INFLUENCE OF MOISTURE AND LOW TEMPERATURE ON THE GERMINATION OF HOP SEEDS[1]

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INTRODUCTION

Though hop plants (*Humulus lupulus* L.) are propagated commercially by the use of cuttings from underground stems, breeding work requires the sexual propagation of the species. The writer became interested in the possibilities of low temperatures in stimulating the germination of hop seeds because of the relation of this problem to varietal improvement. Investigations in progress included the growing of seedlings for observation, selection, and determination of the results of controlled pollination with numerous individual plants. Germination of most seed lots was extremely low, ranging from 0 to 10 percent even after they had remained under apparently favorable germination conditions for several weeks. Seedlings developed from very few of the numerous seeds sown, and the number of plants from which to make selections was therefore small and the difficulties of studying the effects of controlled pollination were greatly increased. For these reasons experiments were undertaken, the results of which are presented here.

The present work is concerned with the germination aspects of treatments of hop seeds, including moisture and temperature factors. The term "dormancy" is used in a general sense, no attempt having been made to study the nature of the seed in its relation to germination. The investigations were made at Corvallis, Oreg.

REVIEW OF LITERATURE

In recent years much has been written concerning the effects of various treatments on the physiological behavior of seeds and plants. The results of subjecting dormant seeds or seeds in early stages of germination to controlled-moisture and low-temperature conditions have been studied both by those interested in germination and by others concerned principally with the effects on subsequent growth.

Crocker (*2*)[2] and Toole (*9*) presented discussions of the general aspects of seed dormancy and indicated certain factors as influential in hastening afterripening. Pertinent literature was also cited by these writers. Nichols (*6*) studied the influence of low temperatures on seed germination and found growth of certain native species to be hastened by exposure to cold. Mirov (*5*) reported that low-temperature treatments stimulated germination in certain coniferous species.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 380.

Haut (*4*) found low-temperature storage at several temperatures to stimulate afterripening and promote germination of pear, apple, cherry, and peach seeds.

Raum (*7*), in a discussion of hop-improvement problems, directed attention to the difficulty of obtaining prompt and complete germination of hop seeds. Sprouting was found to be almost complete, however, if seeds were placed in pots and subjected to outdoor conditions during the winter until April. Bressman (*1*) referred to the use of chilling and scarification in stimulating hop seed germination. The treatment suggested was 10 days at freezing temperatures followed by scarification with a coarse emery cloth.

Though other workers in hop breeding have undoubtedly encountered the problem of initial low germination of hop seeds under certain methods of handling, no references to this subject other than those noted have been seen by the writer.

MATERIALS AND METHODS

In preliminary experiments, 1934, seeds of Late Clusters, Early Clusters, Fuggles, and Red Vine, principal hop varieties of the Pacific coast, were placed upon moist blotters in Petri dishes. Conditions for germination were kept favorable with respect to moisture, and temperatures ranged from 15° to 23° C. The dishes were kept in indirect light. A similar series was incubated in blotters in a standard germination chamber at room temperature. No pretreatments of any kind were given, but midway through the period the seeds and substratum became very moldy. The seeds and Petri dishes were then washed in a dilute solution of an organic mercury disinfectant, new blotters were supplied, and incubation was continued as before.

Incidental tests were made also with several periods of refrigeration at different low temperatures. Eight lots of seeds of each of the four varieties were placed in cold storage at 5° and −12° C. for periods of 2, 4, and 6 weeks at the former and 2 weeks at the latter temperature. The seeds were sown in soil at the termination of the periods, and observations were made on germination.

A more extensive experiment was completed in 1936. Lots of seeds of the four varieties previously studied were tested with four incubation periods before cold storage, five periods of refrigeration, and two temperatures. The combinations of these variables in treatment series comprised 40 tests of each variety, as listed in table 2. The tests were made in triplicate, and three check series were also included. Check 1 consisted of seed lots that received no wetting, incubation period, or cold storage but were sown, without any treatment, direct in the soil. Check 2 was not wetted or incubated in the germinator but was stored 2 weeks at −12° C. Check 3 was treated like check 2 except that the cold storage was at 5°.

Dry seeds were counted out in lots of 100 each. They were then placed in folded, moist paper toweling and incubated in a standard germinator for the periods indicated. Care was taken to avoid exclusion of air in folding the toweling. At the end of the incubation period the lots were immediately placed in refrigeration and left for the desired time. They were then removed, and the seeds were planted one-half inch deep in short rows in greenhouse benches. Lots

of the —12° C. series were allowed to thaw 24 hours at about 70° F. before seeding.

The term "incubation period," as used herein, refers to the period in the germinator during which no seeds germinated. Germination percentages were noted at intervals of 2 weeks from the date of seeding of each lot, percentages being recorded for 2, 4, 6, and 8 weeks after seeding.

In order to obtain further information on the variability of germination among seed lots from different plants, germination tests were made with seeds of the same crop year from 204 individual plants. All lots were first subjected to 2 weeks' refrigeration at 5° C. One series was then sown in field coldframes without bottom heat with outdoor temperatures ranging from 20° to 80° F. A second series was sown in greenhouse beds, with temperatures during germination ranging from 40° to 90° F. Material planted in coldframes was subject to seasonal rains; that in the greenhouse was artificially watered.

It is a common observation among growers that hop seedlings begin to appear in late January or February under field conditions in western Oregon. These develop from seeded hop cones that fall to the ground during harvest or remain on the vines when the latter are cut down following picking. The seeds evidently undergo a rest period in the field and begin to germinate in late winter. In the fall and winter of 1935–36 seeds were gathered September 25, November 25, and January 25 from the same individual plants in the field. The plants represented unnamed seedlings varying widely in morphological characters. After the collection of the last lot the samples of the three collections were seeded in greenhouse benches, and observations were made on germination. This test was not replicated.

In the experiments outlined, care was taken to include only sound seeds, since many apparent "seeds" are only hulls. Seeds of varieties and of individual plants varied in size to some extent. In threshing the seeds from the strobiles by hand the persistent perianth was not removed. No seeds germinated in the incubation period and none were observed to be markedly swelled by this treatment. Unless otherwise noted, 100 seeds were included in each lot.

The soil used in the cultures, both field and greenhouse, was a medium sandy loam of the Chehalis series, and no commercial or other fertilizer was applied. The soil was kept reasonably moist. Seeds were considered to be germinated when the seedlings were beginning the third leaf. No damping-off or other seedling disease was noted in any of the trials. During the period of germination greenhouse temperatures varied between 45° and 90° F., the most frequent range being from 60° to 80° F. The type of refrigeration used was that provided by a small ammonia plant with temperatures kept reasonably constant. No variations of more than 3° C. above or below the temperatures indicated were observed.

RESULTS

PRELIMINARY GERMINATION TESTS

Data for the preliminary tests of germination in blotters and Petri dishes are given in table 1. The figures represent additional seeds that germinated at each successive date. Since there were but 50

seeds in each lot, percentage may be derived by multiplying the figures by 2 in each instance. Though the Petri dishes were kept in daylight and the blotters in a standard germinator, it is obvious that even after long periods seeds may fail to sprout. Very few seeds softened during the period.

TABLE 1.—*Germination of hop seeds of several varieties in Petri dishes and germination blotters* [1]

Variety	Additional seeds germinated after indicated incubation period (days) in—																			
	Blotters										Petri dishes									
	15	20	25	30	35	45	50	60	70	75	15	20	25	30	35	45	50	60	70	75
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Red Vine	4	1	0	0	0	0	1	0	0	0	1	2	1	0	0	0	0	0	0	0
	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fuggles	1	0	0	0	0	3	1	0	0	1	1	0	0	1	0	0	0	1	1	1
	0	0	0	1	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Late Clusters	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	1	0	1
Early clusters	0	0	0	1	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] 50 seeds were used in each test.

Additional preliminary data, derived from subjecting to low temperatures seeds from the same lots as those used in the blotter and Petri-dish tests, indicated that germination was modified favorably thereby. Though the trials were cursory, relative effects of refrigeration periods showed 6-, 4-, and 2-week periods at 5° C., 2 weeks at −12°, and the check to rank in the order named in germination percentage.

MOISTURE AND TEMPERATURE RELATIONS

Table 2 contains data secured from a more extensive study of the interrelation of varieties, periods of incubation before refrigeration, refrigeration periods, and refrigeration temperatures with germination. Though little attention is given to the germination data for other than the final germination date, the values for the earlier dates may be of interest and are therefore included. Each germination value in table 2 represents an average of three replications. Check 1 included seed lots without treatment; checks 2 and 3 were held for 2 weeks at −12° and 5° C., respectively.

In table 3 the averages of each treatment for the last date of observation have been recorded to facilitate comparisons.

In general, variations between replicates were large. Throughout the 40 experimental series, percentage germinations ranged from very low to fairly high values, as the data indicate. Inclusion of all series in a determination of a generalized error, such as the analysis of variance, was therefore inadvisable. It was possible, however, to analyze series 23 to 25, 28 to 30, 33 to 35, and 38 to 40 by such a method (*3*) without serious infringement on data limitations. The results so analyzed are for the eighth-week observations only.

TABLE 2.—*Effect of various periods of incubation and refrigeration (at −12° and 5° C.) on germination of 4 varieties of hops 2, 4, 6, and 8 weeks after seeding*

Series No.	Incubation in germinator	Refrigeration		Germination [1] in indicated number of weeks after seeding—															
		Duration	Temperature	Early Clusters				Late Clusters				Fuggles				Red Vine			
				2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
	Days	*Weeks*	*°C.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	2	1	−12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.33	0.67	0.00	0.00	0.00	0.33
2	2	2	−12	.00	.00	.00	.33	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
3	2	3	−12	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.33	.33	.00	.33	.67	.67
4	2	4	−12	.00	.00	.67	.67	.00	1.67	2.33	2.33	.00	.67	1.00	1.00	.33	2.00	2.00	2.00
5	2	5	−12	.00	.00	.00	.67	.00	1.67	2.67	3.33	.33	.67	.67	1.33	.33	1.67	2.33	3.33
6	5	1	−12	.00	.00	.00	.33	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
7	5	2	−12	.00	.00	.00	.00	.00	.00	.33	.33	.00	.00	.00	.33	.00	.67	1.00	1.33
8	5	3	−12	.00	.00	.00	.00	.00	.00	.00	.33	.00	.00	.00	.00	.00	.33	1.33	1.33
9	5	4	−12	.00	.00	.33	1.33	.00	.00	.00	.33	.00	.33	.33	1.00	.00	.00	.00	2.67
10	5	5	−12	.00	.33	.33	2.00	.00	4.00	5.33	5.67	.33	.33	.67	.67	.00	3.33	6.00	9.67
11	10	1	−12	.00	.00	.00	.00	.33	.33	.33	.67	.00	.00	.00	.33	.33	.33	.67	1.00
12	10	2	−12	.33	.33	.67	.67	.00	.00	.33	.67	.00	.00	.00	.00	1.00	4.33	6.00	7.00
13	10	3	−12	.00	.33	.33	.33	.33	.33	.33	.33	.00	.00	.00	.00	.00	.33	1.33	1.33
14	10	4	−12	.00	.00	.00	.33	.00	.33	.67	.67	.00	.33	.33	1.00	.33	.33	.33	1.67
15	10	5	−12	2.00	9.33	13.00	13.00	3.00	14.67	16.67	16.67	1.00	16.33	20.33	20.33	3.00	27.33	34.33	34.33
16	15	1	−12	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.67	1.00
17	15	2	−12	.00	.33	1.67	1.67	.00	.00	.00	.00	.00	.67	.67	.67	.33	1.00	1.00	1.00
18	15	3	−12	.33	14.33	24.00	24.33	.00	4.00	6.67	7.67	.00	7.67	19.67	24.33	.00	22.67	38.00	42.67
19	15	4	−12	.33	3.33	8.67	8.67	2.00	17.67	25.67	25.67	5.33	19.00	23.00	23.00	1.33	15.67	23.67	23.67
20	15	5	−12	2.67	15.00	20.33	20.33	.33	8.33	14.00	14.00	.33	4.33	14.33	14.33	4.33	19.67	23.00	23.00
21	2	1	5	.00	.00	.00	.00	2.00	2.67	3.00	3.67	.00	.00	.00	.33	.00	.33	1.00	2.00
22	2	2	5	4.67	5.00	5.00	5.00	13.33	15.33	16.33	16.33	.00	.00	.67	1.00	6.00	6.67	7.67	9.00
23	2	3	5	6.00	9.67	10.67	10.67	30.33	39.33	41.33	41.67	5.00	12.00	13.67	13.67	19.33	26.67	27.33	27.33
24	2	4	5	40.00	48.67	49.67	49.67	44.00	52.33	52.67	52.67	35.67	40.67	42.67	42.67	51.00	61.00	61.33	61.33
25	2	5	5	32.00	63.67	64.00	64.67	24.67	45.33	46.33	47.00	29.00	70.33	72.00	72.67	33.33	66.67	67.33	68.00
26	5	1	5	.67	.67	.67	1.33	4.00	5.33	5.33	5.33	.00	.00	.33	1.33	2.67	3.67	4.00	5.00
27	5	2	5	14.67	16.67	17.00	17.00	7.33	9.00	9.33	9.33	.00	3.33	4.00	4.33	11.33	12.33	12.67	13.00
28	5	3	5	13.33	18.67	19.67	19.67	3.67	10.00	10.67	10.67	8.00	12.33	14.33	15.00	1.00	6.00	6.67	7.67
29	5	4	5	.33	6.67	14.67	18.67	.67	8.33	11.67	13.00	.67	7.00	14.00	17.33	.67	6.33	12.67	13.33
30	5	5	5	58.00	77.00	78.33	81.33	60.00	67.00	67.33	69.00	42.33	80.67	81.33	81.67	58.00	68.33	68.33	68.33
31	10	1	5	.00	.00	.00	.33	1.67	1.67	1.67	2.00	.00	.00	.33	.33	1.33	1.33	1.33	2.33
32	10	2	5	13.00	17.67	19.67	20.00	16.33	22.33	23.67	23.67	1.00	5.33	8.33	9.00	14.67	22.67	23.00	23.33
33	10	3	5	21.67	30.00	32.00	32.00	26.67	36.67	37.67	37.67	29.00	38.00	39.67	39.67	21.33	31.67	34.33	34.33
34	10	4	5	9.33	25.33	26.00	26.33	25.00	45.33	46.00	46.33	18.00	32.67	34.00	34.00	25.67	48.33	49.33	49.67
35	10	5	5	23.33	58.67	61.33	61.33	41.33	57.00	57.33	57.33	46.67	62.00	63.00	63.00	30.00	48.67	51.00	51.00

[1] Each value is an average of 3 replications.

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TABLE 2.—*Effect of various periods of incubation and refrigeration (at −12° and 5° C.) on germination of 4 varieties of hops 2, 4, 6, and 8 weeks after seeding*—Continued

Series No.	Incubation in germinator	Refrigeration		Germination in indicated number of weeks after seeding—															
		Duration	Temperature	Early Clusters				Late Clusters				Fuggles				Red Vine			
				2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
	Days	*Weeks*	*° C.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
36	15	1	5	0.00	0.33	0.67	0.67	0.67	1.33	1.67	1.67	0.00	0.33	0.33	0.33	1.67	1.67	2.33	2.33
37	15	2	5	5.33	7.00	7.33	7.33	17.00	18.33	19.00	19.00	1.00	3.00	3.00	3.00	13.33	14.67	14.67	14.67
38	15	3	5	.00	22.00	32.00	32.33	1.33	40.33	44.67	45.67	.33	35.00	38.67	39.67	2.67	30.67	35.33	36.67
39	15	4	5	19.33	41.33	41.33	41.33	23.67	48.00	48.33	48.33	38.67	55.67	55.00	55.00	22.67	45.33	46.33	46.33
40	15	5	5	24.67	46.00	48.00	48.00	21.67	46.67	47.00	47.00	12.67	36.00	37.67	37.67	27.67	44.33	44.67	44.67
Check:																			
1 [2]				.00	2.00	2.00	2.33	.00	2.33	6.33	7.33	.00	.00	.00	.33	.33	.67	5.67	6.33
2 [3]				.00	.67	1.67	1.67	.67	1.67	3.33	3.33	.00	.67	1.33	1.67	14.33	16.00	17.00	17.00
3 [4]				.33	.67	1.33	1.67	.00	.67	1.33	2.00	.00	.33	.67	1.33	1.33	2.67	3.00	4.00

[2] Seeds received no treatment. [3] Seeds were stored 2 weeks at −12° C. without other treatment. [4] Seeds were stored 2 weeks at 5° C. without other treatment.

TABLE 3.—*Mean germination percentages of three tests for varieties, for incubation and refrigeration periods, and for temperatures of refrigeration*

Variety	Incubation in germinator	Germination after indicated refrigeration period (weeks) at specified temperature									
		1		2		3		4		5	
		−12° C.	5° C.	−12° C.	5° C.	−12° C.	5° C.	−12° C.	5° C.	−12° C.	5° C.
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Early Clusters	2	0.0	0.0	0.3	5.0	0.0	10.7	0.7	49.7	0.7	64.7
Late Clusters	2	.0	3.7	.0	16.3	.0	41.7	2.3	52.7	3.3	47.0
Fuggles	2	.7	.3	.0	1.0	.3	13.7	1.0	42.7	1.3	72.7
Red Vine	2	.3	2.0	.0	9.0	.7	27.3	2.0	61.3	3.3	68.0
Average		.25	1.50	.08	7.80	.25	23.35	1.50	51.60	2.15	63.10
Early Clusters	5	.3	1.3	.0	17.0	.0	19.7	1.3	18.7	2.0	81.3
Late Clusters	5	.0	5.3	.3	9.3	.3	10.7	.3	13.0	5.7	69.0
Fuggles	5	.0	1.3	.3	4.3	.0	15.0	1.0	17.3	.7	81.7
Red Vine	5	.0	5.0	1.3	13.0	1.3	7.7	2.7	13.3	9.7	68.3
Average		.08	3.23	.48	10.90	.40	13.28	1.33	15.58	4.53	75.08
Early Clusters	10	.0	.3	.7	20.0	.3	32.0	.3	26.3	13.0	61.3
Late Clusters	10	.7	2.0	.7	23.7	.3	37.7	.7	46.3	16.7	57.3
Fuggles	10	.3	.3	.0	9.0	.0	39.7	1.0	34.0	20.3	63.0
Red Vine	10	1.0	2.3	7.0	23.3	1.3	34.3	1.7	49.7	34.3	51.0
Average		.50	1.23	2.10	19.00	.48	35.93	.93	39.08	21.08	58.15
Early Clusters	15	.0	.7	1.7	7.3	24.3	32.3	8.7	41.3	20.3	48.0
Late Clusters	15	.0	1.7	.0	19.0	7.7	45.7	25.7	48.3	14.0	47.0
Fuggles	15	.0	.3	.7	3.0	24.3	39.7	23.0	56.0	14.3	37.7
Red Vine	15	1.0	2.3	1.0	14.7	42.7	36.7	23.7	46.3	23.0	44.7
Average		.25	1.25	.85	11.00	24.75	38.60	20.28	47.98	17.90	44.35

The results of the use of the analysis of variance are shown in table 4. The data indicate significant differences to exist among refrigeration and incubation periods. The first-order interaction between refrigeration and incubation periods is also significant. Though the comparison of temperatures of refrigeration was necessarily omitted from the analysis, reference to table 2 and comparison of series 1 to 20 with series 21 to 40 make clear at once that a highly significant difference exists. Varietal differences were not significant.

TABLE 4.—*Analysis of variance data on varieties and incubation and refrigeration periods*

Variation due to—	Degrees of freedom	Sums of squares	Mean square	F [1]
Refrigeration periods	2	26,123	13,062	[2] 96.0
Incubation periods	3	2,825	942	[2] 6.9
Varieties	3	141	47	2.9
Refrigeration×incubation	6	17,412	2,902	[2] 21.3
Refrigeration×varieties	6	1,716	286	2.1
Varieties×incubation	9	1,626	181	1.3
Varieties×incubation×refrigeration	18	3,051	170	1.3
Error	96	13,092	136	
Total	143	65,986		

[1] Snedecor (*8*).

[2] Exceeds 0.01 point.

The standard error of a single analysis is 11.66 percent; the standard error of a mean of three tests, 6.73 percent. Since variety differences

were not significant, data for all varieties have been grouped in table 5 for comparison of germination and refrigeration periods. The minimum significant difference for refrigeration general means, based upon the 0.05 point, is 4.75 percent. The 3-, 4-, and 5-week periods, therefore, differ significantly in average germination. For comparing incubation-period means, the least significant difference is 5.50 percent. Accordingly, average germination was significantly lower following 5 days' incubation for all series. It should be borne in mind that these errors apply only to the series included in the analysis of variance.

TABLE 5.—*Mean percentage germination for all varieties for different refrigeration and incubation periods*

Incubation period (days)	Germination after refrigeration for—			Average germination
	3 weeks	4 weeks	5 weeks	
	Percent	*Percent*	*Percent*	*Percent*
2	23.4	51.6	63.1	46.0
5	13.3	15.6	75.1	34.7
10	35.9	39.1	58.2	44.4
15	38.6	48.0	44.4	43.7
Average	27.8	38.6	60.2	42.2

While the check series are not included in the calculation of error, most treatments show unquestionably significant increases in germination when compared with the check lots. The high germination of the Red Vine variety in check 2 is inexplicable.

Within refrigeration and incubation periods he least significant difference, twice the standard error of a difference, is 9.52 percent. On the basis of this error, incubation periods may be compared in relation to the several periods of refrigeration.

Considering lots incubated 2 days in the germinator and refrigerated at 5° C., those left for 4 weeks at low temperatures showed significantly higher germination than those refrigerated for 3 weeks, as an average of all varieties. Lots refrigerated 5 weeks were significantly higher in germination than those remaining at low temperatures for 3 weeks.

The superiority of longer periods of refrigeration following additional days in the germinator was not so marked. Comparing lots incubated in the germinator 10 days before chilling, 5 weeks' refrigeration was better than 4. Among lots incubated in the germinator 15 days before cold storage, refrigeration for 4 weeks was significantly superior to 3 weeks' treatment.

In the lots refrigerated 3 weeks, those incubated 10 and 15 days were significantly higher in germination than those incubated 2 or 5 days. Groups incubated 2 days were also significantly higher than the 5-day incubated groups.

Five-day incubated lots were significantly lower than 2-, 10-, or 15-day lots when refrigerated 4 weeks. The 2-day lot was significantly higher than that incubated 10 days.

When the lots refrigerated 5 weeks after different incubation periods are considered, additional relations are noted. Two-day incubation lots were superior to the 15-day series. Five-day incubation lots were significantly superior to all other groups when refrigerated for 5 weeks. Ten days' incubation was significantly better than 15

days. Fifteen-day incubation groups were significantly lower than others.

The value of F also indicated a significant interaction between incubation and refrigeration periods. Examination of the data in table 5 shows that in lots refrigerated 3 weeks, long periods of incubation were followed by increased germination, while with 5 weeks' refrigeration long incubation periods resulted in decreased germination. Using the extremes as an example,

$$(23.4-63.1)-(38.6-44.4)=-33.9$$

and the least significant difference in this comparison is 13.46 percent when based on twice the standard error of the difference. Since the trends of the differences are not closely consistent, it is possible that uncontrolled factors may have influenced the results.

Considering grouped averages of the entire 40 series, certain generalizations may be made. The greatest increase in germination occurred between the second and fourth weeks after seeding. Lots refrigerated at 5° C. showed a much faster germination rate the first 2 weeks and a somewhat more rapid progress the third and fourth weeks than those stored at −12°. It should be noted that some of the seeds sprouted during the fifth week at 5°, before removal from refrigeration. Most seeds germinating in all lots did so by the end of 4 weeks, with only slight increases thereafter.

Successive weeks in cold storage at either temperature resulted in consistent increases in germination, with no tendency toward decrease indicated, except in series remaining 15 days in the germination chamber. Five weeks at −12° C. gave results very similar to those obtained from storage for 2 weeks at 5°. Seed lots stored at −12° showed consistently increased germination as the periods of incubation were prolonged, and no tendency to decrease is indicated.

COMPARISON OF GERMINATION IN GREENHOUSE AND COLDFRAMES

Germination percentages on 40 lots of seed from individual plants seeded in greenhouse beds and field coldframes are listed in table 6. These are a random sample of 204 lots studied and are listed to indicate plant variations and germination rates. These data, though unreplicated, indicate wide variations in both rapidity and amount of germination in lots of seed from different plants. Many of these differences are undoubtedly significant. Since seed was collected only when the cones were well browned and dry, differences in maturity would appear to be of little consequence in explaining variation among plants.

Average germination for 204 lots in the greenhouse at the end of 60 days was 21.17 ± 0.87 percent, while that in coldframes was 18.85 ± 0.85 percent. The P value for the difference does not quite reach the 0.05 point. The coefficient of correlation between percentages at 60 days, based on 196 plant lots, was 0.19. The P value, according to Fisher's t test (*3*), exceeds the 0.01 point. The slight average increase in greenhouse-germinated seed may have been due to the higher temperatures existing, better controlled moisture conditions, or other factors. There are instances listed where the field-planted seeds greatly exceeded in germination the same lots seeded in the greenhouse.

TABLE 6.—*Germination percentages of seed lots from individual plants sown in greenhouse or field after 14 days' incubation in moist blotters followed by 14 days' refrigeration at 5° C.*

Plant No.	Germination in indicated number of days after seeding in—					Plant No.	Germination in indicated number of days after seeding in—				
	Greenhouse			Field			Greenhouse			Field	
	15	25	40	60	60		15	25	40	60	60
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*		*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
2-4	0	3	9	9	6	25-2	3	5	6	14	41
2-33	7	18	26	30	31	25-3	0	2	5	9	24
4-31	7	18	25	32	16	25-4	2	6	9	17	13
4-33	39	41	41	48	28	25-5	9	20	21	23	6
5-32	12	13	14	25	15	26-1	21	30	35	36	21
6-32	20	29	29	36	33	26-2	4	5	8	10	7
8-10	8	16	18	18	43	26-12	5	10	10	19	15
8-12	19	30	33	33	33	27-3	8	16	18	19	12
9-7	5	14	15	19	9	27-12	13	25	28	28	37
15-32	9	13	15	16	37	27-31	8	17	21	22	10
18-31	16	26	29	29	22	29-3	17	33	44	46	34
19-5	9	14	16	16	24	29-4	22	25	25	25	14
19-13	14	27	34	34	36	29-34	6	11	28	28	19
20-13	6	13	17	21	6	32-4	7	11	20	20	11
20-33	0	3	4	12	7	32-10	10	14	15	15	39
22-10	5	14	17	21	32	35-5	6	10	11	20	18
23-30	9	12	17	22	32	37-4	11	18	21	23	7
24-4	10	25	27	30	28	37-18	6	11	14	14	29
24-32	5	8	15	19	24	37-22	0	4	9	18	5
24-34	1	1	2	17	21	38-4	0	6	8	12	42

EFFECT OF NATURAL CONDITIONS ON STIMULATION OF GERMINATION

Data obtained concerning the effects of weathering on the germination of 16 single-plant seed lots are given in table 7. September 25, the first date of collection, represents a period when the seeds might be considered as nearly mature and browning of cones well started. The November and January collections represent seeds from cones which, with the vines, had fallen to the ground and remained in proximity to the soil for approximately 50 and 110 days respectively, being subject to various weather factors. Pertinent phases of the climatic data for the period during which seeds weathered in the field are given in table 8. These may be of further interest in considering the data of germination.

TABLE 7.—*Percentages of germination of single-plant seed lots allowed to remain in the field for 2 periods following maturity* [1]

Plant No.	Germination, in indicated number of days after sowing, of seeds collected—														
	Sept. 25					Nov. 25					Jan. 25				
	10	25	40	55	70	10	25	40	55	70	5	10	25	35	50
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
2-4	0	0	0	0	0	6	16	16	16	17	26	43	44	44	45
8-12	0	0	0	0	0	0	3	3	3	3	6	11	16	16	16
15-32	0	0	0	0	0	0	1	2	2	2	7	12	26	26	26
18-31	0	1	1	1	2	14	26	26	26	27	2	9	21	23	23
19-5	0	1	1	1	1	1	8	9	9	12	25	37	45	45	46
20-33	0	0	0	0	0	6	12	12	12	12					
22-10	0	0	0	0	0	0	1	1	2	2	11	16	22	23	23
25-4	0	0	0	1	1	2	3	3	4	5	12	16	24	27	28
26-12	0	0	0	0	1	3	4	4	4	4	5	12	13	15	16
27-3	0	0	0	0	0	5	9	9	9	10	10	32	43	43	44
27-31	0	0	0	0	0	2	2	2	2	2	8	9	10	10	11
29-4	0	1	1	1	0	0	9	11	11	11	13	16	21	22	23
38-31	0	0	0	0	0	0	2	2	2	2	7	15	28	29	29
40-33	0	0	0	0	0	0	0	0	0	0	15	31	33	34	34
45-32	0	0	0	0	0	0	0	0	0	0	3	7	24	27	28
47-32	0	0	0	0	0	0	2	2	3	4	6	11	16	19	20
Average	0	0.2	0.2	0.3	0.3	2.4	6.1	6.4	6.6	7.1	10.4	18.5	25.7	26.9	27.5

[1] Data based on 100 seeds of each sown in greenhouse benches.

TABLE 8.—*Summary of weather data for September to December 1935, and January 1936, at Corvallis, Oreg.*

Month	Precipitation		Temperature			
			Maximum		Minimum	
			Mean	Range	Mean	Range
	Days	*Inches*	*° F.*	*° F.*	*° F.*	*° F.*
September	2	1.36	81.7	69–97	51.3	43–59
October	12	[1] 1.95	63.1	36–84	42.6	30–57
November	12	2.26	49.9	36–59	35.5	15–46
December	15	4.76	46.8	39–58	34.3	24–46
January	19	10.82	49.9	44–58	38.1	24–46

[1] 5 inches of snow Oct. 31 not included.

The results show the natural increase in germinating capacity of seeds left to weather in the field and demonstrate the collective influence of the various factors in overcoming dormancy under field conditions. Again variation between lots of seeds from different plants may be noted. In two lots only did seeds collected November 25 fail to germinate better than those obtained September 25, though some differences are small. Seeds gathered January 25 germinated better than those collected November 25 in all lots except one. Seeds from plants 40–33 and 45–32 required longer exposures in the field to induce germination.

DISCUSSION

The results of the experiments have shown that subjection of hop seeds to certain low-temperature treatments exerts a marked beneficial influence on germination percentage. From the data presented it seems probable that longer periods of incubation before storage at −12° C. would have stimulated germination still more. The length of the incubation period seemed to be unrelated to germination following storage at 5°. It also seems likely that periods of refrigeration longer than 5 weeks might have resulted in increased germination.

In the experiment concerned with moisture and temperature treatments, considerable variation in germination existed among varieties. While significant differences were not established, it appears probable that the Red Vine variety might have reacted somewhat differently from the other strains, particularly at storage temperatures of −12° C. More replications would have been desirable to establish variety differences.

The data of table 6 are of special interest in showing wide variations in the germinating habits of seeds from different plants. It should be recalled that hop plants are dioecious. Since the pollination process was uncontrolled, widely different males may have contributed to seed formation in the numerous female plants. Correlation of germination percentages of seed lots from the same plants from year to year would serve to further delineate this character. While treatment of seeds by germinator incubation for 5 days, followed by 5 weeks' storage at 5° C., appeared to be the best of the series used in these experiments, certain seed lots from various female seedlings used in breeding might respond better to other conditions.

From the standpoint of progress in breeding investigations, subjecting seeds derived from controlled pollination to periods of low-temperature storage shortly after maturity allows for greater latitude in utilization and study and more careful control of the material than awaiting germination out of doors. Germination in the former instance may also be more rapid and more complete.

The effects of the treatments on growth after germination were not carefully observed. Differences between lots were probably slight, since the seedlings appeared to be extremely uniform in this respect as growth progressed.

SUMMARY

Germination is very low when recently harvested hop seeds are placed under ordinary conditions of moisture and temperature such as are favorable to the germination of seeds of many plants. Hop seeds undergo a period of dormancy upon maturity of the cone, which, under natural conditions, may be overcome by interaction of weather factors. Seeds from individual plants vary greatly in rate and total amount of germination both under natural conditions and after artificial treatments.

No significant differences were established in reaction among varieties.

The relations of moisture, temperature, and variety to seed germination have been studied. Five days' incubation in the germinator followed by 5 weeks' refrigeration at 5° C. gave the highest germination in the treatments used. Many treatment series exceeded the check lots significantly.

In these experiments storage of moist seeds at 5° C. was greatly superior to storage at −12° in stimulating germination. Three-, four-, and five-week storage periods at 5° resulted in significantly increased germination as the length of the period increased.

Generally longer periods of incubation were accompanied by increased germination in the series refrigerated at −12° C. In the effects upon germination, interrelation of periods of incubation in the germinator and length of the subsequent refrigeration periods at 5° was indicated.

In the general study of incubation and refrigeration periods and temperatures, most germination occurred between 2 and 4 weeks after seeding, regardless of treatment. Hop seeds sprouted at 5° C. after 4 weeks' refrigeration at that temperature.

Total germination of seed lots of 196 plants after 2 weeks' refrigeration at 5° C. and after 60 days in field and greenhouse was significantly correlated and not significantly different.

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