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GENETIC VARIATION IN COLD HARDINESS AND ITS EFFECTS ON THE PERFORMANCE OF PONDEROSA PINE (*PINUS PONDEROSA*) IN MICHIGAN

By

Donald H. DeHayes

A DISSERTATION

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ABSTRACT

GENETIC VARIATION IN COLD HARDINESS AND ITS EFFECT ON THE PERFORMANCE OF PONDEROSA PINE (*PINUS PONDEROSA*) IN MICHIGAN

By

Donald H. DeHayes

A rangewide provenance test of ponderosa pine was established in 1960 and includes two replicated plantations in southern Michigan. From 1975 to 1977 genetic variation in several aspects of cold hardiness and other economically important traits was studied.

Foliage samples were collected from 30 seedlots on 17 different dates from October, 1975 to January, 1977. Freezing tests were performed on needles and tissue damage was assessed using electrical conductivity. Critical temperatures, defined as the highest temperature at which cold damage could be detected, were determined for each seedlot. A procedure for computing such temperatures is described. On six additional dates percent foliar moisture content was determined. Field observations of winter injury were made during several years. Other traits measured were time of leafing out, foliar drying rate from excised twigs, height, and susceptibility to two fungal diseases.

Trees grown from seed collected in British Columbia, Washington, Montana, and Nebraska acclimated to cold fastest, had the lowest critical temperatures in midwinter (-40 to -47°C), suffered little or no cold or disease damage, leafed out earliest, had relatively low foliar

moisture and slow drying rates and were among the tallest trees at age 16 (averaged 20.5 ft tall). Trees from northern Colorado and Utah were similar in several respects but were only 14-15 ft tall at age 16. Trees grown from seed collected in California acclimated to cold slowest, had the highest critical temperatures in midwinter (-22 to -24°C), suffered severe cold and disease damage, leafed out latest, had high foliar moisture, and fast drying rates, and were among the shortest trees at age 16 (averaged 14 ft tall). Trees grown from seed collected in Arizona and New Mexico had critical temperatures of -32°C, were 16.5 ft tall at age 16, suffered severe disease damage, had relatively slow foliar drying rates, and were intermediate in cold damage, time of leafing out and foliar moisture content.

California trees suffered severe cold damage most years because they did not achieve a sufficient depth of hardiness to withstand Michigan winters. In contrast, Arizona, New Mexico, and coastal Oregon trees were sufficiently hardy in midwinter to avoid cold damage. However, they suffered needle injury in years when low temperatures occurred in early winter, before they attained maximum hardiness. Trees from northern origins avoided damage because they acclimated to cold quickly and became very hardy in midwinter.

Winter desiccation was considered as a possible source of winter injury in ponderosa pine. However, the relationship between foliar drying rates and winter injury was not strong. Trees from Washington and British Columbia lost water more rapidly in winter than trees from Arizona and New Mexico but suffered no cold damage. Coastal Oregon trees dried out the fastest, but suffered less cold damage than California or Arizona trees. Thus, it appeared that desiccation was less important than cold temperature in causing damage to ponderosa pine.

Time of leafing out appeared to be related to cold hardening and dehardening. Trees from Washington, British Columbia, Montana, and Colorado hardened to cold fastest in fall, dehardened most rapidly in late winter, and leafed out 10-14 days earlier than trees from southern origins. Despite differences in leafing out phenology, spring frost damage was not a problem in ponderosa pine.

Foliar moisture content varied seasonally as well as among seedlots, decreasing by 15-16% from summer to winter. In general, trees with high moisture content were most susceptible to cold damage. This relationship seemed to hold from summer to winter and also from north to south within the species. Apparently, trees with high foliar moisture content had more intracellular water and thus more water to freeze and cause damage.

Winter injury had an effect on growth rate and age-age height relationships in ponderosa pine. Trees grown from seed collected in California, Arizona, and New Mexico were tallest at early ages, but lost their growth superiority because of repeated winter injury. By age 16, hardy trees from British Columbia, Washington, Montana, and Nebraska were tallest, Arizona and New Mexico trees were average, and California trees were shortest. Repeated winter injury to southern trees, also resulted in an increase in the magnitude of height differences among ecotypes from age four to eight. Error variances decreased gradually with age as average growth rate increased, but were apparently unaffected by winter injury.

Susceptibility to damage from two fungal diseases appeared secondary to winter injury. Trees which were physiologically weakened by repeated cold damage suffered the most twig dieback from disease. Trees which suffered little or no cold damage were least affected by disease.

Other traits studied included mortality leaf length, cone production, stem taper, diameter growth, and incidence of stem forks. Trees grown from seed collected in the Northern Plateau (Oregon, Washington, and British Columbia) were most desirable in all respects. In addition to being the tallest and among the most cold hardy, they had the largest diameters (8.5 in at age 16), the least mortality, the longest needles (7.5 to 8.0 in long), the most cylindrical boles and suffered only a moderate amount of forking. Trees grown from seed collected in the Northern Plateau are recommended for forest and ornamental plantings in southern Michigan.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Ponderosa pine (Pinus ponderosa) is native to the western United States and is the largest ranging species of pine in North America. Its tall, straight trunk and light, easily worked wood make it one of the most important timber species in the world. Ponderosa pine is also highly regarded for its beauty. Its long clean boles, orange plated bark and light green foliage are admired by most western travelers.

Because of its rapid growth rate and adaptability, ponderosa pine is widely used as an exotic. In the Great Plains and eastern United States, it is commonly used in shelterbelt and ornamental plantings and is often considered as a potentially valuable timber or pulp species.

Cold temperature is one of the most important factors limiting the distribution of ponderosa pine. When it is planted as an exotic, winter injury often occurs. Such injury is a problem of major economic importance, often causing severe reductions in growth rate and unsightly damage.

Ponderosa pine is an extremely variable species genetically. As a result, one would expect to discover large differences in cold hardiness among its geographic races. However, in spite of its importance, little direct evidence has been compiled which evaluates intraspecific variation in ponderosa pine for factors which are related to cold hardiness. Such information is essential for the selection and breeding of new adapted races, and for obtaining the maximum potential of this species as an exotic.

The studies reported in this thesis were undertaken to determine genetic variation in several aspects of cold hardiness in ponderosa pine and to determine which aspects are most important in limiting the growth and development of the species in Michigan. Other goals were to study genetic variation in several other economically important traits and to determine the best seed sources for planting in Michigan.

CHAPTER 2

PHYSIOLOGY AND GENETICS OF COLD HARDINESS IN PLANTS: LITERATURE REVIEW

INTRODUCTION

The ability of plants to survive subfreezing temperatures is one of the most important features affecting the distribution, migration and utility of a species. Upon the onset of changing environmental conditions in the fall, many physiological and biochemical processes are initiated which impart a status of cold resistance to plants. However, the impact of cold resistance goes far beyond the physiological and biochemical implications. Freezing damage to our native vegetation and crop plants is an important problem, with major economic impacts. In order to enhance yields, researchers have worked toward achieving a better understanding of the physiological and genetic processes involved in cold hardiness. To date, the physiological aspects are much better understood but there has also been some breeding work. The ultimate advance in cold hardiness improvement will probably not be achieved until physiologists and geneticists combine their skills in an effort to determine the genetic basis for the physiological and metabolic events which result in cold resistance among many higher plants.

In the ensuing discussion, I describe the physiological processes which are believed to be involved in the induction of cold

hardiness in plants. I also speculate on the role of genetic regulation and the possible modes of inheritance of such physiological processes.

FREEZING AND DEATH IN PLANT TISSUES

Freezing in plant tissues is the conversion of liquids in cells and tissue to a solid state with an accompanying loss of heat. Two types of freezing are recognized in plants: solidification of cellular contents into a non crystalline state without an orderly molecular arrangement, and solidification that involves crystallization or an arrangement of liquid molecules into orderly structures (Alden and Hermann, 1971).

The first type of freezing is called vitrification and occurs as a result of extremely rapid freezing of plant tissues. This type of freezing rarely occurs in nature and is mainly of academic interest because cells can survive vitrification to very low temperatures. If freezing is rapid enough to produce submicroscopic ice crystals, no injury occurs in the plant cells (Levitt, 1956).

Most freezing damage that occurs in plants is due to the second type of freezing. In nature the air temperature rarely decreases more than a few degrees per hour. At such low rates of freezing, ice forms first outside cells where water is purest. Hardy woody plants can survive extra-cellular freezing of this type. However, if ice crystals form within the protoplasm, such intracellular freezing usually results in cell death.

The method by which freezing injury occurs has been debated in the literature. Weiser (1970) suggested that during freezing a point is reached when all readily available water has been frozen

extracellularly and only "vital" water remains in the protoplasm. With continued lowering of temperature vital water is pulled away from protoplasmic constituents to the extracellular ice. This sets off a chain reaction of denaturation, additional vital water release, and ultimately death. This proposal allows for an explanation of death based on either mechanical damage, protein denaturation, or dehydration of the cell.

Mechanical damage may occur when intercellular spaces are too small to accommodate ice formation and cells are ruptured by splitting of their walls along the middle lamella. Injury from intracellular ice formation results from mechanical disorganization of the protoplasm by ice crystals (Levitt, 1956). Protein denaturation occurs when cellular water is removed and salts become concentrated in the cells to such an extent that proteins are irreversibly damaged. Severe dehydration shrinks the entire cell, including the cell wall, and causes loose tissue. Often the dehydrated cytoplasm contracts around the nucleus and plasmatic strands break. Survival of cells probably depends on the tenacity of water binding to protoplasmic constituents, or the extent to which bound water is replaced by protective molecules (Heber, 1968).

Levitt (1962) proposed a theory which explains cold damage on the basis of protein denaturation resulting from the formation of disulfide bonds between adjacent sulfhydryl groups of proteins. Since the suggestion of this theory in 1962, considerable evidence has been presented in support of Levitt's idea. He thought that high water stress has an effect upon the sulfhydryl groups of protein. As the layer of water around the protein molecule becomes thinner,

the sulfhydryl groups begin to contact each other in adjacent proteins. Upon oxidation the hydrogen is removed and disulfide linkages are formed. After rehydration, the disulfide linkages hold proteins together in such a way that the developing water layer results in distortions of the protein molecules. Assuming his hypothesis is valid, resistance to cold damage is related to an inhibition of the intermolecular disulfide bonds which form between proteins and ultimately distort them. Heber (1968) found that in cold resistant herbaceous plants sugar replaces water in forming a protective shell around the protein molecules, and thus inhibits formation of disulfide linkages.

THE ROLE OF LIGHT

Light is generally believed to affect the development of cold hardiness through a photoperiodic response. Early theories suggested that photoperiod affected cold resistance of woody plants by inducing dormancy. Tumanov et al (1964) found that black locust (Robinia psuedoacacia) and white birch (Betula verrucosa), which did not enter dormancy because of continuous illumination, failed to develop complete tolerance to cold at hardening temperatures. They therefore concluded that cold hardiness could be induced only after the dormant state had been reached.

More recent studies have shown that dormancy merely accompanies the onset of cold acclimation and that both processes are independently triggered by a photoperiodic mechanism. Zehnder and Lanphear (1966) showed that foliage of Taxus cuspidata developed more hardiness under an 8-hour than under a 16-hour photoperiod. Removal or covering of

the leaves interfered with the development of hardiness, thus suggesting that the leaves are the receptor of the light stimulus which initiates hardening. Williams et al (1972) suggested that reduced photoperiods induce rest and a simultaneous increase in hardiness through a phytochrome mediated response. They demonstrated that dark period interruptions with red radiation suppressed cold acclimation in Cornus and Weigela. When red light was followed by far red light, suppression was relieved. Thus they hypothesized that short days (long nights) allow an accumulation of the Pr form which is subsequently involved in implementing cold hardiness. Irving and Lanphear (1967a, 1967b) found that Acer negundo, Viburnum plicatum tomentosum, and Weigela florida, which remain in a non-growing condition without bud dormancy under a 6 hour photoperiod, developed the same degree of hardiness as dormant plants under an 8 hour photoperiod after hardening for the same period of time. In addition, resistance to frost by Viburnum plants placed under supplemental light to prevent dormancy, equaled the resistance of plants under natural daylengths. These authors therefore concluded that dormancy is not required for hardening and that hardiness was induced by a photoperiodic response similar to dormancy and flower induction. Increasing weeks of short days, followed by a low temperature hardening period, brought about a progressive increase in hardiness. Additional evidence was provided by Young (1961) on grapefruit. Photoperiod was shown to have no effect on bud dormancy in this plant, but plants under short days developed a greater resistance to cold than plants under long days.

A combination photoperiodic and photosynthetic role in cold hardiness was suggested by Steponkus and Lanphear (1968) in their work on the light requirement of Hedera helix during cold acclimation. They found the following effects: 1. Short day light increased development of cold tolerance during the first 6 weeks of hardening, 2. After this time, various photoperiods from 8 to 24 hours had no effect on tolerance, 3. Low intensities of light favored hardening, 4. Reduced CO_2 and darkness during hardening caused a loss of tolerance, 5. Increased photosynthetic area exposed to light increased stem hardiness; and 6. A high concentration of a photosynthesis inhibitor (simetone) decreased hardiness of leaves and had no effect on the stems. They believed that effects 1, 4 and 5 show that the role of light in acclimation is photosynthetic. They could explain the discrepancies noted in 2 and 3 only if a small portion of the photosynthate produced was required for cold acclimation. A photoperiodic response similar to phytochrome induced flowering and dormancy was indicated in effects 2, 3 and 5. The authors concluded that the phytochrome system is at work and it influences carbohydrate metabolism, protein synthesis, and oxidative phosphorylation in promoting the development of cold hardiness.

ACCLIMATION

Woody plants characteristically undergo a series of changes in the fall which enable them to cope with the oncoming freezing stresses of winter. The process by which plants carry out these changes is called acclimation. The acclimation process in plants is generally believed to be carried out in 2 or sometimes 3 phases.

The first stage of acclimation in plants is induced by the onset of short days and is perceived by the leaves (Howell and Weiser, 1970a; Fuchigami et al, 1971; Irving and Lanphear, 1967a, 1967b). Short days in the autumn are detected by a phytochrome clock in the leaves and the Pr form accumulates. Decreasing photoperiods cause the cessation of growth and thereby allow for storage of photosynthates which are the energy producing substrates necessary for acclimation (Fuchigami et al, 1971). Temperature also plays a role, low temperatures often inhibiting the first stage. Once this phase has been induced, very little hardiness is actually attained in the plant. Apparently, the primary role of the first stage is to cease growth and to initiate the metabolic changes which facilitate the plants response to low temperature during the second stage of acclimation.

The means by which the first stage metabolic changes begin is not fully known. However, it has been shown (Fuchigami et al, 1971; Howell and Weiser, 1970a) that upon reception of the short day stimulus, the short day leaf produces a translocatable substance which promotes acclimation. Through a series of modified plant studies, it has been shown that the hardiness promoting factor is only synthesized in short day leaves. Long day leaves produce a substance(s) which inhibits the induction of acclimation. Therefore, under long days and natural fall temperatures, hardiness can be attained only after removal of the long day leaves (Irving and Lanphear, 1967b). Through the use of grafting studies, it has been shown that both inhibitors and promoters of hardiness exist.

The second stage of acclimation is induced by low temperatures. In fact, frost often appears to be the triggering stimulus (Howell

and Weiser, 1970a). It has been further noted that the frost induced phase of acclimation does not involve translocatable factors. Because of this, many authors have suggested that the second stage of acclimation is a physical rather than a metabolic process. However, this is not likely because a number of enzyme mediated reactions are known to be induced by low temperatures (Weiser, 1970).

Weiser (1970) has suggested that the second stage of acclimation may involve a reorientation of macromolecules into stable forms which can withstand severe dehydration. Proteins and hydrophobic residues are known to be temperature sensitive. In the polymerized state, many proteins are biologically active but stress sensitive while depolymerized molecules are inactive but stress resistant. Weiser proposed that there may be a temperature reversible regulation of hydration which results in the conversion of proteins from a polymerized to a depolymerized configuration at low temperature. The ultimate result of such a reaction is the high degree of cold hardiness which is attained during the second stage of acclimation. During the latter stages of this phase hardy plants become very cold resistant, tissue hydration decreases and metabolic activity is slight. It is possible that many of the macromolecular and cellular structures associated with active growth and metabolism are disassembled and in a resting state for the winter.

Tumanov and Krasavtsev (1959) observed that there often is a third stage of acclimation in hardy woody plants which is induced by ultralow temperatures in the range of -50^{o} C. Prolonged exposure of prehardened tissue to temperatures in this range can drive the hardiness to levels which are not ordinarily experienced in nature. This kind of hardiness is quickly lost. They noted that if hardy stems

were thawed for as little as 6 hours their hardiness levels would decrease from -195° to -45°C.

Tumanov and Krasavtsev also suggested that the third phase of acclimation is a physical process associated with reduced intermolecular distances and thermal motion of molecules in frozen cells. Weiser (1970), however, thought this phase of acclimation is related to the amount and degree of orientation of quasi-crystalline water in the cell. He postulated that prolonged low temperatures create a high degree of order among water molecules. This, in turn, increases the tenacity of binding which increases the proportion of bound water and thus increases dehydration resistance or reduces the quantity of available water for destructive crystallization. Upon warming, thermal activity increases and thawed extracellular water quickly reinvades the cell causing rapid decreases in hardiness levels. Burke et al (1974) studied the nuclear magnetic resonance of water in acclimating Cornus stolonifera and provided evidence which supports Weiser's theory. They found that the ability of dogwood to survive low temperature depends primarily on its ability to tolerate diminished quantities of water and high protoplasmic concentrations. They also showed that the tenacity of hydrophobic water binding increased at low temperatures which resulted in increased resistance to dehydration in cells.

The hardiness promoter

The nature of the hardiness promoter which plays a role in the first stage of acclimation is still a mystery. It is known that the hardiness promoting factor is synthesized in leaves exposed to short days. It is also known that the factor is not species specific, i.e.

the hardiness promoting factor from leaves of a hardy species (or genotype) can enhance acclimation of a less hardy species (or genotype) when the two are grafted. After reviewing a majority of the work relating to this area, Weiser (1970) has concluded that the promoting substance is either a inhibitor, a sugar or a regulatory substance.

Growth inhibitor. It is possible that the hardiness promoter may play an indirect role in acclimation by merely stopping growth, an event which is necessary for the onset of acclimation in woody plants. Irving (1969) showed that an inhibitor extracted from short day leaves of Acer negundo was similar to abscisic acid in chromotographic properties. Subsequent treatment of Acer negundo with the extracted inhibitor or abscisic acid increased hardiness. He, therefore, concluded that the hardening process appears to be closely correlated to a build up of abscisic acid levels which are stimulated by short days. It is possible that abscisic acid plays a role in acclimation by causing growth cessation, however, that does not account for the wide spectrum of metabolic changes which occur during acclimation. It is more likely that the hardiness promoter plays an active regulatory role which does not begin until growth ceases.

Sugar. A considerable amount of evidence points to the possibility that sugar may be the translocatable factor. Fuchigami et al (1971) has shown that plants cannot acclimate when they are depleted of photosynthetic substrate. Heber (1968) has shown that sugar protects the enzyme system associated with oxidative phosphorylation in spinach chloroplasts that are subjected to freezing. He believes that the protective influence of sugars may be due to their ability

to retain or substitute water of hydration of proteins via hydrogen binding. Steponkus (1971) showed that Hedera helix protein from cold acclimated tissue exhibited a higher sugar binding capacity than protein from nonacclimated tissue. Furthermore, he also noted a dramatic increase in labelled sucrose in protein from acclimated tissues. Additional evidence was offered by Levitt (1959) that an artificial increase in sugar content increased the cold hardiness in cabbage leaves. He noted that in leaves infiltrated with fructose osmotic potential was higher and the frost killing point was significantly lower than in control leaves. He concluded, however, that an increase in sugars could account for only part of the natural increase in hardiness and that there must be additional factors involved. Many authors have found seasonal increases in sugar content which were highly correlated with increases in cold resistance. Parker (1959) found slight increases in sucrose, glucose and fructose in 6 coniferous species during winter. He also found drastic increases in raffinose and stachyose in the 6 species, which were directly correlated with winter hardiness.

In spite of the collective weight of evidence, no experimental data has been able to show increased hardiness in woody plants as a result of artificial increases in sugar. There is little doubt that some basic level of sugar is necessary for acclimation, but whether or not sugar is the translocatable hardiness promoter has still not been clearly established.

Regulatory hormone. Although there is no experimental evidence, Weiser believes that the hardiness promoter may be a hormone or complex of hormones which play a specific role in regulating acclimation. If

this is the case, then it is probable that ultimate resistance is the product of several distinct processes.

PHYSIOLOGICAL AND METABOLIC CHANGES DURING HARDENING

Water relations

Hypotheses to explain plant survival at low temperatures assume that intracellular ice formation is avoided in hardy tissues. In tender tissues, an increase in the probability of intracellular ice occurs during frost due to a large amount of water within cells. McKenzie et al (1974a) have shown that water content of stems decreases considerably during the initial stage of acclimation. This results as a function of decreased stomatal resistance and increased root resistance to the uptake of water. The initial high rate of leaf transpiration reflects the increase in metabolism associated with the initial short day induced stage of acclimation. McKenzie et al (1974b) also showed that during the first stage of acclimation water permeability of the phloem and cortical parenchyma cells increased significantly, and resulted in an increase in hardiness in Cornus stolonifera.

Relationships between resistance to water flow in plants and hormones are well known. McKenzie et al (1974b) imply that the ratio of cytokinins and ABA may direct this adaptive response of the plant involving water flow. They also suggest that the translocatable hardiness promoter may function primarily by controlling the hydration of overwintering tissue.

Chloroplasts

During hardening two opposing types of changes have been observed in the chloroplasts: in some cases the chloroplasts retain their

integrity, but migrate from a summer position near the cell wall to a crowded position in the cell interior, with some loss in chlorophyll content; in other cases, the chloroplasts have been shown to agglutinate, lose their integrity and merge with each other to become a continuous mass from which chloroplasts reform again as spring approaches (Parker, 1963).

Nucleic acids

Li and Weiser (1968) have shown that nucleic acids in apple twigs begin to show an increase in concentration one week prior to a rapid increase in cold hardiness. Soluble RNA increased 38% in one week, light and heavy ribosomal RNA increased 41% in two weeks just prior to and during the stage of rapid cold acclimation. Following this period there was a slight decrease in these three nucleic acid fractions. DNA concentrations followed the same pattern although the total concentration was much lower. These results suggested that short days and/or low temperatures in the autumn triggers the production of an endogenous growth regulator which induces rapid synthesis of soluble RNA. The soluble RNA participates directly in protein synthesis and also probably functions in the regulation of all RNA and protein synthesis through a repressor-inducer type of system. Such a system may in fact be working because the timing of RNA increases suggest that it may be one of the initial steps in the hardening process.

Further information on the role of nucleic acids in cold hardiness was explained by Kessler and Frank-Tishel (1962) They discovered that dehydration increased the ratio of guanine and

cytosine to adenine and uracil in the RNA of olive leaves. Guanine and cytosine paired to form an additional hydrogen bond between the double helix of the nucleic acids, which was hypothesized to impart greater stability to RNA molecules. They concluded that a high guanine and cytosine content in RNA is related to cold resistance in plants and the ability to synthesize RNA that is rich in guanine and cytosine is a basic part of the cold hardening process in plants.

Proteins

Siminovitch and Briggs (1953) reported that the concentrations of water soluble protein increased in the bark of black locust in the fall along with the development of cold hardiness. In the spring, this concentration declined abruptly with the disappearance of hardiness. Pomeroy et al (1970) found a similar trend in the bark and needles of red pine (Pinus resinosa). Furthermore, Parker (1963) reported a fall increase in water soluble protein in some species. These works ultimately led to the conclusion that the increase in soluble protein may play an important role in the development of frost hardiness.

There are two schools of thought which attempt to explain the paralleling increase in water soluble proteins and development of hardiness. Many studies indicate that the increase in protein results from a breakdown of part of the more complex proteins and not from synthesis of new amino acids and proteins. This view is supported by Heber (1968) who suggested that protein increases associated with the development of tolerance to cold may result from changes in extractability of proteins influenced by seasonal changes in pH.

Others believe that the increase in water soluble proteins

results from protein synthesis. Li et al (1965) found an increase in total protein and a decrease in amino acids upon hardening of Cornus stolonifera. The decline in amino acids probably indicates they were used in synthesizing water soluble proteins.

Siminovitch (1963) showed that an increase in RNA without an increase in DNA occurred preceding the incorporation of labelled glycine into protein, net protein synthesis and cold resistance in the parenchyma cells of black locust bark. The incorporation of labelled glycine into water soluble protein verifies that true protein synthesis is involved. The increase in amino acids and nucleotides necessary for protein synthesis probably occurs as a result of degradation, hydrolysis, and movement of proteins and nitrogen from the leaves into the trunk during autumn. The increased RNA is believed to synthesize specific water soluble proteins that promote frost resistance or transform the protein synthetic capabilities to increase the photoplasmic substances in the bark cells. The changed physical properties of the protoplasm enable it to resist stress from dehydration caused by intracellular freezing.

Amino acids

Several workers have analyzed the amino acid content of water soluble proteins during hardening and dehardening. Pauli and Zech (1964) found that alanine, arginine, asparagine, glutamic acid and histidin contents of water soluble proteins from winter wheat increased to a maximum during hardening and declined during dehardening. These amino acids probably provide proteins with reactive side chains that can disrupt an ice lattice of water molecules formed around nonpolar side chains in the protein. Proteins with polar amino acid side chains increased in hardened twig sections of Cornus stolonifera during hardening

and are believed to preserve water and prevent denaturation of protein when the protoplasm is dehydrated by extracellular formation of ice (Van Huystee et al, 1965).

Li et al, (1965) summarized the cold acclimation changes in 15 amino acids of Cornus stolonifera twigs by grouping them into three general categories. Group 1 decreased during cold acclimation and included the 3 primary amino acids, aspartate, glutamate, and alanine. Group 2 amino acids increased in concentration during cold acclimation and included glutamine, -aminobutyrate, phenylalanine, leucine and isoleucine. Group 3 fluctuated in concentration during acclimation and included serine, threonine, asparagine, B-alanine and cystine.

Enzyme activity

The changes in amino acids, proteins and other organic compounds as plants acclimate to cold must be brought about by changes in enzymes and enzyme activity. Thus, most research in this area has focused on the effect of low temperature on enzyme synthesis and activity.

The role of enzymes in inducing cold hardiness and the enzymes involved are not as yet known. Van Huystee et al (1965) have suggested that hormonal action could influence the enzyme systems responsible for the metabolic changes in cold acclimation. They believed this because changes in hardiness are closely associated with the hormonally regulated cessation of growth in autumn. Roberts (1969), on the other hand, has proposed that the deleterious effects of low temperature may be offset by the substitution of one isozymic form of a protein for a different form or by changing the relative proportions of the isozymes present.

Two enzymes which have yielded results consistent with Roberts' hypothesis are invertase and peroxidase. It has been conclusively demonstrated that invertase is not irreversibly inactivated by killing frosts. This enzyme, which hydrolyzes sucrose to glucose and fructose, has been shown to increase at low temperatures until it exceeds the concentration of some competitive inhibitor and then results in an increased hydrolysis of sucrose and accumulation of reducing sugars. Roberts (1969) found a lower temperature coefficient for invertase extracted from leaves of cold hardened than from leaves of unhardened wheat. He suggested that structurally different forms of the enzyme are synthesized at lower temperatures. He concluded that invertase consists of a number of isozymes with different temperature coefficients and that growth at low temperatures increases the proportion of invertase isozymes with low temperature coefficients.

Evidence for changes in isozymic components have also been reported for peroxidase. Winter clones of Dianthus showed a gradual synthesis of 2 to 4 new peroxidase isozymes during the cold hardening period (McGown et al, 1969). Roberts also reported that more peroxidase isozymes were found in leaves of wheat plants grown at 6°C than in leaves grown at 20°C. McGown et al (1969) concluded that changes in peroxidase isozymes during hardening may regulate permeability and prevent injury at subfreezing temperatures.

Carbohydrates

The fact the reduction of carbohydrate reserves reduces cold hardiness in wintering plants is well known. Starch decreases to a minimum and sugar increases to a maximum as plants become acclimated

to cold. Many authors have reported starch to sugar changes in bark tissue and evergreen leaves of various trees that have developed cold hardiness (evidence cited in Parker, 1963). Although starch to sugar changes are fairly well understood, the conversion of sugars to other sugar complexes and the role of sugars in frost hardiness has not been fully worked out.

Although no attempt will be made here to review the myriad of hypotheses on sugar involvement in cold hardiness, one particular study is worth mentioning. Olien (1967) studied the interference of freezing caused by large water soluble polysaccharide polymers extracted from the cell walls of hardened wheat plants. These substances, which contained large amounts of sugars (xylose and arabinose), interfered with freezing by competing with water molecules for sites in the ice lattice at the liquid interface. The result was that they tended to stop crystal growth, causing an imperfect ice mass to form. Only polymers from cold hardy plants resulted in imperfect crystal formation in the plant.

GENETIC REGULATION OF COLD ACCLIMATION

After reviewing the physiological changes associated with the achievement of cold resistance in plants, one must wonder how plants are able to regulate their metabolism to attain such a state. Because of the large array of metabolic changes involved, it seems likely that the regulatory processes function at the level of nucleic acid transcription. Weiser (1968) has proposed a hypothesis and associated model (1970) which attempts to explain the genetic regulation and associated metabolic changes which occur during cold hardening in plants. He has proposed that short days in autumn are detected by a phytochrome clock

in the leaves and the Pr form accumulates. The Pr phytochrome promotes synthesis of a translocatable hardiness promoter which activates DNA that is normally non-functional during the active growing season. New kinds of mRNA and subsequently structural or enzymatic proteins are produced which bring about the first stage of cold acclimation. The first stage physiologically and metabolically prepares the plant so it is able to respond to low temperature stimuli which trigger the second, temperature activated stage of hardening. During this stage, low temperature induces nontranslocatable but readily reversible alterations in the cells which includes the binding of water to proteins and the resistance of the protoplasm to dehydration.

The model by Weiser offers an excellent summary of the metabolic changes which occur during acclimation. However, his treatment of the genetic regulation involved in cold acclimation seems incomplete in light of the model proposed by Britten and Davidson (1969). They have proposed that four types of genes and activator RNA molecules act in sequence to transfer the message of an environmental stimuli to the resulting metabolic changes known to occur in plants. I have attempted to employ the Britten and Davidson model of gene regulation to explain the sequence of events which occurs from the onset of short days in the autumn to the activation of metabolic changes which result in cold hardiness. These events are outlined in Figure 1. The hypotheses proposed to explain cold hardiness fit the Britten and Davidson model of gene regulation in almost every aspect. However, in stage G a slight discrepancy is noted. Britten and Davidson have proposed that the activator RNA molecules act

directly on the receptor genes; I have suggested that these molecules must code for an enzyme or protein which is involved in the production of a hardiness promoter which in turn functions by activating the receptor genes. Britten and Davidson mention, however, that the role proposed for activator RNA could well be carried out indirectly by protein molecules coded for by these RNA molecules. Thus, I feel the basic structure of the model has remained intact.

FIGURE 1. A hypothetical model of genetic regulation of cold acclimation in plants.

GENETIC REGULATION IN COLD ACCLIMATION

A) Short days act as the external stimuli (also spectral changes in sunlight).

B) The sensor gene cannot directly detect an environmental stimuli. The stimuli must be translated through a chemical form (phytochrome)

C) Short days are detected by a phytochrome clock in the leaves and the P_R form accumulates.

D) The phytochrome turns on a sensor gene, which receives the signal from the environment.

E) The sensor gene activates the set of integrator genes.

F) The integrator genes function in the synthesis of activator RNA.

G) The activator RNA must code for an enzyme or protein which is the hardiness promoter or is involved in producing the hardiness promoter. The Britten-Davidson model allows for such a reaction.

H) The hardiness promoter is translocated throughout the plant.

I) The promoter activates the receptor gene(s).

J) The receptor gene(s) causes the transcription of the producer gene(s) to occur.

K) The producer gene(s) yields new kinds of messenger RNA.

L) The new mRNA codes for structural and/or enzymatic proteins which bring about the first stage of acclimation.

M) The first stage of acclimation predisposes the plant to respond to low temperature stimuli which triggers the second stage of hardening.

GENETIC VARIATION

In species with wide geographic ranges the existence of well defined groups of plants with considerable genetic variability among the groups is quite likely. Such variation due to seed source usually parallels geographic and climatic differences of the physical habitat and is often characterized by extreme forms at the periphery of the range with a clinal intergrading of forms between the extremes. The common method of studying such species variability is the uniform environment plot. This technique has been successfully employed to describe geographic variation in four types of freezing injury that can occur in woody plants. Evert (1967) has classified these types of freezing damage as injury that can occur: 1. in fall before the onset of acclimation; 2. at winter temperatures too low for the maintenance of cellular structure and function; 3. from winter burn or physiological drought; and 4. from spring frosts.

For the purposes of this discussion only the first two types of injury are important. The others are related to physiological processes which are only indirectly associated with the ability or inability of the plant to become acclimated to cold temperatures. In the case of the damage from winter burn, the important determining factors are temperature and moisture conditions in the environment, as well as moisture content and transpiration rates in the plants. Even plants acclimated to cold can suffer this type of damage given specific environmental conditions. Damage from spring frosts is associated primarily with early bud break in the spring before the danger of frost has passed. It is closely associated with the response of specific genotypes to local environmental conditions.

Probably the most common type of cold injury in woody plants occurs as a result of genetic differences in timing of acclimation among climatic races of a species. Such variation has been demonstrated in many woody perennials (Flint, 1972), especially Cornus stolonifera. Smithberg and Weiser (1968) collected cuttings of 25 races of this species from widespread locations in North America and grew them in a uniform environment plot in St. Paul, Minnesota. The results of cold hardening tests showed that all clones became equally hardy (-196°C) by midwinter; however, differences in timing of acclimation were profound. Northern clones acclimated earlier than those of southern or coastal origins and thus escaped winter injury. Clones from Seattle were the last to harden and as a result sustained extensive winter injury. As would be expected, the dates at which clones acclimated to specific temperatures were closely correlated to winter minimum temperature and length of growing season at place of origin. Thus, it was concluded that winter injury occurred because some clones failed to acclimate on time even though all clones ultimately acclimated to levels of cold far beyond any they would encounter in nature.

The second type of cold injury mentioned results from genetic differences in ability of plants to withstand low winter temperatures. Winter damage of this type has been demonstrated for many herbaceous (Kinbacher, 1962; Marshall, 1969; Suneson and Marshall, 1967), as well as woody perennials. A study of genetic variation in this type of winter injury was performed on eastern white pine (Pinus strobus) by Maronek and Flint (1974). They compared timing of acclimation and midwinter killing temperatures of 15 seed sources of eastern white pine growing in western Michigan. They found that northern sources

hardened much earlier than southern sources and also achieved greater midwinter hardiness levels. Northern sources could withstand January temperatures of -90°C while southern sources were damaged at temperatures below -30°C. Variation in killing temperature was clinal and consistently significant between the northern and southern extremes of the sampled range. Although all seed sources achieved sufficient hardiness to withstand normal Michigan winter temperatures, genetic variation did exist in the ability of different genotypes to withstand low temperatures.

Numerous studies have been performed on woody plants which illustrate patterns of genetic variation in visible winter injury. No attempt has been made here to review these studies.

INHERITANCE OF COLD RESISTANCE IN PLANTS

Upon establishing that genetic variation exists in cold hardiness, the next step is to examine the inheritance of the trait. It seems, however, that most researchers have not taken this final step, probably because of early indications of complicated inheritance of this character.

Dantuma (1958), in breeding wheat and barley for winter hardiness, discovered that the crosses made between winter X spring varieties and winter X winter varieties resulted in progeny which did not achieve the winter hardiness of the most resistant parent. In addition, analysis of 139 F_2 progeny from winter wheat crosses showed intermediate inheritance with possible partial dominance. They also noted that the diversity of forms with regard to winter hardiness and the complex of external factors which influence winter hardiness, suggest

that it is a complexly inherited trait. Eunus et al (1962) analyzed the data on the inheritance of winter barley. They observed that no two varieties behaved similarly in the crosses and concluded that winter hardiness was controlled in each variety by a different combination of genes, both additive and non-additive. Furthermore, they found that winter hardiness in barley is controlled by genes ranging from complete dominance to complete recessiveness. Thus, results from the diallel experiment indicate that many genes are involved in winter hardiness inheritance and that it is a complex character. Evidently, genotype X environment interaction is also considerable in such experiments and acts to confuse the issue further.

Reid (1965) analyzed 13 winter X spring barley hybrids for winter survival in the F_2, F_3, and F_4 generations. None of the thirteen crosses were as hardy, on the average, as the hardiest parent but many individual F_4 lines were within the parental survival range. Thus, as one would expect, segregation among F_4 progeny had occurred and resulted in a range of intermediate types between the parents. This study also showed that different winter and spring varieties varied in their ability to produce hardy progeny. Two winter varieties (Kearney and Dicktoo) and 2 spring varieties (Minsturdi and C15890) contributed more hardiness to their progeny than other varieties. Such variation suggests that the genes controlling winter hardiness in barley differ for different varieties. The overall conclusion drawn from the aforementioned studies is that cold hardiness is quantitatively inherited, that is, it is controlled by many genes with small effects.

Evidence for an additive mode of inheritance

Daday and Greenham (1960 and 1964) studied the inheritance of cold resistance among 4 varieties of alfalfa (Medicago sativa). Their investigations demonstrated clear differences within and between varieties in hardiness to cold. From a study of diallel crosses among the 4 varieties, it was found that the average performance (general combining ability) of the parents in crosses were significantly different and that the F_1's were generally intermediate in hardiness between the parents. Studies of specific combining ability yielded non-significant results. The significant differences in general combining ability suggest that this character is controlled by genes with additive effects, i.e. one would expect values of the F_1 generation to be approximately equal to the mean of their parents. This lack of significant differences in specific combining ability indicates that dominance or non-additive genetic effects are less important.

Additional evidence suggesting additive gene effects in the inheritance of cold hardiness was provided by Omran et al (1968) in flax (Linum). Two-hundred F_2 families from each of 2 crosses were studied in the F_3 and F_4 generations for reactions to cold temperatures. A cold hardiness index was calculated from the degree of injury and the percentage of plants injured. Dominance deviations could not be detected in the F_3 or F_4 generations. Narrow sense heritability estimates of cold hardiness indices were generally high. Narrow sense heritabilities are an estimate of the contribution of additive genetic variance to the total variation. Thus, the high heritabilities combined with the fact that dominance deviations could not be detected, indicates that inheritance of

cold hardiness in flax is primarily under the control of many genes with additive effects.

Genetics of cold resistance in wheat

Probably the most comprehensive genetic study of cold resistance was performed by Law and Jenkins (1970) on hexaploid wheat (Triticum aestivum). Through the use of backcross procedures and cytological markers, single homologous pairs of chromosomes from a hardy wheat variety (Cappelle-Desprez) were substituted for their homologues in a cold sensitive wheat variety (Chinese Spring). As a result, it was possible to produce substitution lines for each of the 21 pairs of wheat chromosomes. Moreover, each of these substitution lines differed from the recipient variety (Chinese spring) by a single pair of homologous chromosomes. Subsequently, chromosome assay experiments were carried out to determine which chromosomes carried genes involved in the determination of cold resistance.

The results of the experiment indicated that significant differences occurred between Chinese spring and the substitution lines carrying chromosomes 4D, 5D and 7A of Cappelle-Desprez. These three lines were also significantly different from Cappelle-Desprez which suggests that the levels of resistance expressed by these substitution lines was intermediate. In a duplicate experiment established at a later date the same results were discovered. This substantiated that the differences noted in the first experiment were attributable to the substituted chromosomes and not to segregation of genes for cold resistance in the background.

The data acquired from the first two experiments was subsequently

used to determine whether genes on chromosomes 4D, 5D and 7A behave in an additive fashion or are independent in their action. This was determined by summing the differences between the Chinese spring mean (which includes the mean of the 18 substitution lines and Chinese spring) and the 3 substitution lines carrying chromosomes for cold resistance and comparing this with the difference between Cappelle-Desprez and Chinese Spring. In the absence of between chromosome interactions these differences should be the same. In both experiments the two differences were similar and when tested against their standard error were not significantly different from each other. This evidence is consistent with an additive behavior of the genes on the three substituted chromosomes.

These experiments using intervarietal chromosome substitutions suggest, on first appearance, that the genetic control of cold hardiness in wheat is not overly complex. However, when one considers that in hexaploid wheats triplicated loci are probably involved, the situation becomes more complex. On the simplest hypothesis of one loci for each of the three isolated chromosomes, six other homologous loci could occur. It is therefore likely that in more cold resistant varieties some allelic variation at these loci may occur.

Possible maternal inheritance of cold resistance

The conductivity of electrolytes diffusing out of frozen and thawed tissue of 3 apple varieties hardened under identical conditions indicated that they differed significantly in susceptibility to cold injury. Northern Spy (NS) was a tender variety, McIntosh was intermediate in hardiness and Antonovka (A) was the hardiest

of the three varieties. In an effort to study the inheritance of cold hardiness among apple varieties, Wilner (1965) made all possible crosses among the three varieties including the reciprocals. The F_1 progeny were subjected to a range of freezing temperatures and their relative cold hardiness was ascertained by use of the electrolytic conductivity method. The progenies of the crosses involving the hardier varieties A and Mc generally were more hardy.

Further study of the data showed that progeny of crosses involving hardy female varieties were consistently hardier than the reciprocal crosses. This was verified by 19 of the 21 comparisons made.

Differences in reciprocal crosses have been observed in a number of traits in several plant species, and they usually indicate the occurrence of an extranuclear or cytoplasmic type of inheritance. Several important biological properties have been shown to be inherited through the cytoplasm. The results of Wilner's work offers evidence which suggests that cold hardiness in apples may be cytoplasmic in inheritance and transmitted mainly when brought from the maternal side. The more likely explanation is probably that both nuclear and extranuclear inheritance systems are required for complete genetic expression of an individual.

Genetics of hardiness in fruit trees

Studies elucidating the genetic control of cold hardiness in woody plants are not numerous. However, reports characterizing hardiness differences among cultivars are available and are discussed by Stushnoff (1972). Dorsey and Bushnell (1925) studied the inheritance of cold hardiness in several interspecific hybrid progenies of plums. They

found that the hardiest progenies resulted from crosses in which one or both parents were selected from areas of extreme cold in their natural habitat.

Watkins and Spangelo (1970) determined the genetic variance components for winter survival and injury in apple trees using two diallels. Tip injury and stem damage were estimated to exhibit 90 to 100% additive variance and root damage was estimated at 59 and 82% in the two studies. They concluded that with the possible exception of root damage there was no evidence to show either epistasis or dominance as a major component of genetic variance for cold hardiness. Their evidence is in agreement with an additive mode of inheritance for cold hardiness as shown in crop plants.

CHAPTER 3

CRITICAL TEMPERATURE DETERMINATIONS IN COLD HARDINESS STUDIES USING ELECTRICAL CONDUCTIVITY MEASUREMENTS

INTRODUCTION

Identification of a procedure to differentiate between cold hardy and tender plant material is a prerequisite to conducting research on cold hardiness in plants. Survival or subjective rating of visible winter injury in the field has commonly been used for this purpose. However, the utility of such procedures is limited because of the inconsistency of test winters and confounding effects of field injury related to other causes. Over the past few decades, increased interest in stress physiology of plants has stimulated the use of artificial freezing tests for the purpose of evaluating relative plant cold hardiness. One difficulty associated with such laboratory tests is determining the amount of injury to plant tissue after freezing. Many tissue viability procedures have been devised for use in cold hardiness studies. Their effectiveness and reliability has been discussed by several investigators (Parker, 1953; van den Driessche, 1969a and 1976; Stergios and Howell, 1973; Blazich *et al*, 1974).

Selection of viability tests for cold hardiness depends on many factors, including species, plant tissue, time and the overall objectives of the hardiness research. There is probably no one "best" method for all species or conditions. No matter which procedure is adopted, it is desirable to relate laboratory results to actual field evaluations of cold injury wherever possible. Generally, a viability test which is objective, relatively quick, and capable of utilizing small quantities of plant tissue is preferred. Also, it is desirable that a single meaningful expression of relative cold hardiness can be derived from the viability results, such as a killing temperature, T_{50} or some other definable index of hardiness.

ELECTRICAL CONDUCTIVITY AS A VIABILITY TEST

A procedure which has been effective in evaluating tissue viability after freezing tests is electrical conductivity. This method, as first described by Dexter et al (1932), is based on the principle that live cells quickly lose their ability to regulate their contents when their cell membranes are damaged. As a result, electrolytes diffuse into solution thereby causing an increase in specific conductivity of the solution. Thus, the greater the injury to plant tissue from low temperature, the higher the conductivity of the extract.

In early studies, specific conductivities of leachates from frozen and unfrozen samples were compared. These comparisons were useful, but not exact, because total electrolytes varied for different samples. Stuart (1939) and Wilner (1959, 1960) improved on the procedure by expressing the amount of cell electrolytes released after freezing as a percentage of the total electrolytes released after heat-killing. This measure of injury has been useful for comparing relative cold

hardiness of tissue exposed to various temperatures, but does not provide a single definable expression of cold hardiness. Flint et al (1967) converted the percentage release of electrolytes to an index of injury (I_t) scale where the unfrozen control sample is given a value of zero and the heat killed sample a value of 100. After determining I_t for samples exposed to a series of test temperatures, a temperature corresponding to any selected I_t could be found and used as an expression of hardiness in which to compare samples.

In making cold hardiness comparisons between plants or samples of plant tissue, it is desirable to find a single definable point at which to compare all samples. Killing temperature would be a useful criteria, but it is often difficult to determine when a particular sample of plant tissue is dead. An equally definable criteria is the temperature corresponding to the point of earliest detectable freezing injury. This critical temperature can be easily calculated using the percentage release of electrolytes from unfrozen control samples and samples frozen at various test temperatures.

The objective of the following discussion is to describe a procedure for calculation of critical temperature, where critical temperature is defined as the highest temperature at which freezing injury to plant tissues can be detected.

CRITICAL TEMPERATURE DETERMINATION

The procedure I will describe for calculating critical temperatures presupposes that cold hardiness comparisons are being made among several seedlots and that electrical conductivity is used as a measure of tissue viability. The steps are as follows:

1. Prepare several tissue samples per seedlot.
2. Retain some samples of each seedlot as unfrozen controls.
3. Subject the remaining samples to freezing at a series of low temperatures, replicating each seedlot at each test temperature.
4. Choose test temperatures so that all seedlots will not be damaged at the highest test temperature, but will be damaged at the lowest test temperature.
5. At each of the preselected test temperatures, remove samples from the freezing apparatus and allow them to thaw slowly.
6. After freezing and thawing, determine tissue viability by the electrical conductivity method. To do this, soak each sample in a measured quantity of deionized water to allow leaching of electrolytes. After 24 to 30 hours, measure conductivity of the leachate.
7. Autoclave the samples (including leachate) and measure conductivity again.
8. Calculate relative conductivity according to the formula $100 \times (C_f/C_t)$, where C_f = conductivity of the leachate before autoclaving, and C_t = total conductivity after autoclaving.
9. Perform an analysis of variance on relative conductivities, including data for the three highest test temperatures of each seedlot. Choosing data from only the three highest temperatures minimizes problems of unequal variances in the analysis.
10. From the ANOVA, compute a standard error of the mean conductivity after exposure to any one temperature.

Multiply the standard error by an appropriate multiplier to obtain a "Least Significant Difference" (LSD).

11. Add this LSD to the relative conductivity of the control samples for each seedlot to determine the lowest relative conductivity significantly different from the control.
12. The critical temperature corresponding to this relative conductivity is found by interpolating between the two test temperatures for each seedlot within whose range the calculated conductivity value lies. Interpolation assumes that a linear relationship exists between relative conductivity and test temperature after damage has been initiated. This critical temperature is the highest temperature at which cold injury to each seedlot can be detected.
13. Determine critical temperatures for each replicate of every seedlot and evaluate differences in critical temperatures among seedlots by conventional statistical methods.

Critical temperatures represent the temperatures corresponding to the earliest statistically significant increase in relative conductivity due to cold injury. The extent of cold injury at that temperature, from a biological standpoint, can only be found by evaluating the relationship between critical temperatures and visible cold injury in field plantations or controlled environment studies.

Correspondence between critical temperatures and field injury

I have used electrical conductivity and critical temperatures in comparing the cold hardiness of needles from 30 seedlots of ponderosa pine on 17 different dates. The material was derived from a range wide provenance test in southern Michigan (Wright et al, 1969). The results

revealed large hardiness differences among provenances which were strongly associated with climatic conditions in the region of provenance origin. Field observations of winter injury, made at the end of the 1976-77 winter, were highly correlated ($r = 0.88$) with critical temperatures computed that winter. Thus, at least in a relative sense, critical temperatures seemed to provide a good estimate of cold hardiness differences among seedlots.

I also had the opportunity to evaluate the reliability of critical temperatures when the temperature dropped to -26°C on December 3, 1976 in the test plantation. On that date, I collected needles from six seedlots for the purpose of determining their critical temperatures in laboratory freezing tests. A few days later, I collected foliage samples from the same trees and returned them to the laboratory for close visual inspection of needle cold injury. The results conformed very closely to what was expected based on the laboratory tests. Percent visible needle injury and critical temperatures for the six seedlots are summarized in Table 1. Needles from seedlots with critical temperatures below -26°C on December 3, 1976, suffered no visible winter injury, while needles from other seedlots were damaged. Thus, actual damaging temperatures in the field seemed to be within a few degrees of the critical temperatures as determined in laboratory studies.

The close relationship between laboratory and field data in the case of the ponderosa pine study suggests that critical temperatures provide meaningful information on the cold hardiness of different seedlots. However, the fact that the procedure has worked effectively on ponderosa pine in Michigan does not guarantee that it will perform in a similar fashion on a different species or under a different set

TABLE 1. Relationship between critical temperatures (°C), as determined from laboratory freezing tests on December 3, 1976, and visible cold injury to needles of trees from six ponderosa pine provenances following exposure of trees to -26°C temperatures on December 3, 1976, in a replicated southern Michigan plantation.

Seedlot No.	State of Origin	Critical Temperature	Amount of Needle Discoloration
		°C	%
2012	AZ	-24.9	25
2040	CA	-18.3	63
2053	CA	-17.2	40
2116	UT	-26.1	0
2124	BC	-27.6	0
2197	MT	-41.4	0

of conditions. Nevertheless, preliminary results suggest that the critical temperature procedure may be a valuable means of determining a single definable expression of plant cold hardiness.

CHAPTER 4

GENETIC VARIATION IN SEVERAL ASPECTS OF COLD HARDINESS IN PONDEROSA PINE

INTRODUCTION

Ponderosa pine is one of the most important forest tree species in the world. In the western United States, where it is native, it furnishes more timber than any other species of pine and is the mainstay of the economy in many areas. It is also highly regarded for its beauty. It towers to great heights and produces long, straight, cleanly pruned boles which are admired by tourists as well as lumbermen.

Cold temperature appears to be one of the most important factors limiting the distribution of ponderosa pine. When ponderosa pine is taken from its natural range and moved to northern or inland locations freezing injury often occurs. Such injury causes unsightly damage and reduced growth.

Ponderosa pine, as do other species with large ranges, exhibits considerable genetic variation as a result of long term natural selection over a range of habitats and climates. When several geographic races of the species are grown in a common location, large differences in many morphological and growth traits are observed. Among such genetically variable traits are growth rate, needle length, foliage color and

susceptibility to winter injury. Accounts of such variation were reported by Kempff (1928), Weidman (1939), Wells (1964), and Wright et al (1969).

Studies investigating genetic variation in the development of cold hardiness in woody plant species have revealed that cold resistance may be related to the rate of cold hardening or the depth of hardiness plants can achieve in midwinter. Smithberg and Weiser (1968) found that northern clones of redosier dogwood (Cornus stolonifera) acclimated to cold eralier and faster than clones from warm regions, when all were grown under Minnesota conditions. A Seattle, Washington clone was the last to harden and as a result sustained extensive cold injury to twigs during fall or early winter. However, all clones became hardy enough to withstand temperatures of -90°C in midwinter.

Maronek and Flint (1974) collected needles of 15 provenances of eastern white pine (Pinus strobus) grown in Michigan. They collected them monthly and made laboratory tests to determine seasonal differences in hardiness. They found differences among provenances in rate of cold hardening as well as in ability to withstand extreme temperatures in midwinter. Needles of northern trees hardened most rapidly and could withstand the lowest temperatures in midwinter. In December and January, trees from the southern Appalachians were damaged at temperatures of -30°C whereas trees from Canada could withstand temperatures of -60°C.

OBJECTIVES

The objectives of this study were to detect the presence of genetic variation in ponderosa pine in the following aspects of cold hardiness: depth of hardiness, rate of cold hardening and dehardening and winter desiccation. I also investigated genetic variation in leafing out phenology and its relation to spring frost damage. In addition, I hoped to ascertain the contribution of each of these aspects to the visible winter injury which has occurred to ponderosa pine in Michigan. A final objective was to determine the amount of genetic and seasonal variation in foliar moisture content and to relate that variation to genetic differences among seedlots in cold hardiness.

MATERIALS AND METHODS

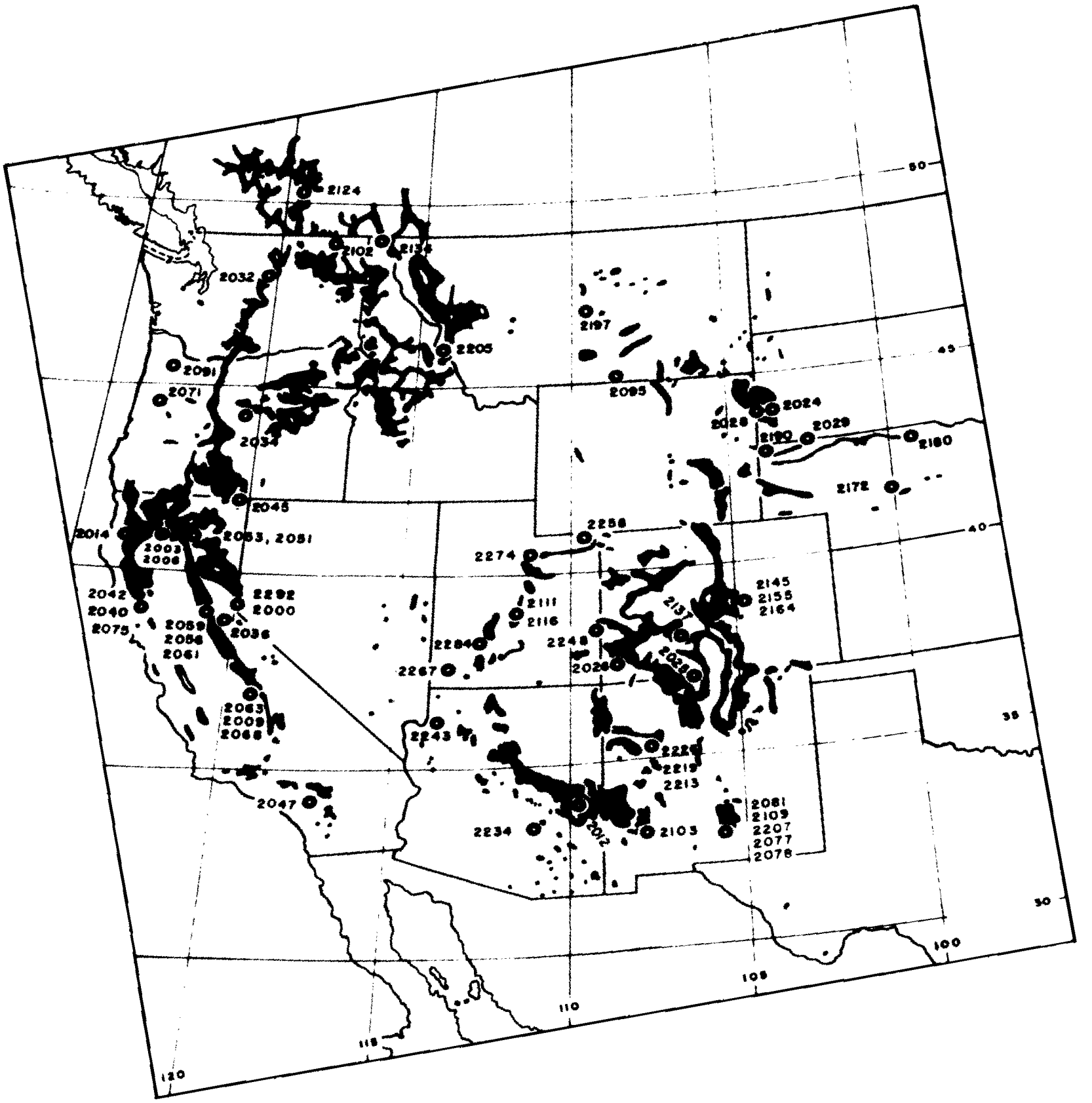
Establishment of plantations

The trees used in my study are part of a range wide provenance study started in 1960 by Dr. O. O. Wells, now geneticist with the U. S. Forest Service at Gulfport, Mississippi, with seed supplied by Dr. R. Z. Callaham, now Director of the Pacific Southwest Forest and Range Experiment Station. The seeds were collected from 60 natural stands located in all parts of the western United States (Figure 2). The seeds were sown in Michigan State University's experimental nursery at East Lansing and grown there for two years. In 1962 they were planted in two permanent test locations at W. K. Kellogg Forest (Augusta, Michigan) and Fred Russ Forest (Dowagiac, Michigan). The plantations follow a randomized complete block design with six tree plots, a 2.5 × 2.5m spacing, and five replicates per plantation. These plantations, which averaged 5.0 and 5.5 m tall respectively in 1976, were the source of material for my studies.

Collection and preparation of samples

On 13 different dates between October, 1975 and February, 1977 I collected twigs from two vigorous trees (the same trees each time) per plot for each of 30 selected seedlots at the Kellogg plantation. A total of ten trees from five replicates were sampled for each seedlot on every date. Each twig was collected from the middle of the crown on the west side of the tree. I immediately placed the twigs in plastic bags, sealed the bags, and stored them in the shade at near-ambient temperature conditions.

FIGURE 2. Distribution of ponderosa pine in the United States and British Columbia, showing the location of stand collections used in this study (map reprinted from Wells, 1962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2009
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2243
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2219
2213
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2078
2234
2103
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45
40
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30
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100

Within 24 hours these samples were returned to the laboratory and prepared for the freezing tests. This preparation started with the removal of 18 needle fascicles per twig (12-15 g of needles). Needles were cut in half and placed, cut end down, in labeled test tubes. The needles were apportioned in such a manner as to result in 12 test tubes per seedlot. Those comprising laboratory replication No. 1 were from the trees in plantation blocks Nos. 1, 2, and 3; those comprising laboratory replication No. 2 were from plantation block Nos. 4 and 5. For each laboratory replication there were six test tubes per seedlot, each to be frozen at a different temperature. This particular method of replication insured that the error terms included biological as well as instrumental error.

On four additional dates, twigs as well as needles were prepared for freezing. After removing 1 cm from each end, the remainder of the twigs were cut into 3 cm segments and placed in test tubes in the same way as the needles. Twigs were sampled for only six of the 30 seedlots for which needle samples were taken.

Freezing methods

After the needles or twigs were placed in the test tubes, the test tubes were stoppered. Then two from each seedlot were placed in a cold room held at 4°C. These were the unfrozen controls. The other ten from each seedlot were placed in a cold room at 0°C and were left to equilibrate for 12 hours. After equilibration, the test tubes were placed in insulated containers and put in a freezer set initially at -20°C. Temperatures within the insulated containers were monitored with a series of thermocouples. Every two hours the temperature control was lowered 4 to 6°C. The exact amount of lowering was

just enough to maintain a 20°C differential between the freezer and the inside of the containers. Freezing rates varied from 2 to 3°C per hour. At each of five preselected temperatures two test tubes per seedlot were removed from the freezer and thawed slowly in the insulated containers. Thawing took place at the rate of about 5°C per hour.

The range of freezing temperatures chosen for each sampling date was such as to result in no damage at the highest temperature but some damage to the hardiest seedlots at the lowest temperature.

Viability evaluations

Tissue viability was determined using a modification of the electrolytic diffusion method as described by Dexter et al, (1932). This method is based on the principle that live cells quickly lose the ability to regulate their contents when their cell membranes are damaged. As a result, electrolytes diffuse into solution thereby causing an increase in specific conductivity of the solution. Thus, the greater the injury to plant tissues from low temperatures, the higher the conductivity of the extract.

After thawing, 25 ml of deionized water was added to each test tube. Samples were soaked for 28 hours at 4°C. While still at 4°C, conductivity of the leachate was measured using a conductivity bridge and a pipette-type conductivity cell. The samples, including the leachate, were then autoclaved at 121°C for 20 minutes to kill all remaining live tissues. Samples were again placed in a cold room at 4°C for six hours after which their conductivities were remeasured.

The conductivities determined in the above manner were termed specific conductivities. They could vary with sample size as well as

amount of damage. For further calculations they were converted to relative conductivities, by expressing each as a percent of the conductivity of the autoclaved sample.

Determination of critical temperature

Critical temperature was defined as the highest temperature at which damage could be detected statistically. To determine it, I first subjected the relative conductivities to an analysis of variance. The error term from the analysis of variance was used to compute a "least significant difference"(LSD) value (at the 1% level). The LSD value, when added to the relative conductivity of control samples for each seedlot, provided the percent conductivity corresponding to the point at which significant cold injury could first be detected. The temperature corresponding to this point was then found by interpolation and represented the critical temperature. Critical temperatures were determined for each replicate of every seedlot.

Foliar moisture content and winter drying rates

In September, 1975 and on five different dates between July, 1976 and January, 1977 I collected 15-20 cm twigs from ten trees (five replications) of each of 30 selected seedlots in the Kellogg Forest plantation. The same trees were used as in the cold hardiness studies. The samples were immediately placed in plastic bags and taken to the laboratory where their fresh weights were determined. They were then placed in a drying oven for 48 hours and reweighed. Foliar moisture content was calculated as a percentage of fresh weight.

Rate of drying of cut branches under laboratory conditions was determined for 15 seedlots sampled on November 23, 1976. The twig samples were collected from blocks 1 to 3; each sample of twigs from

two trees in a plot was kept separate. The cut ends were waxed, the twigs were placed in plastic bags, and the fresh weights of the twigs were determined within two hours. Then the twigs were spread out on laboratory benches in a room kept at 20°C but with uncontrolled relative humidity. The twigs were weighed daily for the first four days and then every two days. After ten days the twigs were oven dried and reweighed to determine relative foliar moisture content.

Leaf phenology

On May 20, 1976, I scored the extent of shoot development for all 60 seedlots planted at Kellogg Forest. I used a scoring system of 1 (= buds in winter condition) to 10 (=leaves 2 in long). These grades corresponded to a time difference of approximately two weeks in the start of growth.

Field scoring of winter injury

Estimates of winter injury were made in the nursery and at various ages in both test plantations. Damage was recorded as the percentage of needles that turned brown as a result of winter cold. Field scorings were made in early spring following damaging winters.

Statistical analysis

For each trait measured, the data were summarized by seedlot and replication. In these summaries, the seedlots were grouped by the ecotypes and varieties recognized in the publications by Wells (1964) and Wright et al (1969). The ecotypes and their boundaries are illustrated in Figure 3.

After such summarization, an analysis of variance was performed for each set of data. There was, for example, a separate analysis, for the critical temperatures obtained at each date. In these analyses, mean

FIGURE 3. Ecotype divisions of ponderosa pine progeny included in this study. Ecotypes are designated by letters as follo

A. NORthern CAlifornia and SOUthern CAlifornia

B. NORthern PLATEAU

C. Arizona-New Mexico

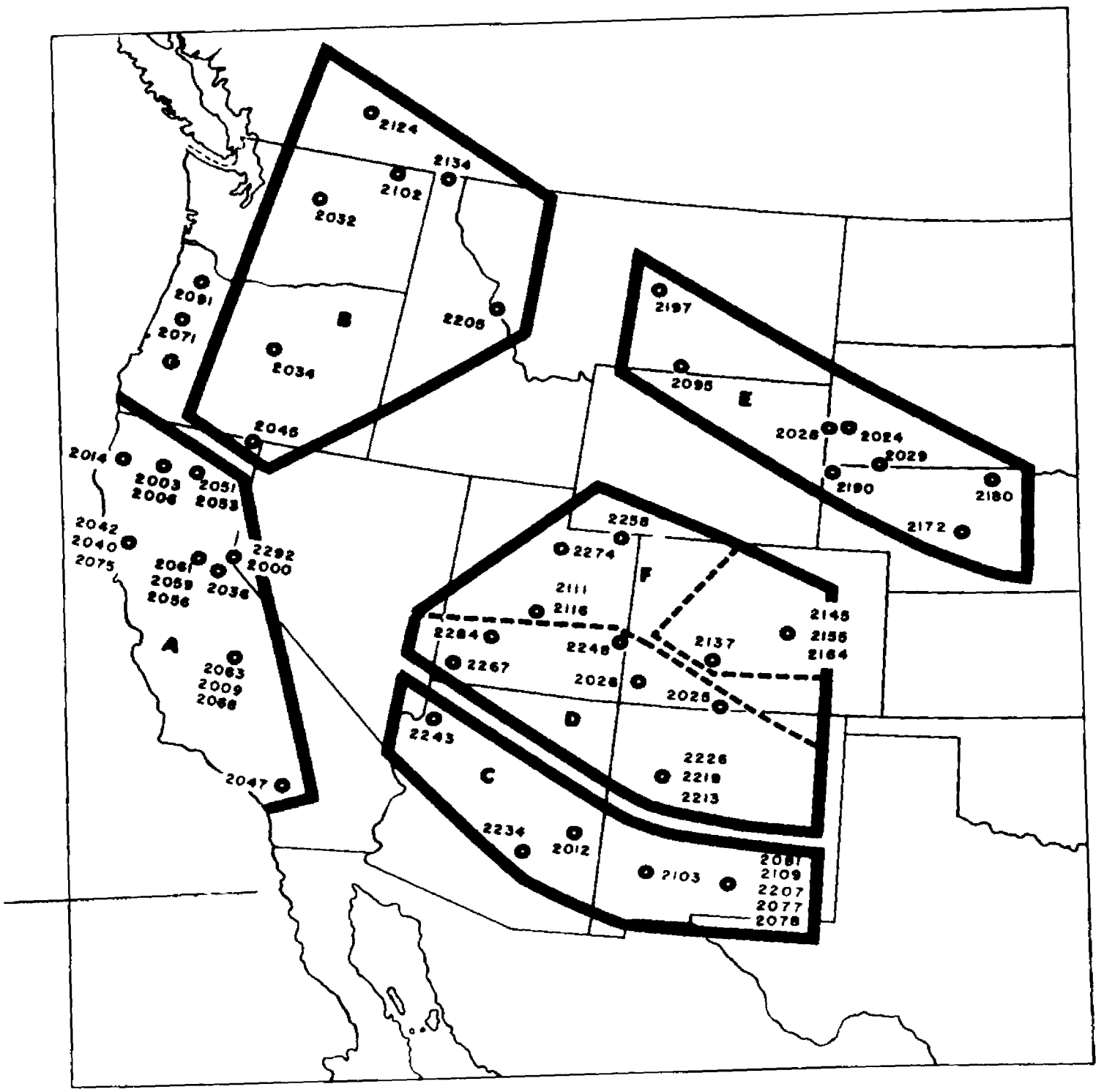
D. SOUthern UTah-northern New Mexico

E. NORthern INTERIOR

F. NORthern COlorado-UTah

G. COASTal ORegon

(map and ecotype boundaries after Wells, 1962).


2124
2134
2102
2032
2091
2071
2034
2208
2046
2014
2003
2006
2051
2053
2042
2040
2075
2292
2000
2061
2059
2056
2038
A
2083
2009
2068
2047
B
C
2243
2234
2012
2103
2081
2109
2207
2077
2078
D
2226
2218
2213
2258
2274
2111
2116
2284
2248
2267
2028
2025
F
2137
2145
2155
2164
2197
2095
E
2028
2024
2029
2190
2180
2172

squares were calculated for ecotype, seedlot within ecotype, replication, and error (seedlot × replication).

As already mentioned, a "Least Significant Difference" (LSD) was calculated for the freezing data for each date. This was calculated from the formula

$$LSD = 2.374 \sqrt{\frac{2(\text{Error Mean Square})}{2}}$$

This LSD was then used to determine the critical temperature at which cold injury could first be detected.

An angular transformation was used before analysis on all data recorded in percentage.

RESULTS

Genetic differences in cold hardiness

Depth of hardiness and rate of hardening. Significant differences in needle critical temperatures were found among the eight ponderosa pine ecotypes on nearly all sampling dates (Table 2). Only in July, 1976, were all trees at approximately the same level of cold hardiness. In general, trees from northern regions could withstand lower temperatures without damage than could southern trees.

In the interior variety (Figure 4), trees grown from seed collected in the Northern Interior (central Montana, South Dakota, Nebraska) hardened to cold faster than those from southern regions, and achieved a greater depth of hardiness by midwinter. Northern trees reached maximum hardiness in January when they could withstand temperatures below -45°C without needle damage. At the other extreme, Arizona and New Mexico trees hardened more slowly and, at maximum hardiness, suffered damage at -31°C. Also, Arizona and New Mexico trees did not increase in hardiness from December to January as did northern trees.

In the coastal variety, a similar pattern was evident (Figure 5). Trees grown from seed collected in the Northern Plateau region (Oregon, Washington, northeastern California and British Columbia) harden to cold rapidly in the fall and achieved a maximum depth of hardiness at -39°C in January. Trees from the two California ecotypes, on the other hand, were slow to harden and were damaged by temperatures of -22°C in early December. As with the Arizona-New Mexico trees, the California trees did not become more hardy in January than in December.

TABLE 2. Critical temperatures (in °C) for needles of trees from eight ponderosa pine ecotypes on 14 dates. Critical temperature is defined as the highest temperature at which cold injury could be detected. Data for July, 1976 is based on six seedlots.

	Critical temperature (°C) on													
Variety & Ecotype	Oct. 30 1975	Dec. 22 1975	Jan. 26 1976	Feb. 27 1976	Mar. 31 1976	Apr. 30 1976	Jul. 7 1976	Aug. 2 1976	Sep. 17 1976	Oct. 28 1976	Nov. 11 1976	Dec. 17 1976	Jan. 10 1977	Feb. 10 1977
var. ponderosa														
Nor. Plateau	-16	-27	-39	-23	-17	-10	- 6	- 7	- 8	-19	-22	-31	-39	-33
Coast. OR	-13	-22	-32	-15	-17	- 9	---	- 7	- 7	-15	-24	-27	-31	-31
Nor. CA	-10	-23	-22	-19	-15	- 6	- 5	- 6	- 7	-13	-17	-21	-24	-22
Sou. CA	- 8	-20	-22	-19	-13	- 6	- 5	- 7	- 7	-12	-15	-20	-22	-23
var. Scopulorum														
Nor. Interior	-19	-38	-45	-28	-19	-15	- 6	- 7	-11	-22[A/]	-28	-39	-47	-41
Nor. CO-UT	-14	-32	-41	-27	-19	-13	- 5	- 7	- 9	-21	-26	-35	-42	-36
Sou. NT-NM	-13	-29	-35	-27	-18	- 9	---	- 6	- 8	-20	-24	-31	-35	-32
AZ-NM	-11	-29	-31	-25	-17	- 7	- 5	- 5	- 7	-17	-22	-30	-32	-31
Least Significant Difference (5% level)	3.5	3.1	5.9	3.4	3.3	2.2	---	.56	.85	2.7	2.4	2.9	4.0	3.9

[A/] Needles not damaged at lowest test temperature

FIGURE 4. Seasonal pattern of cold hardening and dehardening for four interior variety (var. Scopulorum) ecotypes of ponderosa pine growing at Kellogg Forest in southern Michigan.

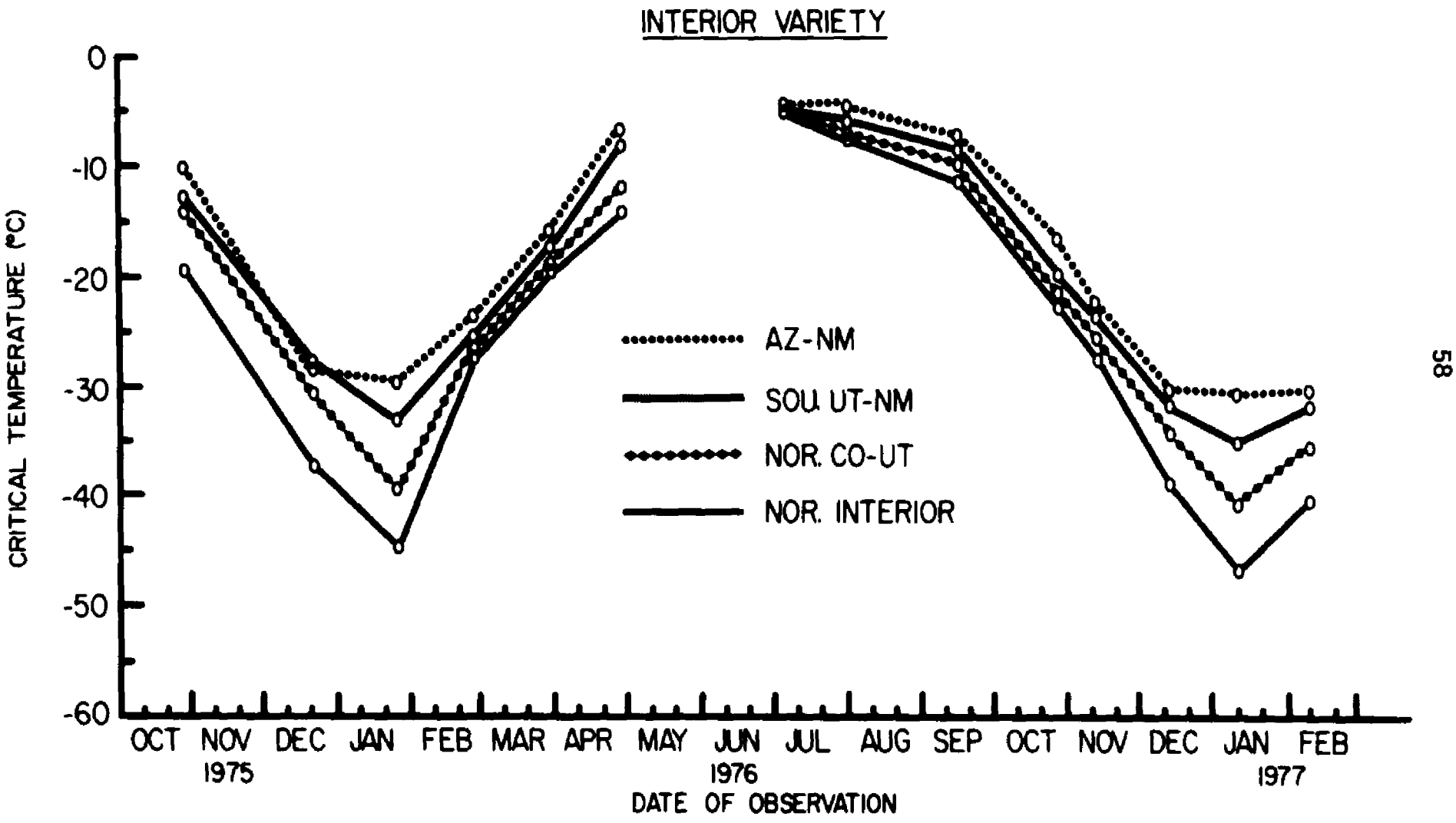
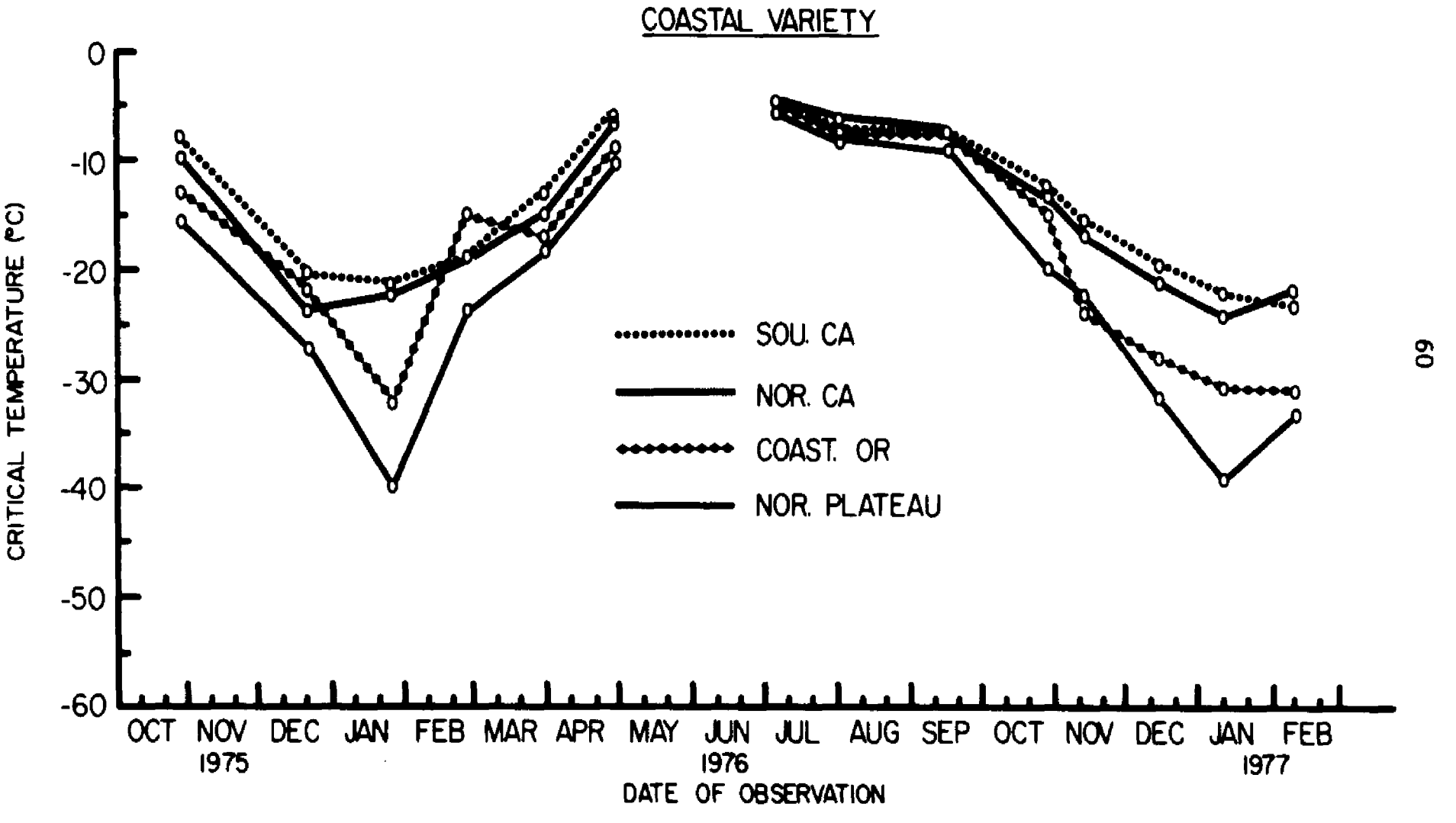

INTERIOR VARIETY
0
-10
-20
-30
-40
-50
-60
CRITICAL TEMPERATURE (°C)
AZ-NM
SOU. UT-NM
NOR. CO-UT
NOR. INTERIOR
OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB
1975
1976
1977
DATE OF OBSERVATION

FIGURE 5. Seasonal pattern of cold hardening and dehardening for four coastal variety (var. Ponderosa) ecotypes of ponderosa pine growing at Kellogg Forest in southern Michigan.


COASTAL VARIETY
CRITICAL TEMPERATURE (°C)
0
-10
-20
-30
-40
-50
-60
SOU. CA
NOR. CA
COAST. OR
NOR. PLATEAU
OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB
1975
1976
1977
DATE OF OBSERVATION

Comparison of hardiness levels on October 30, 1975 and October 28, 1976, indicates that cold acclimation began earlier in 1976 than in 1975. In October, 1976, critical temperature for the 30 seedlots averaged almost 5°C lower than in the previous year. By December, 1976, trees were only 2°C hardier than in 1975 and by January yearly differences were negligible. These differences may be explained by comparing climatic data from Kellogg Forest for the two years. Monthly mean minimum temperatures were 3°C colder and absolute minimums were 6°C colder in October, 1976 than in the previous year. Also temperatures dropped below 0°C on ten days during September and October, 1976 as compared to two days during the same months in 1975.

The geographic differences for ponderosa pine are similar to those reported for eastern white pine by Maronek and Flint (1974). They also found northern seed sources to harden to cold more rapidly and to achieve a maximum depth of hardiness in January. In contrast, southern white pines hardened slower in the fall, hardened less in winter, and reached maximum hardiness in late November.

Rate of dehardening. All trees had dehardened somewhat by middle or late February of both years (Figures 4 and 5). Critical temperatures for northern trees decreased by about 16°C from January to February, 1976. For southern trees, critical temperatures decreased by about 4°C during that period. Despite different dehardening rates, northern trees remained hardier than southern trees.

Dehardening occurred after the advent of relatively warm weather in both years and proceeded more rapidly from January to February, 1976 than the following year. This was apparently due to the warmer weather in 1976 than in 1977. Maximum daily temperatures at Kellogg Forest averaged nearly 10°C higher in February, 1976 than in February, 1977.

Differences within ecotypes. So far I have discussed only differences among ecotypes. There were also large and consistent differences among seedlots from the same region.

Data for individual seedlots within the two most variable ecotypes are given in Table 3. Among the seedlots from the Northern Plateau, No. 2124 from British Columbia was among the hardiest on all dates, having a critical temperature as much as 18°C lower than seedlot No. 2045 from northeastern California. There were also large differences among seedlots from the northern interior region. Seedlot No. 2197 from central Montana was hardiest on almost all dates, while seedlot No. 2095 from southern Montana was among the least hardy.

Hardiness of twigs vs needles. The results of cold hardiness comparisons between needles and twigs for six ponderosa pine seedlots on four dates are summarized in Table 4. On most dates, twigs had slightly lower critical temperatures than needles. In December, the critical temperature was several degrees lower for twigs than for needles. This explains why visible winter injury in the field was usually confined to needles.

Generally, on any one date, those seedlots with the lowest critical temperature for needles also had the lowest critical temperature for twigs.

My data on needle and twig hardiness of ponderosa pine are consistent with those of Parker (1957) who could not detect hardiness difference between acclimating twigs and needles of this species in northern Idaho. By midwinter in Parker's study, hardiness of twigs and needles exceeded the temperature limits of his freezing apparatus so that comparisons could not be made, However, Sakai and Okada (1971) found ponderosa

TABLE 3. Consistency and magnitude of cold hardiness differences among seedlots within two genetically variable ponderosa pine ecotypes.

Ecotype, Seedlot No. & State of Origin	Ranking by critical temperature on: Oct. 30 1975	Jan. 26 1976	Mar. 31 1976	Apr. 30 1976	Sep. 17 1976	Nov. 11 1976	Jan. 10 1977
	- - - 1 = most hardy, 5 = least hardy - - - -						
Nor. Plateau							
2124 BC	1	1	1	2	1	1	1
2032 WA	2	2	4	3	2	2	2
2102 WA	3	3	3	4	4	4	3
2034 OR	4	4	5	5	3	3	4
2045 CA	5	5	2	1	5	5	5
Critical Temperature Range							
from:	-10	-28	-16	- 6	- 7	-18	-31
to:	-23	-46	-19	-12	- 8	-25	-47
Nor. Interior							
2197 MT	1	1	1	3	1	1	1
2190 NB	2	4	2	1	2	2	3
2029 SD	3	2	3	2	3	3	2
2095 MT	4	3	4	4	4	4	4
Critical Temperature Range							
from:	-17	-41	-18	-14	-10	-25	-43
to:	-21	-52	-20	-16	-11	-29	-50

TABLE 4. Differences in cold hardiness between twigs and needles of ponderosa pine seedlots on four dates in 1976.

Seedlot No. and State of Origin	Critical temperatures (°C) on Jul. 7, 76		Aug. 26, 76		Oct. 7, 76		Dec. 3, 76	
	Needles	Twigs	Needles	Twigs	Needles	Twigs	Needles	Twigs
2012 AZ	-5.4	-5.4	-7.8	-8.6	-9.5	-8.1	-24.9	-31.7
2040 CA	-5.4	-5.5	-6.9	-7.1	-7.4	-7.7	-18.3	-17.8
2053 CA	-5.4	-5.4	-7.1	-7.7	-7.2	-7.3	-17.2	-28.4
2116 UT	-5.4	-5.0	-8.4	-9.8	-10.1	-9.1	-26.1	-35.2
2124 BC	-5.6	-5.7	-9.4	-10.3	-8.5	-10.7	-27.6	-40.2
2197 MT	-5.5	-5.8	-10.4	-10.9	-12.7	-13.2	-41.4	-41.4
Mean	-5.4	-5.5	-8.3	-9.2	-9.2	-9.4	-25.9	-32.5
Were differences significant at 1% level?								
Between tissues	No		Yes		No		Yes	
Among seedlots	No	No	Yes	Yes	Yes	Yes	Yes	Yes

pine twigs to be about 10°C hardier than needles in midwinter in a study conducted in Hokkaido, Japan.

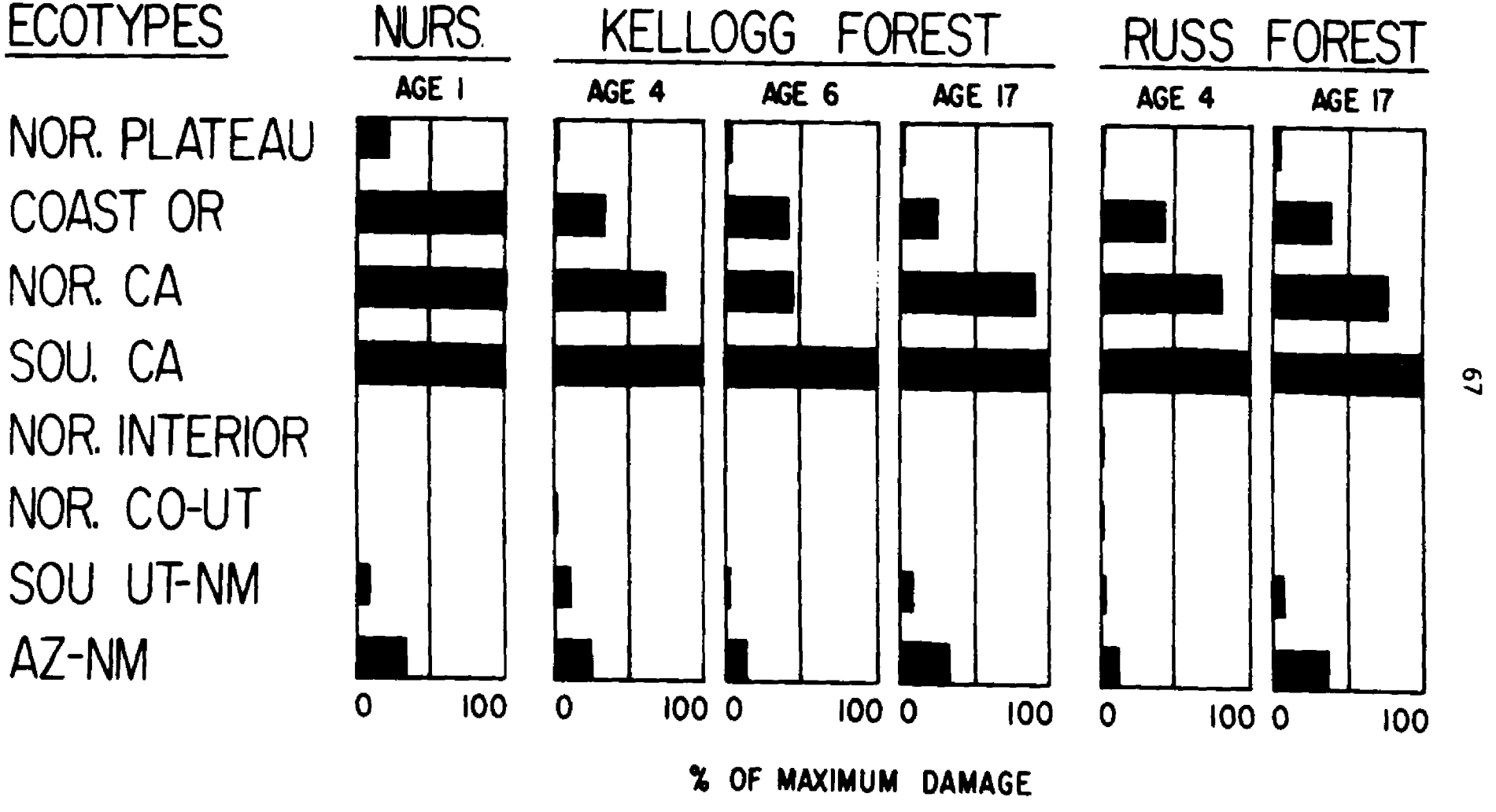
Visible winter injury. Winter injury was first observed in the nursery (Wells, 1964). It has been observed several times since, particularly at Kellogg Forest. Most of the injury has been to needles rather than to buds or cambium.

The injury was most severe on year-old seedlings and at age 17 following the coldest Michigan winter on record. Despite differences in severity from year to year, a similar geographic pattern in susceptibility to winter injury was evident at all sites and in all years (Fig. 5).

Winter injury was nil on interior trees from Colorado northward, while California trees suffered severe damage to their needles in all years that winter injury was a problem. Some California trees also had cambium damage after the cold winter of 1976-77. Coastal Oregon and Arizona-New Mexico trees suffered moderate damage in most years. Trees grown from seed collected in the Northern Plateau (Oregon, Washington, and British Columbia) were moderately damaged in the nursery but recovered sufficiently to be among the least damaged trees at later ages.

A north to south geographic pattern in winter injury has been shown for many forest tree species. In a Douglas-fir (Psuedotsuga menziesii) provenance test in Pennsylvania, Gerhold (1965) observed heavy injury to coastal trees, moderate injury to Arizona and New Mexico trees, and little or no damage on trees from the northern Rocky Mountains. Eiche (1966) also found a north to south pattern in winter injury in a north Swedish provenance test of Scotch pine (Pinus sylvestris). In eastern

FIGURE 6. Relative extent of needle winter injury on trees from eight ponderosa pine ecotypes at different plantations and ages. Age 17 data was collected following the severe winter of 1976-77.


ECOTYPES
NOR. PLATEAU
COAST OR
NOR. CA
SOU. CA
NOR. INTERIOR
NOR. CO-UT
SOU UT-NM
AZ-NM
NURS.
AGE 1
KELLOGG FOREST
AGE 4
AGE 6
AGE 17
RUSS FOREST
AGE 4
AGE 17
0
100
% OF MAXIMUM DAMAGE

hemlock (Tsuga canadensis), Nienstaedt (1958) noted a high correlation between fall frost damage and provenance growing season length for 17 seed sources tested in Wisconsin. Southern trees were apparently adapted to relatively long growing seasons and were damaged most severely by frost occurring in autumn.

In California, Conkle et al (1967) described a unique geographic pattern of winter injury among 43 sources of white fir (Abies concolor). Seedlings from northern California sustained more winter injury than did southern trees. This is probably due to introgression with grand fir (Abies grandis). Grand fir, although a more northern species, grows at much lower elevations than white fir and is consequently less winter hardy. White fir from northern California has more grand fir germplasm and therefore less hardiness than white fir from farther south.

Relationship between critical temperature and field injury. The relationship between critical temperatures and actual field injury were investigated during the winters of 1975-76 and 1976-77.

During the winter of 1975-76, the lowest temperature recorded in the Kellogg Forest plantation was -27°C. This low was recorded in mid January. That winter trees of the California ecotypes suffered a small amount of cold injury to the needles. Trees from other ecotypes had sufficiently hardened by middle January, having critical temperatures of -31°C or below to withstand the -27°C temperature.

The following winter was only slightly colder with a low temperature of -28°C. However, extremely cold weather occurred early in the season. At Kellogg Forest, the temperature dropped to -26°C on December 3, 1976. Foliage samples were collected a few days later and brought into the laboratory for close examination. Winter injury was measured as the

FIGURE 7. Mottling on ponderosa pine needles damaged by temperature of -26°C on December 3, 1976. From left to right the seedlots are: No. 2234 from Arizona, moderate damage; No. 2197 from Montana, no damage; and No. 2036 from California, severe damage.

TABLE 5. Comparison between damage caused by unseasonably cold weather (temperature of -26°C on December 3, 1976) in early winter and cold hardiness as measured by critical temperatures on the same date. The critical temperatures are an average of critical temperatures on November 11, 1976 and December 17, 1976.

Variety and Ecotype	Proportion of foliage injured on December 3, 1976	Critical temperatures (average of values for Nov. 11 and Dec. 17)
	%	°C
var. *ponderosa*		
Nor. Plateau	0	-26.5
Coast. OR	15	-25.5
Nor. CA	45	-19.0
Sou. CA	65	-17.5
var. *Scopulorum*		
Nor. Interior	0	-33.5
Nor. CO-UT	0	-30.5
Sou. UT-NM	5	-27.5
AZ-NM	20	-26.0

amount of mottling present on needles (Figure 7). The results are summarized in Table 5. Also included in that table are the probable critical temperatures reached by December 3 (averages of the critical temperatures measured on November 11 and December 17).

As shown in Table 5, those ecotypes which had hardened sufficiently to attain critical temperatures of -27°C by December 3 were not or only slightly injured by the -26°C temperatures on that date. Trees from California ecotypes suffered very severe mottling as a result of the cold weather. Trees from coastal Oregon and Arizona-New Mexico suffered a moderate amount of damage. Northern Plateau and Arizona-New Mexico trees differed in the amount of apparent winter injury even though both were almost equally hardy according to the laboratory tests.

The results summarized in Table 5 indicate a strong but not perfect relationship between cold hardiness as measured in the laboratory and actual damage from cold under field conditions. Apparently, the "critical temperature" as I measured it in the laboratory is within two or three degrees centigrade of the actual critical temperature of field grown plants.

Seasonal changes in cold hardiness

The seasonal pattern of cold acclimation for needles of four ponderosa pine seedlots is illustrated in Figure 8. That graph is based on cold hardiness data for four seedlots which were studied in more detail during fall, 1976.

The curves for the California and Arizona seedlots were higher than for the other two seedlots at all times. They were much higher during winter, but only slightly higher during summer.

Following others, the seasonal changes in cold hardiness can be divided into stages as follows. The first occurred from July to early

FIGURE 8. Seasonal changes in cold hardiness for four seedlots of ponderosa pine. Numbered arrows designate dates of important climatic changes as follows:

Arrow No. 1: January 18, 1976 - first day below -27°C

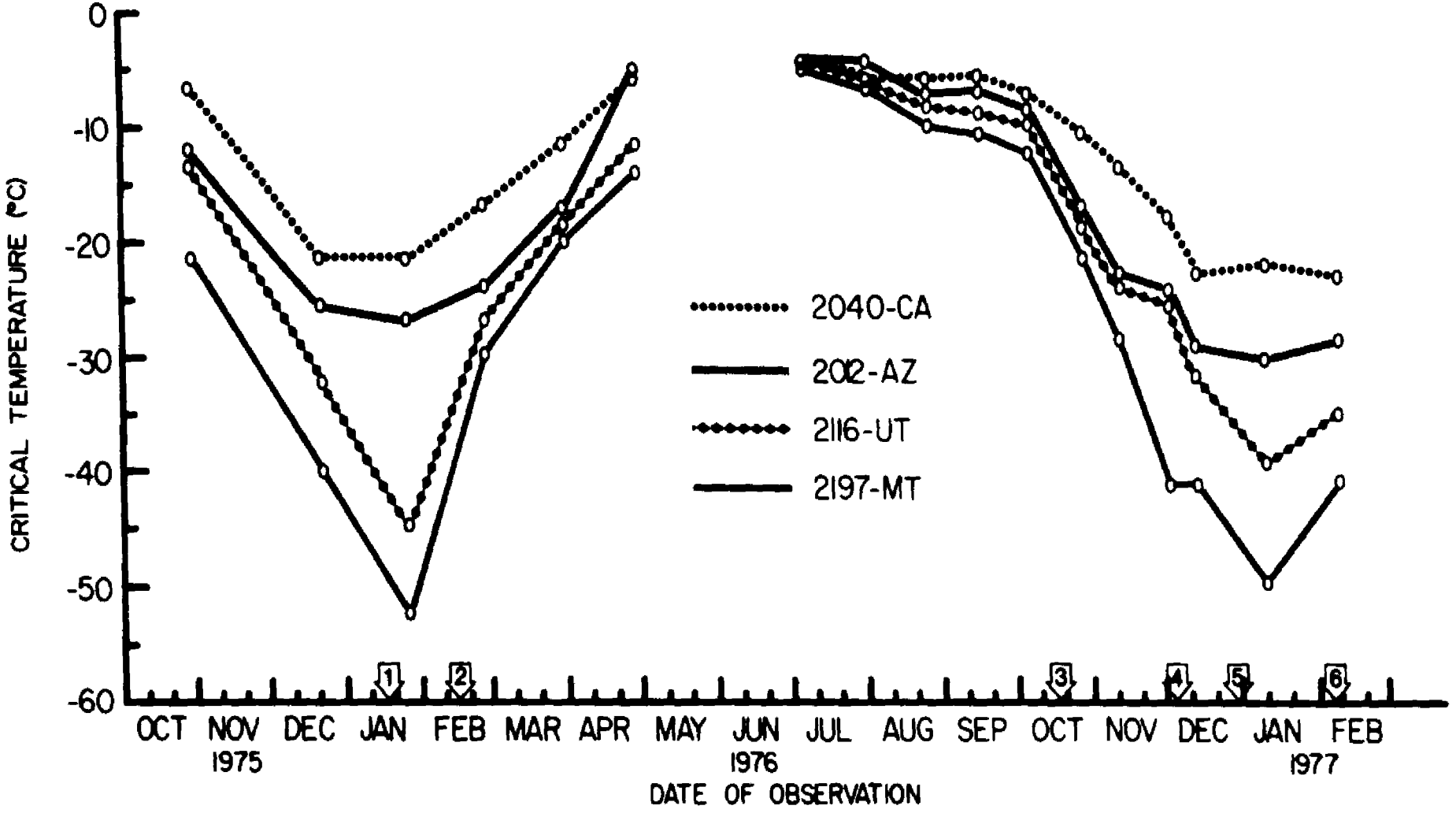
Arrow No. 2: February 15, 1976 - first day above 16°C

Arrow No. 3: October 18, 1976 - first day below - 5°C

Arrow No. 4: December 3, 1976 - first day below -26°C

Arrow No. 5: December 31, 1976 - first day below -28°C

Arrow No. 6: February 7, 1977 - first day above 5°C


0
-10
-20
-30
-40
-50
-60
CRITICAL TEMPERATURE (°C)
2040-CA
202-AZ
2116-UT
2197-MT
1
2
3
4
5
6
OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB
1975
1976
1977
DATE OF OBSERVATION

October, 1976 and was one of gradual hardening. The second occurred from October to mid December and was characterized by an accelerated rate of hardening. The third stage involved different changes in different seedlots and extended from mid December to mid January in both winters. During the third stage, there was no change in critical temperature for seedlots from warm climates. However, there was a further lowering of critical temperatures for seedlots from cool climates.

The rest of the seasonal cold hardiness cycle involved dehardening. The fourth stage occurred in January and February. It was characterized by a loss of the additional hardiness gained in the third stages, leaving the trees with nearly the same hardiness as they had in early December. The fifth stage occurred from the end of February through the end of April. It was a period of rapid dehardening. The fourth and fifth stages seemed to run together for cool climate seedlots, but were more distinct for the warm climate seedlots. Presumably there is a sixth stage from late spring to early summer, but there are no data for that period.

The stages of cold hardiness enumerated above are similar to those reported for hardwoods (Tumanov and Krasavtev, 1959; Irving and Lanphear, 1967b) and other conifers (Glerum, 1973).

Weiser (1970) has done much work in cold hardiness on a variety of species. Many studies conducted by him and his students were carried out under controlled conditions and enabled them to study the environmental factors responsible for the seasonal changes. In a summary paper, Weiser (1970) postulated that the first stage of cold acclimation is induced by shortening photoperiods from summer to fall and is

characterized by relatively minor increases in cold hardiness. His conclusions are supported by studies on Japanese yew (Taxus cuspidata) (Zehnder and Lanphear, 1966) and Douglas-fir (van den Driessche, 1970) and seem to be a reasonable explanation for the first stage observed in ponderosa pine.

The second stage of acclimation is apparently induced by freezing temperatures (Weiser, 1970; Howell and Weiser, 1970a). In my experiment, slight frosts (-2° to -3°C) in September and early October had little effect on the cold hardiness of ponderosa pine. However, there was a rapid increase in cold hardiness of all ponderosa pine seedlots following temperatures of -6° to -8°C during late October, 1976 (arrow No. 3 in Figure 8). In controlled environment studies, van den Driessche (1969b) found no increase in hardiness of seedling Douglas-fir exposed to temperatures of -1°C, whereas Timmis and Worrall (1975) found that evening frosts of -7°C caused rapid hardiness increases in Douglas-fir. In contrast, Zehnder and Lanphear (1966) found that subfreezing temperatures were no more effective than 2°C temperatures for inducing hardiness increases in Japanese yew.

Weiser (1970) speculated that a distinct third stage of cold acclimation occurrs in hardy woody plants subjected to very low temperatures (-30° to -50°C). This stage is characterized by a rapid increase in hardiness that is quickly lost upon return to mild temperatures. Such a third stage appeared to be present in the northern seedlots of ponderosa pine, which became much hardier in January than in December. Whether or not it was triggered by extreme cold weather is debatable. In both seasons the coldest day of the winter (arrow Nos. 2 and 4 in Figure 8) seemed to coincide with rather than precede the period of maximum hardiness.

What triggers the fourth stage, the loss of hardiness which started in January? Several authors (van den Driessche, 1969; Zehnder and Lanphear, 1966) thought that warm weather in midwinter was the environmental factor causing the dehardening. Howell and Weiser (1970b), who worked with trees in controlled environments, found that exposure to warm temperatures was followed by a great decrease in hardiness within a day.

During the 1975-76 winter, the coldest day (-28°C) was January 18, 1976 (arrow No..1 on Figure 8), and the start of weather warmer than 16°C was February 15, 1976 (arrow No. 2). During the next winter, the coldest day (-28°C) was December 31, 1976 (arrow No. 5). That was part of a very cold spell of 54 consecutive days in which temperatures were below freezing. The end of that period of below freezing weather occurred on February 7, 1977 (arrow No. 6) when the thermometer reached +6°C.

As already noted, it has been hypothesized that the development of maximum hardiness is triggered by very cold weather, and that dehardening is triggered by a period of warm weather in midwinter. If the first hypothesis is correct for ponderosa pine, hardiness remained at a near constant level following the December samplings, only to increase dramatically after the very cold days (arrow Nos. 1 and 5 on Figure 8). If the second hypothesis is correct, the period of maximum hardiness lasted for 20 days or more (from arrow No. 1 to arrow No. 2 and from arrow No. 5 to arrow No. 6), and then hardiness decreased after the beginning of warm weather.

Winter drying rates

Winter desiccation can occur in evergreen plants when the soil is frozen and plants begin transpiring in response to warm temperatures or

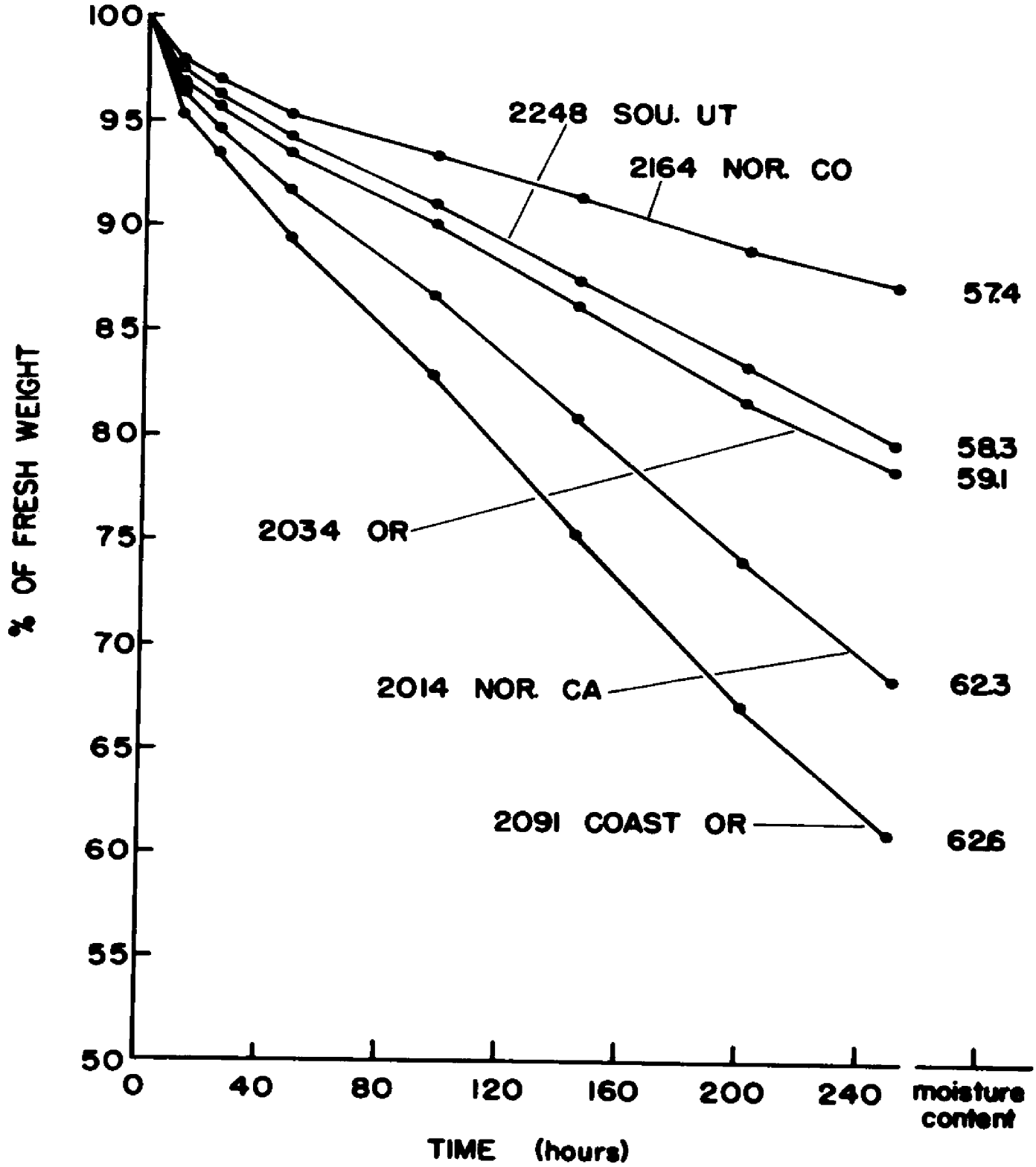
dry winds. Such damage is often thought to be more important than injury from low temperatures and has been a problem on very cold resistant species in some areas (Sakai, 1970). To determine the relative importance of desiccation and low temperature injury, I performed the desiccation experiment described under "Methods."

There were significant differences in drying rates among the 15 seedlots sampled on November 23, 1976. Cut branches of all seedlots of the coastal variety lost moisture more rapidly than did cut branches of any seedlot of the interior variety. This is shown in Figure 9, which includes data for five seedlots representing the extremes for each variety. These differences in drying rate may be related to differences between varieties in foliage color. Trees from the coastal variety (var. ponderosa) are bright green whereas trees from the interior variety (var. scopulorum) are gray-green. The color differences are due to differences in structure of the epidermis, the interior trees having a rough epidermis which probably acts to retard transpiration.

There were also differences within the varieties. Four seedlots from the northern half of the interior variety (represented by 2164 in Figure 9) had slower drying rates than did the four (represented by 2248) from farther south. In the coastal variety, trees from California and coastal Oregon had the fastest drying rates.

I calculated the correlation between moisture content of the fresh needles (based on the difference between fresh weight and oven-dry weight) and drying rate as measured by the slope of the curves in Figure 9. For the 15 seedlots, the correlation was high ($r = 0.91$). In other words, trees with the highest moisture content dried out the fastest. This is opposite to the relationship found in Douglas-fir (DeHayes and

FIGURE 9. Genetic differences in winter drying rates and foliar moisture content of excised twigs from five ponderosa pine seedlots collected on November 28, 1976. Seedlots 2034, 2014, and 2091 are from the coastal variety; seedlots 2164 and 2248 are from the interior variety.


100
95
90
85
80
75
70
65
60
55
50
% OF FRESH WEIGHT
2248 SOU. UT
2164 NOR. CO
2034 OR
2014 NOR. CA
2091 COAST OR
57.4
58.3
59.1
62.3
62.6
0
40
80
120
160
200
240
moisture content
TIME (hours)

Wright, 1976) and Norway spruce (Picea abies) (Saltersdal, 1963). Wilner (1952), working with twigs of different hardwood species, found the same relationship as in ponderosa pine.

Trees from coastal Oregon had much faster drying rates than trees from northern California but suffered much less winter injury. Trees from Arizona and New Mexico had slightly slower drying rates than trees from British Columbia and eastern Oregon, but suffered more winter injury. Such comparisons indicate that cold weather has been more important than desiccation in causing winter injury to ponderosa pine.

Leaf phenology

The changes in cold hardiness in the winter are cyclic phenomena. Leaf development in the spring is part of another cyclic phenomenon. In order to determine whether the two are related, I measured extent of leaf development on May 20, 1976, using grades of 1 (buds still in winter condition) to 10 (leaves 2 in long). These grades correspond to an approximate 14 day difference in the start of growth.

The results, summarized in Table 6, show a north to south trend. Trees from the northern, colder regions began growing the earliest, about 12 to 14 days before trees from Arizona, New Mexico, and California. In other words, the same northern seedlots were first to become hardened in the fall in preparation for winter cold, fastest to deharden in late winter, and first to start growth in the spring.

Hanover (1963), reporting on a ponderosa pine provenance test in northern Idaho also reported that southern seedlots started growth latest. So did Steiner (1975) who reported on several species including ponderosa pine. However, Douglas-fir and black walnut were exceptional, southern seedlots starting growth latest in both species.

TABLE 6. Relative time of bud break of 16 year old ponderosa pine trees from eight ecotypes growing at Kellogg Forest in southwestern Michigan.

Variety and Ecotype	Time of bud break 1 = late, 10 = early
var. ponderosa	
Nor. Plateau	10.0
Coast. OR	5.2
Nor. CA	2.9
Sou. CA	1.0
var. scopulorum	
Nor. Interior	8.7
Nor. CO-UT	8.9
Sou. UT-NM	4.4
AZ-NM	1.6

In other species, such as white spruce (Picea glauca, Nienstaedt and King, 1970), balsam fir (Abies balsamea, Lester, 1970) and Douglas-fir (Steiner and Wright, 1974), differences in date of growth initiation are related to amount of damage from late spring frosts. Generally, those seedlots which start growth earliest suffer the most damage.

This relationship is not true for ponderosa pine. Newly expanded leaves of that species, and most other pines as well, are not damaged by the temperatures of -2° to -4°C which occur so frequently after the start of the growing season in Michigan. Evidence to this has been reported by Sorenson and Miles (1974) on ponderosa pine and lodgepole pine (Pinus contorta) in central Oregon. Frosts of -3°C in early June damaged floral structures but not leaves in both species. Apparently, ponderosa pine needles are able to withstand a few degrees of frost at any stage of development.

Foliar moisture content

The moisture content of all 30 seedlots was measured in September, 1975 and on six dates from July, 1976 to January, 1977. The results are shown in Table 7.

On each date there was a one to four percent difference between the two varieties. Nearly all seedlots of the west coast var. ponderosa had a higher moisture content than any seedlots of interior var. scopulorum.

There were also differences within the varieties, the southern ecotypes of each being more succulent than the northern ones. On most dates there were two to three percent differences among ecotypes from the same variety.

TABLE 7. Ecotype and seasonal differences in foliar moisture content of ponderosa pine growing at Kellogg Forest in southwestern Michigan.

Variety and Ecotype	Foliar moisture content on Sep. 20 1975	Jul. 7 1976	Aug. 26 1976	Oct. 7 1976	Nov. 28 1976	Jan. 25 1977
	%	%	%	%	%	%
var. ponderosa						
Nor. Plateau	65.4	71.0	62.5	61.6	57.9	56.9
Coast. OR	68.6	73.1	66.2	64.8	61.6	57.2
Nor. CA	67.7	73.0	65.7	63.4	60.8	56.9
Sou. CA	68.2	73.7	66.3	64.3	61.1	57.7
var. scopulorum						
Nor. Interior	62.5	69.5	61.2	59.1	56.0	55.8
Nor. CO-UT	64.0	70.4	61.3	59.6	56.9	56.4
Sou. UT-NM	65.8	71.0	62.3	60.0	57.4	56.6
AZ-NM	65.8	72.4	64.1	61.9	58.5	56.7
Least Significant Difference (5% level)	1.4	.9	1.2	.9	.9	.6

Pharis and Ferrell (1966) found differences in foliar moisture content of Douglas-fir varieties even greater than those I found for ponderosa pine. In their work, the west coast variety averaged six percent greater moisture content than the interior variety.

Several authors have found north to south differences in leaf succulence in other conifers. Among the species which have been studied are Scotch pine (Langlet, 1936), Norway spruce (Saltersdal, 1963), jack pine (Pinus banksiana, Teich, 1968) and Douglas-fir (DeHayes and Wright, 1976). In contrast, McLemore et al (1961) found no geographic pattern to the differences in moisture content of loblolly pine (Pinus taeda).

There were also seasonal differences in foliar moisture content (Table 7). In general, moisture content declined from summer to winter. The most rapid decrease occurred between July and August when relative moisture contents for trees of all ecotypes decreased by seven to eight percent. Thereafter, relative moisture content decreased at a steady rate through November. By November, trees from northern ecotypes reached a near constant foliar moisture content, but trees of southern origin continued to decline. In January, 1977, the differences among ecotypes were smaller than at any previous date.

Similar declines in relative foliar moisture content from summer to winter have been shown for several hardwood and coniferous species (Langlet, 1936; Ackley, 1954; Kozlowski and Clausen, 1965; Pharis, 1967; Jameson, 1966). In contrast, Gary (1971) could find only slight changes in moisture content of one year old Engelmann spruce (Picea engelmannii) needles from summer to winter.

Relationship between foliar moisture content and hardiness. Significant negative correlations were found between foliar moisture content

and critical temperatures on all sampling dates (Table 8). In general, seedlots with the highest moisture were least hardy to cold. A similar relationship has been reported by others. Teich (1968), working with jack pine, found a strong relationship between foliar moisture content and cold injury, the most succulent seed sources suffered the most cold damage. Metcalf et al (1970) demonstrated a similar pattern for three cultivars of barley (Hordeum vulgare) and wheat (Triticum aestivum).

The seasonal relationship between moisture content and cold hardiness is illustrated in Figure 10. As moisture content decreased, from summer to winter, hardiness increased. However, the seasonal changes in moisture were not proportional to the seasonal changes in hardiness. For example, the decrease in succulence was greatest in midsummer whereas the changes in hardiness were greatest in the fall.

The pattern of hardiness-moisture content changes in ponderosa pine agrees with that found by McKenzie et al (1974) in stems of red-osier dogwood. Their studies indicated that moisture content changes during summer were caused by decreased stomatal resistance to transpiration and simultaneous increased root resistance to water uptake. They suggested that the short day induced stage of cold acclimation may operate via regulation of processes involved in plant water relations. Furthermore, the initial large decline in tissue moisture content may be a prerequisite for the subsequent large increases in cold hardiness that occur during fall and winter.

The level of tissue moisture content has often been implicated as a factor which is responsible for differences in plant cold hardiness. For instance, McKenzie et al (1974) thought that at high moisture contents there is an increased likelihood of intracellular ice formation and

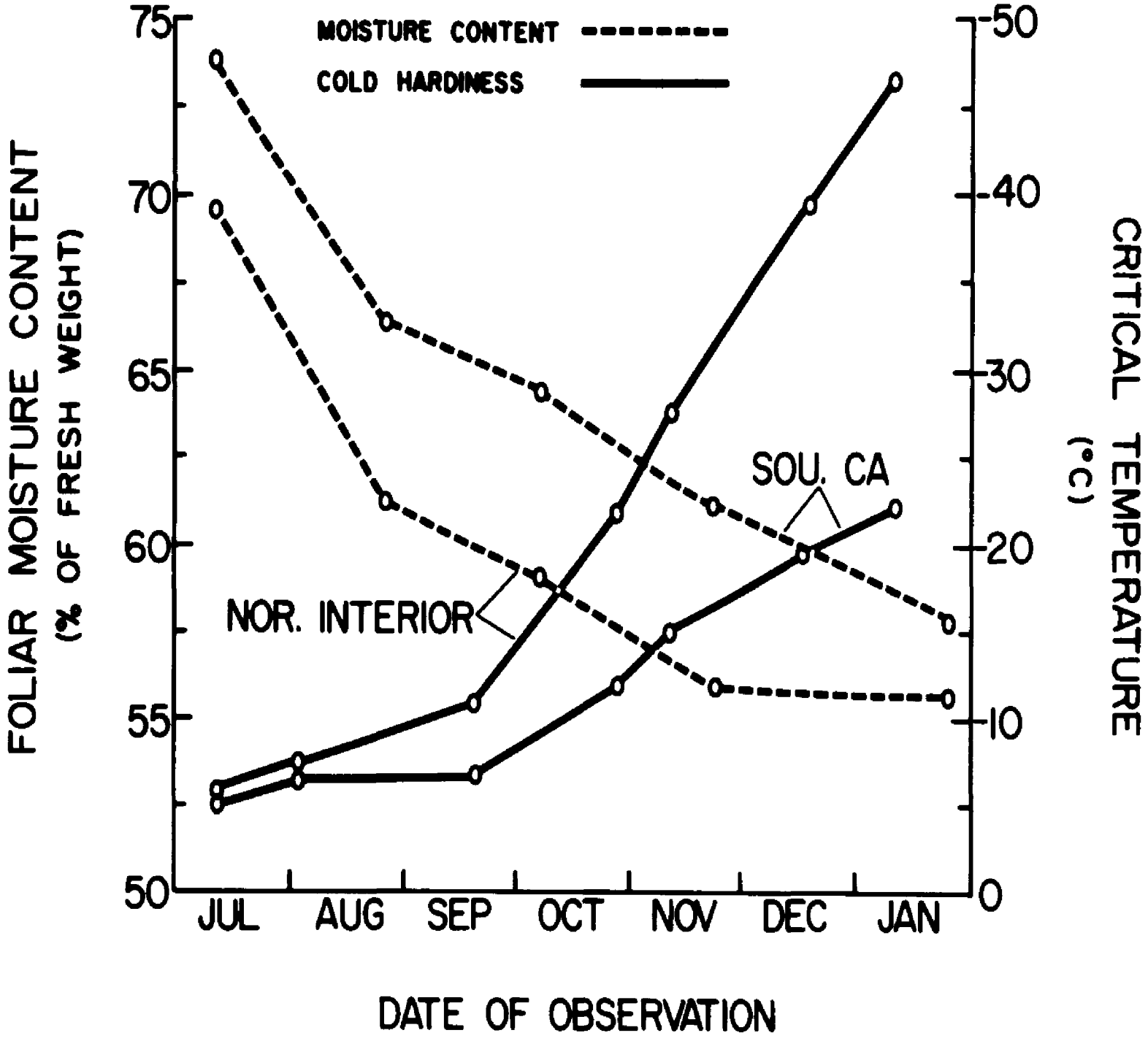
TABLE 8. Simple correlations between foliar moisture content and needle critical temperatures for 30 ponderosa pine seedlots on several dates in 1975 and 1976.

Correlation between		Correlation
Moisture content on:	and Critical temperature on:	coefficient
Sep. 20, 1975	Oct. 30, 1975	-.66**
Jul. 7, 1976	Aug. 2, 1976	-.37*
Aug. 26, 1976	Sep. 17, 1976	-.76**
Oct. 7, 1976	Sep. 17, 1976	-.84**
Oct. 7, 1976	Nov. 11, 1976	-.79**
Nov. 28, 1976	Nov. 11, 1976	-.77**
Nov. 28, 1976	Dec. 17, 1976	-.89**
Jan. 25, 1977	Jan. 10, 1977	-.53**

* Significant at 5% level

** Significant at 1% level

FIGURE 10. Seasonal changes in foliar moisture content and cold hardiness for trees from two ponderosa pine ecotypes growing at Kellogg Forest in southwestern Michigan.


MOISTURE CONTENT
COLD HARDINESS
FOLIAR MOISTURE CONTENT
(% OF FRESH WEIGHT)
CRITICAL TEMPERATURE
(°C)
75
70
65
60
55
50
-50
-40
-30
-20
-10
0
SOU. CA
NOR. INTERIOR
JUL
AUG
SEP
OCT
NOV
DEC
JAN
DATE OF OBSERVATION

cold injury during a frost because of the large amount of water within cells. In a summary paper, Burke et al (1976) supported this viewpoint. They suggested that, at high moisture contents, large quantities of water freeze rapidly and the resulting ice crystals cause tissue disruption. Others have supplied evidence in support of a hardiness-moisture content relationship. Bittenbender and Howell (1975) demonstrated that artificial increases in tissue hydration were accompanied by a decrease in cold hardiness of flower buds of highbush blueberry (Vaccinium australe), while a decrease in moisture content was related to an increase in hardiness. In addition, Li and Weiser (1969) and Chen et al (1975) found that partial dehydration and water stress caused a decrease in tissue moisture content and an increase in cold hardiness in stems of red-osier dogwood.

Based on the evidence discussed, it is tempting to conclude that foliar moisture content is a key factor causing differences in cold hardiness in ponderosa pine. However, the problem is to explain why trees with high moisture content are the most susceptible to cold damage. This is a relationship which seems to hold from summer to winter, and also from north to south within the species.

Differences in osmotic concentration due to the presence of sugars in cell sap can offer protection against freezing to temperatures a few degrees below freezing. However, the concentration of solutes in a plants' sap is never enough to prevent water from freezing at the low temperatures achieved during a Michigan winter.

Weiser (1970) stated that the formation of ice crystals within cells is the primary mechanism of cold damage. Apparently intercellular water can freeze in most hardy tissues without causing too much damage.

If Weiser is correct, then it is possible that the higher the moisture content in general, the higher the moisture content within cells, and the greater the amount of water to be frozen and cause damage.

CHAPTER 5

THE EFFECTS OF WINTER INJURY ON AGE-AGE HEIGHT RELATIONSHIPS IN PONDEROSA PINE

INTRODUCTION

Growth rate is one of the most important characteristics considered in a forest tree improvement program. The expression of growth rate obtained for trees of different seed sources in forest genetics experiments is a combination of inherent growth potential and response of trees to environmental stresses such as winter cold. This chapter evaluates the effects of winter injury on age-age height relationships in the ponderosa pine provenance test.

The relationship between juvenile and later growth rate in provenance tests of many species has been good when winter injury and diseases have not been problems. Nanson (1968) reported strong correlations between height at ages four to ten and ages 40 to 60 years in provenance tests of Scotch pine and Douglas-fir. Others have also demonstrated strong age-age correlations in provenance tests of the same species (Wright et al, 1957; Namkoong et al, 1972).

In ponderosa pine, age-age correlations have been reported by several investigators from many different types of genetic studies. Their results are summarized in Table 9. In general, correlations from provenance tests and elevational zone comparisons were strong when winter

injury was not a problem. For middle and low elevation plantations in California, Conkle (1973) found very strong correlations between heights at ages 3 and 25. However, in a high elevation plantation the age 3 to 25 height correlation was low, presumably because of winter injury which occurred at age 10. Kempff (1928) attributed changes in height ranking among 21 provenances of ponderosa pine to winter injury in a northern Idaho provenance test.

TABLE 9. Summary of phenotypic correlations for height at different ages in genetic variation studies of ponderosa pine.

Last Measured (Age)	Correlations between height at last measurement and at age: 1	2 or 3	5	6 or 7	8	11 or 12	20	25	Author[a/]
	Comparisons of trees from seven elevation zones								
25	---	.92	.98	.98	---	---	.96	---	Co
25	---	.87	.91	.91	---	.96	.98	---	Co
25	---	.14	.04	.02	---	.45	.98	---	Co
	Comparisons of 10 to 35 provenances								
8	.57	.66	.81	---	---	---	---	---	W & P
42	---	---	---	---	---	.69	---	---	S & S
30	---	.81	.51	.67	---	.56	.73	---	S & S
30	---	.40	.85	.83	---	.91	.88	---	S & S
30	---	.53	.75	.79	---	.90	.91	---	S & S
30	---	.51	.54	.61	---	.75	.89	---	S & S
30	---	.92	.61	.75	---	.90	.87	---	S & S
25	.81	---	.84	---	---	---	---	---	Mo
	Comparisons of 71 to 271 half-sib families								
29	---	.05	.19	-.47	-.60	.64	.85	1.00	N & Co
8	.23	.37	.73	---	---	---	---	---	W & P
15	---	.30	---	---	---	---	---	---	C & H

Table 9 continued

	Comparisons of 112 to 448 individual trees								
25	---	.12	.19	.37	---	.62	.93	---	Co
25	---	.25	.60	.70	---	.84	.94	---	Co
25	---	.41	.60	.68	---	---	.93	---	Co
20	---	.08	.02	---	---	.75	---	---	C & D
20	---	.02	.18	---	---	.67	---	---	C & D

[a/] Co = Conkle, 1973

W & P = Wang and Patee, 1973

S & S = Squillace and Siler, 1962

Mo = Moore, 1944

N & Co = Namkoong and Conkle, 1976

C & H = Callaham and Hasel, 1961

C & D = Callaham and Duffield, 1962

MATERIAL AND METHODS

The ponderosa pine study is part of a range wide provenance test initiated in 1960. Seed from 60 provenances was sown in an East Lansing, Michigan nursery using a four replicated randomized complete block design. Two years later, seedlings were transferred to two permanent test plantations at W. K. Kellogg Forest (Augusta, Michigan) and Fred Russ Forest (Dowagiac, Michigan) in southern Michigan. The plantations follow a randomized complete block design with six tree plots and a 2.5 × 2.5 m spacing. Further details of plantation design and establishment are provided by Wright et al (1969).

Height measurements were made in the nursery at ages one and two and at various ages in both test plantations up through age 16. Estimates of winter injury to needles were made following the first year in the nursery and at ages 4, 6, and 17 in the plantations.

Analyses of variance were performed on all nursery and plantation data using plot means as items. From the analyses of variance and expected means squares from height data, variance components due to ecotype and seedlot within ecotype effects were calculated. These components were expressed as a percentage of the total genetic variance (sum of ecotype and seedlot within ecotype components). Coefficients of variation were computed for each age from the error mean squares. Product moment correlations were calculated between seedlot mean heights at age 16 and all previous height measurements.

RESULTS

Changes in relative growth rate

The genetic variation pattern for height in ponderosa pine changed dramatically with age (Figure 11). After the first growing season, the largest trees were those grown from seed collected in California and Arizona-New Mexico. The smallest trees were those grown from seed collected in the northern interior (Montana, South Dakota, Nebraska) and northern Colorado and Utah. By age 16, trees grown from seed collected in the northern Plateau region (Oregon, Washington, British Columbia) and northern interior were fastest growing, Arizona-New Mexico trees were below average, and California trees were smallest.

The greatest changes in relative height growth occurred during the first five years of growth (Table 10). Correlations between height at age 16 and at ages 1 and 2 were negative or near zero for both plantations. After plantation establishment, correlations increased somewhat but were still generally less than juvenile-mature correlations reported in other ponderosa pine provenance tests (Table 9).

The poor correlations were brought about by a sharp decline in growth for the California trees which was accompanied by a steady increase in relative growth of northern Plateau and northern interior trees. By age six, the rankings among seedlots had stabilized and remained constant through age 16.

The changing pattern of growth with age was caused by winter injury which occurred in the nursery and at various ages in both plantations (Figure 6). During the first winter in the nursery, trees from California and coastal Oregon origins suffered severely. Arizona-New Mexico and

FIGURE 11. Change in relative height (expressed as a percentage of plantation or nursery means) with age for trees from fou ponderosa pine ecotypes.

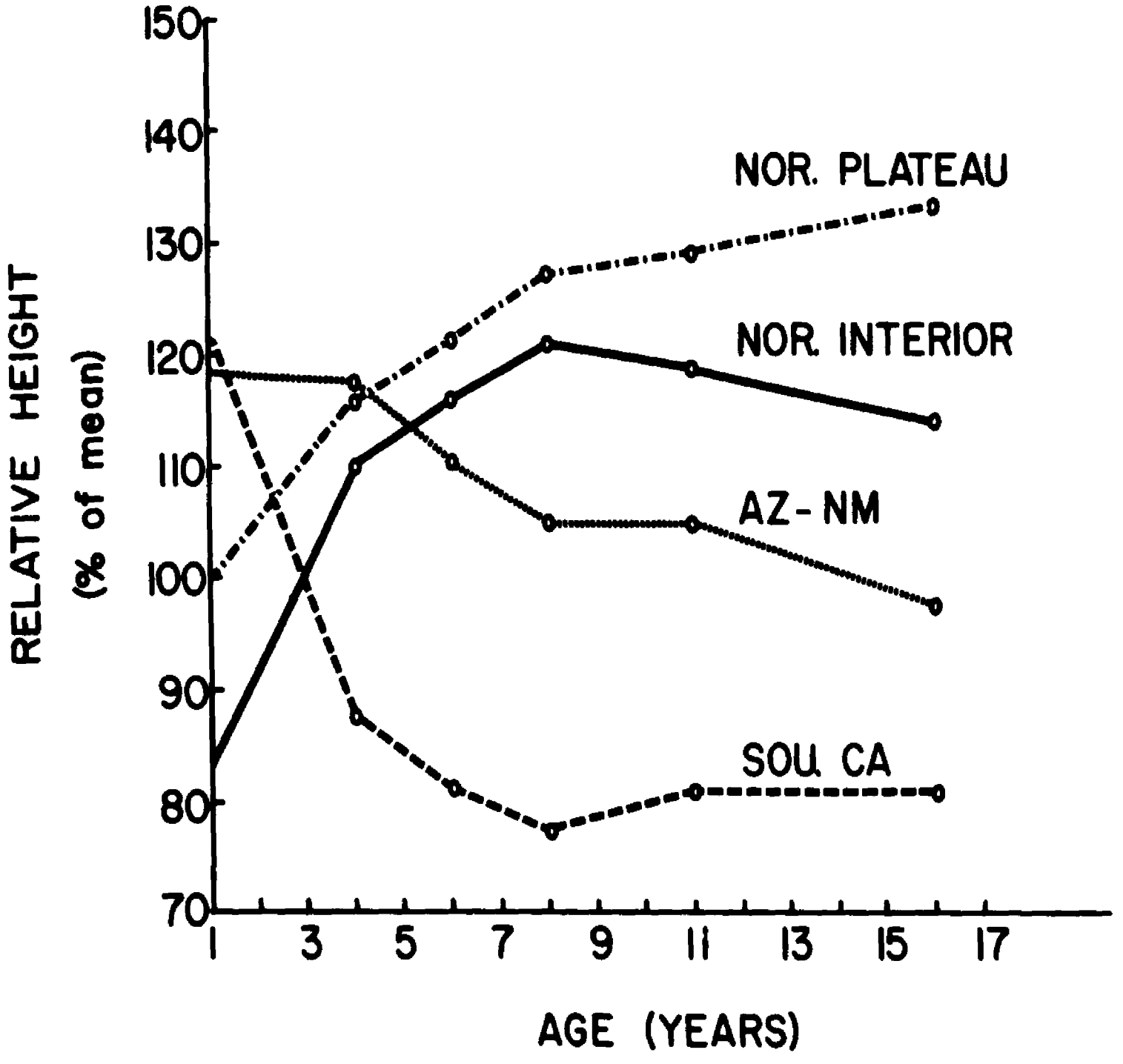

150
140
130
120
110
100
90
80
70
RELATIVE HEIGHT
(% of mean)
NOR. PLATEAU
NOR. INTERIOR
AZ-NM
SOU. CA
1
3
5
7
9
11
13
15
17
AGE (YEARS)

TABLE 10. Age-Age correlations for height among 55 provenances of ponderosa pine at two test plantations in southern Michigan.

Plantation Site	Correlation between height at age 16 and at age									
	1	2	4	5	6	7	8	9	10	13
Kellogg	-.31	.08	.56	.57	.73	.78	.85	.91	.93	---
Russ	-.07	.07	.49	---	---	.65	---	---	---	.97

northern Plateau trees suffered moderate damage. Trees from all other origins suffered little or none. The effects of cold on California trees were so severe that they could no longer maintain their rapid growth rate after age one. Arizona-New Mexico trees recovered sufficiently to continue their rapid growth rate through age four. However, repeated injury in subsequent years in the test plantations resulted in a gradual decline in growth rate. Northern Plateau trees suffered little in the field and became the fastest growing trees in both plantations. Northern interior trees emerged as relatively rapid growers after field planting primarily because of their high degree of cold resistance.

Among the injury-free northern seedlots, height rankings were consistent from year to year. The seedlots which were tallest at age one were tallest thereafter. However, among the heavily damaged California and Arizona-New Mexico seedlots, ranking varied greatly with age. Within the southern California ecotype, the three tallest seedlots at age one, were near average at age two, and among the shortest at age 16.

Thus in the absence of winter injury, age-age correlations in ponderosa pine are expected to be high.

Changes in variance components due to ecotype and seedlot within ecotype

Wells (1964) described eight geographic ecotypes of ponderosa pine, basing his descriptions on nursery measurements. Wright et al (1969) and Kung and Wright (1972) followed the same classification. I did, too, because Wells' original grouping of seedlots into ecotypes was as valid at age 16 as at age 3.

Northern ecotypes maintained the same growth rates as in early years whereas southern ecotypes suffered repeated winter injury and severe

reductions in growth rate. They were shortest at age 16. Thus, the range in ecotype means has increased (Table 11). This increase caused an increase in the proportion of the total genetic variance due to between-ecotype differences (Table 11). At age four, those differences accounted for 67% of the total genetic variance. That ratio increased to 76% by age eight and remained nearly constant thereafter.

Changes in error variance with age

One might expect that repeated winter injury would cause an increase in error variance and, thus, alter experimental precision. To investigate this relationship, I computed coefficients of variation for height measurements made at all ages. These are given in Table 12. The coefficients decreased from age one to two in the nursery despite severe injury the first winter. Apparently, the winter injury suffered on the nursery was so uniform within seedlots as to counteract any increased variability in growth rate from age one to age two.

Coefficients of variation in the test plantations seemed to change in relation to average growth rate rather than winter injury. After field planting, trees grew relatively slowly for the next two years (Table 12) and coefficients of variation increased sharply. Transplanting shock undoubtedly contributed to slow and erratic growth. These effects lessened by age five at Kellogg Forest but not until after age seven at Russ Forest. Thereafter, the variation coefficients declined gradually as average growth rate increased, similar to the finding of Namkoong and Conkle (1976). Winter injury did not seem to effect error variances in the test plantations.

Height differences among seedlots were larger at Kellogg Forest than at Russ Forest as indicated by the higher F values (Table 12).

TABLE 11. Variation with age in range of ecotype means and proportion of total genetic variance due to ecotype and seedlot within ecotype.

Age	Range In Ecotype Means	Proportion of total genetic variance due to	
		Ecotype	Seedlot within ecotype
years	%	%	%
4	35	67	33
5	40	70	30
7	--	73	27
8	50	76	24
9	49	76	24
16	52	75	25

TABLE 12. Changes in the coefficient of variation (C.V.), F value among seedlots, and plantation mean height with age in the nursery and two permanent test plantations.

Site	Pertinent Statistics	1	2	4	5	7	8	9	10	13	16
Nursery	C.V. (%)	15.9	12.3	---	---	---	---	---	---	---	---
	F value	---	---	---	---	---	---	---	---	---	---
	Mean Ht. (cm)	5	16	---	---	---	---	---	---	---	---
Kellogg	C.V. (%)	---	---	19.3	17.1	16.5	17.7	15.3	13.7	---	14.5
	F value	---	---	9.6	11.4	8.9	9.0	10.5	10.7	---	9.5
	Mean Ht. (cm)	---	---	28	50	107	131	177	222	---	490
Russ	C.V. (%)	---	---	18.2	---	19.6	---	---	---	14.9	14.4
	F value	---	---	4.4	---	4.3	---	---	---	5.8	5.5
	Mean Ht. (cm)	---	---	38	---	88	---	---	---	397	520

This was true even though error variances were the same. The larger differences at Kellogg Forest are probably related to the more severe winter injury at that site which tended to maximize genetic differences in height. Winter injury was evident after many winters at Kellogg Forest, but only after the fourth and seventeenth growing seasons at Russ Forest.

CHAPTER 6

IMPROVED PONDEROSA PINE FOR MICHIGAN

INTRODUCTION

Those familiar with the western United States, quickly develop an appreciation for the size and beauty of ponderosa pine. It grows naturally in every state west of the plains and also in parts of Canada and Mexico. This is the most widely distributed species of pine in North America, found from near sea level in the Pacific Northwest to 10,000 ft in Arizona. Its tall straight boles, orange platy back and rich yellow-green foliage frame many of the grandest views associated with the rugged western terrain.

In addition to its aesthetic value, ponderosa pine is a valuable timber tree. Within its natural range, ponderosa pine furnishes more timber than any other pine and is the mainstay of the economy for many areas.

Ponderosa pine grows rapidly, achieves large size and is very adaptable. Trees over 200 ft tall and 8 ft in diameter in the Sierra Nevada Mountains are not uncommon. For these reasons, the species has attracted interest from foresters in the central and eastern United States. It has been used commonly as a shelterbelt tree in the Great Plains, as an ornamental farther east, and is being tested for use in

strip mine reclamation in Pennsylvania (Davidson, 1977). Ponderosa pine also appears to have potential as a timber tree in the East. For example, a 29 year-old stand in East Lansing, Michigan has outgrown native hard pines planted near it and averaged 45 ft tall and 8.5 in in diameter.

Ponderosa pine is an extremely variable species genetically. Trees grown from seed collected in some areas grow three times as fast as seed collected other places. Some seed sources suffer severe damage from winter cold, others are hardy to even the most extreme cold. Also, trees from some areas have long needles, while others have relatively short needles similar to those found on Austrian pine (Pinus nigra). Thus, the success and value of ponderosa pine plantations in the eastern United States depends as much on choosing the proper seed source as on site selection and cultural practices. Work reported in this chapter was undertaken to provide Michigan nurserymen with practical information on the best seed sources to use for timber and ornamental plantings.

MATERIAL AND METHODS

In 1959, Dr. O. O. Wells (presently with the USFS, Southern Experiment Station) received seed collected from 60 natural stands of ponderosa pine located throughout its natural range. Each stand was represented by a bulked seed collection from several average trees which will hereafter be referred to as a seedlot. Information concerning locality of origin as well as climatic data from parental stands accompanied each seedlot. Collection was accomplished through the courtesy of R. Z. Callaham of the U. S. Forest Service.

In May, 1960, the seed was sown in Michigan State University's experimental nursery in East Lansing. The trees were transferred as 2-0 stock to two replicated plantations in 1962.

The two plantations are located 60 miles apart in southwestern Michigan. The W. K. Kellogg Forest plantation is located in Kalamazoo county and contains 57 seedlots and 7 replications. The Fred Russ Forest plantation is located in Cass County and contains 55 seedlots and 5 replications. The trees were planted in a 8 × 8 ft spacing in six tree plots. Weed control consisted of applications of simazene and aminotriazole for three years at the Kellogg plantation and one year at the Russ plantation.

Measurements were made at age one in the nursery and have continued through age 17. Height has been measured at frequent intervals, as has mortality and injury from winter cold. Other traits measured one or more times include bole form, disease damage, time of bud burst, foliar nutrient content, diameter, amount of forking, foliar moisture content, and susceptibility to damage from artificial freezing. Many of the

measurements made through age two and age eight have been reported on previously by Wells (1964) and Wright et al (1969).

Each set of measurement data was subjected to analysis of variance, using plot means as items. Also, simple correlations were calculated for some traits, using seedlot means as items.

THE VARIETIES AND ECOTYPES

For convenience, I used the subdivision of ponderosa pine into varieties and ecotypes as delineated by Wells (1964).

Wells recognized two varieties, as described previously by taxonomists. They are west coast var. ponderosa and interior var. scopulorum. The west coast variety includes the ponderosa pine from western Montana, Idaho, Washington, Oregon, and California. The interior variety includes ponderosa pine from the Rocky Mountain area.

The separation of ponderosa pine into two varieties has a biological foundation. The two differ in several respects and in some traits there is no overlapping. The chief ways in which they differ are summarized in the following list.

As compared with the Interior variety, the West coast variety has:

Greener and longer needles

Erect cone prickles

Browner and more loosely appressed bud scales

Higher foliar concentrations of N, K, P, Ca, and B

A more cylindrical stem form

Higher foliar moisture content

Greater palatability to jack rabbits in Nebraska.

Wells subdivided the varieties into ecotypes or races, based mainly on growth traits such as growth rate and winter hardiness. He delineated his ecotypes in such a manner that differences among ecotypes were large compared to those within ecotypes, at least in most traits. I use his classification partly as a matter of convenience, because it is much easier to understand tables giving the means for eight ecotypes than for all 60 seedlots included in the experiment.

RESULTS

Mortality

Mortality by the end of the 1975 growing season was 43% and 40% for Kellogg and Russ Forests respectively. Mortality rates were low (13%) for the Northern Plateau ecotype and moderate or high for the others (Table 13). Although the cumulative mortality in the two plantations was similar at age 16, the rate at which mortality occurred was different. At Russ Forest nearly all deaths occurred during the first two growing seasons. At Kellogg Forest, where initial mortality was relatively low, death of trees has continued every year since age four, especially for trees of California and Arizona-New Mexico origins. This continued mortality at Kellogg Forest is probably due to repeated winter injury at that plantation.

Growth rate

Height. Height data for the Kellogg and Russ Forest plantations were averaged and are presented in Table 14. At age 16, average heights were 16 and 17 ft at the Kellogg and Russ Forest plantations, respectively. In both plantations, trees grown from seed collected in the Northern Plateau (Oregon, Washington, British Columbia) grew fastest (Table 14). They averaged 22 ft tall at both plantations at age 16. Trees grown from seed collected in the Northern Interior (central Montana, South Dakota, Nebraska) and coastal Oregon were also above average at both plantations. Northern Interior trees averaged 19 ft and 18 ft, whereas coastal Oregon trees averaged 17 ft and 21 ft at Kellogg and Russ Forests respectively. Slowest growing trees were grown from seed collected in southern California, they averaged 13 ft and 15 ft in the two plantations.

TABLE 13. Mortality and mortality increments of 16 year-old ponderosa pine ecotypes growing at Kellogg and Russ Forests in southwestern Michigan.

Variety and Ecotype	Kellogg Forest		Russ Forest	
	Mortality (1975) %	Mortality Increment[a] %	Mortality (1975) %	Mortality Increment[a] %
var. ponderosa				
Nor. Plateau	14	6	12	0
Coast. OR	58	9	67	0
Nor. CA	49	28	42	0
Sou. CA	68	37	44	8
var. scopulorum				
Nor. Interior	40	11	45	0
Nor. CO-UT	32	10	34	2
Sou. UT-NM	39	17	36	3
AZ-NM	45	19	43	5
Mean	43	17	40	2

[a] Mortality increment is expressed as % mortality in 1975 minus % mortality in 1964.

TABLE 14. Growth rate and bole form of ponderosa pine ecotypes.

Variety and Ecotype	Relative height at age 2	Relative height at age 4	Relative height at age 16	Relative diameter age 16	Ratio[a/] Mid-diam. / basal diam.
	- - % of experiment mean - - -				
var. ponderosa					
Nor. Plateau	96	113	130	128	.62
Coast. OR	67	86	113	124	.64
Nor. CA	104	88	98	98	.65
Sou. CA	90	85	83	86	.65
var. scopulorum					
Nor. Interior	96	111	110	99	.54
Nor. CO-UT	81	83	91	86	.59
Sou. UT-NM	106	104	100	103	.55
AZ-NM	148	114	99	101	.56
Average (cm)	16	34	505	16.2	

[a/] Data from LaFarge, 1971

Relative growth rates have changed with time (Table 14). The responsible factor has probably been differential winter injury. Southern California trees were tallest at the end of the first growing season but suffered so much from cold the first winter as to become among the shortest by age two. They continued to decline in growth rate with age. Trees from Arizona and New Mexico suffered slight winter injury in the nursery and more severe winter injury in later years. Thus, although recommended for planting in Michigan at age two, they were no longer among the leaders at age 16. Northern Plateau and Northern Interior seedlots emerged as relatively rapid growers in the plantations primarily because of their high degree of cold hardiness.

Diameter. The data on diameter (measured 1 ft above ground) at age 16 are summarized in Table 14. In general, diameter differed among ecotypes in much the same way as height. Fast growing Northern Plateau trees had the largest diameters (8.6 in and 8.0 in the two plantations), and slow growing southern California and northern Colorado-Utah trees had the smallest diameters. Trees from the Northern Interior ecotype were an exception to this generality. Although among the tallest trees in both plantations, they were below average in diameter. Thus, Northern Interior trees produce a lesser volume of wood than Northern Plateau and Coastal Oregon trees of the same height.

Stem taper

LaFarge (1971) measured stem form in the Kellogg Forest plantation as the ratio of mid-diameter to basal diameter. He found large differences between varieties in stem taper, but no differences within varieties (Table 14). Trees from the western variety were most nearly cylindrical. This strong variety difference was evident despite considerable variation

in height and diameter growth within each variety. Thus, fast growing seedlots from the Northern Plateau had the same taper as slow growing trees from southern California.

Fast growing trees with a high taper ratio are likely to produce more wood than equally fast growing trees with low taper ratios. Trees producing the greatest volume of wood in this study are those grown from seed collected in the Northern Plateau.

Winter injury

Winter injury was first noted at age one in the nursery and has continued to be evident in many years since plantation establishment. Injury appears as needle discoloration during winter and ultimately, as needle browning by spring. Little damage has occurred to buds or cambium and few trees have been killed immediately, but many succumbed after repeated needle damage.

Winter injury estimates in the nursery and plantations are summarized in Figure 6. In the western variety, Northern Plateau trees have been damaged slightly, coastal Oregon trees have suffered moderate damage and California trees have suffered severely. In the interior variety, trees from Colorado, Utah and northward have suffered no damage, whereas trees from Arizona and New Mexico suffered moderate damage.

In order to learn more about the cause of winter injury in ponderosa pine, controlled laboratory freezing studies were performed. Needles were subjected to freezing temperatures at various times of year and damage was measured by electrical conductivity tests. The results are summarized in Table 15. In July, all trees were equally hardy, their needles suffering damage at -5.5°C. Subsequently, however, trees from northern regions acclimated to cold faster than those from southern

TABLE 15. Seasonal differences in cold hardiness of needles among ponderosa pine ecotypes.

Variety and Ecotype	Temperature (°C) at which needle damage occurred on 9/17/76	10/28/76	12/17/76	1/10/77
var. ponderosa				
Nor. Plateau	-7.9	-19.2	-31.1	-38.9
Coast. OR	-6.7	-14.7	-27.1	-30.9
Nor. CA	-6.7	-12.6	-20.9	-24.1
Sou. CA	-6.7	-12.0	-19.8	-22.2
var. scopulorum				
Nor. Interior	-10.7	-22.0	-39.0	-46.6
Nor. CO-UT	-9.1	-21.2	-34.9	-41.6
Sou. UT-NM	-8.0	-20.0	-31.4	-35.0
AZ-NM	-7.2	-17.0	-30.2	-31.7

regions and achieved a greater degree of hardiness by midwinter. Trees from all ecotypes, except northern and southern California, seemed to achieve sufficient midwinter hardiness to tolerate the minimum temperatures recorded at Kellogg and Russ Forests. However, due to a slow rate of cold acclimation, trees from Arizona and New Mexico are susceptible to cold injury in years when low temperatures occur in late November or early December. That happened on December 3, 1976, when the temperatures dropped to -26°C at Kellogg Forest. Field observations made on December 17, 1976, revealed severe damage on California trees and moderate damage on coastal Oregon and Arizona-New Mexico trees. Trees from other regions escaped winter injury. During the winter of 1975-76 the lowest temperature recorded was -27°C in mid January. By this time, coastal Oregon and Arizona-New Mexico trees had achieved sufficient hardiness to tolerate such temperatures and only California trees suffered damage. Thus, timing of cold appears to be an important factor controlling plant susceptibility to low temperature injury.

Disease damage

Twig dieback, caused by a combination of two fungal diseases, was noticed at the Kellogg Forest plantation during summer, 1975. Dr. John Hart (forest pathologist of Michigan State University) thought that the responsible fungi were *Diplodia pinea* and *Cenangium ferruginosum*. *Diplodia* causes dieback of the current season's growth of hard pines. It is frequently serious on Austrian pine, but caused little damage to the ponderosa pine. *Cenangium*, also called "pruning disease", characteristically starts on lower branches and gradually progresses upward. It is often considered to be a secondary pathogen which causes most damage to trees which have been physiologically weakened by adverse weather

conditions. The fungus has been associated with extensive flagging injury on ponderosa pine in the southwest (McKenzie et al, 1948) and with crown dieback on Austrian pine at Kellogg Forest (Wheeler et al, 1976). It has not been a problem on either Austrian or ponderosa pine at Russ Forest.

It proved difficult to separate winter injury from disease damage, and even more difficult to recognize damage from the two fungi. Thus, damage estimates made in 1977 were for both diseases combined.

In general, disease damage was proportional to the amount of winter injury. Northern trees suffered much less (8 to 12% of twigs damaged) than the nonhardy types from California, Arizona and New Mexico (28 to 30% of twigs damaged).

Although the diseases seemed to cause only secondary damage in the provenance test plantation, there was also minor disease damage evident in older plantations of ponderosa pine at Kellogg Forest. These plantations are 39 years old and contain trees that are believed to be of northern origin. There has been no visible winter injury in these plantations, yet a few trees have suffered serious damage and were killed from what appears to be Cenangium.

Stem straightness

Ponderosa pine has the inherent capacity to grow straight. Crooks and forks will develop, however, if the terminal bud or leader is damaged or suppressed. If such damage occurs, the leading shoot is replaced by lateral branches and crooks or forks develop part way up the stem. Depending on the severity of the damage, crooks may either be overgrown within a few years or may persist resulting in a permanently malformed tree which is useless from the timber standpoint.

Stem forking was induced by some unknown agent between the eleventh and fourteenth growing season at both ponderosa pine plantations. I counted the number of trees with forks at age 17. At that time, 36% and 14% of the trees were forked at Kellogg and Russ Forests, respectively.

The geographic pattern in susceptibility to forking was similar for both plantations (Table 16). Trees with the highest propensity to forking were grown from seed collected in Arizona and New Mexico, Northern Interior and coastal Oregon. In the Kellogg and Russ Forest plantations, 69% and 30% of the Arizona and New Mexico trees had forks. Arizona and New Mexico trees not only had the highest percentage of forks, but also the most severe forks (Figure 12). Most of these trees will be permanently malformed. Fast growing Northern Plateau trees and trees from southern Utah parentage were intermediate in forking. The shortest trees, from California, northern Colorado and Utah, had the fewest number of forks.

Generally, trees above average in height at the time of leader damage were most prone to forking, while the shortest trees were most resistant. Such a pattern suggests that an insect which selects the tall trees may be the causal agent. However, since the tallest trees from the Northern Plateau ecotype suffered only intermediate forking, it is likely that differential resistance among ecotypes also exists.

From the standpoint of timber production, trees grown from seed collected in the Northern Plateau gave the most satisfactory growth rate, cold hardiness and stem straightness at both plantations. Most crooked trees from the ecotype would be removed in an early thinning, leaving more than enough straight trees to yield a final stand of high quality sawlogs. Northern Interior trees, which are also fast growing

TABLE 16. Genetic differences among ecotypes in susceptibility to stem forking at Kellogg and Russ Forest plantations.

Variety and Ecotype	Proportion of forked trees at:	
	Kellogg Forest	Russ Forest
	- - - - - - - - - - % - - - - - - - - - -	
var. ponderosa		
Nor. Plateau	34	13
Coast. OR	40	20
Nor. CA	16	8
Sou. CA	22	5
var. scopulorum		
Nor. Interior	48	22
Nor. CO-UT	19	11
Sou. UT-NM	35	15
AZ-NM	69	30
Mean %	36	14

FIGURE 12. Severe stem crook causing permanent damage to a tree o
New Mexico origin growing at the Kellogg Forest planta

and cold hardy, suffered too much forking at Kellogg Forest to produce a fully stocked stand of quality timber trees.

Stem straightness is not as important in ornamental plantings as in timber plantings. Most growers prefer trees that are relatively fast growing and picturesque for ornamentals. Thus, severity of crooks or forks is a more important consideration than the number of crooks. Stem crooks in most Northern Plateau and Northern Interior trees were minor and unimportant from the aesthetic standpoint; they could not be seen at distances greater than 10-15 ft from trees. On the other hand, trees from Arizona and New Mexico had such severe crooks that they would be useless as ornamentals as well as timber trees.

Leaf length

There are large differences in needle length in ponderosa pine. Trees from the coastal variety were uniform in having the longest needles regardless of ecotype. They had needles ranging from 7.0 to 8.0 in in length. In the interior variety, northern seed sources had the shortest needles, ranging from 4.5 to 5.5 in long. Trees from Arizona, New Mexico, and southern Utah were intermediate in leaf length having needles from 6.0 to 7.0 in long.

Needle lengths were so uniform within the coastal variety and the interior variety ecotypes, that it represents a good criteria for identifying ponderosa pine varieties and ecotypes in provenance test plantations. For instance, long needled trees which are fast growing in Michigan clearly belong to the northern Plateau ecotype, whereas short needled trees which are fast growing can be unmistakeably identified as belonging to the Northern Interior.

Leaf length may be an important consideration for those wishing to grow ponderosa pine as an ornamental. Generally, long needled trees are

more picturesque and are considered more desirable for ornamental or roadside plantings. If the longest possible needles are desired, one can combine fast growth with long needles by growing trees from seed collected in the Northern Plateau.

Cone production

Cone production was first noticed at age 13 at Kellogg and Russ Forests and has been confined almost exclusively to trees grown from seed collected in the northern Rocky Mountains. Of 121 trees which produced cones at age 13 or 16, 110 of them were grown from seed collected in the Northern Interior or northern Colorado and Utah. At age 16 at Kellogg Forest, each fruiting tree had an average of 24 cones. Cone production has been very light on fast growing seedlots from the Northern Plateau.

PRACTICAL RECOMMENDATIONS

Choosing the proper seed source is the most important consideration for those wishing to grow ponderosa pine in Michigan. Winter injury has been so severe on trees from some areas that seed dealers and nurserymen must specify exact locations when requesting seed.

In general, the best regions of seed collection for timber or ornamental plantings in southern Michigan are the Northern Plateau (Oregon, Washington, British Columbia, and western Montana) and Northern Interior (central Montana, South Dakota, and Nebraska) regions. Trees grown from seed collected in the Northern Plateau have grown fastest under Michigan conditions and have not been damaged by winter cold. They also have the longest needles, the least mortality and are most resistant to diseases. Trees grown from seed collected in the Northern Interior are also fast growing, have short needles, and are the most cold hardy of all trees tested. However, they seem somewhat prone to forking and have relatively slow diameter growth.

Although the Northern Plateau and Northern Interior generally produced the best trees for Michigan, there were large enough differences in growth rate among trees within these regions to warrant selection of particular desirable stands. Pertinent information on the location of the best stands is provided in Table 17. Seedlot Nos. 2102 (Washington) and 2034 (Oregon) were 17% taller at age 16 than some other seedlots from the Northern Plateau. Within the Northern Interior, seedlot Nos. 2095 (Montana) and 2180 (Nebraska) were tallest by 24% at age 16 in both Michigan plantations.

TABLE 17. Location of the two best stands of ponderosa pine in the Northern Plateau and Northern Interior regions based on performance in two southern Michigan plantations.

Seedlot No.	State	Ecotype	Latitude	Longitude	County	Elevation
2102	WA	Nor. Plateau	48°46'	118°07'	Stevens	1400 ft
2034	OR	Nor. Plateau	44°16'	120°26	Crook	5000 ft
2095	MT	Nor. Interior	45°15'	108°28'	Horn	4500 ft
2180	NE	Nor. Interior	42°45'	99°32'	Rock	2100 ft

By using seed collected from these selected areas, growers can expect growth rates at least as good as that of other hard pines recommended for planting in Michigan. Dr. Donald Dickmann (Department of Forestry, Michigan State University) made growth measurements in four 39 year old hard pine plantations of unknown seed origin at Kellogg Forest. The results are tabulated below. Ponderosa pine was comparable in growth rate to the other three pines that are commonly planted as ornamentals in southern Michigan. Ponderosa pine has other desirable characteristics as well. For instance, it has longer needles than Scotch pine, outgrows red pine on many southern Michigan sites, and appears to be less plagued by disease problems than Austrian pine. Ponderosa pine represents the best choice of the long needled pines for ornamental plantings in southern Michigan.

	Growth of dominant trees	
Species	Height	Diameter
	ft	in
Ponderosa pine	58	12.9
Red pine	57	10.8
Austrian pine	62	12.3
Scotch pine	67	12.3

Seed source selection is the most important but not the only factor to consider when growing ponderosa pine in Michigan. To obtain best results good seed quality, site selection, and care after planting are necessary. If planted on good sites and given good initial weed control,

trees grown from seed collected in the Northern Plateau will become excellent ornamental or forest plantings in southern Michigan.

CHAPTER 7

SUMMARY

A rangewide provenance test of ponderosa pine was established in 1960 and includes two replicated plantations in southern Michigan. From 1975 to 1977 genetic variation in several aspects of cold hardiness and other economically important traits was studied.

Foliage samples were collected from 30 seedlots on 17 different dates from October, 1975 to January, 1977. Freezing tests were performed on needles and tissue damage was assessed using electrical conductivity. Critical temperatures, defined as the highest temperature at which cold damage could be detected, were determined for each seedlot. A procedure for computing such temperatures is described. On six additional dates percent foliar moisture content was determined. Field observations of winter injury were made during several years. Other traits measured were time of leafing out, foliar drying rate from excised twigs, height, and susceptibility to two fungal diseases.

Trees grown from seed collected in British Columbia, Washington, Montana, and Nebraska acclimated to cold fastest, had the lowest critical temperatures in midwinter (-40 to -47°C), suffered little or no cold or disease damage, leafed out earliest, had relatively low foliar moisture and slow drying rates and were among the tallest trees at age 16 (averaged 20.5 ft tall). Trees from northern Colorado and Utah were

similar in several respects but were only 14-15 ft tall at age 16. Trees grown from seed collected in California acclimated to cold slowest, had the highest critical temperatures in midwinter (-22 to -24°C), suffered severe cold and disease damage, leafed out latest, had high foliar moisture, and fast drying rates, and were among the shortest trees at age 16 (averaged 14 ft tall). Trees grown from seed collected in Arizona and New Mexico had critical temperatures of -32°C, were 16.5 ft tall at age 16, suffered severe disease damage, had relatively slow foliar drying rates, and were intermediate in cold damage, time of leafing out and foliar moisture content.

California trees suffered severe cold damage most years because they did not achieve a sufficient depth of hardiness to withstand Michigan winters. In contrast, Arizona, New Mexico, and coastal Oregon trees were sufficiently hardy in midwinter to avoid cold damage. However, they suffered needle injury in years when low temperatures occurred in early winter, before they attained maximum hardiness. Trees from northern origins avoided damage because they acclimated to cold quickly and became very hardy in midwinter.

Winter desiccation was considered as a possible source of winter injury in ponderosa pine. However, the relationship between foliar drying rates and winter injury was not strong. Trees from Washington and British Columbia lost water more rapidly in winter than trees from Arizona and New Mexico but suffered no cold damage. Coastal Oregon trees dried out the fastest, but suffered less cold damage than California or Arizona trees. Thus, it appeared that desiccation was less important than cold temperature in causing damage to ponderosa pine.

Time of leafing out appeared to be related to cold hardening and dehardening. Trees from Washington, British Columbia, Montana, and Colorado hardened to cold fastest in fall, dehardened most rapidly in late winter, and leafed out 10-14 days earlier than trees from southern origins. Despite differences in leafing out phenology, spring frost damage was not a problem in ponderosa pine.

Foliar moisture content varied seasonally as well as among seedlots, decreasing by 15-16% from summer to winter. In general, trees with high moisture content were most susceptible to cold damage. This relationship seemed to hold from summer to winter and also from north to south within the species. Apparently, trees with high foliar moisture content had more intracellular water and thus more water to freeze and cause damage.

Winter injury had an effect on growth rate and age-age height relationships in ponderosa pine. Trees grown from seed collected in California, Arizona, and New Mexico were tallest at early ages, but lost their growth superiority because of repeated winter injury. By age 16, hardy trees from British Columbia, Washington, Montana, and Nebraska were tallest, Arizona and New Mexico trees were average, and California trees were shortest. Repeated winter injury to southern trees, also resulted in an increase in the magnitude of height differences among ecotypes from age four to eight. Error variances decreased gradually with age as average growth rate increased, but were apparently unaffected by winter injury.

Susceptibility to damage from two fungal diseases appeared secondary to winter injury. Trees which were physiologically weakened by repeated cold damage suffered the most twig dieback from disease. Trees which suffered little or no cold damage were least affected by disease.

Other traits studied included mortality leaf length, cone production, stem taper, diameter growth, and incidence of stem forks. Trees grown from seed collected in the Northern Plateau (Oregon, Washington, and British Columbia) were most desirable in all respects. In addition to being the tallest and among the most cold hardy, they had the largest diameters (8.5 in at age 16), the least mortality, the longest needles (7.5 to 8.0 in long), the most cylindrical boles and suffered only a moderate amount of forking. Trees grown from seed collected in the Northern Plateau are recommended for forest and ornamental plantings in southern Michigan.

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