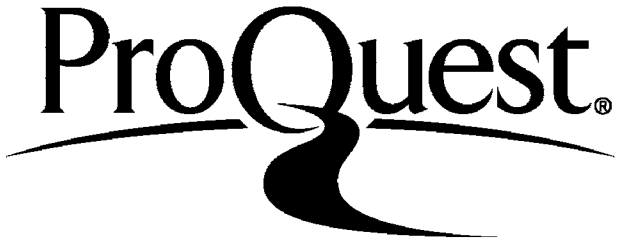


ABSTRACT. Hewlett

The greater part of the work deals with the effects produced by reduction of the oxygen pressure in the atmosphere surrounding seeds kept in germinative conditions. This was studied with the view to the establishment of correlations between the value of the minimum oxygen pressure necessary for germination and 1) the nature of the food reserve or 2) the extent of aeration of the soil of the usual habitat.

After the trial of several forms of apparatus the 'continuous-current' method was adapted to the needs of this problem. Atmospheres with reduced oxygen pressures were obtained by introducing oxygen, produced electrolytically, into a stram of nitrogen supplied from a cylinder.

The seeds used in the investigations combined some of the following characteristics:-

1) Presence of absence of endosperm.

2) Carbohydrate or oil as food-reserve.

3) Soil of habitat usually stiff and poorly-aerated or light and well aerated.

The results indicate that high minimum oxygen values may be expected for seeds storing oils and usually growing in well-aerated soils whilst low minima are connected with the storage of carbohydrates and the usual occurrence in badly aerated soils.

Whilst working on the above it was found necessary to sterilize the seeds. The method recommended by Wilson (A.J.B.1915) was used; but bleaching powder is a strong oxidizing agent and its action upon seed reserves might affect the rate of germination of seeds sterilized in this way. Accordingly a section of the paper embodies attempts made to solve this problem and to determine whether a solution of the specified strength is capable of any action upon the oils stored in seeds.

The results indicate a slight stimulatory effect upon the rate of germination of seeds storing oils after sterilization for short periods of time and that such a solution does not react with some classes of oils found stored in seeds.

C O N T E N T S.

INTRODUCTION.

The work on the germination of seeds in reduced oxygen pressure was begun in the autumn of 1933; it is the outcome of the following considerations. It has long been known that some seeds germinate better when sown in light soils and when covered with only a very thin layer of well-powdered soil and that germination of such seeds may be completely prevented by sowing them in a heavy soil and covering them with a thick layer of soil; again there are other seeds which germinate more satisfactorily when planted in a heavy soil with a thick covering layer of soil. In a number of cases it has been shewn conclusively that the soil conditions in which seeds of many species germinate most freely are those which are most frequently found in the habitats where the particular species usually occurs. This is especially the case when the plant in confined to habitats with very special soil conditions.

Morinaga (1) found that Typha latifolia and Cynodon dactylon gave a higher percentage germination when the oxygen pressure of the air is reduced by dilution with hydrogen or nitrogen - favorable mixtures were obtained by diluting air with 40 - 80 % by volume of these gases.

W. Crocker (2) (3) beleives that the oxygen pressures necessary for the germination of the seeds of many water plants have extremely low minimum values. This he correlates with the fact that under normal conditions the physiological processes of these seeds are carried on under water. Takahashi (4) and others have reported the germination of Oryza in the complete or almost complete absence of oxygen.

The results of these workers indicate that some seeds can germinate in atmospheres with very low oxygen pressures. It is noteworthy that the seeds which have given these results, are of plants which normally grow in water-logged soils containing little free oxygen. Thus it would seem that a low minimum oxygen pressure is often associated with the germination of seeds of plants which are confined to heavy water-logged soils; furthermore, as Morinaga has shown for Typha latifolia, a low oxygen pressure is frequently very beneficial to their germination.

With this in mind it was thought that it would be interesting to make a study of the oxygen minima necessary for the germination of certain seeds growing in various habitats, and also to attempt to find out whether there is any connection between the value of the oxygen minimum and the class of food reserve in the seed.

Accordingly methods for studying the oxygen minima were devised and given trial. Early in the course of experimentation it was found impossible to prevent an active growth of moulds on the seeds. As growing the seeds in an atmos-

phere deficient in oxygen hindered the process of germination to such an extent that the seeds had been completely destroyed by these moulds before a definite result was obtained; some suitable method for sterilizing the seeds had to be found. At first it was decided to use 1 % mercuric chloride, but this is a difficult disinfectant to use because great care has to be taken in the thorough wetting of the seed-coats and also in the subsequent washing of the seeds.

Later it was decided to try, as sterilizing agent, a solution of calcium hypochlorite of approximate strength 2 % titratable chlorine. Calcium hypochlorite has been used with success in the Cornell Laboratories and its use is described fully by Wilson (5). Soon after the use of calcium hypochlorite solution a certain irregularity in the rate of germination of different lots of Pine seeds was noticed. It was thought that these irregulatities might be due to differences in the periods of time used for sterilization of the seeds with the calcium hypochlorite solution.

Consequently it was decided as a preliminary step to make a series of observations upon the effect of exposure of the seeds to calcium hypochlorite solution for varying lengths of time. An attempt was made to find out whether calcium hypochlorite ever produces a stimulatory effect upon the rate of germination - such a stimulatory effect if it occured, might account for the unusually rapid germination recorded by Knudson (6) for Calluna seeds which had been treated with this sterilising agent.

P A R T I.

A STUDY OF THE EFFECTS, PRODUCED BY THE USE OF A SOLUTION OF CALCIUM HYPOCHLORITE AS A SEED-STERILIZER, UPON THE GERMINATION OF CERTAIN SEEDS AND OF THE WAY IN WHICH THESE EFFECTS MAY VARY WITH THE LENGTH OF THE PERIOD OF STERILIZATION; TOGETHER WITH AN ATTEMPT TO EXPLAIN THE CAUSES OF THESE EFFECTS.

GENERAL CONSIDERATIONS.

K. Wilson (5) gives a full account of the use of calcium hypochlorite as a seed-sterilizer, in the raising of sterile seedlings; his paper contains directions for the preparation of a solution of suitable strength together with a table of the optimum and maxinum periods of time needed for the sterilization of a number of kinds of seeds. This list includes examples of starchy cereals and of seeds with oily food reserves. His data show that in most cases the time required to sterilize the seed effectively and that which causes injury to the same seed lie many hours apart. Thus considerable latitude is given with respect to the time during which the disinfectant may be allowed to act. This, together with the fact that calcium hypochlorite decomposes so rapidly that washing the seeds after sterilization is rendered unnecessary, makes a solution of calcium hypochlorite a very useful sterilizing agent for seeds.

Wilson carried out germination tests with seed removed at different times from the solution and compared them with similar tests made with untreated seed. He does not record any differences noticed in the total germination or in the rate of germination of treated seed when compared with those of the untreated seed although he made observations upon the tests during four weeks.

Calcium hypochlorite is a relatively powerful oxidizing agent owing to its ready decomposition with the formation of hypochlorous acid in the presence of moisture. It is due to its oxidizing properties that it is such a successful sterilizing agent; but what is the action of such an oxidizing agent upon the seed-coat and upon the food reserves contained in the seed which is sterilized? If there is an action does it in any circumstances affect the rate of germination and how does its effect vary with the length of time of exposure to the sterilizing agent? These were questions which presented themselves at the beginning of 1934 when the varying rate of germination of Pinus silvestris was observed. It was thought possible that this might be due to insufficient care being taken to immerse the seeds for equal periods of time in the calcium hypochlorite solution during sterilization.

METHOD.

Accordingly attempts were made to determine whether any of the above considerations actually affected the course of the early stages of germination. Tests were made upon seeds with both oily and starchy food reserves. The tests were carried out in the following way.

A suitable number of seeds were selected care being taken to eliminate all those seeds which seemed unlikely to produce good healthy seedlings and also to obtain a representative lot of seed; thus, as far as possible, each lot was comparable to the others in the same experiment. The seeds were sterilized for different known lengths of time by immersion in a solution of calcium hypochlorite, prepared according to the method described by Wilson. In the earlier tests the seed-coats were thoroughly wetted before sterilization by centrifuging the seeds briskly for a short time; later this was found to be unnecessary owing to the comparatively long time the seeds were left in the solution. After treatment with calcium hypochlorite solution the seeds were washed three times with sterile distilled water and were put out on filter paper in petri dishes with a small quantity of distilled water. It was found necessary to set up the tests in a sterilizing room and to autoclave the dishes, filter paper and water used. All the tests were kept at room tem-

perature and each test was considered with special reference to the members of the series to which it belonged and only generally compared with the members of other series of tests. No definite conclusion could be reached by a more than general comparison in the latter case as it was inconvenient to maintain a suitable constant temperature at which to grow the seeds; also it is impossible without great labour to make up two solutions of calcium hypochlorite containing the same percentage of titratable chlorine. Thus for each series of tests the strength of the sterilizing solution was found by titration with standard sodium thiosulphate solution and the temperature was either recorded on a continuous temperature chart or else indicated by readings taken once a day. Usually the seeds were covered with black paper so that they were growing in almost complete darkness.

The tests with treated seed were compared with control tests with untreated seed which were made in each experiment. These latter consisted of seeds soaked in distilled water for known periods of time, some of which corresponded to those used for sterilizing the treated tests. In this way it was hoped to overcome the effects due to absorption of water by the treated seeds during sterilization. In later experiments a control test was made for each member in a series of tests. This precaution was rendered advisable by the results recorded by Shull (7) and others on the effect of varying periods of time of soaking on the germination of seeds.

As far as possible, the tests were observed each day, the number of germinations were recorded and any marked deviation from the normal course of germination which might be due to sterilization was noted. In the cases where smaller seeds were used it was found necessary to open the petri dishes each day and to count the germinations by removing the young seedlings. In experiments with larger seeds this was only necessary when the seedlings obscured the vision and when more water had to be added. In each experiment, when one member had to be opened, all members of the series were opened to give as far as possible equal conditions of aeration etc. to all tests in that series.

Often it is difficult to decide when a seed is germinated as there are so many gradations between a seed which is cracked owing to absorption of water and one which is actually going to produce a seedling capable of normal healthy growth. Germination was considered to have occured when the radicle was just long enough to touch the filter paper i.e. to show geotropic curvature.

EXPERIMENTAL TESTS AND RESULTS.

The results obtained from two preliminary experiments in this series of investigations are shewn in Tables Ia and Ib. Seeds with different classes of food reserve - Pinus silvestris with oily endosperm and Zea mais with starchy endosperm containing a small quantity of oil - were treated with a solution of calcium hypochlorite for the periods of time shewn in the Tables.

The total germination of P. silvestris was very poor, partly due to fungal infection in the later stages brought about by failing to use a sterilizing room when opening the dishes. However, the results seemed to indicate that the seeds sterilized for 35 mins, and also to a lesser degree those sterilized for 1 hour, had germinated at a slightly greater initial rate than the seeds of the control test, whilst sterilization for 2 hours seemed to have exerted a slightly depressing effect.

The total germination of Zea mais was quite satisfactory. The results suggested that sterilization by this agent for any period of time, with the possible exception of 15 minutes, is detrimental to the rate of germination of the seeds and also to the total germination. Several of the seedlings in the 1 hour test exhibited poor root development and did not seem to be quite normal.

No great confidence could be placed in these results because the tests were only roughly carried out and unsoaked seeds had been used for the control tests but they were thought to be sufficiently interesting to justify the continuance of these investigations; there was a definite indication of stimulation of rate of germination by 15 - 60 minutes treatment of the sterilising agent in the case of Pinus having oily reserves, and no such effect using Zea with starch reserves.

TABLE Ia.

Pinus silvestris - Eastern Counties F. M. 1933.

Date of experiment. 27. X. '34.

No: of seeds in each test. 50.

Duration of expt. in days.	Total Number of Seeds shewing germination.				
	Control (unsoaked)	Sterilized with Ca(OCl)Cl solution.			
		15 min:	35 min:	1 hr.	2 hr.
5	1	1	-	1	2
7	4	4	4	3	2
9	6	7	12	10	6
10	8	9	13	13	8
11	12	10	15	13	9
12	15	12	18	14	12
13	16	15	19	14	12
14	17	17	21	17	16
16	17	18	21	19	17
18	17	22	23	19	18

Figure 1

Curves indicating the Rate of Germination of Pinus silvestris after sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite

Data from Table Ia

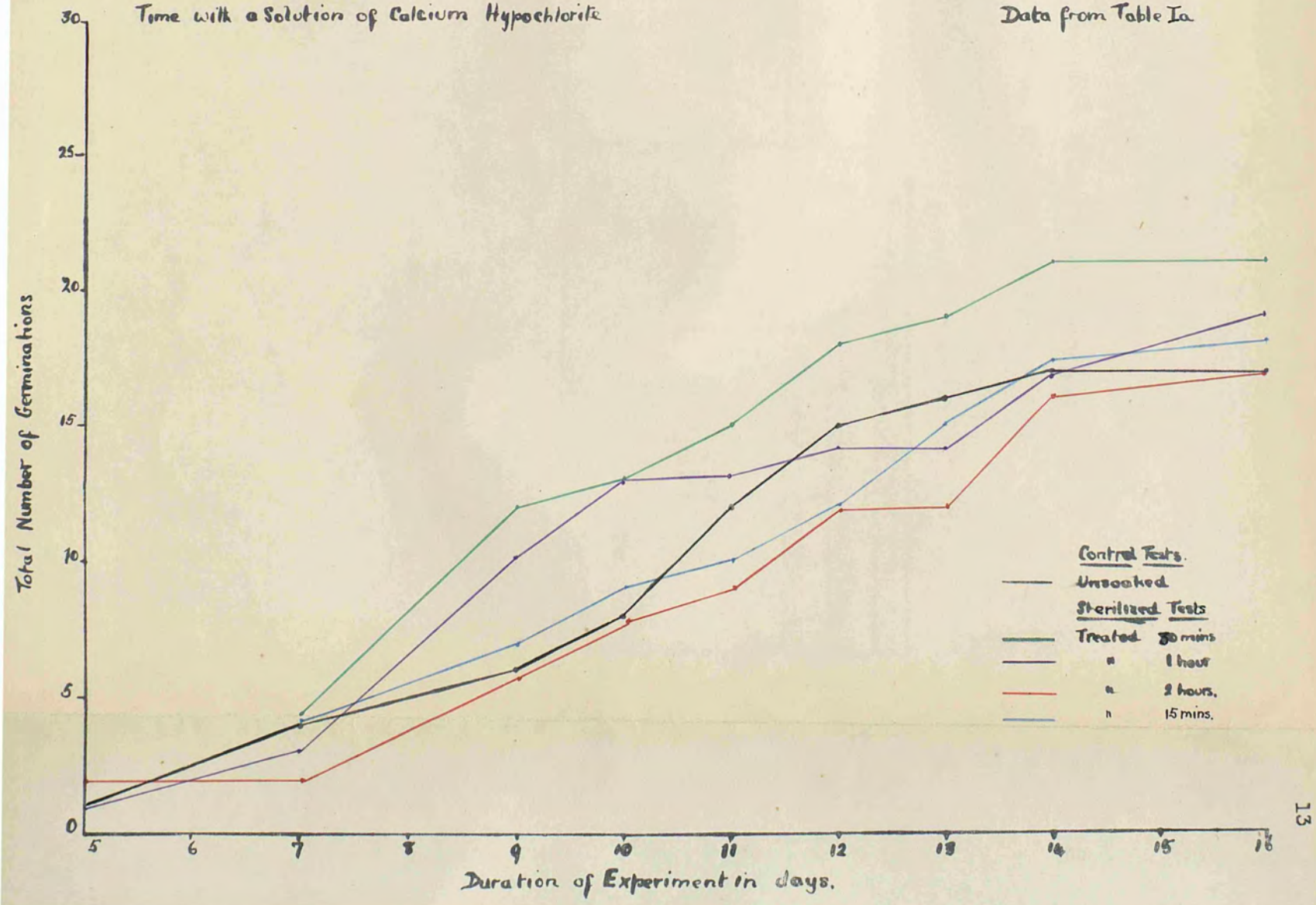


TABLE Ib.

Zea Mais - Carter's White Horsetooth 1933.

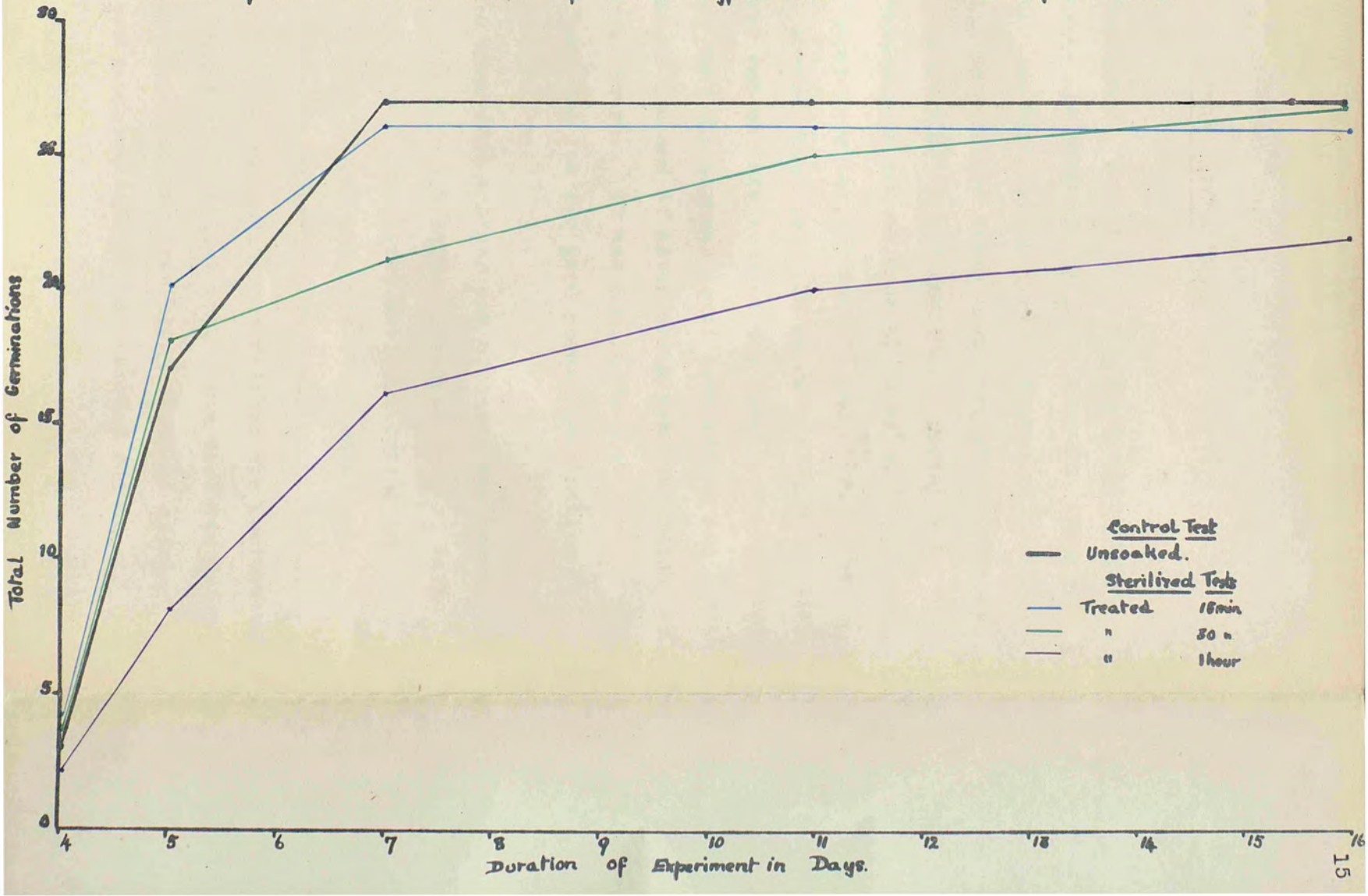
Date of experiment. 27. X. '34.

No: of seeds in each test. 28.

Duration of expt. in days.	Total Number of Seeds shewing germination.			
	Control (unsoaked)	Sterilized with Ca(OCl)Cl solution.		
		15 min:	30 min:	1 hr:
4	3	4	3	2
5	17	20	18	8
7	27	26	21	16
11	27	26	25	20
16	27	26	27	22

Figure 2

Curves indicating the Rate of Germination of Zea Mais after Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite Data from Table II.



PINUS SILVESTRIS.

After the preliminary experiment with Pinus silvestris, another set of tests was made on the same lines in January 1935. The tests were kept in the garden laboratory as it was thought that a temperature lower than that of the laboratory might prove beneficial to germination. During the experiment the temperature ranged from 41° - 61° F. but on an average the temperature lay between 45° and 55° F. The experiment was observed throughout six weeks but again germination was very poor and on this account the results are not worth recording, except that the highest total germination and the most sterile test at the end of seven weeks was that which had been treated for 17 hours. It was concluded that the temperature had been too low for good normal germination to occur.

Later in the same term another set of tests was begun with P. silvestris seed from Scotland obtained from J. Rafn & Sons 1934. These tests were very satisfactory; good germination, as much as 88 and 92%, was obtained and the control tests did not become heavily infected with moulds. Three tests were duplicated to discover whether the members of each pair would behave in a similar way. One test was made with seeds whose seed-coats had been cracked before sterilization, this was compared with a test carried out in

the usual way and treated for an equal period of time. The results obtained from this series of tests are shewn in Table II and are illustrated in Figs. 3, 4, and 6. Fig. 3 indicates the extent of the deviation in rate of germination which may be expected between two tests treated apparently in exactly the same way. Fig. 4 shews the rates of germination of the various tests; as the tests have been in contact with water for different periods of time during sterilization the inferences suggested by these curves must be treated with caution.

This source of error was eliminated by a third series of tests made with a control test for each period of sterilization. Again very satisfactory germination was obtained and the seedlings in all tests seemed healthy. The results are shewn in Table III and are illustrated in Fig. 5 and 6. Fig. 5 was obtained by plotting the number of germinations in any test against time and is meant for comparison with Fig. 4. The curves rise more rapidly than those in Fig. 4 indicating a more rapid rate of germination in all tests; this is due, without doubt, to the higher temperature at which the tests were kept. Fig.6 was obtained by plotting on the same graph the rates of germination of the treated and control tests for each period of time. In this way any increase in the rate of germination due to sterilization with calcium hypochlorite, can be detected immediately.

The results of the above series of tests lead to the following conclusions. The deviation between the rates of

germination of the members of a pair of tests treated in the same way is not great; but one member often shews 4 or 5 more germinations than the other on any day and in extreme cases this deviation may be as much as 10. From Fig. 6 it would seem that no marked stimulation in the rate of germination is exhibited as a result of sterilization for any period. A slight increase in rate is brought out by these curves for the seeds sterilized for 1 hour and to a lesser extent for the 35 minutes and 2 hour periods. This seeming increase in rate is so slight that it lies well within the range of deviation which might be expected from two tests treated in the same way. Periods of sterilization longer than 2 hours have a definite depressing effect upon the inital rate of germination and probably also upon the total germination - though at the end of a month the numbers of germinations in all tests of the second series differed from each other by quantities less than 10. Cracking the seed-coats before sterilization for 6 hours and thus allowing the solution to come into contact with the endosperm and embryo has a definite deleterious effect upon the number of healthy seedlings arising in the test, though it may result in a slightly greater initial rate of germination. This increase is also well within the possible range of deviation.

When Figs. 4 and 5 are compared it is noticed that the curves representing the rates of germination of all tests in Fig. 5 are steeper and lie closer together than those in Fig. 4. The two series of tests represented by these figures

differed only in the temperature at which they were kept; thus it seems probable that the effects of sterilization are more easily discernible at lower temperatures. With this in view it is better not to emphasize too much the similarity which exists between the results indicated in Fig. 6 and the range of deviation between two similar tests, obtained from the earlier series which was carried out at a lower temperature. The results of these tests concur with those obtained in the preliminary series and suggest the occurence of a slight stimulation when seeds of Pinus silvestris are sterilized for periods of time ranging from 35 minutes - 2 hours; but longer periods of sterilization excercise a depressing effect upon the initial rate of germination and probably upon the total germination

TABLE II.

Pinus silvestris. J.Rafn and Sons 1934.

Date of experiment. 29. 3. '35.

No. of seeds in each test. 50. Strength of Ca(OCl)Cl soln. = 1·8 grm Cl_2 per 100c.c soln.

Duration of expt. in days	Tempt.	Total Number of Seeds shewing Germination.									
		Control tests. Soaked.			Sterilized with Ca(OCl)Cl solution.						
		3¾hrs	17½hrs	17½hrs	1hr	1hr	3½hrs	5¾hrs	5¾hrs x	17hrs	17½hr
5	17° C	2	10	4	1	1	2	-	1	2	3
6	16° C	3	11	8	3	4	3	1	3	4	8
7	16° C	10	14	12	4	9	6	6	6	5	11
9		18	24	19	11	18	19	9	11	13	16
10	16½° C	23	29	22	17	22	25	12	14	15	23
11	18° C	27	32	25	22	27	31	17	16	17	26
12	17½° C	31	36	29	25	28	34	22	17	18	27
13	16½° C	34	37	36	30	35	39	27	18	21	30
14	18° C	35	40	40	33	36	39	28	19	23	34
17		39	41	41	34	38	44	34	22	25	35
31		40	43	44	40	40	44	40	23	39	42

x Seed-coats of this test were cracked before sterilization.

Figure 8

Curves illustrating the Extent of the Deviation which may occur between the Rates of Germination of two tests of Pinus silvestris treated in the same way.

Data from Table II

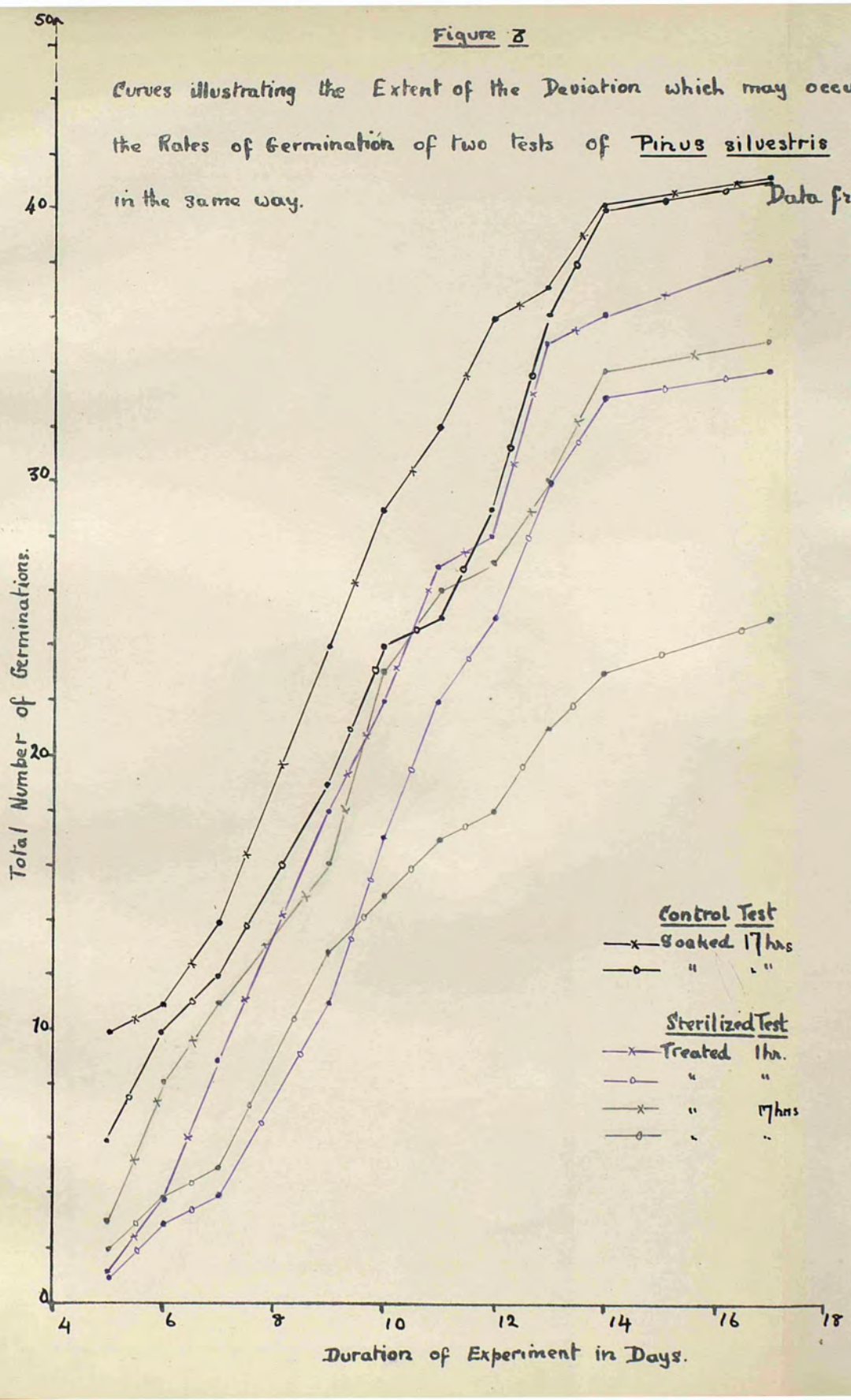


Figure 4

Curves indicating the Rate of Germination of Pinus silvestris after Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite. Data from Table II.

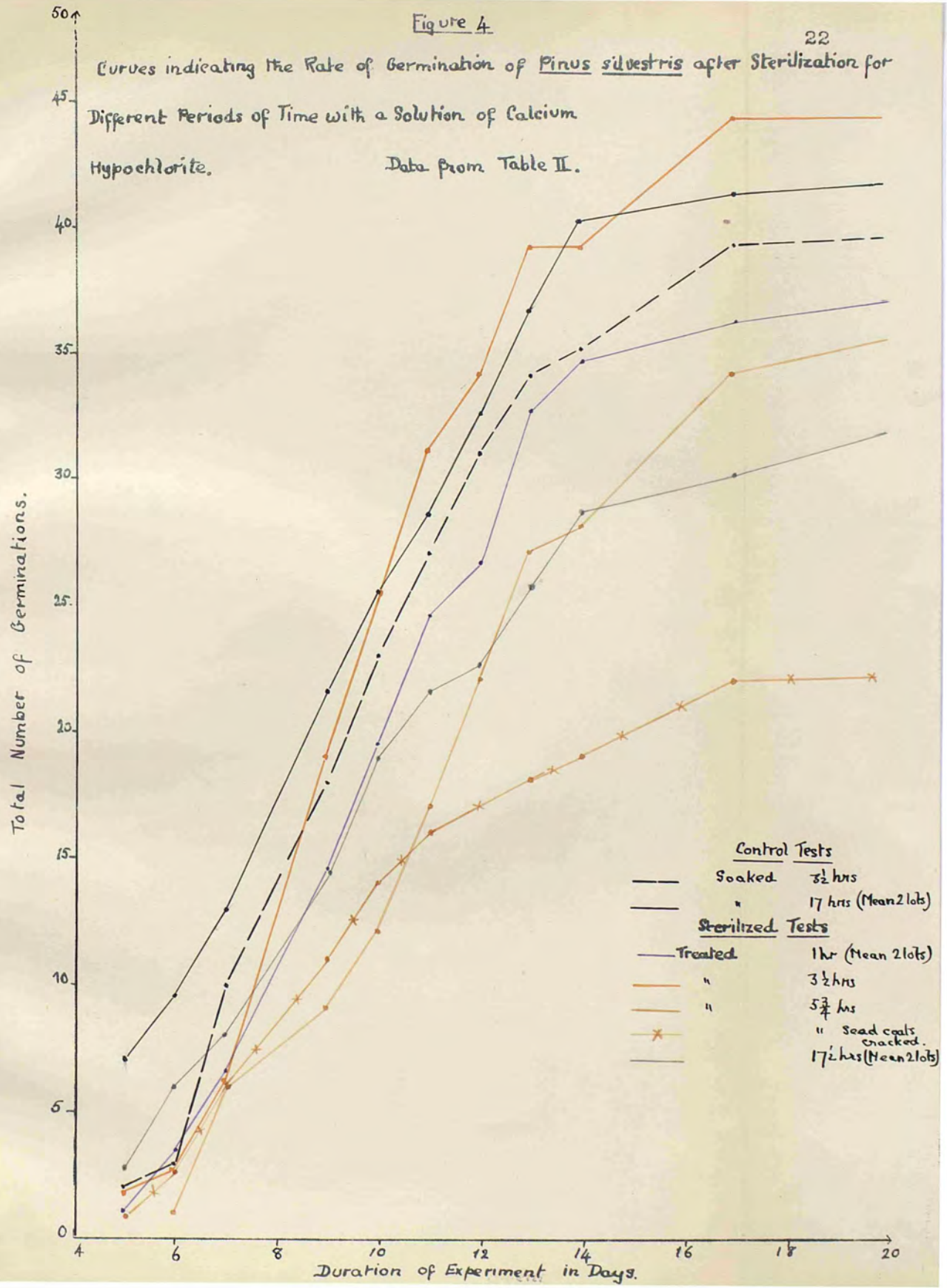


Figure 4 (contin)

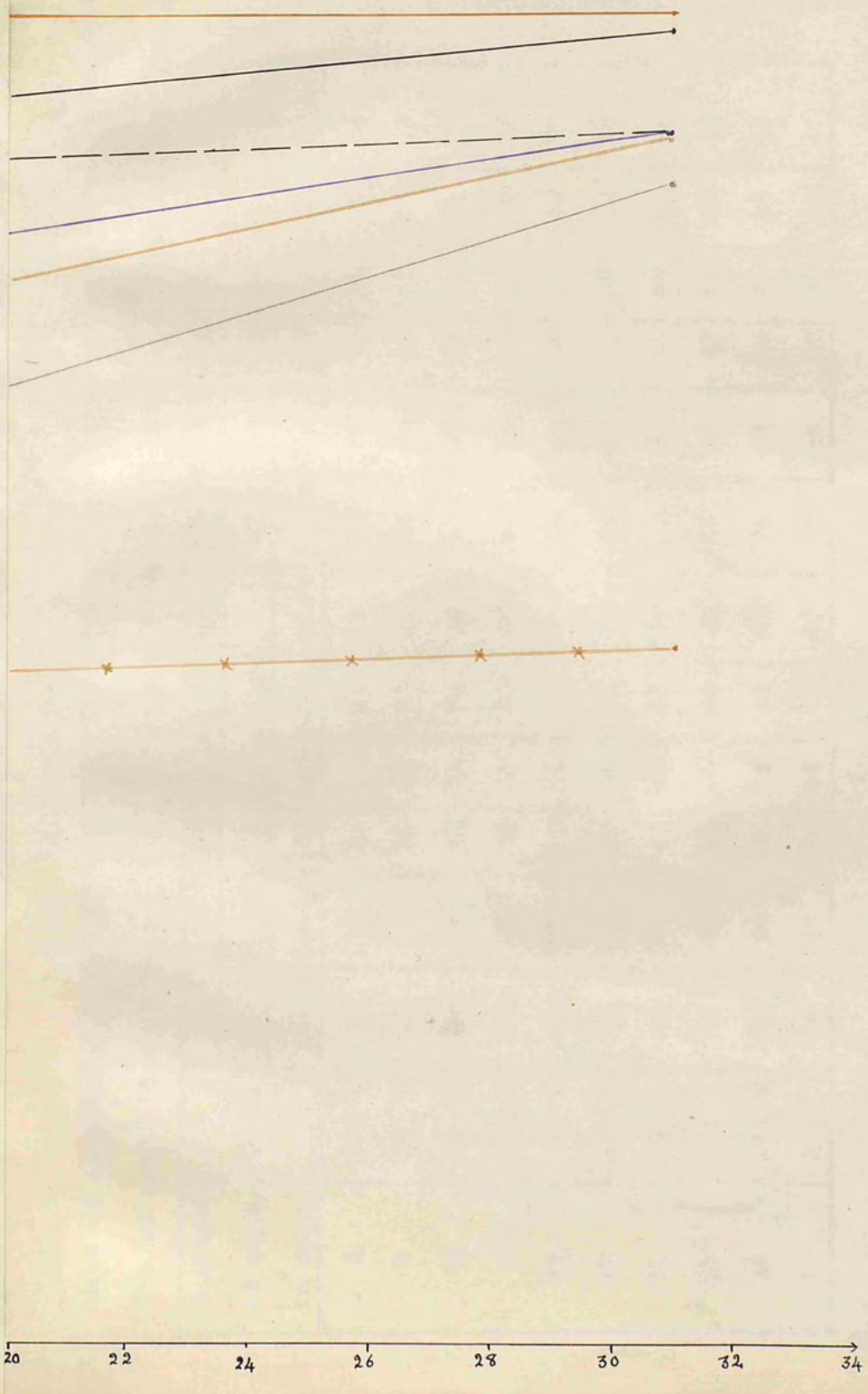


TABLE III.

Pinus silvestris. J. Rafn & Sons 1934.

Date of experiments. 6. 6. '35.

No. of seeds in each test. 50. Strength of Ca(OCl)Cl soln. = 2·2 grm Cl2 per 100c.c soln.

Duration of expt. in days.	Temp.	Total Number of Seeds shewing Germination.											
		Control tests. Soaked.						Sterilized with Ca(OCl)Cl soln.					
		15min	30min	1hr	2hrs	4hrs	6½hrs	15min	30min	1hr	2hrs	4hrs	6½hrs
6	18½°C	15	17	15	9	16	14	18	20	14	16	11	14
7	19 °C	27	26	22	23	27	23	22	25	26	21	21	23
8	20 °C	39	34	31	33	34	34	32	32	34	34	31	28
9	19¼ C	41	38	34	37	38	36	37	36	37	36	34	34
11	20½°C	43	39	40	44	40	38	38	40	40	37	40	37
12	19 °C	43	41	40	44	41	38	38	40	40	38	40	37
13	21 °C	43	41	41	45	43	39	38	41	42	40	40	43
15	21¼°C	43	42	43	46	45	40	43	41	43	43	42	45
16	26¼°C	43	43	44	46	45	42	44	44	44	44	42	45
18	27½°C	44	43	46	46	47	43	44	44	45	45	44	45

Figure 5

Curves indicating the Rate of Germination of Pinus silvestris after Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite.

Data from Table III

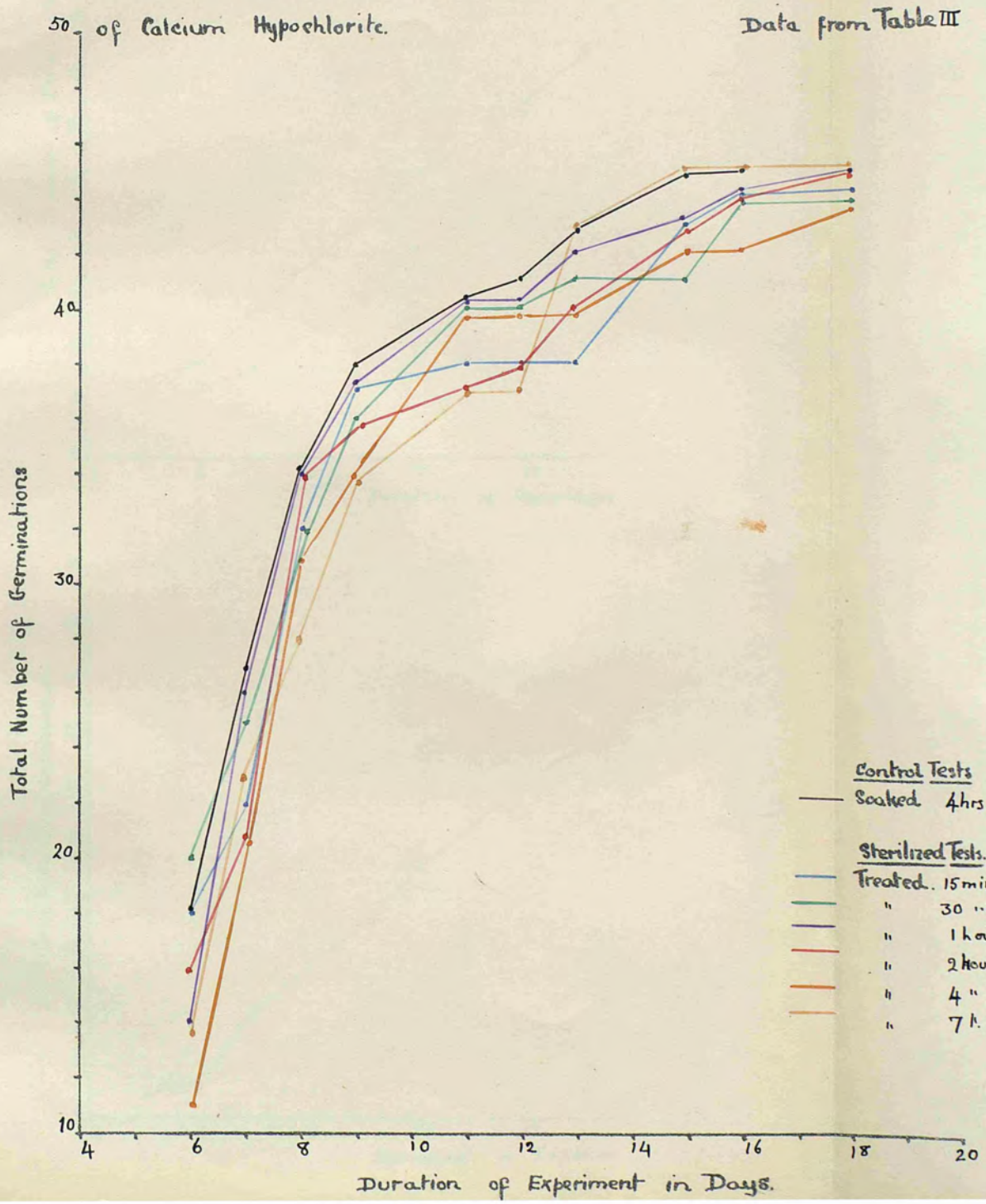
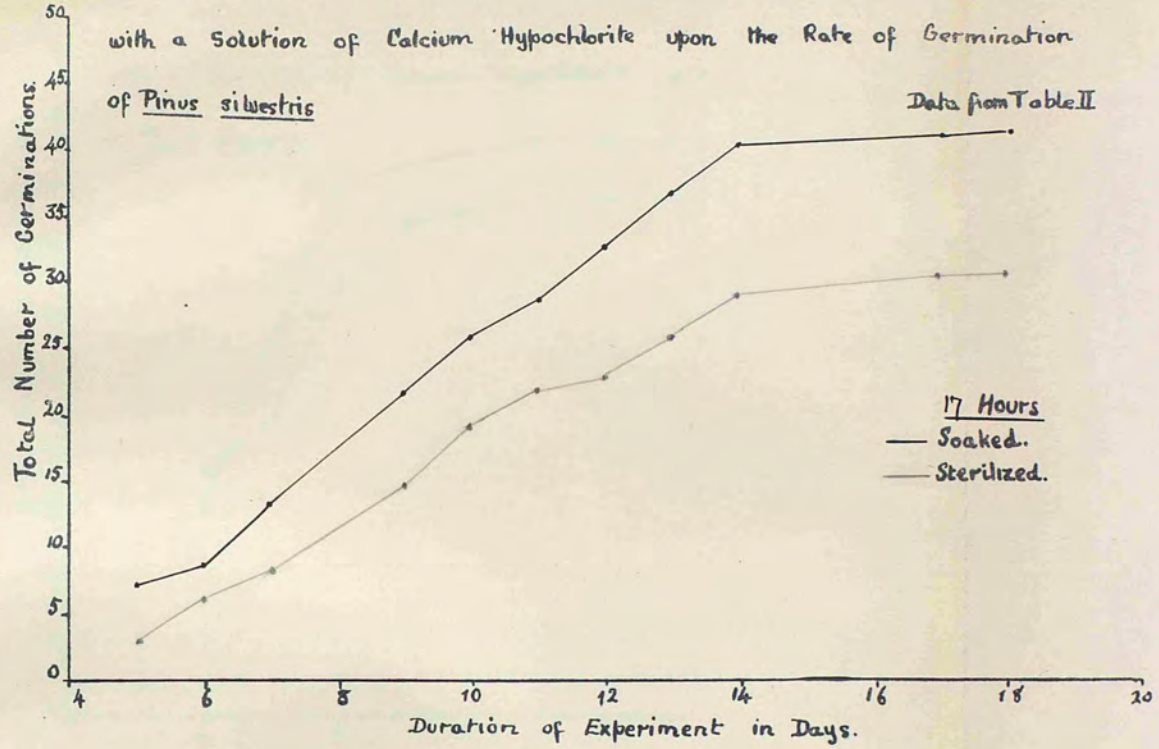


Figure 6.

Curves indicating the Effect of Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite upon the Rate of Germination of Pinus silvestris

Data from Table II



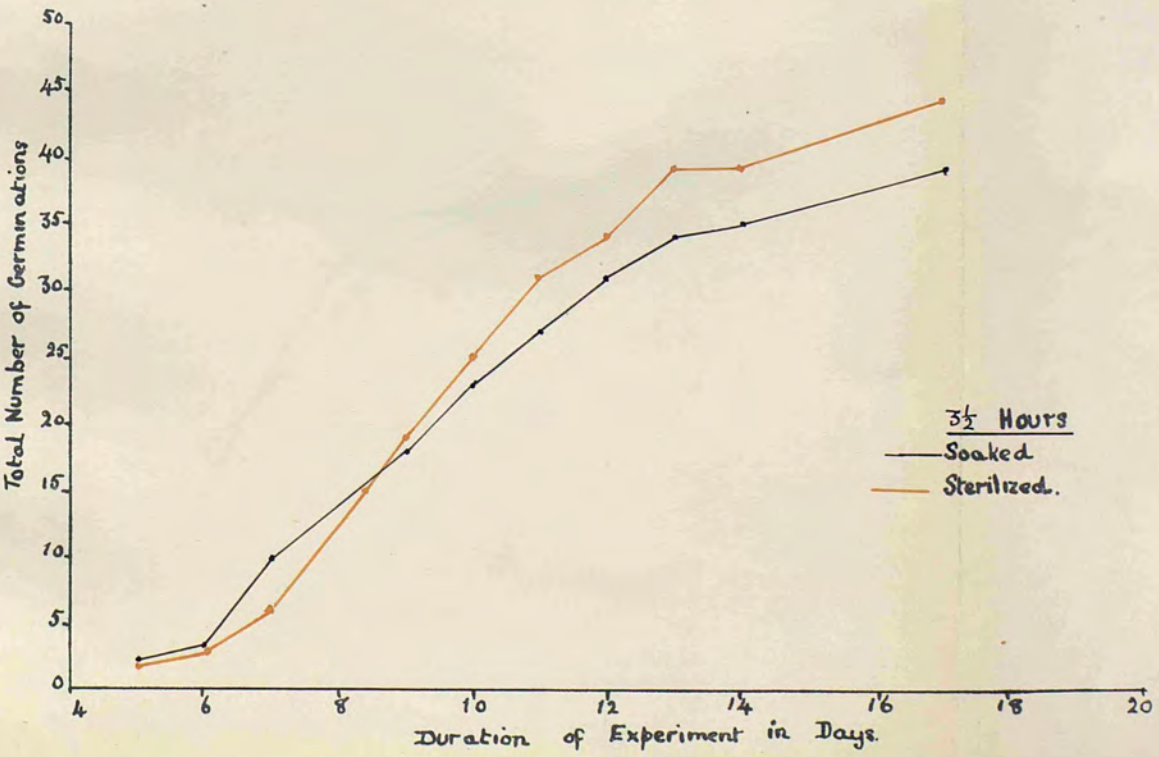
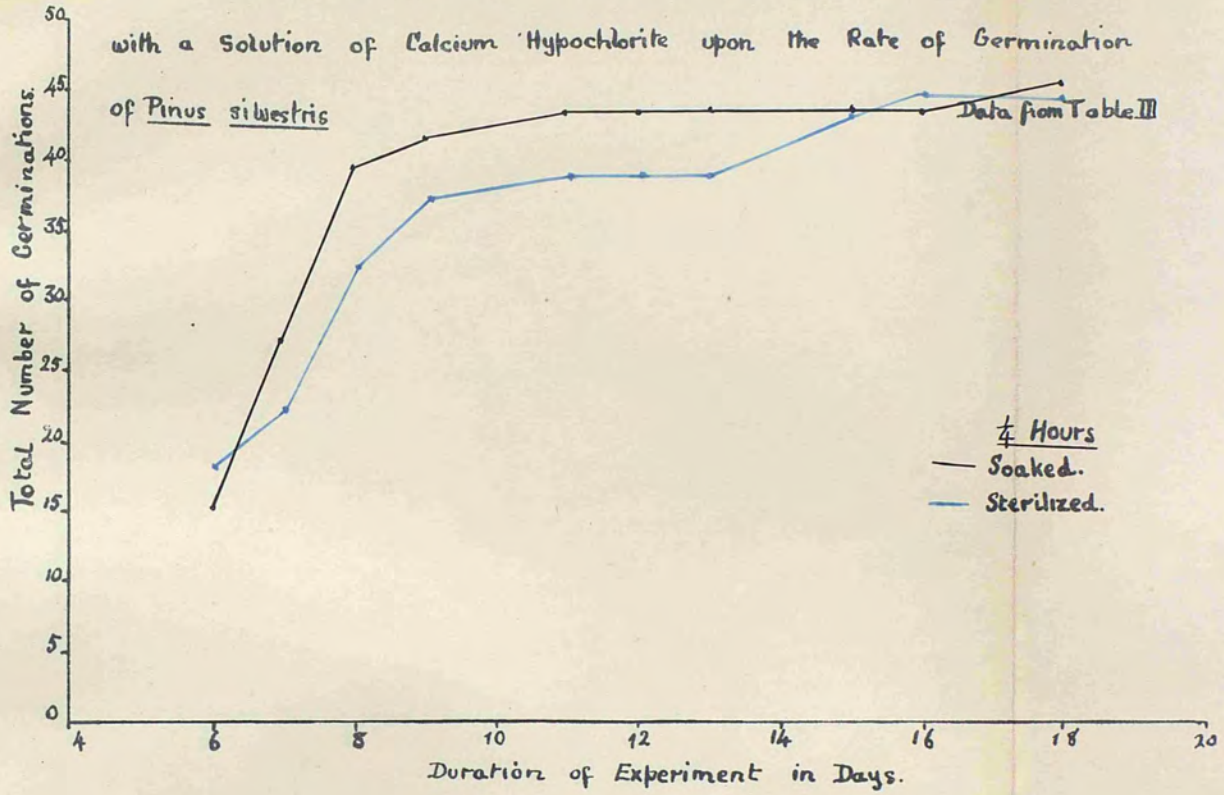


Figure 6. (contin:)

Curves indicating the Effect of Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite upon the Rate of Germination of Pinus silvestris



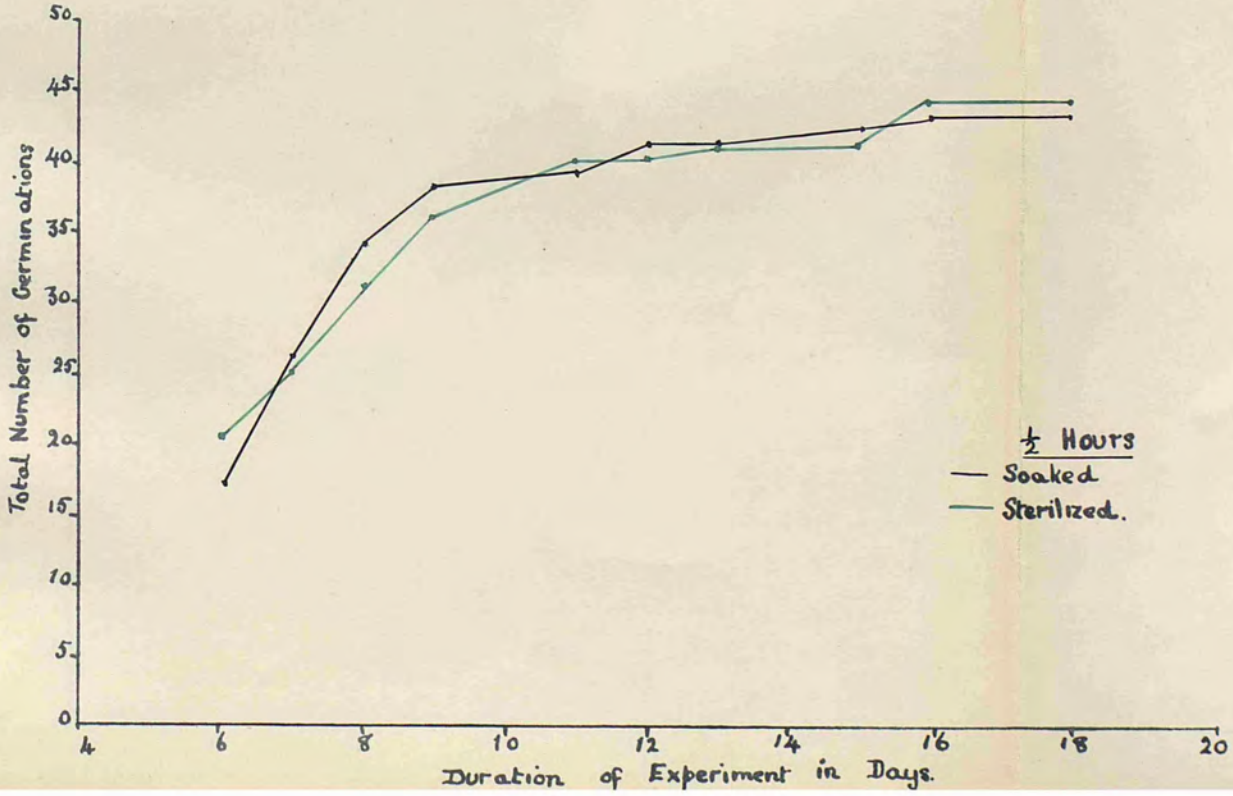
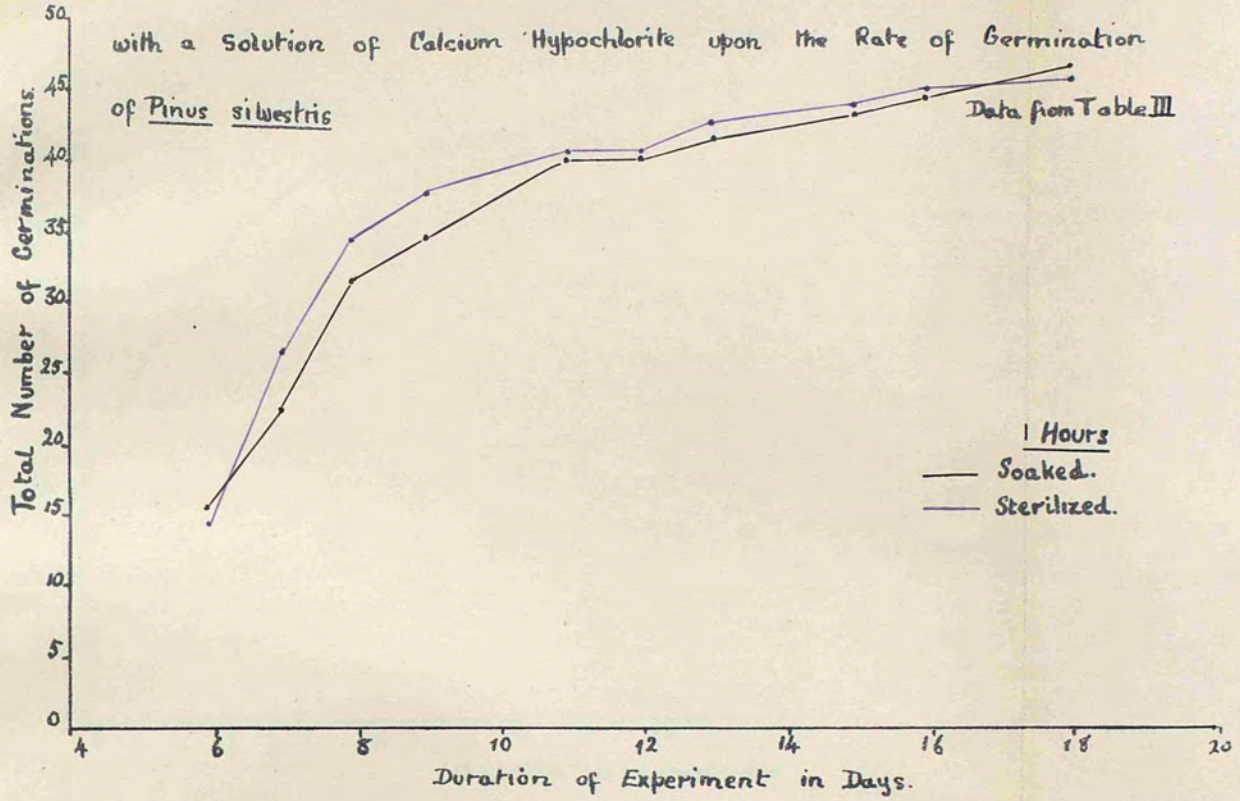


Figure 6. (contin:)

Curves indicating the Effect of Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite upon the Rate of Germination of Pinus silvestris



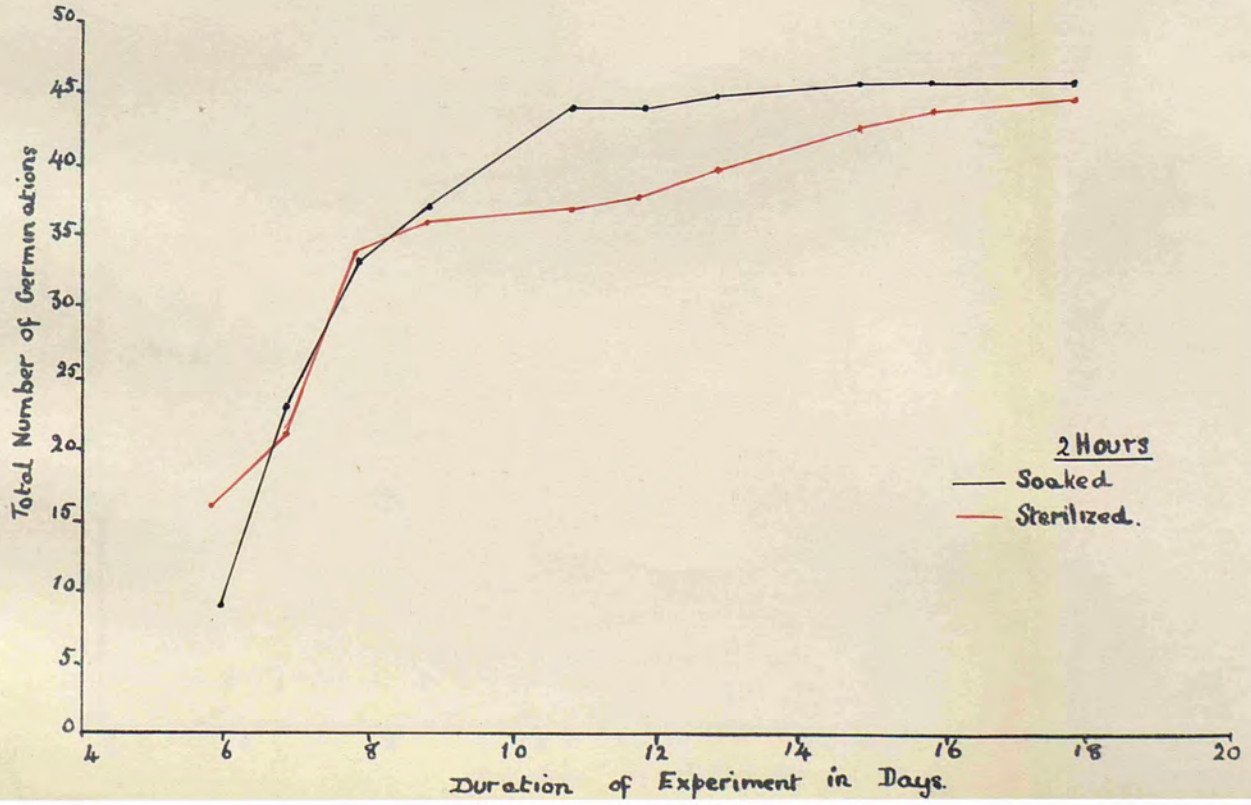
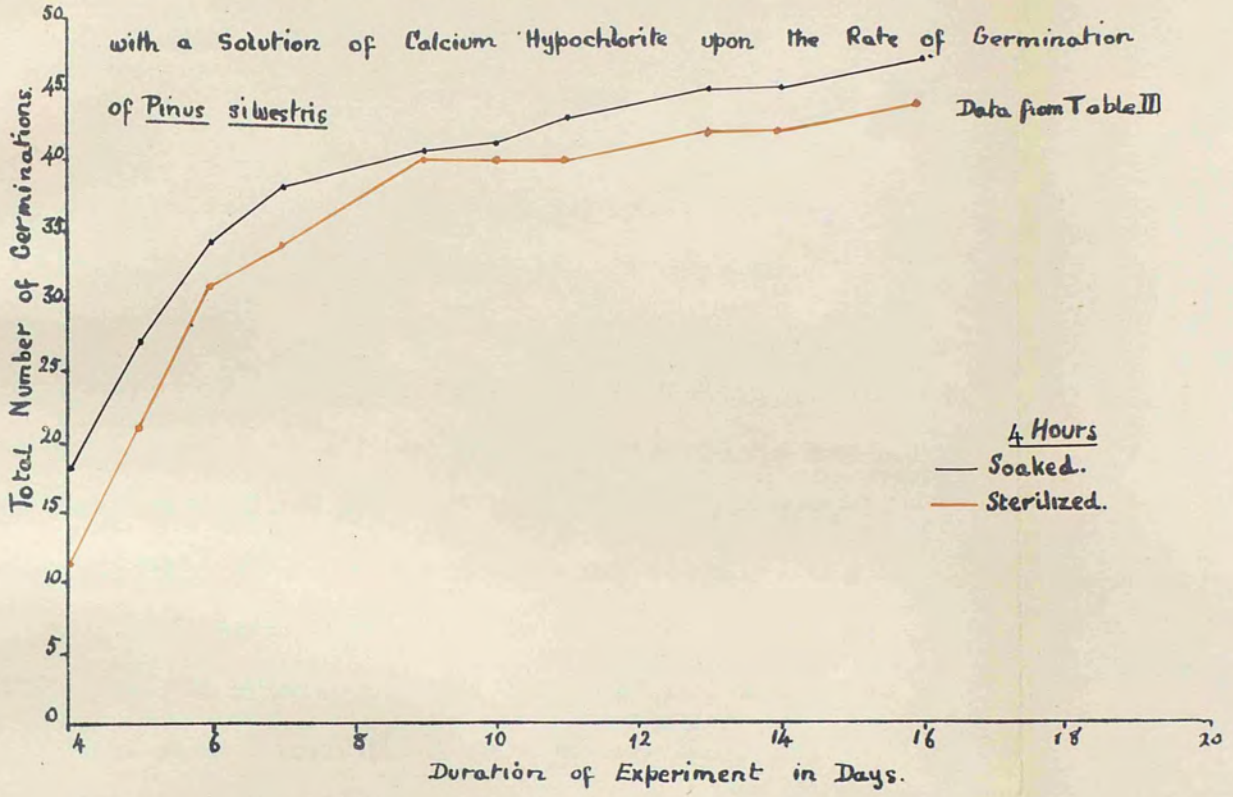
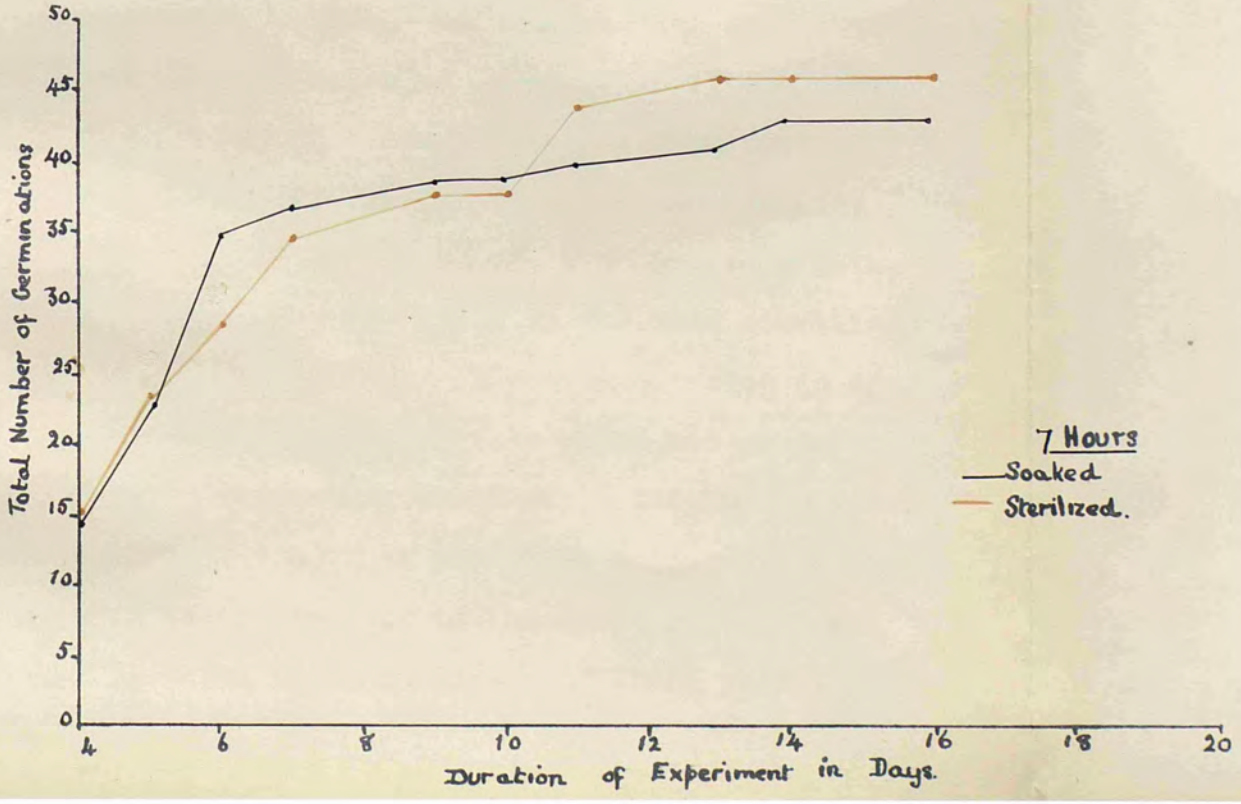


Figure 6. (Contin:)

Curves indicating the Effect of Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite upon the Rate of Germination of Pinus silvestris

Data from Table III





TRITICUM.

Early in January 1935 a series of tests was made to discover the effect of sterilization upon the rate of germination of Triticum - Carter's Yeoman King 1933. The seeds were centrifuged for 5 minutes before sterilization in order to drive off as much air as possible from their seed-coats. A test, sterilized with 1% $HgCl_2$ for 2 minutes, was set up to compare with the other treated tests. The results of this experiment are shewn in Table IV. At the end of seven days the experiment was finished and quantitative measurements were taken of the number of roots per seedling and the average lengths of the first roots of the seedlings in each test. These measurements are illustrated in diagrams 9 and 10, the rates of germination of the tests are shewn in Figs. 7 and 8. Diagram 9 indicates the relative frequency with which a given number of roots per seedling occurs in each test:- thus in square $4\frac{1}{2}$, 6 there are 9 dots, this means that there are 9 seedlings in the test sterilized for $4\frac{1}{2}$ hours which have 6 roots. The dots occuring in the 0 vertical column signify the seedlings which had no root but whose plumular sheaths were enlarged. Diagram 10 shews the average (not mean) length of the first roots of the seedlings in each test:- the dot is the average length and the arrows indicate the range in length of these roots.

This diagram is a rough representation only because in several cases the first root was damaged - it is based on the roots that could be measured. Where two dots occur there were two lengths which were equally frequent in occurence.

The tests were quite satisfactory and on the whole good total germination was obtained, but the 2 hour test was disappointing. The two control tests germinated at practically the same rate although they were soaked for different periods of time; this rendered the use of a control test for each period of sterilization unnecessary. It can be concluded from these results that sterilization with calcium hypochlorite for periods between 30 minutes and 17 hours has no stimulatory effect upon the rate of germination of the treated seeds, but rather depresses the initial rate of germination and reduces the total number of healthy germinations. This depressing effect does not seem to be directly proportional to the length of time of sterilization when shorter periods are used, but for periods as long as $17\frac{1}{2}$ hours it seems to be very marked. Sterilization with 1% $HgCl_2$ for 2 minutes has a similar depressing effect, especially apparent in the initial rate of germination of this test.

Sterilization with calcium hypochlorite affects the early stages of growth of the seedlings as is shewn clearly in diagrams 9 and 10 which illustrates the measurements taken at the end of seven days. The roots produced by the treated seedlings are shorter and greater in number than those produced by untreated seedlings of the same age. This effect

is not produced by sterilization with 1% $HgCl_2$ for 2 minutes. The actual number of seeds shewing some signs of germination on the seventh day is approximately the same in each test, thus it seems that sterilization increases the number of weakly seedlings occuring in the tests.

The results obtained in this series of tests are interesting when compared with the results given by Wilson for Triticum vulgare Vill.. He gives 15 hours as the period necessary for sterilization with a one per cent solution and 20 - 22 hours as that needed to produce injury to the seedlings. In these experiments and in the following ones dealing with the effect of reduced oxygen pressure on germination, seedlings were maintained in a sterile condition for the duration of the experiment, 9 - 10 days, after sterilization for 1 hour with a two per cent solution made up in the way described by Wilson.

TABLE IV.

Triticum. Carter's Yeoman King 1933.

Date of experiment. 28. 1. '35 Centrifuged 5 mins.

No. of seeds in each test. 20. Strength of Ca(OCl)Cl soln. 1·7 grm Cl_2 per 100.c.cs.

Duration of expt. in days	Temp.	Total Number of Seeds shewing Germination.								
		Controls soaked		Sterilized	Sterilized with Ca(OCl)Cl solution.					
		1hr	6hrs	$HgCl_2$ 1%	30min	1hr	2hrs	4½hrs	6hrs	17½hrs x
3	Lab.	18	17	1	14	14	8	15	14	1
4	"	19	18	14	16	18	13	17	17	7
5	"	19	19	17	17	19	14	17	18	8
7	"	19	19	17	17	19	15	17	19	11
"	"	20	19	20	19	20	19	20	20	20

x 17½ hours test was begun 17 hours after other tests.

The lower row of figures for the 7th day gives the total number of seeds which shewed signs of germination.

Figure 7

Curves indicating the Rate of Germination of Triticum after Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite.

Data from Table IV

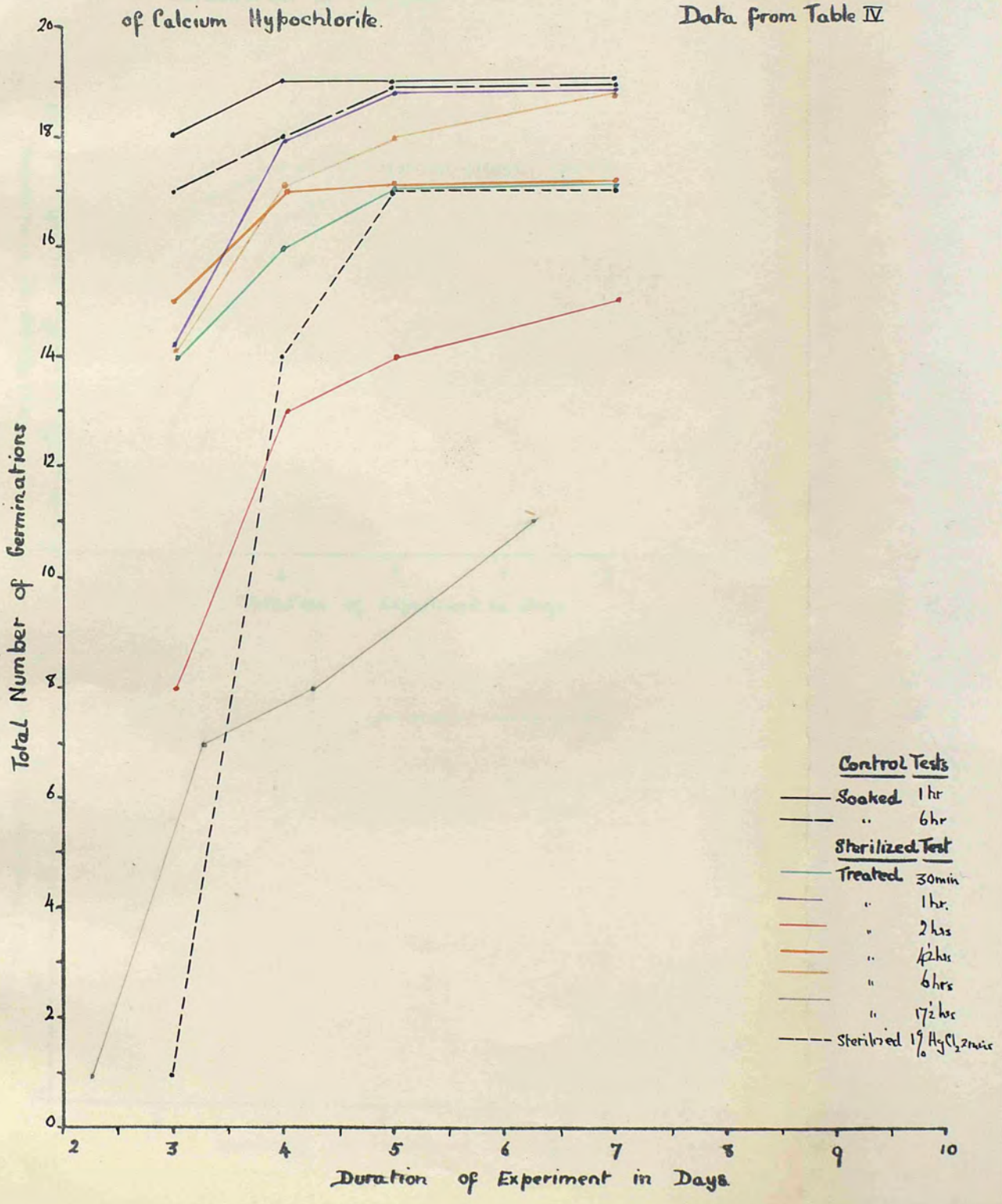
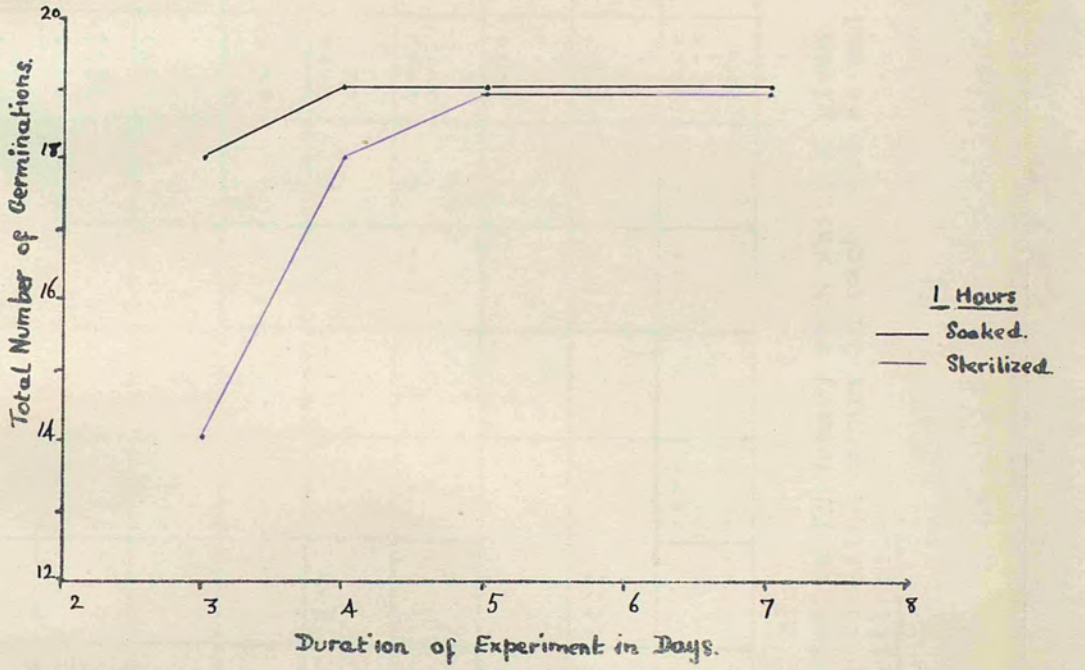
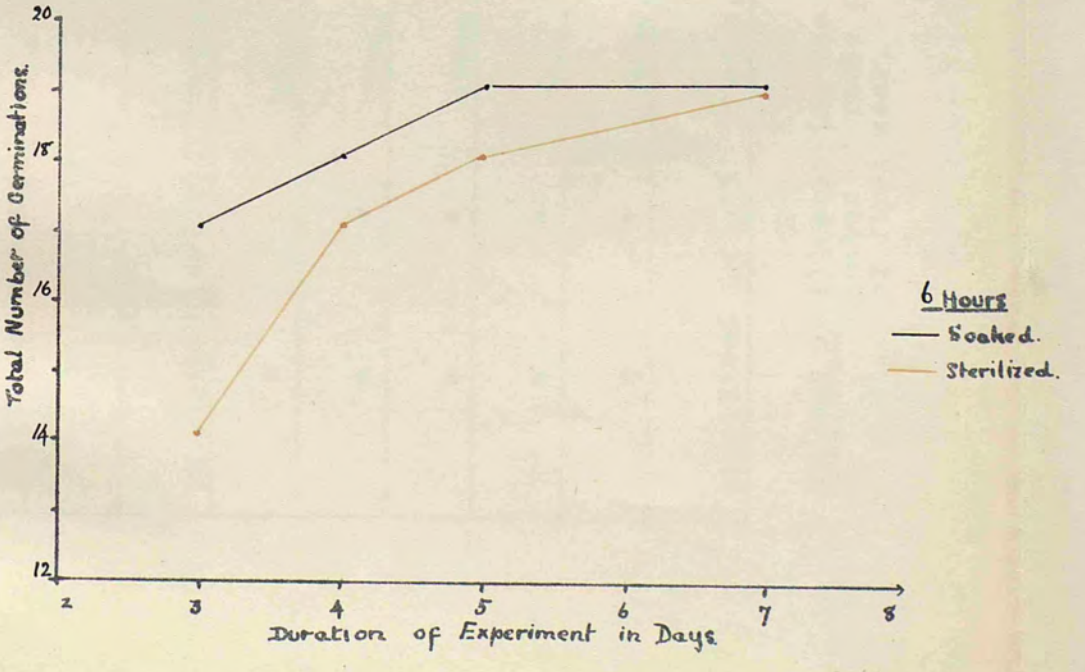


Figure 8

Curves indicating the Effect of Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite upon the Rate of Germination of Triticum

Data from Table IV





Treatment of Tests	Number of roots per seedling.							
	0	1	2	3	4	5	6	7
Control Soaked 1hr.		•			••••	:::: :::: :::		
" " 6 hrs.				••	••••	::::	•	
Sterilized Ca(OCl)Cl 30mins.		•	•		••	:: ::	:::: ::::	•
" " 1hr.			•	•	••••	:: ::	:::: ::::	
" " 2hrs.	•••			••	•	:: ::	:::: :::	
" " 4½hrs.		•		•••	•	::	:::: ::::	
" " 6hrs	•				••••	::	:::: ::::	•
" " 17½hrs.	::::	•		•••		•	•••	
Sterilized $HgCl_2$ 1% 2 mins.		•••		•••		:: ::		

Diagram 9 illustrating the relative frequency with which a given number of roots per seedling occurs in each test at end of first week. Triticum.

Treatment of Test.	Average Length of first roots in each test measured in cms.									
	1	2	3	4	5	6	7	8	9	10
Control Soaked 1 hr.						←	—	—	•	→
" " 6 hrs.										
Sterilized Ca(OCl) Cl 30min.	←	—	•	—	→					
" " 1hr.			←	•	→					
" " 2hrs.	←	—	•	—	—	→				
" " 4½hrs.	←	•	—	—	→					
" " 6hrs.	←	•	—	•	→					
" " 17½hrs.		←	•	—	→					
Sterilized $HgCl_2$ 1% 2mins.				←	•	→				

Diagram 10 illustrating average length and range in length of first root of the seedlings in each test at end of first week. Triticum.

LINUM GRANDIFLORUM.

In April 1935 a similar series of tests was made to investigate the effect of sterilization upon the rate of germination of Linum grandiflorum Carter's 1934. The seed coats wetted satisfactorily so there was no need for centrifuging. In this case it was quite impossible to make reliable counts of the number of germinations without opening the dishes and removing the germinated seeds; the thick mucilaginous coat of the wetted seeds was largely responsible for this difficulty. In this series two tests were duplicated in order to estimate the possible range of deviation between two members of one pair of tests. The results obtained from this experiment are given in Table V and are plotted to shew rate of germination in Figs. 11 and 12. The difference between the number of seeds germinated in the control tests soaked for different times suggested that a second series of tests with control tests for each period of sterilization might prove more illuminating. This was carried out in June 1935 and the results are shewn in Table VI. The temperature of the laboratory was rather higher than in the first series. The results are plotted in Fig.13 which is comparable to Fig. 12 and in Figs. 14 and 15 which were designed to detect any stimulation in the rate of germination due to sterilization with calcium hypochlorite.

Fig. 14 consists of the rates of germination of each pair of sterilized and unsterilized tests plotted together, and Fig. 15 was obtained by plotting against time the difference between the number of germinations in the sterilized and unsterilized tests for each period of sterilization.

The deviation between the numbers of germinations occuring in the members of a pair of tests treated in the same way at any time is indicated in Fig.11; it is not great and in these cases never exceeds 7. The results illustrated in Figs. 14 and 15 suggest that sterilization for periods of time of $\frac{1}{2}$ - 2 hours, brings about a definite increase in the rate of germination of the treated seeds. The greatest stimulatory effect seems to be produced by sterilizing for about $\frac{1}{2}$ hour and then gradually falls off with longer periods of sterilization till with periods of about 2 hours there is practically no difference between the rates of germination of the treated and untreated tests. For periods of sterilization longer than 2 hours, sterilization in this way depresses the rate of germination progressively with the length of time of treatment until 7 hours, further than which there is no data. Treatment for $\frac{1}{4}$ hour does not seem long enough to bring about any increase in the rate of germination. In many cases the difference between the numbers of germinations in the sterilized and control tests are considerably greater than those occuring between two members of a similar pair as indicated in Fig. 11. Thus it would seem that sterilization for short periods of time with a solution of calcium hypo-

chlorite definitely increases the rate of germination. (It must be remembered that the range of possible deviation between two members of a similar pair was found from a different series of tests from that from which the above conclusions are drawn and that the temperature was not the same for both series). The results of the first series of tests do not altogether support these conclusions but as they were not run parallel with control tests they are not thought as significant; they do, however, indicate an increased rate for ½ and 1 hour periods and a general falling off in rate for periods greater than 2 hours.

A curious effect of sterilization by this means is seen when the seeds are sterilized for periods greater than 2 hours - the mucilage of the seed-coats does not swell up. It seems as though a fine powder is precipitated all over the seed; this may prevent the mucilage from swelling. The effect becomes increasingly marked the longer the period of sterilization.

The seedlings in the 5 hour and more especially in the 7 hour test often shewed roots browned at the tips which suggested injury resulting from too long an exposure to the sterilizing agent.

Wilson's times for exposure of seeds of Linum usitatissimum to a one per cent sterilizing solution are 10 hours for effective sterilization and 14 hours for injury to be caused to the seeds. The results obtained above indicate shorter periods of sterilization with a two per cent solution for satisfactory germination of Linum grandiflorum.

TABLE V.

Linum. Carter's Linum grandiflorum 1934.

Date of experiment 9. 4. '35.

Number of seeds in test. 50. Strength of Ca(OCl)Cl soln. = 2·0 grm Cl_2 per 100 c.c soln

Duration of expt. in days.	Temp.	Total Number of Seeds shewing Germination.								
		Unsterilized			Sterilized with Ca(OCl)Cl soln.					
		1hr	1hr	7hr	30mins	1hr	1hr	2¾hrs	5hrs	7½hrs
2	17¼° C	34	28	34	37	30	37	31	33	25
3	16½° C	40	39	44	46	42	46	39	46	39
4	18° C	40	40	45	46	44	46	41	47	46
7	-	42	41	47	47	44	46	43	47	46

Figure 11

Curves illustrating the Extent of the Deviation which may occur between the Rates of Germination of two tests of Linum grandiflorum treated in the same way.

Data from Table V

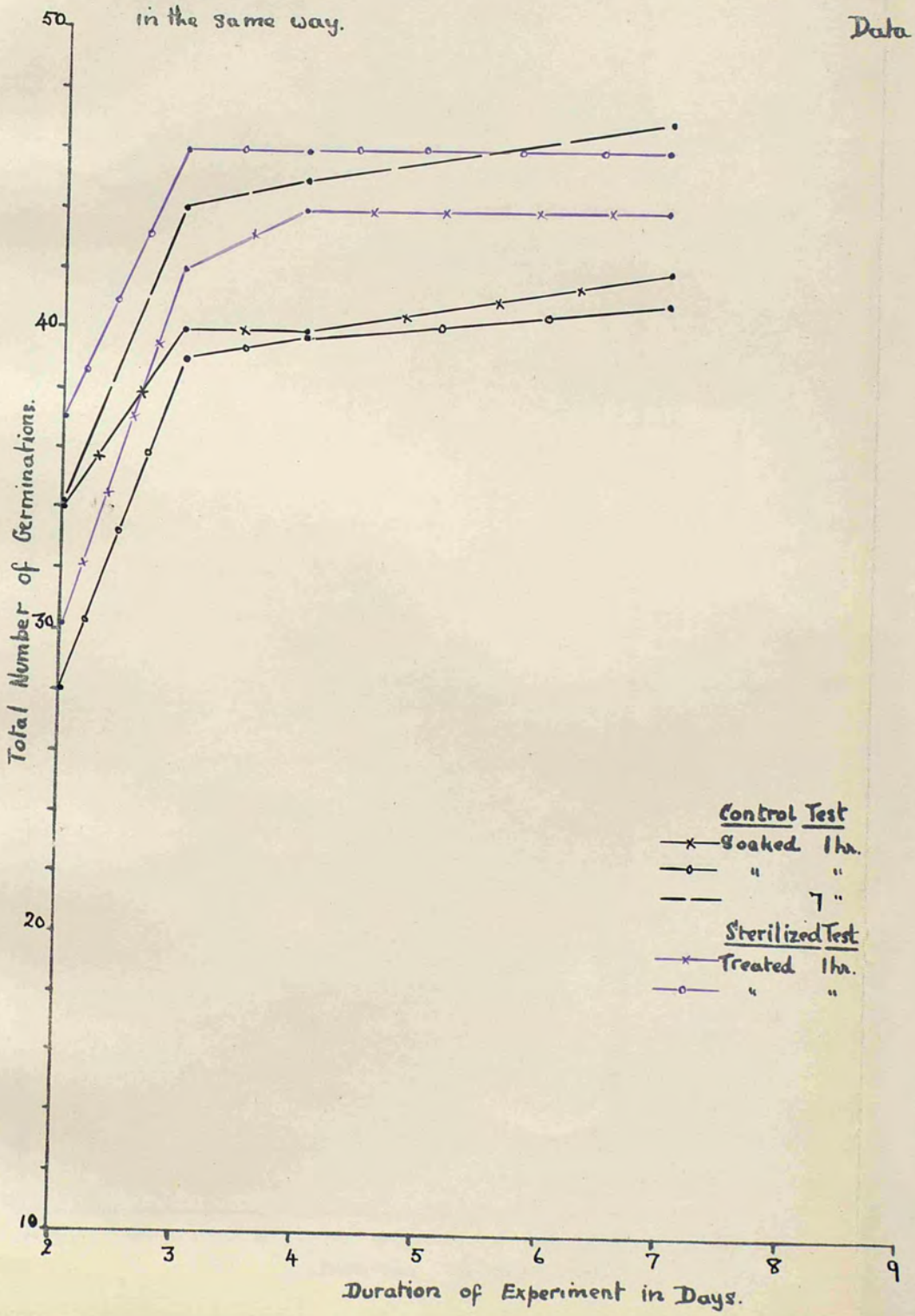


Figure 12

Curves indicating the Rate of Germination of *Linum grandiflorum*. after Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite. Data from Table V.

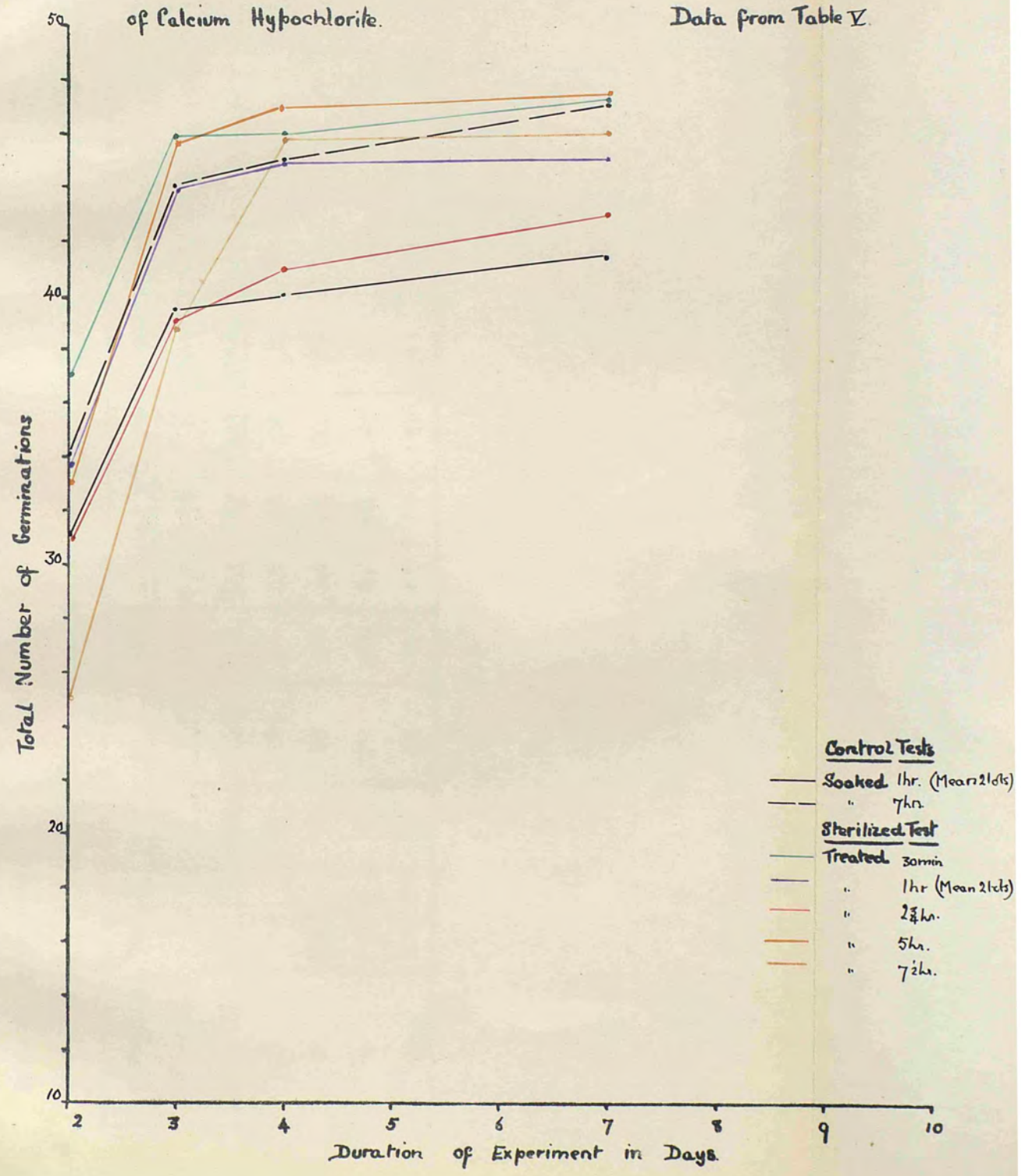


TABLE VI.

Linum. Carter's Linum grandiflorum 1934.

Date of experiment. 3. 6. '35.

No. of seeds in test. 50. Strength of Ca(OCl)Cl soln. = 2·0 grm Cl_2 per 100 c.c. soln

Duration of expt. in days.	Temp.	Total Number of Seeds shewing Germination.											
		Unsterilized soaked.						Sterilized with Ca(OCl)Cl soln.					
		15min	30mins	1hr	2hr	4hr	7hrs	15min	30mins	1hr	2hr	4hrs	7hrs
2	-	18	10	13	16	31	26	15	25	22	15	16	10
3	19° C	41	29	33	34	41	37	35	46	43	35	34	29
4	$18\frac{3}{4}$° C	43	32	37	38	41	43	38	46	46	42	36	37
8	$19\frac{3}{4}$° C	43	36	44	40	43	45	41	47	48	44	42	45

Figure 13

Curves indicating the Rate of Germination of *Linum grandiflorum* after Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite.

Data from Table VI

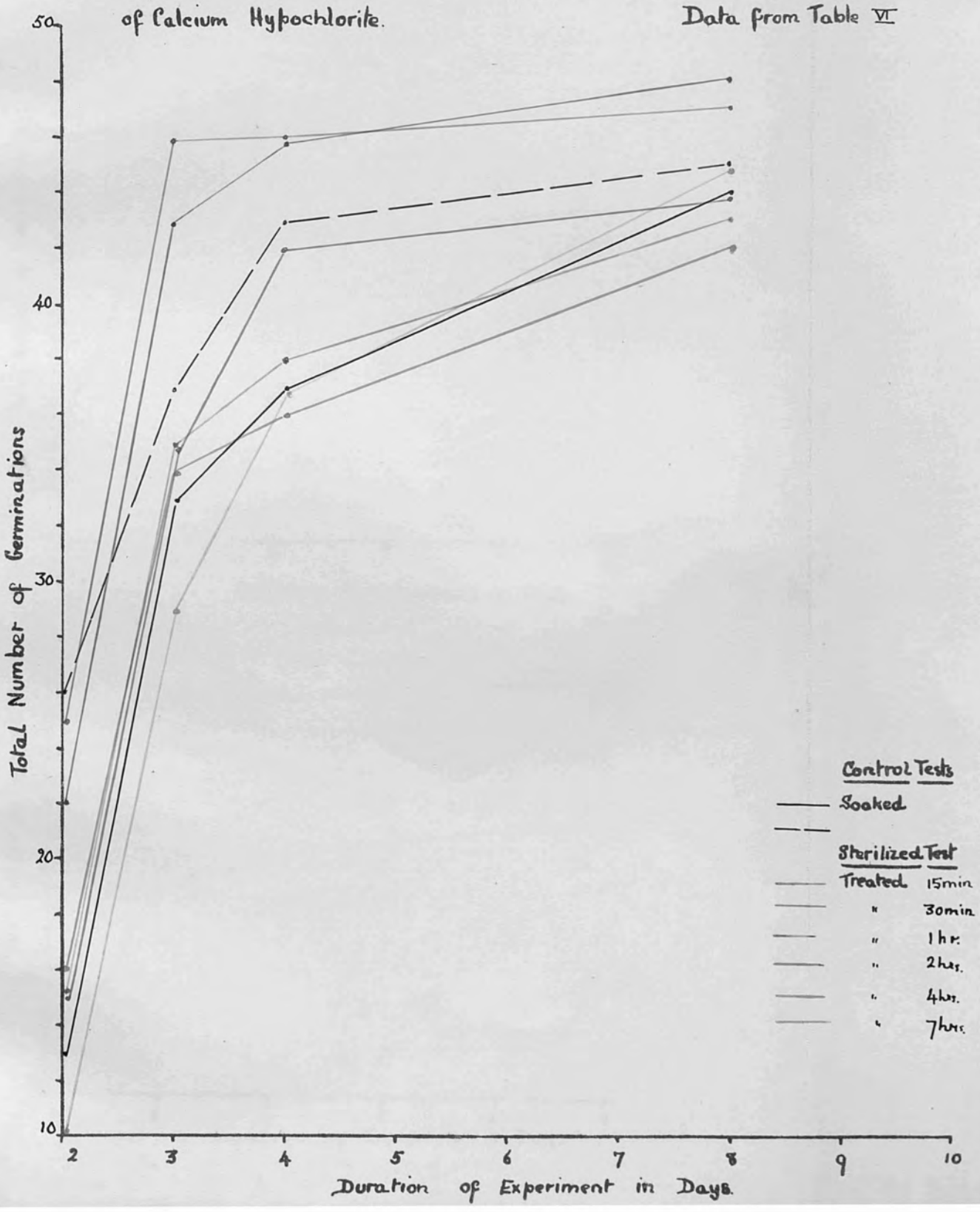
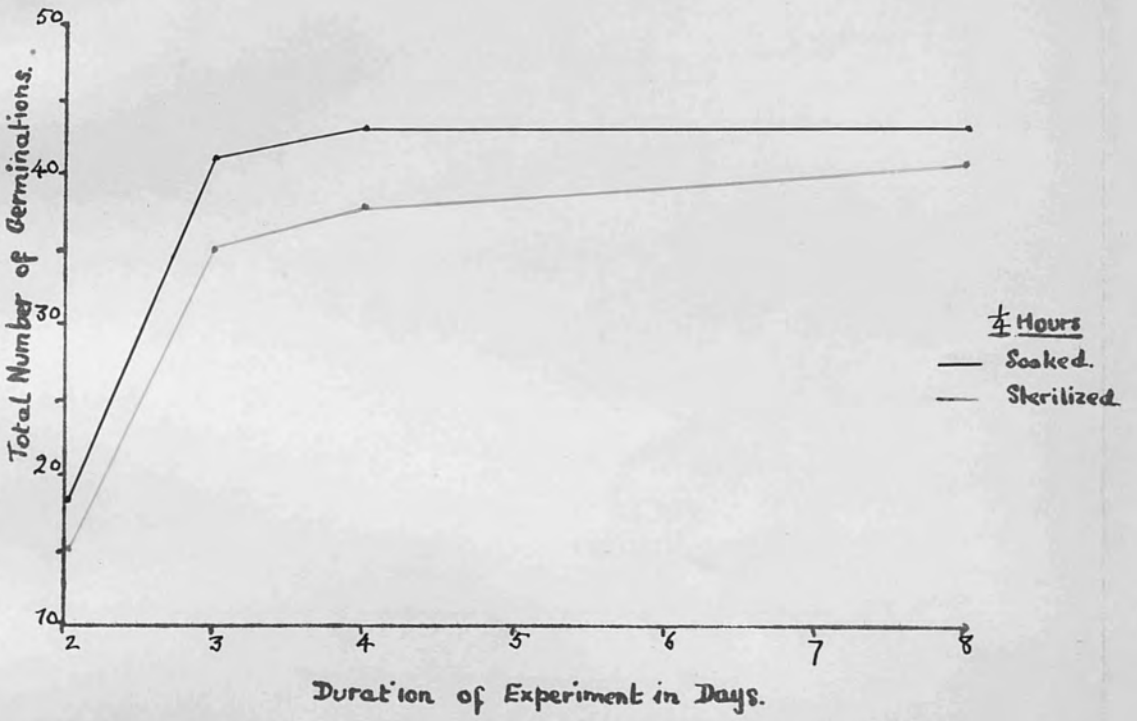


Figure 14

Curves indicating the Effect of Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite upon the Rate of Germination of Linum grandiflorum. Data from Table VI



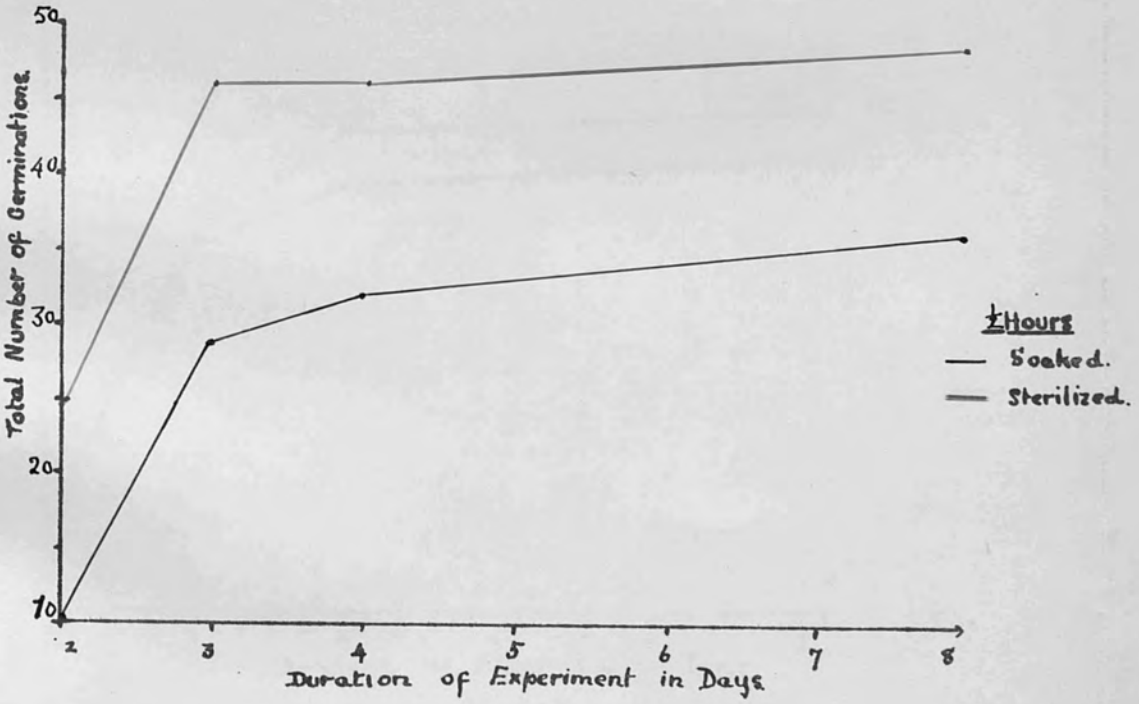
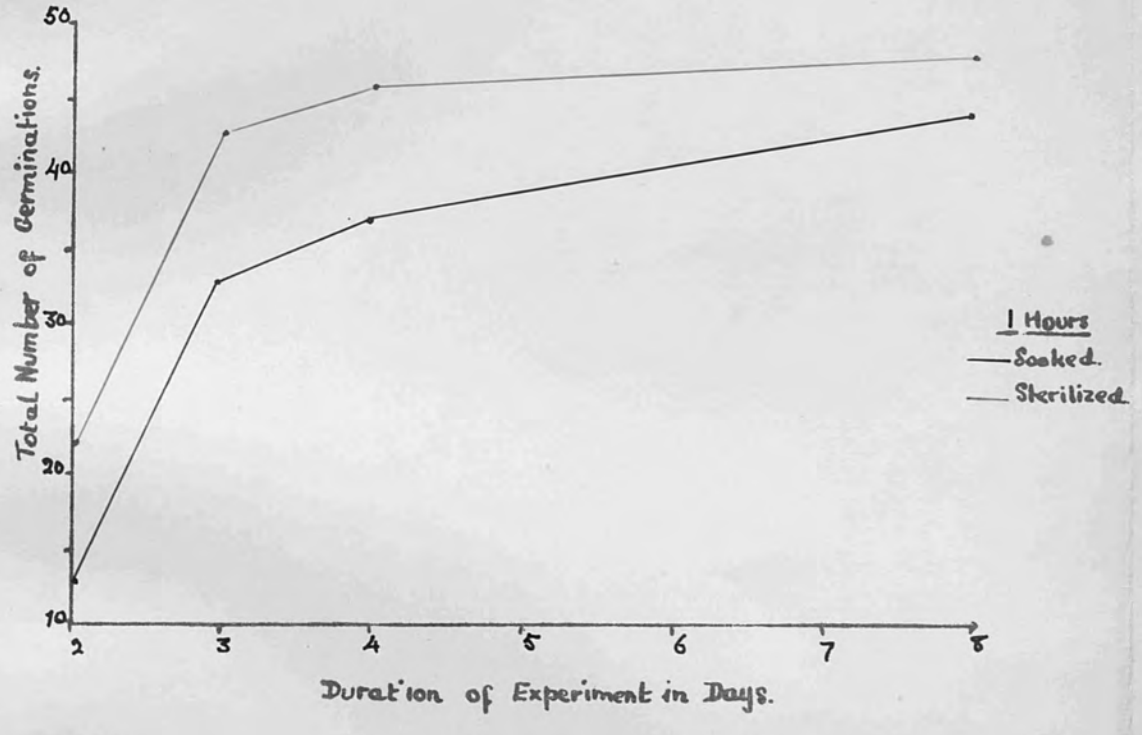


Figure 14 (contin)

Curves indicating the Effect of Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite upon the Rate of Germination of Linum grandiflorum

Data from Table VI



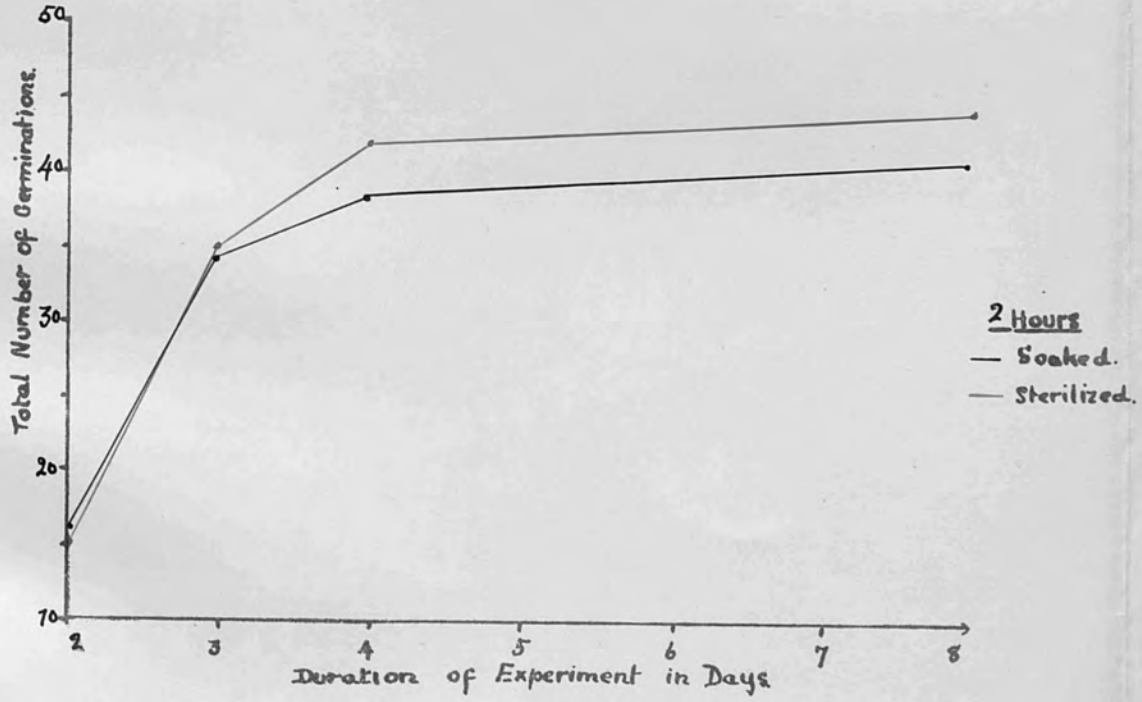
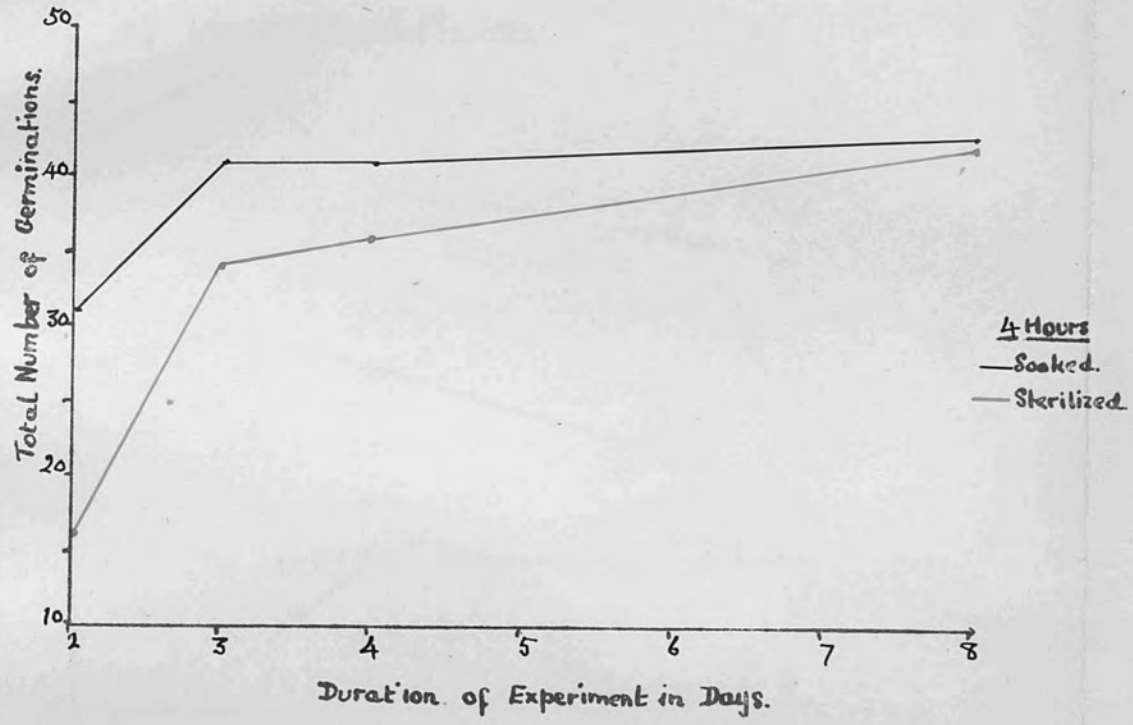


Figure 14 (contin)

Curves indicating the Effect of Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite upon the Rate of Germination of *Linum grandiflorum*

Data from Table VI



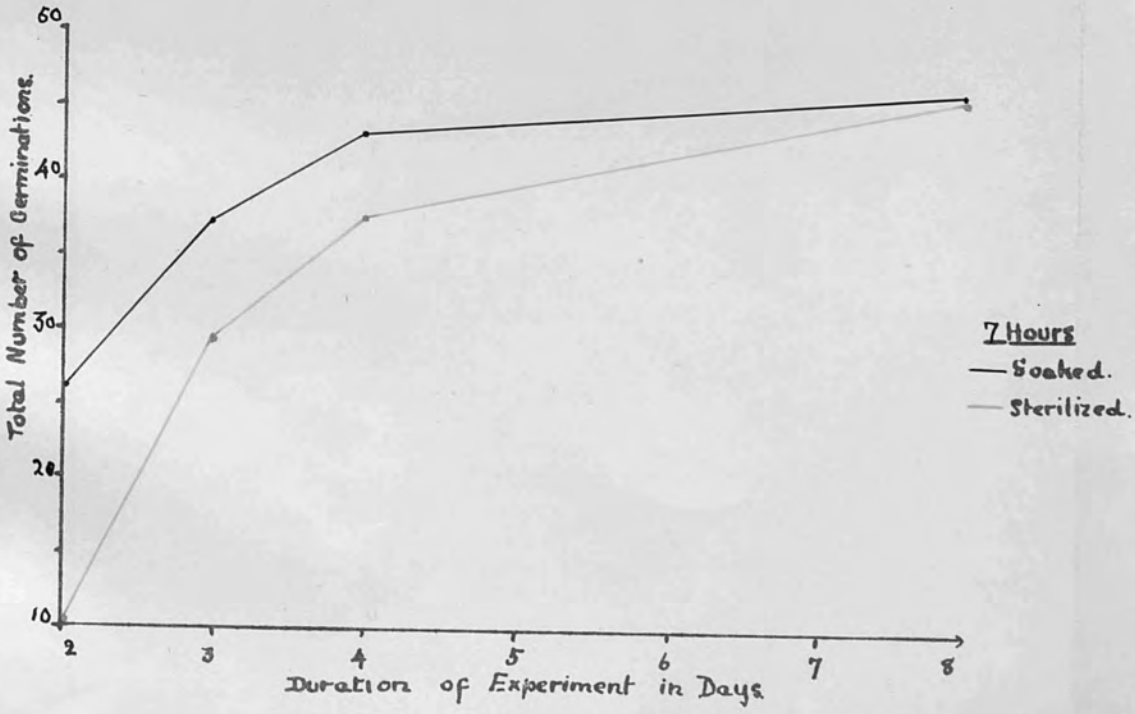
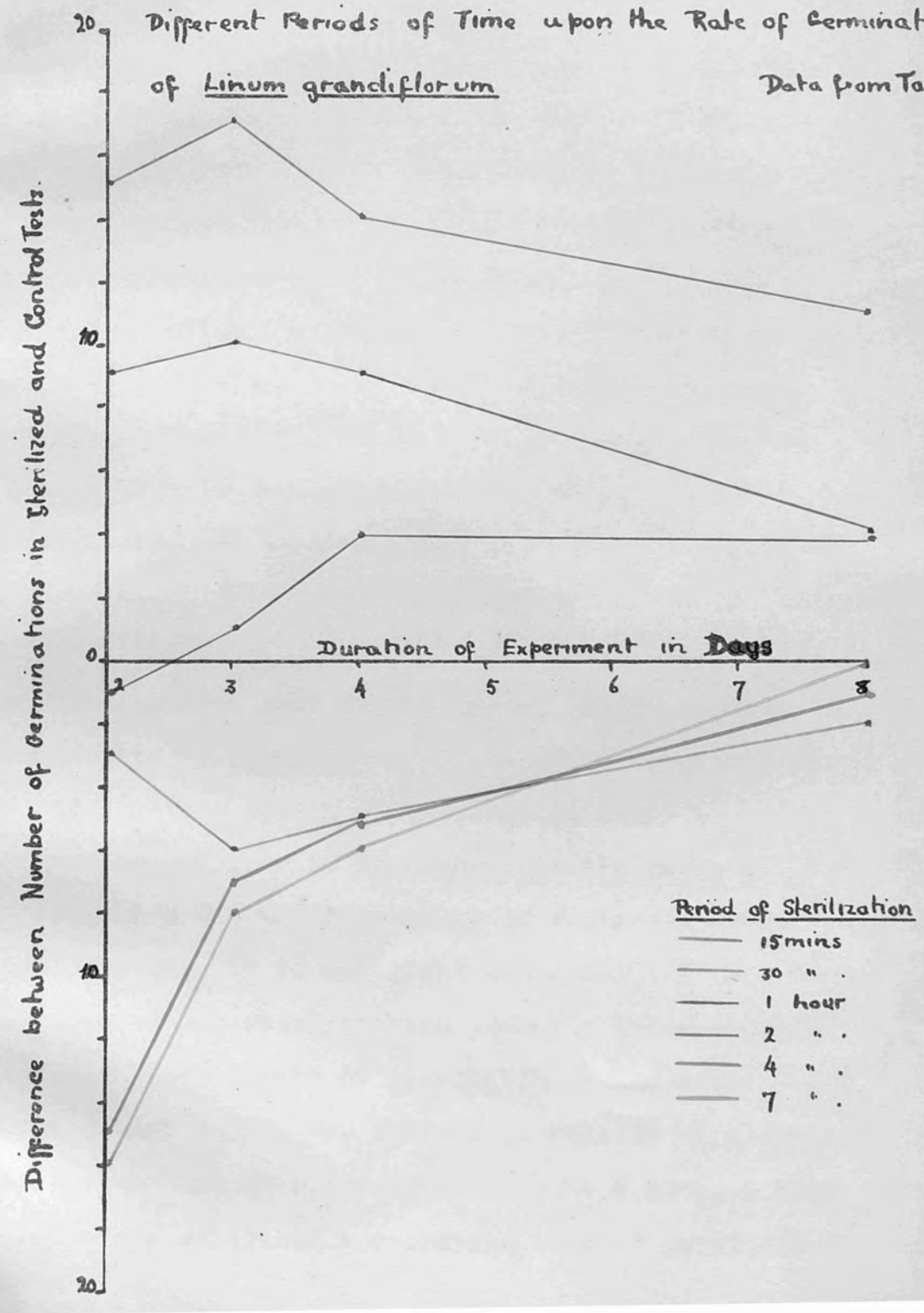


Figure 15.

Curves indicating the Extent of the Stimulatory Effect of Sterilization with a Solution of Calcium Hypochlorite for Different Periods of Time upon the Rate of Germination of *Linum grandiflorum*

Data from Table VI



BRASSICA RAPA.

Tests were carried out in March and April 1935 upon Brassica rapa Carter's 1933 seed. Two series of tests were made and both were kept at approximately the same temperature. In the first series the effect of soaking the seeds in distilled water before sterilization was examined together with the effect of varying periods of treatment. The results are shown in Table VII and are plotted as rate of germination in Fig.16. In the second series two tests were duplicated and a similar series of treated tests were set up to compare with those of the first experiment. The results of these are shewn in Table VIII and are illustrated in Figs. 17 and 18. Good germination was obtained in most of the tests; owing to the normal rapid rate of germination slight differences in this rate are difficult to obtain with accuracy.

The results lead one to conclude that the range of possible deviation between the results of duplicate tests as indicated by Fig. 17 is not great being about 1 to 4 or 5. Short periods of sterilization probably bring about a slight increase in the rate of germination - the seeds treated for $\frac{1}{2}$ hour and 1 hour periods germinated at a greater rate than the control test for 1 hour. The 2 hour, 5 hour and $7\frac{1}{2}$ hour tests exhibited a depressed rate of germination

because the results of the control tests soaked for 1 hour and 17½ hours, suggest that the effect of longer periods of soaking increase the initial rate of germination. The depression in rate increases with the length of time of sterilization. The effect of soaking the seeds before sterilization seems to vary with the length of time of subsequent sterilization. Thus seeds soaked for 17 hours and sterilized for one hour (a period which may stimulate rate of germination) germinate at about the same rate as those treated for the same length of time with calcium hypochlorite, whilst seeds soaked for 6 hours and sterilized for 17½ hours show a much slower rate of germination and poorer total germination than those sterilized for 17½ hours only. It would seem that previous soaking before sterilization for a short period of time is not deleterious but that soaking is definitely harmful before a long period of exposure. As in tests with other seeds the seedlings arising in tests which had been sterilized for more than 2 hours often had roots browned at the tip indicating injury.

The lower row of figures for the fourth day in Table VIII give the number of seeds whose testas were only just cracked or which were abnormal in some way. Thus it seems that the total number of seeds showing signs of germination is approximately the same in each test. This suggests that sterilization increases the number of weakly or abnormal seedlings occuring in the tests. This effect was noticed in the tests made with Triticum.

Wilson using a one per cent solution gives 4 hours and 11 hours as the periods of time, necessary to produce effective sterilization and injury to the seed respectively, for seeds of Brassica rapa.

TABLE VII.

Brassica rapa. Carter's 1933.

Date of expt. 28. 11. '35.

No. of seeds in tests, 100. Strength of Ca(OCl)Cl soln. - 1·8 grm Cl_2 per 100 c.c soln.

Duration of expt. in days.	Temp.	Total Number of Seeds shewing Germination									
		Control tests soaked.		Sterilized with Ca(OCl)Cl solution.							
		1hr	17½hrs	30min	1hr	Soaked 17hr Sterlzd 1 "	3½hr	6½hr	Soaked 6hr Sterlzd 17½hr	17½hr	Sterlzd 1% $HgCl_2$ 2min
1		5	36		2	5					
2	18°C	84	76	80	85	86	74	76	8	14	21
3									16	25	70
4	17°C	95	94	92	91	93	86	88			
5	16°C								24	47	91
6	16°C	97	96	96	100	97	93	97	31	60	96

Figure 16

Curves indicating the Rate of Germination of *Brassica rapa* after Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite.

Data from Table VII

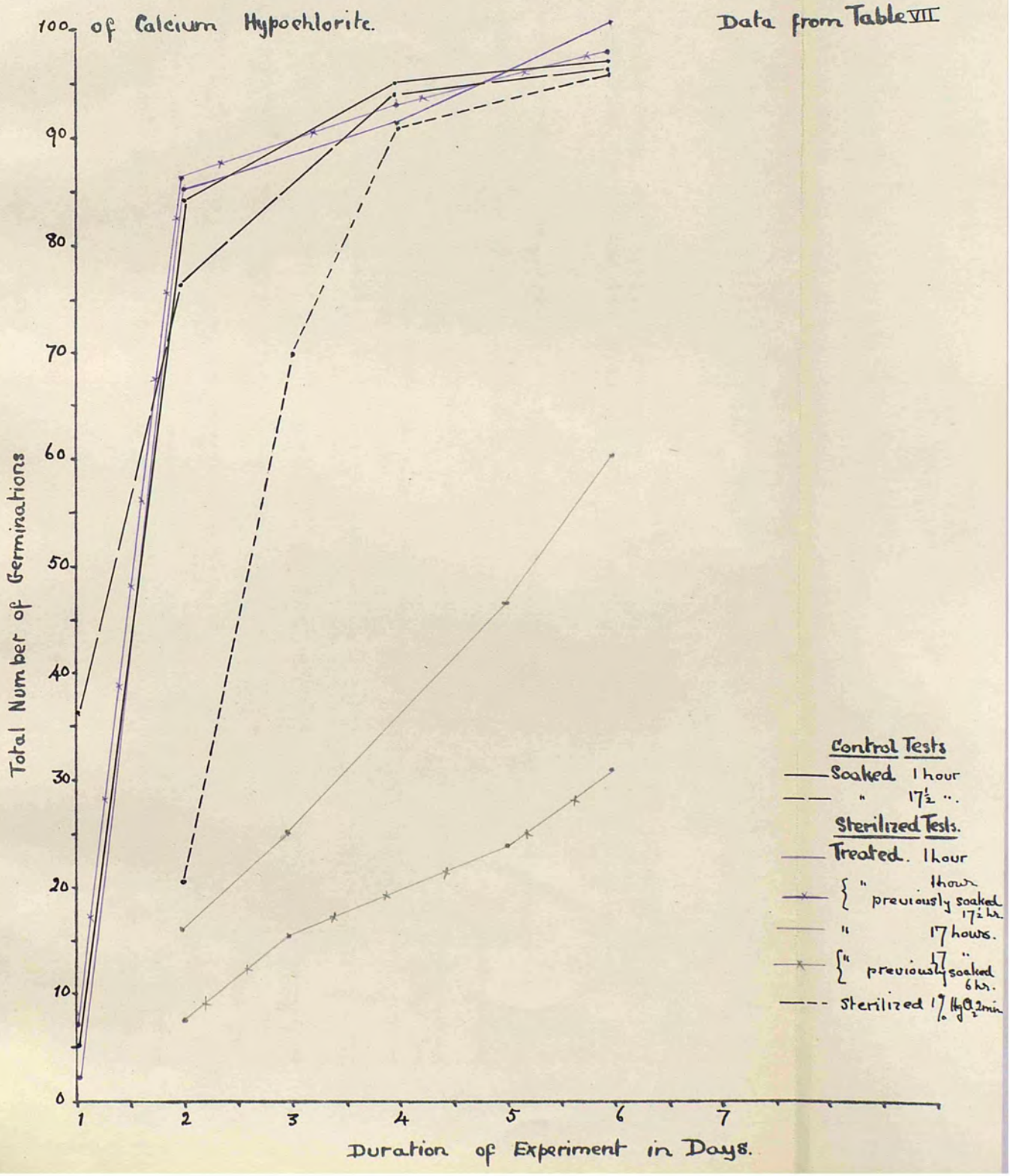


TABLE VIII.

Brassica rapa. Carter's 1933.

Date of experiment. 9. 4. '35

No. of seeds in each test. 100. Strength of Ca(OCl)Cl soln. = 1·9 grm Cl_2 per 100c.c. soln

Duration of expt. in days.	Temp.	Total Number of Seeds shewing Germination.								
		Control tests, soaked			Sterilized with Ca(OCl)Cl solution.					
		1hr	1hr	7hrs	30mins	1hr	1hr	2¾hr	5hrs	7½hr
1	18° C	41	49	55	57	49	44	21	18	10
2	17½° C	80	77	82	84	82	81	80	75	68
3	16° C	88	83	89	89	89	85	83	83	73
4	18° C	89	86	91	90	90	88	84	87	79
x "	"	10	13	7	9	8	6	15	12	20

x The lower row of figures on the 4th day indicate the number of cracked but not fully germinated seeds occuring in the tests in addition to those seeds which have already produced normal seedlings.

Figure 17

Curves illustrating the Extent of the Deviation which may occur between the Rates of Germination of two tests of Brassica napa treated in the same way.

Data from Table VIII

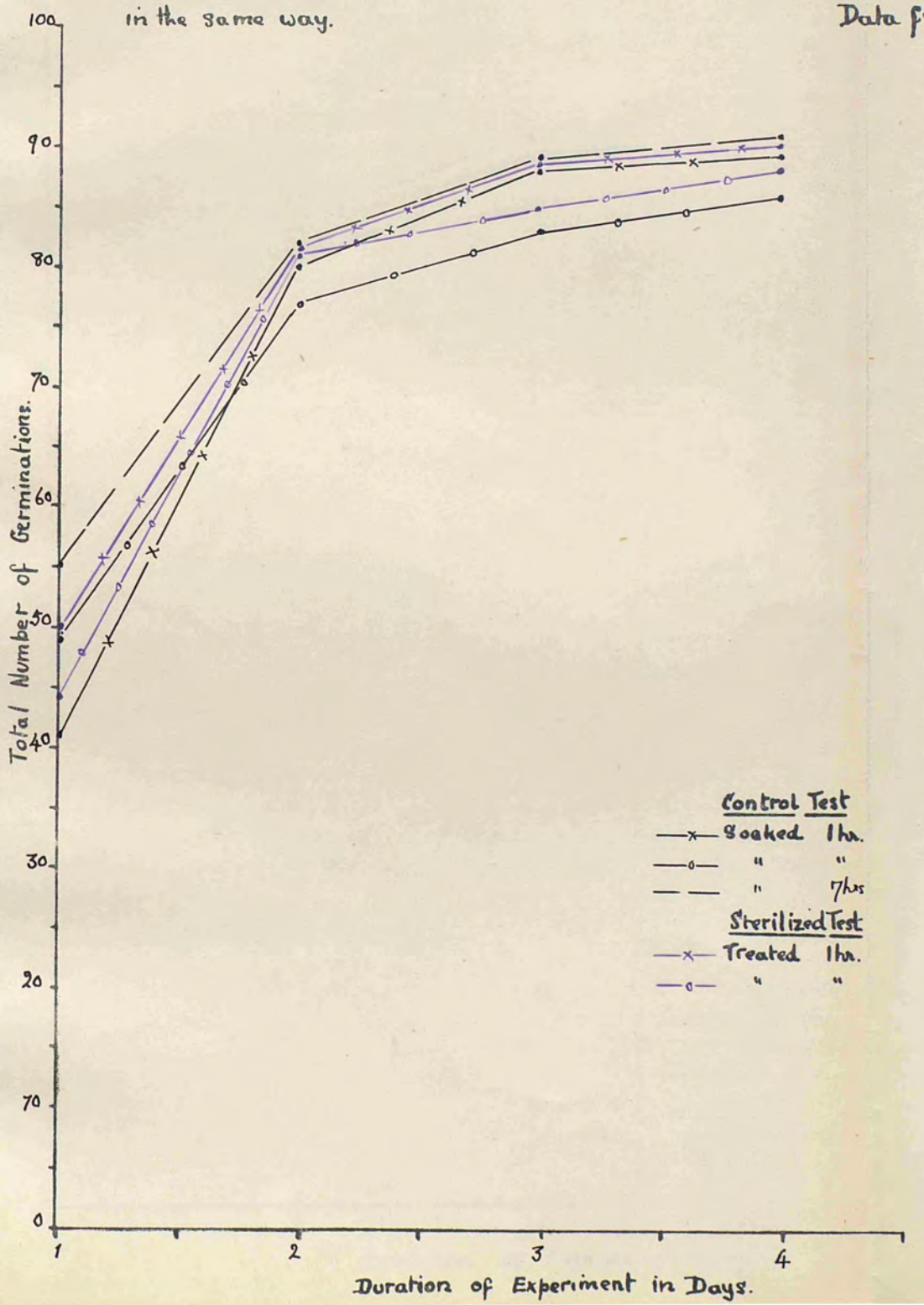
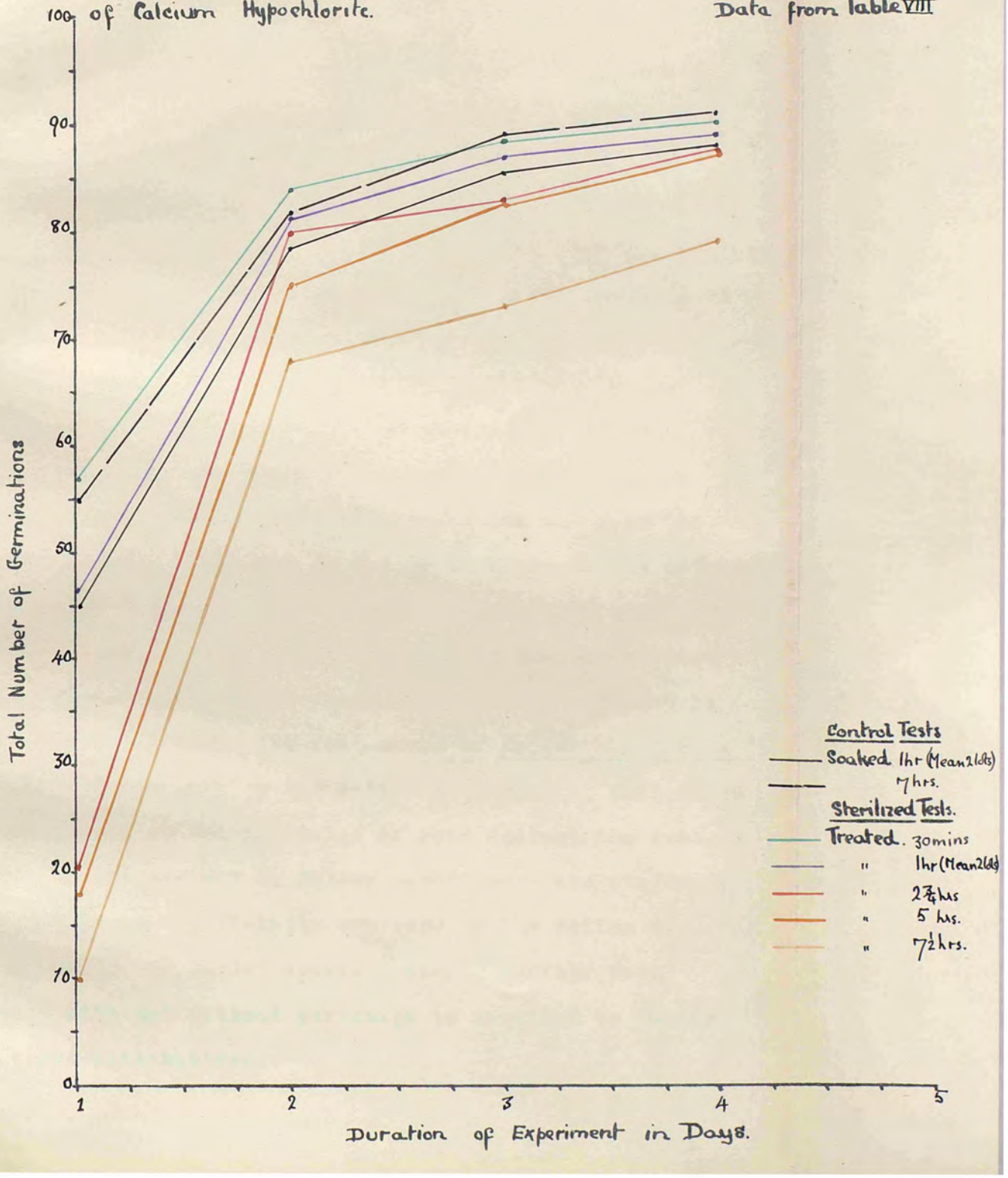


Figure 18

Curves indicating the Rate of Germination of Brassica rapa. after Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite.

Data from Table VIII

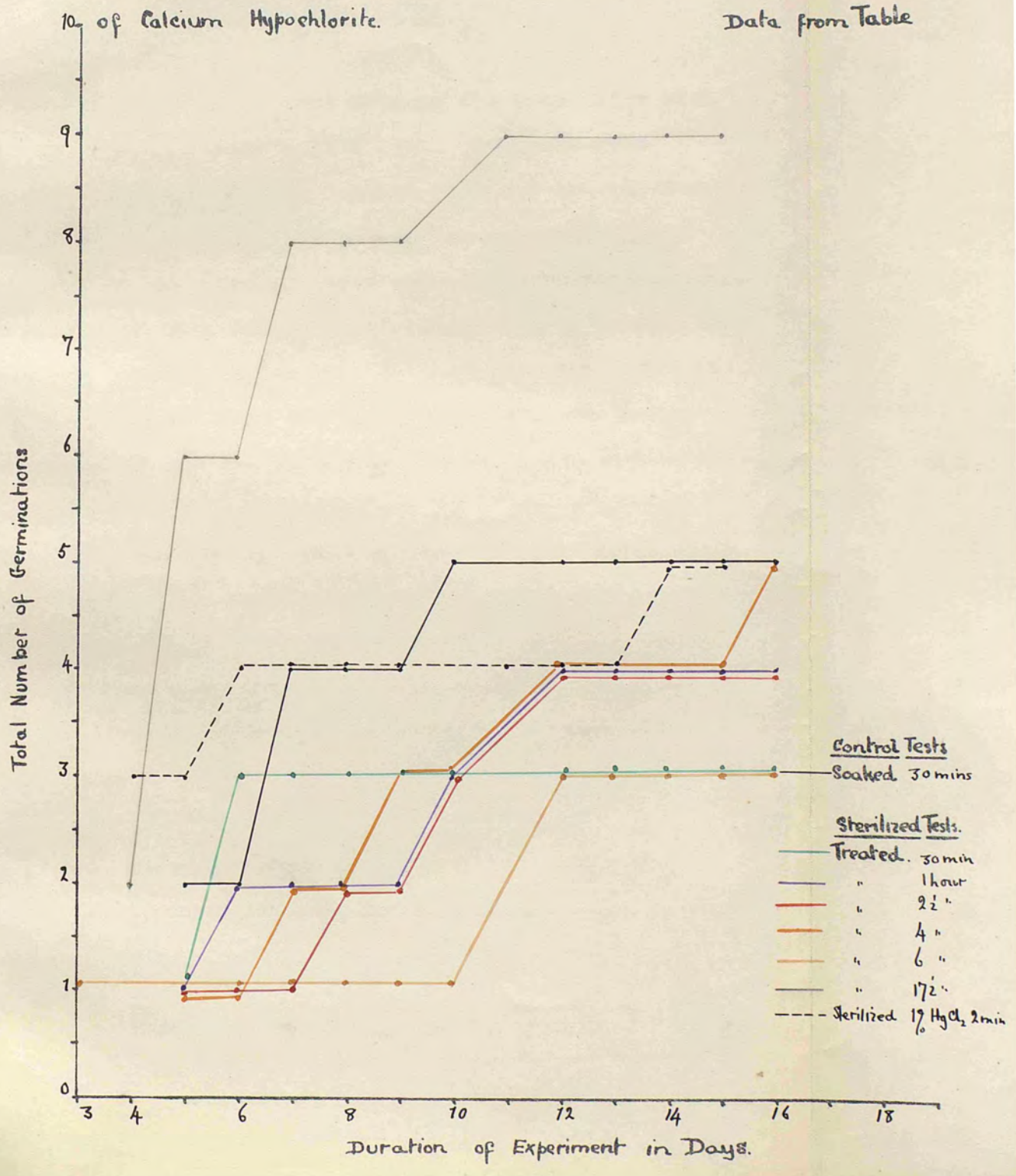


HELIANTHUS ANNUUS.

Fig. 19 illustrated the results obtained from a similar series of tests upon Helianthus annuus. Carter's 1934 seed. The total germination was extraordinarily poor and as, on account of their large size, only 10 seeds were used in each test, no reliable conclusions can be drawn from this experiment. There is, however, one significant feature, which seems to be more than a coincidence; the test treated for 17½ hours was the cleanest culture and shewed a total germination nearly double that of the control test and more than double many of the treated tests. One may conclude that sterilization has a beneficial effect upon the rate of germination and upon the total germination. This may be due to a change in the nature or structure of the seed-coats or the food-reserves contained in the cotyledons. As seeds with uncracked seed-coats were chosen the former alternative seems most likely. It may be that in normal conditions the seed-coats of Helianthus annuus are softened in the soil by the action of bacteria, soil acids or the scratching of the particles of soil against the coat. In sterile conditions and on filter paper these are excluded, and perhaps may be artificially replaced by the action of calcium hypochlorite during sterilization. **Further work using seeds with and without pericarps is required to decide between these alternatives.**

Figure 19

Curves indicating the Rate of Germination of Helianthus annuus after Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite. Data from Table



FAGOPYRUM ESCULENTUM

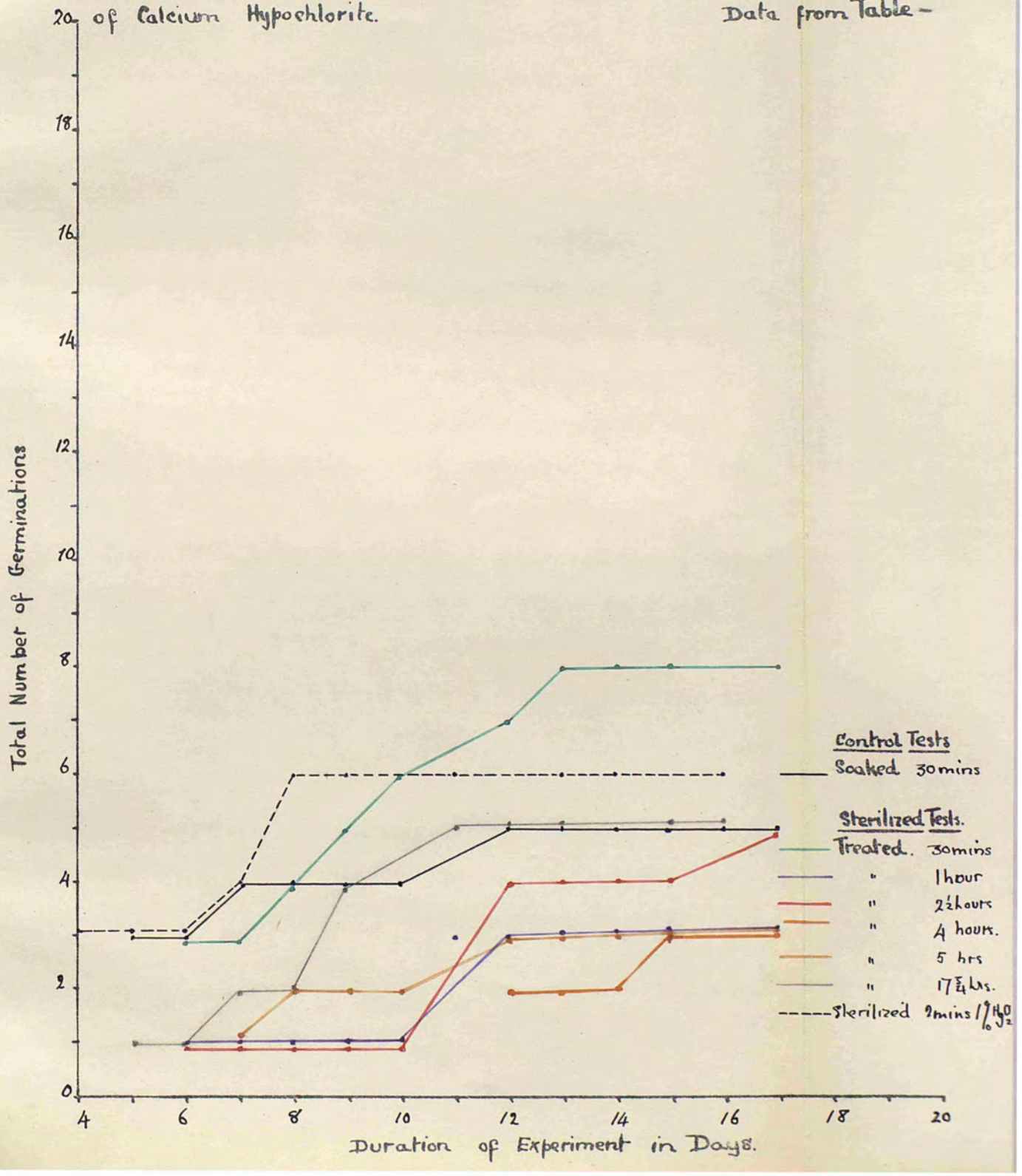
In February 1935 tests were made on the same lines with Fagopyrum esculentum Carter's 1934 seed. 20 seeds were selected for each test and the results obtained are plotted in Fig. 20. Very poor total germination was obtained in all cases and many of the treated tests were infected with moulds. It seems as though this method of sterilization is unsuitable for Fagopyrum, probably owing to the loose pericarp and thin testa; the former makes it difficult to remove air bubbles from the seed-coat and the latter serves as little protection for the embryo from the oxidizing action of the sterilizing solution. The tips of the roots of most of the seedlings in the treated tests were brown and shewed signs of injury. The difficulty is to remove all the air from the pericarp and to find a sterilizing agent which does not injure the embryo. The seeds were centrifuged for 10 minutes in an effort to remove the air from the pericarps, but apparently this was not successful.

Wilson states that he had no success with Fagopyrum esculentum and that it needed an exposure of 40 hours to a one per cent solution to cause injury to the seed.

Figure 20

Curves indicating the Rate of Germination of Fagopyrum esculentum. after Sterilization for Different Periods of Time with a Solution of Calcium Hypochlorite.

Data from Table -



Attempts to Elucidate the Action of Calcium Hypochlorite on Seeds during Sterilization, using Mickroscopical and Chemical Methods.

Whilst the foregoing tests were being carried out attempts were made to discover whether a solution of calcium hypochlorite of this strength acted in any way upon the seed-coats or food-reserves of the seeds being sterilized.

Preliminary microscopical investigations were made upon the seeds of Pinus and Helianthus. Sections cut through Pine seeds sterilized 17 hours and through control seeds soaked for an equal period in water, were treated with Sharlack R and were examined microscopically and compared. However so much oil was stored in the endosperm that it floated out and made it impossible to discriminate between the sections of the two seeds either by the distribution of the oil or by the relative oil content. The same difficulty arose when sections, cut through treated and untreated seeds of Helianthus were examined microscopically. The structure of the seed-coats was scrutinized miscroscopically but again there seemed to be no essential difference between the two sections. The results indicated that nothing could be gained from the microscopical examination of treated and untreated seeds; this led to further work on these lines being abandoned.

The next series of investigations carried out was an attempt to estimate and compare the ether extract of treated and untreated seeds. As a preliminary experiment in this series known weights of Pine seeds were taken and sterilized or soaked in water. The seed-coats were dried with Abs: Alcohol and broken and the seeds were left in contact with cold ether for about a day. The ether was poured off and the residue of the seeds was washed with more ether and, when dry, was weighed. The fat extracted was poured off with the first lot of ether and so was found by difference. Comparison of the results obtained in this way again failed to lead to any definite conclusion.

This led to a more thorough extraction of the oils by ether being carried out by the Soxhlet method. For this experiment two comparable batches each containing 100 seeds of Helianthus were chosen. These were weighed, one lot was soaked in distilled water and the other was sterilized for an equal period of time. The seeds were spread out in large petri dishes and put to dry in an oven, set at 78°C for three hours; they were stored in a dessicator until extracted with ether. This temperature was not high enough to alter the composition of the oils. For extraction, the seeds were ground up in a mortar and were put in an extraction thimble, the mortar being washed out with ether. A few lumps of anhydrous calcium chloride were put in the thimble as the powdered seed was not completely dry; this also served to remove any moisture that might be present in the ether (pure anaesthesia) as the latter had not been previously dried. The

extraction was carried out in the usual way, such as is described by Haas and Hill in their text-book Vol.I. The weights of ether extracts were found by direct weighing:- The results are tabulated below:-

Treatment fo Seeds	Fresh Weight of Seeds	Weight of Ether Extract	Ether Extract expressed as % of Fresh Weight
Soaked 20½hrs	6·8660 grms	2·1620 grms	31·50
Sterilized 19hrs	7·9710 grms	2·4570 grms	30·80

As the percentage ether extracts from treated and untreated seeds were approximately equal, it was concluded that, whatever the action of calcium hypochlorite upon the oily food-reserves of seeds may be, it does not render them insoluble in ether.

The oxidizing reactions of a solution of bleaching powder depend upon the ease with which it gives rise to hypochlorous acid. Calcium hypochlorite, when dissolved in water, is strongly ionized. Thus a solution of this salt can be regarded as containing the following substances in a state of equilibrium:-

$$Ca(OCl)Cl + H_2O \rightleftharpoons CaO + HCl + HClO$$

The calcium oxide, would not exist as such, but would either react with the hydrochloric acid or more water to form calcium chloride or calcium hydroxide.

Hypochlorous acid is very unstable and readily decomposes with the formation of hydrochloric acid and oxygen $HClO = HCl + O$.

This decomposition occurs in the presence of bright sunlight or when in contact with a substance capable of oxidation. In either of these ways one of the constituents of the above equilibrium is removed from the sphere of action and thus the decomposition of the calcium hypochlorite tends to proceed towards completion.

The bleaching action of calcium hypochlorite is caused by the oxidation of the coloured substance by the hypochlorous acid with the subsequent formation of colourless substances. Similarly if any unsaturated organic compound is in contact with a solution of bleaching powder that substance will be oxidized and the oxidation will continue until it is complete or until the calcium hypochlorite is completely decomposed.

With the decomposition of hypochlorous acid either by the liberation of oxygen as such or by the oxidation of some unsaturated compound, the strength of the solution of calcium hypochlorite decreases - the strength is measured by the strength of the available chlorine present. This consideration was utilized in the following experiments designed to indicate whether oxidation of the oils of seeds containing these as food reserves could take place during sterilization of such seeds with a solution of calcium hypochlorite. It was argued that if the amount of available chlorine present in a given volume of the sterilizing solution after sterilation of oily seeds was considerably less than that present in the same volume of the original solution then clear proof of oxidation would be given.

Accordingly a solution of sodium thiosulphate approximately equal in strength to the sterilizing solution was made up ($\frac{N}{1{\cdot}6}$ approx:). The oxidizing action was tested upon 1) an unsaturated oil, e.g. commercial castor oil. 2) whole seeds, containing oils as food reserves e.g. sunflower, and 3) the oils extracted with ether from an oily seed e.g. sunflower. In each case 25 c.cs. of the sterilizing solution were added to the oil and after a period of about 17 hours this was titrated with the thiosulphate solution and the titration value was compared with that obtained for 25 c.cs. of the original solution. The titration was carried out in the usual way using sodium thiosulphate to estimate the liberated iodine:-

$$Ca(OCl)Cl + 2HA\bar{c} = CaA\bar{c}_2 + Cl_2 + H_2O$$

$$2KI + Cl_2 = 2KCl + I_2$$

$$Na_2S_2O_3 + I_2 = 2NaI + Na_2S_4O_6$$

The first experiments with castor oil were carried out by running a layer of this oil into a stoppered bottle and allowing 25 c.cs. of the sterilizing solution to react with it during a given period of time. The control consisted of a similar bottle containing 25 c.cs. of the solution but no oil left for an equal period of time. The bottles were stoppered to prevent the access of carbon dioxide to the solution. Before titration 25 c.cs. of chloroform was added to each; this brought about almost complete separation of the oil from

the aqueous layer. Although this method was repeated several times no great change, in the amount of available chlorine present, was apparent from the titration values. The results are shewn in Table IX.

TABLE IX.

Treatment of Ca(OCl)Cl soln.	Vol. of thiosulphate $\frac{N}{1.6}$ ≡25c.c. Ca(OCl)Cl soln.	Weight of castor oil.
1) 25 c.c. titrated immediately after withdrawal.	25·05 c.c. 25·10 c.c.	- -
2) 25 c.c. mixed with castor oil separated with chloroform and titrated immediately.	24·90 c.c.	-
3) 25 c.c. mixed with castor oil for 4½hrs separated with chloroform and titrated immediately.	24·85 c.c.	1·6680 grm
Same as above, but left in contact with castor oil for 5½ hrs.	24·95 c.c.	1·3274 grm
4) 25 c.c. mixed with castor oil for 16hrs separated with chloroform and titrated immediately.	24·60 c.c. 24·60 c.c.	2·1130 grm 1·4596 grm

In cases where the weight of castor oil used was known, it is seen that the slight change in the titration value bore no relation to the weight of oil used.

Then it was realized that the area of the surface of contact between the aqueous layer and the oil was comparatively small and that the formation of a small amount of an oxidation product over this surface might completely block all further action. Thus it was decided to increase this area by the formation of an emulsion; the mixtures were shaken on a mechanical shaker during the periods of reaction. Separation was brought about by the addition of chloroform before titration. The control consisted of a bottle containing 25 c.cs. of the solution which was shaken for an equal period of time and to which chlorform was added before titration. The results are shewn in Table X.

T A B L E X.

Treatment of Ca(OCl)Cl soln.	Vol. of thiosulphate ≡ 25c.c. Ca(OCl)Cl soln.	Decrease in vol. of thiosulphate needed for treated 25 c.c. Ca(OCl)Cl soln.
1) 25 c.c Ca(OCl)Cl titrated immediately after it was made up.	28·95 c.c. 28·85 c.c.	
2) 25 c.c. mixed with approx. 10 c.c. castor oil shaken for 18hrs separated with chloroform and titrated. Results from 3) used for comparison.	17·30 c.c. 17·20 c.c. 15·45 c.c. Mean = 16·65 c.c.	10·75 c.c.
3) 25c.c. mixed with 10 c.c. castor oil separated with chloroform and titrated immediately	27·10 c.c. 27·70 c.c. Mean = 27·40 c.c.	
4) 25c.c. shaken by itself for 17hrs, chloroform added and titrated.	28·30 c.c.	
5) 25c.c. mixed with 10 c.c. castor oil and shaken for 17hrs separated with chloroform and titrated. Results from 4) used for comparison.	19·65 c.c.	9·65 c.c.

The experiments on the whole seeds of Helianthus and on the oil extracted by cold ether were conducted on the same lines. 25 c.c. of the sterilizing solution were shaken with the whole seed and with the extracted oil and the titration values were

compared with those of a similar volume of sterilizing solution shaken for an equal period of time. The results are shewn below in Table XI.

TABLE XI.

2) Whole seed - Helianthus annuus.	Vol. of thiosulphate ≡ 25c.c. Ca(OCl)Cl soln.
(a) 25 c.c. Ca(OCl)Cl shaken 17hrs	28·30 c.c.
" " " " " with 100 seeds.	1·30 c.c.
(b) 25 c.c. Ca(OCl)Cl shaken 17 hrs with 100 crushed seeds.	2·45 c.c.
	Difference 27·00 c.c.
3) Oil extracted from Helianthus annuus.	
25 c.c. Ca(OCl)Cl shaken 19hrs	25·00 c.c.
" " " " " oil from 100 sunflower seeds	1·20 c.c.
	Difference 23·80 c.c.

These results are far from complete and leave many interesting questions still unsolved, but they shew that a solution of bleaching powder of the strength used for sterilizing seeds is capable of oxidizing unsaturated oils such as occur in the food reserves of oil containing seeds. The titration value of 2b is not too reliable on account of the difficulty of seeing when the titration was complete but

comparison of the results of 2a and 3 shew that the titration values are of the same order thus indicating that the principal action of the sterilizing solution upon the seeds is the oxidation of the oily food reserves; but the decrease in strength of the sterilizing agent used in 2a is greater than that of 3 which suggests that the seed-coats of the seeds may be oxidized also to some extent.

DISCUSSION AND CONCLUSIONS.

The experimental results lead to the conclusion that sterilization of seeds with a two per cent solution of calcium hypochlorite for short periods of time is not detrimental to their rate of germination or to their total germination. Suitable periods of exposure seem to lie between $\frac{1}{2}$ - 2 hours. Longer periods cause decreases in both the rate of germination and in total germination; and these generally become more marked with longer sterilization. The periods of exposure which produce these effects vary with the kind of seed being sterilized. It seems as though seeds with thicker and more resistant seed-coats can withstand longer periods of immersion than those with less protection. This is what would be expected.

Sterilization for short periods of time seems to result in slight increases in the rate of germination of some seeds, e.g. Linum, Brassica and Pinus, whilst no such stimulatory effect is experienced by other seeds e.g. Zea and Triticum. It is interesting to notice that those seeds which exhibit stimulation in this way contain an oil as their food-reserve whilst those which shew no appreciable increase in rate have starchy food reserves.

The work on the action of a solution of calcium hypochlorite upon oily food-reserves was at first disappointing and yielded no clue as to the cause of this slight initial increase in rate of germination. Microscopical investigation

of the distribution of the oil before and after sterilization was impossible to carry out and the amount of ether extract obtained from treated and untreated seed seemed to vary little. Then later a method was devised which shewed that a solution of calcium hypochlorite can bring about the oxidation of oils contained in certain seeds. The results which led to this conclusion were obtained from extracted oils and from whole seeds left in contact with the sterilizing solution for the longest period of time used in the germination tests. They indicate the possibility of extensive oxidation taking place during these periods but that which occurs whilst seeds are being sterilized for shorter periods is without doubt considerably less.

The microscopical examination of the seed-coats of treated and untreated seeds yielded no evidence of alteration, although as already stated the latter series of investigations suggested oxidation to some extent.

The nature of the seed-coats and the size of the seed would seem to be important factors. Thus Linum and Brassica, seeds which shewed a slight increase in rate of germination after short periods of sterilization, are small and have comparatively thin seed-coats when compared with Helianthus, which benefited after 17½ hours sterilization. The seeds which shewed no stimulation, besides having starchy food-reserves have thin papery pericarps, Triticum and Zea or testa Fagopyrum.

The results discussed above bear out Wilson's statement that a solution of calcium hypochlorite is a useful sterilizing agent and needs only reasonable care in its use. The results

indicate that though such long periods as Wilson mentions may be necessary to produce seedlings which remain sterile for months and can be grown on nutritive media, yet satisfactory sterilization may be effected by shorter periods of exposure to a two per cent solution without any danger of causing delay in rate of germination or of injury to the seeds.

Under the usual conditions of sterilization in which whole seeds are used and in which a seed-coat has to be penetrated by the sterilizing agent, the oxidising action of the calcium hypochlorite appears to be slight in the case of the seeds employed, nor has a marked increase in rate of germination of these seeds been found to occur. The microscopical work upon the distribution of oil in the seeds and the structure of the seed-coats after sterilization also indicated that the action of the calcium hypochlorite was slight when compared with the whole mass of the seed upon which it could have acted. At the same time, evidence has been obtained that a considerable degree of oxidation of the unsaturated oils contained in seeds occurs if conditions are suitable, e.g. if a large surface of oil is presented to the calcium hypochlorite. No doubt calcium hypochlorite is capable of oxidizing Calluna oil as readily as Sunflower oil under these circumstances; the matter was not tested practically since sufficient quantity of Calluna seed was not available. Whether it is capable of doing so when the intact seed is used requires further investigation; in short, the results obtained with other seeds leave it an open question as to whether the unusually rapid germination of Calluna seed

sterilized with calcium hypochlorite as recorded by Knudson (6) can be ascribed to the partial oxidation of the storage oil. The small size of the seed, offering a large surface for the hypochlorite to act upon, may be regarded as favouring the occurrance of such action, but until a sample of Calluna seed becomes available for experiment no positive statement either way can be made.

P A R T I I.

A STUDY OF THE EFFECT UPON THE GERMINATION OF CERTAIN SEEDS PRODUCED BY THE REDUCTION OF THE OXYGEN PRESSURE IN THE ATMOSPHERE SURROUNDING THEM WHEN THE OTHER CONDITIONS ARE SUITABLE FOR GERMINATION.

PART II.

General Considerations and Preliminary Methods of Experimentation.

It has been stated above that the greater part of this work was carried out with a view to obtaining some knowledge concerning the capability of seeds to germinate in atmospheres containing proportions of oxygen lower than those which normally occur in the air. Except for this reduction of oxygen the seeds were kept in conditions which were thought adequate for normal healthy germination. The seeds were sewn on moist filter paper, were supplied with sufficient moisture and were maintained at suitable temperatures and at pressures differing little from atmospheric pressure. The sewing of the seeds on filter paper would not have been justifiable if anything more than the effect of reduced oxygen pressures upon the capabilities of various seeds to germinate and upon their behaviour during germination had been required. A short description of the methods of experimentation used at the outset will serve to indicate the difficulties encountered in the attempt to study this problem.

The first apparatus was as simple as possible and was designed to ascertain the nature of the problem and the degree of accuracy necessary rather than to obtain accurate

results. The parts of the apparatus and the way in which they were connected are shewn in Diagram 21. The seeds were scattered on moist filter paper in the wide-necked bottle A, of about 200 c.cs. capacity, fitted with a large cork through which two delivery tubes, one long and one short, were passed. The atmosphere inside the bottle could be changed, or renewed, at will by passing the required gas-mixture through the delivery tubes at a slow rate for a sufficiently long time - e.g. some 6 litres of gas-mixture in 1 hour. The cork was soaked in paraffin wax and every reasonable precaution was taken to ensure the bottle being air-tight.

The gas-mixtute was prepared in the large aspirator B, whose volume was about 12½ litres. This was done by filling the aspiration with water, a known volume was withdrawn with pinch-clip b open to the air thus an equal volume of air was drawn into the aspirator, the remaining volume of water was allowed to run out slowly while pinch-clip b was closed and clip a was open, thus this latter volume of water was replaced by air which had been drawn slowly through the series of wash bottles on the shelf. The first three bottles C contained a strong solution of pyrogallol and the last D was half filled with water. During the passage of the air through the pyrogallol the oxygen and traces of carbon-dioxide, which it contained, were absorbed by the pyrogallol and the caustic potash, in which the pyrogallic acid was originally dissolved, respectively. In this way the second volume of water with-

drawn from the aspirator was replaced by practically pure nitrogen. The approximate composition of the resulting gas mixture was readily obtained by calculation. The gas-mixture was introduced into the seed chamber by connecting the apparatus as shewn in the diagram. The flow of water from the mains was regulated to a gentle rate thus securing a slow and steady flow of the gas-mixture into and through the seed-chamber. A pair of bottles were filled from one mixture.

This apparatus was used to experiment upon cress seed but was soon found to be unsuitable on account of its lack of accuracy. Two of the many sources of error were the lack of an accurate method of calculating the composition of the gas-mixture and the difficulty of making the seed-chamber air-tight. Besides these the extreme slowness of obtaining the nitrogen for the gas-mixture was a serious disadvantage. However, in spite of these faults and others to be enumerated later, the method did indicate that interesting results bearing on the problem might be obtained by the use of more accurate methods.

A due consideration of the sources of error and the disadvantages connected with the working of the method described above led to the assembling of the apparatus shewn in Diagram 22. It was a vast improvement in accuracy; the seed-chambers were sealed with a liquid (cf Morinaga's apparatus (1)) and a device for calculating the composition of the gas-mixture more accurately was incorporated.

The details of the apparatus can be seen from the diagram. The seed-chambers were small rectangular basins inverted in larger basins containing liquid paraffin. They were supported on stands, thus enabling delivery tubes to be inserted under their edges. The seeds were placed on a strip of filter paper, which passed over a slide resting on a low cylindrical glass jar containing water into which the ends of the filter paper dipped; thus the seeds, were supplied with sufficient moisture. The gas-mixture was prepared in the aspirator in a manner similar to that described for the first method, except that the nitrogen was obtained from a cylinder of the liquid gas. After the required volume of air had been drawn into the aspirator, the nitrogen cylinder was connected to the upper tube and the remaining water was run out as before. When the seed-chamber was being filled, two delivery tubes were inserted under its sides and the gas-mixture was pushed slowly through the apparatus by displacement with water from the mains. The atmosphere was renewed about once in every three days.

As very low oxygen pressures were being used in the seed-chambers it was thought advisable to seal them with a liquid in which oxygen was practically insoluble and thus prevent the composition of the gas-mixture being altered owing to slow diffusion of oxygen from the air through the liquid. Thus water was considered unsuitable for this purpose and liquid paraffin was chosen for sealing the seed-chambers. This, then, provided

an absolutely air-tight seed-chamber and ensured the composition of the gas-mixture remaining unchanged as the result of external conditions.

The gas-mixture was prepared as described for the first method and hence a rough idea of the percentage of oxygen contained in it was known. A more accurate value for the percentage of oxygen present was obtained by the estimation of the volume of oxygen present in two samples of the gas-mixture collected in the graduated gas sampling tubes inserted before and after the seed-chamber. Thus when sufficient gas-mixture had been passed through the seed-chamber to replace the old mixture the stop-cocks were closed and samples of the gas-mixture, collected before entering and after leaving the seed-chamber, were removed. The oxygen in these samples was estimated by absorption with pyrogallol. The sampling tubes were clamped in a vertical position with one end immersed in a strong solution of pyrogallol and the lower stop-cock was opened, then as the oxygen was absorbed the pyrogallol rose in the tube. The tube was levelled and the volume of oxygen contained in it was read directly. The volumes of the tubes were known and thus the percentages of oxygen contained in both samples of gas-mixture were easily calculated. The mean of these values was taken as indicating the percentage of oxygen contained in the seed-chamber.

The gas-sampling tubes were shaped as shewn in the diagram

and were designed to give readings correct to 0·05 c.c. in the narrow part of the tube, volume 30 c.c. and readings correct to 5 c.c. in the wider part of the tube, volume 220 c.c. The total volume of the tubes was about 260 c.c. and thus the lower percentages of oxygen could be calculated correct to 0·02 .

However, the estimation of oxygen, present in the tubes, in this way was not straight forward. The rate of absorption is very slow when the pyrogallol has to rise through the narrow bore of a stop-cock and during this time the temperature has to be kept constant else changes in volume will take place and will have to be taken into consideration. Later a method whereby a little pyrogallol was forced into the tube at the beginning of the estimation was designed: this did much to shorten the process, but had to be used with care.

This method was used for the preliminary experiments on the effect of reduced oxygen pressure upon the germination of seeds of Pinus silvestris. These seeds need several days at room temperature before any visible signs of germination occur and when the oxygen pressure is reduced this period is considerably longer. The increasing of the period necessary for germination allows ample time for the spores of fungi and bacteria present on the seed-coats to develop and form large colonies, so much so that after ten or more days it becomes difficult to determine whether the reduced oxygen pressures or the fungal attacks are the cause of non-germination. Accordingly it was

decided that the seeds must be sterilized before being put to germinate, that the seed-chamber must be washed out with conc. HNO_3 and autoclaved distilled water and that the whole operation must be carried out in a sterilizing room. Several sterilizing solutions were tried for Pinus;- those which were found most successful were a freshly prepared solution of calcium hypochlorite (strength 2% titratable chlorine, J.Wilson (5)) and 0·1% mercuric chloride used for 1 minute on seeds which had been centrifuged in order to remove all air clinging to the seed-coats. The advantages connected with the use of calcium hypochlorite solution have already been discussed; however, it was at this stage that doubts, as to its possible action on seeds containing oily food reserves, first arose. It was used for sometime, but when the seeds shewed marked differences in the time necessary for germination it was thought better to use a solution of mercuric chloride which could not exert a stimulatory effect upon the rate of germination as a result of oxidizing properties.

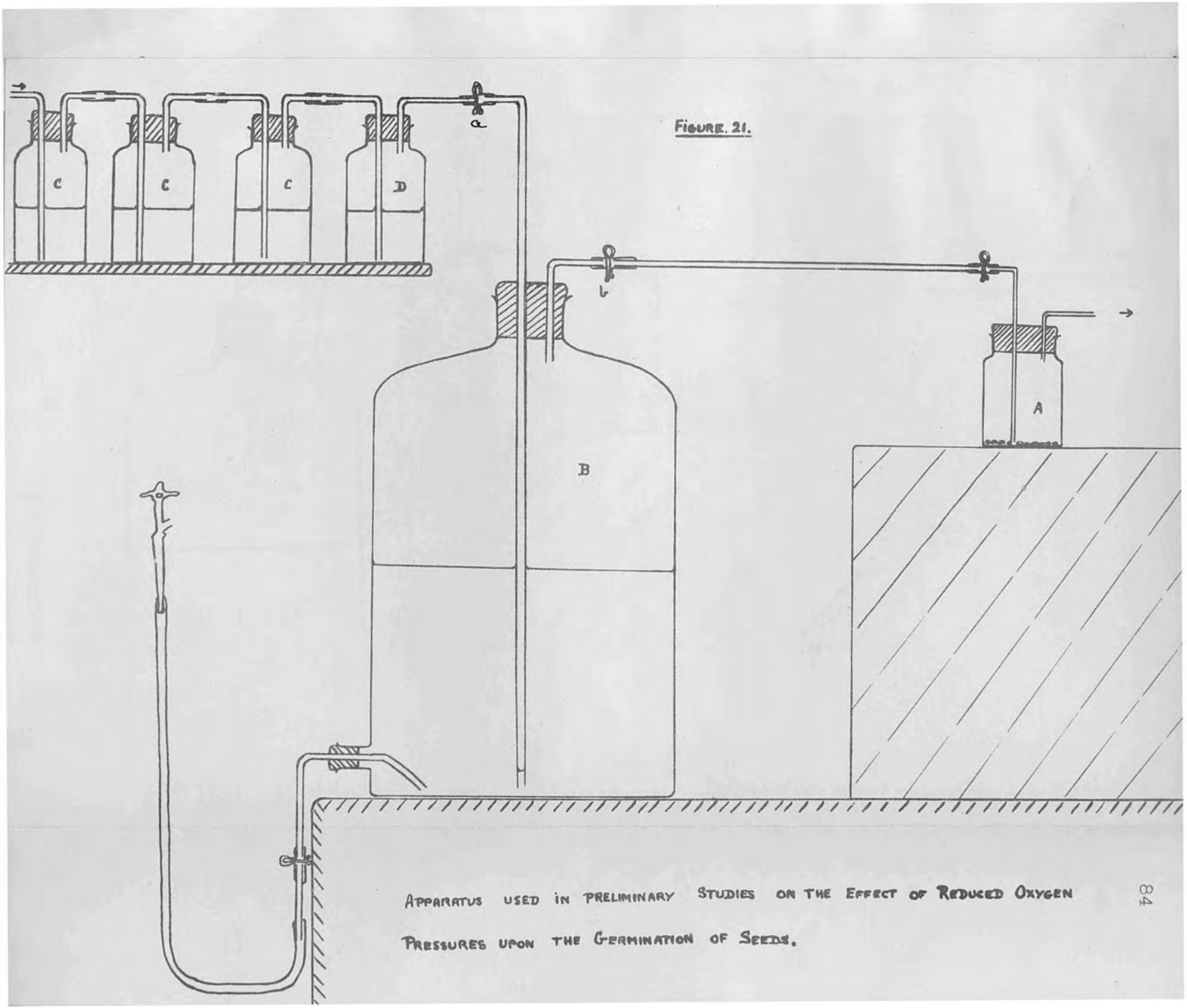
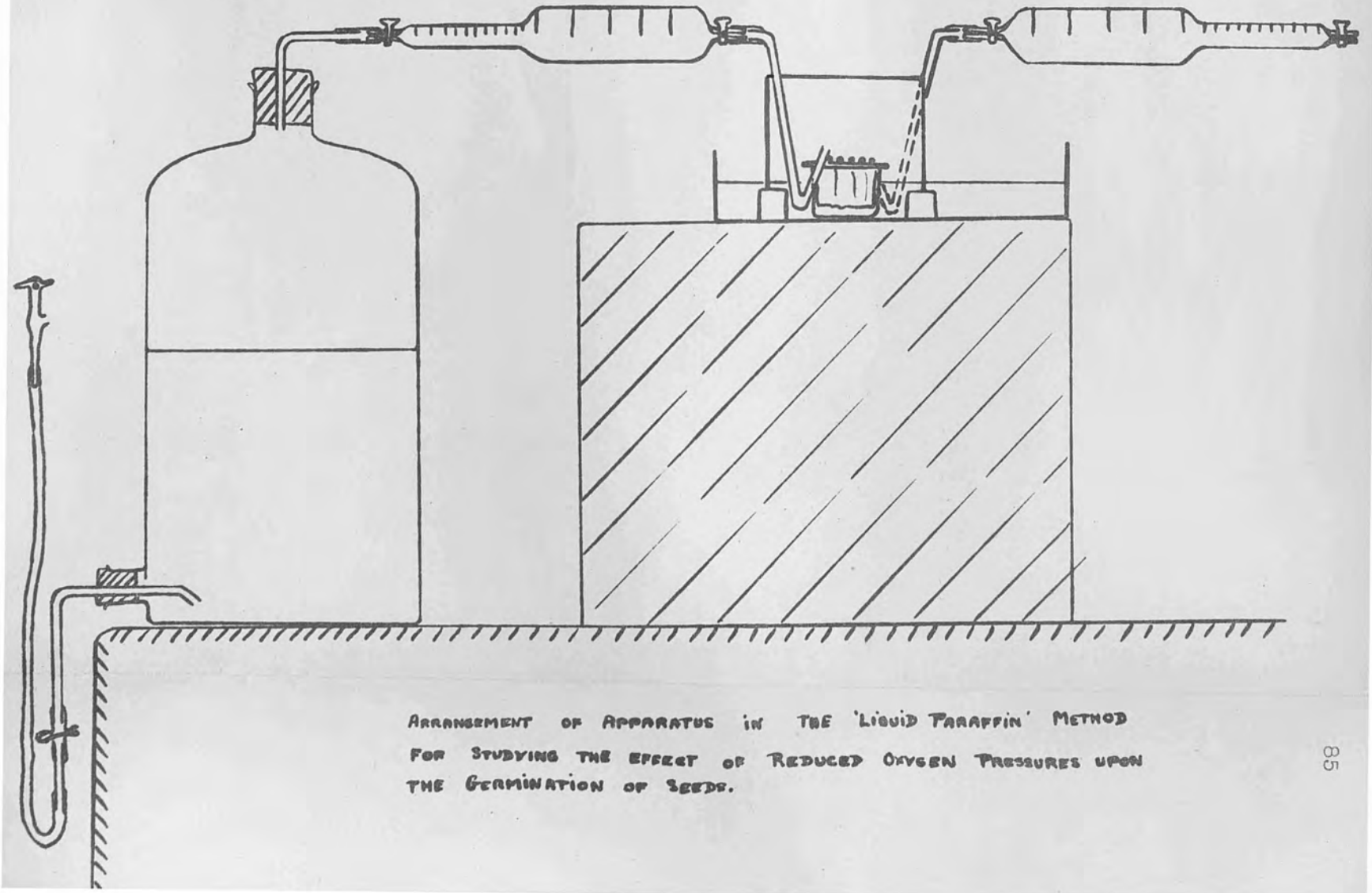


FIGURE. 21.

APPARATUS USED IN PRELIMINARY STUDIES ON THE EFFECT OF REDUCED OXYGEN PRESSURES UPON THE GERMINATION OF SEEDS.

FIGURE 22



ARRANGEMENT OF APPARATUS IN THE 'LIQUID PARAFFIN' METHOD FOR STUDYING THE EFFECT OF REDUCED OXYGEN PRESSURES UPON THE GERMINATION OF SEEDS.

Experimental Details and Results from Preliminary Method.

The liquid paraffin method was used in experiments carried out with seeds of *Pinus silvestris* and *Triticum*. The seeds used were:-

Pinus silvestris. Eastern Counties. F.M. 1933.

Triticum. Carter's Yeoman King. 1933.

In each series of tests a suitable number of seeds were used. These were sterilized with a solution of calcium hypochlorite - *Pinus* Series II and *Triticum* Series IV A and C - or with a 0·1% mercuric chloride solution - *Pinus* Series III and Triticum Series B. Before sterilization the seed-coats were thoroughly wetted by centrifuging for a short time. The tests in Series III were duplicated.

The results obtained from these experiments are shewn in the Tables XII, XIII, XIV, XV and XVI. The total number of germinations in each test is given for definite days after sowing. The results are illustrated in Figs. 23, 24, 25, 26 and 27. In these, percentage germination is plotted against time in days; thus the rate of germination is indicated and the effect of reduction in the oxygen pressure of the atmosphere surrounding the seeds is made clear. Where duplicate tests were set up, the mean percentage

germinations are plotted.

The particular treatment of each test and the days on which the gas-mixtures were renewed are shewn in the Tables. All the precautions, outlined above, were taken when the tests were being prepared.

TABLE XII.

Pinus silvestris Series II.

Number of Seeds in test 20. Controls sterilized. Set up 10. 1. '34

Sterilized Ca(OCl)Cl soln. 30 mins " unsterilized. " " 11. 1. '34

Duration of expt. in days	Controls Sterilized 1 and 2				Controls unsterilized		
		Total Germin.		Mean % Germin.		Total Germin.	% germin.
		CS1	CS2				
4							
5	Filled 1		1	2·5			
6					Filled	1	5
7	Filled 2		6	15	Filled	2	10
8	Filled 1						
	" 2	1	7	20			
9		1	8	22·5	Filled	5	25
10	Filled 1						
	" 2	2	10	30			
11					Filled	8	40
12	Filled 1						
	" 2	6	14	50	Filled	8	40
13						8	40
14		6	14	50	Filled	8	40
15	Filled 1						
	" 2	7	15	55			
16							
17							
18							
19	Filled 1						
	" 2	8	17	62·5			
20							
21						8	40
22		9	17	65			

T A B L E X I I. cont.

Pinus silvestris Series II.

Number of Seeds in test 20. Test III. 6% oxygen. Set up 12. 1. 34.

Sterilized Ca(OCl)Cl soln. 30 mins. Test I 4% " " " 11. 1. 34.

Duration of expt. in days.	Test III 6% oxygen.			Test I 4% oxygen.		
	% oxygen	Total germin:	% germination	% oxygen	Total germin:	% germination.
4				3·9		
5		-				
6						
7	6·0	1	5	4·4	-	-
8		1	5		2	10
9					2	10
10						
11		6	30	4·0	3	15
12		7	35		3	15
13					4	20
14	5·6	7	35	2·8	4	20
15						
16						
17		8	40			
18		9	45	4·5	7	35
19	6·5	10	50		8	40
20					8	40
21						
22		11	55			40

T A B L E XII. cont.

Pinus silvestris Series II.

Number of Seeds in test 20. Test II. 3% oxygen. Set up 11. 1. 34.

Sterilized Ca(OCl)Cl soln. 30mins. Test IV. 2% " " " 20. 1. 34.

Duration of expt. in days.	Test II 3% oxygen.			Test IV 2% oxygen.		
	% oxygen	Total germin:	% germination	% oxygen	Total germin.	~~Mean~~ % germin.
4	2·9			2·1		
5						
6				1·9		-
7	3·6					
8						
9		-				
10				2·4	1	5
11	3·1	2	10	2·6	1	5
12		2	10			
13		4	20			
14	3·4	4	20	2·2	1	5
15						
16					2	10
17						
18	3·5	7	35	2·3	4	20
19		7	35		5	25
20		7	35			
21					5	25
22		7	35			

TABLE XIII

Pinus silvestris Series III.

Number of Seeds in test 20. Controls, sterilized. Set up 25. 1. 34.

Sterilized 0·1% $HgCl_2$ 1 min. Controls, unsterilized. " " 12. 2. 34.

Duration of expt. in days.	Controls Sterilized a and b	Total germin: Ca	Total germin: Cb	Mean % Germin:	Controls unsterilized a and b	Total germin: CUa	Total germin: CUb	Mean % Germin:
5		-	-					
6		1	2	7·5		-	-	
7	Filled a	3	4	17·5		3	1	10
	" b							
8								
9		5	6	27·5		5	4	22·5
10					Filled a			
					" b	6	5	27·5
11	Filled a							
	" b	6	9	37·5		6	6	30
12		8	9	42·5				
13		8	10	45				
14	Filled a							
	" b	8	10	45		11	8	47·5
15								
16		13	11	60		11	8	47·5
17						11	8	47·5
18	Filled a				Filled a			
	" b	14	12	65	" b	11	8	47·5
19		14	13	67·5		12	8	50
20		14	13	67·5				
21		14	14	70		12	8	50
22		14	14	70		12	8	50

TABLE XIIIcont.

Pinus silvestris Series III.

Number of seeds in test 20. Test 4. 6 - 7 % oxygen. Set up 22. 11. 34.

Sterilized 0·1 % $HgCl_2$ 1 min. Test 2. 4 - 5 % oxygen. " " 8. 11. 34.

Duration of expt. in days.	Test 4. a and b 6 - 7 % oxygen				Test 2. a and b 4 - 5 % oxygen.			
	% oxygen	Total germin: 4a	4b	Mean % germin:	% oxygen	Total germin: 2a	2b	Mean % germin:
	6·6 a				4·6a			
5	6·5 b	-	-					
6			1	2·5			-	-
7			2	5	4·6 b		1	2·5
	6·1 a				4·5 a			
8	6·8 b		3	7·5		-	4	5
9			5	12·5				
10								
11		-	6	15	4·8 b	1	5	15
	6·7 a							
12	6·7 b	3	7	25		2	7	22·5
13		4	8	30	4·6 a	2	7	22·5
14		4	8	30	5·0b	3	10	32·5
	6·1 a							
15	6·6 b	6	10	40				
16								
17								
18		6	12	45	4·7 a	8	12	50
19	6·6 a	7	12	47·5				
20						8	12	50
21					4·8 a	8	12	50
					4·8 b			
22		7	12	47·5		8	12	50

T A B L E XIII cont.

Pinus silvestris Series III.

Number of seeds in test 20. Test I. 2 - 3 % oxygen Set up 1. 11. 34.

Sterilized 0.1% $HgCl_2$ 1 min. Test 3. Nitrogen from Cylinder " "14. 11. 34.

Duration of expt. in days.	Test 1. a and b 2 - 3 % oxygen				Test 3. a and b. Filled from N_2 cylinder			
	% oxygen	Total germin: 1a	1b	Mean % germin:	% oxygen	Total germin: 3a	3b	Mean % germin:
5					Filled a			
					" b			
6	2.6 b							
	2.6 a				Filled a			
7					" b			
8	3.2 b		-					
9			1	2.5				
10								
11	2.4 a		2	5				
	2.9 b							
12			2	5	Filled a			
					" b		-	
13			2	5			1	2.5
14	2.8 a		2	5	Filled a		1	2.5
	3.1 b				1.2 b			
15		-	2	5			1	2.5
16							1	2.5
17					0.66 a		1	2.5
					Filled b			
18	2.5 a	1	3	10				
	2.7 b							
19		1	3	10			1	2.5
20		3	3	15			1	2.5
21					1.3 a		3	7.5
					Filled b			
22		3	4	17.5		-	3	7.5

T A B L E XIV.

Triticum Series IV. Control CS 1 Set up. 5. III. 34.

Number of seeds in test 40. Control CS 2 " " 14. III. 34.

Sterilized with Ca(OCl)Cl soln. 30 mins. Test I " " 5. III. 34.

Duration of expt. in days.	Control Sterilized. CS 1.			Control Sterilized CS 2.			Test 1·3 % oxygen		
		Total germin:	% germin:		Total germin:	% germin:	% oxygen	Total germin:	% germin:
1		-	-						
2		15	37·5		-	-		-	-
3	Filled	28	70	Filled	18	45	3·4	6	15
4		33	82·5					24	60
5					25	62·5			
6					31	77·5			
7	Filled	34	85		32	80	3·2	26	65
8		34	85		34	85		27	67·5
9		35	87·5					27	67·5
10		35	87·5		34	85			

TABLE XIV cont.

Triticum Series IV.

Number of seeds in test 40. Test 2. Nitrogen from cylinder Set up 8. 3. 34

Sterilized with Ca(OCl)Cl soln. 30 mins. Test 3. 1% oxygen. " " 14. 3. 34

Duration of expt. in days.	Test 2. a and b. Nitrogen from Cylinder				Test 3. 1% oxygen.		
		Total germin: 2a	Total germin: 2b	Mean % germin:	% oxygen	Total germin:	% germin:
1							
2						-	-
3		-	-	-	Filled	1	2·5
4	1·1 a Filled b	20	19	48·75			
5		26	22	60	Filled	20	50
6		27	24	63·75		23	57·5
7	Filled a Filled b	29	24	66·25	Filled	25	62·5
8						25	62·5
9		29	24	66·25			
10						25	62·5

TABLE XV.

Triticum Series A.

Number of seeds in test 40. All tests set up 19. 3. 34.

Sterilized with Ca(OCl)Cl soln. 4hrs. Gas mixtures renewed when readings were taken.

Duration of expt. in days.	Test A_1 2% O_2		Test A_4 3% O_2		Test A_3 1% O_2		Test A_2 N_2 from cylinder	
	Total Germin:	% Germin:	Total Germin:	% Germin:	Total Germin:	% Germin:	Total Germin:	% Germin:
1	-	-	-	-	-	-	-	-
2	-	-	2	5	-	-	-	-
3	26	65	18	45	18	45	17	42·5
4	27	67·5	21	52·5	20	50	21	52·5
5	28	70	23	57·5	22	55	22	55
6								
7	28	70	23	57·5	22	55	25	62·5
8								
9								
10	28	70	25	62·5	28	70	26	65

T A B L E XVI.

Triticum Series B and C.

Number of seeds in test B, 50.

" " " " " C, 40.

Sterilized with 0·1% HgCl test B 1 - 2 Min.

" " Ca(OCl)Cl soln test C 4 hrs.

Test B. Set up 27. 3. 34.

Test C. " " 27. 3. 34.

Gas mixtures renewed when readings were taken.

Duration of expt. in days.	Test B_2 N_2 from cylinder		B.C. Control		C_6 6% oxygen		C.C. Control	
	Total Germin:	% Germin:	Total Germin:	% Germin:	Total Germin:	% Germin:	Total Germin:	% Germin:
1	-	-	-	-	-	-	-	-
2	-	-	8	16	1	2·5	10	25
3	-	-						
4	15	30	43	86	25	62·5	26	65
5								
6								
7	25	50	46	92	28	70	28	70
8								
9	25	50	47	94	30	75	33	82·5
10								

Explanation of Figure 23. Data from Table XII.
Seeds in all tests, except C Un, sterilized with
Ca(OCl)Cl solution for 30 mins.

Control tests.

Continuous black line. CS. Seeds grown in air.
Dotted black line. CUn. " " " "

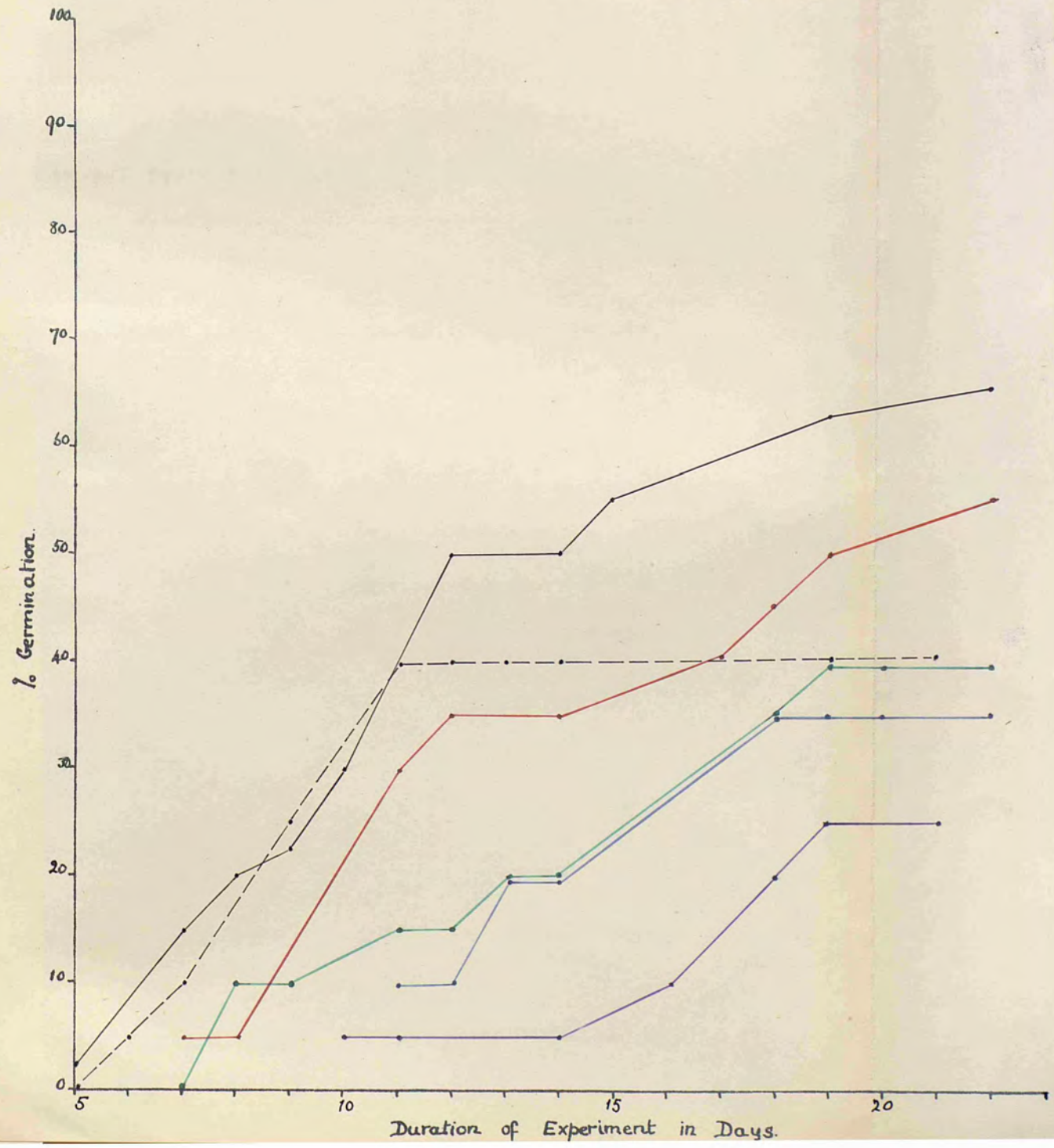
Treated tests.

Red line. Test III. Seeds grown in 6% oxygen.
Green " " I " " " 4% "
Blue " " II " " " 3% "
Mauve " " IV " " " 2% "

Figure 23

Curves indicating the Effect upon the Rate of Germination of Pinus silvestris. caused by Reduction of the Oxygen Pressure in the Atmosphere

Data from Table XII



Explanation of Figure 24. Data from Table XIII.

Seeds in all tests sterilized with 0·1% solution of mercuric chloride.

Control tests.

Continuous black line. CS. Seeds grown in air.

Dotted black line. CUn. " " " "

Treated tests.

Red line. Test 4. Seeds grown in 6 - 7% oxygen

Green " " 2. " " " 4 - 5% "

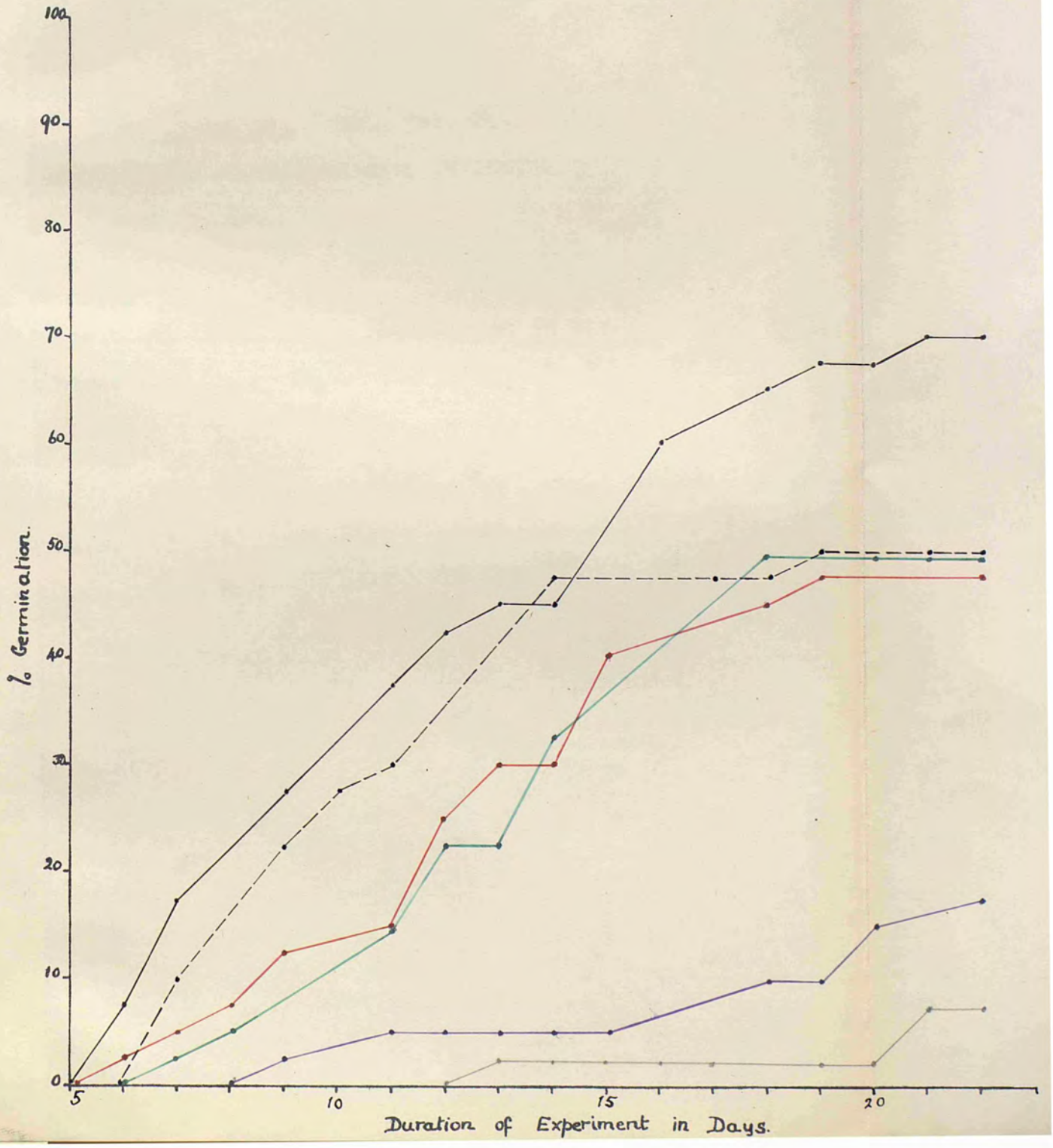
Mauve " " 1. " " " 2 - 3% "

Pencil " " 3. " " " Nitrogen from cylinder, probably about 1% oxygen.

Figure 24

Curves indicating the Effect upon the Rate of Germination of Pinus silvestris caused by Reduction of the Oxygen Pressure in the Atmosphere

Data from Table XIII



Explanation of Figure 25. Data from Table XIV.

Seeds in all tests sterilized with Ca(OCl)Cl solution for 30 minutes.

Control tests.

Continuous black line. CS_1. Seeds grown in air.

Broken black line. CS_2. " " " "

Treated tests.

Blue line. Test 1. Seeds grown in 3% oxygen.

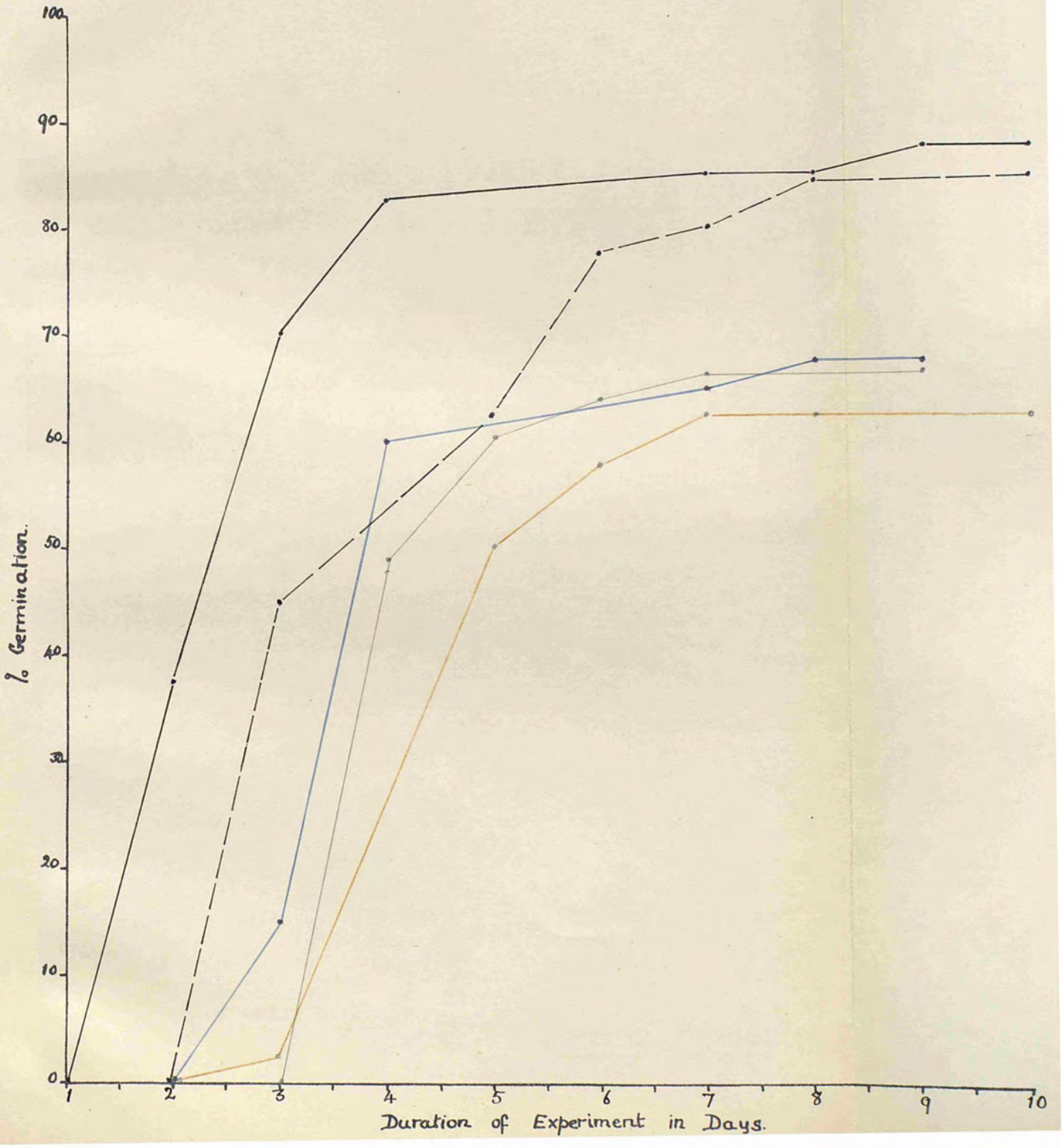
Yellow line. " 3. " " " 1% "

Pencil line. " 2. " " " Nitrogen from cylinder, probably about 1% oxygen.

Figure 25

Curves indicating the Effect upon the Rate of Germination of Triticum caused by Reduction of the Oxygen Pressure in the Atmosphere

Data from Table XIV



Explanation of Figure 26. Data from Table XV.
Seeds in all tests sterilized with Ca(OCl)Cl solution for 4 hours.

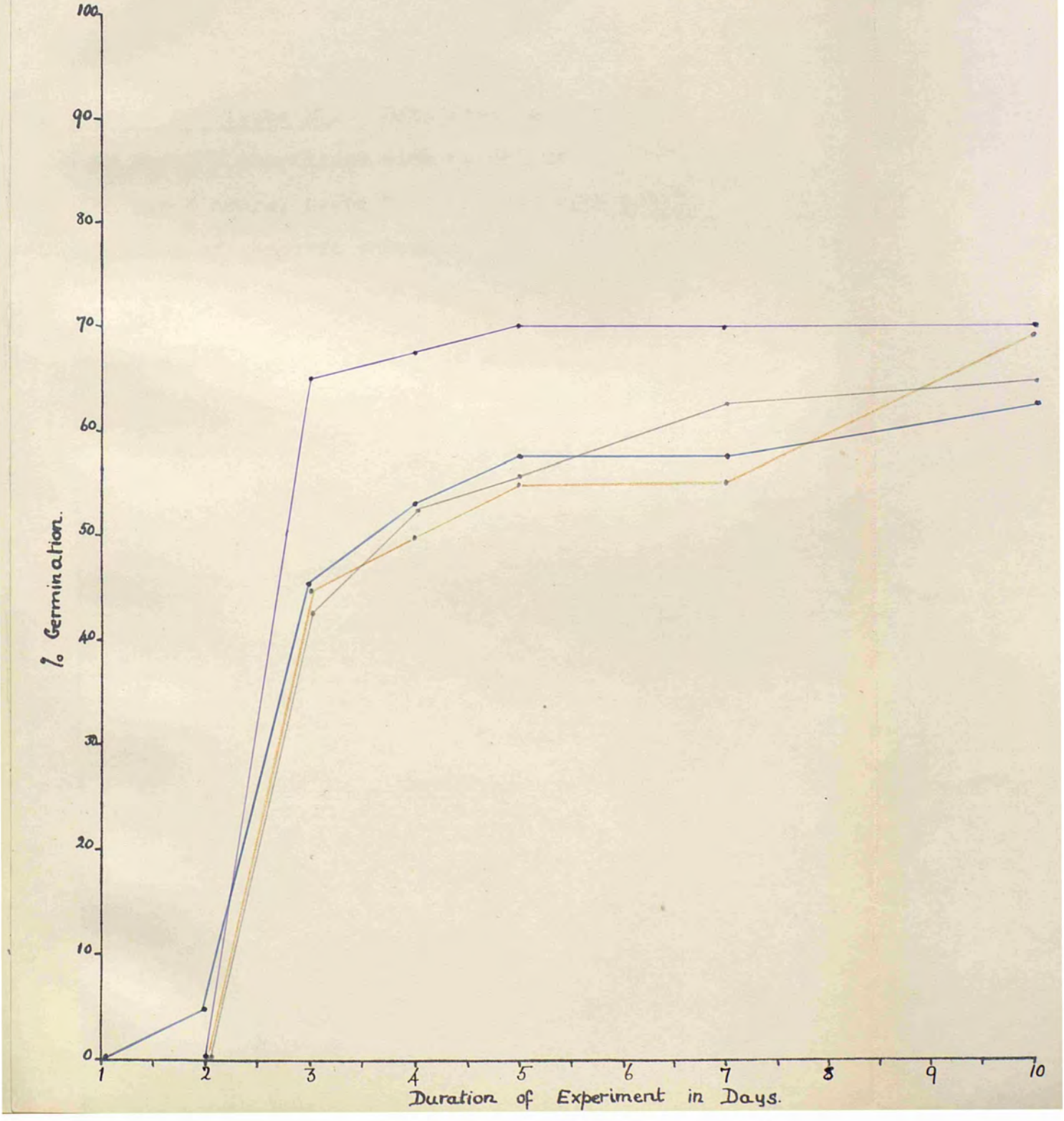
Treated tests.

Blue	line.	Test	A_4.	Seeds grown in	3%	oxygen.
Mauve	"	"	A_1.	" " "	2%	"
Yellow	"	"	A_3.	" " "	1%	"
Pencil	"	"	A_2.	" " "	Nitrogen from cylinder, probably 1%	oxygen.

Figure 26

Curves indicating the Effect upon the Rate of Germination of *Triticum* caused by Reduction of the Oxygen Pressure in the Atmosphere

Data from Table XV



Explanation of Figure 27. Data from Table XVI. Seeds in tests B sterilized with Ca(OCl)Cl solution for 4 hours, tests C sterilized with a 0·1% solution of mercuric chloride.

Control Tests.

Continuous black line. Cc. Seeds grown in air.

Broken black line. Bc. " " " "

Treated tests.

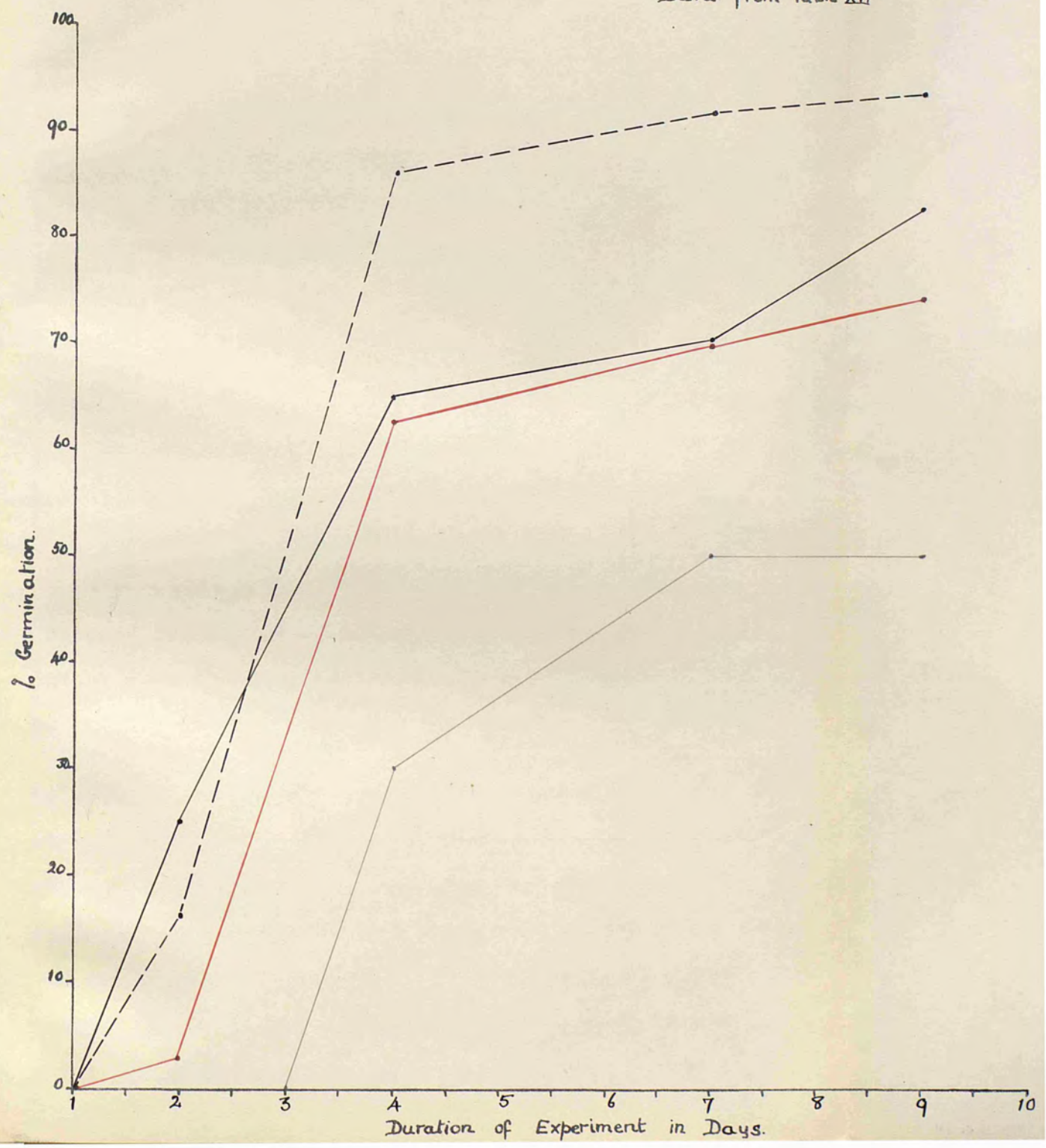
Red line. C6. Seeds grown in 6% oxygen.

Pencil line. B2. " " " Nitrogen from cylinder, probably 1% oxygen.

Figure 27

Curves indicating the Effect upon the Rate of Germination of Triticum. caused by Reduction of the Oxygen Pressure in the Atmosphere

Data from Table XVI



The results obtained by this method may be summarised in the following way:-

1. Reduction in the oxygen pressure in the seed-chamber brings about a retardation in the rate of germination of the seeds of both Pinus silvestris and Triticum. This retardation is exhibited by the treated seeds shewing a lower percentage germination each day throughout the duration of the test, but it is particularly marked in the first stages of the experiment.

2. Generally retardation in rate of germination increases with greater reduction in oxygen pressure. In the lower concentrations of oxygen the first germinations may not occur till several days after germinations are noticed in the control tests. There is however, no direct relationship between the extent of retardation and the degree of reduction; such a direct variation would be most unexpected.

3. The results suggest that there is a minimum value for the oxygen pressure necessary for the germination of the seeds of both Pinus silvestris and Triticum, and that in atmospheres containing less than this minimum the seeds will fail to germinate. The value of this minimum for Pinus silvestris would seem to lie in the region of 2% whilst for Triticum a value of under 1% is indicated. This

latter value was arrived at by estimating the oxygen present in the gas-sampling tubes after filling the seed-chambers with nitrogen (the values obtained are given in the tables and were generally just over 1%); and in these atmospheres 50% or more germination was obtained. In series C a test was set up in an atmosphere totally devoid of oxygen (accomplished by inserting an open tube containing pyrogallol in the seed-chamber); in this no sign of germination occured within twenty-four days, although the seeds remained perfectly healthy throughout. From these two tests it seems safe to conclude that there is a minimum oxygen value necessary for germination of Triticum and that it lies below 1%.

4. It would also seem that slight reductions in the value of the oxygen pressure may have little effect upon the course of germination of seeds and that each kind of seed there is a 'critical' value for the oxygen pressure below which retardation in rate of germination is clearly marked. This value would seem to lie somewhere about 6% for Pinus silvestris and rather lower than 6% for Triticum.

5. At oxygen pressures between the critical and minimum values, there is usually both delay in the appearance of the first signs of germination and a lower final percentage germination.

6. Series A, B and C in the tests with Triticum were set up with a view to investigating differences in behaviour during

germination due to sterilization with mercuric chloride and two different periods of time with calcium hypochlorite, as well as the effect of reduction in value of the oxygen pressure. If any such differences did occur they were negligible and could not be definitely correlated with differences in treatment during sterilization.

The results, summarised above, must be treated with caution and the following considerations borne in mind:- The temperature at which the seeds were grown was not controlled in any way, consequently tests set up on different days were exposed to different temperatures: it is known that temperature affects the early stages of the germination of seeds quite considerably. Then whilst filling the seed-chambers with the gas-mixtures it was impossible to avoid the introduction of small traces of atmospheric air, if it only came from the graduated tube - this latter may account for germination occuring in what would seem from later experiments to be an abnormally low percentage of oxygen.

A third disadvantage inherent in this method is that owing to respiration the composition of the gas-mixtures in the seed-chambers is continually changing - becoming increasingly rich in carbon-dioxide and dificient in oxygen - and when dealing with low oxygen pressures this variation in composition may not be neglected.

It can, however, be concluded that 1) reduction in

oxygen pressure in the atmosphere surrounding seeds in germinative conditions will generally bring about a retardation in rate of germination and possibly failure of germination and 2) the seeds of Pinus silvestris are more sensitive to reduction in the value of the oxygen pressure than the seeds of Triticum.

Later Method and Apparatus.

As already noted the serious objections to the earlier methods of experimentation are 1) that owing to respiration the composition of the gas-mixtures in the seed-chambers was continually changing - becoming increasingly rich in carbon-dioxide and deficient in oxygen and 2) that the temperature did not remain constant.

These were eliminated by using the continuous current method and by immersing the seed-chambers in a thermostat. At the same time it was thought possible that a record of the rate of evolution of carbon-dioxide might shew a definite correlation with the times at which the seeds germinated and that this would prove useful in the recognition of incipient germination.

Continuous currents of mixtures with known percentages of oxygen to pass over the seeds were obtained by means of a slow stream of nitrogen from a cylinder into which varying amounts of oxygen, liberated by electrolysis, were introduced as required. To compare with these, air containing the normal percentage of oxygen, but free from carbon-dioxide was drawn over the seeds by means of an aspirator or water pump.

As seen from figure 28 the seed-chamber was simple and was designed to have small actual volume in order to ensure the constant renewal and removal of the gases in it. It

consisted of a short length of wide-bore glass-tubing (3 cm. diam.) closed at each end by ground glass stoppers into which the entry and exit tubes were fused. The seeds were placed on a slide wrapped round with filter paper which dipped into a little water introduced into the tube. The whole arrangement is seen in the diagram.

Before use, this tube and the slide were washed with concentrated nitric acid, followed by very hot tap water and lastly sterilized distilled water. The filter paper on which the seeds were grown was autoclaved and the seeds themselves were sterilized with calcium hypochlorite or 0·1% solution of mercuric chloride. It was found necessary to carry out the whole of the above process in a sterilizing room in order to prevent fungal or bacterial infection.

This seed-chamber was very convenient; it was small and easily manipulated and as, the seeds were near the glass, the progress of germination could be watched throughout the experiment. It was unnecessary to arrange a device for renewing the water in the tubes as the air-stream passing through it was saturated.

The seed-chamber was immersed in the water of an electric thermostat heated by a make-and-break circuit controlled by a mercury-toluol regulator. It was found to be very reliable and altered only during very hot weather, when it was impossible to use it.

The air, freed from carbon-dioxide, was obtained by the usual method. Air was drawn by an aspirator or water-pump through a soda-lime tower, through a solution of potassium hydroxide, to ensure removal of all carbon-dioxide, through water to wash and saturate the air, over the seeds and through barium hydroxide solution in a Pettenkofer tube - the latter being attached to the aspirator or water-pump. The arrangement of the whole apparatus is shewn in figure 34.

A steady flow of air was maintained by passing the entry tube down to the bottom of the aspirator. Thus the head of water drawing the air over was kept constant as the water level fell inside the aspirator. The rate of flow of the air-stream was regulated by attaching a short length of capillary tubing to the exit by means of longish piece of rubber tubing. The head of water drawing the air over was altered by raising or lowering this capillary in the rubber tubing. The rate of flow of the air-stream could be calculated by measuring in a graduated cylinder the amount of water escaping from the aspirator in a given time.

When the rate of flow of air through the seed-chamber was not required, a water-pump was found to be very useful for supplying a stream of air at a good steady rate. Alteration in the water pressure in the mains was counteracted by inserting a pressure regulator shewn in figure, 30. This regulator made it possible to draw a stream of air through the apparatus

at a convenient rate; it was very reliable and the bubbles of air as they passed through the barium hydroxide were small and regularly formed.

The nitrogen current was obtained from a cylinder fitted with a fine adjustment valve. It is stated that the nitrogen may contain small quantities of oxygen as impurity, this was removed by passing it through pyrogallol and then through water, to wash and saturate it. It was then led through a 'safety-valve' - a T-piece whose end was closed by water and which also served to indicate the pressure inside the apparatus - into the seed-chamber and out through a Pettenkofer tube, containing barium hydroxide solution, and a soda-lime tower - to prevent carbon-dioxide diffusing back into this solution. The arrangement of the apparatus is shewn in figure 35.

It seemed unlikely that the nitrogen should contain any carbon-dioxide and that this should still be present after having passed through pyrogallol dissolved in caustic potash. Blanks were made to see if this was so. The nitrogen was passed through the whole apparatus before the seeds were put in the seed-chamber, and the titration-value was noted. The difference between this value and the one, obtained for the barium hydroxide solution before such treatment, was found to be almost negligible.

The rate at which the nitrogen was supplied was calculated by noting the time taken to collect 100 c.c. of the gas as it escaped from the cylinder - in a burette by displacement

at a convenient rate; it was very reliable and the bubbles of air as they passed through the barium hydroxide were small and regularly formed.

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The rate at which the nitrogen was supplied was calculated by noting the time taken to collect 100 c.c. of the gas as it escaped from the cylinder - in a burette by displacement of water.

When a gas-mixture containing a low percentage of oxygen was required a jar, in which water was electrolysed was inserted in the nitrogen stream between the water-bottle and the safet y-valve. The oxygen was produced by the electrolysis of water acidulated with sulphuric acid, between platinum electrodes and was introduced into the nitrogen stream as shewn in figure 31. The pumping action of the nitrogen bubbling through the two previous bottles assured the thorough mixing of the gases.

The electric currents necessary to liberate the oxygen at suitable rates are very small. It was found that, by connecting a number of wire resistance mats, with a total resistance of 9900 ohms and varying in size from 1250 ohms to 250Ø ohms, in series with the mains and using them in varying combinations, the rate of evolution of the oxygen could be regulated as desired. The rate at which the oxygen was evolved was calculated by inserting an ammeter in the circuit or else by noting the resistance employed; knowing the current passing in the circuit the volume of oxygen liberated in a given time follows from Faraday's laws.

Before any of these experiments with a continuous current of air was started the whole series of connections was tested for leaks by attaching an aspirator to the end of the Pettenkofer tube and closing the entrance tube - if water did not continue to drop from the aspirator after a little time, the apparatus was presumed to be air tight.

The carbon-dioxide evolved by the seeds was absorbed in the barium hydroxide solution contained in the Pettenkofer tube. This solution was prepared by adding exactly 25 c.c. $Ba(OH)_2$ $\frac{N}{10}$ solution from an automatic pipette to about 60 - 70 c.c. distilled water already poured into the tube. After the passage of the air-stream, the whole volume was washed out and titrated with approximately 25 c.c. standard $\frac{N}{10}$ hydrochloric acid.

The barium hydroxide solution was made up in a large bottle fitted with a siphon delivery such as in shewn in figure 32. The delivery tube was connected at first with an automatic burette which delivered 25 c.c. The air entering into the system passed through the soda-lime tube, thus no free carbon-dioxide came in contact with the barium hydroxide. The results obtained with the burette seemed to be rather erratic so after a short time this was replaced by an automatic 25 c.c. pipette. The titrations made with this agreed better but were not absolutely satisfactory. Then it was found necessary to use water freed from carbon-dioxide because it was feared that the small percentage of carbonate ions present in distilled water might affect the accuracy of the titrations. A constant supply of carbon-dioxide free water was obtained by boiling distilled water until no more air-bubbles were liberated. The flask in which it was boiled was closed while still hot with a rubber bung fitted with a soda-

lime tube. Thus all air drawn into the flask as the water cooled was freed from carbon-dioxide.

The water was withdrawn by a simple siphon arrangement such as is shewn in figure 33.

The barium hydroxide solution from the Pettenkofer tube was titrated at intervals of about twenty-four hours. The titration values of the barium hydroxide were compared with that obtained from the blank tests. The difference in these values gave the amount of barium hydroxide neutralized by the carbon-dioxide evolved by the seeds during respiration - hence the volume of carbon-dioxide evolved was calculated. The results were plotted, volume of carbon-dioxide evolved per hour by the seeds against time of duration of the experiment.

The number of seeds germinated was noted at the same time as the titrations were made. These were also plotted against time and a correlation was looked for between these two curves.

Parts of the Apparatus used in the 'Continuous-Current' Method. I

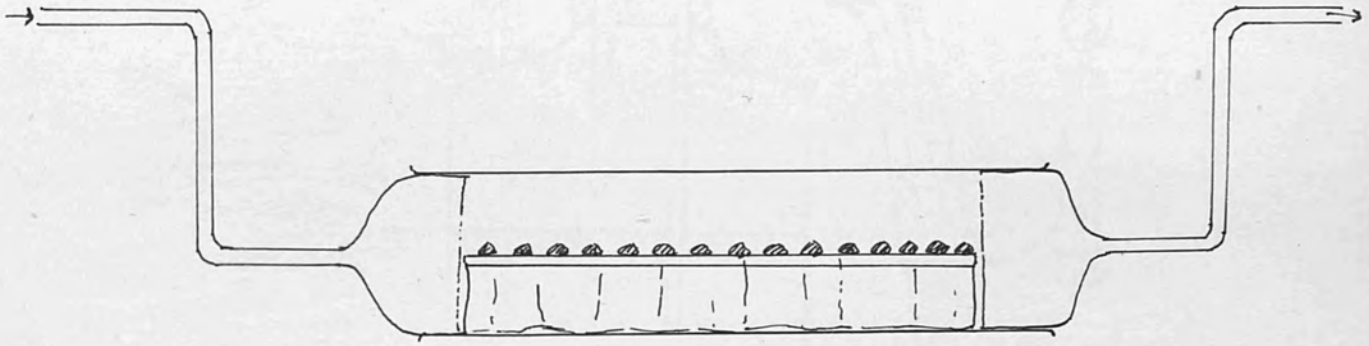


Fig: 28. Seed Chamber.

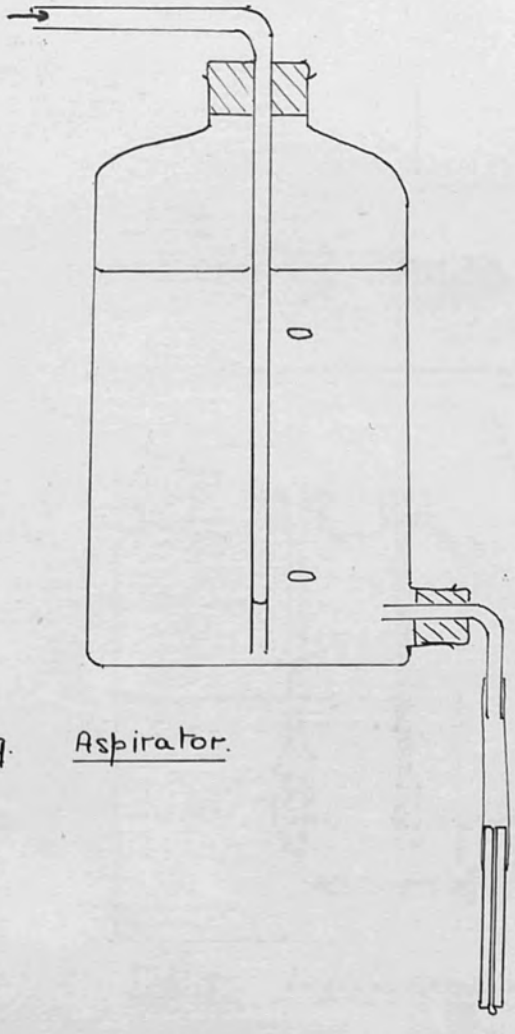


Fig: 29. Aspirator.

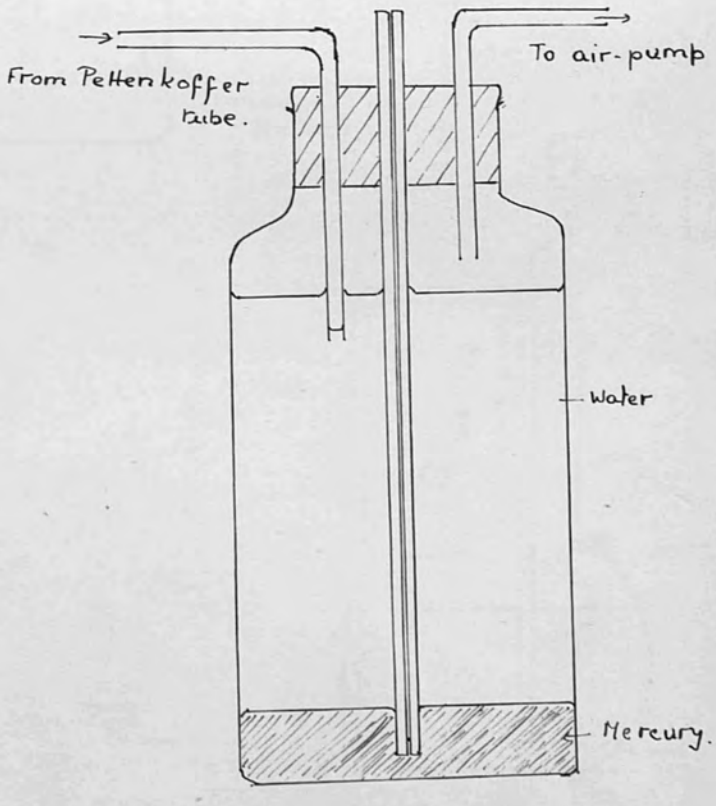


Fig: 30 Pressure Regulator.

Parts of Apparatus used in the 'Continuous Current' Method. II.

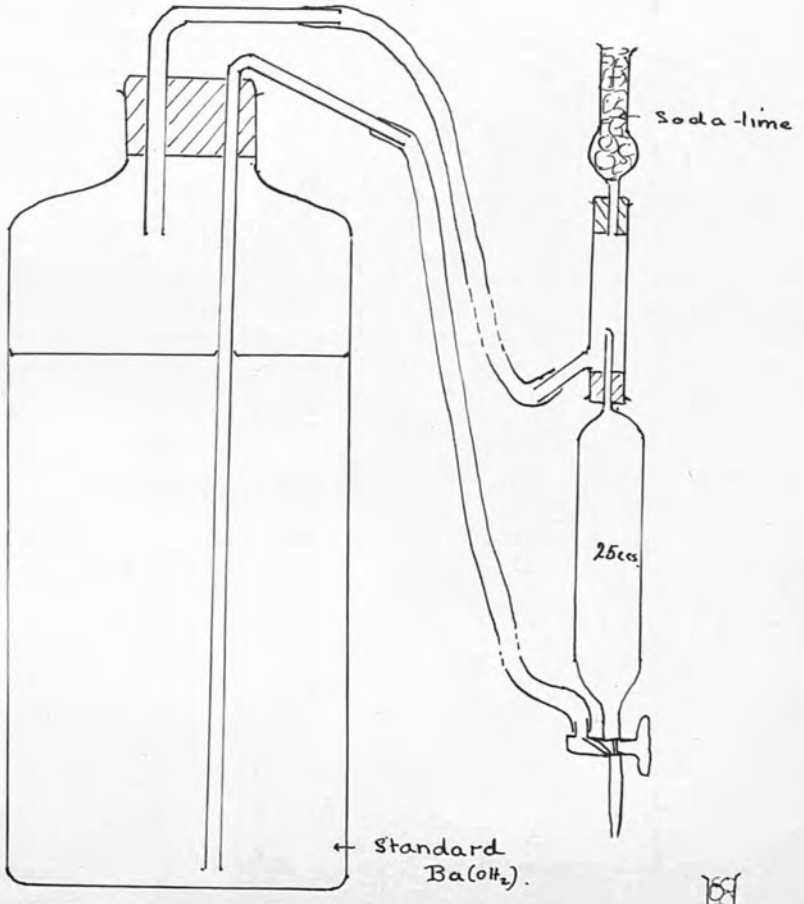


Fig: 32.

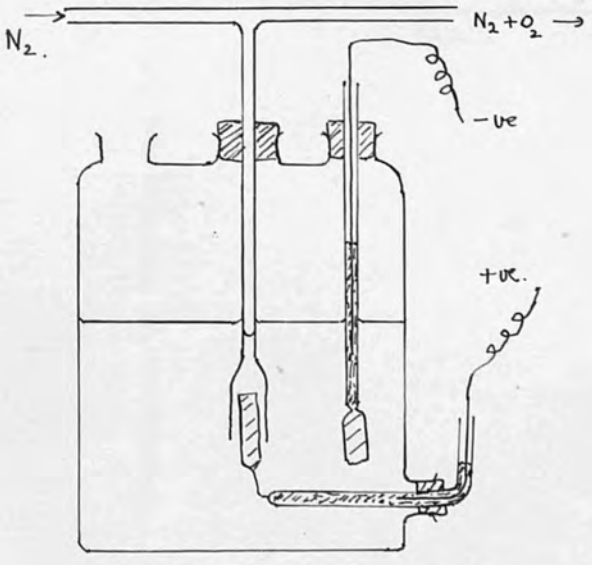


Fig: 31. Oxygen generator.

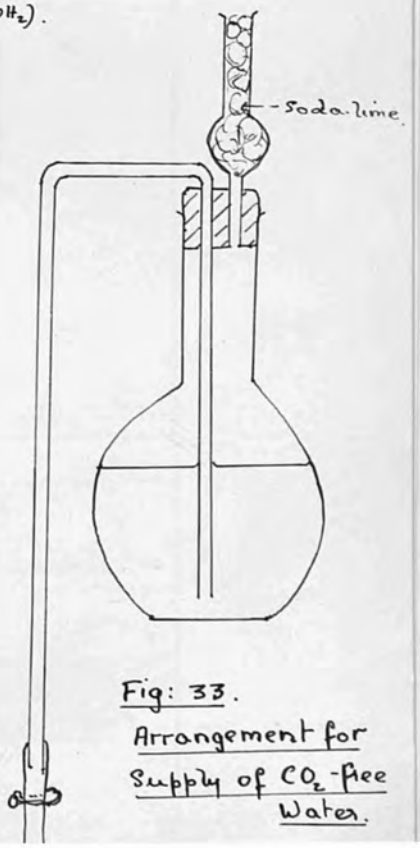


Fig: 33.
Arrangement for Supply of CO_2-free Water.

FIGURE 34.

ARRANGEMENT OF APPARATUS FOR CONTINUOUS CURRENT OF AIR.

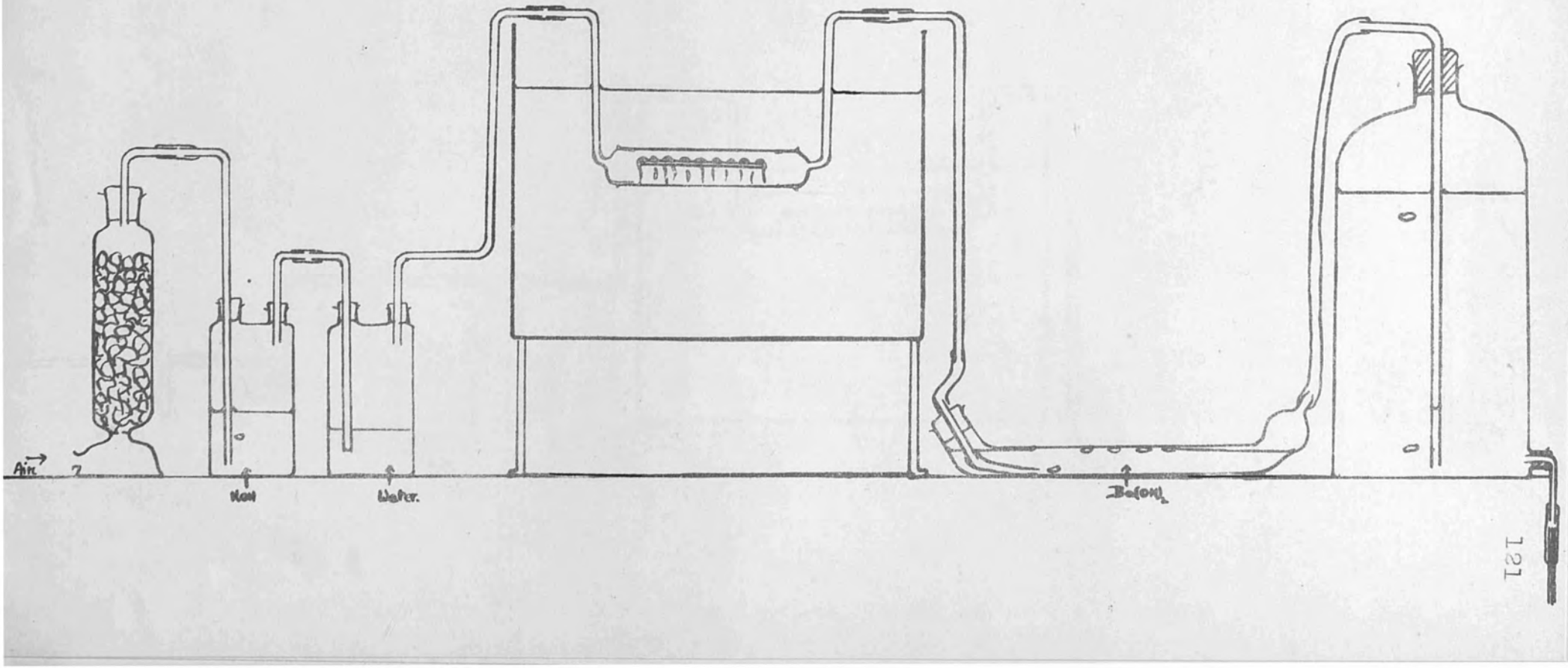
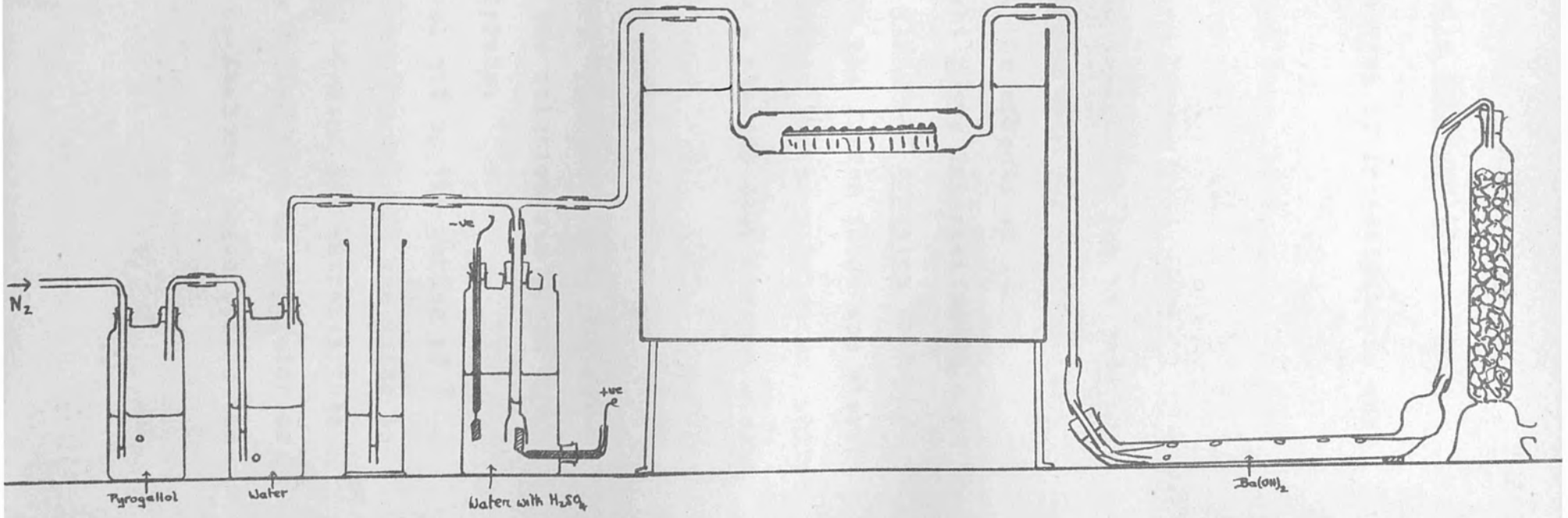


FIGURE 35.

ARRANGEMENT OF APPARATUS FOR CONTINUOUS CURRENT OF NITROGEN OR NITROGEN WITH LOW OXYGEN CONTENT.



Experimentals Details and Results.

The seeds used in this series of investigations were those of:-

Pinus silvestris. Rafn and Sons, 1934.

Brassica rapa. Carter's Rape, 1933.

Triticum. Carter's Yeoman King, 1934.

The main object of the investigation was to determine the effect of the reduction of oxygen upon the germination of *Pinus* and it was thought that the effects of similar treatments on other seeds might prove interesting to compare with it. The seeds of *Pinus* and *Brassica* both contain oily food reserves, in the former these are stored in the endosperm and in the latter in the cotyledons, whilst the seeds of *Triticum* contain a starchy food reserve stored in the endosperm.

In each case a convenient number of seeds was selected and was sterilized with calcium hypochlorite as described in Part I. The strength of the solution was found by titration with sodium thiosulphate.

Before each experiment was set up the series of bottles through which the continuous current was going to pass was tested for leakage by closing the entrance tube and connecting the end of the series with an aspirator or water pump. The continuous current was begun before the

seeds were ready in order to remove any air from the bottles. The Pettenkofer tubes with the barium hydroxide solution were not attached to the seed-chambers for at least 1 hour, and in the later experiments not until 4 hours, after the seeds had been put in the current.

Great care was taken to see that those seeds which were going to be grown in nitrogen or reduced oxygen pressures were set up and connected with the appropriate continuous current as quickly as possible - generally the washing after sterilization, setting up in the seed-chamber, waxing of the stoppers and connecting with the current could all be done in from 10 - 15 mins.

The barium hydroxide was titrated at intervals of about 24 hours and as has already been stressed in all the operations connected with the titration it was found necessary to use carbon-dioxide free water:- i.e. for making up the standard acid, for washing out the Pettenkofer tubes and for diluting the barium hydroxide solution.

Triticum. Carter's Yeoman King, 1933.

Ten seeds were selected for each test and their fresh weight was noted. In each of the eight tests carried out the seeds were sterilized with a solution of calcium hypochlorite (approximate strength, 2% titratable chlorine) for 1¼ hours after which they were washed once with sterilized distilled water. Owing to the loose and papery nature of the pericarp of the seeds and the subsequent difficulty of completely wetting them, they were centrifuged at a low speed before sterilization.

The thermostat was regulated to maintain a temperature of 22°C.

Germination was said to have occurred when the three first roots were long enough to touch the filter paper.

The results obtained are given in the Tables XVII, XVIII and XIX and are plotted in figure 36 - the volume of carbon-dioxide evolved per hour, per grm fresh weight in c.c. against time and also as the rate of germination.

TABLE XVII.

Triticum. Carter's Yeoman King, 1934.

Number of seeds in test, 10.

Temp: of thermostat, 22°C.

Sterilized with Ca(OCl)Cl soln 1½ hrs.

Strength of " " Cl_2 per 100 c.c. = 1·9 grms.

T_1 Seeds weighed 0·5018 grms

T_2 " " 0·4998 "

T_3 " " 0·4166 "

Date	Duration of expt. in hours.	T_1 Air Current. Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	T_1 Air Current. Vol: of CO_2 evolved in c.c. 1hr. per grm Fresh Wght	T_1 Air Current. Number of germin:	T_2 Nitrogen Current VOL: of CO_2 evolved in c.c. 1hr. per 10 seeds	T_2 Nitrogen Current VOL: of CO_2 evolved in c.c. 1hr. per grm Fresh Wght.	T_2 Nitrogen Current Number of germin:	T_3 1% Oxygen Current Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	T_3 1% Oxygen Current Vol: of CO_2 evolved in c.c. 1hr. per grm Fresh Wght.	T_3 1% Oxygen Current Number of germin:
Jan 23rd										
" 24th	19½	0·06	0·01		0·03	0·06		0·06	0·12	
" 25 "	43½	0·16	0·32	8	0·03	0·05		0·04	0·08	
" 26 "	67½	0·32	0·62	9	0·03	0·05		0·05	0·11	
" 28 "	115½				0·02	0·04		0·07	0·17	5
" 29 "	145½				0·02	0·03		0·10	0·25	5
" 30 "	163½				0·02	0·03		0·11	0·25	5

TABLE XVII.

Triticum. Carter's Yeoman King, 1934.

Number of seeds in test, 10.

Temp. of thermostat, 22°C.

Sterilized with Ca(OCl)Cl soln 1¼ hrs.

Strength of " " Cl_2 per 100 c.c. = 1·9 grms.

T_1 Seeds weighed 0·5018 grms

T_2 " " 0·4998 "

T_3 " " 0·4166 "

Date	Duration of expt. in hours.	T_1 Air Current. Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	T_1 Air Current. Vol: of CO_2 evolved in c.c. 1hr. per grm Fresh Wght.	T_1 Air Current. Number of germin:	T_2 Nitrogen Current Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	T_2 Nitrogen Current Vol: of CO_2 evolved in c.c. 1hr. per grm Fresh Wght.	T_2 Nitrogen Current Number of germin:	T_3 1% Oxygen Current Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	T_3 1% Oxygen Current Vol: of CO_2 evolved in c.c. 1hr. per grm Fresh Wght.	T_3 1% Oxygen Current Number of germin:
Jan 23rd										
" 24th	19¼	0·06	0·11		0·03	0·06		0·06	0·12	
" 25 "	43¼	0·16	0·32	8	0·03	0·05		0·04	0·08	
" 26 "	67¼	0·31	0·62	9	0·03	0·05		0·05	0·11	
" 28 "	115½				0·02	0·04		0·07	0·17	5
" 29 "	145¼				0·02	0·03		0·10	0·25	5
" 30 "	163¼				0·02	0·04		0·11	0·26	5

TABLE XVIII.

Triticum. Carter's Yeoman King, 1934.

Number of seeds in test, 10.

Temp. of thermostat, 22°C.

Sterilized with Ca(OCl)cl soln 1¼ hrs.

Strength of " " Cl_2 per 100 c.c. = 1·9 grms.

T_4 Seeds weighed 0·4274grms

T_5 " " 0·4056 "

T_6 " " 0·4074 "

Date	Duration of expt. in hours.	T_4 Air Current			T_5 Nitrogen Current			T_6 Oxygen Current.		
		Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	per grm Fresh Wght.	Number of germin:	Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	per grm Fresh Wght.	Number of germin:	Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	per grm Fresh Wght.	Number of germin:
Jan 30th										
" 31st	19	0·03	0·07		0·01	0·03				
Feb 1st	43	0·10	0·23	7	0·02	0·06		0·02	0·04	
" 2nd	67	0·27	0·63	8	0·01	0·03		0·04	0·10	
" 4th	115				0·01	0·03		0·04	0·11	7
" 5 "	139				0·02	0·04		0·06	0·16	7
					Nitrogen replaced by air.					
" 6 "	163				0·07	0·17	5	0·07	0·17	7
" 7 "	187				0·09	0·22	8	0·11	0·28	7
" 8 "	213				0·38	0·95	10	0·10	0·24	7

TABLE XIX.

Triticum.
Carter's Yeoman King, 1934.

No. of seeds in test, 10. Sterilized Ca(OCl)Cl soln 1¼ hrs. T_7 Seeds weighed 0·4586 grms

Temp. of thermostat, 24°C. Strength of " " Cl_2 per 100 c.c. = 1·9grms. T_8 " " 0·4620 "

Date	Duration of expt. in hours.	T_7 Air current			T_8 4·5% Oxygen current		
		Vol: of CO_2 evolved in c.c. lhr. per 10 seeds	per grm Fresh Wght.	Number of germin.	Vol: of CO_2 evolved in c.c. lhr. per 10 seeds	per grm Fresh Wght.	Number of germin.
Mar 18th							
" 19 "	18	0·04	0·09		0·03	0·07	
" 20 "	42	0·10	0·21	7	0·05	0·11	2
" 21st	66	0·26	0·56	10	0·12	0·29	9

Explanation of Figure 36. Data from Tables XVII, XVIII, XIX.

Black Ink. Tests T_1 - T_3

Continuous line. T_1 Seeds grown in air current.

Dotted " . T_2 " " " nitrogen " .

Broken " T_3 " " " 1% oxygen "

Green Ink. Tests T_4 - T_6.

Continuous line. T_4 Seeds grown in air current.

Dotted " T_5 " " " nitrogen ".

Nitrogen replaced by air on 139th hr.

Broken " T_6 Seeds grown in 0·7% oxygen current.

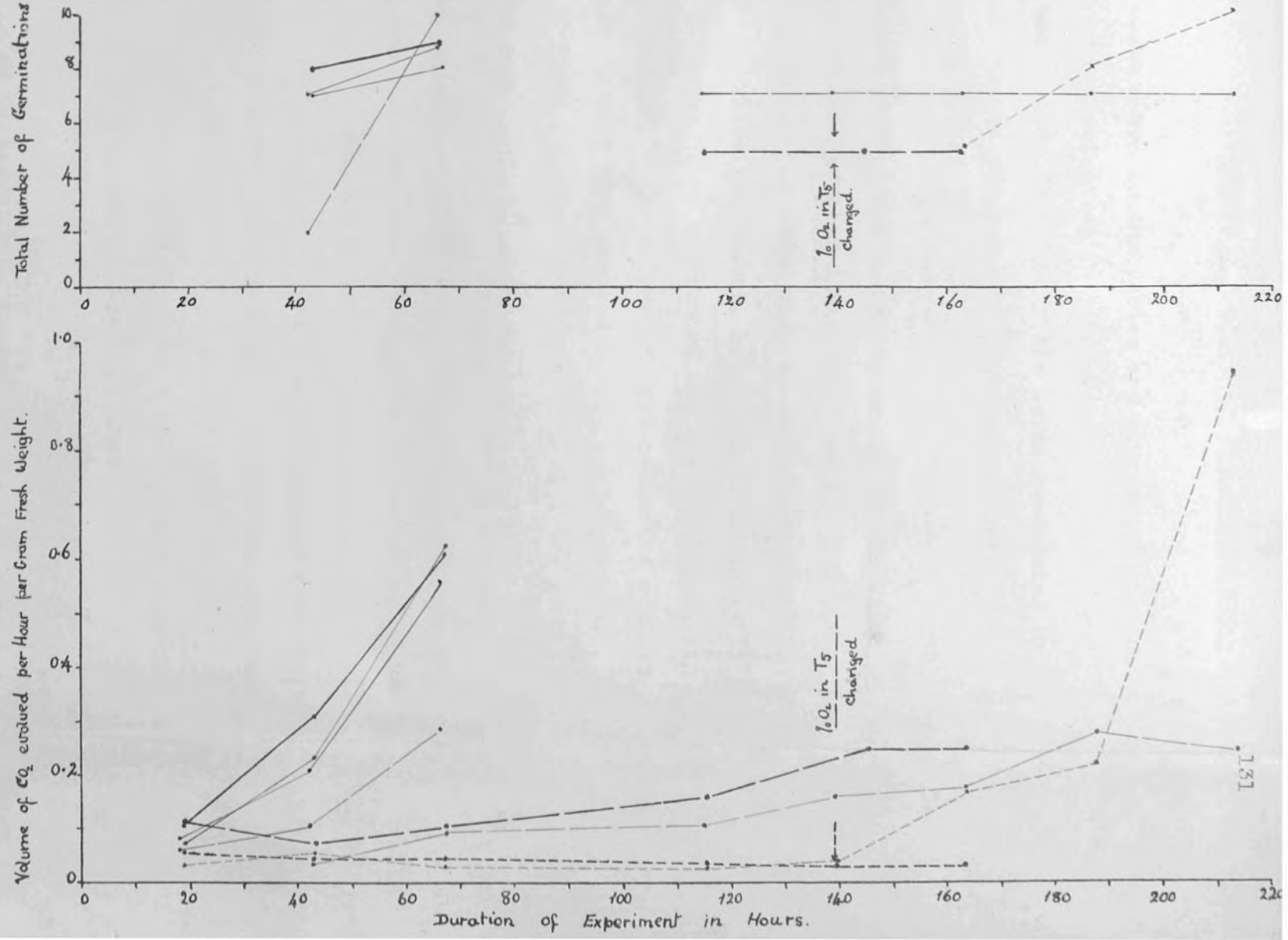
Blue Ink. Tests T_7 - T_8.

Continuous line. T_7 Seeds grown in air current.

Broken " T_8 " " " 4·5% oxygen current.

Figure 36.

Curves indicating the Effect of a Reduction in the Oxygen Pressure of the Atmosphere upon the Rate of Germination of Triticum and upon the Rate of Evolution of Carbon Dioxide during Germination



The results shewn in the Tables and illustrated in the figures can be summarised as follows:-

1) At 22°C and in air, 70 - 80% of the seeds germinated by the end of the second day and total germination occured by the end of the third day.

2) At 22°C and in 4·5% oxygen, only about 20% of the seeds germinated by the end of second day but total germination occured by the end of the third day, as was observed for seeds grown in air. Unlike the latter, seedlings grown in 4·5% oxygen were inclined to be attenuated and their chlorophyll was poorly developed. The volume of carbon-dioxide evolved was approximately half that evolved by similar seeds grown in air.

3) At 22°C and in 0·7 - 1% oxygen, 50 - 70% of the seeds germinated on about the fifth day. The results indicate that the total germination was also obtained by this time as no further germination occured in the next few days. When compared with seedlings grown in air, seedlings grown in these low percentages of oxygen were seen to be very attenuated and practically devoid of chlorphyll. The volume of carbon-dioxide evolved remained throughout at a low value - slightly higher values were obtained for the experiment with 1% oxygen.

4) At 22°C and in Nitrogen. No actual germination occured. The seeds absorbed water and the radicle swelled up and

cracked the pericarp and in some cases emerged a short distance. The volume of carbon-dioxide evolved was very small; it was maintained at a practically constant low level which gradually fell off during the first six days or so.

5) The seeds, which had been in an atmosphere of nitrogen for six days, on being put in air germinated at a rate comparable with that in air and this similarity also existed for the volume of carbon-dioxide evolved. 100% germination was obtained after treatment in this manner.

Pinus silvestris. Rafn and Sons,1934.

Forty seeds were selected for each test and their fresh weight was found. In five of the tests, the seeds were sterilized with a solution of calcium hypochlorite (containing approximately 2% titratable chlorine) for 1⅓ hours, a period of time found to yield satisfactory germination (see Part I); in two other tests the seeds were treated for periods of 17 and 19 hours in order to find out whether longer sterilization affects in any appreciable manner the volume of carbon-dioxide evolved during the early stages of germination. The seed coats became 'wet' fairly easily and sterilization was found to be satisfactory without previous centrifuging. After sterilization all seeds were washed once with autoclaved distilled water.

The thermostat was regulated at a temperature of 23°C. Germination was taken as completed when the radicles were long enough to touch the filter paper and shewed a definite geotropic curvature.

The results are shewn in the Tables XX and XXI and are illustrated in the figures 37 and 38.

TABLE XX.

Pinus silvestris. Rafn & Sons, 1934.

No. of seeds in test, 40. Sterilized with Ca(OCl)Cl soln.

Temp. of thermostat, 23°C. Strength of " " Cl_2 per 100 c.c. 1·8gm

P_1 Seeds weighed 0·3422 grms. Sterilized 1 hr.

P_2 " " 0·3542 " " " "

P_3 " " 0·3992 " " " "

P_4a " " 0·3660 " " 17½"

Date	Duration of expt. in ~~days~~ hours.	P_1 Air			P_1a Air			P_2 2 5% oxygen			P_3 nitrogen		
		Vol: CO_2 evolved 1hr 10 seeds	grm fresh Wt	No. of germin.	Vol: CO_2 evolved 1hr. 10 seeds	grm fresh Wt	No. of germin	Vol: CO_2 evolved 1hr. 10 seeds	grm fresh Wt	No. of germin	Vol: CO_2 evolved 1hr. 10 seeds	grm fresh Wt	No. of germin
Mar. 22nd.													
" 23rd	23½	0·06	0·17					0·03	0·08		0·02	0·05	
" 25th	71½	0·07	0·21	2	0·06	0·16	1	0·02	0·06		0·02	0·05	
" 26 "	95½	0·07	0·21	6	0·05	0·15	4	0·02	0·07		0·01	0·03	
" 27 "	119½	0·10	0·29	12	0·08	0·12	10	0·03	0·08		0·01	0·03	
" 28 "	143½	0·12	0·35	23	0·09	0·23	13	0·03	0·08		0·01	0·03	
" 29 "	167½	0·14	0·41	25			20	0·02	0·07		0·01	0·03	
" 30 "	191½	0·15	0·45	27	0·10	0·28	24	0·02	0·07		0·01	0·02	
								2·5% O_2 replaced by 7·5% O_2					
Ap. 1st	239½	0·23	0·68	28	0·13	0·37	26	0·04	0·10		0·01	0·01	

TABLE XXI.

Pinus silvestris. Rafn & Sons, 1934.

No. of seeds in test, 40. Sterilized with Ca(OCl)Cl soln.

Temp. of thermostat, 23°C. Strength of " " Cl_2 per 100 c.c. 1·8gms

P_4 Seeds weighed 0·3670 grms Sterilized 1 hr.

P_5 " " 0·3990 " " 1 "

P_4a " " 0·3584 " " 19 "

Date	Duration of expt. in hours	P_4 Air			P_4a Air			P_5 5% oxygen		
		Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	per grm Fresh wght.	Number of germin:	Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	per grm Fresh wght.	Number of germin.	Vol: of CO_2 evolved in c.c. 1hr. per 10 seeds	per grm Fresh wght.	Number of germin.
April 3rd					.	.		.	.	
" 4th	28	0·04	0·10		0·05	0·13		0·05	0·13	
" 5 "	48	0·06	0·16		0·05	0·14		0·04	0·11	
" 6 "	71	0·06	0·17	1	0·06	0·16	1	0·04	0·11	
" 8 "	119	0·09	0·25	17	0·08	0·23	12	0·03	0·09	
" 9 "	143	0·12	0·32	20	0·10	0·28	17	0·04	0·10	
" 10 "	167	0·14	0·37	23	0·11	0·30	21	0·03	0·08	
" 11 "	191	0·18	0·49	28	0·13	0·37	25	0·03	0·08	
								5% O_2 replaced by 8·5% O_2		
" 12 "	215	0·25	0·69	29	0·16	0·45	30	0·03	0·09	1
" 13 "	239	0·30	0·81	30	0·17	0·48	31	0·04	0·10	2
" 16 "	313							0·03	0·10	9

Explanation of Figure 37. Data from Tables XX and XXI.

Seeds in all test sterilized with calcium hypochlorite solution and grown in air current.

Black Ink. Tests P_1 - P_1a.

Continuous line. P_1 Seeds sterilized for 1⅓ hrs.

Crossed " P_1a " " " 17½ "

Green Ink. Tests P_4 - P_4a.

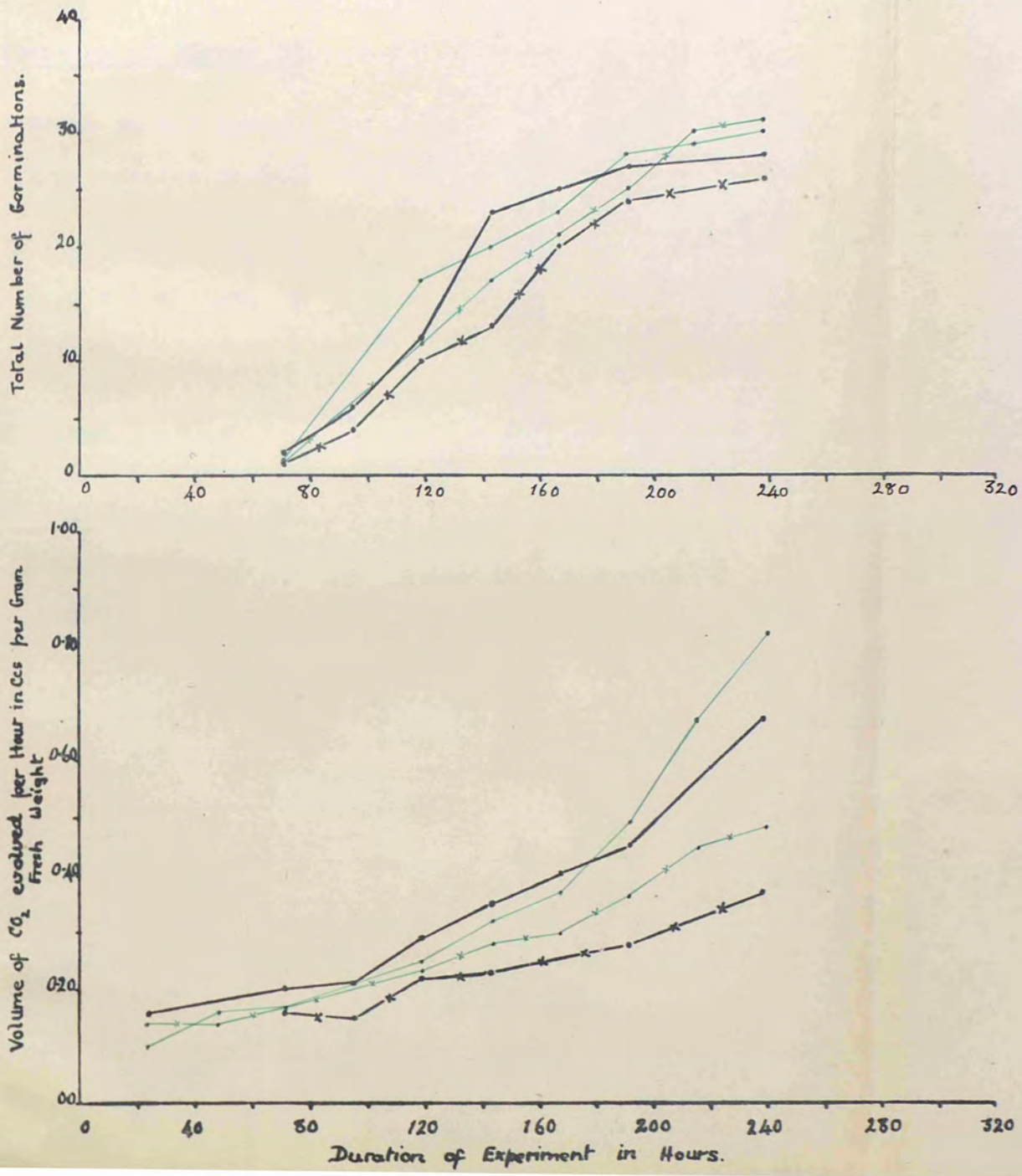
Continuous line. P_4 Seeds sterilized for 1⅓ hrs.

Crossed " P_4a " " " 19 "

Figure 37

Curves indicating the Rate of Evolution of Carbon Dioxide during Germination and the Rate of Germination of *Pinus silvestris* after Steriltzation with a Solution of Calcium Hypochlorite for Periods of 1 Hour and 17 Hours.

Data from Table XX-XXI.



Explanation of Figure 38. Data from Tables XX and XXI.

Seeds in all tests sterilized with calcium hypochlorite solution for 1⅔hrs.

Black Ink. Tests P_1 P_2 P_3

Continuous line. P_1 Seeds grown in air current.

Broken " P_2 " " " " 2.5% O_2 "
on 191st hr increased to 7.5% O_2

Dotted " P_3 Seeds grown in Nitrogen current.

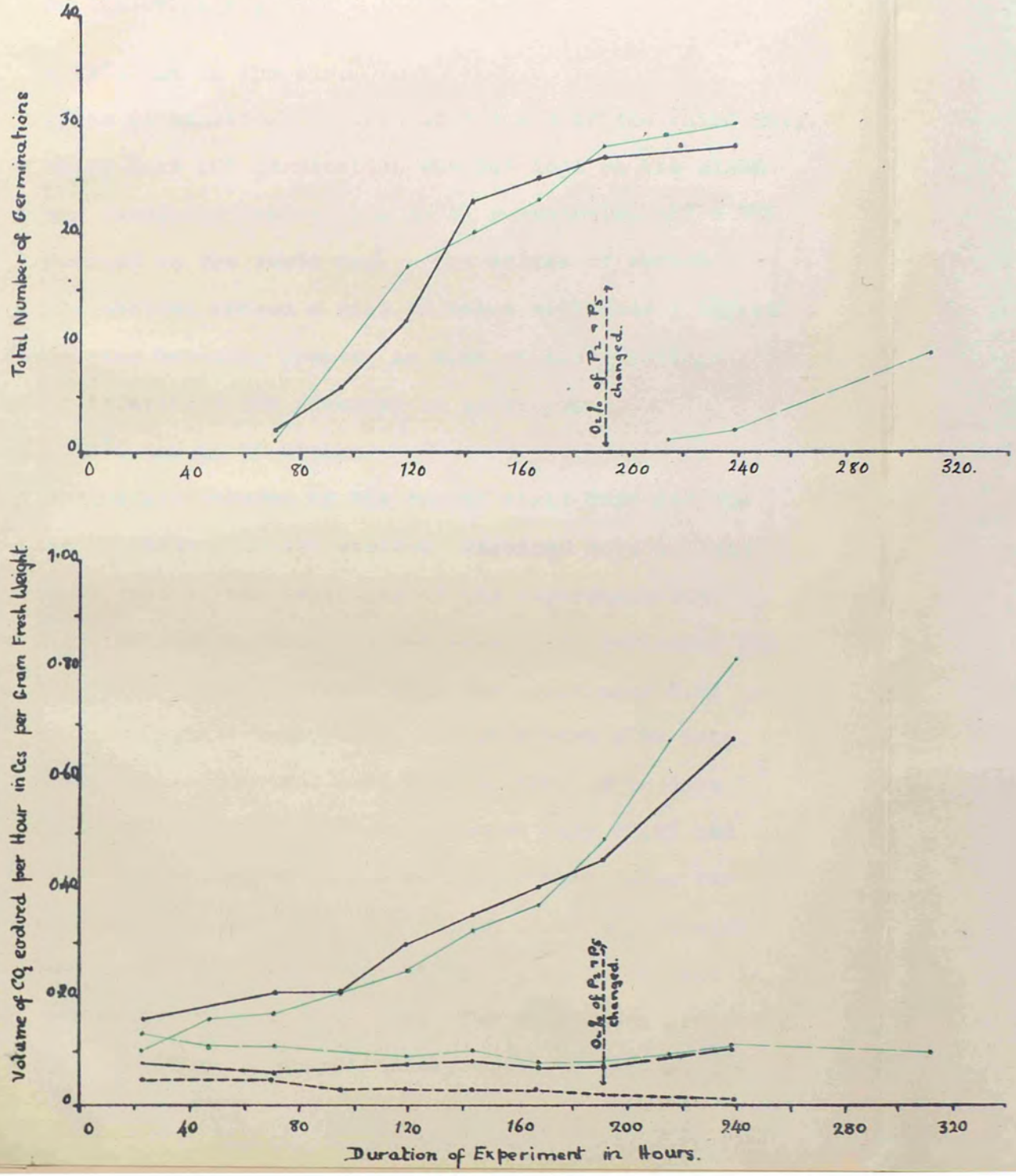
Green Ink. Tests P_4 - P_5

Continuous line. P_4 Seeds grown in air current.

Broken " P_5 " " " 5% O_2 "
on 191st hr increased to 8.5% O_2

Figure 58

Curves indicating the Effect of a Reduction in the Oxygen Pressure of the Atmosphere upon the Rate of Germination of Pinus silvestris and upon the Rate of Evolution of Carbon Dioxide during Germination. Data from Tables XX. XXI.



The results of this experiment may be summarised in the following way:-

1) At 23°C and in the air.

The first germinations occured at the end of the third day, something like 50% germination was obtained on the sixth day and something approaching total germination 65% - 77% was reached on the tenth day. The volume of carbon dioxide evolved shewed a rise in value with time - the rate of increase becoming greater as some of the seedlings became relatively far advanced in development.

2) At 23°C and in 5% Oxygen.

No germination occured by the end of eight days and the volume of carbon dioxide evolved remained more or less equal to that at the beginning of the experiment but shewed a decided tendency to decrease. On replacing the current containing 5% oxygen with one containing 8.5% oxygen germination took place, but at a very slow rate. The rate was much slower than that in air; four days after the first germination had occured only 22.5% had germinated as compared with 60%-the probable value for seeds germinated in air. The volume of carbon dioxide evolved shewed a slight rise but the rate of increase is not comparable with that in air. The value when germination was first noticed is approximately equal to that at the

beginning of the experiment.

3) At 23°C and in 2.5% oxygen.

No germination took place during the first eight days. The volume of carbon dioxide evolved fell to a value slightly lower than that at the beginning of the experiment. When the percentage of oxygen in the continuous current was raised to 7·5% no marked increase in the volume of the carbon dioxide evolved was noticed in the next two days and there was no sign of germination about to take place.

4) At 23°C and in Nitrogen.

No germination occurred during the first ten days and the volume of carbon dioxide fell to a very low value.

5) The effect of longer periods of sterilization upon germination did not seem to be great. The actual rate of germination of the seeds sterilized for periods of seventeen and nineteen hours seemed to be approximately the same as that for seeds sterilized for shorter periods; it is however worth noting that in each pair of tests the one exhibiting the lower germination is the one sterilized longer. The final germination for both members of each pair is approximately the same.

The volume of carbon dioxide evolved by the seeds treated for longer periods was always less than that for seeds treated for the shorter period. This could be correlated with the fact that the seedlings in the tests treated for longer periods did not seem to develop as quickly as the others. (See Part I).

Brassica rapa Carter's Rape 1933.

Fifty seeds were selected for each test and their fresh weight was found. In all save two of the tests carried out, the seeds were sterilized with a solution of calcium hypochlorite for 1⅔ hours, a period of time found to have little effect upon the course of germination (see Part I); in the other two tests, one lot of seeds was treated with the above solution for four hours, whilst the other was sterilized with 0·1% mercuric chloride for two minutes. All seeds were washed once after sterilization with autoclaved distilled water, and those sterilized with mercuric chloride many times.

The thermostat was regulated at a temperature of 22°C.

Germination was taken as completed when the radicyles exhibited geotropic curvature and touched the filter paper.

The results are shown in the Tables XXII and XXIII and are illustrated in the figures 39 and 40.

TABLE XXII.

Brassica Rapa. Carter's Rape, 1933.

No.of seeds in test 50. Sterilized with Ca(OCl)Cl soln. 1⅔hr.

Temp. of thermostat, 22°C. strength of " " grms per 100 c.c. 1·7 grms.

B_1 Seeds weighed 0·2046 grms

B_1a " " 0·2234 "

B_2 " " 0·2288 "

B_3 " " 0·2376 "

Date	Duration of expt. in ~~days~~ hours.	B_1 Air			B_1a Air			B_2 1% oxygen			B_3 Nitrogen		
		Vol: CO_2 evolved 1hr. 10 seeds	evolved 1hr. grm fresh Wt.	No. of germin	Vol. CO_2 evolved 1hr. 10 seeds	evolved 1hr. grm fresh Wt	No. of germin	Vol. CO_2 evolved 1hr. 10 seeds	evolved 1hr. grm fresh Wt	No. of germin	Vol. CO_2 evolved 1hr. 10 seeds	evolved 1hr. grm fresh Wt	No. of germin
Mar. 4th													
" 5 "	18	0·08	0·40	24	0·10	0·43	25	0·07	0·32		0·08	0·35	
" 6 "	42	0·20	0·96	41	0·21	0·96	40	0·03	0·14		0·03	0·13	
" 7 "	66	0·37	1·81	41	0·40	1·81	40				0·02	0·08	
" 8 "	90							0·01	0·04		0·02	0·07	
								1% O_2 replaced by 9·5% O_2			N_2 replaced by Air		
" 9 "	114							0·02	0·10	3	0·05	0·22	38
" 10 "	162							0·09	0·38	43	0·25	1·05	40

TABLE XXIII.

Brassica rapa. Carter's Rape, 1933.

No. of seeds 50.

Temp. 22°C.

B_4 Sterilized with Ca(OCl)Cl 1⅓ hrs.
B_5 " " " " "
B_6 " " " 4 "
B_6a " " 0·1% $HgCl_2$ 2 mins.

Date	Duration of expt. in hrs.	Number of seeds germinated.			
		B_4 Nitrogen	B_5 6% oxygen	B_6 Air	B_6a Air
Mar. 11th					
" 12 "	19			13	3
" 13 "	43			36	45
" 14 "	67		1	43	47
" 15 "	91		12		
" 16 "	115		28		
" 18 "	164½		41		
		Air substituted for N_2			
" 19 "	188½	30			
" 20 "	212	37			
" 21st	237	44			

Explanation of Figure, 39. Data from Table XXII.

Seeds in all tests sterilized with calcium hypochlorite solution for 1⅓hr.

Tests B_1 - B_3

Continuous line B_1 Seeds grown in air current.

Ringed " " B_{1a} " " " " " "

Broken " B_2 " " " 1% O_2 "

on 90th hr increased to 9·5% O_2

Dotted " B_3 Seeds grown in Nitrogen current.

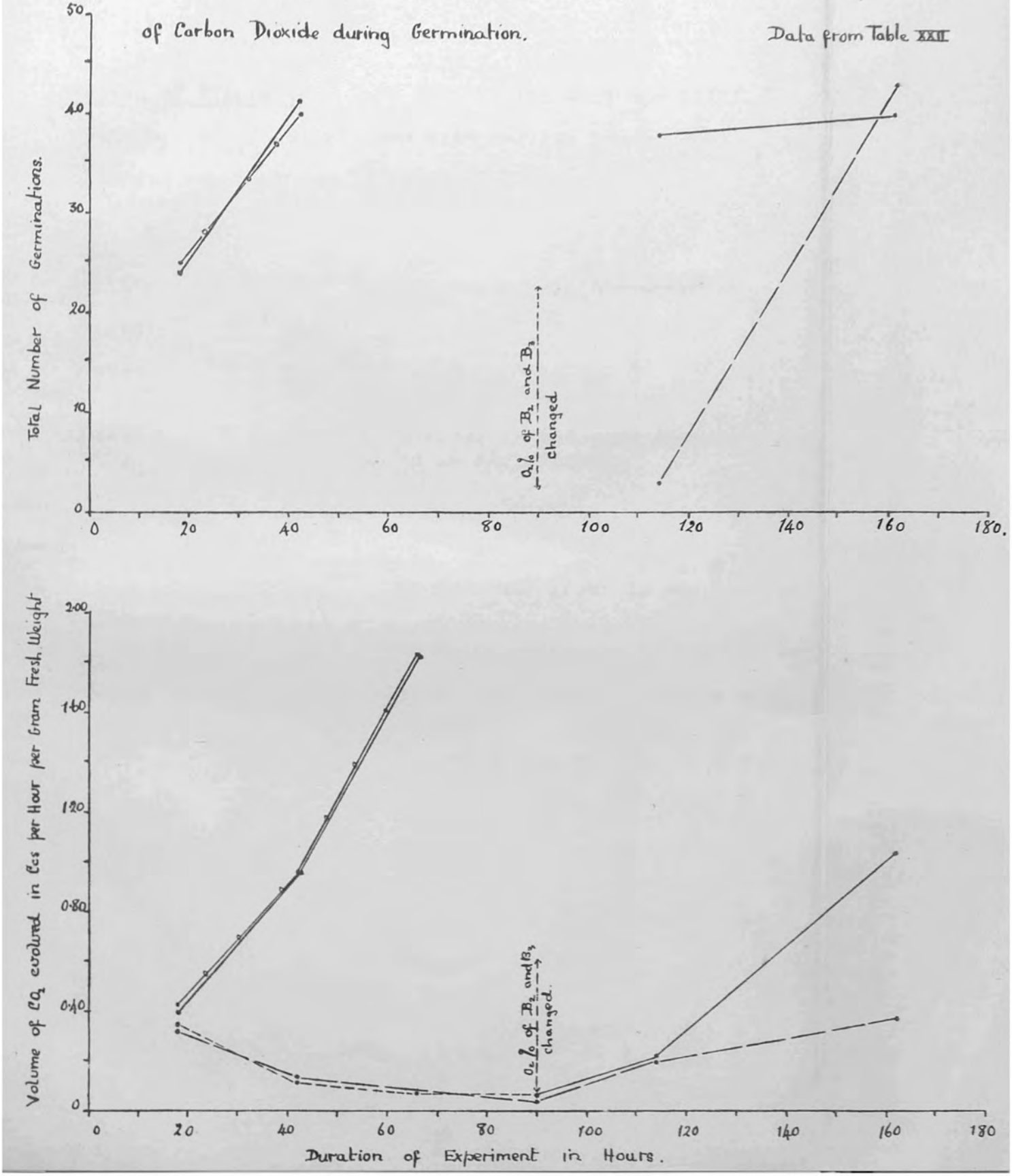
on 90th hr replaced by air current.

(Dotted line replaced by continuous line).

Figure 39.

Curves indicating the Effect of a Reduction in the Oxygen Pressure of the Atmosphere upon the Rate of Germination of Brassica rapa and upon the Rate of Evolution of Carbon Dioxide during Germination.

Data from Table XXII



Explanation of Figure 40. Data from Tables XXII and XXIII.

Tests B_1 - B_5 Sterilized with calcium hypochlorite solution for $1\frac{2}{3}$ hrs.

Black Ink. Tests B_1 - B_3

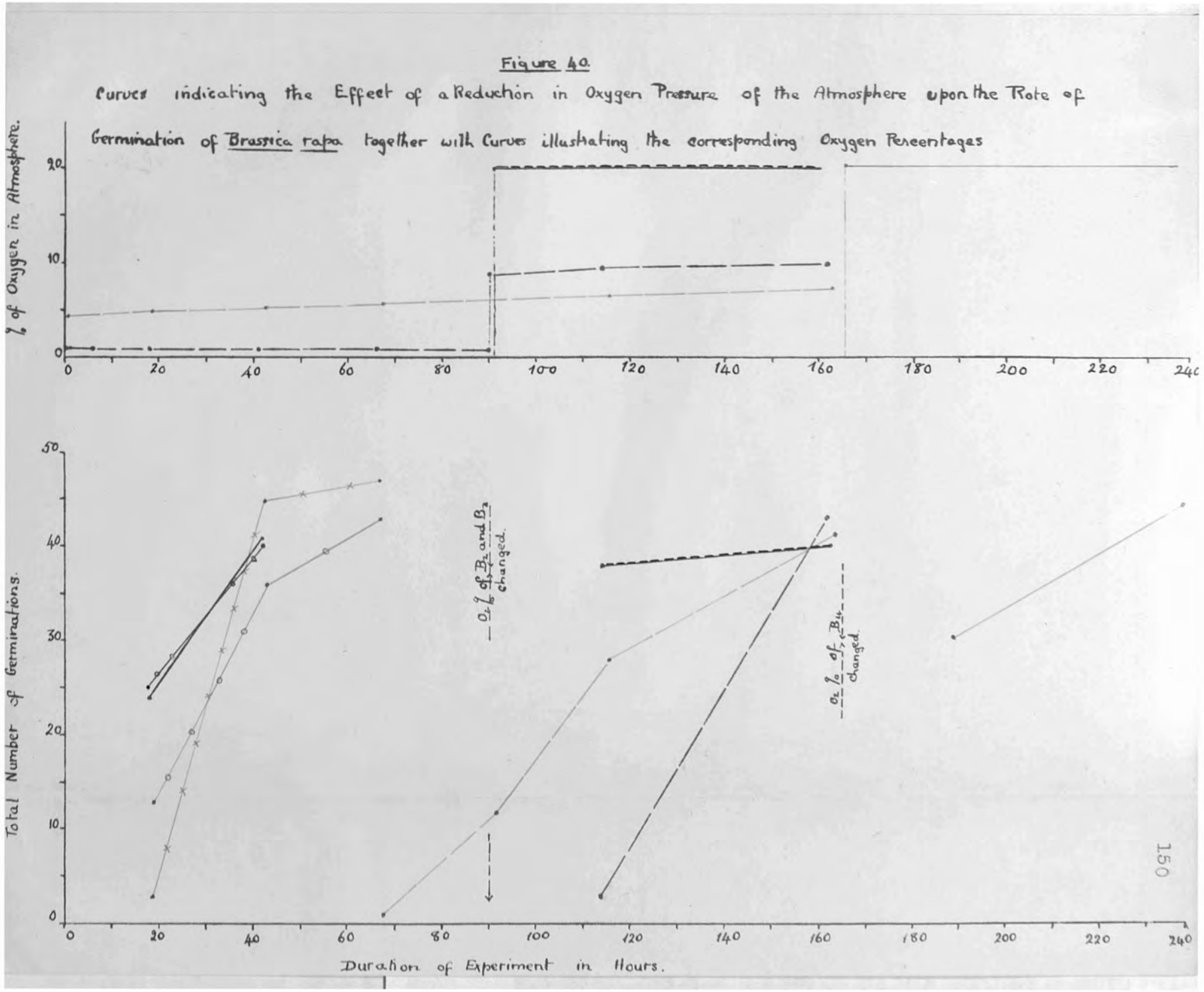
Continuous line	B_1	Seeds grown in air current.
Ringed " "	B_1a	" " " " "
Broken "	B_2	" " " 1% O_2 " increased to 9·5% O_2
Dotted "	B_3	Seeds grown in Nitrogen current changed to air current.

Green Ink. Tests B_4 - B_6

Continuous line	B_6	Seeds sterilized with Ca(OCl)Cl for 4hrs and grown in air current.
Crossed "	" B_6a	Seeds sterilized with 0·1% $HgCl_2$ for 2mins and grown in air current
Broken "	B_5	Seeds grown in 6% oxygen current
	B_4	" " " nitrogen " changed to air current on 164 hr.

Figure 40.

Curves indicating the Effect of a Reduction in Oxygen Pressure of the Atmosphere upon the Rate of Germination of Brassica rapa together with Curves illustrating the corresponding Oxygen Percentages



The results of this series of tests may be summarised as follows:-

1) At 22°C and in air:-
40% - 50% of the seeds germinated by the end of the first day and by the end of the third day 80% - 100% had germinated.

2) At 22°C and in 5% oxygen.
Germination began at the end of the third day and during the next four days rose steadily to a value of over 80%. The rate of germination was much slower than that in air; 80% germination was obtained after three days in air but only after seven days in 5% oxygen.

3) At 22°C and in 1% oxygen.
No germination took place during the first four days. The volume of carbon dioxide evolved fell to a very low value, much lower than that at the beginning of the experiment; this indicated that germination would not take place at this low oxygen pressure.
On replacing the 1% oxygen current with one containing 9·5% oxygen germination occurred at the end of the next day. By the end of the third day after the change the percentage germination was of the same order as that obtained at the end of the third day for seeds germinated in air. The volume of carbon dioxide evolved gradually rose but remained at a

comparatively low value.

4) At 22°C and in nitrogen.

No germination occurred, but when after four or even seven days the nitrogen current was replaced by air, germination followed at a rapid rate; by the end of the first day after the change as many as 60% - 75% had germinated. The rate of germination was equal to or greater than that in air if it is judged from when the air current was first begun. The volume of carbon dioxide evolved fell rapidly at first and remained at a low level; when air was introduced it increased rapidly and the rate of increase was almost as great as that of the carbon dioxide evolved by seeds grown in air.

5) The longer period of sterilization with calcium hypochlorite depressed the initial rate of germination, but the final germination was of the same order as that of seeds treated for the shorter period - 1⅓ hours as opposed to four hours. (See Part I).

6) Sterilization with 1% $HgCl_2$ exhibited a similar depressing effect upon the initial rate of germination but again the final germination reached a high value (See Part I).

Discussion and Conclusion.

The results obtained from both methods of experimentation correspond quite well although the actual values given by the liquid paraffin method indicate lower minima for the oxygen pressures necessary for germination than those obtained from the more accurate 'continuous current' method. The probable reasons for these discrepancies have been noted at the end of the first section of Part II.

They confirm, what would be expected, that in the case of most seeds, a reduction of oxygen in the atmosphere surrounding seeds when placed in germinative conditions brings about a lowering in the rate of germination and may completely prevent this process. The rate of germination of seeds affected in this way is progressively retarded with the extent of the reduction in oxygen, but no direct relationship exists between the degree of retardation and reduction in oxygen. Reduction in oxygen primarily affects the rate of germination but it may also lower the total number of germinations. These two effects of reduction in oxygen are clearly indicated by the figures illustrating the results shewn in the Tables. The gradient of the germination rate curves becomes progressively more gentle as the degree of reduction becomes greater. A similar decrease in the gradient is also apparent in the case of the curves indicating the rate of evolution of carbon-

dioxide by the seeds undergoing treatment.

At the end of the section dealing with the 'liquid paraffin' method two important values for the oxygen pressure were suggested. The minimum oxygen pressure, the value of reduction below which germination will not occur and the critical oxygen pressure, the value of reduction, above which the rate of germination is only slightly affected but below which retardation in rate is apparent. The results obtained from the 'continuous-current' method confirm the existence of the minimum value but indicate difficulty in defining the critical value. Thus even though the rate of germination of treated seeds may seem to be equal to that of seeds grown in air but in otherwise similar conditions, the rate of evolution of carbon-dioxide by the former is generally considerably less than of the latter. This indicates that reduction in oxygen affects the germination of seeds in ways other than those which are actually visible.

It was found that the value of the minimum oxygen pressure differs greatly according to the seed used. In the case of Triticum this value is very low, being somewhere below 1%. In very low oxygen pressures the seeds germinate but produce attenuated seedlings with very poor chlorophyll development. In atmospheres entirely deficient in oxygen the seeds will not germinate. The mimimum value for Pinus silvestris is comparatively very high and would seem to be in the region of 8% (2·5% raised to 7·5% gave no signs of

germination after ten days whilst 5.0% raised to 8.5% gave germinations at a very slow rate after the change to the higher oxygen %). The minimum value for Brassica rapa lies between those of the above seeds and would seem to be between 4% and 5%. 6% is well above the value, giving a steep germination rate curve, whilst 1% is far below the minimum value, as is indicated by the similarity between the curves representing rate of evolution of carbon-cioxide of this and the nitrogen test.

When seeds are grown in nitrogen (the limit of the reduction of oxygen) no germination occurs. Triticum shews indications of it; the radicles swell up and may even crack the pericarp. Seeds vary in respect to the periods of time they may be kept in germinative conditions without oxygen and yet subsequently germinate when the nitrogen is replaced by oxygen. Brassica and Triticum seem to be able to germinate after longer periods in nitrogen than Pinus.

The so-called critical oxygen pressures above which the rate of germination is not appreciably affected vary in a manner similar to the minimum values. For Pinus it has not been determined but must be relatively high, well above 8.5%. The rate of germination of Brassica in 9.5% oxygen differs little from that in air, thus indicating a critical value a little over 9.5%. This value for Triticum would seem to be over 4.5% probably 6%. Thus it is clear that as for the minimum oxygen pressure, the seeds of Pinus are more

sensitive to reduction in oxygen pressure than those of Brassica and Triticum. The values for each quantity in the case of Triticum are lower than those for Pinus and Brassica.

In so far as it is possible to generalise from results derived from a study of only three species, the differences noted above lead to the conclusion that the class of food reserve and perhaps, the organ in which it is stored are of importance in determining the effect of reduction of oxygen pressure upon the germination of seeds. An important correlation is suggested by a study of these minimum oxygen pressures. The high values seem to be connected with the storage of oil in the seeds whilst the lower ones would seem to be associated with the storage of starch. Probably this can be explained by the fact that before the germination of oil storing seeds takes place part of the oil is converted into carbohydrate by the addition of oxygen - the respiratory ~~ratio~~ quotient of oil storing seeds is often considerably less than 1.

As a result of this necessity for a certain minimum oxygen pressure for the germination of seeds it may be that seeds with a high oxygen requirement tend to germinate best in the lighter soils whilst seeds with a low requirement do well in heavier soils. Thus under natural conditions, Pinus regenerates most readily on light sandy soils in which aeration is good; whilst the "soils most suited to the growth of the most valuable wheats are stiff clay loams" (8) in which aeration is in general less good;

this indicates a connection between the value of this mimimum and the degree of aeration of the soil of the most favourable habitat of the seed in question.

As already noticed, the seeds of Brassica shew a marked retention of vitality, when put in germinative conditions but in an atmosphere containing less than the minimum oxygen requirement and readily germinate when restored to an atmosphere containing sufficient oxygen. This, together with the high value of the minimum oxygen requirement, may help to explain the appearance of large numbers of Brassica plants, such as B. Sinapis in England, in newly ploughed pastureland which has been arable land sometime previously. When the soil is ploughed, aeration is improved and the oxygen pressure in the soil is raised to a value above the minimum requirement and the seeds which have retained their vitablity in adverse conditions germinate readily.

In the case of Brassica, it may be recalled that delayed germination is also induced by increased CO_2 pressure (Kidd, F. and West, C. Ann.Bot.31, 1917, p. 457); such a condition might well occur in badly aerated soils. The conditions of culture in the present experiments, however, preclude the accumulation of CO_2, and the effects noted cannot be ascribed to such cause. No doubt CO_2 dormancy and the capacity to germinate after long periods without oxygen under germinative conditions both play a part in the appearance of Brassica plants after ploughing.

SUMMARY.

Part I.

1) A solution of calcium hypochlorite (2% titratable chlorine) is a suitable sterilizing agent for seeds (5).

2) The length of time the seeds are left in contact with it may vary considerably without appreciable effect upon the rate of germination and total germination.

3) Sterilization with this solution may cause a slight increase in the initial rate of germination of oil-storing seeds. This may be due to the slight oxidation of the storage oils.

4) In support of the above statement it has been shewn that a solution of calcium hypochlorite of this strength is capable of oxidizing the oils extracted from Helianthus.

5) A solution of calcium hypochlorite may also increase the initial rate of germination of seeds with thick seed-coats by oxidizing action upon the latter.

Part II.

1) Reduction of the oxygen pressure may result in the retardation or prevention of the germination of seeds

2) For most seeds there are two important values for the reduction of the oxygen percentage. 1) the critical

value, above which the rate of germination is not appreciably altered but below which the rate of germination is retarded 2) the mimimum value below which germination is completely prevented.

3) These values are specific for each kind of seed.

4) Generally speaking, the critical and minimum values are higher for oil-storing seeds than for starch-storing seeds - this is exemplified by Pinus and Brassica when compared with Triticum.

5) The high values noted for the critical and minimum oxygen pressures necessary for the germination of oil-storing seeds may be correlated with the low respiratory ~~ratio~~ quotient of oil-storing seeds during germination.

6) The values for these two quantities may also be a factor in determining the soil most suited to the germination of the seed in question and consequently in determining the type of soil and, to a lesser extent, the habitat in which the particular plant is generally found. Thus seeds with high values for minimum oxygen pressure as exemplified by Pinus and Brassica grow well in aerated soils, and those with low values in heavy poorly aerated soils, as is the case with Triticum.

7) Seeds can retain their vitality in germinative conditions,

but with an oxygen % below the minimum value for varying periods of time. When the percentage of oxygen is increased above this value germination will occur. The length of time of retention of vitality varies according to the seed. It would seem to be very short in the case of Pinus but longer though undetermined in the case of Brassica and Triticum. This may be correlated with the poor germination naturally of Pinus silvestris on heavy or badly aerated soils.

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