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Effects of some alternating temperatures on western larch seed germination

Paul S. Johnson

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EFFECTS OF SOME ALTERNATING TEMPERATURES ON WESTERN LARCH SEED GERMINATION

by

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B.S.F. Montana State University, 1960

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P.S.J.
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INTRODUCTION

Recently more attention has been given to problems concerning the reestablishment and propagation of western larch (Larix occidentalis Nutt.) in the field and nursery. Fundamental to a solution of such problems is a knowledge of the germinative behavior of the seeds of western larch. Both the internal condition of the seed, and its environment may be limiting factors in germination. The readiness of a seed to germinate can usually be induced by some method of pretreatment. However, environmental factors, particularly under field conditions, are often beyond control and unpredictable.

Environmental variables are countless, but as pointed out by Show (43), the factors primarily responsible for both rapidity and total amount of germination of a given lot of seed are: (1) temperature of soil, (2) character of soil, (3) amount of light, (4) amount of available water, and (5) depth of cover. Show also emphasized that the last three factors can be fairly well standardized, both in the nursery and greenhouse, and that the character of the soil is predetermined. Under these conditions, temperature is the uncontrolled variable.

The objective of this study was to simulate field diurnal temperature fluctuations, and study their effect on western larch seed germination. Four seed pretreatments were compared: (1) seeds soaked in 3% hydrogen peroxide (H₂O₂) for 24 hours, (2) same as (1) but soaking was followed by drying the seeds at room temperature for 12 hours, (3) seeds stratified for 40 days, and (4) seeds with no pretreatment (control).
The H$_2$O$_2$ pretreatment was used because of recent interest in its effect as a pretreatment to hasten germination of larch seeds in laboratory and field studies. Stratification is the pretreatment generally suggested for western larch (47), and was used as a comparison.
Although western larch can reproduce itself well on block clear-cut areas during a good seed year (40), the need for supplemental seeding or planting has not been eliminated. Roe (35) reports stocking was correlated with seed supply in his studies on western larch regeneration. Although larch rates as a good seed-producing species (6), and at least a small amount of seed is produced every year (26), a good seed crop occurs only on an average of every four or five years (16). Shearer (40) states that at least one good seed year is required during the regeneration period for adequate seed dispersal to all parts of a 60-acre clear-cut block. Seedbed preparation by scarification or burning is a prerequisite to successful natural regeneration of larch (35). Consequently, the establishment of adequate stocking may be determined by the seed crop during the year of seed bed preparation. Since the chances of this are only about one in four, there is need for further study of larch seed germination behavior to facilitate field and nursery seedling establishment.

Dormancy

Seed of many trees often fail to germinate even when environmental conditions, such as temperature, moisture, oxygen, and light are suitable (47). These seeds are referred to as dormant, and stimulation to induce germination is necessary. Baldwin (3) defines the term stimulation as hastening of germination by artificial means.

According to the Woody Plant Seed Manual (47), there are two main
causes of seed dormancy: "(1) An impermeable or hard seed coat which prevents water and oxygen from reaching the embryo or in some cases prevents the embryo from breaking through the seed coat even though water has been able to pass in; and (2) conditions of the embryo or stored food within the seed which prevent germination." With larch, as with most conifer seeds, certain chemical changes in the stored food or embryo must take place before germination can begin.

Methods of hastening seed germination

Stratification. The most commonly used method of breaking dormancy is stratification, that is, placing the seeds in an environment where abundant moisture and oxygen are available, usually at a temperature between 32° and 41° F. for one to four months (47). This method of pretreatment is similar to the conditions to which the seeds are exposed during the winter on the ground out-of-doors.

Cold stratification is usually carried out by placing the seeds in layers between moist sand or vermiculite, or some other suitable moisture holding, but aerated medium. Hosner (22) compared storing moistened loblolly pine seed in polyethylene bags to stratification in sand as a pregermination treatment. There were no statistically significant differences between the two treatments in either the percentage of total germination or the number of days required to reach 90% of total germination.

Bonner and Galston (7) described the mechanism of stratification as depleting or destroying the "inhibitor of the endosperm making possible germination and growth of the embryo." It was thought that the depletion proceeded more rapidly at low temperature than at high. In
hawthorne (Crataegus), the inhibitor was thought to be related to auxin, for it was shown that treatment of non-dormant stratified seeds with auxin reinduced dormancy. Dormancy could then be broken by repeated cold treatment. In studying the nitrogen metabolism of almond (Amygdalus communis L.) seeds during stratification, Blagovescenskii (5) showed that after the twentieth day of stratification, there occurred a progressive breakdown of proteins into simpler compounds still preserving the properties of proteins. Proteolytic enzyme activity increased at this time also.

In one of the earlier studies on western larch germination in the nursery (48), it was shown that unpretreated seed germinated more promptly and fully the first year when fall sown, than when spring sown. Seeds soaked in water for 5 days gave prompt germination. In field practice, however, this pretreatment had the disadvantage of increasing the difficulty of obtaining uniform broadcast sowing due to the soaking. Another objection was the danger of sprouted seeds suffering damage from exposure during sowing. Stoeckeler and Jones (46) also stated that embryo dormancy can be overcome by fall seeding, but pointed out that stratifying the seed in moist sand or peat for 30 or more days (depending on species) at 40°-50° F. immediately preceding spring sowing resulted in prompt and full germination.

Dementjev (11) placed Siberian larch (Larix sibirica Ledeb.) seeds in muslin bags large enough for the seeds to form a thin layer, and buried them under the snow in December. Seed stored in this manner were moist at sowing time, and germinated in 7 days, yielding 80% more seedlings than seed stored dry.

The rate of Douglas-fir seed germination increased with duration.
of stratification (2). Comparatively rapid germination was obtained at 50° F. after a stratification period of 80 days or longer. It was implied that there may be a possible application of this principle to nursery practice in localities subject to cold spring weather. Neither light nor temperature was found to be a limiting factor in the germination of Douglas-fir seed which had been stratified or overwintered on the ground (1).

**Other physical pretreatments.** Beltram (4) found that larch seeds (species not stated) steeped for 14 days in cold water that was changed every other day all germinated within 8 days in the germinator. A proportion of three liters of water to one liter of seeds was recommended. Also, it was suggested that the soil should be moist at the time of sowing. Schmidt (37) found that soaking western larch seeds in distilled water for 18 days at 33° F. resulted in 96% of total germination within 5 days in a laboratory test. Holmes (20), working with Japanese larch (*Larix leptolepis* (Sieb. & Zucc.) Gord.,) found that soaking gave too little response to be worthwhile. The foregoing indicates there may be considerable differences in the effect of various pretreatments on different species of the genus *Larix*.

Some other physical methods of breaking internal dormancy of some seeds are: radiation, electrical stimuli, and the combined action of temperature and light (3,18). Most of these methods of overcoming dormancy have not been tested using conifer seeds.

**Chemical seed treatments to hasten germination.** Many compounds have been reported to stimulate germination of some seeds under certain circumstances, but relatively few cases of tree seed stimulation have been reported (3). According to Baldwin, Bokorny reported the results
of a large number of chemicals, but very few proved to be effective in stimulating germination, and most hindered germination. Richardson (34) showed that Douglas-fir seed germination was accelerated up to 23 days over non-stratified seed when subjected to light, temperature, and gibberellic acid in a controlled environment. Total germination, however, was not affected.

In testing seeds of European larch (Larix decidua Mill.), Johnson (24) found 1% thiourea and ethylene chlorhydrin treatments increased rate and capacity of germination. Potassium nitrate and red copper oxide treatments did not result in increased rate or capacity of germination. Schmidt (37) showed that soaking western larch seeds in 3% thiourea for 5 minutes or 1 hour did not result in a satisfactory rate of germination for quick laboratory viability tests. Germination was slower than for stratification, steeping, naked stratification, and hydrogen peroxide treatments, but final germination was not considerably different from the other treatments.

The foregoing represents only a partial enumeration of studies using chemicals to stimulate germination of conifer seeds. Many more studies have been undertaken using various chemicals. However, for western larch, and possibly including the entire genus Larix, these investigations appear to be the only ones using chemicals, with the exception of some of the following.

Hydrogen peroxide as a germination stimulant. In 1958 and 1959, Ching (8,9) reported the activation of germination in Douglas-fir seed by hydrogen peroxide (H₂O₂). Hydrogen peroxide was known to be active on different food reserves (starches, proteins, and fats) and proved to break down the fatty food reserves found in the endosperm of conifer
seeds. This subsequently activated the germination processes.

In Ching's experiment (8), seeds from two seed lots were: (1) stratified, (2) soaked in 100 ml. of water, or (3) soaked in 1% H₂O₂ for 6, 12, 24, 36, or 48 hours. It was demonstrated that germination is hastened by H₂O₂ soaking, and that the increase is correlated with duration of soaking. With a seed lot collected at 3,000 to 3,500 feet, twelve hours soaking in 1% H₂O₂ resulted in 50% of total germination in 7 days. With seeds soaked in 1% H₂O₂ for 24, 36, and 48 hours, the time required to obtain 50% of the total germination potential was 2.5, 2.0 and 1.5 days respectively. In seeds soaked with water for 12, 24, 36, and 48 hours, the time required to obtain 50% of the total germination potential was 13.4, 10.5, 8.5, and 8.5 days respectively. Similar findings were obtained for seed collected from 1,500 to 2,000 feet elevation, but the response to treatment was smaller than in seed collected from the high elevation. In general, the data indicated that water pre-soaking could not substitute for stratification, whereas 12 and 24 hour pre-soaking with H₂O₂ increased the germination speed to that of stratified seeds.

Seeds of western larch, Douglas-fir (Pseudotsuga menziesii var. glauca), and subalpine fir (Abies lasiocarpa Hook.) were subjected to 3% H₂O₂ (U.S.P.) as a wetting agent during a germination test by Shearer and Tackle (42). The technique resulted in hastening germination and shortening the test period of larch and Douglas-fir seeds. After 14 days, germinative energy of the H₂O₂-treated seeds was evident, whereas it required 50 to 90 days for control (wetted with water) seeds to attain equal germination. Sub-alpine fir seeds did not respond to H₂O₂ treatment as readily as the other two species. Shearer also used 3% H₂O₂ to

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break the dormancy of subalpine larch (*Larix lyalli* Parl.) (41).

Schmidt (37) found that western larch seeds from several sources soaked in a 3% U.S.P. \( \text{H}_2\text{O}_2 \) solution for 13 hours, resulted in 65, 78, 88, and 93% of total germination in 5, 7, 10 and 14 days respectively. However, sand stratification, naked stratification, and steeping treatments, respectively, required the shortest incubation period to reach 98% or more of the total germination capacity.

Using closely controlled \( \text{H}_2\text{O}_2 \) concentrations, Dhillon (12) showed that western larch seeds soaked in 1% \( \text{H}_2\text{O}_2 \) for 6 hours was the optimum \( \text{H}_2\text{O}_2 \) treatment in terms of rapidity of germination and overall germination among the series of tests he undertook. His investigation included 0, 6, 12, 18, 24, 30, and 36 hour soakings of 0, 1, 2, 3, 4, and 5 percent \( \text{H}_2\text{O}_2 \) solutions. Seeds used in Dhillon's study were from the same source as seeds used in this study.

Ching (8) cites that the first sign of germination of a seed is the rapid increase of respiration which often starts within an hour after the beginning of water inibition. In his studies, he found that seeds soaked with \( \text{H}_2\text{O}_2 \) had a higher respiratory rate than water soaked controls. Subsequently he deduced that \( \text{H}_2\text{O}_2 \) activated germination of Douglas-fir seed by accelerating respiration during the "ante-phase of mobilization", that is, the stage prior to seed coat rupture, characterized by a constant respiratory rate and respiratory quotient, and a temporary stopping of further water uptake. Several mechanisms were thought to account for this activation: (1) Destruction of peroxide by catalase added molecular oxygen, which increased the rate of respiration and facilitated oxidation of fatty substances; (2) Stimulation of lipoxidase by \( \text{H}_2\text{O}_2 \) increased the oxidation rate of unsaturated fatty acids; (3) Peroxidase with \( \text{H}_2\text{O}_2 \)
possibly may have oxidized growth inhibitors (possibly auxin); (4) \( \text{H}_2\text{O}_2 \) acted as an electron acceptor when an appropriate hydrogen donor was present; subsequently the rate of respiration increased. Since the hydrogen peroxide pretreatment seems to have its basis in the effect of oxygen on seeds, a few studies involving \( \text{O}_2 \) and seed germination will be briefly reviewed.

Goo (13) made a physiological study of the seeds of Japanese red pine (\textit{Pinus densiflora} Siev. & Zucc.), Japanese black pine (\textit{P. thunbergii} Parl.), Japanese hemlock (\textit{Cryptomeria japonica}), and Japanese cedar (\textit{Chamaecyparis obtusa}). It was found that \( \text{O}_2 \) consumption by seed increased with the advance of the germination process. Fats decreased in both the embryo and endosperm with the progress of germination, and the nitrogenous compounds (protein) moved from the endosperm to the embryo. With the progress of germination, sugars increased, and starch appeared when the radicle appeared.

Morinaga (29) cites instances in which running water, artificial aeration, or addition of \( \text{H}_2\text{O}_2 \) was successful in germinating some seeds in water, due to an increase of dissolved oxygen. However, he found that with intact cat-tail (\textit{Typha latifolia} L.) and Bermuda grass (\textit{Cynodon dactylon} (L.) Pers.) seeds, reduced oxygen pressure had a very beneficial effect upon germination (31). Shull (44) found the biological role of oxygen very complex, and stated that its effect may not always be due to increasing respiration or oxidation. The role, he stated, may vary in different seeds and plants.

Factors affecting germination other than pretreatment.

Storage. The Woody Plant Seed Manual (47) reports that western larch seed can be kept for 1 or 2 years in sealed containers at room
temperature with an annual loss of about 6 percent of its germination capacity. It was suggested that results would probably be better at a lower temperature storage. Schubert (38) stored western larch seed in air-tight 5-gallon cans at 41° F. for 16 years, after which time the seeds yielded 5% germination capacity. The ideal long-time storage for most coniferous seed, as determined by Heit and Eliason (19), was storage in sealed containers at a temperature of 36.6° F. to 40.2° F., and with a moisture content not exceeding 5 to 8%. The same author noted that Larix seldom gave over 50% germination. Studies at the Danish State seed-testing Station in Copenhagen showed that seeds of European larch could be stored for 7 years without loss of viability if dried to 8% moisture content and kept in air-tight containers (25). It was also pointed out that in a climate like Denmark's, storage could be in an unheated room, fairly well insulated, but not necessarily maintained at a constant temperature.

Periodicity in germinative capacity. In a study on pine and larch seeds, Rehackova (33) concluded that germination showed significant seasonal variations even with strictly controlled storage conditions. The germinative capacity and germinative energy of Scotch pine (Pinus sylvestris L.) and European larch seeds were determined monthly (300 seeds of each per month) for a year and the length of radicle measured. During the year both pine and larch seed were stored in air-tight containers at 46.4° F. In pine, the germinative capacity was relatively high in spring and autumn and relatively low in summer and winter. Larch seed resulted in similar, but less clearly defined periodicity. For both species, high germination capacity resulted in relatively low mean radicle length and vice versa.
In studying pretreatments of cold and wet storage of eastern white pine (*Pinus strobus* L.) Rohmeder (36) found that the effect of the treatment was not always uniformly successful in stimulating germination. Consequently, he recommended that both pretreated and unpretreated seed be used in testing, especially for seed of poorer quality. Jensen and Noll (23), working with Douglas-fir seed from many sources collected and tested over a 12 year period, also noted differences in germinative abilities during certain months of the year. They found that seeds were naturally ready to germinate at certain times of the year, and that cold pretreatments actually hindered germination during these periods. The suggestion was made that both stratified seeds and seeds with no pretreatment be used to determine maximum germinative capacity.

Starckenko (45) studied the germination of Scotch pine, Norway spruce (*Picea abies* L. Karst.), common birch (*Betula verrucosa*), and Siberian peashrub (*Caragana arborescens* Lam.) subjected to variable temperatures of 75.2 to 96.8°F in a Jacobsen germinator. He showed that for all species the germinative capacity is much higher in spring and summer than in autumn and winter, regardless of the age or grade of the seed.

**Previous germination studies employing alternating temperatures.**

Various investigators have studied alternating temperatures in laboratory studies on seed germination, primarily from the standpoint of determining optimum temperature conditions for germination. A review of some of this material may be useful, even though the objective of this study is primarily to get an indication of the probable performance of a given lot of seeds when planted in the field or nursery.
Harrington (17) has reviewed 15 articles in which he found that alternating or fluctuating temperatures were more favorable than constant temperatures for germination of certain seeds. Morinaga (30) also found the same held true for seeds of additional species. Haasis and Thrupp (15) state that such findings might well be expected, since germinating seeds are usually subjected to fluctuating temperatures under natural conditions. Harrington (17) found that temperature changes giving the best germination in the laboratory corresponded closely with field soil temperatures during the period of the most prompt and vigorous production of seedlings. As a result of field tests, he suggested an alternation between 64.4 and 96.8° F. for the germination of many seeds. Harrington also recommended that the upper temperature should be maintained for 6 to 8 hours, and the change to the lower temperature should be fairly rapid. He also stated that securing temperature alternations by transfer between two germinating chambers at fixed temperatures is preferable to the method of heating and cooling a single chamber. This opinion was based on the greater ease, simplicity, and uniformity of temperature control, and the equally good germination results. Baldwin (3) also found daily alternations of temperatures beneficial, possibly because they simulated conditions found in nature with a large diurnal range at seasons when germination occurs. However, he stated that no hypothesis is adequate to explain the effect of alternations.

Morinaga (30) experimented with seeds (primarily grass seeds) subjected to alternating temperatures. He placed seeds in ovens regulated to constant temperatures of 10°, 15°, 22°, 27°, 32°, and 38° C. The alternations were made by daily transfer from one oven to another. Seeds were placed in Petri dishes with moist filter paper. Exposure to the
higher temperature was for 6 hours, and to the lower temperature for 18 hours, but some experiments were carried out to compare the reverse condition. Alternating temperatures were effective in germinating several grass species.

The Livingstons (27) pointed out the complexity of environmental conditions upon an organism. They divided the factors of an environmental complex into water, non-aqueous materials, heat, light, and mechanical conditions. Any one of these was obviously a limiting condition capable of preventing the occurrence of a certain plant in a given area. They also stressed that the variables of an environmental complex are always present in at least two dimensions: that each variable must always be considered with regard to its intensity and also duration. Finally, it was considered that the organism must be realized as in a process of continual internal alteration. Thus a certain intensity and duration of any environmental factor, such as temperature, may be entirely without significant effect upon an unpretreated seed, while the same dimensions of the same factor may be fatal to the same seed when in a more active developmental phase. The Livingstons further stated that the effects produced by any environmental factor cannot be adequately studied unless that factor as well as all the other effective ones are under control by the experimenter.

Haasis (14) enumerated two new experimental variables when working with alternating temperatures: (1) the rate of change of temperature between maximum and minimum, and (2) the time relations of the temperature reversals. Also, Haasis and Thrupp (15) found the rate at which heat passes into or out of a plant varies continually. The temperature of the plant always tends to approach that of the surroundings, and differs from
it only slightly. The temperature of the surroundings, consequently, could then be considered as an index of plant temperature. Environmental temperature, consequently, influences physiological activity.

MacDougal (28) stated that any rational analysis of the effect of environmental complexes upon an organism must be done on the basis of the determinations of the influence of the separate environmental components of which of course temperature is one of the most important.

Show (43) stated that seed in nurseries is probably subjected to a wider variation in temperature than that in the greenhouse, but hesitated to say that this resulted in slower germination. He thought it probable that the accumulated temperatures above 40° F. controlled germination. After examination of thermograph sheets and germination records, Show thought that temperatures down to 32° F. did not retard germination, but appeared to accelerate it unless prolonged.

Davis (10) explained some advantages of alternating temperatures over constant temperatures in the germination of certain seeds. He stated that alternating temperatures in germination of seeds was especially useful in seeds with membranes that restrict gaseous exchange. When such seeds were subjected to high constant temperatures in the germinator, both respiratory intensity and catalase activity increased. Duration of the increase depended upon the temperature and the extent of the restriction of the oxygen supply; later a decline occurred until both respiratory intensity and catalase activity became quite constant regardless of how long the seed remained in the germinator. This condition of the embryo was designated by Davis as fatigue, and was thought to have been capable of passing into a condition of true dormancy. When alternating temperatures were employed, there was a rise in both respiratory intensity and
catalase activity throughout the period of germination. No perceptible
fatigue was apparent. The period of time which was required at each tem­
perature of the alternation depended upon the extent of the restriction
of the membranes and the temperatures used. The higher the upper temper­
ature of the alternation was, the shorter was the time required at that
temperature, and the longer at the lower temperature in order to prevent
fatigue.

The foregoing literature review represents only a partial enumera­
tion of studies which may aid in understanding the present study. However,
the basic concepts involved, particularly the procedures and effects of
seed pretreatments and temperature tests similar to those investigated
here, have been presented.
EXPERIMENTAL PROCEDURE

This study was designed to compare the effect of some alternating temperatures upon the germination of western larch seed. Four seed conditions were compared: (1) seeds soaked in 3% \( \text{H}_2\text{O}_2 \) for 24 hours, (2) seeds soaked in 3% \( \text{H}_2\text{O}_2 \) for 24 hours and dried at room temperature (70°F.) for 12 hours, (3) seeds stratified in moist sand at 38°F for 40 days, and (4) seeds with no pretreatment.

The 3% \( \text{H}_2\text{O}_2 \) concentration and 24 hour soaking duration were used so that a comparison could be made with the only known direct seeding trial using this pretreatment on western larch seed which was carried out during the spring of 1960 at the Coram Experimental Forest in northwestern Montana.\(^1\) It should be pointed out that the larch seeds used at Coram were from the same source and lot used in the present study. However, the \( \text{H}_2\text{O}_2 \) used in the Coram study was a commercial 3% U.S.P. solution of the type obtainable at most drug stores. Variation in concentration of these solutions are not now adequately known, so any rigid comparisons between this study and the Coram study are premature. Dhillon (12), in his study of western larch seed pretreated with \( \text{H}_2\text{O}_2 \), points out the importance of concentration and soaking duration. The concentration of \( \text{H}_2\text{O}_2 \) used in the present study was determined by titration of 30-35% Du-pont "Albone" \( \text{H}_2\text{O}_2 \) with 0.2N potassium permanganate (\( \text{KMnO}_4 \)) and diluted to 3% with distilled water. The method is described by Schumb, Saterfield, and Wentworth (39). Seeds were soaked in \( \text{H}_2\text{O}_2 \) in randomly selected lots

\(^1\) Unpublished data, Intermountain Forest and Range Experiment Station, Missoula Research Center, Missoula, Montana.
of one hundred in 100 ml. beakers, with tops covered, and placed in the
dark.

Half of the seeds subjected to each pretreatment in the Coram di­
eect seeding study were coated with "Endrin" and "Arasan" in a latex binder
as a rodent repellent. The stratified and H₂O₂ pretreated seeds were very
moist at the time of repellent application, necessitating a drying period
of about 12 hours so the latex coating would harden. Therefore, the
"dried out" H₂O₂ pretreatment was used in this study to simulate the con­
ditions of the Coram study. Stratification was used as a pretreatment
because it is the most commonly used, consistently successful method of
pretreatment for western larch seed. Seeds with no pretreatment were used
as the control.

The seed used in the study was collected August 28, 1958, from one
source, which is given below:

Location:  Latitude 48° 13' N
Longitude 113° 43' W
R. 17 W, T. 28 N, Section 15
Near Felix Creek, Flathead National Forest, Montana

Elevation: Approximately 4,000 feet

Collectors: Shearer, Hershberger, Schmidt²

Method of Cone Collection: Primarily from squirrel caches.

Sixteen temperature alternations were used to simulate some of the
temperature fluctuations which might be encountered in the field or nur­
sery in at least part of the natural range of western larch. Temperature

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² Intermountain Forest and Range Experiment Station, Missoula Re­
search Center, Missoula, Montana.
Figure 1

MAP OF WESTERN MONTANA SHOWING SEED SOURCE AREA AND CORAM EXPERIMENTAL FOREST

Scale: 1 inch equals approx. 60 miles

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alternations used were: (°F.)³

| 30° series | 30° - 76° | 30° - 78° | 30° - 80° | 30° - 82° |
| 35° series | 35° - 76° | 35° - 78° | 35° - 80° | 35° - 82° |
| 40° series | 40° - 76° | 40° - 78° | 40° - 80° | 40° - 82° |
| 45° series | 45° - 76° | 45° - 78° | 45° - 80° | 45° - 82° |

Duration of temperatures were approximately 8 hours at the minimum temperature, 6 hours at the maximum temperature, and 10 hours at intermediate temperatures. Temperature alternations are graphically described in the thermograph chart, Figure 2.

Temperature alternations were secured in a refrigerator with a thermostatically controlled heating element which was controlled by an automatic time switch. The 35° and 40° series were placed in a refrigerated room for the 8-hour duration of the low temperature. The 30° and 35° temperature series were run simultaneously, and the 40° and 45° series were run simultaneously at a later time. Since the time required for the temperature in the refrigerator to drop from the maximum to the minimum was practically negligible (see Figure 2), the 35° and 40° series could be manually transferred daily to the refrigerated room without upsetting the temperature treatment pattern. The same applies for reintroducing the 35° and 40° series into the refrigerator for the higher temper-

³ All temperatures referred to from this point on will refer to degrees Fahrenheit, unless otherwise indicated.
Figure 2

THERMOGRAPH CHART OF LABORATORY TEMPERATURE ALTERNATIONS
(30° F. - 82° F.)

The horizontal axis of the chart represents time of day in hours. The vertical axis represents temperature (°F.).
atures. All temperatures were subject to a possible plus or minus 2° F. variation, and any stated temperature represents the mean temperature.

Every five days after the beginning of each low temperature series test, two metal trays containing Petri dishes were placed in the refrigerator. At the beginning of the 30° and 35° as well as the 40° and 45° series, the initial high temperature of the alternating temperature series was 82°. The temperature dropped approximately 2° F. whenever additional trays were introduced into the refrigerator, which occurred every five days. Subsequently, the seeds for all treatments were subjected to a series of alternating temperatures wherein the initial high temperature of each alternation decreased 2° F. every 5 days for the first 15 days. The procedure may be made more clear with an example: The alternation termed 30° - 82° actually consisted of temperatures of 30° - 82° for 5 days, 30° - 80° for 5 days, and 30° - 78° for 5 days. The following table graphically describes the procedure:

Table 1

<table>
<thead>
<tr>
<th>Stated as high of Alternating Temperature</th>
<th>Actual high temperatures encountered for any stated alternating temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>82°</td>
<td>82°</td>
</tr>
<tr>
<td>80°</td>
<td>80°</td>
</tr>
<tr>
<td>78°</td>
<td>78°</td>
</tr>
<tr>
<td>76°</td>
<td>76°</td>
</tr>
</tbody>
</table>

The horizontal bars represent actual high temperatures encountered and the duration of each high temperature for a stated temperature alternation.
The reason for the maximum temperature drop of 2° F. whenever additional trays were introduced into the refrigerator was probably due to the additional surface area which the heating element was required to heat up every 24 hours. Possibly minor changes in the duration of the maximum temperature may have taken place also, but such changes were not discernible on the thermograph sheets. It would have been difficult to notice changes in duration of maximum temperature for periods of less than 30 minutes with the thermograph equipment available. However, even a short period of 30 minutes or less might possibly have accounted for differences in germination. Minimum temperatures and duration of minimum temperatures were very constant, and any variation in this range was probably negligible.

The alternating temperature procedure is admittedly awkward, and arose somewhat accidentally from an original experimental design which did not include any variance in high temperature. The original design was planned to employ a consistently re-occurring high temperature of 82° F. with each of the four low temperatures. But in addition to this, it was planned that seeds would be exposed to temperatures for various alternating temperature durations. Furthermore, seeds of the four pretreatments would be germinated at a constant temperature for comparative purposes. Also, seeds were to be introduced to alternating temperatures at five-day intervals to facilitate the removal of all seeds of the various alternating temperature durations at the same time. These were then to be placed in a bench at a constant temperature.

Because of the abnormally high evaporation rates within the plastic Petri dishes used and the resultant seed desication when in the greenhouse bench, much of the data of the original study was discarded.
Only the portions of the study which were without experimental bias were utilized. Consequently, the germination period had to be limited to 15 days. The legitimacy of using such a short term germination might be questioned, and is discussed under the section of "Discussion and Application of Results" of this study.

Table 7 shows the weekly maximum-minimum temperatures encountered at the Coram Experimental Forest, in northwestern Montana, during the spring and early summer of 1960. This was the site and time of a western larch direct seeding study using \( \text{H}_2\text{O}_2 \) pretreated seeds, stratified seeds, and non-pretreated seeds. A thermograph chart showing some of the temperature fluctuations are shown in Figure 3. The results of the latter study are shown in the "Discussion and Application of Results" section of this paper.

Preliminary to pretreatment and germination, the seed lot was mixed systematically to facilitate randomization. The seeds were then proportionally sampled by spreading them onto a table and dividing the lot into eight equal sections. The seeds were counted out into lots of 100 by removing 12 or 13 seeds from each eighth section of the seed lot until enough seed was obtained. Four replications of 100 seeds each were placed on two layers of moistened Whatman No. 3 filter paper in plastic Petri dishes. The Petri dishes were placed on metal trays and placed in the refrigerator (or cooler room for part of the time). There were 16 Petri dishes per tray, which consisted of the four pretreatments with 4 replications, randomized in a 4 x 4 Latin square. The trays were randomized on the shelves within the refrigerator. Maximum and minimum temperatures were recorded daily with maximum-minimum thermometers, and temperature trends were recorded with a thermograph.
Figure 3

THERMOGRAPH CHART OF FIELD TEMPERATURES
CORAM EXPERIMENTAL FOREST, JUNE 8 - 12, 1960.

The horizontal axis of the chart represents time of day in hours. The vertical axis represents temperature (°F.).
In preparation for stratification, sand was sterilized by heating in quart "Pyrex" dishes to 500° F. for four hours. After the sand was cooled, seeds were placed in the sand in lots of 100 in cotton muslin bags about ¾ inch in diameter with tops bound with rubber bands. Seeds and sand were placed in five quart crocks and stored at 38° F. for 40 days. Eight hundred seeds were stratified every five days so that in the initiation of each temperature test at 5-day intervals, stratified seeds used would all have been stratified for 40 days.

The first seeds were placed in the alternating temperature chamber on November 28, 1960. The first portion of the study, which included the 30° and 35° series was concluded on December 23, 1960. The next tests, the 40° and 45° series, were begun on December 23, 1960, and completed January 22, 1961.

Seeds were counted as germinated if radicles were 1 mm. or greater in length. Germinates were counted every day.
ANALYSIS OF DATA AND RESULTS

The experimental design of this study permitted an analysis of variance. The germination data was analyzed after a 15-day incubation period at the various alternating temperatures stated. The analysis was divided into two major sections: (1) analysis of the 30° and 35° series, and (2) analysis of the 40° and 45° series. The analysis was divided into the two sections because it was possible that the differences in time at which the seeds of the two series were germinated might have caused variations in germination. Also, other minor differences in the two periods might have been present. Examination of the data (Appendix I) shows that time or other factors may have affected the results. Individual analyses of the 30° and 35°, and 40° and 45° series were also calculated when these were thought useful.

Analysis of variance 30° and 35° series.

Results of the analysis of variance for all temperature alternations where 30° or 35° was the low temperature component of the alternation are shown:

Table 2.
ANALYSIS OF VARIANCE, 30° and 35° SERIES

<table>
<thead>
<tr>
<th>Category</th>
<th>D.F.</th>
<th>Variance</th>
<th>Sample F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>127</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Replication</td>
<td>3</td>
<td>31.78</td>
<td>1.15</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>3</td>
<td>10,245.22</td>
<td>372.15**</td>
</tr>
<tr>
<td>High Temp.</td>
<td>3</td>
<td>100.78</td>
<td>3.66*</td>
</tr>
<tr>
<td>Low Temp.</td>
<td>1</td>
<td>192.57</td>
<td>6.99*</td>
</tr>
<tr>
<td>High x Low</td>
<td>3</td>
<td>17.68</td>
<td>0.64</td>
</tr>
<tr>
<td>Prett., x Low</td>
<td>3</td>
<td>16.21</td>
<td>0.59</td>
</tr>
<tr>
<td>Prett., x High</td>
<td>9</td>
<td>105.62</td>
<td>3.84*</td>
</tr>
<tr>
<td>Error</td>
<td>102</td>
<td>27.53</td>
<td>---</td>
</tr>
</tbody>
</table>

* F values significant at the 5 percent confidence level.
** F values significant of the 1 percent confidence level.
Pretreatment F value. Pretreatment F value is highly significant, indicating that there are significant differences in the response of seeds to the various pretreatments. Duncan's Multiple Range Test (Figure 4) shows all pretreatments to be significantly different.

High temperature F value. The high temperature F value is significant. The high temperature category isolates the effect of the high temperature component (75°, 78°, 80°, and 82°).

Low temperature F value. The low temperature F value is highly significant, showing that the difference in germination between the 30° and 35° series is significant. The low temperature category isolates the effect of the low temperature components, 30° and 35°.

Pretreatment x high temperature interaction. This interaction is highly significant, and indicates the possible usefulness of breaking the analysis down by pretreatment, e.g. an analysis for each pretreatment (32). The resultant breakdown analysis would indicate which pretreatments contain high temperature components with significantly different effects. All other categories were insignificant.

Breakdown of analysis, 30° - 35° series.

This section of the analysis consists of a separate analysis of variance for each pretreatment. The following analyses make apparent the pretreatments which are affected by the high temperature of any temperature alternation in the 30° and 35° series.

H2O2 pretreated seeds. Results of the analysis of variance for the 3% H2O2, 24-hour soaking pretreatment are shown below:
Figure 4

MULTIPLE RANGE TEST FOR PRETREATMENTS - 5% CONFIDENCE LEVEL
(30° - 35° SERIES)

Note: If the cross-hatched part of a bar overlaps any part of another bar to its left, the difference between the two bars is NOT significant.*

AVERAGE CUMULATIVE GERMINATION % AT 15 DAYS

<table>
<thead>
<tr>
<th>PRETREATMENT</th>
<th>Control</th>
<th>H₂O₂ (Dried)</th>
<th>H₂O₂</th>
<th>Stratified</th>
</tr>
</thead>
</table>

* All pretreatments are significantly different.
Table 3.
ANALYSIS OF VARIANCE FOR H$_2$O$_2$ PRETREATMENT, 30° and 35° SERIES

<table>
<thead>
<tr>
<th>Category</th>
<th>D.F.</th>
<th>Variance</th>
<th>Sample F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication</td>
<td>3</td>
<td>15.04</td>
<td>0.28</td>
</tr>
<tr>
<td>High Temp.</td>
<td>3</td>
<td>391.38</td>
<td>7.29**</td>
</tr>
<tr>
<td>Low Temp.</td>
<td>1</td>
<td>120.13</td>
<td>2.24</td>
</tr>
<tr>
<td>High x Low</td>
<td>3</td>
<td>7.87</td>
<td>0.15</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>52.71</td>
<td></td>
</tr>
</tbody>
</table>

** F values significant at the 1 percent confidence level.

The high temperature F value is highly significant, indicating there are differences in response of the seeds to the high temperature components of the alternation. A comparison is made between high temperatures (76°, 78°, 80°, and 82°) using Duncan's Multiple Range Test (Figure 5b). All other F values are insignificant.

Analyses for all other pretreatments showed no significant F values for any of the categories.

Analysis of variance, 40° and 45° series.

Results of the analysis of variance for all temperature alternations where 40° or 45° was the low temperature component are shown below:

Table 4.
ANALYSIS OF VARIANCE, 40° and 45° SERIES

<table>
<thead>
<tr>
<th>Category</th>
<th>D.F.</th>
<th>Variance</th>
<th>Sample F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication</td>
<td>3</td>
<td>22.43</td>
<td>0.85</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>3</td>
<td>9,420.47</td>
<td>357.51**</td>
</tr>
<tr>
<td>High Temp.</td>
<td>3</td>
<td>531.70</td>
<td>20.18**</td>
</tr>
<tr>
<td>Low Temp.</td>
<td>1</td>
<td>32.00</td>
<td>1.21</td>
</tr>
<tr>
<td>High x Low</td>
<td>3</td>
<td>77.79</td>
<td>2.95*</td>
</tr>
<tr>
<td>Prett. x High</td>
<td>9</td>
<td>131.13</td>
<td>4.98**</td>
</tr>
<tr>
<td>Prett. x Low</td>
<td>3</td>
<td>11.81</td>
<td>0.45</td>
</tr>
<tr>
<td>Error</td>
<td>102</td>
<td>26.35</td>
<td></td>
</tr>
</tbody>
</table>

* F values significant at the 5 percent confidence level.
** F values significant at the 1 percent confidence level.

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15 DAY GERMINATION BY PRETREATMENT FOR HIGH TEMPERATURES
(30° - 35° SERIES)

\textbf{a/} If the cross-hatched part of a bar overlaps any part of another bar to its left, the difference between the two bars is \textbf{NOT} significant.

\textbf{b/} Horizontal axis represents the high temperature component of the temperature alternations.
Pretreatment F value. Pretreatment F value is highly significant, indicating that there are significant differences in the response of seeds to the various pretreatments. Duncan's Multiple Range Test shows all pretreatments to be significantly different (Figure 6).

High Temperature F value. The high temperature F value is highly significant, indicating there are significant variations between some high temperature components ($76^\circ$, $78^\circ$, $80^\circ$, and $82^\circ$).

High temperature x low temperature interaction. This interaction is significant, showing that there are real differences in germination due to low temperatures for the high temperature components.

Pretreatment x high temperature interaction. This interaction is highly significant, and indicates the possible usefulness of breaking down this analysis by pretreatment, e.g. an analysis for each pretreatment. The resultant breakdown analysis would indicate which pretreatments contain high temperature components with significantly different effects. All other categories were insignificant.

Breakdown of analysis, $40^\circ - 45^\circ$ series.

This section of the analysis consists of a separate analysis of variance for each pretreatment. The following analyses show the pretreatments which are affected by the high temperature of any temperature alternation in the $40^\circ$ and $45^\circ$ series.

H$_2$O$_2$ pretreated seeds. Results of the analysis of variance for the 3% H$_2$O$_2$ 24-hour soaking pretreatment are shown below:
Figure 6

MULTIPLE RANGE TEST FOR PRETREATMENTS - 5% CONFIDENCE LEVEL
(40° - 45° SERIES)

Note: If the cross-hatched part of a bar overlaps any part of another bar to its left, the difference between the two bars is NOT significant.*

* All pretreatments are significantly different.
Table 5.
ANALYSIS OF VARIANCE FOR H\textsubscript{2}O\textsubscript{2} PRETREATMENT, 40\textdegree and 45\textdegree SERIES

<table>
<thead>
<tr>
<th>Category</th>
<th>D.F.</th>
<th>Variance</th>
<th>Sample F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>31</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Replication</td>
<td>3</td>
<td>24.20</td>
<td>1.62</td>
</tr>
<tr>
<td>High Temp.</td>
<td>3</td>
<td>110.95</td>
<td>7.44**</td>
</tr>
<tr>
<td>Low Temp.</td>
<td>1</td>
<td>1.09</td>
<td>0.07</td>
</tr>
<tr>
<td>High x Low</td>
<td>3</td>
<td>459.43</td>
<td>38.81**</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>14.91</td>
<td>---</td>
</tr>
</tbody>
</table>

** F values significant at the 1 percent confidence level.

The high temperature F value is highly significant, indicating there are differences in the response of H\textsubscript{2}O\textsubscript{2} pretreated seeds to the high temperature components of the alternations. A comparison is made between the effects of high temperatures on the germination of this pretreatment using Dencan's Multiple Range Test (Figure 7b).

The high x low temperature interaction is also highly significant, indicating there is a significant difference in germination due to high temperatures for the two low temperatures. The analyses (Appendix II) show high temperatures for both 40\textdegree and 45\textdegree series to be highly significant. However, there are some differences between the 40\textdegree and 45\textdegree series in the effect of individual high temperatures, as shown by Duncan's test.

H\textsubscript{2}O\textsubscript{2} (dried) pretreatment. Results of the analysis of variance for seeds subjected to soaking in 3% H\textsubscript{2}O\textsubscript{2} for 24 hours followed by 12 hours drying are shown below:
15 DAY GERMINATION BY PRETREATMENT FOR HIGH TEMPERATURES 
(40° - 45° SERIES)

\[ a/ \] If the cross-hatched part of a bar overlaps any part of another bar to its left, the difference between the two bars is NOT significant.

\[ b/ \] Horizontal axis represents the high temperature component of the temperature alternations.
Table 6.
ANALYSIS OF VARIANCE FOR H₂O₂ (DRIED) PRETREATMENT

<table>
<thead>
<tr>
<th>Category</th>
<th>D.F.</th>
<th>Variance</th>
<th>Sample F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication</td>
<td>3</td>
<td>4.38</td>
<td>0.12</td>
</tr>
<tr>
<td>High Temp.</td>
<td>3</td>
<td>328.62</td>
<td>9.06**</td>
</tr>
<tr>
<td>Low Temp.</td>
<td>1</td>
<td>42.78</td>
<td>1.18</td>
</tr>
<tr>
<td>High x Low</td>
<td>3</td>
<td>52.86</td>
<td>1.46</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>36.26</td>
<td></td>
</tr>
</tbody>
</table>

** F values significant at the 1 percent confidence level.

The F value for high temperature is highly significant, which shows that there are real differences in the response of H₂O₂ (dried) pretreated seeds to the high temperature components of the alternations. A comparison is made between the effects of high temperatures on the germination of this pretreatment using Duncan's Multiple Range Test (Figure 7c). All other categories are insignificant.

Analyses for all other pretreatments showed no significant F values for any of the categories.

Constant temperature controls.

The data for the original constant temperature controls were discarded because of seed dessication, as explained on page 23. However, some seeds were germinated during the period of October 16, 1960, to December 15, 1960, which might reasonably be used as a substitute for the original controls. Unfortunately, data for the dried H₂O₂ pretreatment was not available. The seeds were from the same source and lot as used in this study, and were germinated in glass Petri dishes on a greenhouse bench at 80° to 84° F. Four hundred seeds per pretreatment were used. No statistical comparison could be made between the latter data, and data of this study, but general comparison may still be useful. Figure 8 illustrates the constant temperature germination data. Other data for germination energy and percent is illustrated in Appendices III to VII.
SEEDS GERMINATED AT CONSTANT TEMPERATURE (82°F)

PRETREATMENT

* 3% H₂O₂, 24 hour soaking
DISCUSSION AND APPLICATION OF RESULTS

In evaluating the germination data, it may be useful to examine the results in relationship to probable field performance. As Holmes (21) pointed out, the ultimate objective of any method of testing seed germination quality is to provide an indication of the percentage of seeds in a given lot that might be expected to produce plants. However, the relationship of germination test results to field germination and plant percent\(^4\) varies considerably with germination conditions in the field, and it is difficult to forecast field results accurately. According to Holmes, Haack investigated this relationship and introduced the term germinative energy\(^5\) in 1909. He believed germination energy a better expression of field germination than germination capacity,\(^6\) on the basis that only those seeds which germinate rapidly and vigorously in a germinator are likely to produce seedlings in the field, where weak or delayed germination is often fatal. The usefulness of germinative energy percent in expressing probable field performance of seed was also confirmed by several other investigators, as pointed out by Holmes.

---

\(^4\) "Plant percent is the number of seedlings surviving at the end of the first growing season, expressed as a percentage of the number of seeds in the sample" (21).

\(^5\) "Germinative energy is the percent of seeds in a sample that have germinated in a germinator up to the time when the rate of germination (i.e. the number of seeds germinating per day), reaches its peak" (21).

\(^6\) Germinative capacity is the total number of seeds that germinate in a germinator, plus the number of sound seeds remaining ungerminated at the end of the test in percent.
The germination energy concept is undoubtedly useful in correlating laboratory germination with plant success in the field or nursery. This may be particularly true when laboratory procedures employ optimum conditions for germination maintained throughout the test period. However, when seeds are subjected to sub-optimum germination conditions, such as to the extreme temperature fluctuations used in this study, germinative energy probably will not be a good index of field germination. For example, germinative energy was greater for stratified seeds held at a constant temperature (36%) during the incubation period than for stratified seeds subjected to alternating temperatures (28%). (See Appendix V and VII). Use of this same lot of seed for the Coram direct seeding study in May, 1960, where seeds were subjected to severe diurnal temperature fluctuations (Figure 3, p. 25), resulted in 42% germination of stratified seeds. It is apparent from the above that germinative energy of seeds used in this study, at least the stratified seeds, might be a gross underestimation of the field performance of this seed. Obviously the exposure of seed to environmental factors which retard germinative energy, such as sub-optimum temperatures, may not always result in appreciatively lowered field germination. However, after a certain time has elapsed, further postponement of conditions suitable for germination will increase the possibility of loss of the seed. This loss may occur through internal or environmental causes. Consequently, a more reasonable index of field germination, under the laboratory conditions used in this study, might be cumulative germination percent after a relatively short period. The action of numerous sub-optimum germination conditions on seeds in the field would tend to delay field germination to a greater extent than even the laboratory germination tests employing only one environmental
factor, such as temperature. Therefore, even laboratory germination tests employing widespread temperature alternations might logically be of shorter duration than the time required to reach peak field germination.

Consequently, the 15-day germination period used in this study was thought to be a reasonable index for correlating field and laboratory germination. This correlation may be less applicable to nursery conditions, where the severity of many environmental conditions is minimized. However, it may be applicable to a direct seeding study, such as was carried on at the Coram Experimental Forest direct seeding study with western larch. The germination for the Coram study at the end of the first summer after seeding is compared with results of the $30^\circ - 76^\circ$, $35^\circ - 76^\circ$, $40^\circ - 76^\circ$, and $45^\circ - 76^\circ$ temperature tests at 15 days in Table 7. It was felt that the $76^\circ$ high temperature component most closely paralleled field conditions.

As shown in Table 7, the $40^\circ - 76^\circ$ and $45^\circ - 76^\circ$ tests most closely approximated actual field germination. Why there was such a large difference between the $30^\circ - 35^\circ$ series, and the $40^\circ - 45^\circ$ series is not known. One explanation might be that the germination differences may have been due to the difference in time since the $30^\circ - 35^\circ$ series was started 30 days earlier than the $40^\circ - 45^\circ$ series. Another cause of the differences may have been differences in duration of high temperature, which could have been as great as 30 minutes less for the $40^\circ - 45^\circ$ series. Such differences could be present, but undiscernable with the temperature recording equipment used. Nevertheless, it is evident that the $\text{H}_2\text{O}_2$ pretreatments are sensitive to temperature effects, possibly to duration as well as intensity of temperature. The stratified and control seeds also showed a decline in germination percent in the $40^\circ$ and $45^\circ$ series, but
the decline was not nearly as great as in the $H_2O_2$ pretreatments.

Table 7.
COMPARISON OF FIELD AND LABORATORY GERMINATION

<table>
<thead>
<tr>
<th>Seed Pretreatment</th>
<th>Coram Study</th>
<th>30-76</th>
<th>35-76</th>
<th>40-76</th>
<th>45-76</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.9</td>
<td>9.2</td>
<td>8.0</td>
<td>4.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Stratified</td>
<td>41.8</td>
<td>47.8</td>
<td>54.0</td>
<td>45.2</td>
<td>46.5</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>10.5</td>
<td>16.0</td>
<td>2.9</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>$H_2O_2$-Dried</td>
<td>5.0$^b/$</td>
<td>24.2</td>
<td>31.0</td>
<td>12.5</td>
<td>13.2</td>
</tr>
</tbody>
</table>

$^A/$ Seeds were sown May 10, and peak germination was reached approximately June 12 on stratified seeds; about June 29 for the remaining seeds. Germination is shown as of the end of the first growing season. A total of 6,750 seeds were sown.

$^b/$ All $H_2O_2$ seeds used in the Coram study were dried.

$^c/$ There were no significant differences between the 40$^o$ and 45$^o$ series.

Allowing the $H_2O_2$ pretreated seeds to dry out, at least for the 12-hour duration, resulted in consistently improved germination over non-dried seeds. Further investigation of this effect, using closely controlled temperature and humidity conditions for drying might be useful.

There was also some evidence that germinative ability of the $H_2O_2$ pretreated seeds subjected to alternating temperatures was largely expended by the fifteenth day after pretreatment, regardless of how many seeds had germinated by that time. Control seeds, in comparison, showed cumulative germination similar to $H_2O_2$ pretreated seeds at 15 days in the laboratory but continued to germinate steadily beyond the fifteenth day.
Consequently, the control seeds might logically result in greater "hold over" germination than the H$_2$O$_2$ pretreated seeds. However, conclusive evidence of this was not obtained. This may be an aspect of this study worthy of further investigation.

The reason for the significant difference between the 30° and 35° series may be attributable to 30° F. being below freezing, and 35° F. being above freezing. There were no significant differences between the 40° and 45° series. The significance of the high x low temperature interaction in the 40° - 45° series was probably due to one relatively high temperature component of the 40° series. It was thought that this relatively high percentage occurred by chance.

From the data presented, it is apparent that H$_2$O$_2$ pretreated western larch seeds represent a considerable risk for field or nursery sowing in view of their sensitivity to sub-optimum germination temperatures. However, as Dhillon (12) found, 3% H$_2$O$_2$ soaking for 24 hours was not the best H$_2$O$_2$ pretreatment for this lot of western larch seed. A study employing some of the better H$_2$O$_2$ pretreatments, as determined by Dhillon, under field conditions might prove worthwhile. Since very quick germination results can be obtained under optimum laboratory conditions with H$_2$O$_2$, further application of it under field conditions should not go uninvestigated. In view of present knowledge, however, stratification appears to be more consistently successful as a pregermination stimulant for nursery or field use.

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7 "Hold over" germination is germination occurring during the year after sowing.
SUMMARY AND CONCLUSIONS

Western larch seeds soaked in 3% H₂O₂ for 24 hours exhibited lower germination after 15 days exposure to some alternating temperatures than when germinated under a constant temperature of 82° F. This was generally true for seeds dried at room temperature for 12 hours after soaking, and for non-dried seeds. However, non-dried seeds generally showed lower germination percent at 15 days. No statistical analysis was used in comparison of alternating and constant temperature germination.

Sixteen combinations of alternating temperatures were used, employing 30°, 35°, 40°, or 45° F. for the eight-hour low range; 76°, 78°, 80°, or 82° F. for the 6-hour high range, and a ten-hour intermediate range (Figure 2, p. 21).

Analysis of variance indicated that the H₂O₂ pretreated seeds subjected to the greater high temperature components displayed higher germination after 15 days than those exposed to lower high temperatures. There was a significant difference between the effect of the 30° and 35° low temperature components, the 35° series showing higher germination. The 40° and 45° low temperature series showed no significant difference in germination, and was analyzed separately from the 30° - 35° series, since it was carried out at a different time. The H₂O₂ pretreated seeds of the 30° and 35° low temperature ranges, which were dried, showed no significantly different responses to differences in high temperature components of the alternations.

Stratified seeds and seeds with no pretreatment showed no significant differences in their response to temperature variations. In all
tests, stratified seeds exhibited the highest germination after 15 days. The 15-day germination response from highest to lowest germination percent for all pretreatments was: (1) stratified, (2) dried $H_2O_2$, (3) $H_2O_2$; and (4) no pretreatment (control). All pretreatments showed significantly different germination percent after 15 days.

The following conclusions were made:

(1) Western larch seeds soaked in $3\% H_2O_2$ for 24 hours will probably display very low germination ability after exposure to field diurnal temperature fluctuations in the spring.

(2) The high temperature component of the alternations is the most critical temperature factor for germination of the $H_2O_2$ pretreated seeds. The greater the high temperature component, the greater is the germination percent of $H_2O_2$ pretreated seeds. Duration of high temperature may also be important, although conclusive evidence of this was not obtained.

(3) The minimum temperature of diurnal temperature fluctuations in the field will probably not lower the germination of $H_2O_2$ pretreated seeds, except where the temperature drops below freezing.

(4) Stratified and control seeds are not affected by temperature alternations within the range of normal spring diurnal temperature fluctuations.

(5) Stratified seeds will show greater germination after 15 days under field temperature conditions, than any of the other pretreatments used in this study.

(6) Seeds with no pretreatment and seeds soaked in $3\% H_2O_2$ for 24 hours may show a very similar percentage of germination at the end of the first growing season.
(7) Seeds with no pretreatment will probably continue to germinate during the second growing season. In contrast, the 3% \( \text{H}_2\text{O}_2 \) pretreated seeds will probably show little or no germination during this time.

(8) Allowing \( \text{H}_2\text{O}_2 \) pretreated seeds to dry out at room temperature for 12 hours after soaking will probably improve germination in comparison to non-dried \( \text{H}_2\text{O}_2 \) pretreated seeds.
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APPENDIX I

TIME EFFECT ANALYSIS

\[ 30^\circ \text{ series } = X_1 \]
\[ 35^\circ \text{ series } = X_2 \]
\[ 40^\circ \text{ series } = X_3 \]
\[ 45^\circ \text{ series } = X_4 \]

\( n \) = number of items per series
\( N \) = total number of items

The sum of squares (SS) for treatments can be divided into three individual sums of squares as follows:

\[ SS_1 = \frac{(X_1)^2}{n} + \frac{(X_2)^2}{n} - \frac{(X_1 + X_2)^2}{2n} = 192.57 \]
\[ SS_2 = \frac{(X_3)^2}{n} + \frac{(X_4)^2}{n} - \frac{(X_3 + X_4)^2}{2n} = 72.34 \]
\[ SS_3 = \frac{(X_1 + X_2)^2}{2n} + \frac{(X_3 + X_4)^2}{2n} - \frac{\text{Sum}(X)^2}{N} = 3173.13 \]

Each of these equations has 1 degree of freedom, and is mutually independent. In \( SS_1 \) and \( SS_2 \), there is no effect due to time. Hence, this sum has 2 degrees of freedom, and comparison within a term will indicate presence of treatment effects. \( SS_2 \) is a compounding of treatment and time effects. If \( SS_1 \) and \( SS_2 \) show treatment effects (which they do), and \( SS_3 \) is "about" the same size, this would indicate no time effect. However, \( SS_3 \) is definitely not "about" the same size as \( SS_1 \) or \( SS_2 \), and indicates the possibility of time effects.

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\( ^8 \) Personal interview with Dr. Howard Reinhardt, Assistant Professor of Mathematics, Montana State University, November, 1960.
### APPENDIX II

#### BREAKDOWN ANALYSIS OF 40° SERIES FOR THE H₂O₂ PRETREATMENT

<table>
<thead>
<tr>
<th>Category</th>
<th>D.F.</th>
<th>Variance</th>
<th>Sample F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication</td>
<td>3</td>
<td>34.67</td>
<td>2.28</td>
</tr>
<tr>
<td>Hi-Temp.</td>
<td>3</td>
<td>396.00</td>
<td>26.02**</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>15.22</td>
<td></td>
</tr>
</tbody>
</table>

** F value significant at the 1% confidence level.

** Duncan's Multiple Range Test:**

By Duncan's test, the only two high temperature components which were NOT significantly different were 76° and 78° at the 5% confidence level.

#### BREAKDOWN ANALYSIS OF 45° SERIES FOR THE H₂O₂ PRETREATMENT

<table>
<thead>
<tr>
<th>Category</th>
<th>D.F.</th>
<th>Variance</th>
<th>Sample F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication</td>
<td>3</td>
<td>9.06</td>
<td>0.69</td>
</tr>
<tr>
<td>Hi-Temp.</td>
<td>3</td>
<td>174.23</td>
<td>13.34**</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>13.06</td>
<td></td>
</tr>
</tbody>
</table>

** F value significant at the 1 percent confidence level.

** Duncan's Multiple Range Test:**

By Duncan's test, the 76° and 78° high temperature components, and the 80° and 82° components were NOT significantly different at the 5% confidence level. All other pairs of temperatures were significant at the 5% confidence level.
APPENDIX III

CUMULATIVE GERMINATION

Key:
- HP = H₂O₂ pretreatment
- HPD = H₂O₂ dried pretreatment
- S = Stratification
- C = Control
APPENDIX IV

CUMULATIVE GERMINATION FOR H$_2$O$_2$ PRETREATMENT

(By High Temperature Component for Each Low Temperature Series)

30° Series

35° Series

40° Series

45° Series

Days

Days

Days

Days

% Germination

% Germination

% Germination

% Germination

0

0

0

0

5

5

5

5

10

10

10

10

15

15

15

15

20

20

20

20

30

30

30

30

40

40

40

40

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APPENDIX V

GERMINATION ENERGY

30° - 76°

30° - 82°

40° - 76°

40° - 82°

Key: S = Stratification
HP = H₂O₂ pretreatment
HPD = H₂O₂ dried pretreatment
C = Control

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APPENDIX VI

CUMULATIVE GERMINATION FOR SEEDS

GERMINATED AT A CONSTANT TEMPERATURE OF 82° F.

* 3% H$_2$O$_2$, soaked for 24 hours.
APPENDIX VII

GERMINATION ENERGY FOR SEEDS

GERMINATED AT A CONSTANT TEMPERATURE OF 82° F.

* 3% H₂O₂, 24-hour soaking.