GENETIC VARIATION IN FOLIAR MOISTURE CONTENT AND RATE OF MOISTURE LOSS AMONG INTERIOR RACES OF DOUGLAS-FIR

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ABSTRACT

GENETIC VARIATION IN FOLIAR MOISTURE CONTENT AND
RATES OF MOISTURE LOSS AMONG INTERIOR RACES OF
DOUGLAS-FIR

By

Donald DeHayes

Douglas-fir is a valuable Christmas tree and ornamental throughout much of western and northern United States. Through the use of provenance tests, the genetic variability of the species has been determined. These tests have yielded useful information as to the fastest growing and most suitable races for specific planting purposes. My objectives were to determine the genetic variability in foliar moisture content and foliage drying rates among interior races of Douglas-fir and to learn the evolutionary significance of such variation.

Seeds were collected in 1961 from various parts of the species' natural range and were sown in Michigan State University's experimental nursery. Four permanent test plantations containing 68 seedlots were established between 1965 and 1967 in Michigan. During the summer of 1973, I collected current-growing and year-old foliage from trees growing at the 4 provenance plantations in Michigan. I determined foliar dry content for each 4-tree plot. Analyses of variance were performed to determine the significance of differences in dry matter contents.

In a supplementary study, I compared rates of moisture loss from Arizona and Inland Empire seedlots. Branches were removed from 2 seedlots of these races and were dried under normal room conditions for 27 days. Moisture loss was determined by periodically weighing the branches. The significance of differences were tested by an analysis of variance and t-test.

The largest differences in moisture content were found between current-growing and year-old foliage. The year-old foliage had about 10% higher dry matter content. Although current and year-old foliage differed in moisture content, the relative trends among the races were similar.

One seedlot from slightly east of the White Pass in central Washington had the lowest foliar dry matter content, 29.6% and 38.7% for current and year-old foliage respectively. This seedlot probably represents an area of overlap between the coastal and interior Douglas-fir varieties. It owes its high moisture content to its affiliation with the coastal variety.

The Arizona Douglas-fir seedlots had the next lowest dry matter content. They averaged 32.2% and 42.5% dry matter content for current growing and year-old foliage respectively. Trees with the highest dry matter content were from central Washington and the Inland Empire. They averaged 3-4% less moisture than the Arizona trees. A study in Oregon also showed that Arizona seedlots had the highest moisture content of interior Douglas-fir seedlots.

The Arizona trees also lost moisture at a significantly slower rate than the Inland Empire. The rates and differences in moisture loss were greatest during the first 2 days of drying. At this time, Arizona branches had lost 17% of their fresh weight, while the Inland Empire branches lost 30% of their fresh weight. Differences in rate of drying were clearly reflected in foliage appearance. Inland Empire branches turned brown more rapidly.

Differences in drying rates may be related to waxes which coat the needles of Arizona Douglas-fir trees. My results indicate that the waxy surface of the tree leaves may be of selective value in retarding moisture loss from the Arizona trees. Natural selection may also have played a role in promoting racial differences in foliar moisture content. Selection has probably resulted in two modes of adaptation. In the hot dry southern Rockies, selection has resulted in Arizona trees with high moisture content as well as slow rates of moisture loss. In the colder northern Rockies, selection for high dry matter content may be an adaptation to colder temperatures at place of origin.

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CHAPTER 1

INTRODUCTION AND OBJECTIVES

Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) is one
of the most widely distributed conifers in western United
States and has great potential for planting. It can be planted
successfully in large portions of western and northern United
States. The extreme variation in foliage color and growth
rate, obtainable from several geographical seed sources,
allows the nurseryman, Christmas tree grower, landscape
designer, or homeowner to select the appropriate type for
his personal preferences or intended use.

Douglas-fir is recognized as one of the most important
timber trees in the world. It comprises about 50% of the
standing timber of western forests, and more than 25% of the
standing timber of the entire nation. In sawtimber volume
there is more Douglas-fir standing today than in any other
species. Of the 51 billion board feet of softwood cut for
the market in 1973 more than eight billion was Douglas-fir
(Wharton, 1974).

Douglas-fir is also an excellent Christmas tree and in
western United States is the most commonly used tree for that
purpose. Recently, geographic origin studies on interior
Douglas-fir have yielded useful information on its Christmas
tree possibilities in the East. The tree's soft, graceful

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foliage, its excellent needle-holding capacity, and its natural pyramidal habit are important factors contributing to its large potential in the Christmas tree market.

Variation within a species creates opportunities for locating better types, and raises problems in avoiding undesirable types. Foresters have found that it may be just as detrimental to use the wrong race of a species as to use the wrong species. The term provenance, as used in forestry, refers to the particular place where trees are naturally growing or to the place of origin of seeds or trees. Forest geneticists, by testing seed of different provenances, have been able to study genetic variability within species. The results of such tests have been the identification of genetically distinct types which are suited specifically to various environmental situations. Further study of specific provenances within the distinct types will, hopefully, furnish the evidence necessary to identify the most genetically superior seed sources.

Forest geneticists have recently established provenance tests to study the genetic variability among interior origins of Douglas-fir. These tests have largely been responsible for the identification of fast growing and hardy ecotypes, thus making it a feasible Christmas tree and ornamental possibility in much of the nation. The information obtained up until this point has been derived from genetic studies concentrated on growth rate, foliage color, and frost damage. These results have been of tremendous benefit to the growers

of southern Michigan; they have already been able to cut the rotation of Christmas trees in half. However, as more information on growth rate is gathered, it has become apparent that data on other tree characteristics were needed.

A sufficient supply of water is one of the most important factors enabling a plant to carry out its life processes and more than 70% of the protoplasm of actively functioning plant cells is made up of water. A severe lack of water may result in death or extreme reduction in protoplasmic activity. In addition, the cell sap and cell walls of most living cells contain large amounts of water. In brief, water functions in a plant as a: (1) raw material for food manufacture; (2) solvent of gases, mineral nutrients, and food; (3) medium of transportation of raw materials and food; (4) a medium for maintaining turgor in living cells.

OBJECTIVES

The primary objectives of this study were to determine the amount of genetic variability in foliar moisture content of Douglas-fir and to learn the evolutionary significance of such differences. A secondary objective was to learn differences in drying rate of cut branches from two geographically and morphologically extreme Douglas-fir races.

As in most genetic studies, the ultimate goal was to learn the practical significance of existing differences. The effects of foliar moisture content on moisture loss, foliage appearance, and fire susceptibility are practical considerations important to christmas tree growers and the public alike.

CHAPTER 2

SPECIES DESCRIPTION

"Its somber shape, its serrated crowns and sharp
lancepoint tips and long swaying boughs become
printed like a lasting eidolon on all our memories
of the Pacific Northwest. And even in the desert
states of the Southwest we meet it again, on high
peaks, with gratitude for its dim, cool groves,
after the glare and heat of the rocky wastes below...
With experience one comes to recognize a Douglas-tree
in the field from almost as far as it can be seen...
The dense, compact crowns, the lusterless, dark-blue
green of the foliage; the darkly, deeply furrowed old
boles, the mast-like stems, and the grand down sweeping
of the boughs, all go to make the character of this
species. But one feature there is which is peculiarly
distinctive, and that is the way that numberless long
slender twigs clothed on a spiral of needles, hang
vertically from the branches. Though the general habit
of the tree is not what gardeners call weeping, these
long pendants have a sort of sorrowful grace. When
the summer winds blow lightly through the forest they
stir this shawl-like fringe in an idle, ferny way; when

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winter rains come driving through the forest, level
and endless from the storm bound Pacific, then these
long pennants lie out waving upon the gale in a way
that gives the whole tree a wild and streaming look."

A description of Douglas-fir by Donald Culross Peattie
(1956).

Douglas-fir was first discovered on the Pacific Coast
by Archibald Menzies in 1792. It received its common name
in the honor of the Scotch botanist, David Douglas, who first
sent seed to England. After being classified as a Pinus,
Abies, Taxus, Tsuga, and Picea, it was finally separated into
a new genus in 1867. It was given the scientific name
Pseudotsuga meaning "false hemlock". The genus is a member
of the Pinaceae, the largest family of conifers. Six species
are recognized:: four are native to various regions of China,
Formosa, and Japan and the other two are native to Western
North America. Of these six, Douglas-fir is the most familiar
and economically important.

Douglas-fir is mighty in stature. It towers up to
heights as great as 400 ft and may reach 17 ft in diameter.
Except for the Sequoias, it is the tallest tree in North
America. It is generally a long lived species as well. Ages
in excess of 500 years are common and many have exceeded 1000
years.

Douglas-fir typically grow into tall trees, having thick,
deeply furrowed, dark brown bark. Buds are long and narrow,

sharply pointed, and reddish-brown in color. The needle-like leaves are one inch long and are flattened and flexible with short petioles. The leaves range in color from yellow-green to bluish-green and are persistent up to 8 years. Male strobili are borne along the twigs, while ovulate strobili are terminal on short twigs. Cones are 3-5 inches long and easily identified by the distinctive three-pointed bracts protruding beyond the rounded cone scales. The narrowly shaped cones hang down from the tree, mature in one season, and persist on the tree into the following year. Seeds are long-winged and light reddish-brown in color and are often produced as early as 10 years. The number of cotyledons varies from six to twelve.

Douglas-fir consists of two widely recognized forms. The coastal form grows on the islands and mainland of the Pacific Coast, and the interior form is distributed through-out the Rocky Mountains. The north-south range of Douglas-fir extends over 3,000 miles from the southern half of British Columbia, through the Pacific Northwest and Rocky Mountains, and well into the higher elevations of Mexico (Figure 1). Its east-west range extends from eastern Montana and Colorado to the Pacific Coast.

Typically, Douglas-fir is found in mild humid regions with annual temperatures ranging from 40° to $65^{\circ}F$ with extremes of -30° to $110^{\circ}F$. Annual precipitations varies from 15 to 100 inches but the summers are usually dry. Douglas-fir have their best developments on soils of

Figure 1. Natural range of Douglas-fir (shaded area) and
            location of stands (dots) from which seed was
            collected and grown. Douglas-fir races as
            recognized by Wright et al. (1970) are illu-
            strated.
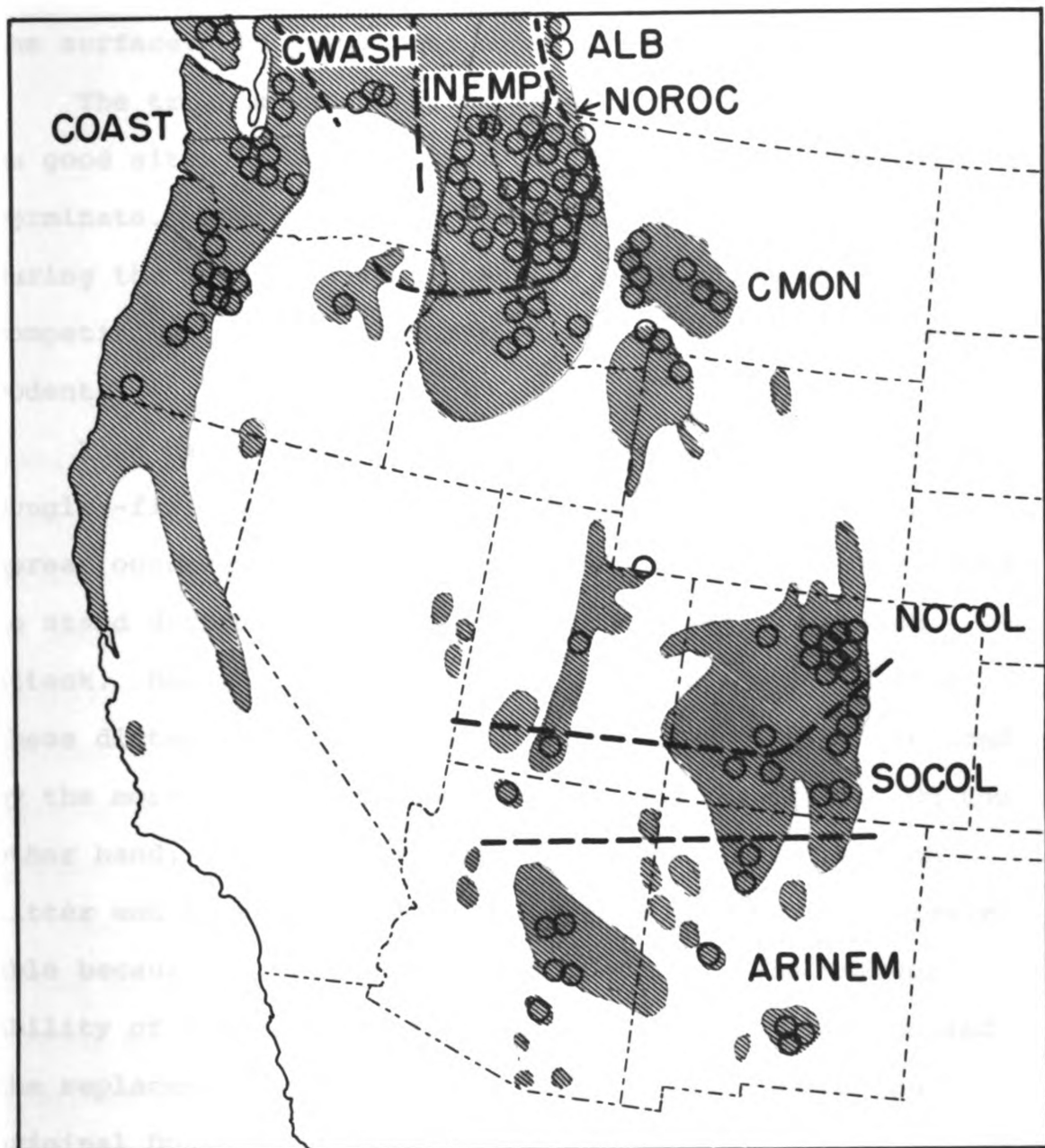
sedimentary and volcanic origin. They will not thrive on poorly drained soils or soils with an impervious layer near the surface. It grows best on soils with pH 5 to 5.5.

The tree is generally rated as a prolific-seeder, and on good sites, a large number of the viable seed will germinate. However, about 75% of the seedlings usually die during the first two years because of heat injury, drought, competition, frost, insect, root rot, dampening off, and rodents.

Fire has played a varied role in the regeneration of Douglas-fir. In the coastal varieties, the species widespread occurrence in even-aged stands is largely attributed to stand destruction by fire, clear cutting, or insect attack. However, it is believed, that if protected from these disturbances, Douglas-fir would gradually be replaced by the more tolerant hemlock, cedar, and true firs. On the other hand, since the interior trees regenerate best on litter and moist soil, soils blackened by fire are unfavorable because of high surface temperatures. The unfavorability of the burned over soil is believed to have caused the replacement by lodgepole pine of all but 5% of the original Douglas-fir in Colorado and Wyoming.

CHAPTER 3

REVIEW OF PAST LITERATURE

GENETIC VARIATION IN DRY MATTER CONTENT OF

SCOTCH PINE: A REVIEW OF LANGLET (1936).

The first and most extensive study on the genetic
variation in foliar dry matter content within a species was
undertaken by Langlet (1936). Langlet spent four years
(1929-1933) determining dry weights for 582 Swedish seedlots
of Scotch pine (Pinus sylvestris), and for several other
seedlots obtained throughout the natural range of the species.
Langlet's work deserves special recognition because of its
relevancy to my Douglas-fir study, and because of the physio-
logical implications which his data suggest.

Langlet's data, in general, suggest a strong latitudinal
trend in foliar dry matter content. Trees from northern
Sweden approached 40% dry weight, while southern Swedish
seedlots ranged from 31-32% dry weight (Figure 2). Northern
regions with few warm days provided trees with the highest
dry weights, i.e. the lowest moisture contents. Data from
the range-wide study follow the same patterns. Seedlots
from northern parts of Norway and Finland had 10-12% higher
dry weight percents than seedlots from Italy and Spain
(Table 1).

Figure 2. Variation in foliar dry weight percent of Scotch
pine in relation to temperature at place of
origin. Number of 6° (C) days is defined as the
number of days per year with mean temperatures
greater than 6° (C). Trees grown from seed
collected in regions with few warm days gener-
ally had higher dry weight percents. (Langlet,
1936).
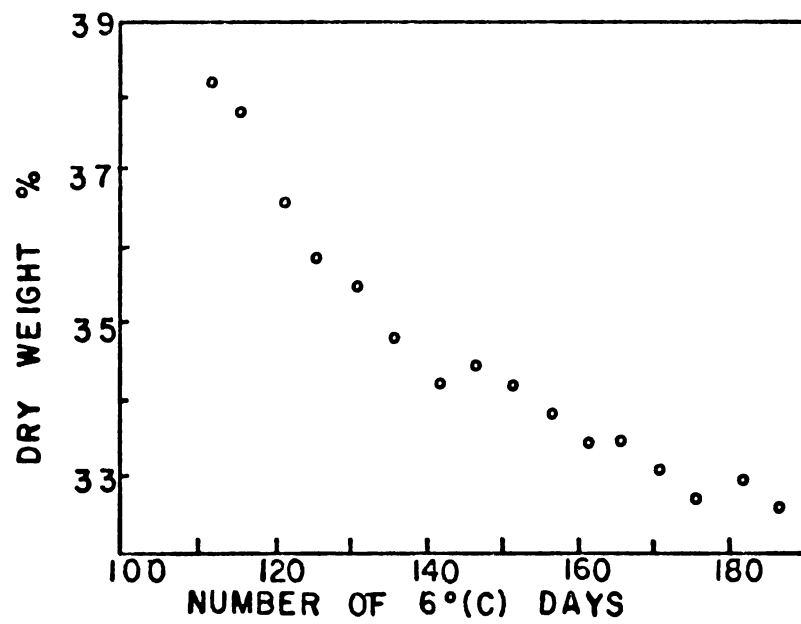
Table 1. Dry matter contents (% fresh weight) of Scotch
pine foliage from different parts of its natural
range (Langlet, 1936).

Country of Origin	# of Seedlots	Dry Matter Content
		% fresh weight
Finland	25	37.6
Norway	26	34.9
Russia	16	34.2
Scotland	3	30.1
Lithuania	7	32.2
Germany	3	31.7
Ukraine	7	30.3
Poland	11	29.7
France	3	29.3
Central France	1	29.0
Italy	1	29.1
Spain	2	27.0

Langlet also studied seasonal variation in dry matter content. His seasonal data showed the same relative latitudinal trend. Dry weights increased toward the end of the growing season, dropped during December, and reached maximums during early spring.

In an effort to explain the seasonal variation in dry matter content, Langlet determined the seasonal variation in foliar sugar (glucose) content of several Scotch pine seedlots. The seasonal pattern in sugar content paralleled closely the pattern for dry matter content. From fall to winter, the sugar content increased for both northern and southern seedlots. The consistent winter increase in sugar suggests a relation between sugar storage and adaptation to cold conditions.

To better document the variation in sugar content, Langlet periodically determined sugar content and dry weight from September to June for four northern and two southern seedlots. Table 2 contains the results of this study. In fall and early winter, trees from northern regions accumulate more sugar than trees from southern regions. A possible explanation for this phenomenon could be the early occurrence of frost in northern areas. The accumulation of sugar may either aid in a rapid acclimation to cold conditions or provide a store of energy which may be required for the functioning of another cold resistance mechanism. During December, sugar and dry matter content dropped for trees from both northern and southern regions. This drop may be

Table 2. Seasonal variation in foliar sugar and dry matter content for northern and southern seedlots of Scotch pine.

Month	Northern seedlots (Northern Sweden)		Southern seedlots (Hungary and Germany)	
	Dry matter	sugar	Dry matter	sugar
	----------------% fresh weight--------------------			
September	35.3	7.8	26.8	5.0
November	34.5	17.1	26.4	16.2
December	33.8	14.5	26.7	13.7
February	34.9	17.9	27.8	22.8
April	36.5	15.3	28.2	20.2
June	36.2	10.8	35.3	9.4

related to the completion of the plant's acclimation to early winter cold, and an initiation of enzymatic activity once again preparing the plant for the extreme cold of winter. The rise in sugar content with the onset of winter was much greater in trees from the southern regions. This may be related to the fact that temperature decreases from December to February are greater in the southern regions than in the already cold northern regions. Finally, the decline in sugar content arrives with the onset of spring. The low sugar contents during spring in the northern trees are probably related to their earlier break from dormancy than trees from southern regions.

VARIATION IN DRY MATTER CONTENT

Within-species variation: Few studies have been done
on genetic variation in dry matter content since Langlet's
(1936) Scotch pine study. One of the more relevant studies
since then was undertaken by Pharis and Ferrell (1966). They
determined foliar dry matter contents of 5 and 16 month old
Douglas-fir seedlings growing under well-watered conditions
in a greenhouse at Corvallis, Oregon. The study compared 4
inland and 3 coastal seedlots. For seedlings of both ages,
coastal and Arizona seedlots had significantly higher moisture
content than seedlots from Montana, northeastern Washington,
and Utah. Whether these differences in moisture content
indicate adaptations which are physiologically significant
to the plant in coping with their environment or whether
seedlings with low moisture content merely have smaller cells
or thicker cell walls is not known.

In 1963, J. W. Hanover compared foliage dry matter con-
tent of western white pine (Pinus monticola) trees which were
resistant and susceptible to infection by the blister rust
fungus. He found that dry matter content varies with age of
needles and groups of trees growing in contrasting environments.
However, no significant differences were found in the dry
matter content of susceptible and resistant strains. The
slightly higher dry weight found in the susceptible tissue
was believed to be due to the effects of rust infection on
susceptible trees and was unrelated to resistance. Schütt
and Hoff (1969) pursued this study and also found no

differences in dry matter content between the different
strains. They realized, however, that foliage dry matter
comparisons of different western white pine trees are com-
plicated by a large within-tree variability for which light
is the controlling factor. Needles from the sunny portion
of the crown had significantly less dry matter content than
shaded needles.

A study by Philpot (1963) compared dry matter content
of ponderosa pine (Pinus ponderosa) trees growing at various
elevations in the central Sierra Nevada Mountains. His
results showed no significant differences in dry matter
content of trees growing at different elevations. He did
discover a relationship between dry matter content and part
of the crown sampled. Dry matter content of the foliage
increased from the tip of the crown to the bottom.

Saetersdal (1963) compared drying rates of excised plants
of various provenances of Norway spruce and coastal Douglas-
fir. In general, Douglas-fir seedlots from Bella Coola,
British Columbia had the lowest moisture content and the
slowest drying rate. Seedlots from the mountains of
Washington had the next slowest drying rate, while the low-
land provenances from the Olympic Peninsula dried the fastest.
In Norway spruce moisture content and drying rate were highly
correlated with latitude of origin. The southernmost German
seedlots had a significantly higher moisture content and
slower drying rate than the northern Norwegian seedlots.
Saetersdal suggests that slow cuticular drying rates may

result in resistance to desiccation during late winter when the ground is frozen and plants are subjected to sunny conditions.

Diurnal variation: Because of the daily lag of absorption behind transpiration, a daily cycle in water content occurs in plant tissues (Kramer, 1949). Kozlowski and Peterson (1960) suggested that internal moisture distribution and absorption of water from the atmosphere are additional factors initiating diurnal changes in moisture content.

The leaves of most trees show a daily cycle in water content, whether growing on a dry or moist soil. In general, minimum water content occurs in the early afternoon or about the time of maximum transpiration; maximum water content usually occurs near midnight and decreases toward morning. (Kramer and Kozlowski, 1960). In keeping with the patterns of transpiration, one would expect maximum water content to occur just before sunrise. The speculated reason for the early morning decrease is a redistribution of carbohydrates in the plant during the night which initiates translocation of water from the leaves to other organs (Kramer and Kozlowski, 1960). Although the water content of leaves varies with species, age, and season, a similar diurnal cycle has been reported by several investigators (Ackley, 1954; Kozlowski and Peterson, 1960; Zaerr, J. B., 1971; Gibbs, 1935; Jones, 1972; Cleary and Waring, 1965).

Studies refuting a diurnal variation pattern in foliar moisture content have also been reported. Working with

Engekmann spruce (Picea engelmanni) in northern New Mexico,
Gary (1971) found no significant diurmal differences in
moisture content of needles up to 4 years of age. The small
differences existing were attributed to variation within
crowns.

Jameson (1966) collected foliage from 10 juniper
(Juniperus spp.) and 10 pinyon pine (Pinus edulis) trees at
2-hour intervals from daylight to darkness in the summer and
winter. There was a significant midday depression in moisture
content of the juniper trees. The pinyon pine results re-
vealed no diurnal differences in moisture content during the
summer. In the winter, however, pinyon pine foliage had
slight increases in moisture content in the morning, with a
significant afternoon drop. Jameson speculated that pinyon
pine leaves may possess some inherent mechanism to retard
moisture loss that would normally occur during summer.

Seasonal variation: Seasonal changes in foliar moisture
content also occur, the water content of trees usually de-
creases toward the end of the growing season. Often a slight
rise in moisture content is noted during early winter, again
followed by a steady decrease until the onset of the new
growing season. Studies by Ackley (1954) have shown that
the seasonal reduction in moisture content is caused by
increased leaf dry weights rather than a decrease in moisture
content. Another theory concerning seasonal variation has
been proposed by Jameson (1966). He suggests that seasonal
variation occurs because of differential behavior of the

stomates; i.e., stomates of some species may close during dry periods in order to retard transpiration and allow moisture content to increase. Evidence supporting Ackley's theory is offered by several authors (Gary, 1971; Kramer and Kozlowski, 1960; Pharis, 1967).

Studies by Kozlowski and Clausen (1965) have indicated similar seasonal patterns for various angiosperm and gymnosperm species. However, different degrees of seasonal change were noted between angiosperms and gymnosperms. Dry weight changes in gymnosperms are believed to result from rapid carbohydrate translocation out of older needles and into growing needles. Thus, there is a decrease in dry weight in the older needles early in the growing season.

GENETIC VARIATION IN DOUGLAS-FIR

Provenance research in Douglas-fir began in 1912 when
T. T. Munger of the United States Forest Service started
an experiment in northwestern Oregon and western Washington.
Seed were collected from 14 localities and two years later
were outplanted at 4 plantations, using the same arrangement
at each site. Munger and Morris (1936 and 1942), reported
on this study and found a consistent superiority in height
growth in trees grown from seed collected in Granite Falls
and Darrington, Washington. Morris (1957), working on two
of the previously established test sites, discovered a strong
genetic control over time of bud bursting in Douglas-fir.
Trees from localities with warm spring days and nights
started growth earlier than trees from localities with warm
days but cool spring nights.

The results of this early provenance test encouraged
many others in the Pacific Northwest to pursue studies on
the genetic trends in Douglas-fir. Irgens-Moller (1957)
collected Douglas-fir seed from 7 localities at various
elevations in western Oregon and sowed them in a greenhouse
under controlled conditions. Photoperiod studies revealed
that trees grown from seed collected at high altitudes were
the latest to start growth under natural daylength conditions
and were significantly affected by long day treatments. Long
day treatments hastened bud burst.

In 1958, Irgens-Moller studied genotypic variation in
time of growth cessation of trees grown from seed collected

in British Columbia, Washington, and Oregon. Trees native
to the coast stopped growth one to four weeks later than
trees from interior British Columbia, and higher coastal
elevations. A positive correlation was found between growth
cessation and height of trees; those stopping growth earliest
attained less growth. The differences in time of growth
cessation were ascribed to different responses to the
natural decrease in day length during the summer. The study
indicated that sensitivity to daylength changes is of
selective advantage to plants native to areas where the
climatic changes from summer to winter are severe and abrupt.

Ching and Bever (1960) started a provenance study in
Corvallis, Oregon. They collected seed from trees growing
in 14 locations ranging from southern Oregon to Vancouver
Island. Nursery performance of the seedlots was evaluated
with respect to variation in time of bud burst, bud set,
frost damage, height and needle length. Seedlings from
Vancouver Island, British Columbia, and the Shelton Area
of Washington were generally faster growing and had the
longest needles. Southern seedlots burst bud the earliest,
set buds the latest, and suffered the greatest amount of
frost damage. They suggested that differences in selective
pressures from locality to locality have led to the evolution
of genetically distinct types with regard to those traits
studied.

Improvement of Douglas-fir was also undertaken abroad.
A provenance test was started in Rotorua, New Zealand,

containing 30 seedlots of Douglas-fir from west of the
Cascade Range and Sierra Nevada Mountains. Sweet (1965)
reported that the fastest growing seedlots were from Santa
Cruz, California. He suggested that Douglas-fir from this
area had probably developed superior growth as a result of
a warmer and longer growing season.

In the eastern United States, early studies indicated
poor survival of coastal Douglas-fir seedlots. Interior
seedlots, however, were relatively hardy and varied widely
in performance. One of the first provenance tests of
interior seedlots was undertaken by Baldwin and Murphy (1956)
in New Hampshire. They sowed seed collected from New Mexico,
Idaho, Washington and Montana. Trees from New Mexico had the
least mortality, greatest height, and longest shoots. Further-
more, all four Douglas-fir seedlots grew faster and had less
mortality than native balsam fir (Abies balsamea) of the same
age on the same site.

Byrnes (1958) grew several Douglas-fir seedlots at 24
test sites in Pennsylvania. He categorized the seedlots as
coastal, southern interior (southern and central Rocky
Mountains), and northern interior (northern Rockies and
British Columbia). Southern interior trees were superior
in survival and growth.

Gerhold (1965) planted several of the same seedlots of
Douglas-fir that I studied in Pennsylvania. His results
showed coastal sources to be the tallest but the most vari-
able in survival; Arizona and New Mexico seedlots were the

tallest of the Rocky Mountain origins and had good survival;
trees grown from seed collected in northern Idaho and north-
western Montana had good height, growth and excellent survival.

A New York provenance test was established in 1968 by
Heit. He found that seedlings of Arizona and New Mexico
origins had the fastest growth rate. His studies also showed
that seeds from the coast, northern Rockies, and British
Columbia require a prechilling treatment to break dormancy.

A genetic study on interior populations of Douglas-fir
was established by J. W. Wright and W. I. Bull of Michigan
State University in 1962. The test contained 128 seedlots
which were planted in four plantations in Michigan and
Nebraska. Wright et al. (1970 and 1971) reported that seed-
lots from Arizona-New Mexico were the fastest growing and
had the bluest foliage. Northern varieties tended to be
slower growing and very hardy. Trees grown from seed col-
lected in the Inland Empire were vigorous and very hardy.
Wright et al. suggests that by careful seed source selection
and proper cultural methods Douglas-fir could be grown as a
Christmas tree in Michigan on rotations of 6-10 years.

CHAPTER 4

MATERIALS AND METHODS

PLANTATION MATERIAL

Seed procurement: In 1961, J. W. Wright and W. I. Bull
of Michigan State University obtained seed from 128 natural
stands of Douglas-fir (Figure 1). Two years later, H. D.
Gerhold of Pennsylvania State University obtained seed from
an additional 41 natural stands and shared the seed with
Michigan State University. Seed collection was promoted
through the courtesy of the United State Forest Service and
various local agencies within the sampled regions.

In both cases, seeds from any given locality were col-
lected from each of about 10 average trees in a native stand
of at least 10 acres in size. Cones collected within a 5
mile radius were lumped together. Both series of collections
covered the entire natural range of the species. Seeds were
accompanied by data on latitude, longitude, elevation, soil
type, aspect, slope, collection date, etc.

Nursery practice: Seeds from the 1961 collection were
sown in the spring of 1962; seeds collected in 1963 were sown
in the spring of 1964. The seeds were sown in the Michigan
State University experimental nursery in East Lansing. In
each case the seedlots were replicated and a randomized

complete block design was used. Each nursery plot consisted
of a single 4 foot row in which 20 seeds were sown. The
rows were spaced 1 foot apart. Seedlings from the 1961
collection were transplanted once or twice while in the
nursery and were field planted as either 1-2 or 1-2-2 stock.
Seedlings from the 1963 collection were not transplanted and
were field planted as 2-0 stock.

Outplanting procedure: In 1965 and 1966, four permanent
test plantations were established in southern Michigan. In
all plantations a 6x6 ft. spacing and 4 tree plots were used
.except MSFGP 4-67. Weed control was obtained by applying
amino-triazole (1 gal./acre) in 2-foot strips the autumn
prior to planting. After planting, simazine was applied at
the rate of 4 lbs. per acre. All sites were covered with
dense grass and alfalfa sod before planting.

Of the 128 seedlots collected in 1961, 68 were included
in test plantations. Of the 41 seedlots collected in 1963,
28 were included in test plantations. Most of the seedlots
which were not planted were of West Coast origins and suf-
fered high mortality in the nursery from winter cold.

From a comparison standpoint, it is desirable that every
seedlot be represented in every replicate in all plantations.
However, nursery stock was too limited to permit this in all
cases, or in every plantation. Therefore, some seedlots are
represented in 1-2 plantations only or in less than the full
number of blocks in a plantation.

Further details for individual plantations are as follows:

MS FGP 16-65/67

> Planted 4/15/65 and 4/15/67 at W. K. Kellogg Forest,
> Kalamazoo Co., 65 miles SW of East Lansing: 67
> seedlots; 340 plots; 5 replicates; randomized
> complete block design; mortality 9%; average
> height 7 ft. in 1973. Site a level hilltop; sandy
> loam soil of the Bellefontaine series; weed control
> with simazine resumed in 1968 and continued each
> subsequent year.

MS FGP 6-65

> Planted 4/27/65 at Camp Kett, Osceola, 150 miles
> NW of East Lansing; 36 seedlots; 153 plots; 5
> replicates; randomized complete block design;
> mortality 25%; average height 5.5 ft. in 1973.
> Site rolling with 5-20% north slopes; sandy loam
> soil of the Montcalm series.

MS FGP 9-66

> Planted 4/19/66-4/20-66 at W. K. Kellogg Forest;
> 22 seedlots; 76 plots; 4 replicates; randomized
> complete block design; mortality 60%; average
> height 4.0 ft. in 1973. Site level: sandy loam
> soil of the Bellefontaine series.

MS FGP 4-67

> Planted 4/67 at Michigan State University's experi-
> mental nursery in East Lansing, Ingham County; 12
> seedlots; 12 plots; 25-tree plots; 2 ft. spacing;
> not replicated; mortality 3%; average height 4.5 ft.

in 1973. Site open and level; sandy loam soil of
the Miami series; weed control continued annually.

The reason that plantation MS FGP 16-65/67 was planted
in 2 separate years was related to the size of the planting
stock. In 1965 there were large differences in growth rate
among seedlots. In 1965 the tallest seedlots averaged 5-12
in. tall and were ready for field planting. Others averaged
2-5 in. tall and were not ready for field planting. The slow
growing seedlots were lined out on the East Lansing nursery
and field planted as 1-2-2 stock in alternate rows two years
later.

Further details are contained in a publication by Wright
et al. (1970).

SAMPLING TECHNIQUE

I collected foliage samples during the summer of 1973.
Samples consisted of either current year's foliage or year-
old foliage. In all plantations I collected foliage from
all living trees in each plot. All material collected from
one plot was combined and placed in a labelled, tared paper
envelope and then sealed in a tared plastic bag to prevent
moisture loss.

Current year's foliage was collected from plantations
MS FGP 16-65/67 at the Kellogg Forest and 5-65 at Camp Kett.
The samples consisted of 10-45 gm. of needles and attached
twigs. The time lag between removal and sealing in the
plastic bags was approximately one minute.

Year-old foliage was collected from plantations MS FGP
16-65/67 at Kellogg Forest, 9-66 at the Kellogg Forest, and
4-67 at the East Lansing nursery in the same manner as de-
scribed above. The time lag between removal and sealing in
the plastic bag was about two minutes.

The dates on which the samples were collected from each
plantation and the number of replicates sampled on each day
are listed in the following tabulation:

| Rep. # | Current-year's foliage | | Year-old foliage | | |
	16-65/67	5-65	16-65/67	9-66	4-67
1	June 26	Aug. 23	July 13	Aug. 17	Aug.13
2	June 26	Aug. 23	Aug. 3	Aug. 17	--
3	July 9	Aug. 23	Aug. 3	Aug. 17	--
4	July 9	Aug. 23	Aug. 6	Aug. 17	--
5	July 13	Aug. 23	Aug. 6	Aug. 17	--

As shown above, samples were collected on different days, but all samples from one replicate were collected the same day. An analysis of variance showed that differences in moisture content between replicates were significant (1% level), either because of site variation or day-to-day variation. Since an entire replicate was sampled in one day, sampling time did not affect the genetic results.

To standardize collection procedures, each sample was removed from the middle portion of the crown and from the south side of a tree. In order to account for daily and diurnal fluctuations in moisture, accurate records of sampling time were recorded to the nearest minute. A general description of the weather conditions on each sampling day were also compiled.

All samples were returned to the laboratory for weighing within 24 hours after they had been sealed in the plastic bags. The envelopes containing the plant tissue were then placed in an oven at $70^{\circ}C$, and remained until they reached constant dry weight (approximately 36 hours). Again I weighed the samples and their moisture content was computed as a percent of their fresh weight according to the following formula:

$$\text{Dry weight in \%} = \frac{\text{dry weight}}{\text{fresh weight}} \times 100.$$

Adequacy of techniques: Within about 5 minutes after I collected samples, noticeable quantities of moisture had accumulated inside the plastic bags. This led me to believe that small quantities of moisture were probably lost during

the 1 or 2 minute lag time before sealing the plastic bags.
This moisture escape could not be prevented or measured. I
estimated that the amount of moisture lost was only .1-.2%
of the total sample weight.

Once samples were sealed in the plastic bags moisture
loss did not occur. By a series of weighings on a
top loading balance, it was determined that no measurable
quantity of moisture could escape following the procedures
used.

RATE OF MOISTURE LOSS

A separate study was designed to determine the rate of
moisture loss between two races (ARINEM and INEMP), and the
effect of inherent moisture content on rate of moisture loss.
This study involved trees growing at Kellogg Forest (planta-
tion MS FGP 16-65/67) only. One 2-3 ft. branch was removed
from each tree in two seedlots of each race. The 30 branches
(18 from ARINEM trees and 12 from INEMP trees) were collected
from the middle portion of the south side of the crowns of
vigorous trees in two replicates.

The branches were sealed in tared plastic bags and
brought to the laboratory where their fresh weights were
determined. They were then placed on a laboratory table and
allowed to dry at normal room temperatures (28°C). The
samples were weighed daily for the first 4 days, and perio-
dically thereafter until 27 days after cutting. Also re-
corded was the effect of moisture loss on foliage appearance.

Dates were recorded on which 50% and 100% of the foliage on each branch turned brown.

A t-test was used to determine the significance of differences in moisture loss. It compared the differences between mean percent moisture loss of the two races studied. The degrees of freedom were 1 and 28 for race and within race, respectively. The data on the change of foliage appearance was analyzed by the Chi-square test. Each day's data were arranged in a 2 by 2 table with 1 degree of freedom for each day.

GROWTH RATE AND FOLIAGE COLOR

Growth rate and foliage color are also important characteristics and were therefore measured. Height measurements were made at Camp Kett (MS FGP 16-65/67) and Kellogg Forest (MS FGP 5-65) at the end of the 1973 growing season (age 12 from seed). Foliage color was measured at Kellogg Forest (MS FGP 16-65/67) on September 4. In scoring color I used live tree standards with 0 as the greenest and 20 as the bluest foliage. In previous work color data taken with live tree standards proved more reproducible than that with paper standards.

ANALYSIS OF VARIANCE AND CORRELATIONS

ANALYSIS OF VARIANCE

Variation due to year of planting: The Kellogg Forest plantation (MS FGP 16-65/67) was planted in two different years. During the interim, the trees were subjected to different environmental conditions. Ten seedlots were planted in each of both years, and were thus a means for comparing the performance of the trees planted in different years. Analyses of variance were done to determine the possible significance of differences due to year of planting. In these analyses, the degrees of freedom were 9, 1, and 9 for seedlot, year, and seedlot X year interaction, respectively.

The analysis of variance showed that year of planting did not result in differences in dry matter content or foliage color. Accordingly, year of planting was ignored in subsequent analyses of the data for these traits.

The results of the analyses showed, however, that those trees planted in 1965 were significantly taller (1% level) than those of the same seedlot planted in 1967 (F value = 12.1). The seedlots planted in 1965 averaged 1.07 feet taller than those planted in 1967. As a result, 1.07 ft. was added to the height of each tree planted in 1967 before calculating racial means and the significance of racial differences.

Genetic variation: An analysis of variance was performed

for each trait (dry matter content, foliage color, and growth rate) using plot means as items. In seedlots which were represented in, at least, 2 but not in all 5 replicates, replacement values were computed for the missing plots. This was done by using the seedlots mean, adjusted upward or downward as necessary, to compensate for differences among replicates. By using this method, daily variation as well as between replicate variation was considered. One degree of freedom was subtracted from the degrees of freedom for error for each missing plot.

The Kellogg Forest plantation (MS FGP 16-65/67) contains 67 seedlots (of which 10 were planted in both years) belonging to 9 races as recognized by Wright et al. (1970), 5 replicates (blocks), and 340 total plots. Degrees of freedom were as follows: block--4, seedlot--66 (= 8 for race + 58 for seedlot within race), seedlot X replicate (= error)-- 268, year of planting--1, total--339. The levels of significance were determined by testing the seedlot and seedlot within-race mean squares against the seedlot X replicate mean squares, and the race mean square against the seedlot within-race mean square.

The Camp Kett (MS FGP 5-65) and other Kellogg Forest plantation (MS FGP 9-66) contained 36 and 22 seedlots, respectively, belonging to 7 races. Similar analyses were done for data collected at these plantations. Of course, the degrees of freedom differed and it was not necessary to account for differences in year of planting.

An analysis of variance was used to detect possible significant differences in rate of drying. Thirty branches belonging to 2 races were collected. The degrees of freedom were as follows: race--1, seedlot within race--2, plot within seedlot--4, tree within plot (= error)--22, total--29.

Variation with time: Additional analyses were calculated to determine whether differences in sampling time within a replicate were important. Plot means were used as items.

Spearman's Rank Correlation Coefficient

Rank Correlations were run to compare moisture content results for different studies and plantations. The various sets of data were arranged in order of increasing moisture content, and the Spearman's coefficient (R) was computed directly from the differences between the ranks of two paired variables. The variables tested were moisture content data between: current-growing and year-old foliage, current foliage at different plantations, and means for Arizona seed-lots from all plantations. Degrees of significance were determined by comparing the computed coefficient (R) for the number of listed values (N), with the significance levels listed in the rank correlation table.

CHAPTER 5

RESULTS

GEOGRAPHIC TRENDS IN DRY MATTER CONTENT

For the purpose of comparison and clear presentation
the sampled origins were divided into 9 geographical races
(Figure 1) and given code names according to the classifica-
tion of Wright et al. (1971). Their grouping was based on
similarities and differences in several characteristics. My
data supported their grouping in general, but I found it
desirable to split their CWASH and ARINEM races.

The races and their distribution are as follows:

ALB------------Alberta

CMON-----------central Montana, Wyoming

NOROC---------northern Rockies, west central Montana

INEMP---------Inland Empire, northern Idaho and
              northwestern Montana

CWASH

   Northern-----north-central Washington

   Tieton Road--south-central Washington, Yakima Co.

COAST---------Western slope of the Cascade mountains
              of Oregon, Washington, and British
              Columbia

NOCOL---------northern Colorado and adjacent Utah

SOCOL----------southern Colorado and adjacent Utah and

          Arizona

ARINEM

  New Mexico---central and southern New Mexico

  Arizona-------central and southern Arizona

CURRENT YEAR'S FOLIAGE

<u>Differences between races</u>: The racial differences in
dry matter content of current years foliage are shown in Table
3. A single seedlot from Tieton Road in Yakima Co., Washington
had the most succulent foliage, i.e., the lowest dry matter
content. Its exceptional performance will be discussed in
length later.

At Kellogg Forest, Colorado-Utah trees (NOCOL and SOCOL
races) had the next lowest dry matter content. This was
probably related to a very damaging frost ($25^{O}F$) at Kellogg
Forest on May 18-19. When sampling this plantation in late
June and July, it was quite apparent that the foliage collected
from the NOCOL and SOCOL trees was fresher and more succulent
than foliage of other races. Studies by Kim Steiner showed
that the southern races leafed out earliest and were most
severely damaged by the frost (Table 4). As a result, the
foliage which I sampled was on shoots which had arisen from
adventitious buds. These shoots were only 1-2 in. long and
bore needles only half as long as those produced in previous
years. Studies by Pharis (1967) have shown that new foliage
of Douglas-fir contains much less dry matter than foliage

Table 3. Differences among geographic races of Douglas-fir
in the dry matter content of fresh foliage.

Region of Origin	Dry matter content of				
	Current-year foliage collected at		Year-old foliage collected at	Foliage collected at Corvallis, or[B]	
	Kellogg 16-65/67	Camp Kett 5-65	Kellogg and East Lansing[A]	5-mo-old Foliage	16-mo-old Foliage
	% of fresh weight				
ALB	33.1	--	43.0	--	--
CMON	33.7	34.3	43.5	43.2	45.2
NOROC	34.0	--	43.2	--	--
INEMP	34.7	35.1	43.3	43.2	47.0
CWASH					
Northern	34.0	35.5	43.7	--	--
Tieton Rd.	29.6	--	38.7	--	--
COAST	--	--	--	37.7	41.6
NOCOL	30.6	35.2	44.2	--	--
SOCOL	30.8	34.4	43.5	41.8	--
ARINEM					
New Mexico	33.2	34.5	43.4	--	--
Arizona	32.1	32.5	42.7	38.2	41.7

[A] Data for year-old foliage determined as means from plantations MSFGP 16-65/67, 9-66, 4-67.

[B] Data from Pharis and Ferrell (1966).

Table 4. Racial differences in susceptibility to frost
damage and time of leafing out of Douglas-fir grow-
ing on plantation MS FGP 16-65/67 (Kellogg Forest)
in Michigan. [C]

Region of Origion	Frost damage (6/73) % buds killed	Time of leafing out (spring,73) 1 = early 10 = late
ALB	71	5.1
CMON	59	6.2
NOROC	35	8.4
INEMP	29	8.1
CWASH	57	4.8
NOCOL	88	3.0
SOCOL	74	3.3
ARINEM	59	2.9

[C] Data courtesy of Kim Steiner.

which is only a few weeks old. As a result, low dry matter content of NOCOL and SOCOL foliage may have been a consequence of leafage rather than genotype of the trees. A further effect of the frost damage was to increase the variability of dry matter content of foliage from damaged seedlots. The frost damage resulted in error mean squares 2-10 times greater for NOCOL and SOCOL races than for others.

Other seedlots at Kellogg Forest were also damaged by the frost. However, due to the lesser damage and the larger tree size, shoots arising from adventitious buds were not collected in these seedlots.

In the Camp Kett plantation, where frost damage was much less noticeable, Colorado-Utah trees had normal dry matter contents. This would probably have been the case at Kellogg Forest, also, in a normal year. The Camp Kett data shows that Arizona seedlots had the lowest dry matter content, having 3% greater moisture content than the more northern INEMP group. At the Kellogg Forest, the Arizona trees also had low dry matter contents. An analysis of variance showed that racial differences in foliar dry matter content for both plantations were highly significant (Table 5).

Tieton Road vs. Northern CWASH: The Tieton Road seed-lot had previously been placed in the CWASH race by Wright et al.(1971). It was included because morphological studies showed it was somewhat similar to others in this race and because it had been postulated that the crest of the Cascade Range was the dividing line separating CWASH from the COAST

Table 5. Analysis of variance for dry matter content data
of current years foliage of Douglas-fir at planta-
tions MS FGP 16-65/67 (Kellogg Forest) and MS FGP
5-65 (Camp Kett).

Source of Variation	Kellogg Forest			Camp Kett		
	df	ms	f	df	ms	f
Seedlot	66	25534	2.63***	35	679.4	1.64*
between region	8	10479	7.18***	6	1978.5	4.82***
within region	58	1460	1.51*	29	410.7	<1
Replicate	4	18113	18.68***	4	2230.0	5.37***
Year of planting	1	11.77	1			
Total error	268	1003		113	593.5	
Total	339			152		

* significant at 5% level

*** significant at 1% level or better

race.

As a result of its exceptionally low dry matter content, further studies were made of its area of origin. The parental stand was located a few miles east of White Pass (elevation 4500 ft.), which is located at the crest of the Cascade range. Travellers through White Pass report Douglas-fir to be growing there, so the Tieton Road seedlot may represent an area of overlap between the COAST and CWASH races. This is further indicated by data on other characteristics, as shown in the following tabulation. All differences between the Tieton Road and other CWASH seedlots indicate the Tieton Road trees tend to be more similar to the COAST race as described by Wright et al., 1970: Gerhold, 1966: Byrnes et al., 1958.

Seedlot origin	Height	Color	% buds killed by frost damage (3 yr.av.)	Time of leafing	Dry matter	
					Current foliage	old foliage
	ft.	0=green 20=blue	%	1=early 10=late	% of fresh wt.	
CWASH	6.9	11	26	4.8	34.0	43.7
Tieton Rd.	7.2	8	37	1.9	29.6	38.7

Arizona seedlots: Excluding trees from the two highly damaged races, Arizona seedlots had the highest moisture content of all interior races. That agrees with the results of a study done at Corvallis, Oregon by Pharis and Ferrell (1966). They compared foliar dry matter content of 5 and 16

month old seedlings of five Douglas-fir races, each repre-
sented by 1-3 seedlots. For seedlings of both ages, the
Arizona and COAST races had the lowest dry matter content
(Table 1).

A rank correlation was run comparing the dry matter
contents of the Arizona seedlots growing at both Kellogg
Forest and the Camp Kett plantation. The results showed
that the data from the two plantations were independent. The
lack of correlation could be attributed to plantation X seed-
lot interaction or differences in date of foliage sampling.

Variation within races: Figure 3 illustrates the varia-
tion in dry matter content within the between races,based
upon Kellogg Forest data (plantation MS FGP 16-65/67). The
seedlot means ranged from 25% to 36%. The races with the
largest variability were NOCOL (range=8%) and SOCOL (range=
8%) and SOCOL (range=6%). An analysis of variance showed
that differences in moisture content within races were signif-
icant at the 5% level (Table 3). The significance was prob-
ably largely attributed to variation within the highly damaged
NOCOL and SOCOL races.

The within race data from the Camp Kett plantation
(MS FGP 5-65) was somewhat less variable than the Kellogg
Forest data. The range was from 30% to 37%, as shown in
Figure 4. The NOCOL and SOCOL races were not severely
damaged by frost at Camp Kett and thus showed comparatively
little variation. An analysis of variance showed that this
variation was not significant.

Figure 3. Differences in current-years foliage dry matter content between and within races of Douglas-fir growing at Kellogg Forest (plantation MS FGP 16-65/67) in southern Michigan. Dots represent seedlot means.

ALB

CMON

NOROC

INEMP

CWASH   Northern

Tieton Rd.

NOCOL

SOCOL

ARINEM   N. Mexico

Arizona

% OF FRESH WEIGHT

25   30   35   40   45

Figure 4. Differences in current-years foliage dry matter
content between and within races of Douglas-fir
growing at Camp Kett (plantation MS FGP 5-65)
in southern Michigan. Dots represent seedlot
means.
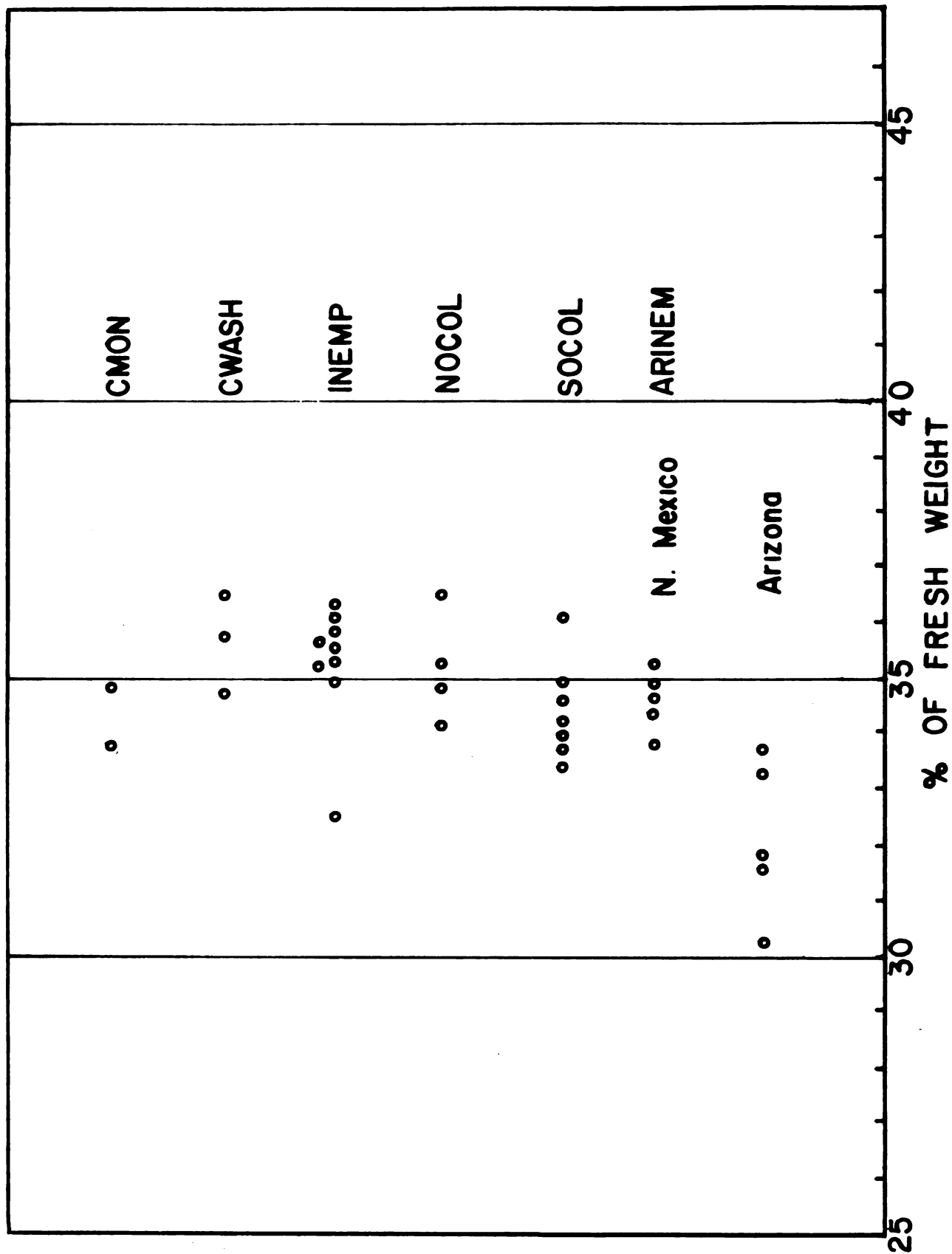
YEAR-OLD FOLIAGE

The purpose of sampling year-old foliage was to compare
dry matter content of current and year-old foliage, and to
test the effect of frost damage on the dry matter content
of the NOCOL and SOCOL races. The results as shown in Table
1, reveal higher dry matter content for the year-old foliage
in general, and considerably higher dry matter content for
the NOCOL and SOCOL origins in comparison with others. Since
the year-old foliage was not damaged by 1973 frosts, the
relatively high dry weights for these races supports the
previously mentioned belief that current foliage data for
NOCOL and SOCOL races was not representative of their normal
dry weights.

Differences between races: The racial variation in
dry matter content of year-old foliage was considerably less
than that in the current year's foliage. Data from three
plantations (MS FGP 16-65/67, 9-66, 4-67) were averaged and
are presented in Table 1. The Tieton Road seedlot had the
foliage with the lowest dry matter content. The Arizona seed-
lots were also low in dry matter content. An analysis of
variance showed that trees from these races were significantly
(2.5% level) higher in moisture content than all other races
growing at the Kellogg Forest (plantation MS FGP 16-65/67).
These results concur with the data from the current-year's
foliage.

Differences within races: The plotted seedlot means
in Figure 5 shows that dry matter content of year-old foliage

Figure 5. Differences in year-old foliage dry matter content

between and within races of Douglas-fir growing

at Kellogg Forest (plantation MS FGP 16-65/67)

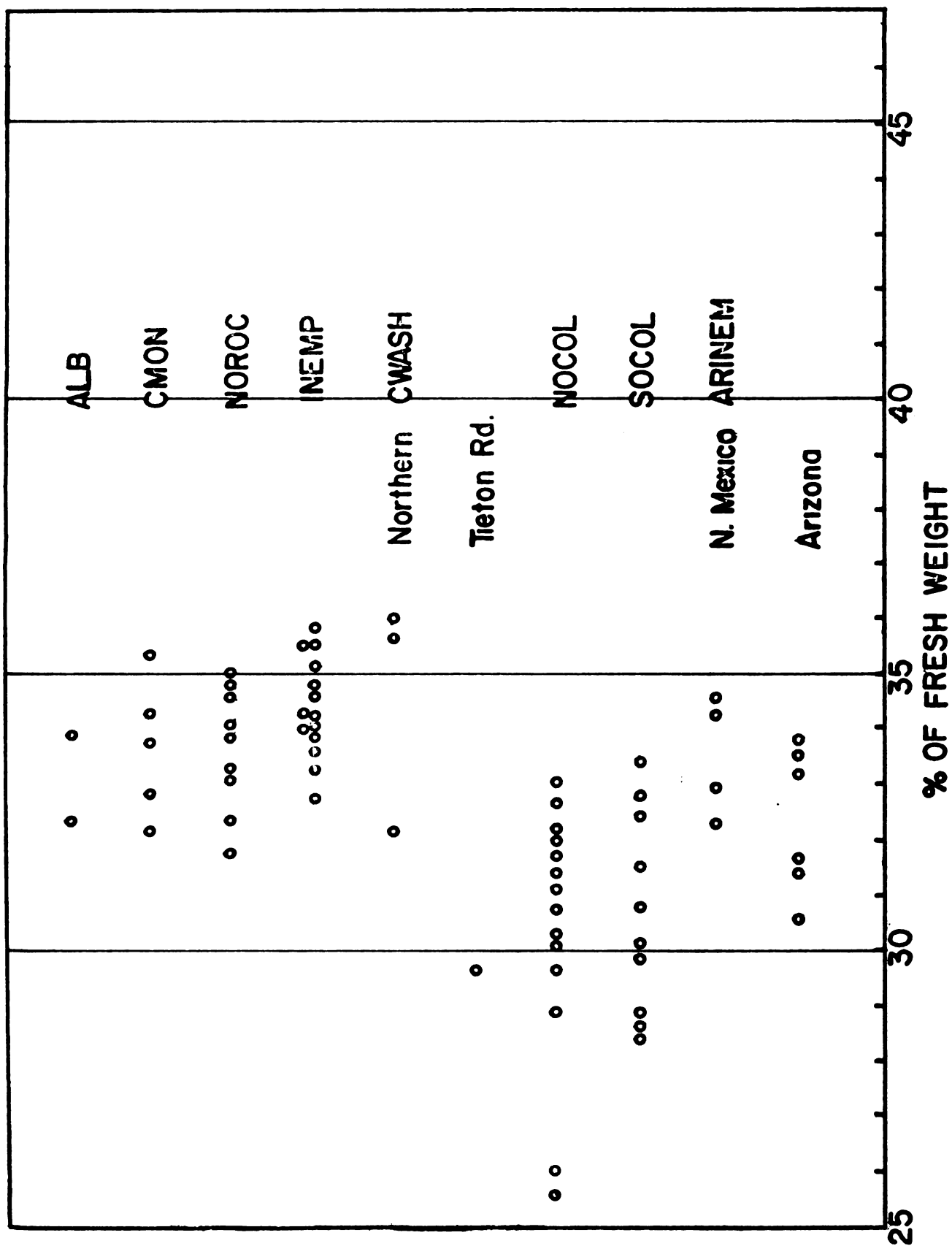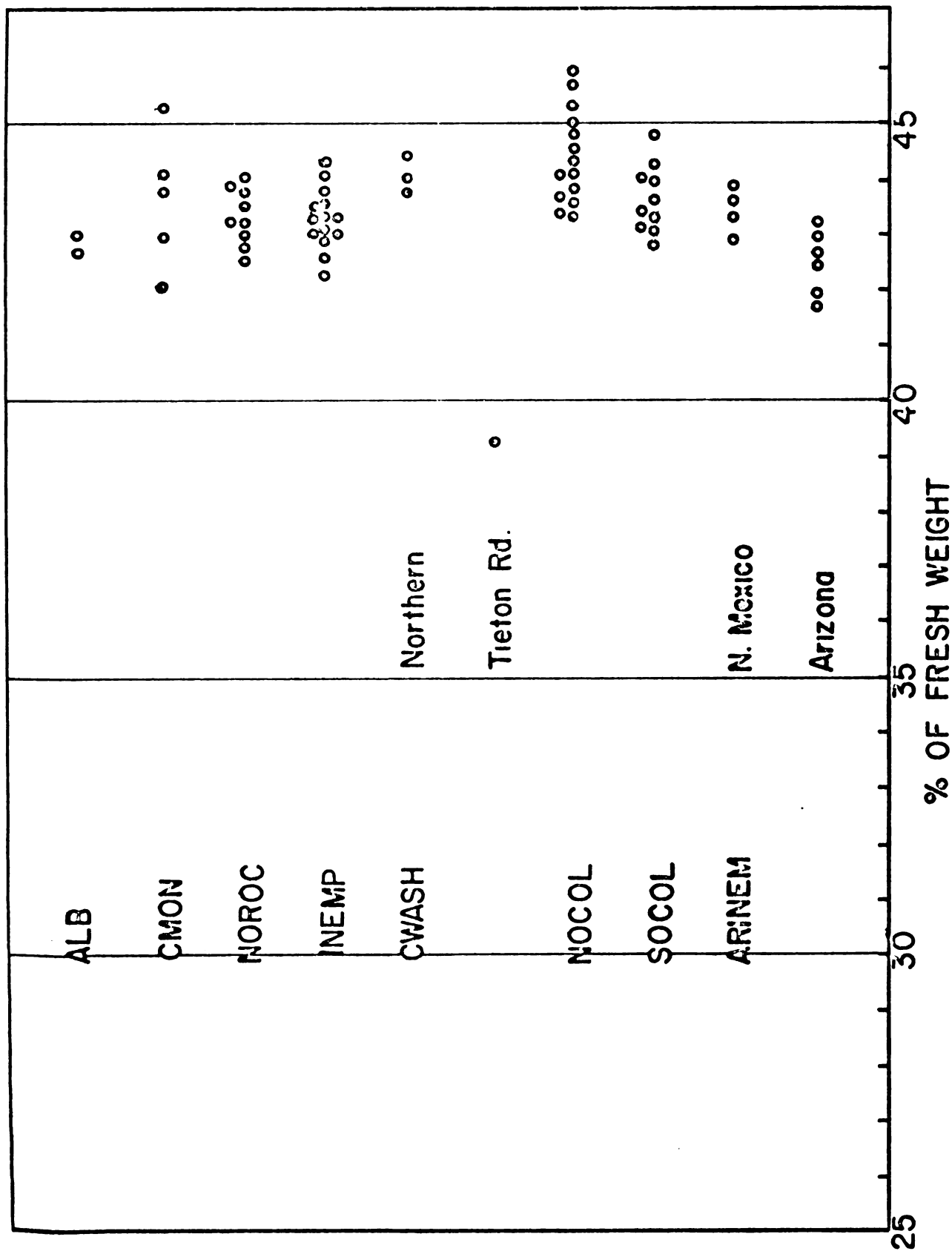in southern Michigan. Dots represent seedlot

means.

% OF FRESH WEIGHT

was less variable than that in current-year's foliage. With the exception of the Tieton Road seedlot, the range in dry weight was from 41% to 46%. The dry matter content differences within races were not significant.

Rank correlations were run comparing dry matter content trends between year-old (plantation MS FGP 16-65/67) and current-year's foliage (plantations MS FGP 16-65/67 and 5-65) of Arizona seedlots. In various combinations of testing all sets of data, the correlation coefficient was not significant. As a result, the value of selecting for high moisture content among different stands within Arizona is not, at this stage of research, a valuable asset.

DRY MATTER CONTENT OF CURRENT VS. YEAR-OLD FOLIAGE

In comparing all moisture content data, the largest differences were between current and 1 year old foliage. It is important to emphasize that these differences are not necessarily associated with differences in actual amounts of moisture. Kramer and Kozlowski (1960) explain that as plant tissues become more mature, their cell walls thicken and the amount of dry matter increases. Therefore, a decrease in moisture content (expressed as a % dry weight) will occur without appreciable loss in water content. Evidence to support this is provided by Korstian (1933) who reported leaf moisture contents ranging from 52% (% fresh weight) in one year old leaves to 78% in newly formed leaves of evergreen Magnolia (Magnolia grandiflora). Ackley (1954) found water content of Bartlett pear leaves to decrease from 73% (% fresh weight) in May to 59% in August, while at the same time actual moisture content increased. More recently, Kozlowski and Clausen (1965), found the same general trends in the leaves and buds of several species, including red pine (Pinus resinosa) and eastern white pine (Pinus strobus).

Another relation noted was the decreasing variability with increasing age of the foliage. The results reveal that the moisture content of the current growing foliage collected at Kellogg in early summer was considerably more variable than it was a few months later at Camp Kett. In the same manner, the 1 year-old foliage sampled in the middle of the summer was less variable than either set of current foliage

data. These differences are probably associated with metabolic changes within the plant that occur early in the growing season. As a result, spring was probably a poor time to sample trees for moisture content.

I ran rank correlations to compare racial trends in dry matter content for current and year-old foliage. The current foliage data from the Camp Kett plantation showed a moderate correlation ($r=.63$) with the trends in year-old foliage at Kellogg Forest. Similarly, a moderate correlation ($r=.69$) was found between current and year-old foliage at the Kellogg plantation(MS FGP 16-65/67). The primary consistency corroborated by all sets of data was that trees grown from seed collected in Tieton Road (Washington) and Arizona contained the highest moisture content.

GENETIC VARIATION IN RATES OF MOISTURE LOSS

Rates of moisture loss were compared for branches re-
moved from trees of ARINEM and INEMP races, which represent
extremes in foliar moisture content and have the greatest
economic potential in southern Michigan. The samples were
composed of branches from 18 trees belonging to 2 seedlots
from Arizona and of branches from 12 trees belonging to 2
seedlots from northern Idaho.

The patterns of moisture loss are graphically illustrated
in Figure 6. The rates of moisture loss and the differences
in moisture loss between the two races were greatest during
the first two days. At the end of the second day, the
branches from the ARINEM race had lost 17% of their fresh
weight, while the branches from the INEMP race had lost 30%
of their fresh weight. During the following four days, both
races lost moisture at about the same rate.

After 6 days of drying, branches from the INEMP race had
a slight reduction in moisture loss. At the same time, the
branches from the ARINEM race continued to lose substantial
amounts of water. As the number of days increased, the
curves for the two races began to approach each other. After
20 days of drying, the INEMP branches reached a constant
weight (any loss after this was probably due to needle drop),
while the ARINEM branches continued to lose moisture. The
experiment was stopped after 27 days and the ARINEM branches
had not yet reached a constant weight.

Size of branch did not affect rates of moisture loss.

Figure 6. Differences in rates of moisture loss of cut branches of ARINEM and INEMP Douglas-fir races growing at Kellogg Forest (Plantation MS FGP 16-65/67) in southern Michigan.
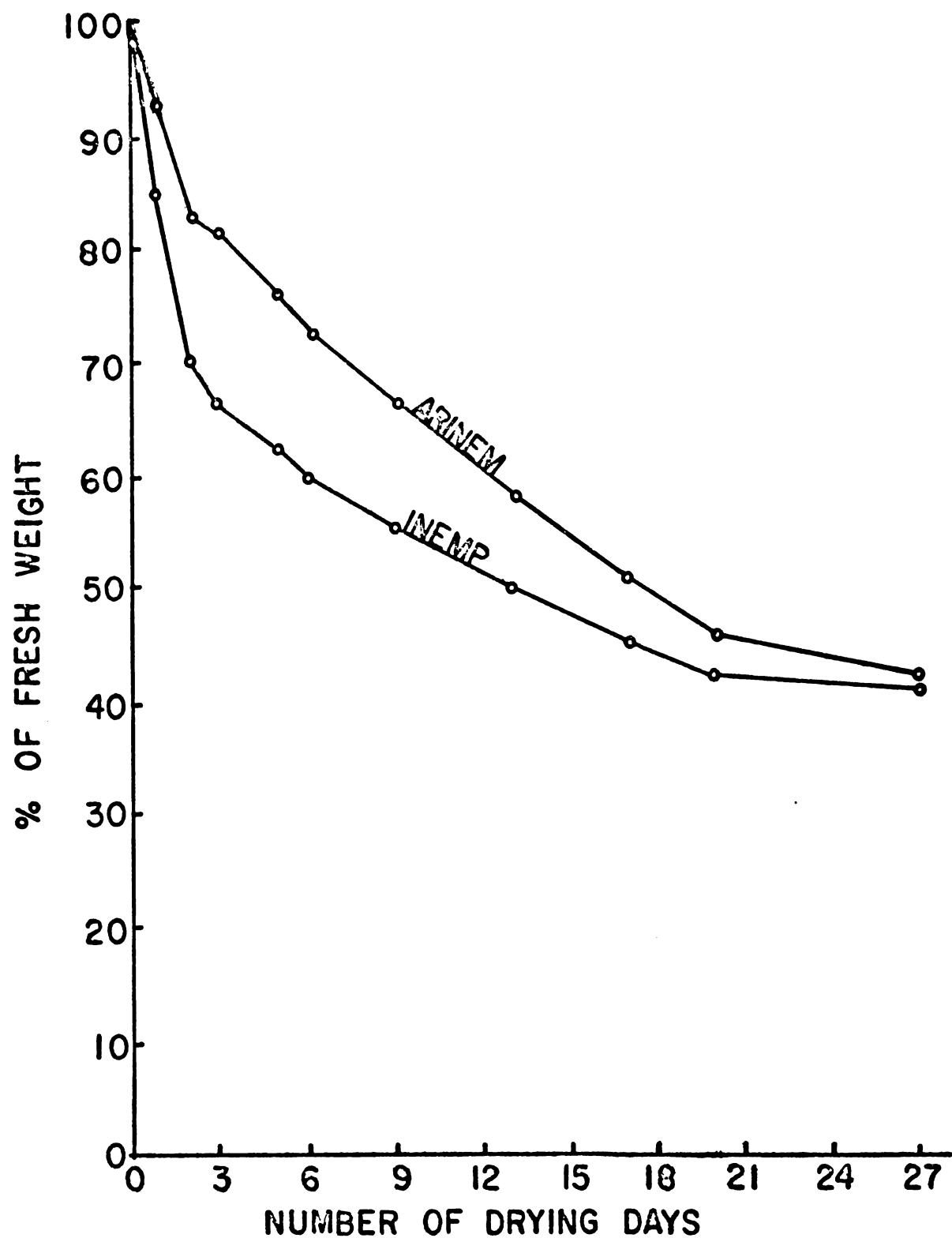
The smallest and largest branches from each race lost similar percentages of their fresh weights. Even the smallest ARINEM branches had not reached a constant weight on the last day of measurement.

An analysis of variance was performed on the second days data. Differences between races were significant at better than the 1% level (Table 6). For other days data t-tests were used to compare differences in moisture loss between the two races. This test was used after 2, 6, and 9 days of drying, respectively, and indicated significant differences (1% or 2% levels) for each day.

Table 6. Analysis of variance for rate-of-drying data for
ARINEM vs. INEMP Douglas-fir races growing in
Michigan. Data analyzed after 2 days of drying.

Source of Variation	df	ms	f
Race	1	586.7	10.04***
Seedlot within race	2	41.1	.7
Plot within seedlot	4	61.20	1.05
Error (tree/plot)	22	58.41	
Total	29		

*** Better than 1% level.

DISCUSSION

The evidence indicates that trees from the ARINEM race
have evolved some mechanism for conserving water. Whether
the water conservation mechanism is related to the 3% lower
dry weight in the ARINEM race is not known. One might have
expected more rapid rates of moisture loss in the ARINEM race
due to the higher moisture content. On the other hand, the
retarded moisture loss might be the factor responsible for
the higher moisture content.

A study by Palpant (1973), compared rates of moisture
loss of Douglas-fir of Colorado origins with central Montana,
Idaho and British Columbia origins. The study was made under
two humidity regimes and, in both cases, the northern Rocky
Mountain origins dryed faster than the Colorado origins. This
evidence agrees with my results that genetic variation in
rate of moisture loss exists and southern Douglas-fir origins
lose moisture more slowly.

It is important at this point to compare the respective
foliage color of the two seed sources examined in this study.
Results of several color analyses, including one in this
study, concur that ARINEM Douglas-fir are significantly bluer
than other races. INEMP seedlots are among the most green
of all Douglas-fir types. The color differences are attrib-
uted to waxes, which coat the needles of many evergreens.
The blue glaucous appearance of the ARINEM race is due to
the light reflecting characteristics of the waxy foliage
surface. The structure, arrangement and density of wax

largely determine the degree of claucousness (Hanover and Recovsky, 1971).

It is no coincidence that the ARINEM races of Douglas-fir are bluer than the more northern races. This same tendency is found in several other species whose range extends into the southern Rocky Mountains (Kung and Wright, 1972). The southern Rockies have almost twice as many hours of sunshine per year as the northern Rockies. This region is also characterized by a low precipitation-evaporation ratio. The waxy surface of the tree leaves has probably been of selective value in several ways. It may act to reduce water loss and gaseous exchange from the plant surface. Also, photo-destruction of auxin is rapid under blue light. Therefore, reflection of blue light would be an effective auxin control mechanism in areas of intense solar radiation (Kung and Wright, 1972).

Results of this study suggest that waxy foliage surfaces may, indeed, play a role in modification of moisture loss. It must be emphasized, however, that trees compared in this study are of different genotypes, as well as different foliage color. As a result, the possibility that other genetically fixed mechanisms may be involved in modifying rates of moisture loss can not be eliminated.

The relation between glaucousness and moisture retention has been questioned in a study by Recovsky (1973). He found no significant differences in moisture loss of glaucous and nonglaucous foliage of blue spruce (Picea pungens). Recovsky

subjected blue spruce foliage to three treatments of various temperatures and humidity. His results for all treatments showed that glaucous foliage lost slightly more water early than the non-glaucous foliage. After time the pattern reversed. His results were essentially the opposite of those found in this study. Recovsky selection of trees was based solely on foliage color with no regard to genotype.

In conclusion, it can only be stated that differences in the rates of moisture loss between the ARINEM and INEMP races growing at the Kellogg Forest are considerable. The reason for these differences, if not related to the prescence of waxes, could be related to any of several anatomical or environmental adaptations. The need for further research on this matter is evident.

EFFECT OF MOISTURE LOSS ON FOLIAGE APPEARANCE

While branches from the ARINEM and INEMP races were
drying, I kept records on the effects of moisture loss on
the appearance of foliage. The differences in drying rates
were clearly reflected in the rate of foliage browning. The
tabulation below lists the percentage of branches from each
race which had at least 50% of their foliage turn brown after
drying for several days. ARINEM branches retained a richer
color for a considerably longer time than the INEMP branches.
The most pronounced differences were found in the first 8
days of drying. At this time, all of the ARINEM branches
had maintained a rich color while 42% of the INEMP branches
had turned brown.

	% of branches turning brown after drying for:				
Race	3 days	8 days	11 days	20 days	27 days
ARINEM	0	0	11	39	88
INEMP	8	42	50	75	100

A Chi-Square analysis was used to test the significance
of these results. The analysis was done after each measured
day of drying. The analysis indicated significant differences
in foliage appearance between the two races for branches that
were drying from 6-11 days.

VARIATION IN PERCENT DRY WEIGHT DUE TO TIME OF COLLECTION

The leaves of most tree species show a diurnal cycle in moisture content, whether growing on a dry or a moist soil. The chief cause of diurnal variations is the lag of water absorption behind water loss (Kramer and Kozlowski, 1960).

Variation within replicates: Due to diurnal variation in moisture content found in many species, it was necessary to perform a test to detect if time of collection affected moisture content. In order to facilitate such an analysis, time of collection was recorded as each plot was sampled. Each replicate was then divided into 5 one-half hour time periods according to the order in which the samples were collected. By subtracting the percent dry weight for each plot from the seedlot mean, a pattern of positive and negative deviations was established. The deviations for each one-half hour time period were then compared in an analysis of variance.

The analysis was done for current foliage and 1 year old foliage moisture content data at Kellogg Forest. The results showed that time of collection did not significantly influence the moisture content of samples from the same replicate. Detected differences were therefore attributed to error.

The value of the analysis was in assuring that the moisture content results were due to genetic variation, and not related to transpirational differences associated with time of collection.

CHAPTER 6

EVOLUTIONARY CONSIDERATIONS

ISOLATION

The Douglas-fir provenance study was designed to yield practical and evolutionary information about racial variation within the species.

Populations occupying climatically different regions tend to adapt in such a way as to become genetically different. However, a prerequiste to such adaptation is isolation so as to prevent migration of genes between them. Absolute range gaps (such as mountain ranges, deserts, etc...) are the most effective barriers. As a result, racial differentiation is most apparent in areas such as the western United States and southern Europe where the ranges are disrupted. Distance can also act as an effective barrier to the migration of genes within a continuous population. Several experiments have shown that pollen travels relatively short distances in quantity. Thus, isolation may be essentially complete if two populations are separated by a distance of 20 miles and there is no consistent one-way air or water movement (Wright, 1962).

Population density is related to the distance needed to effectively isolate a population. If population density is

low there are fewer paths by which genes can migrate and genetic differentiation becomes more pronounced. Small equal sized populations separated by 2 or 3 miles may often be considered as isolated.

Douglas-fir range gaps: In Douglas-fir the most effective range gap is the desert-grassland region stretching from Mexico to Canada and separating the West Coast from the interior forests. The Cascade and Sierra Nevada Mountains effectively cut off precipitation from the interior. As a result, the intermountain area has prevented migration ever since its existence.

The boundaries of the northern interior range are outlined by the Salmon River on the south, the Flathead river on the east, and the eastern Washington desert in the north. Trees lying within these boundaries grow at lower elevations than in the surrounding areas. These differences in elevations are seemingly sufficient to effectively separate the Inland Empire region from central Montana and other northern Rocky Mountain populations.

The boundary separating the northern and central Rockies is the desert area along the Snake River of southern Idaho and the treeless area in the Wyoming basin. Within the central Rockies, the racial distinctions are not great. The trees are separated by small elevational differences, which follow a pattern of increasing latitude and decreasing elevation. A pattern which tends to compensate itself with regards to temperature and length of growing season. The

races in the central Rockies are possibly portions of clines, rather than distinct races in themselves.

The approximate boundaries separating the races of the southern Rockies are the Painted Desert and the treeless region south of Zion Canyon (Kung and Wright, 1972). A racial boundary seems to exist along the Colorado-New Mexico border even though it cuts through forested regions.

NATURAL SELECTION

Most racial differences are associated with climate at the place of origin, which suggests that natural selection is a major factor promoting racial differentiation.

In most cases, the elevational zone occupied by a species varies so that similar climatic conditions occur throughout the entire range of a species. This phenomena does not hold for the entire range of Douglas-fir. At the same elevations, the climate of the coastal Douglas-fir region is warmer than that of the interior; it is warmer and moister in the Inland Empire than it is in surrounding areas; and, it is warmer and dryer in Arizona and New Mexico than in the northern parts of the range. Furthermore, Douglas-fir grows at surprisingly low elevations in northern Idaho and Arizona and New Mexico, which further accentuates the climatic contrasts. The result of these distinct climatic regimes has been the evolution of specific adaptations.

Natural selection favors the fastest growing trees in a habitat, providing that those trees are fit in other respects.

The lower elevation and more southerly location produce a much warmer climate for Douglas-fir of the ARINEM race. Natural selection has resulted in adaptation to these conditions, resulting in a type which grows one month longer and taller in Michigan than other types. In the north, the lower elevation of the Inland Empire results in a warmer and moister climate there than is found farther south or east. Adaptation to this climate has resulted in INEMP trees with faster growth than trees from the northern Rockies and central Montana (Wright, 1971).

SELECTION AND MOISTURE CONTENT

Natural selection has probably played an important role in determining the moisture content of Douglas-fir races. The differences are believed to be related to the varied climatic conditions throughout the interior Douglas-fir range. In the hot, dry southern Rockies selection for drought resistance would be a valuable asset, whereas in the colder northern Rockies cold resistance may be of primary importance. Both of these factors are believed to have played a role in selection for foliar moisture content.

DROUGHT RESISTANCE

The effect of foliar moisture content on drought resistance has been debated for several years. Early views suggested that drought resistant plants have low rates of water loss, but this was discarded when it was discovered that many xerophytes transpire rapidly when supplied with water. Emphasis then shifted to the idea that ability to withstand dehydration is the primary factor involved in drought resistance. At present, it is suggested that drought resistant plants must have protoplasm with some capacity to endure dehydration combined with the morphological and anatomical characters which decrease the rate of water loss and postpone the development of critical internal water deficits.

Drought resistance can be differentiated into two specific types as suggested by Levitt (1956), that is, those plants which endure drought and those which avoid

drought. Those plants which endure drought are able to withstand a dry internal environment resulting in severe tissue dehydration. Drought avoidance, on the other hand, may involve some aspect of the plant's physiology or morphology which enables it to avoid a dry internal environment. Resistance often involves both ability to endure drought and avoidance of drought.

Many non-succulent, drought-resistant species are able to endure drought. That is, they lose exceptionally large quantities of water so that their protoplasm is subjected to very negative potentials, and yet they are not killed. These plants are true xerophytes and exhibit the property of hardiness to drought. True xerophytes are capable of maintaining protoplasmic elasticity to high levels of desiccation.

Succulent species, such as cacti, are drought avoiders. They avoid drought by storing water in their succulent tissue. Enough water is stored in their tissue, and its rate of loss is so extremely low that they exist for long periods of time without added moisture. Many non-succulent species avoid drought by various anatomical modifications such as a very deep root system, reduction in leaf size, sunken stomates, or heavy pubescence on the leaf surface. Avoidance of drought has often been explained on the basis of water conservation. Water savers are able to restrict transpiration long before wilting occurs, and they may succeed under conditions of more extreme drought than plants

which are water spenders. Many of the more moderate xero-
phytes and even some mesophytes are water savers. As a
result, the superior drought resistance of a species or a
variety has been found to be partly or even solely due to
water conservation (Kramer and Kozlowski, 1960).

This adaptation for water conservation has accounted
for the differences in habitats of many conifers. Studies
by Johnson Parker (1951 and 1954) compared relative rates
of moisture content and moisture loss on various species of
northern Rocky Mountain conifers. His studies showed that
the leaves of dry site ponderosa pine (Pinus ponderosa) con-
tained greater moisture and lost water less rapidly than
moist and wet sites species of Douglas-fir and western
arborvitae(Thuja plicata).

Similar results have also been found within species
that occupy large and varied environments. Meuli and Shirley
(1937) showed genetic variation in drought resistance of
green ash (Fraxinus Pennsylvanica). Drought resistance in-
creased significantly from south to north in the prairie
plains states. Trees showing the greatest resistance to
drought were consistently associated with regions of driest
climate and longest drought periods. Similar evidence was
accumulated by Kriebel (1957 and 1963) in studying the
genetic variation in sugar maple (Acer saccharum). Trees
from the cool, damp climate of the northern Appalachian
forests produce progeny more susceptible to drought condi-
tions than trees from the hot and dry climates of southern

and midwest habitats.

In 1966, a study was undertaken by Ferrell and Woodward to determine the effects of seed origin on drought resistance of Douglas-fir. Trees from six interior and coastal seed sources were subjected to various drought conditions, and their relative resistance was measured in terms of a "time to death" index. In all cases, interior seed sources were more drought resistant than coastal sources. In two of three drought experiments, Arizona seedlings lived significantly longer than seedlings from other origins. In a third test, the seedlot from northeastern Washington survived longer than the Arizona seedlings, but the differences were not significant.

In seeking further evidence on drought resistance in Douglas-fir, this study was pursued by Pharis and Ferrell (1966). Their first study compared the foliar moisture content of 6 interior and coastal seedlots. The results showed that the Arizona and coastal seedlings (Vancouver Island and Corvallis, Oregon) had significantly higher moisture content than other interior seedlings. For the Arizona seedlings this can be postulated as an adaptation to a relatively dry habitat. The coastal region, however, is well watered, (except in summer). These trees may, therefore, not require a moisture retention mechanism to survive. This lack of adaptation was again clearly shown in the "time to death" and "lethal soil-moisture content" indices of drought resistance. The results of these tests

showed that the interior seedlings were significantly more drought resistant than the coastal seedlings. In the time to death test, the northeast Washington seedlot proved slightly hardier than the Arizona seedlots. In the soil moisture lethal point study, it was discovered that the Arizona seedlings were able to survive at the lowest soil moisture content of all seedlots.

It is this adaptation for drought resistance among dry habitat plants that is believed to be, at least, partly responsible for the high moisture content of the Arizona Douglas-fir seed source. The results of my study verify that the southern ARINEM race of Douglas-fir have an inherently higher moisture content and slower rate of moisture loss than more northern sources. It is probable that in dry habitats selection favored types that are able to utilize and conserve limited moisture.

Similarly, experimental evidence has shown that southern Arizona seed sources have a significantly slower rate of moisture loss than a more northern race. It is also interesting to note that surface waxes, evident on the foliage of southern seedlots only, are believed by many to be an adaptation to water conservation. This point, however, has not been satisfactorily proven in all cases. Trees from Arizona seedlots have probably adapted a mechanism for retarding transpiration and thus conserving moisture.

Further evidence supporting drought resistance in southern seed sources was discovered in Douglas-fir provenance

tests in Michigan and Nebraska. In all Michigan plantations, the ARINEM and INEMP races were the fastest growing (Wright et al., 1970). However, on the dry prarie soils of Nebraska only the ARINEM seedlots grew fast. INEMP seedlots decreased in vigor with time.

The evidence thus far accumulated suggests that natural selection has responded to the dry climates of the Arizona region by providing Douglas-fir from that area with adaptations for high moisture content and moisture conservation.

Based on the results of Pharis and Ferrell, it appears that natural selection has not played the same role in the northern and coastal Douglas-fir races, as in the southern races. Their results present two inconsistencies: (1) coastal seed sources (Corvallis and Valsetz, Oregon and Vancouver Island) although comparatively non-drought resistant, contain an equal or at times a greater amount of foliar moisture than the dry habitat Arizona seed sources, and (2) in some cases, north-eastern Washington seedlots were equal in drought resistance, but contained less foliar moisture than Arizona seed sources.

It is feasible to postulate that these inconsistencies may be a result of any or all of the following circumstances:

1) The coastal seed sources are clearly a separate taxonomic variety, and are probably subjected to unique genetic, as well as, environmental modifications.

2) Although the coast receives an abundant supply of moisture on an annual basis, a very small amount (2-4%)

occurs during the summer; the time in which the studies were performed. As a result, the higher summer moisture content among coastal sources may represent an adaptation to the relatively xeric conditions present during those months.

3) Another factor, not drought related, may be wholly or partially responsible for moisture content adaptation.

The first two explanations are clear possibilities, which can only be substantiated by further experimental evidence. The third explanation is subjective, and therefore allows for further selective interpretation.

COLD RESISTANCE

Another climatic factor which may be related to variations in percent dry weight is temperature. Literature explaining the physiological or environmental relationship between the two variables is comparatively scarce; nevertheless, data showing correlations between high dry matter content and low temperature is available.

Langlet's (1936) study of variation in dry matter content of Scotch pine showed significant correlations between dry weight and winter temperature at place of origin (Figure 1). Trees from cold northern latitudes had the highest percent dry weights. This relation held true for the Swedish provenances, as well as range wide provenances. The differences among Swedish seedlots were large even though precipitation is fairly constant throughout the country.

Langlet and others have studied the seasonal variations

in percent dry weight as well. Although the results for different species vary slightly, all the data indicate an increase in dry weight with the onset of colder seasons. In other studies, Langlet showed that the seasonal increase in dry weight was at least partly due to increases in sugar (glucose) content in the plant. Further investigations by Langlet showed positive correlations between high dry matter content and high sugar content during the winter. The trees from the colder northern origins had higher dry weights and higher sugar content. It is, therefore, feasible that selection for high dry matter content is an adaptation which enables trees to endure winter cold.

A relationship between temperature at place of origin and dry matter content is also evident in Douglas-fir. It has been found that trees from cold northern parts of the Douglas-fir range contain significantly higher dry weights than trees from warm southern and coastal sources. The dry weight data presented by Pharis and Ferrell agree with this relationship.

Such evidence suggests that selection for dry matter content might be a response to temperature at place of origin, rather than precipitation as previously suggested. If this is the case, an adaptation for high dry matter content in colder climates would result in the advantage of cold resistance found in the northern parts of the Douglas-fir range. At the same time, low dry matter content, found in warmer southern and coastal races, occurs at the expense of

cold resistance. This suggestion is supported by the fact
that coastal origins of Douglas-fir suffered severely from
winter cold when planted in Michigan (Wright, 1971).

Assuming this theory to be valid, then the low dry
matter content of coastal sources and the high dry matter
content of northeastern Washington seedlot (Pharis and
Ferrell, 1966) are explainable with respect to variation
with temperature at these respective origins.

CONCLUSION

Attempts to document variations in moisture relations within and between species have often led to studies of drought resistance. As a result, evidence linking moisture relations and drought resistance is generally overwhelming. However, a closer look into the exceptions to this evidence suggest that another factor, namely temperature, is equally involved. It is likely that both temperature and drought hardiness play a varying role in the evolution of dry matter content in different geographical areas.

The results of the foregoing discussion point out that selection processes operate to develop different adaptations in various environments in nature. In general, Douglas-fir originating from the southern Rocky Mountains contains more moisture and have developed a means of retarding moisture loss. These adaptations are probably a response to the droughty conditions and warm temperatures. The northern interior Douglas-fir races possess higher dry weights, which is likely an adaptation to the cooler climates and high moisture availability. Coastal Douglas-fir is subjected to a completely different set of environmental conditions than the interior variety. It is probable that high moisture content in trees of this region represents a lack of adaptation to cold and a storage mechanism for hot dry summers.

Selection and isolation have combined to segregate Douglas-fir into several races. Because Douglas-fir is a wide ranging species, its components are subjected to

variable environments and a vast array of competing and
associated plant species. Such variation requires flexible
adaptive adjustment for survival insurance. Presumably,
the basic mechanism for coping with the physical and biolo-
gical environment is genetic differentiation or adaptation.
This, in turn, facilitates selective adaptation and the pro-
motion of genetic strains within species.

CHAPTER 7

PRACTICAL IMPORTANCE AND APPLICATION

IMPORTANCE

As of 1972, Douglas-fir comprised only 4% of the total
Christmas trees sold in Michigan. It also demanded the
highest price (approximately $1.25 per foot wholesale). The
reason for the relatively low production is because, until
recently, eastern growers have found Douglas-fir to be a
slow grower.

Recent genetic studies have offered information which
refutes the slow growing reputation Douglas-fir has attained
for the past several years. The results of these studies have
been the identification of races with optimum growth, foliage
color and hardiness characteristics when planted in southern
Michigan (Wright, et al., 1970 and 1971). Correct use of
these published results have resulted in rotation ages half
as long as had previously been experienced.

One of the aims of studying the variation in dry matter
content was to bring a better and safer Christmas tree to
growers and the public. The effect of moisture content on
the longevity and appearance of Christmas trees has been
drastically overlooked in the past, despite the complaints of
the public and salesman of dried out trees. In 1972, the

Christmas tree harvest in Michigan began as early as
September 11 on some plantations. In these cases, the trees
either lied around in lots or in houses for 3 months. The
result being undesirable and unsaleable trees in many in-
stances. In an effort to reduce the drying problem, many
growers delayed cutting until October 9, but still many
Christmas trees had to be sprayed with pigment to be sale-
able.

The results of this study offer evidence which could
minimize drying problems in Douglas-fir Christmas trees.
The 3-4% higher moisture content of Arizona seedlots is of
tremendous significance and will greatly enhance the longe-
vity of trees from that origin. This, of course, is of
practical benefit to those who store or ship trees.

Of practical benefit to growers and the public is the
effect of dry matter content on the appearance of the tree.
The studies have shown that trees of high dry weight tend
to dry faster and lose their color more rapidly than low
dry weight trees. This would be a detriment to the seller
and produce a displeasing tree for the public. In addition,
the association of high moisture content with fire resistance
is an apparent one which is of extreme interest to the public.
It is this problem of fire susceptibility which has gradually
leaned the public towards fireproof artificial trees.

An additional value of moisture content data might be
found in connection with site requirements. Douglas-fir is
known to be a demanding species with respect to moisture and

soil requirements. Due to the fact that soils of Michigan are sandy to sandy loam at best and have a relatively low moisture holding capacity, it would appear that the high moisture varieties would be better adapted to handle such conditions.

It is important to emphasize that in addition to racial selection, care must be taken to avoid dessication in cut trees. Palpant (1973) has shown that by storing Norway spruce (Picea abies) in a cool refrigerated environment, moisture content of the tree could be maintained. This is of particular importance in shipping trees. In trees left outside, studies have shown that a cool, moist, and shaded site with light winds had the greatest effect in maintaining moisture content. The use of anti-desiccants did not improve moisture retention in Norway spruce (Palpant, 1973).

An important implication of drying rate may be found during late winter when complete cessation of water uptake occurs. This occurs when plant roots are confined to the frozen stratum of the earth, and plants are subjected to sunny conditions and dry winds. During such conditions, Saetersdal (1963) has found that slow drying seedlots of Norway spruce (southern German seedlots) are more resistant to winter drought damage than faster drying northern seedlots. Winter drought damage occurs when plants transpire, and are unable to withdraw water from frozen soil. It results in browning of foliage and may possibly lead to death of the tree. Under conditions of winter drought, the

differences between Douglas-fir seedlots in drying rate may
be of importance. Based on drying differences, ARINEM seed-
lots may be expected to survive better than INEMP seedlots
under such conditions.

APPLICATION

Moisture content data can only be of value to the
Christmas tree industry if other growth characteristics are
also suitable. Growth rate and hardiness are probably the
most important considerations in choice of seed source for
ornamental or Christmas tree use. The value of moisture
content data comes in choosing between fast growing and
hardy types, and in improving the quality of the tree.

Table 7 represents a compilation of growth data on two
Douglas-fir plantations in Michigan. Differences between
races in all characteristics listed were statistically
significant. Arizona and New Mexico origins are fastest
growing, have the bluest foliage, and low dry matter content,
but they do suffer considerably from late spring frosts.
Nevertheless, they have been very successful in Michigan and
command top price, despite their reputation for frost damage.
SOCOL trees have the same desirable foliage as the ARINEM
race, but they are slower growing and suffer a great deal
more frost damage. At very young ages the SOCOL trees were
among the fastest growing, but they have since fallen off the
pace due to repeated yearly frost damage.

The northern origins are more hardy than the southern

Table 7.   Racial differences in height, foliage color, and
           frost damage in Douglas-fir growing at plantations
           MS FGP 16-65/67 (Kellogg Forest) and 5-66 (Camp
           Kett) in Michigan. Height data for trees 12 years
           old from seed.

Region of origin	Height (ft.)		Foliage color 20= blue 0= lt. green	frost damage % buds killed	
	Kellogg	Camp Kett	Kellogg	Kellogg (3 yr.av.)	Camp Kett (2 yr.av.)
ALB	6.5	---	14.1	36.5	---
CMON	6.3	4.0	10.0	29.0	15.0
CWASH					
Tieton Rd.	7.2	---	8.0	37.0	---
No. CWASH	6.8	5.2	11.0	26.0	22.0
NOROC	8.0	---	6.5	19.0	---
INEMP	8.8	5.6	7.3	18.0	12.0
NOCOL	5.2	4.1	12.1	53.3	24.0
SOCOL	7.7	4.9	15.4	44.0	32.0
ARINEM					
Arizona	10.6	5.9	16.4	35.0	33.0
New Mex.	10.4	7.4	17.3	37.5	24.0

ones, but seem to be lacking in growth rate, have a less desirable foliage color, and a high dry matter content. Of these, only the INEMP race is fast enough growing for large scale planting. Although hardier than the ARINEM race, the INEMP race is slightly slower growing, has greener foliage, and dries out much faster than the Arizona trees.

It is important to remind growers, that no one type of seed will be best for all conditions or all uses of the tree. The grower must define his planned use of the tree and evaluate local climatic conditions before choosing a seed source. Site selection and care are also important factors. Even the best seed sources will not flourish in frost pockets or very dry sites. It is believed that growers in southern Michigan can achieve maximum results with a mixture of ARINEM and INEMP Douglas-fir seed sources, in combination with good cultural practices. The ARINEM race seems to offer the best quality tree, but the hardiness of the INEMP trees provides the insurance which is essential to the success of any large scale operation.

LITERATURE CITED

LITERATURE CITED

Ackley, W. B., 1954. Seasonal and diurnal changes in the
    water content and water deficits of Bartlett pear
    leaves. Plant Physiol. 29:445-448.

Baldwin, H. I., and D. Murphy, 1956. Rocky Mountains Douglas-
    fir succeeds in New Hampshire. Fox Forest Note 67: 2pp.

Byrnes, W. R., H. D. Gerhold, and W. C. Bramble, 1958.
    Douglas-fir varietal tests for Christmas tree plantations
    in Pennsylvania. Progra. Rep. Penn. Agr. Exp. Sta. No.
    198. 6 pp.

Ching, K. K., and D. Bever, 1960. Provenance study of
    Douglas-fir in the Pacific Northwest region. 1. Nursery
    performance. Silvae Genetica 9:11-17.

Cleary, B. D., and R. H. Waring, 1965. Seasonal and diurnal
    variation in the international moisture stress of mature
    Douglas-fir in the Siskiyou Mountains. Proc. of Western
    For. Gen. Associ., (3rd session) abstract.

Ferrell, W. K., and E. S. Woodward, 1966. Effects of seed
    origin on drought resistance of Douglas-fir. Ecology
    47:499-503.

Fuchigami, L. H., D. R. evert, and C. J. Weiser, 1971. A
    translocatable hardiness promoter. Plant Physiol. 47:
    164-167.

Gary, H. L., 1971. Seasonal and diurnal changes in moisture
    content and water deficits of Engelmann spruce needles.
    Bot. Gaz. 132:327-333.

Gerhold, H. D., 1965. Douglas-fir growth and winter injury
    demonstration. Thirteenth No. East For. Tree Impr. Conf.,
    Am. Christ. Tree Growers Jour.:Nov.

_____, 1966. Growth and winter injury in Douglas-fir
    in a 3 year provenance demonstration. Northeast For.
    Tree Impr. Conf. Proc. 13:50-52.

Gibbs, R. D., 1958. Patterns in the seasonal water content
    of trees. In K. V. Thimann (ed.), The Physiology of
    Forest Trees, Ronald Press, New York: 43-69.

Hanover, J. W., 1963. Comparative biochemistry and physiology of western white pine resistant and susceptible to infection by the blister rust fungus, Washington State University, Ph.D. thesis: 173 pp.

_____, and D. A. Reicosky, 1971. Surface wax deposits on foliage of *Picea pungens* and other conifers. Amer. Jour. of Botany 58:681-687.

Heit, C. E., 1968. Propagation from seed, part 17. Testing and growing Douglas-fir from different sources. Amer. Nurseryman, Nov. 15 issue.

Irgens-Moller, Helge, 1957. Ecotypic response to temperature and photo-period in Douglas-fir. For. Sci. 3:78-83.

_____, 1958. Genotypic variation in the time of cessation of height growth in Douglas-fir. For. Sci. 4: 325-330.

Jameson, D. A., 1966. Diurnal and seasonal fluctuations in moisture content of pinjon pine and juniper. U. S. For. Ser., Rocky Mtn. For. Range Exp. Sta. Res. Note RM-67: 7 pp.

Jones, J. R., 1972. Moisture stresses in Arizona mixed conifer seedlings. U.S.D.A. For. Serv. Res. Paper RM-86: 10 pp.

Korstian, C. F., 1933. Physiochemical properties of leaves and sap as indices of the water relations of forest trees. Union Intern. Inst. Recherles For., Congress of Nancy, Comptes Rendus: 312-325.

Kozlowski, T. T. and A. E. Peterson, 1960. Variations in moisture content of dormant buds, For. Sci. 6.

_____ and T. J. Clausen, 1965. Changes in moisture content and dry weights of buds and leaves of forest trees. Bot. Gaz. 126:20-26.

Kramer, P. J., 1949. Plant and Soil Relationships. McGraw-Hill Publ. Co., New York: 274-278.

_____, and T. T. Kozlowski, 1960. Physiology of Trees. McGraw-Hill Publ. Co., New York: 342-367.

Kriebel, H. B., 1957. Patterns of genetic variation in sugar maple. Ohio Agr. Exp. Sta. Res. Bull. # 791:17-22.

_____, 1963. Selection for drought resistance in sugar maple. World Consultation on For. Gen. and Tree Impr., Doc. # 319:5 pp.

Kung, F. H. and J. W. Wright, 1972. Parallel and divergent evolution in Rocky Mountain Trees. Silvae Genetica 21: 65-72.

Langlet, O., 1936. Studier overtallens fysiologiska variabilitet och dess samband med klimatet. Ett bidrag till kannedomen om tallens ekotyper. Stockholm: Statens Skogsforsoksanstatt Meddelanden, no. 29:219-420.

Levitt, J., 1956. The Hardiness of Plants. Academic Press, Inc., New York: 278 pp.

_____, 1962. "A sulfhydryl-disulfide hypothesis of frost injury and resistance in plants." Jour. of Theoretical Biology 3:355-391.

Meuli, L. F. and H. L. Shirley, 1937. The effect of seed origin on drought resistance of green ash in the prarie plains states. Journ. of For. # 35:1060-1062.

Morris, W. G., R. R. Silen and H. Irgens-Moller, 1957. Consistency of bud bursting in Douglas-fir. Jour. of For. 55:208-219.

Munger, T. T. and W. G. Morris, 1936. Growth of Douglas-fir trees of known seed source. U.S.D.A., Tech. Bull. 537: 40 pp.

_____, 1942. Further data on the growth of Douglas-fir trees of known seed source. Pac. Northwest For. Exp. Sta., April 10:12 pp.

Olien, C. R., 1967. "Freezing stresses and survival." Annual Rev. of Plant Physiol. 18:387-408.

Parker, J., 1951. Moisture retention in leaves of conifers of the northern Rocky Mountains. Bot. Gaz. 113:210-216.

_____, 1954. Available water in stems of some Rocky Mountain conifers. Bot. Gaz. 115:380-385.

Palpant, E. H., 1973. Christmas tree desiccation - Is there a solution? Am. Christmas Tree Grow. Jour. 37:11-12.

Pharis, R. P. and W. K. Ferrell, 1966. Differences in drought resistance between coastal and inland sources of Douglas-fir. Canad. Jour. of Bot. 44:1651-1659.

Pharis, R. P., 1967. Seasonal fluctuations in the foliage moisture content of well-watered conifers. Bot. Gaz. 128:179-185.

Philpot, C. W., 1963. The moisture content of Ponderosa pine and whiteleaf manzanita foliage in the central Sierra Nevada's. U.S. For. Ser. Res. Note PSW-39, Pacific So. West For. and Range Exp. Sta.: 7 pp.

Reicosky, D. A., 1973. Developmental and physiological aspects of surface waxes of blue spruce. Michigan State University, Ph.D. thesis: 84 pp.

Saetersdal, L. S., 1963. The rate of drying of young excised plants of various provenances of Norway spruce and Douglas-fir. Meddelser fra Vestlandets Forstlige Forsøksstasjon # 38: 88 pp.

Schütt, P., and R. J. Hoff, 1969. Foliage dry matter of Pinus monticola its variability with environment and blister rust resistance. U.S.D.A. For. Ser. Res. Note INT-102:6 pp.

Shirley, H. L. and Meuli, L. J., 1939. Influence of moisture supply on drought resistance of conifers. Jour. of Agr. Res. 59: 1-21.

Silen, R. R., 1965. A 50 year racial study of Douglas-fir in Western Oregon and Washington. Proceed. of West. For. Gen. Assoc., (1st session).

Sweet, G. B., 1965. Provenance differences in Pacific Coast Douglas-fir. Silvae Genet. 14:46-56.

United States Forest Service, 1965. Silvics of forest trees of the United States. U.S. Dept. of Agr. Handbook 271: 782 pp.

Weiser, C. J., 1970. Cold resistance and acclimation in woody plants. Science, 169:1269-1278.

Wharton, D., 1974. Douglas-fir: Our most valuable tree. Am. Forests, 80:30-34.

Wright, J. W., F. H. Kung, R. A. Read, W. A. Lemmien, and J. N. Bright, 1971. Genetic variation in Rocky Mountain Douglas-fir. Silvae Genetica 20:54-60.

Wright, J. W., 1962. Genetics of forest tree improvement. FAO Forestry and forest products study # 16:399 pp.

Wright, J. W., R. A. Read, F. H. Kung, J. N. Bright, and W. A. Lemmien, 1970. Better Douglas-fir for Eastern United States. Am. Christmas Tree Gro. Jour., 14:23-28.

Zaerr, J. B., 1971. Moisture stress and stem diameter in young Douglas-fir. For. Sci., 17.

Zavithovski, J. and W. K. Ferrell, 1968. Effect of drought upon rates of photosynthesis, respiration, and transpiration of seedlings of two ecotypes of Douglas-fir. Bot. Gaz. 129:346-350.

APPENDIX

APPENDIX A1

SUGAR AND COLD RESISTANCE

During freezing water is removed from plant cells, resulting in high water stress in the plant. Levitt (1959) has proposed 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 (Figure 7). Upon oxidation the hydrogen is removed and the 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. Resistance to cold damage is related to an inhibition of these intermolecular disulfide linkages which form between the proteins and ultimately distort them. Heber and Ernst have found that in cold resistant herbaceous plants sugar replace water in forming a protective shell around the protein molecules. This, if it were true for trees, could account for the increase in sugar content at the expense of water during the colder months of the year.

Charles Olien (1967) studied the interference of freezing caused by large water soluble polysaccharides polymers extracted from the cell walls of hardened plants. These substances, which contained large amounts of sugar (xylose and

Figure 7. A schematic illustration of Levitt's sulfhydryl-

disulfide hypothesis of frost injury and resistance

in plants. (Based upon J. Levitt, 1962).

Unfrozen

protein   water
helix   between
           proteins

-SH   HS-

S-S

HS-
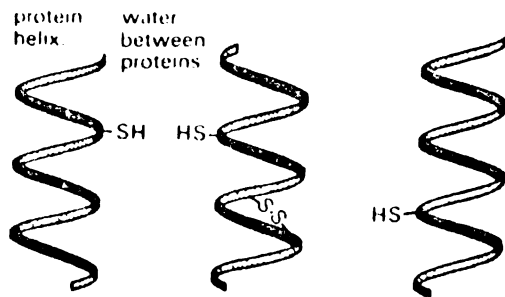
Water Removed by Freezing:

-S-S-

S-S-S
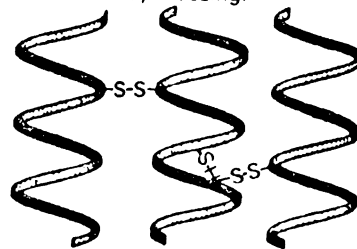
Water Returned at Thawing:

-S-S-

SH

S-S-

distorted proteins

arabinose), interfered with freezing by competing with water molecules for sites in the ice lattice at the liquid inter-face. The result was that they tended to stop crystal growth, causing an imperfect ice mass to form. Only polymers from cold hardy plants or plant varieties resulted in imperfect crystal formation in the plant.

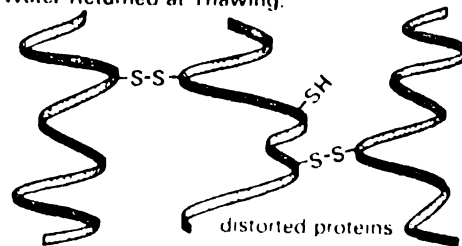In attempting to explain cold resistance much has been learned by studying the plants acclimation to cold. Weiser (1970) has found that the leaves are the site of perception of short days which initiates the first stage of acclimation. He proposes that short day induced leaves are the source of a translocatable hardiness promoting factor which moves from the leaves to the stems. The substance of the hardiness promoter is not known; however, it is believed to be either a growth inhibitor, a sugar, or a regulatory hormone. In work done by Fuchigami et al. (1970), it was discovered that abscissic acid (a growth inhibitor) and gibberelin (a growth hormone) did not induce or enhance acclimation of different races of red-osier dogwood (Cornus stolonifera). Weiser (1970) has compiled considerable circumstantial evidence which suggests that some basic level of sugar is probably required for acclimation: (1) leaf discs of cabbage and leaves of gardenia increased in frost resistance when infused with sugar, (2) plants cannot acclimate when they are depleted in photosynthetic substrate, (3) sugar protects the enzyme systems associated with oxidative phosphorylation in isolated spinach chloroplasts subjected to freezing, (4) considerable data

indicates that starch is converted to sugar in plant tissues exposed to low temperatures. Although this evidence seems considerable, no experimental evidence shows that feeding sugar to woody plant tissue has increased their resistance. Attempts to do this with red-osier dogwood have been unsuccessful.

Langlet's (1936) dry weight and sugar content data offer relevant information to the problem of cold resistance in plants. His data show significant correlations indicating that sugar is in some way related to reinforcing the plant for the cold of winter.

APPENDIX A2

STATISTICAL FORMULAS

1. Analysis of Variance (Machine Formulas):

Source	Degrees of freedom	Sums of Squares	Mean Squares
Seedlot	$n-1$		SS/d.f.
Replicate	$r-1$		SS/d.f.
Error	$(n-1)(r-1)$		SS/d.f.
Total	$nr-1$		

Seedlot #	Replicate #			Sum	
	$\underline{1}$ $\underline{2}$ $\underline{3}$			$-$	n = # of seedlots
501	A	A	A	B	r = # of replicates
502	A	A	A	B	A = plot means
503	A	A	A	B	B = seedlot sum
504	A	A	A	B	C = replicate sum
505	$\underline{A}$	$\underline{A}$	$\underline{A}$	$\underline{B}$	D = grand total
	$C_1 C_2 C_3$			D	

Sums of Squares (SS):

Seedlot SS $= 1/r\ B^2 - \dfrac{(D)^2}{nr}$

Total SS $= A^2 - \dfrac{(D)^2}{nr}$

Replicate SS $= \dfrac{1}{n}\ C^2 - \dfrac{(D)^2}{nr}$

Error SS = Total SS - (Seedlot SS + Replicate SS)

2. Least Significant Difference (LSD):

$$LSD = t\alpha(v) \sqrt{\frac{2 \text{ (error mean square)}}{n}}$$

v = error d.f.

n = # of plots per race

t = t value from table

α = desired level of significance

---

3. T-test: for even samples

$$t = \frac{(Y_1 - Y_2) - (u_1 - u_2)}{\sqrt{1/n \ (s_1^2 + s_2^2)}}$$

Y = sample mean

u = parametric mean (0)

s^2 = variance of sample mean

n = number of observations

for uneven samples

$$t = \frac{(Y_1 - Y_2) - (u_1 - u_2)}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1) s_2^2}{n_1 + n_2 - 2} \quad \frac{n_1 + n_2}{n_1 n_2}}}$$

d.f.= $n_1 + n_2 - 2$

---

4. Spearman's coefficient of rank correlation:

$$r = 1 - \frac{6 \sum\limits^{n} (R_1 - R_2)^2}{n (n^2 - 1)}$$

R = ranks of the variables

n = number of observations

---

5. Chi-square test (two by two table):

$$x^2 = \frac{(\text{Observed frequency}_1 - \text{expected frequency}_1)^2}{\text{Expected frequency}}$$

$$+ \frac{(\text{observed frequency}_2 - \text{expected frequency}_2)}{\text{Expected frequency}}$$

degrees of freedom = 1

APPENDIX A3

ANALYSIS OF VARIANCE

Table 1A. Analysis of variance of current-years and year-old
foliage moisture content data from plantations
MSFGP-16-65107 (Kellogg Forest), MSFGP-5-65 (Camp
Kett), MSFGP-9-66 (Kellogg Forest), and MSFGP-4-67
(East Lansing). Levels of significance indicated
by asterisks.[A]

Kellogg Plantation - MSFGP 16-65167

| | | Current-Growing Foliage | | Yr-old Foliage | |
Source of Variation	df	MS	F	MS	F
Seedlot	66	2553.4	2.63**	467.50	2.76***
between region	8	10,479	7.18***	1007	2.56**
within region	58	1,460	1.51**	393	2.32***
Replicate	4	18,113	18.68***	10,049	59.37***
Error	268	969.7		169.25	
Total	339			339	

Camp Kett Plantation - MSFGP 5-65

| | | Current-Growing Foliage | |
Source of Variation	df	MS	F
Seedlot	35	679.4	1.64*
between race	6	1978.5	4.82***
within race	29	410.7	.99

92

Camp Kett Plantation (continued)

	df	MS	F
Replicate	4	2230	5.37**
Error	113	414.9	
Total	152		

Kellogg Plantation - MSFGP-9-66 & 4-67 (combined)

Year-Old Foliage

Source of Variation	df	MS	F
Seedlot	21	517.7	2.06*
between race	6	83.19	.12
within race	15	691.57	2.35**
Replicate	4	1381.75	5.51**
Error	59	251.44	
Total	85		

*   significant at 5% level
**   significant at 1% level
*** better than 1% level

Table 2A. Analysis of variance of height and foliage color data from plantation MSFGP 16-65/67. Levels of significance indicated by asterisks.

Source of Variation	df	Height		Foliage Color	
		MS	F	MS	F
Seedlot	68	263.79	8.38***	71.43	6.64***
between race	7	1902	24.95***	594	34.62***
within race	61	75.8	2.41***	16.05	1.49**
Replicate	4	34.75	1.10	1.18	.11
Error	235	31.48		10.75	
Total	307				

Table 3A. Analysis of variance showing significance of year
of planting at the Kellogg Forest (MSFGP 16-65/67)
on current-growing and year-old foliage moisture
content data and on height and foliage color.
Differences in year of planting significantly
affected height growth.

| | | Moisture Content | | | |
| | | Current-Years Foliage | | Year-Old Foliage | |
Source of Variation	df	MS	F	MS	F
Seedlot	9	36.64	5.22*	2.69	1.12
Year of Planting	1	11.77	1.68	.17	.07
Error	9	7.02		2.31	
Total	19				

| | | Height and Color | | | |
| | | Height | | Color | |
Source of Variation	df	MS	F	MS	F
Seedlot	11	278.63	5.27**	63.18	6.26**
Year of Planting	1	637.07	12.06**	30.0	2.97
Error	11	52.84		10.1	
Total	23				

Table 4A. Analysis of variance showing the effect of time

of collection (within and between replicates) on

current growing and year-old foliage moisture con-

tent at plantation MSFGP 16-65/67 (Kellogg Forest).

		Current-Years Foliage		Year-Old Foliage	
Source of Variation	df	MS	F	MS	F
Seedlot	76				
Time effect					
between replicates	4	142.79	14.05**	93.76	53.58**
within replicates	20	9.94	.94	2.43	1.36
Error	242	10.58		1.78	
Total	342				

Table 5A. Error mean squares for moisture content data from each Douglas-fir race at the Kellogg Forest plantation (MSFGP 16-65/67). (Both current-growing and year old foliage). High error mean squares for NOCOL and SOCOL (current growing foliage) is due to variability caused by frost damage.

Race	df	Error Mean Current Foliage	Year-Old Foliage
ALB	6	1314	461
CMON	22	1036	417
NOROC	40	279	159
INEMP	70	427	239
CWASH	17	1592	175
NOCOL	37	2649	572
SOCOL	43	2262	361
ARINEM	33	1163	331
Total	268		

APPENDIX A4

CLIMATIC PATTERNS

Table 6A. Climatic variation throughout the Douglas-fir range. Various climatic regimes for the environments occupied by each interior Douglas-fir race are given. (Baker, F., 1944. Mountain climates of the western United States. Ecol. Monographs 14:223-254).

Race	Elevation	MinT	MaxT	Average T		Annual		Rainfall during				Average
				Jan.	July	Precip.	Snowf.	Dec. Feb.	Mar. May	June Aug.	Sept. Nov.	Annual Sunshine
	---ft.---	--------of--------				---in.---		--% of total--				--hrs.--
CWASH	3300	-19	95	22	59	55	300	48	18	7	28	2400
NOROC	3900	-31	102	23	65	32	170	37	25	17	21	2600
INEMP	3300	-22	97	22	62	38	180	34	26	13	27	2600
CMON	5400	-35	100	20	61	18	120	16	19	42	23	2600
NOCOL	7500	-31	94	25	63	18	75	11	25	40	19	3000
SOCOL	7800	-21	91	25	65	22	70	18	22	41	19	3200
ARINEM	7200	- 5	85	39	55	17	40	16	15	41	28	3700