



Ecotypic variation in *Pinus contorta* Dougl. var. *latifolia* Engelm. (lodgepole pine)
by David Anthony Perry

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF
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Abstract:

Seed was collected from ten lodgepole pine trees in each of five stands located in Utah, Montana, Idaho, Washington, and British Columbia. Seed was planted and seedlings grown in four daylength regimes--14, 15-1/2, 16-1/2, and 22 hours—in each of two temperature regimes—warm and cool. Other seedlings were grown at three levels of moisture stress--0, 3, and 5 bars. Mass, height, and dates of budset and budburst were recorded. Significance of variations among and within stands was determined by analysis of variance.

Stands tended to be uniform in their mass values and dates of budset and budburst. There was significant variation in height, with tallest seedlings coming from parent stands with the longest growing season. There were no significant differences which were latitudinally correlated in the way stands reacted to daylength, however, seedlings from high-elevation sources appeared to be favored in cool temperatures and short daylengths. Seedlings from parent trees in summer drought areas resisted moisture stress somewhat better than other seedlings.

Within-stand variation was of two types: (a) that among parent trees and (b) that among progeny from a single parent tree. With the exception of the Montana source, stands formed a north-south line of decreasing type (b) and increasing type (a) variation.

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ABSTRACT

Seed was collected from ten lodgepole pine trees in each of five stands located in Utah, Montana, Idaho, Washington, and British Columbia. Seed was planted and seedlings grown in four daylength regimes--14, 15-1/2, 16-1/2, and 22 hours--in each of two temperature regimes--warm and cool. Other seedlings were grown at three levels of moisture stress--0, 3, and 5 bars. Mass, height, and dates of budset and budburst were recorded. Significance of variations among and within stands was determined by analysis of variance.

Stands tended to be uniform in their mass values and dates of budset and budburst. There was significant variation in height, with tallest seedlings coming from parent stands with the longest growing season. There were no significant differences which were latitudinally correlated in the way stands reacted to daylength, however, seedlings from high-elevation sources appeared to be favored in cool temperatures and short daylengths. Seedlings from parent trees in summer drought areas resisted moisture stress somewhat better than other seedlings.

Within-stand variation was of two types: (a) that among parent trees and (b) that among progeny from a single parent tree. With the exception of the Montana source, stands formed a north-south cline of decreasing type (b) and increasing type (a) variation.

INTRODUCTION

Pinus contorta, lodgepole pine, is one of the most widespread of North American pines. Its geographic range extends from the divide between the Klondike and McQuesten Rivers at about 64° N. latitude to the Sierra San Pedro Martin in Baja California at about 31° N. latitude, and from the Pacific coast to the Black Hills (Tackle, 1959).

Along the Pacific coast lodgepole pine is a small tree, 7 to 9 meters in height, which is often characterized by a contorted bole and dense, irregular crown. In contrast, the inland form is a medium-sized tree with a clear, cylindrical bole and small crown (Harlow and Harrar, 1958). Some taxonomists distinguish two varieties of lodgepole pine, Pinus contorta var. contorta, the coastal form, and Pinus contorta var. latifolia, the inland form (Tackle, 1959).

In an extensive study of morphological characteristics, Critchfield (1957) suggested that the species be divided into four subspecies, contorta (coastal region), bolanderi (Mendocino white plains), latifolia (Rocky Mountains), and murrayana (Sierra Nevada). Critchfield also noted that within the inland subspecies low and high elevation populations formed groups with distinct morphological characteristics.

The environmental conditions under which the various regional forms of lodgepole pine grow vary widely. The Sierra Nevada and coastal populations grow in zones of high precipitation, while in the Rocky

Mountains and other areas in which ssp. latifolia is found¹ summers may be dry and include a period in which soil moisture becomes deficient. Coastal areas have a very narrow seasonal temperature range, while inland it is generally quite wide. At high elevations frost may occur at any time (Tackie, 1959).

It is generally agreed that in the Rocky Mountains fire has played a large rôle in the development of ecosystems. The serotinous cone of lodgepole pine, which remains closed at maturity and is opened by temperatures of 40° C. or greater (Clements, 1910) represents an adaptation to fire and promotes the recolonization of burns by this species. Once established, the high degree of shade intolerance in lodgepole seedlings very often prevents the stand from perpetuating itself and, in the absence of fire, other species, usually Douglas fir at low elevations and subalpine fir and Englemann spruce at high elevations take over the site as the original lodgepole stand ages and dies.

Although the principal niche of lodgepole pine in the Rocky Mountains is as a major component of the fire sere, in some areas it may form stands which persist in the absence of fire. In Colorado Moir (1969) found that deep soils between 2500 and 2850 meters

¹The area in which the Rocky Mountain form of lodgepole pine is found includes not only the Rocky Mountains but the intermountain region, the Washington Cascades, and the Blue Mountains of Oregon (Critchfield, 1957).

elevation might have 60 to 100-year old lodgepole stands with no advanced reproduction of other conifers. He related this to high summer soil surface temperatures and summer drought. In Yellowstone Park, on sites with combination of low rainfall and nutrient-poor soils, lodgepole pine forms pure stands which appear to persist over long periods. Despain (1973) related this to the inability of spruce and fir to become established on these sites. Similarly, in the northwest Oregon Cascades lodgepole pine forms pure, relatively stable stands on very droughty and nutrient-poor soils (Stephens, 1966).

There are few physiological limitations to lodgepole pine's ability to occupy various habitat types. In colonizing burns it encounters a wide variety of edaphic, topographic, elevational, and moisture conditions (Tackle, 1959). Its role seems to be that of a pioneer tree taxon on sites where other species, either temporarily or permanently, seem unable to become initially established. If by its presence, conditions are ameliorated sufficiently for other trees to establish, lodgepole pine apparently cannot compete with them and is ultimately replaced. If conditions remain too stringent for others, it maintains occupancy of the site.

Although morphological variations in lodgepole pine have been well studied and such factors as rates of photosynthesis and growth have been compared between populations (Sweet and Wareing, 1963a; Buchert, 1971), little is known about the degree to which populations of lodge-

pole pine have adapted to specific factors of the environment. There are many conflicting reports of the conditions under which it grows, and Edwards (1954) states that "it is a most adaptable species, reacting in many different ways to the factors of the locality where it grows." Does the species have a high degree of phenotypic plasticity, or is it characterized by a great number of ecotypes? Edwards (1954) reviews provenance studies that have been conducted in Europe.² These experiments demonstrated growth differences among provenances, as well as a tendency for individuals to perform better in climates similar to the place of their origin than they do in other climates. He concludes that lodgepole pine is differentiated into ecotypes which form an ecocline "ranging from the coastal shore pine to the most continental form of lodgepole growing east of the Rocky Mountains, and distributed between 64° and 36° N. latitudes." Edwards cites other researchers, however (Whitford and Craig, 1918; Davidson and Abercrombie, 1927), who maintain that the varieties within the species are "environmental forms," presumably implying that differences are due to phenotypic plasticity rather than genetic differentiation.

²Provenance is defined by Hanson (1962) as "the place of origin of seeds or other propagules." The term provenance study is used by foresters to describe studies which compare the performance of trees of the same species but different geographic origin, when tested under common conditions.

This paper compares growth and phenology among lodgepole pine seedlings from five provenances, when subjected to conditions of varying daylength, temperature, and moisture stress. The objectives of the research are to determine (a) if there are significant differences in seedling response among samples of the various provenances and (b) whether such differences, if they exist, are dependent on the environmental factors tested.

THE CONCEPT OF THE ECOTYPE

Although this paper is solely an analysis of phenotypical differences among various populations of Pinus contorta, "lodgepole pine," and does not purport to determine genotypical variation, the role which the genotype undoubtedly plays in ecotypic variation warrants a brief examination of the genetic factors which contribute to the nature of variation in a species.

Early studies of infraspecific variation relating to habitat variation were of European forest trees. Langlet's (1971) historical review mentions work by Duhamel du Monceau (circa 1745), de Vilmorin (circa 1820), Kienitz (1879), Cieslar (1887-1907), and Engler (1905-1913). These investigations were in the nature of what foresters know today as provenance studies. Seed was collected from trees of contrasting locales and planted in one or several common gardens (See Heslop-Harrison, 1964, for a discussion of the limitations of the common-garden

method.) The observation that differences in plant growth and other characteristics often were related to habitat variation were and maintained in a common environment led to the concept that different habitats are occupied by different genotypes of a species, each genotype tending to "fit best" the set of environmental factors characteristic of its particular habitat.

Although this idea existed as early as the mid-eighteenth century, it was not effectively articulated until Turreson published "The genotypical response of the plant species to the habitat" in 1922. Turreson transplanted individuals of several European herbaceous species from varied habitats to a common garden. As a result of his observations he proposed a terminology to deal with various levels of genotype-environment interaction. In fact this was more than a terminology; it was a defining of new modes of thought concerning the genotypical organization of living systems. Among the terms proposed was ecotype, which Turreson defined as the genotypical response to a particular habitat.³ In 1923 Turreson introduced the term "genecology" for the study of ecotypic

³Other terms proposed by Turreson were:

- Coenospecies: the total set of genetic combinations possible to the gene pool of the Mendelian species.
- Ecosppecies: the set of genotypes within the coenospecies which have been elicited by environmental requirements, i.e., those found in nature (roughly equivalent to the Mendelian species).
- Ecophene: the set of non-genetic modifications of a particular ecotype.

variation in plants. Heslop-Harrison (1964) summarizes the basic propositions of genecology as follows:

(1) wide ranging plant species show spatial variation in morphological and physiological characteristics; (2) much of this infraspecific variation can be correlated with habitat differences; (3) to the extent that ecologically-correlated variation is not simply due to plastic response to the environment, it is attributable to the action of natural selection in moulding locally adapted populations from the pool of genetical variation available to the species as a whole.

A controversy soon developed, and continues today (Spurr and Barnes, 1973) over the validity of the ecotype concept. The school which developed in opposition maintained that, since most environmental factors vary in a continuous fashion, genotypes would also tend to vary continuously. This 'clinal' viewpoint was stressed in the studies of Gregor (1936, 1938, 1939, 1950, 1961), Langlet (1959); and Callahan (1962), among others. Expressing the opposing viewpoint, Clausen et al. (1940, 1945, 1948, 1948a) reported that population changes were rather abrupt in the species studied by them.

The idea of continuity is a difficult one to deal with. Modern physics has demonstrated to the satisfaction of most people that, with the possible exception of time, there is no such thing as a continuous phenomenon in nature. Our perception of continuity is a function of the level at which we choose to look and the sensitivity of our measuring devices. The closeness with which some trait ("T") is able to vary with some environmental factor ("F") is dependent on the relative sizes of minimum ΔT and minimum ΔF . Although it is theoretically possible for a

nucleic acid molecule to respond to energy changes at the quantum level, the instabilities that this would introduce into the genome would probably prohibit maintenance of an organized system. Thus, it seems reasonable that a single gene is active over a range of environmental values, and that genomes do not vary continuously in their response to the environment. Mather (1943) indirectly made this point when he observed that the finest detectable level of variation would occur in those traits controlled by several genes, and hypothesized that, since much genetic variation appears to be continuous, traits which show ecotypic variation are polygenically controlled.⁴

It seems likely that, because of the limitation on the number of genetic combinations available, discontinuous variation in environmental response does exist. Whether this variation appears as ecotypical or ecoclinical depends perhaps on many factors, chief among them the size of

⁴Clausen and Heisey (1958a) estimated that a minimum of 100 loci controlled 19 characters in Potentilla glandulosa. In contrast to Mather, they hypothesized that a high number of loci involved in a trait would increase the chance of linkage between genes involved in several traits, thus creating trait "complexes" and increasing the chance of discontinuous variation.

Langridge (1963) states that adaptations of the physiological trigger type are probably based on simple genetic systems. He cites research which has shown photoperiodism to be under the control of one gene in tobacco (Allard, 1914), maize (Singleton, 1950), and rice (Chandraratna, 1955); and cold requirement for flowering under single gene control in sugar beet (Abegg, 1936) and Hyoscyamus (Lang, 1948). Langridge goes on, however, to express mild despair about the ability to analyze the genetic basis of climatic adaptation because "a major part of the genome is concerned in any adaptive process that is a reasonably general feature of the organism."

the discontinuity compared to the degree of homeostasis of individuals (Levins, 1963), the heterogeneousness of the habitat (Levins, 1963), and the degree of gene flow within the population (Heslop-Harrison, 1964). Where a given trait is distributed in space normally around its adaptive norm, as would be expected if gene exchange is random and selective forces nondisruptive, mean response to a given environmental factor may well appear to be continuous. If gene exchange is restricted to selection disruptive, homogeneous ecotypic subsets may develop within a population.⁵

Table 1 (Heslop-Harrison, 1964) shows the relationship between the nature of gene recombination in a species and various other features. Forest trees, it would seem, fit in the category of free gene recombination and clinal variation. However, the evidence for this is contradictory and inconclusive.

Pollen distribution by pine is probably not extensive, although reports in the literature vary widely. Colwell (1951) found that Pinus coulteri pollen is generally transported only 10 to 30 feet downwind, and very little goes over 150 feet. At the normal density of forest

⁵Thoday and Gibson (1962), in a set of classic experiments with Drosophila melanogaster, demonstrated that disruptive selection would produce racial divergence despite a high level of intercrossing. They cited this experiment as evidence that distinct populations could be formed by an intermingling of habitat types, despite a high degree of intimacy between the populations.

Table 1 (from Heslop-Harrison, 1964)

Relationship between Some Components of the Genetic System
and Longevity, Community Type, Physiological Features and
Variation Pattern

Free gene recombination resulting
from a high recombination index
and outbreeding.

Associated with:

- (a) Protracted lifespan of individuals reaching maturity
- (b) High and selective seedling mortality; dispersal capacity moderate to poor
- (c) Membership of closed climax communities in stable or but slowly changing habitats
- (d) High range of physiological tolerance in the adult, frequently dependent upon precisely adjusted developmental rhythms but not necessarily associated with phenotypic plasticity.
- (e) Continuous, clinal variation

Restricted gene recombination
resulting from a low recombination
index or inbreeding.

Associated with:

- (a) Short life span of individuals
- (b) Low seedling mortality during colonization of suitable habitats; efficient seed dispersal
- (c) Membership of impermanent communities in ephemeral, fluctuating or shifting habitats
- (d) Avoidance of adverse conditions in the seed state, with or without an associated capacity for adaptive plastic response to environmental stress.
- (e) Discontinuous variation

stands an effective breeding radius of 30 to 50 feet would result in considerable genetic drift (Wright, 1943).⁶ Rehfeldt and Lester (1969) felt that forest tree populations may subdivide themselves into distinct breeding units as one general method of obtaining a fitness-flexibility compromise, and cited research by Squillace and Bingham (1958), Habeck (1958), Muller (1952), and Benson et al. (1967) as evidence. Heslop-Harrison (1964) concluded that ". . . in a continuously dispersed, wind-pollinated species the average area within which there is an appreciable chance of two individuals mating will normally be quite small in relation to the total range." Koski (1967), on the other hand, in a study conducted in the pine stands of northern Finland, found significant pollen exchange over relatively long distances. He concluded that gene exchange between stands located tens of kilometers apart was efficient, and that breeding within stands approached panmixis. Although this resulted, he said, in a great deal of genetic variation between individuals, the formation of local races was seriously hampered by the gene flow.

The effectiveness of pollen distributed long distances from its

⁶In lodgepole pine stands, where the majority of seed following fire will come from those trees which are serotinous, drift in individuals of the parent stand might conceivably result in a patchwork of progenies from single trees, each patch being fairly homogeneous but varying from other patches in a random fashion. This would be most evident for stands in which the preceding generation had a low proportion of serotinous trees.

parent tree will eventually be negated by differences in phenology.⁷ However, pathways for gene migration do exist throughout any area over which the species is relatively continuous, i.e., no discontinuities larger than can be bridged by pollen (Heslop-Harrison, 1964). A particularly appropriate example of gene migration is the probable introgression of jack pine (Pinus banksiana Lamb.) genes into lodgepole pine populations. Although closely related and freely interbreeding, the terpene composition of the resin in these two pines is very different (Mirov, 1961). Evidence of terpenes commonly found in jack pine in lodgepole pine turpentine has been found in Montana (Lotan and Joye, 1970), British Columbia (Zavarin et al., 1969), and Colorado (Mirov, 1956). Sierra Nevada trees sampled by Zavarin et al. did not show notable shifts toward jack pine resin composition.⁸

Regardless of gene flow, extremes of selection will result in

⁷Flowering times may overlap over fairly large areas, however. Koski (1970) reported overlap in the flowering of Scots pine in northern and southern Finland four out of ten years.

⁸Turpentine composition of lodgepole pine at various points gives a measure of how far away from the point of contact of the two species (central Alberta) jack pine genes have moved and therefore, if the period in which they have been in contact is known, an estimate of the range of effective pollen transfer. If one accepts the estimates of Yeatman (1967) and Schoenike (1962) that the two species have been in contact only since the late post glacial, gene spread to Colorado during this period would require an effective rate of pollen spread of roughly 1000 feet per year. However, Argus (1966) and Zavarin et al. (1969) feel that the two pines have been in contact for a much longer period.

divergent populations (Levins, 1963; Heslop-Harrison, 1964). An unanswered question is the degree of environmental difference required to negate the effects of gene flow. Local ecotypic variation in forest trees has been reported for northern white cedar between well-drained and swampy areas (Habeck, 1958), western white pine between dry and moist sites (Squillace and Bingham, 1958), and Douglas fir between north and south slopes (Hermann and Lavender, 1968). The question is confounded considerably by the high degree of random variation which is apparently found in most forest stands. Rather than being merely adaptive "noise," however, the degree and nature of random variation may present a question equal to or surpassing in interest that concerning environmentally correlated local variation, since it is probably the source of much ecotypic differentiation (Stebbins, 1950), and represents a buffering capacity within the population against temporal environmental changes (Rehfeldt and Lester, 1969).

CHAPTER 2

HYPOTHESES

Hypothesis I

There is significant variation among stands in the response of lodgepole pine seedlings to different (a) temperatures, (b) daylengths, and (c) moisture stress regimes.

Hypotheses II

There is no significant variation within stands in the response of lodgepole pine seedlings to different (a) temperatures, (b) daylengths, and (c) moisture stress regimes.

CHAPTER 3

PROCEDURE

Seed Collection

Seed was collected from five open-cone and five closed-cone trees in each of four stands located in the Uinta range of Utah and Wyoming; the Gallatin range of southwestern Montana; the Nez Perce National Forest of central Idaho; and the Colville National Forest of northeastern Washington. Seed from ten closed-cone trees located in central British Columbia was provided by the British Columbia Forest Service. A small amount of seed was collected from four trees in an exceptionally low-elevation stand on the Colville National Forest. Location, elevation, and habitat type of each stand are listed in Table 2, along with site index, which is the average height in feet of dominant trees at 100 years of age, and is considered to be one measure of site quality (Davis, 1954). Parent tree data are given in Appendix I.

Stands were selected so as to represent as far as possible uniform habitat factors. This was difficult to do on a factor-by-factor basis because of the myriad possibilities for factor interaction and the many ways in which they could vary over the range of seed collection. In order to approximate as closely as possible the desired uniformity, stands were chosen from the Abies lasiocarpa/Pachistima myrsinites habitat type, or its ecological equivalent where that habitat type was not represented (Daubenmire and Daubenmire, 1968).

Table 2
Locations, Elevations, Habitat Types, and Site Indexes
of Stands Sampled

Stand	Location	Elevation (Meters)	Habitat Type	Site Index (Alexander, 1966)
Washington #1	Sherman Pass, Colville N.F., Lat. 48° 36' N.	1,600	<u>Abies lasiocarpa/</u> <u>Pachistima myrsinites</u>	90
Washington #2	Colville N.F., Lat. 48° 36' N.	750	<u>Abies lasiocarpa/</u> <u>Pachistima myrsinites</u>	100
Montana	Butte Meadows, Gallatin N.F., Lat. 45° 26' N.	2,200	<u>Abies lasiocarpa/</u> <u>Pachistima myrsinites</u> ^a	60
Utah	Gilbert Creek, Wasatch N.F., Lat. 40° 54' N.	2,500- 3,500	<u>Abies lasiocarpa/</u> <u>Pachistima myrsinites</u> ^a	45
Idaho	Little Slate Creek, Nez Perce N.F., Lat. 45° 40' N.	1,550	<u>Abies grandis/</u> <u>Xerophyllum tenax</u>	90
British Columbia	Cariboo Land District Lat. 53° 25' N.	700	Unknown, but probably <u>Abies lasiocarpa/</u> <u>Clintonia uniflora</u>	100

^aThese habitat types did not fit well into any of those proposed by Daubenmire. There was no Pachistima myrsinites present. However, Galium triflorum was present, indicating that they would fit better into the Abies lasiocarpa/Pachistima myrsinites than in the Abies lasiocarpa/Vaccinium scoparium habitat type.

Seed was collected from dominant or codominant trees located a minimum of 60 m. apart. In most cases all parent trees were taken from an area no greater than five to ten ha. in extent, with no appreciable change in elevation or severe aspect differences. In the Uintas stand, however, low frequency of serotinous trees required seed collection over a considerable altitudinal range.

Seed was collected from both current-year cones and cones up to four years old where both were available, however all tests were done with one- to four-year-old seed from closed-cone trees and current-year seed from open-cone trees. The exception was the British Columbia seed, the age of which was not known.

Serotinous cones were opened by immersion in a water bath at 60° C. (Lotan, 1972). Extracted seed was dewinged and stored at 1° C.

The term "family" is used through this paper to designate the progeny from one wind-pollinated parent tree.

Temperature and Photoperiod Tests

Seedlings were grown in a greenhouse under four daylengths in each of two temperature regimes.

In all tests seedlings were exposed to the same period of natural daylength, generally eight hours per day. During the balance of the 24-hour period each treatment was covered by a black plastic hood and its photoperiod extended to the desired length by two gro-lux fluorescent

bulbs. Each hood was fitted over the top of the fluorescent light fixture and draped down over its treatment bench in the manner of a tent. It was loose fitting enough around the base of the treatment bench to ensure adequate ventilation, and yet effectively excluded all external light. Temperatures under the hood varied little from those outside the hood, but humidity was maintained at a higher level.

Daylengths used were (World Almanac, 1969);

14 hours - the mid-June photoperiod at 30° N latitude, the southern limit of the species.

15-1/2 hours - the mid-June photoperiod at 45° N latitude.

16-1/2 hours - the mid-June photoperiod at 50° N latitude.

22 hours - the mid-June photoperiod at 65° N latitude, the northern limit of the species.

Daylengths were chosen to include a range of photoperiods common to the seed collection zone as well as a range similar to that experienced by the species as a whole. The mid-June figure was picked as a convenient one within the period of maximum growth activity.

Each set of daylength treatments was grown under one of two temperature regimes. Generally these temperature regimes have been classified as warm and cool. Precise temperature control in the greenhouse, however, was not always possible. Cooling in the greenhouse wing selected for the cool temperature treatment turned out to be less efficient than in the warm temperature wing, with the result that day-

time temperatures in June, July, and August often peaked higher on the cool side than on the warm side.

The duration of high temperatures during the day was always considerably less on the cool than on the warm side, however, and nighttime temperatures were consistently 5° to 10° lower. The pattern of temperature variation for the two treatments is briefly summarized in Tables 3 and 4.

Temperatures in the greenhouse, especially during the cooler months, tended to vary with changes in the outside temperatures, and thus changes from day to night were usually smooth and somewhat gradual rather than abrupt.

Seed was stratified by soaking in cold water for 24 hours, then storing moist in plastic bags at 1° C. for two weeks (Lotan, 1972). In each treatment, seed from each parent tree was planted in rows, each row containing approximately 15 seeds spaced 1.5 to 2 cm. apart. Each parent tree was replicated three times per treatment, making a total of 150 rows per treatment. These 150 rows were in turn arranged into four rows, so one entire treatment was contained in a space 1.2 x 1.5 meters. Assignment of replications to planting spaces was done by random selection of replication numbers from a hat.

Seedlings were grown in a mixture of 20% sand, 40% peat, and 40% vermiculite. In the first few weeks nutrients were applied by watering once a day with full strength Shive's solution (Shive and Robbins, 1938)

Table 3

Approximate Temperature Range per Treatment (°C)

Treatment	Dec. through May	June through August	August through Nov.
Warm	30° day - 20° night	30° day - 20° night	30° day - 20° night
Cool	20° day - 5° night	30° day - 12° night	20° day - 5° night

Table 4

Average Hours per Week in Which the Temperature Exceeded 27°C and 32°C and in Which the Temperature was Lower Than 15°C and 10°C

Treatment	Dec.- April		May- Aug.		Sept.- Nov.		Dec.- May		June- Aug.		Sept.- Nov.	
	Hours Above		Hours Above		Hours Above		Hours Below		Hours Below		Hours Below	
	27°	32°	27°	32°	27°	32°	15°	10°	15°	10°	15°	10°
Warm	44	16	41	11	12	0	1	0	10	0	19	1
Cool	1	0	27	11	4	1	46	18	49	5	99	47

diluted 4:1. This regime resulted in a salt buildup and increased pH and so was changed to an application of two quarts Shive's solution once per week to each treatment. Seedlings were watered with tap water whenever the soil began to dry, generally twice a day in the summer and once a day in winter, spring, and fall.

Seedlings in both the warm and cool treatment were planted in mid-January. In February cool-side seedlings began to show symptoms of what was thought to be a damping-off fungus, and within two weeks their condition was such that it was decided to dig them up and replant in fumigated soil. Thus cool-side and warm-side seedlings were seven weeks out of sequence with each other, and a soil difference between treatments was introduced. In order to evaluate the effect of fumigation on seedling performance, a small test was set up in which approximately 50 seedlings from the Washington, Montana, and Utah stands were grown in soil from the cool treatment, and the same number of seedlings from the same parent trees were grown in soil from the warm treatment. Temperature and daylength were the same for both sets of seedlings. Each soil type was replicated three times.

Before describing the phenological measurements which were made, it would perhaps be useful to describe the development of pine seedlings. Following germination, the first foliage to appear consists of a whorl of cotyledons. Development of the stem soon results in the appearance of juvenile leaves, which are spirally arranged, linear, and single

(i.e., not in groups defined by a single fascicle) (Harlow and Harrar, 1958). Juvenile leaves are followed by acicular, fascicled adult leaves which develop in the axils of the juvenile leaves (Harlow and Harrar, 1958). The timing of the appearance of adult leaves is not clear. Harlow and Harrar (1958) state that, depending on the species, it may be one to several seasons. Mirov (1967), on the other hand, says that adult needles may appear during the first season. Under certain conditions many pines undergo a period of dormancy in midsummer which is broken in late summer or early fall (Mirov, 1967), and observations of lodgepole pine (Morsby, 1972) indicate that this secondary flush of growth may result in the production of adult needles. In the present study appearance of adult needles in relation to terminal bud activity varied with temperature. In the warm treatment adult needles were never produced before a terminal bud was set, and their appearance in the axils of juvenile leaves invariably signaled resumption of terminal growth. In contrast, cool treatment seedlings produced adult needles well before appearance of a terminal bud.

Cessation of subapical meristem activity, and consequently shoot elongation, is usually marked in pines by the appearance of a terminal bud, which is an unextended portion of stem containing the apical meristem and primordial leaves and their internodes, and which is covered by primordial leaves (Romberger, 1963). Because it is an obvious and easily recognized feature the terminal bud is often taken as a sign of

dormancy in the plant, but this is not necessarily an accurate interpretation. Activity of the various plant meristems--apical, subapical, cambial and root--is very seldom simultaneous, and other regions of the plant may be actively growing, or at least producing primordia, at the same time that the terminal bud has signaled cessation of subapical meristematic activity (Romberger, 1963; Mirov, 1967). Nevertheless, the terminal bud does have value as an indicator of dormancy in at least one part of the plant. It is quickly and easily seen and provides a reliable common basis for comparing developmental activity between seedlings. For these reasons it has been used as a measure of comparative phenology in this study.

With these comments as background, it is now appropriate to describe the phenological measurements made in this study. At two-week intervals for a period of 18 weeks the number of seedlings with terminal buds and the number with adult needles were recorded. In both temperature regimes the appearance of a terminal bud was taken to indicate the beginning of one phase of dormancy, and in this paper the length of time from planting to appearance of the terminal bud is defined as the growing period. The growing period of a given family has been taken as the number of weeks from planting till 50% of its seedlings have terminal buds.

In the warm temperature treatment appearance of adult needles, either from the apical meristem or axillary buds, was taken to indicate

cessation of subapical meristem dormancy. The use of axillary bud break to indicate subapical meristem activity was considered valid because the author's observations consistently revealed that, in these seedlings, appearance of new needles from axillary buds very closely preceded terminal bud burst (they did not of necessity precede it, however). The number of weeks from budset to appearance of adult needles is defined here as the dormant period, and the dormant period of a family is taken as the number of weeks from 50% of seedlings with terminal buds to 50% of seedlings with adult needles. In some daylength treatments, especially 22 hours, trees tended to set and break buds in very rapid sequence, one tree producing as many as three to four terminal buds in the course of the experiment. Data given here, however, is concerned only with appearance of the initial terminal bud and its subsequent burst.

In the cool temperature treatment adult needles appeared before budset and therefore were not appropriate as a measure of cessation of dormancy. In fact, once buds were set in these temperatures, they showed no signs of bursting during the duration of the experiment, approximately three months after the time of bud set.

It should be stressed that the terms, "growing period" and "dormant period," as used here refer only to subapical meristem activity. The total physiological state of the plant cannot be safely extrapolated from just this information.

Seedlings were lifted from the beds by hand. Essentially the method was one of running the hands down underneath each row and, with gentle movements of the fingers, loosening the soil medium from around the roots until the seedlings could be freed without forcing. Roots were considerably intertwined and, despite all precautions, some of the fine roots were torn and lost. It is difficult to estimate the amount of root lost in this manner but, in terms of mass, it was probably quite small.

Before lifting, the height of each seedling from ground level to terminal bud tip or growing tip was recorded to the nearest millimeter. After lifting, roots were washed and seedlings dried for 24 hours at 100° C. to 105° C. Total and top mass for each row (one replication of the seedlings from one parent tree) were measured to the nearest .01 grams.

Moisture Stress Tests

Seed from four parent trees in each of the stands used in the daylength-temperature tests, as well as four parent trees from the low-elevation Washington stand (herein called Washington #2), were used in the moisture stress tests. Polyethylene glycol 6000 was mixed with distilled water and nutrients to give solutions with osmotic pressures of 5.2, 3.2, and .2 bars. Amounts of polyethylene glycol 6000 necessary to give 5 and 3 bars pressure were 215 grams per liter and 160 grams

