EASTERN HEMLOCK SEEDS AND SEEDLINGS

RESPONSE TO PHOTOPERIOD AND TEMPERATURE

Jerry S. Olson   Forest W. Stearns   Hans Nienstaedt

Foreword

The Problem

Forest ecology seeks ultimately a better understanding of the forest as a whole in terms of the interactions of its living components with each other and with the environment.

A forest is complex: it is necessary to separate the components into units which can be studied intensively.

The Approach

The research reported herewith is a small part of a larger study which deals with the ecological life history of one species — eastern hemlock. It treats with the responses of seeds and seedlings when grown in controlled environments. Since the parent trees were located over the entire range of the species, it has been possible to isolate and evaluate the effects of both heredity and environment.

The Objective

Broadening the base to include other species and their reactions, one on another, will eventually provide a sound basis for forest management.

Practical Application

The present study has already shown how to avoid many of the difficulties which formerly beset nurserymen in growing hemlock from seed.

Who Made This Study?

When the experimental work was performed, Dr. Jerry S. Olson and Dr. Hans Nienstaedt were members of the Station staff in the Departments of Forestry and Genetics, respectively; Dr. Forest W. Stearns was a member of the Forestry Department staff while on sabbatic leave from Purdue University in 1956.

As of the date of this publication Dr. Olson is employed at the Oak Ridge National Laboratory, Oak Ridge, Tennessee; Dr. Nienstaedt is in charge of the Northern Institute of Forest Genetics, Lake States Forest Experiment Station, Rhinelander, Wisconsin; Dr. Stearns is at the Vicksburg Research Center, Southern Forest Experiment Station, Vicksburg, Mississippi.

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Cover Photo: The Carrington Phelps Forest

This was the last "virgin" forest tract of appreciable size in Connecticut. Some 300 acres in area, it was located at North Colebrook. The following notes are from an article by George E. Nichols in Torreya XIII, 9, Sept. 1913. Composition (in per cent): Hemlock and beech, 55; sugar maple, 12; yellow birch, 10; red oak, 6; chestnut, 7; white ash, black cherry, red maple, and white pine, 4. Diameters to 55 inches; heights to 115 feet; ages to 350 years; last evidence of severe disturbance — a fire in 1781. The stand was cut in 1912-13.

A. F. Kerr (files of this Station, dated 1910) estimated that volumes in this forest ranged up to 45,000 board feet per acre.   *Photo by W. O. Filley.*

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INTRODUCTION

Because of the significance of the eastern hemlock for forest ecology and its intangible and economic values, the species has been studied intensively at this Station for the past 6 years. The present report covers mainly the results of laboratory and greenhouse experiments. By using seed from selected sources, and controlling growing conditions with respect to light, temperature, and nutrient, it has been possible to estimate the separate and combined effects of these factors on seeds and seedlings. The early stages of growth are especially critical for the establishment and survival of hemlock. Knowledge of the factors that regulate the growth of the young plants should also help us to understand the growth of the tree throughout its life.

Indeed, the growth responses by hemlock were so striking as to arouse interest in its physiology. This is one of the first plants to show response of *seeds* to duration of light and dark (photoperiodism) during germination. Photoperiod also had a strong influence on breaking *bud* dormancy and forcing growing plants back into dormancy. Day and night temperature, chilling, and nutrient also had important effects on growth, and modified the response of seeds and seedlings to light.

THE LIFE CYCLE OF EASTERN HEMLOCK, *Tsuga canadensis* (L.) Carrière

The life cycle consists of a vegetative or sporophytic stage, during which the tree grows, and a sexual or gametophytic stage during which male and female cells are produced; these cells unite to start a new sporophyte generation. The sporophytic (tree) stage may extend over several hundred years, with the tree producing sexual structures periodically; the sexual stage is completed within a few months.

Development of Cone and Seed

The nucleus of each cell of the parent tree or sporophyte has *two* sets of 12 chromosomes. During the sexual stage, male and female strobili or "flowers" are developed in specialized buds. Within each of these strobili certain cells divide so that the resulting cells have nuclei with only *one* set of 12 chromosomes. These are the first cells of the male and female gametophyte, from which the embryo develops, as described below. The sequence, based on observations by Echols and others, and by ourselves during this study, traces the progress of development from the time the strobilate buds are formed, through the gametophytic stages, and finally to the fully developed seed. Dates are approximate for New Haven, Connecticut.

1. Buds for male and female strobili are developed the preceding summer on the parent tree (Figure 1).

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2. After remaining inactive until late March, certain cells in the male strobili undergo meiosis, or reduction division, to produce the male gametophyte cells, each with one set of 12 chromosomes. As the buds swell in April and early May, these cells develop into mature pollen grains.

In early May, the female gametophyte is produced within the female strobilus by reduction division of certain cells inside the ovule (Figure 1). During the following two months, the cells of this gametophyte divide to form (a) one or more unfertilized egg cells which may later be fertilized by a nucleus from a pollen cell, and (b) other cells from which the endosperm develops. All cells of the female gametophyte have one set of 12 chromosomes per nucleus. Two ovules lie on each scale of the miniature cone and are exposed to air when the cone scales spread during the second week in May (Figure 3B).

Surrounding the female gametophytic cells are two layers of tissue previously derived from the cone scale of the parent tree, the nucellus and integument. The wing is also derived from the cone scale. Seed and wing are initially attached to the cone scale (Figure 3D-F) but later become freed from it.

3. After pollen is carried to the young cone by wind about the second week in May, it sends out a pollen tube (Figure 2C) which, over a period of about 6 weeks, grows into the ovule. Two 12-chromosome nuclei are produced from the original nucleus of the pollen grain and are then drawn into the ovule. One of these joins the nucleus of an egg cell of the female gametophyte to constitute a fertilized egg cell (Figure 2D) whose nucleus then has two sets of 12 chromosomes which carry the genetic contributions of both parents to the new generation. The remaining cells of the female gametophyte, which still have only 12 chromosomes per nucleus, develop into the endosperm which nourishes the growing embryo before and during germination.

4. After fertilization in late June, and continuing during July and August, the fertilized egg develops into the embryo. Embryo and endosperm grow at the expense of the nucellus which persists only as a remnant, the endosperm membrane. The integument gives rise to the outer and inner seed coats which enlarge as endosperm and embryo develop (Figures 2C-E).

5. By September, cone and seed are mature but they are still green, wet, and oily. By early October much of the excess moisture has been lost, the color has changed from green to tan and finally to brown, and the cones begin to open (Figure 3G, H). Most of the seed is shed during the first few periods of dry windy weather thereafter. Some seed may hang in the cones through the winter and be dispersed more gradually, but much of this is sterile seed which developed without an embryo.

At maturity, the embryo is a white cylindrical structure about 3 mm. long and .5 to .7 mm. in diameter (Figure 4B). The embryo (Figure 4A) extends almost the full length of the seed, but its diameter is only about one-third that of the seed. At the end of the seed which bears the wing, the embryo has incisions which separate the seed leaves or cotyledons (normally three, occasionally four or more, rarely two) below which is the hypocotyl. At the other end, a small notch divides the true root from the stem portion of the hypocotyl.

New seed is partially dormant and incapable of rapid germination in the autumn when shed. This dormancy is normally overcome by winter chilling. Thus seed can germinate rapidly in the spring as soon as the temperature

again becomes warm enough to permit growth. Over the years the sporophyte passes through seedling, sapling, and mature tree stages. When about 15 years old, or much later in some cases, the tree produces strobili and another sexual cycle is begun. (We would welcome information on earlier flowering.)

Germination and Growth

The description below assumes that conditions for germination and growth are favorable. Following this presentation is a discussion of the factors which regulate these processes.

The first indication of germination is the splitting of the seed coat for one-half to two-thirds of its length, exposing the grey reticulate membrane which surrounds the endosperm. This layer may bulge slightly from the gap. If all conditions are favorable, growth of the root will begin. The appearance of the pointed, bright red root tip is a positive sign of active germination.

The root grows at the rate of 2 or 3 mm. a day for the first several days after which the stem portion of the hypocotyl also begins to grow. The entire structure curves abruptly where it emerges from the seed (Figure 6A). Within the seed, digestion of the food reserves proceeds as the endosperm softens and becomes liquified.

When grown in the light, much of the true root portion of the hypocotyl is bright red. The pigmented cells cover the root tip densely while the older portion of the root has a loose, rather shaggy covering of the same filamentous, pigmented cells. When grown in soil or in the dark, only the tip portion of the root is red although unpigmented filamentous cells are present over the remainder of the root surface (Figure 6A).

An abrupt change from the filamentous covering to a green shiny epidermis marks the transition to the stem portion of the hypocotyl. This region reaches its full length of 2 or 3 cm. within 1 to 3 weeks and then begins to straighten (Figure 4H, I). It shows a marked curvature toward light. The root also continues to elongate; at the end of a month it may be 2 to 4 cm. long; at the end of 3 months, 6 to 10 cm. or more, depending on nutrient conditions.

Normally there is a pause in development after the cotyledons open and it is at this point that germination might arbitrarily be considered to end. At the junction of the cotyledons lies the growing point from which stem and later leaves develop. The leaves first appear as a tiny tuft of pale yellowish needles which gradually enlarge and darken. During the early stages, needles are typically laid down in threes around the stem at about the same level so that they appear whorled. This is indicated by the high percentage of seedlings with 3, 6, or 9 needles (Table 1). The stem between the needles (internode) elongates as the leaves grow. On later and faster-growing portions of the stem, grouping by three's is less evident. Toward the end of the first annual growing cycle, some of the needles may show only as short green stubs surrounding the bud which forms at the stem tip. As growth slows down and finally ceases, some of the leaf primordia become highly modified into bud scales. These surround and protect the primordia which will give rise to the next year's growth. Early buds on vigorous shoots occasionally put out a second flush of growth but normally late summer buds will not resume growth unless the buds are chilled for a month or more at temperatures slightly above freezing. In nature such chilling takes place during the late autumn months

and, by the time the chilling requirement is completed, temperatures will usually have become too low to permit a resumption of growth until the following spring when rising temperatures permit the initiation of another cycle of growth; this is terminated by formation of buds later in the summer. Only by keeping successive annual growth cycles in adjustment to the local climate can hemlock attain maturity.

Table 1. Frequency distribution of needles and stem length in relation to daylength

Needles above cotyledons	Hours of light and darkness Day 8 / Night 16	12 / 12	16 / 8	Stem length above cotyledons	Hours of light and darkness Day 8 / Night 16	12 / 12	16[1] / 8
No.	*Percentage of seedlings*			Mm.	*Percentage of seedlings*		
0	4	6	....	0	14	19	....
1	....	3	....	1	37	40	....
2	1	1	....	2	26	24	8
3	42	57	....	3	16	8	13
4	6	....	....	4	7	5	31
5	7	1	....	5	....	4	18
6	39	27	2	6	....	....	16
7	....	....	8	7	....	....	10
8	....	1	5	8	....	....	5
9	....	4	44				
10	....	....	10				
11	....	....	10				
12	....	....	10				
13	....	....	11				
14	....	....	2				

[1]Mean stem elongation for five seed sources on 16-hour day, 8-hour night: No. 34, Quebec, 2.93 mm.; No. 24, Maine, 4.46 mm.; No. 7, Connecticut, 4.71 mm.; No. 50, Indiana, 4.54 mm.; No: 17, Tennessee, 6.60 mm.

Factors Regulating Germination and Growth

Adjustment of seed germination and stem growth to climate is regulated mainly by sensitive responses to temperature and the length of day and night. Separate and combined effects of these variables were estimated in a long series of experiments. Hemlock seeds from various parts of the species' range were germinated and, in some cases, grown for a year or more under controlled conditions of light and temperature in growth chambers. Some experiments included 2-year-old wild seedlings brought indoors to controlled conditons.

This experimental approach not only permitted the separation of effects which would be confounded with one another outdoors; it permitted a reduction in variability due to other factors which affect germination or growth in the field. The observations below bring out the principal results of these experiments.

Control of germination

Stratification[1] was the most important factor affecting the course of germination. Stratified seed (upper curves, Figure 5) germinated much better

[1]Chilling at temperatures between 32° and 44° markedly hastens the germination of *moistened* hemlock seeds and the opening of buds. In this paper the chilling of moistened seed, by whatever means accomplished, will be referred to hereafter as "stratification." The term "chilling" will be applied to plants which have already formed buds.

FIGURE I. LIFE CYCLE OF TSUGA CANADENSIS

REDUCTION DIVISIONS {FEMALE— MALE—

OVULE AND POLLEN DEVELOP AS BUDS SWELL

POLLEN CARRIED TO OVULES BY WIND

BUDS

FEMALE—

MALE—

MAR. APR.

FEB. MAY

JAN. JUNE

DEC. JULY

NOV. AUG

OCT. SEPT.

POLLEN TUBE GROWING INTO OVULE

POLLEN NUCLEUS FERTILIZES EGG

CONE, SEED AND EMBRYO GROW

SEED SPREAD BY WIND

CONE AND SEED MATURE AND DRYING

FIGURE 2. IDEALIZED SEED DEVELOPMENT

OVULE—

CONE SCALE — BRACT

BRACTS

POLLEN

FUTURE SEED WING

A. APRIL 15

B. APRIL 25

FERTILIZED EGG

WING

INTEGUMENT NUCELLUS FEMALE GAMETOPHYTE

C. MAY 20

BRACT

CONE SCALE

D. LATE JUNE

ENDOSPERM, from FEMALE · GAMETOPHYTE

ENDOSPERM MEMBRANE, from NUCELLUS

OUTER INNER } SEED COAT, from INTEGUMENT

WING

ROOT STEM GROWING

HYPOCOTYL TIP COTYLEDONS
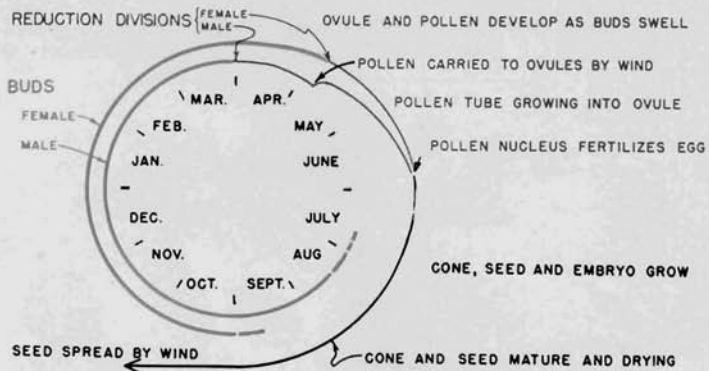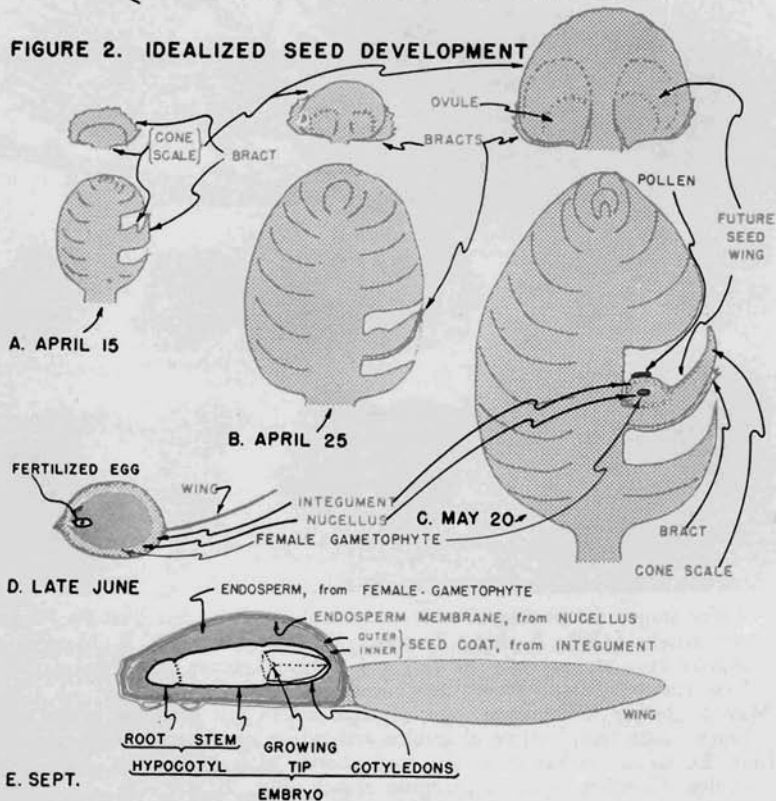
E. SEPT.

EMBRYO

Figures 1 and 2. Green represents parent sporophytic (tree) stages (Figure 1) and tissue (Figure 2); red, gametophytic (sexual) stages and tissues after reduction divisions; black, successive stages of the new sporophyte terminating with the fully developed embryo.

Fig. 3. Eastern hemlock cone development.

A-C. Three stages in opening of male and female strobili, described by Nienstaedt and Kriebel (35); A, May 7, pollen beginning to shed; B, May 9, cones slightly open and receptive to pollen; C, May 15, cones closed and developing. Note rosette of light-colored new needles.

D. May 8 (before pollination); tips of cone scales just showing behind pointed bracts; note faint outline of ovules and wings on detached cone scale.

E. May 23. Cross section of young cone shortly after pollination. Note rounded ovules. Compare with diagramatic sketch, Fig. 2C.

F. June 4 (after pollination but before fertilization); cone scales have grown out over bracts; seeds and wings are separating from cone scale as they grow.

G. Late September, mature cones; those unopened are still green in color; those with spreading scales have turned tan; size depends on mutual competition.

H. Brown cones in October. Note that smallest cones are from extreme northern and southern locations: 60. Grayson, Ala., 800'; 62. Robbinsville, N. C., 4600'; 61. Cheshire, Conn.; 63. Mont Tremblant, Quebec.
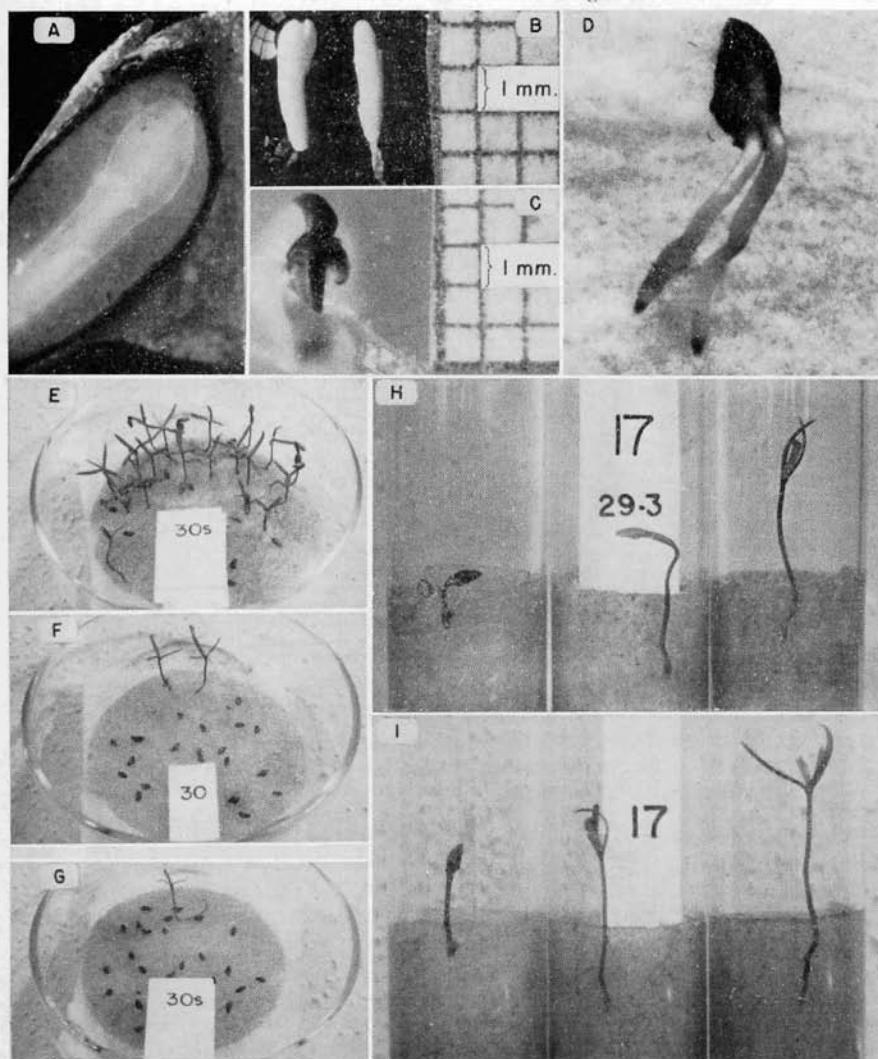
Fig. 4. Hemlock germination.

A. Cross-section of embryo surrounded by oily endosperm, endosperm membrane
    layer and brown seed coats. See Figure 2E.
B. Embryos excised from seed.
C. Excised embryo on agar showing three dwarfed cotyledons surrouding rudi-
    mentary epicotyl. Root tip (in agar) shows as a dark blur; such embryos
    lived only a short time.
D. Rare example of two embryos developed in a single seed.
E,F. Higher germination of stratified seed (30s) compared with unstratified seed
    (30) at 62° under 16 hours of light.
G. Poor germination of stratified seed (30s) at 80°; unstratified seed failed to
    germinate at 80° and is not shown.
H,I. Growth of seedlings during a 1-week period at several early stages of de-
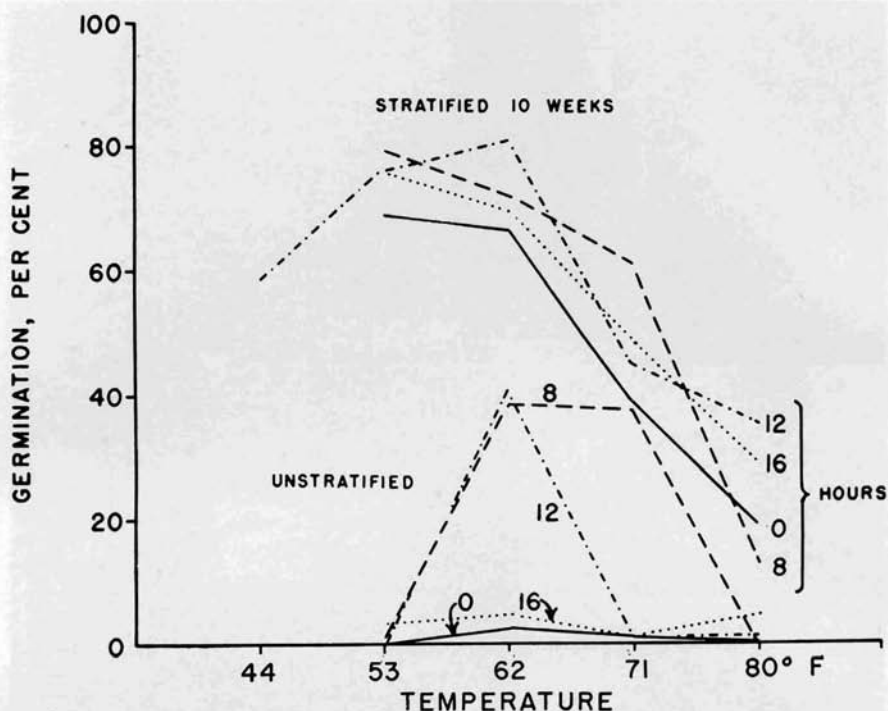   velopment.

Figure 5. Effect of stratification, temperature, and duration of light on germination of hemlock seed, 7 weeks after start of test. Numbers indicate hours of light in each 24-hour day.

than unstratified seed (lower curves) at temperatures[2] ranging from 53° to 80°, put percentage germination as of 7 weeks decreased as temperature increased. The duration of exposure to light had little effect on the germination of stratified seed although there appeared to be a tendency for somewhat better germination at 80° on the longer light periods.

Unstratified seed germinated poorly under 0 and 16 hours of light at all temperatures. There was, however, a marked improvement in germination at 62° under 8 and 12 hours of light and at 71° under 8 hours of light, indicating that under these conditions a favorable duration of light (photoperiod) compensates for the lack of stratification.

A constant temperature of 62° was generally satisfactory for the germination of stratified or unstratified seed of all sources but it was found that temperatures which fluctuated between about 70° during the day to 55° at night were also favorable. Constant temperatures of 55° or below tended to slow down germination; above 70°, to depress germination levels.

Seeds from different sources did not respond the same to these factors (Table 2). Unstratified southern seed showed a much stronger stimulation by photoperiod than northern seed. This geographic difference may also reflect a lesser requirement of southern seed for stratification, thereby adapting it to milder winters. Stratified Tennessee seed germinated best at temperatures above

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[2]All temperatures are in degrees Fahrenheit.

62°; Indiana seed between 53° and 62°; and Connecticut, Maine and Quebec seed at 53° or below, as might be expected if the life cycle of trees from different regions had become adapted to the climate of the region from which they came. Just as crop varieties have been selected by man for different climates, a widespread native species like eastern hemlock evidently contains different genetic types which have undergone a natural selection; this helps to explain its adaptation to diverse regions.

Table 2. Genetic variation in germination response as of 7 weeks

Environmental factor	Seed source				
	Tennessee 17	Indiana 50	Connecticut 9	Maine 24	Quebec 34
Stimulation by 8- or 12-hour photoperiod (unstratified seed at 62°)	high	medium	medium	low	low
Temperature with high germination (stratified seed)	above 62°	53° to 62°	53° or below		

Control of stem growth

Temperature and photoperiod also have a regulating effect on seedling growth. Seedlings which have completed their growth and formed buds in late summer will ordinarily not break dormancy until after they have been chilled[3] for a month or more at temperatures below 40°. However, if unchilled plants are subjected to daylengths of 16 hours or more, they can be forced to break dormancy without chilling, although more slowly than if they had been chilled. Hence photoperiod can compensate to some extent for lack of chilling.

Once dormancy is broken a constant temperature of about 60° is quite favorable for growth but, as in germination, the effects of much higher or lower constant temperatures are adverse; below 50° growth slows down, above 80° plants are stunted. Also, as in germination, it was found that temperatures which fluctuated between 80° and 90° during the daytime and 60°, or even somewhat lower, at night, were also favorable.

Photoperiodic effects are shown in Table 1 by differences in the number of needles formed above the cotyledons and by stem elongation under 8, 12, and 16 hours of light. Under 8-hour daylength some seedlings formed buds without producing any needles above the cotyledons (Figure 6H) and no seedlings put out more than 6 needles. More needles were formed on a few plants under 12 hours of light, but under a 16-hour daylength no seedling had fewer than 6 needles and most had 9 (Figure 6I, Table 1). Several developed 12 or more before growth was stopped by bud formation. Under 8 and 12 hours of light no seedling grew more than 5 mm. above the cotyledons and 14 and 19 per cent, respectively, made no stem growth at all. On 16 hours of light no seedling elongated less than 2 mm. and 49 per cent grew 5 mm. or more. Even more pronounced differences in growth were shown in further experiments with more closely spaced photoperiods. The best growth was under a 20-hour day, 4-hour night treatment (Pot d, Figure 7); growth was markedly reduced if the dark period was longer than 8 to 9 hours.

[3]See footnote. page 8.
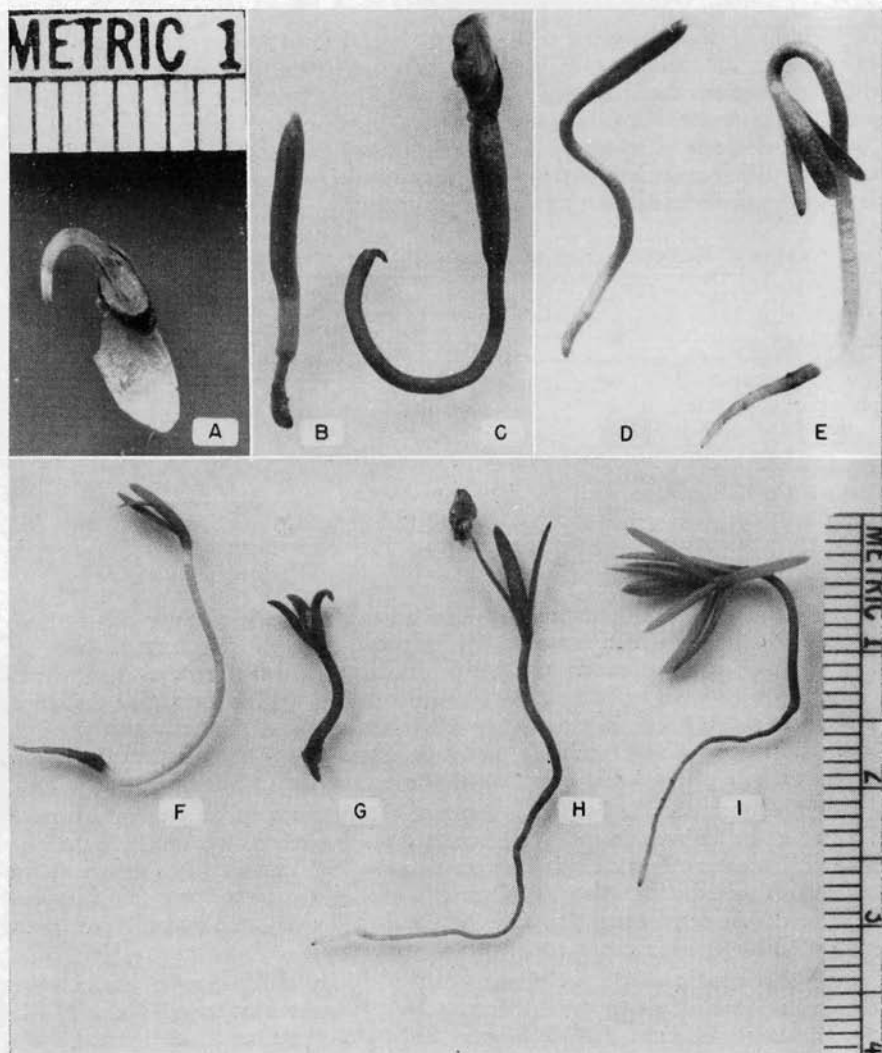
Fig. 6 . Normal and abnormal germination of eastern hemlock.

A.   Germination in darkness; normal except for persistence of seed wing.

B-G.   Abnormalities induced by treatment with chemicals.

B, C.   *Urea*, .5 per cent damages the root tip: B in the light, C in the dark.

D, E.   *Thiourea*, .3 per cent, stunts roots slightly in the dark, E, (color tan instead of normal pink); more strongly in the light, D. Stronger concentrations lead to more severe damage like that in B. Pale color in E and F is for seedlings grown in darkness.

F, G.   *Coumarin*, .05 per cent, also shows less damage in the dark, F, than in the light, G.

H, I.   More advanced stages of normal growth. H, effect of short (8-hour) days with no growth beyond cotyledons; I, of long (16-hour) days with several whorls of needles showing beyond the cotyledons.
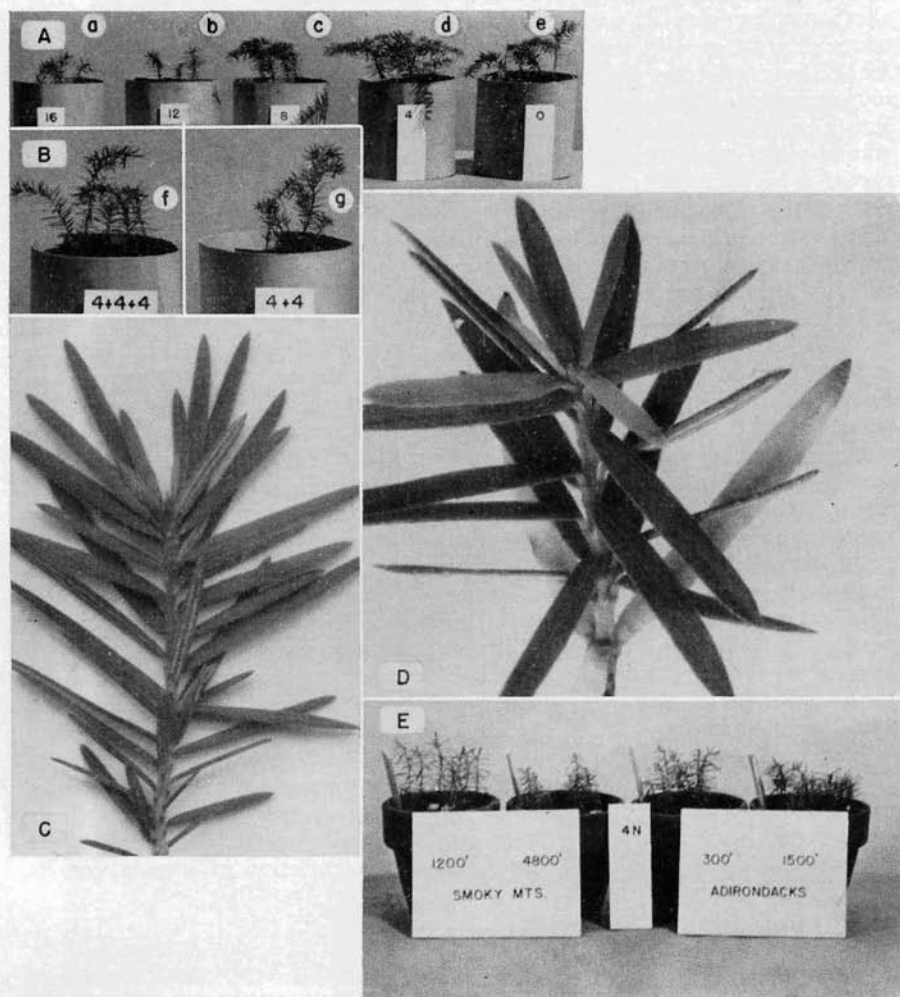
Figure 7. Stem growth and bud formation.

A. Effects of photoperiod. Short nights lead to better growth than long nights. Numbers indicate hours of continuous darkness per 24-hour cycle.

B. Daily interruption of long dark periods by dim light permits plants to grow like those under a long day, short night regime (pots 4½" wide as in A).

C. Plant still elongating. There is no terminal bud; leaves near the apex show all gradations in length.

D. Terminal bud on dormant plant. Gradation to small needles no longer present.

E. Effect of latitude and altitude of seed source on amount of elongation of plants on a favorable 20-hour day, 4-hour night (4N) cycle.

Breaking a long dark period into two or more *short* dark periods with a dim light (too low for appreciable photosynthesis) has essentially the same effect as a *long day, short night*. Pots b and g, Figures 7A and B, both had 12 hours of light at 300 foot candles daily; Pot b was in continuous darkness for 12 hours whereas the 12-hour dark period for Pot g was interrupted for four hours with incandescent light below 10 foot candles (4+4). Similarly Pots a and f each had 8 hours of light daily at 300 foot candles but Pot a was in continuous darkness for 16 hours; the dark period for Pot f was interrupted twice for 2 hours (4+4+4) with the same dim illumination as in Pot g. As in many other biological effects of light, this suggests that the total duration of light or darkness throughout a 24-hour period is less important in controlling growth than the *manner* in which light and total darkness are distributed over the period, and that the duration of darkness probably has more importance than the duration of light.

Seed source also had a marked effect on the timing of bud formation and hence on the amount of growth that can be attained in any given season. Table 1 also gives the mean length of stem for five seed sources under 16-hour daylength, and shows that seedlings of Tennessee parentage had more than twice as much elongation as seedlings of Quebec parentage; seedlings from intermediate latitudes fell between these extremes. Much larger experiments have shown the same kind of relation among seedlings from 30 sources, representing a wide sampling over the entire range of the species. For any given daylength and temperature condition, northern types consistently tended to go dormant earlier and to make less growth than southern types (Figure 7E). Within any given latitude, mountain types tended to go dormant earlier than nearby lowland types. Thus northern or mountain types of eastern hemlock seem best adapted to early termination of growth and hardening off where the growing season is short; southern types, especially those from lowland areas with long growing seasons, seem adapted to more prolonged growth and fuller utilization of the season. However, as a result of tardy hardening off, seedlings of southern origin may suffer frost injury when grown in the north. Evidently nature has selected genetic types whose patterns of stem growth, as well as of seed germination, are in harmony with the local climate. Such physiological diversity within a seemingly homogeneous species helps to account for its wide climatic range.

Nutrient deficiency

Although hemlock can do quite well on nutrient levels usually found in nature, it does show a quite marked response when nutrients are deficient. In the experiments shown in Figure 8, some hemlock seeds which had already begun to germinate were placed between fiberglass sheets and the vertical walls of plastic boxes and provided with distilled water or nutrient solution. All plants on complete nutrient solution developed good roots and tops and were dark green, but under 12 hours of light, early bud formation caused premature cessation of top growth (Figure 8T). Under 16 hours of light (8U) plants made far more top growth than is usual in soil; conditions of photoperiod and nutrient were nearly ideal. Plants in Figures 8V and 8W, also on a 16-hour photoperiod, were provided with a nutrient solution that was complete except for reduced quantities of nitrogen. The series 8U, V, W, clearly shows the effects of decreasing even one of the major nutrient elements. Plants in Figure 8X, supplied only with distilled water, had very limited top and
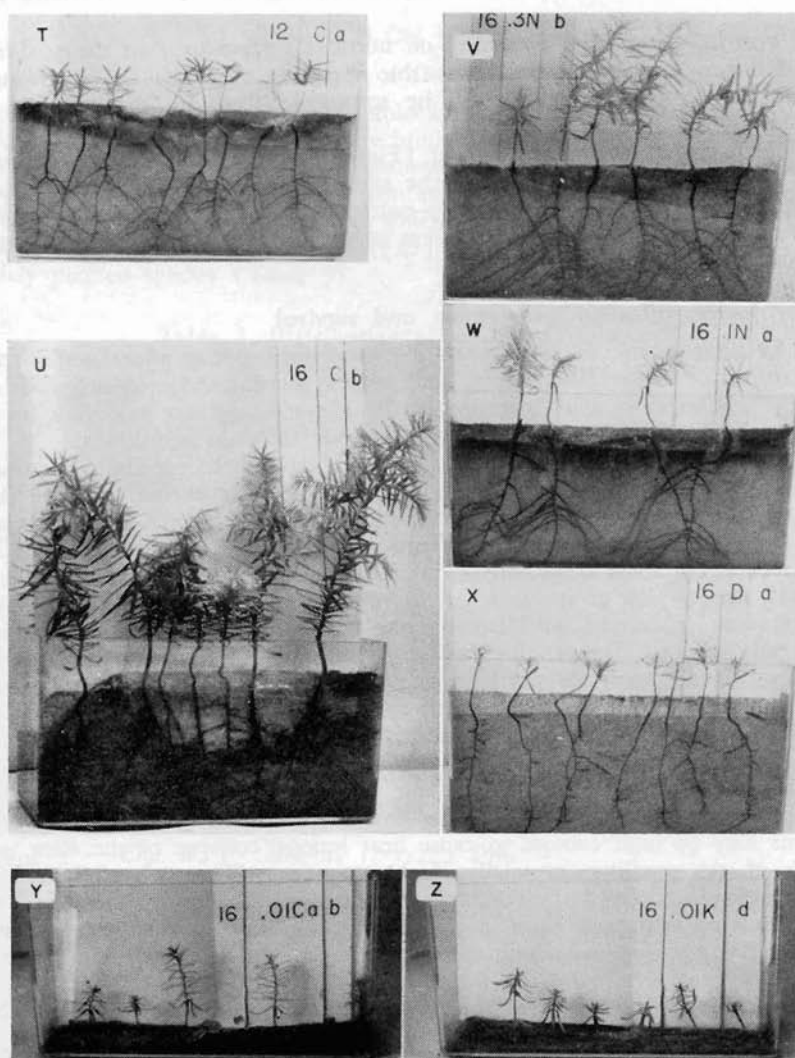
Figure 8. Effects of nutrients on first-year growth of hemlock.

T,U. Plants supplied with complete nutrient solution, (C); T on a 12-hour photo-period, U on a 16-hour.

V,W. All plants on a 16-hour photoperiod. Nutrient solutions complete except for nitrogen (N) which was reduced to 30 per cent of complete in V and 10 per cent of complete in W.

X. Plants on a 16-hour photoperiod supplied only with distilled water (D).

Y,Z. Plants on a 16-hour photoperiod. Nutrient solutions complete except that calcium (Ca) in Y and potassium (K) in Z were reduced to 1 per cent of the level in complete nutrient solutions. Calcium deficiency indicated by terminal rosette of dark, short needles, and somewhat variable stunting; potassium deficiency by general stunting, chlorosis, and gradual shortening of younger needles.

root growth; these were subsisting on nutrient carried over in the seed and on minor impurities introduced in the fiberglass or from the air. Natural nutritional conditions undoubtedly lie somewhere between the extremes of this series.

Plants from another experiment (Figure 8Y, Z) illustrate the deficiency symptoms resulting from lowering the amounts of calcium and potassium to 1 per cent of the levels in complete nutrient solutions. Deficiencies of either of these elements, and to a less extent of certain others, hasten the onset of dormancy and stunt growth.

Other factors affecting germination and survival

In addition to the factors just discussed, others may affect germination or survival of the seedling during its first few months. Adverse situations are most likely to exist when germination is delayed, or when seed falls on an unfavorable seed bed, or under a combination of these conditions.

Moisture is essential for germination and growth of seedlings. Naturally shaded seedbeds are usually sufficiently moist in early spring. At this time temperatures are most favorable for germination, and for the rapid development of a root system and partial hardening of stem tissues. A seedling established under these conditions is usually able to survive.

If, due to lack of stratification, incomplete stratification, or other causes, germination is delayed until later in the spring, the probability of survival is greatly reduced. Temperatures will then be too high for good germination and even shaded seedbeds may have become somewhat dried out. Development of the root and shoot are consequently retarded; stem tissues are still succulent and are particularly susceptible to attack by damping-off fungi during warm, humid periods. On open seedbeds[4] temperature and moisture conditions unfavorable for germination and seedling growth occur much earlier than on shaded seedbeds. Under full sunlight, temperatures at the surface of litter or humus may be high enough to cause heat lesions, collapse of the stem, and death of the seedling, especially if the stem is still succulent. Sometimes germination is delayed until autumn and the seedling is not hardened off sufficiently to withstand frost injury; germination may also be delayed until the following spring. Such long delays expose the seed to destruction by many agencies.

Sometimes seed is buried so deeply in the litter of the forest floor that the seedling cannot force its way through.

Seedlings may be stunted or killed when daylight intensity falls below about 100 foot candles, as under very dense coniferous shade. Seedlings established on more open seedbeds respond vigorously to light of much higher intensity.

Insects may infest seeds while in the cone, and insects, birds, and other animals often consume large quantities of seed on the ground, or damage or destroy seedlings.

In nature the hazards to survival are often very great but are, in large measure, compensated for by abundant seed crops which are produced at intervals of 2 or 3 years. In nursery propagation many of the natural hazards can be avoided but certain others are introduced; these can be largely nullified by proper conduct of the nursery operation.

[4]Nursery seedbeds, a special kind of open seedbed, are discussed on page 65.

RESEARCH ON HEMLOCK
Review of Literature

Early botanists classified the hemlocks with either the pines, spruces, or firs until a century ago when Carrière brought them together in the new genus *Tsuga*, which he named after its Japanese representatives. A technical monograph by Flous (22) reviews the confused taxonomy of the genus and the detailed anatomy of the 16 species which she recognized. Less technical descriptions by Dallimore and Jackson (15) and Bailey (2) deal with the most widely planted species (Table 3).

Table 3. Distribution of species of *Tsuga*

America

    Eastern, *T. canadensis* (L.) Carrière[1]

    Carolina, *T. caroliniana* Engelmann[1]

    Western, *T. heterophylla* (Rafinesque) Sargent[1]

    Mountain, *T. Mertensiana* (Bongard) Sargent[2]
    [= *T. Hookeriana* (Murr.) Carrière]

Asia

    Himalayan, *T. dumosa* (Don) Eichler
    [= *T. Brunnoniana* (Wall.) Carrière]

    Japanese, *T. Sieboldii* Carrière[1]

    Northern Japanese, *T. diversifolia* (Maxim) Masters[1,2]

    Formosan, *T. formosana* Hayata

    Chinese, *T. chinensis* (Franch.) Pritl.[2]

[1] Used in eastern American or European ornamental planting.
[2] Flous (22) recognized additional species within the range of this species.

A monograph on eastern hemlock, *Tsuga canadensis* (L.) Carrière, was prepared 70 years ago by Prentiss, and later revised slightly with Griffith (45), but was not published. U. S. Forest Service publications by Frothingham (23) and Hough (26) incorporate parts of this early description and present a great deal of later information. Lloyd (32) briefly described seed and seedling growth.

Murrill (34) described the fertilization of the hemlock egg in great detail. Echols (18) recently studied the earlier stages of sexual development; he found that reduction divisions began outdoors around March 27, 1954 at New Haven. Strobili on branches on the north side of a tree lagged 2 days behind those on the south side in this and subsequent stages of gametophyte development, suggesting a sensitive temperature control by solar heat.

Santamour and Nienstaedt (47) showed that daylength as well as temperature affects the processes leading up to pollen shed. Branches brought indoors in February and kept at about 75° on natural daylengths could be forced to shed pollen in slightly less than a month. By mid-March, the time

required to shed pollen indoors was only 19 to 23 days under natural day-length conditions, but could be reduced to only 11 to 15 days by artificial 20-hour daylengths. The time required was reduced to 4 days for branches collected April 27 for both daylengths, so only a week was gained as compared with natural pollen shed in the second week of May. The same authors (47) reported that pollen stored for a year at 50 per cent relative humidity and at temperatures ranging from 34° to 60° had a viability of 86 to 92 per cent; when stored at 10 per cent relative humidity within this temperature range, viability varied from 31 to 74 per cent. Storage at room temperatures, including hot summer temperatures, reduced viability to 39 and 28 per cent at 50 per cent and 10 per cent relative humidity, respectively, which indicates that the pollen stage of the life cycle is a fairly hardy one.

In making artificial pollinations, Nienstaedt and Kriebel (35) found striking differences in the number of viable seed per cone in various types of pollination bags. While some of the poor viability was probably due to excess humidity in impermeable bags and lack of light in black cloth or heavy paper bags, the most interesting result was a correlation between low yield and temperatures above 100°, which occurred in several types of bags in mid-May when most of the gametophyte development is taking place. High cone temperature may account for extremely low seed yield from cones collected in outliers of the species' range in Alabama and eastern North Carolina.

Although field studies by many observers have given much ecological information about hemlock, very little controlled laboratory experimentation has been attempted with this genus.

Toumey and Stevens (55) reported unpredictable germination of eastern hemlock in seed tests and nurseries, and suggested that the seeds have a chilling requirement that can be satisfied by sowing them in the autumn. Baldwin (3) demonstrated that artificial chilling usually, but not always, resulted in high germination. In 1934 he reported (4) that in 2 or 3 days water is quickly absorbed (32 per cent in 3 days) and that catalase activity increased 20 per cent, except in poor lots of seed. He concluded that "while the exact cause of sluggish germination was not made apparent from these tests, it is probable that differences in degree of maturity of different [seed] crops, or previous treatment of seed during collection, extraction, and storage may be partly responsible for the erratic germination of this species." Heit and Eliason (25) confirmed the effectiveness of moist chilling in hastening germination, and said that light may also have an effect. Allen (1), who studied western hemlock and other western conifers, also noted the effects of chilling, light, and temperature.

We have recently shown (48) that, under some conditions, light is necessary for rapid germination of eastern hemlock seed and that the daily proportion of light and darkness is important. Temperature requirements were also found to be quite specific, more so for unchilled than for chilled seed.

We have also reported some aspects of photoperiodism, day and night temperatures (thermoperiodism), and seed source on seedling growth and bud dormancy (36, 37, 40, 41), and report further thereon in this Bulletin. Western hemlock from British Columbia, grown in Norway, also showed a photoperiodic response (46).

The influence of light intensity on photosynthesis, respiration, and growth of eastern hemlock has not been covered in the present work. However,

Ferchau (21) and Bourdeau (10) have recently used infrared gas analyzers to show that the light intensity at which photosynthesis exceeded respiration (the compensation point) is unusually low, less than 100 foot candles.

Krajina (27) included western hemlock along with other Pacific conifers in greenhouse studies of mineral nutrition. This and previous work by others (57) on western red cedar and Douglas-fir provide comparisons with the preceding preliminary results on the nutrition of eastern hemlock.

The handling of cones and seed is discussed in references (5), (6), and (56), and in the last section of this paper.

Materials and Methods

Seed collection

Eastern hemlock seeds used in the experiments later described came from more than 60 cone collections made by ourselves and others at known locations. These collections were from both high and low elevations over the range of the species; many were from the extreme margins of the range (Figure 24). The percentage of sound seed was low at both the northern and southern extremes of the range. Seed weight increased with increase in the length of growing season (Figure 21B). Trees growing side by side sometimes showed marked differences in the time of change of cone color from green to tan, presumably due to genetic factors.

Where possible, seeds from four or more trees in each stand were kept separate for field plantings. Almost all batches of seed used in indoor experiments were composites from a given geographic location. In most of our own collections, cones were taken from the tops of superior trees. A special ladder was strapped around the trunk, section by section, to reach each crown.

Good seed years have usually occurred at least once every 3 years, for example, 1950, 1953, and 1956 in New England. Yields tend to be very poor the first season after a good year, but vigorous trees may produce moderate crops in the second season.

Table 4 shows the degree of opening on October 15 of cones collected at intervals between September 14 and October 15 when dried at 4 different temperatures. Moisture lost during drying by green cones picked in mid-September amounted to 160 per cent of their air-dry weight; by tan cones picked in mid-October, to 100 per cent of air-dry weight. Green cones lost this excess moisture in 2 weeks at 80° and in 3 weeks at 53°. At the same temperatures, tan cones lost excess moisture in 1 and 2 weeks, respectively. If cones are picked green and dried quickly, they may turn pink or purple rather than tan and fail to open well unless subjected to repeated cycles of re-wetting and drying at 100°.

Growing conditions

Except in certain early experiments in the greenhouse and in later nursery trials designed to test laboratory predictions, seeds and seedlings were grown under artificial light in rooms or cabinets with controlled temperature. Growing conditions were generally as follows, but further details will be noted under individual experiments.

Light was mainly from fluorescent tubes. Their intensities of 300 to 500 foot candles (about 3,000 to 5,000 lux) were well above the compensation point (less than 100 foot candles) and permitted vigorous growth when other

Table 4. Opening of hemlock cones

Date of collection	Temperature of drying			
	53°	62°	71°	80°
	Degree of opening by Oct. 15			
Sept. 14	slight	slight	slight	none
Sept. 20	moderate	moderate	slight	none
Oct. 2	high	high	high	medium
Oct. 8	high	high	high	high
Oct. 15	Open at time of picking			

conditions were favorable. In some experiments, incandescent bulbs (5 to 40 foot candles) were kept on for part of the time when fluorescent bulbs were off.

Heating and cooling blowers controlled by thermostats were available in several growing rooms. Lead-covered electric heating cables and small fans run by phonograph turntable motors were installed in smaller glass-topped boxes within the larger rooms. These boxes were cooled by heat loss to the cooler room.

Records of temperatures, obtained from 30-gauge thermocouples imbedded in seeds, showed fluctuations around the mean during the heating-cooling cycle varying from 1° to 4° for nominally constant temperature. Under some conditions, there was an additional rise of 1° or 2° in mean temperature when the lights were turned on. Such fluctuations were small in comparison with those obtained by moving plants from one chamber to another in experiments which simulated natural daily temperature oscillations.

Seeds were stratified in thin layers between sheets of fiberglass in plastic boxes, or in folded cloth surrounded by sphagnum moss, kept at 35° to 40° for varying lengths of time prior to germination. Other seeds were germinated without stratification. In most experiments, seeds were germinated in glass or plastic petri dishes on pads of fiberglass or filter paper moistened with distilled water. In certain experiments seeds or excised embryos were grown on agar. Treatments with added chemicals will be described later. Counts were normally made every 2 or 3 days during the first few weeks and less frequently later.

Seedlings for nutrition experiments were grown on fiberglass sheets bent up along the edges of plastic boxes containing mineral nutrient solution, either complete, using the formula of Walker, Gessell, and Haddock (57), or reduced in level of one element. For other experiments seedlings were grown in clay pots containing vermiculite and sand or soil, supplemented with nutrient solution according to the above formula. To prevent early damping off and subsequent root rot, pots were treated periodically with 8-hydroxyquinoline sulfate, 1 to 4,000 or 1 to 2,000.

Germination experiments were generally based on dishes with 25 seeds. The more important treatments were replicated in either the same or separate experiments. Most of the seedling experiments averaged 25 seedlings per treatment.

Table 5. Effects of photoperiod, and stratification at 40°, on hemlock germination at 62°

Per cent germination after 2 weeks

Source	Unstratified[1] Hours of light						Stratified 5 weeks Hours of light						Stratified 10 weeks Hours of light					
	0	8	12	14	16	20	0	8	12	14	16	20	0	8	12	14	16	20
17 Tennessee							4	12	16	....	24	....	52	64	76	64	64	64
50 Indiana							0	0	0	....	0[2]	....	12	28	28	40	24	32
9 Connecticut		None for any source					12	8	16	....	12	....	44	44	40	48	44	44
24 Maine							16	20	32	....	16	....	44	72	64	52	60	60
34 Quebec							20	28	12	....	20	....	60	68	76	72	64	72
							10.4	12.0	15.2	....	14.4	....	42.4	55.2	56.8	55.2	51.2	54.4

Per cent germination after 7 weeks

Source	Unstratified[1] Hours of light						Stratified 5 weeks Hours of light						Stratified 10 weeks Hours of light					
	0	8	12	14	16	20	0	8	12	14	16	20	0	8	12	14	16	20
17 Tennessee	0	80	58	4	4	40	52	48	56	....	52	....	56	72	80	68	68	68
50 Indiana	0	46	66	0	0	0[2]	52	92	92	....	8[2]	....	60	76	88	88	84	84
9 Connecticut	2	44	50	0	4	20	40	56	52	....	44	....	72	48	56	48	60	44
24 Maine	0	6	10	4	6	40	52	68	64	....	44	....	72	80	84	72	60	80
34 Quebec	0	16	24	8	10	52	84	80	76	....	60	....	72	84	92	80	72	96
	0.4	38.4	41.6	3.2	4.8	30.4	56.0	69.6	68.0	....	41.6	....	66.4	72.0	80.0	70.4	69.6	74.4

[1]Averages of two experiments.

[2]Note that source 50 commonly lags behind other sources under several conditions.

Germination: Controlling Factors and Interactions

Stratification and photoperiod

On page 12 the effect of environmental factors on germination were briefly discussed. Table 5 summarizes in somewhat greater detail the influence of these factors on germination after 7 weeks, of seed from 5 sources at a constant temperature of 62°. At this temperature, contrasts among different combinations of stratification and photoperiod were most striking.

Stratification for 5 weeks at 40° was long enough to start some germination within 2 weeks after seeds were moved to 62°, except those from source 50 which normally lagged behind other batches of seed. After 7 weeks at 62°, germination was much higher and showed comparatively little effect of photoperiod, with seed from source 50 again a notable exception.

After 10 weeks of stratification, a high percentage of seeds had germinated within 2 weeks for sources other than 50. After 7 weeks, germination was still higher. Photoperiod seemed to have little effect at either 2 or 7 weeks.

The effect of photoperiod on the germination of unstratified seed after 7 weeks is also evident in Table 5. Photoperiods of 0, 14, and 16 hours resulted either in no germination or very low germination of seed from all sources. By contrast there was appreciable stimulation under 8 and 12 hours of light. This stimulation was greater for seed from southern than from northern sources. Twenty hours of light stimulated germination to a somewhat lesser extent. We are left with the unanswered question as to why germination does not increase progressively as duration of light increases.

In other experiments, stratification for 16 weeks at 40° permitted 90 to 100 per cent germination in the first 2 weeks for seed from some sources. After 20 weeks, seeds started germinating without being taken out of stratification.

Unfortunately, losses due to attack by fungi also increase after stratification for several months at 40°. Such losses may explain why final germination of stratified seed was sometimes slightly below the level eventually attained by unstratified seed. They may also explain one anomalous case in which germination of seed stratified 10 weeks was consistently below that of seed stratified 5 weeks.

> How can one decide on the best compromise between too long or too brief a period of stratification? If, for example, one had only 12 weeks available before reaching a decision as to density of planting, how should this time be allocated between the stratification period and the period of testing for germination? Following stratificaton for 5 weeks at 40°, germination after 7 weeks at 62° was usually, but not always, higher than germination after 2 weeks at 62° of seed that had been stratified for 10 weeks at 40°. Presumably still higher germination would result if the stratification period at 40° were decreased to 8 weeks and the period at 62° were increased to 4 weeks.
>
> Consider a second example in which there is plenty of time to stratify the seed but one wishes to get the fastest possible germination in the field or laboratory. In this case, stratification might be extended to 3 or 4 months, if preliminary tests showed that contamination by fungi was not serious. Temperatures would have to be held consistently below 40° to prevent germination during stratification, unless provisions were made for unusually careful handling during later transfer to field or experimental conditions.
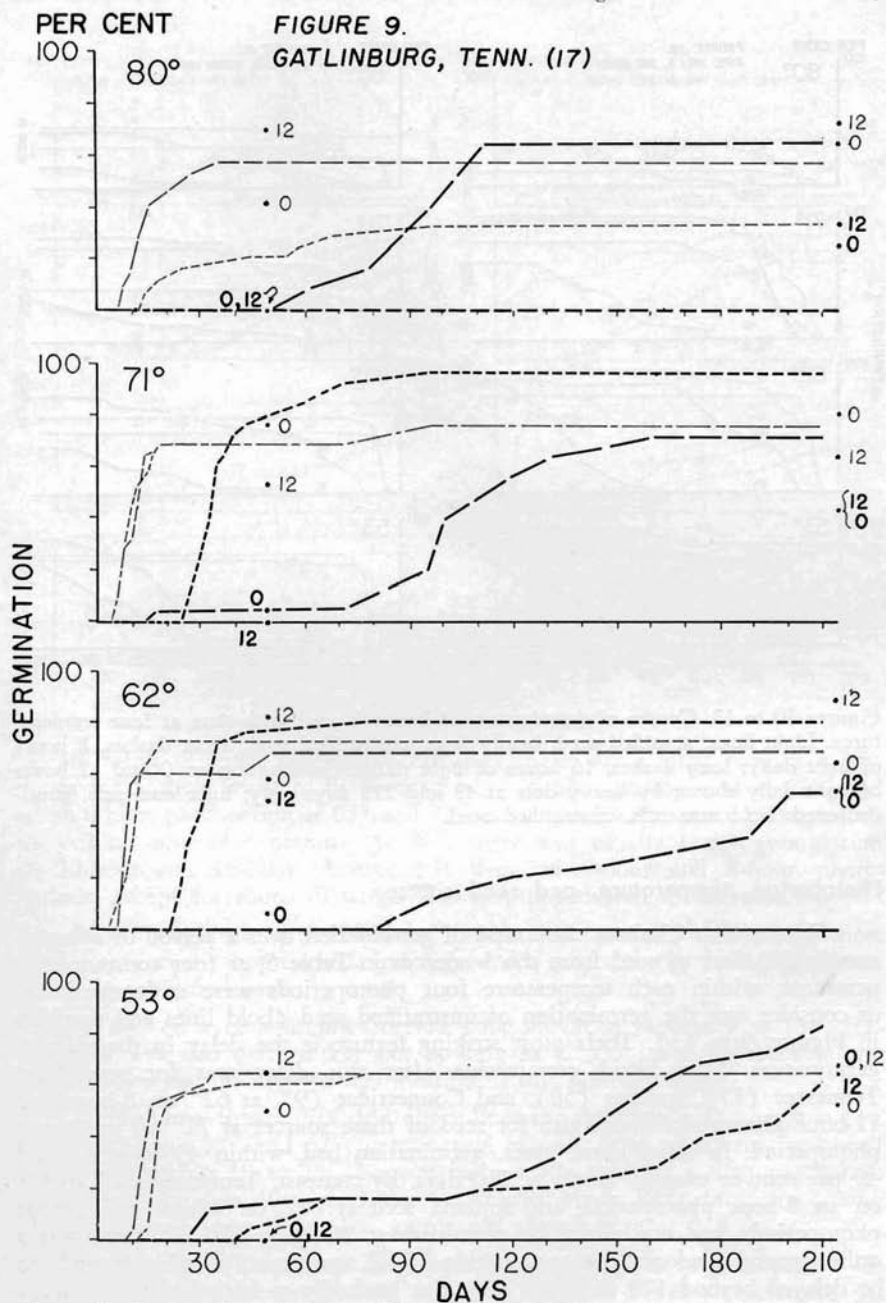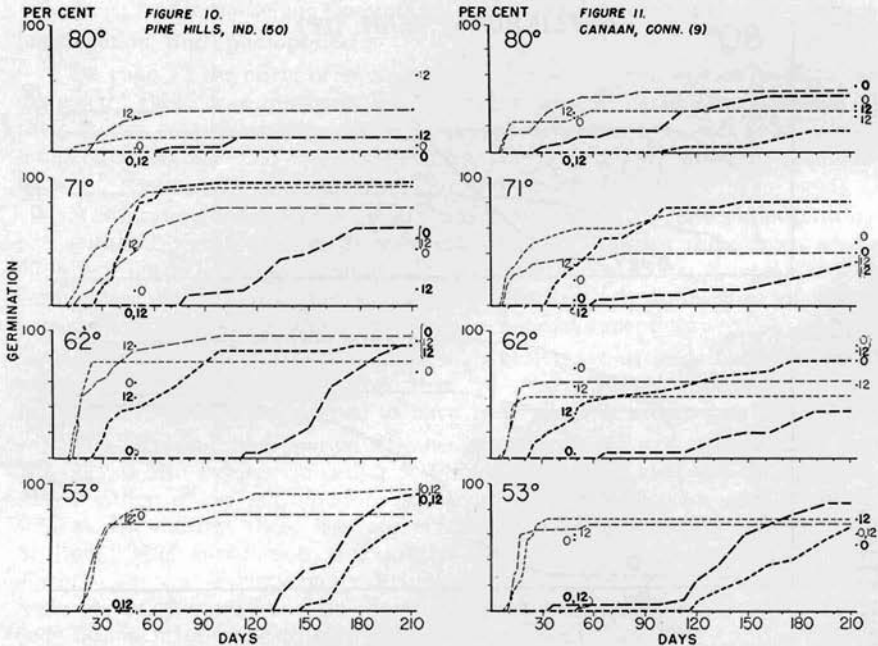
Figure 9. Course of germination of hemlock seed with time at four temperatures. Light lines, stratified seed; heavy lines, unstratified seed; short dashes, 8 hours of light daily; long dashes, 16 hours of light daily. Germination on 0 and 12 hours of light daily shown by heavy dots at 49 and 213 days only; light numerals, stratified seed; bold numerals, unstratified seed.

Figures 10 to 13. Course of germination of hemlock seed with time at four temperatures. Light lines, stratified seed; heavy lines, unstratified seed; short dashes, 8 hours of light daily; long dashes, 16 hours of light daily. Germination on 0 and 12 hours of light daily shown by heavy dots at 49 and 213 days only; light numerals, stratified seed; bold numerals, unstratified seed.

Photoperiod, temperature, and seed source

Figures 9 to 13 show the course of germination over a period of approximately 210 days of seed from the 5 sources in Table 5, at four constant temperatures; within each temperature four photoperiods were maintained. Let us consider first the germination of unstratified seed (bold lines and numbers in Figures 9 to 13). Their most striking feature is the delay in the start of germination. Most rapid germination after this delay was for seed from Tennessee (17), Indiana (50), and Connecticut (9), at 62° on 8-hour and 12-hour photoperiods, and also for seed of these sources at 71° on an 8-hour photoperiod. In all of these cases, germination had, within 49 days, reached 40 per cent or more of levels at 210 days. By contrast, Tennessee seed at 80° on an 8-hour photoperiod, and Indiana seed at 80° on 0-hour and 8-hour photoperiods, had not started to germinate at 210 days. At 53° there was a quite general tendency for germination of all sources on all photoperiods to be delayed beyond 120 days and then rise gradually to high levels.

Comparisons limited to germination on 8-hour and 16-hour photoperiods at 100 days show that, at 53°, no seed from Indiana (50), Maine (24), and Quebec (34) had germinated, and that seed from Tennessee and Connecticut had attained less than 25 per cent of levels at 210 days.

FIGURE 12. ORONO, ME. (24)

FIGURE 13. MONT TREMBLANT, QUE. (34)
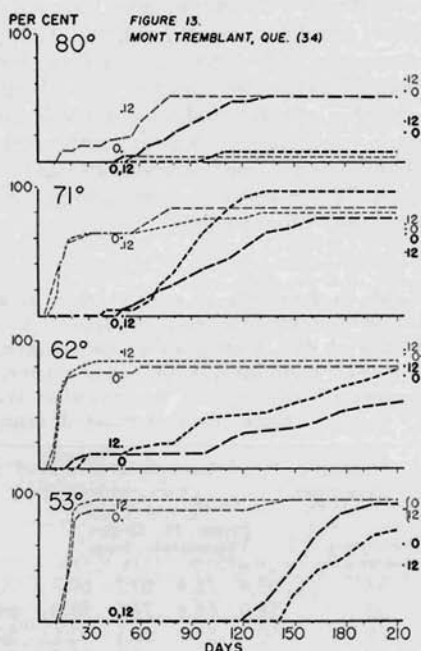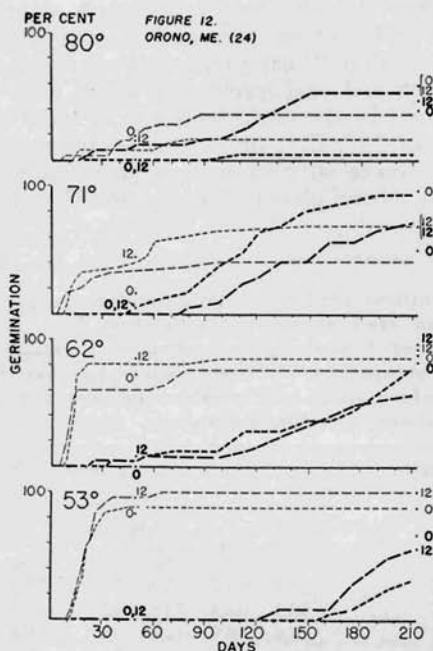
In terms of levels attained at 210 days, there was a fairly consistent tendency for highest levels to be reached on a 16-hour photoperiod at 53°, and on an 8-hour photoperiod at 62° and 71°. Levels attained at other photoperiods showed no consistent pattern. At 80°, there was usually better germination on 12-hour and 16-hour photoperiods than on 0-hour and 8-hour photoperiods, except for source 9 which also germinated well in darkness.

Even though the germination of unstratified seed was delayed, in most cases it eventually approached and sometimes exceeded the levels finally attained by stratified seed.

At 44° on a 12-hour photoperiod (not shown in Figures 9 to 13), germination was also delayed but not as long as at 53°, probably because the seed was actually undergoing stratification at this temperature.

Turning now to *stratified* seed (light lines and numbers in Figures 9 to 13) we find that, at 53° and 62°, germination of seed from all sources was well under way within 10 to 15 days and had usually attained a high percentage of final levels within 49 days. There was comparatively little difference among photoperiods for any given seed source and temperature at 210 days.

At 71°, some differences in germination attributable to photoperiod are evident. Tennessee seed responded much as it did at lower temperatures. Its germination at 49 days approximated that at 210 days on all photoperiods. For the other sources there was some lag beyond 49 days on all photoperiods in the attainment of levels at 210 days. The latter, however, were about as high as at 53° and 62°.

At 80° Tennessee seed reached about as high levels at 210 days as at lower temperatures on 0-hour, 12-hour and 16-hour photoperiods, and had attained a high percentage of these levels within 49 days; on an 8-hour photoperiod germination lagged beyond 49 days and final levels were below those on other photoperiods. Final levels attained by the other four sources at 80° were much lower than at temperatures of 53°, 62°, or 71°. Attainment of these levels lagged beyond 49 days for almost all photoperiods. In common with Tennessee seed, germination on an 8-hour photoperiod was lower than on other photoperiods.

Table 6. Analysis of germination of stratified seed of eastern hemlock in relation to temperature, photoperiod, and seed source. Stratification for 10 weeks at 40°. Germination values are as of 7 weeks. Percentage germination for each combination of temperature, photoperiod, and seed source has been converted to degrees by angular transformation; all means and averages have been computed from these transformed values[1]

A. Response for 4 temperatures and 4 photoperiods for 5 seed sources and their averages.

Temperature, degrees F.	Source 34. Quebec Photoperiod, hours					Source 24. Maine Photoperiod, hours				
	0	8	12	16	Mean	0	8	12	16	Mean
53	63.4	78.4	69.7	69.7	70.3	66.4	69.7	78.5	78.5	73.3
62	58.0	66.4	73.6	58.0	64.0	53.1	63.4	73.6	50.7	60.2
71	50.7	53.1	46.1	53.1	50.8	23.6	36.9	41.6	35.4	34.4
80	16.4	10.8[2]	39.2	23.6	22.5	26.6	16.4	23.6	23.6	22.6
Mean	47.1	52.2	57.2	51.1	51.9	42.4	46.6	54.3	47.0	47.6

	Source 9[3]. Connecticut Photoperiod, hours					Source 50. Indiana Photoperiod, hours				
	0	8	12	16	Mean	0	8	12	16	Mean
53	71.1	76.8	58.2	66.7	68.2	48.9	50.8	66.4	66.4	58.1
62	76.8	52.7	59.1	62.7	62.8	50.8	60.7	69.7	66.4	61.9
71	30.9	59.2	40.1	43.5	43.4	16.4	63.4	41.6	41.6	40.8
80	34.2	40.5	30.8	46.5	38.0	11.5	16.4	34.4	26.6	22.2
Mean	53.2	57.3	47.0	54.8	53.1	31.9	47.8	53.0	50.2	45.8

	Source 17[3]. Tennessee Photoperiod, hours					Mean of 5 sources Photoperiod, hours				
	0	8	12	16	Mean	0	8	12	16	Mean
53	71.1	55.4	58.2	58.2	60.7	64.2	66.2	66.2	67.9	66.1
62	52.7	64.5	72.0	61.2	62.6	58.3	61.5	69.6	59.8	62.3
71	67.9	61.2	50.0	61.2	60.1	37.9	54.8	43.9	47.0	45.9
80	42.2	25.2	61.2	58.2	46.7	26.2	21.9	37.8	35.7	30.4
Mean	58.5	51.6	60.4	59.7	57.5	46.6	51.1	54.4	52.6	51.2

B. Mean response at 44° for a 12-hour photoperiod for each seed source.

°F Temp.	(34) Quebec	(24) Maine	(9)[3] Conn.	(50) Ind.	(17)[3] Tenn.	Average
		(12-hour	photoperiod	only)		
44	60.7	50.8	62.7	16.4	39.6	46.0

[1]Figures 14A and 14B were plotted from data in Tables 6A and 6B (see text).

[2]Maximum likelihood estimate of the angle for 0 per cent germination.

[3]Adjustment for non-viable seed from sources 9 and 17 was made by assuming 22 viable seeds instead of 25 for source 17, and 19 instead of 25 for source 9.

Table 6 (continued). Analysis of variance and regression on temperature

C. Analysis of variance

Environmental or "plot"	effects	of		Source or "subplot"	differences	
	DF	MS			DF	MS
				Between sources	4	347**
Temperature				Interaction of sources with		
Linear	1	15,282**		Temperature, linear	4	525**
Quadratic	1	680		Temperature, quadratic	4	133
Cubic	1	184		Temperature, cubic	4	72
Photoperiod	3	219		Photoperiod	12	112
Photoperiod x temp.[4]	9	152*		Photoperiod x temp.[5]	36	66
Photoperiod[6] within 53°	3	12		Photoperiod[6] within 53°	12	67
Photoperiod within 62°	3	127		Photoperiod within 62°	12	69
Photoperiod within 71°	3	246		Photoperiod within 71°	12	97
Photoperiod within 80°	3	290*		Photoperiod within 80°	12	77

D. Variance due to regression on temperature, by individual sources

Coefficient	Source				
	34	24	9	50	17
Linear	4,908	6,337	2,420	3,320	398
Quadratic	482	2	0	497	233
Cubic	13	143	157	152	8

*Significant   [4]Plot error
**Highly significant   [5]Subplot error
[6]An alternate partitioning of the degrees of freedom and sums of squares in the two preceding rows.

Statistical analysis for stratified seed

Because the effects of temperature, photoperiod, and source were much less pronounced for stratified seed than for unstratified seed, the statistical analysis in Table 6 was made in order to test their significance. Prior to this analysis, figures for percentage germination were converted to angles in degrees by the inverse sine transformation, after adjusting for non-viable seed in the case of sources 9 and 17 (see footnote, Table 6).

The transformed data and their relevant means are given in parts A and B of Table 6. In the first five sub-tables of part A, germination data are shown separately for seed of each of 5 sources for each temperature and photoperiod; the data are averaged in the margins for each temperature over all 4 photoperiods (row means) and for each photoperiod over all 4 temperatures (column means). In the sixth sub-table (lower right corner) average germination of 5 sources for each photoperiod and temperature is given, together with row and column means. In part B, germination for 44° is limited to a 12-hour photoperiod; these data were not used in the analysis in parts C and D.

Figure 14A, in which the germination has been related to the 5 constant temperatures, averaging the 5 seed sources, was plotted from the data of the 6th sub-table in the lower right corner of part A, and from the average for 44° on a 12-hour photoperiod (46.0) of part B. In Figure 14B, the same data have been plotted again against temperature on the same coordinates but separating the individual sources, averaged over the 4 photoperiods, representing in this case the row means in part A, plus the data for 44° at a single 12-hour photoperiod in part B.
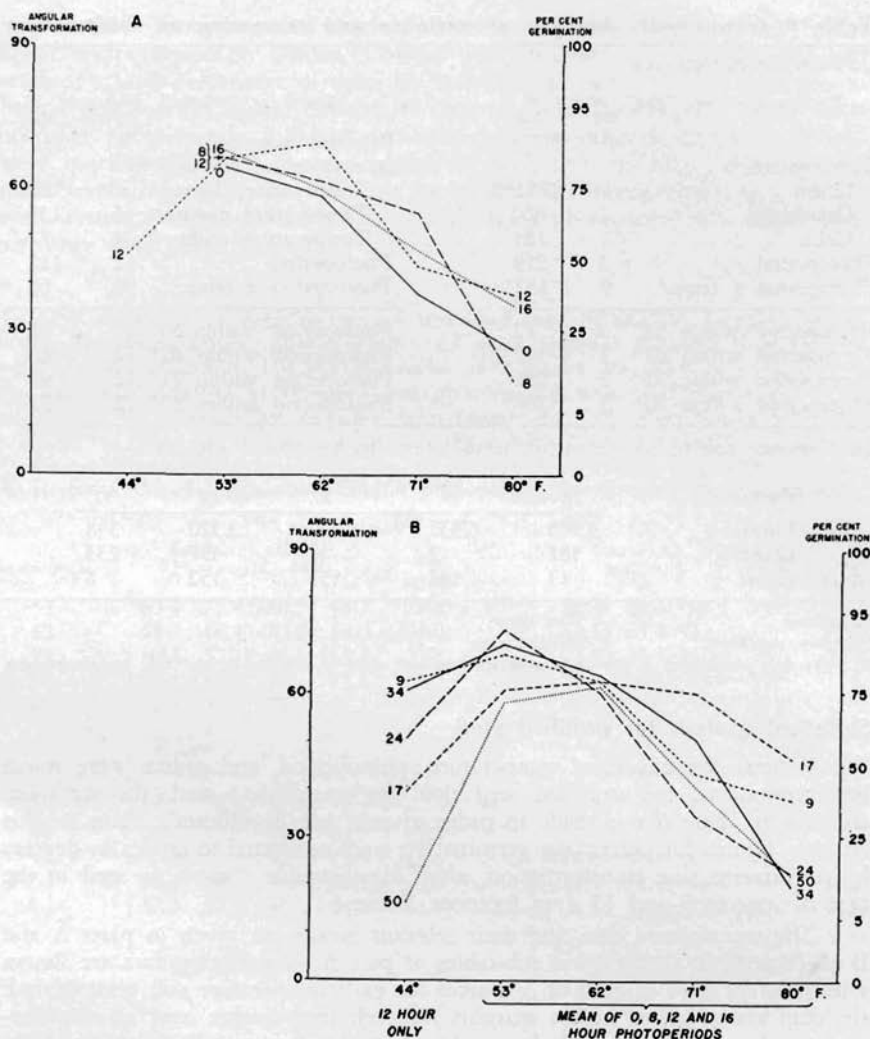
Figure 14. Differences in temperature response by stratified hemlock seed from five sources as of 7 weeks. A. Data averaged over five seed sources for each photoperiod. Numerals indicate duration of photoperiod. B. Data averaged over four photoperiods for each seed source, except for 44° which was for a 12-hour photoperiod only. Numerals represent seed-source numbers. Figures 14A and 14B plotted from the means in Table 6A and 6B.

Table 6C includes a general analysis of variance of all factors and interactions, and an alternative analysis which considers effects of photoperiod separately at each temperature. In both, the environmental effects are considered in the left column and effects of source and interactions of environment and source are in the right. The latter are treated as "subplot" differences in a split-plot experiment.

The highly significant effect of temperature is due mostly to the linear trend (15,282**) of decreasing germination with increasing temperature (Figure 14). This linear trend in the temperature response differed significantly (525**) between seed sources and reflects the differences between the linear regression coefficients for the 5 sources in Table 6D.

The average or direct effect of photoperiod was not significant (219) but it did differ significantly with temperature (152*, plot error). Although the mean squares for photoperiod within temperatures in the lower left part of Table 6C increase with increasing temperature, only that for 80° is significantly larger than its interaction with seed sources in the lower right part of Table 6C.

Turning to inherent differences between seed sources or "subplot" comparisons, we find a significant difference in both average response of seed sources (347**) and in their interaction with the linear trend on temperature (525**) but not in their interaction of source with photoperiod (112). In the lower right part of Table 6C we find that the variation between sources in their response to photoperiod is independent of temperature in contrast to the progressive change for photoperiod alone (lower left part).

The influence of temperature on the effect of photoperiod on germination and on the shift in its optimal length (Table 6A) is reflected in the significant interaction of light and temperature (152*, the plot error) as compared with the light-temperature-source interaction of 66 (the subplot error). About half of this latter variance, 32.8, is attributable to the variability of binomial sampling as estimated for the angular transformation; the other half represents additional unassigned variability between samples or third-order interactions.

Looking more closely at the temperature-source interaction, the curves in Figure 14B and the italicized numbers in Table 6A show how the optimal temperature for germination varies with source. Maine (24) and Quebec (34) seed had considerably higher germination than Tennessee (17) seed at 53° and 44°. On the other hand, these northern sources show especially low or long-delayed germination at 80°. By contrast, germination of stratified seed from Tennessee was poorer than that from northern sources at 44° and 53°, and higher at 71° and 80°; its optimal temperature was higher than that of any other source (Figure 14B).

Interpretation of differences among sources

The inferred adaptation of northern seed to cold soils is probably important where growing seasons are very short and where frozen soils warm up slowly, particularly under the shade of evergreen forests which are more common in the north. The adaptation of southern seed to generally higher temperature seems natural in the south where soils warm up earlier in the deciduous forest mixtures that let the spring sunlight reach the ground.

Adjusted percentage germination for stratified seed from the northern hardwood region of Connecticut (9) was about as high as for seed from Quebec at 44° and 53° and considerably better than seed from the latter source at 80°. Stratified seed from Indiana (50) showed an optimal temperature very near to that of Tennessee seed but its germination at 44°, 71°, and 80° was much lower than that of Tennessee seed (Figure 14B).

Without stratification, Tennessee seed (Figure 9) reached high levels of germination much more quickly than Maine and Quebec seed (Figures 12 and 13), perhaps because the former has become adapted to lesser chilling

requirement. Most rapid attainment of high levels by Tennessee seed at favorable temperatures ($62°$ or $71°$) was under an 8- to 12-hour photoperiod, which would prevail in late winter and very early spring when hemlock seeds start germinating in the south. This suggests that photoperiod may be compensating to some extent for lack of chilling.

Under many conditions, a few seeds germinated much earlier or much later than the great majority in the same lot. This suggests that there is considerable genetic variability within a local population. The evolution of different races (ecotypes) presumably has occurred through many generations by selection of those individuals which were best adapted to their local environment.

Thermoperiodism of seeds

Although the preceding experiments involved nominally constant temperatures, with fluctuations of $\pm1°$ or $\pm2°$ for the most part, seeds obviously

Table 7. Effects of daily temperature alternations on the number of days required for 50 per cent of final germination as of 212 days, 12 hours of light and 12 hours of darkness throughout

Night tempera-ture	Seed source	Unstratified seed					Seed stratified 10 weeks				
		44°	Day temperature			80°	44°	Day temperature			80°
			53°	62°	71°			53°	62°	71°	
			Number of days					Number of days			
80°	17		>72²			118	17¹				18
	50		>72			96	54				47
	9		>72			111	10¹				16¹
	24		>72			154	26				75¹
	34		>72			114	24				33¹
71°	17	27			125		14			12	
	50	37			149		19			20	
	9	33			131		20			12	
	24	33			149		17			20	
	34	30			167		14			14	
62°	17			27		48			11		14
	50			36		54			17		20
	9			55		>72			13		14
	24			121		>72			11		14
	34			97		>72			11		20
53°	17		132		28	·39		20		14	18
	50		166		37	57		33		23	34
	9		132		34	47		21		18	19
	24		175		30	56		24		18	20
	34		140		30	47		20		18	20
44°	17	112			42	37	41		18	21	
	50	117			>72	>72	58		23	34	
	9	103			>72	·59	34		24	25	
	24	116			>72	>72	41		23	23	
	34	111			>72	54	37		26	19	

[1] Final germination was markedly reduced; hence the small number of days to reach 50 per cent germination may not mean favorable conditions.

[2] Seeds were removed from alternating to a constant temperature of $60°$ for the last 140 days of the test. Lots marked >72 were thus shifted before their germination had reached 50 per cent.

are subjected to greater temperature oscillations in nature, and these have long been known to stimulate germination. Figures in each half of Table 7 show the difference between unstratified and stratified seed in the time required for half of the final germination to be completed. Each group of five numbers represents a single temperature treatment for the five sources arranged in climatic order with the most southern (17) at the top in each case. The groups on the diagonal, shown in boldface, (from 53° day, 53° night to 80° day, 80° night) were derived from final germination data on a 12-hour day and 12-hour night constant temperature shown in Figures 9 to 13. Germination data for 44° day, 44° night (not plotted on these figures) were taken at about the same time from an additional experiment. Other data, not in boldface, were obtained in the same experiment from plants which were moved daily between warm and cool chambers. The groups to the right of those in boldface include seeds that were normally kept at the higher temperature during 8 hours of the light period. These alternations simulate diurnal temperature fluctuations encountered in natural habitats. The groups to the left of those in boldface represent the opposite condition in which the seeds were at the higher temperature for 16 hours which included the 12-hour dark period.

One striking result was the hastening of germination of unstratified seed by 71° day, 53° night temperatures, even for northern seed which showed relatively little response to favorable photoperiod. The 44° day, 71° night combination produced almost identical results. This similarity suggests that the value of oscillating temperatures does not depend strictly on whether the high (or low) temperature occurs during the light or dark phase of the diurnal cycle.

Both of these combinations, and also the 80° day, 62° night treatment, gave conspicuously more rapid germination than did constant temperatures of either 80° or 71°. Such relief from unfavorably high temperatures, normally at night, is probably important in the ecological relations of plants.

Similarly the conspicuous sluggishness of germination at a constant temperature of 44° was partially overcome by letting the seeds spend part of each day at 62° or 71° and part at 44°. Perhaps some of the benefit from these temperature oscillations comes from gradual completion during the cool part of the diurnal cycle of some processes which normally occur during stratification.

Stratified seed germinated so rapidly at 62° constant temperature that no further improvement was gained by alternating temperatures; also there was relatively little delay if part of each day was spent at temperatures considerably higher or lower as long as there is some relief from such extremes.

Long-term temperature shifts

The striking decrease in germination at 80° raised a question as to whether this effect could be reversed by subsequent low temperature. First, unstratified seeds which had virtually no germination after 6 weeks at 80°, on 8 or 12 hours of light, were moved to 62° with 8 hours of light. Under these favorable conditions germination increased after about 2 weeks while stratified seed similarly treated showed an increase that was more prompt but less abrupt (48, Figure 3).

In another treatment, duplicate lots of 25 seeds from source 50 were shifted from 62° to 80° and *vice versa*. Transfer was made from 62° to 80° after 5, 11, or 15 days (Figure 15A) and from 80° to 62° after 5 or 11
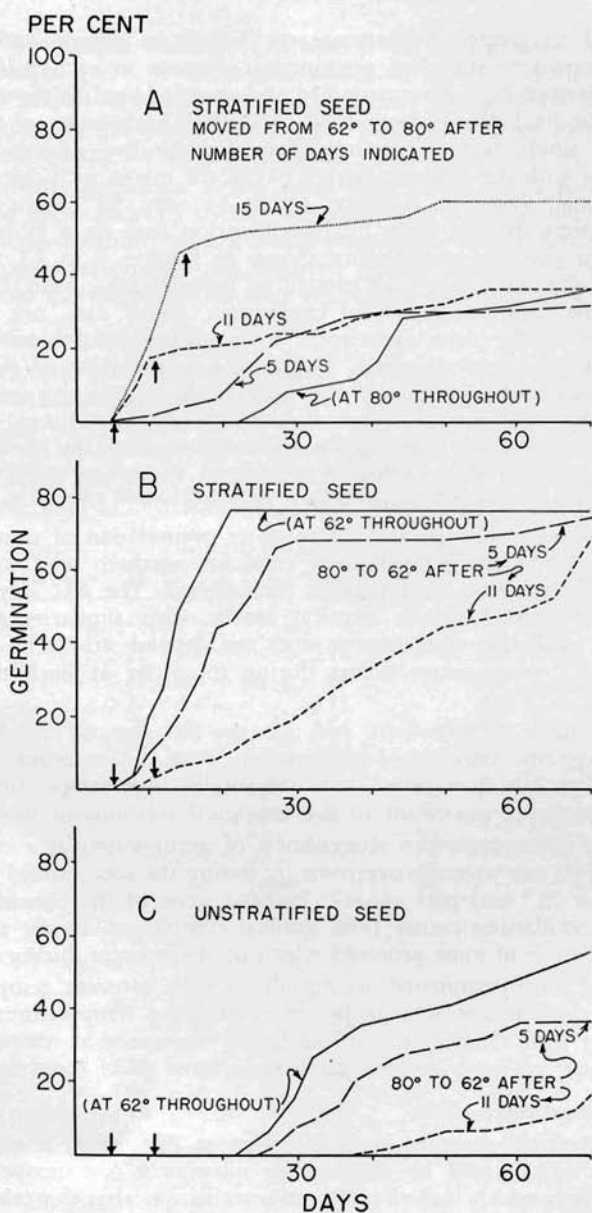
PER CENT

Figure 15. Effects of temperature change on germination of hemlock seed. Heavy arrows indicate days at which change was made.

days (Figure 15 B and C). The results were compared with the germination of seeds kept constantly at the two temperatures.

Stratified seed, started at 62° and shifted to 80° after 11 or 15 days, began germinating normally, but suddenly slowed down as soon as the shift

to higher temperature was made. The germination of seeds which were shifted after 5 days was greatly delayed but finally reached about the same level as that reached by seeds which were shifted after 11 days. Seeds kept at 80° throughout the experiment eventually reached about the same level as that attained by seeds which were shifted after 5 and 11 days (Figure 15A). Results on unstratified seeds moved from 62° to 80° are not shown; none germinated before being moved and subjected to an unfavorable temperature; none germinated thereafter during the test period.

Germination of stratified seed, (Figure 15B) moved from 80° to 62° after 5 days, was delayed slightly and that moved after 11 days, markedly. Both lagged behind seed started and kept at 62°, but the final germination for the three lots was approximately the same. The pattern of germination curves for unstratified seeds (Figure 15C) was essentially similar, but indicates that the delay caused by keeping seeds at 80° for 11 days was even greater, and the germination level after 10 weeks was notably reduced.

Thus, for both stratified and unstratified seed an initial temperature of 80° not only postpones the start of germination by the duration of the period at this temperature; it also has a subsequent depressing effect on germination after the seeds are moved to 62°. This effect is considerably worse for all days at 80° than for 5 days. Presumably some internal change tends to block germination, and the change is only slowly reversible when seeds are returned to favorable conditions.

Drying

In the previous experiments, stratified seeds usually were exposed to drying for less than an hour in the process of handling. This leaves unanswered the important question of whether the advantages of stratification are lost if the seeds are more thoroughly dried in handling, and if so, under what conditions of drying.

In a preliminary test, seeds which had been stratified for 12 weeks and which were already showing some germination, were dried for 0, 12, 16, and
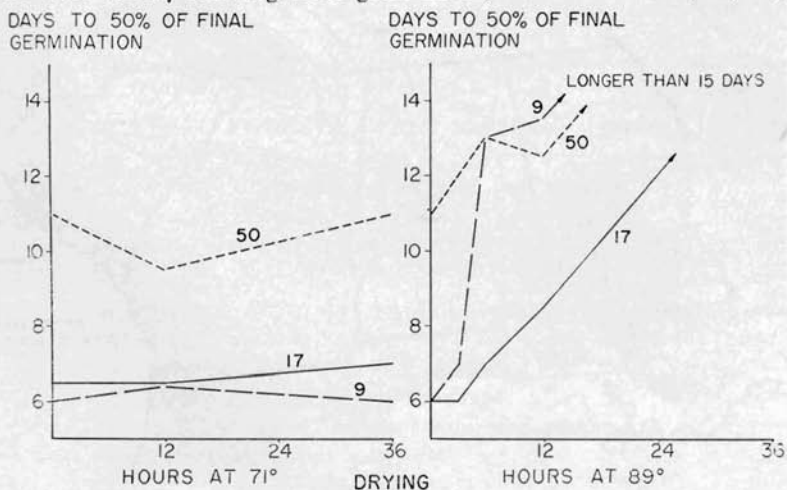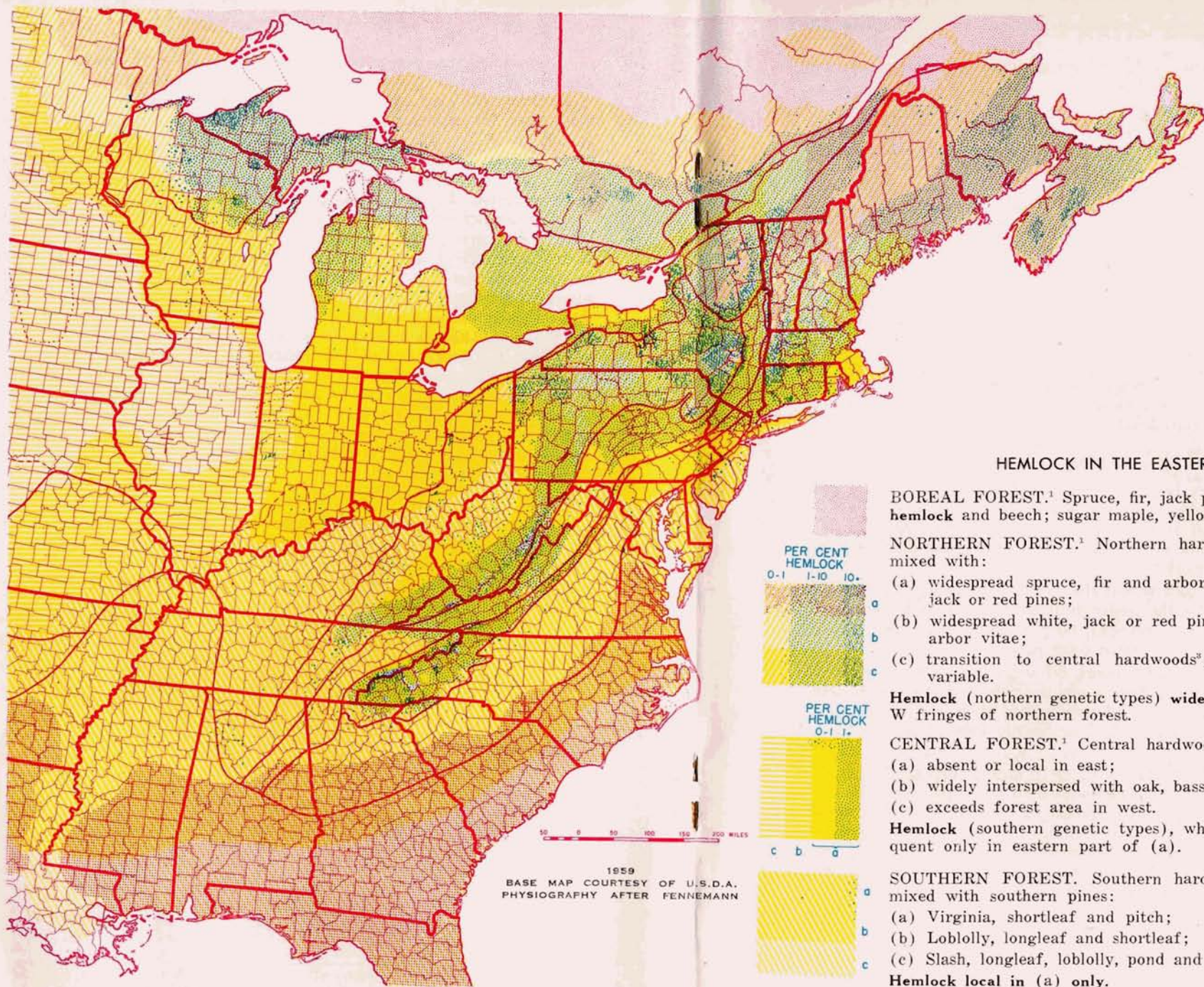

Figure 16. Effects of drying hemlock seed at 71° and 89° for number of hours indicated. Numerals on graph are seed-source numbers.

HEMLOCK IN THE EASTERN FOREST

BOREAL FOREST.[1] Spruce, fir, jack pine, paper birch; **lacks hemlock** and beech; sugar maple, yellow birch rare or absent.

NORTHERN FOREST.[1] Northern hardwoods[2], alternating or mixed with:

(a) widespread spruce, fir and arbor vitae; variable white, jack or red pines;

(b) widespread white, jack or red pine; variable spruce, fir, arbor vitae;

(c) transition to central hardwoods[3]; with species in (b) variable.

Hemlock (northern genetic types) **widespread** except in N and W fringes of northern forest.

CENTRAL FOREST.[1] Central hardwoods[3]; prairie:

(a) absent or local in east;

(b) widely interspersed with oak, basswood;

(c) exceeds forest area in west.

Hemlock (southern genetic types), white and pitch pine frequent only in eastern part of (a).

SOUTHERN FOREST. Southern hardwoods[4], alternating or mixed with southern pines:

(a) Virginia, shortleaf and pitch;

(b) Loblolly, longleaf and shortleaf;

(c) Slash, longleaf, loblolly, pond and sand.

Hemlock local in (a) only.

PER CENT HEMLOCK
0-1 1-10 10+

PER CENT HEMLOCK
0-1 1+

1959
BASE MAP COURTESY OF U.S.D.A.
PHYSIOGRAPHY AFTER FENNEMANN

50 0 50 100 150 200 MILES

[1]Southern phases of spruce-fir forests occur at high elevations in the southern Appalachians.
[2]Northern hardwoods: northern genetic types of sugar maple, beech, yellow birch, red oak, and basswood.

[3]Central hardwoods: white, red, black, scarlet, chestnut, and other oaks, hickories, tulip tree, and southern types of sugar maple, beech, and others.
[4]Southern hardwoods: many of the hardwoods in (3) plus southern red and additional oaks, extensive black and red gum, magnolia, and others.

36 hours at 78°. This treatment delayed germination only a few days and decreased 7-week germination slightly.

Further tests were made on stratified seed from sources 50, 17, and 9 which were on the verge of germinating. These were dried at temperatures of 71° and 89° for periods up to 36 hours at 40 per cent relative humidity. The results are shown in Figure 16, expressed in days to reach 50 per cent of final germination after the drying treatments indicated. (As usual, seeds from source 50 germinated more slowly than those from 17 and 9 under the same conditions.) Drying at 71° caused no significant delay in germination whereas drying at 89° caused a marked delay for seeds from all sources, the delay increasing with the duration of drying.

At the apparently safe temperature of 71°, somewhat longer drying periods did not lower the level of maximum germination (80 to 95 per cent) of other lots of seed, but did delay the attainment of this level by 5 to 15 days for 3 and 5 days drying, respectively, as compared with negligible delay for drying only 1 day. Part of the effect of drying is doubtless a result of dehydration, which has to be reversed when seeds are again moistened. But drying at high temperature seems to have a more specific inhibiting effect, like high temperature effects already considered.

Damage to germinating seed by dessication

Seeds which had already begun germinating suffered much more acutely from drying. There was visible shrinkage in diameter of the root within 10 minutes after exposure to air at 40 per cent relative humidity. After an hour some of the roots had shrunk to one-fourth of their original diameter; they turned brown in 1 to 15 hours.

If seeds were returned to a humid chamber after 1 hour, they began to swell within 1 to 2 hours. Of the seeds which were only slightly germinated (less than 2 mm. of hypocotyl emerged), 80 per cent had recovered normal diameter within 5 hours, but the brown color persisted.

Of the seeds dried for 2 and 6 hours, 60 and 80 per cent, respectively, appeared to be severely damaged. After a month in the humid chamber, some of those subjected to only 2 hours of drying seemed to have recovered. Seedlings dried for 6 hours were either dead or heavily infested with a fungus (*Botrytis* sp.).

Seedlings which had grown further (2 to 10 mm. of hypocotyl emerged) showed 80 to 90 per cent of the plants with dead root tips within 10 days, for both 2- and 6-hour drying periods. Almost all root tips were dead within 30 days. In spite of this damage, many seedlings maintained green cotyledons for 30 days in the humid chamber where there was no need for water from their roots. Some seedlings with dead root tips put out one to three new root tips above the injury. In nature such seedlings would almost certainly have died.

Ecological considerations

What is the importance of these environmental influences on the natural life cycle of eastern hemlock? Stratification clearly has the most consistent effects in hastening germination over a wide variety of conditions, and tends to erase the effects of other factors. In nature chilling is likely to be adequate, except possibly at or beyond the southern limit of the range. Artificial strati-

fication may be needed in horticultural production of this species in the deep south, or in northern nurseries if seed is sown in the spring.

The effects of light are greatest on southern seed, with favorable photoperiod perhaps compensating for lack of chilling. In nature, there may be favorable seedbeds (moist moss carpets, damp mineral soil, seepage areas, and the like) where seeds can be exposed on the surface without drying out and where germination may be accelerated by light. For the larger number of seeds that are buried in litter as they fall, light has no effect; stratification and temperature alternations must be responsible for hastening of germination.

Tsuga canadensis seems to have an unusually narrow range of favorable temperature, especially for unstratified seeds. Moderate fluctuations around the optimal temperature, and within the range of 55° to 70°, sometimes retard germination slightly and sometimes stimulate it. In many soils, however, temperature may reach much higher, sometimes lethal, levels within less than a half hour after exposure to direct sunlight. Seedbeds which heat up are also likely to dry out. Both heat and drought are important in determining special seedbed and cover conditions under which hemlock seed has a fair probability of germination and survival.

Even excluding conditions that quickly damage the seed or seedling, delayed germination can also be critical for ultimate survival, in that the seedlings are in a less vigorous stage and less capable of withstanding later unfavorable situations of drought or frost.
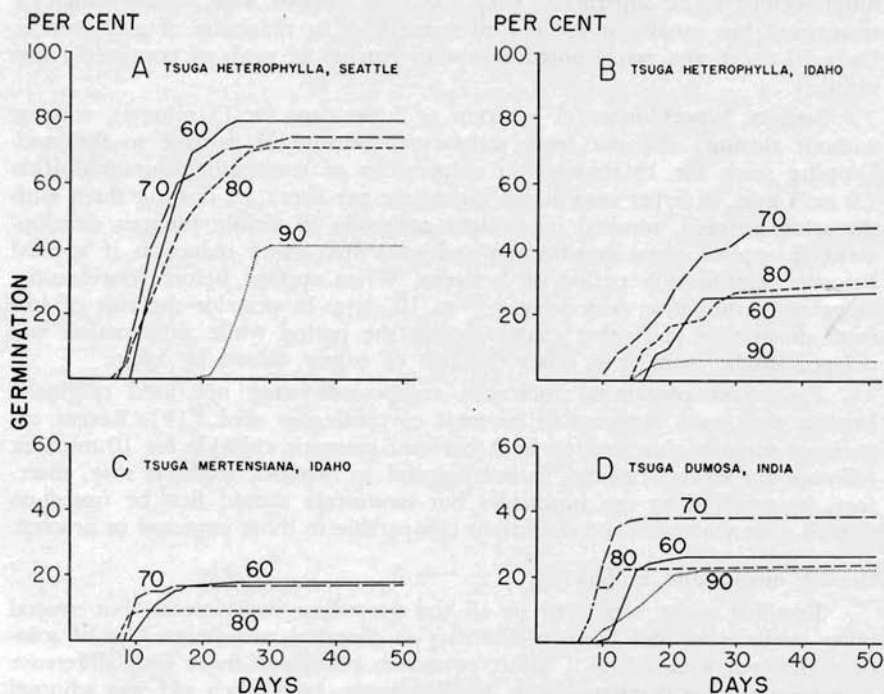

Figure 17. Rate of germination of unstratified seed of several species of *Tsuga* at temperatures indicated by numbers adjacent to the curves.

Comparisons with other species of *Tsuga*

Although the seeds of many species show some enhancement of germination by stratification, the chilling requirement of eastern hemlock seed is especially exacting. Also, the range of temperature favorable for germination is somewhat narrower than that of many tree seeds, including those of other species of *Tsuga*. Even unstratified seed of western and mountain hemlock (Figures 17A to C) germinated over a wide range from 60° to 80°. Himalayan hemlock (Figure 17D) germinated fairly well even at 90°. Maximum germination was never high in these lots of seed. Later experiments have shown relatively slight effects of stratification and photoperiod for these species, compared with those for *Tsuga canadensis*. Ching (12) has recently confirmed the relatively small effect of stratification on the germination of western hemlock seed.

Pathogens and their control

Many fungi appeared in the humid germination dishes, some on seed and some on the medium. E. M. Stoddard and Frances Meyer of this Station identified species of the following genera — *Chaetomium, Stibella, Trichoderma, Botrytis, Pestalozzia, Cephalothecium,* and *Gonatobotrys*. The last was the most common fungus borne internally; it developed a black sclerotial growth which later produced a feathery mass of spores. Few if any seeds infected with this fungus germinated and no control was provided by surface treatment. Most fungi seemed to be superficial. They could be reduced with certain fungicide treatments, but usually at the risk of some delay or reduction of germination. Bacterial decay was rarely noted except in batches of seeds of consistently low viability.

Sodium hypochlorite (.5 per cent or 5 per cent for 15 minutes, with or without rinsing) did not seem satisfactory because of damage to the seed. Dipping seeds for 15 minutes in suspensions of tetramethylthiuramdisulfide (.5 or 3 gms. of 5 per cent active ingredient per liter), or dusting them with the same material, resulted in a slight reduction in visible fungous development if applied after stratification and somewhat more reduction if applied before a stratification period of 5 weeks. When applied before stratification, however, germination was delayed 7 to 10 days. In practice the risk of loss from dessication and other causes during the period while germination was delayed might more than offset the risk of injury caused by fungi.

Fungicides containing mercurial compounds were not used originally because they were reported to be toxic to coniferous seed (13). Recent experience suggests that soaking in .1 per cent mercuric chloride for 10 minutes, followed by several rinsings, is not harmful to hemlock seeds. It may, therefore, be practical to use fungicides but treatments should first be tested on a small scale with seed and conditions comparable to those expected in practice.

Growth media and buffers

Distilled water was used in all the preceding experiments, but several other media were also tried, preliminary to chemical treatments. Use of solutions buffered with .01 or .1 molar potassium phosphate made little difference in germination as compared with distilled water, even when pH was adjusted over a range from 4.5 to 8.0; the less acid solutions had very slight, if any, advantage. High (1 molar) concentrations prevented germination. Potassium

nitrate (.02 molar) is frequently used to stimulate germination of other seeds (53) but was not conspicuously better than water for hemlock (Table 8); neither were more complex nutrient solutions. Germination in bacto-agar (2 per cent) differed little from that in water and buffer at 62° but was higher in some cases at 80°, for reasons that are not yet clear.

Table 8. Preliminary results on effects of chemicals on hemlock seed germination after 9 weeks

Temperature and stratification	Hours of light	H₂O	KNO₃	Chemical[1]			
				T	T&C	C	U
				Per cent germinating			
Unstratified	0	0	8	88	68	68	40
	8	36	....	....	....	68	68
62°	12	76	84	0	0	68	68
	16	0	....	0	0	12	84
Stratified	0	92	88	96	68	68	84
	8	88	....	....	....	92	84
62°	12	92	88	92	96	76	100
	16	76	80	76	76	12	76
Unstratified	0	0	0	4	0	....	....
	12	0	4	0	0	....	....
80°	16	0	12	0	0	....	....
Stratified	0	8	16	48	24	....	....
	12	36	32	36	28	....	....
80°	16	56	60	36	52	....	....

[1]KNO₃, .02 molar; T, Thiourea .5%; C, Coumarin .05%; U, Urea .5%

Promoters and inhibitors

The effects of chemicals on germination have a two-fold interest. It would be helpful, in practice, if special stratification or light treatments could be by-passed through simple treatments of unstratified seeds to make them germinate well even in the dark. The environmental influence of light and temperature presumably involve the action of growth-promoting or growth-inhibiting substances inside the seed. A better understanding of these responses might result if they could be induced experimentally by applications of known compounds.

Only preliminary information on this problem is presented here; this suggests not only that increases in the percentage of plants starting germination are possible but also that the embryos may be damaged. (Compare the damaged seedlings noted below with normally developed seedlings shown in Figure 6H and I.)

Urea and coumarin have both been considered as inhibitors of germination (20, 38). Yet at concentrations shown in Table 8, both increased the percentage of unstratified seeds which *started* germination in the dark and under 16 hours of light at 62°, at which temperature there was no germination of untreated seeds. High germination of stratified seed was not further increased by either material. Unfortunately, this increase in per cent germination by both substances was accompanied by severe damage to the embryo root, both in the

dark and in the light. With urea treatment in darkness, one or two millimeters of the hypocotyl tip turned brown or black while the rest remained normal for several weeks (Figure 6B); in the light, the remainder of the hypocotyl tended to turn bluish or greenish black instead of the normal red (6C). With coumarin treatment, the root tip first became blunted and took on a pale bluish tint. In time it turned brown (Figure 6F and G). A normal root later grew out of the blunt portion in some seedlings.

As expected from its general reputation in breaking dormancy of other species (52), thiourea stimulated germination of unstratified hemlock seeds in the dark, markedly at 62° and slightly at 80° (Table 8). There was also quite appreciable stimulation of stratified seeds in the dark at 80°. Under other photoperiods there was little stimulation at either temperature. Combined thiourea-coumarin treatment resulted in germination similar to that with thiourea alone.

Thiourea also damaged the roots at .5 per cent concentration, if left in contact with the seeds during germination. Damage was usually greater in the light (Figure 6D) than in darkness (Figure 6E). Both with and without coumarin, thiourea tended to turn the hypocotyl bright red; frequently the root tip was blunted and swollen out of normal proportion. In later experiments, .25 and .3 per cent concentrations resulted in only minor stunting (Figure 6E) and a tan discoloration of the root covering, which normally is pale pink.

Data from experiments with unstratified seeds at 62° (not included in Table 8) showed rapid germination under 8 hours of light for both untreated seeds and seeds treated with thiourea; germination under 12 hours of light was irregular and under 16 hours generally low, as in Table 8.

Thiourea concentrations of up to 4 per cent, followed by very thorough rinsing, were later used for seed treatment for 15 minutes without injury. Hence there is still a possibility that correct adjustment of concentration, rinsing, and timing may provide a convenient means of bypassing the need for special stratification or light treatments.

Preliminary trials at 62° with 16 hours of light showed no promoting effects with gibberellin or kinetin, substances which have had very marked effects on other plants. Indoleacetic acid (.01 to .1 mg. to the liter) in 2 per cent agar had a somewhat irregular stimulatory effect at 80° and deserves further study.

Excised embryos

Not infrequently, the seedcoats of unstratified seeds split, thus exposing the whitish endosperm and its membranous sheath, but the germination process does not proceed further. Presumably some process is blocking germination. The following experiments were performed to find out whether the behavior of excised embryos was similar to that of intact seeds. Unstratified seeds were surface-sterilized with .1 per cent $HgCl_2$ for 10 minutes, then rinsed 5 times and left overnight in distilled water before excising the embryos and placing them on nutrient agar[5]. Within a few days, about three-fourths of the embryos showed definite swelling, and the root tip took on the typical

red color; cotyledons on a small number of embryos turned green (Figure 4C). The most striking result was that there was no statistically significant difference between high and moderate temperaure, 80° compared with 62°, or between long or short daylengths, 16 compared with 8 hours (Table 9). (Variations in sterilization treatments and nutrient supplements were tried out on some of these seeds, without apparent effect). These results suggest that some inhibiting material within the endosperm or other seed tissue outside the embryo is at least partially responsible for delayed or low germination of intact unstratified seed and that stratification or favorable light and temperature conditions tend to overcome the inhibition.

Table 9. **Number of excised hemlock embryos beginning growth of red root tips or cotyledons within 10 days**

Photoperiod	Temperature	
	62°	80°
8 hours		
Number germinating	55	50
Number in test	68	68
16 hours		
Number germinating	56	53
Number in test	68	68
Mean of all tests 212/272, per cent		77.9
Chi square (M=3)		1.814
Probability of chance deviation exceeding this chi square		0.61

Even though most embryos started growing, none continued normal development (see stunted seedling in Figure 4C). It is not yet known whether this is due to an unfavorable effect of agar on the embryo root, whether some essential substance was not made available in the nutrient medium, or whether the embryo itself still lacked certain prerequisites for normal growth.

Interpretation of Germination Processes

We have already mentioned adaptive values of some of the responses of hemlock seeds to environmental factors, and noted some internal processes by which these adaptations might be achieved (p. 35). Without attempting to pursue the biochemical details, we can at least summarize the incomplete knowledge and cite a few references on the physiology of the fundamental problem of seed germination and dormancy.

We have reviewed the recent discoveries of the effects of photoperiod on the germination of seeds, and some of the related work on non-photoperiodic responses to light (48). Wareing (58) has also surveyed photoperiodism of seeds and seedlings of woody plants in more detail. Koller (28), Evenari (19), and Toole *et al* (54) cover this and other aspects of seed germination in recent comprehensive reviews that supplement older standard books on seeds (14, 5). Further references will be found in the bibliographies of papers cited in this section.

Toole *et al* (53) proposed a general hypothesis for the physiology of germination which emphasizes the changing balance of three types of reactants: (1) substrate or food substances providing energy, (2) respiratory intermediates and enzymes, and (3) materials for the synthesis of new plant material. All three are connected through various reactions, including at least one that is sensitive to light. Enzymatic substances which catalyze these reactions may temporarily combine with substrates, but must later be released in order to be used again.
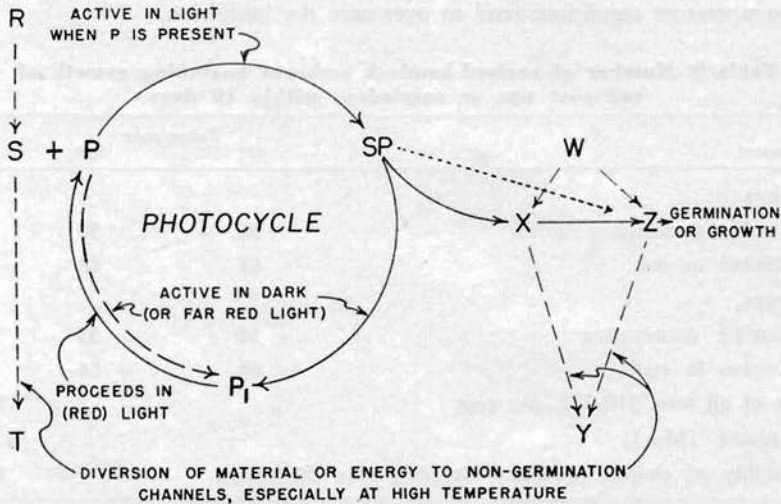

Figure 18. Speculation on chemical control of germination or growth by light and temperatures (adapted from Toole, et al, 53; Liverman and Bonner, 31; Wareing, 58).

Role of light

One possible explanation of the action of light on germination outlined in Figure 18, assumes (a) that one of these enzymatic substances is a pigment P, which (b) unites with substrate S to form the combination SP; (c) that this breaks down to form a modified pigment P_1, and a product X, which (d) subsequently changes to Z, providing either materials or energy required for germination. R and W represent sources for S and for X or Z, respectively. T and Y represent pathways consuming these materials without leading to germination. The relation of many responses to various wavelengths of radiation (9, 54) suggests that the modified pigment P_1, may absorb energy from light (especially red light from 5800 to 7000 Å) and thereby be changed back to the original form P, so that the process can be repeated. In the dark, this process would proceed only as far as P_1 after the reserve of P was depleted, and the so-called "photocycle" (31, 58) would fail to be completed. "Far-red" radiation (exceeding 7000 Å) commonly reverses the action of red light, possibly through changing P to P_1, directly (54, 58) or by way of SP (31).

Seeds with little or no light requirement are assumed to have enough X or Z without benefit of this photocycle (53), possibly by other pathways which have been denoted by W in Figure 18.

Only a single exposure of red light is necessary for high germination of certain light-requiring seeds, such as lettuce and *Lepidium* (53). From his work on unstratified seed of *Betula pubescens* Wareing (58) states "At 20° C. [68° F.], sufficient Z to cause germination is evidently formed following a single light exposure, but at 15° C. [59° F.] repeated light exposures . . . are necessary for the accumulation of Z to a certain threshold value." And further ". . . at 15° C., there is no summation [accumulation] with successive cycles when the dark period exceeds a certain duration, presumably because some product decays progressively as the length of the dark period is increased."

The behavior of unstratified seed of *Tsuga canadensis* during germination is similar in some respects to Wareing's results with *Betula* and is consistent with his hypothesis. But with *Tsuga,* repeated cycles of light of appropriate duration and intensity are needed at all temperatures; also, we noted an apparent shift, with temperature, of the duration of light which is optimal (Figures 9 to 13). At 53°, germination, although delayed, is somewhat better on a 16-hour than on an 8-hour photoperiod, suggesting a more rapid accumulation of Z on the former. At 62° and 71°, germination generally tends to be faster and to reach higher levels on 8-hour than on 16-hour photoperiod. This may be because 8 hours of light daily is long enough for a sufficiently rapid accumulation of Z whereas 16 hours may result in the accumulation of excessive levels of certain substances regulating germination, assuming that *either* too much or too little may be unfavorable (31).

This last assumption may help to explain why the favorable photoperiod shifts from 8 hours to 16 hours at 80°. If X or Z should be diverted to other channels Y, at a rate which increased rapidly with temperature, an increased ratio of day to night may be necessary to compensate partially for this depletion. If, on the other hand, there is decreased diversion at 40° to 44°, this may help to explain improvement in germination by stratification.

Obviously there may be many variations in details of the relations conjectured in Figure 18. Liverman and Bonner (31) have suggested close connection between a photocycle and auxin metabolism, but the bearing of their studies on the germination of seeds, and the role of pigments discussed by Borthwick *et al* (9), are still not clear (30). According to the approach of Bünning (11), cycles like that outlined here may activate endogenous rhythms in the metabolism of the seed or growing plant and the action of the cycle may depend on maintaining a favorable coincidence of internal and external (environmental) cycles.

Role of temperature and respiration

As long as seeds remain inactive but alive, respiration proceeds slowly, presumably through channels that are not coupled with subsequent germination processes. Possibly the diversion of X or Z to Y is involved in such a channel. Another possibility is that the substrate S (either present initially or formed from a precursor R) is consumed through a channel T, instead of participating in the photocycle. Toole *et al* (53) propose that the many reactants involved in germination are initially in a sensitive equilibrium, which shifts only slowly as food reserves are depleted *unless some change in light or temperature alters the rates of some reactions much more than others.* Prolonged high temperature may increase the respiration rate, without channeling energy into germination. But a *temporary* rise in temperature may upset the previous balance in levels of several reactants, suggesting that "this new balance of reactants, while

unfavorable for germination at the high temperature, is favorable at the reaction rates involved at the lower temperature" (53), *Tsuga canadensis* and many other seeds have not responded so markedly to a single temperature change as was observed in the case of *Lepidium,* discussed by Toole, *et al;* but such seeds can benefit from repeated temperature alternations, perhaps through the same kind of mechanism.

Role of enzymes and growth regulators

Recently, enzymes for more than one pathway of respiration have indeed been demonstrated with lettuce seed, with indications of a shift from one pathway to another early in the course of germination (33). Perhaps some of the environmental influences on germination act by helping to shift these pathways in the manner outlined by Toole *et al* or through more specific control by changing the regulating substances.

Paper chromatography has been used to separate growth inhibiting substances which might initially be helping to suppress the enzymatic processes leading to germination. Poljakoff-Mayber *et al* (44) also show that these inhibitors disappear soon after imbibition of light-treated lettuce seeds, and are replaced by growth-promoting substances, which may include 3-indoleacetic acid. Both the loss of inhibitors and the formation of growth-promoting substances could thus have a role in regulating a shift in respiratory pathway.

The hypothetical schemes discussed thus far avoid stating where in the seeds these processes occur. The fact that excised embryos of *Tsuga canadensis* and *Betula pubescens* can start growth under a wider variety of conditions than can the intact seeds strongly suggests that the endosperm or other external portions of the seed play an important role in inhibition. In many seeds, puncturing or other physical disturbance helps overcome this inhibiting effect, as does increased oxygen tension. Hence some oxidation process may act by disposing of an inhibitor, thus permitting liberation of the materials necessary for germination.

Shimon Klein and John Price (personal communication) exposed lettuce seeds to a beam of deuterons of varying energies in order to penetrate either the seedcoat alone, the endosperm, or the embryo itself. Penetration through the seedcoat had little effect; penetration through the endosperm was sufficient to permit relatively high germination. Radiation penetrating into the embryo also permitted good initial germinaton but the embryos whch emerged could not continue growth because of damaged roots. Possibly the same effects which lead to injury of the embryo merely neutralize an inhibiting system when they occur outside the embryo, and thereby encourage germination indirectly.

We find some parallels with this result in our application of chemicals to hemlock seeds. Certain treatments with thiourea, urea, and coumarin increased initial germination in the dark, but also tended to damage the embryo. Damage by chemicals, as from radiation, was most evident in the root tip.

Bud Dormancy and Stem Elongation

The influence of chilling, photoperiod, seed source, and temperature on the breaking of bud dormancy, stem elongation, and return to dormancy were investigated on seedlings of two different ages:

(a) Wild seedlings collected in the New Haven area which had completed their second year of growth.

(b) Seedlings which were grown from seed collected over the range of the species and which were in their first year of growth.

The influence of chilling and photoperiod on the breaking of dormancy and of elongation and return to dormancy, were studied on the wild stock which was kept at a constant temperature of $60° \pm 2°$, but under different photoperiods.

Data on the effect of seed source on elongation were taken on first-year seedlings from 30 seed sources which were maintained at a constant temperature of $62° \pm 2°$, but with photoperiods varied.

The effects of temperature and seed source were studied on seedlings from both groups (a) and (b) which were all growing under a 16-hour day, 8-hour night under different temperature regimes.

The breaking of dormancy

Photoperiod and chilling were first found to be effective in controlling bud dormancy by comparing groups of 2-year-old wild hemlock seedlings which were transplanted to greenhouse conditions from a forest near New Haven (40, 41). All plants brought into the greenhouse on December 1, 1952 broke dormancy and developed growing shoots between December 25 and January 25. Some plants brought indoors in October but kept on a 20-hour photoperiod also broke dormancy, but much more slowly than plants which had been exposed to normal autumn chilling. None of those brought indoors in October and kept on short wintertime photoperiods broke dormancy. Meanwhile, other plants dug in October were given 5 to 15 weeks of artificial chilling in a $40°$ room with either 8- or 16-hour photoperiods, in preparation for the following experiment.

On March 9, both unchilled and artificially chilled plants were assigned at random to a new series of controlled photoperiods at $62° \pm 2°$. As before, chilled plants broke dormancy promptly, with no apparent differences related to duration of chilling or photoperiod during chilling, and only minor differences among the photoperiods (Figure 19B).

Unchilled plants on a 16-hour light, 8-hour dark treatment began breaking dormancy more gradually than chilled plants and all were growing within 86 days (Figure 19A). In other compartments on the same bench, with identical fluorescent light of about 300 foot-candles, additional plants were given 4 or 8 more hours of supplementary incandescent light of approximately 20 foot-candles, thereby decreasing the dark period to 4 or 0 hours, respectively. Buds of these plants began breaking dormancy more promptly than those on 8 hours of darkness and all were growing within 35 and 43 days, respectively (Figure 19A).

By contrast, unchilled plants on 12 or 16 hours of darkness still failed completely to break dormancy, as in the greenhouse. Many developed chlorotic leaves and purplish buds, and 60 per cent had died after 4 months under treatments which were very favorable for initiation of growth of chilled seedlings.

Additional treatments on the same benches involved interruption of long nights by dim incandescent light. With one group of plants, a 12-hour dark period was interrupted after 4 hours with 4 hours of 20 foot-candle illumination, creating two 4-hour dark periods. Another group of plants had a 16-hour dark period interrupted twice for 2 hours to create three 4-hour dark periods. Plants in both groups broke dormancy within 4 to 6 weeks — about as rapidly as under 0- and 4-hour dark periods. These treatments thus have the same results as many similar experiments with flowering which show that the dark period is especially important in photoperiodic processes (30).
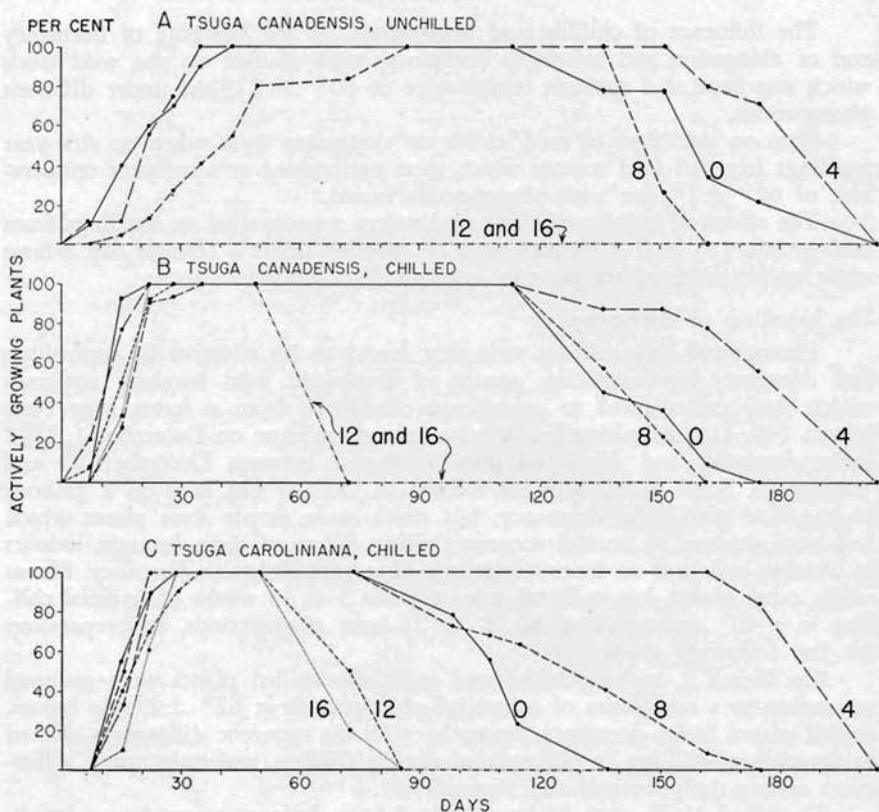
Figure 19. Changes in percentage of actively growing plants throughout the annual cycle of stem growth as influenced by length of the dark period. Numbers for different lines give hours of darkness out of each 24.

One-year-old seedlings of *Tsuga caroliniana,* locally grown but of unknown seed origin, were included in the same experiment (Figure 19C). Carolina hemlock differed slightly from eastern hemlock in its chilling-photoperiod relations; a few unchilled seedlings began growing in the greenhouse under the relatively short-day and long-night conditions of late February. This suggests either that the chilling requirement was less than for *Tsuga canadensis* or that the photoperiod compensation for lack of chilling is greater in at least some individuals of this species. The latter alternative seems less likely since 8- and 4-hour night lengths were less effective in breaking dormancy of unchilled seedlings than these photoperiods were for *T. canadensis.* Only in continuous light and in interrupted dark treatments did a large percentage of the plants break dormancy. As with eastern hemlock, *Tsuga caroliniana* consistently broke dormancy very rapidly when removed to higher temperatures after 15 weeks of chilling.

A later experiment was designed to determine more exactly the duration of chilling required to break dormancy of eastern hemlock. Table 10 shows that plants kept under 12-hour photoperiod failed to break dormancy if they

had only 4 days of chilling or none at all; that plants chilled 2 weeks started very much more slowly than plants chilled 8 weeks. The bottom line of Table 10 shows that 20 hours of fluorescent light of about 400 foot-candles can break dormancy of unchilled plants. However, as the footnotes indicate, there was less elongation than for plants with 2 or 8 weeks of chilling, due to less prompt breaking of dormancy. The middle line refers to a treatment with 12 hours of fluorescent light supplemented by 8 hours of incandescent light of only 5 to 10 foot-candles. This is even dimmer than the 20 foot-candles that was used to break dormancy of buds in the earlier experiment by interrupting long dark periods. Such dim supplementary light was not sufficient to produce the marked stimulation shown by 20 hours of stronger light. Presumably the critical intensity of light needed for penetrating the bud scales and stimulating growth lies somewhere in the neighborhood of 10 to 20 foot-candles, although the effectiveness is probably also modified by the variable exposure of individual buds and by the spectral quality of the light.

These experiments indicate that there is no exact critical duration of chilling required for breaking dormancy. Two or three weeks seems to be long enough to stimulate growth of buds under long photoperiods, but not sufficient to have more than a very slow effect on plants on photoperiods of 12 hours or less. Thus light and chilling act together in breaking bud dormancy, but chilling had the more marked effect.

An interesting sidelight on the breaking of dormancy appeared when seedlings from the seed-source experiment discussed later were placed outdoors. Some buds began to break dormancy by late November, at a time when this never occurs with normal plants. Under indoor experimental conditions, these particular plants had already formed buds the preceding May instead of in July or August which is normal for field grown plants. Thus the buds had a longer period for maturation which may lessen the need for chilling.

The similarities of bud and seed dormancy phenomena are striking. Chilling has the more drastic effect on both, but favorable photoperiod can compensate for lack of chilling. This compensation seems to be greater in *Tsuga canadensis* than has been reported for many other trees. For example, Olmsted (39), working with unchilled seedlings of *Acer saccharum*, found that after

Table 10. Interaction of chilling and photoperiod in breaking dormancy of hemlock buds; duration of test — 74 days for plants with 8 weeks of chilling, 130 days for the remainder

Photoperiodic regime: (hours out of 24)				Duration of chilling			
Dark	Light			0 days	4 days	2 weeks	8 weeks
	Strong	Weak					
12	12	0	active	0	0	2	13[1]
			total	20	20	19	16
4	12	8	active	0	0	5	14[2]
			total	18	20	16	20
4	20	0	active	16[1]	15[1]	19[2]	193[3]
			total	20	19	19	206

[1] No plants over 14 mm.; lack of activity in some plants probably due to *Rhizoctonia*.
[2] Most plants well elongated, but none over 34 mm.; few side branches.
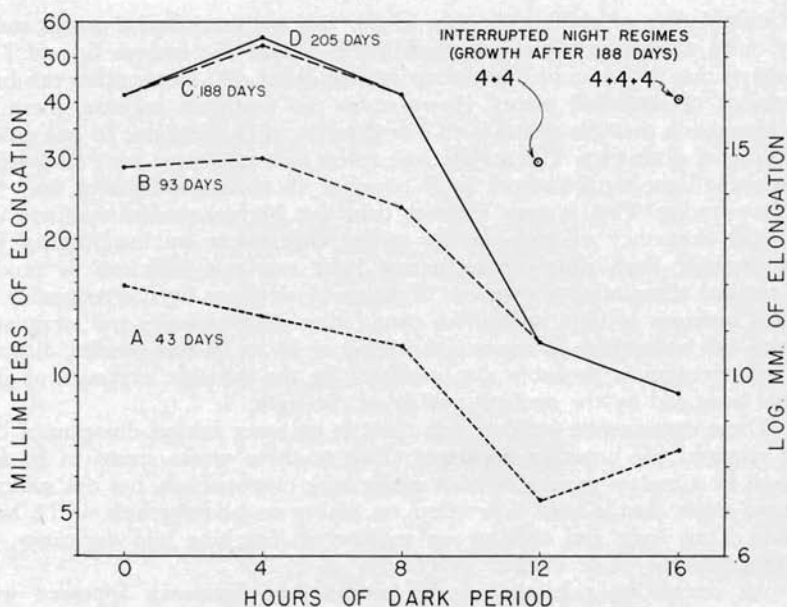[3] Plants up to 56 mm; many side branches.

Figure 20. Influence of different lengths of dark period on the elongation of hemlock stems at different periods in the growth cycle. Note that the interruption of a 12-hour (4+4) or a 16-hour (4+4+4) night resulted in far more growth than uninterrupted nights of equal duration. See also Figures 7A and 7B.

4 months, 64 per cent broke dormancy on a 20-hour day, 4-hour night and 35 per cent on a 10-hour day, 14-hour night. Under conditions approximating these, the percentages for hemlock were 100 and 0 (Figure 19A).

    Gustafson (24) showed that long-day and short-night conditions helped break dormancy in *Pinus resinosa* seedlings. In a series of studies by Wareing (reviewed in 58), mature but unchilled winter buds of *Fagus sylvatica, Betula pubescens,* and *Larix decidua* resumed growth under long photoperiods; those of *Pinus silvestris, Quercus robur, Acer pseudoplatanus,* and *Robinia pseudo-acacia* did not. However, in the second group of species, long photoperiods lead to a resumption of growth of *immature* buds. In fact, the normal pattern of growth in oak involves early formation of buds and later resurgence of one or more additional "flushes" of growth under long summer photoperiods. Evidently dormancy in these species gradually becomes "deeper" as the buds harden off. Presumably this is due to the gradual accumulation of growth inhibiting substances in the buds late in the growing season. Differences in behavior among genera, of the same genus in different geographic locations (58), of different species, or of the same plant at different times of the year should not be surprising; all may be due to slightly different balances of growth promoting and growth inhibiting substances.

Elongation and bud formation

    After bringing together various aspects of breaking bud dormancy, we are now ready to follow the ensuing stem growth until it is terminated by formation of new buds.

Figure 19 shows a rapid increase in percentage of growing plants as dormancy is broken, and a more gradual decrease as plants return to dormancy. The three long-day and short-night treatments permitted much more persistent growth. Under them it took at least 113 days before the first buds were formed on either chilled or unchilled plants. As with the breaking of dormancy, a 4-hour dark period was most favorable for growth: over 200 days elapsed before the last plant was dormant. On 8 and 0 hours of darkness, some unchilled plants continued to grow until 161 and 200 days, respectively; chilled plants, 161 and 175 days, respectively. Under a short-day and interrupted-night regime (not shown in Figure 19) some plants remained growing nearly as long as those on short dark periods, 147 and 133 days, for single and double interruption, respectively. There was much variability in timing among individuals on short-night or interrupted-night treatments.

By contrast, some of the chilled plants under uninterrupted nightlengths of 12 and 16 hours started to form buds in less than 50 days and all were dormant by the 75th day. Under these conditions there was less variability among plants in returning to dormancy than under shorter nightlengths. Unchilled plants on 12- and 16-hour uninterrupted nightlengths failed to break dormancy.

Figure 20 shows elongation after 43, 93, 188, and 205 days. The differences in total elongation under various light treatments (Figure 7A and B) are primarily due to striking differences in duration of growth. Among chilled plants, which all began growth at about the same time (Figure 19), those under nightlengths of 0 to 8 hours were already elongating more rapidly than

Table 11. Hemlock stem elongation added to 2-year-old plants[1]

	Photoperiod, hours		Elongation mean log. mm.	90 per cent confidence limits
	Light	Dark		
Short night	24	0	1.627	±.106
	20	4	1.738	±.111
	16	8	1.632	±.669
Long night	12	12	1.077	±.132
	8	16	.984	±.063
Interrupted night	4+4		1.475	±.117
	4+4+4		1.658	±.101

Analysis of Variance

Source of Variance	Degrees of Freedom	Mean square	F
Between photoperiods	6	1.23182	22.21[2]
Within photoperiods	99	.04286	
Between pots	22	.05545	1.41 1.00
Within pots	77	.03926	1.00

[1]Values for Curve D and interrupted night regimes, Figure 20, plotted from the means in this table.
[2]Differences between photoperiods are significant with P less than 0.001.
Differences between pots are not significant.

those under continuous darkness for 12 or 16 hours even while all plants were still active (Curve A, Figure 20 for 43 days). But these differences in elongation were greatly accentuated with time (Curves B to D, Figure 20). After 93 days, there was no further elongation on 12- and 16-hour continuous nightlengths; after 188 days, elongation was continuing only on a few plants under 4-hour nightlength conditions (Curve D, Figure 20).

Elongation as of 188 days under interrupted-night regimes (symbols, upper right, Figure 20; Figure 7B) was approximately equal to that under 8-hour continuous-night conditions for double interruption (4 + 4 + 4) and somewhat less for single interruption (4 + 4). Elongation under both types of interruption was appreciably less than under a 20-hour day, 4-hour night. The duration of high intensity light was 8 hours for the 4 + 4 + 4 treatment and 12 hours for the 4 + 4; this eliminates duration of a high light intensity as a variable in the comparisons of Pot f with a and of Pot g with b in Figures 7A and 7B.

Because of the variability in timing of bud formation and in inherent vigor, the standard deviation is larger for the treatments with greater mean elongation, but is approximately constant when elongation is expressed on a logarithmic scale (right axis, Figure 20). Hence in the analysis of final height growth (Table 11), the variance can be pooled, giving an estimate of .04286, corresponding to a standard deviation of .21 logarithmic units. Statistical analysis showed that variance between pots with the same treatment was not significantly greater than variance of individuals within the same pot ($F = 1.41$ for 22 and 77 degrees of freedom). Confidence limits on the means of all seedlings on a given treatment have been computed for a 90 per cent probability of covering the expected mean value.

The analysis of variance in Table 11 also confirms the statistical significance of the difference between means for various light treatments shown in Figure 20, in spite of the small number of plants involved.

The maturation of the new growth is a gradual process. The actively growing plants have needles of many lengths, tapering down to the newly emerging primordia arranged in a rosette surrounding the twig tip (Figures 3C and 7C). Under favorable photoperiods, new needles continue to form from the tip meristem as the older ones gradually mature and become spaced out along the stem by the elongation of the tender, growing internodes. Further down the stem, the twig begins to stiffen slightly and then the stem takes on a whitish cast due to surface hairs. By the middle of the growing season (early July) we may begin to find a more advanced stage of maturation along the older, basal portion of the twigs, which will gradually move upward as the rest of the twig matures. The whitish color gives way to rusty brown as the twig is stiffened by lignification. By autumn, the whole twig has turned shiny and tan in color as a corky outer layer develops. The color remains lighter than the brown of the previous year's twigs until the following growing season.

On seedlings under long photoperiods, very close observation of the growing tip is needed to see the first signs of bud formation. Within the clustered bundle of recently formed needles, leaf primordia, which otherwise would have become needles, become bud scales instead, very small and pale green at first. As the last true needles elongate, the previous gradation in size is broken; one or two needles may retain an intermediate size, even to maturity, but the rest grow to normal length. Gradually these needles spread so that

the enlarging bud can clearly be seen (Figure 7D). In the normal process of maturation, the bud turns from green to pale tan to brown; the surrounding needles, from pale yellowish green to blue green.

On seedlings under short photoperiods, the whole process of stem and bud maturation not only occurs earlier, but is much more abrupt. Needles diverge and turn dark green at an early stage of bud formation. There is less elongation of each internode before it hardens off, and many fewer internodes are in the flexible meristematic stage at any one time.

Effect of seed source on elongation

The preceding experiment showed the general relation of growth to photoperiod and chilling on 2-year-old stock. In 1954, a much larger experiment was performed with shorter intervals between photoperiods to determine the variation in growth in the same environment among seedlings from many parts of the species' range. Stratified seeds from 30 sources (Table 12) were germinated in pots in the greenhouse. Before appreciable elongation of the epicotyl had occurred, duplicate pots representing each source were distributed at random in the growth chambers under conditions similar to those of the preceding experiments. All chambers had the same duration of overhead illumination (12 hours out of 24) at about 400 foot-candles, but differed in the amount of supplementary illumination (less than 10 foot-candles) to make total light periods of 12, 13, 14, 15, 16, and 20 hours; totally dark periods were thereby reduced to 12, 11, 10, 9, 8, and 4 hours, respectively. All chambers were maintained at $62° \pm 2°$.

In connection with Figure 7E we already noted that, under any given photoperiodic treatment, seedlings from northern or high elevation seed sources tend to form buds earlier than those from southern or low elevation sources. As a result, they elongate less before they finally form terminal buds. One statistical analysis of the relation, to be presented in detail elsewhere (37), is summarized in Figure 21. The logarithm (z) of the mean epicotyl elongation of all plants on a given nightlength at first appeared to be significantly dependent upon seed weight (Figure 21A) as well as growing season (Figure 21C). But partial regression analysis showed that this apparent relation was accounted for by the regression of seed weight on growing season in turn (Figure 21B); the effect of seed weight independent of growing season was not significant in accounting for variability over and beyond that accounted for by frost-free season alone (e.g. Figure 21C). This positive regression of growth on frost-free season was found under all conditions of nightlength and temperature, although the exact values of the regression coefficients (slopes) varied significantly (Figure 21D).

The systematic decrease in mean value or general level of the lines in Figure 21D with increasing nightlength confirms the importance of this factor in hastening bud formation and stopping elongation. Specifically it showed a relatively greater difference in growth between 8- and 9-hour nights than for any other hourly interval along the series. Evidently there is a "critical nightlength" in the neighborhood of 8 to 9 hours at which we find a maximum change from active shoots to dormant buds. Changes to nightlengths of longer or shorter duration also have some influence on growth and dormancy, so that there is no absolute threshold. Even in such classic photoperiodic phenomena as flowering (30) the effects of varying nightlengths are not always abrupt.
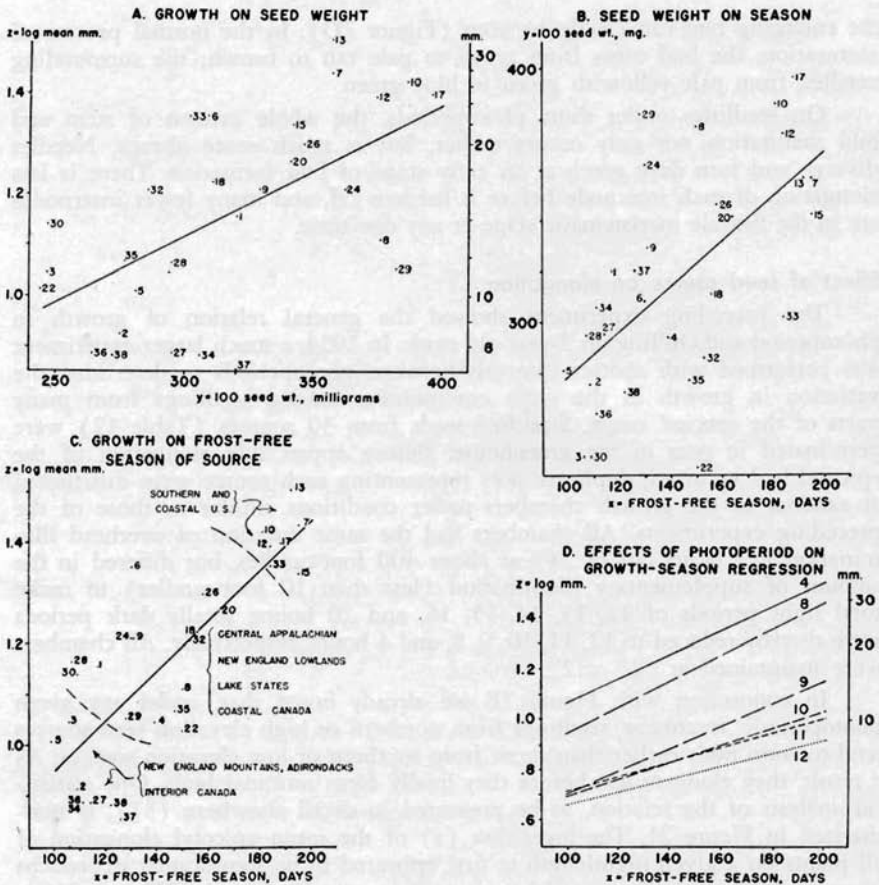
Figure 21. Regressions relating z, the logarithm of mm. of elongation above cotyledons, y, the weight of 100 seeds in mgs., and x, the frost-free season of the region of the parent source, to one another. A, B, and C represent simple regressions of z on y, y on x, and z on x, for 30 sources at a single night length of 8 hours. D shows how the regression of z on x varies with nightlength, both in mean level and slope.

Table 12 shows a further analysis of this experiment, in which the logarithmic transformation was made on individual measurements of epicotyl elongation instead of on arithmetic means. Means of these logarithmic values are given for nightlengths of 4, 8, 9, and 12 hours for all 30 seed sources in columns a, b, c, and d, respectively. Vertically, sources are arranged in order of decreasing frost-free season. As in the other analysis for Figure 21D (37), they show positive regression coefficients b_{zx} on frost-free season for all nightlengths. Variance accounted for by regression B^2_{zx} is highly significant compared with residual variance or scatter around the line.

A separate analysis (not shown here) in which latitude and altitude were included as predictors, in addition to length of frost-free period, did not improve the predictability attributable to frost-free period alone, so that these two additional variables have been omitted from further computations.

Table 12. Effect of source and nightlength on epicotyl elongation of first-year hemlock seedlings

Source (Number, location and elevation, in feet)	Frost free season, days[z]	4 (a)	Night length, hours[z] 8 (b)	9 (c)	12 (d)	(1) $a+b-c-d$	Index (2) $\dfrac{a-b}{a+c-d}$	(3) $\dfrac{-a+b}{+c-d}$
			Mean log elongation					
Long								
15 Spencer, Tenn., 1,000	195[z]	1.426	1.278	1.078	.846	.780	.280	.084
7 Branford, Conn., 20	195	1.385	1.397	.982	.739	1.061	.231	.255
13 Garden City, N. C., 1,300	192[z]	1.533	1.492	1.201	.921	.903	.321	.239
17 Gatlinburg, Tenn., 1,200	188	1.400	1.364	1.002	.791	.971	.247	.175
33 Stevensville, Mich., 600	184	1.325	1.290	.899	.860	.856	.074	.004
14 Garden City, N. C., 2,300	184[z]	1.490	1.382	1.179	.903	.790	.384	.168
10 Colt's Neck, N. J., 100	181	1.481	1.402	1.224	.911	.748	.392	.234
Medium								
20 W. Augusta, Va., 1,900	164[z]	1.297	1.223	.826	.828	.866	.072	−.075
26 Ithaca, N. Y., 950	158	1.342	1.254	1.064	.771	.761	.381	.205
18 Newfound Gap, Tenn., 4,800	156	1.328	1.187	.889	.730	.896	.300	.018
32 Loudonville, Ohio, 1,000	153	1.336	1.179	.942	.796	.777	.303	−.011
22 St. Margaret's Bay, N. S., 100	150[z]	1.122	.966	.814	.591	.683	.379	.067
8 Eastford, Conn., 500	150[z]	1.332	1.045	.711	.758	.908	.240	−.334
4 Crown Point, N. Y., 400	139	1.319	.980	.713	.622	.964	.430	−.248
Short								
9 Canaan Mt., Conn., 1,100	131	1.196	1.127	.928	.713	.682	.284	.146
24 Orono, Me., 120	130	1.290	1.159	.901	.759	.789	.273	.011
6 Onteora Park, N. Y., 2,300	130[z]	1.264	1.272	.787	.623	1.126	.156	.172
29 Mille Lacs Lake, Minn., 1,000	128	.995	1.080	.764	.635	.676	.044	.214
37 Fredericton, N. B., 164	126	1.003	.824	.571	.515	.741	.235	−.123
38 Petewawa, Ont., 550	122	1.045	.851	.734	.587	.575	.341	−.047
1 Keene, N. H., 500	117	1.145	1.095	.788	.760	.692	.078	−.022
35 Loretteville, Que., 700	117	1.157	.982	.882	.814	.443	.243	−.107
27 Gould (Thessalon) Ont., 700	114[z]	1.028	.849	.690	.667	.520	.202	−.156
Very short								
34 Mt. Tremblant, Que., 1,500	111[z]	.986	.808	.712	.599	.483	.291	−.065
36 Wanakena, N. Y., 1,550	111	.975	.859	.722	.741	.371	.097	−.135
2 Franconia Notch, N. H., 1,950	109[z]	.966	.871	.743	.572	.522	.266	.076
30 Laura Lake, Wis., 1,600	109	1.116	1.076	.810	.764	.618	.086	.006
28 Ishpeming, Mich., 800	107	1.130	1.119	.827	.788	.634	.050	.028
3 Brandon Gap, Vt., 1,950	106[z]	1.181	1.008	.793	.689	.707	.277	−.069
5 Newcomb, N. Y., 2,100	98[z]	.966	.984	.655	.638	.657	.001	.035
A Mean $\bar{z}$		1.219	1.113	.861	.731	.7400	.2352	.0248
B Regression on season $b{\cdot}x$		.00480	.00508	.00405	.00214	.00369	.00162	.00219
C Variance due to regression $B^{x}{\cdot}zx$		.65294	.73227	.46395	.13015	.38672	.07437	.13547
D Variance around line		.00789	.01316	.01166	.00742	.01951	.01297	.01786
E Variance ratio of slope (F)[a]						19.82	5.73	7.59

[z]Values interpolated between existing stations where no very nearby record was available.

[a]Expected values: at $P = 0.025$, $F = 5.61$; at $P = 0.01$, $F = 7.64$; at $P = 0.001$, $F = 13.50$.
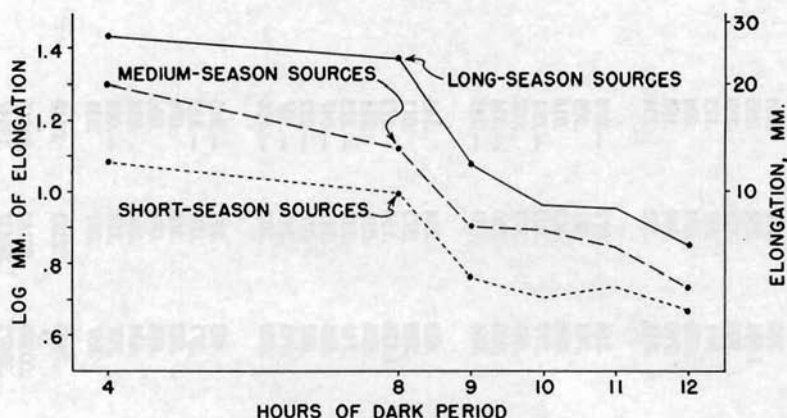
Figure 22. Growth as a function of the dark period for seedlings from source regions of long, 181-195 days, medium, 139-164 days, and short, 98-131 days, of frost-free season, represented by 7, 8, and 15 sources, respectively.

Based upon Table 12, the trend of elongation as a function of hours of nightlength is illustrated in Figure 22, for long, medium, and short (including very short) seasons. There is a general similarity in form of these curves but also an interesting shift in pattern. For long-season sources the difference in mean elongation between 9- and 12-hour nightlengths, relative to that between 4- and 8-hour nightlengths, is greater than for medium and short-season sources. This could be biologically significant in permitting some plants of southern genetic types to remain active under natural nightlengths which produce buds on almost all plants of more northern populations. The statistical significance of this difference in response is indicated by the following factorial analysis.

There are three possible comparisons of elongation under the different nightlengths shown in Columns a, b, c, and d, Table 12: (1) the overall difference between short and long nights by the algebraic summation, $a+b-c-d$; (2) the average trend of elongation with nightlength within both short- and long-night treatments by the summation $a-b+c-d$; (3) the interaction of (1) and (2) by the summation $-a+b+c-d$. These three summations are shown as indices on the right side of Table 12 for each seed source. Index (3), which compares the difference within the relatively short nightlengths, is an indirect measure of the "critical nightlengh" for each seed source. Their averages over all 30 sources are given on line A. The regression coefficients of all three indices for individual sources on length of frost-free season are given on line B, and the statistical tests of their significance on lines C to E.

The difference in log-growth (.740) *between* long- and short-night treatments (Index 1) is over three times as great as the difference (.235) *within* groups (Index 2), as was already suggested in Figures 21D and 22. The positive regression of (Index 1) and (Index 2) on season shows that the span of growth under contrasting nightlengths is less for northern than for southern types, because the former start going dormant early even when nightlength is relatively favorable for continued growth. Index (3) is always

positive for long-season sources; it tends to become smaller, and in some cases negative, as length of season decreases. This index is plotted in Figure 23. It has a positive regression on season (b = 0.00219), which accounts for a significant variance (0.13547) when compared with the residual variance of 0.01786. This confirms a real tendency for southern genotypes to have a slightly longer critical nightlength for bud formation compared with northern types, as well as to remain active longer under any given nightlength.

This conclusion bears on a controversy concerning whether the natural change from active growth to dormancy in trees is regulated by internal factors (58) or by external factors such as the changing relation of day and night, which follows the regular seasonal change in the environment as a signal for termination and hardening off of new growth (42). In Connecticut, at least, buds begin to become conspicuous around mid-August, when the nightlength has already increased to 9 hours and is increasing by several minutes each day. The case for a general environmental effect of photoperiod is better for hemlock than for many other species which terminate growth much earlier, while nightlength is still near its minimum for the year. The preceding inference, that seedlings of northern parentage tend to have a slightly shorter critical nightlength, also might partially explain why they tend to go dormant slightly earlier than those of southern parentage when both are growing under similar nursery and plantation conditions.

Photoperiod is probably only one of several factors which make northern seedlings stop growing earlier than southern ones, because they did this even under 20-hour photoperiods, which should have been about as favorable as possible for maintaining growth. As Wareing (58) suggests, there is evidently also some internal process tending to change the growing shoot to a dormant one regardless of photoperiod. In addition, there are more subtle differences in response to photoperiod from one geographic race to another as Pauley and Perry (42) have emphasized.

As with genetic differences in seed response, genetic differences in the control of stem growth have presumably arisen through survival of those types which can live and thrive in diverse environments over the range of the species.

Effect of temperature on elongation

The preceding comparisons of the effects of chilling, photoperiod, and seed source were made for constant temperatures of 60° or 62° ±2°. In the same experiments, the effects of different temperatures were also studied under a favorable (16-hour day) photoperiodic regime.

Chilled 2-year seedlings from the experiments described on page 50 attained an average total elongation of 44 mm. at 60° constant temperature. At other constant temperatures chilled seedlings from the same source had average elongations as follows: at 75°, 42 mm.; at 45°, 6.5 mm. (needles pale green); at 90°, 12.5 mm. This unfavorable effect of high temperature was overcome to some extent if plants were removed to a lower temperature at night (28 mm. at 75° night temperature; 51 mm. at 60° night temperature). With 90° day, 60° night temperatures, terminal elongation was slightly better than for 60° constant temperature with the interesting difference that there was considerably more elongation of lateral shoots on plants under a 90° day, 60° night temperature alternation than under 60° constant temperature; a 75° day, 60° night was intermediate for both terminal and lateral elongation.

Table 13. Effects of day and night temperatures on elongation[1] of first year hemlock seedlings

Night temper- ature	Degrees Estimated frost-free season of source	Day Temperature						
		44°	53°	62°	71°	80°	89°	98°
Degrees					*Millimeters*			
98	Long	......	......	......	......	......	......	
	Medium	......	......	......	......	......	......	**All**
	Short	......	......	......	......	......	......	**Died**
	Very short	......	......	......	......	......	......	
89	Long	......	......	......	......	......	13.0	......
	Medium	......	......	......	......	......	7.5	......
	Short	......	......	......	......	......	8.5	....
	Very short	......	......	......	......	......	7.4	......
80	Long	......	......	21.8	......	19.3	......	16.9
	Medium	......	......	16.8	......	14.4	......	12.8
	Short	......	......	13.5	......	12.3	......	10.8
	Very short	......	......	14.5	......	11.8	......	11.7
71	....	......	......					......
62	Long	......	......	24.9	......	25.9	......	16.4
	Medium	......	....	16.1	......	14.7	......	14.0
	Short	......	......	8.6	......	15.1	......	11.2
	Very Short	......	......	10.6	......	11.7	......	11.4
53	Long	......	......	......	......	19.0	......	......
	Medium	....	......	......	......	14.9	......	......
	Short	......	......	......	......	11.5	......	......
	Very short	......	......	......	......	10.4	......	......
44	Long	3.0	6.3	8.0	......	......	......	......
	Medium	2.8	4.8	6.3	......	......	......	......
	Short	2.8	2.9	4.8	......	......	......	......
	Very Short	2.8	3.5	4.7	......	......	......	......

[1]Measurements are for a full season's growth, given in mm. of elongation of epicotyl of seedlings from regions of long (181-195 day), medium (139-164 day), short (114-131 day) and very short (98-111 day) frost-free seasons, based respectively on the means of 3, 4, 3, and 5 sources from each zone.

[2]Duration of day temperature was about 8 hours out of each 16-hour light period daily.

Seedlings from 15 of the 30 sources used in the seed source experiments described on page 53 were subjected to a wider variety of day and night conditions. The experiments indicated that best growth occurred in the range of 62° to 80°, with some benefit from diurnal temperature alternation within this range, Table 13. The greatest total elongation was attained by seedlings from long-season sources growing on an 80° day, 62° night temperature or at 62° constant temperature. Even the unnatural alternation of 80° night, 62° day was better than 80° constant temperature.

A constant temperature of 98° killed all plants; a combination of 98° day with 80° night temperature decreased total growth only moderately (Table 13). An examination of the records on timing of growth indicated one reason why such high temperatures did not cause a more drastic decrease in final elongation. This is the tendency for warm temperatures to delay bud formation, thus compensating to some extent for decreased internodal elongation.

This tendency introduces one more element into the general interpretation of the timing of bud formation under field conditions. A combination of favorable photoperiod and warm, naturally fluctuating temperatures in midsummer would be especially favorable for keeping plants in active growth. Early bud formation in at least some plants at 62° constant and other cool-temperature combinations suggests that the normal decrease in temperature in late summer is another factor, in addition to increasing nightlength, which tends to hasten bud formation; these factors reinforce each other in nature.

Differences referable to source were evident in temperature as well as light treatments; earlier termination of growth by seedlings from short-season sources (lower figures in each group in Table 13) accounts for their lesser elongation. (But differences among sources in duration and amount of growth were less at 98° day, 80° night and 98° day, 62° night than they were at cooler temperatures.) The great difference between plants from long- and short-season sources in hastening of bud formation at 62°, even under favorable 8-hour nightlengths in the original experiment (Figures 21 to 23), suggests
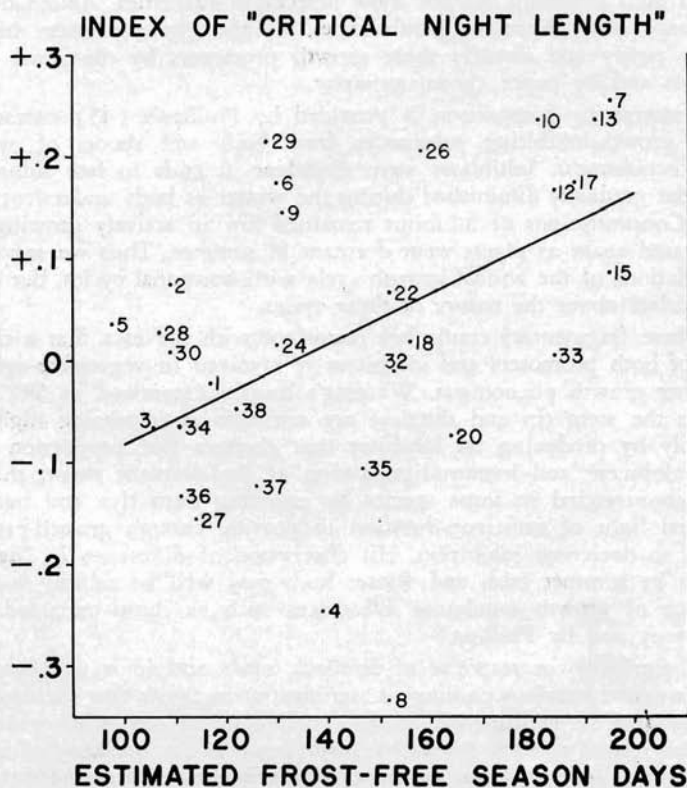
Figure 23. Trends in index of critical nightlength for seed from 30 sources. Points and regression line from Column 3, Table 12.

that seedlings from short-season sources are naturally more sensitive to cool temperatures than those from long-season sources. This would represent one more kind of genetic difference which adapts them to the cool climate of their native habitat and tends to start hardening them off early, even while night-lengths are relatively short. The more persistent growth of seedlings from long-season sources at cool temperature suggests that temperature is a less important regulator than is photoperiod.

The considerations of the preceding paragraph resolve what might at first seem a paradox in Table 13, in that final total growth of plants from long-season sources exceeds that of plants from short season sources by a greater amount at cool temperatures than at warm temperatures. The opposite was true for germination; seed from warm climates germinated better than those from cool climates at high temperatures, and poorer at cool temperatures.

Mechanisms governing stem growth

The presence of growth-promoting substances in crude ether extracts from active shoots and chilled buds of *Tsuga canadensis* was indicated by posi-tive curvatures in tests made at this Station with de-seeded oat coleoptiles. Unchilled buds generally did not show detectable quantities. Abundant resins, and perhaps other interfering substances, in the crude extracts hampered efforts to purify and identify these growth promoters by the usual changes in solvents and by paper chromatography.

An interesting comparison is provided by Phillips's (43) extraction of phenolic growth-inhibiting substances from buds and shoots of sycamore, *Platanus occidentalis*. Inhibitors were abundant in buds in late summer and autumn but gradually diminished during the winter as buds underwent natural chilling. Concentrations of inhibitor remained low in actively growing shoots but increased again as plants went dormant in summer. Thus we see interest-ing correlations of the annual growth cycle with hormonal cycles, but can still only speculate about the nature of these cycles.

These fragmentary results are consistent with the idea that a changing balance of both promoters and inhibitors is involved in vegetative cycles and many other growth phenomena. Wareing's studies (reviewed in 58) suggest that both the stem tip and the leaf are sensitive to increasing nightlength, presumably by producing an inhibitor that changes leaf production to bud scale development and hastens lignification of the dormant shoot; this effect may be counteracted in some species by exposing stem tips and buds daily to artificial light of sufficient duration to provide enough growth-promoting substance to overcome inhibition. His observaton of difference in "depth" of dormancy by summer buds and winter buds may well be related to shifting importance of growth regulating substances such as those extracted in the present study and by Phillips.

The similarity in response of hemlock seeds and buds to photoperiod, temperature, and previous chilling or stratification suggests that the mechanism which regulates the germination of seeds (discussed in connecion with Figure 18) is also related to such vegetative phenomena as the breaking of bud dor-mancy, growth, and return to dormancy. Experimental and theoretical studies of many separate aspects of such problems are currently very active, but they have yet to account for and relate the whole complex of environmental res-ponses which we have surveyed.

General Conclusions

We can see many points of similarity in the response of seeds and seedlings of *Tsuga canadensis* to their environment. Both show marked dormancy in their natural condition in the autumn. Chilling for a month or more at temperatures slightly above freezing permits them to grow when returned to favorable temperatures. The chilling requirement is not absolute; repeated daily cycles of light and darkness of appropriate duration can also lead to resumption of growth although somewhat less promptly than for seeds or buds which have been previously chilled.

Response to duration of light and darkness (photoperiodism) is also evident in the return of growing stems to a dormant condition by the formation of new terminal buds. The duration and amount of growth decrease gradually as nightlength increases; the most marked hastening occurs when nightlengths increase beyond 8 to 9 hours (slightly longer for seedlings of southern than northern parentage). Irrespective of photoperiod, there seems to be a tendency for northern types to go dormant and harden off earlier, and to make less growth than southern ones, and for mountain types to grow less vigorously than nearby lowland types. These tendencies combine to help the annual growth cycle to conform to the climate at the point of seed origin.

Both seeds and seedlings show a fairly narrow range of temperatures suitable for optimal growth, and there is usually some benefit from temperature oscillations within and beyond this range. For most rapid germination, the range is between 55° and 70°; for elongation between 60° and 80°. Germination of southern seed is better than that of northern seed in the upper end of the range indicated; that of northern seed is better at the lower end. Given a favorable photoperiod, germination of unstratified seed is better for southern than for northern types.

Although long-day and short-night conditions are consistently best for stem growth, there is a marked change in the photoperiod optimal for the germination of unstratified seeds with changing temperature. Within the optimal temperature range, for example, 8-hour photoperiods are relatively favorable; at higher and possibly also at lower temperatures they are relatively unfavorable.

Both seed and seedling responses seem to have evolved in directions that adjust different genotypes to the varying environments encountered by *Tsuga canadensis* in different parts of its range. This adjustment is probably also typical of many wide-ranging species.

Physiological interpretations of environmental and genetic differences seem to fit within the general framework of current research on seed germination and photobiology in general. However, the strong interactions of photoperiod, temperature, and previous chilling, and the stimulatory and inhibitory responses to chemicals like thiourea and coumarin, all pose special problems that may test the adequacy of general theories about the regulation of plant growth.

In nature the growth of any plant is governed by net response to environmental factors. These are never constant, they interact and are confounded with one another, and are difficult to assess in the field. By use of controlled environments, it has been possible to analyze, literally "take apart," the effects of some of the more important environmental factors, and to show how responses to these vary with genetic type. Some ways in which these individual
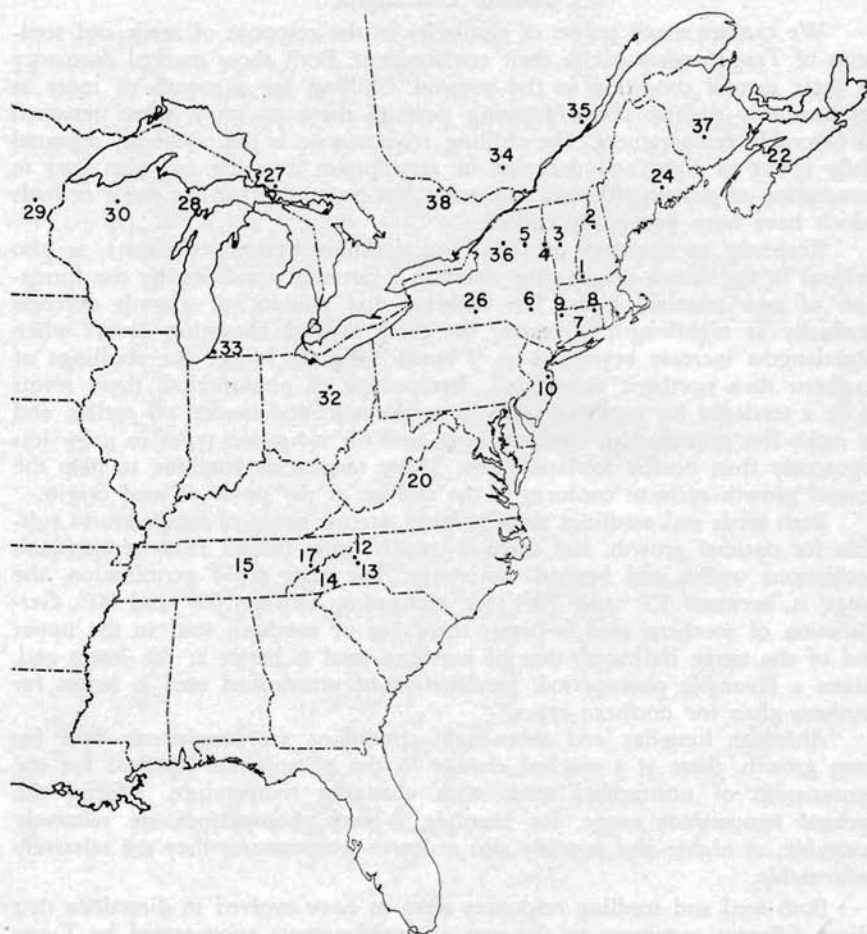
Figure 24. Map of hemlock seed sources listed in Table 12 which were used for indoor experiments with first-year seedlings.

pieces of information fit into a whole picture of the natural life history of the species can be suggested now, but further synthesis of both the laboratory and field aspects of the problem is needed. Only when this kind of information is brought together for many other species can we hope to achieve the more remote and challenging goal of an understanding of the forest as a whole.

As often happens in the pursuit of basic research, new information has incidentally provided the answers to a number of practical questions which are discussed in the next section.

THE GROWING OF HEMLOCK

As with domestic plants and animals, the best trees require both superior heredity and a favorable environment. These factors are particularly important in the propagation of trees because of their long life span; they apply both to trees for forest growth and for ornamental purposes although the methods used and the permissible costs differ greatly.

In much of northeastern United States, hemlock can usually be introduced by planting on sites where natural seedling establishment may be difficult, or on extensive areas where a natural seed supply has been eliminated by fire or other disturbance. The demand for forest nursery stock has kept ahead of the supply in recent years in Connecticut; over half a million nursery trees are also currently being grown for ornamentals. Probably this species would now be planted even more widely if growers were not plagued by unpredictable behavior in the nursery.

Large-scale production will be primarily from seed because it is cheap and permits the growing of large amounts of stock. If the trees are to be grown for timber production, progeny from seed from superior stands of unusually tall, straight trees with narrow crowns, little trunk taper, and light branches will increase the chances of passing on these desirable characteristics to future generations; likewise stand degeneracy can result if seed is consistently collected from poor individuals.

For ornamental plantings, trees with special characteristics such as compactness, unique branching habits, and leaf size and color are desired. Occasional trees with these features often occur in nature or in nurseries as sports or mutants[6]; rarely do they produce seed progeny similar to themselves. Hence they usually must be propagated vegetatively[7] by grafts, or by rooted cuttings, which will have the same characteristics as the parent stock. Our own experience and that of Mr. E. C. Childs, Norfolk, Connecticut, and of others cited in the footnotes indicates that improved rooting of hemlock is obtained through the use of hormone powders or solutions, and that success appears to vary markedly with the horticultural type being propagated, and with the season.

Seed Collection and Storage

Seed should preferably come from areas with a climate like that prevailing where the trees are to be grown so that they will be well adapted to it. If seed is purchased, remember that its age and source have a bearing on germination and development.

Cones are most readily collected from the tops of trees felled in normal cutting operations in late September of a good seed year. If the trees cannot be felled, small quantities of cones can be collected by using special sectional ladders and a pole pruner. Cones collected just as they are turning tan will open readily in the sun or in a dry room. They should be spread out in thin layers for a week or two and stirred occasionally so that they will dry quickly

6 For a discussion of horticultural varieties and forms see articles by J. C. Swartley in the Cornell Plantations, Vol. 2, pp. 3-6. 1945 and in American Nurseryman Vol. 83, No. 7, pp. 7-10; No. 8, pp. 11-13. 1946.

7 Additional references on vegetative propagation: Doran, W. L., American Nurseryman, vol. 74, pp. 18-19, 1941. Journal of Forestry, vol. 50, pp. 126-129, 1952; Thimann, K. V. and Behnke, J., Maria Moors Cabot Foundation Publ. No. 1, Harvard Forest, Petersham, Mass., 1947.

and not become contaminated by molds which tend to develop on damp cones. Green cones are not only subject to molds but are often very hard to open; difficulty of opening may be partially overcome by subjecting cones to repeated cycles of drying at $100°$, alternated with moistening.

Care in excluding or removing leafy twigs makes cleaning easier. Seeds can be de-winged by rubbing them by hand or by machine. They should be cleaned as thoroughly as possible by a fan or special blower adjusted to winnow out hollow seed and fine debris. Low viability that is commonly reported for hemlock may be due to the difficulty of separating the poor seed from the good in cleaning.

Our experience shows that seed can be stored for 2 to 4 years in jars or plastic bags in a refrigerator running at a few degrees above freezing, but different lots of seed, even from the same locality, varied considerably in their retention of viability so that periodic testing is necessary. Temperatures below freezing have been used successfully ($26°$ for eastern hemlock at the nursery of the Connecticut Park and Forest Commission at Voluntown and $0°$ for western hemlock at the Boyce Thompson Institute, Yonkers, New York).

Stratification hastens the breaking of seed dormancy and permits the rapid germination of hemlock and many other seeds. Germination of unstratified hemlock seed is usually greatly delayed. Seed from northern sources may require longer stratification than that from southern sources.

Wind-dispersed seed or seed sown in the autumn in the nursery is naturally stratified and is ready for quick germination in the spring. Here the stratification process is subject to little control and losses due to rodents, birds, fungi, and other causes may run very high. Where possible, it is better practice to stratify during the winter under conditions that permit at least some degree of control, and to sow the seed early in the spring. The best method of stratifying large amounts of seed is to place them in thin layers, alternating with damp sand or peat moss, in flats which are kept for 2 to 3 months in a cold room or refrigerator maintained at a temperature above freezing and below $40°$. A method which is often used but which is less subject to control is to rodent-proof the flats, place them on well-drained ground, and cover them with enough straw to keep the temperature within the desired range. The temperature of the seeds should be checked occasionally, and straw added or removed as needed; a maximum-minimum thermometer is helpful.

Seed Testing

Seeds may be cut open with a razor blade or finger nail to provide an estimate of the percentage of filled, firm, oily seeds with embryos. Since the percentage germination is usually less than such tests indicate, actual germination tests are desirable for estimating rate of sowing. These may be made by placing duplicate or triplicate lots of 50 seeds each in glass or plastic dishes on absorbent tissue that is kept moist enough so that a little water oozes off the tissue when the dish is tilted. The tissue should be kept moist during the tests.

If the seed has already been stratified, the dishes should be placed where the temperature can be maintained at $62° \pm 8°$. This may be in a suitable unheated room or in a closed box fitted with a lead heating cable and a household thermostat for maintaining the desired temperature range. Light is not essential for successful germination of stratified seed.

If the seed has not been stratified, the dishes of seed prepared for germination can be placed in a refrigerator for 2 to 3 months, prior to testing.

If for any reason seed cannot be stratified, germination tests in the prepared dishes can still be made, but it will be necessary to maintain a more rigid control of both light and temperature. Optimum conditions are 8 to 12 hours of light, equivalent to natural daylight under fall and winter conditions, alternating with 16 to 12 hours of total darkness, with corresponding alternating temperatures of about 70° and 55°, respectively. If natural daylight is not suitable, fluorescent or incandescent lights controlled by a time switch set for 8 hours of light can be substituted.

Continuous temperatures in the upper 70's are definitely unfavorable for hemlock germination.

Nursery Practice

Nursery work begins with the establishment of the seedbed where the seedlings may either be left for 2 or 3 years and directly planted into the field, or be transplanted after the second year and left in transplant beds for 1 or 2 years. Usually 3-year seedlings (3-0) or 3-year transplants (2-1) are sufficiently well rooted and of suitable size for out-planting.

Of the several nursery operations, the establishment and care of seedbeds is the most critical. Availability of labor and ease of working up the beds offer some advantages for fall sowing and some seed beds will undoubtedly be put in at this time. However, fall-sown beds are subject to losses from causes which are not easily controlled, and it is probably better to shape the beds in the fall when the ground works easily but to delay sowing until spring. If fall sowing is planned, small lots of seed from crops of previous years can be stratified for testing in a refrigerator for 2 to 3 months, beginning in June or July, and tested for germination in September and October in time to serve as a guide to the amount of seed to sow in November or early December. (Note — All dates given are for southern New England.) If seed is collected in the same year it is to be sown, a germination test without stratifying can be made if the test is started promptly. If there is any delay, only a cutting test can be made.

For spring sowing, the suggested procedure is to place enough seed for testing in stratification about November 1, and withdraw it for germination tests about January 1. By February 1, germination should be sufficiently well along to indicate the amount of seed to be stratified for the main planting. If this is stratified about February 1, it should then be ready for field sowing between April 1 and 15.

When seed is stratified too long, it is likely to start germinating while still in stratification. Because such seed is quite susceptible to drying out and to mechanical injury, it is not satisfactory for routine nursery work. However, seeds already germinated during stratification get off to an unusually rapid start after planting. With careful handling, small lots of such seed can be used advantageously in experimental work and in making sowings in woodland where a fast start is necessary for good establishment before the soil dries out in the spring. Another way of getting pregerminated seed for such uses is merely to take seed that has been stratified for the normal period and leave it at 60° for about a week before planting.

Seedbed soil should be loose and friable so that it does not cake, and the surfaces of beds should be convex and elevated several inches to provide good drainage. A common practice is to cover fall-sown beds immediately with a pine needle mulch which is removed about April 1st and replaced with lath or tobacco shade tent cloth supported a foot above the bed surface.

Spring sown beds are immediately covered with the cloth. During the first season, the surface of the beds should never be allowed to dry out.

Seed which is sown after May 1, even if stratified, may not germinate that spring if soil temperatures are too high. Some of it may germinate in the fall, often so late that the tender seedlings are killed by frost; some may not germinate until the following spring. After the ground freezes at the end of the first and second years, it is usually desirable to mulch with needles or salt hay to prevent heaving during the winter and following spring. During the second and third years the beds are usually left uncovered but should be watered thoroughly during periods of drouth.

For very special lots of hemlock seedlings or cuttings, the growth of several normal growing seasons can be compressed into a shorter period. If, after the growth for a year has been completed and the buds have formed, the stock is chilled for 4 to 6 weeks below $40°$, it can then be moved back to the growth room or greenhouse under favorable temperature and light conditions with the expectancy that buds will break dormancy and the trees again resume growth without the long over-winter delay.

The Use of Chemicals

Thiourea and several other chemicals stimulate hemlock seed germination under certain conditions. However, there is also a danger of damaging the embryos unless the right concentration and appropriate rinsing are used. Since the desired germination can be obtained without chemicals, the practice is not yet recommended for routine use.

Essentially the same conclusion was reached regarding the use of various fungicides to prevent superficial molds because of the risk of delaying germination. If preliminary tests indicate serious contamination, products like Tersan or Arasan (50 per cent active) as dust or in 0.5 to 3.0 per cent solutions may be tried out, preferably on a small scale at first.

Inherently good seed usually survives superficial contamination with molds on the seed coat if other conditions are favorable for rapid germination. If seed is infected internally, it is probably not viable and no treatment is effective.

During the first few months of life, seedlings in the nursery are very subject to damping off by the fungus *Rhizoctonia*. A treatment of the beds in early September to a depth of 6 inches with formaldehyde at the rate of 2 quarts of 2 per cent solution per square foot should fully sterilize the soil and kill the weed seed to a depth of 1 foot. *Two weeks should elapse between treatment and sowing, longer if temperature is below $50°$. After the first week the top 6 inches of soil should be turned over several times.* September treatment permits sowing either in the late fall or the following spring.

After the seed has germinated, damping off by *Rhizoctonia* can be quite well controlled by treating the soil with 8-hydroxyquinoline sulfate, 1:2000 or 2/3 teaspoonful per gallon of water, applied at the rate of 1 pint per

square foot two or more times during the season; more frequent treatments may be needed during humid weather while the stems are green and soft. *Rhizoctonia* may also attack the small active roots of older hemlock trees and cause wilting and sometimes death, especially during dry periods. Further injury to the roots can be checked by the same treatment if diagnosis is made in time.

Nutrition

Hemlock seedlings can survive at a low level of nutrition. However, experiments and nursery experience have demonstrated that growth can be increased several-fold by maintenance of nutrition at moderate levels. Maintenance of high levels of nutrition, or the continuance of fertilization and watering into the late summer or fall season are to be avoided because plants will continue growth too late in the season and will not be sufficiently hardened off before cold weather sets in. This is particularly true for plants from southern sources which tend to keep growing late in the fall.

Experience at this Station has repeatedly demonstrated that over-fertilization of larger hemlocks, and many other plants in commercial nurseries and home plantings, increases their susceptibility to *Rhizoctonia* as a root-destroying fungus. If the roots are not too severely injured, the plants will recover if the soil is treated with 8-hydroxyquinoline sulfate, but it is better to lessen the probability of infection by keeping nutrition at a moderate level than to have to treat a heavily infected plant.

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