1/ eg. 3-1 means shoot No. 3, first cutting taken adjacent to main stem;  
3-2 is the cutting on the same shoot after adjacent to 3-1

2/ Generally both buds produced shoots. The capital letter denotes the shoot which developed first, the small letter the one which grew later.

\* Indicates clones advanced to next cycle of selection.

Table 5. Polyploidy breeding with Fuggle: third cycle of selection after colchicine treatment

Shoot number and cutting	Cytological results
A-2-2-I	diploid
A-2-2-II	"
B-1-1-I *	tetraploid carrier
B-1-1-II	diploid
B-1-2-I	"
B-1-2-II	"
B-1-3-I	"
B-1-3-II	"
B-1-4-I *	tetraploid carrier, good
B-1-4-II	not analyzed
b-1-1-I	diploid
b-1-1-II *	tetraploid carrier, very good
b-1-2-I *	" " " "
b-1-2-II *	" " " " nearly all cells tetraploid
b-1-3-I	diploid
b-1-3-II *	tetraploid carrier, very good, nearly all cells tetraploid
B-2-1-I	diploid
B-2-1-II	"
B-2-2-I	"
B-2-2-II	not analyzed
B-2-3-I	diploid
B-2-3-II	"
B-2-4-I *	questionable, some tetraploid tissue
B-2-4-II	diploid
b-2-1-I	"
b-2-1-II *	tetraploid carrier, very good
b-2-2-I	diploid
b-2-2-II	not analyzed
b-2-3-I *	tetraploid carrier, very good
b-2-3-II *	" " " "
b-2-4-I *	" " very good
b-2-4-II *	" " " " nearly all cells tetraploid
C-2-3-I	diploid
C-2-3-II	"
C-3-3-I	not analyzed
C-3-3-II	" "
G-2-1-I	too young, not analyzed
G-2-1-II	" " " "
G-2-2-I	" " " "
G-2-2-II	" " " "
H-2-1-I	diploid
H-2-1-II	not analyzed
H-2-2-I *	tetraploid carrier, very good
H-2-2-II	diploid
H-2-3-I	"
H-2-3-II *	tetraploid carrier, very good

Table 5 -- cont.

Shoot number and cutting	Cytological results
H-2-4-I	diploid
H-2-4-II *	questionable, tetraploid tissue (H2-4-II-3)
h-2-1-I	diploid
h-2-1-II	"
h-2-2-I	"
h-2-2-II	"
h-2-3-I	"
h-2-3-II	"
h-2-4-I *	tetraploid carrier, very good
h-2-4-II	diploid
h-3-1-I *	tetraploid carrier
h-3-1-II	diploid
h-3-2-I	"
h-3-2-II *	tetraploid carrier
h-3-3-I	not analyzed
h-3-3-II	diploid
H-4-1-I	"
H-4-1-II	"
H-4-2-I	"
H-4-2-II	"
H-4-3-I	"
H-4-3-II	"
H-4-4-I *	tetraploid carrier, very good
H-4-4-II	not analyzed
k-3-1-I	diploid
k-3-1-II *	tetraploid carrier, very good
k-3-2-I	diploid
k-3-2-II	"
k-3-3-I	"
k-3-3-II	"
L-2-3-I	"
L-2-3-II	not analyzed
l-1-1-I	diploid
l-1-1-II	"

\* Indicates clones advanced to next cycle of selection.

13 clones from 10 different cuttings were advanced to the third cycle of selection in midsummer (Table 4). At this time three clones, namely B-1, (b-1); B-2, (b-2); and H-2, (h-2) looked especially promising. Tetraploid tissue was found in several root tips of both shoots on each clone.

Most cuttings again developed shoots from each of the two axillary buds which were designated I and II (Table 5).

Several clones in the above table (especially b-1-2-II, b-1-3-II, and b-2-4-II) appeared to contain a large amount of tetraploid tissue. Other clones (those marked "very good") also carried considerable amounts of tetraploid tissue. Several cuttings from each of the promising clones were rooted, transplanted to peatmoss pots, and subjected to vernalization during November-December, 1966. They will be grown in one gallon cans in the greenhouse for further selection.

In comparing cuttings from Table 4 with daughter clones listed in Table 5, it can be noticed that some of the apparently good tetraploid carriers in the follow-up generation apparently produced only diploid tissue (e.g. B-1; h-2). Several factors may be responsible for this apparent discrepancy. The diploid tissue may have produced roots more rapidly than the tetraploid tissue in the same cutting and thus a non representative sample was available for cytological examination. It is also known from other crops that in tissue of mixed ploidy, such as diploid-tetraploid, a competitive disadvantage exists for the tetraploid tissue which can lead to a "running out" of the polyploid tissue upon vegetative propagation. It appears, however, that this is not the case with hops, since a number of cuttings remained good tetraploid carriers in subsequent cycles. (e.g. b-1; b-2; H-2)

Cytological examination of hop root tip tissue using the new Venetian turpentine technique in most cases gives a clear cut identification of the polyploid tissue. Even with non-dividing cells, tetraploid tissue in many cases can easily be recognized since the cells are almost twice the size of their diploid counterparts on the same slide. Cell size alone without chromosome counts however cannot be used to identify tetraploid tissue since cell size in diploid root tip tissue may vary considerably.

At present it appears that a pure tetraploid Fuggle clone may be obtained during the coming year after at least one more screening cycle. Should a pure clone become available early in the year, it will be grown to flowering and crossed with a male Fuggle-like seedling to produce a Fuggle-like triploid which will be sterile, and hopefully, higher yielding than diploid Fuggle. Triploids in future work will also be used as a starting point in the production of trisomics as discussed on page 168.

#### Collection of genotypes for genetic studies:

A number of atypical hop seedlings appeared in the progeny of several crosses in the greenhouse in the spring of 1966. These included albino seedlings, seedlings with mosaic leaves, seedlings with yellowish leaves or with lanceolate, narrow, or deeply lobed leaves, stunted (dwarfed) seedlings, seedlings with a green or deep red hypocotyl, green or red leaf petiole, and others. The "mutant"

seedlings were transplanted to one gallon cans later in the spring and some of the more vigorous ones were later transplanted to the field. They will be phenotypically checked again in the spring of 1967 and the most promising ones will be included in a genetic block in the new hop yard.

The remainder was grown in the greenhouse during the summer of 1966 and re-classified in the fall (Table 6). They are now undergoing vernalization in a cold frame and will be transplanted to the genetic block in the spring of 1967. Some of the more easily recognizable traits (e.g. leaf color, stem color, etc.) will be used in the future for genetic studies.

Table 6: Genotypes collected for genetic studies, Spring, 1966.

Tentative gene symbol( )	Cross No.	Pedigree	Remarks
lanceolate leaf (1a)	65033	47-40 x OP	trisomic (2n+1) about 20" tall at midsummer, many growing points, lanceolate leaves, fine stems, hooked hairs, low in vigor, sex unknown
dwarf (dw)	"	"	very small (2-3" high at midsummer) very low in vigor, very few furry hairs, distorted leaves
green hypocotyl (gh)	"	"	looks normal, green hypocotyl
lobed (1o)	65036	48-8 x OP	striped leaves, deeply lobed, little tendency to climb, grew well during summer. In late summer many small branches developed from several nodes. Female; height about 3 feet.
red hypocotyl (rh)	65030	40-2 x OP	hypocotyl deep red; vigorous; normal
1o, rh	"	"	deeply lobed leaves, red hypocotyl, normal
rh	"	"	vigorous, red hypocotyl
rh	"	"	" " "
gh	"	"	green hypocotyl
red vine (rv) ?	"	"	red vine, looks normal otherwise, leaf petioles also colored
1o	"	"	deeply lobed leaves similar to wild American hops
yellow leaves (y)	65027	OB852 x 110-S	yellow leaves, lower stem portions red.

Table 6 -- cont.

Tentative gene symbol ( )	Cross No.	Pedigree	Remarks
y	65027	OB852 x 110-S	yellow leaves, lower stem portions green
y	"	"	yellow leaves, lower stem portions green
y	"	"	yellow leaves, lower stem portions green
y	"	"	yellow leaves, lower stem portions green
y	"	"	yellow leaves, lower stem portions green
y	"	"	yellow leaves, heart shaped

Among the seedlings kept in the greenhouse, three were remarkable because of their low vigor. One had small, striped and deeply lobed leaves. It grew somewhat more vigorous during mid-summer, reaching a height of about three feet and later developed many small shoots on a number of nodes. It produced a single cone, and is probably a female plant. The second one never grew more than two to three inches tall, and had irregularly shaped leaves, and no distinct growing point. It appeared to grow somewhat better in late fall during shorter day length, and lower greenhouse temperatures. The third mutant appeared to be a dwarf initially that started to grow somewhat more vigorous in mid-summer, reaching a height of about 20 inches. It had many small shoots and pale green lanceolate leaves and a few hairs (figure 1). Softwood cuttings were made in September, and a few small roots developed, which were fixed in Carnoy's solution for cytological examination. The plant was identified as a trisomic, having 21 chromosomes ( $2n + 1$ ) (figure 2). The extra chromosome was tentatively identified as one of the larger ones of the chromosome complex with a median centromere. The plant never reached flowering stage, and therefore the sex could not be determined. This is the first known case that a trisomic has been identified in hops, and a manuscript describing the plant and the cytological techniques used for its identification is in preparation.

Trisomics in other species have been used to identify linkage groups. It is anticipated that a complete set of male and female trisomic hops can be developed for genetic studies. Most frequently in other species, trisomics were obtained from the progeny triploid x diploid crosses. One natural triploid hop (C56008) which was identified in our breeding material last year (1965 report page 75) rarely sets seed. 28 seeds were obtained from an open-pollinated location in 1966. They will be germinated in the spring of 1967 in the hope of obtaining additional trisomics. An effort will also be made to pollinate C56008 with a number of different males in order to see whether seed set could be



Figure 1. Phenotype of the trisomic hop plant with "lanceolate" leaves. Height in cm.

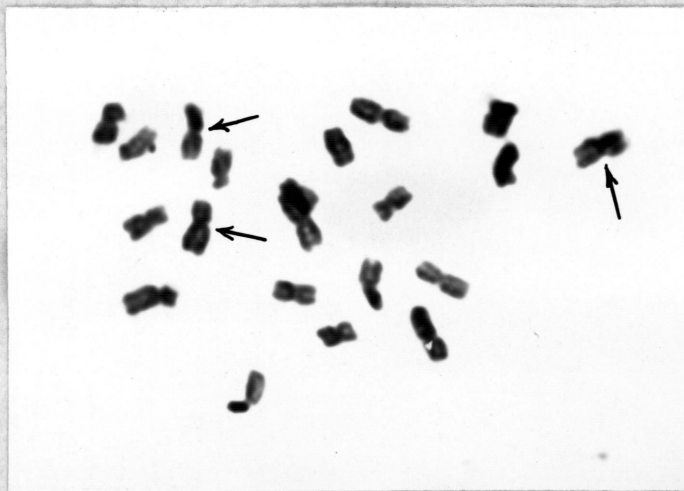


Figure 2. Metaphase cell from a root tip smear showing 21 chromosomes (approx. 5000 X). The suspected trisomic chromosomes are indicated by arrows.



increased by using different male pollinators. The search for other naturally occurring trisomics and triploids will also continue. The mutant seedlings that were transplanted to the field in the spring of 1966 will be re-evaluated in the spring of 1967, and the most promising ones included in the genetic block. A large number of genotypes that exhibited interesting morphological characteristics such as cone size and shape, leaf color, leaf shape, etc. were selected from the 1963 nursery in the old hop yard to be included in the genetic block in the new yard (Table 7). They will be maintained in single hills and used in crosses for future genetic studies.

#### Spider mite resistance study:

Segregating progenies from three crosses were grown in a 13 x 100 ft. greenhouse soil bed during the summer of 1966 under natural spider mite infestation in order to obtain preliminary information on mite resistance. Seedlings were germinated in greenhouse flats and transplanted on June 3-4 to a 2' x 2' spacing. A total of 285 hills were planted, representing 265 genotypes from the three crosses plus 20 parental checks at appropriate intervals. One cross (6505) had only nine seedlings, while cross 6512 was represented by 130 seedlings, and cross 6511 by 126 seedlings. Commercial fertilizer (12-12-12) at the rate of 450 lbs./acre was applied before planting. The young hop plants were trained on a ten foot trellis under simulated field conditions. Plants were rated visually based on the amount of leaf damage due to insect feeding on July 29, August 11, and Sept. 19. The most promising genotypes were harvested on October 10 and the crowns are presently in cold storage. They will be planted in the field this spring for further studies and crossing. A few male and female genotypes that apparently were completely susceptible were also saved for future genetic studies.

The rating on July 29 was obtained when some of the plants were still too young to show good infection. The August 11 rating was the most meaningful one and selections were largely based on that classification. Many plants that previously had exhibited a good level of resistance appeared to be heavily infested on September 19. This was due probably to the extremely high population of spider mites at that time. A build-up of green aphids occurred during September, particularly in cross 6511 in the South section of the soil bed. Some genotypes that showed less aphid infestation as compared to adjacent plants were also saved and are included in Table 8. The information on aphid resistance which was obtained at harvest time may be only of limited value due to the localized infection concentrated mainly in one area of the soil bed and near the greenhouse windows.

Some genotypes under very heavy spider mite infestation looked surprisingly good (Table 8). Figure 3 shows three plants adjacent to each other grown an identical distance from the greenhouse window. The plants on the left and on the right were severely infested (10-1 and 12-1) while the center plant (11-1) maintained a high level of resistance throughout the growing season. Plant 11-2 adjacent to 11-1 also was heavily infested. Most plants in the soil bed, even those which were severely damaged by spider mites, had a well-developed root system. Considerable differences among genotypes were noticed with regard to the number of lenticelles and color of the crown buds.



Table 7 Genotype collection potentially useful for genetic studies, seedling nursery, old yard.

	Row	Cross No.	Plant No.	Remarks
1963 nursery	37	62037	29	"pagoda" cones, tips of bracts bent upwards
	38	62046	19	large cones
		62040	27	small cones, late
		"	29	very large cones
		62042	33	very small cones
		62029	37	"pagoda" cones
		"	38	dwarf, dark green foliage, narrow, deeply lobed leaves, sex? Looks like male
		"	41	hermaphrodite
		"	42	hermaphrodite "pagoda" cones, very long
		"	43	cones small, clustered (look like elm seed clusters)
		"	45	very small cones, poorly developed
	39	62020	3	hermaphrodite
		"	5	hermaphrodite, long pointed cones
		"	10	" " " "
		62027	21	very large cones, "pagoda"-like, pointed
		"	22	very small cones
		"	25	very small cones, slightly hermaphroditic
		"	26	hermaphrodite, distorted, small cones
		62028	28	very large cones
		"	35	very small cones, late, no seed set
		"	36	male, poorly developed inflorescence, looks like dwarf
		"	40	male, dark foliage, deeply lobed
		62030	50	male, red vines
	40	62002	4	medium, compact pointed cones
		"	5	large cones, round, compact
		"	10	large cones, loose, very long bracteoles
		"	17	very long cones
		"	20	very large cones
		"	23	very large cones, round
		"	28	very long, "pagoda"-like cones
		62018	35	very large, square cones
		62019	38	very small round cones
		"	41	hermaphrodite
		"	42	very small cones
		62021	46	hermaphrodite
		"	48	very long cones
	41	62007	8	"pagoda" cones
		62034	9	male, dark green foliage, five deep lobes, large leaves
		"	11	very long, square cones
		62034	15	med. to small round cones
		62010	20	male, leathery leaves, pronounced ribs
		"	26	female, mostly 3 lobes on leaves

Table 7 cont.

	Row	Cross No.	Plant No.	Remarks
1963				
nursery	41	62010	42	red stripes on vines, small round cones
		"	46	red vine, male, yellow areas on leaves different from mosaic pattern
	42	62003	7	hermaphrodite
		"	28	male with a few female cones (well developed) seed set (use for selfing) hermaphrodite
		62015	37	was hermaphrodite at Prosser, shedding pollen
	43	62008	14	mosaic leaves, female
		"	15	med. round cones
	44	62005	28	ruffled, long cones
		"	34	very long, pointed square cones, female
		"	39	long, loose, very long bracts & bracteoles, ruffled appearance
	45	62013	37	large, pointed, "pagoda"-like cones
		"	38	large cones, pointed, bracteoles, long.
		"	48	very long cones, pointed, square
		"	52	"pagoda" cones, large, round
	46	62011	2	med. to small cones, ruffled appearance of bracts and bracteoles
		"	44	small cones, round
		"	48	med. cones, round
		"	49	very large, square, pointed cones
	47	62000	15	male, red vines
		"	48	light green foliage
		"	49	dwarf? only 1 foot high at end of season
		"	50	red vines, female
	48	62003	3	dwarf plant
		"	4	" "
		"	5	" "
		"	17	"pagoda" cones, large cones
		"	20	"pagoda" cones, med. to small cones
		"	23	med. size cones, pointed, compact, hermaphrodite
		"	35	dwarf? 3 ft. tall at end of season, leaves not lobed
		"	36	" " " " " " " " " " " " " "
		"	38	" " " " " " " " " " " " " "
		"	43	hermaphrodite, mostly male, female cones and end of sidearm, poorly developed seed set
1962				
nursery	49	61074	8	very large cones, square, very pointed
		61084	16	ruffled cone, "pagoda"-like, large lobed leaves
		61085	18	yellowish leaves, male
		"	22	large, "pagoda" cones, shiny foliage, 5 lobed leaves
		"	25	ruffled large "pagoda"-like cones, dark foliage
		"	30	yellow foliage, large ruffled cones
		61086	31	speckled foliage (yellow)
		"	33	" " particularly at bottom. Fem. large cone
		"	36	yellow to white foliage, mostly at bottom, large cones.

Table 7 cont.

	Row	Cross No.	Plant No.	Remarks
1961				
nursery	51	60051	15	large cones "pagoda"-like
		60022	16	large cones, ruffled, "pagoda"-like, very strong aroma
		"	17	very large cones, "pagoda"-like, very strong aroma
		60088	32	"pagoda" cones, very pronounced
		"	38	very small cones, berry like
	52	60028	2	small round cones, hermaphrodite, lower mostly male, upper mostly female, seed set
		"	3	hermaphrodite mostly male, cones at end of sidearm, seed set
		"	4	hermaphrodite mostly male, cones at end of sidearm, seed set
		"	15	hermaphrodite, mostly male, seed set, cones at end of sidearm and branches
		"	16	hermaphrodite, mostly male, seed set, cones at end of sidearm and branches
		"	31	long, ruffled cones, slender
		60009	37	med. size, pointed cones, "pagoda"-like
		"	38	hermaphrodite, mostly male, seed set, cones at end of sidearm and branches
		"	39	hermaphrodite, mostly male, seed set, cones at end of sidearm and branches
		60045	52	small round compact cones
	53	60028	4	small round cones
1959				
nursery	54	58029	18	small ruffled "pagoda" cones
	55	58041	7	small round cones, a few male flowers
		"	16	very leathery leaves, males, large tissue sections, partially dead
		"	21	very many tightly clustered cones (med.) size hermaphrodite, mostly female
		"	31	med. size, compact, pointed cones
	56	LC x OP	12	small cones, red vine, red coloration on strig
		"	16	"pagoda" cone, large
		"	24	red strig, red vine, female
		"	50	ruffled, "pagoda" cone
1960				
nursery	57	59037	6	cones rectangular, medium size
		"	12	cones rectangular, medium size
	58	59037	16	pointed cones
1962				
nursery	59	61027	10	ruffled, large cone, "pagoda"-like
		"	25	male, dark green foliage, unknown pedigree, probably seedling volunteer

Table 7 cont.

	Row	Cross No.	Plant No.	Remarks
1962				
nursery	60	61009	20	red vine, med. to small round cones
		61015	28	very long slender cones
	61	61030	1	large cones, ruffled, "pagoda"-like
		"	2	large cones, ruffled, "pagoda"-like
		61033	17	female, "pagoda" cones aphid resistant?
		"	18	female, pointed, square cone, susc. to aphids
		"	19	female, pointed, square cone, susc. to aphids
		61034	20	female, "pagoda" cones aphid resistant?
	62	61054	29	red vine, shiny foliage, male
		61059	44	male, bot. leaves yellow, virus?
	63	61075	19	ruffled cones
		61078	47	round cones, med. size

Table 8. Parents and selections from the spider mite resistance study grown in a greenhouse soilbed, June - October, 1966

Pedigree	Source or Cross No.	Row & Plant No.	Sex	Spider mite infection <sup>1/</sup>			Remarks
				7/29	8/11	9/19	
<u>Parental checks: 2/</u>							
119-1,2	19058M			3.60	4.00	3.50	
120-1,2	19060M			1.90	3.50	3.60	
Bull.	I64100			2.20	3.60	4.80	
B.G.	I19001			2.90	4.30	4.80	
<u>Segregating progeny:</u>							
Bu x 120-1,2	6512	2-5	?	1-	3	5	
		3-1	Fem.	2	3	3-4	
		-2	"	0-1	2	3-4	
		-6	"	1	2+	5	
		4-1	"	1+	2	4	
		-3	"	3	3	3	poor
		5-2	"	2	2	5	poor, many leaves lost
		-4	"	2	3+	3-4	good
		6-1	"	1	1+	3-	good
		-2	?	2	2	5	good
		-6	Fem.	3	3+	3	early
		7-2	Male	1+	2	2-3	very good, little infec.
		-6	Fem.	2	4	3	
		8-5	"	0-1	1	4-	many lower leaves dead, aphids.
		9-4	"	5	5	5	poor, completely hermaph.
		-5	"	2+	2	4-5	lower leaves dead, some aphids.
		-6	"	3+	4	4	early, lower leaves dead very few aphids.
		11-1	?	1	2+	3	good
		-5	Fem.	1+	3	4	
		12-6	Male	3	3+	4	older leaves dead, seems to have some resistance
		14-6	Fem.	4	4+	3	good
		15-6	Male	1	3+	4-5	poor
		16-2	Fem.	2	3	4	older leaves dead
		-5	Male	2-3	3	3-4	older leaves dead
		-6	Fem.	1	2+	4	good
		17-1	Male	3+	5	5	completely susceptible
		-5	"	1	2	4+	
		-6	?	1+	3+	3	
		18-4	Fem.	0-1	1	3-	* very good
		-5	Male	1	3+	4	good
		19-3	Fem.	2	3	4+	good
		20-2	Male	3	4	4+	few lenticelles
		21-3	Fem.	1-	1	2	* very good, all adjacent plants heavily infested small, young
		22-4	?	1-2	2+	3	
BG x 120-1,2	6505	25-3	Fem.	1	2+	4-	very good, some aphids
		-5	"	2-	3+	4	older leaves dead, many aphids. Pink crown buds.

Table 8 - cont.

Pedigree	Source or Cross No.	Row & Plant No.	Sex	Spider mite infection <sup>1/</sup>			Remarks
				7/29	8/11	9/19	
Bu x 119-1,2	6511	27-1	Fem.	2+	3	2	* Very good, some aphids
		-6	"	1	2	3	many aphids
		28-1	"	0-1	1	3	young, good, few aphids
		-4	"	1+	4	3	
		-6	?	0-1	2+	4	many aphids
		29-2	Fem.	1	2+	4-5	good
		-5	"	2+	2+	3	good, few aphids, many have some aphid resist.
		30-4	Fem.	1	2+	5	good, few aphids
		-6	"	4	4+	5	few aphids
		31-2	"	2	3+	4	
		-3	"	2	3+	4	
		32-1	"	3	4	4	good
		-5	"	4	4	3	many aphids
		-6	Male ?	1	2	3+	some aphids
		33-2	Male	2	3	3	questionable
		-3	Fem.	1+	2	4+	
		-6	"	3	3	3	very good, many aphids but minimal leaf damage
		34-1	"	3	3	4+	good, few aphids
		-3	"	1	2	5	many aphids
		-4	"	3+	3	3	many aphids
		35-1	"	3	3	4-5	
		-2	"	2	2	1-2	good, young
		-5	"	2	2	4	heavy aphid infestation
		-6	?	2+	3	3	heavy aphid infestation
		36-6	Fem.	1	3+	4	heavy aphid infestation
		37-1	"	3+	3	2	* very good, few aphids, pink crown buds
		-2	"	3	4	4	good, no aphids, pink crown buds
		38-1	Male	1	2	3	* <u>very</u> good, no aphids
		-4	Fem.	1-2	2+	4	few aphids
		-5	Male	2+	4	5	very susceptible
		-6	Fem.	3	5	5	few aphids
		39-2	Male	2	3+	2+	no aphids
		-3	Fem.	1	2+	1-2*	<u>very</u> good, no aphids
		-6	"	3	5	5	<u>very</u> suscep. no aphids
		40-1	"	2	2+	4	some aphids
		-5	"	1	2+	2+	very good, no aphids
		41-4	"	1-2	3+	2-	very good, no aphids
		-6	"	2-	3	4+	no aphids
		42-2	?	1-2	2+	1	heavy aphid infestation
		-6	Fem.	1	2	4+	few aphids
		43-5	"	1-2	3	4	few aphids
		44-3	"	2	2	4	many aphids
		44-5	"	1	2+	3+	few aphids
		45-1	Male	1-2	3	2	heavy aphid infestation plant dead
		-6	Fem.	1	2	4	very few aphids

Table 8 - cont.

<u>Pedigree</u>	<u>Source or Cross No.</u>	<u>Row &amp; Plant No.</u>	<u>Sex</u>	<u>Spider mite infection</u> <sup>1/</sup>			<u>Remarks</u>
				<u>7/29</u>	<u>8/11</u>	<u>9/19</u>	
Bu x 119-1,2	6511	46-1	Fem.	0-1	1	3	many aphids
		-3	"	1-2	3+	2	many aphids
		-5	"	1	2	5	good, some aphids
		-6	"	1	2	3	good, few aphids
		47-3	"	0-1	2	2	many aphids

1/ Classified from 0-5. 0 = no feeding, no injury. 5 = leaf tissue killed, plant dying.

2/ Score based on five 1-hill replications, except for 19060M (Aug. 11) where only 4 replications were scored.



Figure 3. Various degrees of leaf damage due to spider mite infestation. Very little damage (center plant) as compared to heavy infestation (plants on left and right). Note dying plant on the right.



Grafting study:

The grafting experiment described in the last report (page 76) was not repeated in the field last summer. Environmental conditions in the field were unfavorable for grafting. Another grafting study is presently under way in the greenhouse. It is too early at this time to give any results, particularly with respect to an influence on sex expression.

Morphological studies of the female hop inflorescence:

Hop cones represent the commercial hop crop due to the large number of resin glands concentrated at the base of the bracteoles and bracts. The resin glands are generally believed to be saucer shaped structures of secretory cells carrying the resin in a bubble like structure. Macroscopic differences between certain varieties and between seeded and seedless hops with respect to number of resin glands are readily apparent in mature cones. Little information however is available on cone development and resin gland development.

Cones of Fuggle, Late Cluster, and Brewers Gold were collected during the summer of 1966 at the following stages:

Not burred out, non pollinated,  
 Burred out, non pollinated,  
 One day after pollination,  
 Seven days after pollination,  
 Fourteen days after pollination.

Additional non pollinated cones in the seedless yard were also collected to correspond to the collection time of pollinated cones. Cones were fixed in FAA (70%) dehydrated, embedded in "tissuemat", and will be sectioned in the near future.

Meiosis in hops:

Little work has been done on meiosis of hops. A large amount of microsporocytes was collected in 1960 by Y. Puri mainly to evaluate various fixatives and to learn about the proper time of collection. Preliminary information on this material was included in the 1965 report (page 78). The material has not been completely analyzed, and a summary appears in Table 9. Carnoy's, Newcomer's, or Farmer's fixative in most cases gave satisfactory results. Newcomer's solution seemed to give slightly better preparations and less background staining than the other two fixatives. It should be remembered, however, that this material was stored in the original fixative for over five years, which is considered too long for excellent results. Samples that were fixed in Navashin's fixative or FAA were totally unsuitable for cytological examination. All material stored in Navashin's fixative was partially frozen, dark, and cytologically indistinguishable. FAA likewise gave unsatisfactory fixation and none of this material is included in the table.

Many of the samples were collected too late in the season, since most florets had already completed meiosis. No difference was noted between material collected at 8:30 or 11 a.m. and good stages of meiosis could be found at all sampling times in the morning. Likewise no apparent difference was observed

Table 9. Meiosis in hop: time of collection of microsporocytes and evaluation of fixing solutions.  
(Collected by Y. Puri, 1960; evaluated summer 1966)

Date of collection	Time	Genotype	Pedigree	Fixative	Remarks
7/7/60	8:30-9 AM	C19008M	SemschS x 8-2 Br.Yard	Carnoy's	Good meiosis, dark background, staining
				Farmers	Good meiosis I, meiosis II, good staining, light background
				Newcomer's	Good stages, good staining
	9-9:30 AM	C19054M	EKG x BavS	Carnoy's	Good meiosis I, good staining
				Newcomer's	Many florets too old; poor stages of pachytene in youngest florets
				Farmers	Good meiosis I, many florets at quartet stage or containing mature pollen grains
	9:30-10 AM	C19058M	EG x OP	Carnoy's	Good pachytene and meiosis II esp. in quartets sample slightly too old
				Newcomer's	Very good meiosis I, good staining
				Farmers	Good meiosis I, quartets
	10 AM	C51114M	LandhS x GC1 x FuS x C19008M	Newcomer's	Good meiosis I, but dark background, older florets in quartet stage
				Carnoy's	Good meiosis I, and quartet stage, dark background staining
				Farmers	Meiosis I all stages, good staining, light background
7/8/60	9:15-9:40AM	C19060M	EKG x BavS	Carnoy's	Meiosis I, all stages, dark background staining
				Newcomer's	Good pachytene, diplotene, poor staining
				Farmers	Good meiosis I, all stages; quartets, dark background staining.
	C19052M	I19209 x FuS	Carnoy's	Good pachytene, dark background	
			Farmers	Good meiosis I, dark background	
			Newcomer's	Good meiosis I, many florets too old	
				Newcomer's	Good meiosis I, good staining

Table 9 - cont.

Date of collection	Time	Genotype	Pedigree	Fixative	Remarks
7/8/60	10:30 AM	C19009M	I19209 x FuS	Farmers Carnoy's Newcomer's	Pachytene, Telophon I, many florets too old Good metaphase I, good staining Good metaphase I, good staining
7/16/60	9 AM	C19058M	EG x OP	Carnoy's Newcomer's	Most florets too old, poor staining quartets, mature pollen Most florets too old, staining good
	9:45 AM	C19051M	BurgS x FuS	Newcomer's Carnoy's	Most florets too old Many florets too old, good stages of meiosis I in smallest florets
7/18/60	9 AM	C19085M	Landh-S x (Gol, Cl. x FuS)	Carnoy's Carnoy's	Most florets too old, quartet stage Most florets too old, youngest florets at pachytene
7/19/60	9 AM	C19062M	EKG x BavS	Carnoy's	Too old, mature pollen in most anthers
		I19005M	LC S	Carnoy's	Too old, poor staining
		C19060M	EKG x BavS	Newcomer's	Good metaphase I in youngest florets, <u>very</u> good staining
		C19058M	EG x OP	Newcomer's	Many florets too old, good staining
		C19170M	Unknown x [EKG x (EG x KGS)]	Newcomer's Carnoy's	Pachytene or quartets, difficult to find metaphase stage Good meiosis I
		C19053M	Belg Burv x BurvS	Newcomer's Carnoy's	Pachytene, Telophon I, very good staining Meiosis I, all stages
	10:30 AM	C19044M	I19209 x FuS	Newcomer's Carnoy's	Good meiosis I, occasionally a lagging univalent at metaphase I, bridge or late dividing univalent at telophon I, micronuclei at quartet stage Quartet stage, too old

Date of collection	Time	Genotype	Pedigree	Fixative	Remarks
7/21/60	10 AM	I58015M	WA - Utah	Newcomer's	Very good meiosis I, very good staining
		C19182M	Bu x (Belg 31 S x Belg 31)	Newcomer's	Very good meiosis I, very good staining
		I58006M	WA - Utah	Newcomer's	Very good meiosis I, very good staining
7/22/60	11 AM	I58010M	WA - Utah	Carnoy's	Good meiosis I, dark background staining
		I58006 M	WA - Utah	Carnoy's	Meiosis I, quartets, dark
		C52045M	C19063 x C19058M	Carnoy's	Good meiosis I, poor staining, dark
		C52046M	C19213 x OP	Carnoy's	Good meiosis I, some florets too old
		C19036M	LC x FuS	Carnoy's	Good meiosis I, many florets too old, dark background staining
		C19058M	EG x OP	Carnoy's	Good meiosis I, dark background staining
	11:30 AM	C19041M	EG x OP	Carnoy's	Meiosis I, dark background staining, many florets too old
		C19085M	Landh-S x (GoCl x FuS)	Carnoy's	Good meiosis I, good staining
		C19051M	BurS x FuS	Carnoy's	Meiosis I, <sup>poor</sup> very poor staining, dark

between the various male genotypes represented in this study with the possible exception of C19044M (collected on July 19, 1960) which occasionally showed univalents at metaphase I and a bridge and micro-nuclei at Telophase II. The time for sampling however cannot be considered adequate. Next summer additional material will be collected over a 24-hour period in order to learn whether or not a pronounced peak of meiotic activity exists in hops.

A number of good stages of meiosis from microsporocytes were photographed (figure 4). In several cases, individual chromosomes at pachytene were found completely separated from the usual cluster and a morphological study of pachytene chromosomes is planned for the future. Most frequently, pachytene and quartets were found in the material, while the later stages of meiosis I (diakinesis, metaphase I or telophase I) or stages of meiosis II were more difficult to find. This may not be of significance, since most of the material available was too old, and good stages of meiosis frequently could only be found in the smallest and youngest florets. Generally no abnormalities were noted in any of the genotypes. At metaphase I, usually ten bivalents were observed.

No work has been done on meiosis on the female inflorescence and generally cytologists have avoided working on female flowers if male flowers were available. Since hop is a dioecious plant with the female being the commercially important one, an attempt will be made to develop techniques for studying meiosis in the female inflorescence.

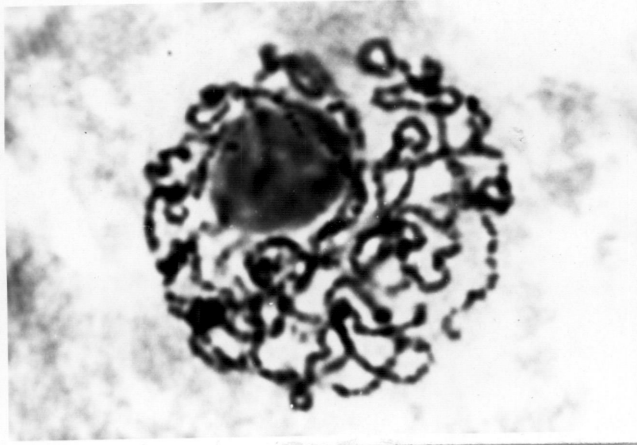
#### Crosses for genetic purposes:

Several crosses were made in 1966 to initiate genetic research of disease and insect resistance. Problems were encountered during the crossing season which resulted in poor seed set for several crosses, probably due to a high temperature build-up in the parchment bags. An attempt will be made during the 1967 crossing season to shade a portion of the breeding block. New bagging materials will also be investigated.

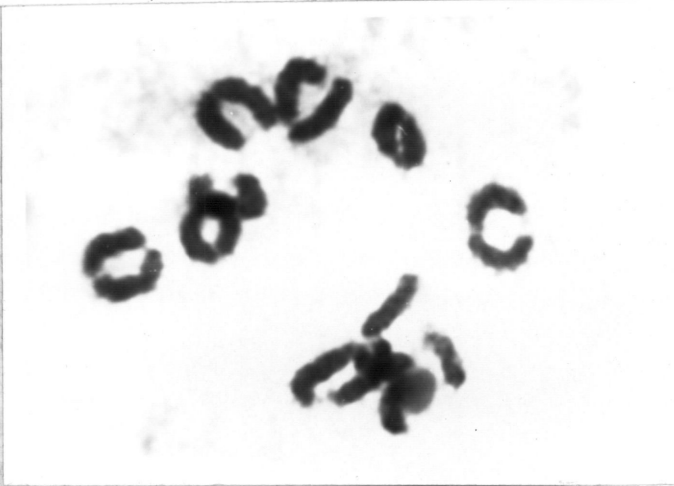
The successful crosses for the genetic program are listed in Table 10. They are also included in Table which lists all the crosses made in 1966. The seed will be pre-treated and transplanted in greenhouse flats in the spring of 1967. Seedlings of cross No. 666 later will be transplanted to the field.

Table 10. Crosses for genetic studies made in 1966.

Cross No.	Parentage	Pedigree	Type
665	C19151 x C19041M	[Fu x (RV x XS)] x EG x XS	mildew res.
666	C19209 x C19173M	Fu x (	inbreeding
667	C19113 x OP	[Bu x (Sam x XS)] x OP	trisomic ?
668	C56008 x OP	XS x [Fu x (EG x ECS)]	trisomic
673	I19001 x C63023M	BG x [BG x (BG x C19062M)]	vigor obs.
674	I19001 x Fu-1-1(66)	BG x Fu sex aberrant	mutants
675	I19001 x Fu-2-4(66)	BG x Fu sex aberrant	mutants
676	I19001 x C63022M	BG x (BG x I58015)	vigor obs.



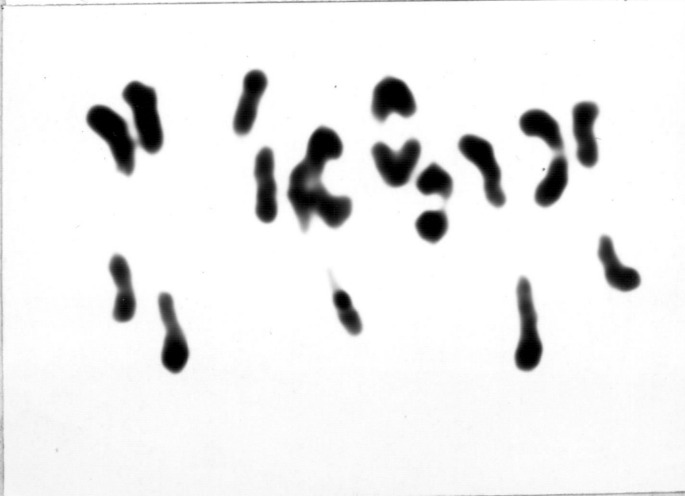
(a)  
Pachytene,  
nucleolus upper center



(b)  
Diakinesis  
10 bivalents,  
nucleolus visible  
lower center



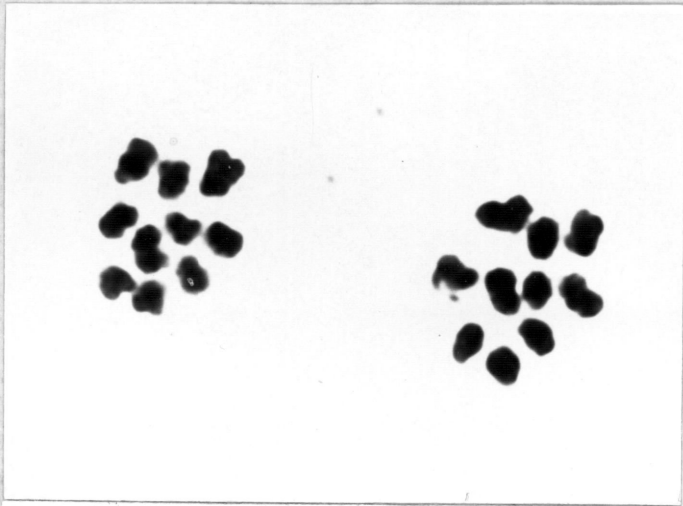
(c)  
Metaphase I  
10 bivalents



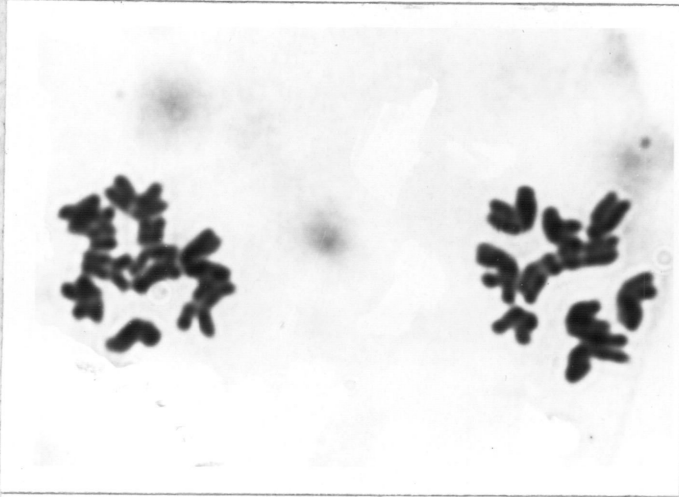
(d)  
Late Metaphase I or  
early Anaphase I  
2 groups of 10 chromosomes  
moving to opposite poles

Figure 4 Meiosis in the male hop, Humulus lupulus L.

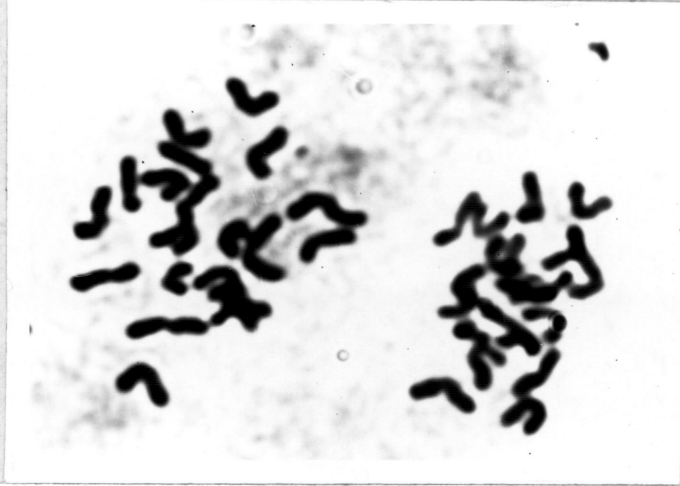




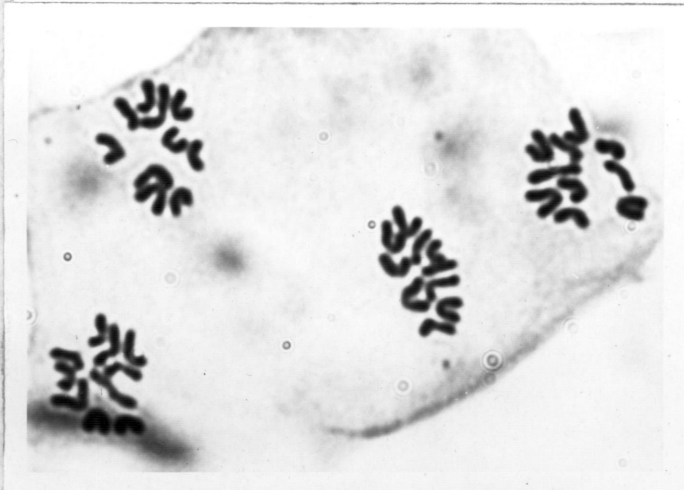
(e)  
Telophase I  
10 chromosomes at each  
pole



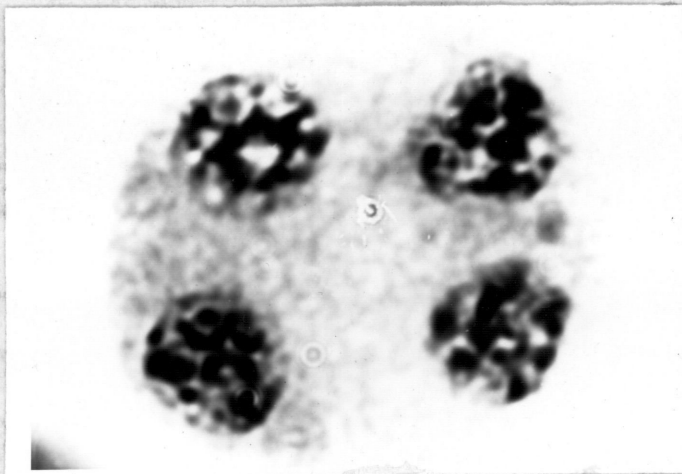
(f)  
Metaphase II  
Chromosomes dividing  
longitudinally



(g)  
Anaphase II  
Chromosomes moving  
to opposite poles



(h)  
Telophase II  
4 groups of 10 chromosomes  
on each pole



(i)  
Quartet  
giving rise to 4 pollen  
grains (n = 10)



Plans for the coming year:

1. Continuation of the Fuggle polyploidy program to produce a tetraploid Fuggle.
2. Search for additional mutants for genetic studies and especially trisomics ( $2n + 1$ ).
3. Study of cone morphology and resin gland development.
4. Pollination of C56008 (triploid) with various males to produce  $2n + 1$  seedlings.
5. Screening of parental material for spider mite resistance and evaluation of mite resistance crosses made in 1966.
6. Study of the time of collection of microsporocytes in relation to meiotic activity and development of techniques for studying meiosis in the megasporocyte.
7. Establishment of a genetic block in the new hop yard.
8. Study of inbreeding depression in two crosses by brother-sister mating.
9. Work on chromosome morphology and study of individual pachytene chromosomes.
10. Grafting study of male scions on female rootstock and vice versa in relation to sex expression.
11. Making of additional crosses for studying inheritance of spider mite resistance and downy mildew resistance.

Seedless Observation Block

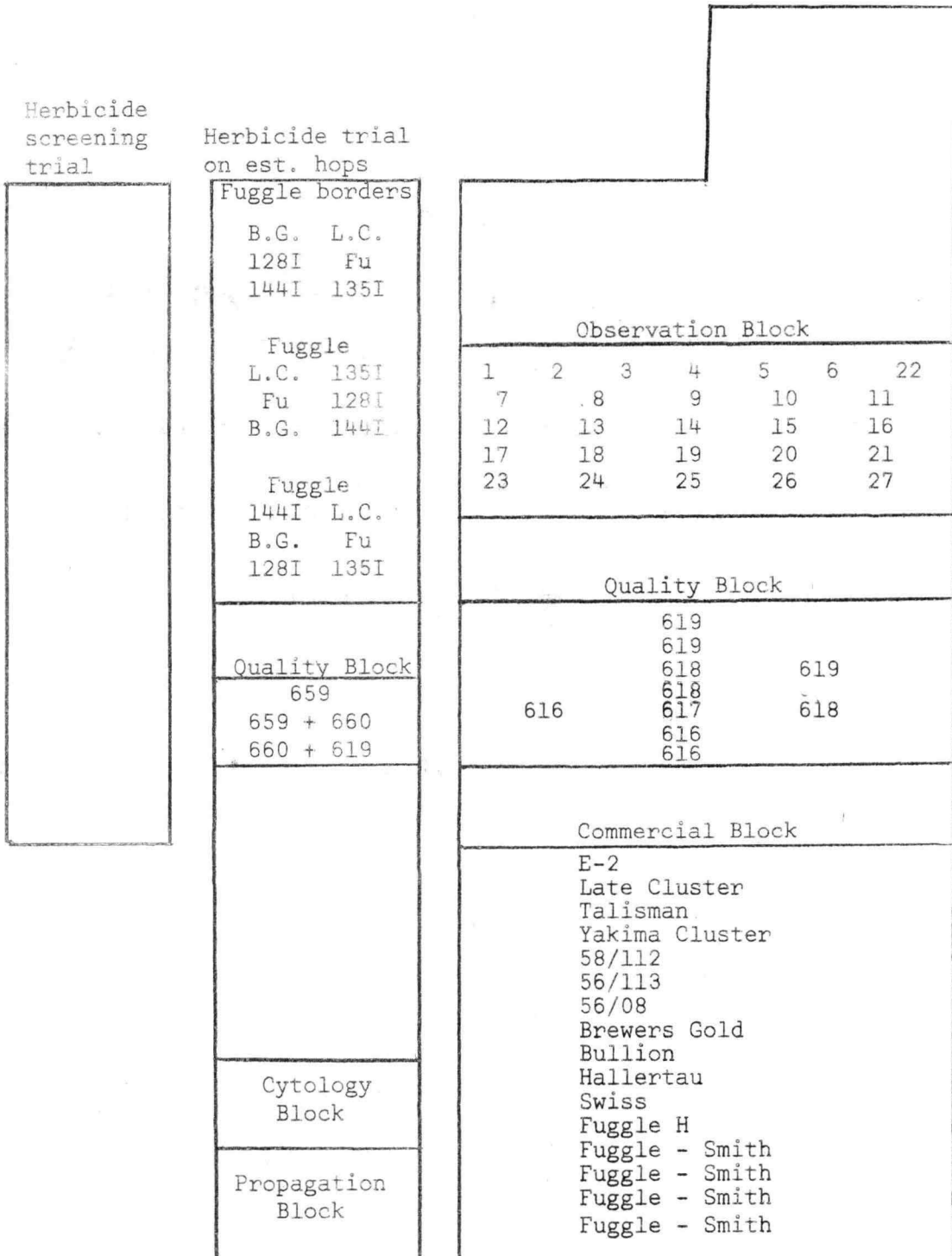
<u>Plot</u>	<u>Acc. No.</u>	<u>Ident.</u>	<u>H's planted</u>	<u>Missing H's</u>	<u>H's/Plot</u>
1	--	HG-NW 11-6-8	1	0	1
2	--	HG-NW 11-6-120	1,2	0	2
3	I 60/42	Shinshuwase	1,2	0	2
4	59/06	OB 841	1,2,3	0	3
5	57/11	OB 813	1,2,3,4	0	4
6	I 19/137	50-S	1,2,3,4	0	4
7	63/04	6341-34	0	1,2,3,4	4
8	63/05	6342-39	1,2	3,4	4
9	63/09	6348-9	0	1,2,3,4	4
10	--	6249-24	1,2,3	0	4
11	63/07	6344-44	0	1,2,3,5	5
12	19/20	BB 307	0	0	4
13	63/21	6348-1	0	2,3,4	4
14	63/20	6347-42	0	1,2,3	4
15	63/19	6347-40	0	0	4
16	63/18	6347-35	3,4,5	0	5
17	63/01	6337-28	3,4	0	4
18	63/02	6339-14	0	0	4
19	63/06	6344-9	2,3	0	4
20	63/08	6348-8	3,4	0	4
21	63/10	6348-21	0	0	5
22	--	GA 4-3	1,2,3,4,5	0	4
23	--	R 201-2	1,2,3,4	0	4
24	--	NW 12-20	1,2,3,4	0	4
25	--	R 117-2	1,2,3,4	0	4
26	--	HG-NW 11-6-31	1,2,3,4	0	4
27	19/110	15-S	1,2,3,4,5	0	5

Quality Block

<u>Cross No.</u>	<u>Pedigree</u>	<u>No. of plants</u>
616	Brs Gold x 6339-9	296
617	Brs Gold x 6344-36 (63/13M)	53
618	Brs Gold x 6347-32 (63/23M)	233
619	Brs Gold x 6348-15 (63/25M)	347
659	6347-42 (63/20) x 6348-15 (63/25M)	142
660	6347-42 (63/20) x WA 73-3 (I 60/30M)	28

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Outline of Seedless Yard, Smith 1967



Details of High Alpha-acid Crosses from 1966 <sup>1/</sup>

<u>Cross No.</u>	<u>Fem.</u> <sup>2/</sup>	<u>Male</u> <sup>2/</sup>	<u>No. cones</u>	<u>No. sound seed</u>	<u>No. abort. seed</u>	<u>No. sound seed/cone</u>	<u>% aborted</u>
616	BG	63N-39-9	147	714	101	5	19
617		C63/13M(44-36)	47	102	35	2	25
618		C63/23M(47-32)	140	765	119	5	13
619		C63/25M(48-15)	149	408	101	3	20
620		I60/30M(73-3)	79	578	51	7	9
657	C63/20(47-42)	C63/13M(44-36)	39	204	99	5	32
658		C63/23M(47-32)	16	74	18	5	20
659		C63/25M(48-15)	83	500	214	6	30
660		I60/30M(73-3)	43	255	104	6	29

<sup>1/</sup> Crosses 657 and 658 were cold treated and planted in the greenhouse on Mar. 29.

All other crosses were planted in 2 1/2 x 3" pots and left in the Smith Yard on Jan. 14.

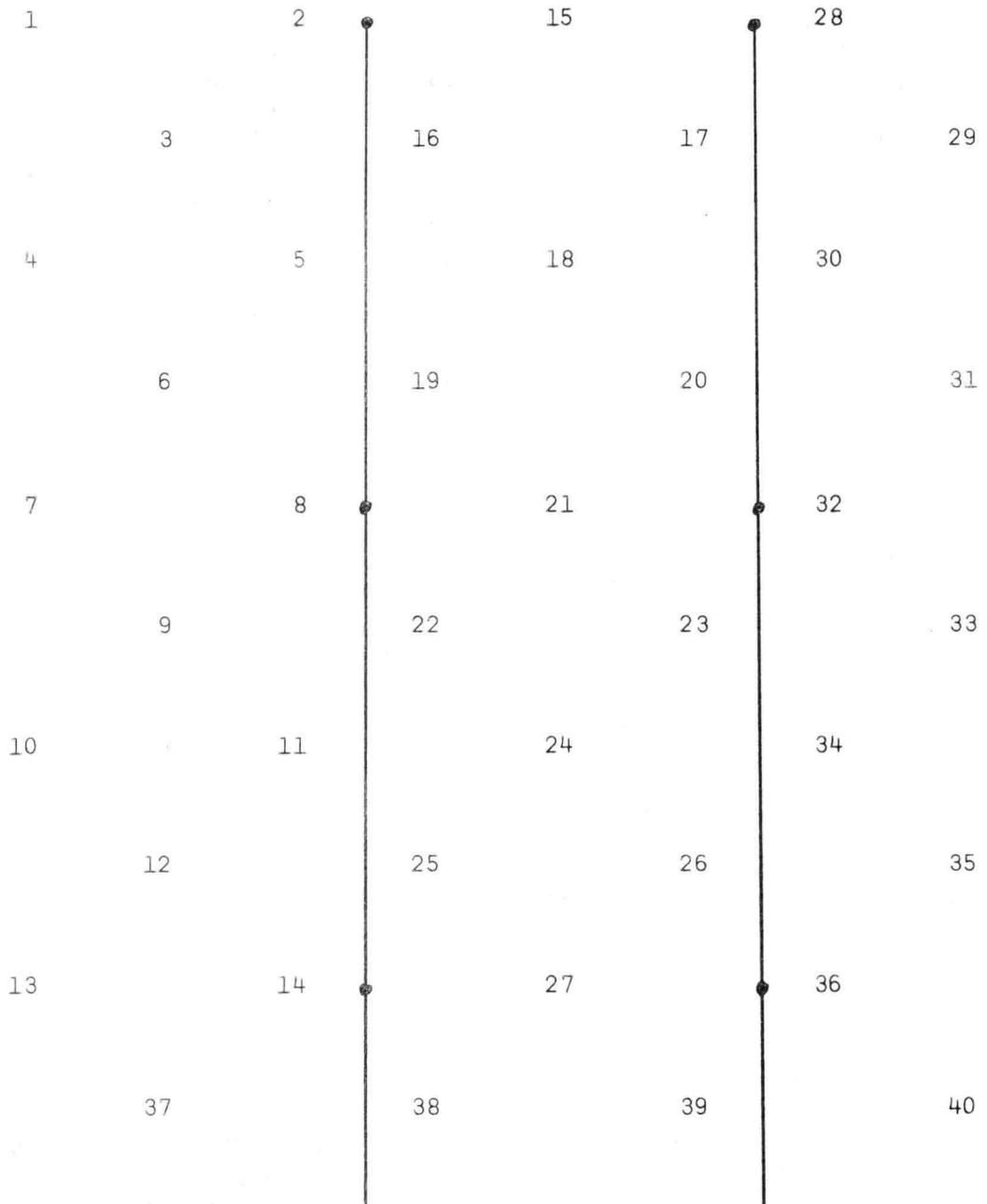
Crosses on C63/10(48-21) resulted in 0 to 10 seed per cross and were discarded as being unreliable.

Crosses were made on C63/19(47-40), but the vine broke early in the season and all crosses were lost.

<sup>2/</sup> Numbers in parenthesis refer to the 1963 nursery row and plant.

Testing of new irrigation equipment (summer 1966):

Two hour set was run at  $40 \pm 5$  psi with following arrangement (30' between lines):



## 6" diameter can data (two 1-hr. sets):

Can No.	water		Can. No.	water	
	mls/can	in./A/2 hr.		mls/can	in./A./2 hr.
1	70-60	.42	21	155-165	1.03
2	185-160	1.12	22	155-195	1.13
3	90-100	.61	23	160-255	1.34
4	105-75	.58	24	115-220	1.08
5	110-110	.72	25	130-190	1.03
6	95-105	.65	26	160-255	1.34
7	65-50	.37	27	60-110	.55
8	140-120	.84	28	95-100	.63
9	50-95	.47	29	80-95	.57
10	40-55	.31	30	105-165	.87
11	75-105	.58	31	115-145	.84
12	50-95	.47	32	180-210	1.26
13	15-50	.64	33	80-110	.62
14	75-85	.53	34	80-165	.79
15	165-165	1.06	35	70-125	.63
16	175-180	1.14	36	110-175	.93
17	145-190	1.08	37	10-60	.22
18	160-215	1.21	38	45-95	.46
19	240-225	1.51	39	-125	.80
20	230-255	1.56	40	30-45	.25

Can No.	in.A/2 hr.	Can No.	in/A/2 hr.
15	1.06	22	1.13
16	1.14	23	1.34
17	1.08	24	1.08
18	1.21	25	1.03
19	1.51	26	1.34
20	1.56		
21	1.03		

$\bar{x}$  = 0.60 acre inches per hour  
at 40 psi.

Calculations: Area of can = 18.8496 in.<sup>2</sup>  
1 in.<sup>3</sup> = 16.42 ml

$$\frac{\text{mls}}{16.42 \times 18.8496} = \frac{\text{mls}}{309.5} = \text{acre inches}$$

0.00323 x mls = acre inches of water.

System: Rainbird #30 W TNT sprinklers with single 5/32" nozzle. At 40 psi at lateral valve the sprinklers are designed to discharge an average of 4.45 gpm with a precipitation rate of 0.48 inches/hour. At 45 psi they should yield 4.7 gpm and 0.51 inches/hour. At 50 psi they should yield 4.9 gpm and 0.53 inches/hour.

