Natural Variation in *Abies* of the Southern Appalachians

John F. Robinson
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by John F. Robinson entitled "Natural Variation in *Abies* of the Southern Appalachians." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

<div align="right">

E. Thor, Major Professor

</div>

We have read this thesis and recommend its acceptance:

H. R. DeSelem, E. R. Buckner

<div align="right">

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

</div>

(Original signatures are on file with official student records.)

June 24, 1968

To the Graduate Council:

    I am submitting herewith a thesis written by John F. Robinson, entitled "Natural Variation in <u>Abies</u> of the Southern Appalachians." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

<u>   Eyvind Thor   </u>
Major Professor

We have read this thesis and
recommend its acceptance:

<u>Edward R. Buckner</u>

<u>H. R. De Selm</u>

Accepted for the Council:

<u>   Hilton A. Smith   </u>
Vice Chancellor for
Graduate Studies and Research

NATURAL VARIATION IN ABIES OF THE SOUTHERN APPALACHIANS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

John F. Robinson

August 1968

ACKNOWLEDGMENT

The writer wishes to express sincere appreciation to Dr. E. Thor of the Department of Forestry for his counsel and assistance as his major professor.

Appreciation is also expressed to Dr. H. R. DeSelm of the Department of Botany and Professor E. R. Buckner of the Department of Forestry who have both served on his committee and have made valuable criticisms of the manuscript.

He also wishes to express appreciation to Dr. Alan Heilman of the Department of Botany for his assistance with microtechnique,

Thanks are also due to the members of the Faculty and Staff of the Department of Forestry for their full cooperation and assistance when needed, with special mention of Miss Mary Andre', Laboratory Technician. Paul Barnett's assistance with photography is also gratefully acknowledged.

The personnel of the U. S. Forest Service, U. S. Park Service, and Pennsylvania Department of Forests and Waters, as well as other individuals, were especially helpful in locating suitable fir stands for this study.

Special thanks are due to Dot, the writer's wife, for her excellent typing of this thesis.

ii

ABSTRACT

Determinations were made of several foliage, seed, and cone characteristics from material collected throughout the ranges of Abies fraseri in high elevations of Tennessee, North Carolina, and southern Virginia; Abies balsamea var phanerolepis in West Virginia and northern Virginia; and Abies balsamea from its southernmost distribution in Pennsylvania and southern New York.

Natural variation was investigated to determine relationships among these taxa, especially with reference to possible hybridity of A. balsamea var phanerolepis in West Virginia and northern Virginia. Much variation was found among species groups and among stands within groups. Variation patterns suggested sampling from a north-south cline. Stand values of many characteristics overlapped, in many cases obscuring taxonomic boundaries. High correlation with north-south geographic location was shown for many characteristics.

Variation was no greater within the intermediate fir stands (A. balsamea var phanerolepis) than within Fraser fir stands for 12 of the 13 characteristics analyzed, and distribution of hybrid index values of the intermediate fir was normal. The theory of hybrid origin of intermediate fir in West Virginia and Virginia was not generally upheld. Only one characteristic, "leaf scar width" showed wider variation within the intermediate stands than within Fraser or balsam fir.

iii

"Total hypodermal cells" discriminated well between Fraser fir and the other firs, but not between balsam fir and intermediate fir. The traditional cone bract length-scale length ratio was the only characteristic which distinguished absolutely among the three taxa with no overlapping of values. This ratio is calculated from "bract length" and "scale length" which very more or less inversely to each other from north to south. The ratio exaggerated differences due to variation of either characteristic. Differences among <u>Abies</u> <u>fraseri</u>, <u>A</u>. <u>balsamea</u>, and <u>A</u>. <u>balsamea</u> var <u>phanerolepis</u> may be less distinct than heretofore believed.

TABLE OF CONTENTS

LIST OF TABLES

TABLE

LIST OF FIGURES

ix

I. INTRODUCTION

The genus _Abies_ is represented in Eastern United States by two
species, _Abies fraseri_ (Pursh) Poir, and _Abies balsamea_ (L.) Mill.

Abies balsamea is extensively distributed in Canada from Labrador
westward to Less Slave Lake, southward through Newfoundland, the Maritime
Provinces, Quebec, and Ontario. In the United States its distribution
extends southward into the Lake States, New England, and New York
(Sargent, 1949). Its southernmost extension is characterized by dis-
junct distribution through Pennsylvania, northern Virginia, and West
Virginia, where it occurs in boggy habitats at fairly high elevations
(E. L. Little, 1953; Bakuzis and Hansen, 1965).

Farther south along the Appalachian Mountain chain, in the higher
elevations of southwestern Virginia, eastern Tennessee, and western
North Carolina, the closely related _Abies fraseri_ replaces _Abies
balsamea_. In contrast to the extensive range of _Abies balsamea_, _Abies
fraseri_ has a limited disjunct distribution in this area. The most
southerly occurrences of Fraser fir are on Clingman's Dome, Tennessee,
and in the Richland Balsams in North Carolina. These are the southern-
most of a series of peaks 5700 feet and above which extend northeastward,
including Roan Mountain, Tennessee; Mount Mitchell, North Carolina,
Grandfather Mountain, North Carolina; and Mount Rogers, Virginia, where
the northernmost occurrence of Fraser fir is found.

1

The most prominent feature which distinguishes Abies fraseri from Abies balsamea is the relative length of the cone scales and bracts. Abies balsamea has a bract much shorter than the cone scale. The bract is fully enclosed within the cone scale and does not project from the cone. Abies fraseri has a bract much longer than the cone scale, being exserted from the cone and reflexed downward. Fraser fir cones are also shorter and more rounded than balsam fir cones (Engelman, 1878; Core, 1934; Little, 1953; Bakuzis and Hansen, 1965). Other less prominent distinguishing characteristics are the number of hypodermal sclerenchy-matous cells on the upper side of the leaf, and number of rows of stomata on the underside of the leaf (Engelman, 1878; Anderson, 1897; Dorner, 1899; Zon, 1914; Fulling, 1936; Bakuzis and Hansen, 1965).

The Abies which occurs in West Virginia and Shenandoah National Park in northern Virginia has cones in which the relative length of bracts and scales is intermediate between Abies fraseri and Abies balsamea. The bracts are mostly exserted but not to the degree found in Abies fraseri, nor are they as sharply recurved (Core, 1934). The fact that the geographic distribution of this population is also intermediate between the ranges of typical Abies balsamea and Abies fraseri has led to speculation that it may be hybrid origin (Core, 1934; Fulling, 1936; Ramseur, 1961). Fulling (1936) described the Abies in Shenandoah National Park at Hawksbill Mountain as a new species, "Abies intermedia, the Blue Ridge fir." His classification was criticized by Fosberg (1941) as being based on a character of sporadic occurrence throughout a population. Fosberg associated this population with A. balsamea var phanerolepis (Fernald), described in 1909 from mountaintops in Maine.

The primary objective of this study is to provide a description of _Abies_ populations in the Southern Appalachian Mountains by measuring natural variation found in: (1) Fraser fir, (2) the southernmost populations of balsam fir in Pennsylvania and New York, and (3) the intermediate populations of _Abies_ in West Virginia and Virginia. Various morphological characteristics have been studied from foliage, seed, and cone samples collected throughout the ranges of these populations. Quantitative measurements were taken of those foliage, seed, and cone characteristics which a preliminary investigation and literature review showed to be most important in discriminating between _Abies balsamea_ and _Abies fraseri_. Data on all measured characteristics were subjected to analyses of variance to estimate within tree, among tree, among stand, and among population group variance components. From this information and use of graphs, two hypotheses concerning the origin and present occurrence of the intermediate populations were evaluated:

1. They originated from hybridization between _A. balsamea_ and _A. fraseri_ during range sympatry during the Pleistocene.

2. They are relicts of a once continuous ancestral population of _Abies_.

II. LITERATURE REVIEW

Taxonomic

The most obvious distinguishing characteristic between Fraser fir
and balsam fir is the relative lengths of bracts and scales of the cones.
Abies fraseri has exserted bracts which are reflexed downward, whereas
Abies balsamea has shorter bracts which are hidden under the scales
(Core, 1934). Engelman (1878) described Abies fraseri as follows:
"readily distinguished from Abies balsamea by shorter, more oval cones
with largely exserted and reflexed bracts." Lester (1964) suggested
that stalk length of the bracts may be the most important characteristic
affecting bract exsertion. Pursh (1814) in his original description of
Abies fraseri described the cones as being only one-fourth or less the
size of those of Abies balsamea.

Many leaf features have been used to distinguish Abies fraseri
from Abies balsamea. However, they are generally less reliable than
cone characteristics because most leaf characteristics are quite vari-
able, being strongly affected by environment. Anderson (1897) described
the normal leaves of Abies balsamea as having more stomata on the lower
leaf surface than on the upper. The upper leaf surface has one band of
stomata with 3 - 10 stomatal rows, and the lower leaf surface has two
bands, one on each side of the midrib, with 8 - 10 stomatal rows in
each band. Fulling (1934) separated balsam fir from Fraser fir on the
basis of number of stomatal rows in each band on the lower surface,

4

stating that Abies balsamea has 4 - 8, while Abies fraseri has 8 - 12,

Engelman (1878) stated that Fraser fir has 8 - 10 - 12 stomatal rows in

each white stomatal band on the underside of the leaf.

Another feature for distinguishing Abies fraseri from Abies

balsamea is the number and continuity of sclerenchymous hypodermal cells.

The hypodermal layer in Abies fraseri was described by Engelman (1878)

as continuous on the upper surface and more crowded on the edges than

the discontinuous hypodermal layer in Abies balsamea. This observation

was supported by Dorner (1899), Zon (1914), and Fulling (1934).

Other conifer leaf characteristics commonly exhibiting variation

include needle length and width; position, number, and size of resin

canals; leaf color; leaf ranking along the twig; leaf length and shape;

acuteness of leaf tip; and width-thickness ratio (Harlow, 1931; Gathy,

1957; Vidakovic, 1957; Schutt and Hattemer, 1958; Ching, 1959; Thorbjornsen,

1960). Ching (1959) used number of stomates per unit length of row to

distinguish Douglas fir (Pseudotsuga taxifolia) from bigcone Douglas fir

(Pseudotsuga macrocarpa) and their hybrid progeny. Mergen (1957) reported

that number of stomates per lineal measure in slash pine (Pinus elliottii)

and some of its hybrids is relatively independent of environment. Shape

of parenchyma cells was used by Gathy (1957) with variable results, and

Oosting (1956) reported that the shape of parenchyma cells is highly

variable; cells are elongated vertically under intense light, but have

a spongy formation under weak light. Amount of supporting tissue is

also affected by light intensity. Harlow (1931) noted that number of

resin canals in pine is variable under different light intensities. He
advocated, instead, use of resin canal position in relation to the epi-
dermis. Recent work by Roller (1966), however, with Abies balsamea,
Abies lasiocarpa, and Abies fraseri revealed that position of resin
canals is highly variable during needle development. Position is
affected by tree age, needle age, and crown position. He found no
significant differences among species in resin canal position.

Zavarin and Snajberk (1964) analysed terpene hydrocarbons of
bark blister resins, and found wide differences between Fraser and
balsam firs.

Intermediate Populations

Abies sp. occurs in West Virginia in four general locations:
Blister Swamp in Pocohontas County at approximately 3640 feet in eleva-
tion; Blister Run in Randolph County at 3650 feet; Canaan Valley in
Tucker County at 3250 feet; and Stony River Dam in Grant County at 3500
feet (Brooks, 1920, U.S.D. Agric., 1928; Core, 1934; Wherry, 1934;
Allard and Leonard, 1952; Clarkston, 1966). These are remnants of much
larger populations of Abies extant less than 100 years ago throughout
much of northern West Virginia in boggy habitats at higher elevations,
which have since been reduced by fire, agriculture, and logging (Allard
and Leonard, 1952).

Core (1934) described the West Virginia Abies at Blister Run and
at Canaan Valley as intermediate between Abies balsamea and Abies
fraseri, "differing markedly" from Abies balsamea because of "more acute

leaves, larger seeds, and broader scales," but more closely related to
Abies balsamea because the bracts were shorter than the scales. He also
stated that two races may be represented in West Virginia because there
are several distinct differences between specimens collected from Cheat
Bridge (Blister Run) and those collected from Canaan Valley. He con-
sidered the fir of West Virginia as of possible hybrid origin between
Abies balsamea and Abies fraseri. In later correspondence[1] he stated
that many or most of the West Virginia trees resembled Abies balsamea
var. phanerolepis in that the awns of the bracts were exserted. Some
of the trees resembled Abies balsamea, some Abies fraseri, and some
were intermediate. Wherry (1934) also considered the stand at Cheat
Bridge intermediate and referred to it as Abies balsamea var.
phanerolepis. Brooks (1920) described the West Virginia Abies as A.
fraseri and earlier Millspaugh (1913) identified it as Abies balsamea.

Fulling (1936) described the Abies at Hawksbill Mountain in
northern Virginia as "Abies intermedia, the Blue Ridge fir." He pro-
posed it as a new species because of the following features: inter-
mediacy in bract length between Fraser fir and balsam fir; scales
auriculate at the base resembling neither balsam nor Fraser fir; ex-
serted bracts not recurved as are Fraser fir bracts; number of stomatal
lines on the leaves overlapped both other species. His description

---

[1]Personal communication: Dr. E. L. Little, 1963, 1966, with Dr.
E. Thor, Associate Professor of Forestry, The University of Tennessee,
and Mr. Russell Walters, Research Forester, U.S.F.S., Berea, Kentucky.

lent support to the hybrid theory. Ramseur (1961) described the West Virginia and Virginia _Abies_ populations as limited to seven small, well-isolated stands. He compared herbarium specimens of cones, scales, bracts, and seeds from these stands with specimens of _Abies fraseri_ and _Abies balsamea_. Using Anderson's (1936) hybrid index method of evaluation, he concluded that variation fell within the limits which could be expected of a hybrid, and that their ranges may have come together during the Pleistocene enabling such hybridization to occur. Fosberg (1941) criticized Fullings new species as based on a character of sporadic occurrence and associated this Virginia population with Fernald's (1909) var. _phanerolepis_. He stated that several cones of _A. balsamea_ and _A. balsamea_ var. _phanerolepis_ from farther north also showed occasional auriculate scales. Heimburger and Holst (1955) considered the Virginia _Abies balsamea_ morphologically the same species as occurs in eastern Canada, but possibly of a different ecotype.

Fernald (1909) described _A. balsamea_ var. _phanerolepis_ as follows: "all but the uppermost scales are equalled or exceeded by the obvergent awn of the subtending bract so that the awns appear wide spreading and stand out from one to five millimeters from the cone."

The distribution of _A. balsamea_ var. _phanerolepis_ was described by Little (1953) as "ranging from Newfoundland and Labrador to Ontario and Maine and the higher mountains of New Hampshire, Vermount, and New York." He also placed the West Virginia and northern Virginia populations into this category, but stated that these may represent an intermediate population between _Abies balsamea_ and _Abies fraseri_. In 1965

he identified as Abies balsamea var. phanerolepis cone and foliage specimens from the vicinity of Hawksbill and Skyland Mountains, Virginia (Shenandoah National Park) (Telerico, 1965). This population was described by Fulling (1936) as Abies intermedia. Myers and Borman (1963), investigating variation in Abies balsamea cones and leaves, used cone scale/bract ratio to measure intergradation between Abies balsamea var. balsamea, and Abies balsamea var. phanerolepis. They found a complete series of morphological forms connecting the varietal extremes. The distribution of these forms was continuous from low to higher altitudes in the Northeastern United States with phanerolepis occurring at higher elevations. They also found an east-west morphological cline with phanerolepis at the eastern extreme. Leaf length was negatively correlated with altitude with longest leaves becoming shorter from median altitudes to alpine-tundra. Their investigations included northern Virginia and West Virginia specimens which fell in the var. phanerolepis extreme of the cline.

Origin of Abies Fraseri

Ecologists believe that during the glacial advances in the Pleistocene the boreal forests extended much farther south than their present distribution. The Southern Appalachians provided an avenue to the boreal forest elements in their migration southward during cooler climates associated with southward advance of the glaciers. A continuous population of Abies probably extended from the present locations of the boreal forests in Canada and Northeastern United States, southward

through Pennsylvania, the higher elevations of West Virginia, Virginia, Tennessee, and North Carolina. Presence of pollen deposits of northern genera (Larix, Picea, Thuja, or Abies) in North and South Carolina, Louisiana, and Texas indicate that the southernmost extension of Abies was farther than the present southern limits of Abies fraseri in Tennessee and North Carolina (Brown, 1938; Cain, 1944; Potzger and Tharp, 1947; Oosting and Billings, 1951; Braun, 1955; Whittaker, 1956; Whitehead and Barghoorn, 1962). As the climate again warmed, the boreal forest retreated. The Abies sp. individuals at lower elevations in southern latitudes were the first to be replaced by species better adapted to the warmer climate. As warming continued, selection against individuals at lower elevations became more intense until the firs became isolated on the highest mountains where Abies fraseri occurs today. Whittaker (1956) has pointed out that in the Great Smoky Mountain National Park, Abies fraseri occurs on or adjacent to high peaks 6000 feet and above, and in favorable areas down to an elevation of 4500 feet. He noted that south of Clingman's Dome in the Smokies, even though considerable areas are present above 4500 feet which are ecologically suited to Abies fraseri, no fir occurs. He explained this by showing that the highest points in the park south of Clingman's Dome are only 5500 and 5600 feet in elevation and that zonal upward movement during a xerothermic period eliminated fir from these elevations. Later climatic cooling allowed the migration of Abies fraseri down to its present lower altitudinal limits only from those higher elevations where

it survived the xerothermic period. That Mount Rogers, Virginia, is the northernmost of these refugia and also the lowest in elevation at 5720 feet is further evidence of this probable event.

Origin of West Virginia and Virginia Abies

There are two plausible hypotheses concerning the origin of the intermediate Abies populations in West Virginia and Virginia. The first is that they represent relicts of a once continuous population of Abies which extended from the present boreal forest of Canada and Northeastern United States southward along the Appalachian and Blue Ridge Mountains to its southernmost extension. The second hypothesis is that the intermediate population originated from hybridization between A. balsamea and A. fraseri during an overlapping of their ranges in one of the colder climates associated with the glacial advances of the Pleistocene or an earlier epoch. As both the Tertiary and the Cretaceous periods were characterized by much warner climates than now exist (Braun, 1955), it is more likely that such sympatry would have occurred during the Pleistocene, if indeed it occurred at all, because no southern migrations of Abies would likely have occurred during these warmer periods. Klaehn and Winieska (1962) showed experimentally that Mediteranean species of Abies cross readily and assumed that North American species will probably cross. They reported an artificial hybrid between A. fraseri and A. balsamea.

Tests of Hybridity

One of the most widely known and accepted methods of assessing
hybridity of a natural population is by Anderson's (1936) hybrid index
(Stebbins, 1950). A number of various morphological or anatomical
features which show differences between two parental species are
measures to show distribution of values within each species or popula-
tion group. The putative hybrid population is measured in the same
manner. Each individual is scored for each characteristic as a, like
species A; b, like species B; or i, intermediate. Assuming values for
a, i, and b, the scores for individuals may be computed. The frequency
distribution of the scores is used to evaluate populations, with bimodal
distribution considered as evidence of hybridity. This was shown by
Anderson in Tradescantia (1936), adapted by Stebbins and Matzke in
Quercus (1947) and by Kriebel (1962) with Pinus strobus x P. griffithii
individuals.

In a natural population where introgressive hybridization has
occurred, the variances, ranges of values, and standard deviations of
quantitative characteristics would be expected to be greater than in
either parental group (Stebbins, 1950). Clausen (1962) supported this
when he adapted Anderson's index to evaluate natural introgressive
hybridization between Betula papyrifera and Betula pumila var
glandulifera.

III. PROCEDURE

Sample Design and Plot Locations

Both Fraser fir and the intermediate fir populations in West Virginia and Virginia have very limited, disjunct distributions. Sampling was done throughout their ranges. Balsam fir was sampled from isolated stands at the southern limit of its range.

Samples were classified at three levels: group or species, stand or local population, and individual trees. Three groups were tentatively recognized: Fraser fir, intermediate fir in West Virginia and Virginia, and balsam fir. Only five stands of the intermediate group could be found of a size suitable for wood,[1] foliage, and cone collections; therefore, to obtain equal sampling intensity five stands of Fraser fir and five stands of balsam fir were also sampled. Five observations per tree were shown to be adequate to determine within-tree variability in a regional study of Pinus taeda (Thorbjornsen, 1960), and Squillace (1966) obtained 10 observations per tree in a study of Pinus elliottii. Considering the limited range of Southern Appalachian fir, it was estimated that 10 trees per stand and five observations per tree would be sufficient to estimate the within-tree and among-tree components of variance for this study. The mathematical model is:

$$X_{ijkl} = M + G_i + S_{ij} + T_{ijk} + e_{ijkl}$$

---

[1]Wood characteristics will be discussed in another report.

13

where

M refers to mean,

G to groups,

S to stands, and

T to trees.

A nested analysis of variance was used to estimate components of variance

at each level (Snedecor, 1956) as shown in Table 1.

Table 1. Nested analysis of variance with estimated variance
components at four mean square levels

Source of Variation	Degrees of Freedom	Expectation of Mean Square
Groups	$g-1$	$\sigma^2 + n\sigma_t^2 + nt\sigma_s^2 + nts\sigma_g^2$
Stands in Groups	$g(s-1)$	$\sigma^2 + n\sigma_t^2 + nt\sigma_s^2$
Trees in Stands	$gs(t-1)$	$\sigma^2 + n\sigma_t^2$
Determinations in Trees	$gst(n-1)$	σ^2

Null hypotheses tested were:

1. $\sigma_{group}^2 = 0$; F estimated $\dfrac{\sigma^2 + n\sigma_t^2 + nt\sigma_s^2 + nts\sigma_g^2}{\sigma^2\ n\sigma_t^2 + nt\sigma_s^2}$

2. $\sigma_{stand}^2 = 0$; F estimates $\dfrac{\sigma^2 + n\sigma_t^2 + nt\sigma_s^2}{\sigma^2 + n\sigma_t^2}$

3. $\sigma_{tree}^2 = 0$; F estimates $\dfrac{\sigma^2 + n\sigma_t^2}{\sigma^2}$

Data were processed at The University of Tennessee Computing Center.

Foliage plot locations are shown in Figure 1 and listed in Table 2. Unfortunately all stands were completely barren of cones in 1966. Sampling was necessarily limited to foliage and wood during that year. In 1967, cones were collected from some of these stands, but an incomplete cone crop required modifications of stand locations. In Fraser fir, no cones were present at Mount Mitchell, North Carolina; Clingman's Dome, Tennessee; Roan Mountain, North Carolina; or the higher elevations at Richland Balsams. Three other stands were substituted, one at 4000 feet elevation in the Great Smoky Mountain National Park near Alum Cave, and one at Heintooga Overlook approach off the Blue Ridge Parkway (5050 feet) also in the Great Smoky Mountain National Park. The Richland Balsams were sampled at Rinehart Gap, North Carolina, along the Blue Ridge Parkway at 5400 feet elevation. Only two stands of the intermediate fir had cones: Hawksbill Mountain in Shenandoah National Park, and Blister Run at Cheat Bridge, West Virginia. All balsam fir stands had cones, but the investigator arrived too late at Bear Meadow to collect unshattered cones and also missed most trees at Tamarack Swamp. Statistical analysis of cone and seed data was accomplished by comparing the two intermediate stands with first one set of two Fraser fir and two balsam fir stands and then with another set of two Fraser fir and two balsam fir stands. A nested analysis of variance was used similar to that described for the foliage data.
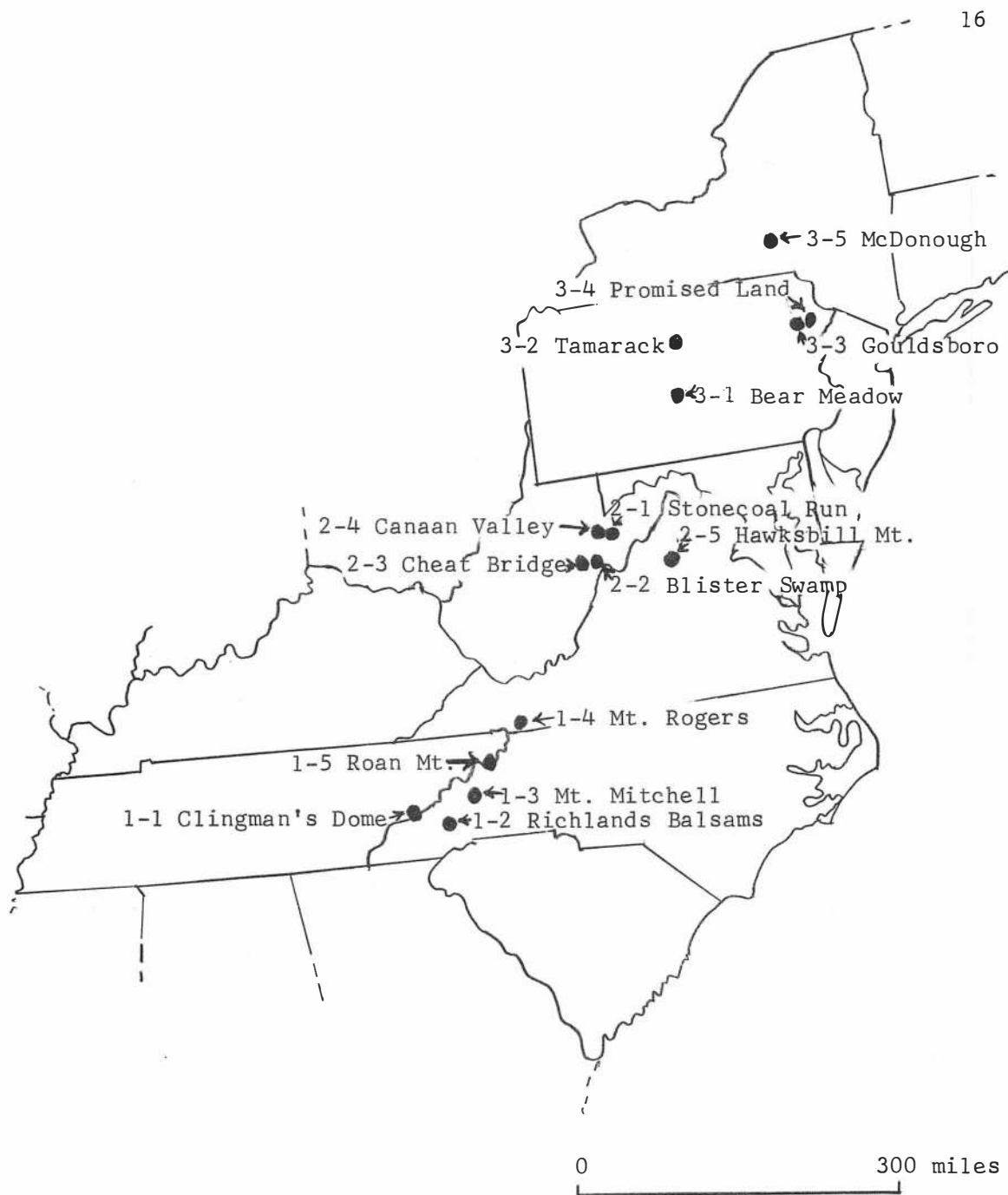
Figure 1. Foliage sample plot locations.

Table 2. Location and elevation of foliage sample plots

Group	Plot No.	Location	Appx. Elev.
Fraser Fir	1-1	Clingman's Dome, Tennessee, Great Smoky Mountain National Park	6500
	1-2	Richland Balsams, North Carolina, at highest point of Blue Ridge Parkway	6000
	1-3	Mount Mitchell, North Carolina	6600
	1-4	Roan Mountain, Tennessee-North Carolina. Plot is on North Carolina side.	6000
	1-5	Mount Rogers, Virginia	5000
Intermediate Fir	2-1	Cheat Bridge (Blister Run) near Durbin, Randolph County, West Virginia	3650
	2-2	Blister Swamp, Pocohontas County, West Virginia	3640
	2-3	Canaan Valley, headwaters of Blackwater River, Tucker County, West Virginia	3250
	2-4	Along Stonecoal Run, Tucker County, West Virginia	3650
	2-5	Hawksbill Mountain, Shenandoah National Park, Virginia	3800
Balsam Fir	3-1	Bear Meadow near Boalsburg, Centre County, Pennsylvania	1600

Table 2. (continued)

Group	Plot No.	Location	Appx. Elev.
	3-2	Tamarack Swamp, near Tamarack, Clinton County, Pennsylvania	1730
	3-3	Near Gouldsboro, Wayne County, Pennsylvania	1950
	3-4	Promised Land State Park, Pike County, Pennsylvania	1800
	3-5	Near McDonough, Chenango County, New York	1650

Collection, Preparation, and Observation

Trees were climbed and branch samples taken in August and September, 1966. Three-year old foliage from branches exposed to the sun in the upper one-half of the crown was selected in an effort to reduce within-tree variability due to leaf age or light exposure. Sample trees were selected only on the basis of: (1) dominants, codominants or inter-mediates,[2] (2) healthy, unaffected by disease or insects, (3) safe to climb, (4) relatively straight clear stem at breast height,[3] and (5) at least five inches in diameter.

All branch material was labelled at time of collection and placed in plastic bags. When sampling from a stand was completed, the bags were placed in an ice chest. Upon return to The University of Tennessee, a small amount of damp spaghnum moss was placed in each bag which was kept in cold storage at 40 degrees Fahrenheit. Morphological data were taken during fall and early winter from five three-year old needles selected at random from one twig of each tree. Some deterioration had occurred before measurements were completed and a lower storage tempera-ture or freezing would probably have been more desirable.

Other leaves were preserved in a 70 percent formol-acetic-acid solution for later anatomical investigation. Approximately two

---

[2]Scarcity of suitable trees at Hawksbill Mountain required sampling some intermediates.

[3]This was a requirement for wood samples to minimize knots and compression wood.

millimeters were cut from the tip and the base of each of five leaves
from each tree to allow penetration of the preservative. After preser-
vation, the leaves from each tree were tied together with thread into
bundles and labeled. The bundles were put through a series of 70, 85,
95 percent alcohol, and embedded three or four days each in alcohol-
ether, 2 percent, 8 percent, and 14 percent celloidin. They were then
placed in paper trays, hardened in chloroform overnight and stored in
1:1 95 percent alcohol-glycerin solution (Emig, 1959). Sections were
cut from the center of the leaves at 30 microns by sliding microtome.

All foliage characteristics measured are listed in Table 3.
Morphological measurements were made directly or by ocular micrometer
and a dissecting binocular microscope at 10, 20, or 40 magnifications.
Where necessary, ocular micrometer units were converted to millimeters
with a stage micrometer (Sass, 1958). Width-thickness ratios were de-
termined from cross sections placed on slides and projected with a
"Rayoscope" at 22 magnifications. Observations at 125 magnifications
were made of the number of hypodermal cells which fell within a net
reticule (0.1 mm x 0.1 mm) superimposed upon the leaf cross section at
three locations (Figure 2). Each net-reticule square was positioned
with one side flush with the outer edge of the leaf. Position 2 was
located directly above the center of the resin canal on the upper leaf
surface. Position 1 and 3 were located at the leaf angle and midrib,
respectively, where the largest clusters of hypodermal cells occurred.
This method counted hypodermal cells occurring several layers deep at

Table 3. Foliage characteristics

Characteristic	Description
	Morphological
Leaf-Scar Width	Diameter in units of ocular micrometer at right angle to twig
Leaf-Scar Length	Same as above, but parallel to twig
Leaf-Scar Shape	Leaf-scar width / Leaf-scar length
Color of Twig Pubescence	Brown, intermediate, or grey; a subjective determination
Leaf Length	In millimeters
Leaf Width	Taken at maximum point (mm)
Leaf Shape	Leaf length / Leaf width
Lower Stomatal Rows	No. of stomatal rows in stomatal band on one side of midrib on lower leaf surface
Stomatal Frequency	No. of stomata per 1.9 mm of lineal length in center of stomatal row in one of the stomatal bands on underside of leaf
Lineal Proportion	Lineal proportion of upper leaf surface containing stomata
Taper Factor	Bluntness or acuteness of leaf tip determined by measuring distance to tip of leaf from a point at which the leaf is 0.85 mm wide
Notch	Leaf tip notched or not notched
Upper Stomatal Rows	No. of stomatal rows on upper leaf surface
Width-Thickness Ratio	Width-thickness determined on cross section

Table 3. (continued)

Characteristic	Description
	Anatomical
Hypodermal Cells (1)	No. of cells within ocular net reticule positioned at leaf angle
Hypodermal Cells (2)	No. of cells within ocular net reticule positioned over resin canal at hypodermal layer, plus hypodermal cells in position (1)
Hypodermal Cells (3)	No. of hypodermal cells within ocular net reticule at midrib on lower hypodermal layer
Total Hypodermal Cells	Sum of all three positions
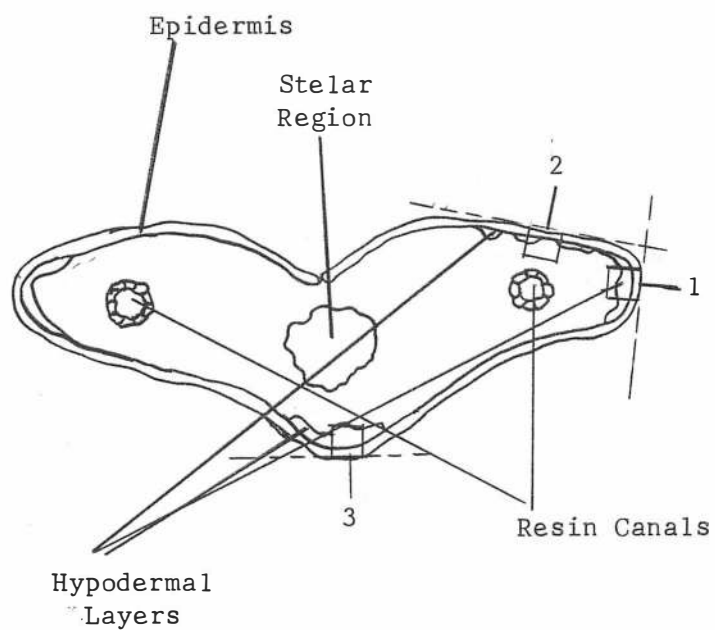
Figure 2. Schematic cross-section of leaf of _Abies_ sp. showing the 3 locations of net-reticule 0.1 x 0.1 mm squares for hypodermal cell count.

the leaf angle and midrib as well as the extent of the immediate layer of hypodermal cells just below the epidermis.

Cone and seed characteristics measured are shown in Table 4. Cones were collected in late August and September 1967 just before seedfall. Where possible 100 cones per tree were collected for later progeny testing. Cones from each tree were placed in a labeled cloth bag upon collection. At the end of the collection period the cones were spread in drying trays. Balsam fir cones disintegrated readily, but the intermediate and Fraser fir cones remained firm and had to be broken manually. Seeds were cleaned by hand rubbing, screening, and fanning (Roe, 1948).

Less within-tree variation is expected in reproductive character-istics (Stebbins, 1950). Thorbjornsen (1960) found that five cones per tree adequately measured within-tree variation in loblolly pine. Thus the number of observations taken per tree on cone length was reduced to four. These were measured directly with calipers to the nearest milli-meter. Because of known taxonomic significance, eight observations per tree of scale and bract lengths were obtained and recorded to the nearest millimeter (two scales with bracts attached from four cones of each tree). The Fraser fir scales and bracts had to be soaked in 50 percent alcohol overnight to soften the long recurved bracts so they could be held flat in order to measure them directly. The awns of these bracts were frequently broken or bent so the awn lengths of Fraser, balsam, or intermediate fir were not included as part of the bract

Table 4. Cone and Seed Characteristics

Characteristic	Observations Per Tree	Description
Cone Length	4	Total cone length (mm).
Scale Length	8	Total scale length from central axis to outer edge (mm).
Bract Length	8	Total length of bract from central axis to outer edge in mm (excluding awn).
Bract-Scale Ratio	8	Bract length/scale length.
Seed Color	1	Determined by Reflectometer in relative units of reflected light intensity.
Seed Length	8	Measured with a desk-type dial guage to the nearest .001 inches.
Seed Width	8	Same as above.
Seed Length/Width Ratio	8	Self-explanatory.

lengths. Both scale and bract lengths were measured from the point of attachment to the central axis. Myers and Borman (1963) indicated that eight observations of bract-scale length ratio in <u>Abies</u> <u>balsamea</u> were ample. Eight observations per tree of seed length and seed width were also taken using a desk type dial thickness gauge to the nearest one-thousandth of an inch. One observation per tree of seed color was taken by placing a measured amount of seed from each tree into a white cup and measuring the relative amount of reflected light with a reflec-tometer. Great care had to be taken to remove trash from the seeds, keep all external light sources constant, and keep the reflectometer a constant distance from the surface being measured.

IV. RESULTS AND ANALYSES

Foliage Characteristics

Analysis of variance of all vegetative characteristics are presented in Table 5, showing mean squares at all levels (group, stand, tree, and determinations). Variance components in percent of total variation for groups, stands, trees, and determinations were computed from mean squares and presented in Table 6.

From Table 6, the characteristics were ranked according to their combined group and stand variation to determine which descriminated best between Fraser fir and balsam fir, and what their relative weight should be in a hybrid index. For example, 76 percent of the variation of "total hypodermal cells" is attributable to differences among groups and 3 percent to differences among stands. The two combined account for 79 percent of the total variation. Each characteristic was evaluated in this manner and ranked accordingly.

Of the characteristics which were closely correlated, only the best were selected. For example, "hypodermal cells (1)," "(2)," and "(3)" were not chosen because collectively they made up "total hypodermal cells" which was the best hypodermal characteristic. Likewise "needle shape" was selected over the closely correlated "needle length" and "needle width;" "scar width" was used in lieu of "scar shape" and "scar length;" and the "width-thickness ratio" was considered better than "width" or "thickness." "Stomatal frequency" and "taper factor"

27

Table 5. Analyses of variance for vegetative characteristics

Characteristic	Source of Variation	df	Mean Square
Scar Length	Group	2	512.66*
	Stand	12	117.68**
	Tree	135	20.56**
	Error	600	0.58
Scar Width	Group	2	690.10**
	Stand	12	82.16**
	Tree	135	16.11**
	Error	600	0.70
Scar Shape	Group	2	0.13 N.S.
	Stand	12	0.06**
	Tree	135	0.02**
	Error	600	0.00
Needle Length	Group	2	656.11**
	Stand	12	90.10**
	Tree	135	35.07**
	Error	600	0.61
Needle Width	Group	2	8.23**
	Stand	12	0.46**
	Tree	135	0.12**
	Error	600	0.01
Needle Shape	Group	2	1060.01**
	Stand	12	25.74*
	Tree	135	11.60**
	Error	600	0.37
Lower Stomatal Rows	Group	2	257.06**
	Stand	12	18.48**
	Tree	135	4.12**
	Error	600	0.74*
Taper Factor	Group	2	374.64**
	Stand	12	77.27**
	Tree	135	17.51**
	Error	600	0.58

Table 5. (continued)

Characteristic	Source of Variation	df	Mean Square
Stomatal Frequency	Group	2	46.13 N.S.
	Stand	12	36.78**
	Tree	135	9.35**
	Error	600	2.93
Upper Stomatal Rows	Group	2	285.29**
	Stand	12	40.11**
	Tree	135	9.00**
	Error	600	1.12
Lineal Proportion	Group	2	4.45**
	Stand	12	0.34**
	Tree	135	0.05**
	Error	600	0.00
Hypodermal Cells (1)	Group	2	1287.53**
	Stand	12	21.75 N.S.
	Tree	135	12.19**
	Error	600	1.93
Hypodermal Cells (2)	Group	2	5770.31**
	Stand	12	61.95**
	Tree	135	23.57**
	Error	600	3.66
Hypodermal Cells (3)	Group	2	2401.29**
	Stand	12	34.17**
	Tree	135	13.13**
	Error	600	3.00
Hypodermal Cells (Total)	Group	2	15615.69**
	Stand	12	160.25**
	Tree	135	54.78**
	Error	600	7.76
Thickness	Group	2	169.52 N.S.
	Stand	12	110.98**
	Tree	135	29.64**
	Error	600	2.24

Table 5. (continued)

Characteristic	Source of Variation	df	Mean Square
Width-Thickness Ratio	Group	2	14.59**
	Stand	12	1.35**
	Tree	135	0.33**
	Error	600	0.05

N. S. = Nonsignificant.

* = Significant at 95 percent level of probability.

** = Significant at 99 percent level of probability.

Table 6. Components of variance in percent for
leaf characteristics

Component	Characteristic	Characteristic	Characteristic
	Scar Length	Scar Width	Scar Shape
Group	20	32	3
Stand	24	18	8
Tree	49	41	38
Determination	7	9	51
Total	100	100	100
	Needle Length	Needle Width	Needle Shape
Group	21	48	59
Stand	10	10	4
Tree	63	33	32
Determination	6	9	5
Total	100	100	100
	Lower Stomatal Rows	Taper-Factor	Stomatal Frequency
Group	36	6	1
Stand	11	22	11
Tree	25	61	27
Determination	28	11	61
Total	100	100	100
	Upper Stomatal Rows	Linear Proportion	Hypodermal Cells (1)
Group	23	46	55
Stand	14	16	2
Tree	31	25	22
Determination	26	13	21
Total	100	100	100

Table 6.   (continued)

Component	Characteristic	Characteristic	Characteristic
	Hypodermal Cells (2)	Hypodermal Cells (3)	Total Hypodermal Cells
Group	73	63	76
Stand	2	3	3
Tree	13	14	11
Determination	12	20	10
Total	100	100	100
	Thickness	Width-Thickness Ratio	
Group	2	29	
Stand	17	14	
Tree	58	30	
Determination	23	27	
Total	100	100	

had only 12 and 28 percent, respectively, of their variation attributable
to group and stand differences and were not selected for inclusion in
the hybrid index. The seven best characteristics, which were used to
construct the hybrid index, are presented in Table 7. The combined
percent of variation at group and stand levels is presented in Column
2 of Table 7. Each characteristic was weighted according to the values
in Column 3. Weights of "linear proportion," "lower stomatal rows,"
and "upper stomatal rows" were reduced by one each because they all
measured stomatal properties and it was felt that their original
weights of 6, 5, and 4 would overemphasize stomatal characteristics.
The adjusted weights are presented in Column 3.

In an adaptation of Anderson's (1936) index, a hybrid index was
developed using techniques similar to those described by Clausen (1962).
The overall mean of all trees measured was determined for each character-
istic. All individual tree values were converted to percentages of this
mean.

In the case of four characteristics, balsam fir values were low
and Fraser fir values were high; but "upper stomatal rows," "needle
shape," and "linear proportion," values for balsam fir were high while
they were low for Fraser fir. These values had to be reversed to make
the overall index value consistently composed of balsam fir-like values
at the lower end of a scale and Fraser fir-like values at the higher end.
This was accomplished by using the formula:

$$x' = (2 - x/\bar{x})$$

Table 7. Seven foilage characteristics weighted according
to the combined percent of variance due
to group and stand levels

1 Characteristic	2 Combined Percent of Variance of Group and Stands	3 Adjusted Weight	4 Weight Factor
Total Hypodermal Cells	79	8	.229
Needle Shape	63	6	.171
Linear Proportion	62	5	.143
Scar Width	50	5	.143
Lower Stomatal Rows	47	4	.114
Width/Thickness Ratio	43	4	.114
Upper Stomatal Rows	37	3	.086

for those characteristics which had to be reversed. For each individual tree the x' or x values of each characteristic were converted to percent of the mean and multiplied with the weight factors in Table 7, Column 4. The products were summed to form a hybrid index value for each tree. These index values were plotted in Figure 3 by species groups. Hybrid index values of the three taxonomic groups are distributed normally.

Figure 4 shows the hybrid index values of each stand with the ranges and means, plus and minus two standard errors of the means (Dice and Leraas, 1936). With one exception the stands are arranged from north to south.[1] See Figure 1, page 16, for geographic location of numbered stands. No significant differences exist among four balsam stands and the intermediate fir at Stonecoal Run, Canaan Valley, and Blister Swamp. The intermediate fir at Cheat Bridge has an index value closer to balsam fir than to Fraser fir, but is not significantly different from the southernmost balsam fir stand. The Hawksbill fir, while not significantly different from fir at Cheat Bridge, is significantly different from all the stands of both Fraser and balsam fir. Fraser fir values are quite distinct from those of intermediate or balsam fir. The variability of Fraser fir and intermediate fir is almost identical; stands of both groups have greater variation in

---

[1]Because the stands at Bear Meadow and Tamarack, Pennsylvania are geographically close together, Tamarack was placed after Bear Meadow even though it is farther north than the stands at Gouldsboro and Promised Land State Park in eastern Pennsylvania.
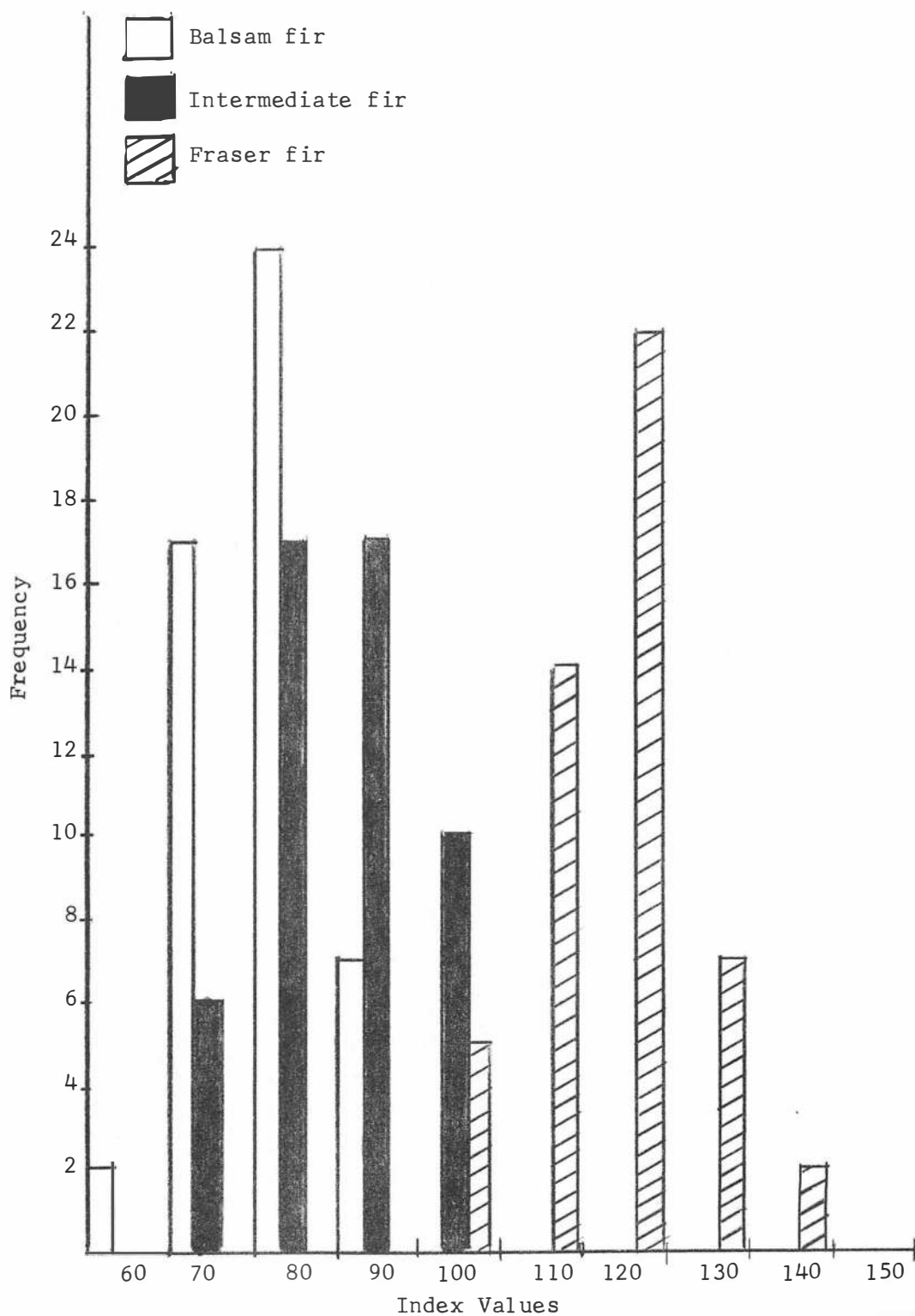
Figure 3. Frequencies of hybrid index values for individual trees, based on seven foliage characteristics, in the three species groups.
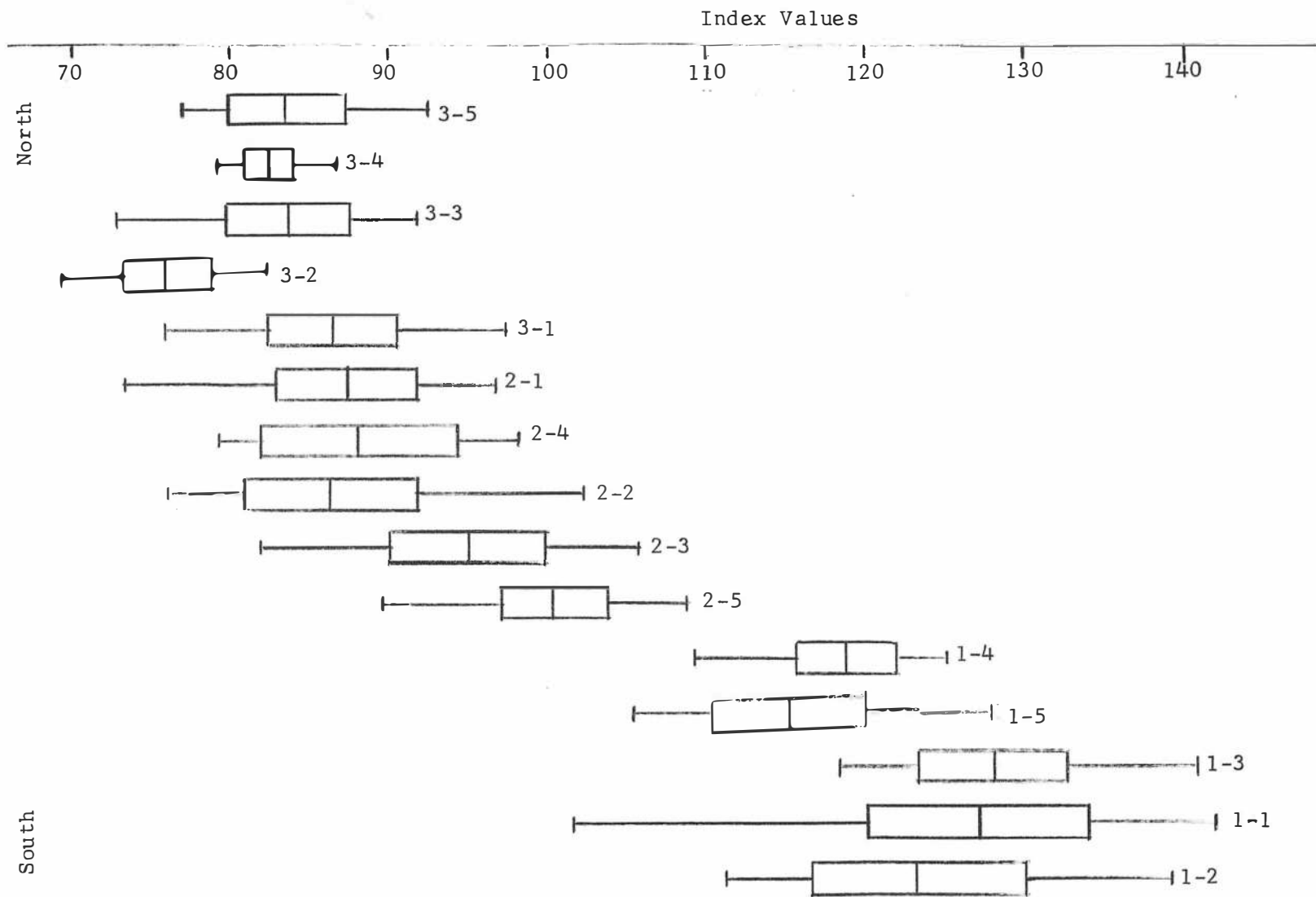
Figure 4. Hybrid index values based on seven foliage characteristics arranged by stands from north (up) to south (down).

index values than that found for balsam fir.

In Figures 5 through 11 individual leaf characteristics are ploted as in Figure 4, showing variation from north to south. From Figure 5 and Table 5, page 28, it can be seen that of all leaf characteristics, "total hypodermal cells" discriminates best between balsam and Fraser fir. Figure 5 also shows that for this characteristic the intermediate populations vary only slightly from balsam fir with gradual changes along a north-south gradient. Figure 12 illustrates how stand means of "total hypodermal cells" vary among geographic locations. In Figure 13, photomicrographs of leaf cross-sections of balsam, Fraser, and intermediate fir illustrate hypodermal cells at leaf angles.

In "number of stomatal rows on upper leaf surface" (Figure 6, page 40), "linear proportion of upper leaf surface with stomata" (Figure 7, page 41), and "width-thickness ratio" (Figure 8, page 42), gradual or no differences appear between balsam and intermediate fir, while Fraser fir values, especially for the four southernmost stands, appear to be more distinct. "Number of stomatal rows on underside of leaf" (Figure 9, page 43) shows a gradual change in values from north to south with no indication of species or groups boundaries. "Needle shape" (Figure 10, page 44) also changes from north to south, but here a grouping of values distinguishes boundaries between the three population groups.

Total number of hypodermal cells in within three net reticule areas
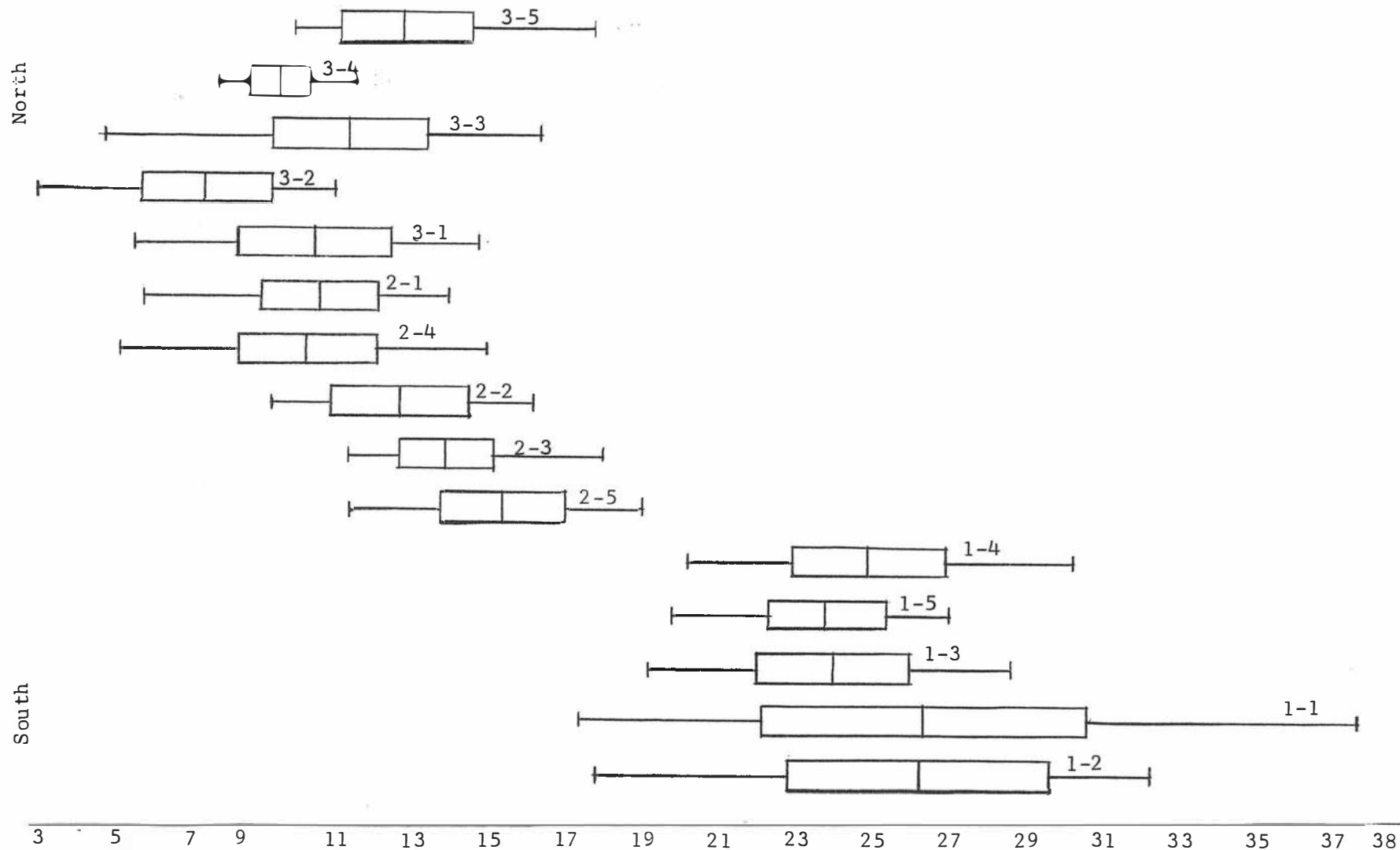
Figure 5. Total hypodermal cell count, arranged by stands from north (up) to south (down).
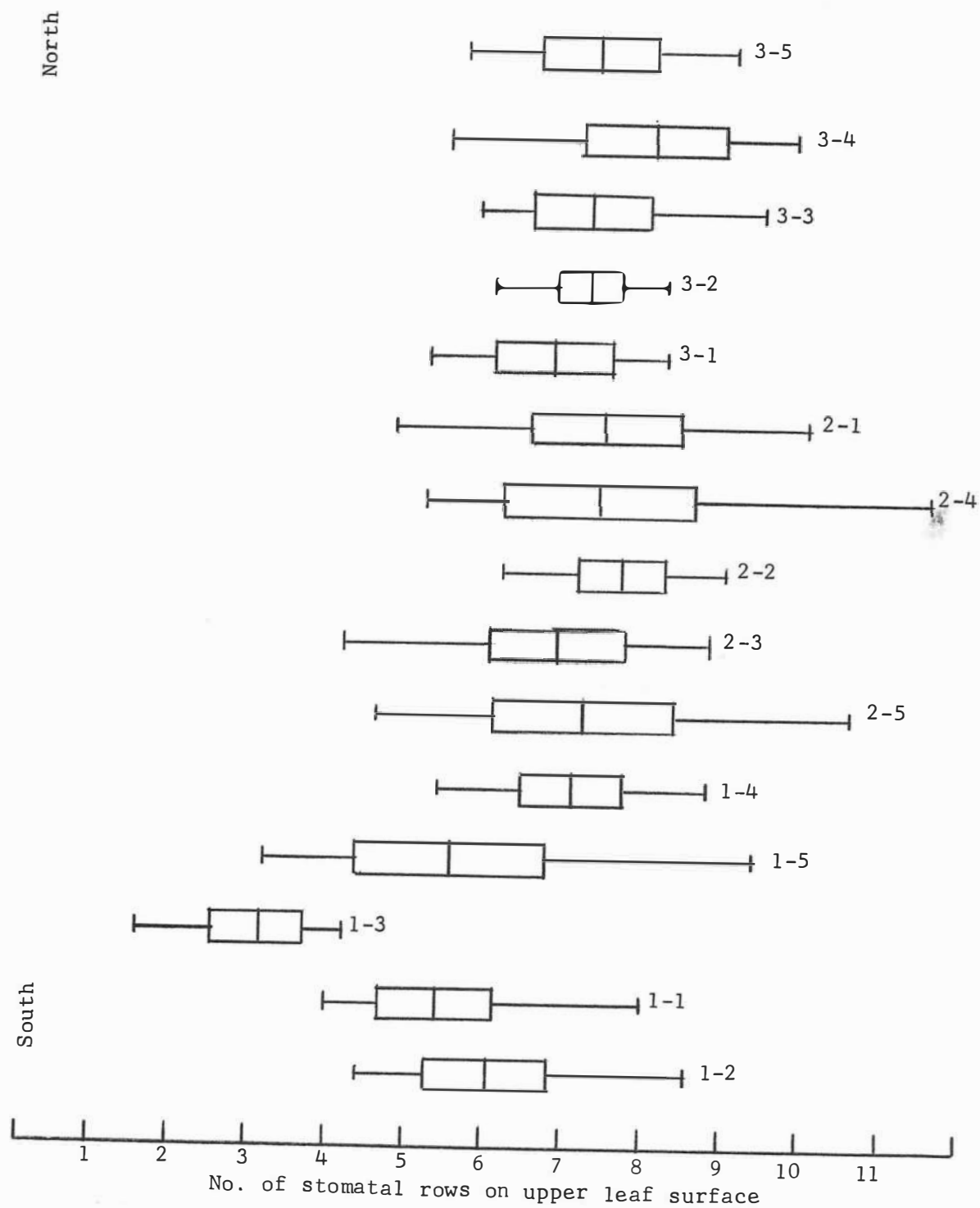
Figure 6. Number of stomatal rows on upper leaf surface, arranged by stands from north (up) to south (down).
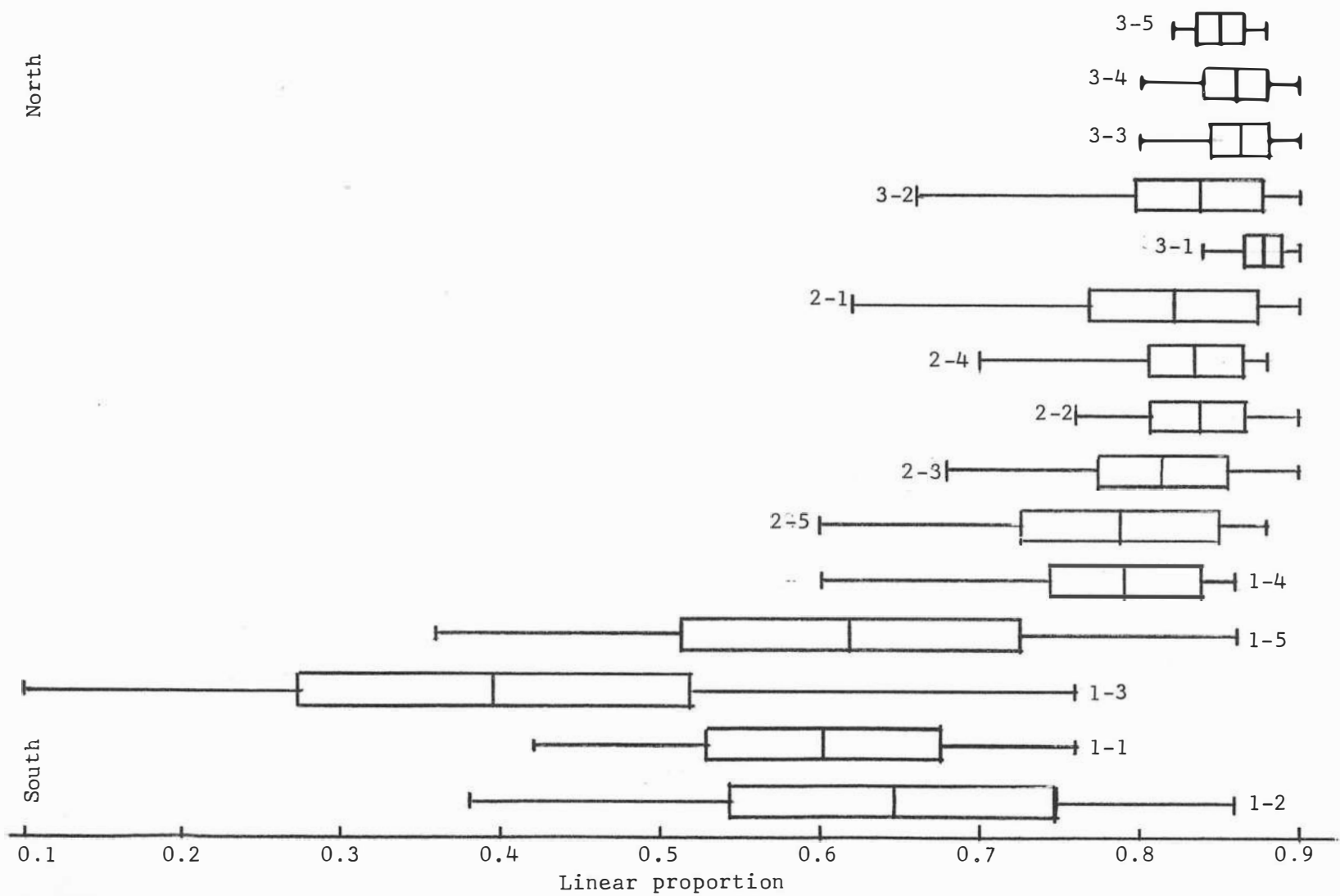
Figure 7. Linear proportion of upper leaf surface with stomata, arranged by stands from north (up) to south (down).
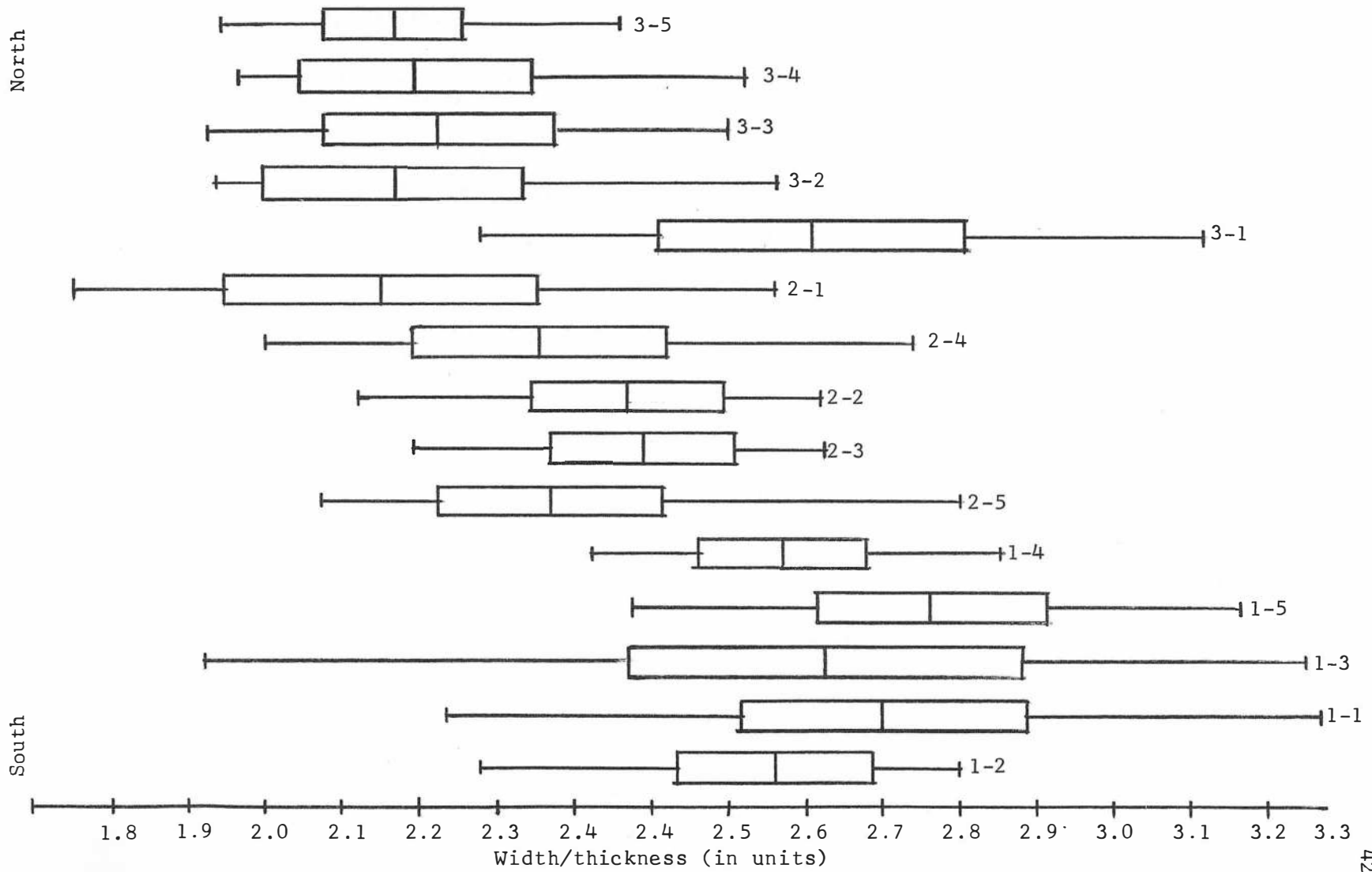
Figure 8. Width-thickness ratio, arranged by stands from north (up) to south (down).

3-5

3-4

3-3

3-2

3=1

2-1

2-4

2-2

2-3

2-5

1-4

1-5

1-3

1-1

1-2

5.0   6.0   7.0   8.0   9.0   10.0   11.0

No. of stomatal rows in one band on underside of leaf
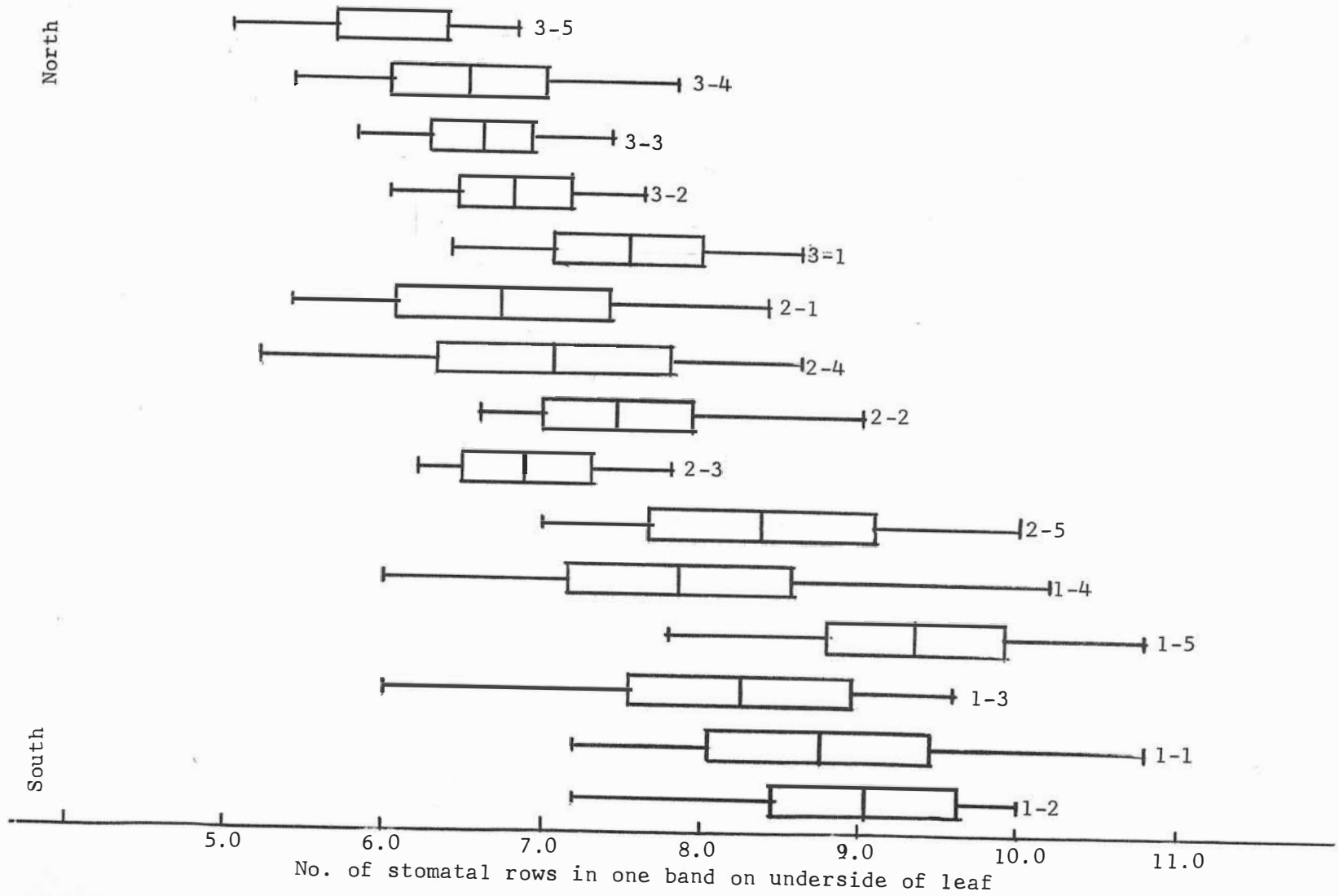
Figure 9. Number of stomatal rows in one band on underside of leaf, arranged by stands from north (up) to south (down).

Figure 10. Needle shape (length/width), arranged by stands from north (up) to south (down).

Figure 11. Leaf scar width in relative units, arranged by stands from north (up) to south (down).
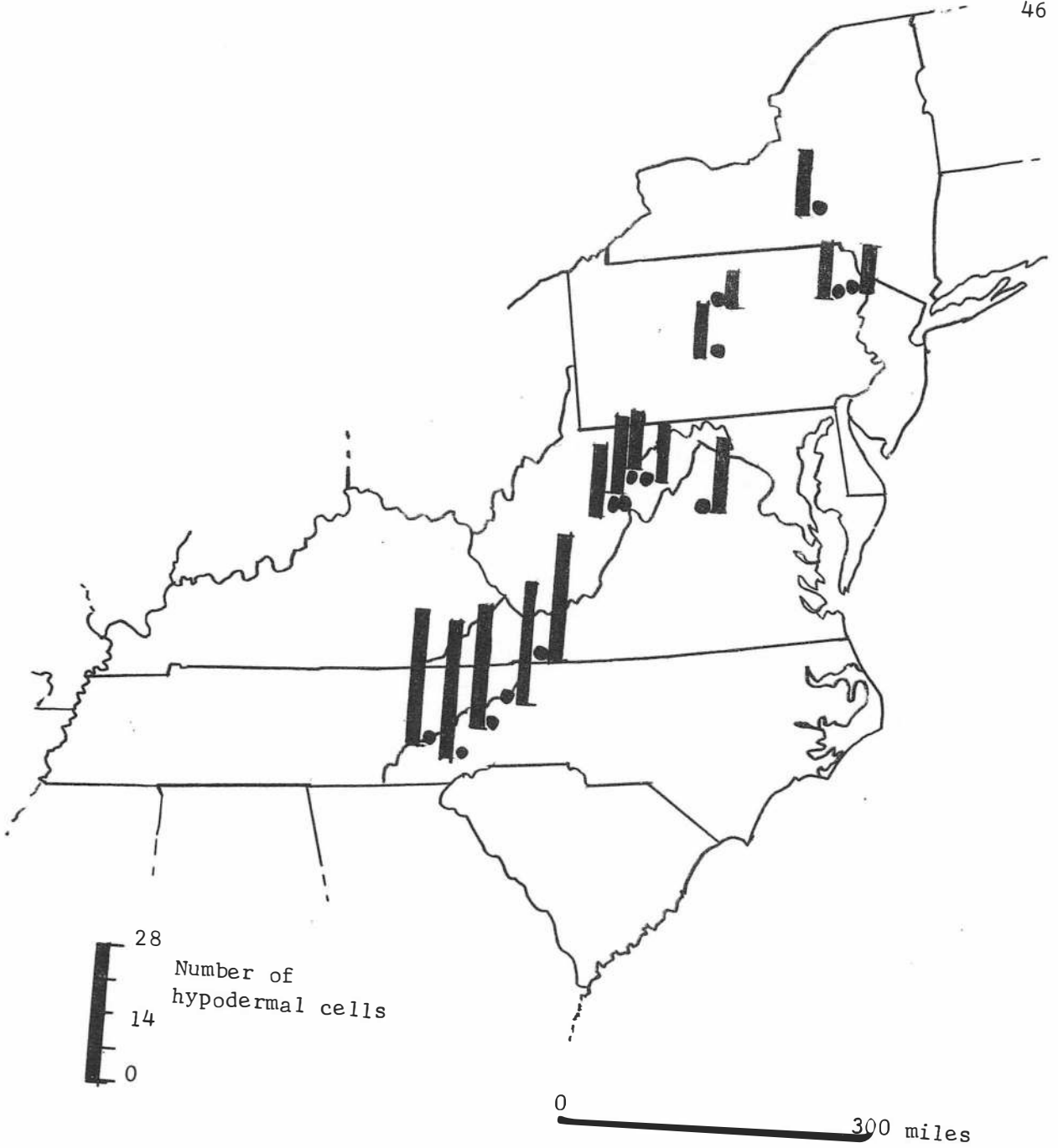
Figure 12. Stand means of "total hypodermal cells" by geographic location.
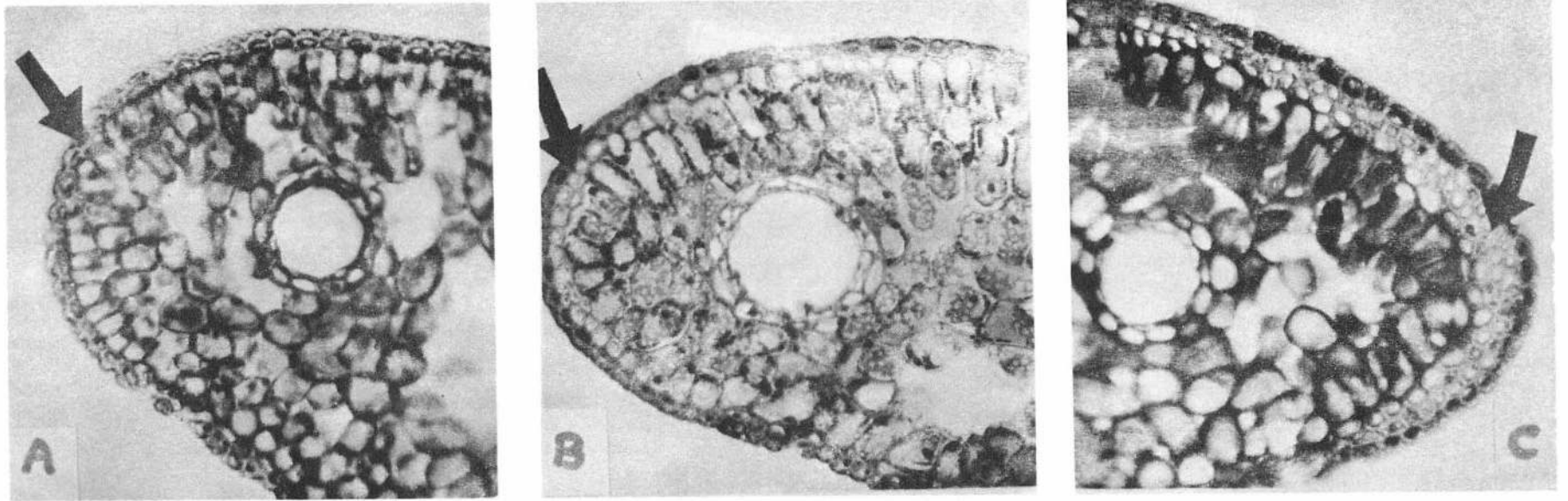
Figure 13. Leaf cross-sections of: (A) balsam fir; (B) intermediate fir; (C) Fraser fir showing hypodermal sclerenchymous cells at leaf angles.

For most characteristics, the variation in balsam fir was less than in either Fraser fir or intermediate fir. Fraser fir variation was usually as gr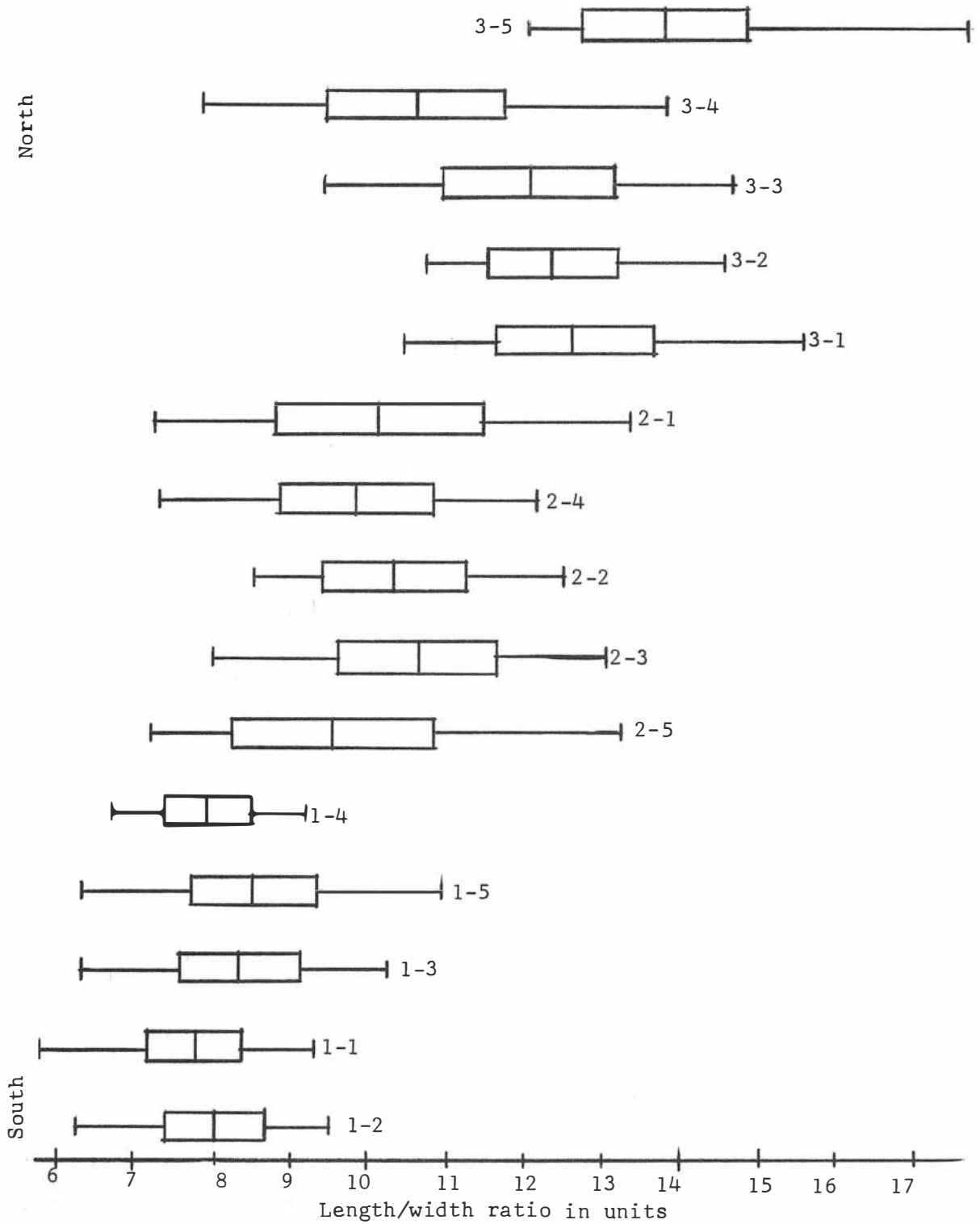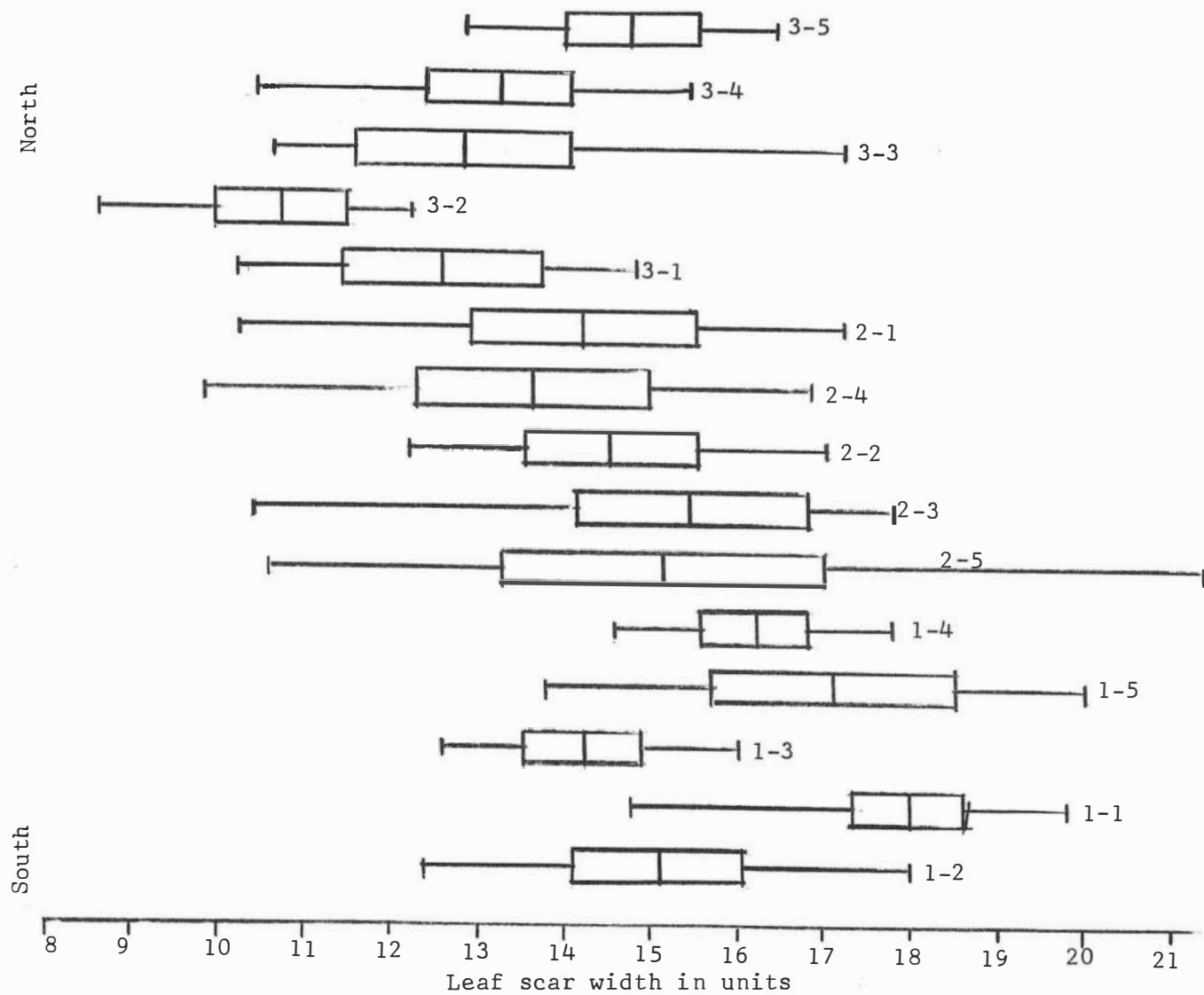eat or greater than that of intermediate fir. This was somewhat unexpected, as one would imagine that (1) the long-time isolated Fraser fir and intermediate fir populations would have somewhat limited gene pools and show less variation than balsam fir, or (2) hybridization would have resulted in more variation in the intermediate populations. However, all balsam fir stands sampled were also isolated and fairly small, being from the southernmost disjunct distribution of the species, and probably also possessed limited gene pools.

The only characteristic which showed most variation in the intermediate populations was "leaf scar width." The within-stand variation at Hawksbill was especially large, and other intermediate stand values were also quite variable (Figure 11, page 45).

The most striking difference in variation between Fraser fir and balsam fir was in "linear proportion of stomatal rows on the upper leaf surface" (Figure 7, page 41). Fraser fir was highly variable, especially in the four southernmost stands; intermediate fir much less variable; and balsam fir quite uniform. This characteristic is a measure of stomatal frequency and likely reflects a response to moisture availability. Fraser fir occurs at high elevations in southern latitudes where moisture is abundant throughout the year, mostly in the form of rain or fog (Shanks, 1954). In contrast, much precipitation throughout the balsam and intermediate fir ranges occurs as snow which must melt

in order to become available to the plant. In addition these regions receive less precipitation, are less humid, and have greater high and low temperature extremes than that found farther south (Oostings and Billings, 1951). Thus, moisture stress probably has been more of a long-term limiting factor in the northern regions, allowing less variability in stomatal properties.

Without detailed knowledge of physiological responses to environmental differences, it is difficult to properly evaluate variation in morphology and anatomy. Yet, analyses of variance revealed significant differences among groups and Figures 5 through 11 (pages 39 through 45) show this variation to be correlated geographically. These results indicate that much variation may be attributed to long-term genetic responses to regional environmental differences.

Cone and Seed Characteristics

A nested analysis of variance with unequal samples and subsamples presents difficulties in calculating coefficients for each variance component (Snedecor, 1956). The University of Tennessee Computing Center had no nested analysis program for unequal samples, therefore, two analyses were made for each characteristic using a program for equal samples. In each set of analyses, the balsam fir and Fraser fir were limited to two stands:

A. Fraser fir stands 1 and 2, the two intermediate stands, and balsam fir stands 2 and 4.

B. Fraser fir stands 3 and 4, the two intermediate stands, and

balsam fir stands 3 and 4.

The stands were arbitrarily grouped with no regard to their

geographic locations. Balsam fir stand 1 at Tamarack, Pennsylvania

was excluded because of an insufficient number of sampled trees. Loca-

tions of these stands are as follows:

Fraser fir stand 1 ------ Great Smoky Mountains National Park

near Alum Cave, Tennessee, elevation

4000 feet.

Fraser fir stand 2 ------ Mount Rogers, Virginia, elevation

5000 feet.

Fraser fir stand 3 ------ Along approach to Heintooga Overlook

in Great Smoky Mountains National

Park, North Carolina, elevation 5050

feet.

Fraser fir stand 4 ------ Rhinehart Gap on Blue Ridge Parkway

in Richland Balsams, North Carolina,

elevation 5400 feet.

Intermediate fir stand 1 - Cheat Bridge, West Virginia, elevation

3650 feet.

Intermediate fir stand 2 - Hawksbill Mountain, Virginia, eleva-

tion 3800 feet.

Balsam fir stand 1 ------ Tamarack, Pennsylvania, elevation

1730 feet.

Balsam fir stand 2 ------ Gouldsboro, Pennsylvania, elevation
                          1950 feet.

Balsam fir stand 3 ------ Promised Land State Park, Pennsylvania,
                          elevation 1800 feet.

Balsam fir stand 4 ------ Near McDonough, New York, elevation
                          1650 feet.

Analyses of variance of all cone and seed characteristics are
shown in Tables 8 and 9. Reduction of stands in each group to two reduced
the degrees of freedom making the F tests less sensitive than were the
F tests for foliage characteristics. This may be seen by comparing
Tables 8 and 9 with variance components in percent of total variation
shown in Tables 10 and 11. Cone length, for example, shows no signifi-
cant differences at the group level in Tables 8 and 9, yet Tables 10
and 11 indicate that this level accounts for 28 and 32 percent, respec-
tively, of the total variation.

Figures 14 through 19 show the ranges and means, plus and minus
two standard errors of the mean for six cone and seed characteristics
by stands. Even though "cone scale length," cone bract length," and
"bract length-scale length ratio" are closely correlated, all were
plotted because of their known taxonomic significance. "Seed length"
and "seed length-width ratio" were not plotted because of the low per-
cent of variation accounted for at group and stand levels. Because
cone and seed data were not available from many of the stands where
foliage data were obtained, no attempt was made to include cone and seed
characteristics in a hybrid index.

Table 8. Analyses of variance for cone and seed characteristics among
the two intermediate stands, Fraser fir stands 1 and 2,
and balsam fir stands 2 and 4

Characteristic	Source of Variation	df	Mean Square
Scale Length	Group	2	1027.41N.S.
	Stand	3	122.10**
	Tree	54	16.16**
	Error	420	1.03
Bract Length	Group	2	1434.98*
	Stand	3	93.58**
	Tree	54	14.61**
	Error	420	0.79
Seed Length	Group	2	19.189N.S.
	Stand	3	5.532*
	Tree	54	1.771**
	Error	420	.123
Seed Width	Group	2	6.108N.S.
	Stand	3	.662*
	Tree	54	.226**
	Error	420	.059
Bract Length/Scale Length	Group	2	21.9111**
	Stand	3	0.0664N.S.
	Tree	54	0.0316**
	Error	420	0.0032
Seed Length/Seed Width	Group	2	2.9199N.S.
	Stand	3	0.6247N.S.
	Tree	54	0.4196**
	Error	420	0.1146
Cone Length	Group	2	2183. N.S.
	Stand	3	255. N.S.
	Tree	54	164. **
	Error	180	25.

Table 8. (continued)

Characteristic	Source of Variation	df	Mean Square
Seed Color	Group	2	193.1 N.S.
	Stand	3	87.5**
	Tree	54	9.1

N.S. = Nonsignificant.

* = Significant at 95 percent level.

** = Significant at 99 percent level.

Table 9. Analyses of variance for cone and seed characteristics
among the two intermediate stands, Fraser fir stands 3
and 4, and balsam fir stands 3 and 4

Characteristic	Source of Variation	df	Mean Square	
Scale Length	Group	2	1091.14*	
	Stand	3	73.27**	
	Tree	54	16.15**	
	Error	420	0.82	
Bract Length	Group	2	2056.96**	
	Stand	3	46.24**	
	Tree	54	10.73**	
	Error	420	0.87	
Seed Length	Group	2	16.024 N.S.	
	Stand	3	2.060 N.S.	
	Tree	54	1.690**	
	Error	420	.104	
Seed Width	Group	2	7.290*	
	Stand	3	.362 N.S.	
	Tree	54	.307**	
	Error	420	.056	
Bract Length/Scale Length	Group	2	29.2404**	
	Stand	3	0.0322 N.S.	
	Tree	54	0.0296 **	
	Error	420	0.0032	
Seed Length/Seed Width	Group	2	5.3040 N.S.	
	Stand	3	0.8788 N.S.	
	Tree	54	0.4344 **	
	Error	420	0.1380	
Cone Length	Group	2	4490.	N.S.
	Stand	3	1097.	**
	Tree	54	191.	**
	Error	180	26.	

Table 9. (continued)

Characteristic	Source of Variation	df	Mean Square
Seed Color	Group	2	103.2*
	Stand·	3	10.1 N.S.
	Tree	54	5.1

N.S. = Nonsignificant.

* = Significant at 95 percent level.

** = Significant at 99 percent level.

Table 10. Components of variance in percent for cone and seed
characteristics among two intermediate stands, Fraser fir
stands 1 and 2, and balsam fir stands 2 and 4

Component	Characteristic	Characteristic
	Cone Scale Length	Cone Bract Length
Group	58	71
Stand	13	8
Tree	19	14
Determination	10	7
Total	100	100
	Seed Length	Seed Width
Group	19	29
Stand	10	5
Tree	44	18
Determination	27	48
Total	100	100
	Bract Scale Ratio	Seed Length Seed Width Ratio
Group	95	8
Stand	1	2
Tree	2	22
Determination	2	68
Total	100	100
	Cone Length	Seed Color
Group	28	24
Stand	3	35
Tree	40	41
Determination	29	0
Total	100	100

Table 11. Components of variance in percent for cone and seed
characteristics among two intermediate stands, Fraser fir
stands 3 and 4, and balsam fir stands 3 and 4

Component	Characteristic	Characteristic
	Cone Scale Length	Cone Bract Length
Group	66	83
Stand	7	3
Tree	19	8
Determination	8	6
Total	100	100
	Seed Length	Seed Width
Group	22	33
Stand	1	1
Tree	51	24
Determination	26	42
Total	100	100
	Bract Scale Ratio	Seed Length Seed Width Ratio
Group	96	13
Stand	0	3
Tree	2	18
Determination	2	66
Total	100	100
	Cone Length	Seed Color
Group	32	45
Stand	17	5
Tree	31	50
Determination	20	0
Total	100	100
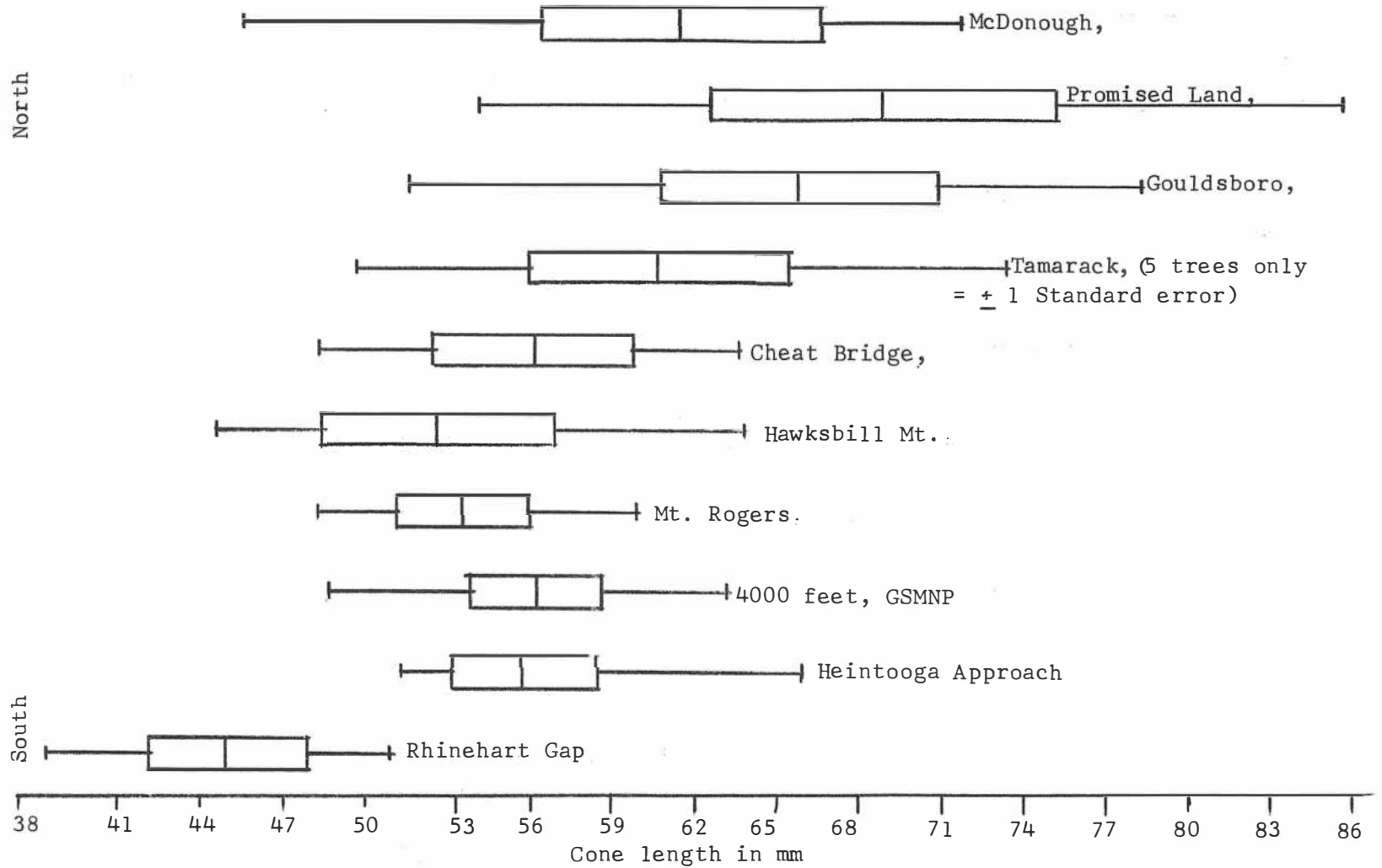
Figure 14. Cone lengths in millimeters, arranged by stands from north (up) to south (down).

North

South

| McDonough |
| Promised Land |
| Gouldsboro |
| Tamarack, (5 trees only = ± 1 Standard error) |
| Cheat Bridge |
| Hawksbill Mt. |
| Mt. Rogers, Va. |
| 4000 feet, GSMNP |
| Heintooga Approach |
| Rhinehart Gap |

40   45   50   55   60   65   70   75   80   85

Seed widths (.001 inches)
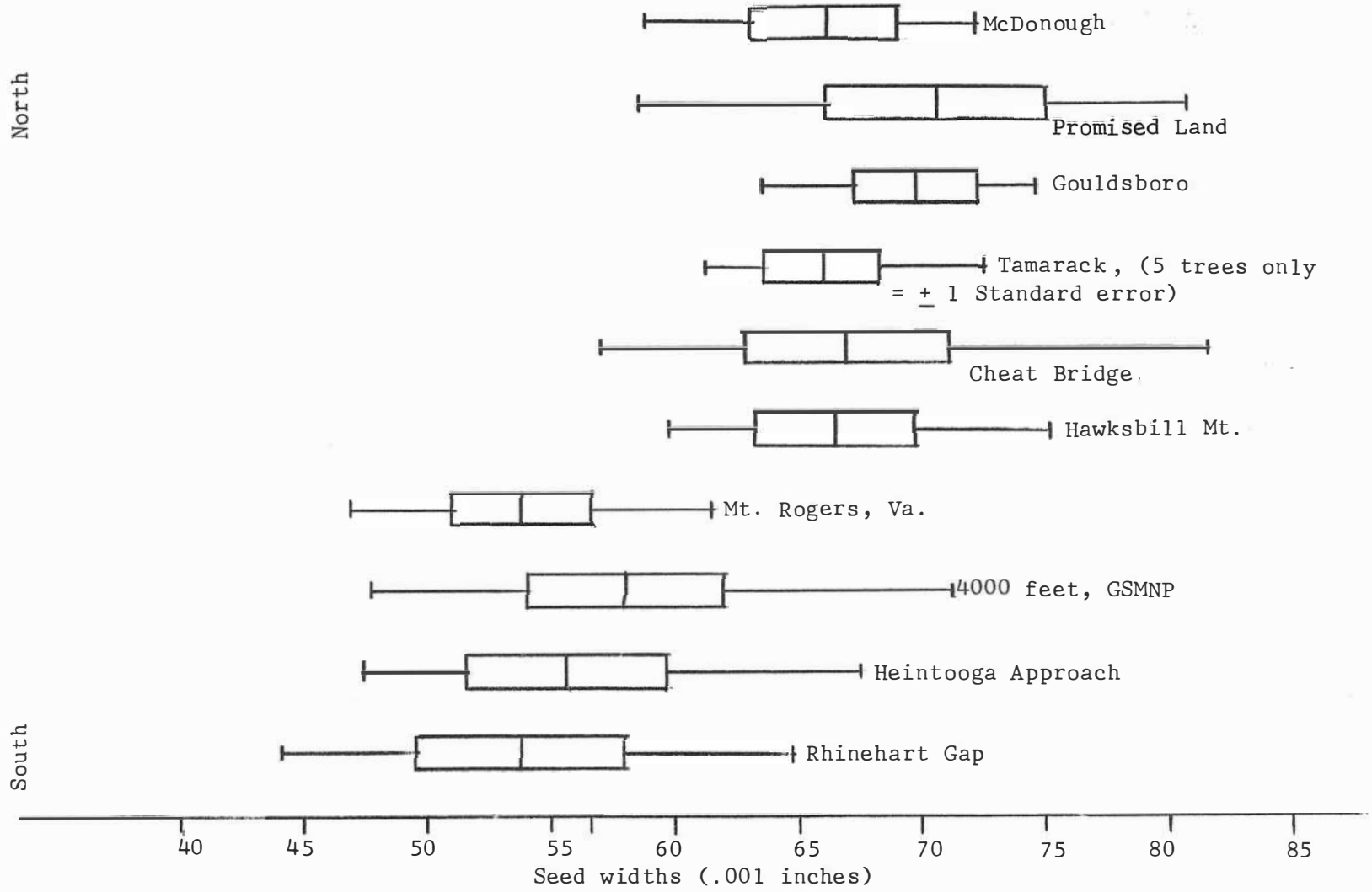
Figure 15.   Seed widths in thousandths of an inch, arranged by stands from north (up) to south (down).
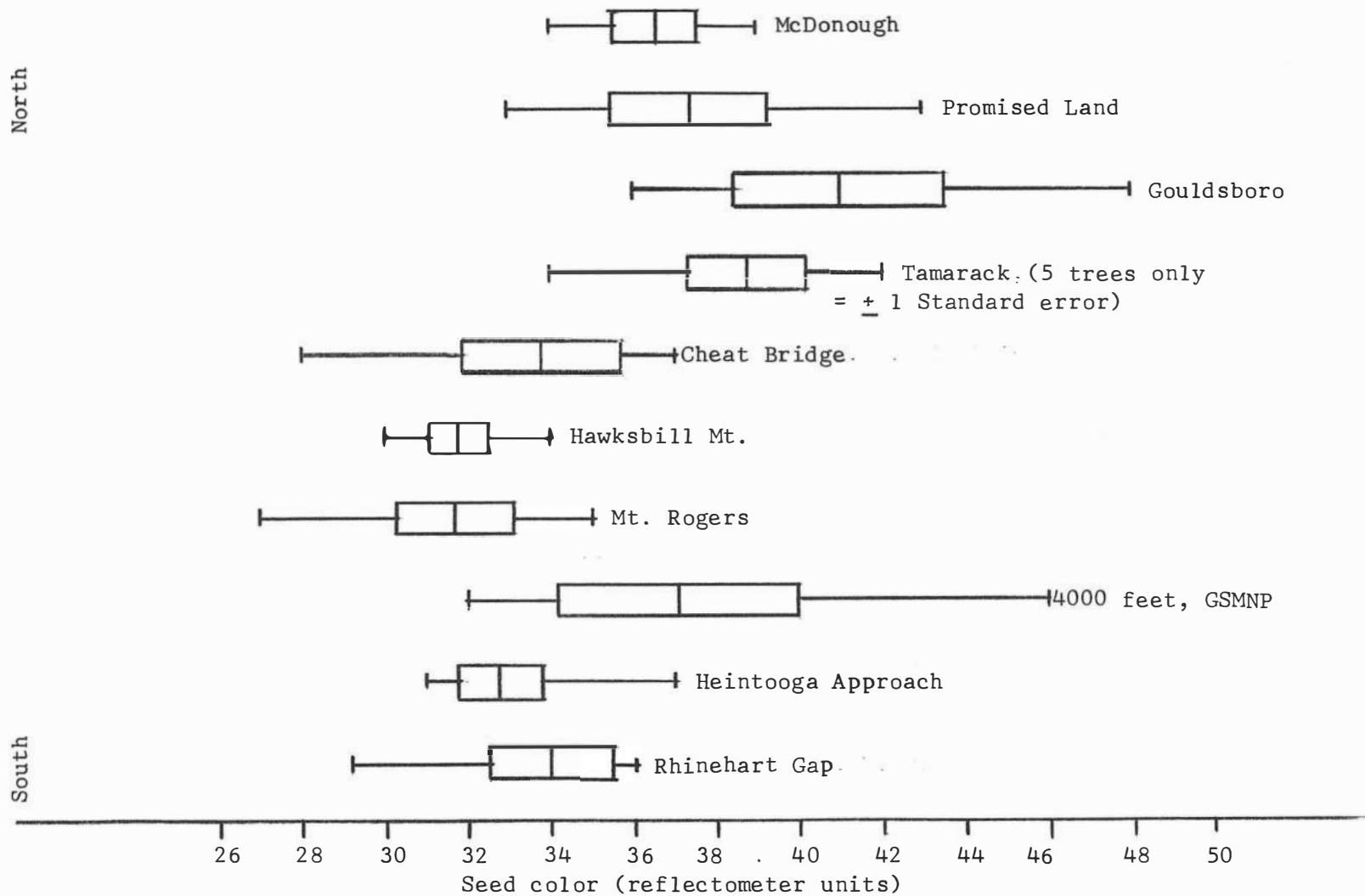
Figure 16.   Seed color in reflectometer units, arranged by stands from north (up) to south (down).

Figure 17. Bract-scale ratios (bract length/scale length), arranged by stands from north (up) to south (down).

Figure 18. Cone bract lengths in millimeters, arranged by stands from north (up) to south (down).

North

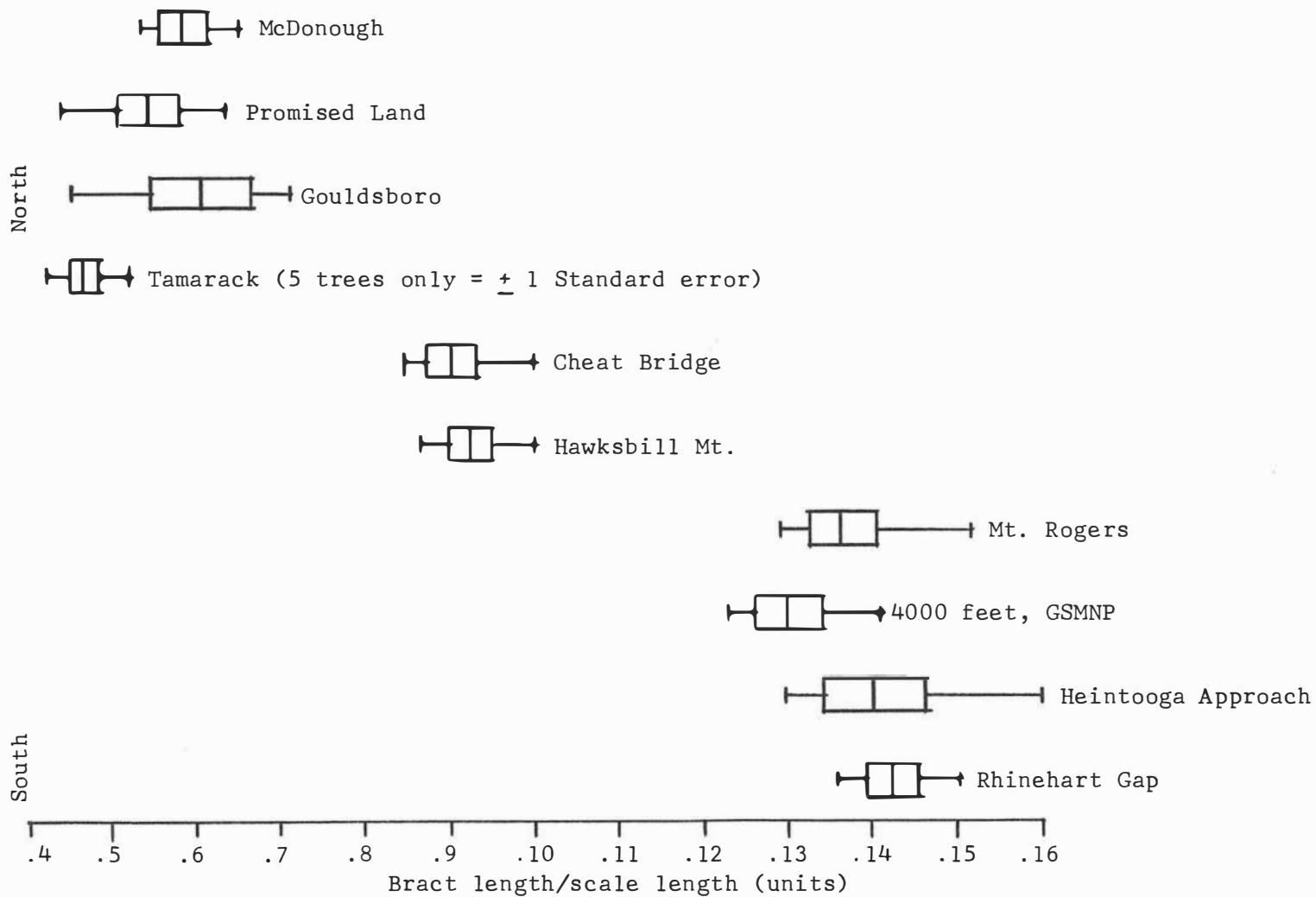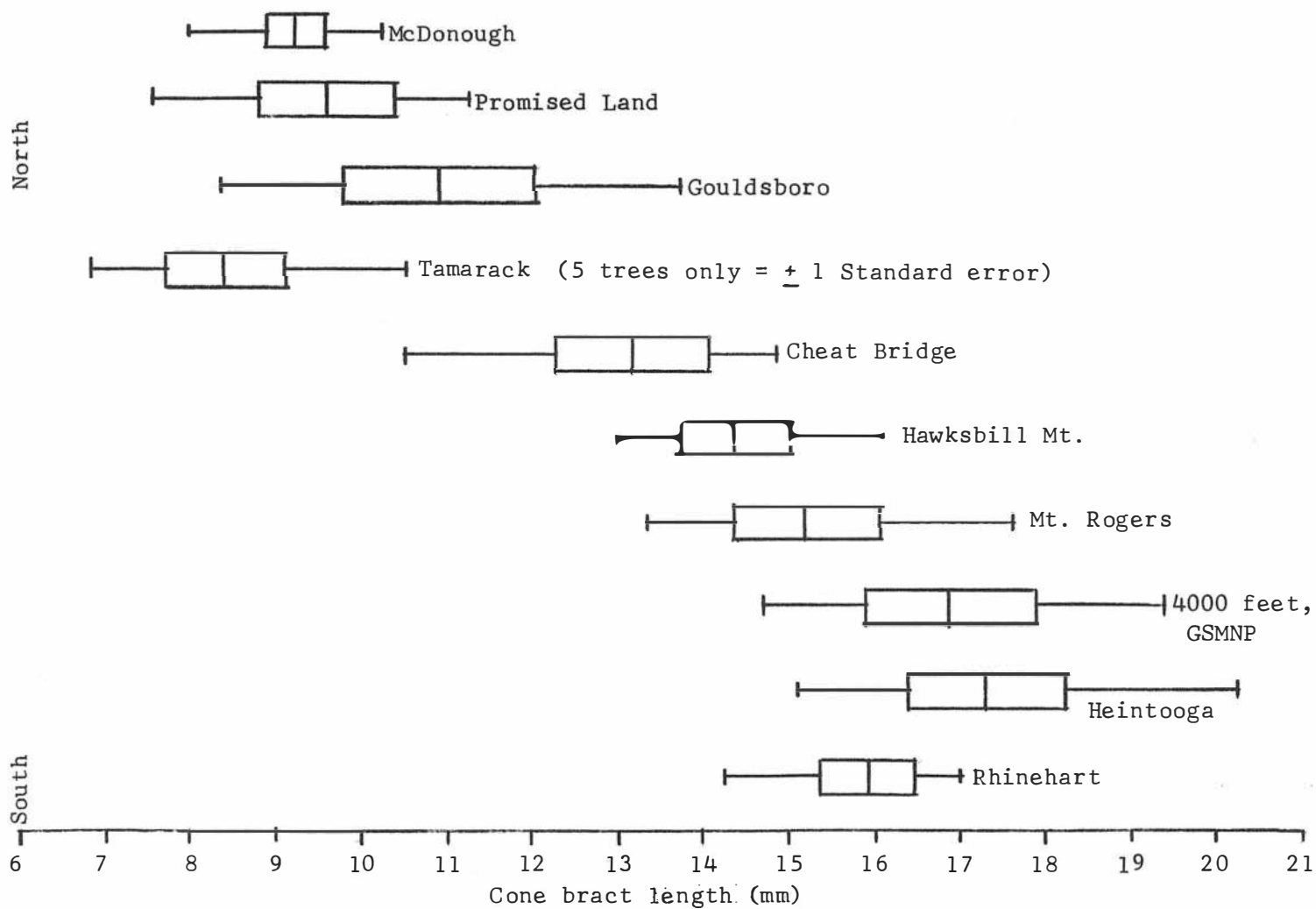South
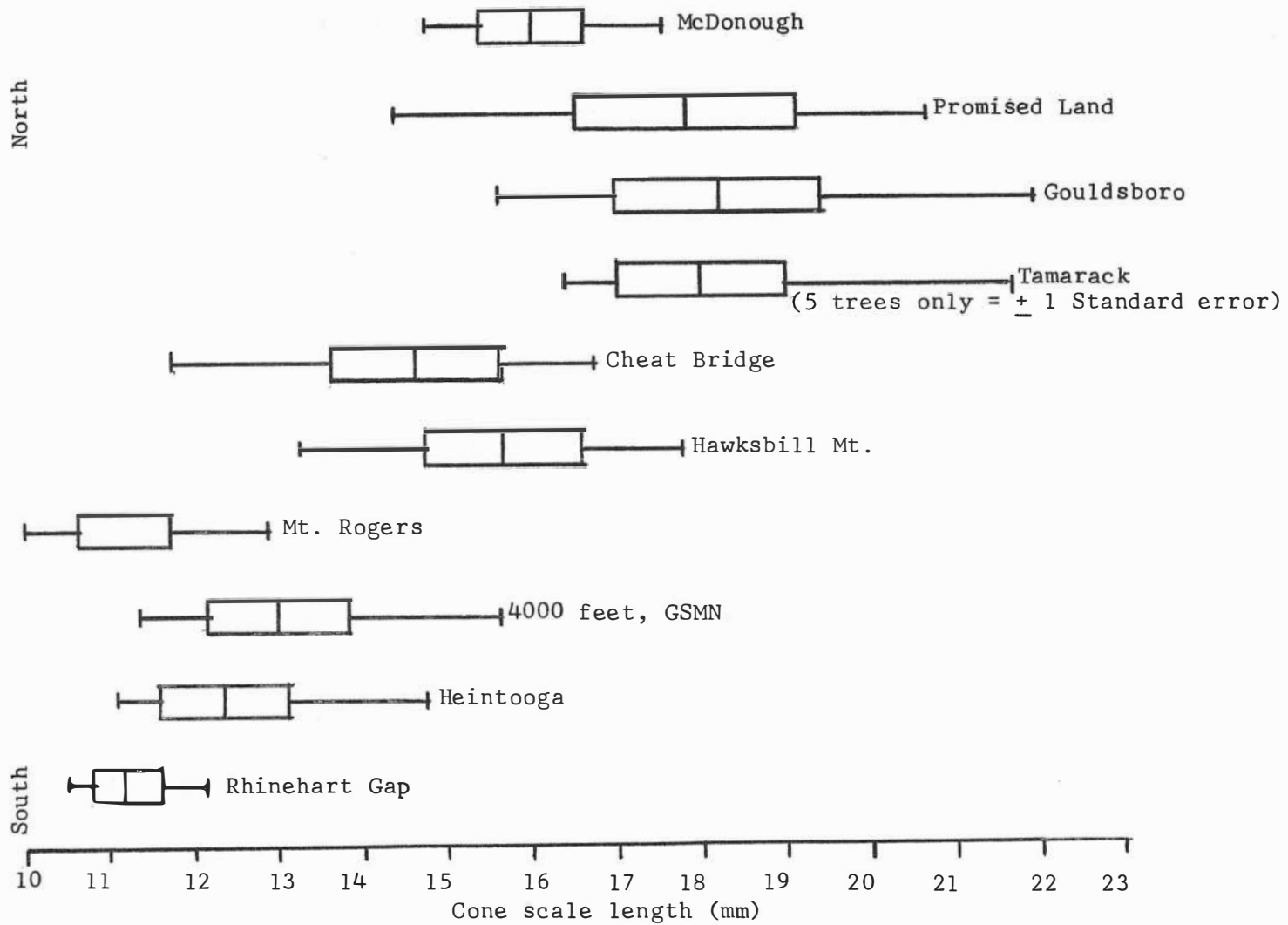
Cone scale length (mm)

Figure 19.   Cone scale lengths in millimeters, arranged by stands from north (up) to south (down).

"Cone lengths" (Figure 14, page 58) show little or no differences between Fraser fir and the intermediate fir. Balsam fir values are slightly higher. Fraser fir have more narrow seed than those found in other stands (Figure 15, page 59), and "seed color" variation (Figure 16, page 60) is more random with intermediate fir values closer to Fraser fir than to balsam fir.

Figure 17, page 61, shows that the cone "bract-scale ratio" falls neatly into the three taxonomic groups presently recognized as Abies fraseri, A. balsamea, and A. balsamea, var phanerolepis. Yet examination of the two characteristics from which this ratio was calculated, i.e., "scale length" and "bract length" which are independently plotted in Figures 18 and 19, pages 62 and 63, reveals different variation patterns. Figure 20 depicts stand means for bract length and scale length according to geographic location.

Considered independently, scale lengths or bract lengths show more overlapping of stand values and more gradual variation across taxonomic boundaries. Bract lengths increase gradually from north to south while scale lengths increase from south to north. Overlapping of double standard errors of stand means (Figure 18 and 19, pages 62 and 63) indicates bract lengths of intermediate fir at Hawksbill Mountain are not significantly different from bract lengths of either the Fraser fir at Mount Rogers or the other intermediate stand at Cheat Bridge. Bract lengths of the intermediate stand at Cheat Bridge are, however, significantly different from those of any stand of either Fraser fir or balsam
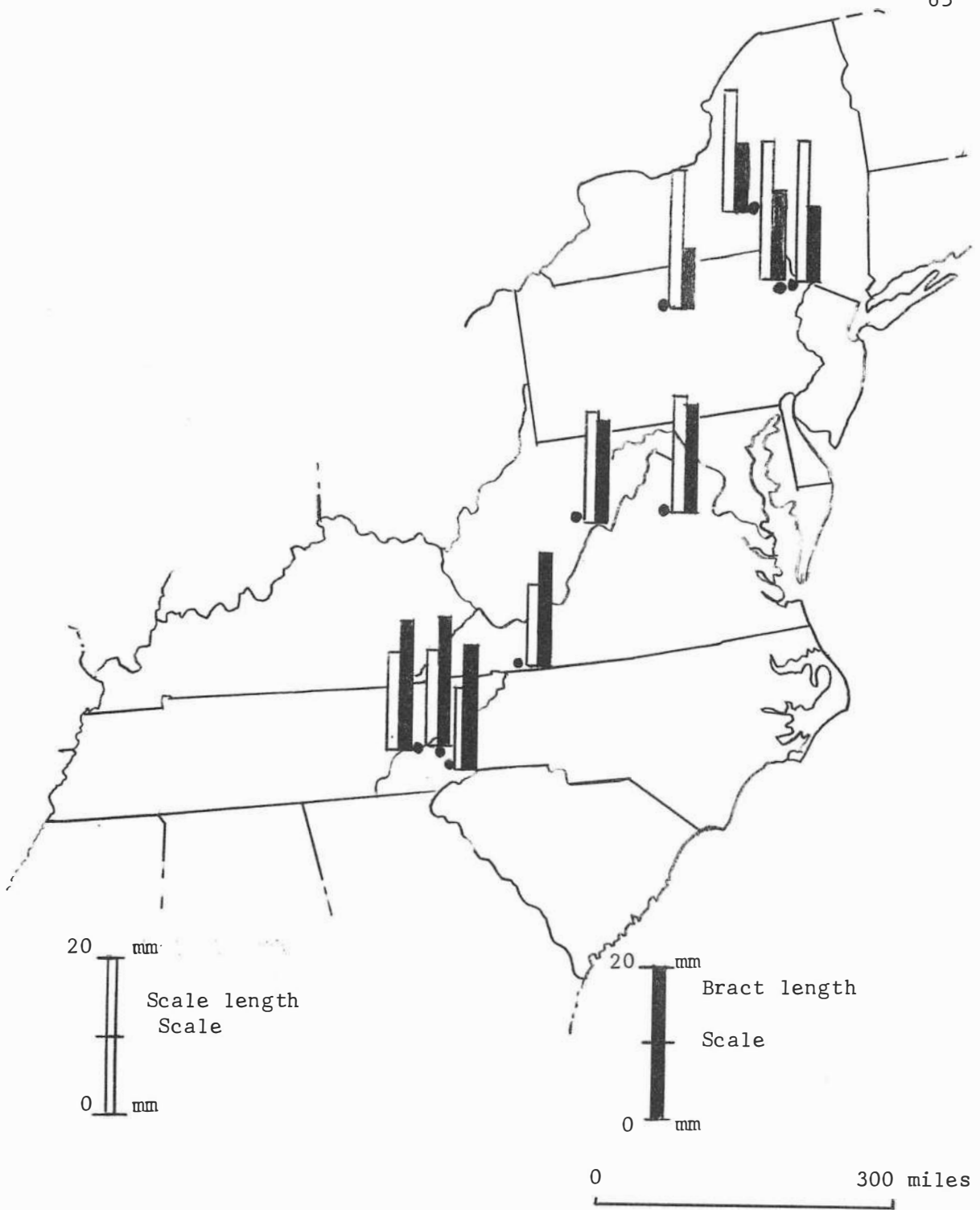
Figure 20.   Stand means for bract length and scale length by
geographic location.

fir. Cone scale lengths "group" a little more, but the Hawksbill fir is not significantly different from two of the balsam fir stands and Cheat Bridge fir is not significantly different from one of the Fraser fir stands.

Discontinuous Data

In addition to the characteristics which were measured and analyzed statistically, five leaf apexes per tree were scored as notched or not notched and pubescence on one twig per tree was scored as grey, intermediate or brown.

Sixty-two percent of the Fraser fir, 20 percent of the intermediate, and 10 percent of the balsam fir had notched leaf apexes.

Fifty-four percent of the Fraser fir trees, 20 percent of the intermediates, and 10 percent of the balsam firs had brown twig hairs. Over a third of the trees in all groups were classed as intermediate in hair color.

Geographic Correlation

From the northernmost stand of balsam fir at McDonough, New York, distances to other stands were scaled in inches on a map along probable migration routes and plotted as the independent variable against stand means for each characteristic. Stands ranked from north to south in the same order as are plotted in Figures 4 through 11 (pages 37 through 45), for foliage characteristics, and Figures 14 through 19 (pages 58 through 63), for cone characteristics. Tables 12 and 13 show simple

Table 12. Simple correlation coefficients (r), and coefficients
of determination (r^2) for mean stand values of seven foliage
characteristics with southward map distance from McDonough,
New York, along probable migration routes

Characteristic	df	r	r^2
Needle Shape	13	-0.95	.90**
Hybrid Index	13	+0.91	.83**
Lower Stomatal Rows	13	+0.88	.77**
Hypodermal Total	13	+0.87	.76**
Width-Thickness Ratio	13	+0.79	.62**
Linear Proportion	13	-0.77	.59**
Leaf Scar Width	13	+0.71	.50**
Upper Stomatal Rows	13	-0.65	.42**

** = Significant at 99 percent level of probability.

Table 13. Simple correlation coefficients (r), and coefficients of determination (r^2) for mean stand values of six cone characteristics with southward map distance from McDonough, New York, along probable migration routes

Characteristic	df	r	r^2
Bract-Scale Ratio	8	+0.97	.94**
Bract Length	8	+0.96	.92**
Scale Length	8	-0.89	.79**
Seed Width	8	-0.85	.72**
Cone Length	8	-0.80	.64**
Seed Color	8	-0.63	.40*

* = Significant at 95 percent level of probability.

** = Significant at 99 percent level of probability.

correlation coefficients (r), and coefficients of determination (r^2)
for foliage and cone characteristics.

On the basis of the strong correlation of morphological variation
with geographic location, one would expect variation patterns to show
gaps between Fraser fir, intermediate fir in West Virginia and Virginia,
and balsam fir. Small distances separate stands within each group, but
large distances separate groups. Such gaps for most characteristics
do not appear. "Bract-scale ratio" and "needle shape," two of the three
characteristics which show the strongest correlation with geographic
location, do show variation gaps between the three taxons. Both are
ratios of characteristics which vary inversely to each other in north-
south direction.

The seven foliage characteristics in Table 12 (page 67), were
chosen on the basis of their ability to discriminate between balsam and
Fraser firs, and the cone characteristics in Table 13 (page 68), like-
wise are important taxonomically. As balsam fir occurs farther north
than Fraser fir, strong correlations between these characteristics and
north-south location were expected. Nevertheless, the simple correlation
coefficients and coefficients of determination emphasize the strong
relationship between geographic location and morphologic variation.

V. DISCUSSION AND CONCLUSIONS

Origin of Intermediate Fir

Theoretically, it is expected that the ranges and standard errors of any particular characteristic in a population containing hybrids, parentals, and introgressive forms would be greater than the ranges and standard errors of this characteristic measured in either parental group. Such an intermediate population would represent a merging or intermingling of two relatively diverse gene pools thus affording greater variability among individuals of that population. On the other hand, if this intermediate population is in fact a relict of a once larger continuous population where no hybridization has occurred, then less variation in characteristics would be expected than from a hybrid population.

A test of hybridity in this case must consider variability because regardless of whether the intermediate population is of hybrid origin or of relict origin without hybridization, the means of various characteristics measured would still be expected to be intermediate.

Data in this study indicate that the intermediate fir is most likely not of hybrid origin. In 12 of the 13 characteristics plotted, intermediate fir variation was no greater than variation in Fraser fir. Also, hybrid index values of the intermediate fir are distributed normally (Figure 3, page 36), whereas a bimodal or skewed distribution with wide variation is expected of a hybrid swarm (Anderson, 1936; Stebbins, 1950; Clausen, 1962). The possibility of hybrid origin is

70

of most of these characteristics with north-south geographic location suggests that they may be important in selection, as several important environmental factors change from north to south.

The large among stand differences of leaf scar widths in Fraser fir could be explained by random drift. Although the effective breeding population of Fraser fir stands would be comparatively high, these populations, being farther south, have been isolated longer than have intermediate or balsam fir stands, permitting random fixation of a characteristic which has low selection value.

Wide within stand variation of leaf scar width in four of the intermediate stands and the balsam fir stand at Promised Land is difficult to explain (Figure 11, page 45). Possible hybrid origin of the intermediate stands would explain the wide within stand variation of the four intermediate stands at Hawksbill, Cheat Bridge, Canaan Valley, and Stonecoal Run, but Balsam Swamp does not show wide within stand variation. Wide variation of the balsam fir stand at Promised Land cannot be explained by possible hybridity. Of interest is that the Hawksbill and Promised Land fir stands contained the fewest trees. The Hawksbill stand was under extreme ecological stress as evidenced by many dead and dying trees and by dominance of Quercus prinus and presence of Pinus virginiana in adjoining stands. Both these species are adapted to warm dry climates. Dead and dying fir trees were also apparent in the Promised Land stand.

Taxonomy

For most characteristics there are significant differences between intermediate fir and balsam or Fraser fir. However, there is much overlapping of values from Fraser to intermediate to balsam fir. Many characteristics change gradually from north to south suggesting differences due to sampling from points on a north-south cline. Foliage characteristics, as summarized by the hybrid index stand values (Figure 4, page 37), change gradually from balsam fir to intermediate fir with no apparent discontinuity between taxa. Fraser fir is distinct from the intermediate fir and balsam fir largely because of "total hypodermal cells" which discriminates well between Fraser fir and the other firs.

Cone data were unfortunately incomplete. Cones were missing from the intermediate stands at Blister Swamp, Canaan Valley, and Stonecoal Run and the balsam fir stand at Bear Meadow. "Bract length" and "scale length" differences between intermediate fir and balsam or Fraser fir were greater than foliage differences, but intermediate stands were not always significantly different from all balsam or Fraser fir stands.

"Bract-scale ratio" was the only characteristic which discriminated absolutely among the three taxa with no overlapping of values. Although readily observed and convenient for taxonomic categorization, this characteristic may be questionable in a study of variation because it is calculated from "bract length" and "scale length" which vary more or less inversely to each other. Thus it exaggerates differences due to variation of either characteristic considered separately. If variation

in "bract length" or "scale length" is considered independently, differences among <u>Abies</u> <u>fraseri</u>, <u>Abies</u> <u>balsamea</u> var. <u>phanerolepis</u> and <u>Abies</u> <u>balsamea</u> are not very distinct.

Variation Patterns

Depending on the characteristic, individual tree variation accounted for from 11 to 63 percent of total variation. All characteristics tested showed highly significant within stand differences. Significance of within stand variation of seed color could not be estimated because only one observation per tree was taken, leaving no degrees of freedom for error. High among tree variation has been reported in most variation studies (Zobel, Thorbjornsen and Hansen, 1960), and is not unexpected in fir. On the basis of variation at this level, tree breeders could expect to make gains for many characteristics using individual tree selection.

Large variance components at group and stand levels were found for most characteristics. Considering the discontinuous distribution of Southern Appalachian fir, with no genetic exchange among stands, significant stand and group differences would be expected.

The variation patterns of most characteristics suggest sampling from a continuous clinal gradient. It must be noted that this sampling is based on ten trees per location and some sampling errors are likely. Also, in a study of natural variation no control is possible over the environment. Morphological characteristics will necessarily reflect both

hereditary and environmental effects. As the distribution of fir in the Southern Appalachians is discontinuous, so too must we expect the variation patterns to be discontinuous. Connecting intermediate forms between stands no longer exist.

High correlation of mean stand values of some characteristics with north-south location (Tables 12 and 13, pages 67 and 68) adds support to the theory that the disjunct Abies subpopulations of the Southern Appalachians are relicts of a once continuous ancestral fir population where clinal variation existed along a north-south gradient.

Seed collected during this study has been nursery planted by the United States Forest Service. Subsequent heritability tests will perhaps reveal the extent of genetic control over the phenotypic variation measured.

VI. SUMMARY

Determinations were made of many foliage, seed, and cone character-
istics from material collected throughout the ranges of: Abies fraseri
in high elevations of Tennessee, North Carolina, and southern Virginia;
Abies balsamea var phanerolepis in West Virginia and northern Virginia;
and from the southernmost distribution of Abies balsamea in Pennsylvania
and southern New York.

Natural variation was investigated to determine relationships
among these taxonomic groups, especially with reference to possible
hybridity of A. balsamea var phanerolepis in West Virginia and northern
Virginia.

Variance components among species groups, stands within groups,
trees within stands, and determinations within trees were computed for
seventeen foliage characteristics and six cone and seed characteristics.
Much variation among species groups and among stands within groups was
shown. Variation patterns suggested sampling from a north-south cline.
Stand values of many characteristics overlapped, in many cases obscuring
taxonomic boundaries. High correlation with north-south geographic
location was shown for many characteristics.

Variation was no greater within the intermediate fir stands than
within Fraser fir stands for 12 of the 13 characteristics analyzed, and
distribution of hybrid index values of the intermediate fir was normal.
Variation in both Fraser fir and intermediate fir was greater than in

balsam fir. Thus, the theory of hybrid origin of A. balsamea var
phanerolepis in West Virginia and Virginia was not generally upheld.
Almost all data supported the theory that intermediate fir is a relict
of a once continuous population of Abies where clinal variation existed,
although other interpretations may be possible. One characteristic,
"leaf scar width" did show wider variation within the intermediate
stands than within Fraser or balsam fir, as would be expected of hybrid
populations.

"Total hypodermal cells" discriminated well between Fraser fir
and the other firs, but not between balsam fir and intermediate fir.

The traditional cone bract length-scale length ratio was the only
characteristic which distinguished absolutely among the three taxa with
no overlapping of values. This ratio is calculated from "bract length"
and "scale length" which vary more or less inversely to each other from
north to south. The ratio exaggerated differences due to variation of
either characteristic. Bract scale ratio may be misleading in determin-
ing relationships of Abies taxa. Differences among Abies fraseri,
A. balsamea, and A. balsamea var phanerolepis may be less distinct than
heretofore believed.

LITERATURE CITED

LITERATURE CITED

Allard, H. A. and E. C. Leonard. 1952. The Canaan and the Stony River
    Valleys of West Virginia. Castanea 17:1-60.

Anderson, A. P. 1897. Comparative anatomy of the normal and diseased
    organs of Abies balsamea affected with Aecidium elatinum. Bot.
    Gaz. 24:309-344.

Anderson, E. 1936. Hybridization in American Tradescantias. Ann. Mo.
    Bot. Gard. 23:511-525.

Bakuzis, E. F. and H. L. Hansen. 1965. Balsam Fir. Univ. Minn. Press,
    Minneapolis. 445 pp.

Braun, E. L. 1955. The phytogeography of unglaciated eastern United
    States and its interpretation. Bot. Rev. 21:297-375.

Brooks, A. B. 1920. West Virginia trees. W. Va. Univ. Coll. of Agr.
    Exp. Sta. Bull. No. 175. 242 pp.

Brown, C. A. 1938. The flora of Pleistocene deposits in the West
    Florida Parishes, West Feliciana Parish, and East Baton Rouge
    Parish, Louisiana. Lous. Geol. Surv. Bull. 12:59-94.

Cain, S. A. 1944. Pollen analysis of some buried soils, Spartanburg
    County, South Carolina. Bull. Torrey Bot. Club 71:11-22.

Ching, Kim K. 1959. Hybridization between Douglas fir and bigcone
    Douglas fir. For. Sci. 5:246-254.

Clarkston, Roy B. 1966. The vascular flora of the Monongahela National
    Forest, West Virginia. Castanea 31:31-32.

Clausen, Knud E. 1962. Introgressive hybridization between two
    Minnesota birches. Silvae Genet. 11:142-150.

Core, Earl L. 1934. The blister pine in West Virginia. Torreya
    34:92-93.

Dice, Lee R. and Harold J. Leraas. 1936. A graphic method for compar-
    ing several sets of measurements. Contrib. Lab. Vertebrate Genet.
    No. 3, Univ. Mich. 3 pp.

Dorner, Herman B. 1899. The resin ducts and strengthening cells of
    Abies and Piceae. Ind. Acad. of Sci. Proc. pp. 116-129.

80

Emig, William H. 1959. Microtechnique Text and Laboratory Exercises. Published by author. Colorado Springs, Colo. 92 pp.

Engelman, George. 1878. A synopsis of American firs. J. St. Louis Acad. of Sci. Trans. 3:1-10.

Falconer, D. S. 1960. Quantitative Genetics. Ronald Press Co., N. Y. 365 pp.

Fernald, M. L. 1909. A new variety of Abies balsamea. Rhodora 11:201-203.

Fosberg, F. R. 1941. Observations of Virginia plants. Part I. Va. J. of Sci. 2:106.

Fulling, E. H. 1934. Identification by leaf structure of the species of Abies cultivated in the United States. Bull. Torrey Bot. Club 61:497-524.

_____. 1936. Abies intermedia, the Blue Ridge fir, a new species. J. So. Appal. Bot. Club.1:91-94.

Gathy, Par. P. 1957. A propos de l'hybride natural Abies concolor (Gord.) Engelm x Abies grandis Link. Silvae Genet. 6:186-190.

Harlow, W. M. 1931. The identification of the pines of the United States, native and introduced by needle structure. Bull. N. Y. St. Coll. of For., Syracuse Univ. Tech. Publ. No. 32. 19 pp. 17 plates.

Heimburger, C. C. and M. Holst. 1955. Notes from a trip to the southern United States, January 1953. For. Chron. 31:60-73.

Klaehn, F. U. and J. A. Winieski. 1962. Interspecific hybridization in the genus Abies. Silvae Genet. 11:130-141.

Kriebel, Howard B. 1962. Verifying species hybrids in the white pines. Proc. For. Gen. Workshop. So. For Tree Impr. Comm. Publ. No. 22. pp. 49-54.

Lester, Donald T. 1965. NC-51 provenance research by the Winsconsin Agricultural Experimental Station. Proc. 4th Centr. St. For. Tree Impr. Conf. Oct 1-3, 1964. pp. 35-37.

Little, E. L. 1953. Check List of Native and Naturalized Trees of the Unites States. U.S.D.A. Agr. Handb. 41. 472 pp.

Mergen, Francois. 1957. Genetic variation in needle characteristics of slash pine and in some of its hybrids. Silvae Genet. 7:1-9.

Millspaugh, C. F. 1913. Living flora of West Virginia. W. Va. Geol. Surv. Rep. No. 5(a). 491 pp.

Myers, Oval Jr. and F. H. Bormann. 1963. Phenotypic variation in _Abies balsamea_ in response to altitudinal and geographic gradients. Ecol. 44:429-436.

Oosting, H. J. 1956. The Study of Plant Communities--An Introduction to Plant Ecology. Freeman and Co. San Francisco, Calif. 440 pp.

Potzger, J. E. and B. C. Tharp. 1947. Pollen profile from a Texas bog. Ecol. 30:75-82.

Pursh, Frederick. 1814. Flora Americae septentrionalis or a systematic arrangement and description of the plants of North America. White, Cochrane, and Co. London. 2:639-640.

Ramseur, George S. 1960. The vascular flora of high mountain communities of the southern Appalachians. J. Elisha Mitchell Sci. Soc. 76:82-112.

_____. 1961. A hybrid index for the Mid-Appalachian _Abies_ (fir or balsam). Assoc. Stheast. Biol. 8:31.

Roe, Eugene. 1948. Balsam fir seed, its characteristics and germination. Lake St. For. Exp. Sta. Pap. 11. pp. 1-13.

Roller, Kalman J. 1966. Resin canal position in the needles of balsam, alpine, and Fraser firs. For. Sci. 12:348-355.

Sass, John E. 1958. Botanical Microtechnique. Iowa St. Univ. Press. Ames. 228 pp.

Schutt, Von P. und H. H. Hattemer. 1958. Die Eignung von Merkmalen des Nadelquerschnitts fur die Kiefern Bastarddiagnose. Silvae Genet. 8:93-99.

Shanks, R. E. 1954. Climates of the Great Smoky Mountains. Ecol. 35:354-361.

Snedecor, G. W. 1956. Statistical Methods. Collegiate Press. Ames, Iowa. 523 pp.

Squillace, A. E. 1966. Geographic variation in slash pine. For. Sci. Monogr. No. 10. 56 pp.

Stebbins, G. L., Jr. 1950. Variation and Evolution in Plants. Columbia
Univ. Press. N. Y. 641 pp.

_____, E. B. Matzke, and C. Epling. 1947. Hybridization in a
population of Quercus marilandica and Quercus ilicifolia. Evol.
1:79-88.

Telerico, Robert L. 1965. Examination of the fir at Skyland and
Hawksbill for the balsam wooly aphid (Adelges piceae Ratz.) on
the Shenandoah National Park. Region Seven Detection Report.
Supp. 1. So. Insect and Disease Control Zone. U.S.D.A. For. Ser.
Harrisonburg, Va. Unpublished. 1 p.

Thorbjornsen, E. 1960. Variation in loblolly pine. Ph. D. Dissertation.
N. Car. St. Coll. Raleigh. 188 pp.

U.S.D.A. Bureau of Soils. 1928. Field operations of the Bureau of
Soils. Maps. Series 1922. U. S. Gov't Print. Off. Wash. D. C.

Wherry, Edgar T. 1934. Temperature relations of the bunchberry, Cornus
canadensis L. Ecol. 15:440-443.

Whittaker, R. H. 1956. Vegetation of the Great Smoky Mountains. Ecol.
Monogr. 26:1-80.

Whitehead, D. R. and E. S. Barghoorn. 1962. Pollen analytical investi-
gations of Pleistocene deposits from Western North Carolina and
South Carolina. Ecol. Monogr. 32:347-369.

Wright, J. W. 1962. Genetics of Forest Tree Improvement. Food and
Agric. organiz. of the United Nations. 399 pp.

Zavarin, Eugene and K. Snajberk. 1965. Chemotaxonomy of the genus
Abies - I. Survey of the terpenes present in the Abies balsams.
Phytochemistry 4:141-148.

Zobel, B., E. Thorbjornsen, and F. Henson. 1960. Geographic, site,
and individual tree variation in wood properties of loblolly pine.
Silvae Genet. 9:149-158.

Zon, Raphael. 1914. Balsam Fir. U.S.D.A. Bull. 55. 68 pp.

VITA

The author was born in Cambridge, Massachusetts on August 18, 1931. He came to Oak Ridge, Tennessee with his family during World War II and graduated from Oak Ridge High School in 1949.

He enrolled in pre-forestry at The University of Tennessee, transferred to North Carolina State University, where he received his B. S. in Forest Management in 1955, graduating with honors.

After serving two years as a lieutenant in the Army, the author was employed by Woodlands, International Paper Company, and worked in the Coastal Plain of North and South Carolina.

He resigned from International Paper Company in 1965 to become Post Forester at For Knox, Kentucky. In July 1966 he returned to The University of Tennessee to pursue a Master's Degree in Forestry, specializing in tree improvement.

The author married the former Miss Dorothy Dry in 1951, and they have two children: Dottie Deval, born in 1954, and Allan Frederick, born in 1961.

The author is a member of the Society of American Foresters.