CONIFEROUS TREE SEED GERMINATION WITH PARTICULAR REFERENCE TO THE EFFECTS OF TEMPERATURE, SEED MOISTURE AND STRATIFICATION ON GERMINATION BEHAVIOUR OF WESTERN HEMLOCK SEED

by

WILLEM BIENTJES

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF FORESTRY

in the Faculty

of

Forestry

We accept this thesis as conforming to the standard required from candidates for the degree of MASTER OF FORESTRY.

Members of the Faculty of Forestry

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1954

ABSTRACT

In a study of the problems met in testing germination of coniferous tree seed, special attention is given to the variability between different seedlots of the same species. Known and suspected causes for this variability are discussed. These include seed source, genetic constitution of individual trees, seed maturity, treatments during the commercial extraction process and storage conditions. Loss in seed viability may occur during anyone of several phases of the extraction process, such as cone storage, kiln drying and dewinging.

In the discussion of the germination test procedure the importance of a moist-cold pretreatment or "stratification" is indicated. It is shown, that many species of coniferous seed are subjected to such treatment in nature. The desirability of stratification of coniferous seed as a standard pretreatment prior to incubation is emphasized. The effect of stratification on temperature and light requirements is discussed for Douglas fir in particular. The usual methods for stratification and their disadvantages are described. In order to overcome some of the disadvantages of the older stratification methods, particularly the lack of control over seed moisture content, the "naked stratification" method was developed at the University of British Columbia. This method has been applied successfully to Douglas fir seed and seed from several other species.

A detailed description is given of an experiment, designed to develop a standard germination test for western hemlock seed. This involved determination of an optimum incubation temperature, seed moisture content and stratification period. The "naked stratification" method was used. The effect of incubation temperature, seed moisture content and stratification on the final germination percentage and the rate of germination was evaluated, using analysis of Variance and the t-test. The results showed, that for germination of western hemlock seed the following treatments are most favourable. Stratification for 7 - 9 weeks with a seed moisture content of 50 - 75 percent,--which is obtained after approximately 33 hours of soaking,--followed by incubation at a constant temperature of 20°C. Under these conditions the germination test can be concluded in approximately 20 days. Different incubation temperatures influenced both final germination percentage and rate of germination. Different seed moisture contents and stratification periods generally did not affect the final germination percentage, but did have a pronounced effect on the rate of germination. Stratification did not seem to change the temperature requirements of western hemlock seed. Differences in the germination behaviour of the four seedlots tested were observed.

TABLE OF CONTENTS

	Page
INTRODUCTION	
VARIABILITY OF MATERIAL	1
Seed Source	1
Genetic Constitution of Individual Trees	7
Year of Seed Collection	11
Date of Seed Collection	12
Differences in Processing	17
Storage Conditions	21
Seasonal Influences on Germination	23
THE GERMINATION TEST	24
Moist-Cold Pretreatment	25
Effect of Stratification on Temperature Requirements	27
Effect of Stratification on the Light Requirements	28
Methods of Stratification	30
Soaking Injury of Seeds	32
Bio-chemical Changes During Stratification	33
EXISTING INFORMATION ABOUT DISTRIBUTION, SEEDING HABITS AND GERMINATION CHARACTERISTICS OF WESTERN HEMLOCK	35
Seeding Habits	36
Previous Experiences with the Testing of Hemlock Seed	38
THE EXPERIMENTAL PROCEDURE	40
Seed	40
Sampling Procedure	41
Testing Procedure	44
Incubatore	45
Treatments	45

	Page
Stratification Periods	49
Incubation Temperatures	49
Counting the Germinating Seeds	50
Interpretation of the Results	51
STATISTICAL ANALYSIS OF DATA	53
Influence of the Main Effects	62
Influence of the First-Order Interactions	66
Influence of the Second-Order Interactions	82
SUMMARY OF RESULTS	92
CONCLUSIONS	96
RECOMMENDATIONS FOR THE TESTING OF WESTERN HEMLOCK SEED	98
APPENDIX I	99
APPENDIX II	101

LIST OF ILLUSTRATIONS

Figure 1: Germination behaviour of western hemlock,trees 1 and 2, collected Sept. 15 and Nov. 26, 1953.	10
Figure 2: Germination behaviour of western hemlock seed-lots 4907, 4908, 5101, 5102 and germination behaviour of seed collected from trees 1, 2 and 3, University Forest, Haney, B.C.	16
Figure 3: Range map for western hemlock	37
Figure 4: Seed-sampler	42
Figure 5: View of seed-sampler	43
Figure 6: Rate of Moisture Absorption for Seedlot 4907	46

		Page
Figure 7:	Rate of Moisture Absorption for Seedlots 4907, 4908, 5101 and 5102.	48
Figure 8:	Effect of the length of stratification period on the germination rate at twelve days.	64
Figure 9:	Lot 4907, germinated without soaking or stratification at 25°C, alternating temperatures and 20°C.	68
Figure 10:	Lot 5102, germinated without soaking or stratification at 25°C, alternating temperatures and 20°C.	68
Figure 11:	Lot 4907, soaked for 11 hours; stratified for 3 weeks and incubated at 25°C., alternating temperatures and 20°C.	72
Figure 12		
A:	Influence of Seedlot-Soaking Period Interaction on Rate of Germination.	74
B:	Influence of Seedlot-Seed Moisture Content Interaction on Rate of Germination.	74
Figure 13:	Influence of the stratification period - soaking period interaction on the final germination percentage.	78
Figure 14:	The influence of the stratification period - soaking period interaction on the germination rate.	80
Figure 15:	The effect of increasing seed moisture content during stratification on the rate of germination at 25°C., alternating and 20°C. incubation temperatures.	82
Figure 16:	Relationship between incubation temperature and stratification period for seedlots 4907, 4908, 5101 and 5102.	84
Figure 17:	Seedlot 4907. The influence of different incubation temperatures (25°C., 20°C, alternating) on the germination behaviour of seed, stratified for 7 weeks.	
A:	Soaking period - ½ hour.	86
B:	Soaking period - 2 hours.	86
C:	Soaking period - 11 hours.	87
D:	Soaking period - 33 hours.	87

Page

Figure 18: Seedlot 5101. The influence of different incubation temperatures (25°C., 20°C., alternating) on the germination behaviour of seed, stratified for 7 weeks.

A: Soaking period - ½ hour. 88

B: Soaking period - 2 hours. 88

C: Soaking period - 11 hours. 89

D: Soaking period - 33 hours. 89

Figures 19 and 20: Seedlot 4907. The influence of different incubation temperatures (25°C., 20°C., alternating) on the germination behaviour of non-stratified seed.

19: Seed was not soaked. 102

20: Seed soaked for 33 hours. 102

Figure 21: Seedlot 4907. The influence of different incubation temperatures (25°C., 20°C., alternating) on the germination behaviour of seed, soaked for 33 hours and stratified for 9 weeks. 103

Figure 22: Seedlot 4907. The influence of different soaking periods (0, ½, 2, 11 and 33 hours) on the germination behaviour of non-stratified seed, incubated at 20°C. 103

Figure 23: Seedlot 4907. The influence of different soaking periods (½, 2, 11 and 33 hours) on the germination behaviour of seed stratified for 9 weeks and incubated at 20°C. 104

Figure 24: Seedlot 4907. The influence of different stratification periods (0, 3, 5, 7 and 9 weeks) on the germination behaviour of seed, soaked for 33 hours and incubated at 20°C. 104

Figures 25 and 26: Seedlot 4907. The influence of different soaking periods (33, 48, 72 and 96 hours) on the germination behaviour of seed, incubated at 20°C.

25: Stratified for 3 weeks. 105

26: Stratified for 7 weeks. 105

Figures 27 and 28: Seedlot 5102. The influence of different incubation temperatures (25°C., 20°C., alternating)

Page

on the germination behaviour of non-stratified seed

27: Seed was not soaked. 106

28: Seed soaked for 33 hours. 106

Figure 29: Seedlot 5102. The influence of different incubation temperatures (25°C., 20°C., alternating) on the germination behaviour of seed, soaked for 33 hours and stratified for 7 weeks. 107

Figure 30: Seedlot 5102: The influence of different soaking periods (0, ½, 2, 11 and 33 hours) on the germination behaviour of non-stratified seed, incubated at 20°C. 107

Figure 31: Seedlot 5102. The influence of different soaking periods (½, 2, 11 and 33 hours) on the germination behaviour of seed, stratified for 7 weeks and incubated at 20°C. 108

Figure 32: Seedlot 5102. The influence of different stratification periods (0, 3, 5, 7 and 9 weeks) on the germination behaviour of seed, soaked for 33 hours and incubated at 20°C. 108

TABLES

Table I: Date of collection, seed weight and percent filled seed after thorough cleaning for western hemlock trees 1 and 2. 9

Table II: Information about seed used. 41

Table III: Rate of Moisture Absorption for Seedlot 4907. 47

Table IV: Seed Moisture Content After Soaking. 47

Table V: Apparent germination per cent after 42 days of incubation. 54

Table VI: Rate of germination after 12 days of incubation. 55

Table VII: Analysis of Variance for the Germination Rate at 12 days. 59

Table VIII: Analysis of Variance 60

INTRODUCTION

Importance of Seed Research

It is a remarkable fact that, in most of the works and articles dealing with research in forestry, seed research is seldom mentioned. This, however, should not lead to the conclusion that forest tree seed research is unimportant, or that research in this field has been neglected. Baldwin (1942) describes its importance as follows: "The increased practice of artificial reforestation and also the growth of the ornamental nursery business, requires reliable information for data on harvesting, storage and germination of seed of various species. A thorough knowledge of the behaviour of tree seeds is required, not only for successful propagation of trees in nurseries for ornamental or reforestation purposes, but also for the intelligent control of forestry operations aiming to assure natural reproduction. The latter should be stressed as of fundamental importance in silviculture. In studies of natural reproduction of forests the start should be made with the seed, and seed production of stands."

Sources of Information for Seed Research

A great difficulty which faces everyone who wants to obtain information about previous work done in the seed research field or other fields of research, is the inaccessibility of information. Publications appear in many different journals, often obscure ones. Keeping up to date is therefore a time consuming task. The forester or nursery man will often have neither time nor opportunity to look up information that could be very useful to him.

To remedy such defects in the methods of disseminating the results of scientific study an occasional synthesis and digestion of the scattered information should be made and concentrated in a single publication. Among the classics in the field are Nobbe's "Handbuch der Samenkunde" (1876), Detmer's "Vergleichende Physiologie des Keimungsprocesses der Samen" (1880) and Harz's "Landwirtschaftliche Samenkunde" (1885). The vast literature on seed which has appeared since that time defies summary in one volume, and recent treatises have covered special groups only, like Wittmack's "Landwirtschaftliche Samenkunde" (1922) and Lehmann and Aichele's "Keimungsphysiologie der Graeser (Gramineen)" (1931). Schmidt's "Unsere Kenntnisse vom Forstsaatgut" (1930) is a more recent attempt to assemble information on tree seed and genetic problems. Some forest seed information is contained in Toumey and Korstian's "Seeding and Planting in the Practice of Forestry" (1931) and in the revised edition of 1942. Baldwin's "Forest Tree Seed of the Northern Temperate Regions" (1942), with special reference to North America is probably the most complete forest tree seed handbook available for this continent. Barton and Crocker's "Twenty Years of Seed Research at Boyce Thompson Institute for Plant Research" (1948) covers completely the work done at this research institute, and contains some important information on forest tree seed. "The Woody Plant Seed Manual", prepared by the United States Forest Service and published by the U. S. Department of Agriculture in 1948, contains some general information about cone collecting and seed processing. The main content is detailed information on cone collection, seed processing and germination characteristics of the North American conifers and dicotyledons; it is a very valuable reference for forester and nursery man.

In 1951, Rohmeder published his "Beitraege zur Keimungsphysiologie der Forstpflanzen", in which the results of fifteen seed physiological problems of very practical importance are thoroughly discussed. Noteworthy, is that Rohmeder describes his experiments in sufficient detail to enable repetitions and checks to be made by anyone interested.

The most general outline on seed physiology is Crocker and Barton's "Physiology of Seeds" (1953). It contains basic information on many different kinds of seed and some information about storage and stratification of forest tree seed is included. A number of publications of the Boyce Thompson Institute for Plant Research are a useful source of detailed information for anyone working in the seed research field.

This enumeration is not complete. There are many other journals and research publications that contain seed physiological information. The aim has been to cover only briefly the most important sources of condensed information.

Some of the Problems in Seed Research

The handling of large amounts of seed requires a considerable amount of information. Following the different steps in order of handling, these questions arise:

What is the best time of collection for cones of a certain species?

How can cones be stored and shipped without loss of seed viability?

Are the existing methods of extraction, dewinging and cleaning of seed in any way harmful to its viability?

What are the possibilities for successful storage of seed?

How can the seed quality be evaluated?

These questions represent only some of the problems encountered in tree seed research. Furthermore, a close interrelation exists between the numerous factors affecting seed viability and germination behaviour, which complicates the problems to a large extent. For instance, prematurely collected seed may give satisfactory germination, but may not store very well under the best storage conditions known to date. There are indications that several phases of the usual extraction and cleaning process may be harmful to seed viability or may alter the course of germination.

Storage conditions have only been investigated incompletely, and little is known of the influence of storage conditions on the course of germination.

The evaluation of seed quality, which appears to be so simple, is in reality very complicated. This particular part of seed research, more commonly called "seed testing", is of great importance. Many seed problems cannot be investigated without the existence of a reliable evaluation test. With seed prices as high as they are today, a reliable germination test is needed to obtain information about the quality of purchased seed.

VARIABILITY OF MATERIAL

A complication which is often overlooked, is the variable behaviour of different seedlots of the same species under identical germination conditions. Reasons for this difference in behaviour can be many, such as:

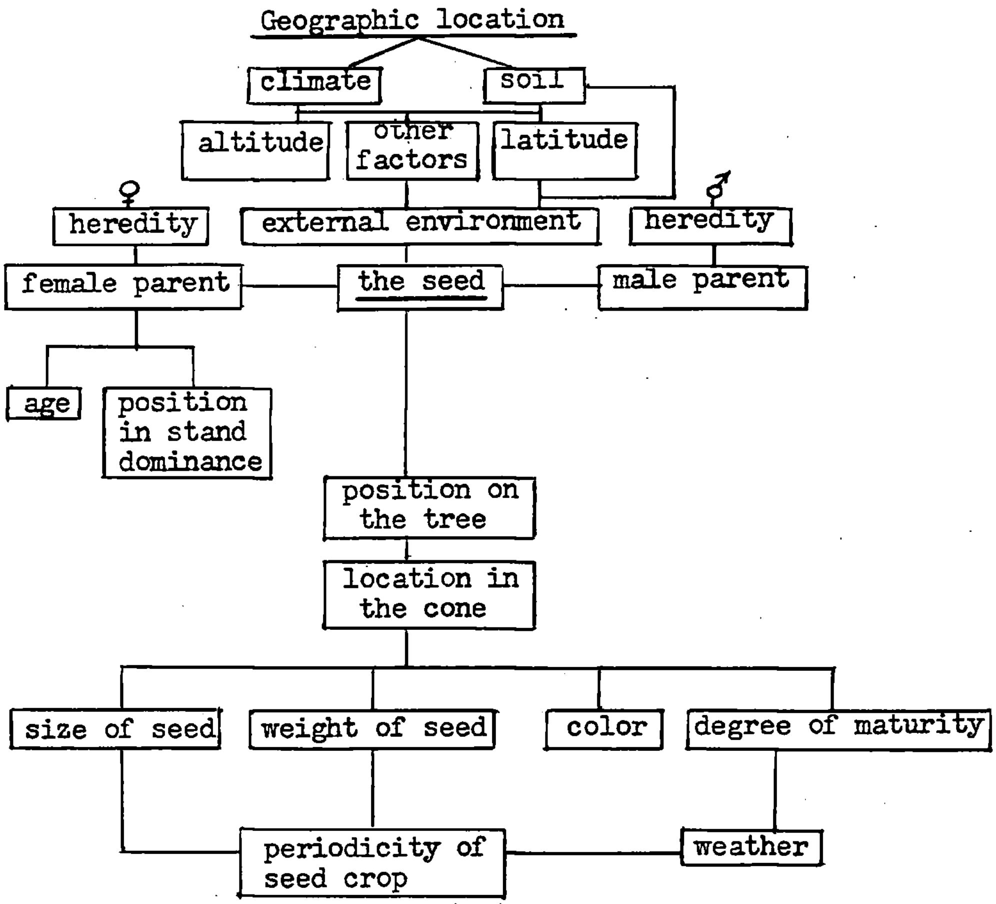
1. Seed source, which may include geographical races and altitudinal influences.
2. Genetic constitution of individual trees.
3. Year of seed collection.
4. Date of seed collection.
5. Differences in processing in the various extraction plants.
6. Storage conditions.
7. Seasonal influences on germination.

The above mentioned factors are only the more obvious ones and this summary is by no means complete. The influence of many factors is not clear, and other factors still unknown probably influence seed behaviour.

Of seed received at the laboratory for testing, the investigator usually knows only the year of collection and the general area of collection. The rest of the seed's history is usually unknown to him and often impossible to trace back. A short discussion of what is known about the influence of the above mentioned factors, will follow.

1. Seed Source

The following diagram by Baldwin (1942) gives an idea of the complex of factors included under seed source.



Seed collected in a geographic location of limited size is considered to be of the same source or provenance. Actually one should be more specific and call it a certain geographic or climatic source. Seeds collected from different trees, growing side by side, may be of different origin, seen from the hereditary point of view. Cross-pollination results in a variety of parentage of seeds on the same mother tree and seeds from the same parents may differ genetically. (Baldwin, 1942) Thus it is obvious that every seed collection, no matter how limited in size it may be, is a mixture of differently behaving individuals.

Important characteristics of tree seed are germinative capacity, germination behaviour, the early size of seedlings it produces and the degree to which it possesses desirable hereditary traits. Environment influences size of seed, germinative capacity, and possibly germination behaviour. Size of seed influences in turn the early size of the resulting seedlings. The hereditary qualities of the seed on the other hand, are only indirectly influenced by the environment.

(a) Effect of age and condition of mother tree on size of seed and germination

In a review of the literature, Baldwin (1942) comes to the conclusion that "No general rules can be given. The work done shows conflicting results."

For Douglas fir Hofmann (1921) found that seed from young trees was larger and gave a higher germinative capacity than seed from old trees. Seed from high altitudes showed poorer germination than that from low altitudes. Pearson (1910), investigating the effect of condition of parent (aside from age) in *Pinus ponderosa*, states that fire-scarred trees produced seed with a higher germinative capacity than did healthy trees. In a later publication (1931) he concludes that extremely vigorous trees produce few seed. Allen (1942) collected seed from Douglas fir trees less than twenty years of age, a practice which used to be questioned seriously. Indication was obtained, however, that young trees are capable of producing fertile seed in important quantities if conditions for cross-pollination are favourable. Young trees (8 - 18 years) seldom bear male flowers in quantity. This means that the pollen supply of young stands may be insufficient for satisfactory pollination and an outside pollen source must then be available, usually in the form of older trees. If this last requirement is fulfilled, young stands can be a source of easily available good seed.

(b) Effect of cone-size on size and germinative capacity of seed

Baldwin (1942) concludes that "apparently the relation between size of fruit and size of seed is not the same in all cases".

Eliason and Heit (1940), working on *Pinus sylvestris*, report a greater percentage of good seed in large cones. According to Baldwin (1942) Gast and Wright report a definite increase in the size of the seed with increased cone size in *Pinus strobus*. McIntyre (1929) finds no relation between size of cone and seed viability in *Pinus pungens*, *P. rigida*, *P. rigida* var. *serotina* and *P. banksiana*. In the case of *P. echinata* they find that small cones have seed of larger size and greater germinative capacity than large ones.

Allen (1942) reports for Douglas fir, that unpollinated cones grew as rapidly as cross-pollinated cones and reached the same mature size. The unpollinated cones produced apparently normal seeds which contained no embryos. Thus, for Douglas fir, no relationship exists between the cone size and seed quality, but fertilization is the important factor related to seed quality.

There are indications, that for Western hemlock the same situation exists as for Douglas fir. In a poor seed year, such as 1953, Western hemlock cones were collected at the University Forest at Haney, B. C., which contained normal-looking seed. Examination of this seed showed, however, that up to ninety percent of the seed was empty.

(c) Effect of position of seed in the cone and location of cone in the tree crown on size and germination of seed

Baldwin (1942) concludes that "no general rules can be given as to which part of the cone or which part of the crown provides the seed which are best in regard to size or germination behaviour".

According to Baldwin (1942), Gast and Wright (unpub.) found that seed from the basal portion of the cones of Pinus strobus were larger than those from the apical portion. Munns (1921) reports for Pinus jeffreyi an earlier germination of seeds from the lower portion of the cone, compared to seed from the apical portion of the same cone.

McIntyre (1932) and Stone (1933), however, could not find a difference in germinative capacity of seeds from different parts of the cone in Pinus pungens and P. virginiana respectively.

Acatay (1938) reports the largest and heaviest seeds in the upper third of the crown in Carpinus and Acer, both seed and fruit weight increasing with height above ground. For Picea excelsa and Larix europaea the same author finds no difference in the germination of seeds from the top and bottom of the crown. For Pinus sylvestris he reports that the highest percent germination comes from cones near the base of the crown.

The evidence presented here seems conflicting, but suggests that differences may exist between different species. Baldwin (1942) points to the fact that Abies species bear cones only in the very top of the crown, and that Viburnum alnifolium produces fertile flowers only in the center of the influorescence. He suspects that similar factors causing this behaviour may operate in many trees, which would lead one to expect differences in seed size and germination behaviour according to the position of the cone in the tree crown. Many other chance factors may come into the picture, however. Prevailing wind direction during pollination and degree of shadow caused by neighbouring trees may be of importance, for example. It may well be that cones in different positions in the crown mature at different times.

When collected at the same time, differences in maturity for these different positions may be reflected in the germination behaviour. When, however, each cone is collected when "ripe", these differences can be expected to disappear. This problem certainly needs more thorough investigation, before a satisfactory answer can be found. It is obvious, however, that such investigations must be designed very carefully, otherwise differences may show up that are caused more by faulty design than by a difference in position of cones in the tree crown.

(d) Differences in germination behaviour for different geographical races of the same species

Pseudotsuga taxifolia: The coast and Rocky Mountain forms of this species show a distinct difference in germination behaviour. The Rocky Mountain or interior form shows a much faster germination than the coastal form. To obtain reasonably fast germination, stratification is not necessary for interior seed, in contrast to coastal seed, which germinates slowly without stratification. When both coastal and interior seed are stratified under identical conditions, interior seed still germinates faster than coastal seed.

This difference in behaviour is in some respects contrary to expectation. Interior Douglas fir grows in a climate, where the winters are much longer and colder and the summers warmer and dryer than on the West coast, where the coastal form is native. In the first instance, therefore, one would expect interior Douglas fir seed to benefit more from a moist-cold pretreatment than the coastal form. The reverse has, however been found.

Possibly there could be some truth in the following reasoning. During stratification, temperatures just above freezing are the effective ones and not the temperatures below freezing. On the West coast, temperatures just above freezing will occur, alternating with freezing temperatures, over the whole winter period. In the interior, however, just above freezing temperatures are confined mainly to the beginning and the end of the winter period. The total time that temperatures effective for stratification will occur, is probably longer on the West coast than in the interior. Possibly this climatic difference is connected with the observed difference in germination behaviour.

Marcet (1951) mentions investigations, carried out at the Forestry Institute at Zurich, where germination behaviour was studied for different origins of Scots pine, larch, and spruce in an attempt to find a short method of distinguishing between the different sources.

In investigations of this nature it is of utmost importance that the investigator be satisfied that the only difference between the seedlots is their difference in origin. Very often this supposition cannot be proven because of unknown factors that may influence germination behaviour. This makes the results of such investigations rather questionable. At any rate, one has to be extremely careful in drawing conclusions.

2. Genetic Constitution of Individual Trees

The genetic constitution of the individual tree is complex and can only be investigated by very detailed methods over a long period of years. Although a population consists of a broad framework of identical inheritable traits, variations will occur. These variations can be anatomical, physiological or morphological, and in some instances can be of great economical value (branching habit, resistance to disease). Some traits may influence survival chances of a tree within a certain region. Whether variation in seed characteristics and germination behaviour between individual trees within a limited locality is genetically determined has not been investigated properly yet. The following observations may indicate an influence of the genetic constitution of the mother tree on seed characteristics and germination behaviour.

During the autumn of 1953, exploratory investigations were started at the Faculty of Forestry of the University of British Columbia, on the germination behaviour of western hemlock seed, collected at the University Forest at Haney, B. C., from different individual trees at more or less regular intervals. At the laboratory the cones were dried at room temperature (approximately 70°F.); the seed was extracted, dewinged by hand and empty seed removed in a vertical air column. This primitive but very effective method produced seed with a negligable number of empty seeds and dewinging damage was reduced to a minimum. Figure 1 shows the germination behaviour for trees 1 and 2 for two dates of collection (germination at 20°C. for non-stratified seed). It is obvious that seed from tree 1 shows a remarkably rapid germination, especially for the later collection. Obvious also is the similarity between the shape of the cumulative germination curves. The main difference is in the start of the germination. The cones of the late collection of tree 1 had lost most of their seed. Seed yield was therefore low. Cones from tree 2 yielded more seed, although they also had shed a considerable amount of their content. Another interesting observation was made on tree 1. The late collection showed seeds with a protruding shrivelled radicle. In other words, germination in the cone had taken place somewhere in the period from September to November. In the September collection no "pre-germination" was observed. This pre-germination probably accounts for the ten percent lower final germination of the late collection. Table I gives additional information about the seed from trees

1 and 2.

Table I:

Date of collection, seed weight and percent filled seed after thorough cleaning for western hemlock trees 1 and 2.

Tree	Date of collection	1000 seed-weight	% filled
1	Sept. 15, 53.	1.633 gr.	99.
2	" "	2.703 "	97.
1	Nov. 26, 53.	1.409 gr.	100.
2	" "	2.649 "	100.

Tree 1 shows a very much lower seed weight than tree 2. Seed shape was also markedly different. Where most western hemlock seed is rather plump, seed from tree 1 was elongated with pointed ends.

Reasons for this difference in characteristics are impossible to give at this stage. Influence of the genetical constitution of the mother tree is suspected however. Two morphological characters, seed shape and seed weight, combined with a physiological character, rapidity of germination, are obviously different in this tree from other western hemlock trees.

The pre-germination observed in tree 1 must be considered a loss in this tree's seed production capacity. It is also probable that a certain percentage of the seed, disseminated during the fall, will germinate immediately and the resulting seedlings will more than probably not survive winter conditions. The reproduction capacity may be considerably curtailed by the seed behaviour.

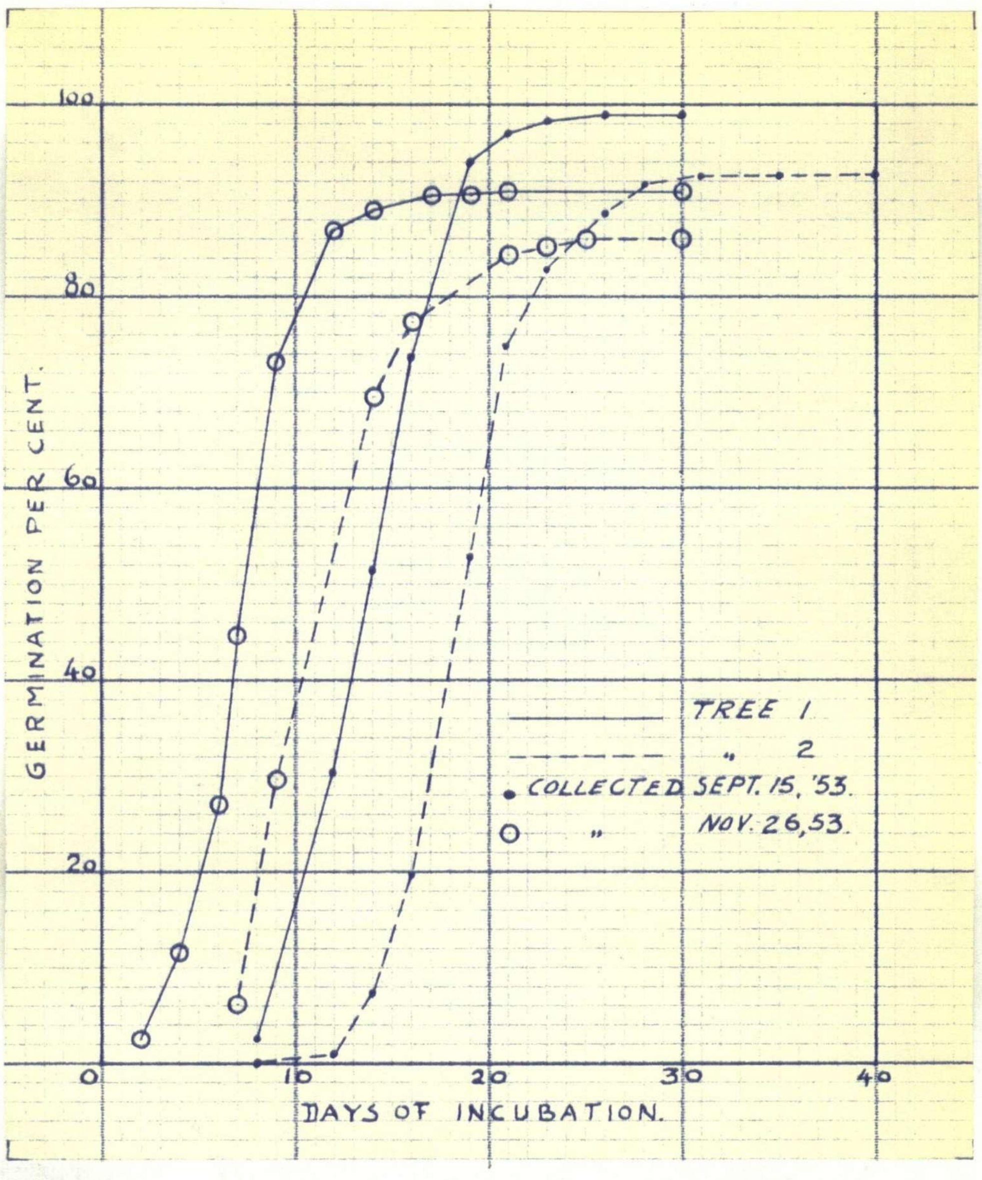


Figure 1: Germination behaviour of western hemlock, trees 1 and 2, collected Sept. 15 and Nov. 26, 1953.

3. Year of Seed Collection

Periodicity of seed bearing is a very well known fact among trees, especially the coniferous species. Others, like poplars, willows and some maples, bear plentiful crops each year. Outside of forestry, periodicity in fruit-bearing is a common phenomenon in fruit and nut culture. Baldwin (1942) describes two kinds of periodicity, an inner or spontaneous periodicity and induced periodicity. A classic example of spontaneous periodicity is bamboo, Chusquea abietifolia, which at intervals of about thirty-three years seeds simultaneously over a wide area and then dies. Induced periodicity presupposes a readiness for fruiting which awaits external conditions favourable for fruit setting. Fruiting occurs, when the inner (spontaneous) and induced periodicity (favourable external conditions) coincide. Weather conditions may influence the initiation and development of reproductive buds as well as influencing pollination in the year following bud initiation. Induced periodicity is therefore highly complex and usually combined with some degree of spontaneous periodicity. The situation just described seems very common among our coniferous species. Weather conditions during the time of pollination may mean the difference between a bumpercrop and a crop failure, if reproductive buds in great quantities are present. The reasons for the periodic occurrence of reproductive buds, however, are still very obscure.

In different years forest trees will thus produce very different amounts of seed. Our question now is, whether a difference in germination behaviour does exist between seed produced in different years. Very little is known about this question, but some indications, mainly related to storage ability of coniferous seed, more in particular Douglas fir, seems to exist. Allen (1949-53), during the past five years, has conducted a storage experiment which seems to indicate that Douglas fir seedlots, which showed a good initial germination capacity, retain their viability better under different storage conditions than seedlots with a low initial germination percent. These seedlots deteriorate faster and show a much greater susceptibility to improper storage conditions. For instance, seedlots with eighty percent or higher germination capacity showed little loss and little difference in germination capacity after three years of storage at room temperature, 32°F. and 0°F. Seedlots however with only about forty or fifty percent initial germination capacity showed a considerable loss of viability after three years of storage. The highest losses occurred at room temperature and the lowest in most cases at 0°F. In several other cases, however, 32°F. and 0°F. seemed to be equally good. Many uncertainties still adhere to this problem, however. The experiment was carried out mostly with commercial seed of practically unknown history. Mistreatment somewhere during collection or processing may play a role. It is therefore suggested by Allen to carry out future storage experiments with very carefully "ideally" processed seed, collected over a period of consecutive years, to include years of meagre as well as bumper crops.

4. Date of Seed Collection

Many investigations have been carried out to compare germination behaviour of seed collected at different dates. The objective in each case was to establish a criterion for seed maturity which would indicate the ripeness of cones for collection. For some species a suitable criterion seems to have been established; for many others, however, reliable information is still lacking. Baldwin (1942) defines maturity as follows: "seed is mature when growth of the seed on the parent plant ceases, and it enters into a resting stage and is ready for dispersal". It is obvious that cone collections should be made before seed dispersal begins in order that as high a yield as possible may be obtained. The question is a matter, however, of how early cones can be harvested without impairing the seed quality. It is known that the stage of maturity affects germination behaviour, keeping quality in storage, and possibly the dormancy of the embryo. (Crocker and Barton, 1953) The ability of coniferous tree seed to store is of utmost importance, due to the irregularity of good seed years.

Maki (1940) reports a marked influence of date of collection on the germination capacity of ponderosa pine seed. Four cone collections, made between July 27 and September 5, showed highly significant differences respecting germination capacities. The collection of July 27th showed no germination at all. Seed, collected August 6th, produced a few seedlings. From there on the germination capacity increased rapidly until September 5th, after which date a general opening of the cones occurred and satisfactory sampling was no longer possible. These results indicate that actual gains in seed quality are realized by permitting as much of the ripening as is practicable to take place while the seed is still in the cone on the tree.

Over the same period, Maki reports a decrease of cone specific gravity from 0.92 (July 27) to 0.74 (Sept. 5) and also an increase of "self extracted" seed (seed which fell out of the cone on its own accord after drying in partial sunlight) from 0 to 91.6 percent. From these data he concludes that ponderosa pine should have reached an acceptable degree of maturity when the specific gravity of the cone is about 0.85. Although this experiment has shown that at this specific gravity the germination capacity is reasonable, no information was obtained about the keeping quality of the seed in cold storage.

Finnis (1950) carried out a maturity study on Douglas fir. He reports a general increase of germination capacity in samples collected over the period July 26 to September 6. Later collections showed no increase. He was unable, however, to establish a maturity-specific gravity relationship. More promising as a maturity index seems to be the ratio between embryo length and seed length. Finnis states that the growth in embryo length during early August was particularly noticeable.

Crossley (1953), investigating the possibilities of cone colour as a maturity index for white spruce, Picea glauca (Moench) Voss var. albertiana (S. Brown) Sarg., collected cones at several dates and obtained results similar to those found by Finnis for Douglas fir. Germination capacity increased as the collecting date was later. It was also found that the samples collected on August 5th were always several days slower to commence germination than any of the samples collected at later dates. To investigate this further, seed from the August 5th collection and seed from later collections was planted and the seedlings allowed to grow for thirty days. At this time there was no apparent difference in seedling health, but the seedlings obtained from the later collections did germinate three or four days earlier and averaged twenty percent greater height growth than the others. Whether this difference will remain or will disappear, was not investigated.

For western hemlock, some information was obtained during the Fall of 1953, as shown in Figure 1, page 10. The cumulative germination curves for the individual trees are very similar in shape, but seed from the earlier collections starts to germinate later than that from later collections. For all collection dates tested, the germination capacity was high, being eighty percent or more. This high germination percentage could be obtained after careful separation of filled and empty seed. Before separation, about thirty percent of the seed was filled. This high germination for all collection dates is in contrast to the findings of Maki, Finnis and Crossley who found an increase in germination capacity with later collections. In the present study, western hemlock collections were started quite late, and by that time apparently most of the filled seeds were capable of germination. Were collections started early in August, results similar to those listed above would probably have been obtained.

The germination behaviour of seed of the individual trees does not suggest marked embryo dormancy. It is in several respects different from the behaviour of several hemlock seedlots received at this laboratory and which were used for the main study of this thesis. This seems to be the right place to make a closer comparison between these seedlots. To eliminate the differences between the final germination capacities for the different seedlots, the cumulative germination curves are transformed as follows: The final germination percent is set at 100 and each intermediate germination count is expressed as a percentage of the final germination capacity. In this manner a set of curves is obtained which all reach the 100 percent level and therefore allow a direct comparison for germination behaviour. The results are shown in Figure 2.

The four seedlots used for this study (4907, 4908, 5101, 5102) form quite distinctly a separate group from trees no's 1, 2 and 3. The latter group shows a steeper slope, which means a higher rate of germination. Tree 1, which shows a still faster and earlier germination than the others, must be regarded as an exception.

The history of the seven seedlots pictured above is, however, very different. Seedlots 4907 and 4908 represent the 1949 crop. Since 1950, this seed has been in cold storage (32°F). Lot 4907 is of low level, 4908 of high level origin. Seedlots 5101 and 5102 represent the 1951 crop. This seed has been in cold storage since 1952. These four seedlots have all been processed commercially; in other words, have been subjected to a series

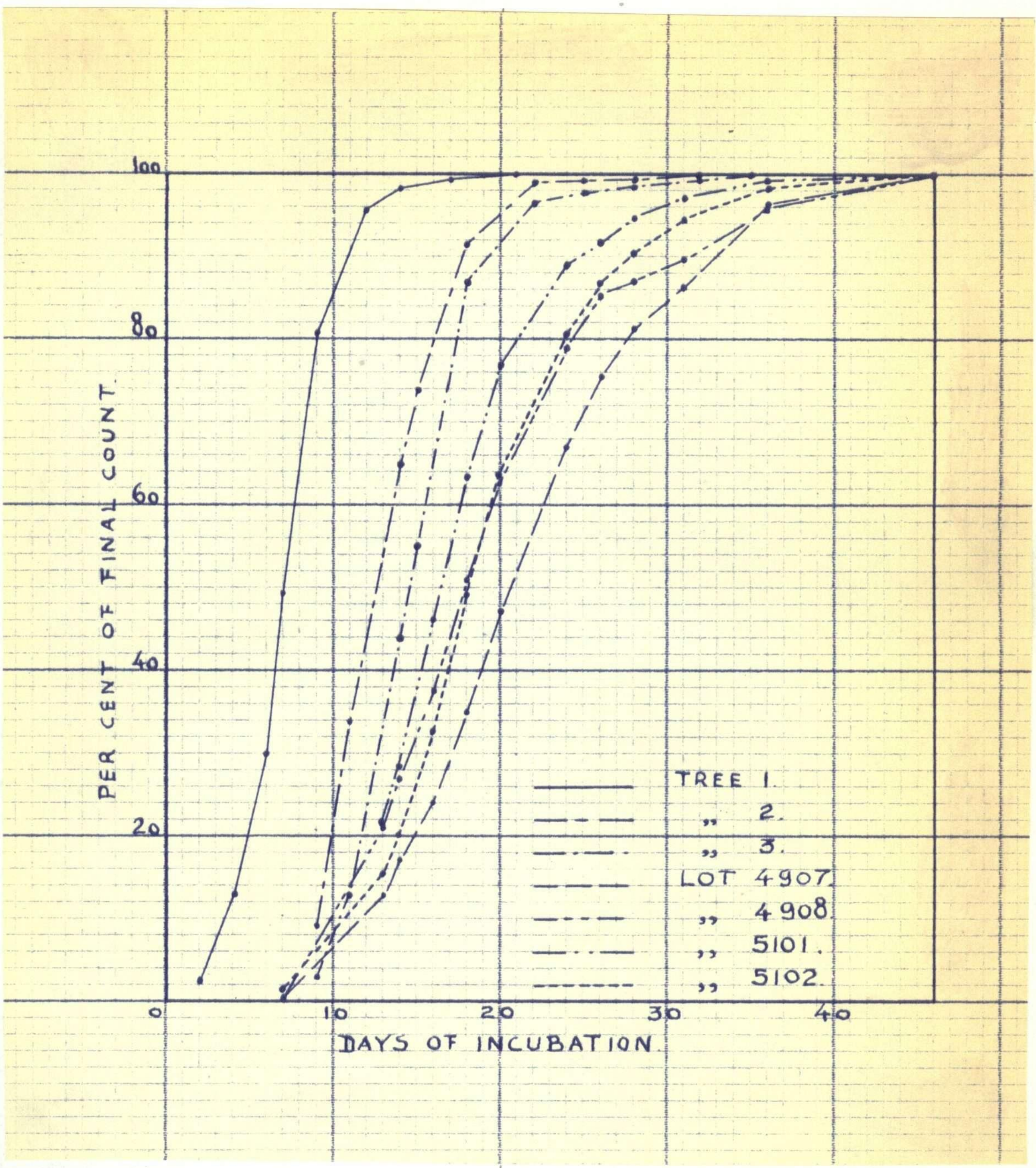


Figure 2: Germination behaviour of western hemlock seed-lots 4907, 4908, 5101, 5102 and germination behaviour of seed collected from trees 1, 2 and 3, University Forest, Haney, B. C.

of treatments about which virtually nothing is known.

About the seed from trees 1, 2 and 3 on the other hand, much more is known. In the first place, these seed lots represent seed from a single tree each, whereas the four previously discussed seedlots are mixtures from different trees. Locality, collection date and treatments are fully known. We know that this seed has been handled as gently as is practically possible. Cones were dried at room temperature and dewinging was done by hand. Until being tested this seed was stored at room temperature in open glass containers.

It is thus obvious that the history of these seedlots is quite different. Somewhere in this history some factor, or, more probably, combination of factors was responsible for the differences in germination behaviour. To date these factors have not been identified. They might be one or more of a number, such as source, collection date, processing, (which in itself is a complex of factors), storage influences, ageing influences, etc., to mention only a few possibilities. Hereditary characteristics may also play a certain role, as seems to be the case in tree 1.

It must be stated here, that the comparison as shown in Figure 2 is not completely true. The different seedlots were not tested at the same time. But the germination conditions were kept as comparable as possible with the available equipment.

5. Differences in Processing

The seed extraction process can roughly be divided into the following ten steps or phases:

1. Collection of fresh cones in the forest.
2. Transportation of cones to the extractory.
3. Preliminary cone storage at the extractory.

4. Kiln-drying period.
5. Actual extraction.
6. Dewinging of the seed.
7. Cleaning of the seed.
8. Preliminary storage of the processed seed.
9. Cold storage of same seed.
10. Handling and packing of seed prior to shipment to the purchaser.

The effects of the different processing steps on seed viability and germination behaviour are only very incompletely known.

The available information about the influence of the date of cone collection on germination behaviour has been discussed before. Some information is available about the effect of cone storage on seed germination. Huss (1950) showed clearly in several investigations the detrimental effect on seed viability of improper cone storage. Moist cones must be allowed to dry out promptly in order to prevent heating, moulding of cones, and subsequent loss of viability. Storing cones in piles, where the inner part of the pile dries only very slowly for lack of aeration, is an especially harmful procedure.

The question of moulding of cones comes up at the same time. Does mould on cones affect the quality of the seed within these cones? Huss (1950) reports that mildew on pine cones appears to have no noticeable influence on the germination capacity of the seed. Toumey and Korstian (1931) reported, that the moulding of green cones seems to injure the seed but little, but makes extraction more difficult. A small scale experiment undertaken at the University of British Columbia (1952, unpub.) comparing the germination behaviour of Douglas fir seed from mouldy and mould-free cones, did not give conclusive results.

Relatively little is known about the kiln-drying process. Kiln temperatures are usually higher than the outside temperatures which occur during the period of seed dispersal. Therefore, this procedure must be regarded as unnatural, and the lowest temperatures that will produce the desired results should be used. Cones from different species have different drying requirements. Pine cones are often hard to open and require prolonged drying at rather high temperatures (130°-140°F.). Spruce and western hemlock cones on the other hand open easily at lower temperatures (70°-80°F.). In some cases, however, cones will only open up completely after alternate wetting and drying. The influence of these different kiln treatments on germination behaviour should be thoroughly investigated.

When trying to evaluate the influence of kiln drying on seed germination, it must be kept in mind, that the heat employed serves two purposes. First, heat reduces the relative humidity of the air passing over the cones to remove moisture and cause them to open. In the second place, heat reduces the seed moisture content to a point where seed can be stored successfully over longer periods. Thus, a combination of two factors influences the seed during kiln treatment; heating and drying. Several investigations have shown that a close relationship exists between the effects of temperature and moisture on seed viability. Willis (1917) carried out numerous kiln-drying experiments on Douglas fir and concluded: "if a cone is wet, or if the relative humidity of the air is high, an air temperature which otherwise would be harmless to seed may prove exceedingly dangerous".

The use of a gradually rising temperature seems advisable, especially when drying green cones. Pre-curing at air temperature of freshly harvested cones also is desirable. It decreases the danger attached to storage of green cones and the kiln-drying process. Partly dry cones are less sensitive to higher temperatures, and shorter kiln-periods can be employed.

Kiln-drying must be regarded as a critical phase in seed processing. When carried out carelessly, serious losses in seed viability may occur.

The influence of the actual extraction of seed from the cones on the germination behaviour is almost completely unknown. In general this treatment, mostly carried out in a cone-tumbler, is regarded as harmless to seed quality. Recent investigations in Sweden by Huss (1950), however, have shown that repeated weak blows or shocks to seed can lower the seed quality. Some of the treatments used by Huss were: throwing cones on the floor or against a stone wall; throwing loose seed against a wall or into a wooden box; rotating loose seed for different lengths of time in tin and glass containers. He found that losses in seed viability in the case of pine and fir seed occurred as a result of all these treatments. In the cone-tumbler, seed and cones are subjected to a similar treatment and therefore it seems advisable to investigate the influence of this kind of treatment on the germination behaviour of our North American tree seed species.

Dewinging is probably the most harmful phase of seed processing. It was again Huss (1950) who studied this phase intensively. Schmidt and Hildebrandt (1930) and Eliason and Heit (1940) report earlier on dewinging damage to seed. Huss investigated the influence of several types of rotary dewingers on the seed viability of Sweden's three main coniferous species, Pinus sylvestris, Abies alba and Picea excelsa. He summarizes the results of his investigations in the following six points:

1. A dewinger with a greater speed of rotation causes the germination capacity to decrease faster than one with a lower speed.
2. A considerable quantity of wing residue gives the seed a certain protection in the dewinger.

3. Under the same dewinging conditions, seed of different species shows a difference in resistance to injury.
4. The germination capacity seems to decrease faster in the beginning of the dewinging period than at the end.
5. Microscopically, no damage could be found on the embryo and the endosperm.
6. Embryos which had lost their ability to develop as a result of a certain treatment, did not react to staining with Triphenyl Tetraxolium Bromide (Grodex). They must be considered unable to germinate normally.

Although it is not directly related to the subject of this study, it seems important to mention the effect of dewinging on the subsequent seedling development. Huss extended his germination experiments into the nursery and found that:

1. Seedling height decreases with increasing roughness of handling of the seed.
2. Seed, which passes through certain types of dewingers without apparent loss to its germination capacity, produces seedlings of smaller size than those produced by the hand-dewinged controls.
3. The reduction in "plant percent" caused by mechanical dewinging is three to five times higher than the reduction in "germination percent".

6. Storage Conditions

Experimentation and experience to date have shown that favourable storage conditions for coniferous tree seed are: low temperature and a constant low seed moisture content. The literature about this subject is fairly extensive and no attempts will be made to review it. Only the most important points concerning storage and its influence on seed quality will be reviewed.

(a) Storage temperature

Low temperatures retard respiration and biochemical processes of the seed and therefore help to prolong the lifespan. How much the temperature must be lowered for most successful storage is still an open question. Barton (1953) showed that a temperature of -4°C. is superior to 5°C. At the University of British Columbia (Allen, 1949-53) it was shown that a storage temperature of 0°F. was superior to 32°F. in several cases, but the results were not consistent. Whether or not still lower temperatures can be used to advantage, will depend on the advances in refrigeration techniques.

(b) Seed moisture content

Constant low seed moisture content can be maintained by the use of sealed containers. Storage in sealed containers is general practice today, and at any temperature shows better results than storage in open containers. Barton (1953) conducted a storage experiment on several coniferous tree seeds. For three different species of Abies (A. grandis, A. nobilis, A. magnifica shastensis) a seed moisture content of eleven percent seems to be the most favourable. Higher and lower moisture contents tend to decrease the lifespan in storage. A great variation in the germination capacity from year to year was apparent, however, which makes the results less conclusive.

For Pinus echinata and P. taeda Barton showed the favourable effect of storage in sealed containers under reduced air pressure. The seed moisture content was described as "air dry". Open containers invariably gave the poorest results.

Apparently air humidity (in open containers) or seed moisture content (in sealed storage) is more important than the storage temperature. However, in practice, only the proper combinations of seed moisture content and temperature are likely to result in successful storage. Whether dormancy is at all affected by cold storage is as yet unknown.

7. Seasonal Influences on Germination.

In nature, coniferous seed usually germinate in the Spring. The effect of this fixed habit is possibly retained when seed is stored over a longer period. Schmidt (1930) tested *Pinus sylvestris* seed of the same seedlot at monthly intervals, and reports especially rapid germination in the Spring. In Summer and Fall, the germination energy decreased, but increased again the following Spring. Baldwin (1935) carried out a similar experiment on *Picea rubra* and reports a slight increase in germination rate in the Spring. The observation period, however, was too short to permit a definite conclusion.

In many cases, the influence of a longer photo-period, light intensity and higher temperatures may have caused these results. But under controlled conditions, where external seasonal influences could hardly have been a factor, the same behaviour was observed. It is doubtful, however, whether photo-period was included in the controlled conditions. If not, this may have caused the observed behaviour. Schmidt (1929) also observed an increase of catalase activity each Spring. The above observed phenomenon may be of importance in cases where non-stratified seed is tested at various times of the year. Stratification of the seed probably eliminates this variation.

THE GERMINATION TEST

In the previous chapter, the emphasis has been laid upon the variability in germination behaviour between seedlots of the same species. Known and suspected causes for this variability have been discussed, but it is evident that very little reliable information is available.

It was also indicated that within a particular seed lot a considerable variability exists in the behaviour of the individual seeds. Some seeds will germinate shortly after incubation, some will need from several weeks to several months of incubation before they germinate, and some seeds will not germinate at all, even though they retain their viability throughout the incubation period.

Incubation temperature will have a very pronounced effect on this germination behaviour. For each species the optimum incubation temperature or, in the case of alternating temperatures the optimum range, must be determined. Much work has been done in this field and no attempt will here be made to review the available information.

But even under the most favourable germination conditions, a long period may elapse before all seeds capable of germination will have germinated. For testing purposes a long incubation period is very undesirable for the following reasons:

a. Constant attention of the seed analyst is required to maintain the optimum germination conditions throughout the test period.

b. Development of mould is favoured.

c. Germination is often incomplete.

d. Statistical analysis of data is of no value, when complete germination has not been obtained.

e. Results are often needed as soon as possible.

Moist-Cold Pretreatment

No treatment has proven to be as successful in reducing the length of the incubation period as the moist-cold pretreatment or "stratification". This means that the variation in germination behaviour among the individual seeds is reduced. This pretreatment also reduces the variation in germination behaviour between different lots of seed. In the north temperate zone, nature regularly provides the seeds with this treatment. They fall to the ground in the Autumn and are kept moist and cold over winter by a snow mulch, moist leaves, etc. But, several species do not always shed all their seed in the Autumn and dissemination may be distributed over a period of several months or longer. Western hemlock is one of these species. Although seeds which fall to the forest floor in the Autumn obviously are subjected to a natural stratification treatment, the question arises as to whether or not seed retained in the cones over winter are subjected to natural stratification. A positive answer can not be given at the present time. The following observations on cone moisture content of hemlock however, may lead to the speculation that seed, retained in the cone during the winter, is also subjected to natural stratification. Up to October 1st, cone moisture content was high (approximately 100%). Between October 1st and 7th, the moisture content fell sharply (to 40-60%) and afterwards decreased slowly but steadily until the middle of November (to 20-40%). After this date the moisture content was entirely dependent on weather conditions and at one time went up as high as 95 percent again. This may lead to the conclusion that the moist cone provides the seed with enough water to effect stratification. At present, this is merely a hypothesis. It would be of interest to investigate this problem thoroughly. On the other hand, this problem does not seem to be of great practical importance, as only a relatively small number of seeds remain in the cone all winter.

The occurrence of a stratification period in nature poses at once the question as to whether or not the moist-cold pretreatment should be an integral part of the standard germination test. It seems logical to include stratification in the germination test of those species of seeds which are known to be subjected to a natural stratification treatment. A study of the dissemination behaviour of the different species is therefore desirable. Abies species, whose cones disintegrate during the Autumn, present a clear cut situation. All seed falls to the forest floor and is subjected to a moist-cold treatment during the winter. And, in general, Abies species respond well to stratification. The coastal form of Douglas fir may show a long dissemination period, somewhat similar to western hemlock, but usually has shed most of its seed by December. This species reacts very well to stratification. Non-stratified seed on the contrary often germinates slowly. The interior form of Douglas fir, on the other hand, does not need stratification, but the rate of germination is increased markedly by it and no injurious effects have been noticed. For many of the pine species, a reliable germination test is impossible without stratification.

Mirov (1936) studied the germination behaviour of seeds of California conifers and their reaction to stratification. His experience indicates that although seed of Jeffrey, ponderosa, lodgepole, Monterey, and some other pines can be germinated well without stratification, stratification almost always effected a higher and more rapid germination. A large group of the Californian conifers produce seed, however, that does not germinate normally unless stratified. Even seed from conifers growing in the warmer parts of this continent (Southern-pine region) responds to the stratification treatment. Mirov concludes that it is better to stratify all coniferous seeds for testing purposes than to use non-stratified material.

Some lots of western hemlock germinate quite rapidly without stratification; other lots, however, germinate slowly when not stratified, and long stratification periods (2-3 months) are required to obtain rapid germination.

Although much work remains to be done in this particular field, experience indicates that a moist-cold pretreatment is desirable for seed that is subjected to similar conditions in nature prior to germination.

The Effect of Stratification on Temperature Requirements

Moist-cold pretreatment influences the temperature requirements for germination in many cases. Stratification reduces the minimum temperature for germination. At the University of British Columbia, it was observed that after long stratification periods (3 months and more) some seeds of coastal Douglas fir and western hemlock would germinate at a temperature as low as 33°F. Rohmeder (1951) obtained the best germination results for *Sorbus aucuparia* by keeping the seed moist at 4°C. for a period of twenty-three months. After this period, ninety-two percent of the seed had germinated at this low temperature. At the University of British Columbia (1953), the following experiment was carried out on coastal Douglas fir seed. Three different incubation temperatures were used;

1. first three days of incubation at 30°C., followed by 25°C. for the remainder of the period (30/25).
2. 25°C. throughout the incubation period.
3. 20°C. throughout the incubation period.

The seed was stratified for two different periods of time, one week and six weeks. After one week of stratification, the best germination was obtained by the 30/25 temperature combination. The second best germination was obtained at 25°C. throughout and poorest germination was obtained with the 20°C. temperature. After six weeks of stratification, however, all three incubation temperatures produced practically identical germination curves. The three temperatures were equally good under those conditions. Some Douglas fir seedlots show this indifference toward incubation temperature even after one week of stratification. In determining optimum germination temperatures, it must be clearly indicated whether they are for stratified or non-stratified seed.

Allen (1941) showed that Douglas fir seed, stratified for fifty days, was indifferent to germination temperature, within the range used. (20°C. to 35°C.) For non-stratified seed, however, temperature had a very pronounced effect on germination behaviour.

These two effects, increased in sensitivity to temperature and increased germination rate, brought about by moist-cold pretreatment, are of definite advantage in the development of standard germination tests. Probably not all coniferous seed will react in the same way to stratification. Each species should be investigated separately in this respect.

For western hemlock, Allen (1941) found also an increased indifference toward germination temperatures with stratified seed. The effect is less pronounced, however, than for Douglas fir.

<u>The Effect of Stratification on the Light Requirements</u>

It is a well-known fact that the germination of some seeds is favoured by light; other seeds are favoured by darkness, and a third group seems to be indifferent to light. Much attention was paid to the light factor several years ago and a considerable amount of literature concerning this subject was published. No attempt will be made to cover this phase of seed research here; only the possible connection between stratification and the light factor will be briefly discussed.

Allen (1941) reports that new seed of coastal Douglas fir (unstratified!) shows a definite response to light exposure. Seed germinating in darkness attains the same germination percent as those germinating in light only after exposing to light for a few minutes each day. Old seed of Douglas fir from Colorado shows no response to light treatment. This may be due either to seed origin or seed age. Evidence suggests that the light sensitiveness decreases with increased seed age. Nordstrom (1953) reports the same influence of seed age on light sensitiveness for *Pinus silvestris.*

For unstratified western hemlock seed Allen (1941) reports that, during the first thirty days this species germinates best in darkness. At the end of forty days, however, germination in both light and darkness attain the same end result.

Allen (1941) also showed for unstratified Douglas fir seed that, under certain conditions, temperature has a much greater influence upon the rate of germination and the total germination percent reached than has exposure to light. Exposure to light has little or no effect upon the germination at low temperatures (15° to 27°C.) but has a significant effect at high temperatures (15° to 38°C.). A close interrelation between the effects of light and temperature is thus indicated with temperature generally being more limiting in the case of unstratified Douglas fir seed.

In many instances, stratification seems to affect germination in the same way as a light treatment. Stratification increases the germination rate of Douglas fir seed markedly. After stratification, this seed does not respond to light anymore. The need for light seems to be eliminated by stratification.

The germination of unstratified western hemlock seed is apparently favoured by darkness as shown above. Stratification favours hemlock seed germination to a much greater extent than darkness.

Thus it appears that light can either increase or retard the rate of germination. Stratification, however, always increases the germination rate of the coniferous seeds that have been investigated.

When investigating the influence of light or darkness on germination behaviour, the investigator must be absolutely certain that the only difference in germination conditions is the presence or absence of light. But this presence or absence of light very easily changes the temperature conditions. Differences in germination behaviour then may be the result of temperature rather than light. This complication has not always been recognized and in many of the older investigations, differences attributed to light may have been related to temperature influences.

Methods of Stratification

The term "stratification" originated from the old practice of layering seed in between a moisture-holding medium, such as sand or granulated peat. The low temperature was provided by burying the mixture outside during the winter months, or keeping it in a cold cellar. Later on, refrigerators were used to provide the low temperature and more attention was paid to the most effective temperature or temperature range for stratification. A temperature range of 0^{o}-$10^{o}C$. seems to be effective in most cases. When germination is tested in sand flats, a common practice has been to prepare the seed for germination and to place the moistened flat in the refrigerator for the required length of time. All these methods have the disadvantage, however, of the considerable bulk of the moisture-holding medium. Allen (unpub.) stratified seed in moistened filter paper envelopes, stacked on end in a glass jar. A considerable saving in space was thus obtained.

All the above mentioned methods, however, have a great disadvantage. The seed moisture content during the stratification period is not controlled. Allen suspected that differences in seed moisture content during stratification might account for part of the observed variation in germination behaviour. The following procedure was developed and tried to eliminate the medium during stratification. Seed was soaked for different periods of time to obtain different moisture contents. The seed was dried and then refrigerated without a moisture-holding medium. This method is referred to as "naked stratification". Stratification containers are tin cans, small glass bottles or vials. The containers are covered in such a way as to provide aeration. In the refrigerator, however, a gradual drying out of the seed takes place. The degree of drying depends on various factors, such as size and shape of container, amount of aeration provided, and the amount of seed in a container. For Douglas fir, the most effective range of seed moisture content proved to be sixty to seventy percent initially, decreasing to thirty to forty percent over a six-week stratification period. An initial moisture content of sixty to seventy percent was reached after soaking at room temperature for about thirty hours. Longer soaking periods seem to be harmful to Douglas fir seed, although there are indications that this setback is at least partly eliminated by longer stratification. A constant seed moisture content during stratification, somewhere within the range of the values mentioned above, would probably be equally effective. No reliable method has as yet been worked out, however, for maintaining seed moisture content at a predetermined level throughout the stratification period. The effect of seed moisture content during stratification on the germination behaviour of western hemlock seed will be discussed in the next section.

The experiments so far carried out on Douglas fir seed have shown a great influence of seed moisture content during stratification upon the subsequent germination behaviour. They have shown the necessity of establishing the optimum moisture content or optimum range of moisture content in connection with the optimum stratification period, for each species. Without this information, standardization of the pretreatment is impossible.

Soaking Injury of Seeds

As the required initial seed moisture content is obtained by soaking the seed in tap water for a specified period, some of the observations made at the Boyce Thompson Institute on soaking injury of seeds will be related here. (Barton, 1952). Bean, corn and oat seeds were soaked at room temperature (21 to 26°C.) in tap water. In part of the experiment, the tap water was oxygenated, in another part carbon dioxide was passed through, and in the third part the soaking water was not aerated.

Oxygenated water showed a deleterious effect upon subsequent germination; carbon dioxide prevented injury in many cases and non-aerated water proved to be less injurious than oxygenated water. These findings indicate that any disorganization of the metabolism of the seed resulting from soaking is neither due to a deficiency in the oxygen supply, nor to an accumulation of carbon dioxide, two explanations of soaking injury given by previous workers. A striking reduction in the rate of moisture absorbtion by seeds under treatment with carbon dioxide was observed. This indicated the possibility of a direct relationship between the amount of moisture absorbed and injury from soaking.

Polyvinyl pyrrolidone (PVP), a synthetic polymer, which has been used as a retardent for drug effects and which had been reported to retard or inhibit absorption of water by branches of eucalyptus and lilac, was used in subsequent experiments at the Boyce Thompson Institute to reduce the moisture absorption by the seeds. PVP in five and ten percent solutions reduced soaking injury in the presence of oxygen, but had no effect in the presence of carbon dioxide. In the first case moisture absorption was markedly reduced; in the second case (CO_2) the moisture absorption was practically unchanged, regardless of the concentration of PVP, and the germination percentages also remained unchanged.

These experiments indicate the importance of seed moisture content and may have some relation to the influence of seed moisture content on the effectiveness of stratification. It is far too early, however, to draw any conclusions in this respect, as the described experiments were carried out on seed very different from coniferous seed. But it may be worthwhile to keep the above mentioned results in mind when the influence of seed moisture content on the effectiveness of stratification is further investigated.

Bio-chemical Changes During Stratification

Very little is known definitely about the changes taking place during stratification in coniferous seeds. According to their behaviour, coniferous seed can be classified as seed with a non-dormant embryo, which is benefftted by stratification. (Crocker, 1948). According to the nature of the reserve foods, they are classified as seeds high in fatty and oily substances (5-20% starch; 50-80% fats; 15-30% proteins) (Crocker and Barton, 1953). In seeds with dormant embryos, during stratification, both hydrolitic and oxidizing enzymes are formed or activated; sugars, amino acids and other soluble organic compounds are formed from more complex and less soluble compounds. Perhaps similar changes take place in coniferous seed. Low temperatures in plants in general lead to the formation of soluble sugars and other soluble substances (Crocker, 1948). Likewise, it is possible that changes occur in the seed coats. Stratification also gives favourable circumstances for the leaching of inhibiting substances. In this respect it is interesting to note that Rohmeder (1952) found that turpentine extracted from Abies alba seed inhibited germination of European spruce seed. He also found that juices of many fleshy fruits inhibited the germination of spruce and pine seed and also of the seed within the fruits. In the case of Abies, it is possible that the inhibiting effect of turpentine is removed by either leaching or volatilizing or a combination of both. On the other hand, there are good reasons to oppose the hypothesis of leaching of inhibitors. The optimum temperature range for stratification is rather narrow (0-10°C.) and low. Leaching will also take place at higher temperatures, probably even at a higher rate. But stratification is not effective at these higher temperatures. If leaching of inhibitors is a factor in stratification, it does not seem to be one of very great importance.

Pack (1921, 1925) (Crocker and Barton, 1953) made a rather thorough study of the changes occurring during stratification and early germination of several Juniperus species. The pH of the embryo was 8.4 to 8.8 and changed to 4.4 to 6.0 after 90 days of stratification. Titratable acids measurably increased during stratification and markedly increased after germination. Fatty substances decreased and at the same time carbohydrates increased. Amino acids increased markedly with stratification and still more during germination, showing a breakdown of the proteins. Catalase increased during stratification and was a good measure of the progress of after-ripening. Other investigators, however, found catalase in rather high quantities even in dead seeds. Stratification of Juniperus seed led to the accumulation of more soluble and more readily available compounds for the nutrition of the embryo.

The role of growth hormones in relation to promptness of germination was studied by several authors. The results, however, were quite conflicting. Haddock (1942) found no correlation between the auxin content and promptness of germination of seeds of Pinus jeffreyi and P. lambertiana. Also stratification did not modify the auxin content. Auxin content was higher in immature than in mature seeds and increased in the embryo and endosperm during the early phases of germination.

EXISTING INFORMATION ABOUT DISTRIBUTION, SEEDING HABITS AND GERMINATION CHARACTERISTICS OF WESTERN HEMLOCK

Western hemlock was chosen as the object of this study for the following reasons:

1. It is often difficult to obtain a rapid and reliable germination test in the laboratory.
2. It is a species of growing importance for lumber and pulpwood in the Pacific Northwest.

Before describing the actual experiment, the distribution, seeding habits and previous experiences with this species will be briefly discussed.

Distribution

The genus Tsuga comprises about ten species that are native to the temperate portions of eastern and western North America, Japan, Formosa, China and the Himalayan Mountains. Four species occur in North America, of which three are found in Canada. Two species, western hemlock

Tsuga heterophylla (Raf, Sarg.) and mountain hemlock T. Mertensiana (Bong. Carr.) are confined to parts of British Columbia and northwestern United States. Eastern hemlock, T. canadensis (L. Carr.) ranges from Cape Breton Island westward to Lake Superior. T. caroliniana occurs in the southeastern United States.

Western hemlock occurs from Northern California to Alaska in a narrow strip along the Pacific Coast on the humid western slopes. It is native also to the interior wet belt, which includes parts of British Columbia, Washington, Idaho and Montana.

Seeding Habits

Male and female strobili are borne on the same or on different branches of the same tree. The male strobili are placed axillary or terminally along previous year's shoots, singly or in groups; small female strobili, which develop into cones, are borne at the end of the previous year's lateral shoots. The small pendulus cones, 3/4 to 1-inch long, ripen during the first year, but remain on the tree after release of the seed for approximately another year. The cone colour changes from yellowish green to purplish or brown when it ripens. Each cone scale bears two seeds, but only the seeds in about the central half of the cone are fertile (Woody Plant Seed Manual, 1948). Hemlock seeds are brown, ovate, oblong, compressed and nearly surrounded by their much longer wings. They are dispersed by wind.

Western hemlock is a fairly constant and prolific seed producer. Its seed germinates well on almost any kind of seedbed, but particularly under shade on moist decaying logs, stumps or mineral soil. Survival of seedlings is high, even in a dense forest, if moisture is abundant. Exposed

Figure 3: Range map for western hemlock
(U.S.D.A. Misc. Pub. 287, 1938)

south slopes are unfavourable to both seed germination and seedling survival (Westveld, 1949).

Garman et al report on the seasonal distribution of seed dispersal for western hemlock. Garman (1951) reports that although seed dispersal begins early in September, most of the seed is shed during the next March and April and trails along until about August. Pickford (1929), however, reports the greatest seed fall up to the end of January, with a sharp drop from then on. However, Pickford agrees with Garman that the total amount of seed is shed over about a twelve-month period. It seems probable that the weather conditions during late summer, fall and winter will influence the distribution. Good weather will effect earlier cone opening and greater seed dispersal during the autumn. Rainy weather will retard cone opening and seed dispersal. Dry, cold weather during the winter may result in the release of some seed. The remainder of the seed will be released during the spring and early summer. Pickford (1929) states that an appreciable percentage of hemlock seed is distributed in early spring. The distribution pattern probably changes from year to year, in connection with the weather conditions.

This question of seed dispersal was briefly touched before, in connection with the discussion of "natural stratification" of the seed. In the case of western hemlock seed, with its long period of dispersal, possible effect on germination behaviour of over-wintering in the cone can not be neglected. A rather large proportion of the seed may over-winter this way and will thus not be subjected to moist-cold treatment on the forest floor.

Previous Experiences with the Testing of Hemlock Seed

Information on the testing of western hemlock seed is very scarce.

More information is available for eastern hemlock, however, and a review of this may be of value.

The Woody Plant Seed Manual (1948) provides the following information: "Although seed of Tsuga heterophylla usually germinates well without stratification, some lots need pretreatment. A stratification period of ninety days (in moist sand at 41°F.) is recommended for this species and for T. Mertensiana." As for germination temperatures, the Manual gives these recommendations: "68°F. (night) to 86°F. (day) for T. canadensis and T. Mertensiana; 52° to 60°F. for T. heterophylla."

Heit and Eliason (1940) experienced their greatest difficulties with eastern hemlock. Even stratified seed did not give satisfactory results. They suggest, that a cool temperature, 15° to 18°C. may give better results than any higher temperature. The seed also appears to be sensitive to excessive moisture during germination.

Baldwin (1930) secured better germination after stratification for 1 to 1½ months and with abundant moisture available to the germinating seeds. But he also mentions the failure of some lots to germinate properly, even after prolonged periods of stratification.

The influences of light, temperature and stratification on the germination behaviour of western hemlock seed, reported by Allen (1941) were discussed earlier.

In 1952, a temperature experiment was carried out on western hemlock seed at the University of British Columbia (Allen, 1949-53). The two seedlots used were also included in the main study of this paper. The following incubation temperatures were used:

1. 25°C. Constant.
2. Alternating temperatures with a maximum of 25°C. (8 hours)

and a minimum of approximately 18°C. (16 hours).

3. 20°C. Constant.

All seed was stratified for six weeks in filter paper envelopes at 33°F.

The best results were obtained with the 20°C. incubation temperature. Alternating temperatures resulted in a lower rate of germination and Constant 25°C. gave the poorest results. In another experiment, seed stratified for six weeks was germinated at 15°C. Results obtained were poorer than at any of the three higher temperatures.

From the publications cited above, the following conclusions can be drawn:

1. Seed of western and eastern hemlock seems to be sensitive to temperature; the optimum being in the neighbourhood of 20°C.
2. Seed of western and eastern hemlock responds to stratification. Relatively long stratification periods are indicated.
3. Considerable differences in ease of germination seem to exist between different seedlots.

THE EXPERIMENTAL PROCEDURE

It is known that stratification and a rather low incubation temperature are favourable to the germination of western hemlock seed. In the following experiment, the effect of different incubation temperatures and of different seed moisture on the effectiveness of stratification are discussed. This section describes the experimental procedure followed.

The Seed

Four different lots of western hemlock seed were tested in the experiment. The numbers indicating the different seedlots consist of four digits; the first two indicating the year of collection, the last two indicating the order of arrival at the laboratory. In Table II the available information about the seedlots is given.

Table II: Information about seed used

Lot Number	Source and elevation	Company seed was obtained from	Crop Year
4907	Clatsop County, Oregon. 0-500 ft.	Crown Zellerbach Corporation.	1949
4908	Oregon, high elevation.	Manning Seed Co.	1949
5101	Sequim, Wash. 500-1000 ft.	Manning Seed Co.	1951
5102	Elma, Wash. 0-500 ft.	Manning Seed Co.	1951

Additional information, such as exact location of collection, time of collection, extraction procedure, storage conditions prior to shipment, etc. was not available. Upon arrival at the laboratory, the seed was put in cold storage in airtight glass containers at 33°F. Seed lots 4907 and 4908 have been in cold storage approximately two years longer than seed lots 5101 and 5102.

Sampling Procedure

Reduction of the seedlots to the proper sample size was done mechanically rather than by hand, in the hope that this would provide a reasonably representative sample. Figures 4 and 5 show respectively a section and an overall view of the seed sampler, built at the laboratory. The seed flows into the sampler through a glass funnel, is spread out when it hits the cone centred underneath the funnel stem, bounces back and forth on three layers of round wooden dowels and then is received in two identical

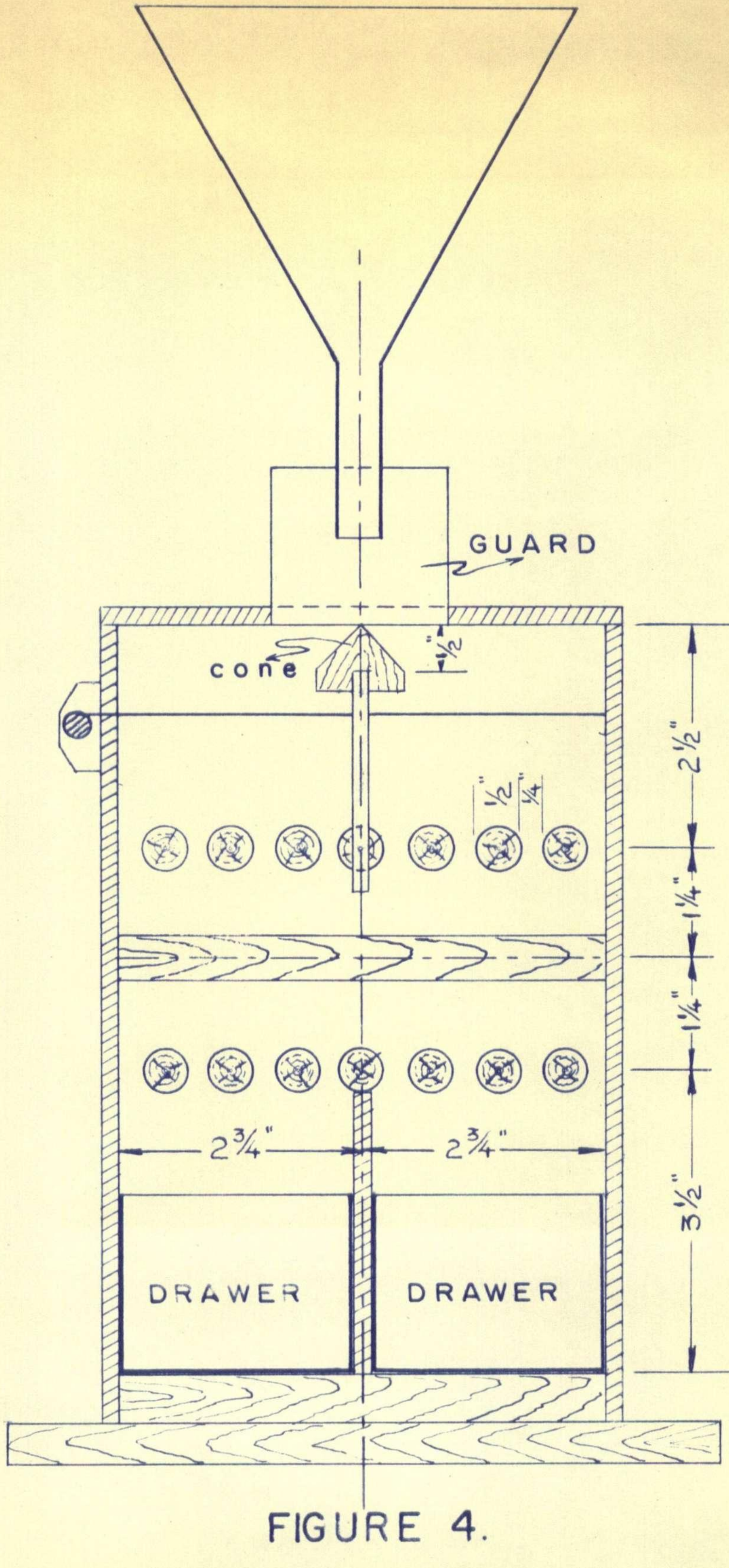


FIGURE 4.

SEED-SAMPLER

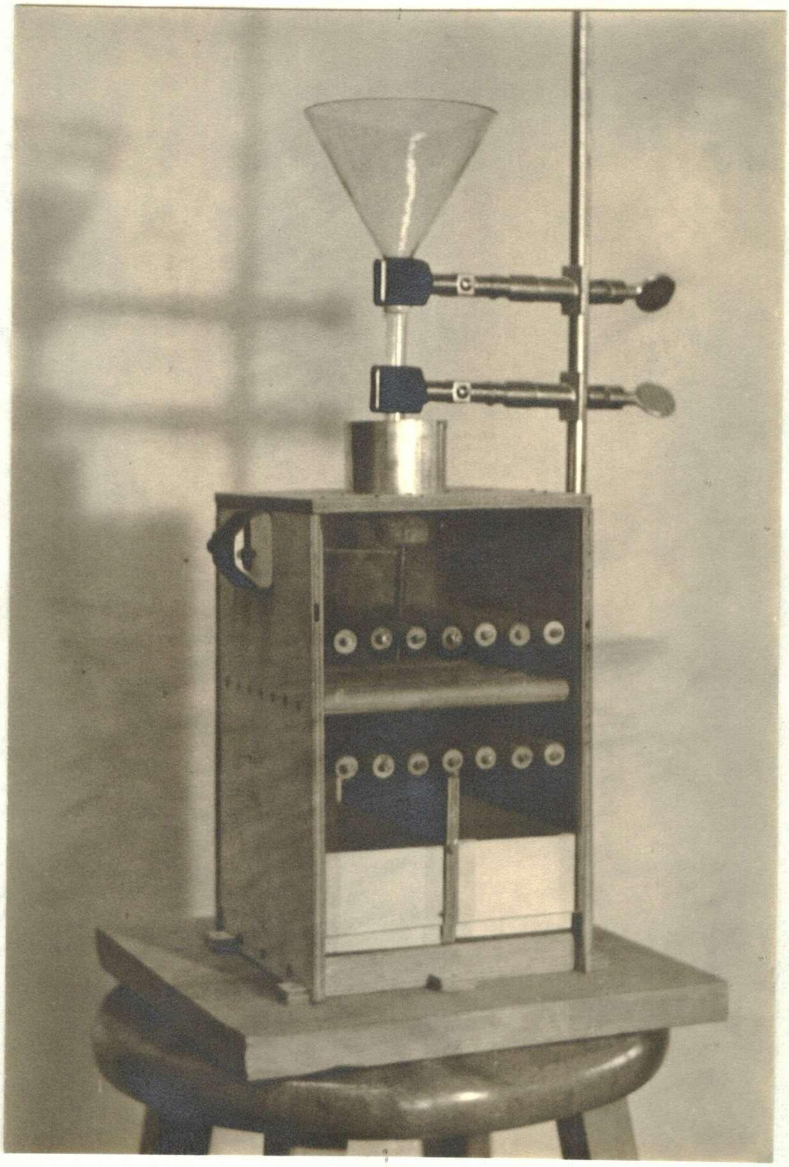


Figure 5: View of seed-sampler.

drawers. In this way the sample is divided in two halves each time the seed passes through the sampler. Alternately, seed from the drawer on the left hand side and right hand side is passed through the sampler again until the seedlot is reduced to a suitable sample size. The front wall of the sampler is made of plexiglass, which enables observation of the action inside.

Each sample thus obtained is then subjected to a standardized procedure to be described.

In the actual germination test, four replicates of 100 seeds each were used for each test. These replicates were counted out by hand from the mechanically obtained samples.

The Testing Procedure

The actual germination took place on vermiculite[1] in petri-dishes, 6 cm in diameter. A measured volume of vermiculite was placed in each dish and 10 cc of tapwater added. This amount of water will just saturate the vermiculite, without leaving free water. Each dish was then divided into two halves by means of a wax-coated cardboard strip. A sterilized linen pad was placed in each half on top of the vermiculite and pressed down to assure proper contact. The sole purpose of the linen pad was to make the small brown seeds more easily visible. In each half of the dish, 100 seeds were placed. On the dish edge three furnace chain links were placed to permit proper aeration. These links lifted the lid approximately 1/16 inch. Experience has shown that, if the dish sections are not separated, condensation water will sometimes seal the rim, prevent aeration and thus interfere with normal germination.

---

1. Trade name - "Terralite".

The Incubators

The petri dishes were placed in order of treatment on the incubator trays. Thelco electric incubators of the dry type with gravity convection (Model 2A) were used. In order to reduce evaporation from the moistened vermiculite, four tins filled with water were placed directly over the heating element inside the incubator. The incubator temperature was thermostatically controlled.

The Treatments

In order to study the effect of seed moisture content during stratification on the subsequent germination behaviour, the seed was brought to different moisture levels by soaking for different periods of time at room temperature. After surface drying, the seed was stratified without medium, so-called "naked stratification", in glass vials at 33°F. The vials were stoppered with cotton to permit limited aeration during the stratification period.

As an aid in deciding on the length of the different soaking periods, an exploratory test was made for the purpose of determining the rate of moisture absorption in relation to soaking time on seedlot 4907. A weighed amount of air-dry seed was soaked at laboratory temperature (21°-25°C.) for various periods of time and weighed again after surface drying on paper towels. Moisture content was calculated on ovendry weight basis, assuming that air-dry seed contained eight percent moisture. The results are shown in Table III and graphically presented in Figure 6.

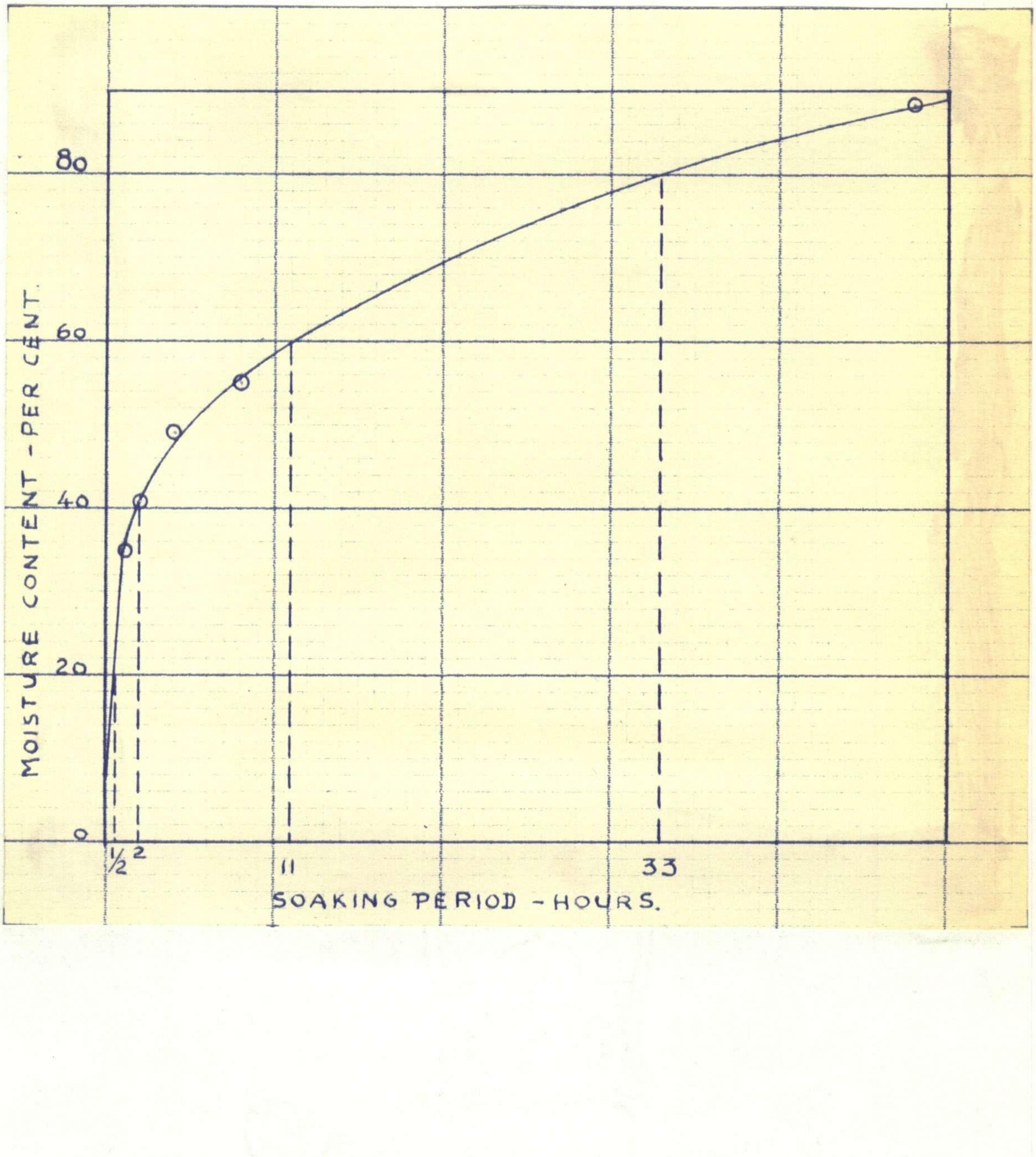


Figure 6: Rate of Moisture Absorption for Seedlot 4907

Table III

Rate of Moisture Absorption for Seedlot 4907.

Soaking time in hours	Moisture content in % (ovendry wt. basis)
0	8.0
1	35.0
2	40.8
4	49.1
8	55.1
48	88.5
72	93.2
96	100.0

From this graph, the soaking periods required to obtain moisture contents of 20, 40, 60 and 80 percent were read. These were respectively 1/2, 2, 11 and 33 hours. By using these soaking periods, it was hoped to attain a more or less evenly distributed range of seed moisture contents.

For the experiment, the four seed samples were soaked for the periods mentioned above, surface dried and weighed. The moisture contents obtained by the four seed lots are shown in Table IV and Figure 7.

Table IV

Seed Moisture Content After Soaking

Soaking period in hours	Moisture content in percent			
	4907	4908	5101	5102
0	7.4	8.5	6.9	7.1
1/2	29.6	23.0	23.2	22.1
2	39.1	26.5	26.1	30.8
11	56.8	36.5	37.1	41.8
33	75.0	49.5	53.0	57.6

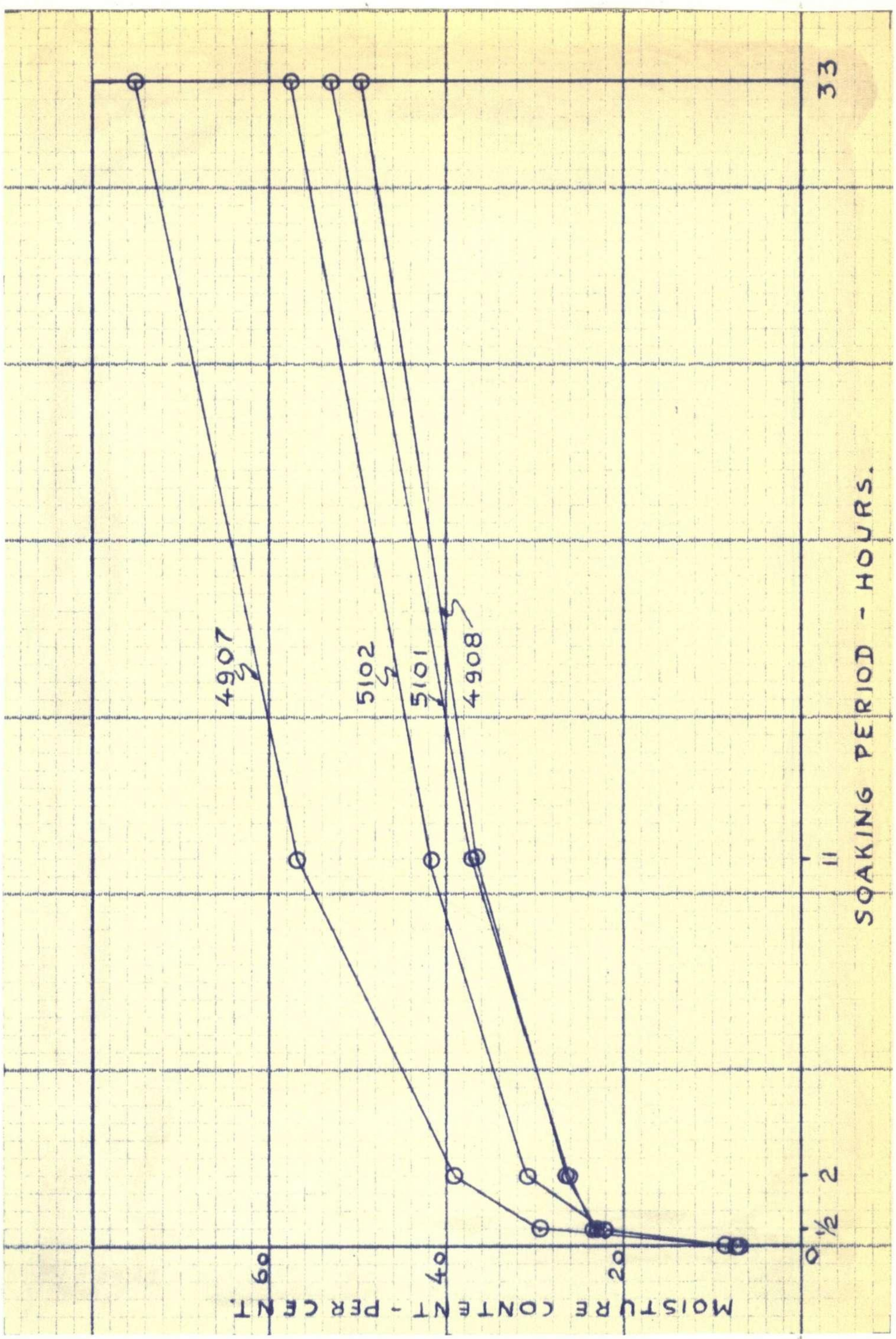


Figure 7: Rate of Moisture Absorption for Seedlots 4907, 4908, 5101 and 5102.

From Figure 7 it was obvious that the desired range of seed moisture content was not obtained, except for seedlot 4907. Unfortunately, this seedlot which was used for the preliminary determination of the rate of moisture absorption, showed a behaviour quite different from the other seedlots. Moisture absorption for 4908, 5101 and 5102 was considerably lower. Variation between the seedlots was already indicated.

After being soaked and surface dried, the seeds were stored in glass vials, three inches high and 7/8 inches in diameter. The necks of the vials were plugged with cotton. The vials were placed in the refrigerator in upright position.

The Stratification Periods

Since different authors have recommended different periods of stratification for a proper course of germination, it was decided to investigate four periods: two, four, six and eight weeks. Because of interference of other work, these periods had to be extended by one week, the actual periods being three, five, seven and nine weeks.

The Incubation Temperatures

The following germination temperatures were used:

1. 25°C. constant.
2. Alternating temperatures with a maximum of 25°C and a minimum of approximately 18°C.
3. 20°C. constant.

A constant temperature of 25°C was easily maintained with the available incubator. The alternating temperatures were obtained with the aid of a time switch, which turned on the current at 9 A.M. and switched it off at 5 P.M. The thermostat was set at 25°C. Between 11 A.M. and noon, the incubator would reach 25°C., remain at this temperature until 5 P.M. and then slowly drop during the night to laboratory temperature (approximately 18°C.). Radiators in the laboratory were turned off during the night. A constant temperature of 20°C. could not be obtained in the laboratory. Therefore, an incubator was placed in a cool, unheated storage room. Here a temperature of 20°C. was maintained without difficulty during the winter and early spring.

Temperature control in the incubators was not as accurate as it should have been. Fluctuations up to 3°C. were noticed.

Although a narrower temperature range would have been preferred in these experiments, for instance 22.5°, 20° and 17.5°C., it was felt that these could not be maintained with sufficient accuracy with the available equipment.

Counting the Germinating Seeds

Germination in the commonly accepted botanical use of the term has occurred when the radicle has protruded beyond the seed coat. (Toumey and Stevens, 1928). Not every such seed, however, will develop into a normal seedling; abnormalities occur regularly. One of the most common abnormalities is the inverted embryo. In this case the cotyledons instead of the radicle are oriented towards the micropyle. When such an embryo resumes growth, the cotyledons emerge first. The little root cannot establish itself and soon the seedling withers and dies. Another abnormality, quite regularly noticed in the course of the experiment, was a swollen, club-like radicle. These seedlings would die within a few days, as several trials to grow them indicated.

Cracking of the seed coat does not mean in all cases that the embryo will resume growth. Non-viable seed will absorb water and swell up. Sometimes the radicle will even protrude several millimeters, without the seed being viable. All of these abnormalities are termed "false germination".

A seed was considered germinated as soon as the radicle tip emerged, showed geotropic curvature and otherwise looked normal. By requiring geotropic curvature as one of the characteristics, the danger of including seeds showing "false germination" into the germination count, was largely eliminated. Presumably only normal radicles from viable seed exhibit geotropic curvature.

Germinated seedlings were counted at regular intervals and removed. Abnormal seedlings were not included in the count, but were separately noted. A test was considered completed if no germination occurred during six consecutive days. Otherwise the test was concluded after forty-two or, in some cases, forty-five days.

The Interpretation of the Results

There are many ways of expressing germination results. The most simple way is by means of the "apparent germination percent", which is the germination percent based on the total sample, including empty seed. (Baldwin, 1942).

The "real germination percent" is based on the number of filled sound seeds at the beginning of the test, not on the total number of seeds used. A final cutting test is required and the number of empty seeds is deducted from the total. This final cutting test, however, poses a serious problem. The apparently sound seeds that did not germinate are easily recognized upon cutting. But it is often hard to distinguish between empty seeds and seeds with decayed contents. Often the best one can do is to make a guess. But most serious of all is the problem of how to take the decayed seeds into account. When both empty and decayed seeds can be classified in the final cutting test, deduction of only the empty seeds from the total, as the definition indicates, may still be misleading. What was the condition at the start of the test of the seeds showing decay at the close of the test? It is impossible to find an answer to this question.

Calculating the real germination percent on the basis of the total number of seeds germinated plus the number of apparently sound seeds found by cutting after the test was finished, does not solve the problem either. The best solution seems to be to take a sample of the same size as used for germination and apply the cutting test on this sample. If the sample is large and sampling is done properly, it seems that in this way the closest estimate for the real germination percent can be obtained. For a sample of four hundred seeds, however, the results are probably not very reliable.

Due to the difficulties encountered with the final cutting test, the original idea of using "real germination" as the expression of the germination results, had to be abandoned. Not only was a proper classification into the different categories practically impossible, but the size of the experiment made this task too time consuming.

Graphs

The easiest and quickest way to compare germination behaviour of different seedlots or treatments is to compare this behaviour in curve form. The curves shown are cumulative curves, showing the total percent germination at the end of a given incubation period.

As mentioned before, four replicates of one hundred seeds were used for each test. The average germination percent from these replicates was used to plot the curves.

Controls and Treatments

Three variables were studied in this experiment:--germination temperature, seed moisture content (soaking period) and stratification period. Two types of controls were used:--dry controls and soaked controls.

For the dry controls, air-dry seed was incubated at the three temperatures without soaking and stratification. Temperature was the only variable in this case. The soaked controls were not stratified, but were brought to different moisture levels by soaking for the four different periods of time. Temperature and soaking period were the variables in this case. In the actual experiment, three variables were present for each seedlot tested.

The germination behaviour is thus the result of the interaction between temperature, seed moisture content during stratification, and stratification period. In order to obtain a measure for each variable separately, comparisons are made by keeping two variables constant and so evaluating the influence of the third variable, under the conditions supplied by the two constant ones.

STATISTICAL ANALYSIS OF DATA

The two main points of interest in a germination test are:

(a) the final germination percentage.

(b) the rate of germination.

The results of the experiment are tabulated in Table V for the final germination percentage. However, the final germination percentage could not be given in all cases. If after forty-two days of incubation germination was still in progress, the percent reached at forty-two days was considered to be the final germination percentage and the test was discontinued at this point.

The speed of germination was expressed as follows: The germination count at twelve days was expressed as a percent of the final germination count. If for instance the twelve-day count showed fifteen percent germination and the final count sixty percent, then the rate of germination

Table V: Apparent germination per cent after 42 days of incubation.

SEEDLOT NUMBER	SOAKING PERIOD IN HRS.	NO STR'N			3 WKS. STR'N			5 WKS. STR'N			7 WKS. STR'N			9 WKS. STR'N		
		25°C.	ALT.	20°C.	25°C.	ALT.	20°C.	25°C.	ALT.	20°C.	25°C.	ALT.	20°C.	25°C.	ALT.	20°C.
4907	1/2	48	57	64	52	69	68	51	55	67	46	61	67	51	56	61
	2	52	57	66	51	74	72	63	66	71	57	65	70	61	65	69
	11	48	58	64	62	65	75	64	71	71	66	70	73	68	66	66
	33	54	62	70	64	71	68	62	64	67	64	66	72	62	72	71
4908	1/2	50	54	57	52	54	54	46	52	51	41	48	50	50	52	55
	2	50	53	54	50	50	55	46	48	54	41	56	51	50	49	52
	11	57	53	62	55	57	55	51	60	56	50	54	53	51	56	54
	33	54	54	58	50	55	53	49	56	55	51	56	57	52	54	54
5101	1/2	78	78	81	81	84	84	79	83	84	72	76	82	78	78	85
	2	84	86	86	83	85	86	82	85	84	75	81	85	82	85	87
	11	86	88	88	84	87	88	82	88	86	81	89	88	80	85	87
	33	82	86	83	86	88	86	84	88	91	86	86	86	83	90	93
5102	1/2	73	80	82	70	74	78	70	74	73	69	73	76	67	76	77
	2	72	76	79	70	79	73	70	76	76	71	73	78	70	74	81
	11	76	78	78	74	77	75	74	70	77	69	81	78	76	74	80
	33	78	79	81	76	81	78	77	75	78	75	80	86	78	80	84

Non-soaked controls.

4907	-	40	62	66
4908	-	50	52	59
5101	-	79	83	85
5102	-	70	72	79

The percentages shown are means of four replicates.

Table VI: Rate of germination after 12 days of incubation.

SEEDLOT NUMBER	SOAKING PERIOD IN HRS.	NO STR'N			3 WKS. STR'N			5 WKS. STR'N			7 WKS. STR'N			9 WKS. STR'N		
		25°C.	ALT.	20°C.	25°C.	ALT.	20°C.	25°C.	ALT.	20°C.	25°C.	ALT.	20°C.	25°C.	ALT.	20°C.
4907	1/2	13	8	5	16	2	11	16	8	11	13	6	10	14	10	8
	2	13	7	9	30	10	19	24	18	21	29	14	22	13	13	22
	11	15	8	8	49	24	38	47	38	52	43	32	55	48	48	48
	33	22	12	17	59	24	50	51	47	64	68	58	80	67	63	80
4908	1/2	10	8	17	13	10	26	8	4	12	10	5	8	11	12	29
	2	8	11	8	19	11	31	6	6	24	17	8	15	12	13	32
	11	5	10	14	36	28	44	26	24	40	33	34	51	34	33	50
	33	14	12	18	55	38	60	51	47	59	51	55	70	67	63	68
5101	1/2	9	6	15	14	10	24	14	8	25	18	13	23	11	21	27
	2	5	10	16	14	9	22	10	13	25	16	18	30	17	20	36
	11	7	6	16	25	18	28	22	30	45	42	37	52	35	40	63
	33	9	13	21	52	49	75	52	66	86	74	77	80	68	77	81
5102	1/2	10	8	21	16	10	25	11	13	19	12	16	22	18	16	30
	2	7	9	20	15	14	32	10	13	25	18	24	35	18	25	43
	11	9	9	20	33	32	52	24	37	56	40	46	57	46	53	63
	33	20	19	33	53	50	81	55	72	80	70	80	90	72	72	88

Non-soaked controls.

4907	-	14	8	12
4908	-	15	9	20
5101	-	14	9	17
5102	-	15	11	15

The percentages shown are means of four replicates.

is expressed as $\frac{15}{60} \times 100 = 25\%$. This rate of germination is shown in Table VI.

The choice of the twelfth day of incubation for the expression of the germination rate is based upon the following considerations: A cummulative germination curve can be divided into three sections:

(a) The lower section, where germination starts and the germination rate gradually increases. This section curves upwards.

(b) The middle section, where the rate of germination has reached a maximum. This section is practically a straight line.

(c) The top section, where the rate of germination decreases gradually and where the slope of the line gradually approaches zero.

These parts can be recognized in most curves, representing a variety of conditions under which germination takes place. The middle section, representing the period of fastest germination, is the most representative part as far as germination rate is concerned. Somewhere along this section a point must be fixed to express germination rate. The chosen point, twelve days, lies in the beginning of this section for non-stratified seed and practically at the end for stratified seed. Therefore, twelve days is considered to be a reliable point to be used in the expression of germination rate.

In analyzing the experimental data, a slight complication occurs. The experimental results are expressed in percentages and a comparison of these proportions is required. Normally this should be carried out using the chi-square test. However, in an experiment of this size, the chi-square test cannot be applied easily. It is much more desirable to use the analysis of variance and the t-test to establish the significance of the experimental results. Such an analysis is, however, complicated by the differing accuracies of the percentages. A low percentage can be determined with greater accuracy than a medium percentage. The variance increases with the mean observed percentage, until fifty percent is reached. Between fifty and one hundred percent, a decrease of the variance takes place again. In other words, the mean and the variance are not independent of each other.

As a consequence of this changing accuracy it is necessary to employ a transformation to the percentages, in order to make the variance independent of the mean. This transformation is the $\sin.^{-1}\sqrt{p}$, i.e.the angle whose sine is the square root of the percentage. (Quenouille, 1950). This quantity is expressed in radians.

The transformation just described was applied to the experimental results and on these transformed data the analysis of Variance is applied.

An analysis of Variance, using all replicates in the experiment, would supply the most accurate information about the factors influencing germination behaviour. The experiment under discussion was carried out on 96,000 seeds and thus 960 replicates. An analysis of Variance on this number of data, carried out twice--once for the final germination percentage and once for the germination rate--would be very time consuming.

Therefore, it was considered to base the analysis of Variance on the <u>means</u> of four replicates, thus reducing the number of data to 240. Is this procedure justified, however? The analysis, based on means, shows an error term (highest-order interaction) of 8.28 and 7.90. (See Table VIII). For seedlot 4907, the error term was calculated using the transformed individual replicate values. The value of this error term was 34.44. To make this error term comparable to the one based on means, this value must be divided by 16. In this way, an error of 2.15 is obtained. Thus it is shown that the error, based on means, is approximately four times larger than the error based on individual replicate values. From Table VIII can be seen, however, that the influence of the single factors and their interactions can be shown to a 0.1 percent level of significance in most cases. This gives confidence that the followed procedure is correct and sufficiently accurate. A smaller error term would probably not yield any additional information.

The arc sine transformation for the mean of four replicate values (which are percentages) can be carried out in two different ways.

(a) The mean percentage, calculated as the arithmetic mean of four replicate values, is transformed to its arc. sine value..

(b) The value of each replicate is transformed and the arithmetic mean of the four transformed values calculated.

Slightly different values will be obtained by these methods. In order to decide which method is to be followed, both transformation methods were carried out on a limited number of data (data for lot 4907) and an analysis of variance applied on both sets of transformed values. In method "b", however, not the arithmetic means of the four transformed values was calculated, but the analysis was simply carried out on the sum of the four transformed values. The "F" values are not affected by this procedure, the remainder, however, is sixteen times greater, The results are given in Table VII.

From Table VII it is clear that the two sets of "F" values are only slightly different. Their significance is not affected. The ratio between the two remainder values is 238/15 = 15.87. If methods "a" and "B" would produce identical values, this ratio would be 16. This ratio indicates once more the unimportance of the differences obtained by the two methods.

Method "a", the transformed mean percentage, will be used on account of its greater simplicity.

Table VII

Analysis of Variance for the Germination Rate at 12 Days:

(a) using the transformed mean percentage;

(b) using the sum of four transformed replicate values.

Source	Degrees of freedom	Value of F a	Value of F b
Total	59		
Stratification periods	4	112.9 ***	117.1 ***
Incubation temperatures	2	62.5 ***	62.0 ***
Soaking periods	3	451.5 ***	468.4 ***
Str'n period x Temperature	8	9.0 ***	8.3 ***
Str'n period x Soaking period	12	23.3 ***	23.7 ***
Temperature x Soaking period	6	3.4 *	3.0 *
Remainder	24	(15)[1]	(238)[1]

Table VIII shows the results of the analyses of variance for the final germination percentage and the rate of germination at twelve days. Several conclusions can be drawn from this table:

1. Germination rate at twelve days shows highly significant differences for the second order interactions. The final germination percentage does not show significant differences for these interactions.

   This means, that although different combinations of seedlot, stratification period, incubation temperature and soaking period do cause significant differences in the rate of germination, the final germination percent is not affected by these.

Comparing the percentage of variation caused by simple factors and their interactions for the final germination percent and the germination rate, the

1. Circled values are mean square for the remainder.

Table VIII

Analysis of Variance

Effect of seedlot, incubation temperature, soaking period and stratification period on final germination percent and germination rate at 12 days.

Source of variation	Degree of freedom	Final germination %		Germination rate at 12 days.	
		F	% of variation	F	% of variation
Total	239				
seedlots (S)	3	1,658.26 ***	85.33	120.94 ***	1.92
stratification (T)	4	3.04 *	0.21	937.51 ***	19.89
temperature (Te)	2	131.74 ***	4.52	575.43 ***	6.10
soaking period (P)	3	59.25 ***	3.05	3,413.70 ***	54.33
S x T	12	6.60 ***	1.36	16.34 ***	1.04
S x Te	6	5.08 ***	0.52	63.91 ***	2.03
S x P	9	4.85 ***	0.76	22.10 ***	1.05
T x Te	8	2.13 *	0.29	22.58 ***	0.96
T x P	12	2.49 **	0.51	156.84 ***	9.99
Te x P	6	1.97	0.20	5.82 ***	0.18
S x T x Te	24	1.64	0.68	4.48 ***	0.57
S x T x P	36	1.35	0.83	4.40 ***	0.84
S x Te x P	18	0.71	0.22	2.85 **	0.27
T x Te x P	24	0.71	0.29	3.52 ***	0.45
S x T x Te x P	72	8.28[1]	1.23	7.90[1]	0.38

*** significant at 0.1% level.
** significant at 1.0% level.
* significant at 5.0% level.

1. Mean square of error term.

following differences become evident:

2. Of the variation in the final germination percentage, eighty-five percent is attributed to the different seedlots alone. This needs no further explanation; it simply means that the germination capacity of the four seedlots used is quite different.

   In the germination rate calculations, this difference in final germination percent has been eliminated, as explained before. The variation due to seedlot is therefore reduced very considerably from 85.33 percent to 1.92 percent. But nevertheless, the different seedlots show significant differences in their speed of germination at twelve days.

3. The different soaking periods have a much greater influence on the germination rate than on the final germination percentage. (54.33% versus 3.05%).

4. The interaction stratification period--soaking period has a greater influence on the germination rate than on the final germination percentage (9.99% versus 0.51%).

5. In general, it can be stated that the germination rate is more sensitive to the different factors than the final germination percentage. Different factors (or combinations of factors) may cause a significant difference in germination rate, without causing a difference in the final germination capacity.

The analysis of variance does not provide information as to which factors or combination of factors are most favourable for the germination of western hemlock seed. To gain this information, the t-test is applied to the subtotal differences for the different factors and their interactions. To facilitate the computations and comparisons, the"least significant difference" (L.S.D.) is calculated for different levels of significance, according to the formula:

$$L.S.D. = t\sqrt{2fs^2}$$

t = t value at a predetermined level of significance (5%, 1% or 0.1%) for the number of degrees of freedom corresponding to the error term.
f = number of observations in the subtotals under comparison.
s^2 = independent error term from the analysis of variance of transformed percentages.

When using the ordinary t-value in the above mentioned formula, the least significant difference can only be used to compare two adjacent group totals, when these are listed in order of increasing or decreasing size.

Often, however, a comparison between adjacent groups will be of no value. A comparison between two or more predetermined groups is required. The least significant difference in this case is larger, and is obtained by replacing "t" in the formula for the "Studentized" range "t_R". The level of "t_R" which is selected determines whether the risk of making a wrong statement about paired comparisons between groups is five percent or one percent.

In the following part, this procedure will be followed for those factors and interactions which show significant differences in the analysis of variance.

The Influence of the Main Effects

Seedlots

(a) Final germination percentage:

seedlot:	5101		5102		4907		4908
totals:	6981		6348		5555		4904
ranked differences:		633 ***		793 ***		651 ***	

L.S.D. for ranked data 0.1% level = 109.

Highly significant differences are shown between the seedlots. Seedlot 5101 shows the highest, 4908 the lowest germination capacity, 5102 and 4907 take second and third place respectively.

(b) Germination rate at twelve days:

seedlot:	5102		5101		4907		4908
totals:	3672		3412		3264		3109
differences:		260 ***		148 ***		155 ***	

L.S.D. for ranked data 0.1% level = 106.

Reasons for this difference in germination rate at twelve days are difficult to give. Seed maturity is possibly a factor of importance. Influence of seed age is indicated by the fact that lots 4907 and 4908 show a lower rate of germination than lots 5101 and 5102, which were collected two years later. Many other factors, such as treatments during processing, storage, etc. may be of importance, but so little is known about the seed's history, that it is impossible to determine the causes for the differences in germination rate.

The differences in germination behaviour, shown in the analysis of variance, substantiate again the variability between different seedlots from the same species. It shows the necessity to test many different seedlots, before reliable conclusions can be drawn about the germination behaviour of a certain species. It also shows the necessity to compare the behaviour of fresh seed with seed that has been stored for different periods of time.

Stratification periods

(a) Final germination percentage.

str'n period:	3 wks.		9 wks.		5 wks.		0 wks.		7 wks.
totals:	4807		4778		4744		4740		4719
differences:		29		34		4		21	

L.S.D. for ranked data, 5% level = 56.4

No significant differences between stratification periods are shown. It is also impossible to find a certain trend when the totals are arranged from high to low. Therefore, it can only be concluded that for western hemlock, the duration of the stratification period has no influence upon the final germination percentage.

(b) Germination rate at twelve days:

str'n period:	9 wks.		7 wks.		5 wks.		3 wks.		0 wks.
totals:	3206		3069		2773		2722		1687
differences:		137 ***		296 ***		51		1035 ***	

L.S.D. for ranked data 5% level = 55.2. 0.1% level = 95.0.

The influence of the stratification period on the rate of germination is clearly indicated. Rate of germination increases with increasing period of stratification. The greatest increase occurs during the first three weeks of stratification. A stratification period longer than three weeks increases the germination rate still further, but the rate of increase is considerably smaller.

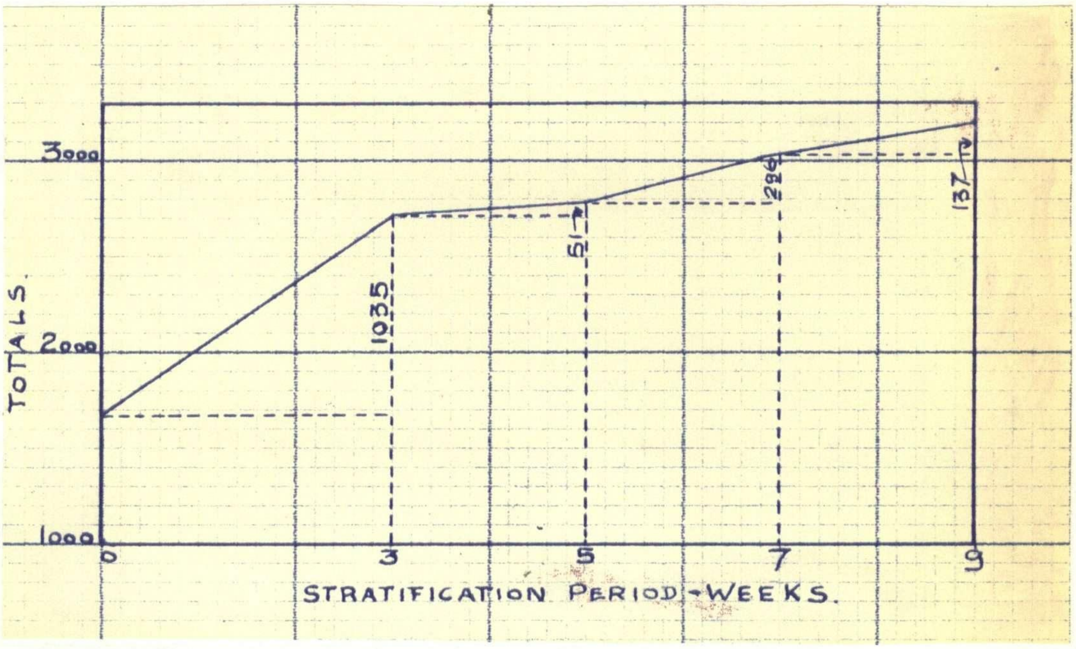


Figure 8: Effect of the length of stratification period on the germination rate at twelve days.

The value of stratification in the testing of western hemlock seed is the increased germination rate; the final germination percentage is hardly influenced.

Incubation temperatures

(a) Final germination percentage:

incubation temperature:	20°C.		Alternating		25°C.
totals:	8183		8000		7605
differences		183 ***		395 ***	

L.S.D. for ranked data, 0.1% level = 125.9.

Incubation temperatures have a very pronounced effect on the final germination percentage. A temperature of 20°C. produces the best results, 25°C. the poorest and alternating temperatures produce intermediate results.

The beneficial effect of a low incubation temperature (20°C.) compared to a high temperature (25°C.) and alternating temperatures (20°C. - 25°C.) is thus clearly established.

(b) Germination rate at twelve days:

incubation temperature:	20°C.		25°C.		Alternating
totals:	5169		4262		4026
differences:		907 ***		236 ***	

L.S.D. for ranked data, 0.1% level = 122.6.

The highest germination rate at twelve days is obtained with the 20°C. incubation temperature; a temperature of 25°C. produces intermediate results and alternating temperatures give the lowest rate.

The order of effectiveness of temperatures for the germination rate at twelve days and the final germination percentage is not the same. While in both cases a temperature of 20°C. is most effective, 25°C. comes next in the case of germination rate. It must be stated here, however, that the rate of germination is not constant throughout the incubation period, but changes during incubation. The period of twelve days is arbitrarily set and is in several cases somewhat deceiving. When a temperature of 25°C. gives a higher rate of germination at twelve days, compared to alternating temperatures, it does not mean that this situation will exist throughout the incubation period. A crossing of the curves may take place, thereby changing the situation in favour of the alternating temperature. This complication will be discussed in more detail below.

In general, however, it can be concluded that a temperature of 20°C. produces the highest final germination percent and the highest rate of germination.

Soaking periods. (Seed moisture content)

(a) Final germination percentage:

soaking periods:	33 hrs.		11 hrs.		2 hrs.		½ hr.
totals:	6118		6049		5886		5735
differences:		69 *		163 ***		151 ***	

L.S.D. for ranked data, 5% level = 63.0; 0.1% level = 109.0

The soaking period has a definite influence on the final germination percentage with 33 hours showing the best results. The increase in germination percentage is greatest up to 11 hours of soaking. Beyond 11 hours the rate of increase is much smaller.

(b) Germination rate at twelve days:

soaking periods:	33 hrs.		11 hrs.		2 hrs.		½ hr.
totals:	5024		3683		2528		2222
differences:		1341 ***		1155 ***		306 ***	

L. S. D. for ranked data, 0.1% level = 106.0.

Increasing soaking periods effect a highly significant increase in the rate of germination. This increase is of much greater magnitude than the increase in final germination percentage caused by soaking. An increase in soaking period from ½ to 33 hours causes an average increase in the final germination percentage of six percent. The same increase in soaking period causes an increase of 126 percent in the germination rate.

The Influence of the First-Order Interactions

Seedlot x stratification period

(a) Final germination percentage:

Str'n period (weeks)	4907 totals	4907 diff's	4908 totals	4908 diff's	5101 totals	5101 diff's	5102 totals	5102 diff's
0	1049		1006		1393		1292	
	1)	93 **		-16		18		-28
3	1142		990		1411		1264	
		-22 2)		-16		-5		-20
5	1120		974		1406		1244	
		8		-20		-37		24
7	1128		954		1369		1268	
		-12		26		33		12
9	1116		980		1402		1280	

1) highest values for each seedlot underlined.

2) difference is not significant.

L.S.D. for non-ranked data, 5% level = 67.5. 1% level = 78.5.

Comparing the different seedlots, stratification shows a significant increase of the final germination percent only for lot 4907. In the discussion of the influence of the main effects, stratification did not show an influence on the final germination percentage. The different behaviour of the lot 4907 was lost where the effect of stratification was evaluated for all four seedlots together. The following reasons can be given for this difference in behaviour between the seedlots:

1. Lot 4907 does not reach its final germination capacity without stratification.
2. Lot 4907 needs more than forty-two days to complete germination, when the seed is not stratified.

Figure 9 shows that at forty-two days the germination curve is still going upward, especially for 25°C. Figure 10 shows lot 5102 germinated under the same conditions. At forty-two days germination is much more complete for this seedlot.

Figure 9:

Lot 4907, germinated without soaking or stratification at 25°C, Alternating temperatures and 20°C.

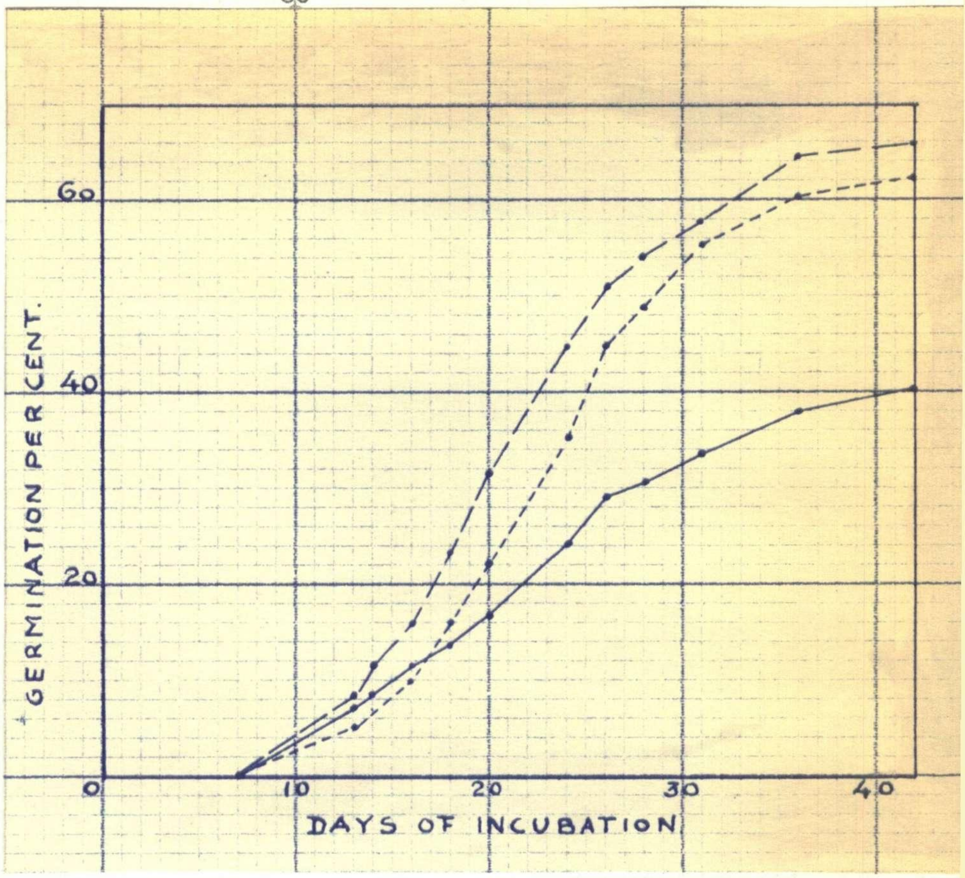
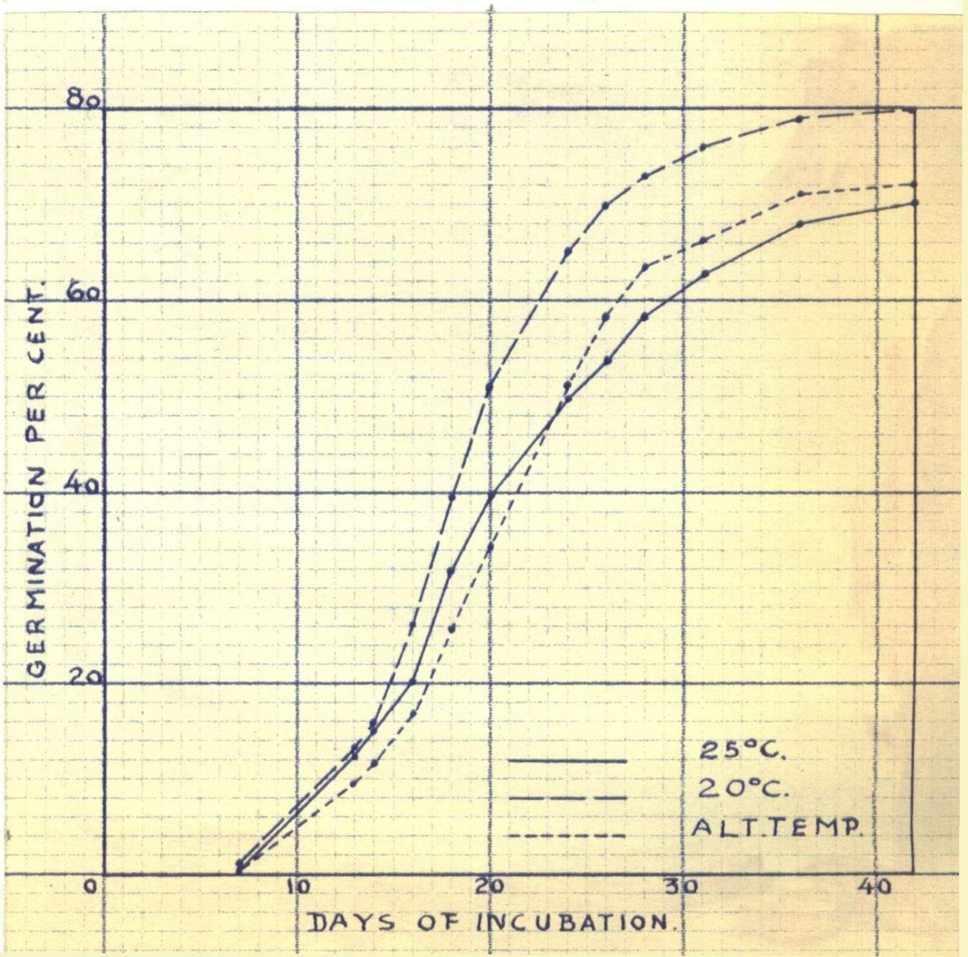


Figure 10:

Lot 5102, germinated without soaking or stratification at 25°C, Alternating temperatures and 20°C.



Another interesting fact is that the highest germination percentages for all seedlots occur either without stratification or after three weeks of stratification. An explanation for this fact may be found in the occurrence of mould during prolonged stratification. Moulds will kill damaged seeds or seed with cracked seed coats, which without the presence of mould would remain viable. However, this reduction in viability is nowhere significant.

(b) Germination rate at twelve days:

Str'n period (weeks)	4907		4908		5101		5102	
	totals	diff's	totals	diff's	totals	diff's	totals	diff's
0	411		405		400		471	
		227 **		287 **		255 **		266 **
3	638		692		655		737	
		80 **		-86 ** ?		61		-4
5	718		606		716		733	
		31		55		97 **		113 **
7	749		661		813		846	
		-1		84 **		15		39
9	748		745		828		885	

L.S.D. for non-ranked data, 5% level = 63.2; 1% level = 73.5.

Stratification effects a highly significant increase in the germination rate of all four seedlots. The greatest rate of increase is affected by the first three weeks of stratification. The subsequent six weeks of stratification do increase the germination rate significantly, but to a much lesser degree than the first three weeks. Lot 4908 shows a behaviour at five weeks of stratification which cannot be explained. A significant drop occurs at five weeks, which, however, is followed by an increase at seven weeks and nine weeks. Apart from this irregularity, all seedlots seem to react in much the same way upon increased duration of stratification.

<u>Seedlot x incubation temperature</u>

(a) Final germination percentage:

Temper-atures	4907		4908		5101		5102	
	totals	diff's	totals	diff's	totals	diff's	totals	diff's
25°C.	1728		1579		2255		2043	
		144 **		75		89		87
Alt.	1872		1654		2344		2130	
		83		17		38		45
20°C.	1955		1671		2382		2175	
totals		227 **		92		127 **		132 **

L.S.D. for non-ranked data, 5% level = 95.0; 1% level = 108.8

For all seedlots, 20°C gives the highest germination percentage, 25°C. the lowest and Alternating temperatures produce intermediate results.

Lot 4907

Alternating temperatures produce results, highly significantly better than constant 25°C. The final germination percentage obtained with 20°C. is not significantly better than the one obtained with alternating temperatures. Incubation at 20°C. is, however, highly significantly better than incubation at 25°C.

Lot 4908

No significant differences do exist. The same pattern is found, however, as for the other seedlots.

Lot 5101

Alternating temperatures are not significantly better than 25°C. Incubation at 20°C. is not significantly better than incubation at alternating temperatures but highly significantly better than incubation at 25°C.

Lot 5102

Same situation as for 5101.

(b) Germination rate at twelve days:

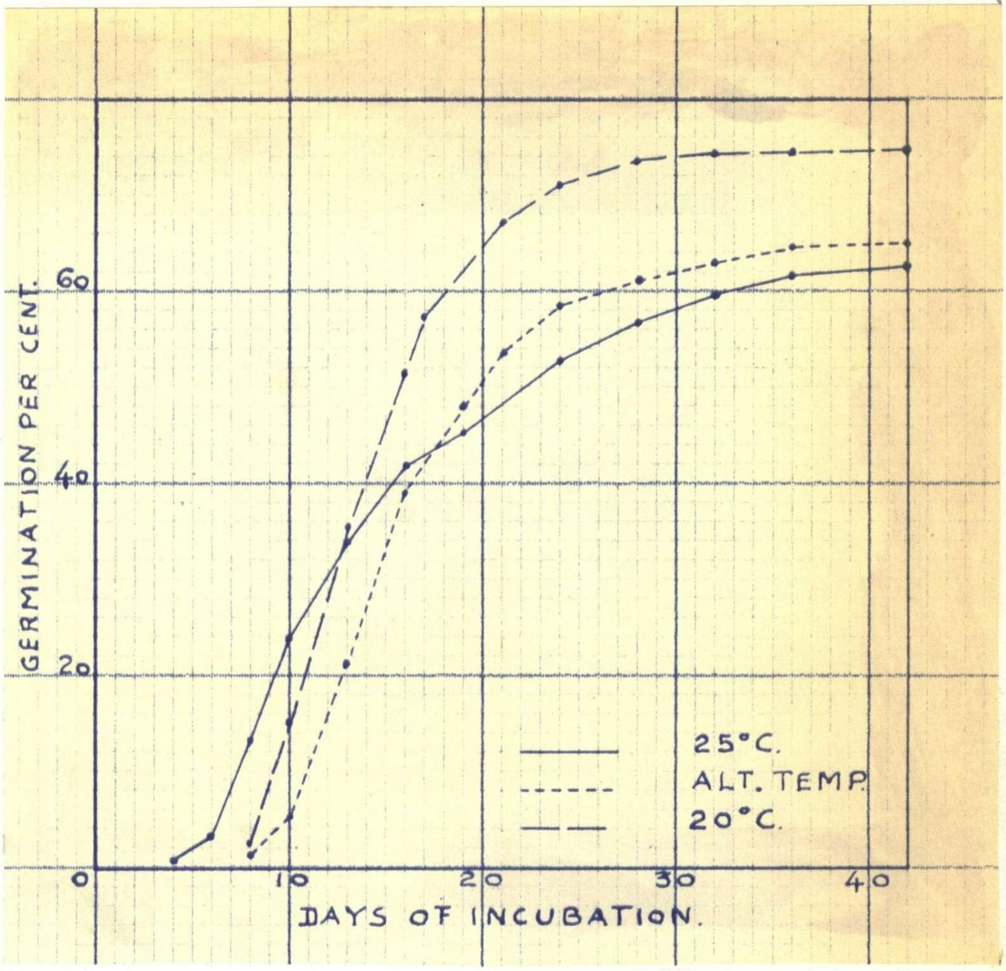
Temperatures	4907		4908		5101		5102	
	totals	diff's	totals	diff's	totals	diff's	totals	diff's
25°C	1188		985		1019		1070	
		-256 **		-71		+ 24		+ 67
Alt.	932		914		1043		1137	
		+ 212 **		+296 **		+307 **		+328 **
20°C	1144		1210		1350		1465	

L.S.D. for non-ranked data, 5% level = 92.9; 1% level = 106.4

In general, 20°C produces the highest rate of germination at twelve days. Incubation at 20 C. is highly significantly better than at both alternating temperatures and 25°C. The one exception to this rule is lot 4907. For this seedlot both 20°C. and 25°C. are highly significantly better than alternating temperatures. The difference between 20°C. and 25°C. is only small. The reason for this apparent difference in behaviour between seedlots has been briefly touched before. Although lot 4907 shows the highest germination rate at twelve days when incubated at 25°C., this situation does not remain. In several cases the 25°C.-curve will cross both the 20°C. and alternating temperatures curves and thus end up with the lowest germination rate, as is shown in Figure 11. This figure shows that at 25°C. germination starts earlier than at 20°C. and alternating temperatures. After $12\frac{1}{2}$ days the 25°C and 20°C. curves cross and after 18 days the 25°C. and alternating temperatures curves cross. After 18 days of incubation, 25°C. shows the lowest germination rate of the three temperatures used. As will be seen later, this crossing of curves will take place much earlier when longer stratification periods are employed.

Figure 11:

Lot 4907, soaked for 11 hours; stratified for 3 weeks and incubated at 25°C., alternating temperatures and 20°C.



Seedlot x soaking period

(a) Final germination percentage:

Soaking periods (hours)	4907 totals	4907 diff's	4908 totals	4908 diff's	5101 totals	5101 diff's	5102 totals	5102 diff's
½	1308		1203		1668		1556	
		88 ★		-9		66		6
2	1396		1194		1734		1562	
		28		66		45		24
11	1424		1260		1779		1586	
		3		-13		21		58
33	1427		1247		1800		1644	

L.S.D. for non-ranked data, 5% level = 78.5; 1% level = 90.7.

A soaking period of two hours produces significantly better results than ½ hour for seedlot 4907 only. Soaking for 11 hours produces highly significantly better results than soaking for $\frac{1}{2}$ hour for seedlots 4907 and 5101. Soaking for 33 hours produces highly significant differences compared to soaking for $\frac{1}{2}$ hour for seedlots 4907, 5101 and 5102.

In general, longer soaking periods result in an increased final germination percentage. Lot 4908, however, shows a behaviour impossible to explain.

(b) Germination rate at twelve days:

Soaking periods (hours)	4907		4908		5101		5102	
	totals	diff's	totals	diff's	totals	diff's	totals	diff's
$\frac{1}{2}$	478		521		605		618	
		159 **		53		24		70
2	637		574		629		688	
		326 **		291 **		238 **		300 **
11	963		865		867		988	
		223 **		284 **		444 **		390 **
33	1186		1149		1311		1378	

L.S.D. for non-ranked data, 5% level = 76.7; 1% level = 88.6.

Although a two-hour soaking period does increase the germination rate compared to $\frac{1}{2}$ hour soaking, this increase is significant only for lot 4907. Soaking periods of 11 and 33 hours, however, do increase the germination rate highly significantly.

The 33 hours soaking period has proved itself beneficial in increasing the final germination percentage and highly increasing the rate of germination.

Referring back to Table IV, where the actual seed moisture contents are given for the different soaking periods, it is observed that lot 4907 shows a considerably higher rate of moisture absorption than any one of the other seedlots used. Plotting the rate of germination against the duration of soaking, as shown in Figure 12-A, divides the four seedlots into two

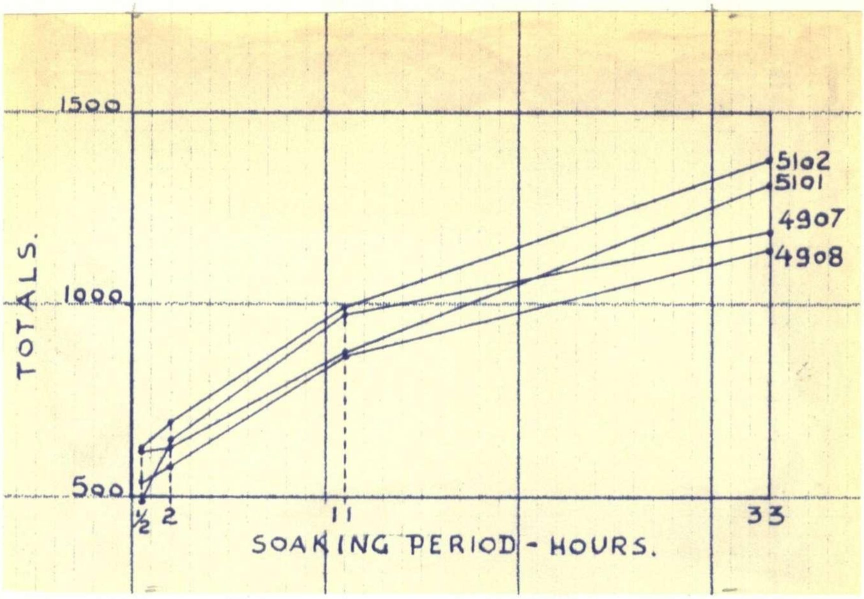


Figure 12-A: Influence of Seedlot-Soaking Period Interaction on Rate of Germination.

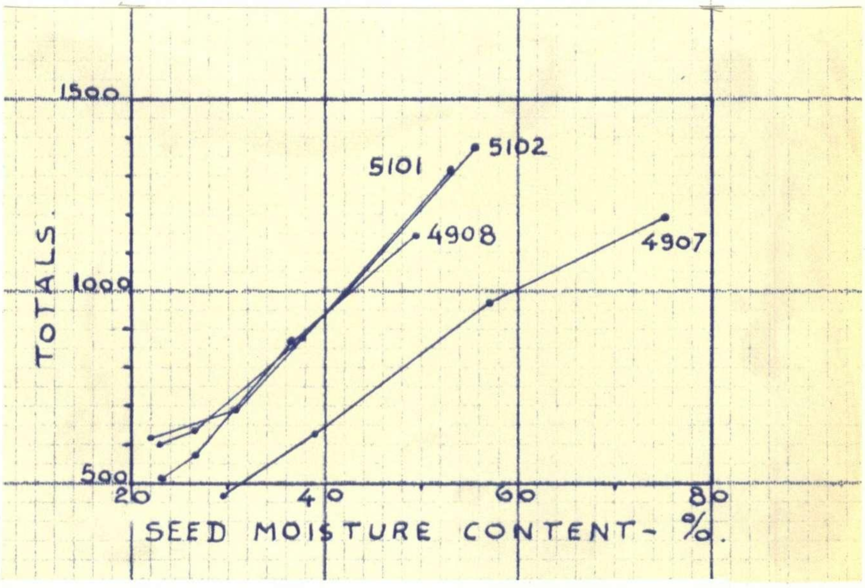


Figure 12-B: Influence of Seedlot-Seed Moisture Content Interaction on Rate of Germination.

groups. Seedlots 4907 and 4908 show a distinct slowing down of the germination rate when soaked longer than 11 hours. Seedlots 5101 and 5102 show only a slight slowing down beyond 11 hours of soaking.

When the rate of germination is plotted against the actually obtained moisture contents, however, as shown in Figure 12-B, three seedlots, 4908, 5101 and 5102, fall into one group and are very close together. Seedlot 4907 deviates quite distinctly from this group and shows a lower increase of the rate of germination with increased seed moisture content. Obvious, also, is the practically linear relationship between germination rate and seed moisture content, for seed moisture contents higher than about thirty percent.

This graph shows clearly the difference in the relationship between seed moisture content during stratification and the rate of germination for lot 4907, compared to lots 4908, 5101 and 5102.

In the analysis of the main effects, it was found that the seedlots could be arranged according to their germination rate from high to low, as follows: 5102, 5101, 4907, 4908. This same order can be found in Figure 12-A, when the highest germination rates obtained are compared. If, however, the comparison is made for equal moisture contents, then lot 4907 shows a considerably lower germination rate compared to the other three seedlots. The differences between 5101, 5102 and 4908 are only small.

It is difficult to find the causes for this deviating behaviour of lot 4907. The higher moisture absorbtion rate suggests either a higher osmotic activity or a more permeable seed coat, or possibly a combination of the two factors. Western hemlock seed coats are very thin, however, which reduces the likeliness of the seed coat alone causing the observed difference in moisture intake. Increased osmotic activity indicates a higher content of soluble substances in the endosperm. This may indicate a higher percentage of sugars in the seeds of lot 4907, which in turn may indicate early collected, "immature" seed. At this stage, however, it is impossible to decide whether or not maturity is a factor in the behaviour of this seedlot. Too many unknowns are still attached to the problem of maturity and very little is known about the seed's history.

Stratification period x temperature

(a) Final germination percentage:

Temp.	0 weeks		3 weeks		5 weeks		7 weeks		9 weeks	
	total	diff's	total	diff's	total	diff's	total	diff's	total	diff's
25°C.	1521		1539		1526		1485		1534	
		60		97 **		67		109 **		62
Alt.	1581		1636		1593		1594		1596	
		57		-4		32		46		52
20°C.	1638		1632		1625		1640		1648	
total		117 **		93 *		99 **		155 **		114 **

L.S.D. for non-ranked data, 5% level = 82.2; 1% level = 94.7.

The same trend, as found for the influence of the different temperatures as main effects, is found within each stratification period. The best results are obtained with 20°C., intermediate results with alternating temperatures and the poorest results with 25°C. Alternating temperatures are significantly better only in the 3 and 7 weeks stratification periods. But the consistency of the trend between temperatures compels us to assume that alternating temperatures are better than 25°C. in all cases. A temperature of 20°C. is highly significantly better than 25°C. in all cases.

It is also seen from the table that no change takes place in the sensitiveness towards temperature with increased length of stratification. Totaling the differences between the temperatures in order of increasing stratification periods gives: 117, 93, 99, 155, 114. These figures do not even suggest that increased length of stratification decreases the sensitiveness towards different temperatures.

(b) Germination rate at twelve days:

Temp.	0 weeks		3 weeks		5 weeks		7 weeks		9 weeks	
	total	diff's	total	diff's	total	diff's	total	diff's	total	diff's
25°C.	532		929		839		986		976	
		-25		-200 **		6		-48		31
Alt.	507		729		845		938		1007	
		141 **		335 **		244 **		207 **		216 **
20 C.	648		1064		1089		1145		1223	

L.S.D. for non-ranked data, 5% level = 80.1; 1% level = 92.4.

In all cases, 20°C. gives the highest rate of germination at 12 days and is highly significantly better than both 25°C. and alternating temperatures. The differences between alternating temperatures and 25°C. are not consistent. Alternating temperatures or 25°C. may show the highest germination rate.

Increased length of stratification shows a general trend to increase the germination rate at all temperatures. The same effect was found for stratification periods as a main effect. Incubation temperature and stratification period seem to affect the germination rate independently from each other.

Stratification period x soaking period.

(a) Final germination percentage:

Soaking periods	0 weeks		3 weeks		5 weeks		7 weeks		9 weeks	
	total	diff's	total	diff's	total	diff's	total	diff's	total	diff's
½ hr.	1157		1181		1142		1113		1142	
		19		6		38		45		43
2 "	1176		1187		1180		1158		1185	
		25		30		29		59		20
11 "	1201		1217		1209		1217		1205	
		5		5		4		14		41
33 "	1206		1222		1213		1231		1246	
totals		49		41		71 *		118 **		108 **

L.S.D. for non-ranked data, 5% level = 67.5; 1% level = 78.5.

This interaction is shown in graph form in Figure 13:

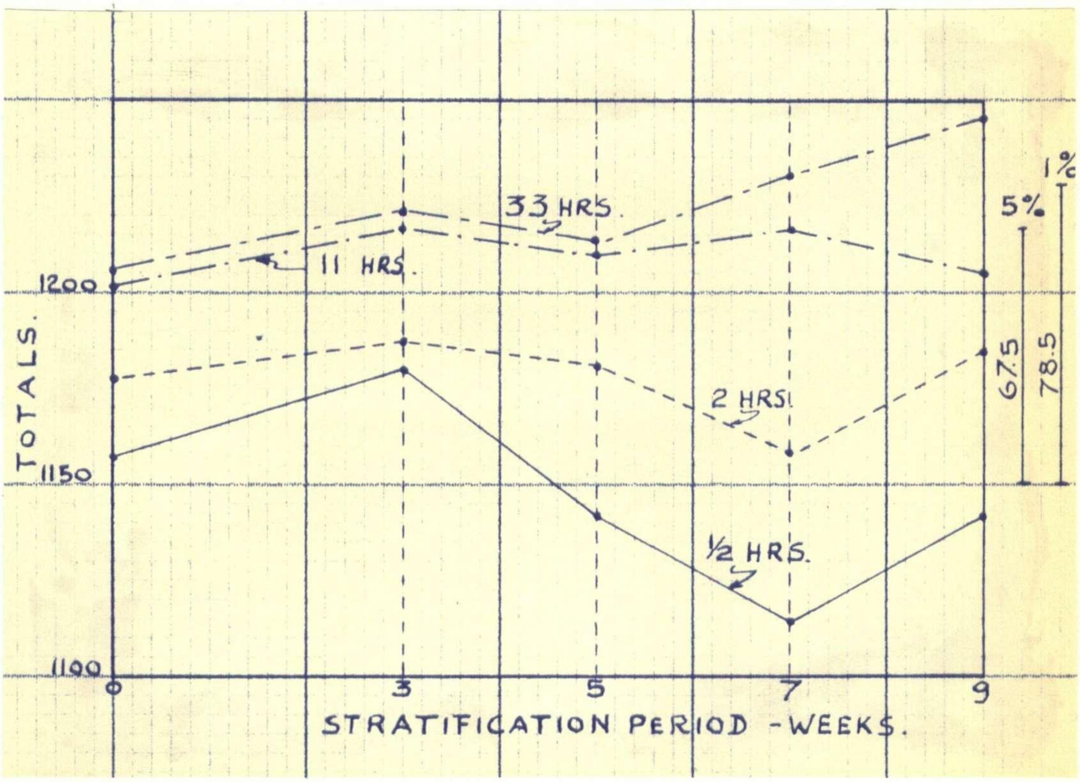


Figure 13: Influende of the stratification period x soaking period interaction on the final germination percentage.

In the analysis of the main effects, no significant influence of stratification period could be found upon the final germination percentage. Soaking period as a main effect, however, showed the highest germination percentage at the longest soaking period. The interaction, however, shows a puzzling variation, especially for the ½ hour and 2 hours soaking periods. Soaking for 11 and 33 hours produces much more uniform results throughout. Particularly mysterious is the drop in germination percentage beyond 3 weeks of stratification for seed soaked for ½ and 2 hours, followed by an increase at 9 weeks of stratification. All that can be concluded here is that 33 hours of soaking produces the best and most uniform results.

(b) Germination rate at twelve days:

Soaking period	0 weeks		3 weeks		5 weeks		7 weeks		9 weeks	
	total	diff's	total	diff's	total	diff's	total	diff's	total	diff's
½ hr.	397		459		424		435		507	
		-10		68 *		63 *		119 **		67 *
2 "	387		527		487		554		573	
		4		215 **		292 **		312 **		332 **
11 "	391		742		779		866		905	
		121 **		252 **		304 **		348 **		316 **
33 "	512		994		1083		1214		1221	

L. S. D. for non-ranked data, 5% level = 63.2; 1% level = 73.5.

This interaction is shown in graph form in Figure 14. On this graph the influence of longer soaking periods (higher moisture contents!) on the effectiveness of stratification is noticed at once. Soaking periods of ½ and 2 hours effect a relatively small increase of the germination rate, compared with 11 hours and 33 hours of soaking. A soaking period of 33 hours shows the best results.

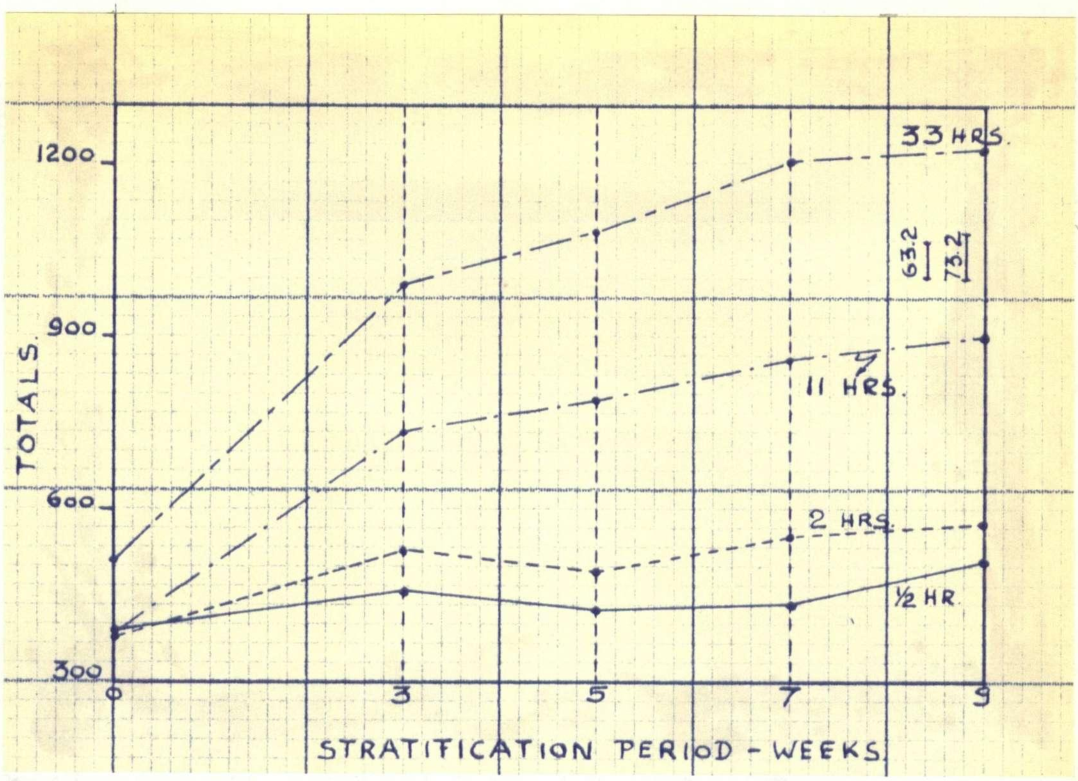


Figure 14: The influence of the stratification period x soaking period interaction on the germination rate.

It is also obvious that the first three weeks of stratification result in the greatest increase of the germination rate. Beyond three weeks this increase is much less but steady for seed soaked for 11 and 33 hours.

This graph shows clearly the close interrelation between soaking periods (seed moisture content) and stratification on the rate of germination. A moist-cold pretreatment is only effective at relatively high seed moisture contents (50 - 75%).

It is interesting at this point to come back to the discussion concerning the changes taking place in a seed during stratification. If leaching of inhibiting substances is of any importance, this should show up in seed that has been soaked but not stratified. At 0 weeks of stratification, the germination rate increases with 115, when ½ hour and 33 hours of soaking are compared. This increase is highly significant and could support the theory of leaching of inhibitors. After soaking and three weeks of chilling, however, the same difference has increased to 535; after nine weeks of chilling to 715. If leaching is of any importance at all, it is of minor importance compared to the increase in germination rate, effected by 9 weeks of moist-cold pretreatment. This is roughly six times as effective as soaking without subsequent chilling.

Incubation temperature x soaking period

This interaction shows no significant differences in the table of Analysis of Variance, for the final germination percentage. Therefore, this interaction will only be investigated further for the rate of germination at twelve days:

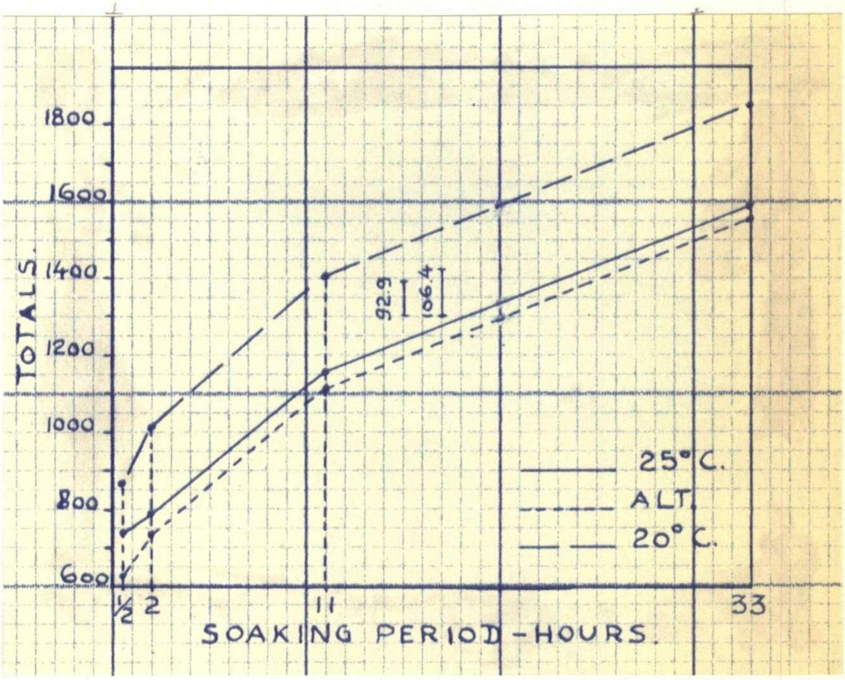
Soaking period	25°C.		Alternating		20°C.	
	totals	diff's	totals	diff's	totals	diff's
½ hr.	730		622		870	
		51		109 **		146 **
2 "	781		731		1016	
		374 **		386 **		395 **
11 "	1155		1117		1411	
		441 **		439 **		461 **
33 "	1596		1556		1872	

L.S.D. for non-ranked data, 5% level = 92.9; 1% level = 106.4

For all incubation temperatures employed, an increase of the soaking period (increase in seed moisture content) results in an increase of the rate of germination. At 25°C. incubation temperature, the 2 hours soaking period shows only a slightly higher germination rate than for ½ hour of soaking. For alternating temperatures and 20°C., this difference is highly significant, however. The most favourable temperature in all cases is 20°C. This temperature produces highly significantly better results than both alternating temperatures and 25°C. Alternating temperatures produce generally slightly lower results than 25°C., but only in the case of seed, soaked for ½ hour, is this difference significant.

<u>Figure 15</u>:

The effect of increasing seed moisture content during stratification on the rate of germination at 25°C., Alternating and 20°C. incubation temperatures.



In Figure 15 the previous table is presented graphically. The temperature lines are practically parallel, except between ½ and 2 hours of soaking. This means that the influence of different incubation temperatures is independent of the seed moisture content during stratification.

The Influence of the Second-Order Interactions

In the analysis of variance, the second-order interactions show significant differences only for the rate of germination at 12 days. Therefore, second-order interactions will only be analyzed for the rate of germination and not for the final germination percentage.

Seedlot x stratification period x incubation temperature

Seedlot	0 weeks			3 weeks			5 weeks			7 weeks			9 weeks		
	25°	Alt	20°	25°	Alt	20°	25°	Alt	20°	25°	Alt	20°	25°	Alt	20°
4907	164	122	125	265	149	224	248	214	256	263	210	276	248	237	263
4908	121	131	153	230	188	274	190	173	243	216	197	248	228	225	292
5101	111	120	169	211	183	261	200	218	298	261	253	299	236	269	323
5102	136	134	201	223	209	305	201	240	292	246	278	322	264	276	345

L.S.D. for non-ranked data, 5% level = 29.7; 1% level = 36.4.

The table shows that the highest germination rate is obtained at a temperature of 20°C., after 9 weeks of stratification, for seedlots 4908, 5101 and 5102. Lot 4907 shows its highest value after 7 weeks of stratification, again at 20°C. This value is not significantly higher, however, than the one for 9 weeks of stratification. These highest germination rates are significantly better for all seedlots, except 4907, than those obtained at either 25°C. or alternating temperatures, within the same stratification period.

To simplify the overall grasp of this table, it is graphically presented in Figure 16. The advantage of 20°C. over the other incubation temperatures used is easily seen. Lot 4907, however, seems to deviate in this temperature requirement from the other three seedlots. Incubation at 25°C. seems to be the best for the first 5 weeks, after which period 20°C. takes over. However, 20°C. is not significantly better than 25°C. at any point. For all four seedlots, 25°C. shows an unexplainable irregularity. Remarkable is the drop in germination rate after three weeks of stratification. At 20°C., this irregularity is much less. Lot 4908 shows a very irregular course with increased stratification period. For all three temperatures employed, this irregular course is quite similar, however.

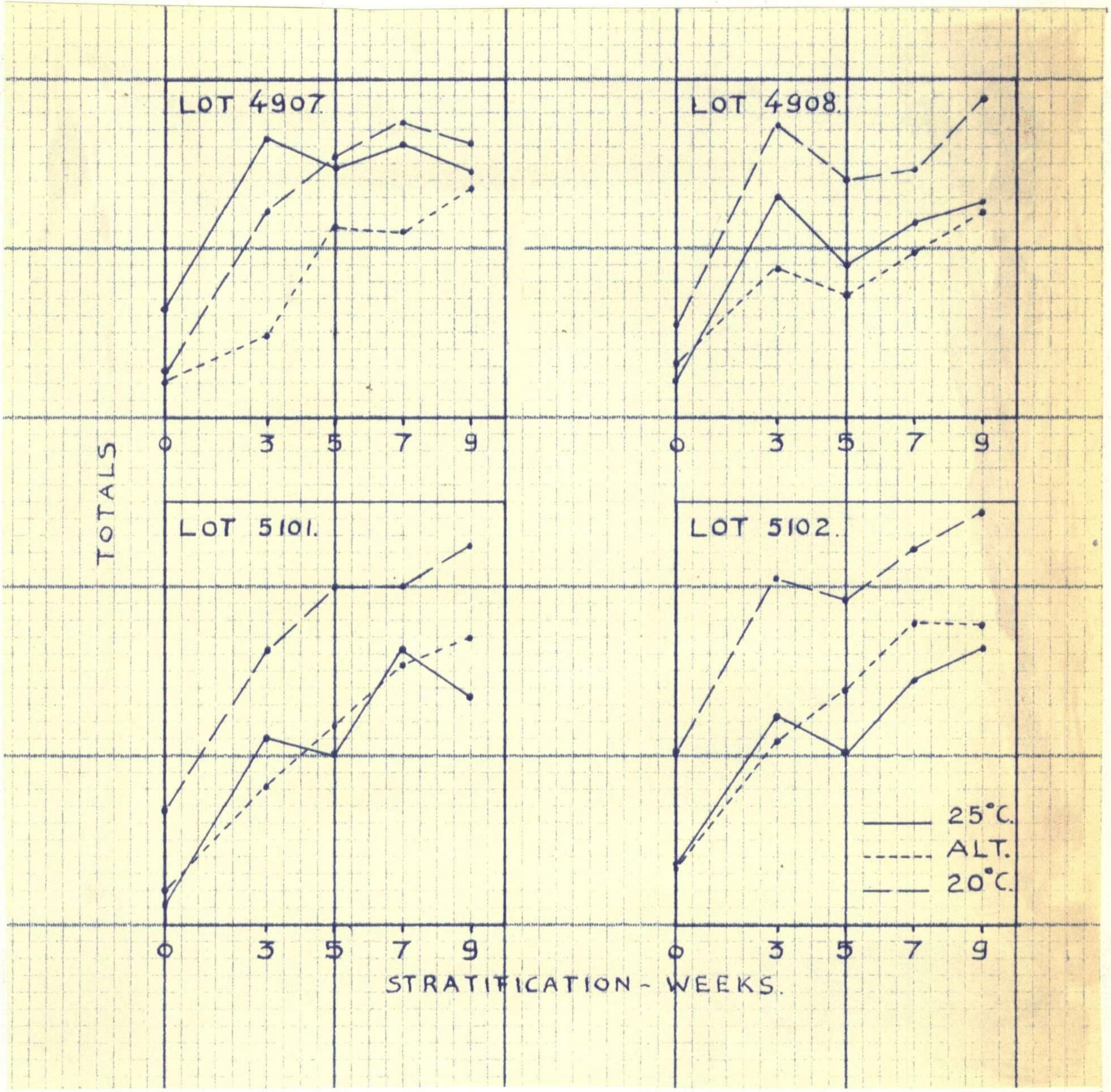


Figure 16: Relationship between incubation temperature and stratification period for seedlots 4907, 4908, 5101 and 5102.

A closer look at this apparent different behaviour towards temperature of seedlot 4907 seems justified at this place. The cumulative germination curves in Figure 16 show that incubation at 25°C. initiates germination earlier than incubation at either 20°C. or alternating temperatures. The 25°C. curve crosses, however, both the 20°C. and alternating temperature curves, and ends up with the lowest germination capacity. The final germination percentage at 25°C. is often also considerably less than for 20°C. or alternating temperatures. This difference is greater for a low seed moisture content and decreases with increasing seed moisture content. For the other seedlots used, the final germination percentages for the three incubation temperatures are much closer together, irrespective of the seed moisture content. Also these seedlots do not show the early and faster start of germination at 25°C. The combination of these two factors (early start of germination and low final germination percentage) causes the germination rate at 12 days to be deceiving for seedlot 4907. When for instance for 25°C. the 12 day count is 15 percent, the final count 50 percent, then the rate of germination is expressed as $\frac{15}{50} \times 100 = 30$ percent. If, however, the final germination percentage for 20°C. is 70, with the same 12 day percentage, a situation that is not unusual, then the rate is expressed as $\frac{15}{70} \times 100 = 21.4$ percent. For the other seedlots, the germination percent at 12 days for 20°C. is usually considerably higher than that for 25°C. The difference in final germination percentage is usually small and therefore the expression for the germination rate much more reliable.

This variation in behaviour between the seedlots is shown in Figures 17 and 18. It will be noted, that for seedlot 4907 incubation at 25°C. depresses the germination curve much stronger than incubation at 20°C. For seedlot 5101, the differences between the curves for different incubation temperatures are generally smaller.

Figure 17: Seedlot 4907. The influence of different incubation temperatures (25°C., 20°C., and alternating) on the germination behaviour of seed, stratified for 7 weeks.

A: Soaking period - ½ hour.

B: Soaking period - 2 hours.

FIGURE 17-A.

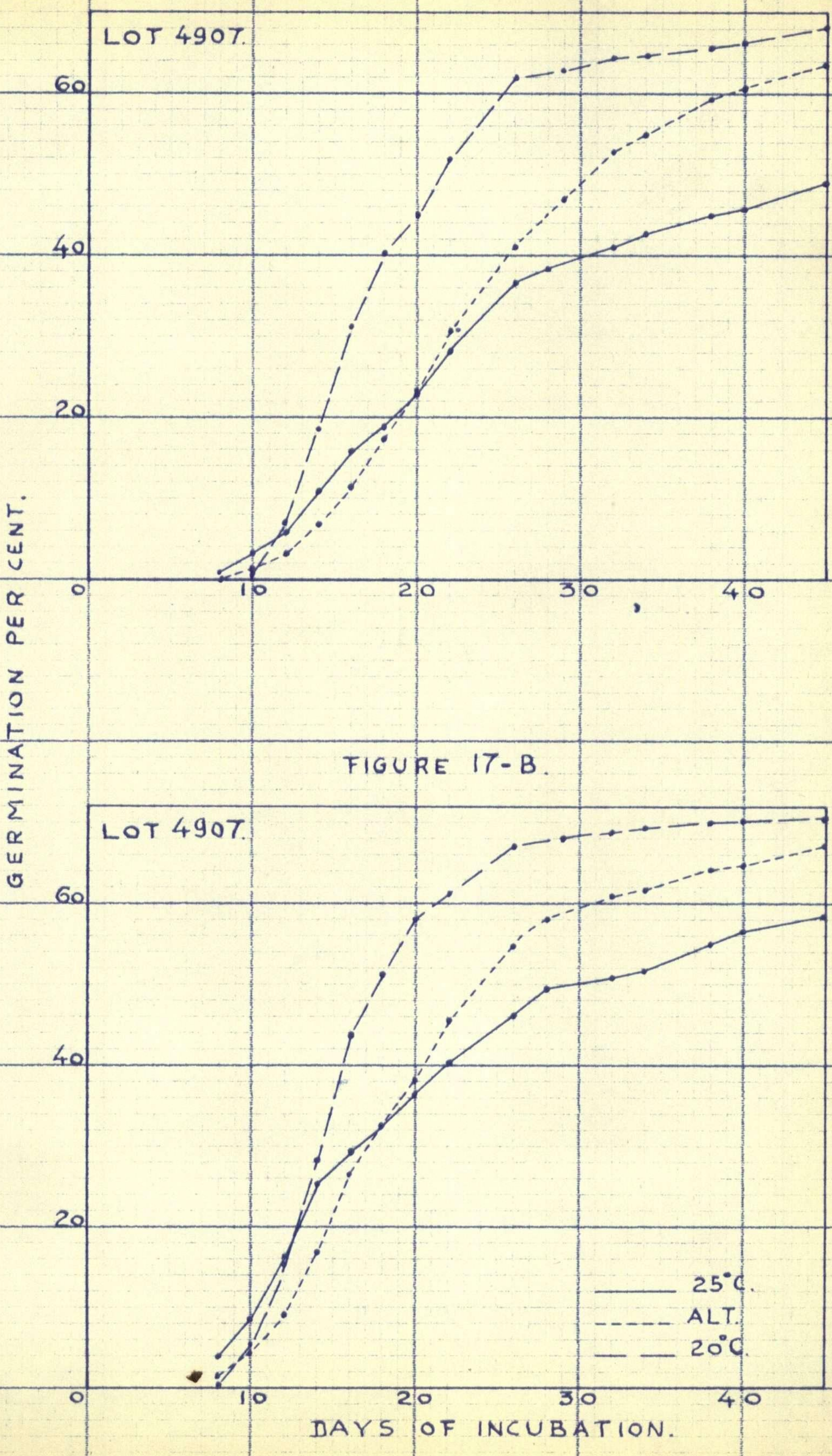

LOT 4907.
60
40
20
0
10
20
30
40
GERMINATION PER CENT.
FIGURE 17-B.
LOT 4907.
60
40
20
0
10
20
30
40
25°C.
ALT.
20°C.
DAYS OF INCUBATION.

Figure 17: Seedlot 4907. The influence of different incubation temperatures (25°C., 20°C., alternating) on the germination behaviour of seed, stratified for 7 weeks.

C: Soaking period - 11 hours.

D: Soaking period 33 hours.

FIGURE 17-C.

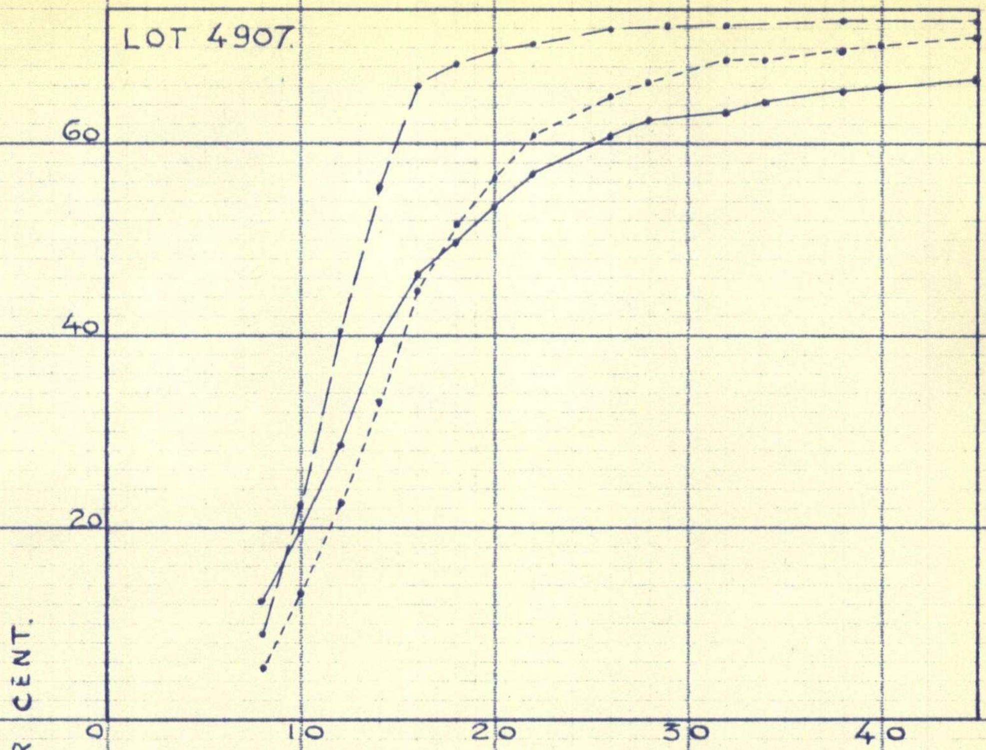


FIGURE 17-D.

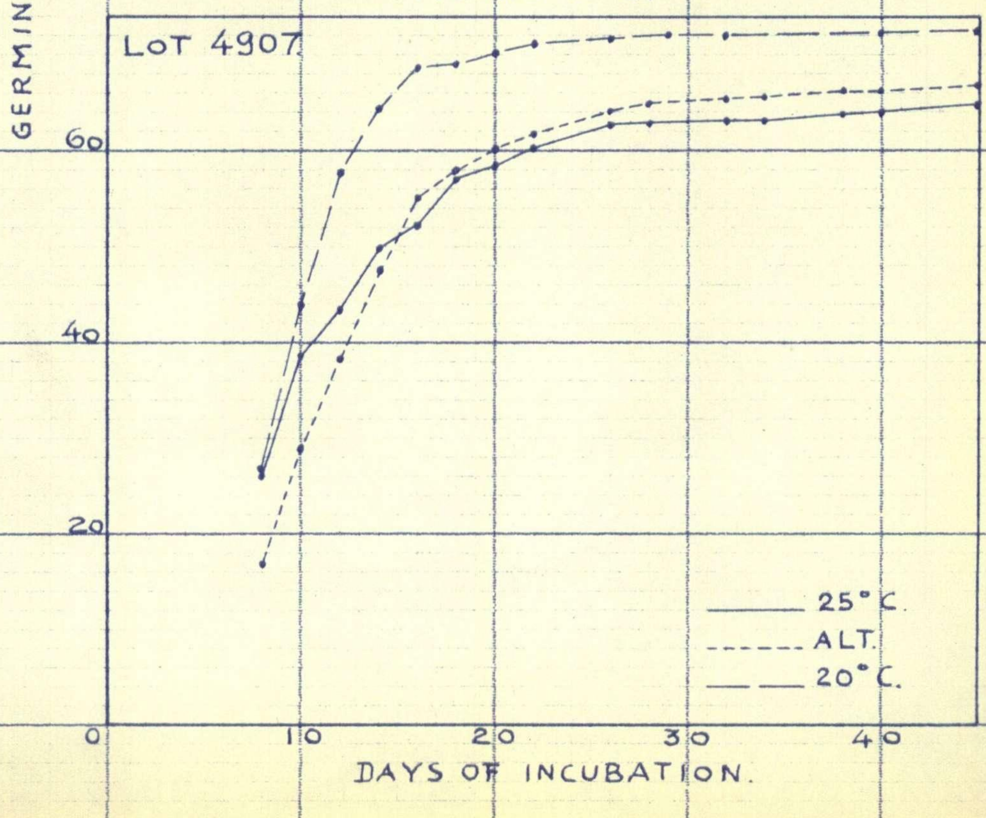


Figure 18: Seedlot 5101. The influence of different incubation temperatures (25°C., 20°C., alternating) on the germination behaviour of seed, stratified for 7 weeks.

A: Soaking period - ½ hour.

B: Soaking period - 2 hours.

FIGURE 18-A.

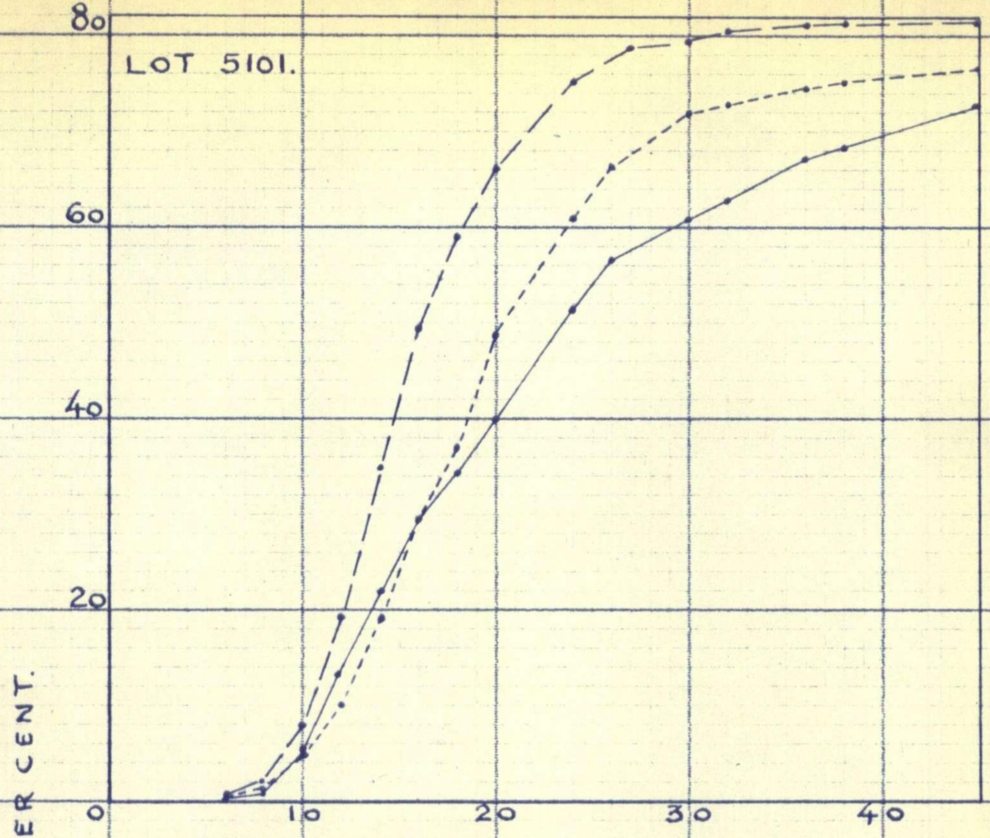


FIGURE 18-B.

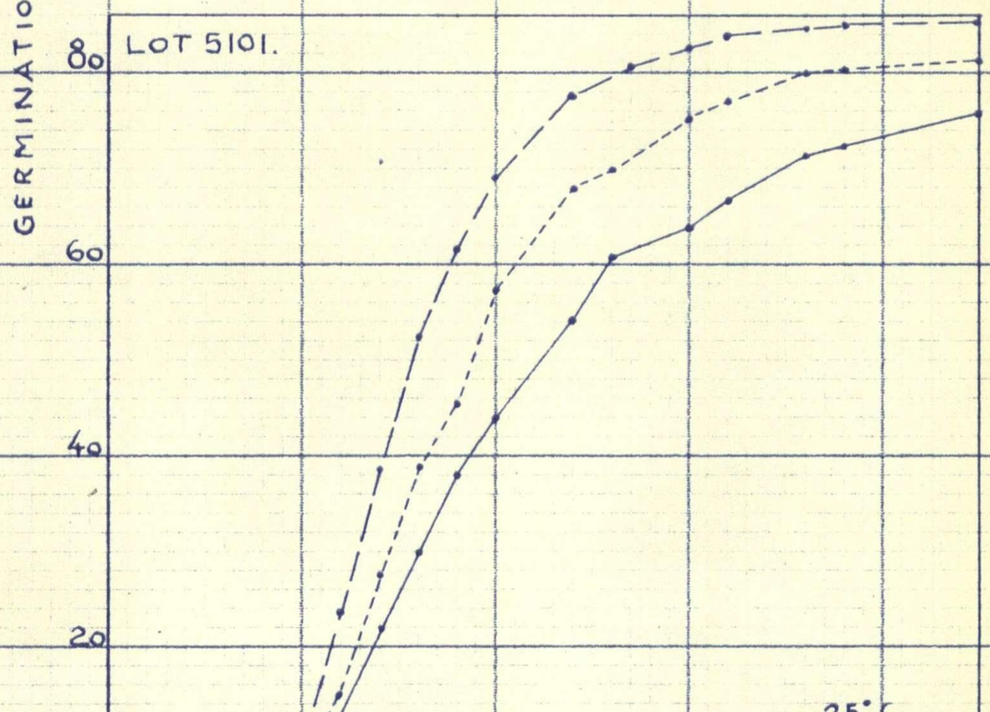


Figure 18: Seedlot 5101. The influence of different incubation temperatures (25 C., 20 C., alternating) on the germination behaviour of seed, stratified for 7 weeks.

C: Soaking period - 11 hours.

D: Soaking period - 33 hours.

FIGURE 18-C.

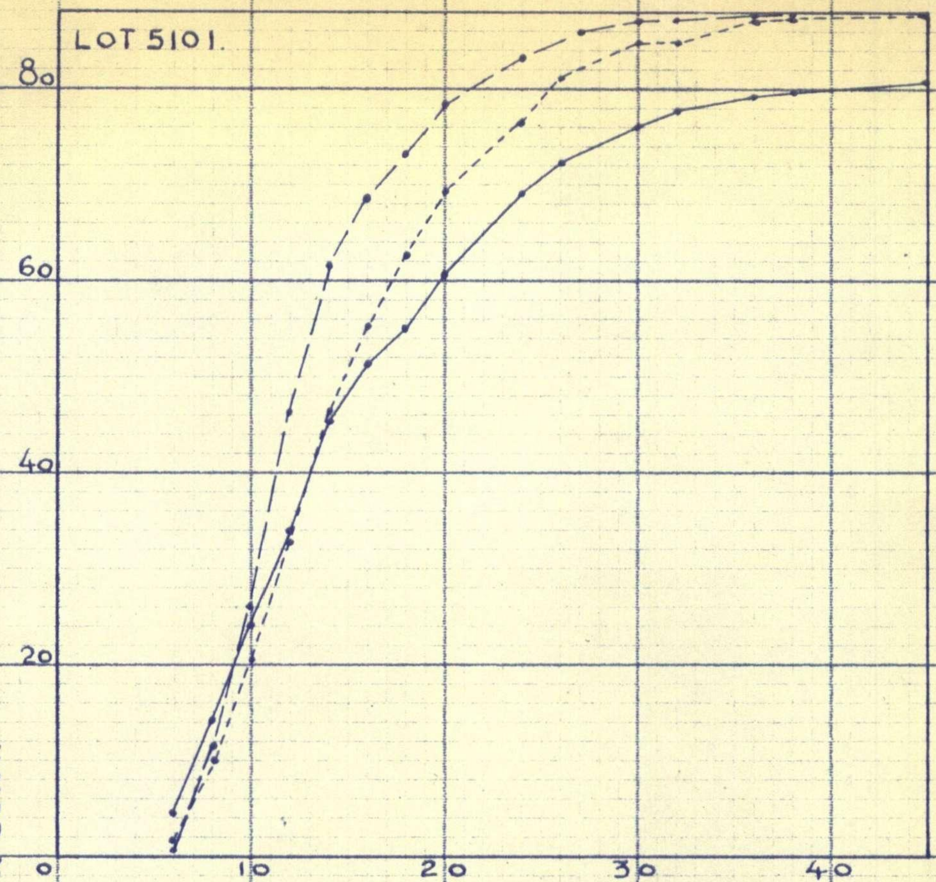
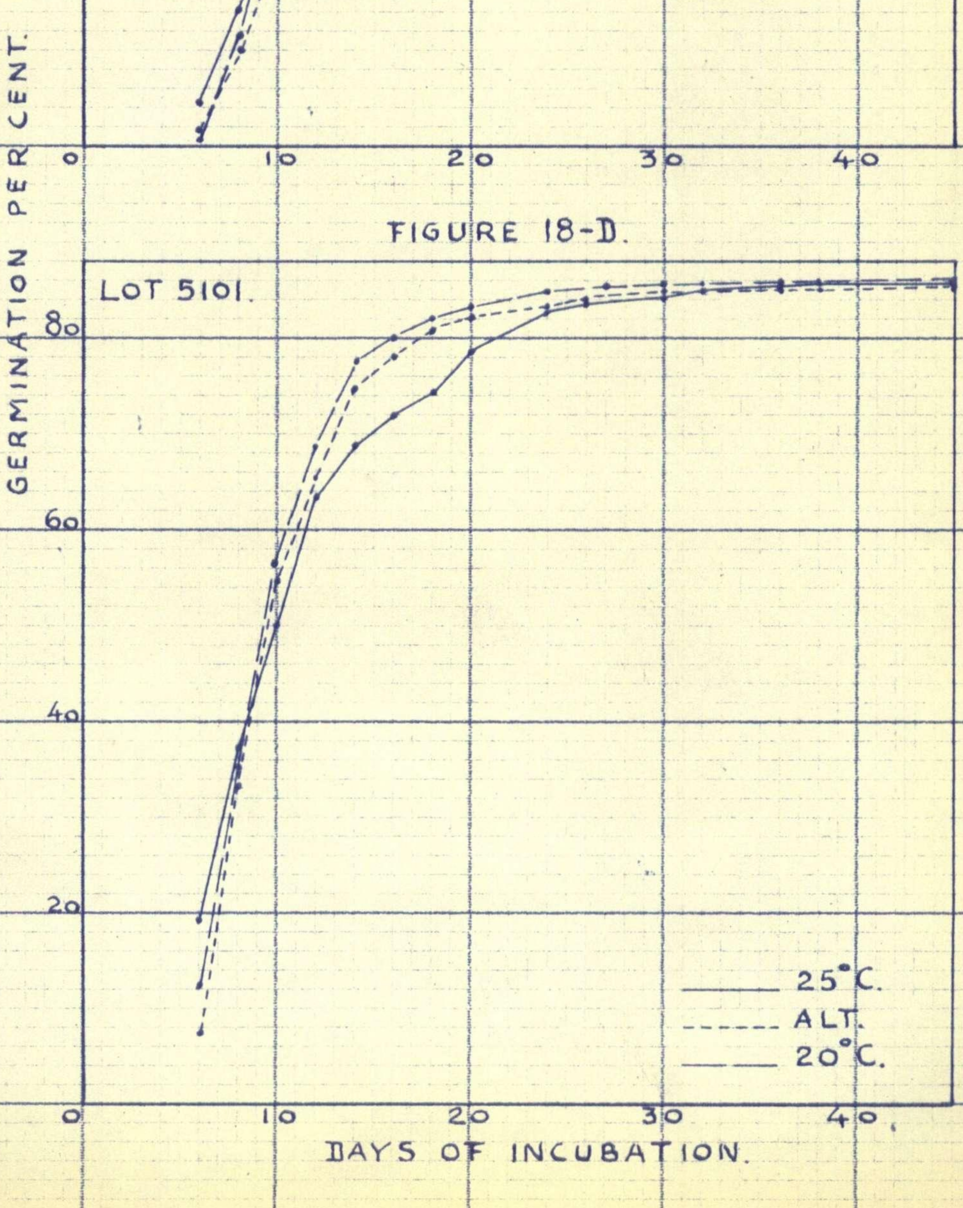


FIGURE 18-D.



Seedlot x stratification period x soaking period

Seed Lot	0 weeks Soaking - (hrs.) ½	2	11	33	3 weeks ½	2	11	33	5 weeks ½	2	11	33	7 weeks ½	2	11	33	9 weeks ½	2	11	33
4907	90	95	98	128	91	134	195	218	104	142	223	249	94	144	216	295	99	122	231	296
4908	103	92	92	118	121	138	194	239	85	103	174	244	85	110	203	263	127	131	202	285
5101	96	96	94	114	122	118	151	264	119	121	181	295	131	142	218	322	137	152	223	316
5102	108	104	107	152	125	137	202	273	116	121	201	295	125	158	229	334	144	168	249	324

L.S.D. for non-ranked data, 5% level = 22.6; 1% level = 29.7.

The underlined figures indicate the highest results obtained for each stratification period. For each seedlot this highest rate of germination is obtained with soaking for 33 hours. Increasing stratification periods effect an increase in the rate of germination. This increase is greatest during the first three weeks of stratification. For non-stratified seed, only the 33 hours soaking periods increase the germination rate to any extent. A significant increase can be shown for lots 4907 and 5102. In general it can be said that all four seedlots show the same reaction to the soaking period--stratification period interaction.

Seedlot x incubation temperature x soaking period

Soaking period	4907 25°C	Alt	20°C	4908 25°C	Alt	20°C	5101 25°C	Alt	20°C	5102 25°C	Alt	20°C
½ hr.	194	131	153	165	139	217	185	172	248	186	180	252
2 "	240	176	221	177	158	239	177	189	263	187	208	293
11 "	343	283	337	264	263	338	263	259	345	285	312	391
33 "	411	342	433	379	354	416	394	423	494	412	437	529

L.S.D. for non-ranked data, 5% level = 35.3; 1% level = 42.6.

The highest values reached for each seedlot are double underlined. The highest values for each seedlot at each soaking period are underlined singly. An incubation temperature of 20°C., combined with 33 hours of soaking, proves to be the best for all seedlots. This highest value obtained is in all cases highly significantly better than the value directly above it (11 hours soaking) or directly to the left of it (alternating temperatures). An exception again is lot 4907. The reasons for the different behaviour of this seedlot have been discussed.

For each soaking period within a seedlot, 20°C. proves to be superior to 25°C. or alternating temperatures. Seedlot 4907, however, forms the exception again.

Stratification period x incubation temperature x soaking period

Soaking period	0 weeks			3 weeks			5 weeks			7 weeks			9 weeks		
	25°C	Alt	20°C	25°C	Alt	20°C	25°C	Alt	20°C	25°C	Alt	20°C	25°C	Alt	20°C
½ hr.	131	113	153	158	111	190	142	115	167	149	126	160	150	157	200
2 "	116	125	146	181	133	213	141	142	204	185	161	208	158	170	245
11 "	120	116	155	255	211	276	231	240	308	272	262	332	277	288	340
33 "	165	153	194	335	274	385	325	348	410	380	389	445	391	392	438

L.S.D. for non-ranked data, 5% level = 29.7; 1% level = 36.4.

Within each stratification period, 20°C. proves to be the most favourable temperature. For soaking periods of ½, 2 and 11 hours the highest germination rate is obtained after 9 weeks of stratification at 20°C. For 33 hours of soaking, the highest value is obtained after 7 weeks of stratification. However, the value for 9 weeks is only slightly lower.

Soaking periods of ½ and 2 hours show a certain fluctuation with increasing stratification periods. Soaking periods of 11 and 33 hours, however, show a steady increase in germination rate with increasing stratification periods.

SUMMARY OF RESULTS AND CONCLUSIONS

The Influence of Seedlots, Stratification, Incubation Temperatures and Seed Moisture Content, Considered Individually, on the Germination Behaviour of Western Hemlock Seed.

Seedlots:

Highly significant differences between the final germination percentage and between the germination rates at 12 days exist between the four seedlots used. In order of decreasing final germination percentages, they can be arranged as follows: 5101, 5102, 4907 and 4908. For the germination rate, this order is somewhat different; 5102, 5101, 4907 and 4908.

Stratification:

The final germination percentage is not influenced by the length of the stratification period. The germination rate, however, is considerably accelerated by stratification. The greatest increase takes place during the first three weeks of stratification. A stratification period of 7 to 9 weeks, however, is required to obtain a satisfactory rapid course of germination.

Incubation Temperatures:

Both final germination percentage and germination rate are influenced by different incubation temperatures. In both cases, an incubation temperature of 20°C. gives significantly better results than 25°C. or alternating temperatures. For the final germination percentage, alternating temperatures are significantly better than 25°C. For the germination rate, however, this order is reversed. The cause of this reversal is the fact that 25°C. produces initially a higher rate of germination than alternating temperatures. As germination proceeds, however, this difference becomes gradually smaller and disappears at the intersection of the two curves. Beyond this point, alternating temperatures produce a higher rate of germination than incubation at 25°C. (See Figure 17).

Soaking periods (Seed moisture content)

Increasing seed moisture contents result in an increase of both the final germination capacity and the germination rate. Soaking the seed for 33 hours produces the best results. During this soaking period, a seed moisture content of 50 ÷ 75 percent is obtained, varying for the different seedlots as shown in Figure 7.

The Influence of the First-order Interactions

Seedlot x stratification period

Although stratification generally does not effect the final germination percentage, a significant increase was found after three weeks of stratification for lot 4907. This difference must be attributed to incomplete germination in 42 days without stratification.

The effect of stratification on the germination rate shows the same pattern for each seedlot used, except lot 4908, which shows unexplained irregularities. For each seedlot, however, 9 weeks of stratification produces the highest germination rate.

Seedlot x incubation temperature

For all seedlots, incubation at 20°C. produces the best results for both the final germination percentage and the germination rate. A temperature of 25°C. generally produces the poorest results and alternating temperatures are intermediate.

The different seedlots seem to differ in sensitivity toward the temperatures employed. For lot 4907, the curves for 20°C. and 25°C. incubation are much farther apart than for any of the other seedlots (See Figures 9 and 10).

Seedlot x soaking period

In general increasing seed moisture contents produce an increase in both final germination percentage and germination rate. The increase of the germination rate is much more pronounced than the increase of the final germination percentage. At equal seed moisture contents, seedlot 4907 shows a considerably lower rate of germination than any of the other seedlots used (See Figure 12-B).

Stratification x Incubation temperature

Increasing the length of the stratification period does not seem to change the temperature requirements for germination of western hemlock seed. An incubation temperature of 20°C. produces the best results for all stratification periods. The influence of temperature on germination seems to be independent of the influence of stratification on the germination behaviour.

Stratification x soaking period

Stratification for a period of 5 weeks or longer, combined with a high seed moisture content, obtained after 33 hours of soaking, increases the final germination percentage.

The stratification period--seed moisture content interaction has a much greater influence on the germination rate than on the final germination capacity. High seed moisture contents (50 - 75 percent), combined with long periods of stratification produce the highest germination rates. Long stratification periods combined with low seed moisture contents (30 ÷ 40 percent) do not increase the germination rate appreciably. (See

Figure 14). The close interrelation between stratification period and seed moisture content is clearly shown.

Incubation temperature x soaking period

Increasing seed moisture contents result in increasing rates of germination. The influence of seed moisture content is independent of the incubation temperature used.

The Influence of the Second-order Interactions

Seedlot x stratification x incubation temperature

As was stated before, incubation temperature reacts independently from stratification period. Although the different seedlots generally react in the same way to the three temperatures used, seedlot 4907 shows a little different behaviour from the other three seedlots.

For three of the four seedlots used, 9 weeks of stratification produces the highest germination rates. Lot 4907 shows the maximum rate after 7 weeks of stratification. The difference with the 9 weeks result is, however, not significant.

Seedlot x stratification x soaking period

Generally, all four seedlots used show the same reaction to the stratification period—seed moisture content interaction. The highest germination rates are obtained after 7 or 9 weeks of stratification at high seed moisture contents, obtained after 33 hours of soaking.

Seedlot x incubation temperature x soaking period

For all seedlots used, the highest germination rates are obtained when seed with a high moisture content (50 - 75 percent, obtained after 33 hours of soaking) is incubated at 20°C. Seedlot 4907, however, shows a significant increase in germination rate after 2 and 11 hours of soaking (39 - 57 percent seed moisture).

Stratification x incubation temperature x soaking period

An incubation temperature of 20°C. is superior in all cases. A stratification period of 9 weeks produces the highest germination rates for seed, that was soaked for ½, 2 and 11 hours. The highest germination rate is produced by stratification for 7 weeks and soaking for 33 hours.

CONCLUSIONS

1. Seedlots

The four western hemlock seedlots used show significant differences in both the final germination percentage and the rate of germination. A cutting test shows the following percentages of filled seed.

lot 4907 - 96 percent
lot 4908 - 93 percent
lot 5101 - 98 percent
lot 5102 - no seed available.

The differences in final germination percentage cannot be explained on the basis of the number of empty seeds.

It is impossible to give specific reasons for the observed differences. Degree of maturity at the time of collection, treatments during the commercial extraction procedure, seed age and storage conditions, may all have a certain influence on the germination behaviour. Concerning the relative importance of these factors, very little is known.

Seedlot 4907 has a few characteristics very different from those for the other three seedlots:

(a) The rate of moisture absorption is considerably higher than that of any of the other seedlots.

(b) The rate of germination is considerably lower than that of the other seedlots, when compared at equal seed moisture contents.

The suspicion that this seed was collected immature, was mentioned before.

2. The Influence of Different Incubation Temperatures

Of the three different incubation temperatures used--constant 25°C., alternating temperatures and constant 20°C.-- a temperature of 20°C. produces the highest final germination percentage and the highest rate of germination.

The effect of the different incubation temperatures on the germination behaviour is not changed by stratification.

3. The Influence of Seed Moisture Content During Stratification

Stratification does not increase the final germination percentage, except for lot 4907. The most important influence of stratification is on the rate of germination. The seed moisture content during stratification is of utmost importance to the germination behaviour. Stratification at moisture contents up to 30 percent (40 percent for seedlot 4907) is ineffective. However, stratification at moisture contents ranging from 35 to 57 percent, increases the germination rate considerably. A moisture content of 50 to 57 percent (75 percent for lot 4907), obtained after 33 hours of soaking, produced the highest germination rates obtained in this experiment.

4. The Influence of the Length of the Stratification Period

The greatest increase in germination rate takes place after 3 weeks of stratification. Longer stratification periods cause a further increase in germination rate, but to a much smaller degree. A stratification period of 9 weeks, the longest one used, produces the highest rate of germination. The difference between the germination rates for 7 and 9 weeks of stratification, however, is comparatively small. Germination has finished in about 21 days, after 9 weeks of stratification.

RECOMMENDATIONS FOR THE TESTING OF WESTERN HEMLOCK SEED

Seed Moisture Content

Bring the seed to a moisture content of 50 to 60 percent (oven-dry weight basis) by soaking for 30 to 35 hours in tap water at room temperature. Surface dry the seed and stratify at 33°F.

Stratification period

Stratify seed for eight weeks. Germination will be finished in three to four weeks.

Incubation Temperature

An incubation temperature of 20°C. constant produces better results than any higher temperatures used. This incubation temperature is probably very close to the optimum germination temperature.

APPENDIX I

The discussion of the influence of different seed moisture contents on the effectiveness of stratification showed that a high seed moisture content, obtained after 33 hours of soaking, produced the best results.

The question arises whether the seed moisture content, obtained in this soaking period, represents the optimum, or is close to the optimum moisture content. In an attempt to answer this question, seed from lot 4907 was soaked for 33, 48, 72 and 96 hours. The method of stratification was the same as previously described. Incubation was carried out only at 20°C. Unfortunately, seedlot 4907 had to be used. This was the only seedlot with sufficient seed left to carry out this additional experiment.

In contrast to the main experiment, where soaking was carried out at room temperature, soaking for this experiment took place in an incubator, set for a constant temperature of 25°C. The reason for this was that the laboratory temperature in the course of this experiment dropped considerably at night, and 25°C. was thought to be closer to the conditions of soaking in the main experiment, although this assumption was later found to be incorrect. For seed soaked 33 hours, the germination percentage was generally five to ten percent lower in this experiment, as compared with the main experiment.

The following seed moisture contents were obtained for the different soaking periods:

33 hours	83.7 percent
48 "	87.9 "
72 "	93.3 "
96 "	100.0 "

The 33 hours soaked "control" obtained a moisture content 8.7 percent higher than that for seed soaked for the same period in the main experiment. This higher moisture content was undoubtedly caused by the higher average temperature at which soaking took place.

The germination results are presented in graph form (Figures 25 and 26). After three weeks of stratification, the curves representing the different soaking periods are fairly regularly distributed.

Seed soaked for 33 hours shows the best germination. After 5, 7 and 9 weeks of stratification very little differences exist among the curves for 33, 48 and 72 hours of soaking. Seed soaked for 96 hours shows a much lower germination capacity.

From this additional information it may be concluded that <u>for seedlot 4907</u> a moisture content of 75 percent is very near the optimum moisture for stratification. No conclusions regarding optimum seed moisture content may be drawn for the other seedlots, on account of the differences in the rate of moisture absorption.

APPENDIX II

The following graphs will illustrate the influence of the different treatments on the germination behaviour. First, the influence of the different incubation temperatures is shown on the germination behaviour of air-dry seed, 33 hours soaked seed, and 33 hours soaked and subsequently stratified seed.

Selecting the best incubation temperature, which is 20°C., the influence of soaking without stratification at this incubation temperature is shown. Then the influence of the different soaking periods on the germination behaviour of stratified seed is shown. Finally, the influence of the different stratification periods on seed, soaked for 33 hours and incubated at 20°C. is illustrated.

Figures 19 and 20: Seedlot 4907. The influence of different incubation temperatures (25°C., 20°C., alternating) on the germination behaviour of non-stratified seed.

19: Seed was not soaked.

20: Seed soaked for 33 hours.

FIGURE 19.

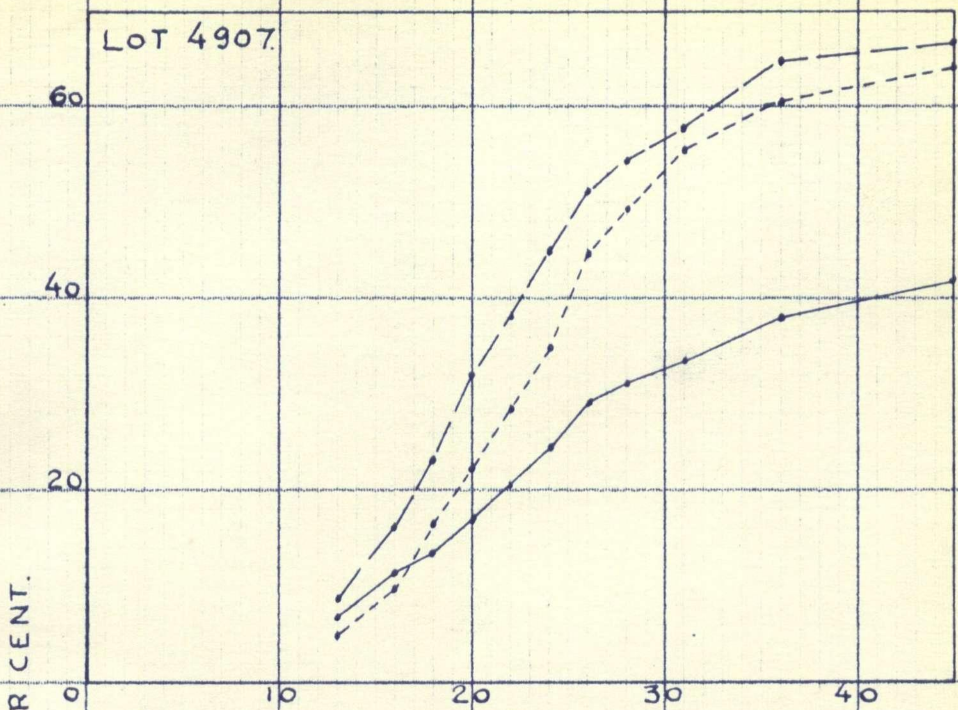
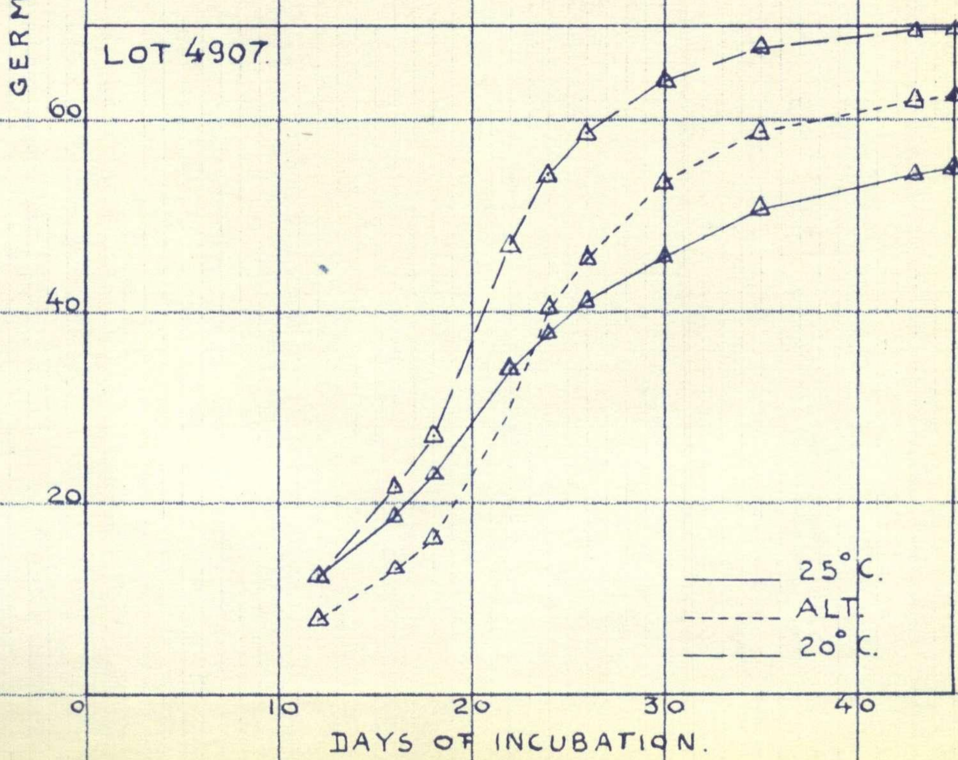


FIGURE 20.



opposite page 103

Figure 21: Seedlot 4907. The influence of different incubation temperatures (25°C., 20°C., alternating) on the germination behaviour of seed, soaked for 33 hours and stratified for 9 weeks.

Figure 22: Seedlot 4907. The influence of different soaking periods (0, $\frac{1}{2}$, 2, 11 and 33 hours) on the germination behaviour of non-stratified seed, incubated at 20°C.

FIGURE 21.

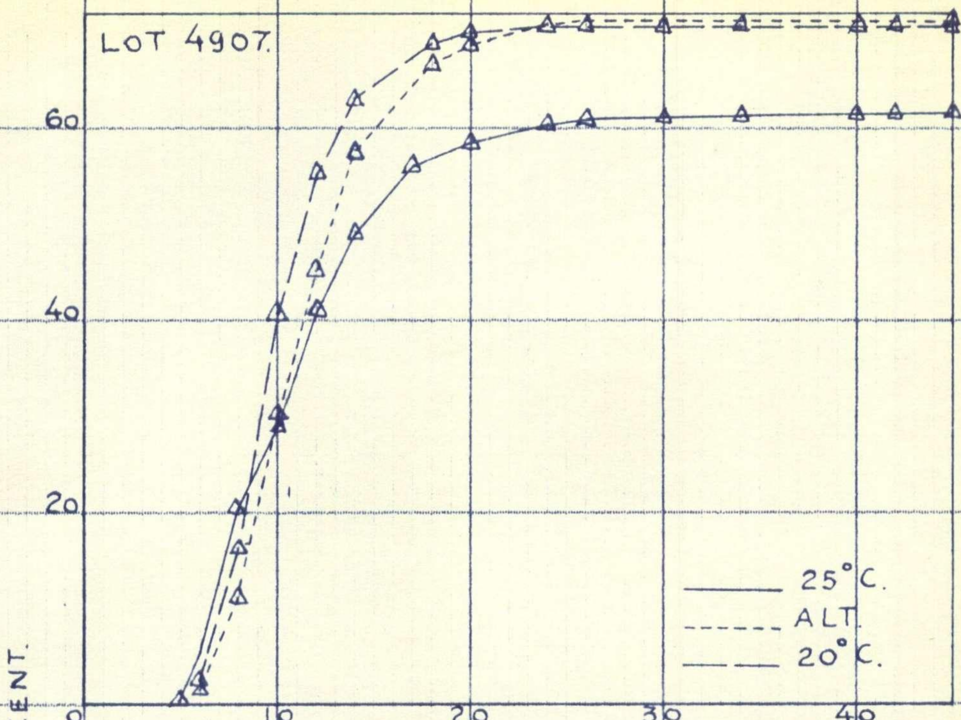


FIGURE 22.

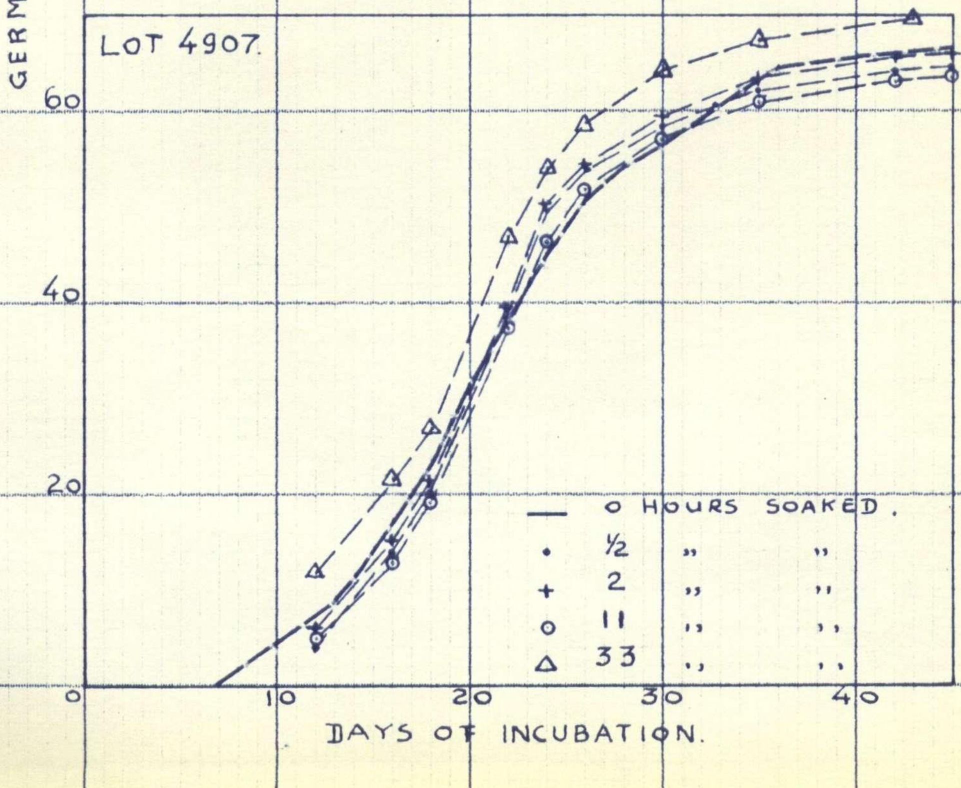


Figure 23: Seedlot 4907. The influence of different soaking periods ($\frac{1}{2}$, 2, 11 and 33 hours) on the germination behaviour of seed stratified for 9 weeks and incubated at 20°C.

Figure 24: Seedlot 4907. The influence of different stratification periods (0, 3, 5, 7 and 9 weeks) on the germination behaviour of seed, soaked for 33 hours and incubated at 20°C.

FIGURE 23.

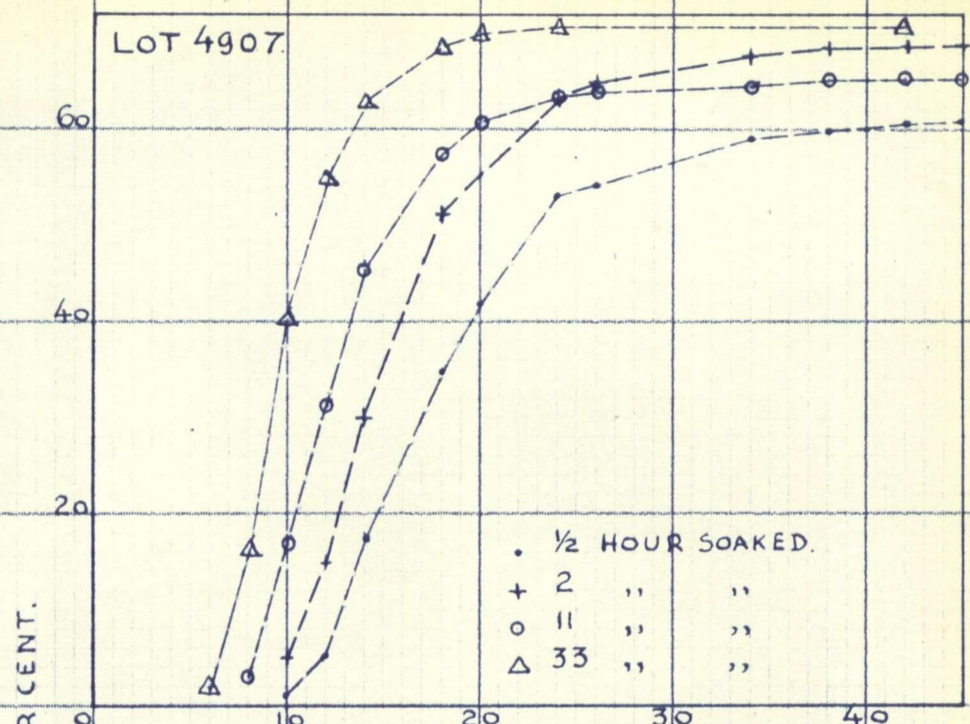
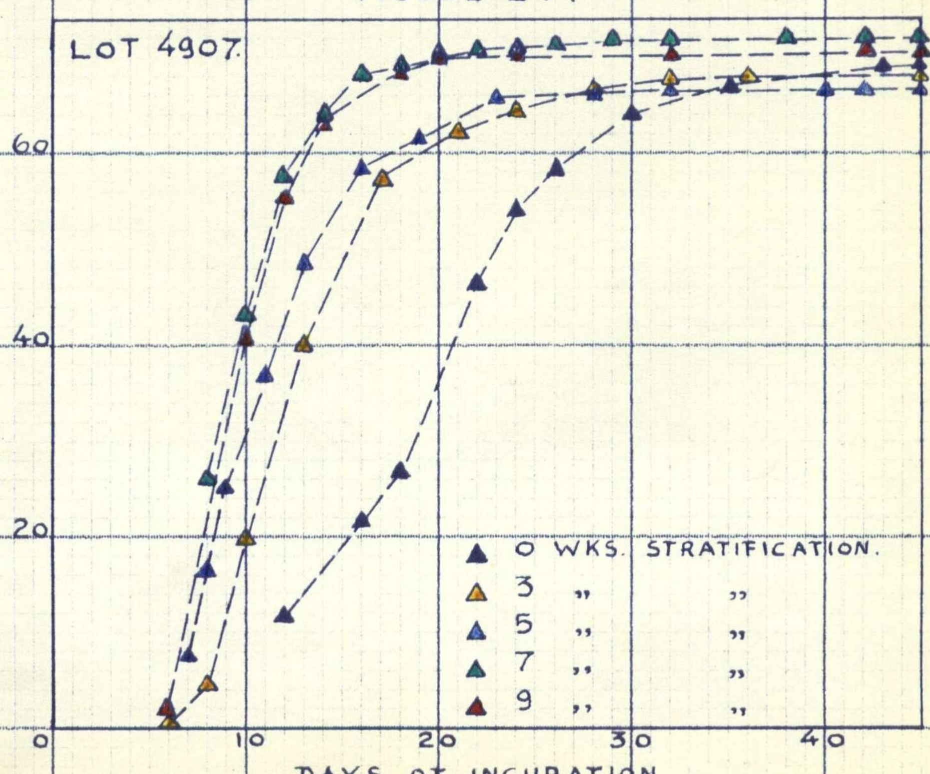


FIGURE 24.



Figures 25 and 26:	Seedlot 4907. The influence of different soaking periods (33, 48, 72 and 96 hours) on the germination behaviour of seed, incubated at 20°C.
25:	Stratified for 3 weeks.
26:	Stratified for 7 weeks.

FIGURE 25.

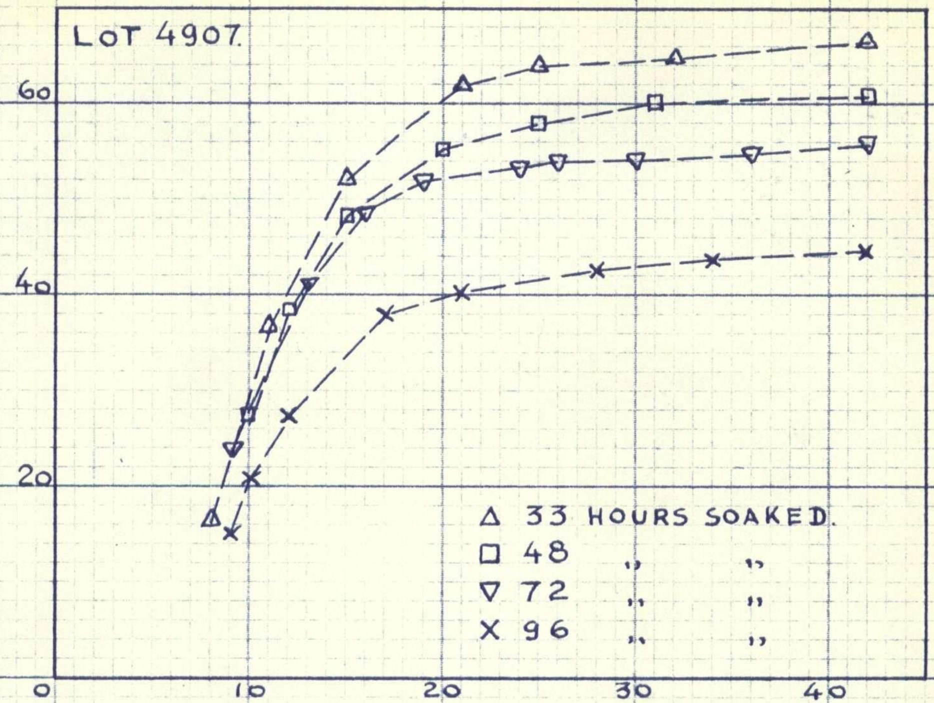
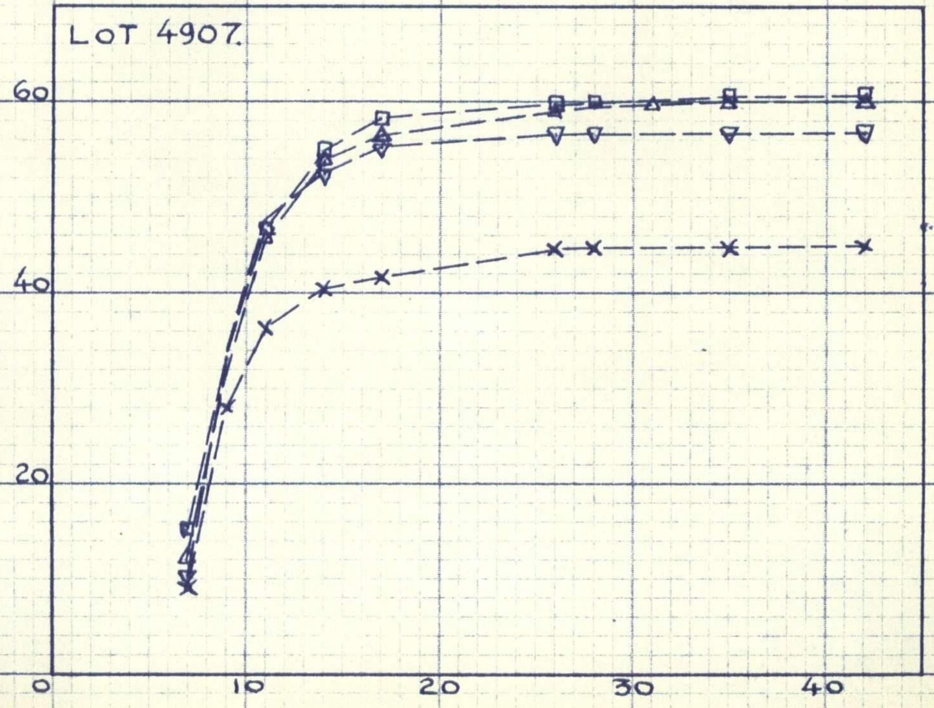


FIGURE 26.



Figures 27 and 28: Seedlot 5102. The influence of different incubation temperatures (25°C., 20°C., alternating) on the germination behaviour of non-stratified seed.

27: Seed was not soaked

28: Seed soaked for 33 hours.

FIGURE 27.

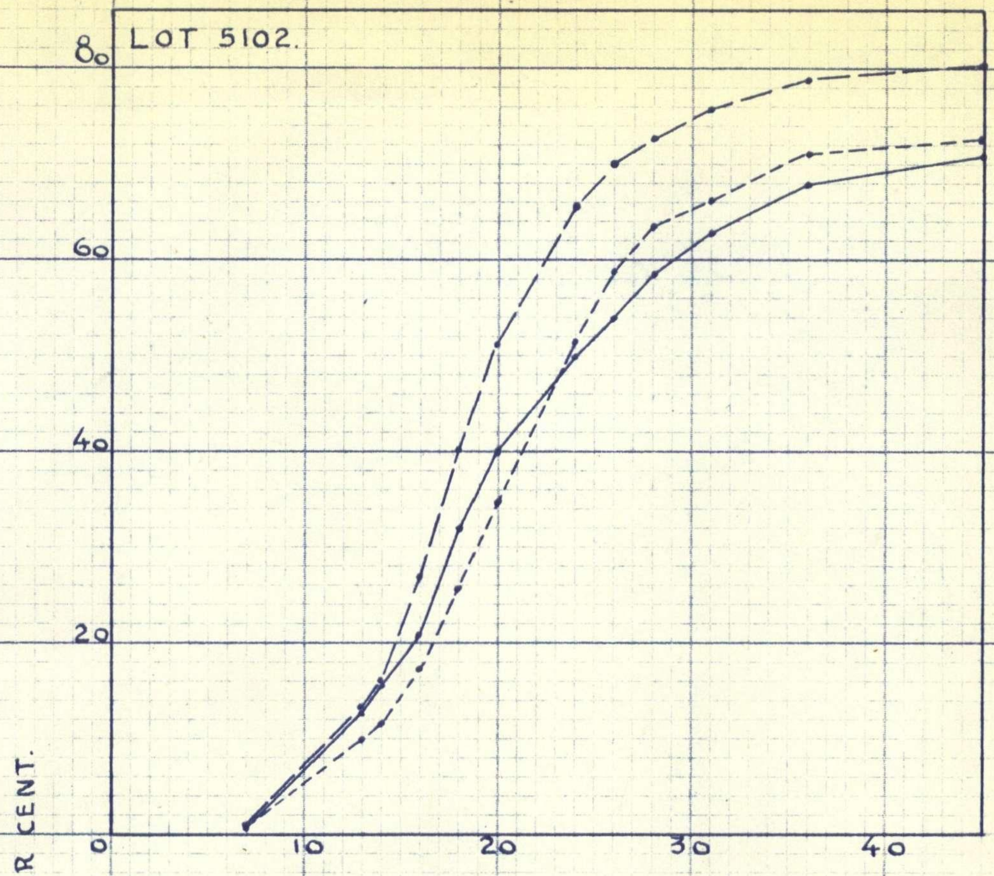


FIGURE 28.

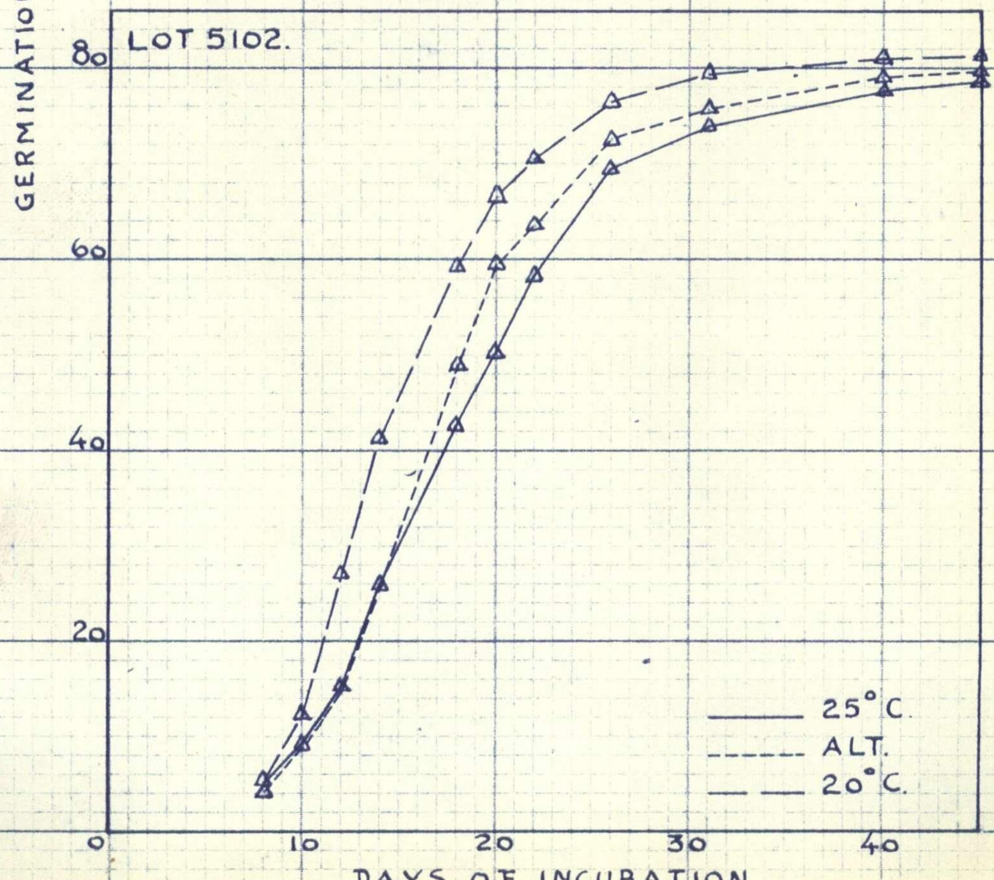


Figure 29: Seedlot 5102. The influence of different incubation temperatures (25°C., 20°C., alternating) on the germination behaviour of seed, soaked for 33 hours and stratified for 7 weeks.

Figure 30: Seedlot 5102: The influence of different soaking periods (0, $\frac{1}{2}$, 2, 11 and 33 hours) on the germination behaviour of non-stratified seed, incubated at 20°C.

FIGURE 29.

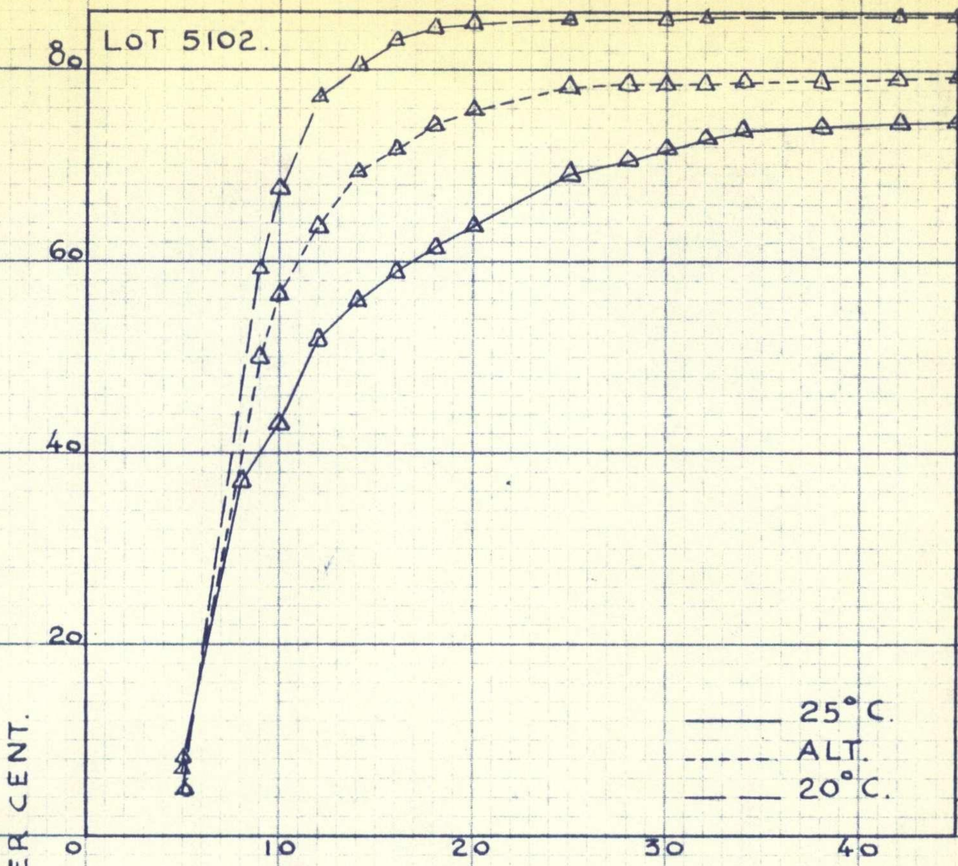


FIGURE 30.

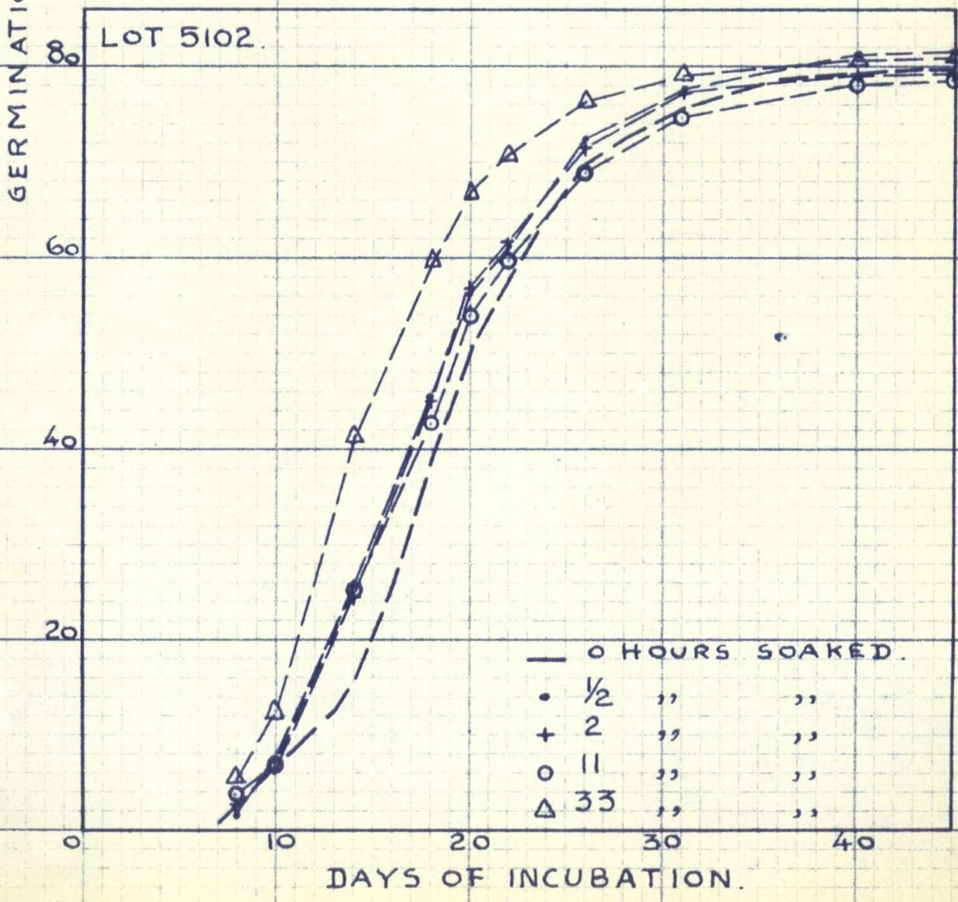


Figure 31: Seedlot 5102. The influence of different soaking periods ($\frac{1}{2}$, 2, 11 and 33 hours) on the germination behaviour of seed, stratified for 7 weeks and incubated at 20°C.

Figure 32: Seedlot 5102. The influence of different stratification periods (0, 3, 5, 7 and 9 weeks) on the germination behaviour of seed, soaked for 33 hours and incubated at 20°C.

FIGURE 31.

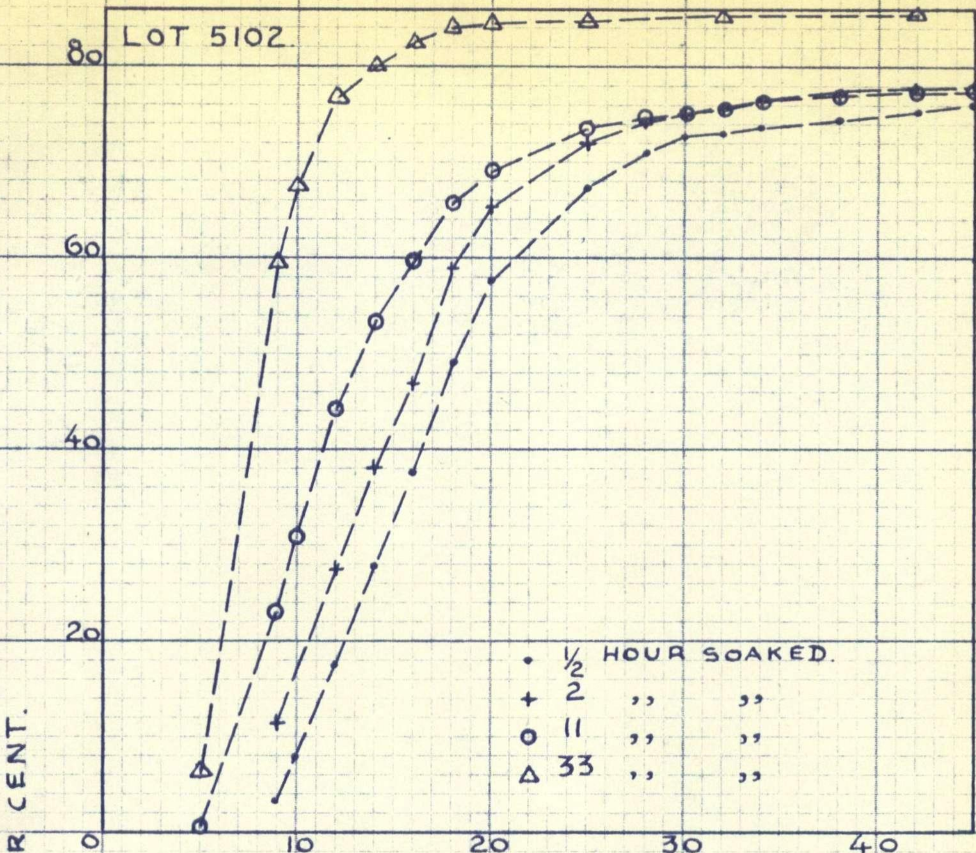
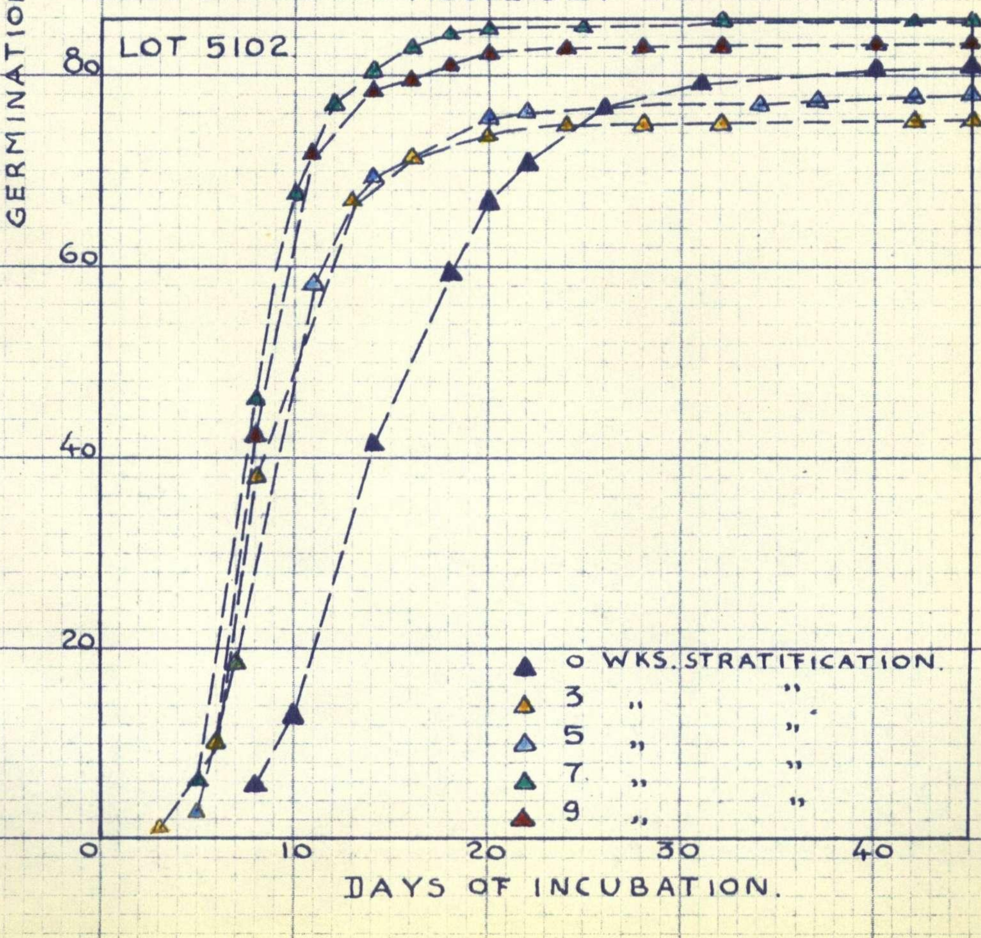


FIGURE 32.



REFERENCES

Acatay, A. (1938). Untersuchungen ueber Menge und Gute des Samenansatzes in Verschiedenen Kronenteilen Einheimisher Waldbaume (Tharandter Forstl.) Jahrb. 89: 265-364. In Baldwin, 1942.

Allen, George S. (1941). A Standard Germination Test for Douglas Fir Seed. The Forestry Chronicle, 17: 75-78.

---------------- (1941). Light and Temperature as Factors in the Germination of the Seed of Douglas Fir. The Forestry Chronicle, 17: 99-109.

---------------- (1942). Parthenocarpy, Parthenogenesis, and Self-Sterility of Douglas Fir. Jour. For. 40: 642-644.

---------------- (1942). Douglas Fir Seed from Young Trees. Jour. For. 40: 722-724.

---------------- (1949-53). Studies on Coniferous Tree Seed. University of British Columbia, unpublished data.

Baldwin, H. I. (1930). The Effect of After-Ripening Treatment on the Germination of Eastern Hemlock Seed. Jour. For. 28: 853-857.

---------------(1935). Seasonal Variations in the Germination of Red Spruce. Amer. Jour. Bot. 22: 392-394.

-------------- (1942). Forest Tree Seed of the Northern Temperate Regions with Special Reference to North America. Chronica Botanica Company, Waltham, Mass.

Barton, Lela V. (1952). Relation of Different Gases to the Soaking Injury of Seeds. II. Contrib. Boyce Thompson Inst. 17 (1): 7-34.

----------------(1953). Seed Storage and Viability. Contrib. Boyce Thompson Inst. 17 (2): 87-103.

Crocker, W. (1948). Growth of Plants. Twenty Years' Research at Boyce Thompson Institute. Reinhold Publishing Corporation, New York.

Crocker, W. and Barton, Lela V. (1953). Physiology of Seeds. An Introduction to the Experimental Study of Seed and Germination Problems. Chronica Botanica Company. Waltham, Mass.

Crossley, D. I. (1953). Seed Maturity in White Spruce. Silvicultural Research Note No. 104. Forestry Branch, Dept. of Resources and Development, Ottawa.

Heit, C. E. and Eliason, E. J. (1940). Coniferous Tree Seed Testing and Factors Affecting Germination and Seed Quality. New York State Agricultural Experiment Station.

Finnis, J.M. (1950). Seed Maturity in Douglas Fir. Research Note No. 18, British Columbia Forest Service, Victoria.

Garman, E. H. (1951). Seed Production by Conifers in the Coastal Region of British Columbia, Related to Dissemination and Regeneration. Technical Publication T.35. British Columbia Forest Service, Victoria.

Gast, P.R., and Wright, J.W. (1941). Size and Germination of Northern White Pine Seed as Influenced by Tree and Cone Characters (Unpub.). In Baldwin, 1942.

Haddock, P.G. (1942). Summary of Dissertation for the Degree of Doctor of Philosophy. University of California.

Hofmann, J. V. (1921). Adaptation in Douglas Fir. Ecology 2: 127-131. In Baldwin, 1942.

Huss, E. (1950). Om Avuningsskador da Skogsfro. On the de-winging damages of seeds. Meddelanden Fran Statens Skogsforskningsinstitut. Band 39. NR. 3.

Maki, T. E. (1940). Significance and Applicability of Seed Maturity Indices for Ponderosa Pine. Jour. For. 38: 55-60.

Marcet, E. (1951). Pollenuntersuchungen an Fohren Verschiedener Provenienz. Dissertation for the Degree of Doctor of Technical Science. Zurich, Switzerland.

Mirov, N. T. (1936). A Note on Germination Methods for Coniferous Species. Jour. For. 34: 719-723.

Munns, E. N. (1921). Effect of Location of Seed upon Germination. Bot. Gaz. 72: 256-260. In Baldwin, 1942.

McIntyre, A. C. (1929). A Cone and Seed Study of the Mountain Pine (Pinus Pungens Lambert). Am. Jour. Bot. 26: 402-406. In Baldwin, 1942.

---------------- (1932). Seeding Habit of Pitch Pine. Forest Leaves 3: 109-111. In Baldwin, 1942.

Nordstrom, L. (1953). A Method of Stimulating the Germination of Pine Seed (Pinus silvestris). Bulletin of the Royal School of Forestry, Stockholm, Stockholm, Sweden, Nr. 12-19: 91-107.

Pack, D. A. (1921). After-Ripening and Germination of Juniperus Seeds. Bot. Gaz. 71: 32-60. In Baldwin, 1942.

----------- (1921). Chemistry of After-Ripening, Germination and Seedling Development of Juniper Seeds. Bot. Gaz. 72: 139-150. In Baldwin, 1942.

----------- (1925). Dispersion of Lipoids. Bot. Gaz. 79: 334-338. In Baldwin, 1942.

Pearson, G. A. (1910). The Influence of Age and Condition of Trees Upon Seed Production in Western Yellow Pine. Mimeo. Report U.S.F.S. In Baldwin, 1942.

-------------- (1931). Source of Seed. Jour. For. 29: 982-984.

Pickford, A.E. (1929). Studies of Seed Dissemination in British Columbia. The Forestry Chronicle, V, No. 4: 8-16.

Quenouille, M.H. (1950). Introductory Statistics. Butterworth-Springer Ltd., London.

Rohmeder, E. (1951). Beitraege zur Keimungsphysiologie der Forstpflanzen. Bayerische Landwirtschaftsverlag, Muenchen.

Schmidt, W. (1930). Die Spiegelung der Jahreszeit in der Samenaktivitaet. Forschungen und Fortschritte. 25: 325-326. In Baldwin, 1942.

Schmidt, W. and Hildebrandt, W. (1930). Rationelle Fortsaatgutreinigung. Zeitschr. F. Forst-u. Jagdw. 62 1-18; 94-114. In Baldwin, 1942.

Stone, L.H. (1930). A Cone, Seed and Seedling Study of Virginia Pine (Pinus virginiana). Ms. Thesis, Penn. State College, State College, Pa. In Baldwin, 1942.

Toumey, J.W. and Korstian, C.F. (1931). Seeding and Planting in the Practice of Forestry. New York.

Toumey, J.W. and Stevens, C.L. (1928). The Testing of Coniferous Tree Seeds at the Yale University 1906-1926. Yale Univ. School of Forestry, Bull. 21.

Westveld, R.H. (1949). Applied Silviculture in the United States. John Wiley & Sons, Inc. N.Y.

Willis, C.P. (1917). Incidental Results of a Study of Douglas Fir Seed in the Pacific Northwest. Jour. For. 15: 991-1002.

Woody Plant Seed Manual, (1948). Prepared by the Forest Service, U.S. Dept. of Agriculture. Misc. Pub. No. 654.