

1959  
ANNUAL REPORT

of

HOP INVESTIGATIONS  
(CRE5, OAES 36)

REAT-  
36

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HOP INVESTIGATIONS  
(CRe5, OAES 36)

by

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Crops Research Division  
Agricultural Research Service  
United States Department of Agriculture

in cooperation with the

Oregon Agricultural Experiment Station  
Corvallis, Oregon

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## INTRODUCTION

S. N. Brooks

This 1959 annual report of investigations carried out by the regional hop project headquartered at Corvallis, Oregon includes data collected and summarized during the period March 1, 1959 to February 29, 1960. It includes data in some cases also which were collected by personnel at the Irrigation Experiment Station at Prosser, Washington and the Extension Service at Sacramento, California. 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 a limited number of copies of the report.

Some of the line projects are conducted cooperatively by investigators located at Oregon State College. 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.

In addition to support of hop research given by public agencies annual grants are made by the United States Brewers Foundation to partially support several phases of work carried on at Corvallis, Oregon. In 1959 the phases receiving industry funds were (1) Hop pest control, (2) Hop disease control, (3) Evaluation of experimental hop lines, and (4) Irrigation-fertility research. All phases except Hop pest control are included in this report.

In the spring of 1959 line project outlines were revised and brought up to date. Whereas previously work had been conducted under five line projects,

it is now conducted under four. These include four main phases of work, namely: (1) breeding, (2) pathology, (3) agronomy, and (4) chemistry. Before revision hop investigations included these same four phases plus a line project dealing with development of field, greenhouse and laboratory techniques. This has been incorporated into the other four line projects, and any work of this nature that is done is made a part of the line project to which it is most closely related. The revised line project outlines are included in the Appendix at the end of the report.

On November 5 and 6 a conference was held at Corvallis, Oregon for the purpose of reviewing the USDA regional hop research program and its relationship to the States' programs. Those present were: P. F. Knowles and J. M. Ogawa representing California; A. M. Finley representing Idaho; W. H. Foote and H. E. Morrison representing Oregon; H. P. Singleton, C. E. Nelson and C. B. Skotland representing Washington; and L. M. Pultz, J. O. Culbertson, C. E. Horner, S. T. Likens and S. N. Brooks of ARS.

During the two days, the entire USDA program on hops was reviewed in detail. The individual programs carried out in the states were also reviewed -- both those phases dealing with cooperative research as well as separate projects. The whole program (state and federal) was outlined from the standpoint of how it appears now or will appear in the foreseeable future providing there will be no expansion or change of direction. Weaknesses as well as the strong points in the overall program were discussed during the conference. The needs to be considered in an expanded research program were outlined.

No attempt will be made here to evaluate the conference nor to speculate what will be its impact on future hop research. The technical men present at the conference were of one mind on all major phases of the program after they had been discussed. Proceedings of the conference prepared by Dr.

Pultz and Dr. Culbertson appear in the Appendix of this report. These are essentially as approved by the conferees.

There were several changes in personnel during 1959. Mr. Charles Zimmermann was added to the USDA staff on March 31 as a research agronomist. He will be working part-time for an indefinite period of time while attending graduate school at Oregon State College. On the project he will concentrate mainly on the agronomic phases, but will help on the breeding and other phases when needed.

Mr. Don Skoe joined the College staff as graduate research assistant on July 1, 1959. He will be working out a thesis problem in downy mildew research under the direction of Dr. C. E. Horner until completion of his M.S. degree in 1960.

Mr. Yesh Pal Puri was placed on the College staff as Junior Agronomist on July 1, 1959. He will be employed on the project until December 31, 1960. His particular interests are pollen studies, flowering behavior and embryo development in the hop. Already significant findings have been made which are included in this report under the breeding line project.

Weather conditions generally were favorable for hop production in 1959. A dry winter and spring in California checked downy mildew and growing and harvesting conditions were good. The Oregon Fuggle crop was the best on record. Growing conditions in Washington and Idaho also were good and no difficulty was encountered in harvesting early varieties. Fall rains did hamper late harvest in Oregon, Washington and Idaho, however. A summary of the climatological data collected near Corvallis, Oregon are given in the following table.



Climatological data taken at Hyslop Agronomy Farm, near Corvallis, Oregon, in 1959, and during previous years.

Month	Avg. Max. Temp. (°F)		Avg. Min. Temp. (°F)		Avg. Mean Temp. (°F)		Precipitation (inches)	
	1959	25 yr.* Avg.	1959	25 yr.* Avg.	1959	25 yr.* Avg.	1959	25 yr.* normal
<u>1958</u>								
Oct.	65.5	65.4	41.5	43.8	54.5	54.6	2.68	3.62
Nov.	53.5	53.1	38.9	38.3	46.2	45.8	8.49	5.42
Dec.	51.0	47.9	38.3	36.0	44.0	41.9	4.15	7.21
<u>1959</u>								
Jan.	47.6	46.0	35.9	33.2	41.7	39.6	10.52	6.26
Feb.	48.8	51.0	35.7	35.1	42.2	43.1	4.56	4.74
Mar.	54.3	55.9	35.8	37.7	45.1	46.8	3.99	3.91
Apr.	61.2	63.0	39.1	40.9	50.2	51.6	.84	2.05
May	63.5	69.0	42.7	45.1	53.1	57.0	2.20	1.83
June	71.4	73.8	49.1	49.6	60.3	61.7	1.31	1.14
July	83.7	80.7	51.4	52.2	67.6	66.4	.32	.36
Aug.	81.0	81.3	50.7	51.9	65.9	66.7	Tr.	.44
Sept.	70.0	76.7	47.7	49.1	58.9	63.0	1.60	1.35
Yearly total							40.66	38.33
Yearly mean	62.6	63.7	42.2	42.7	52.5	53.2		

\* Years 1933-1957 inclusive

Month	Rel. humid. @8AM (%)		Evapora- tion (in.)		No. clear		No. ptly. cloudy		No. cloudy		No. rainy		Avg. wind velocity (MPH)	
	6yr.(1)	27 yr.(2)	18(3)	18(3)	18(3)	18(3)	18(3)	18(3)	18(3)	18(3)	18(3)	18(3)	6 yr.	6 yr.
	1959 Avg.	1959 Avg.	1959 yr.	1959 yr.	1959 yr.	1959 yr.	1959 yr.	1959 yr.	1959 yr.	1959 yr.	1959 yr.	1959 yr.	1959 Avg.	1959 Avg.
<u>1959</u>														
Apr.	73.0	81.2	3.58	2.68	4 7	8.8	14 11.3	9 9.0	14 13.8	1.69	2.14			
May	70.7	76.2	3.86	4.10	3 9	10.0	13 12.4	9 8.4	19 10.5	.95	1.22			
June	74.1	76.3	5.53	4.67	6 8	8.3	9 12.2	15 9.1	14 9.2	.85	1.27			
July	63.2	70.5	9.13	6.55	1 20	17.7	7 10.2	4 3.0	4 2.7	1.61	1.52			
Aug.	65.5	73.2	8.05	5.83	4 16	16.0	8 8.8	7 5.9	0 3.0	1.45	1.55			
Sept.	78.5	80.2	3.40	3.86	4 14	14.4	15 9.1	11 5.1	14 5.6	1.61	1.68			
Total					62	75.2	66	64.0	55	40.5	65	44.8		
Mean	70.8	76.3	5.59	4.61									1.36	1.56

- (1) = 1953-58 inclusive
- (2) = 1932-58 "
- (3) = 1941-58 "

Hops: Acreage, yield, production, season average price received by growers, and value,  
Average 1948-57, annual 1958 and 1959 <sup>1/</sup>

State	Acres harvested			Yield per acre			Production			Price per pound		Value	
	Average 1948-57	1958	1959	Average 1948-57	1958	1959	Average 1948-57	1958	1959	1958	1959	1958	1959
	- acres -			- pounds -			- Thousand pounds -			- cents - -		Thousand dollars	
Idaho,	1,447	3,500	3,500	1,846	1,620	1,940	2,755	5,670	6,790	54	50	3,062	3,195
Wash.	13,880	19,000	18,600	1,670	1,490	1,640	23,193	28,310	30,504	56	49	15,854	14,465
Ore.	9,920	5,000	5,200	1,150	1,080	1,340	11,110	5,400	6,968	54	46	2,916	3,205
Calif.	7,490	5,900	5,800	1,510	1,530	1,610	11,421	9,027	9,338	60	57	5,416	5,323
U.S.	32,737	33,400	33,100	1,490	1,449	1,619	48,478	48,407	53,600 <sup>2/</sup>	56.3	50.2	27,248	26,188

<sup>1/</sup> Taken from Oregon Crop and Livestock Reporting Service, USDA and Oregon State College, Dec. 17, 1959

<sup>2/</sup> Includes hops produced but not harvested because of economic conditions: Idaho 400,000 pounds,  
Washington 984,000 pounds.

Total production of hops in the United States amounted to 53.6 million pounds in 1959 which is 11% above average. Included in this total are 1.4 million pounds grown in Idaho and Washington which were not harvested because of economic conditions. Total value of the crop amounted to 26.2 million dollars at an average price received by growers of 50.2¢ per pound. Production data are shown in the foregoing table.

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CR e5-1 (OAES 36:FC) BREEDING AND EVALUATING  
NEW AND IMPROVED VARIETIES OF HOPS.

S. N. Brooks

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.

Several changes were made in the breeding program in 1959. One of the preliminary yield trials was discontinued at Corvallis since it had already been used as a basis in choosing material for advanced and off-station testing. An additional off-station trial was established in Oregon on the ranch of Charles Johnston of Woodburn. The breeding schemes were not changed but increased attention was given to the newly initiated backcrossing program. Due to the interest of other states in testing backcross material, this scheme will undoubtedly be given major emphasis in future years.

The first year's data were acquired from the off-station testing program in Oregon and Washington. The results from these trials were encouraging. It begins to appear that downy mildew resistant lines yielding adequately in the Yakima Valley have been isolated. Likewise for Oregon, but this was not as much of a question mark. The real problem now is the matter of quality.

Basic research related to the breeding program was increased in 1959. An additional year's data were obtained in phenotypic characterization of a group of male lines. In addition, an 18 month study was initiated in July whereby considerable information has and will be accumulated regarding pollen dispersal and germination, flowering behavior, and embryo development in the hop. These two studies are vitally important to the breeding program.

## BREEDING AND SELECTION

Results of Crosses Made in 1958

In 1958 six groups of crosses were made among new and old material in the breeding block. Seed was collected and seedlings were grown according to procedures described in the 1958 report. Seedling progenies were subjected to a downy mildew spore suspension inoculation by C. E. Horner and data recorded on systemic infection and severity of leaf infection. A complete listing of these data is furnished in the section Seedling Reaction of 1958 Crosses to Downy Mildew along with details of procedure and other observations pertinent to the greenhouse screening program.

A portion of the data obtained by C. E. Horner were grouped into the crossing groups in order that a summary could be made according to purpose of cross. These data in some cases also provided a basis for formulating future crosses.

Group I, downy mildew inheritance study:

Eight progenies of the crosses obtained in this group were subjected to seedling inoculation with downy mildew in the greenhouse. Following are the results of the screening:

1958 Cross	Classification	No plants	% systemically infected	Visual rating*
58004	Res. x res.	226	11.5	2.5
58006	Res. x res.	214	3.3	1.5
58009	Res. x res.	43	0	2.0
58005	Res. x susc.	34	8.8	2.0
58006	Res. x susc.	39	15.4	2.0
58007	Res. x susc.	186	8.1	1.5
58015	Susc. x res.	37	0	2.0
58031	Susc. x res.	60	13.3	2.5

\* Horner's severity rating: 0=none, 1=light, 2=moderate, and 3=severe disease development

Group II, repeat of previously used good crosses:

Seedling progenies were obtained from 5 of these crosses. The crosses along with percentages of the seedlings systemically infected with downy mildew are as follows:

Cross	Sys. D M	Cross	Sys. D M	Cross	Sys. D M
58001	0	58012	6.2	58057	2.0
58010	0	58034	16.7		

Group III, crosses of progeny tested males and females:

Thirteen progenies were obtained from the 16 seed lots obtained in this group and were subjected to screening in the greenhouse. Rather wide differences were obtained in progeny performance as evidenced by the following data:

Cross	Sys. D M	Cross	Sys. D M	Cross	Sys. D M
58002	4.2	58045	15.0	58054	1.9
58013	5.9	58047	11.1	58056	2.6
58032	2.4	58048	2.2	58058	9.6
58033	3.8	58051	3.9		
58044	12.0	58052	8.0		

Cross 58002 exhibited an exceptional degree of resistance to downy mildew on the basis of average progeny performance.

Group IV, progeny test of new males:

Greenhouse inoculation was made on 10 progenies from males of unknown breeding behavior. Percentages of systemically infected seedlings ranged from 2.0 to 24.0. None of the progenies appeared to be exceptional, however, several seedlings were transferred to the 1959 seedling nursery. On the basis

of these tests male lines 317-4 (C 51051 M) and 518-5 (C 52041 M) will be discarded. Line 519-2 (C 52042 M) appeared to have good potential as a source of mildew resistance and was used in 1959.

Group V, open-pollination progeny test of new females:

Out of 15 seed lots obtained from open-pollinated females, 13 progenies were tested for reaction to downy mildew in the seedling stage. These progenies include the one arising from seed of Late Clusters produced in Idaho and sent by Dr. Romanko. They do not include the progeny of 18-S (C 19113) or 128-I. Several of the new females so tested were retained in the breeding block for future breeding and several were discarded.

Data obtained from the downy mildew screening progeny test of unproven females, (spring, 1959) and disposition of the individuals in breeding block.

Breeding block No.	Cross	% D M * systemic infection	Disposition
104-1	58003	14.3	Discarded
114-3	58011	0	Saved, insufficient test
215-3	58016	21.8	Discarded
301-1	58017	11.2	Saved, very strong seedlings
301-3	58018	9.1	Discarded
302-1	58019	7.1	Discarded
302-3	58020	15.1	Discarded
306-1	58021	13.0	Discarded
306-2	58022	--	No test
306-3	58023	9.0	Discarded
308-3	58028	11.6	Discarded
405-3	58039	0.6	Saved, highly resistant progeny
504-3	58046	--	No test
510-1	58050	14.7	Discarded
18-S	58055	--	Not tested, saved because normally sterile (128-I)
--	58059	28.0	Late Cluster progeny from Idaho

Group VI, backcrossing program:

Three back cross progenies were screened for resistance to downy mildew. These were progenies of 121-2 and 421-1 on Brewers Gold and 123-S on Backa. These were treated in the same way that the other seedling progenies were treated, but all seedlings which appeared to be resistant were saved -- no selection was made for vigor.

General notes, all groups:

Cross	Seedling vigor	Remarks	Cross	Seedling vigor	Remarks
58001	+		58031	+	
2	++		32	+	
3	++	Albino seedlings	33	++	
4	+++		34	+	
5	+++		35	+	Abnormal seedlings
6	+++		36	++	
7	+++		37	++	
8	+++		38	--	
9	+++		39	+	Albino and virescent seedlings
10	++		40	+	
11	--		41	--	
12	+		44	+	Virescent seedlings
13	+		45	--	Virescent seedlings
14	+++		47	--	
15	+		48	+	
16	++		50	+	Albino and virescent seedlings
17	+++		51	+	
18	+++		52	+	
19	++		54	+	
20	+		56	+	
21	--	Virescent seedlings	57	+	
23	+		58	+	
24	+		59	+	
25	++		22	--	
26	++		43	--	
27	+		46	--	
28	+		49	--	
29	++		53	--	
30	♦		55	+	



Evaluation of greenhouse screening program:

In last year's report (p.23) C. E. Horner tabulated data from the screening program indicating that the degree of systemic infection was reduced from 1956 to 1958. He suggested that as parents are progeny tested and those producing a high percentage of susceptible seedlings are eliminated the average degree of systemic infection should be reduced.

The results obtained in 1959 do not follow the trend indicated in previous years. Average percentage of systemic infection in 1959 (excluding Late Cluster progeny from Idaho) was 9.9 compared with 2.2 in 1958 and 7.3 in 1956. It seems reasonable that we should expect year to year variation -- effectiveness of inoculation will undoubtedly vary from year to year resulting from differences in greenhouse environment and condition of inoculum.

Fortunately 9 progenies were evaluated both in 1958 and 1959. The following table shows the differences in performance from one year to the next. In only 2 cases was the percentage of systemic infection greater in 1958 than 1959, and the incidence was low for both cases each year. In 5 cases where systemic infection occurred each year there was from 2 to 9 times as much systemic infection in 1959 as in 1958.

Cross	% systemic infection		Cross	% systemic infection	
	1958	1959		1958	1959
101-2 x 120-1	1.0	0	402 x 419-5	0	7.1
102-3 x 320-1	0	4.2	404 x 419-5	1.6	9.1
108 x 124-S	.6	0	511-3 x 418-5	1.0	8.0
203 x 119-S	.9	5.9	73-S x 108-S	.9	2.0
401 x 219-5	.4	3.8			

It can be concluded from the foregoing analysis that although the 1959 data did not support the suggestion of a decreasing incidence of systemic downy mildew infection in hybrid progenies, they did not altogether refute it. Of importance in this respect is the apparent conclusion that one must be care-

ful in comparing years. Until such time as trends can be established on the basis of longer term results, comparisons should more likely be confined to "within year" observations.

The data for the past 4 years were summarized so that comparisons could be made between random crosses and selected crosses with respect to effectiveness of the screening program in isolating lines which produce higher degrees of resistance in their progenies.

Results of Greenhouse Downy Mildew Screening of Hop Seedlings, 1956-1959.

Crosses made	Progenies tested	No. progenies	No. plants	Overall score of progeny <sup>1/</sup>	% plants systemic infection	% progeny saved for nursery	No. of exceptional progenies
<u>Performance of progenies from crosses selected for downy mildew resistance:</u>							
1955	1956	35	2,927	2.84	6.1	--	--
1956	1957	74	8,143	2.27	3.0	4.05	--
1957-II	1958	11	2,655	1.91	1.9	1.13	2
-III	1958	38	6,351	1.76	1.7	1.35	7
1958-II	1959	5	148	1.50	5.0	7.43	0
-III	1959	13	783	1.77	6.4	4.60	1

Performance of progenies from lines of unknown breeding value:

1955	1956	25	4,108	2.83	7.9	--	--
1956	1957	113	23,234	2.48	3.3	2.10	--
1957-V	1958	64	16,465	2.20	2.5	0.93	2
-IV	1958	4	589	2.50	0.9	0.85	1
1958-V	1959	12	2,919	2.29	9.0	1.92	1
-IV	1959	10	1,273	2.20	11.7	1.96	0

- <sup>1/</sup> = Scale of 0 - 3, none, light, moderate, and severe disease development respectively.
- II = Progenies from crosses previously having resistant seedlings or insufficient test previously.
- III = Progenies from progeny tested males and females.
- V = Open-pollinated progeny test of new females (obtained after 1950).
- IV = Unselected mating progeny test of new males (obtained after 1950).

In the foregoing table it can be seen that there was essentially no difference in 1956 between progenies of selected and unselected material. This was the first year of this program and there was little basis available for selection at the time. Crosses selected on the basis of 1956 testing showed a somewhat lower percentage of systemic infection (3.0 as compared to

3.3) and a higher percentage of superior individuals in their progenies (4.05 compared to 2.10) than did unselected crosses.

In 1958 the same type of relationship existed except that group IV showed a very small incidence of systemic infection. Only 4 progenies were available in this group which may not furnish a representative sample. In comparing groups II and III with group V, a lower incidence of systemic infection, a less severe disease development and a higher number of exceptional seedlings were indicated for the selected crosses.

The same comparison for 1959 indicates that the selected crosses (groups II and III) had a lower percentage of systemically infected plants, a lighter degree of disease development and a higher percentage of good seedlings than the unselected crosses (groups V and IV). Group II had only 5 progenies and perhaps is not adequately represented. The results from this group coincide with those of Group III, however.

The conclusion that the screening program is being at least partially effective in providing a tool for selecting superior breeding stock appears to have firm foundation in these data. Screening techniques will continue to be modified as improvements are developed.

Seedling Reaction of 1958 Crosses to Downy Mildew

(G. E. Horner)

Seedlings from 52 crosses and open pollinated sources were evaluated for downy mildew resistance by greenhouse tests. Previous tests (1956 report pp. 15-23; 1957 pp. 32-38; 1958 pp. 19-24) demonstrated that most of the susceptible seedlings could be eliminated from further testing by greenhouse inoculation with downy mildew. In those tests differences in degree of resistance were observed both among crosses and among individuals within certain crosses.

Procedure:

Seed from the 1958 crosses was germinated and seedlings were planted out in flats by S. N. Brooks. When seedlings were about 10 weeks old they were heavily inoculated with downy mildew. Inoculum consisted of sporangia collected from several varieties and lines of hops to include possible different races of downy mildew, although the existence of races has never been demonstrated.

Water suspensions of downy mildew spores were prepared by washing systemically infected "spikes" then filtering to remove the larger particles of soil and plant debris. Inoculation was accomplished by spraying 20 ml. of a uniform spore suspension on the undersides of the leaves and on the growing tips of seedlings in each flat. Humid conditions were maintained by covering the inoculated seedlings for 48 hours with cheesecloth saturated frequently with water.

Good infection was obtained and disease development was rapid and severe. Approximately 6200 seedlings were available for evaluation. The number of seedling from each cross was recorded. A record was made of the number of systemically infected seedlings and each cross was rated for mildew severity on a scale of 0 to 3 representing none, light, moderate and severe infection. Selection of plants to be saved was based on both mildew resistance and seedling vigor and type.

Results:

A total of 6225 plants representing 52 crosses were evaluated for resistance. The following table shows the disease reaction of seedling from the 1958 crosses to downy mildew.

Mildew Severity Ratings of 1958 Crosses

Cross Number	No. plants tested	No. Systemically infected	Percent Systemic infection	Mildew severity rating *	No. plants kept
1	10	0	0	1.5	1
2 **	72	3	4.2	1.0	9
3-O.P.	293	42	14.3	3.0	2
4	226	26	11.5	2.5	3
5	34	3	8.8	2.0	4
6	39	6	15.4	2.0	3
7	186	15	8.1	1.5	6
8	214	7	3.3	1.5	4
9	43	0	0	2.0	2
10	47	0	0	1.0	5
11-O.P.	30	0	0	3.0	2
12	16	1	6.2	1.5	2
13	34	2	5.9	1.5	2
14	321	49	15.3	2.0	6
15	37	0	0	2.0	1
16-O.P.	321	7	2.2	2.0	9
17-O.P.**	303	34	11.2	2.0	8
18-O.P.	308	28	9.1	2.0	5
19-O.P.	322	23	7.1	2.0	2
20-O.P.	324	49	15.1	2.5	2
21-O.P.	54	7	13.0	3.0	2
23-O.P.	100	9	9	2.5	2
24	50	12	24.0	3.0	1
25	324	49	15.1	3.0	3
26	104	12	11.5	2.0	2
27	51	1	2.0	1.5	3
28-O.P.	301	35	11.6	2.5	4
29 (Back cross)	87	13	14.9	2.5	47
30 (Back cross)	15	4	26.7	2.5	4
31	60	8	13.3	2.5	37
32	82	2	2.4	2.0	3
33	53	2	3.8	1.5	4
34	24	4	16.7	2.0	1
35	42	3	7.1	2.5	2
36	201	24	11.9	2.5	3
37	33	3	9.1	2.0	1
38	38	2	5.3	1.5	2
39-O.P.**	318	2	.6	1.0	12
40	109	17	15.6	2.0	2
41 (Back cross)	61	26	42.6	3.0	35
44	25	3	12.0	2.0	2
45	40	6	15.0	2.0	2
47	9	1	11.1	2.0	0
48	89	2	2.2	2.0	3
50-O.P.	245	36	14.7	2.0	6
51	51	2	3.9	1.5	4
52	100	8	8	2.0	3
54	54	1	1.9	2.0	2
56	38	1	2.6	1.5	1
57	51	1	2.0	1.5	2
58	136	13	9.6	2.0	3
59 Selfed L.C.	100	28	28	3.0	0
Totals	52	6225			276

\* Based on a scale of 0=none; 1=light; 2=moderate and 3=severe infection  
 \*\* Denotes crosses producing many vigorous as well as resistant seedlings

Discussion and Conclusions:

Over 70,000 hop seedling have been tested in the greenhouse during the past 4 years for resistance to downy mildew. From these about 1,600 resistant seedling have been selected for further evaluation. Field observations on the selected seedlings for 3 consecutive seasons indicate that relatively few of the seedlings selected as resistant by greenhouse screening become severely infected.

From the tabulated data above it is apparent that there are differences among progenies in resistance to downy mildew infection. Resistance to systemic infection is considered most important. Degree of systemic infection ranged from 0 to 42 per cent. The highest susceptibility was shown by backcrosses of certain highly susceptible varieties.

Cross number 59 is of special interest because it is believed to be the result of a selfed Late Cluster hop. A male vine originated in a field of susceptible Late Cluster hops in Idaho and pollinated female flowers on the same and adjacent hills. The seedlings of this "cross" showed 28% systemic infection -- the second highest percentage recorded in 4 years. In a later section in this report results will be given of the reaction of cross 59 seedlings to direct crown inoculation with downy mildew.

From the 1959 greenhouse screening tests, 276 plants representing 50 of 52 crosses were selected for further testing. Of the 276, 123 were seedlings of backcrosses of special interest to the agronomist, and 153 selected for mildew resistance.

On the basis of 4 years' results and observations it appears conclusive that the greenhouse screening program is effective in eliminating most of the downy mildew susceptible plants.

1959 Seedling Nursery

Following the greenhouse screening program the selected seedlings were transplanted in a field nursery where they will be allowed to grow through the season of 1960. The nursery was planted with a 4 x 8 ft. spacing in 7 rows. The rows were numbered from the West side of the block and the plants were numbered from the South end.

<u>Row I</u>		<u>Row II</u>		<u>Row III</u>		<u>Row IV</u>	
<u>Cross</u>	<u>No. plants</u>	<u>Cross</u>	<u>No. plants</u>	<u>Cross</u>	<u>No. plants</u>	<u>Cross</u>	<u>No. plants</u>
58001	1	58015	1	58031	37	58034	1
2	9	16	9	39	12	35	2
3	2	17	8	40	2	36	3
4	3	18	5			37	1
5	4	19	2			38	2
6	3	20	2			44	2
7	6	21	2			45	2
8	4	23	2			48	3
9	2	24	1			50	6
10	5	25	3			51	4
11	2	26	2			52	3
12	2	27	3			54	2
13	2	28	4			56	1
14	6	32	3			57	2
		33	4			58	3
<u>Row V</u>		<u>Row VI</u>		<u>Row VII</u>			
58029	50	58041	34	58059	40		
		30	4	55	11		

Selections Saved in 1959

Fourteen selections were saved from the 1958 seedling nursery of 274 plants. Selection was made on the basis of vigor, richness of lupulin, aroma, cone type and, in some cases, genetic curiosity. One of the selections is a male and the remaining 13 are females. The females will be planted in 4-hill plots in an observation nursery -- the male will be entered in the breeding block for a test of its breeding behavior.

The male plant was assigned accession No. C 58011 M. It is a high gland count individual with fair vigor. The females were assigned 1958 accession numbers also. These appear, along with descriptive notes on each individual in the next section dealing with the observation block.



1958 Observation Block

There have been some 34 female clones saved from crosses made since 1953. In addition 3 selections have been maintained following preliminary testing in early and late maturity yield trials terminated in 1956. Two other individuals have been increased from the breeding block or from the disease nursery. All of these individuals were placed into an observation block in 1958. The selections saved in 1959 will be transferred to this block in the spring of 1960.

<u>Number in Observation Nursery</u>	<u>Accession Number</u>	<u>Remarks</u>
Ob-817 (4 pl.)	C 19233	2010 lbs./A., med. $\alpha$ -acid, long arms, some mildew in LM trial, 1952-56.
-818 (4 pl.)	C 19020	2160 lbs./A., 5.0-5.7 $\alpha$ -acid, long arms, some mildew in EM trial, 1952-56.
-819 (4 pl.)	C 19032	1840 lbs./A., med. high $\alpha$ -acid, long arms, little mildew in LM trial, 1952-56.
-820 (4 pl.)	C 19022	Heavy set of cones, med. $\alpha$ -acid, some mildew in breeding block.
-821 (3 pl.)	C 51026	Rich lupulin, strong aroma.
-805 (1 pl.)	C 54049	Very good vigor, sweet, some mildew (tested in D N).
-812 (1 pl.)	C 55055	Mild, low lupulin content, no mildew (tested in D N).
-826 (1 pl.)	C 56008	Strong aroma, sweet, long pointed cones, poor vigor.
-830 (1 pl.)	C 56012	Med. aroma, good vigor, dark colored cones.
-831 (1 pl.)	C 56013	Med. strong aroma, long arms, high proportion of small cones.
-833 (1 pl.)	C 56016	Med. aroma, compact seedless cone, poor vigor.
-834 (1 pl.)	C 56017	Mild aroma, good set of large cones.
-836 (1 pl.)	C 56019	Med. aroma, good set of large cones, hopped down well, shatters.
-838 (1 pl.)	C 56021	Poor vigor, ragged appearance, seedless.
-801 (4 pl.)	C 57001	Med. aroma, good vigor, large cones, late.
-802 (1 pl.)	C 57002	Med. aroma, large bright cone, attractive appearance.
-803 (1 pl.)	C 57003	Med. aroma, large shatter-resistant cones, early.
-804 (1 pl.)	C 57004	Med. aroma, good set of small heavy cones, early.
-806 (1 pl.)	C 57005	Mild aroma, heavy set of pale colored cones.
-807 (1 pl.)	C 57006	Med. aroma, bright lupulin, small seedless cones.
-808 (1 pl.)	C 57007	Med. aroma, pleasant, rich lupulin, long arms.

Ob-809 (1 pl.)	C 57008	Med. aroma, tight cones, good vigor and long arms.
-810 (1 pl.)	C 57009	Large round cones, hopped down well.
-811 (1 pl.)	C 57010	Med. aroma, firm cones, poor vigor.
-813 (1 pl.)	C 57011	High resin and oil content, good set of cones, some shatter.
-814 (1 pl.)	C 57012	Small cones, large smooth vines, late.
-815	C 58001	Good vigor, late.
-816	C 58002	Sweet aroma, purple seeds, early, good vigor.
-822	C 58003	Rich lupulin, firm cone, hard picking, good vigor.
-823	C 58004	Rich, green pointed cones, very good vigor.
-824	C 58005	Med. lupulin, very good vigor.
-825	C 58006	Good vigor, late.
-827	C 58007	Mild aroma, rich lupulin, good vigor.
-828	C 58008 H	Female intersex, large glands, possibly triploid (saved for genetic studies).
-829	C 58009	Medium aroma, tight cones, good vigor, long arms.
-832	C 58010	Primitive (?) type cones (saved for genetic studies).
-835	C 58012 H	Rich lupulin, excellent firm cone, fair vigor, female intersex.
-837	C 58013	Strong aroma, large firm cone.
-839	C 58014	Med. strong aroma, fair vigor.

Crosses Made in 1959

Seed was collected from 81 controlled parentage and open-pollinated sources in 1959. A percentage of crosses made out of the total attempted was not calculated. The degree of success was much higher in 1959 than it was in 1958 in spite of similar climatic conditions during much of the breeding season.

Crosses were made in groups much the same as last year. In order to avoid confusion the groupings were maintained as near as possible from year to year. For example, groups IV and V have included the progeny tests of males and females, respectively, the past 3 years.

Group I:

15 crosses were made among resistant and susceptible lines used in the downy mildew inheritance study. These crosses are as follows:

<u>R x R</u>	<u>R x S or S x R</u>		<u>S x S</u>
59003	59004	59020	59019
59006	59005	59029	59030
59043	59042	59032	59031
59070	59068		
	59069		

Group II:

14 crosses were made as repeats of crosses which have produced resistant and otherwise desirable seedlings in previous years. This is similar to Group II in 1957 and 1958. The crosses were: 59001, 59007, 59011, 59013, 59033, 59034, 59035, 59050, 59051, 59055, 59063, 59074, 59026 and 59039.

Group III:

This group constitutes the one in which progeny tested females and males are brought together into crosses. It should supply the highest overall degree of downy mildew resistance seedling progenies. The 27 crosses in this group were:

59002, 59008, 59012, 59021, 59022, 59036, 59038, 59040, 59047, 59048, 59049, 59052, 59053, 59056, 59057, 59058, 59059, 59060, 59061, 59062, 59064, 59065, 59066, 59067, 59071, 59072, 59073.

Group IV:

11 seed lots were obtained from crosses involving male lines for which breeding behavior information was not available. The males were crossed on non-selected females -- those having sufficient plants to accommodate additional crosses. The male progeny test crosses were as follows:

<u>Males</u>	<u>Progeny test crosses</u>
520-5 (C 52044 M)	59014, 59027
219-4 (C 51061 M)	59015, 59046
518-2 (C 52040 M)	59016, 59025, 59044, 59045
419-5 (C 52048 M)	59024
519-2 (C 52042 M)	59017, 59018

The crosses involving 519-2 were made inadvertently since this individual had been tested in 1958. Male lines 517-5 and 419-5 still have not received adequate testing and should be used in 1960.

Group V:

Open-pollinated progenies were obtained from 114-3, 306-2, and 504-3 for progeny testing. Evaluation of these 3 lines will essentially complete the backlog progeny testing of females. As new lines are moved into the breeding block they, of course, will be tested for breeding behavior.

Group VI:

4 crosses were made, one each on Late Clusters, Brewers Gold, Hallertau and Backa, which are initial crosses in a back crossing program with these varieties. Eventually 15 back crosses will be carried out involving 5 females (commercial varieties) and 3 male lines. To date only 5 of these have been started. Early Clusters will come of age in the breeding block in 1960. Special effort will be made to get the remaining back crosses under way in 1960.

## Crosses for 1959

<u>Cross No.</u>	<u>Group</u>	<u>Pedigree</u>
59001	II	101-2 (501-2) C 50075 x 120-1,2 (523) C 19060 M
59002	III	102-3 (502-1) C 50091 x 320-1,2 (323) C 19047 M
59003	I	106 (506) C 19032 x 121-2 (525) C 19062 M
59004	I	106 (506) C 19032 x 317-1,2 (317) C 19041 M
59005	I	106 (506) C 19032 x 321-1 (325) C 19049 M
59006	I	106 (506) C 19032 x 421-1,2 (225) C 19040 M
59007	II	108 (508) C 19033 x 124-S C 19172 M
59008	III	111-2 (511-2) C 50054 x 419-1,2 (221) C 19037 M
59009	V	114-3 (514-1) C 52020 x O.P.
59010	VI	122- I 19208 x 421-1,2 (225) C 19040 M
59011	II	203 (403) C 19022 x 318-1,2 (319) C 19043 M
59012	III	203 (403) C 19022 x 320-4,5 (324) C 19048 M
59013	II	203 (403) C 19022 x 418-1,2 (219) I 19006 M
59014	IV	205 (405) I 19004 x 520-5 (124) C 52044 M
59015	IV	209 (409) C 19026 x 219-4 (422-2) C 51061 M
59016	IV	209 (409) C 19026 x 518-2 (119) C 52040 M
59017	IV	209 (409) C 19026 x 519-2 (121) C 52042 M
59018	IV	210 (410) C 19027 x 519-2 (121) C 52042 M
59019	I	212 (412) C 19028 x 317-1,2 (317) C 19041 M
59020	I	212 (412) C 19028 x 521-4,5 (126) C 19010 M
59021	III	215-2 (415-2) C 52013 x 417-1,2 (217) C 19036 M
59022	III	301-1 (301-3) C 50040 x 519-2 (121) C 52042 M
59023	V	306-2 (306-2) C 51104 x O.P.
59024	IV	307 (307) C 19020 x 419-5 (222) C 52048 M
59025	IV	307 (307) C 19020 x 518-2 (119) C 52040 M
59026	II	307 (307) C 19020 x 519-2 (121) C 52042 M
59027	IV	307 (307) C 19020 x 520-5 (124) C 52044 M

<u>Cross No.</u>	<u>Group</u>	<u>Pedigree</u>
59028	VI	311(311) I 19001 x 121-2 (525) C 19062 M
59029	I	314 (314) C 19076 x 121-2 (525) C 19062 M
59030	I	314 (314) C 19076 x 317-1,2 (317) C 19041 M
59031	I	314 (314) C 19076 x 321-1 (325) C 19049 M
59032	I	314 (314) C 19076 x 421-1,2 (225) C 19040 M
59033	II	315-3 (315-1) C 19077 x 119-1,2 (521) C 19058 M
59034	II	315-3 (315-1) C 19077 x 319-2 (321) C 19045 M
59035	II	(315-1) 315-3/C 19077 x 110-S C 19173 M
59036	III	316-1 (316-3) C 52005 x 420-4,5 (224) C 19039M
59037	VI	322- I 56001 x 421-1,2 (225) C 19040 M
59038	III	403 (203) C 19011 x 318-4,5 (320) C 19044 M
59039	II	404 (204) C 19014 x 519-2 (121) C 52042 M
59040	III	405-3 (205-1) C 54010 x 419-5 (222) C 52048 M
59041	III	405-3 (205-1) C 54010 x 519-2 (121) C 52042 M
59042	I	409-2 (209-2) C 19067 x 317-1,2 (317) C 19041 M
59043	I	409-2 (209-2) C 19067 x 421-1,2 (225) C 19040 M
59044	IV	412 (212) C 19017 x 518-2 (119) C 52040 M
59045	IV	414 (214) C 19018 x 518-2 (119) C 52040 M
59046	IV	416 (216) I 19003 x 219-4 (422-2) C 51061 M
59047	VI	422 I 56002 x 123-S C 19182 M
59048	III	501-3 (101-1) C 50008 x 320-4,5 (324) C 19048 M
59049	III	501-3 (101-1) C 50008 x 417-1,2 (217) C 19036 M
59050	II	501-3 (101-1) C 50008 x 520-1,2 (123) C 19009 M
59051	II	504-2 (104-2) C 54003 x 221-1 (425-2) C 51114 M
59052	III	504-2 (104-2) C 54003 x 320-4,5 (324) C 19048 M
59053	III	504-2 (104-2) C 54003 x 419-1,2 (221) C 19037 M
59054	V	504-3 (104-1) x O.P.

<u>Cross No.</u>	<u>Group</u>	<u>Pedigree</u>
59055	II	507-1 (107-3) C 54007 H x 217-1,2 (417) C 19050 M
59056	III	507-3 (107-1) C 54005 x 120-1,2 (523) C 19060 M
59057	III	507-3 (107-1) C 54005 x 217-1,2 (417) C 19050 M
59058	III	511-3 (111-1) C 53007 x 217-1,2 (417) C 19050 M
59059	III	511-3 (111-1) C 53007 x 318-1,2 (319) C 19043 M
59060	III	511-3 (111-1) C 53007 x 418-5 (220) C 52047 M
59061	III	513-2 (113-2) C 50017 x 119-4,5 (520) C 19057 M
59062	III	513-2 (113-2) C 50017 x 219-5 (422-1) C 51060 M
59063	II	514-2 (114-2) C 53013 x 521-2 (125) C 52045 M
59064	III	7-S C 19102 x 119-S C 19180 M
59065	III	15-S C 19110 x 110-S C 19173 M
59066	III	23-S C 19118 x 217-1,2 (417) C 19050 M
59067	III	23-S C 19118 x 110-S C 19173 M
59068	I	25-S I 19120 x 317-1,2 (317) C 19041 M
59069	I	25-S I 19120 x 321-1 (325) C 19049 M
59070	I	25-S I 19120 x 421-1,2 (225) C 19040 M
59071	III	35-S C 19124 x 108-S C 19172 M
59072	III	46-S C 19134 x 517-1,2 (117) C 19008 M
59073	III	50-S I 19137 x 120-1,2 (523) C 19060 M
59074	II	73-S C 19152 x 108-S C 19172 M
59075		Composite Wild Am. females x O.P.
59076		526-3 I 58014 x O.P.
59077		523-3 I 58003 x O.P.
59078		209 (409) C 19026 x 525-1 I 58009 M
59079		209 (409) C 19026 x 526-4 I 58015 M
59080		209 (409) C 19026 x 527-1 I 58017 M
59081		416 (216) I 19003 x 526-4 I 58015 M

Miscellaneous crosses:

Crosses 59075 through 59081, 7 in all, involve progenies from wild American hops introduced from Utah in 1958. Seedlings from these crosses will be screened in the ~~same~~ way that the rest of the seedlings are screened, but particular attention will be placed on resistance to systemic crown infection by downy mildew.

The seeds from all crosses were placed in cold storage in a moist condition early in January. On or about February 15 they will be planted in flats in the greenhouse where they will be germinated. Initial selection will be made in the seedling stage in the greenhouse. The selected seedlings will then be removed to the field to make up the 1960 nursery.



## EVALUATION

Corvallis-Prosser Yield TrialObjectives:

See 1956 Annual Report, p. 25.

Duration of Experiment:

See 1956 Annual Report, p. 25. The trial at Corvallis was discontinued at the close of the 1959 growing season.

Procedure:

See 1956 Annual Report, p. 25.

Experimental Results:

Data were obtained on yields, moisture dry down percentages, and cone size from the trial at Corvallis, Oregon. Limited quality data were obtained also from several of the lines in test. Yield data were obtained from the trial at Prosser, Washington. Samples were received for chemical analysis. They were in excellent condition except that the hops were so low in lupulin that some difficulty was encountered in analyzing them for alpha-acid. This is discussed further in CR e5-5 under "Evaluation of Strobiles".

Observations were made on some of the lines grown near Sacramento, California, but no yield data were obtained. No samples were available for chemical analysis.

Production data obtained from all trials are reported in the following table.

Hand evaluation of a portion of the lines grown at Corvallis was provided by the Hop Committee of USBF. Unfortunately there was some misunderstanding regarding the importance of chemical data on the samples submitted for hand evaluation, and major emphasis was placed on samples to be submitted for actual

Data obtained on experimental hop lines in Oregon, Washington and California since 1954.

<sup>2/</sup>  
Sacramento  
California

Line	Corvallis, Oregon								Prosser, Washington <sup>1/</sup>					Relative vigor <sup>4/</sup>
	Average yield		Matur- ity	Seeded 1959		Seedless 1959		Average yield		Matur- ity	Relative cone size <sup>3/</sup>	Machine pick- ability		
	Lbs/a 1959	Lbs/a 1956-9		Avg. cone length (mm.)	Avg.wt. of 100 cones (grms)	Avg. cone length (mm.)	Avg.wt. of 100 cones (grms)	Lbs/a 1959	Lbs/a 1957-9					
L.Clusters	1360	1480	Medium	37	24.1	28	19.0	2450	2410	Med.late	4	Fair	1.0	
Fuggles	--	--	Early	29	19.0	20	10.1	--	--					
Brewers Gld.	1490	1360	Med.late	34	28.5	18	12.8	--	--					
103 I	2370	1810	Medium	32	21.5	29	17.4	1950	1790	Medium	1	Poor	2.9	
104 I	2360	1720	Late	32	22.5			1160	950	Med.late	1	Poor		
107 I	1320	1750	Med.early	41	26.1	24	13.2	1380	1260	Medium	3	Poor	3.5	
108 I	1730	1770	Med.early	31	22.1	21	14.6	2180	1990	Med.late	1	Good	2.7	
109 I	1170	1390	Medium	33	23.3			1230	1190	Med.late	1	Fair		
112 I	1520	1790	Med.early	32	21.1	18	7.6	1280	1220	Med.late	3	Fair	3.2	
123 I	1650	1850	Medium	38	27.5			2560	2010	Medium	5	V.good		
124 I	1080	1300	Medium	32	19.4			1420	1220	Med.late	3	Poor		
127 I	1520	1210	Medium	32	14.7			930	600	Med.late	1	Fair		
128 I	--	--	Med.late	21	14.9	23	13.1	--	--					
132 I	1980	1620	Medium	34	23.2			1600	1180	Medium	1	Fair		
135 I	1800	1420	Med.late	27	17.4	17	8.4	1310	960	Late	1	Fair	2.3	
138 I	930	1080	Medium	33	23.2			1510	1140	Medium	3	V.good		
139 I	750	1330	Medium	26	15.6	23	9.4	1460	1470	Med.late	1	Good	2.5	
144 I	1430	1380	Med.early	31	19.1	24	10.1	1700	1470	Med.late	3	Good	3.0	
Hallertau	--	--				21	14.4							
Backa	--	--				19	12.4							
Mean	1530	1520		32	21.3	22	12.5	1610	1390					
LSD(5%)	580	380												
CV(%)	13	9												

<sup>1/</sup> Data provided by C.E.Nelson.

<sup>2/</sup> Observations furnished by W.G.Golden, Jr.

<sup>3/</sup> 1 small, 3 med., 5 large.

<sup>4/</sup> 1 excellent, 2 good, 3 medium, 4 poor.

brewing trials. As a result, chemical data are not available for all samples evaluated. A summary of USBF hand evaluation is furnished along with chemical quality data in the following table.

Quality evaluation of Corvallis samples, 1959.

Line	Seeded samples					Seedless samples			
	Chemical eval. 1/			Hand eval. 2/		Chemical eval. 1/		Hand eval. 2/	
	% $\alpha$ acid	% $\beta$ acid	mls/100g. oil	Score	Rank	$\alpha$ acid	mls/100g. oil	Score	Rank
Late Clusters	7.53	4.28	0.50	43.5	5	9.46	0.49	46.5	2
Fuggle	5.03	1.61	1.07	43.8	4	6.44	0.64 3/	30.8	8
Hallertau	5.49		0.53	38.5	7	8.85	1.09	32.5	6
Backa						5.75	0.78	30	9
Brewers Gold						10.14	1.50	49	1
103-I				38	8	2.55	0.69	27.5	12
107-I	4.39	2.36	0.78	27	11	5.98	0.72	26.2	13
108-I	4.22	3.88	0.25	47	2	6.20	1.02	38.8	4
112-I	4.85	8.08	0.95	33	10	8.28	0.93	28.5	11
128-I 4/				47.5	1	10.57	1.89	39.5	3
135-I				40.5	6	2.35	0.72	34.5	5
139-I				33.5	9	4.82	0.24	29.2	10
104-I	2.33	3.33	0.79	44.5	3	2.48	0.67	31.8	7

- 1/ Data supplied by S. T. Likens; all on a dry wt. basis.  
 2/ Made by USBF Hop Committee; highest score possible was 60.  
 3/ Some oil lost due to lack of condensation early in distillation.  
 4/ Two samples grown; one in seeded yard, but both samples were seedless.

Discussion and conclusions:

Yields in the trials located at both Corvallis and Prosser have been somewhat disappointing almost every year. The experimental lines grown at Prosser have not yielded up with Late Clusters, although the potential of some of them appears to be very good. Yields have generally been lower at Corvallis than they were for comparable lines grown in previous trials here. At Prosser, lines 108-I, 123-I and 103-I appear to be the only ones which might be considered to have satisfactory adaptation to that area. At Corvallis, 103-I and 104-I have been the best yielders. The quality of 104-I is poor.

It is felt that these trials have served their purpose -- that of furnishing data on which selection of lines for more advanced testing could be based. The trial at Corvallis will not be grown in 1960. Growing the trial at Prosser will depend upon the decision of the research personnel there.

## Off-station Cooperative Trials

### Objectives:

- (1) To test the performance under grower conditions in several hop growing areas of lines which have been selected on the basis of preliminary trials.
- (2) To produce a sufficient amount of strobiles from each of the lines in test for full-scale brewing tests.

### Procedure:

The procedures followed in these trials will depend upon the facilities of the cooperating growers, and any agreements made between them and the research agency involved. Growers will be selected on the basis of their interest and willingness to cooperate in the program, the type of soil they have under trellis, and their geographic location. Some consideration will be given also to individual abilities in growing hops as well as facilities available, but the purpose of testing lines under representative conditions using representative methods will not be ignored. Efforts will be made to carry out harvesting, drying and baling operations with the most care possible in order to furnish attractive, high quality hops for brewing analysis. Wherever funds and personnel permit, maturities of the individual lines will be determined by sampling and subsequent chemical analyses so that true varietal comparisons can be made with respect to quality. Harvesting will be done at maturity.

Only one trial was carried through to completion in Oregon in 1959. Two other trials were grown, but the plantings were young and somewhat variable, and they were not completely harvested. The early harvest from the one trial was dried in a wood-fired kiln and the later harvest was dried in an oil-fired kiln.

Although all 3 trials were harvested for yield determination in Washington, only one was carried through to drying and baling the hops from each line separately. All of the varieties in each trial were harvested on one date

rather than at the maturity of each line.

### Results:

Alpha-acid, cohumulone, and oil data for the lines grown in Oregon in addition to yield data from one trial are given in the following table. The build-up of chemical components was followed in this trial and an attempt was made to harvest the varieties at maturity, but this was only partially successful. This point is discussed further in CR e5-5 "Evaluation of Strobiles."

Alpha-acid and oil data were obtained for 3 trials in Washington. These data are given along with yield data from all Washington trials in the second table.

Brewing data will be provided at a future date on samples grown in Oregon. No samples from Washington were submitted for brewing trials due to apparent immaturity.

All chemical data were provided by S. T. Likens. All agronomic data from Washington were provided by C. E. Nelson.

### Discussion and Conclusions:

All of the trials included in this section of the report were in their first year of production. They have not furnished sufficient data as yet from which to make final decisions regarding the potential worth of any of the varieties. It is hoped that in 2 or 3 years decisions can be made to either release for commercial production or to discard the varieties included in the trials.

The growing of these trials in 1959 provided much needed experience, both for the grower-cooperator and the experimenter. These trials were the first of their kind to be grown, having evolved as the next logical step in the breeding program. The mistakes made in 1959 should provide sufficient incentive to provide greater attention to detail in 1960 and subsequent years. It has been agreed that the Oregon trials will be harvested closer to the proper stages

Quality and production data obtained from Oregon  
off-station hop variety trials in 1959.

Chemical data (all on dry weight basis)

<u>Line</u>	<u>Fresh oil ml./100 g.</u>	<u>Dry oil ml./100 g.</u>	<u><math>\alpha</math>-acid percent</u>	<u><math>\beta</math>-acid percent</u>	<u>Cohumulone ratio</u>	<u>Cohumulone percent</u>
112 I Kerr	1.69	.41	4.66	2.24	21.9	1.02
128 I Kerr	3.26	.82	10.06	4.28	32.3	3.42
135 I Kerr	1.89	.62	2.15	2.97	41.8	0.90
144 I Kerr	2.06	.52	4.16	4.27	26.2	1.09
Crosby Schwab.		.65	2.62			
Fuggle Kerr		.67	2.50			
Crosby Schwab.		.81	4.95		20.0	0.99
		.73	4.40			
			4.49			

<u>Line</u>	<u>Yield in lbs./a at Kerr's, Salem</u>			<u>Date machine harvested</u>		<u>Pounds avail. for brew.</u>
	<u>Rep.I</u>	<u>Rep.II</u>	<u>Avg.</u>			
112 I	1540	1490	1510	9/18	Over-ripe, shattered badly	200
128 I	1780	1710	1740	9/18	Slightly over-ripe, excellent picker, some mildew in cones	230
135 I	1690	1360	1520	9/18	Slightly over-ripe, some shattering	213
144 I	1860	1670	1760	9/8	Ripe, good picker, shattered 1/2 as much as Fuggles, some mildew in cones	327
Fuggle	1440	1180	1310	9/8	Over-ripe, shattered badly	355

Trials located also at Crosby's near Woodburn, Schwabauer's near Hubbard, and Johnston's near Woodburn. Those at Crosby's and Schwabauer's were not completely harvested due to variable growth and bad weather at harvest time. The one at Johnston's was planted in 1959.

Chemical and production data obtained from  
Washington off-station variety trials in 1959.

<u>Line</u>	<u>Trial</u>	<u>Chemical data (dry wt. basis)</u>			
		<u>α-acid percent</u>	<u>Dry oil ml./100 g.</u>	<u>Cohumulone ratio</u>	<u>Cohumulone Percent</u>
103 I	Brulotte	1.66	.28	39.8	.66
	Strausz	.74	.19		
	Aries	2.06	.28		
107 I	Brulotte	5.64	.39	23.8	1.34
	Strausz	1.54	.24		
	Aries	2.12	.35		
108 I	Brulotte	4.36	.34	28.6	1.25
	Strausz	1.61	.12		
	Aries	--	--		
135 I	Brulotte	3.22	.43	38.6	1.24
	Strausz	1.61	.20		
	Aries	1.93	.23		
139 I	Brulotte	4.13	.48	35.6	1.47
	Strausz	2.52	.25		
	Aries	1.45	.23		

Yield in lbs. / A. (kiln-dry)

<u>Line</u>	<u>Aries Toppenish</u>	<u>Strausz Mabton</u>	<u>Brulotte Moxee City</u>	<u>Remarks</u>
103 I	2078	2112	2187	Good picker, med. size open cones
107 I	1599	1590	1333	Fair picker, like L.C., large open cones
108 I	--	2465	2169	Best picker, med. size open cones
135 I	1657	1849	1485	Good picker, med. size open cones
139 I	1699	1524	1556	Poor picker, small firm cones
Early Clusters	--	--	1700 (estimated)	

Machine harvested 9/9/59

of maturity of each line. Maturity will be determined by periodic sampling and chemical analyses. The laboratory at Corvallis stands ready to provide a like service for determination of the proper time for harvesting the Washington trials. Sampling procedures need only to be worked out.

Data obtained in 1960 from the trials as well as quality information based on the 1960 crop should be much more indicative of the performance of the lines.



Variation in Male Hops

Objectives:

See 1958 annual report, page 27.

Reasons for undertaking the work:

See 1958 annual report, page 27.

Nature and extent of previous work:

See 1958 annual report, page 27.

Procedure:

The procedures followed in 1959 were not greatly different from those followed in 1958 (see 1958 annual report, page 28).

A study of variances based on last year's data indicated that a smaller number of flowers could be used for resin gland counts. Therefore, 10 flowers were used per plant rather than 25. Resin gland counts were made on 4 plants of each line rather than 3 as in 1958 since the study of variances indicated more information could be gained by increasing replications than by increasing the number of observations within replications.

Alpha and beta hop acids were determined by the spectrophotometric method. A description of this method can be found in "Evaluation of Male Lines" of the report for CR e5-5.

Experimental results:

Hop acid analyses for 4 replications for 1958 were completed in 1959. These are included in this report along with the 1959 data on hop acid analyses. Resin gland counts were made on 4 replications also. Data on gland counts, hop acid analyses and flowering along with correlation coefficients for 1958 are presented in the following tables. Time did not permit complete analysis and interpretation of all of the 1959 data. Flower samples are available from both years for size determination but they were not weighed.

Table 1. Data obtained in the male line study in 1958 and 1959 (Chemical data supplied by S. T. Likens)

n.	Line	Percent alpha-acid			Percent beta-acid			Percent total hop acid			No. resin glands per flower			Ratio $\alpha/\beta$ - acid			Wt. of 25 flowers(mg)			Days from May 31 to: initial bloom-full bloom					
		1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.	1958	1959	Av.	1958	1959	Av.	1958	1959	Av.	1958	1959	Av.			
1	106-S	0.49	0.51	0.50	0.99	1.12	1.06	1.48	1.63	1.56	11	21	16	0.49	0.48	0.48				24	29	26	30	35	32
2	110-S	0.87	0.70	0.78	1.36	1.30	1.33	2.24	2.01	2.12	22	28	25	0.64	0.54	0.59				38	39	38	44	44	44
3	123	0.87	0.61	0.74	1.65	1.62	1.64	2.52	2.24	2.38	31	34	32	0.53	0.38	0.46				36	36	36	40	41	40
4	217	0.94	0.64	0.79	2.30	1.54	1.92	3.24	2.18	2.71	35	23	29	0.44	0.44	0.42				44	44	44	50	51	50
5	125	0.33	0.22	0.28	0.66	0.54	0.60	0.99	0.76	0.88	19	22	20	0.48	0.41	0.44				45	60	52	50	68	59
6	221	0.71	0.47	0.59	1.47	1.20	1.34	2.18	1.67	1.92	27	21	24	0.48	0.40	0.44				45	44	44	50	54	52
7	324	0.90	0.87	0.88	0.62	0.74	0.68	1.52	1.61	1.56	21	26	24	1.43	1.16	1.30				40	50	45	44	56	50
8	224	0.65	0.58	0.62	0.47	0.51	0.49	1.12	1.09	1.10	19	16	18	1.40	1.15	1.28				36	49	42	40	55	48
9	317	1.47	0.78	1.12	2.94	2.96	2.95	4.42	3.75	4.08	56	49	52	0.50	0.27	0.38				46	56	51	50	64	57
10	319	0.39	0.27	0.33	0.66	0.46	0.56	1.05	0.73	0.89	16	17	16	0.57	0.57	0.57				44	54	49	48	62	55
11	322	0.46	0.12	0.29	1.88	1.34	1.61	2.34	1.46	1.90	30	29	30	0.24	0.08	0.16				48	48	48	53	60	56
12	320	1.89	1.28	1.58	1.26	1.00	1.13	3.15	2.28	2.72	42	36	39	1.51	1.28	1.40				54	59	56	56	64	60
13	323	1.28	0.98	1.13	0.98	0.61	0.80	2.26	1.59	1.92	40	28	34	1.31	1.74	1.52				40	48	44	46	54	50
14	424	0.26	0.08	0.17	0.86	0.62	0.74	1.12	0.70	0.91	29	17	23	0.30	0.12	0.21				38	47	42	41	52	46
15	518	0.57	0.12	0.34	1.96	1.95	1.96	2.54	2.07	2.30	37	36	36	0.30	0.06	0.18				47	51	49	54	58	56
16	425-1	0.30	0.39	0.34	1.09	1.36	1.22	1.40	1.75	1.58	21	30	26	0.28	0.29	0.28				52	63	58	57	70	64
17	521	2.40	1.80	2.10	3.01	3.29	3.15	5.41	5.09	5.25	47	80	64	0.80	0.56	0.68				44	62	53	50	68	59
18	523	1.95	1.14	1.54	1.93	1.71	1.82	3.88	2.85	3.36	60	52	56	0.99	0.68	0.84				47	48	48	52	59	56
19	417	0.54	0.28	0.41	1.38	1.49	1.44	1.91	1.78	1.84	29	32	30	0.39	0.20	0.30				40	52	46	46	62	54
20	524	0.93	0.48	0.70	1.84	1.86	1.85	2.78	2.34	2.56	42	28	35	0.52	0.25	0.38				47	43	45	51	53	52
Mean		0.91	0.62	0.76	1.47	1.36	1.42	2.38	1.98	2.18	32	31	32	0.68	0.55	0.62				43	49	46	48	56	52
LSD(5%)		0.22	0.27		0.27	0.36		0.44	0.47		9.2	7.0		0.14	0.35					4.7	5.0		4.8	5.5	
CV(%)		17	31		13	18		13	17		177	16		15	45					7.7	7.2		7.0	6.9	

Table 2. Genetic estimates computed for several characteristics,  
Male Lines, 1958 and 1959.

Characteristic	Mean	Genetic		Genetic CV (%)	1/ Heritability (H)	Genetic 2/Range potential in (S) means	
		(Vg)	(Vg)			(S)	(S)
Days to initial bloom, 1958	43	38.61		14.5	.780	11.3	30
" " " 1959	49	73.78		17.5	.856	16.4	34
Days to full bloom, 1958	48	36.40		12.6	.761	10.8	27
" " " 1959	56	78.98		15.9	.840	16.8	35
Wt. of 25 flowers(gms) 1958							
" " " 1959							
Resin glands per flower, 1958	32	162.77		40.4	.597	20.3	49
" " " 1959	31	193.14		44.8	.886	26.9	64
% $\alpha$ -acid, 1958	0.91	0.355670		65.5	.938	1.19	2.14
" " " 1959	0.62	0.181533		68.7	.835	0.80	1.72
% $\beta$ -acid, 1958	1.47	0.522825		49.2	.936	1.44	2.54
" " " 1959	1.36	0.566512		55.3	.899	1.47	2.83
% total hop acids, 1958	2.38	1.386314		49.5	.934	2.34	4.42
" " " " 1959	1.98	1.038843		51.5	.905	2.00	4.39
Ratio $\alpha$ -/ $\beta$ -acid, 1958	0.68	0.170157		60.6	.943	0.82	1.27
" " " " 1959	0.55	0.183741		77.9	.750	0.76	1.68

$$1/H = H = \frac{V_g}{V_g + V_e} = \text{heritability in broad sense}$$

$$2/S = 2.06 \times H \times \sqrt{V_g + V_e}$$

Table 3. Phenotypic and genotypic correlation coefficients, 1/  
Male Lines, 1958.

	Percent $\alpha$ -acid	Percent $\beta$ -acid	Percent total hop acid	Ratio $\alpha$ - $\beta$ - acid	Number <u>2/</u> resin glands per flower	Days from May 31 to initial bloom
Percent $\beta$ -acid	.59** .57					
Percent total hop acid	.87** .87	.91** .91				
Ratio $\alpha$ - $\beta$ - acid	.52* .52	-.30 -.30	.08 .08			
Number resin glands per flower <u>2/</u>	.78** .79	.78** .79	.87** .88	.13 .13		
Days to initial bloom (from May 31)			.33 .33		.58* .60	
Days to full bloom (from May 31)			.36 .36			.99** .99

1/ Upper figures are phenotypic correlation coefficients; lower figures are genotypic correlation coefficients.

2/ All correlations with resin gland number are based on 3 observations for 17 lines. All of the rest are based on 4 observations for each of 20 lines. All are "among lines" correlations.

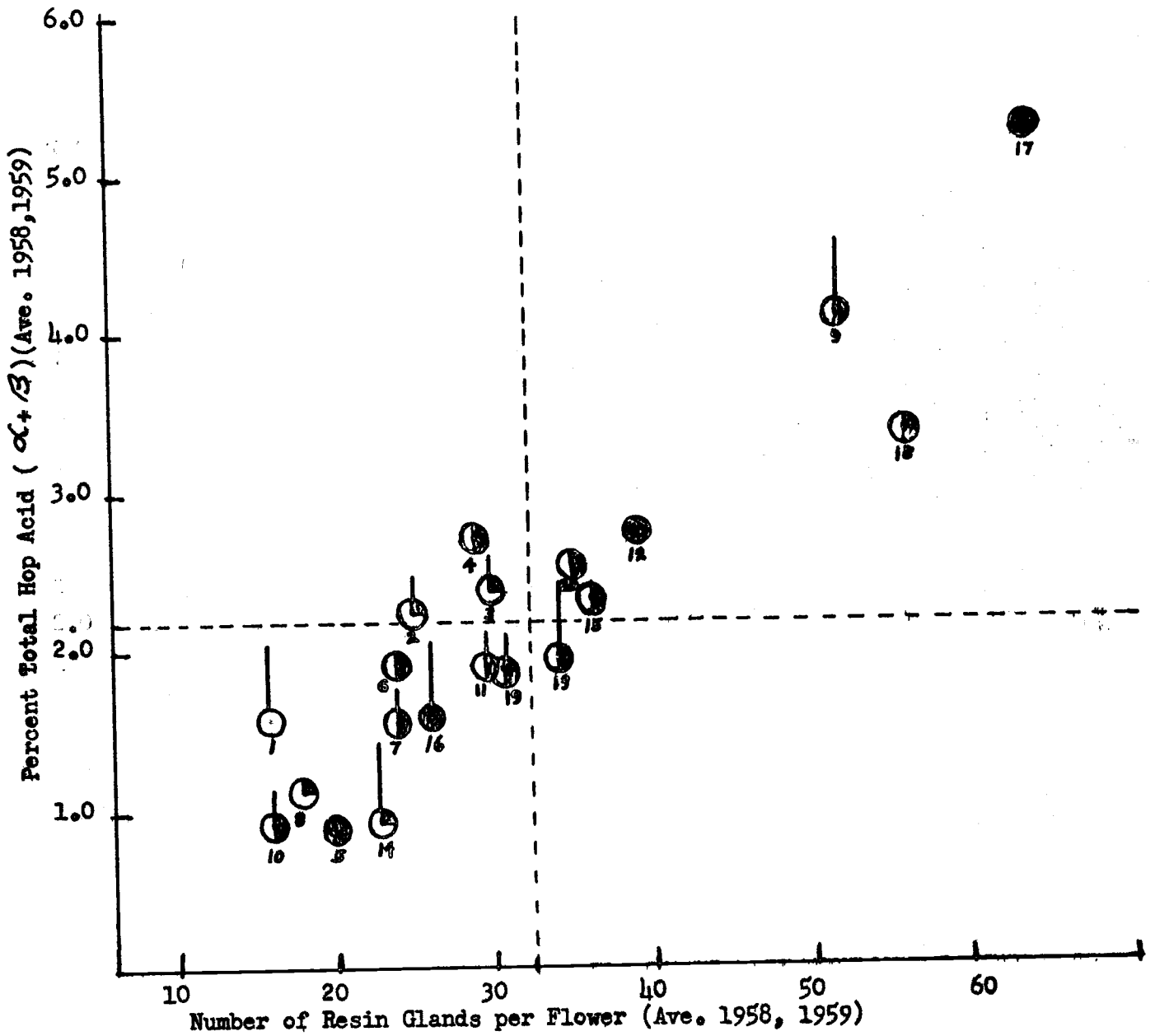
r of .444 with 18 DF significant at 5% level.

r of .561 " " " " " 1% "

r of .482 with 15 DF significant at 5% level.

r of .606 " " " " " 1% "

Fig. 1. Association <sup>1/</sup> of total hop acid, resin gland number, date of initial bloom and reaction to downy mildew in male lines, 1958-1959.



Days to Initial Bloom

- < 33 Very early (1)
- ◐ 34-42 Early (4)
- ◑ 43-51 Medium (11)
- > 52 Late (4)

Reaction to Downy Mildew

- Very susceptible (5)
- ◐ Moderately susceptible (6)
- ◑ Slightly susceptible (9)

<sup>1/</sup> See Anderson, Edgar. Genetics in Plant Breeding. Brookhaven Symposia in Biology No. 9, 1956.

Table 1 shows that wide differences were exhibited for all characters measured in 1958 and 1959. There was some disagreement from one year to the next from the standpoint of average values. Alpha-acid, beta-acid and total hop acid percentages were lower in 1959 than in 1958. Likewise, the number of resin glands per male flower was lower in 1959. As a general rule, the plants flowered somewhat later in 1959 than in 1958.

Although time did not permit calculation of two-year summaries it is evident that lines maintained their relative ranking quite well in 1959 according to that established in 1958. This is very encouraging. As long as it is possible to observe plants one year and be fairly certain that classification can be made into high, medium or low groupings for any particular character, it is not too important what the absolute values are. However, accurate estimates for any quantitative trait in any line can be gained only by making observations for several years.

As a general rule, more relative variation was present for several characters in 1959. For example, the CV's for  $\alpha$ -acid and  $\alpha/\beta$ -ratio were extremely high. Were not the absolute ranges for these characters several times in magnitude the sizes of the means, there would be serious cause for concern. It should be remembered that we are working with extremely dilute concentrations of the chemical components, and the standard error of the analytical method may be equal to at least half of the mean value obtained for a line (e.g.,  $0.20 \pm 0.10$ ). When the difference between lines varies from 0.20% to 2.00%, this is not too serious.

Heritabilities (broad sense) calculated from variance components held in close agreement for both years (see Table 2). The lowest H value was obtained for resin gland number in 1958 and this was .597. The expected or potential gains from selecting the upper 5% of the population varied from

about 0.2 to about 1.5 times the means for the several characters studied. These data indicate that wide genetic differences exist in this group of male lines, making it possible to select lines which exceed the group mean by more than double in some cases.

It was deemed advisable to calculate observed and genotypic correlation coefficients among several characters. The correlations among alpha-acid, beta-acid, and total hop acid were all positive. Most of the 6 were of sufficient magnitude to provide some measure of prediction. An exception to this statement might be that the correlation between alpha- and beta-acid was only .59, although this is significant. The correlations of resin gland number with the 3 hop acid measurements were quite high. Over half of the correlations were too low to be of predictive value.

There were essentially no differences between the observed coefficients and the genotypic coefficients. This indicates a low degree of genetic x environmental interaction. This fact is also encouraging, since it indicates that selection can be based on phenotypic observation with no correction needed for masking environmental effects.

The method of Anderson was used to describe the association, if any existed, among total hop acid, resin gland counts, maturity and reaction to downy mildew (downy mildew reaction was based on long-time observations). Figure 1 shows extremely good association between total hop acid content and resin gland numbers. Maturity did not appear to be related to either. Although the association of downy mildew reaction with either gland number or hop acid content was not clear cut, there was a suggestion of an association with both. Most of the moderately or very susceptible lines fell in the areas of low resin gland numbers and low hop acid contents.

This study will be carried on for another year, mainly for the purpose of studying several morphological characters rather than quantitative characters. A more detailed analysis of character association will be based on next year's data.

Flowering Behavior and Embryo Development  
in the Hop, Humulus Lupulus L.

(Y. P. Puri and S. N. Brooks)

In a breeding program the plant breeder is striving for a better variety. This can best be done by crossing different varieties, species and genera. However, attempts can be defeated by one or more of the following difficulties, (1) disharmony in the time of flowering of the two parents; (2) failure of pollen to germinate on stigma; (3) slow growth of pollen tubes; (4) inability of male and female gametes to effect fertilization; (5) inability of embryo to develop.

An understanding of flowering habits including duration of flowering stages, variation in flowering time between males and females and order of flowering are important in an attempt to obtain progenies from desired genotypes. Disharmony in the time of flowering in the two parents can partially be overcome by altering environmental conditions (temperature and photoperiods). A second solution to the problem might involve storing the pollen from one season to another, which will enable the plant breeder to cross early and late genotypes. Knowledge regarding the optimum conditions (temperature and humidity) of storage which will allow the pollen to remain viable for a long time is essential. In addition being able to recognize the right stage of maturity of pollen and knowing how to collect it in large quantity are equally important.

A practical tool for testing the viability and fertilizing capacity of pollen involves the use of nutrient agar medium. For such studies optimum conditions should be determined for pollen germination and tube growth which will duplicate natural conditions. In an agar medium the optimum level of sugar, agar, and nutrient elements play an important role. The environmental conditions (temperature, light and relative humidity) of the incubator are very important.



Pollen germination:

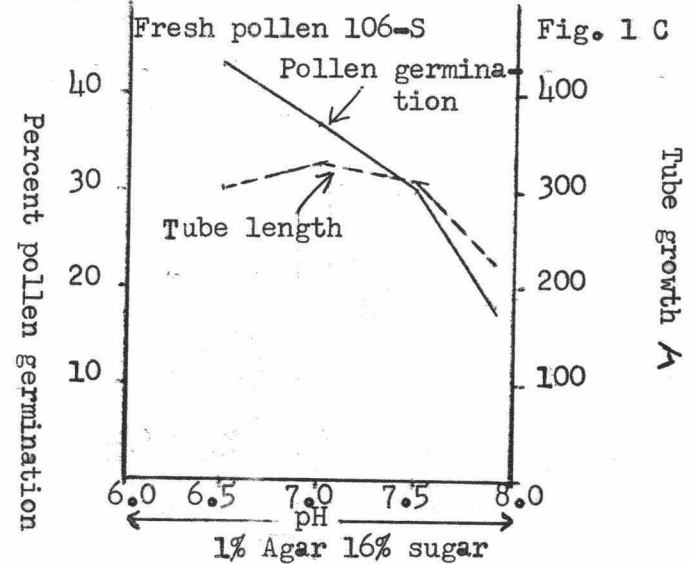
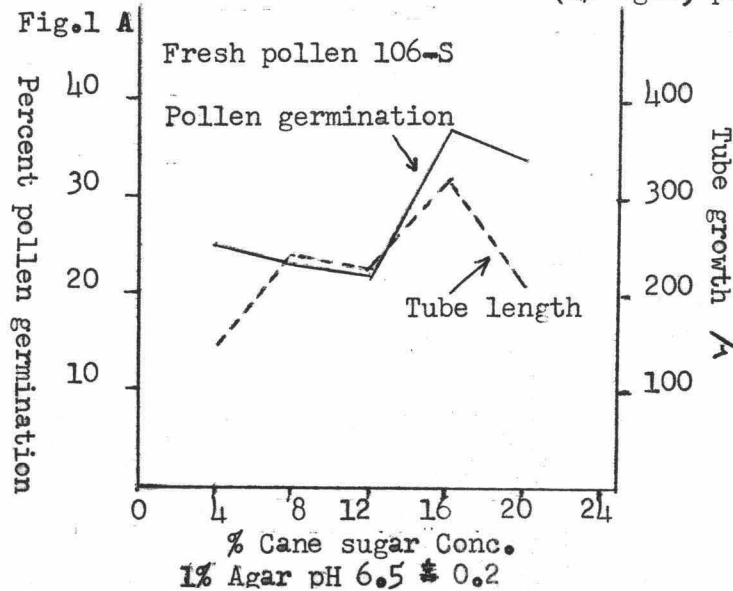
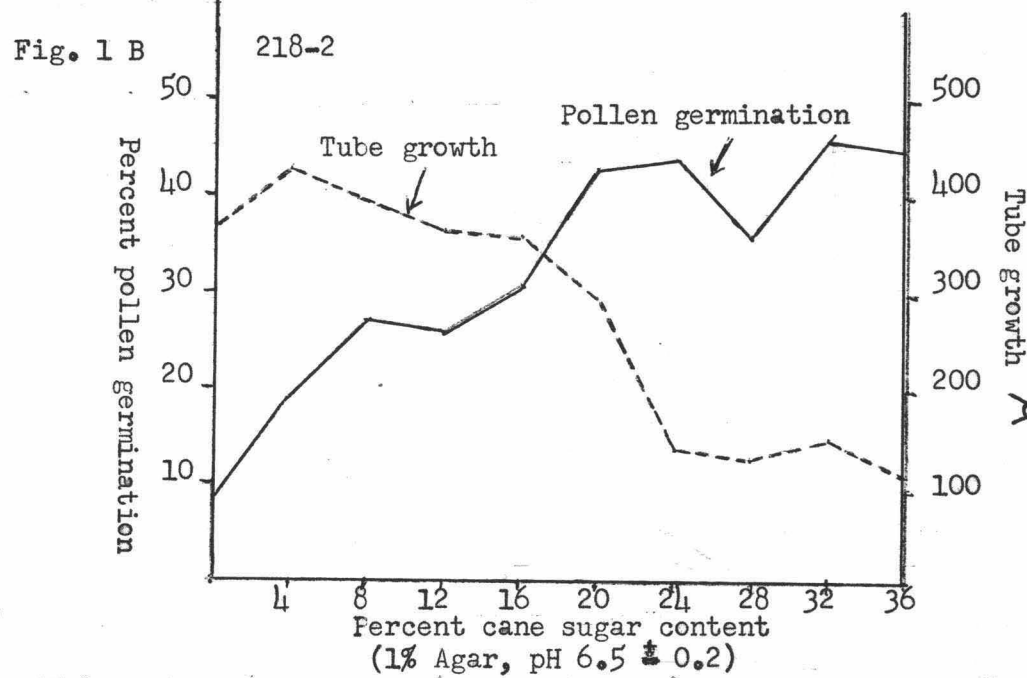
Preliminary pollen germination studies were conducted to determine the optimum level of sugar, agar and pH. In all the pollen germination studies, pollen was germinated on a solidified, thin layer of medium placed on a microscope slide. Pollen was dusted over the solidified medium on the slide with a cotton swab. These slides were placed on wet paper towels in a covered petri dish. After a period of incubation, germination counts were made at random under 300 x magnification. A minimum of 250 pollen grains were counted on each of 2 slides for the germination counts. 25 pollen tubes were measured at random with an ocular micrometer on each of 2 slides.

Table 1. The effect of temperature on pollen germination and tube growth (1% agar, 10% sugar and pH  $6.8 \pm 0.2$ ). Incubation for 20 hours. Fresh pollen of 106-S used.

Treat. No.	Treatment	Average % pollen germination	Pollen tube growth (micron)	
			Range	Average tube length
1	5°C	5.27	30- 54	47
2	10°C	13.70	72-216	138
3	15°C	14.80	66-318	154
4	20°C	29.10	72-270	128
5	30°C	32.96	54-252	116
6	20°-30°C	36.80	104-270	152

The effect of different temperatures of the incubator on the pollen germination and tube growth is presented in table 1. The effects of different levels of sugar and agar and hydrogen ion concentration on the pollen germination and tube growth are presented in figure 1. 1% agar, 16% sugar and pH  $6.5 \pm 0.2$  appeared to furnish quite satisfactory conditions for conducting further pollen germination studies. A temperature of the incubator of 30°C constant or 20°-30°C alternating (20°C 16 hours night and 30°C 8 hours day) appeared to be optimum.

Figure 1 - The effect of different levels of sugar and agar and hydrogen ion concentration on the pollen germination and tube growth of H. lupulus



Total pollen germination did not exceed 43 % in any case. The low pollen germination was attributed to faulty agar medium or partial sterility of pollen of male lines grown at Corvallis. A very recent article on germination of experiments of hop pollen on artificial culture medium by Yoshitada Mori published in Bulletin of Brewing Science, confirmed the above finding except for pH. Mori reported that the most suitable conditions for germination of hop pollen was: 1% agar-agar solution having 0.4 ml cane sugar, adjusted to pH 5.2 at a temperature of 30°C.

Several genotypes produced pollen grains of different sizes, and number of germ pores per pollen grain also varied. Some of the pollen grains had 4 or 5 germ pores while others had 3 and 2 (Figures 2 and 3)

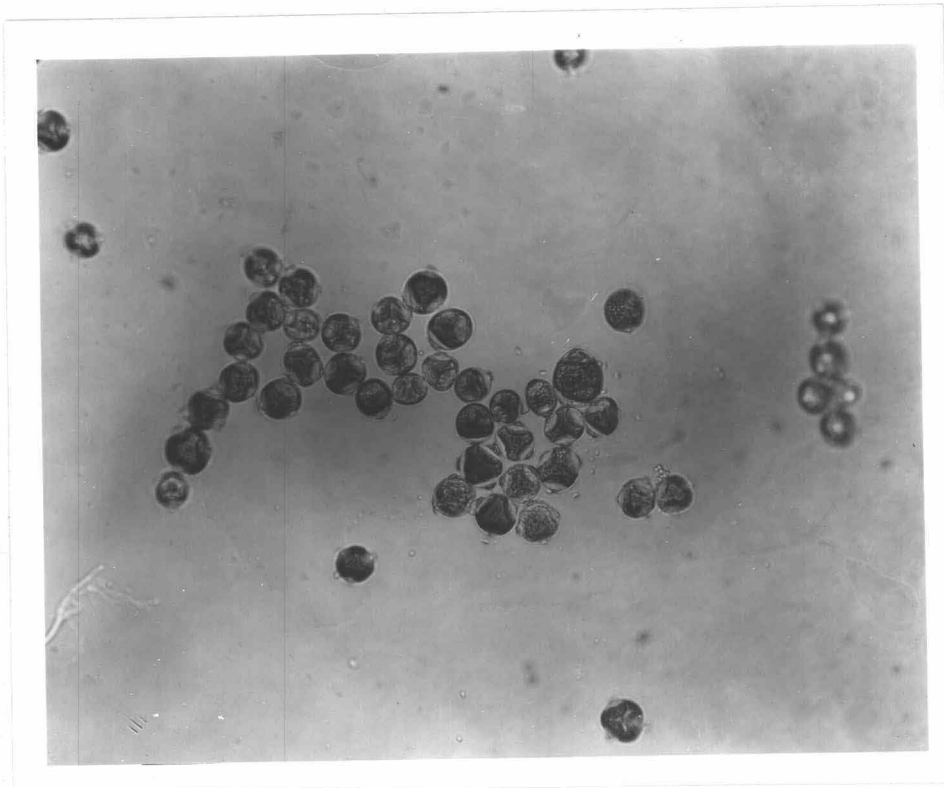


Figure 2. Pollen grains showing variability in size and number of germ pores. (on agar medium; x 400)

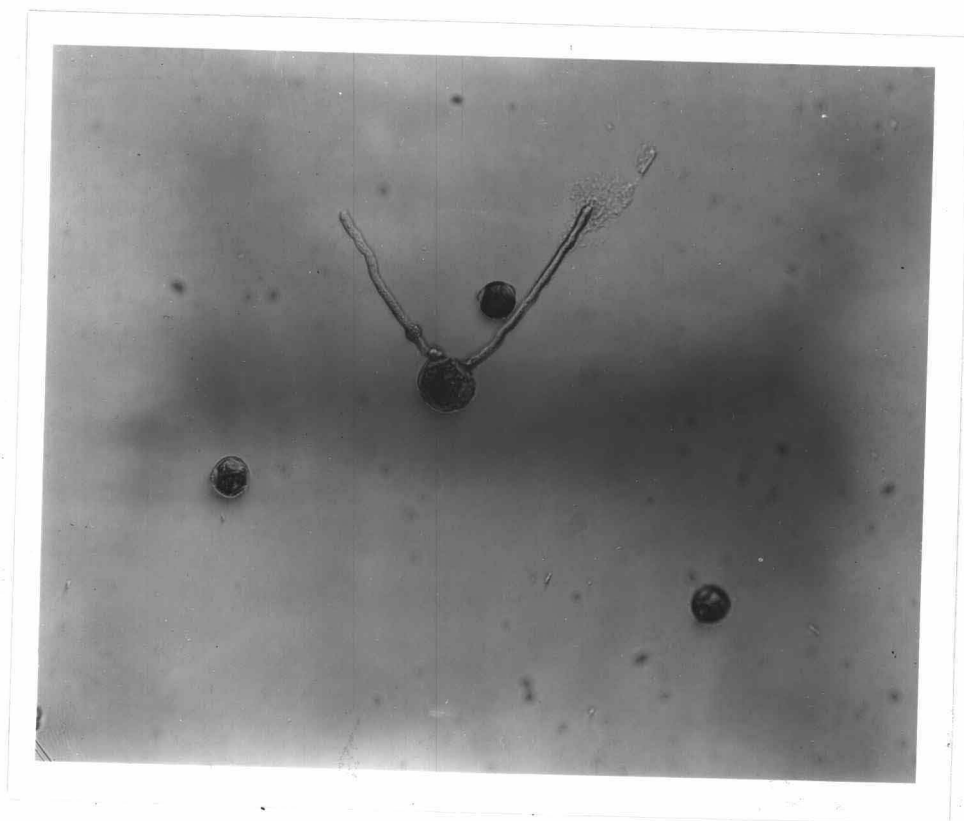


Figure 3. Variation in size and number of germ pores per pollen grain. Two tubes produced by one pollen grain with 5 germ pores. (on 1% agar, 16% sugar and pH  $6.5 \pm 0.2$ ; x 400)

Capacity to germinate and produce large tubes appeared to be related with size and number of germ pores.

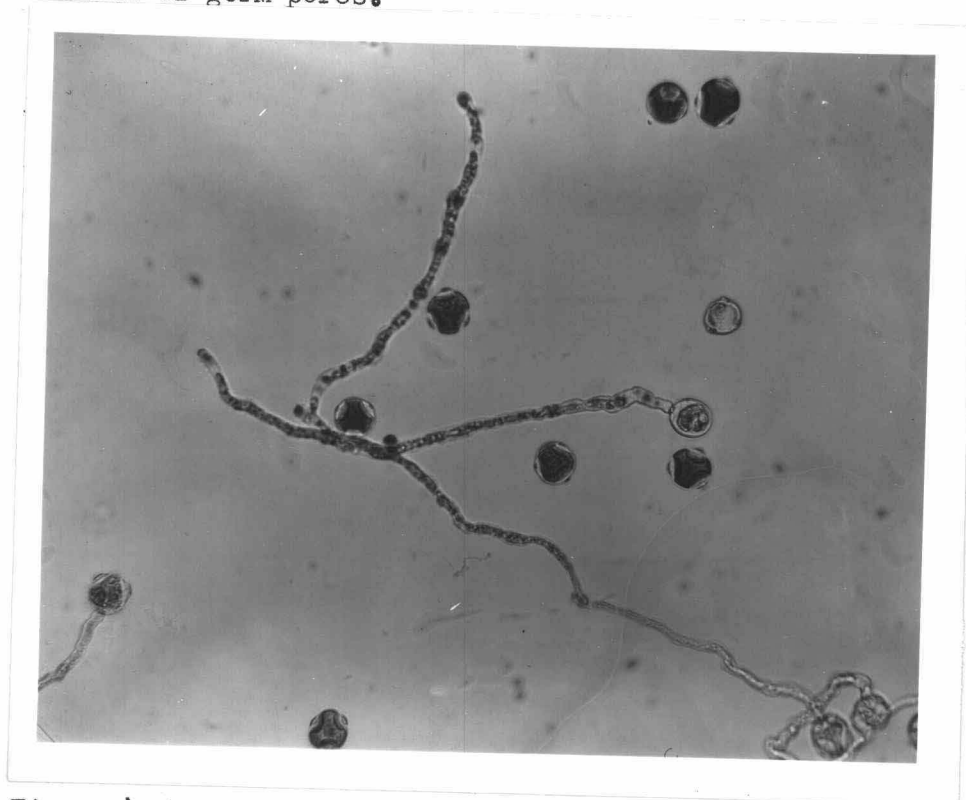


Figure 4. General view of the pollen grains showing germination and tube growth after 89 days of storage at  $0^{\circ}\text{C}$  and 35-40% R.H.

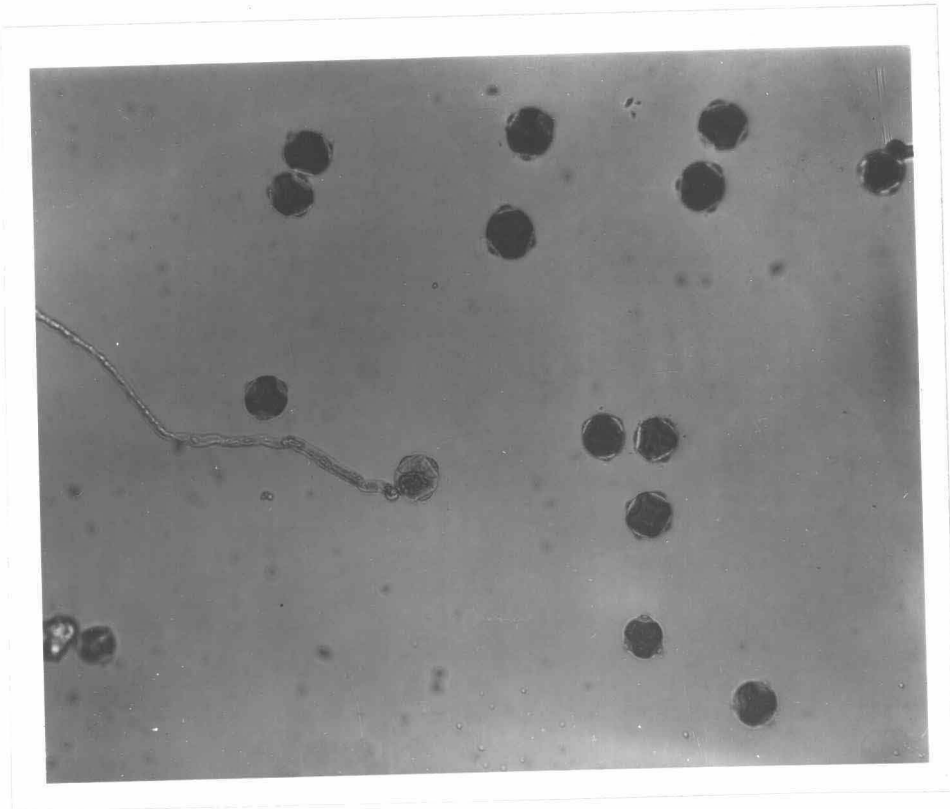


Figure 5. Pollen grain with 4 germ pores shows viability (on agar medium; x 400)

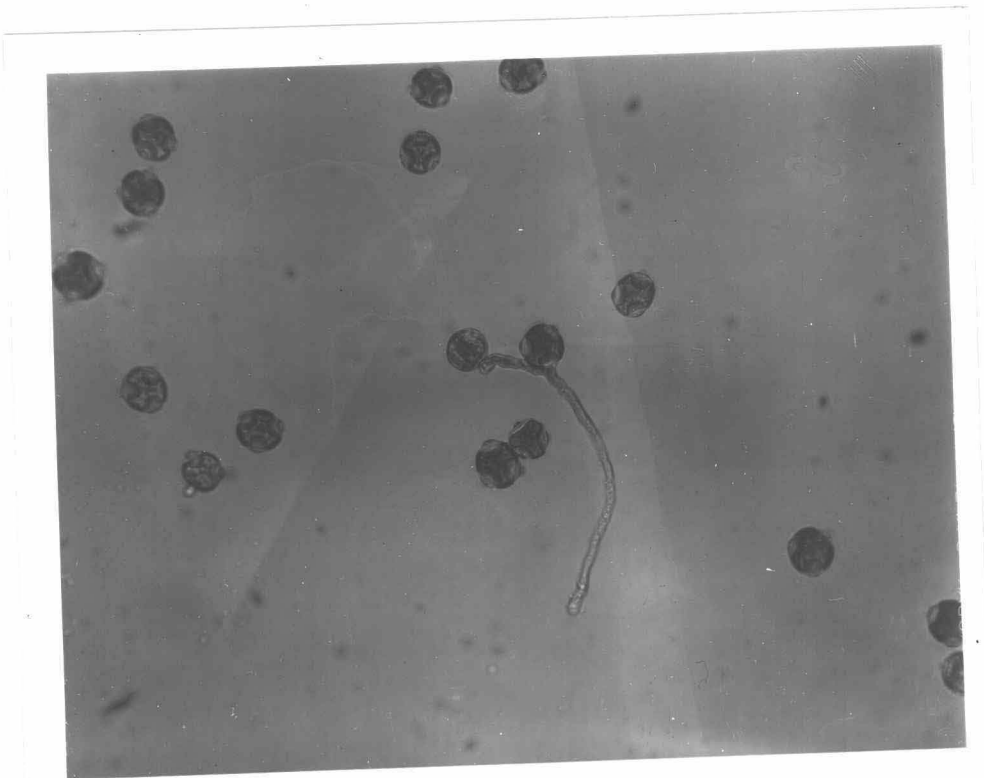


Figure 6. Pollen grains showing the protrusion of pores and the subexineous thickenings extended deeply into the cell. (On agar medium x 400)

The pollen grains with 3 germ pores appeared to germinate most readily and produce longer tubes. It is speculated that variation in size and number of germ pores of pollen might be due to the production of a large proportion of unbalanced gametes (not determined).

The genotypic differences in pollen germination and tube growth under artificial conditions (nutrient agar medium) are given in table 2.

Table 2. Genotypic differences in pollen germination and pollen tube growth under artificial conditions: 1% agar, 16% sugar and pH 6.5  $\pm$  0.2; incubation at 15°-25°C. for 22 $\frac{1}{2}$  hours; fresh pollen dusted from the inflorescences.

	Genotype	% Pollen germination	Pollen tube growth (microns)	
			Range	Avg. tube length
1	221-2	16.2	198-546	306
2	218-2	23.3	159-555	370
3	219-2	35.4	207-581	370
4	103	1.4	85-338	178
5	123	23.0	203-615	364
6	106-S	36.8	104-270	128

An effort was also made to increase the germination and tube growth of stored pollen by the use of hormones. No significant results were obtained (data not given). These hormones were not water soluble and germination studies will again be conducted by using water soluble hormones.

#### Cytological studies of male genotypes:

Cytological studies were conducted to determine the cause of partial sterility in the male lines. Male buds were collected and fixed in Carnoy's fluid. Technique of smearing and iron acetocarmine stain was used. In this study an attempt was made to determine **univalents**, bivalents and trivalents per pollen mother cell and the haploid number of chromosomes per microspore in each genotype. An effort was also made to detect the chromosomal aberrations per pollen mother cell.

In the present preliminary study a few of the first meiotic division stages were found in pollen mother cells of genotypes 106-S, 218 and 125. In genotype 106-S the pollen mother cells had only bivalents and these were 10 pairs of chromosomes. A few of the P.M. cells had bridges. In the genotypes 218 and 125, a few of the P.M. cells were found in pachynema and metaphase I stages.

In these three genotypes it was quite common to find P.M. cells of an anther in the pachynema stage and others in quartets. This indicated that anthers consisted of different stages of maturity. Some sterility could be attributed to the irregularity in meiosis and some could be due to other factors in meiosis as mentioned above. Similar results were found by Winge. He reported that the nuclear divisions took place basipetally in such a manner that the base of a pollen sac (anther) consisted of nuclei in synopsis, whereas nuclei near the apex had already completed the meiotic division and lay in a kind of resting stage. He also stated that in the hops the pollen sacs (anthers) opened by an apical slit, and the apical pollen grains naturally fell out before the basal. He also counted 10 and 8 chromosomes in a few of the pollen mother cells in the anaphase I.

In the present study, the material for cytological studies was collected late in the season. Therefore, right material showing all stages of meiosis could not be collected. Similar studies will again be conducted in the summer of 1960.

#### Pollen storage:

The data on longevity of pollen when stored under different humidity conditions and 0°C. temperature are presented in table 3.

Table 3. Pollen storage. Media used: 1% Agar, 16% Sugar and pH 6.5 ± 0.2

	Treatment	Days of storage	% Pollen germination	Pollen tube length (microns)	
				Range	Average
1	0°C, saturated CaCl <sub>2</sub> 6H <sub>2</sub> O (38-39% R.H.) 106-S loose pollen	0	36.4	129 - 489	329
		7	23.1	129 - 400	239
		12	25.0	181 - 589	374
		35	18.9	138 - 472	313
		42	14.7	65 - 224	144
		76	3.7	54 - 229	112
		121	1.9	86 - 212	142
2	0°C, saturated CaCl <sub>2</sub> 6H <sub>2</sub> O (38-39% R.H.) 218-2 loose pollen	8	31.1	78 - 396	271
		20	21.0	211 - 645	349
		44	6.3	78 - 397	167
		89	1.6	74 - 345	172
3	-10°C, saturated CaCl <sub>2</sub> 6H <sub>2</sub> O 218-2 loose pollen	44	15.2	29 - 192	133
		89	2.4	91 - 338	199
4	0°C, no humidity control in household refrigerator 218-2 loose pollen	44	9.8	54 - 215	179
		89	2.4	112 - 516	267
5	0°C, saturated CaCl <sub>2</sub> 6H <sub>2</sub> O (38-39% R.H.) 218-2 Inflorescence	44	4.7	63 - 208	139
6	0°C, saturated CaCl <sub>2</sub> 6H <sub>2</sub> O (38-39% R.H.) 218-2, Florets	44	0.7	-- --	--
7	0°C, saturated CaCl <sub>2</sub> 6H <sub>2</sub> O (38-39% R.H.) 221-2	0	16.2	198 - 546	306
		5	11.6	185 - 495	297
		50	1.0	86 - 135	117
8	0°C, saturated CaCl <sub>2</sub> 6H <sub>2</sub> O (38-39% R.H.) 219-2	0	35.4	207 - 581	370
		5	22.9	177 - 413	280
		50	3.0	125 - 432	248
9	0°C, saturated CaCl <sub>2</sub> 6H <sub>2</sub> O (38-39% R.H.) 218-2	0	23.3	155 - 555	370
		5	9.0	144 - 435	293
		50	4.7	138 - 594	305
10	0°C, saturated MgCl <sub>2</sub> 6H <sub>2</sub> O (35-40% R.H.) 218-2 Loose pollen	44	14.9	108 - 330	222
		89	6.5	123 - 324	236
11	0°C, saturated MgCl <sub>2</sub> 6H <sub>2</sub> O (35-40% R.H.) 218-2 Inflorescence	44	1.4	-- --	--
12	0°C, saturated MgCl <sub>2</sub> 6H <sub>2</sub> O (35-40% R.H.) 218-2 Florets	44	--	-- --	--



52  
Table 3 - cont.

Treatment	Days of storage	% Pollen germination	Pollen tube length (microns)	
			Range	Average
13 0°C, saturated NaNO <sub>3</sub> (77-80% R.H.) Loose pollen	44	--	--	--
14 0°C, saturated NaNO <sub>3</sub> (77-80% R.H.) Inflorescence	44	--	--	--
15 0°C, saturated NaNO <sub>3</sub> (77-80% R.H.) Florets	44	--	--	--

The freed pollen of 106-S genotype collected on June 7, 1959 was stored for 121 days and germinated 3 percent with an average tube length of 142 microns. The freed pollen collected from 218-2 on August 13, 1959 was stored for 89 days and germinated 6.5 percent with an average tube length of 236 microns. Pollen could not be stored successfully in the inflorescence and florets. The saturated solutions of MgCl<sub>2</sub> 6H<sub>2</sub>O (producing 35-40% R.H.) seemed to be better than saturated solution of CaCl<sub>2</sub> 6H<sub>2</sub>O (producing 38-39% R.H.) for pollen storage at 0°C.

Pollen shedding and dispersal:

With wind-pollinated crops, it is important to understand pollen shedding and dispersal habits in order to maintain genetic purity. In such studies the identification of pollen is very important. It is a well known fact that wind-borne pollen travels many miles, but the information whether pollen remains viable (capable of fertilization) is not available.

Studies of the time of day of pollen shedding, density of pollen in the air (Breeding Block) and pollen dispersal were carried out. The time of day of pollen shedding and density of pollen was determined hourly by exposing vaseline coated slides attached to wind-vanes at 4 stations in the

breeding block. The average of these 4 slides was reported as the number of pollen grains present in the air at that particular hour. The temperature and humidity was recorded every hour by means of a psychrometer in the center of the breeding block.

The average number of pollen grains relative to temperature, percent relative humidity and time of day on July 22 and 25, August 6 and 7, 1959 are presented in Figures 7, 8 and 9. The data from 3 days were averaged, and this relationship is shown in figure 10. A more direct relationship between pollen density and temperature and humidity can be seen in Figures 11 and 12, respectively.

The number of pollen grains in the air on July 22, 1959 was low and the number increased on July 25, 1959. This indicated that the frequency of opening<sup>of</sup>/male florets was low on July 22, 1959 and increased on July 25, 1959. On August 6, 1959 the number of pollen grains in the air was less compared to July 25, 1959. This indicated that the heavy male floret opening and pollen shedding period was between July 23 to August 6, 1959.

Pollen shedding and density were related to temperature and humidity. The average number of pollen grains per hour in the air increased with the increase of temperature and decrease of relative humidity. This was only true up to the time of maximum pollen shedding. At this time dehiscence of a majority of the anthers of mature florets was brought about by environmental conditions, namely temperature and relative humidity.

The collection of pollen for the hybridization program should be made by picking the inflorescences in the early morning and keeping their bases in water. They should be placed in the room where low humidity and high temperature will be maintained. The optimum temperature and relative humidity should be determined in order to obtain pollen with high viability.

Figure 7 - Number of pollen grains of male hops relative to temperature, percent relative humidity and time of day on July 25, 1959 at Corvallis, Oregon (Avg. of 4 slides).

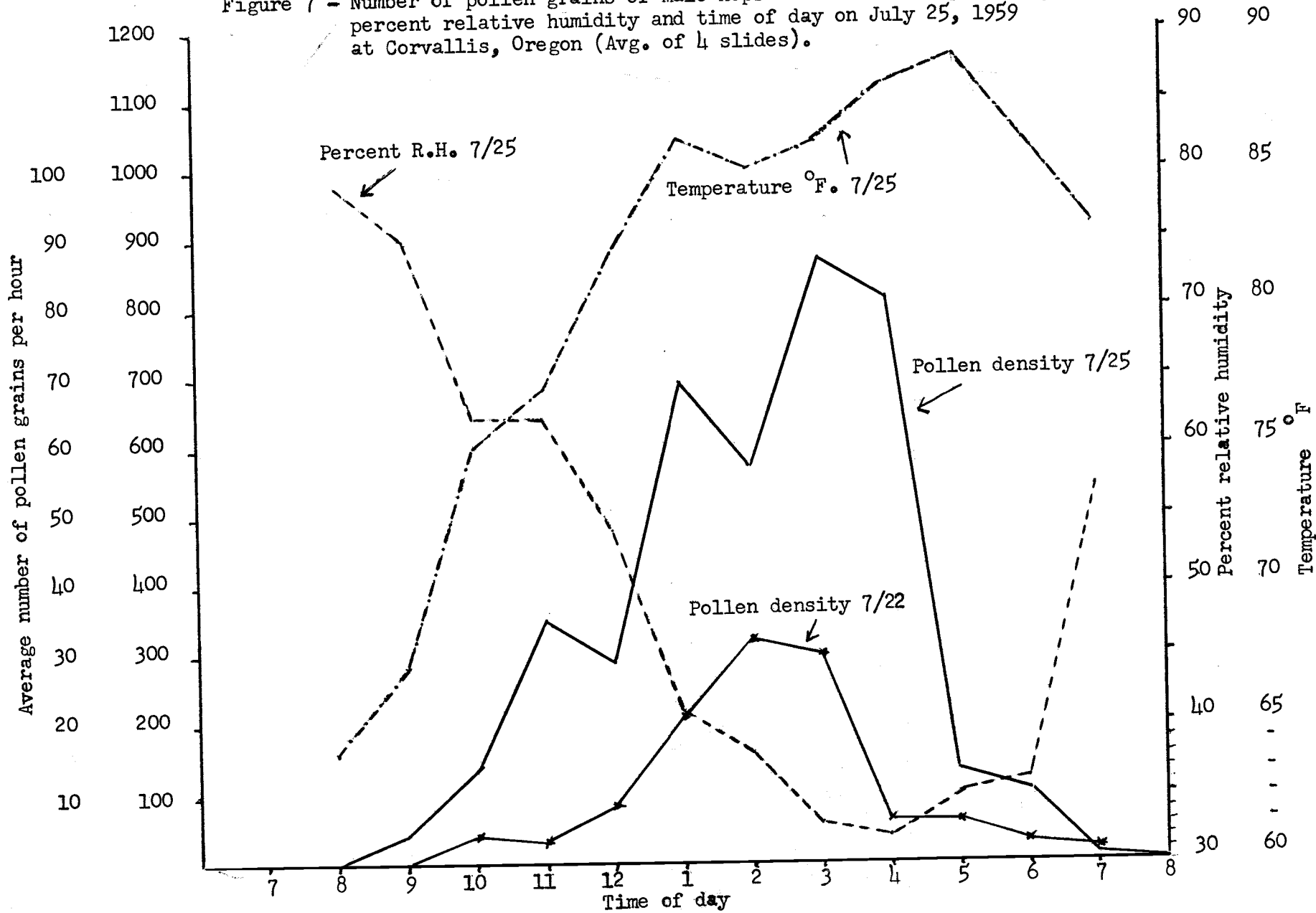


Figure 8 - Number of pollen grains of male hops relative to temperature, percent relative humidity and time of day on August 6, 1959 at Corvallis, Oregon. (Avg. of 4 slides)

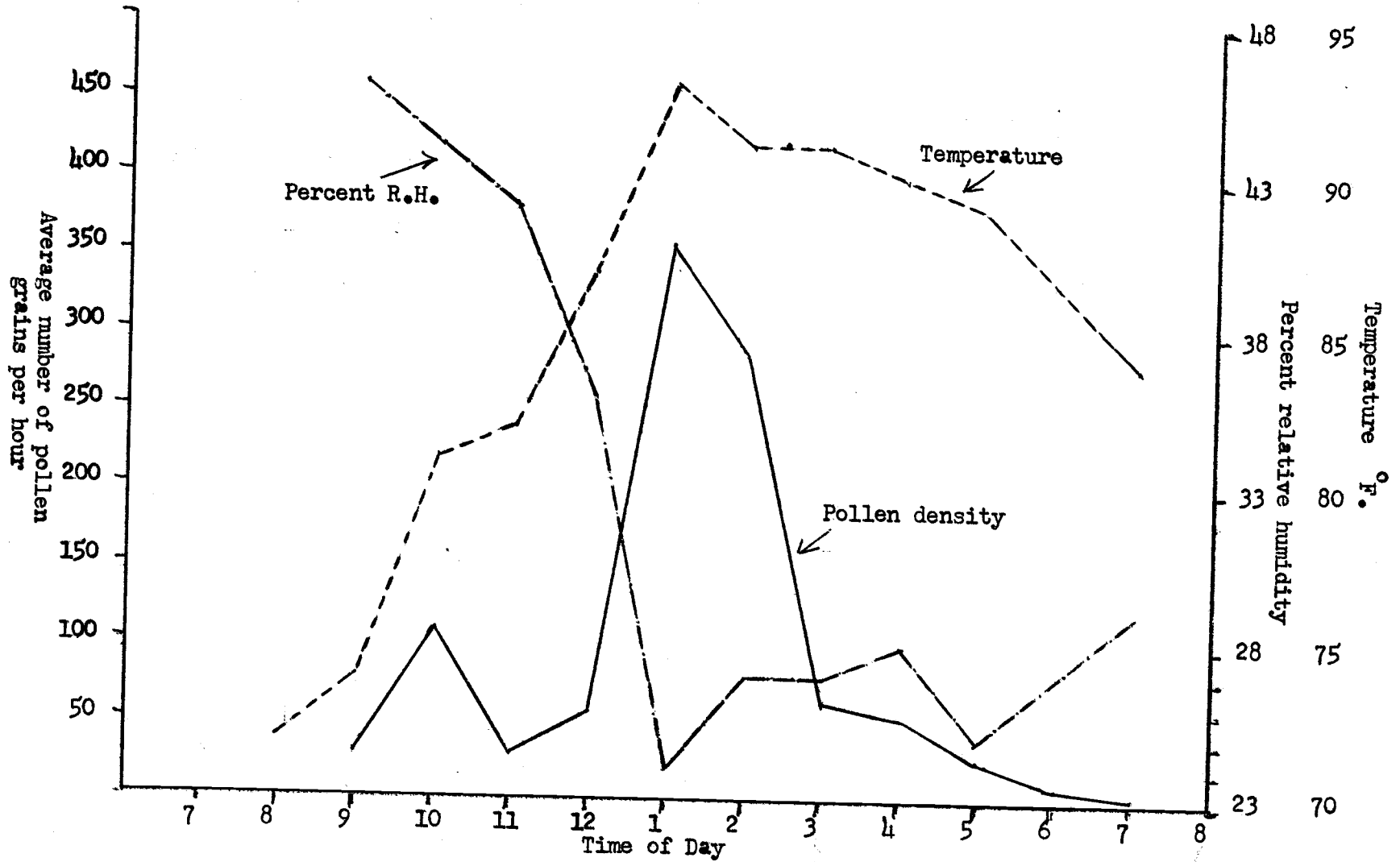


Figure 9 - Number of pollen grains of male hops relative to temperature, percent relative humidity and time of day on August 7, 1959 at Corvallis, Ore. (Avg. of 4 slides)

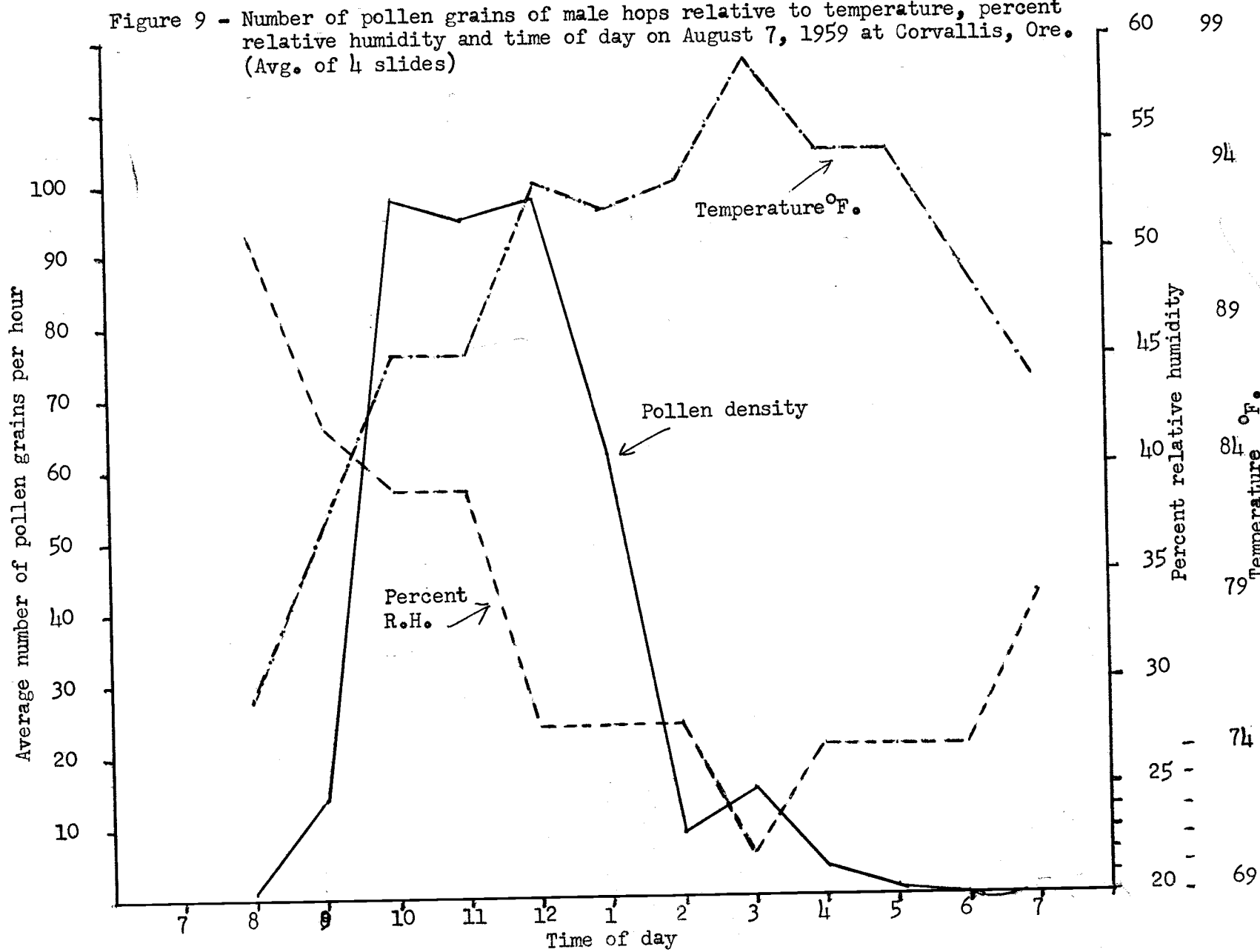


Figure 10 - Relationship between temperature, percent relative humidity, time of day and density of pollen shedding in male hops (Avg. of 3 days: 7/25/59, 8/6/59 and 8/7/59)

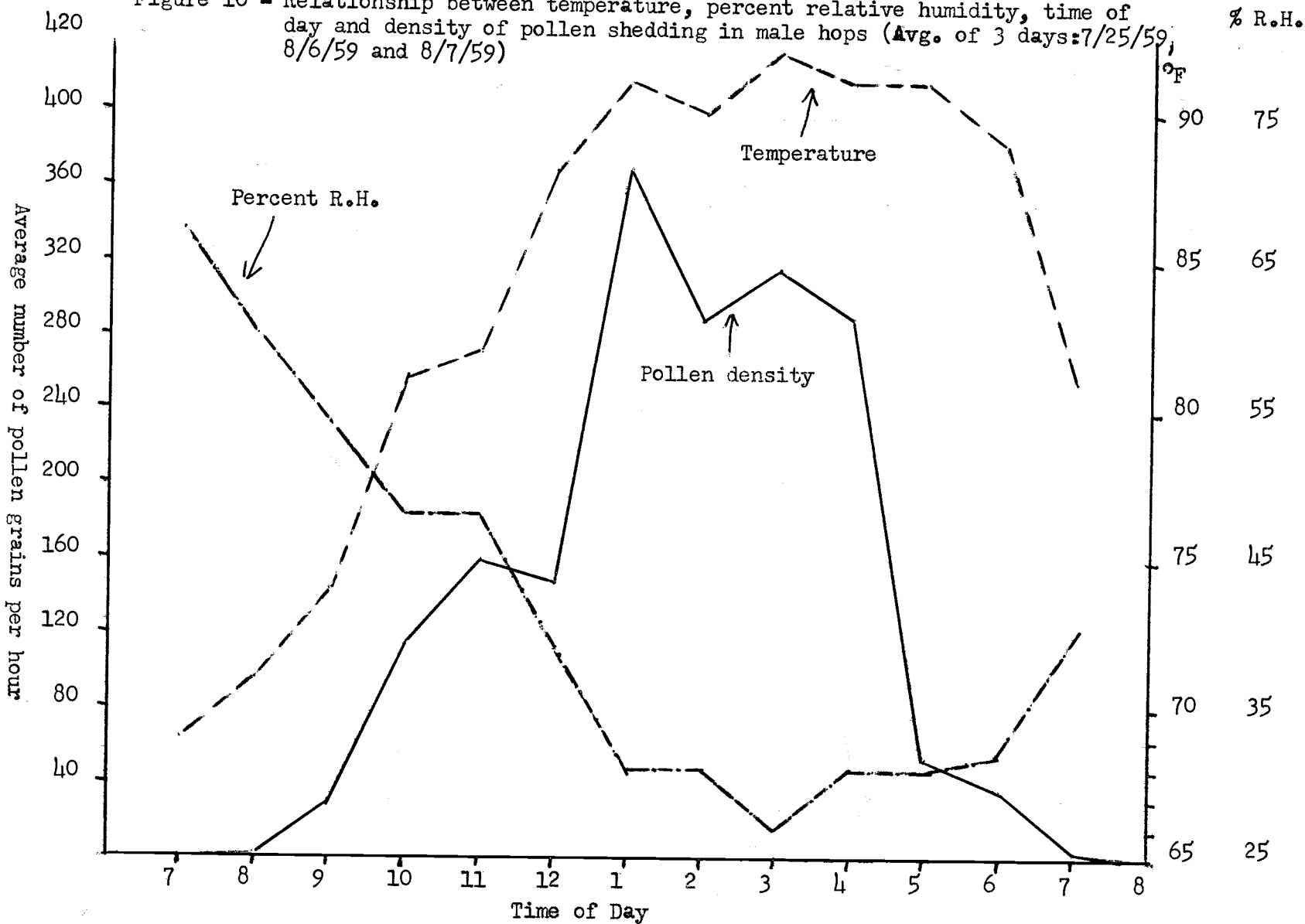


Figure 11 - The relationship between temperature and average number of hop pollen grains per hour.

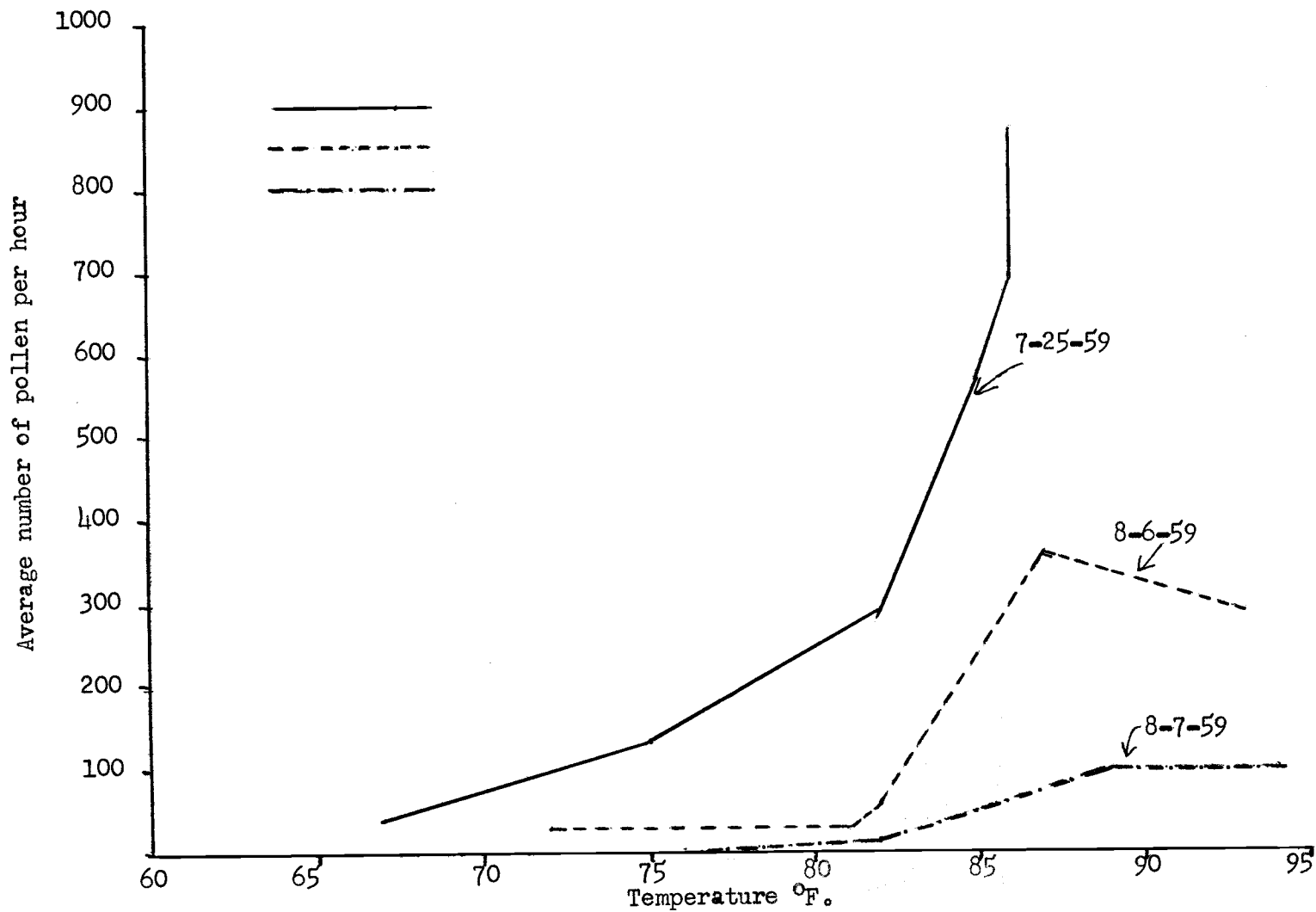
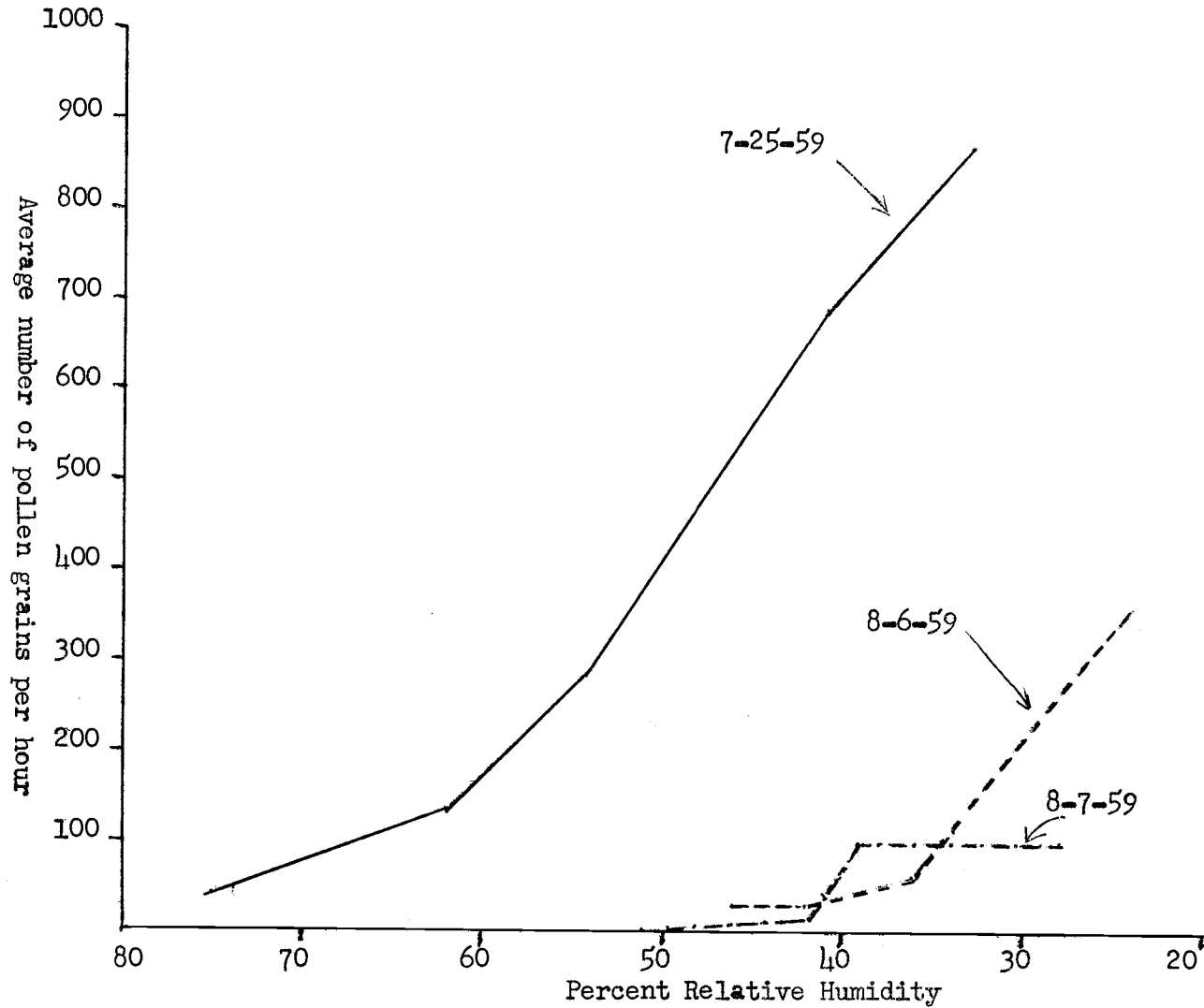


Figure 12 - The relationship between percent relative humidity and average number of pollen grains of hop per hour.





Pollen shedding started after 7:00 A.M. and reached a peak at 1:00 P.M. on the average. After 7:00 P.M. the pollen density in the air became negligible all 3 days.

Pollen dispersal and density from the breeding block at several distances is shown in table 4.

Table 4. Pollen dispersal

Distance from the field (meters)	No. of pollen collected per sq. inch of the slide 7 A.M. to 1 P.M. exposure	No. of pollen collected per sq. inch of the slide 1 P.M. to 7 P.M. exposure	Density of pollen per sq. inch area of slide exposure 12 hrs.
55	261	346	607
90	50	251	301
225	--	--	153
1210	--	--	25

Female flower development:

The stages of female development from the time of inflorescence initiation to cone formation are described in table 5. This table also presents the information regarding stigma receptivity under natural conditions. Data showing stigma receptivity under vegetable parchment bags are given in table 6. The stigma did not remain receptive after July 28, 1959 under the bags nor under natural conditions. These observations indicated that from a breeding standpoint all crosses should be made as early as possible.

Table 5. Female flower development and pollen receptivity under natural conditions.

Dates	BB 404 Stages of Inflorescence	BB 403 Stages of Inflorescence	BB 210 Stages of Inflorescence
6-25-59	Initiated	Initiated on 6-18-59	Initiated on 6-18-59
7-17-59	Stigma not visible	Stigma not visible	Stigma not visible
7-19-59	Stigma started to emerge out	Stigma started to emerge out	Stigma not visible
7-21-59	In majority of cones stigma enlarged, and color green. Receptive to pollen	In majority of cones stigma enlarged. The tips of stigma at the base of cone turned brown	Stigma not visible
7-22-59			Stigma started to emerge out and receptive.
7-25-59	Cone enlarged, tips of the stigma at the base of the cone turned brown. Rest of the 2/3 of cone had green stigma and receptive.	Cones enlarged, bracts at base of cone developed. Rest of the 2/3 cone had green and receptive stigma. Cone completely burred.	Stigma enlarged and visible in whole cone. Started to turn brown at the base. Rest of the 2/3 cone receptive.
7-28-59	Cone enlarged further. Tips of the stigma of whole cone except at the apex turned brown. Bracts of cone developed more. Only apex receptive to pollen.	Similar to 404	Completely burred. Initiation of cone formation. (Bracts developed at the base of cone). Stigma completely brown except at the apex of cone.
7-31-59	2/3 of the cone from the base formed. 1/3 of the cone from the apex not fully developed. Stigma completely turned brown.	Similar to 404	3/4 of the cone formed. Apex of the cone still had green or receptive stigma.
8-3-59	Cones completely formed	Similar to 404	Stigma completely brown in the whole cone. Cone developed.
8-6-59			Cone formation with enlarged bracts.

Table 6. Stigma receptivity under vegetable parchment bags.

<u>Genotype</u>	<u>Plant No.</u>	<u>Date of pollination</u>	<u>Average No. of seeds formed per cone</u>	
402 (202)	1	7-25-59	6.7	
	1	7-25-59	2.0	
C 19013		7-28-59	3.3	
		7-31-59	0.8	
		8-3-59	--	
	2		8-3-59	--
			8-5-59	--
			7-25-59	5.1
			7-28-59	--
			7-31-59	--
			7-25-59	1.8
	3		7-28-59	7.7
			7-31-59	--
			8-3-59	--
			8-3-59	--
8-5-59			--	
4			7-25-59	6.7
	7-25-59	11.7		
	8-5-59	--		
	222	1	7-25-59	8.0
7-28-59			3.3	
7-28-59			7.6	
2		7-22-59	--	
		7-25-59	--	
		7-28-59	2.0	
3			7-22-59	--
			7-25-59	9.6
			7-28-59	--
	8-3-59		--	
4		7-22-59	--	
		7-28-59	--	
		8-3-59	--	

Seed maturity studies:

Seeds were collected 49, 54, 59, 64, 69, 73, 78, 84, 90 and 96 days after the initiation of inflorescence from genotype 404. Fresh and dry weights per 100 seeds were determined immediately. The dry weight per 100 seeds was recorded after removing the resin glands from the seed coat by rinsing in 50% alcohol. The maturity studies also included seed germination and seedling vigor determination.

The effect of different stages of maturity on seed germination is presented in figure 13. The influence of seed maturity on fresh and dry weight, dry weight after removing resin, moisture content and percent germination of seed and seedling vigor is shown in table 7. The seed became mature about September 7, 1959 according to commercial harvesting standards. The dry weight of seed reached a maximum at 90 days after initiation of inflorescence. The highest total seed germination and speed of germination was obtained from the seeds collected 96 days after initiation of inflorescence. The highest seedling vigor was obtained from the seeds collected 90 and 96 days after inflorescence initiation.

Figure 13 - The effect of different stages of maturity on seed germination (H. lupulus).

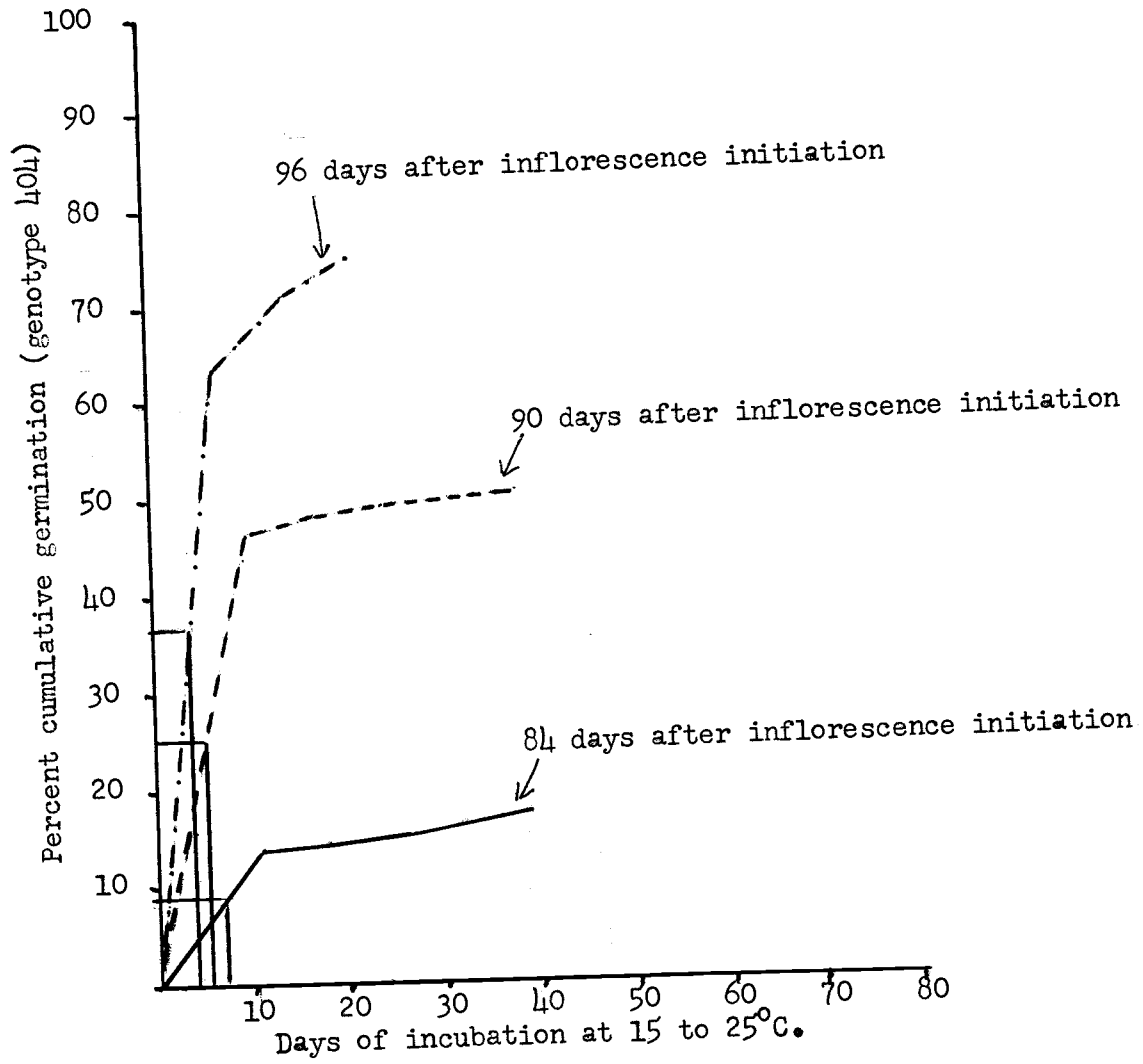


Table 7. The effect of seed maturity on the fresh and dry weight, moisture contents, percent germination and seedling vigor indices. (Genotype 404) Date of initiation of inflorescence = 6-25-59

Days after initiation of inflorescence	Av. fresh wt. per 100 seeds mg.	Av. dry wt. per 100 seed plus resin mg.	Percent moisture	Av. wt. per 100 seed without resin mg.	Seed germination			Seedling vigor indices			
					Speed of germination percent germination	days of incubation	percent total germination	Root length cm	Seedling length cm	Dry wt. per seedling mg.	
8-12-59	49	27.31	5.72	78.79	--	--	--	--	--	--	
8-17-59	54	43.96	7.80	82.33	--	--	--	--	--	--	
8-22-59	59	40.87	10.34	74.70	8.61	--	--	--	--	--	
8-27-59	64	42.77	11.42	73.31	11.45	--	--	--	--	--	
9-1-59	69	42.72	18.96	55.62	18.35	--	--	--	--	--	
9-5-59	73	49.19	21.88	55.56	22.49	--	--	0.8	--	--	
9-10-59	78	42.82	27.01	36.68	25.30	--	--	0.4	1.0	4.2	0.10
9-16-59	84	44.57	27.88	37.44	26.60	9.0	7	17.6	1.9	5.2	0.23
9-22-59	90	41.97	28.22	32.75	28.17	25.0	5	50.4	2.0	6.4	0.27
9-28-59	96	35.09	27.12	22.74	26.40	37.0	4	74.8	2.6	7.1	0.25

CR e5-2 (OAES 36:Bot.) HOP DISEASES;  
THEIR ETIOLOGY, EPIPHYTOLOGY, AND CONTROL

C. E. Horner

HOP DOWNY MILDEW

Field Tests with Streptomycin

Objectives:

To determine comparative effectiveness of 1 and 2 applications of streptomycin spray for downy mildew control.

Nature and extent of previous work:

- 1956 Annual Report p. 64
- 1957 Annual Report pp. 47-48
- 1958 Annual Report pp. 46-51

Procedure:

The experiments were conducted on the Melvin King and Charles Lathrop hop yards at Grants Pass, Oregon. Both yards were planted to the mildew susceptible variety Late Clusters. Plots consisted of 500-600 hills each, with 4 replications. Sprays of 1000 ppm streptomycin were applied with hose guns from a tractor-drawn spray tank. Approximately 20 gallons of spray per acre was required to wet all foliage. The first application was made when new shoots were 4-16 inches long, with the majority about 8 inches. The second application was made about 7 days after the first training when most vines were 4-6 feet tall.

Two weeks after applications, data were recorded on the number of infected hills and infected shoots. Disease incidence was low and conditions were unfavorable for rapid disease development in the test area.

Results:

Summary data on incidence of infected hills and shoots are recorded in the following tables.

Table 1. Comparison of 1 and 2 applications of 1000 ppm streptomycin spray for downy mildew control. King Yard, Grants Pass, Oregon. 1959.

Treatment	Rep.	1 application			2 applications		
		Number infected Hills	spikes	Treatment Totals	Number infected Hills	spikes	Treatment totals
1000 ppm strep.	1	10	28	46 hills	4	11	28 hills
	2	13	34	105 spikes	11	27	68 spikes
	3	10	16		5	13	
	4	13	27		8	17	
1000 ppm strep. + 1% glycerol	1	10	18	41 hills	3	7	20 hills
	2	15	42	92 spikes	9	20	47 spikes
	3	10	21		4	10	
	4	6	11		4	10	
Control	1	23	56	102 hills	20	49	78 hills
	2	21	47		17	40	184 spikes
	3	33	87	254 spikes	21	51	
	4	25	64		20	44	

Data in the table above show a reduction in infected hills of 55 per cent and in spikes of 59 per cent resulting from 1 application of 1000 ppm streptomycin spray. After two applications infected hills were reduced 64 per cent and infected shoot incidence reduced 63 per cent compared to untreated controls. The addition of glycerol at 1% of the spray solution appeared to increase effectiveness slightly. However, statistical analysis of the data was not completed at the time of this writing and no conclusion can be made now on the effectiveness of glycerol or on one versus two applications.

A similar test was conducted in another hop yard with the following data obtained.



Table 2. Effect of 1000 ppm streptomycin spray on incidence of downy mildew. Lathrop Yard, Grants Pass, Oregon. 1959.

<u>Treatments</u>	<u>Rep.</u>	<u>Number of Infected</u>	
		<u>Hills</u>	<u>Spikes</u>
Streptomycin 1000 ppm spray	1	7	22
	2	5	16
	3	21	59
	4	19 Total 52	111 Total 111
Untreated control	1	18	49
	2	23	70
	3	30	98
	4	37 Total 108	125 Total 342

The data in table 2 above show, once again, that streptomycin spray reduced the incidence of both infected hills and infected shoots. It should be recorded that a comparison was made between the plot treatment area and an equal number of adjacent hills treated by the grower with 2 applications of zineb dust. In the zineb-treated area 91 infected hills and 301 infected shoots were recorded compared to 52 hills and 111 spikes in the streptomycin plots, and 108 hills and 342 spikes in the untreated controls.

The 1959 season was not favorable for hop downy mildew build-up and spread. Consequently disease incidence in the experimental area was low, and control tests did not show the striking results achieved in some previous seasons.

#### Discussion and Conclusions:

The 1959 results with streptomycin sprays for hop downy mildew control, though less striking than in previous years, show significant control with either 1 or 2 applications. Because of low disease incidence in 1959 control was less striking than in 1957, for example, when disease incidence and severity were high. Variable results obtained by plant pathologists in Washington and California have pointed out the necessity

of conducting carefully planned and uniform testing procedures and have emphasized the importance of proper timing of spray applications to obtain satisfactory control. Published recommendations for the use of streptomycin for hop downy mildew control will be withheld pending further data on timing of application and residue status.



The low degree of infection developing in inoculated controls was disappointing, therefore the experiment was repeated using 300 healthy cuttings from the same lot. When ready for inoculation the young plants were sprinkler irrigated to thoroughly wet the soil. Inoculation was carried out at night and each hill was covered with a plastic hot cap immediately after inoculation. Hot caps were removed the following day. Four days after inoculation a single spray of 1000 ppm streptomycin and 1% glycerol was applied.

Excellent infection was obtained by the above method of inoculation. Data were recorded weekly for 1 month on the number of systemically infected shoots. Typical data is given in the table below.

Table 2. Eradicant action of streptomycin spray applied four days after inoculation.

<u>Treatment</u>	<u>Number infected shoots</u>				<u>Total</u>	<u>Avg.</u>
	<u>R1</u>	<u>R2</u>	<u>R3</u>	<u>R4</u>		
Inoculated plus streptomycin	0	0	0	0	0	0
Inoculated only	21	14	21	20	76	19
Not inoculated	0	0	0	0	0	0

In this test a single application of 1000 ppm spray applied 4 days after inoculation completely prevented establishment of systemic infection by the hop downy mildew fungus. It is important to distinguish here between infection and systemic infection. Infection occurred even in treated plots as was clearly shown by sporulation of the fungus on leaves. The leaf infection and sporulation was markedly reduced by streptomycin, but it was clear that infection occurred, at least on the leaves.

Discussion and Conclusions:

A single application of 1000 ppm streptomycin spray 4 days after inoculation markedly reduced or completely prevented the establishment of systemic infection by downy mildew in young hop shoots. Leaf infection occurred but was reduced in incidence and severity. These results have practical application in the control of hop downy mildew. Frequently weather or other factors make it impossible to dust or spray hops during or immediately after conditions favorable for downy mildew spread and infection. Streptomycin could be used at least up to 4 days after such conditions. Streptomycin would be superior to zineb or other protectant type fungicides in that streptomycin would eliminate downy mildew infection after it had taken place, whereas protectant fungicides would not.

### Resistance to Systemic Infection

Hop downy mildew has two distinct infection phases -- localized and systemic. Localized infection occurs on leaves, stipules, petioles and sometimes on stems. Infection is limited to a small area around the site of invasion. Systemic infection occurs in young hop buds, young shoots, young or mature rhizomes, roots, and crowns. Systemic crown infection (Figure 1) is extremely important in the disease cycle of hop downy mildew on the West Coast. So far as is known systemic crown infection is entirely responsible for overwintering of the fungus in Washington and Idaho and largely responsible for overwintering in Oregon and California. In addition the principal losses from downy mildew in Washington and Idaho result from root and crown rot following infection.

Fuggle hops are considered to be resistant to downy mildew. The nature of this resistance has never received critical study. It has been commonly observed in England that the leaves and cones of Fuggle are subject to infection. Is it resistant because the perennial crown does not become infected and thus does not serve as an inoculum source? Field observations point to this as a strong probability. Systemically infected "spikes" arising directly from an infected crown of Fuggle hops have never been observed by the writer.

There are two important reasons why a redirection of the program of screening seedlings and breeding material for downy mildew resistance is justified. (1) Resistance to systemic crown infection is a prerequisite of new varieties for general production in any of the West Coast areas. (2) The techniques to accomplish screening for resistance to systemic crown infection have been developed.

Objectives:

To develop effective techniques for determining resistance of hop seedling and mature plants to systemic crown infection by downy mildew. To test seedlings, breeding parents and promising selected lines for resistance to systemic crown infection.

Procedure:

Specific procedures varied with the individual experiments and are described with them.

Experiments and results:

A number of methods were tested for effectiveness in establishing systemic infection. Rhizome cuttings were dipped and soaked in spore suspension at normal pressures and under vacuum. Also, application of spores to artificial wounds and to buds was tested. Injection of active zoospores by hypodermic needle was tested extensively, and proved to be the most rapid and effective method. The influence of temperature on infection and systemic disease development was tested. The best temperature was found to be 65° F. After these preliminary tests were completed the following technique was used to conduct several experiments.

Freshly produced sporangia were washed from leaves with distilled water, filtered in a Buchner funnel, then resuspended in soil extract water obtained by washing 100 g. of soil with 200 ml. distilled water. After 1-2 hours at 20°C. sporangia were germinating to release 2-16 motile zoospore per sporangium. At this time inoculation was accomplished by injecting a standard dosage into the phloem area of crowns or roots with a calibrated microsyringe supplied with a micrometer screw and scale.

Experiment # 1

About 60 seedlings of cross # 59 (see section on Reaction of 1958

Crosses to Downy Mildew under (Re5-1) were trimmed of excess foliage and soil pushed away from the top of the crown area. Seedlings were about 4 months old and had formed crowns averaging  $1/4$  inch in diameter. Each crown was injected with 0.05 ml. of a suspension containing 50,000 active zoospores per ml. Soil was replaced over the injection area and the plants held on the greenhouse bench with temperature controls set at 65°F.

One month after inoculation seedlings were dug, washed out and sliced vertically, then examined for the presence of downy mildew symptoms. Of the 52 seedlings available for analysis, 50 were systemically infected. Symptoms were typical of systemic crown infection as observed many times in the field. Figure 2 shows typical symptoms on seedlings.

Seedlings from cross 59 were used for this test because they were supposedly a result of a selfed Late Cluster and could be expected to be susceptible. The high percentage infection obtained indicated that this method of inoculation would be useful for screening purposes provided it would not be so severe as to mask or overcome resistance of a type which would be practical under field conditions.

The experiment was repeated using seedlings of open pollinated Late Clusters. The source of pollen was not known, but most of the pollen available would be from "resistant" males since they predominated. Seventy seedlings were inoculated and after six weeks were dug and 64 examined. Twenty nine were systemically infected in the crown, while 35 showed no evidence of systemic infection. These results suggested that either resistance was carried by about half the seedlings or that the method was not consistent enough to be reproduceable.





Figure 1. Systemic crown infection by hop downy mildew. Infected crown on left shows diffuse brown necrosis of phloem. Contrast with white phloem in healthy crown on right.

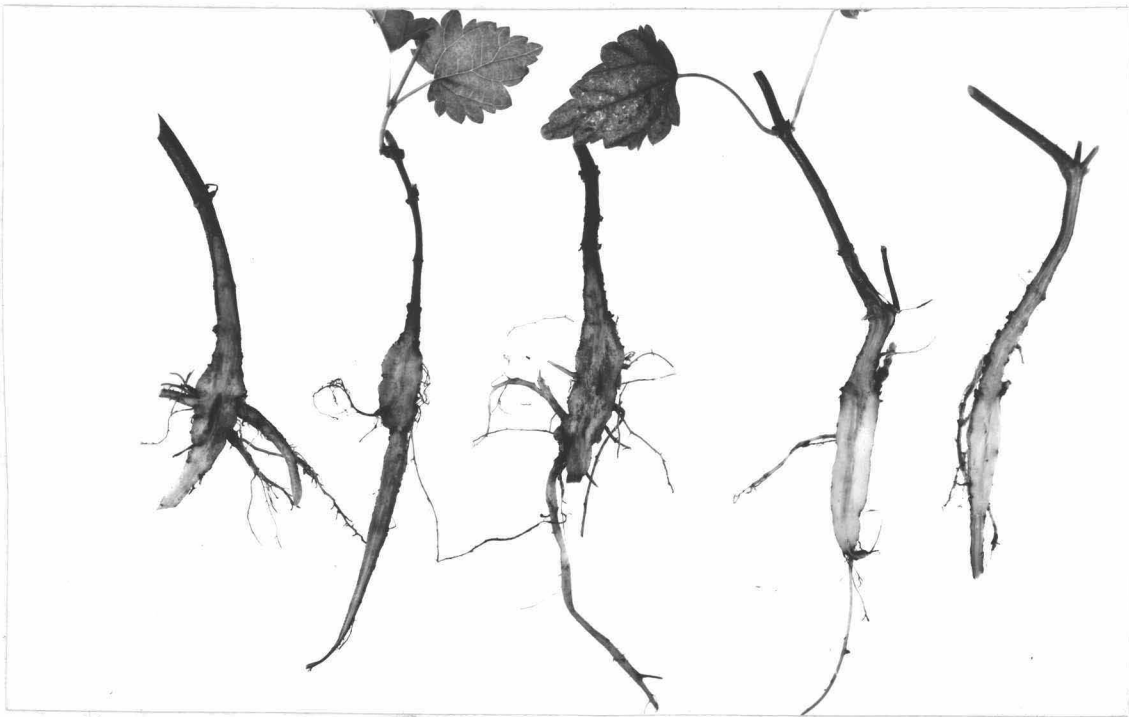
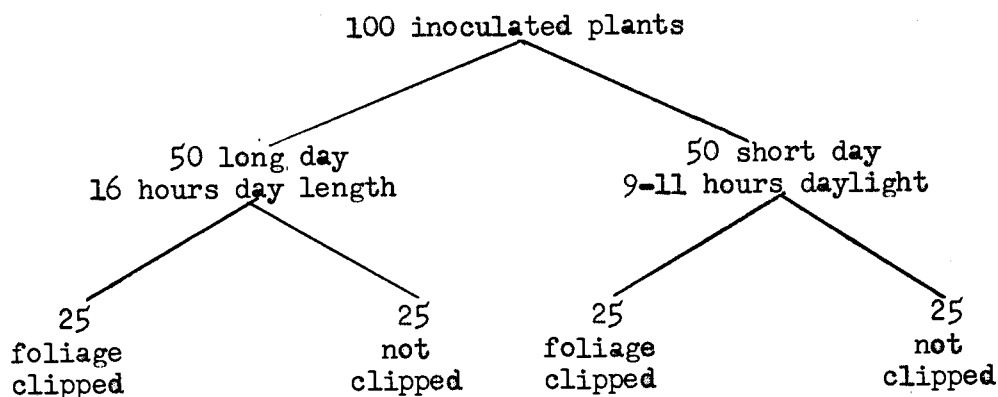


Figure 2. Systemic crown infection in artificially inoculated hop seedlings about 4 months of age. Three seedlings on left show complete invasion of crown and root tissue one month after inoculation. Two seedlings on right are healthy.

## Experiment # 2

An experiment was conducted to determine if Fuggle hops, which possess good "field resistance", are resistant to root and crown infection; and to determine if day length, by influencing plant growth, would effect development of systemic infection.

Basal shoot cuttings were obtained from Fuggle hops growing in our plots. No attempt was made to identify cuttings by clone. Cuttings were rooted in sand, then potted and allowed to grow for about 3 months. Crowns then averaged about one half inch in diameter. One hundred crowns were inoculated with zoospores of downy mildew by injection. The following treatment scheme was then set up.



A group of 40 plants were injected with sterile water then arranged similarly to serve as controls. Plants on long day remained vegetative and those under the "clipped" category had their foliage clipped off weekly. Plants under short day went into dormancy after about 1 month.

Two months after inoculation all plants were dug, washed and the crowns sliced longitudinally for examination. Data were recorded on the number of plants with visible symptoms of downy mildew infection as shown in the following table.

Treatment	Number infected *					Totals by treatments	Totals by day length
	R1	R2	R3	R4	R5		
Long day, clipped	0	0	2	2	0	4	5/50
" " not clipped	1	0	0	0	0	1	
Short day, clipped	3	0	2	2	1	8	14/50
" " not clipped	1	0	1	3	1	6	
Controls	0	0	-	-	-	0	0

\* Each value is the number infected of 5 plants examined.

Of 100 inoculated Fuggle crowns, 19 became systemically infected with downy mildew. This was unexpected, since systemic crown infection of Fuggle had never been observed in the field. Nearly 3 times as many plants became infected under short day as compared to long day (14 and 4 of 50 respectively). Of particular interest are the results obtained from the long day -- not clipped treatment where only 1 of 25 plants became infected. Clipping appeared to have less effect than day length. It should be emphasized that this experiment was of an exploratory nature and, unfortunately, not designed to separate all the treatment effects by analysis. The results, however, do suggest that resistance to systemic infection in Fuggle has a physiological basis conditioned by factors associated with growth and dormancy. There could, of course, be purely mechanical factors responsible for some of the observed resistance of Fuggle in the field. The method of inoculation used here would prevent such factors from expression.

### Experiment # 3.

The technique of crown inoculation described above was used to assay 23 varieties and lines for resistance to systemic downy mildew infection. The amount of plant material available from some lines was limited, consequently the number of plants available for inoculation was frequently less than desired.

Shoot tip cuttings were rooted in sand, then transferred to pots and allowed to grow for about 3 months through late summer and early fall on a 16 hour day length. Crowns were then inoculated and supplementary light

discontinued. Six weeks after inoculation plants were dug and examined for systemic mildew infection. In general 15 plants of each genotype were available for analysis, however, fewer (9-14) were available from some. The following table shows number and percentage infection among 23 varieties and lines.

Reaction of 23 varieties and lines of hops to crown inoculation with downy mildew.

<u>Variety or number</u>	<u>Ratio of infected to inoculated plants</u>	<u>Percent infection</u>
107-I	0/15	0.0
103-I	0/15	0.0
112-I	1/15	6.7
135-I	1/15	6.7
106	1/11	9/1
221-2	2/14	13.3
317	3/15	20.0
Br. Gold	3/15	20.0
139-I	3/15	20.0
Backa	3/14	21.4
Bullion	4/15	26.7
317-2	4/15	26.7
144-I	5/15	33.3
212	5/15	33.3
108-I	6/15	40.0
Fuggle	7/15	46.7
505	7/15	46.7
409	8/15	53.3
Hallertau	5/9	55.5
314	11/15	73.3
422	8/10	80.0
Late Cluster	14/15	93.3
128-I	15/15	100.0

Infection ranged from 0 to 100 per cent among the 23 varieties tested. Two varieties known to be highly susceptible (Late Cluster and 128-I) showed 93 and 100 per cent infection respectively. Field observations for several years on the performance of 107-I, 103-I, 112-I and 135-I show them to be highly resistant, which is in agreement with the results of this test. On the other hand, Fuggle, which possesses good field resistance, showed 47 per cent crown infection in this test.

Broad conclusions can not be made from the limited data available, but the results strongly suggest that at least 4 of the lines of hops now in advanced agronomic tests (107, 103, 112 and 135) have a very high degree of resistance to systemic crown infection by downy mildew.

Because of the wide range of resistance shown among the varieties and lines, one could speculate that resistance is due to multiple factors. The breeding method used (modified recurrent selection) should, then, result in the accumulation of "minor genes" for resistance. The data available support this hypothesis. If this be true, it is indeed fortunate because much evidence exists that varieties with "multiple factor" resistance to plant pathogens remain resistant for a much longer period in the presence of a potentially variable pathogen than do varieties with single factor resistance.

CR e5-4 (OAES 36:FC) IMPROVING YIELD AND QUALITY  
OF HOPS BY PRODUCTION AND MANAGEMENT PRACTICES

S. N. Brooks and C. E. Zimmermann

Numerous agronomic trials have been conducted on hops during the past 50 years. They have involved studies on irrigation, cultivation, plant spacing, fertilizers, time of pruning, the use of cover crops and many other factors. Cropping practices, varieties, equipment and management practices are seldom static in any agricultural industry, and there is a continued need for modification in cultural operations or practices. The purpose of this line project is to provide for improvement in these respects.

In 1959 agronomic or cultural work was confined to 4 lines of work (1) irrigation-fertility effects, (2) date of pruning and training, (3) a study with gibberellic acid, and (4) a test of a chemical gametocide. A 5th line of work was conducted on comparing different heights of trellis with 6 varieties of hops, but it was not harvested due to uneven growth. Most agronomic work is confined to the Fuggle variety since it makes up about 60% of Oregon's hop acreage.

Fuggles Irrigation-Fertility Experiment

Objectives:

See 1957 Annual Report, p. 67.

Reasons for undertaking the work:

See 1957 Annual Report, p. 67.

Nature and extent of previous work:

See 1957 Annual Report, p. 67.

Location and duration of experiment:

See 1957 Annual Report, p. 67.

Procedure:

A detailed description of plot lay-out and general procedures is given in the 1957 Annual Report. Only modifications or additions to the general procedures will be listed here.

Fertilizers were applied on April 15. First training of the vines was begun on May 18 and completed by May 20. Irrigation application was modified in 1959 due to uneven application the previous 2 years using perforated pipe. A "rain-bird" type of irrigator was made which delivers water more uniformly over the plots and has the added advantage of operating satisfactorily even with moderate wind conditions. However, the new irrigator applies water too rapidly (1.6 in. per hr.) and cannot be run for very long at a time due to ponding. Irrigation scheduling in 1959 was as follows:

Inches irrigation water applied during season:

Treat- ment	Date	Replication				Treat- ment	Date	Replication			
		I	II	III	IV			I	II	III	IV
B	7/7	5.00	5.00	5.00	5.00	C	7/27	5.00	5.00	5.00	5.00
D	6/23	1.98	2.25			E	7/3	3.73			
	6/27			1.84	3.03		7/8		2.77		
	7/9	2.64	2.78	1.86			7/11			3.11	3.11
	7/14		2.17	1.99	3.33		7/15	3.52			
	7/17	1.88					7/22		3.04		
	7/21	2.74	3.51	2.64			7/24	4.03		3.28	3.45
	7/24				4.10		7/30		3.09		
	7/28	2.91	4.09	2.76	3.40		8/5	4.29	2.74	3.06	3.35
	8/4	3.35	4.23	2.91	3.59		8/12	3.63	3.29		
	8/11	4.01	4.67				Total	19.20	14.93	9.45	9.91
	8/13			3.13	3.29		No.	5	5	3	3
Total		19.51	23.70	17.13	20.74		Ave.	3.84	2.99	3.15	3.30
No.		7	7	7	6						
Ave.		2.79	3.39	2.45	3.46						

Experimental results:

Data obtained in 1959 are included in the following tables.

Yields produced in the Fuggles Irrigation-Fertility Experiment in 1959.

	Yield (lbs./acre)							Ave.
	(1) N <sub>0</sub> P <sub>1</sub> K <sub>1</sub>	(2) N <sub>1</sub> P <sub>1</sub> K <sub>1</sub>	(3) N <sub>2</sub> P <sub>1</sub> K <sub>1</sub>	(4) N <sub>3</sub> P <sub>1</sub> K <sub>1</sub>	(5) N <sub>1</sub> P <sub>0</sub> K <sub>1</sub>	(6) N <sub>2</sub> P <sub>0</sub> K <sub>1</sub>	(7) N <sub>2</sub> P <sub>1</sub> K <sub>0</sub>	
A - no irrig.	910	1500	1650	1470	1260	1420	1360	1370
B - irrig.burring	1330	1790	2200	2050	1950	2010	1590	1840
C - irrig.coning	1440	1970	2170	2140	1810	2200	1980	1960
D - high moisture	1900	2480	2500	2260	2230	2330	2120	2260
E - med.moisture	1570	2310	1990	2090	2210	2270	1580	2000
Ave.	1430	2010	2100	2000	1890	2040	1730	1890

LSD (5%) = 360 for irrigation; 190 for fertilizers.  
CV (%) = 6.1 for irrigation; 3.6 for fertilizers.

Alpha-acid and oil contents in the hops  
in 1959 (data supplied by S. T. Likens).

Treatment	% alpha-acid				Ave.	Oil in mls./100 grams				Ave.
	(1) N <sub>0</sub> P <sub>1</sub> K <sub>1</sub>	(3) N <sub>2</sub> P <sub>1</sub> K <sub>1</sub>	(6) N <sub>2</sub> P <sub>0</sub> K <sub>1</sub>	(7) N <sub>2</sub> P <sub>1</sub> K <sub>0</sub>		(1) N <sub>0</sub> P <sub>1</sub> K <sub>1</sub>	(3) N <sub>2</sub> P <sub>1</sub> K <sub>1</sub>	(6) N <sub>2</sub> P <sub>0</sub> K <sub>1</sub>	(7) N <sub>2</sub> P <sub>1</sub> K <sub>0</sub>	
A (none)	4.68	5.14	4.71	5.24	4.94	.68	.77	.68	.78	.73
D (high)	4.36	4.42	4.54	4.51	4.45	.69	.67	.59	.72	.67
E (med.)	4.33	4.43	4.45	4.54	4.44	.66	.65	.69	.74	.68
Ave.	4.45	4.66	4.57	4.76	4.61	.68	.70	.65	.75	.69

Discussion and conclusions:

Yields of Fuggle hops were exceptionally good in 1959. The average yields in the Willamette Valley were the highest on record. The average yield in this experiment was 1890 lbs. per acre with the highest treatment average being 2500 lbs. per acre. Average yields for many other treatments ran well over 2000 lbs. per acre. This is in strong contrast with last year's average yield of 750 lbs. per acre and a top treatment average of 1140 lbs. per acre.

Significant yield differences were indicated in 1959, both for irrigation treatments and fertilizer treatments. No irrigation x fertilizer



interaction was demonstrated. With respect to irrigation, the highest yield was obtained from the high moisture treatment. All irrigation treatments out-yielded the non irrigated treatment. There was no yield response to nitrogen fertilizers beyond that caused by an application of 67 lbs. of N per acre. There was no yield response to 75 lbs. of  $P_2O_5$  per acre, but 75 lbs. of  $K_2O$  per acre accounted for an increase in yield of 300 lbs. per acre on the average.

Similar to the findings of last year, irrigation caused a reduction in alpha-acid content of approximately 10% (from 4.94% to 4.12% in 1959) on the average. However, no significant differences due to fertilizers were apparent in 1959. Neither irrigation nor fertilizer treatment affected oil content to any demonstrable extent.

The problem of variable water application was solved in 1959 by the prefabrication of an experimental plot irrigator, but a new problem reared its head. This was the one of non-uniform water penetration into the soil. The hop hills normally are mounded up during the course of the season. This causes the water to run away from the hills toward the centers. Since the moisture measuring devices were close to the hills, often in the edges of a dry cone of soil at each hill, false indications of the soil moisture status of the soil were given in many cases. As a result, more water was often added to treatments D and E than was actually required to keep the soil within the desired moisture content limits. This will be solved next year by burying the gypsum stakes further away from the hills or by periodic soil sampling across the centers between the rows.

Date of Pruning and Training.Objectives:

See 1956 Annual Report, p. 104.

Reasons for undertaking the work:

See 1956 Annual Report, p. 104.

Procedure:

The procedures were modified to a large extent in 1959. Objectives were modified also. Instead of varying severity of pruning on Fuggle hops, all plots were given a moderate pruning. Pruning was accomplished at different dates, following which the vines were trained up at two different dates for each date of pruning. One pair of treatments included training of first and later growth vines from plots which were not pruned. Next year these plots will be pruned in the fall. Another pair of treatments had first and later growth vines trained up on plots which were pruned in early April where the earliest vines were pruned off. A third pair of treatments included a late pruning which was delayed until considerable vegetation had grown out. Following pruning, either early or later growth vines were trained up. Following is a description of the treatments for 1959:

<u>Entry</u>	<u>Pruning Treatment</u>	<u>Training Treatment</u>	<u>Remarks</u>
1	Fall (not pruned, 1959)	Early vines (5/7)	Vines over 2 ft. long trained
2	" " "	Late vines (5/15)	Vines under 2 ft long trained
3	1/ Early growth (4/3)	Early vines (5/15)	Vines over 1½ ft. long trained
4	" " "	Late vines (5/21)	Vines under 1½ ft. long trained
5	Late growth (4/23)	Early vines (5/27)	Vines over 2 ft. long trained
6	" " "	Late vines (6/1)	Vines under 1½ ft. long trained
1/	Standard treatment (check).		

Experimental results:

Data were obtained on yield sidearm length, cone weight and length and date of burring. Alpha-acid data were furnished also by S. T. Likens.

These are given below:

Data obtained in the Date of Pruning and Training trial on Fuggle, 1959.

Entry-Prune-	Train	Pounds	Percent	Grams	Cone length (mm.)	Sidearm length (inches)	Date initial burring	
		hops per acre	alpha acid	per 100 cones				
1	Fall <u>1/</u>	Early vines	1420a	4.32 b	12.0 b	26	35a	7/3
2	Fall <u>1/</u>	Late vines	1440a	4.68ab	12.0 b	26	20 b	7/7
3	<u>2/</u>	Early gr. Early vines	1360a	4.15 b	11.3 b	25	23ab	7/5
4	Early gr.	Late vines	1350a	4.44 b	11.9 b	25	23ab	7/10
5	Late gr.	Early vines	1320a	5.09a	12.0 b	26	20 b	7/14
6	Late gr.	Late vines	780 b	5.06a	13.6a	27	15 b	7/15
Mean			1280	4.62	12.1	26	23	
LSD (5 %)			380	0.59	1.3	N.S.	11	
CV (%)			25	8.5	7.2	4.9	31	

1/ Not pruned, 1959; treatment simulated.

2/ Standard treatment (check).

#### Discussion and conclusions:

Significant differences in yield, sidearm length, and cone size among treatments were indicated in 1959. Yield was reduced by very delayed training (training on 6/1), but there was no apparent difference among the other treatment means. Delayed training accounted also for a downward trend in sidearm length: plants trained on 6/1 had very short laterals. There were no differences in cone length, but the weights of the cones for the 6/1 training were significantly greater than those for all of the other treatments.

Yield data for 1959, although somewhat variable, were in line with yield data from a previous Date of Pruning experiment. It was demonstrated previously that no yield reduction was experienced by pruning as late as 4/25

and training up the earliest vines. It was not until training was delayed following late pruning that yields were reduced, according to the 1959 data. The previous experiment did not include delayed training as a treatment.

Alpha-acid percentages in 1959 were increased by delayed pruning and training. The late training of the non-prune treatment as well as both trainings of the late prune treatment were high in alpha-acid percentage. These results do not coincide with previous trials which did not show any difference in alpha-acid percentage due to delayed pruning.

As was expected the later trained plants were slower coming into the burr stage. However, no differences in stage of maturity were detected at harvest time.

Effects of Gibberellic Acid on Fuggle HopsObjectives:

See 1958 Annual Report, p. 60.

Reasons for undertaking the work:

See 1958 Annual Report, p. 60.

Nature and extent of previous work:

Until this year there was little or no information regarding the use of gibberellic acid on hops. A recent note in Nature, Jan. 2, 1960, by A. S. Nash and P. D. Mullaney described some preliminary work with this material on hops grown in Australia. When applied at full-bloom at a concentration of 12.5 ppm, gibberellic acid caused a 40% increase in yield but an 80% reduction in alpha-acid. Concentrations up to 50 ppm caused a decrease in the "time in burr stage" of triploid hops, hastening maturity by as much as 10 days. C. B. Skotland at Prosser, Washington is conducting experiments along this line using the "Clusters" types. Details of his work are not known.

Procedure:

Procedures were modified to some extent in 1959. The highest concentration used was 100 ppm as compared to 500 ppm in 1958. The period of time over which applications were made was lengthened to include pruning and post-harvest. The post-harvest treatment will furnish no data until 1960 since it was first made on 10/6/59. Late applications in 1959 were made by sprinkling the solutions over the plants rather than by spraying them on. This was done to avoid wind-drift. Less thorough coverage was made in this manner, but due to the high degree of absorption and translocation of gibberellic acid by the plant, it was considered to be satisfactory for the purpose intended in this trial.

A soluble powder of pure gibberellic acid was used in the trial.

Following are detailed notes pertaining to the treatments:

<u>Entry</u>	<u>Treatment</u>	<u>Date of treatment and remarks</u>
1	Check	
2	100 ppm at pruning	- pruned 4/6 and 4/7, treated 4/10
3	5 ppm at 5' ht.	- treated 5/27, symptoms noticeable by 6/1
4	50 ppm at 5' ht.	- treated 5/27, symptoms noticeable by 6/1
5	100 ppm at 5' ht.	- treated 5/27, symptoms noticeable by 6/1
6	100 ppm at 10' ht.	- treated 6/10, symptoms pronounced by 6/15
7	100 ppm at burring	- treated 7/7
8	100 ppm at coning	- treated 7/28
9	100 ppm post harvest	- not done until 10/6/59

Notes:

Treat. 2 slightly more advanced by 4/21, chlorotic, 35 gal. solution per acre applied.

$1\frac{1}{2}$  gal. per 30 hills sprinkled on treatments 3, 4, and 5.

Last hill of second rep. of treatment 6 was only lightly covered - ran out of solution, later had same appearance as rest of treatment.

$3\frac{1}{2}$  - 5 gal. per 30 hills used for treatments 7 and 8

Experimental results:

Data were obtained on yield, sidearm length, alpha-acid and oil contents, cone size and flowering time. These data were analyzed as two experiments since a comparison of rates and a comparison of stages of growth existed in the series of treatments.

Discussion and conclusions:

Significant differences were indicated for yield in 1959, both for application at different stages of growth and for different concentrations at the 5ft. stage. The highest yield was obtained from a treatment having 5 ppm applied early in the season (5 ft.). This particular treatment looked outstanding all season. The symptoms of chlorosis and spindly growth were not nearly

Data obtained in the Gibberellic Acid trial on Fuggle in 1959.

Stages of growth:

<u>100 ppm at:</u>	<u>Yield (lbs.per acre)</u>	<u>Sidearm length (inches)</u>	<u>Grams per 100 cones</u>	<u>Cone length (mm)</u>	<u>Mls. oil per 100 grams</u>	<u>% Alpha- acid</u>	<u>Date initial burr</u>
2 prune	1440 b	32 b	14.6ab	29a	0.70a	4.28 bc	7/3
5 5 ft.	1400 b	27 b	12.0 d	25 c	0.70a	5.17a	7/13
6 10 ft.	1510 b	29 b	9.1 e	22 d	0.58ab	4.14 bc	7/15
7 burr	1030 c	37ab	13.4 bc	26 c	0.46 b	3.81 c	7/7
8 cone	1860a	34 b	14.9a	28 b	0.60ab	4.58 b	7/8
Mean	1450	32	12.8	26	0.61	4.40	--
Stat.sign.**		**	**	**	**	**	--

Concentration:

at 5 ft.ht.

1 0 (check)	1550 b	32 b	12.7 cd	27a	0.68	4.37	7/7
3 5 ppm	1990a	46a	12.4 cd	27a	0.73	4.87	7/6
4 50 ppm	1580 b	34 b	11.9 d	24 b	0.68	4.52	7/10
5 100 ppm	1400 b	27 b	12.0 d	25 b	0.70	5.17	7/13
Mean	1630	35	12.3	26	0.70	4.73	--
Stat.sign.**		**	**	**	NS	NS	--

Note: Data for Sidearm length and wt. of 100 cones analyzed as one experiment; multiple ranges and statistical significance based on all means in both groups.

so marked as with 50 ppm and higher concentrations. The main symptom appeared to be a real boost in growth with no adverse effect becoming apparent. Although the sidearms were caused to elongate by this treatment, they appeared to be normal in other respects. It should be mentioned also that treatment with 5 ppm at the 5 ft. high stage of growth did not produce significant differences in cone weight or length, oil content, alpha-acid content, nor in date of burring. On the other hand, concentrations of 50 or 100 ppm at this stage of growth produced no increase in yield, no change in sidearm length, somewhat shorter cones and delayed flowering.

With regard to applications of 100 ppm made at different stages of growth, a yield reduction was caused by treatment at burring time, and a yield increase was caused by treatment at coning. Treatment at pruning, 5 ft., or 10 ft. produced no differences in yield. Sidearm length was increased by treatment at burring time only: sidearms for this treatment were thin and weak. Treatment at burring time also reduced oil and alpha-acid contents. Treatment at the 5 and 10 ft. stages produced lighter, shorter cones and delayed flowering. Alpha-acid content appeared to be greater for the treatment of 100 ppm at the 5 ft. stage when compared with treatment at other stages.

All gibberellic acid treated plants came into cone prior to 7/27, whereas the non-treated plants came into cone on 7/28. The treatment which came into cone the earliest (7/11) had 100 ppm at burring. Treatment 2 (100 ppm at pruning) began to cone out on 7/23. The rest of the treated plants were coning on 7/25 or 7/26, those treated at the 10 ft. stage being the latest.

The results obtained in 1959 do not coincide too well with results obtained from similar treatments in 1958, but some generalizations can be made based on two year's results. Treatment with 100 ppm concentration at



the 5 ft. stage tended to delay flowering by 2 to 6 days. Treatment at the 10 ft. stage also delayed flowering from 0 to 8 days. Treatment at the burring stage reduced yields and alpha-acid contents.

### Test with a Chemical Gametocide.

#### Objectives:

To test the effectiveness of Sodium  $\alpha, \beta$  dichloroisobutyrate, a chemical gametocide, in limiting seed set in female hop plants grown in the presence of males.

#### Nature and extent of previous work:

A detailed account of the gametocidal effectiveness of this material on several kinds of crop plants, its biological and chemical properties are given in "Progress Report on FW-450 Chemical Gametocide", a Rohm and Haas publication dated June, 1959. In general, FW-450 has been used to induce male sterility in some species of dicotyledonous plants without adversely affecting female fertility. In other dicots and in most monocots, the material has induced similar levels of male and female sterility. In some cases high rates of ovule sterility have occurred.

#### Procedure:

An 8 oz. bottle of FW-450 (a technical grade water-soluble salt of the material, 100% active ingredient) was obtained on request from Rohm and Haas Company. Two concentrations of a water solution, 0.5% and 1.0% were applied as a spray to plants at weekly intervals during the flowering season. Two plants were used for each treatment and a check. Treatments consisted of 2 plants which were sprayed once on 7/7; 2 plants which were sprayed twice, on 7/7 and 7/14; and 2 plants which were sprayed thrice, on 7/7, 7/14 and 7/21.

The plants were in the initial burr (flower) stage on 7/7 and remained in burr for over 2 weeks. Thorough coverage of the plants was made at each spraying. Many male plants were present in close proximity to the test plants. Cone samples were picked and threshed by hand.

Experimental results:

Data were obtained on phytotoxicity, seed-set and seed wt. The seeds were treated for germination counts, but these data are not available at this writing.

<u>Treatment</u>	<u>Plant</u>	<u>Ave.wt. of 100 cones (grams)</u>	<u>Wt. of harvested sample (grams)</u>	<u>Wt. of threshed seed (grams)</u>	<u>% seed by wt.</u>	<u>Ave. wt.of seed (mg.)</u>	<u>% germination</u>
Check	1	9.8	40.0	4.71	11.8	1.9	
	2	14.2	50.4	6.56	13.0	2.1	
0.5% once	1	10.0	43.0	2.55	5.9	2.6	
	2	10.5	51.0	1.58	3.1	2.6	
1.0% once	1	9.5	42.0	0.16	0.4	2.4	
	2	8.2	45.0	0.07	0.2	1.4	
0.5% twice	1	7.4	47.5	0.91	1.9	2.5	
	2	12.0	40.0	2.68	6.7	2.6	
1.0% twice	1	8.7	42.0	0.01	tr	0.5	
	2	8.2	48.5	0	0	--	
0.5% thrice	1	8.8	30.6	1.46	4.8	2.1	
	2	13.0	39.0	3.46	8.9	2.6	
1.0% thrice	1	6.5	44.0	0	0	--	
	2	5.5	43.0	0.02	tr	1.8	

Observations following treatment:

<u>Date</u>	<u>Treatment</u>	<u>Foliage burn</u>	<u>Cone damage</u>
7/17	1.0% once	Slight to mod.	
	1.0% twice	Moderate	
	0.5% once	Slight	
	0.5% twice	Slight	
Harvest	1.0% all		Mostly severe
	0.5% once		None to slight
	0.5% twice		Mostly moderate
	0.5% thrice		Slight to severe

Discussion and conclusions:

It was apparent from the rather sketchy data and observations obtained in 1959 that 1.0% solutions are much too strong for the hop plant, causing severe phytotoxicity even when they were applied only once. On the other hand, 0.5% solutions did not cause very severe phytotoxicity when they were applied only once or twice. Cone damage was apparent, to one degree or another, wherever the material was applied. Yield data were not obtained, but it looked as if yields were reduced by all treatments. Not only was there some cone damage or cone loss encountered with each treatment but cone weights were reduced.

Hops from the check plants contained approximately 12% seeds by weight. Spraying thrice with 0.5% solution was no better (if as good) as spraying once with 0.5% solution: some reduction in seed content was obtained with all schedules of 0.5% solutions. Spraying once with 1.0% solution reduced seed content to near zero.

Up to a point, larger seeds were obtained from the sprayed plants. Perhaps this was due to better nutrition for each seed since there were fewer in number per inflorescence. An exception to the rule occurred where severe cone damage was accompanied by apparent seed damage, and smaller seeds were obtained from plants sprayed twice or thrice with 1.0% solutions.

CR e5-5 (OAES 36:AC) CHEMICAL INVESTIGATIONS  
RELATIVE TO THE EVALUATION OF HOPS.

S. T. Likens

The main purpose of this line project is to support the breeding program by chemical evaluation (as related to brewing value) of promising experimental lines. To accomplish this the chemical evaluation program embraces nearly all phases of the entire program by (1) quality characterization of new material, (2) maintaining a record of the effect of the influences of various testing programs on the brewing value of promising lines and (3) establishing the quality potential of both male and female parent stock.

Coincident with this, chemical evaluation of brewing quality is required for all agronomic trials to detect any favorable change and to insure against recommendation of treatments which would have an adverse effect.

Since the project is of regional nature, it is felt that the laboratory facilities should be available to research personnel from other areas for chemical evaluation of material from experiments which are of other than local nature. This will be done to the extent that time, personnel and funds permit.

Besides efforts in support of other line projects, it is the responsibility of CR e5-5 to (1) carry out experiments to elucidate the nature of hop quality, (2) to determine the effects of various processing techniques on quality, (3) investigate the relationship between plant fertility and mineral composition (leaf analysis) and (4) make investigations into chemical methods as necessary to accomplish the general objectives.

## EVALUATION OF STROBILES

Experimental LinesObjectives:

The chemical evaluation of quality factors in experimental lines constitutes the only objective assessment of their brewing value. It is the purpose of this phase of CR e5-5 to rank experimental varieties as to their  $\alpha$ -acid and oil content and to expose any changes brought about by various conditions under which they are grown and processed.

Nature and extent of previous work:

This phase of CR e5-5 is in support of the regional breeding program (CR e5-1) and has consequently been of a continuous nature.

Procedure:

Hop samples are taken from trials involved in testing experimental varieties and analyzed for  $\alpha$ -acid and oil content. These results are presented in summary form in connection with other varietal characteristics as well as in detail in this section.

Experimental results and discussion:

Off-station trials: Several experimental varieties are in advanced testing in commercial yards (see CR e5-1) in Oregon and Washington. In order to determine (a) the characteristic build-up of quality components of each variety, and (b) the practical usefulness of such information, samples were taken at intervals throughout the season and analyzed for  $\alpha$ -acid and oil content. The results of these efforts are recorded in table 1.

Table 1. The accumulation of quality components and related factors in four experimental varieties. (Ray Kerr ranch; Salem, Oregon; 1959) Results presented on a bone-dry basis.  $\alpha$ -acids by the spectrophotometric method.

Collection date	8/10	8/22	8/28	9/2	9/8	9/11	9/17	9/17*
<u>112-I</u>								
% D.M.		18.9	18.4	21.0	22.2	24.8	25.0	19.2
mls oil/100 g		0.69	1.58	1.28	1.25	1.29	1.28	1.69
% $\alpha$ -acid		5.58	5.98	5.30	3.97	5.34	5.18	4.66
% $\beta$ -acid		2.12	3.23	2.55	2.44	2.04	2.18	2.24
Cohumulone ratio								21.9
Cohumulone percent								1.02
<u>128-I</u>								
% D.M.	15.9		20.0	24.8	23.4	23.4	23.5	20.4
mls oil/100 g	0.21		1.99	2.07	2.99	lost	3.18	3.26
% $\alpha$ -acid	4.23		9.22	10.75	11.91	11.76	11.70	10.06
% $\beta$ -acid	2.32		3.89	4.33	4.33	4.39	4.09	4.28
Cohumulone ratio								32.3
Cohumulone percent								3.42
<u>135-I</u>								
% D.M.			21.8	21.6	22.2	23.6	23.2	20.0
mls oil/100 g			1.42	1.55	1.55	1.86	1.94	1.89
% $\alpha$ -acid			2.61	1.71	2.88	2.85	2.15	2.15
% $\beta$ -acid			2.77	3.39	2.62	2.87	2.79	2.97
Cohumulone ratio								41.8
Cohumulone percent								0.90
<u>144-I</u>								
% D.M.	13.6		19.6	20.7	19.2	**		
mls oil/100 g	lost		1.43	2.24	2.06			
% $\alpha$ -acid	2.31		3.18	3.49	3.73	4.16		
% $\beta$ -acid	3.31		4.30	4.53	3.80	4.27		
Cohumulone ratio						26.2		
Cohumulone percent						1.09		

\* Machine picked at harvest: Rainy day: Wet hops.

\*\* Harvest date was 9/9 - No green sample for D.M. or green oil.

On the basis of analysis, 112-I was picked approximately 2 weeks later than optimum, 128-I about 1 week late, 135-I about right, and 144-I about right.

Upon the basis of this single year's data it would surely be concluded that the accumulation of quality factors is different for the varieties, both in date and rate. For example, note that 112-I maintains a rather constant  $\alpha$ -acid and oil content through the season, while 128-I continually

Table 2. Summary of quality data on kiln dried off-station material, 1959.

Variety	Location	Condition	M.C. at analysis	Mls oil per 100 g	% $\alpha$ -acids		Cohumulon ratio
					Spectro	CCD	
Fuggles	Kerr	(O) loose	10.60		4.95	2.55	20.0
	Crosby	(O) loose	11.75	0.81	4.40		
	Schwabauer	(O) loose	8.80	0.73	4.49		
Hallertau	Kerr	(O) loose	9.80	0.53	5.49	4.01	18.7
103-I	Brulotte	(W) bale	9.10	0.28	1.66	1.39	39.8
	Strausz	(W) loose	7.10	0.19	0.74		
	Ayres	(W) loose	9.10	0.28	2.06		
107-I	Brulotte	(W) bale	8.95	0.39	5.64	2.90	23.8
	Strausz	(W) loose	7.40	0.24	1.54		
	Ayres	(W) loose	7.70	0.35	2.12		
108-I	Brulotte	(W) bale	8.30	0.34	(4.36, 4.92)	2.95	(28.6, 25.9)
	Strausz	(W) loose	7.75	0.12	1.61		
	Ayres	(W) loose	--	--	--		
112-I	Kerr	(O) loose	13.05	(0.41 (1.69))*	4.66	3.59	21.9
128-I	Kerr	(O) loose	12.90	(0.82 (3.26))*	10.06	7.45	32.3
135-I	Brulotte	(W) bale	11.60	0.43	3.22**	2.47	38.6
	Strausz	(W) loose	7.00	0.20	1.61		
	Ayres	(W) loose	5.30	0.23	1.93		
	Kerr	(O) loose	10.60	(0.62 (1.89))*	2.15	1.97	41.8
139-I	Brulotte	(W) bale	7.75	0.48	4.13	3.00	35.6
	Strausz	(W) loose	7.20	0.25	2.52		
	Ayres	(W) loose	6.85	0.23	1.45		
144-I	Kerr	(O) loose	12.55	(0.52 (2.06))*	4.16	2.68	26.2
	Crosby	(O) loose		0.65	2.62		
	Schwabauer	(O) loose	9.65	0.67	2.50		

\* Oil content of green hops (dry weight basis). Machine harvested.

\*\*  $\alpha$ -acid analysis was run again on Jan. 14, approximately 2 months later, and found to contain 3.28%  $\alpha$ -acid.

increases in both factors. If these differences are found to be real after sufficient years of testing, such information would undoubtedly influence USDA recommendations regarding uses of each variety and methods of deciding their harvest dates.

If possible this type of data should be collected from all varieties in off-station testing.

In addition to maturation data of material in off-station testing in Oregon, quality data have been collected on samples of all the commercially grown, harvested and processed varieties in both Oregon and Washington. This includes  $\alpha$ -acid, and oil for each grower who harvested, and cohumulon for each lot which was scheduled for brewing trials. The results are listed in table 2.

Table 3 shows a 4 year summary of cohumulon ratios for those varieties in advanced testing. Samples were taken from the quality trial (Corvallis, East Farm) in 1956, 57 and 58. 1959 samples were from off-station material as indicated. While the data leave something to be desired in annual precision, they nevertheless indicate the ranges characteristic of each variety.

It is interesting to note the constancy of cohumulon ratios for 135-I from the three locations (Quality Trial at Corvallis, Kerr at Salem and Brulotte in Yakima Valley, Wash.)

Prosser yield trial: Four replications of 15 varieties from the Prosser Station were analyzed for  $\alpha$ -acid and oil. As received, the samples were in excellent condition and showed every sign of careful picking, drying and storage for shipping. Emil Nelson, who collected the samples, verified this. However, examination of the cones showed a paucity of resin granules and analysis (see table 5) indicated low  $\alpha$ -acid and hop oil contents.



Table 3. Four year summary of cohumulon ratios in experimental varieties in advanced stages of testing.

	<u>1956</u>	<u>1957</u>	<u>1958</u>	<u>1959*</u>
Hallertau		21		19 (O)
Late Cluster	34	39	39	
Fuggles	23	30	26	20 (O)
103-I	40	30	23	30 (W)
107-I	21	34	24	24 (W)
108-I	30	40	40	29 (W)
112-I			18	22 (O)
128-I	32	33	31	32 (O)
135-I	41	50	49	39 (W) = 42 (O)
139-I	36	44	39	36 (W)
144-I	20	26	24	26 (O)

\* 1956, 1957 & 1958 samples from Quality Trial; Corvallis, Ore.  
 1959 sample from commercial fields. (O) = Oregon, Ray Kerr.  
 (W) = Washington, Brulotte.

Table 4. Summary of 1959  $\alpha$ -acid data on varieties in advanced testing.

	<u>Kerr,</u>	<u>Brulotte,</u>	<u>Strausz,</u>	<u>Ayres,</u>	<u>Crosby,</u>	<u>Schwabauer,</u>	<u>Prosser,</u>	<u>Lewis- Brown</u>
Fuggles	4.95				4.40	4.49		6.44
Hallertau	5.49							8.85
103-I		1.66	0.74	2.06			1.26	2.55
107-I		5.64	1.54	2.12			1.71	5.98
108-I		4.36	1.61				1.50	6.20
112-I	4.66						2.13	8.28
128-I	10.06							10.57
135-I	2.15	3.22	1.61	1.93			0.85	2.35
139-I		4.13	2.52	1.45			1.81	4.82
144-I	4.16				2.62	2.50	1.26	2.48
L.C.							2.69	9.46

Table 5. Summary: average values for 4 replications, Prosser, 1959.

<u>Entry</u>	<u>% Alpha *</u>	<u>mls oil/100 gm dwb</u>
103-I	1.26	0.18
104-I	0.09	0.16
107-I	1.71	0.25
108-I	1.50	0.18
109-I	1.47	0.46
112-I	2.13	0.55
123-I	3.15	0.26
124-I	1.63	0.21
127-I	2.40	0.31
132-I	1.04	0.14
135-I	0.85	0.31
138-I	1.52	0.29
139-I	1.81	0.31
144-I	1.26	0.19
Late Cluster	2.69	0.15

\* Determined Spectrophotometrically: This method was found to yield unreliable values, see text, this section and section on "Invest. into Anal. Methods."

Besides the scarcity of the resin granules, it was discovered that the spectrophotometric method failed to yield reliable results for  $\alpha$ -acid due to the presence of an interfering compound not previously encountered in hops. The conductometric method was found to give more reliable results but the lack of time prevented re-analysis of all samples by that method. This difficulty is discussed in more detail under "Cr e5-5, Investigations into Analytical Methods."

Lewis-Brown trial:

The varieties currently in advanced testing are also grown in seedless condition at the Lewis-Brown Farm at Corvallis. Growing conditions were apparently optimum during 1959 and  $\alpha$ -acid data indicates that the trial indicates the potential of the varieties. Table 6 lists the analytical results.

Table 6. Quality data from Lewis-Brown farm (seedless) 1959.

<u>Entry</u>	<u>% <math>\alpha</math>(dwb)</u>	<u>mls oil/100 gm.(dwb)</u>
Backa	5.75	0.78
Brewer's Gold	10.14	1.50
Hallertau	8.85	1.09
Late Cluster	9.46	0.49
Fuggles	6.44	0.64 *
103-I	2.55	0.69
107-I	5.98	0.72
108-I	6.20	1.02
112-I	8.28	0.93
128-I	10.57	1.89
135-I	2.35	0.72
139-I	4.82	0.24
144-I	2.48	0.67

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\* Some oil lost

Storage tests:

1959 completes the third year of storage tests for the experimental varieties. Table 7 lists the data for the 1958 crop.

Table 7. Summary of results of storage tests, O.S.C. hop yards, 1958 crop.

Variety		Storage time at 38°F.				% loss in 11 mo.	
		0	3 mo.	6 mo.	11 mo.		
Late Clusters	α-acid	5.89(3)	5.54(3)	5.26(3)	5.03(3)	15	33
	oil	0.49(3)	0.54(1)	0.40(1)	0.33(3)		
Fuggles	α-acid	3.80(4)	3.80(3)	3.98(3)	3.48(4)	8	34
	oil	0.59(4)	0.72(2)	0.26(3)	0.39(4)		
Brewer's Gold	α-acid	7.94(4)	4.75(3)	4.26(4)	2.44(4)	69	66
	oil	2.60(4)	1.55(1)	1.21(4)	0.88(4)		
Bullion	α-acid	7.73(2)	4.70(2)	4.18(2)	2.60(2)	66	68
	oil	(2.11)(2)	1.18(1)	1.01(2)	0.68(2)		
103-I	α-acid	3.08(3)	2.34(1)	1.93(3)	1.19(3)	61	54
	oil	0.78(3)	0.59(1)	0.49(3)	0.36(2)		
104-I	α-acid	3.08(4)	2.02(4)	2.04(4)	1.31(4)	57	61
	oil	1.17(4)	0.75(2)	0.65(4)	0.46(4)		
107-I	α-acid	4.16(4)	3.49(3)	3.17(4)	2.40(4)	42	41
	oil	0.83(4)	0.38(2)	0.59(3)	0.49(4)		
108-I	α-acid	4.48(4)	--	4.30(4)	3.52(4)	21	37
	oil	(0.49)(4)	--	0.47(4)	0.31(4)		
109-I	α-acid	5.02(4)	4.84(3)	4.37(4)	3.81(4)	24	53
	oil	1.30(3)	1.09(2)	1.14(3)	0.61(4)		
112-I	α-acid	4.94(4)	5.03(3)	5.20(4)	4.28(4)	13	42
	oil	0.80(4)	0.75(3)	--	0.46(4)		
123-I	α-acid	5.77(4)	5.40(3)	5.15(4)	4.14(4)	28	51
	oil	0.75(4)	0.75(1)	0.64(3)	0.37(3)		
124-I	α-acid	5.63(4)	5.57(2)	5.52(3)	3.45(3)	39	54
	oil	0.95(3)	0.79(2)	0.66(3)	0.44(3)		
127-I	α-acid	5.29(4)	5.19(3)	4.85(3)	3.54(3)	33	36
	oil	(0.94)(4)	0.88(3)	0.77(3)	0.60(4)		
128-I	α-acid	9.08(4)	8.05(4)	7.41(4)	5.21(3)	43	53
	oil	2.16(4)	1.69(3)	--	1.01(3)		
132-I	α-acid	3.43(4)	3.38(2)	2.81(4)	2.44(4)	29	0
	oil	0.21(4)	0.28(2)	0.19(2)	0.26(3)		
135-I	α-acid	1.54(4)	1.91(3)	1.61(4)	0.96(4)	38	31
	oil	0.85(4)	0.68(3)	0.75(3)	0.59(3)		
138-I	α-acid	4.49(4)	4.67(1)	4.44(3)	3.55(3)	21	39
	oil	0.64(4)	0.62(1)	0.45(3)	0.39(3)		

Table 7. Summary of results of storage tests, O.S.C. hop yards, 1958 crop.-cont.

Variety		Storage time at 38° F.				% loss in 11 mo.	
		0	3 mo.	6 mo.	11 mo.		
139-I	$\alpha$ -acid	3.27(4)	3.46(4)	3.48(4)	2.43(4)	26	43
	oil	0.47(4)	0.38(3)	0.29(3)	0.27(4)		
144-I	$\alpha$ -acid	3.42(3)	3.42(2)	3.53(3)	3.00(3)	12	25
	oil	0.61(3)	0.45(2)	0.40(3)	0.46(3)		
					$\bar{x}$	33.9	43.2
					S	18.6	16.0
					$\bar{x} \pm S$	15-52	27-59

The tests indicate, for the most part a general decline in both  $\alpha$ -acid and oil content from the beginning of the trial. Overall the losses in quality factors for this year's test were high, averaging 33% for  $\alpha$ -acid and 44% for oil.

A 3-year summary (Table 8) of quality losses in 38° storage shows that the initial values for both  $\alpha$ -acids and oils are in good agreement from year to year. The final values, however, are very different for the three years.

Of special interest are the two English varieties Bullion and Brewer's Gold which lost only 5 and 6% of their  $\alpha$ -acid the second year (1957) while the third year (1958) they lost 66 and 69%. The complete data, (Table 8), which gives the values for several replications sampled at 0, 3, 6 and 11 months, shows a remarkable uniformity of  $\alpha$ -acid values for the 1957 Bullion and Brewer's Gold. The complete data for 1958 shows, without doubt, that the major loss -- nearly half -- of the  $\alpha$ -acid was lost during the first 3 months.

The uniformity of data between replications obviates the possibility that the annual differences noted are a matter of sampling techniques. In attempting to assign responsibility for annual differences in storageability of these varieties, it seems necessary to believe that there was a difference in the condition of the samples at the time they were

Table 8. 3 year summary of storage data for experimental varieties in advanced stages of testing. ( $\alpha$ -acid; %, D.W.B.; Spectrophotometric. Oil content; mls/100 g D.M.; Hydrodistillation.)

Variety	Year	No. reps.	$\alpha$ -acid			oil content		
			0 mo.	11 mo.	% loss*	0 mo.	11 mo.	% loss*
L.C.	1956	(3)	5.9	5.4	7	.35	.29	17
	1957	(3)	5.4	5.4	1	.48	.39	19
	1958	(3)	5.9	5.0	15	.49	.33	33
Fugg.	1956	(3)	4.1	3.2	22	.49	.18	63
	1957	(4)	5.3	5.4	0	.75	.67	10
	1958	(4)	3.8	3.5	8	.59	.39	34
B.G.	1956	(3)	8.5	5.2	39	2.89	1.47	49
	1957	(3)	8.0	7.5	3	2.89	1.76	39
	1958	(4)	7.9	2.6	67	2.60	.88	66
Bull.	1956	--	--	--	--	--	--	--
	1957	(2)	7.6	7.2	5	2.14	1.16	46
	1958	(2)	7.7	2.6	66	2.11	.68	68
103-I	1956	(4)	4.0	2.3	42	1.43	.90	39
	1957	(4)	3.5	3.4	2	1.38	.93	33
	1958	(3)	3.1	1.2	61	.78	.36	54
107-I	1956	(4)	4.6	3.9	16	.91	.76	16
	1957	(4)	4.0	3.9	3	1.04	.86	17
	1958	(4)	4.2	2.4	42	.83	.49	41
108-I	1956	(4)	4.6	3.6	22	.89	.65	27
	1957	(4)	4.3	3.7	13	.70	.63	10
	1958	(4)	4.5	3.5	21	.49	.31	37
112-I	1956	--	--	--	--	--	--	--
	1957	--	--	--	--	--	--	--
	1958	(4)	4.9	4.3	13	.80	.46	42
128-I	1956	(1)	10.5	8.8	22	2.13	1.57	26
	1957	(2)	8.0	7.9	2	2.22	2.00	11
	1958	(3)	9.1	5.2	43	2.16	1.01	53
135-I	1956	(3)	2.7	2.3	15	.75	.57	24
	1957	(4)	2.4	2.1	13	1.20	1.00	17
	1958	(4)	1.5	1.0	38	.85	.59	31
139-I	1956	(4)	4.1	3.5	15	.57	.41	28
	1957	(4)	3.8	3.6	6	.60	.52	13
	1958	(4)	3.3	2.4	26	.47	.27	43
144-I	1956	(4)	4.8	2.6	35	1.49	1.47	1
	1957	(4)	4.9	4.3	12	1.66	1.30	22
	1958	(3)	3.4	3.0	12	.61	.46	25
$\bar{x} \pm S$	1956				13-41			11-39
	1957				0.3-14			6-34
	1958				15-52			27-59

\* represents loss in 11 months storage at 38°F.

Table 9. Loss of  $\alpha$ -acid in Bullion and Brewer's Gold 1957-1958.

Variety	Year	Months stored	Replication				Avg.	% loss
			1	2	3	4		
Bullion	1957	0	7.88	--	--	7.32	7.60	
		3	7.84	--	--	7.67	7.76	
		6	7.65	--	--	6.86	7.26	
		11	7.40	--	--	6.99	7.20	
	1958	0	7.59	--	7.84	--	7.73	
		3	4.60	--	4.80	--	4.70	
		6	3.92	--	4.43	--	4.18	
		11	2.53	--	2.67	--	2.60	
Brewer's Gold	1957	0	7.59	--	8.88	7.68	8.04	
		3	7.51	--	8.44	7.87	7.93	
		6	7.44	--	8.86	7.14	7.80	
		11	7.47	--	8.32	6.78	7.52	
	1958	0	8.19	8.55	7.25	7.98	7.94	
		3	4.91	--	4.49	4.84	4.75	
		6	4.24	5.27	3.40	4.12	4.26	
		11	2.64	2.22	3.26	2.45	2.64	

placed in storage. It follows that such a difference would be either (1) the condition of the resin granules at the time of harvest or (2) a difference in the treatment of these granules between harvest and storage, i.e., during drying and "packaging" for storage. If the difference is due to growth conditions, detection of the mechanism and remedy would be difficult. However, if processing variations are involved it will be possible to locate and correct faulty techniques. An experiment along these lines should be established immediately since poor storage qualities can cause an otherwise satisfactory variety to be rejected.

From Agronomic TrialsObjectives:

The purpose of this phase of CR e5-5 is to evaluate any changes in brewing value (as determined by chemical evaluation) resulting from variations in agronomic conditions.

Nature and extent of previous work:

This work has indicated that, in general, practices which are normally encountered in hop production have no practical influence on brewing values. This has included NPK fertilizer trials with the varieties Fuggles and Late Clusters, time of application of N fertilizers, use of chemical defoliant and the time and severity of pruning. 1958 data indicated that an increase in  $\alpha$ -acid was associated with a limited water supply. 1959 data supports this but accompanying yield losses are shown to more than offset the increased brewing value. The effect of gibberellic acid is in its second year of evaluation.

Procedure:

Samples are taken from the agronomic trials for dry-down determination. Subsamples are taken from these after they have been dried and weighed. These subsamples are analyzed for  $\alpha$ -acid and oil content. The results are evaluated on the basis of standard statistical tests.

Experimental results and discussion:

Irrigation-Fertility, Fuggles: Samples have been taken from 4 replications of 3 irrigation treatments, A, D and E, (no irrigation, high moisture and medium moisture) including 4 fertility treatments under each irrigation level.

Although an increase in oil content was indicated in 1958 for the treatment receiving no irrigation (A), this year's data failed to detect a moisture level effect. Neither did the 1959 data support the 1957 indication that the absence of N fertilizer decreased the oil content. No consistent

trend has been found for the effects of moisture level or fertility status on the hop oil content of Fuggles. A third year's data may clarify the situation, however it can be tentatively concluded that neither a wide range of available moisture nor a practical range of fertility status affects the oil content of Fuggles to an extent that would have economic consequences.

Table 1. Hop oil content. Irrigation-Fertility (Fuggles) 1959.  
(average of 4 reps.)

	<u>N<sub>0</sub>P<sub>1</sub>K<sub>1</sub></u>	<u>N<sub>2</sub>P<sub>1</sub>K<sub>1</sub></u>	<u>N<sub>2</sub>P<sub>0</sub>K<sub>1</sub></u>	<u>N<sub>2</sub>P<sub>1</sub>K<sub>0</sub></u>	<u>Average</u>
No Irrig.	0.68	0.77	0.68	0.78	0.73
High moisture	0.69	0.67	0.59	0.72	0.67
Med. moisture	0.66	0.65	0.69	0.74	0.68
Average	0.68	0.70	0.65	0.75	0.69

Table 2.  $\alpha$ -acid. Irrigation-Fertility (Fuggles) 1959.  
(average of 4 reps.)

	<u>N<sub>0</sub>P<sub>1</sub>K<sub>1</sub></u>	<u>N<sub>2</sub>P<sub>1</sub>K<sub>1</sub></u>	<u>N<sub>2</sub>P<sub>0</sub>K<sub>1</sub></u>	<u>N<sub>2</sub>P<sub>1</sub>K<sub>0</sub></u>	<u>Average</u>
No Irrig.	4.67	5.04	4.71	5.24	4.94
High moisture	4.37	4.42	4.54	4.51	4.45
Med. moisture	4.33	4.42	4.46	4.55	4.44
Average	4.45	4.66	4.57	4.77	4.61

Alpha acid has shown a significant increase (1%) associated with low available moisture both during 1958 and 1959. It would appear that withholding water would produce a crop with sufficiently higher brewing value to be economically attractive. However the accompanying yield loss (see table 3) clearly shows an overall disadvantage.

Table 3. Summary of 2 years' data on  $\alpha$ -acid, yield and available moisture.

	Average for High and Med. Moisture		No Irrigation		Difference due to Withholding water	
	<u>% <math>\alpha</math> acid</u>	<u>Yield*</u>	<u><math>\alpha</math> acid</u>	<u>Yield</u>	<u><math>\alpha</math> acid</u>	<u>yield</u>
1958	3.21	880	3.55	550	+ 10%	- 38%
1959	4.44	2130	4.94	1370	+ 11%	- 36%

\* lbs. dry hops per acre.



(see CR e5-4)

Gibberellic Acid: This experiment has been revised/to test (1) the rate of application and (2) application at different stages of growth. The results of chemical evaluation for brewing value are listed in table 4 as the averages for 4 replications.

Table 4. Effect of Gibberellic Acid on hop quality (Fuggles 1959)

Part I: Rate of application of G.A. when vines were 5 ft. high.

<u>ppm G.A. applied</u>	<u>Entry</u>	<u>% <math>\alpha</math>-acid*</u>	<u>oil content*</u>
0	1	4.37	0.68
5	3	4.87	0.73
50	4	4.52	0.68
100	5	5.17	0.70

Part II: 100 ppm G.A. at different stages of growth

<u>Growth stage</u>	<u>Entry</u>	<u>% <math>\alpha</math>-acid</u>	<u>oil content</u>
Pruning	2	4.28	0.70
5 ft.	5	5.17	0.70
10 ft.	6	4.14	0.58
Burring	7	3.81	0.46
Coning	8	4.58	0.60

\*  $\alpha$ -acid by spectro. method. Oil content in mls/100 g. Each value represents average of 4 replications.

Analysis of variance did not indicate any effect on  $\alpha$ -acid or oil content when G.A. was applied when the vines were 5 ft. high. The high  $\alpha$ -acid value at 100 ppm is the result of one unusually high value for one observation which could easily have been a result of sampling.

Reductions of 10-15%  $\alpha$ -acid content when 100 ppm G.A. was applied at burr is in agreement with the 1958 data. A reduction in oil content is indicated for G.A. at burring and possibly for application at 10 ft. and at coning. From the standpoint of brewing value it appears that application of Gibberellic Acid near burring time should be avoided, but application at any other time could be permitted if yield or other advantages justified it. Another year's supporting data will be required to make this conclusion firm.

Date of Pruning and Training: The time and severity of pruning experiment concluded in 1958 showed that the condition tested had no effect on  $\alpha$ -acid content. The current experiment was re-designed to test the date of pruning in combination with the stage of growth to be trained i.e. first or second crop of shoots. For details see CR e5-4. this report.

The following table shows the results of chemical evaluation as averages of 4 replications. The early Spring pruning and training most nearly approximate normal cultural practices. Late pruning and training either the first or second crop of shoots gave  $\alpha$ -acid values significantly

Table 5. Summary of quality data from pruning and training. Fuggles, 1959.

Treatment		<u>% <math>\alpha</math>-acid</u>	<u>Mls oil/100 g.</u>
<u>pruning condition</u>	<u>training</u>		
Fall pruning	1st crop	4.32	0.67
	2nd crop	4.68	0.60
Early Spring growth	1st crop	4.15*	0.72
	2nd crop	4.44*	0.65
Late Spring growth	1st crop	<u>5.09</u>	0.69
	2nd crop	<u>5.06</u>	0.68

\*These two treatments approximate normal management practice and are considered "control" or check plots for purposes of comparison. Underlined values found significant at 1% level of confidence.

(5% level) higher than the early Spring pruning and training. This data is not consistent with observations on the "moderate pruning", April 25 treatment of the Time and Severity of Pruning experiment of 1956, 1957 and 1958 (see those A.R.s).

Samples from Other StationsObjective:

To provide analytical assistance to regional research personnel in evaluation of brewing value of experiments which have objectives of general interest.

Nature and extent of previous work:

This is the first year that this type of cooperative effort has been incorporated as a regular phase of CR e5-5.

Procedure:

The number of analyses handled this way will be limited by the laboratory and personnel facilities. All data will be given to the investigator responsible for the experiment. Results of analysis will be listed as a matter of record in the Hop Research Annual Report with full research credit to the originating investigator.

Experimental results:

Selections from Early Cluster and Late Cluster yards:

C. B. Skotland (plant pathologist, Irrig. Exp. Sta., Prosser, Wash.) made 21 selections from an E.C. yard and 20 selections from an L.C. yard. These were harvested with a portable picker, dried in onion sacks in a commercial kiln and held in cold storage until analysis. Results of  $\alpha$ -acid analyses are listed in table 1.

Table 1.  $\alpha$ -acid content of early and late selections of C. B. Skotland (Irrigation Experiment Station, Prosser, Wash.)

<u>Late</u>	<u><math>\alpha</math>-acid</u>	<u>Late</u>	<u><math>\alpha</math>-acid</u>	<u>Early</u>	<u><math>\alpha</math>-acid</u>	<u>Early</u>	<u><math>\alpha</math>-acid</u>
1	6.55	11	7.28	1	8.42	11	6.47
2	8.24	12	6.58	2	7.37	12	6.96
3	7.46	13	5.51	3	8.63	13	2.42
4	6.93	14	7.15	4	6.45	15	6.97
5	7.26	15	7.35	5	6.07	16	6.55
6	5.95	16	7.18	6	6.99	17	7.23
7	6.92	17	6.21	7	6.22	18	6.20
8	7.14	18	7.16	8	5.93	19	6.59
9	7.30	19	4.02	9	6.04	20	5.86
10	7.46	19A	3.81	10	7.22	21	7.69
		20	7.70				

## Gibberellic acid treatments:

C. B. Skotland (Prosser) treated plants of the variety Late Clusters with 100 ppm gibberellic acid on two dates. He comments that at harvest (Aug. 27) those which had been sprayed were riper than the checks. Results are listed in table 2.

Table 2.  $\alpha$ -acid content of Gibberellic Acid Treatments  
(C. B. Skotland, Irrigation Exp. Sta., Prosser, Wash.)

	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>Mean</u>
Check plots	5.42	4.77	5.75	4.08	5.70	5.14
100 ppm 7/14/59	2.25	3.20	3.01	2.22	3.62	2.86
" " 7/23/59	3.76	3.50	3.63	3.77	4.68	3.87

## PHYSICAL AND CHEMICAL CHANGES IN STROBILES DURING MATURATION.

Objective:

See Annual Report 1958, page 83.

Reasons for undertaking this work:

See Annual Report 1958, page 83.

Nature and extent of previous work:

See Annual Report, 1958, page 83

Procedure:

See Annual Report, 1958, page 83.

Experimental results:

The data accumulated under this phase of CR e5-5 during 1959 was extremely erratic and any conclusions drawn from such data would be unreliable.

Additional demands on the laboratory brought about by the serial sampling of the experimental lines in advanced testing (off-station) were greater than present facilities could handle.

In order to obviate the possibility of such a loss in 1960, new cold storage and deep freeze space have been arranged for. If it becomes necessary to hold samples for analysis, sufficient room will be available. In addition to this, equipment has been purchased so that  $\alpha$ -acid analyses can be made directly on green samples and apparatus for the direct determination of dry matter by toluene distillation has been purchased. Furthermore, arrangements are being made to finance extra help for sample collection during the peak season. Every effort is being made to insure that the 1960 sampling and analytical schedule will not "swamp" the available facilities.

A summary table of the data collected in 1959 will be found in the appendix of this report.

## INVESTIGATION INTO ANALYTICAL METHODS

Objectives:

To evaluate, modify or extend analytical methods as is necessary to accomplish the overall objectives of CR e5-5.

Reasons for undertaking this work:

This work is of continuous nature. As the field of chemical evaluation of the quality factors in hops expands, it becomes necessary to evaluate available methods of analysis for their accuracy and precision relative to the hop research program. If found lacking, they must be revised or modified. As new lines of work are added in the hop research program it is frequently necessary to adapt existing chemical methods to this specific crop.

Nature and extent of previous work:

This is discussed, as required, in conjunction with the individual experiments.

Experimental results:

The determination of  $\alpha$  and  $\beta$  hop acids in male hop flowers:

The method for determining  $\alpha$  and  $\beta$  hop acids in male hop flowers by anion exchange isolation was reported in the 1958 annual report along with preliminary data indicating the possibility of a relatively fast ultra-violet light absorption method (A.R. 1958, pages 34, 80-82 and 100-103).

Using the following U. V. method, 26 male flower samples were compared with results obtained by the ion-exchange method. Results are listed in table 1.

Tentative U. V. method for  $\alpha$  and  $\beta$ -acids in male flowers:

Extract a male flower sample with sufficient methanol to give a solution with the equivalent of approximately 200 mg M.F./ml methanol. Shake 10 mls of this with 10 mls of 2%  $H_2SO_4$  and 10 mls light petroleum ether for 2

minutes. Repeat the extraction 2 times, combine the petroleum ether extracts and dilute them to 50 mls. Put an aliquote of this into a volumetric flask, (so that when made to volume the U.V. readings at 325 mu<sup>and 355 mu</sup> are 1.000 to 1.500), and evaporate to dryness with a stream of nitrogen. Dilute to volume with alkaline methanol (0.002 N, NaOH) and read at 275 mu, 325 mu and 355 mu against a blank made in the same manner. Apply the equations of Alderton et. al., (Anal. Chem., 26: 893(1954)):

$$\text{Mg M.F./liter} = \frac{\text{Wt. samp. (mg.)}}{\text{Total mls MeOH extract}} \times \frac{\text{aliq. extract (MeOH)}}{\text{vol. pet. ether from partition}}$$

$$\times \frac{\text{Aliq. pet. ether}}{\text{vol. alk. MeOH.}} \times 1000$$

$$\text{Mg } \alpha\text{-acid /liter} = -51.56 A_{355} + 73.79 A_{325} - 19.07 A_{275}$$

$$\text{Mg } \beta\text{-acid /liter} = 55.57 A_{355} - 47.59 A_{325} + 5.10 A_{275}$$

$$\% \alpha\text{-acid} = \frac{\text{mg M.F./l}}{\text{mg } \alpha\text{-acid / l}} \times 100$$

$$\% \beta\text{-acid} = \frac{\text{mg M.F./l}}{\text{mg } \alpha\text{-acid/l}}$$

The  $\alpha$ -acid values for the 26 samples ranged from 0.2% to 2.5%.

The average by ion-exchange was 0.956% compared with 0.878 by U.V. The t-test indicates that, while this is a relatively small difference, it is highly significant. Inspection of the graphs of  $A_{332}$  plotted against fraction number revealed that in many cases a slight irregularity was present in the  $\alpha$ -acid peak of the ion-exchange method (see A.R. 1958, page 82). Since this was small and its quantitative contribution difficult to estimate, it was not accounted for in the calculations. It is possible that the inclusion of such an impurity in calculating  $\alpha$ -acid by the ion exchange method would be of approximately the proper magnitude to account for the difference between the two methods. Upon this assumption, the spectrophotometric method is believed to provide the more accurate estimates of  $\alpha$ -acid in male hop flowers.

Table 1. Comparison of Ion-exchange and U.V. methods for  $\alpha$ - and  $\beta$ -acids in Male Hop Flowers.

Sample No.	Entry-Rep.	$\alpha$ -acid			$\beta$ -acid		
		I.E.	U.V.	Diff.	I.E.	U.V.	Diff.
1	1-1	.51	.38	.15	1.04	.84	.20
2	1-2	.47	.43	.04	.87	1.01	-.14
3	201	1.34	1.01	.33	1.54	1.36	.18
4	2-2	1.19	.79	.40	1.37	1.42	-.05
5	3-1	.91	.72	.19	1.76	1.51	.25
6	3-2	.96	.87	.09	1.73	1.66	.07
7	4-1	1.16	1.05	.11	2.58	2.68	-.10
8	4-2	.81	.87	-.06	2.08	2.03	.05
9	5-1	.22	.16	.06	.47	.40	.07
10	5-2	.35	.30	.05	.64	.68	-.04
11	6-1	.62	.70	-.08	1.60	1.58	.02
12	6-2	.92	.70	.22	1.46	1.50	-.04
13	7-1	1.36	1.11	.25	.72	.69	.03
14	7-2	.60	.91	-.31	.70	.63	.07
15	8-2	.60	.48	.12	.35	.39	-.04
16	9-1	1.14	1.31	-.17	3.08	2.97	.11
17	11-1	.35	.51	-.16	1.83	1.88	-.05
18	12-1	1.71	1.78	-.07	1.25	1.16	.09
19	13-1	1.40	1.28	.12	1.22	1.09	.13
20	14-2	.34	.25	.09	.82	.88	-.06
21	15-1	.71	.70	.01	1.89	1.88	.01
22	16-2	.42	.33	.09	1.05	1.14	-.09
23	17-1	2.54	2.32	.22	2.74	2.82	-.08
24	18-1	2.35	2.09	.26	1.99	1.99	0
25	19-1	.56	.57	-.01	1.46	1.59	-.13
26	20-1	1.33	1.20	.13	1.95	2.23	-.28
Totals		24.87	22.82	2.05	38.19	38.01	0.18
Means		.9565	.8777	.0788	1.273	1.266	.007
Std.error of obs.				.1116			.0382
" " " mean				.0219			.0074
t. value				3.6021**			.9319 N.S.

\*\* indicates significance at the 1% level of confidence.



The range of  $\beta$ -acids was from 0.4% to 3.0%. The average of 1.273%  $\beta$ -acid by the ion-exchange method was 0.007% higher than by the U.V. method. The t-test could not distinguish the methods.

Upon the basis of these data it has been concluded that the spectrophotometric method, employing the regression equations of Alderton, et al is reliable and sufficiently sensitive to detect varietal differences in the  $\alpha$  and  $\beta$  acid potentials of male hop plants.

The stability of Methanol extracts of male hop flowers at  $-5^{\circ}\text{F}$ :

Since subsampling of male flower samples yields extremely variable "brewing value" results, it has been found necessary to extract the entire sample at one time and to take any subsequent sub-samples from that extract. In order to insure that the extracts can be stored ( $-5^{\circ}\text{F}$ .) without fear of loss of  $\alpha$  or  $\beta$  acids, samples have been run at various intervals from 3-9 months as indicated in table 2.

Table 2. Storage of Male Flowers at  $-5^{\circ}\text{F}$ . (Spectro. method)

<u>Sample</u>	<u>Rep.</u>	<u>Date extracted</u>	<u>Date analyzed</u>	<u>% <math>\alpha</math>-acid</u>	<u>% <math>\beta</math>-acid</u>
ML 521-1,2	1	2/1/59	2/1/59	0.16	0.40
			5/15/59	0.19	0.52
ML 317-1,2	1	2/1/59	2/1/59	1.31	2.97
			5/15/59	1.60	2.93
ML 420-4,5	5	5/11/59	5/11/59	0.76	0.42
			1/16/60	0.64	0.37
ML 318-4,5	5	5/11/59	5/11/59	1.78	1.34
			1/16/60	1.78	1.18
ML 120-1,2	3	3/31/59	3/31/59	1.91	1.96
			1/16/59	1.72	2.01

Experiments concerning the partitioning of methanol extracts of male flowers with light petroleum ether.

1. Number of extractions necessary to remove all  $\alpha$  and  $\beta$ -acids:

Exactly 10 mls of methanol extract of MF 15-2, 10 mls 2%  $\text{H}_2\text{SO}_4$  and 10 mls petroleum ether were shaken 2 minutes and allowed to separate. The petroleum ether was collected and made to volume. This was repeated a second and third time with the same aqueous phase. Appropriate dilution

with alkaline methanol were made and spectral absorption curves from 250 mu to 400 mu were made with a Carey Recording Spectrophotometer. Absorptions at 275 mu, 325 mu and 355 mu are recorded in table 3.

Table 3. Repetitive Extraction of Methanol Extracts with Petroleum Ether. (Absorption values from solution containing 1.206 mg M F/ml)

	Wavelength		
	<u>355 mu</u>	<u>325 mu</u>	<u>275 mu</u>
1st extraction	1.253 1.316	0.958 1.030	0.282 0.331
2nd extraction	0.038 0.029	0.036 0.020	0.025 0.028
3rd extraction	0.018 0.015	0.018 0.017	0.013 0.017

absorption curves of extraction #1 were typical for  $\alpha$  and  $\beta$ -acid solutions while extractions 2 and 3 were apparently U.V. absorbing residues or impurities and bore no resemblance to  $\alpha$  or  $\beta$ -acid curves. It was concluded that a single extraction was sufficient.

2. Shaking time required to reach equilibrium in the partitioning of methanol extracts with petroleum ether.

Four 10 ml. aliquotes of the methanol extract of M F 15-2 were prepared as above. These were shaken 10, 30, 60 and 120 seconds respectively and the petroleum ether layers were each made to 50 mls. Identical aliquotes of each were diluted with alkaline methanol and their U.V. absorption curves obtained. Absorption data (in table 4) indicates that at least 1 minute shaking is required to get all the  $\alpha$  and  $\beta$  acids extracted.

Table 4: Shaking Time Required for Methanol-acid: Petroleum Ether Partition.  
(Absorption values for a solution containing 0.804 mg M F / ml)

	Wavelength		
	<u>A 355</u>	<u>A 325</u>	<u>A 275</u>
10 seconds shake	0.828	0.650	0.199
30 " "	0.848	0.665	0.200
60 " "	0.860	0.660	0.205
120 " "	0.860	0.668	0.220

3. Absorption curves indicating the U.V. absorbing nature of the residue not partitioned into petroleum ether:

Another method of detecting the presence of  $\alpha$  or  $\beta$  acids not extracted into petroleum ether is the "differential absorption method." This is accomplished by preparing two solutions of equivalent male flower content but one of which has been partitioned into petroleum ether and the other is a dilution of the original extract. The one which has been partitioned plus that which remains in the aqueous phase must be equal in U.V. absorption to the original extract. Therefore if the partitioned sample is used as a "blank" and the original extract is scanned with a recording spectrophotometer the resulting curve represents the U.V. absorbing spectrum of the unextracted material left in the aqueous phase.

This was done for two male flower samples (MF 3-3(59) and MF 4-1(59)) using a single extraction with 90 second shaking as indicated from experiments 1) and 2) above. In each case the spectrum of the methanol sample showed the impurity at 275 mu and the partitioned sample did not. The differential curves showed that the impurity absorbing at 275 mu was left behind in the extraction and that no absorption corresponding to  $\alpha$  or  $\beta$  acids was present in the unextracted residue.

4. Stability of  $\alpha$  and  $\beta$  acids in petroleum ether solutions of male flower extracts:

In order to determine the stability of pet. ether solutions after

partitioning, one set of 5 samples was partitioned and the pet. ether solutions were stored at room temperature in the dark for 24 hours at which time the same samples were again partitioned. The pet. ether solutions were then scanned and the fresh solutions were compared with those which had stood 24 hours. The data seen in table 5 indicates that, while duplication of  $\alpha$ -acid is poor percentagewise, the absolute magnitude of the errors involved are well within the range of error encountered in duplicate analyses.

Table 5. Stability of petroleum Ether Solutions of Male Flowers at Room Temperature and in the Dark.

<u>Sample</u>	<u>Storage (hrs)</u>	<u>% <math>\alpha</math></u>	<u>% <math>\beta</math></u>
15-1	0	.08	1.84
	24	.16	1.75
15-2	0	.01	2.12
	24	.01	2.19
15-3	0	.06	1.82
	24	.09	1.89
16-2	0	.25	1.26
	24	.29	1.28
16-3	0	.43	1.61
	24	.52	1.66

From these data it has been concluded that petroleum ether solutions show sufficient stability when protected from light, that any liberties can be taken with them during the working day without fear of loss of  $\alpha$  or  $\beta$ -acids.

Summary of experiments with spectrophotometric method for  $\alpha$  and  $\beta$  acids in male flowers:

1. The regression equations of Alderton et. al. have been found suitable for use with the spectrophotometric method described.
2. Methanol extracts of male flowers have been found to be stable up to 9 months when stored at  $-5^{\circ}\text{F}$ .
3. A single partitioning of methanol extracts with petroleum ether is sufficient to remove all  $\alpha$  and  $\beta$ -acids if the shaking time exceeds 60

seconds.

4. Petroleum ether solutions resulting from partition with methanol extracts of male flowers are sufficiently stable, when kept in the dark at room temperature, to permit storage for any period during the working day, or overnight if necessary.

Observations on extracts of samples from Prosser:

Although the varieties in yield test at Prosser had obviously had excellent care and handling, their cones were small and resin glands were underdeveloped. Petroleum ether extracts of most contained components which (when in alkaline methanol) exhibited unusual U.V. absorption. Readings at 275 mu were frequently higher than those at 325 mu and 355 mu. The construction of the regression equations for calculation of  $\alpha$  and  $\beta$  acids is such that the 275 mu reading (usually about 1/3 of the 325 and 355 mu readings) is a measure of foreign absorption and is consequently associated with a negative constant. When the 275 mu readings are too large, as in the case of many Prosser samples there is an over-correction; occasionally to the extent that negative values are found for  $\alpha$  and  $\beta$  acids. This was the case with many 1959 Prosser samples.

The best method presently available for the analysis of old hops or hops low in  $\alpha$ -acid appears to be the relatively new conductometric method. In order to get an estimate of the degree of failure of the spectrophotometric method, several Prosser samples were run by that method. A comparison of results is shown below. Although the  $\alpha$ -acid values by the conductometric method are quite low, they are more realistic in most cases.

Table 6. Comparison between the spectrophotometric and conductometric methods of determining alpha acid in dry hops. (Prosser, 1959)

Variety and Rep.	Spectrophotometric % Alpha	Conductometric % Alpha
103-I, 4	----	0.85
104-I, 1	0.23	1.10
104-I, 3	0.16	1.63
104-I, 4	0.09	1.61
108-I, 1	0.40	1.48
108-I, 2	1.74	2.79
108-I, 3	1.85	2.68
124-I, 3	0.86	1.89
144-I, 1	1.27	2.15
144-I, 2	----	1.41
Late Cluster, 1	3.54	3.60
Late Cluster, 2	3.09	2.62
Late Cluster, 3	1.15	3.70
Late Cluster, 4	2.29	3.52

\* The spectrophotometric method gave a minus answer.

The conductometric method for  $\alpha$ -acid analysis.

The following four references are listed as indicative of the conductometric method and its usefulness and not intended to be complete.

1. The Conductometric Determination of  $\alpha$ -acids on the Hop Farm., Hartong, B.D. and Isebaert, L., Wall. Lab. Comm., 22: 17-23 (1959).
2. Conductometric Hop Analysis, Goedkoop, W., and Hartong, B. D., J. Inst. Brew., 63: 368-90 (1957).
3. Rapid Determination of  $\alpha$ -acids and Moisture of Green Hops, Isebaert, L., Brauwelt, 98: B: 663-65 (1958). Abstract I-276, J. Sci Food and Agr., Vol. 10, No. 5, May 1959).

4. Experience with a Conductometric Method of Determination of  $\alpha$ -acids in Hops in Regard to Brewing Value, Hartong, B. D., Jansen, H. E., and Mendlik, F., Brau Wissenschaft, 12: 2: 43 (1959). (Abstracted in Wall. Lab. Comm., 22: 79: 344 (1959).

The method employed in this laboratory in 1959 appears to be suitable although no serious study has yet been made. It is as follows:

Kiln dried hops:

5 grams of ground hops are shaken 20 minutes with 100 mls chloroform then centrifuged. (Fast filtration through cotton is satisfactory, if collected so that evaporation of  $\text{CHCl}_3$  is minimized, e.g. in volumetric flask.) 20 mls are transferred to 250 ml beaker, diluted with sufficient methanol to cover the electrodes of the dip cell of the conductivity apparatus and stirred with an automatic stirrer until a constant reading is reached. Titration of the  $\alpha$  acids is then carried out with 0.25 ml increments of freshly prepared 2% lead acetate in methanol, recording the resistance after each increment. 4% lead acetate is better if hops are better than average i.e. over 5%  $\alpha$ -acid. If resistance is measured, then the reciprocal, (conductivity), must be calculated. The increments of lead acetate are plotted vs. conductivity and a straight line drawn through the two arms. The intercept of these lines represents the mls of lead acetate equivalent to the  $\alpha$ -acid present and,

$$\% \alpha\text{-acid (dry basis)} = \frac{\text{mls lead acetate} \times \% \text{ lead acetate} \times 0.944 \times 100}{\% \text{ dry matter}}$$

Green hops:

30 grams of fresh hops are homogenized 5 minutes in a Waring blender (or equivalent) with 100 mls chloroform. This is centrifuged or filtered through cotton and a 15 ml aliquote is diluted with sufficient methanol to cover the

electrodes. This is titrated with 2% lead acetate as with dried hops. Calculation of conductivity and construction of the graph is the same.

Calculation of  $\alpha$ -acid is:

$$\% \alpha\text{-acid (dry basis)} = \frac{\text{mls lead acetate} \times \% \text{ lead acetate} \times 0.21}{\% \text{ dry matter}}$$

In the case of green hops it is imperative that the dry matter content of the sample is known at the time it is weighed. This requires that a second sample be taken simultaneously for this determination.

General comments:

Several solvents can be used but chloroform has the advantage of being non-flamable and less toxic than benzene. (Even  $\text{CHCl}_3$  should be used only in a well ventilated place.)

The percentage error involved in duplicate analyses appears to be less than  $\pm 5\%$ .

Complete equipment for the determination might cost from \$500. to \$700.

One man could run 10-20 determinations daily.



A P P E N D I X

2. Service and Units  
ARS - CR - Oilseed and Industrial Crops Research Branch
3. Line Project Title  
Breeding and evaluating new and improved varieties of hops.
4. Work Project Title  
Industrial crops production, breeding, disease and quality investigations.
5. Project Leader and Address  
S. N. Brooks, Corvallis, Oregon; J. O. Culbertson, Beltsville, Maryland  
(Supervisory)
6. Location of Work  
Corvallis, Oregon; Prosser, Washington
7. Estimated Duration  
5 years
8. Objective: To develop hop varieties or strains possessing disease resistance, quality, and yielding ability acceptable to both the grower and brewer.
9. Justification: High level production of hops in Oregon, Washington, California, and Idaho is dependent upon the development of new and improved varieties of hops resistant to infection by the downy mildew and root rot organisms. Downy mildew has been a serious disease on hops since the 1930's particularly on the two most widely grown varieties. Only one variety possesses any degree of resistance to downy mildew, and it is relatively low yielding, is not adapted to high temperature, is shattered badly by the picking machine when ripe, and is acceptable to a relatively small segment of the brewing industry. Previous work has established that many of the characteristics in which improvement is desired are highly heritable. Sufficient genetic variability exists within the species to permit development of varieties adapted to a wide range of environmental or harvesting conditions and having the desired physical and chemical quality characteristics. Sources of genetic diversity for the breeding program consist of four widely grown varieties and approximately 75 introductions, none of which have exhibited immunity to downy mildew. Crosses have been made between the genetic types in the breeding program, and some of them have survived severe selection for resistance to downy mildew, and for quality and yielding ability. New germ plasm has been introduced into the breeding program, consisting of living plant material of wild hops native to the Rocky Mountain area. These individuals were introduced on the basis of reports from England which indicated that crosses of domestic and wild American hops gave a high proportion of progenies which were resistant to crown infection by downy mildew. This work is closely coordinated through the Federal hop project with headquarters at Corvallis, Oregon, as well as through the Oregon Agricultural Experiment Station interdepartmental hop research project (OAES 36), and key individuals at the other State institutions. Those phases having immediate application are coordinated also through

the National Hop Research Committee in cooperation with the Hop Growers of America, Inc. The development of new varieties of hops is related to the agronomic, chemical, and disease line projects (CRE5-2 rev., -4 rev., and -5 rev.), since new varieties must be evaluated for quality, disease resistance, and growing characteristics.

10. Plan of Work: Crosses will be made to combine resistance to the important diseases, desirable chemical and physical properties, and acceptable agronomic, picking and growing characteristics, into varieties which are adapted to the growing areas in the four hop-producing States. The crossing scheme will follow a modified recurrent selection program to a large extent. A limited backcrossing program will be carried out for the purpose of incorporating already accepted quality characteristics into adapted and disease-resistant lines. All of the crosses will be made at Corvallis, Oregon, where from 10,000 to 50,000 seedlings will be grown in the greenhouse each year for preliminary downy mildew evaluation. Following an artificial epiphytotic in the greenhouse, the selected seedlings will be transplanted in the field where they will undergo selection for vigor and type. The desirable individuals will be vegetatively increased into four- or five-hill observation blocks which will be grown at Corvallis, Oregon; Sacramento, California; Prosser, Washington; and Parma, Idaho. The most desirable lines, based on the observation trials will be increased further and advanced into replicated yield trials in each of the four hop-producing States. Following yield and other evaluation in preliminary and advanced yield trials, a smaller number will be released under memoranda of understanding to hop growers for final testing under actual field and management conditions. During the testing phase, experimental lines will be given additional evaluation at Corvallis for reaction to downy mildew disease. Samples of strobiles will be obtained for preliminary chemical evaluation which will be carried out in the laboratory at Corvallis, Oregon. The selections will be advanced into actual brewing trials conducted in cooperation with the brewing industry. Basic studies to determine the mode of inheritance of agronomic, pathologic, and chemical quality characteristics are now in progress and will be continued. In addition, the flowering behavior and breeding behavior will be determined. This project will require approximately 1 professional man-year per year of ARS personnel.

11. Cooperations: Research under this project is conducted in cooperation with the Agricultural Experiment Stations in Oregon, Washington, California, and Idaho, as well as with the Agricultural Extension Service of California. A memorandum of understanding exists with the Oregon Agricultural Experiment Station, which furnishes offices, laboratory, greenhouse and field space, and varying amounts of professional and sub-professional assistance and labor. Cooperation with other States is informal. The United States Brewers Foundation furnishes financial support for much of the work of regional significance which is done at Oregon State College.

USDA Line Project Description

CRE5-2 (rev. April, 1959)  
(supersedes CRE5-3 in part)

2. Service and Units  
ARS - CR - Oilseed and Industrial Crops Research Branch
3. Line Project Title  
Hop diseases; their etiology, epiphytology, and control.
4. Work Project Title  
Industrial crops production, breeding, disease and quality investigations.
5. Project Leader and Address  
C. E. Horner and S. N. Brooks, Corvallis, Oregon; J. O. Culbertson, Beltsville, Maryland (Supervisory).
6. Location of Work  
Corvallis, Oregon; Prosser, Washington
7. Estimated Duration  
5 years
8. Objective: (a) To develop practical methods of hop disease control. (b) to obtain basic knowledge of the life cycle and host-parasite relations of hop diseases as a basis for developing effective control measures.
9. Justification: Hop diseases are frequently limiting factors in production. In 1957 California growers estimated a loss of 30 percent (about 1 million dollars) from downy mildew alone. In Washington, root and crown rot which follows systemic downy mildew infection, results in extensive losses of up to 30 percent or more of the hills annually. When downy mildew devastated the hop industry in the cool, moist-climate valleys of Western Oregon and Washington in the 1930's and 1940's, production of the popular, mildew-susceptible varieties shifted to the irrigated desert valleys where it was thought that downy mildew would not be a problem because of the hot dry climate. However, downy mildew has become firmly established there, and persists by virtue of systemic crown and root infections which cause severe crown and root rot. Early research on control of the hop downy mildew was aimed largely toward developing methods of control by protective fungicides. Zineb (zinc ethylene bisdithiocarbamate) was the most effective organic fungicide tested and was approved for use on hops with a tolerance of 60 parts per million. In 1955, the entire downy mildew control research program was re-evaluated in the light of more recent knowledge of the disease. It was decided that future research on downy mildew control would be directed toward: (1) control of systemic infection by eradicant fungicides or systemic antibiotics and fungicides, and (2) continued effort in developing resistant varieties (part of CRE5-1 rev.) with emphasis on improving greenhouse and field testing for resistance. Substantial progress has been made toward achieving those objectives. The use of streptomycin for controlling early spring systemic infection is being developed; and greenhouse and field testing methods for screening hop seedlings for resistance to downy mildew are being successfully employed. Virus diseases in hops are known to be widespread and some are very destructive. A number of virus-like conditions of hops have been described in the United States but no definitive work has yet been done. Since hops are entirely vegetatively

propagated, a knowledge of hop virus diseases is imperative to the successful application of disease control by selection of virus-free planting stock.

Verticillium wilt of hops has caused limited losses, but is present in many hop-growing areas and poses a potential threat to the industry. This disease causes extensive losses of hops in England. It would be desirable to know the varietal reaction of American varieties to wilt, and especially to know the status of resistance to wilt of new selections being considered for release as new varieties

Closely related work is conducted by the Oregon, Washington, Idaho, and California Experiment Stations. All of the work is coordinated through the National Hop Research Committee which meets each year. Information is furnished the Extension Service, and growers are supplied information through the Hop Growers of America, a national growers organization. Knowledge and results of this project are closely correlated with hop breeding (GR5-1 rev.).

10. Plan of Works: Randomized and replicated field tests will be conducted to determine the best rates and time of application of streptomycin for control of systemic downy mildew infection. Laboratory and greenhouse experiments will be conducted to test systemic fungicides and antibiotics for their effectiveness in eradicating systemic mildew infection. Studies will be made to determine the factors effecting infection and progression of systemic downy mildew infection in hop roots and crowns. A study of virus diseases of hops will be started to determine what viruses are present, their symptoms, methods of spread, reaction on different hop varieties, and control. The reaction of varieties and selections to Verticillium wilt will be determined. This project will require about 0.3 professional man-years per year of ARS personnel.

11. Cooperation: Research under this project is conducted under a memorandum of understanding in cooperation with the Oregon Agricultural Experiment Station, which furnishes office, laboratory, greenhouse and field facilities. Informal cooperation exists with the Washington Experiment Station, which aids with field tests and collection of data. The United States Brewers Foundation furnishes financial assistance, through the Oregon Agricultural Experiment Station.

USDA Line Project Description

CR5-4 (rev. April, 1959)  
(supersedes CR5-3 in part)

2. Service and Units  
ARS - CR - Oilseed and Industrial Crops Research Branch
3. Line Project Title  
Improving yield and quality of hops by production and management practices.
4. Work Project Title  
Industrial Crops production, breeding, disease and quality investigations.
5. Project Leader and Address  
S. N. Books, Corvallis, Oregon; J. O. Culbertson, Beltsville, Maryland  
(Supervisory)
6. Location of Work  
Corvallis, Oregon
7. Estimated Duration  
5 years
8. Objective: To develop more effective cultural and management practices for growing hops through research on the effects of various fertilizer, irrigation, pruning and trellising treatments on the yield, quality, incidence and severity of diseases, and net returns per acre.
9. Justification: Numerous agronomic trials have been conducted on hops in cooperation with the Oregon Agricultural Experiment Station since 1910. These investigations have involved studies of irrigation, cultivation, plant spacing, number of vines per plant, fertilizer trials, stripping and suckering, crowning and pruning, time of hoeing, and the use of various cover crops. Many of these trials have been of preliminary nature and repetition or continuation of some of these studies is desirable in drawing inferences based on more complete experimental data. In years of unfavorable market conditions many growers have tried to reduce their annual expenditures by eliminating some of the standard or traditional practices which have been time-tested. Modification of some practices has been found to be undesirable. Progress in mechanization has caused new problems in some cases. When hops were hand-picked, they were allowed to mature completely; with machine-picking it is necessary that they be cut from the ground at harvest time. Premature harvesting has caused loss of vigor of the hills with subsequent die-out, poor stands and declining yields. Cultural and management practices vary from one area to the next, depending upon grower preference and upon soil and climatic conditions peculiar to the different areas, and need to be tested, developed, or modified, in order to promote the most economical production for each area. Due to differences in varieties and their response to climatic and soil conditions, the cultural and management practices standard for one variety are not always the best for another. The recent introduction of European varieties has necessitated a new series of experiments designed to determine the best cultural practices for those varieties. This work is related to the other line projects (CR5-1 rev., -2 rev., and -5 rev.) in that cultural practices must be modified to suit the needs of new varieties. Cultural and management practices can have an effect on the prevalence of diseases and insect pests as well as on the

quality of the hops produced. It will be coordinated through the Federal regional hop research program with headquarters at Corvallis, Oregon, as well as through the Oregon Agricultural Experiment Station hop research project. Results will be brought to the attention of the Hop Growers of America, Inc., through the National Hop Research Committee. The work will be designed to supplement and complement agronomic work done in the States of Washington, Idaho, and California.

10. Plan of Work: Cultural and management practices will be carried out on a replicated trial basis at Corvallis, Oregon. Trials will consist of four- or five-hill plots as well as individual hills of potted greenhouse plants, depending upon the nature of the study involved. The lines of work undertaken will involve rates and dates of application of nitrogen, phosphorus, potash, sulphur, lime and minor element fertilizer materials; the effects of various fertilizer elements in connection with yield and quality of hops. Additional trials will be designed to determine the best methods of placement of fertilizer materials. The effects of time of pruning on the commercial varieties being grown in the Willamette Valley will be determined with regard to yield and quality responses. Additional information will be obtained relative to the number of plants per acre and vines per plant, particularly for any new varieties which are introduced to the growing area or which are developed in the breeding program. The effects of height of trellis will be studied in order to determine the height best suited to each of the commercial varieties being grown. Other cultural practices and methods of crop handling that may have an influence on yield and quality of the hops will be investigated as the needs arise. Experimental techniques will be modified and developed in order to determine the responses of the crop to cultural and management practices. Approximately 0.7 man-years per year of ARS personnel will be devoted to this project.

11. Cooperation: Research under this project is conducted in cooperation with the Oregon Agricultural Experiment Station under a formal memorandum of understanding. The State Experiment Station furnishes offices, laboratory, greenhouse and field space, and a varying amount of subprofessional assistance. The United States Brewers Foundation furnishes financial support of some of the work. Informal cooperative work is done with growers and industry representatives for obtaining data and for evaluating hop samples.

2. Service and Units  
ARS - CR - Oilseed and Industrial Crops Research Branch
3. Line Project Title  
Chemical investigations relative to the evaluation of hops.
4. Work Project Title  
Industrial crops production, breeding, disease and quality investigations.
5. Project Leader and Address  
Sam T. Likens and S. N. Brooks, Corvallis, Oregon; J. O. Culbertson,  
Beltsville, Maryland (Supervisory)
6. Location of Work  
Corvallis, Oregon; Prosser, Washington
7. Estimated Duration  
5 years
8. Objective: (a) To characterize experimental hop varieties or strains by chemical methods. (b) To evaluate change in chemical composition of plant parts affected by cultural practices. (c) To modify or improve analytical methods or techniques as may be required.
9. Justification: Increasing emphasis on chemical assay as a basis of quality evaluation of hops by the brewing industry requires that knowledge of these characteristics, in parental breeding stock and in their progenies, be available to the agronomist as an aid in breeding and selection. Chemical analysis is used to detect quality changes resulting from variations in cultural and processing practices. Such information is necessary in the formulation of recommendations to growers. Preliminary data show that chemical analyses of plant parts can be useful in assessing the nutrient status of hop plants. Off-station trials need to be conducted before these data can be applied to grower recommendations. Results of chemical tests provide an aid to the identification of unknown varieties. The hop chemical research laboratory at Corvallis, Oregon, is the only one serving the hop-growing industry of the Pacific Northwest from which results are used directly in the development of new varieties and improved cultural and processing practices. The work done by this laboratory does not represent a duplication of work done by any other organization in the United States. This work is actively supported by the Brewing Industries Research Institute, the United States Brewers Foundation, and the Oregon Agricultural Experiment Station. More recently the Irrigation Experiment Station at Prosser, Washington, the Parma Branch Experiment Station of Idaho, and the Experiment Station and Extension Service of the University of California have entered into active cooperation with the project. The work at Oregon State College is coordinated through an Experiment Station hop research project (OAES36). The work with the other institutions and with the Hop Growers of America, Inc., is coordinated through the regional Federal research projects (CR e5-1 rev. and CR5-4 rev.) as well as through the National Hop Research Committee.



10. Plan of Work: Samples of strobiles and other plant parts will be collected at various intervals during the growing season from experimental lines and varieties in the breeding, agronomic, and disease studies conducted in Oregon, Washington, California, and Idaho. These samples will be subjected to various chemical determinations, and the data therefrom will be analyzed in accordance with accepted statistical procedures. Quality evaluation of strobile samples from cultural and processing trials will be made predominantly by alpha-acid and oil yield determinations. Characterization of both male and female inflorescences from parental breeding stock will be made by analysis for alpha and beta hop acids, humulone-cohumulone ratios, oil yield and fractionation of oils into their components by gas chromatography. A study of the relation of several chemical and physical characteristics of three commercial varieties to their maturation is in progress and will be continued. Chemical analysis will be applied to samples taken from experiments dealing with heritable characteristics and genetic relationships. Preliminary analysis of root cuttings will be made to determine if easily identifiable chemical entities can be found which can be used in identification of rootstock material. Tissue analysis for the evaluation of nutrient status will be extended to off-station trials. Chemical methods and techniques will be modified and improved as may be necessary in making the chemical determinations. This project will require approximately one professional man-year per year of ARS personnel.

11. Cooperation: Research under this project is conducted in cooperation with the Agricultural Experiment Stations in Oregon, Washington, California, and Idaho, as well as with the Agricultural Extension Service of California. A formal memorandum of understanding exists with the Oregon Agricultural Experiment Station; cooperation with the other agencies is informal. The State agencies furnish office, laboratory, greenhouse and field space, and varying amounts of professional and subprofessional assistance and labor. The United States Brewers Foundation furnishes financial support of much of the work, particularly that of regional or national significance. Informal cooperative work is done with growers and industry representatives for obtaining and/or evaluating plant material.

1. BREEDING AND EVALUATION

1. Disease resistance

- A. Increase emphasis on breeding and evaluation for resistance to systemic crown infection by downy mildew. ARS-OREGON, WASHINGTON, IDAHO, CALIFORNIA.
- B. Obtain and employ in breeding, wider genetic diversity of hop germ plasm. ARS-OREGON.
- C. Develop earlier testing of large numbers of mildew-resistant genotypes in Washington, Idaho, and California. ARS-OREGON, CALIFORNIA, IDAHO, WASHINGTON.
- D. Develop early testing of selected material for resistance to Verticillium wilt and virus diseases. ARS-OREGON, WASHINGTON.
- E. Continue variety improvement along present lines using recurrent selection as the breeding method. Also, continue backcrossing program, using Late and Early Clusters as recurrent parents, to obtain disease-resistant "Cluster" types. ARS- OREGON.

2. Quality

- A. Breed special-purpose varieties to meet demands of brewers, using Hallertau, Backa, and Brewers Gold in backcrossing program. The varieties represent different "quality" classes that need to have resistance transferred to them.
- B. Maintain quality of existing varieties in New Lines having other desirable characteristics. ARS-OREGON.
- C. Select for good storage characteristics. ARS-OREGON.

3. Agronomic characteristics

- A. Select for high yield potential, pickability (machine), and wide range of maturity. ARS-OREGON.

II. VARIETY PURIFICATION

- 1. Continue mass selection of better types, free of virus and other diseases, from heterogeneous material in yards. WASHINGTON, CALIFORNIA.
- 2. Investigate methods of propagating such materials. CALIFORNIA.

III. DISEASES

1. Downy mildew

- A. Study life history and epidemiology on individual state basis because of wide environmental differences. ARS-OREGON, WASHINGTON, IDAHO, CALIFORNIA.
- B. Develop methods of control by chemicals and by modification of cultural practices. ARS-OREGON, WASHINGTON, IDAHO, CALIFORNIA.

2. Virus Diseases

- A. Determine identity. ARS-OREGON, WASHINGTON, IDAHO, CALIFORNIA.
- B. Determine method of dissemination (aphids), WASHINGTON
- C. Study effects on yield. WASHINGTON, ARS-OREGON.

IV. INSECT PESTS

- 1. Continue evaluation of several effective chemicals currently recommended for control of major hop pests -- aphids and spider mites. OREGON.
- 2. Evaluate breeding lines for resistance to insect attack. ARS-OREGON.
- 3. Investigate soil pests (nematodes, symphillids, grubs, etc.) OREGON, CALIFORNIA.

V. MANAGEMENT

- 1. Study nutritional requirements on individual area basis. ARS-OREGON, WASHINGTON, CALIFORNIA, IDAHO.
- 2. Investigate irrigation practices, requirements, and consumptive use of water. ARS-OREGON, WASHINGTON.
- 3. Develop improved cultural practices for existing and new varieties.
  - A. Pruning and training. ARS-OREGON, WASHINGTON, IDAHO.
  - B. Spacing and number of vines, and trellis height. ARS-OREGON.
- 4. Weed control by herbicides. CALIFORNIA.

VI. QUALITY

- 1. Define quality and components of quality in definite terms. ARS-OREGON.
- 2. Study quality in relation to maturity. ARS-OREGON, CALIFORNIA.
- 3. Study effects of processing and harvesting. CALIFORNIA, ARS-OREGON.
- 4. Study effect of locality (environment) on quality factors. ARS-OREGON.
- 5. Develop techniques for determining quality components. ARS-OREGON.
- 6. Characterize quality components of existing varieties and promising lines. ARS-OREGON.

VII. BOTANY OF HOPS

- 1. Determine inheritance of disease resistance, quality components and other characteristics. ARS-OREGON.
- 2. Study flowering behavior. ARS-OREGON.
- 3. Study fertilization and embryo development. ARS-OREGON.

After reviewing present research on hops and recommending changes within existing authorization to strengthen it, the Committee gave consideration to problems that the states are not able to deal with adequately at the present time.

It was agreed that in the event of expansion, the responsibilities of ARS and the States as outlined in the Memorandum of Understanding would apply. Crossing, preliminary screening for disease resistance, and chemical evaluation of quality would be conducted by ARS and the Oregon Agricultural Experiment Station. Large expansions in primary testing of disease-resistant selections, plant disease research, or research in other lines in each of the States, would be done by either State or Federal personnel, depending upon availability of funds and agreements made at the time of expansion.

#### WASHINGTON AGRICULTURAL EXPERIMENT STATION (H. P. Singleton)

The program in Washington should be strengthened to better serve those regional areas that regularly grow Early and Late Cluster hops as follows:

##### I. Breeding and Agronomy

The addition of one professional man and the regular component of subprofessional and labor help together with the necessary special equipment to:

A. Expand the regional breeding program by carrying on a primary testing study with from 300-800 crosses annually from Corvallis, chiefly back crosses of mildew resistant selections on Early and Late Clusters.

B. Study cultural and management practices under irrigation conditions.

##### II. Plant Pathology

The addition of one professional man and the regular component of subprofessional and labor help and special equipment to:

A. Search for germ plasm resistant to hop viruses.

B. Develop the techniques suitable for large scale testing of hops for resistance to virus diseases.

C. Determine the identity and epidemiology of hop viruses.

D. Cooperate in the breeding and selection of virus disease resistant hop varieties.

E. Participate in a greatly expanded program developing downy mildew resistant hops.

F. Investigate the identity, epidemiology and control of other hop diseases.

The Washington Agricultural Experiment Station through the Irrigation Experiment Station will provide at no cost to the U. S. Department of Agriculture:

1. Land for the program and will erect the yard.
2. Irrigation water.
3. A moderate amount of greenhouse and screen house space.
4. Office and laboratory space.
5. Build a small hop drier and baler.
6. Light, heat, water and other facilities.

CALIFORNIA AGRICULTURAL EXPERIMENT STATION (P. F. Knowles)

- A. Purpose: To permit an expansion of research on hops by the University, with particular emphasis on the following:
1. Phytopathology and control of downy mildew.
  2. Methods of commercial propagation of better types in hop yards.
  3. Control of insect pests.
  4. Improved cultural practices.
- B. Personnel: Two research assistants to work full-time during the summer (mid-June to mid-September) and half-time during the academic year (mid-September to mid-June). The research assistants to work toward M.S. or Ph.D. degrees. Additional equipment and supplies will be required if the program is expanded.

IDAHO AGRICULTURAL EXPERIMENT STATION (A. M. Finley)

The Idaho Agricultural Experiment Station is presently conducting a minimal research program on the control of downy mildew of hops by chemicals and cultural practices. There is need for an expansion of this program to include the testing of improved plant types for adaptability, resistance to downy mildew and virus diseases. Facilities, including a small propagating house, experimental yard and research laboratory, are available for such a program, but it will be necessary to obtain additional support of a full-time subprofessional research assistant before it can be enacted.

U. S. Department of Agriculture in Cooperation with the OREGON AGRICULTURAL EXPERIMENT STATION

The Committee agreed that, in the event of expansion of the hops research in California, Idaho, and Washington, it would be necessary to increase crossing,

screening for disease resistance, and quality evaluation carried on by the ARS in cooperation with the Oregon Agricultural Experiment Station. Additional personnel with supporting funds would be required in the following fields of work:

A. Breeding and Agronomy

- 1. One subprofessional assistant.

B. Plant Pathology

- 1. One subprofessional assistant.

C. Chemistry and Quality Evaluation

- 1. One subprofessional laboratory technician.
- 2. One professional chemist (trainee).

