This is to certify that the

dissertation entitled

THE GENETIC STRUCTURE OF SYMPATRIC POPULATIONS
OF BLUE AND ENGELMANN SPRUCE

presented by

Peter Robert Schaefer

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Forestry

James W. Hanover
Major professor

Date June 30, 1983

THE GENETIC STRUCTURE OF SYMPATRIC POPULATIONS OF BLUE AND ENGELMANN SPRUCE

By

Peter Robert Schaefer

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Forestry

1983

ABSTRACT

- THE GENETIC STRUCTURE OF SYMPATRIC POPULATIONS OF BLUE AND ENGELMANN SPRUCE

By

Peter Robert Schaefer

Blue spruce is considered to be a relatively recent derivation of Engelmann spruce. The geographic ranges of these species are nearly identical within the range of blue spruce, however the species are generally separated by altitude. Blue and Engelmann spruce have been crossed artificially with very limited success. There is very little evidence of their crossing in nature. The purpose of the present study was to identify the morphological and chemical traits which may be used to distinguish between these species, and to use these traits with the Hybrid Index technique and Discriminant Function Analysis to determine whether blue and Engelmann spruce cross in nature.

The study area was located in the Scotch Creek Drainage in southwestern Colorado. Thirty-eight blue and 24 Engelmann spruce from opposite ends (low and high elevation, respectively) of the study area were used as the blue and Engelmann spruce reference individuals. One hundred forty-seven spruce from intermediate elevations within the study area were analyzed to detect possible hybridization between the species.

Forty-four morphological traits and 20 chemical (cortical oleoresin components) traits were analyzed on mature spruce and their progeny.

Seven qualitative and 12 quantitative morphological traits, and 13 chemical traits appeared to be useful in distinguishing between mature blue and Engelmann spruce. Seedlings of the two species could not be reliably differentiated by either the morphological or chemical traits used in the study. The differences between mature tree and seedling cortical oleoresin composition were quantitative rather than qualitative. The large increase in β-pinene concentration in Engelmann spruce seedlings was the most striking finding.

Four discriminant functions based on morphological and chemical traits and a hybrid index were used to classify the trees from the intermediate elevations. The results indicated that blue and Engelmann spruce do hybridize under natural conditions, although such hybridization is very limited in occurrence. Putative hybrids appear to resemble Engelmann spruce more than blue spruce. The selection of reference populations for use in studies of natural hybridization, and the speciation of blue and Engelmann spruce are discussed.

ACKNOWLEDGMENTS

I would like to sincerely thank Dr. J.W. Hanover (Chairman) for his guidance and assistance throughout my years at Michigan State University. I would also like to thank the members of my quidance committee, Drs. J. Hancock, D. Keathley, D. Reicosky and D. Sprugel. The assistance of Drs. P. Moore and D. Keathley during the collection phase of the study was very much appreciated.

My wife, Mary, has supported me in every way throughout this research project, and to her I owe a very special thank you. I would also thank my parents and family for many years of support and encouragement.

TABLE OF CONTENTS

Page

Page

IV. Evidence of Natural Hybridization Between Blue and Engelmann Spruce in Southwestern Colorado

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

THE GENETIC RELATIONSHIPS AMONG THE NORTH AMERICAN SPRUCES WITH SPECIAL REFERENCE TO BLUE AND ENGELMANN SPRUCE

Introduction

Questions concerning the origin of, and genetic relationships among, the North American spruces have received an increasing amount of attention among forest biologists since the publication of Wright's (1955) paper summarizing information on crossability and distribution. As a result of this attention the genetic relationships among white (*Picea glauca* Moensch) and Engelmann (*Picea engelmannii* Parry) spruce, red (*Picea rubens* Sarg.) and black (*Picea mariana* (Mill) B.S.P.) spruce, and to a lesser extent white and Sitka (*Picea sitchensis* (Bong.) Carr.) spruce are now fairly well documented. Several studies involving blue (*Picea pungens* Engelm.) and Engelmann spruce have been reported, however the results have not been as conclusive as those from investigations of the previously mentioned species.

Although research concerning various species relationships has resulted in several hypotheses of spruce origin and evolution on the North American continent, relatively little is actually known. This situation is aggravated by large gaps in the fossil record, and the inability to identify fossil spruce pollen grains at the specific level.

The Fossil Record

The distribution of conifers has a strong association with the Pacific basin, with a majority of the relic, endemic, and disjunct genera concentrated on lands bordering the east and west of the Pacific in both hemispheres (Li, 1953). The present distribution of coniferous families in either the northern or southern hemisphere (rarely both) suggests the existence of two separate areas of development that have probably been separated since the late Paleozoic (220+ million years BP) (Li, 1953; Florin, 1963).

The descriptions of spruce in the fossil record are generally based on palynological evidence. These spruce pollen fossils generally can not be separated into species which makes their interpretation difficult. Other than the discovery of fossilized cones by Hills and Ogilvie (1970), spruce megafossils seem to be generally restricted to seeds, which are also difficult to assign to taxa below the genus level. One hypothesis for the paucity of spruce leaves and branches in fossil deposits was advanced by MacGinitie (1953, 1969). He suggested that spruce grew at high elevations (relative to most other species of the time) and therefore at greater distances from the sites of deposition of fossil flora and fauna.

Whether the use of extant species names for fossil remains is justified depends larely on the completeness of the record of these remains. MacGinitie (1969) stated that there must be a consistent range of material through several localities and numerous specimens to justify the use of a living species name.

The first evidence of spruce in the fossil record dates back to the Cretaceous (Florin, 1963), 65-135 million years ago. Apparently the earliest records of spruce in western North America date back to the late Paleocene. Occurrences in Alaska (LaMotte, 1952; Florin, 1963), the High Arctic (Florin, 1963), Montana (LaMotte, 1952) and North Dakota (Leopold and MacGinitie, 1972) have been reported. Although this evidence is by no means conclusive it does indicate that the proposed age of _Picea_ _chihuahuana_ Martinez in North America may have been overestimated (65+ million years BP, (Gordon, 1968)).

There is ample evidence of spruce in the floras of epochs more recent than the Paleocene. Axelrod (1968) found evidence of at least two spruce species in strata from the Snake River Basin of Idaho dating to the Eocene. _Picea_ microfossils were also represented in the Eocene Green River flora of northwestern Colorado and northeastern Utah (MacGinitie, 1969). _Picea_ microfossils were also represented in flora from the Oligocene, Miocene and Pliocene in Nebraska, New Mexico, Utah, Colorado, Wyoming, Idaho, Montana, the Pacific Northwest and Alaska (MacGinitie, 1953, 1962, 1969; Love, 1959; Watts and Wright, 1966; Colinvaux, 1967; Hills and Ogilvie, 1970; Wells, 1970; Wright, 1970; Leopold and MacGinitie, 1972; Axelrod and Bailey, 1976).

Wolfe (1972) suggested that a rich coniferous forest dominated by *Picea*, *Pinus* and *Tsuga* covered the arctic slope of Alaska as late as the late Miocene.

Two spruce species were recognized by MacGinite (1953) in flora from the lower to middle Oligocene. These were listed as *Picea magna* and *Picea lahontense*. The seeds of the former were larger and similar to extant species of central and southern China. The seeds of *Picea lahontense* were most similar to spruce of the central and southern Rocky Mountains. These two species were also discovered in strata of more recent origin in Oregon. MacGinitie (1953) suggested that it is doubtful that conifers can be assigned to species solely on the basis of seed morphology, and all that can be safely inferred are similarities.

The composition of North American conifer forests in the late Miocene was described in some detail by Wolfe (1969). He suggested the presence of Sitka and white spruce in Alaska, Brewer (*Picea breweriana* S. Wats.) spruce in the Columbia Plateau - Cascade Range and Brewer spruce and *Picea magna* in Nevada. By the late Miocene the flora of the Pacific Northwest was modern in aspect. MacGinitie (1969) suggested that it was highly unlikely that exactly the same species are present today as were present in the late Miocene. He felt that adaptations have surely changed in response to changing conditions. However the conservative nature (Wright, 1955) and long life of spruce may have kept this genus from changing as much as MacGinitie might have suspected. It is also possible that changes in adaptive physiology have not resulted in large morphological changes.

That environmental conditions have changed drastically at various times since the late Miocene is evident. Periods of orogenic activity occurred several times during the Tertiary, culminating in a massive uplift of the western United States and Canada during the Pliocene (Axelrod, 1958; Floring, 1963; MacGinitie, 1969; Axelrod and Bailey, 1976). The climate throughout this period was generally much more equable than that of today, so the periodic upheavals of the western mountains may have played a central role in the speciation of spruce in western North America. It should be noted that a north-south system of mountains has been present in western North America since the late Cretaceous (Florin, 1963). However, these early mountains rose from much lower basins (perhaps as low as 500 feet in Colorado (MacGinitie, 1969)) and were badly weathered during the Eocene and early Oligocene. The significant upheavals, relative to spruce evolution, apparently began in the middle to late Oligocene (Florin, 1963; Axelrod and Bailey, 1976).

The Arrival of Extant Spruce Species in North America

It is still a matter of conjecture as to when the spruce species of recent times reached the North American Continent. Migrations of forest trees across Beringia (the Bering Land Bridge) ceased at least two million years ago (Hopkins, 1959, 1967; Haag, 1962; Colinvaux, 1967), and probably somewhat earlier for coniferous species. Apparently these progenitors of North American conifers had migrated and speciated at least four to 10 million years ago, as eastern Asia and North America had almost no coniferous species in common by that time (Hopkins, 1967). Beringia was above sea level during various periods of the Pleistocene, but the land supported tundra only. Though the land bridge remained essentially ice-free, the climate would not support forest vegetation. It is clear that the progenitor(s) of the North American spruces must have reached this continent something over four to ten million years ago, before the severing of contact between the coniferous forests of Asia and North America.

Several authors have suggested that spruce arrived on the North American continent in two or more migrational waves (Wright, 1955; Gordon, 1968, 1976; LaRoi and Dugle, 1968; Nienstaedt and Teich, 1972; Roche and Fowler, 1975). The order of appearance would seem to be *Picea chihuahuana* - *Picea breweriana*, *Picea mariana* - *Picea rubens*, and most recently *Picea glauca* - *Picea sitchensis* - *Picea engelmannii* - *Picea mexicana* Martinez. *Picea mexicana* is included in the last group because taxonomically it is most similar to these species (Dallimore and Jackson, 1967; Nienstaedt and Teich, 1972). *Picea chihuahuana* and *Picea breweriana* appear to be close taxonomically to each other and to certain Chinese species (Wright, 1955). Picea chihuahuana may have arrived in North America during the Cretaceous (Gordon, 1968).

Wright (1955) considered red and black spruce to be the result of an earlier migration than that which produced the northwest spruces, and others agree (Gordon, 1976; LaRoi and Dugle, 1968; Nienstaedt and Teich, 1972; Roche and Fowler, 1975). Nienstaedt and Teich (1972) citing personal communication with Fowler, and Roche and Fowler (1975) suggested that red and black spruce may have entered the North American continent from the east before separation of the continents (70+ million years ago). This suggests the possibility that the red - black spruce group may have reached the North American continent as early as did the *Picea chihuahuana* - *Picea breweriana* group. The northwest spruces were apparently the last to reach North America. There is fairly good evidence suggesting that these spruces had their origin on the North American continent something over 13 million years ago (Hills and Ogilvie, 1970).

Evidence of *Picea* in the late Cretaceous fossil flora of the eastern United States (Florin, 1963) supports the view of Roche and Fowler (1975) that red and black spruce arrived in North America before separation with Europe in the late Cretaceous. Florin (1963) proposed several migration routes for members of the Pinaceae, including a route between northwest Europe and northeast North America. If the northeast group reached this continent by such a route then they have been separated from their Eurasian ancestors for over sixty million years. It is probable that they have been separated from the line producing the northwest group for an even longer time.

Wright (1955) proposed that *Picea jezoensis* provided the link between western North American and Asian spruces since it is taxonomically similar to Sitka and Engelmann spruce and it crosses with white spruce. However, the recent discovery of a cache of spruce cones on Banks Island, Canada has led to speculation that they may belong to the ancestral species representing the link between the North American and Asian species of spruce (Hills and Ogilvie, 1970). The age of the formation from which these cones originated is estimated to be late Miocene or early Pliocene (not more than 15 million years BP). *Picea banksii* n. sp. was the name assigned to this extinct species. The cones of this spruce were most similar to those of white spruce from the Cypress Hills, Alberta. They could be differentiated from white spruce cones only by their larger size. This similarity in cone morphologies coupled with the fact that white spruce appears to have the most generalized gene pool of the North American spruces (based on crossability) encouraged the authors to place white spruce in a pivotal position in spruce evolution in northwestern North America. They felt that white spruce or a close ancestor (*Picea banksii*) provided the link with Asia rather than *Picea jezoensis* (Sieb. Zucc.) Garr.. Fowler (1966) and Nienstaedt and Teich (1972) also proposed that white spruce was the connecting link, based on crossability data.

The Tertiary history of the flora of North America is extremely complex and our understanding of past events is made more difficult by the meager evidence available. Perhaps the Pre-Pleistocene history of Picea in North America can be summarized as follows:

1) Picea arrived in at least three migrational waves, two from northeast Asia and one from northwest Europe.
2) Picea chihuahuana - Picea breweriana and Picea rubens - Picea mariana apparently reached the continent at about the same time (70+ million years BP) from the west and east respectively.
3) Picea banksii or a similar ancestor, which then speciated into Picea glauca - sitchensis - engelmannii - pungens reached North America 14 to to 30 million years ago.
4) Picea mexicana may be related to the previous group.
5) Contact with Europe was broken about 65 million years ago, while contact with Asia was lost four to 10 million years ago.
6) Speciation of the northwestern spruces has probably resulted from climatic and topographic disturbances since the middle Oligocene, which culminated in the late Pliocene.
7) The extant species of spruce were probably all at least partially differentiated before Pleistocene glaciation.

Since the inception of the Pleistocene the North American continent has been subjected to at least four major periods of glaciation. The drastic environmental changes associated with this epoch have undoubtably affected the relationships among the North American spruces. Citing Wright (1955) and Daubenmire (1968), Hills and Ogilvie (1970) suggested that recent evolution in *Picea* was probably related to two major effects of Pleistocene glaciation; the first being changes in geographical distributions of species, and the second being the repeated isolation and coalescing of populations. The current distribution of *Picea* throughout North America offers unique opportunities for studying the processes of evolution under natural conditions.

Genetic Relationships Among the North American Spruces

There are nine extant species of North American spruces, six of which can be assigned to two groups based on morphological and chemical data and species crossability patterns. The three spruces which can not be assigned to a cohesive group are the relic species *Picea chihuahuana*, *Picea mexicana* and *Picea breweriana*. Each of these spruces occupy very small ranges (Figure 1), and are few in number. Brewer spruce is restricted to the higher elevations of the Siskiyou Mountains and neighboring coast ranges in southern Oregon and northern California (Harlow and Harrar, 1969; Little, 1971). *Picea chihuahuana* and *Picea mexicana* occur in small disjunct mountain populations in northwestern and northeastern Mexico, respectively (Gordon, 1968). Wright (1955) and others (Gordon, 1968, 1976; LaRoi and Dugle, 1968; Nienstaedt and Teich, 1972; Roche and Fowler, 1975) have

Figure 1. The geographic ranges of <u>Picea breweriana</u>, <u>P. chihuahuana</u> and <u>P. mexicana</u>.

grouped red and black spruce together in a northeast species complex, and Sitka, white, Engelmann and blue spruce in a northwest species complex. White and black spruce form the connecting link geographically between these complexes, however there is no conclusive evidence of their crossing in nature (Parker and McLachlan, 1978).

The Northeast Spruce Complex

Red and black spruce are very similar morphologically and chemically (Gordon, 1952, 1976; Morgenstern and Farrar, 1964; Von Rudloff, 1966, 1967; Manley and Fowler, 1969; Manley, 1971, 1972; Wilkinson and Hanover, 1972), with perhaps the morphological differences being the more distinctive (Wilkinson and Hanover, 1972). The species can be separated on the basis of cone diameter, length and ratio of diameter to length, seed length (Gordon, 1976), foliage color, twig color (Gordon, 1976; Morgenstern and Farrar, 1964), twig surface (Gordon, 1976; Morgenstern and Farrar, 1964; Gordon, 1952), cone shape, scale margin, cone persistence, cone color, seedwing color, seed color, and crown shape (Gordon, 1976).

The range of black spruce is extensive (Figure 2). It is found as far south as Pennsylvania in the east, and central British Columbia in the west, and it is transcontinental from eastern Canada to northwest Alaska. Black spruce extends towards the limits of tree growth in northern Canada and Alaska (Fowells, 1965; Harlow and Harrar, 1969). Red spruce has a much smaller range (Figure 2). It is found

Figure 2. The geographic ranges of red and black spruce.

throughout much of the northeastern United States and adjacent Canada and as far south as southwestern North Carolina and southeastern Tennessee in the Appalachian Mountains (Fowells, 1965; Harlow and Harrar, 1969). Numerous accounts of natural hybridization in areas where these two species grow in close proximity have been reported (Wright, 1955; Morgenstern and Farrar, 1964; Morgenstern, 1969; Manley and Fowler, 1969; Manley, 1971, 1972; Gordon, 1976).

Morgenstern and Farrar (1964) used a hybrid index technique (Anderson, 1949) based on qualitative characters to detect natural hybridization between red and black spruce. They felt that sexual barriers scarcely existed between red and black spruce and that natural hybridization has played a significant role in the relationship between these species. The authors stressed the role of disturbed sites (hybrid habitats) in this relationship and suggested that conditions favorable for introgression occurred during the retreat of the final glacial advance, the receding of the Champlain sea, and more recently, human disturbances of the ecosystem. Morgenstern and Farrar concluded that there was widespread introgressive hybridization among populations in the overlapping areas.

Manley (1972) also used a hybrid index based on qualitative traits to differentiate between red and black spruce and detect possible hybrids. He reached three conclusions: 1) red and black spruce form natural hybrids and have been gradually introgressing since glaciation initiated contact between these species (also suggested by Morgenstern and Farrar, 1964); 2) extensive hybridization in the Maritime provinces of Canada was related to the recent disturbances of fire and logging; 3) extensive hybridization has not resulted in widespread introgression of parental species in their characteristic habitats. The lack of widespread introgression was the result of strong selection pressure favoring parental types in their typical habitats (Manley, 1972).

The most recent study of the relationship between red and black spruce was that of Gordon (1976). Gordon discussed several problems with the studies of Morgenstern and Farrar (1964), and Manley (1972). Among the problems with the 1964 study were poor structure and in some cases poor documentation of seed collections, the use of a hybrid index based on population means, and the exclusion of several mixed populations consisting of each pure species. Gordon stated that the conclusion that all populations across the overlapping range were influenced by introgression was not justified, and that the extent of hybridization between red and black spruce was still unclear. Gordon was not as critical of Manley's study, however he suggested that the methods used have tended to overemphasize the occurrences of hybridization.

In his study, Gordon used both discrete and continuous traits which were generally non-overlapping between the species. He then subjected this data to factor analysis to differentiate between the pure species and hybrids. He also sampled from a large part of the natural range of each species. Coupled with the study of natural hybridization was a companion study of artificial hybridization between Picea rubens, Picea mariana and Picea omorika (Pancic) Purkyne.. Gordon found that both red and black spruce crossed much easier with Picea omorika than they did with each other (crossability of 36% - 71% and 1% - 3% respectively). This is in contrast to the claim of Morgenstern and Farrar (1964) of almost no sexual barriers between red and black spruce. It also suggests a possible link of these two spruces to the Old World through Picea omorika. Von Rudloff (1975a) stated that on the basis of monoterpene composition both red and black spruce are very similar to Picea omorika. Natural hybridization of red and black spruce definitely occurs, but the frequency of successful hybridization and the chance of hybrids moving into established parental populations are relatively small and much less than previous studies had suggested (Gordon, 1976). Gordon reached the following conclusions concerning the relationship between black and red spruce in northeastern North America.

1) Both genetic and ecological factors operate to separate red and black spruce.
2) Many of the hybrids are weak and a low number survive.

3) Apparently most hybrids drop out of stands as they age.
4) Hybrids seem to survive best in a "hybrid habitat", but this is hard to find, and competition from hardwoods usually leads to the demise of the hybrids in these areas.
5) Ecological barriers exert a strong selection pressure favoring the pure species on their typical habitat. The hybrid is not as successful as either parent species in their respective habitats.
6) There is little evidence for significant gene flow between red and black spruce.
7) Red and black spruce are not a species pair in the sense that white and Engelmann spruce are. The genetic and ecological barriers are not as restraining between the latter species pair and they are introgressed, whereas red and black are not.

The Northwest Spruce Complex

Opportunities for natural hybridization also exist among the species of the northwest spruce complex. At least six of the possible ten species combinations for the four northwest spruces have been reported. These combinations include Sitka and white spruce (Little, 1953; Wright, 1955; Garman, 1957; Daubenmire, 1968; Roche, 1969; Hanover and Wilkinson, 1970; Nienstaedt and Teich, 1972; Roche and Fowler, 1975; Copes and Beckwith, 1977; Von Rudloff, 1977), white and Engelmann spruce (Wright, 1955; Garman, 1957; Horton, 1959;

Taylor, 1959; Habeck, 1964; Weaver, 1965; LaRoi and Dugle, 1968; Ogilvie and Von Rudloff, 1968; Habeck and Weaver, 1969; Roche, 1969; Wilkinson _et al_, 1971; Nienstaedt and Teich, 1972; Daubenmire, 1974; Fowler and Roche, 1977; Thompson and Kujit, 1976; Strong, 1978), Sitka and Engelmann spruce (Wright, 1955; Garman, 1957; Ogilvie and Von Rudloff, 1968), white, Sitka and Engelmann spruce (Garman, 1957; Ogilvie and Von Rudloff, 1968), Engelmann and blue spruce (Wright, 1955; Habeck, 1964; Weaver, 1965; Habeck and Weaver, 1969; Daubenmire, 1972; Taylor _et al_, 1975; Fowler and Roche, 1977; Strong, 1978; Mitton and Andalora, 1981), and white, blue and Engelmann spruce (Habeck, 1964; Strong, 1978). Hybridization and introgression are generally alluded to in all of the various combinations except that of blue and Engelmann spruce. Controlled pollinations between most of the bi-specific combinations have resulted in viable seed (Johnson, 1939; Richens, 1945; Johnson and Heimburger, 1946; Wright, 1955; Yablokov, 1960; Santamour, 1967; Hanover and Wilkinson, 1969; Fechner and Clark, 1969; Kossuth and Fechner, 1973; Kudray and Hanover, 1980). Pure types of all of these species can be distinguished from one another by a wide array of morphological and chemical characters. In general, female cone characteristics have been the most useful in morphological analyses (Horton, 1959; Taylor, 1959; Weaver, 1965; Daubenmire, 1968, 1972, 1974; Taylor _et al_, 1975; Strong, 1978), while the monoterpenes have proven themselves a very useful taxonomic tool not only in spruce

(Von Rudloff, 1962, 1964, 1966, 1967, 1972, 1975a, 1975b, 1975c, 1977; Von Schantz and Juvonen, 1966; Ogilvie and Von Rudloff, 1968; Habeck and Weaver, 1969; Hanover and Wilkinson, 1969; Wilkinson et al, 1971; Wilkinson and Hanover, 1972; Forrest, 1980a; Bongarten and Hanover, 1982), but in several other tree species as well (Mirov, 1948; Santamour, 1965; Zavarin and Snajberk, 1965, 1972; Hanover, 1966a, 1966b, 1966c, 1971, 1974, 1975; Mirov et al, 1966; Flake et al, 1969; Zavarin et al, 1969, 1978; Pauly and Von Rudloff, 1971; Tobolski and Hanover, 1971; Flake and Turner, 1973; Hunt and Von Rudloff, 1974, 1979; Lawrence et al, 1975; Squillace, 1976a, 1976b; Flake et al, 1978; Adams et al, 1980; Forrest, 1980c; Zavarin et al, 1980; Adams et al, 1981; Squillace et al, 1981).

In reviewing the literature it becomes readily apparent that the relationship between white and Engelmann spruce in western Canada and adjacent northwestern United States has received more attention than any other interspecies relationship in the genus. White spruce has a transcontinental distribution across northern North America (Figure 3). It grows to the limits of tree growth in the north and is found as far south as the northcentral and northeastern United States, as well as an isolated stand in the Black Hills of South Dakota and a limited distribution in northwestern Wyoming (Fowells, 1965; Harlow and Harrar, 1969). It is also found in isolated stands in the Cypress Hills of Alberta and the Sweetgrass Hills of Montana (Daubenmire,

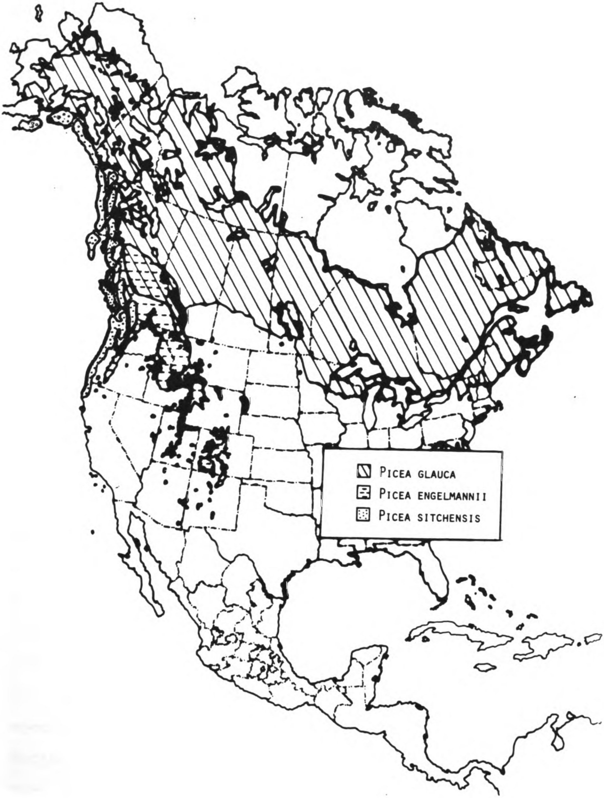

Figure 3. The geographic ranges of white, Engelmann and Sitka spruce.

1974; Thompson and Kujit, 1976; Strong, 1978). Engelmann spruce has a more limited but still rather extensive range covering nine western states and two Canadian provinces (Fowells, 1965)(Figure 3). It is found along the east slopes of the Coast Range from British Columbia to northern California. Engelmann spruce is a major component of the Rocky Mountain high elevation vegetation from Arizona and New Mexico north to southwestern Alberta (Fowells, 1965; Harlow and Harrar, 1969). These two spruces hybridize readily in areas of range overlap, resulting in significant hybrid swarms. The literature is replete with accounts of natural hybridization between these species (Wright, 1955; LaRoi and Dugle, 1968; Daubenmire, 1974; and others).

Wright (1955) supported the idea that white and Engelmann spruce hybridize readily in the area of range overlap, and that the populations in these areas represent unstabilized introgression products. Garman (1957), on the other hand, felt that these species may be two branches of a common stock, separated by the glacial epoch and brought together relatively recently through migration. Taylor (1959) tended to agree with Garman, suggesting that white and Engelmann spruce are possibly subspecies of a single species. Horton (1959) supported Wright's view and followed Wright in referring to the hybrid swarms as Picea glauca var. albertiana. Taylor found that in Alberta white spruce predominated in valleys below 5000 feet, Engelmann spruce was found above 6000 feet and the intervening slopes supported the hybrid swarm.

LaRoi and Dugle (1968) analyzed a total of 68 white and Engelmann spruce using morphological characters and paper chromatographic analysis of leaf extracts. The geographic range of white spruce was well represented, with the best representation in the west. The sample of Engelmann spruce was not as broad, as it was concentrated for the most part in the area of range overlap between the two species. The 15 morphological traits considered were analyzed using the morphological index of Garman (1957). The chromatographic spots were analyzed with Normal Association Analysis and an ordination analysis. Their results indicated the presence of hybrid and introgressed populations, and they found that Engelmann and white spruce can be readily differentiated both morphologically and chemically throughout most of their respective ranges. These results led the authors to conclude that such evidence was not sufficient to establish a common ancestry for white and Engelmann spruce, nor was it sufficient to propose that these two taxa comprise a single species. However, LaRoi and Dugle also state that their results did not conflict with the hypotheses of either Garman (1957) (common origin of white and Engelmann spruce), or Taylor (1959) (subspecies of a single wide ranging and adaptable species) concerning the relationship between white and Engelmann spruce. In essence the authors have neither accepted nor rejected the hypotheses of Garman and Taylor, and they seem unwilling to provide an alternative. However, they did suggest that the specific status of white and Engelmann spruce should probably be maintained. LaRoi and Dugle also suggested that western varieties of white spruce may be quite different from eastern varieties. Supporting evidence for this view has been provided by Wilkinson et al (1971).

In their study along the Bow River in western Alberta Ogilvie and Von Rudloff (1968) examined the monoterpene composition of five spruce populations (total of 30 trees) along an elevational gradient. They also analyzed several cone and twig characteristics. Using the morphological data the authors were able to distinguish four groups of spruce, which were similar to those determined by other researchers. Grouped by elevation, the populations were comprised of trees exhibiting 1) pure white, 2) white - intermediate, 3) white - Engelmann - intermediate, and 4) pure Engelmann traits. The terpene data generally corroberated the conclusions from the morphological data, however the high elevation Engelmann spruce were extremely variable.

Habeck and Weaver (1969) also used a combination of morphological traits and monoterpene composition to investigate the relationship between white and Engelmann spruce in Montana, Alberta and British Columbia. They found patterns of altitudinal variation similar to those of Ogilvie and Von Rudloff (1968). They also noted that the appearance of white spruce characteristics in spruce populations at low elevations increased from western Montana northward into eastern British Columbia and western Alberta. In an analysis of the monoterpenes of white spruce Wilkinson et al (1971) also found evidence of introgression between white and Engelmann spruce.

The question of hybridization and introgression between white and Engelmann spruce was also addressed by Roche (1969) in British Columbia. He suggested that white and Engelmann spruce hybridize freely and that the hybrid populations occupy a niche intermediate between that of the parental species. He described the transition zone between subalpine Engelmann and montane white spruce as just such a habitat. This ability to survive in intermediate habitats should allow the genus to expand into a wider array of habitats than either of the parental forms could invade (Roche, 1969). Roche felt that the hybrid swarms represent every degree of backcrossing and intercrossing, and since the variation in intermediate populations was small, he concluded that the altitudinal cline represented by such populations was of long standing. This is contrary to Wright's (1955) view of relatively recent contact and hybridization between these species.

Daubenmire (1974) appeared to argue in favor of both of the preceding hypotheses. He suggested that contact and hybidization may have been initiated at least as early as late Wisconsin time south of the ice sheet. As the glacial ice retreated the two spruce species rapidly advanced to the north. Species contact and subsequent hybridization followed the retreating ice, and therefore introgression of these two species is not as advanced in the north as it is in the south.

Daubenmire's (1974) study was probably the most extensive concerning the relationship between white and Engelmann spruce. Twelve populations distributed throughout the range of Engelmann spruce, 14 populations distributed over the sympatric ranges of white and Engelmann spruce and seven populations of western white spruce were sampled. At each site he collected cone and branch samples from the first 30 trees encountered. Introgression was evident from all collections at all altitudes in areas of sympatry. Previous authors considered the populations at elevational extremes to be either pure Engelmann or pure white spruce (Garman, 1957; Horton, 1959; Ogilvie and Von Rudloff, 1968; Roche, 1969). Daubenmire further stated that he could find no evidence in the field supporting Roche's (1969) belief that the hybrid populations have expanded the range of the genus into habitats inhospitable to either parent species.

Recent studies (Thompson and Kujit, 1976; Strong, 1978) have demonstrated the existence of white and Engelmann spruce and their hybrids in the Sweetgrass Hills of north-central Montana. Daubenmire (1974) also received a sample of cones from this area and he corroberated the existence of white, Engelmann and white x Engelmann spruce in this area. The significance of this discovery lies in the contribution such information can make toward explaining the Pleistocene and later history of spruce in the western United States and Canada. This is a discussion which will be considered later in this paper.

The preceding discussion allows for the following conclusions concerning the present relationship between white and Engelmann spruce.

1) White and Engelmann spruce are two distinct species which have been in contact at one time or another since at least as early as the late Wisconsin.
2) White and Engelmann spruce hybridize freely where their ranges are in contact.
3) There is evidence of gene exchange in virtually all of the populations in areas of sympatry.

The relationship between Sitka spruce and white spruce has also been investigated during the past three decades (Little, 1953; Wright, 1955; Garman, 1957; Daubenmire, 1968; Roche, 1969; Hanover and Wilkinson, 1970; Roche and Fowler, 1975; Copes and Beckwith, 1977; Von Rudloff, 1977). Sitka spruce is the largest representative of the genus, growing to heights in excess of 200 feet and diameters greater than four feet (Harlow and Harrar, 1969). The range of Sitka spruce extends along the west coast for about 1800 miles from northern California to Alaska (Figure 3) (Fowells, 1965; Harlow and Harrar, 1969). The ranges of Sitka spruce and white spruce overlap in both Alaska and British Columbia.

In 1953 Little published a report of a natural hybrid between white and Sitka spruce in Alaska in the area of Kenai Lake. He assigned the name Picea x lutzii Little to this hybrid. He cited several authors who have reported successful artificial crosses between these two species as evidence that they will hybridize given the opportunity to do so (Eklundh, 1943; Larsen, 1934, 1937; Richens, 1945; Thaarup, 1945).

Garman (1957) found evidence of hybridization between white and Sitka spruce along the Skeena and Nass river basins of British Columbia. He also felt that Engelmann spruce was involved in these areas but later researchers have not been able to verify this (Daubenmire, 1968; Roche, 1969).

Daubenmire (1968) based his analysis of possible hybridization between white and Sitka spruce along the west coast of Canada and the northern United States on the morphology of ovuliferous cones and the twigs and needles of each species. Thirty trees from each of ten populations were sampled. His results indicated that the Skeena River collections appear to be white x Sitka spruce hybrids. These hybrids resembled Sitka spruce at the lower end of the drainage and white spruce at higher elevations further inland. Daubenmire could find no evidence of Engelmann spruce in the Skeena and Nass river basins.

Using seedling phenological traits as well as several cone characteristics of mature trees, Roche (1969) also found evidence of hybridization between white and Sitka spruce. The cone characters were analyzed using line of shape and discriminant function analysis. The results of the discriminant analysis were hampered somewhat by the resemblance of hybrid white x Sitka spruce to Engelmann spruce in terms of cone scale morphology. Most of Roche's conclusions were therefore based on the line of shape analysis. He suggested that there is considerable evidence of introgressive hybridization between white and Sitka spruce in the areas of the Skeena and Nass river valleys. Evidence from seedlings and mature trees was corroborative.

Hanover and Wilkinson (1970) and Von Rudloff (1977) have also provided evidence of natural hybridization between white and Sitka spruce. Hanover and Wilkinson examined the leaf phenolics of white, Engelmann and Sitka spruce and found that several compounds were species specific and varied little within the species. Trees from the lower Skeena population were similar to Sitka spruce while those from the upper Skeena populations were similar to white. Von Rudloff analyzed the volatile oils of Sitka spruce needles and found that three trees from southwestern Yukon Territory were intermediate in their volatile oil composition between Sitka and white spruce.

Copes and Beckwith (1977) determined the degree of introgression between white and Sitka spruce using mean distinctiveness values of isoenzyme fequencies of freshly germinated seed. They concluded that the hybrids were generally more like white than Sitka spruce.

Several reports of natural hybridization between white and black spruce appear in the literature (Little and Pauley, 1958; Larsen, 1965; Roche, 1969), but most of these reports are unconfirmed. Until recently the Rosendahl spruce of Little and Pauley (1958) has been considered a genuine hybrid by most researchers. One serious shortcoming of the study by Little and Pauley was that they only considered three trees in their analysis. These trees were growing together, a white spruce growing on one side and a black spruce on the other of the supposed hybrid. They found that the hybrid was intermediate in 23 traits, similar to white in 18, similar to black in seven and different from either parent species in two traits. From these results they suggested that this tree was probably a first generation hybrid. Three percent of the seed from this tree was considered viable.

Von Rudloff and Holst (1968) supported the hybrid status of the Rosendahl spruce. They analyzed the volatile leaf oils of five white, five black and the Rosendahl spruce and found that the putative hybrid had intermediate levels of camphor and to a lesser extent camphene, limonene, borneol and α-terpineol. The optical rotation of these oils and the total yield of oil was also shown to be intermediate. However, the yield of volatile oils has been shown to be influenced by environmental factors (Von Rudloff, 1966, 1972).

Perhaps more trees should have been sampled to better represent the variation present in each species in the stand.

Recent evidence has cast some doubt on the hybrid nature of the Rosendahl spruce (Parker and McLachlan, 1978). These authors examined cone and twig characteristics of seven white, six black and 28 spruce from mixed stands on disturbed sites. Each of the traits studied was first plotted in a frequency histogram to provide an indication of its diagnostic ability. The selected traits were then used in an aggregated hybrid index (Hubbs _et_ _al_, 1943) to distinguish between the pure species and any hybrids if present. Parker and McLachlan found no evidence of hybrids or otherwise introgressed spruces. Their results show that the Rosendahl spruce is not an F_1 hybrid of white and black spruce, but that it is actually a white spruce. The extreme difficulty with which artificial crosses can be made between these species (Fowler, 1983) supports the conclusions of Parker and McLachlan. Several trees did exhibit partial intermediacy, but the authors dismiss this as a result of phenotypic or ecotypic variation. They cite Anderson (1939) to support this view, suggesting that hybrids intermediate in one trait should also be intermediate in others. In their study of blue and Engelmann spurce Taylor _et_ _al_ (1975) found that morphological traits appeared to segregate independently of each other and also generally independent of chemical traits. Assuming a lack of strong selective pressure relative to these traits, Taylor _et_ _al_ suggested that introgression would be expected to result in various combinations of mixed traits. Considered in this light some of the trees exhibiting partial intermediacy in Parker and McLachlan's study might be hybrids. Although their study is the most comprehensive and conclusive to date, Parker and McLachlan have not put the case of hybridization completely to rest. Their study, as well as the others involving these two species, illustrates some of the difficulties encountered in the interpretation of studies of natural hybridization. It also emphasizes the importance of crossability studies in investigations of species relationships and the detection of natural hybridization.

Up to this point the discussion has been limited to a fairly brief review of the major research involved with interspecific relationships among the spruces of North America, with the exception of one species combination. The remainder of the discussion will be concerned with the question of speciation in blue and Engelmann spruce, and will begin with a description of the ecology and habitats of these species.

The geographic range of blue spruce lies almost entirely within that of Engelmann spruce in the southern and central Rocky Mountains (Figure 4). Their ranges are generally separated altitudinally, as blue spruce is typically found at elevations of 2000 to 3000 meters and Engelmann spruce is generally found above this elevation in the central and southern Rocky Mountains.

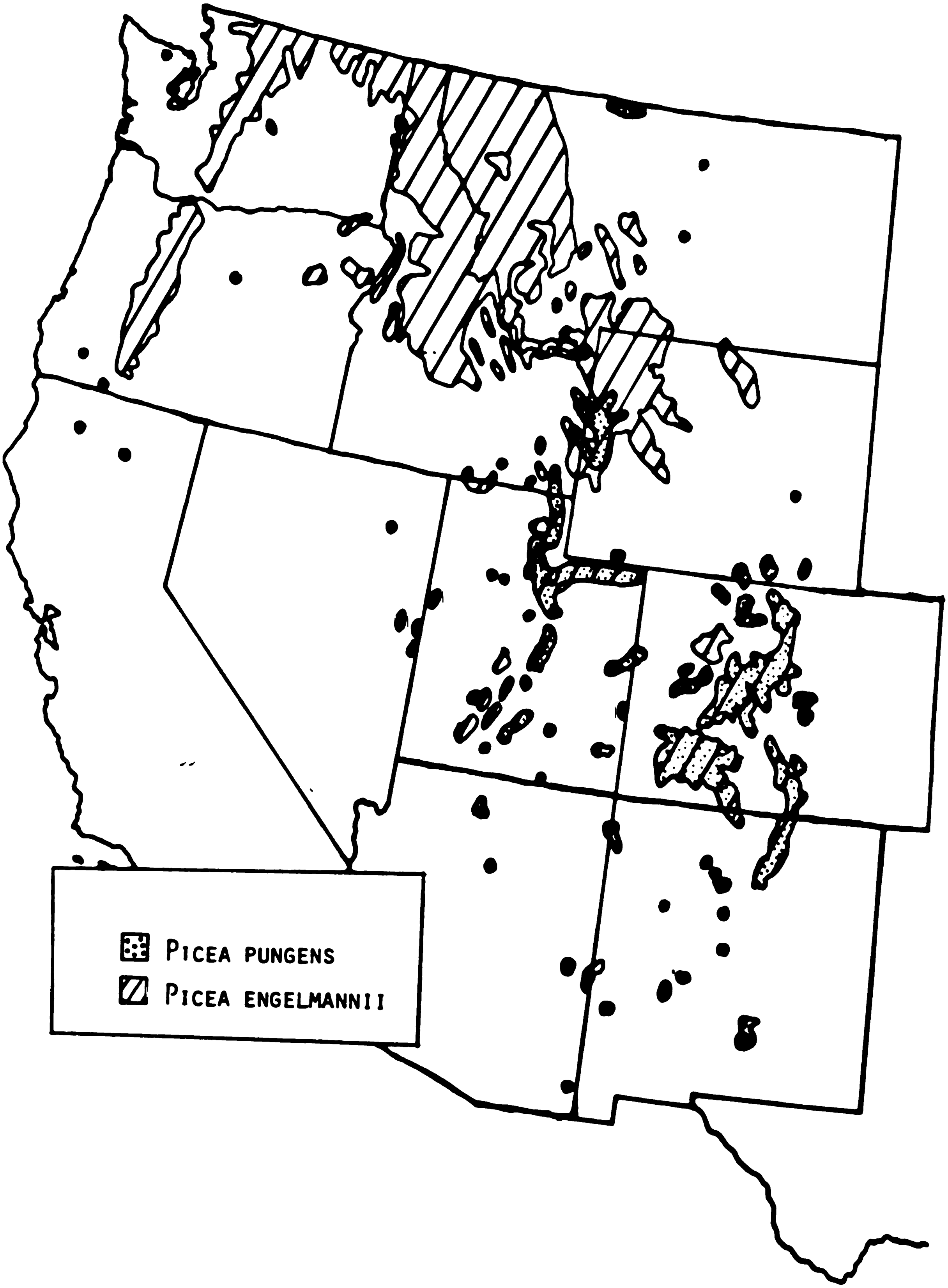

Figure 4. The geographic ranges of blue and Engelmann spruce.

The ecology and typical habitats of blue spruce have not received a great deal of attention in the past. Much of the following discussion of blue spruce ecology is from Fechner (1983). Blue spruce is generally considered a species of the montane zone. The climate is moist and cool, with fifty percent of the precipitation occurring during the growing season. Blue spruce grows in soils that are typical of the mixed conifer forest of the Rocky Mountains. These soils are rich and fairly fertile. Although they are wet at the end of winter they can become too dry for initial seedling survival during early summer drought periods in the southern part of the range. Fall moisture deficits occur throughout most of the range of blue spruce, but these are less limiting to seedling establishment than early summer deficits (Alexander, 1974; Jones, 1974).

Blue spruce typically occurs in small open stands along streambottoms or valley floors and on the slopes to either side, often in association with narrow leaf cottonwood (*Populus angustifolia* James). Blue spruce may also be found bordering meadows at higher elevations. Away from the streams Douglas-fir (*Pseudotsuga menziezii* (Mirb.)Franco) and ponderosa pine (*Pinus ponderosa* Laws.) are probably the most common associated tree species. At higher elevations these species are replaced by trembling aspen (*Populus tremuloides* Michx.), subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) and on occasion, Engelmann spruce. White fir (*Abies concolor* (Gord. & Glend.)Lindl. ex Hildebr.) is also an associated species on more mesic sites. Blue spruce rarely extends more than ten or fifteen meters from the drainage bottom up north-facing slopes. These slopes often contain dense stands of Douglas-fir at lower elevations, and lodgepole pine, or a mixture of subalpine fir and Engelmann spruce at higher elevations. Blue spruce is intermediate in tolerance and would have difficulty becoming established under dense coniferous stands. Blue spruce stands reach their best development along valley bottoms and on gentle south-facing slopes.

Blue spruce is both a climax and a seral species depending on the location in its geographic range and on its associated species. In the southwest it is most often associated with Douglas-fir and ponderosa pine and it represents a topo-edaphic climax (Daubenmire and Daubenmire, 1968). Blue spruce may occur as a minor seral species on cooler sites dominated by white and subalpine firs, or it may even occur as a pioneer species in a hydrarch successional sequence. A warm growing season with abundant moisture is optimum for this species, yet it can withstand drought better than any other spruce species, it can withstand very cold temperatures (-40°C), and it is less susceptible to high isolation and frost damage than its associated species.

Blue spruce produces heavy seed crops every two or three years. Most seeds fall within 300 feet of the stand's edge. Seedlings can become established on most seedbeds, however mineral soil is generally required for establishment under natural conditions. The growth rate of the seedlings is very slow.

The geographic range of Engelmann spruce is much more extensive (as described earlier) than that of blue spruce. However, within the geographic area covered by blue spruce the ranges of both species are nearly identical. As I have mentioned previously, the ranges are generally separated altitudinally.

Engelmann spruce occupies the upper valleys and plateaus of the Rocky Mountains which corresponds to the subalpine zone (2750m to timberline) and the upper montane zone (2300m to 2750m). Alexander (1974) refers to the altitudinal distribution of Englemann spruce as the Rocky Mountain Subalpine, the highest forested area in the states of Wyoming, Colorado and northern New Mexico. Engelmann spruce is often found in pure, dense stands or mixed with subalpine fir. The climate is cold and humid, with annual temperature extremes ranging from -45°C to > 32°C, and heavy snowfall (Fowells, 1965). Summer is the driest season in much of the range, however east of the continental divide, and in the mountains of southern Colorado, Arizona, New Mexico and Utah, late summer rainfall is heavy (Fowells, 1965).

As in the mixed conifer zone the soils of the subalpine zone are quite variable (Alexander, 1974; Fowells, 1965). Engelmann spruce reaches its best development on moderately deep, silt and clay loam soils. Good growth also occurs on alluvial soils where an accessible water table is more important than soil structure. In both of these cases the parent materials vary widely (Fowells, 1965). Coarse, dry, shallow soils are not conducive to good growth. Heavy clay surface soils and saturated soils are also not preferred.

The Engelmann spruce - subalpine fir forest is generally considered the climax vegetation of the subalpine zone. Engelmann spruce is tolerant and subalpine fir is very tolerant (Schmidt et al, 1973). Mountain vegetation is a function of many factors other than elevation. Topographic, edaphic, biotic and climatic factors are all very important in determining the presence of various vegetation types (Alexander, 1974). Fire, insects and logging are the most effective in displacing the spruce-fir type with other species. In most cases the spruce-fir type will eventually reoccupy the site, but this may require as many as 300 years depending on what type of vegetation initially occupies the disturbed site (Alexander, 1974). Engelmann spruce has difficulty invading heavy grass communities. Common tree species associates of Engelmann spruce in the central and southern Rocky Mountains are subalpine fir at higher elevations, and Douglas-fir, aspen, white bark pine (Pinus albicaulis Engelm.), limber pine (Pinus flexilis James), bristlecone pine (Pinus aristata Engelm.), white fir, and occasionally blue spruce at middle and lower elevations (Fowells, 1965).

Light seed crops can generally be expected in Engelmann spruce with good crops every two to six years. Seed may be spread as much as one-eighth mile by the wind (Fowells, 1965; Alexander, 1974). Seedlings can become established on almost any seedbed common to forest conditions. Seedlings will survive in moderate to dense shade, but they suffer heavy losses in strong sunlight (Fowells, 1965; Ronco, 1970; Alexander, 1974), or high temperatures (Fowells, 1965; Alexander, 1974). Seedling growth is very slow under natural conditions.

Blue spruce is somewhat of an enigma among the spruces of the northwest complex. As previously mentioned, Sitka and white spruce and Engelmann and white spruce produce hybrid progenies in areas of range overlap, and Sitka and Engelmann spruce will hybridize under artificial conditions. Apparently blue spruce will not readily cross with any of these three species, either in nature or artificially. Limited success has been achieved in crosses with white spruce (Yablokov, 1960; Santamour, 1967; Hanover and Wilkinson, 1969; Kudray and Hanover, 1980), and some hybrid seed has been produced from crosses with Engelmann spruce (Fechner and Clark, 1969; Kossuth and Fechner, 1973). In its natural range blue spruce seems to have rather strict habitat preferences, yet it can be planted worldwide, over a much broader range of environments than any of the other northwestern spruces. Studies concerning the relationship of blue and Engelmann spruce indicate that relatively little is known regarding the position of blue spruce in the northwest spruce complex (Wright, 1965; Habeck, 1964; Weaver, 1965; Habeck and Weaver, 1969; Daubenmire, 1972; Taylor et al, 1975; Strong, 1978; Mitton and Andalore, 1981).

Weaver (1965) found no stands of blue and Engelmann spruce that exhibited high variability and many intermediates. He suggested that reproductive isolation between the species was a result of spatial isolation or differences in flowering time, rather than meiotic problems. In a later study primarily concerned with white and Engelmann spruce, Habeck and Weaver (1969) suggested that the trees from the southernmost stand of Engelmann spruce in the study were blue x Engelmann spruce hybrids.

Fechner and Clark (1969) approached the blue - Engelmann spruce problem from the crossability perspective, as did Kossuth and Fechner (1973). In the first study the authors obtained an average of 2.2 seeds/cone (1-2%) using the Engelmann spruce as the female parent. No viable seeds were obtained using blue spruce as the female parent. The hybrids were intermediate in germination characteristics, but they did not differ from Engelmann spruce in cotyledon number and hypocotyl color. One-half of the hybrid seedlings had abnormalities and died. This indicates that few natural hybrids would survive, since they would be subjected to conditions far more rigorous than those found in the greenhouse. Fechner and Clark suggested that Engelmann spruce would probably be the female parent if natural hybridization does occur. A major drawback to this study was that only two trees were used as females, one of each species. The results of several studies clearly show the effect of individual parents on seed yields (Hanover and Wilkinson, 1969; Kudray and Hanover, 1980; Bongarten and Hanover, 1982; Fowler, 1983). The wide variation that exists in the crossability of individual parents demands that crosses between many combinations of parent trees should be attempted to properly depict the crossability between blue and Engelmann spruce.

The work of Kossuth and Fechner (1973) generally confirmed the previous study of Fechner and Clark (1969). However, as in the previous study Kossuth and Fechner only used one tree of each species for the female parent. They also used a very limited pollen sample consisting of a two tree mix of blue spruce pollen and a single tree for the Engelmann spruce pollen. They compared the ovular development of Engelmann x blue and blue x Engelmann crosses with that of wind pollinated blue spruce. Their results showed that incompatibility rather than embryo inviability was the primary mechanism preventing high crossability between the species. The studies of both Fechner and Clark (1969) and Kossuth and Fechner (1973) indicate that there is strong, but not complete, incompatibility between blue and Engelmann spruce. In fact their results indicate that compatibility between Engelmann and blue spruce may be similar to that between white and blue spruce (about 2 viable seeds per cone) (Hanover and Wilkinson, 1969; Kudray and Hanover, 1980).

This can not be confirmed until a more thorough study of cross compatibility of blue and Engelmann spruce is made.

Daubenmire (1972) made the first attempt at an extensive study of the relationship between blue and Engelmann spruce. Thirty trees from each of five blue spruce, 12 Engelmann spruce and four mixed blue - Engelmann spruce populations were analyzed using morphological data and the hybrid index technique (Anderson, 1949). Daubenmire reported no absolute differences between the species, resulting in a slight overlap of the hybrid index values for populations considered to be pure blue and pure Engelmann spruce. No evidence of hybridization was found in this study. Blue and Engelmann spruce which were growing intermingled appeared as distinct as trees from allopatric populations. These results coupled with those of Fechner and Clark (1969) led Daubenmire to suggest that blue spruce (with its smaller geographic range and reduced variability in reproductive structures) is a relatively recent derivation of Engelmann spruce. Blue spruce arose by a mutation which immediately caused it to be incompatible with, and to have a different ecological amplitude than, Engelmann spruce.

There is reason to believe that Daubenmire's hypothesis concerning the origin of blue spruce may not be correct. Mikkola (1969) investigated interspecific sterility among nine spruce species, including white and Sitka spruce of the northwest spruce complex. Some degree of incompatibility was observed in all interspecific crosses, with embryogenesis reaching many different stages. Mikkola suggested that the evolution of incompatibility in spruce has generally followed the pattern of geographical speciation. He cited Lewis (1966) in suggesting that complete barriers to gene exchange tend not to be the result of selection for reproductive isolating mechanisms, but rather that these barriers are more likely the accidental by-products of evolution. The occurrences of a sudden major change in compatibility is not acceptable to this view, and such changes were not observed by Mikkola. The results of Kossuth and Fechner (1973) substantiate Mikkola's findings, as Kossuth and Fechner identified four principle stages of developmental irregularities.

In a follow-up study to that of Daubenmire (1972), Taylor _et al_ (1975) analyzed the same populations adding three chemical markers (leaf phenolics) and a discriminant analysis to the study. The chemical markers allowed the identification of two suspected hybrid trees which had been earlier (Daubenmire, 1972) classified as blue spruce. Eleven other trees were classified as either chemical and/or morphological intermediates. The authors suggested that hybridization between blue and Engelmann spruce does occur, but it does so infrequently. This interpretation was questioned by Mitton and Andalora (1981) since trees in allopatric populations were also found to be intermediate. They were not convinced that the intermediacy found in the study of Taylor _et al_ (1975) was a result of hybridization. Taylor _et al_ were aware of this discrepancy and hypothesized a range extension of blue spruce during the Xerothermic period, with hybrid traits persisting in what was thought to be a pure Engelmann spruce population.

Taylor et al (1975) noted that even if less than two percent of the artificial crosses were successful it is not unreasonable to expect the production and establishment of an occasional hybrid under favorable ecologic conditions. Taylor et al supported Daubenmire's (1972) views concerning the evolution of blue spruce, noting that complete genetic incompatibility would be the most efficient isolating barrier between sympatric species. That localized environmental factors can be instrumental in separating races of species has been well documented (McNeilly, 1968; Erlich and Raven, 1969; Antonovics and Bradshaw, 1970; Hamrick and Allard, 1972). It seems that Taylor et al have not considered this possibility.

Both the study by Taylor et al (1975) and Daubenmire (1972) revealed less variation in the sympatric populations than in the allopatric populations. Taylor et al speculated that natural selection may favor any distinction leading to niche specialization, resulting in reduced character intergradation in sympatric populations. They also noted that this pattern of variability may be the result of chance allelic distribution.

Recently Mitton and Andalora (1981) reported on a study of genetic and morphological relationships between blue and Engelmann spruce in the Colorado Front Range. They analyzed a total of eighty-eight trees in one blue, three sympatric and one Engelmann spruce population along an elevational transect. Nine morphological traits and one enzyme polymorphism were used to distinguish between the species. No clusters were evident from a principle components analysis of the morphological data, yet none of the expected heterozygous polymorphisms were observed. Since the enzyme polymorphism could separate the two species it was used to classify individual trees as either blue or Engelmann spruce. Thus separated, a discriminant analysis was performed based on the morphological characters. All nine characters were included in the analysis regardless of their discriminatory power. The discriminant function was not able to completely separate the blue and Engelmann spruce groups, a problem also encountered by Taylor et al (1975). Mitton and Andalora did not feel that this was an indication of hybridization, however. They felt that the morphological traits used were not adequate to separate the species, and that morphological similarity between blue and Engelmann spruce is not a result of hybridization, but may result from either convergent evolution or the influence of environmental variation upon these characters. These authors did not use any cone characters in their analysis. Cone characters have been found to be the most diagnostic for separating many of the North American spruces. The inclusion of such traits in their study may have improved the diagnostic ability of their discriminant function. Gordon (1976), and Parker and McLachlan (1978) point out that the inclusion of non-diagnostic characters in an investigation of natural hybridization can only serve to make the results less interpretable.

The presence of small stands of blue spruce south of Flagstaff, Arizona, coupled with the discovery of white, Engelmann and blue spruce in the Sweetgrass Hills of north-central Montana indicates that blue spruce at one time occupied a more extensive range than it does today (Thompson and Kujit, 1976; Strong, 1978). Thompson and Kujit suggested that these hills were colonized by belts of coniferous forest following the receding glacier. There is strong evidence for the occurrence of spruce in the Great Plains during Wisconsin and earlier times, however the microfossils have not been identified to the species level (Löve, 1959; Watts and Wright, 1966; Kapp, 1970; Wells, 1970; Wright, 1970). Apparently the coniferous forests of the Great Plains were rapidly replaced by grassland between eight and ten thousand years ago, leaving relic populations of spruce in the Sweetgrass Hills, the Cypress Hills of Alberta, and the Black Hills of South Dakota. Strong (1978) suggested that all three spruces (white, Engelmann and blue) have introgressed in the Sweetgrass Hills. A comprehensive study including representative populations from the normal range of each species will be required to confirm Strong's proposal.

Although the results of the various studies concerning the genetic relationship between blue and Engelmann spruce conflict, one aspect of this relationship is clear. Blue and Engelmann spruce do not hybridize to the extent that white and Engelmann or white and Sitka spruce do. If they did the studies presented here would have detected such hybridization. Still, the evidence presented to date suggests that these two species will hybridize when given the chance, albeit with a low success rate. It is evident that such hybridization will not be easily detected by the same methods used in studies of white and Engelmann or white and Sitka spruce. Future research should place more emphasis on intensive samples of blue and Engelmann spruce populations in areas of sympatry, and controlled crosses between the two species to generate known hybrids. Such studies would have the advantage of being better able to detect hybrids if they exist and would lead to a better understanding of the effect of local environments on the blue - Engelmann spruce relationship. Compatibility studies involving many parents of each species are badly needed.

CHAPTER II

A MORPHOLOGICAL COMPARISON OF BLUE AND ENGELMANN SPRUCE IN SOUTHWESTERN COLORADO

Abstract

Thirty-eight blue and 24 Engelmann spruce from opposite ends (low and high elevations, respectively) of the study area were analyzed. Forty-four morphological traits were measured. Seven qualitative and 12 quantitative traits appeared to be useful in distinguishing between blue and Engelmann spruce. A morphological index and stepwise selection form of discriminant function analysis were very successful in separating the species. The selection of reference populations for use in studies of natural hybridization is discussed.

A Morphological Comparison of Blue and Engelmann Spruce in Southwestern Colorado

Introduction

Blue (*Picea pungens* Engelm.) and Engelmann (*Picea engelmannii* Parry) spruce are thought to be closely related species. Within the geographic area covered by blue spruce the ranges of both species are nearly identical, although the species are generally separated altitudinally. Very few spruce species in Wright's (1955) study exhibited closer affinities for one another than blue and Engelmann spruce. Daubenmire (1972) could determine no absolute differences between these species in any morphological traits. Jones and Bernard (1977) suggested that the overlap of certain morphological traits of blue and Engelmann spruce was probably responsible for several reports of natural hybridization between these species. The authors concluded, however, that consideration of several traits will lead the experienced observer to the correct identification of blue and Engelmann spruces.

The most useful morphological traits for taxonomic studies in *Picea* appear to be those associated with female cones (Horton, 1959; Taylor, 1959; Weaver, 1965; Daubenmire, 1968, 1972, 1974; Taylor *et al*, 1975; Strong, 1978). A wide array of other traits have also been used successfully in studies of spruce, however their diagnostic ability varies with the species under consideration. Twig pubescence, bark texture, needle sharpness and stiffness, the length and distribution of resin sacs in the needle, along with female cone characteristics are most often used to distinguish between blue and Engelmann spruce (Marco, 1931; Reed and Freytag, 1949; Daubenmire, 1972; Taylor et al, 1975; Jones and Bernard, 1977; Buchert, 1981; Mitton and Andalora, 1981).

Investigations concerning the detection of natural hybridization between blue and Engelmann spruce have been hampered by an inability to completely separate pure forms of each species. Daubenmire (1972) used the hybrid index technique of Anderson (1949), but could not completely separate these species using three morphological traits. Taylor et al, (1975) applied the hybrid index technique as well as discriminant analysis to ten morphological traits and obtained more decisive results than Daubenmire. However, some intermediate forms appeared in allopatric populations. Recently, Mitton and Andalora (1981) analyzed 9 morphological traits that could be easily measured on trees of any age or at any time of year. They were no more successful than previous authors in separating blue and Engelmann spruce.

The observation of an area of extensive range overlap in the Scotch Creek Drainage of southwestern Colorado encouraged the present author to investigate the possibility of natural hybridization between blue and Engelmann spruce.

It is clear that traits must be found which can be used to unequivocably separate pure forms of blue and Engelmann spruce before investigations concerning natural hybridization between the species can be undertaken. The purpose of the present study was to analyze and evaluate a wide array of morphological traits for their utility in differentiating between blue and Engelmann spruce found at extreme ends (low and high elevation, respectively) of the Scotch Creek Drainage.

The Study Area

The Scotch Creek Drainage is part of the Dolores River watershed in southwestern Colorado. It is located in Dolores County about 3 miles south of Rico (Figure 5a). Elevation of the drainage is 2590m at the lower end and rises to over 3050m at the upper end. The valley floor is quite narrow, with steep (up to 110%) adjacent slopes. The drainage has an east-west orientation, however there are many smaller tributaries which feed Scotch Creek from the north and south, adding to the overall topographical complexity of the drainage (Figure 5b).

Scotch Creek joins the Dolores River in a wide, low, flat area separated from the rest of the drainage by state highway 145. This area supports dense shrub growth as well as blue spruce and aspen (Populus tremuloides Michx.) in fair numbers. The spruce are not large (max. ht. 50 ft., diam. 14 in.), and probably became established after construction of the original highway through the area.

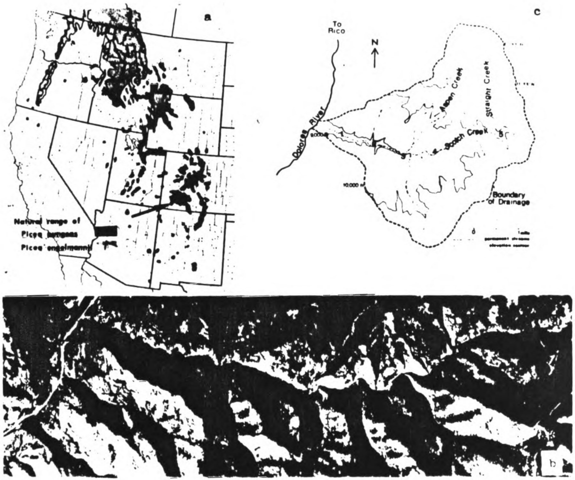

Figure 5. a) Location of the Scotch Creek drainage, b) topography of the drainage, c) diagram of the study area.

The spruce within the lower end of the drainage itself tend to be much larger (often > 20" DBH, > 80' tall), although smaller representatives are also present. The slopes to either side of the creek are very rugged, with large rock outcrops and cliffs, and steep talus slopes. The occurrence of spruce is limited to single trees or very small groups on these slopes, with greater numbers found along the valley floor in small stands. Associated with spruce in the flat area between the road and the creek are a fair number of shrubs and aspen.

The upper end of the study area was terminated at 2970m, as the areas to the east of this have been clearcut. At this point the road is well above the valley floor along the southern (north-facing) slope. The slope is generally very steep (90+%) and very difficult to negotiate as a result of a great many fallen trees (by natural causes). The area is cool and moist and supports a fairly dense, mixed stand of Engelmann spruce and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.). Aspen is also present, especially on more open, west-facing, gentler slopes. Shrub growth is very limited.

Materials and Methods

The trees for this study were selected in the fall of 1981. The only requirement was that they bear a crop of at least 30 current year's cones to provide adequate seed for a progeny test. The trees selected for the present study were from the extreme lower and extreme upper ends of the study area (Figure 5c). It was anticipated that cones from the first 50 trees meeting the fecundity requirement would be collected in each area. This goal was not achieved as a result of a relatively poor seed year. Cones were collected from 38 blue spruce at the lower end and from 24 Engelmann spruce at the upper end of the drainage. Four cones from each tree were set aside for measurement purposes prior to seed extraction. These cones were soaked until they closed before measurements were made. One scale was removed from the center of each cone. A total of 17 cone and cone scale traits were measured (Table 1). The average value of the 4 cones or cone scales for each tree was used in all analyses.

Seed data included the number of sound seeds per cone, 100 seed weight, percent germination and germination rapidity. For the germination study 15 seeds from each tree were placed on water-saturated filter paper in a separate petri dish for each tree. Each dish was randomly placed on a growth frame under flourescent lights at a temperature of 25°C. The experiment was replicated twice. The study was terminated after 20 days. At that time no seeds had germinated for three days. Percent germination was determined for each tree in each replication and the average value was then determined. A seed was considered to have germinated

Table 1. Description of cone and cone scale characters. All were based on 4 randomly selected cones/tree.

Character	Description
Cone length	(cm)
Cone width	Measured at the middle of the cone (cm)
Cone size	(length x width)/10 (Taylor *et al*, 1975)
Cone ratio	length/width (Taylor *et al*, 1975)
Cone scale length	(mm)
Cone scale width	(mm)
Cone scale size	(length x width)/10 (Daubenmire, 1972; Taylor *et al*, 1975)
Cone scale ratio	length/width (Daubenmire, 1972; Taylor *et al*, 1975)
Seed length	Measured from the imprint left in the cone scale (mm)
Seed width	"
Seed wing length	"
Seed wing width	"
Seed + wing length	"
Free scale length	Distance from the tip of the seed wing imprint to the tip of the cone scale (mm) (Daubenmire, 1972; Taylor *et al*, 1975)
Percentage of free scale	(Free scale length/cone scale length) x 100 (Daubenmire, 1972; Taylor *et al*, 1975)
Scale apex shape	1 (rounded), 2 (intermediate), 3 (truncate)
Scale apex margin	1 (entire, 2 (intermediate), 3 (erose)

when its radicle was as long as the seed itself. Germination rapidity was calculated as:

$$\text{Rapidity(days)} = \frac{\Sigma \text{ seedlots(days to germ. x \# of germ. seed)}}{\text{Total \# of germinated seed}}$$

(Hanover and Wilkinson, 1969).

Seeds were sown in the greenhouse in a randomized complete block design, with 6 trees per plot and 4 replications. The seedlings were grown under accelerated-optimal-growth conditions (Hanover _et al_, 1976). These conditions were maintained for six months, at which time the seedlings were allowed to enter their dormant stage. After one month in the greenhouse the seedlings were scored for hypocotyl color (1 = green, 2 = intermediate, 3 = red), and the number of cotyledons for each seedling was determined. When seedling growth had ceased height measurements were made to the nearest centimeter.

Foliage collections were also made from each parental selection, and 6 morphological traits were scored in the field. Fourteen foliar traits were analyzed (Table 2). Except for bud scale orientation all measurements are based on 4 needles per tree from which an average value was determined.

A device was developed to quantify needle sharpness and stiffness (Figure 6). Needle sharpness was determined by continuously adding weight (sand) to the balance arm until the needle punctured the clear plastic wrap membrane (.052 mil.). Needle sharpness was then recorded as the weight of

Table 2. Description of foliage characters. All needle characters were based on 4 randomly selected needles from 2 year old twigs.

Character	Description
# of stomatal lines	Maximum # of stomatal lines summed over all 4 sides of the needle
Needle length	(mm)
Needle width	Measured at the middle of the needle, perpendicular to the axis between the dorsal and ventral needle surfaces (mm)
Needle thickness	Measured at the middle of the needle along the axis between the dorsal and ventral needle surfaces (mm)
Needle length/width	----
Needle width/thickness	----
Needle angle	Angle of departure of the needle from the twig
Needle curvature	Needles were shortened to 15 mm by trimming each end equally. The distance from the bottom edge (at the center of the needle) to the line connecting the ends of the needle was recorded (mm).
Needle sharpness	Amount (gm) of sand required to force needle through plastic wrap membrane (see text)
Needle stiffness	Amount (gm) of sand required to deflect needle 1 mm from the starting position (see text)
Needle sharpness index	Needle stiffness/sharpness
Twig color	Scored from 1 (tan or pale yellow) to 5 (orange)
Twig pubescence	Scored from 1 (densely pubescent) to 3 (glabrous)
Bud scale orientation	Scored from 1 (appressed) to 3 (reflexed)

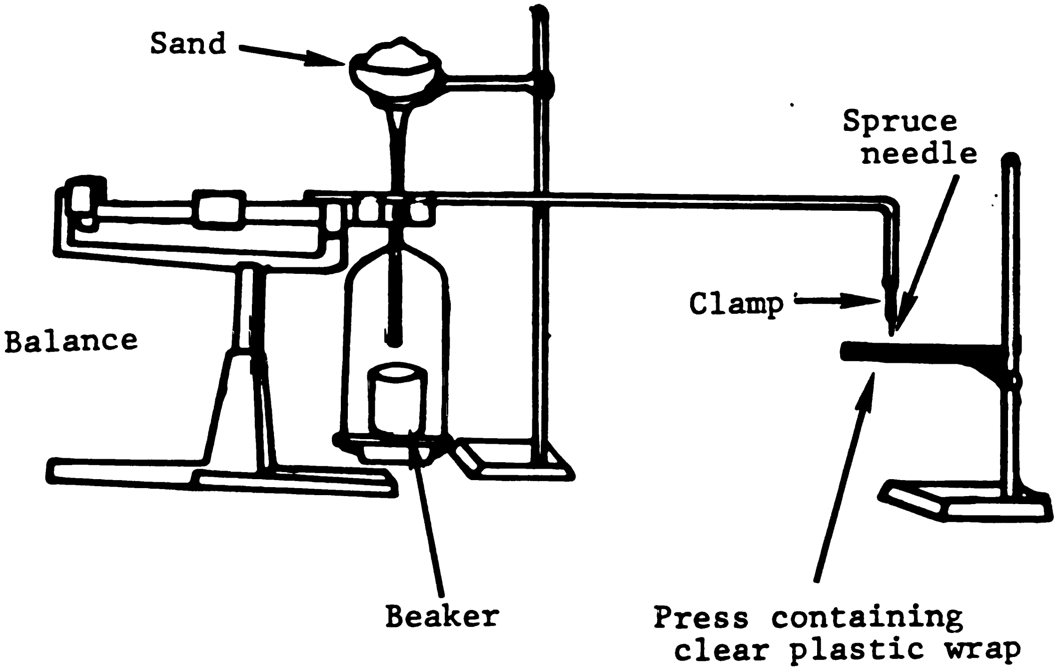

Figure 6. Diagram of the device used to measure needle sharpness and stiffness.

sand (grams) required to puncture the membrane. The less weight required, the sharper the needle. Needle stiffness was measured using the same device except that the needle was placed horizontally between two supports (spaced 1 cm apart), and the back (rounded) edge of a single edged razor blade was attached to the balance arm to apply pressure across the center of the needle. Needle stiffness was recorded as the weight of sand required to deflect the center of the needle 1 mm from the starting position. This technique yielded consistent results for measurement of both needle characteristics.

The six morphological traits recorded in the field were each scored on subjective scales (Table 3). Diameter classes were recorded as a quick indicator of tree age, realizing that in some cases the local site conditions may have a greater effect on diameter than tree age would.

Statistical Approach

An approach similar to that of Parker and McLachlan (1978) was used to determine the diagnostic ability of the morphological traits in the present study. The qualitative characters were plotted in frequency histograms to determine how well species could be distinguished by each character. The means, standard deviations, and ranges of the quantitative characters were also plotted for each species. Differences between species for those traits that appeared to be diagnostic were tested for statistical significance by Analysis of Variance procedures.

Table 3. Description of the morphological characters scored in the field.

Character	Characters
Bark texture	Scored from 1 (smooth (young tree) or scaley (mature tree)) to 5 (scaley (young tree) or rough-ridged (mature tree))
Bark color	Scored from 1 (red or red-brown) to 5 (gray or gray-brown)
Foliage color	Scored from 1 (yellow-green) to 5 (Steel blue)
Epicormic branching	Scored from 1 (none) to 5 (extensive)
Branching habit	Scored from 1 (non-planar) to 3 (distinctly planar)
Diameter class	1 (6"), 2 (7" - 12"), 3 (13")

Discriminant function analysis and Anderson's (1949) hybrid index technique were used to test the diagnostic ability of the selected variables. Only qualitative traits were used with the hybrid (or morphological) index, while only quantitative traits were included in the discriminant analysis. Discriminant function analysis is a multivariate technique that maximizes the differences between groups while minimizing within group differences, and has proved to be a useful tool in studies of species relationships among forest trees (Clifford and Binet, 1954; Mergen _et_ _al_, 1965; Namkoong, 1966; Ledig _et_ _al_, 1969; Dancik and Barnes, 1975; Flake _et_ _al_, 1978; Kudray and Hanover, 1980). The first step in such studies is to define a discriminant function based on 2 or more reference populations. This function is then used to classify individuals of unknown species assignment. A very useful form of discriminant analysis, which often increases the parsimony of the model, involves a stepwise procedure in which variables may be included or excluded at each step depending on their discriminating power. This method selects the subset of variables which best discriminate the groups under consideration. The stepwise form of discriminant analysis was used successfully by Dancik and Barnes (1975) with birch and by Kudray and Hanover (1980) in a study of artificial white x blue spruce hybrids. This form of the analysis was chosen for use in the present study. The procedure is described by Klecka (1976).

Results and Discussion

The initial step of graphing the distributions of the morphological traits revealed that 7 of 12 qualitative characters appear to be of diagnostic value in distinguishing blue from Engelmann spruce (Figures 7a-g). Jones and Bernard (1977) considered bark texture and color, degree of epicormic branching, crown form, and twig color to be diagnostic in comparisons of blue and Engelmann spruce. The results presented here agree with their conclusions, with the exception of bark color. Foliage color, bud scale orientation, seedling hypocotyl color, number of cotyledons and seedling height were found to have little diagnostic ability. Measures of twig pubescence were used in the studies of Daubenmire (1972), Taylor et al (1975) and Mitton and Andalora (1981). Twig pubescence density would appear to be a useful trait in the present study as well.

Figures 7a-g. Frequency histograms of qualitative morphological characters of blue and Engelmann spruce that were considered diagnostic. Open bars = blue spruce, lined bars = Engelmann spruce.

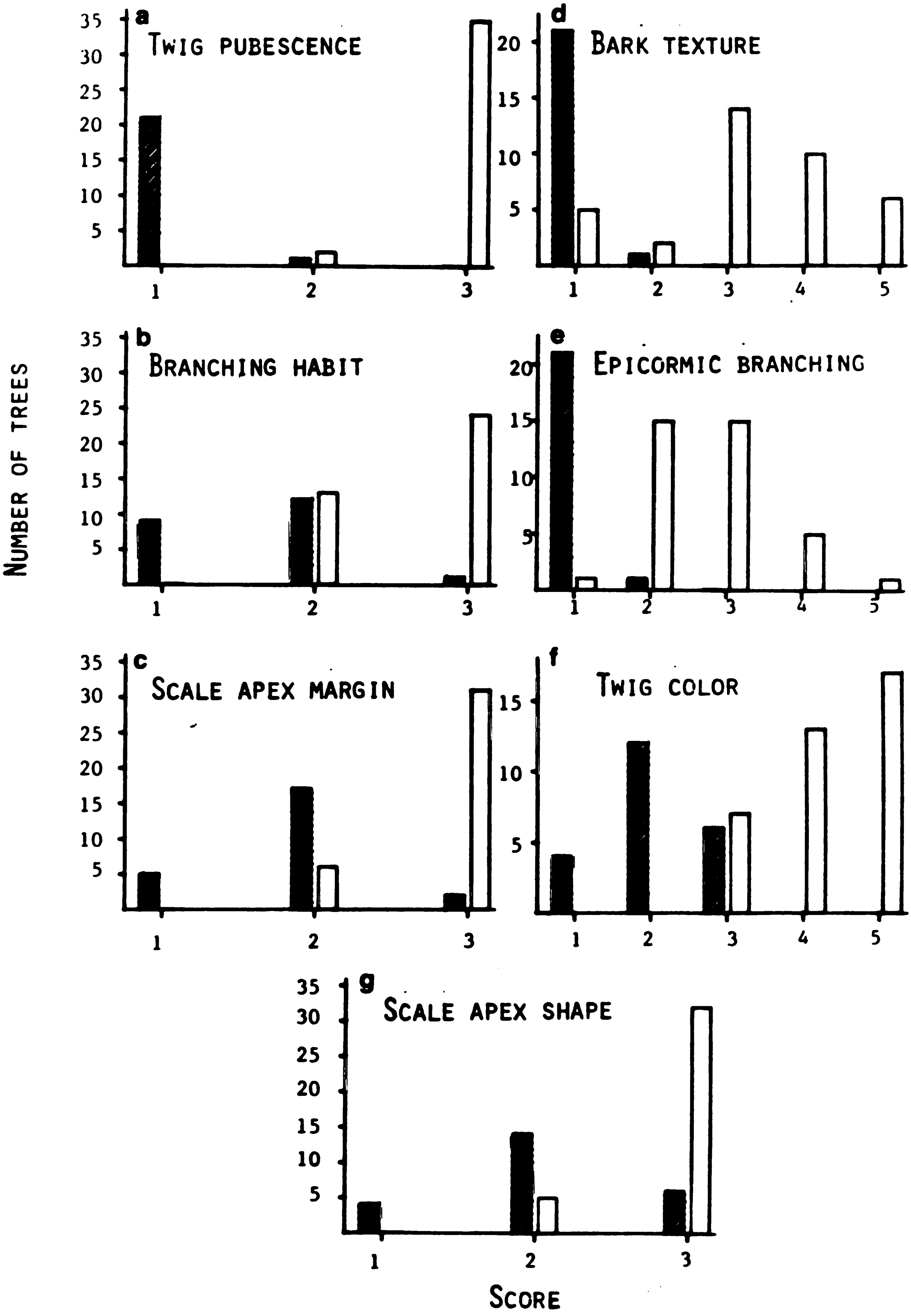
a
Twig pubescence
b
Branching habit
c
Scale apex margin
d
Bark texture
e
Epicormic branching
f
Twig color
g
Scale apex shape
Number of trees
Score
35
30
25
20
15
10
5
1
2
3
4
5

Figure 7. a-g

Parker and McLachlan (1978) proposed four categories for determining the diagnostic ability of quantitative traits. In the present study only two categories were considered, diagnostic and non-diagnostic. A character was considered to have diagnostic value if the overlap of its range between the two species was not greater than 30% of the total range of both species combined. If the range of one species included the mean of the other the trait was considered non-diagnostic, even if the range overlap was less than 30%. On this basis 12 of the 31 quantitative morphological characters were determined to be of value in differentiating the species (Figures 8a-l). Species differences for all of the diagnostic traits were highly significant (Table 4).

Daubenmire (1972) suggested that certain measurements of the ovuliferous scale were most useful in separating the species. He proposed a taxonomic guide to distinguish between blue and Engelmann spruce based on these observations and the nature and density of twig pubescence. The study of Taylor _et al_ (1975) generally agreed with that of Daubenmire. They determined that the percentage of free scale (see Table 1 for description) and the nature and density of twig pubescence separated the species. There was still some slight overlap in these two traits, however. It is apparent from their data that the length of the median cone, cone size, cone scale length and free scale length may also have diagnostic ability (see Table 1 for description).

Figure 8a-1. Means (vertical lines), ranges (horizontal lines) and standard deviations (horizontal bars) for 12 quantitative morphological characters of potential diagnostic value. B = blue spruce, E = Engelmann spruce.

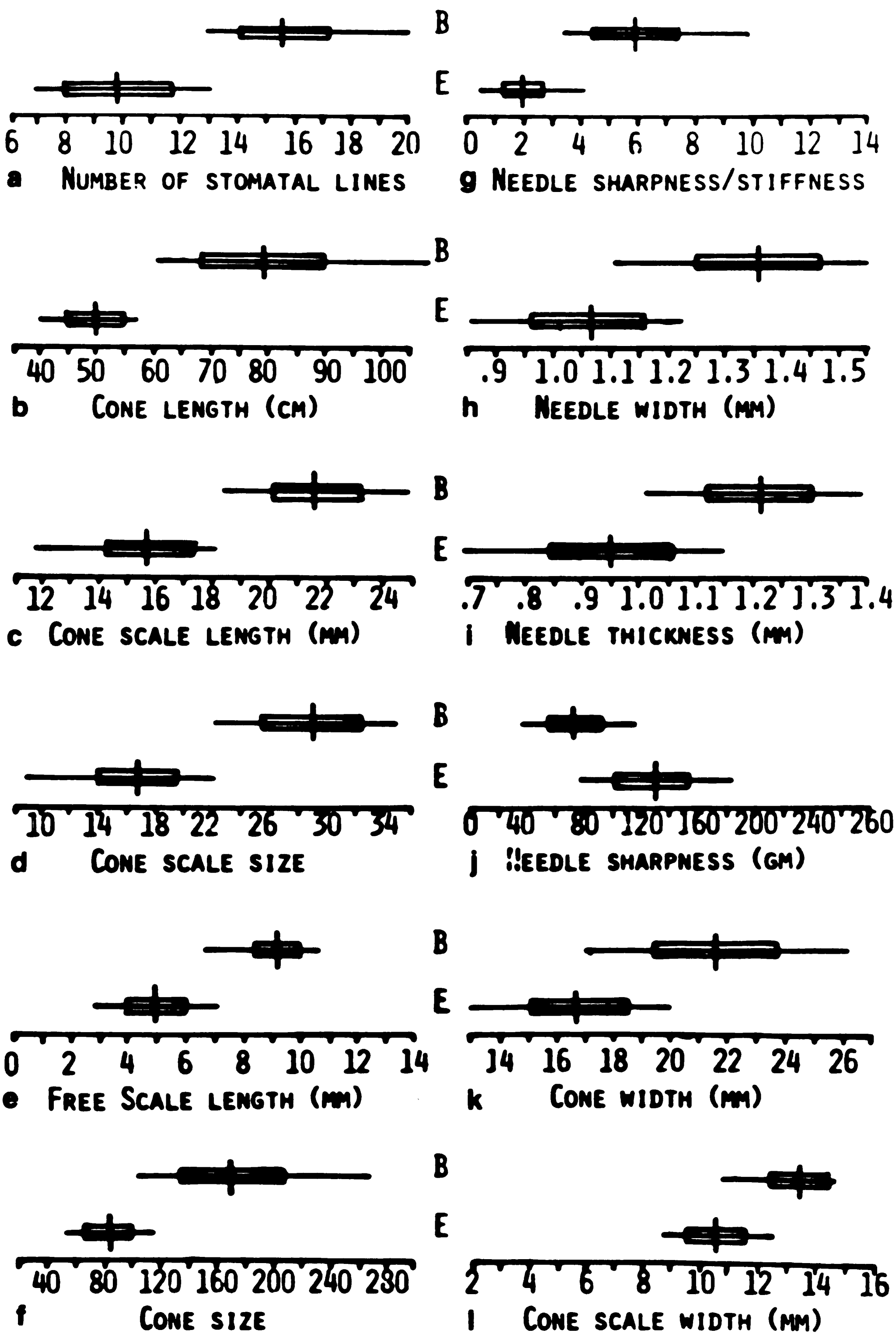
B
E
6 8 10 12 14 16 18 20
a Number of stomatal lines
0 2 4 6 8 10 12 14
g Needle sharpness/stiffness
40 50 60 70 80 90 100
b Cone length (cm)
.9 1.0 1.1 1.2 1.3 1.4 1.5
h Needle width (mm)
12 14 16 18 20 22 24
c Cone scale length (mm)
.7 .8 .9 1.0 1.1 1.2 1.3 1.4
i Needle thickness (mm)
10 14 18 22 26 30 34
d Cone scale size
0 40 80 120 160 200 240 260
j Needle sharpness (gm)
0 2 4 6 8 10 12 14
e Free Scale length (mm)
14 16 18 20 22 24 26
k Cone width (mm)
40 80 120 160 200 240 280
f Cone size
2 4 6 8 10 12 14 16
l Cone scale width (mm)

Figure 8. a-l

Table 4. Degrees of freedom and mean squares showing the significance of the variation between blue and Engelmann spruce for 12 quantitative characters of potential diagnostic value.

Character	DF		Mean Square	
	Between groups	Error	Between groups	Error
# stomatal lines	1	56	422**	2.65
Needle sharpness index	1	56	206**	1.61
Needle sharpness	1	56	39784**	457
Needle width	1	56	1.109**	.0112
Needle thickness	1	56	.8855**	.0093
Cone length	1	60	13000**	85
Cone scale length	1	60	520**	2.28
Cone scale size	1	60	2212**	10.87
Free scale length	1	60	242**	.9075
Cone size	1	60	114135**	886
Cone width	1	60	320**	4
Cone scale width	1	60	111**	.9463

** Significant at the .01 level of probability

The results of the present study are generally in agreement with those of Daubenmire (1972) and Taylor et al (1975) regarding the usefulness of cone scale and twig pubescence measurements in comparisons of blue and Engelmann spruce. However, several other measures also appear to distinguish the species. Absolute free scale length appears to differ more between the species than the percentage of free scale. Daubenmire (1972) noted that 60 mm is often designated as a critical cone length separating the shorter Engelmann spruce cones from the longer blue spruce cones. Individual trees in almost all of the Engelmann spruce populations had cones exceeding this length. He also found individual blue spruce with cones less than 60 mm long. In the present study the use of average tree values for cone lengths has resulted in a complete separation of the species. The average length of Engelmann spruce cones was always less than 60 mm and the average length of blue spruce cones was always greater (Figure 8b).

It is important to note that all of the spruce analyzed in the present study occur at the same latitude. Previous studies have generally involved trees from a rather wide range of latitudes. This fundamental difference between the present study and earlier investigations may account in part for the apparent increase in diagnostic value for many of the traits evaluated in the present study when compared to evaluations based on a broad range of latitudes.

Cone width and cone size also have diagnostic potential, as do the 5 needle traits, number of stomatal lines, needle width, thickness and sharpness, and needle stiffness/sharpness (see Table 2 for description). Needle sharpness was used by Mitton and Andalora (1981), however this trait was measured on a qualitative scale. They found that the variation between blue and Engelmann spruce was significant with blue having the sharper needles. The quantitative measure of needle sharpness in the present study indicates that blue spruce needles are indeed sharper than those of Engelmann spruce and that this difference is highly significant.

The Potential for Species Differentiation Using Discriminant Analysis and the Morphological Index Technique

The results of the morphological index exemplify the importance of determining the diagnostic ability of traits before including them in the analysis (Figure 9). The scores assigned to each trait in the index have been described in Tables 1, 2 and 3. A tree exhibiting purely Engelmann spruce characters would receive a score of 7 on the index, while a tree exhibiting purely blue traits would receive a score of 27. The characters included in the morphological index exhibit strong diagnostic ability when taken as a group and should be of use in future studies of natural hybridization between blue and Engelmann spruce in the Scotch Creek study area.

The discriminant analysis was also very useful in separating the blue and Engelmann spruce populations

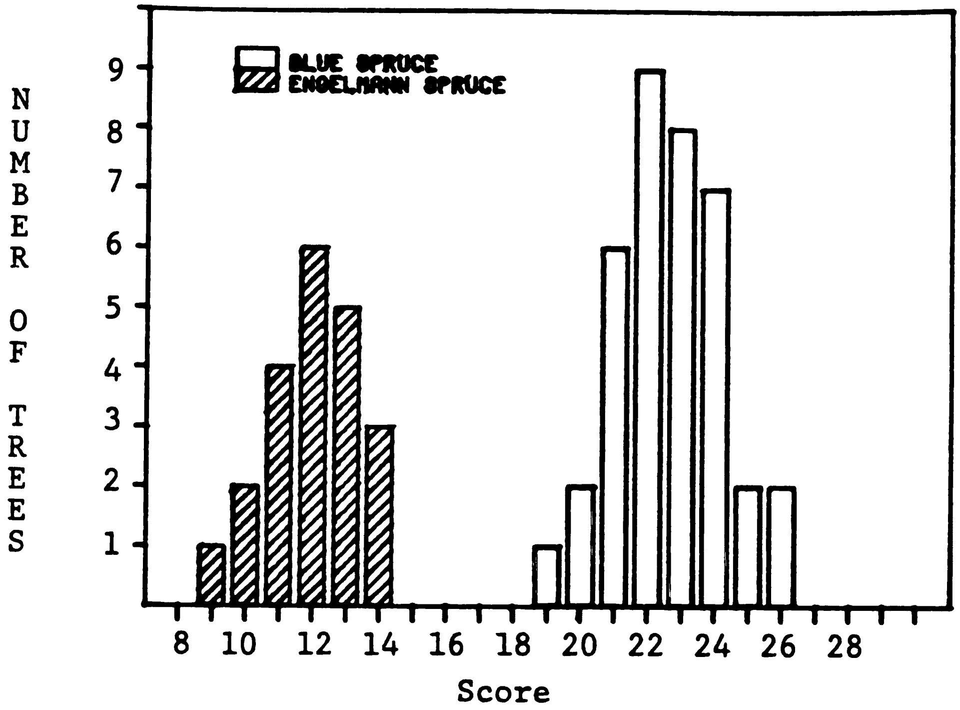

Figure 9. Morphological index of blue and Engelmann spruce based on 7 qualitative characters.

(Figure 10). The stepwise procedure used in the analysis was very effective in simplifying the discriminant function, as only 3 of the original 12 variables remained in the function. These variables were the number of stomatal lines, free scale length and needle sharpness. The inclusion of any of the remaining variables provided little, if any, improvement in differentiating the species.

Various measures of the number of stomatal rows have been used in comparisons of several spruce species. Morgenstern and Farrar (1964) and Gordon (1976) found such measures to be of little use in their studies of red and black spruce. Garman (1957) and Daubenmire (1968) felt that such measures were useful in comparisons of Engelmann and white spruce and Sitka and white spruce, respectively. Daubenmire's (1974) measure of dorsiventrality was not useful in studies of Engelmann and white spruce, however. Daubenmire (1972) also used a measure of dorsiventrality for the number of stomatal rows in blue and Engelmann spruce, but he apparently did not include this value in his analysis. Three measures of the number of stomatal rows were included in the study of Mitton and Andalora (1981). The discriminant function including these and several other morphological variables was unable to completely separate blue and Engelmann spruce populations. The present study suggests that a single measure of the number of stomatal rows is of potential use in distinguishing between these species.

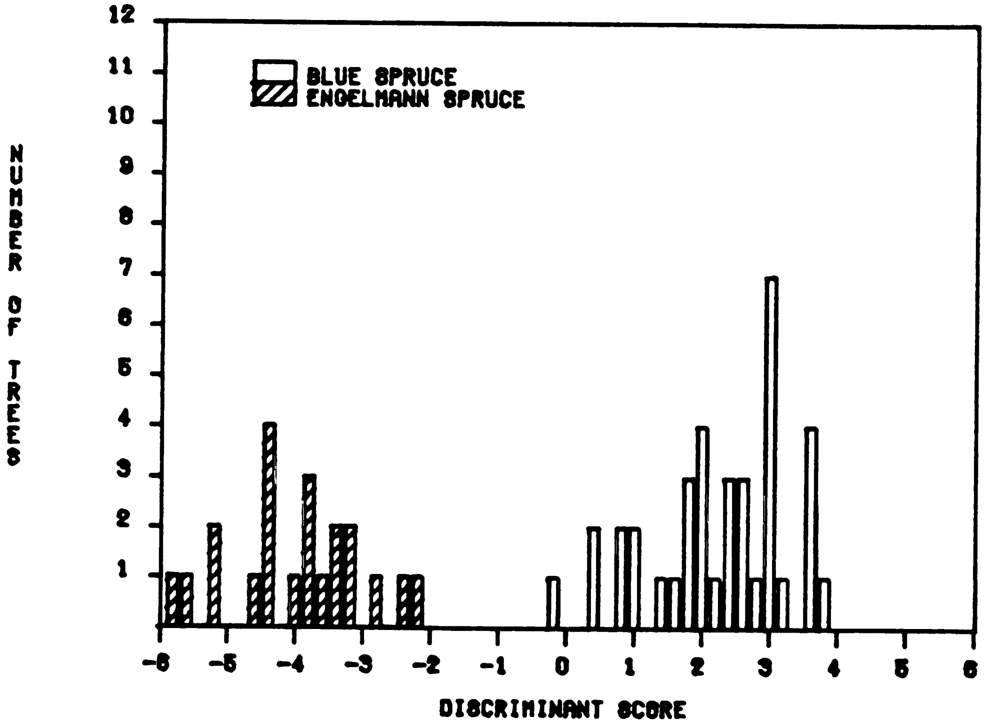

Figure 10. Discriminant analysis of blue and Engelmann spruce based on quantitative morphological characters.

It also has the advantage of being relatively easy to measure, because no determination of the needle surface being examined is required.

Daubenmire (1972) and Taylor _et_ _al_ (1975) both considered the percentage of free scale a diagnostic character. The results of the present study indicate that free scale length is more useful than the percentage of free scale in differentiating blue and Engelmann spruce. Free scale length has the advantage of being easier to determine than the alternative measurement.

To author's knowledge this is the first study in spruce to make use of a quantitative measure of needle sharpness. The importance of this character in distinguishing between blue and Engelmann spruce is underscored by its inclusion in the discriminant function.

The results of this study indicate that the discriminant function defined using quantitative morphological traits will be useful in detecting possible hybridization between blue and Engelmann spruce in the study area.

The ability of the morphological index and the discriminant analysis to differentiate between blue and Engelmann spruce is somewhat at odds with previous studies of these species (Daubenmire, 1972; Taylor _et_ _al_, 1975; Mitton and Andalora, 1981). Two possible reasons for this present themselves when comparing the present study with those previous. The first possibility is that the present study included only traits with potential diagnostic value in the analyses. Mitton and Andalora (1981) apparently did not critically evaluate the morphological characters in their study for diagnostic ability before including them in a discriminant analysis. Taylor et al (1975) accounted for characters with little diagnostic value by assigning smaller weights to these than to diagnostic characters. It may have been more useful to simply eliminate the non-diagnostic traits from further analysis.

The second reason concerns the reference populations used in the various studies. Adams (1982) noted two problems associated with obtaining samples of reference individuals. He suggested that individuals from sympatric populations may be introgressed, and yet individuals from allopatric (possibly quite distant) populations may be quite differentiated from the actual parents involved in hybridization. The present study used populations just beyond (about 1.5 kilometers) the range of overlap of the two species. These populations proved to be distinctly different from one another, and they should provide a better reference for each taxa within the remainder of the study area than would more distant populations. The studies of Daubenmire (1972) and Taylor et al (1975) involved collections from populations covering a broad geographic range. Their attempts to detect natural hybridization may have been hampered using as reference groups allopatric populations that were generally a great distance from the areas of suspected hybridization. The problem of which reference populations to use can not be adequately resolved without a thorough analysis of intraspecies variation for each of the species under consideration (Adams, 1982). Until such information is available researchers must use their best judgment as to which individuals should be used as reference material.

Summary

A total of 19 morphological traits (7 qualitative, 12 quantitative) were found to exhibit diagnostic ability in this study. All of the 7 qualitative traits were included in a morphological index, which separated the two spruce populations very well. Only 3 of the original 12 diagnostic quantitative traits were included in the discriminant function, as they contained most of the discriminatory power available from the use of all of the variables. Both the discriminant analysis and hybrid index techniques appear to be of potential use in future analyses of natural hybridization within the study area.

CHAPTER III

MONOTERPENE CONCENTRATIONS IN CORTICAL OLEORESIN OF BLUE AND ENGELMANN SPRUCE

Abstract

Twenty-two compounds were detected in the cortical oleoresins of mature blue and Engelmann spruce. Twelve monoterpenes were identified. Species differences were generally quantitative, with most statistically significant. Blue spruce oleoresin contained higher levels of tricyclene, α-pinene, camphene and bornyl acetate, while Engelmann spruce oleoresin contained higher levels of β-pinene, 3-carene, terpinolene and several unknown compounds. There were many significant differences between seedling and mature tree monoterpene content. The large increase in β-pinene concentration in Engelmann spruce seedlings was the most striking. The monoterpenes of cortical oleoresin collected from mature blue and Engelmann spruce have significant diagnostic potential in taxonomic studies of these species, while seedling cortical monoterpenes appear to be less useful.

Monoterpene Concentrations in the Cortical Oleoresin of Blue and Engelmann Spruce

Introduction

In recent years the monoterpenes have received a great deal of attention in taxonomic studies of coniferous species. Their value as a taxonomic tool stems from the fact that they are under strong genetic control by relatively few genes (Hanover, 1966b, 1971; Squillace, 1971, 1976a, 1976b; Hiltunen _et al_, 1975; Von Rudloff, 1975a; Squillace _et al_, 1981), and they are influenced little by environmental factors (Hanover, 1966b, 1971; Von Rudloff, 1975a; Squillace, 1976a). As a result of rapid improvements in gas-liquid chromatography (GLC) they are also fairly easy to analyze. Where morphological analysis leads to ambiguities, the monoterpenes may be useful for clarification (Mirov, 1948; Santamour, 1965; Hanover, 1974; Von Rudloff, 1975a).

Most of the North American spruces have been analyzed for their monoterpene content, although most of these studies have generally been concerned with the composition of leaf oils. Von Rudloff has analyzed the leaf oils of white, blue, red, black, Engelmann and Sitka spruces (Von Rudloff, 1962, 1964, 1966, 1967, 1972 1975b, 1975c, 1977). He stated that the leaf oils of these species are well suited for chemosystematic studies (Von Rudloff, 1975a). Von Schantz and Juvonen (1966) studied the monoterpene content of several tissues for many spruce species of Asia, Europe and North America. Differences among species as well as among tissues within species were evident from their results. The monoterpene content of cortical oleoresins has been analyzed in white spruce (Picea glauca Moench.)(Wilkinson et al, 1971), red spruce (Picea rubens Sarg.)(Wilkinson and Hanover, 1972), blue spruce (Picea pungens Engelm.)(Rottink and Hanover, 1972; Reed and Hanover, 1983), white x blue spruce hybrids (Hanover and Wilkinson, 1969) and (white x blue) x red spruce hybrids (Bongarten and Hanover, 1982).

The monoterpenes of either the cortical oleoresin or leaf oils are considered to be more useful for taxonomic studies than the xylem monoterpenes (Hanover, 1966b; Squillace, 1976b), as the monoterpene composition in the former tissues is more complex, affording a greater possibility for genetic variation. These tissues are also more reliable for genetic analysis because they are closer to the sites of terpene synthesis. The monoterpene composition of the cortical oleoresin varies little with position in the crown or with season (Squillace, 1976b; Forrest, 1980b, 1980d; Moore, 1980). Isolation of cortical oleoresins is also much simpler than extraction of oleoresins from spruce needles.

The monoterpenes of blue and Engelmann (Picea engelmannii Parry) spruce have not been compared in the same study. Von Rudloff has analyzed the terpenes of the leaves, twigs and buds of blue spruce (Von Rudloff, 1962, 1975b), and the terpenes of the leaves of Engelmann spruce (Von Rudloff, 1964;

Ogilvie and Von Rudloff, 1968). In the analysis of twig terpenes he did not separate the cortical tissue from the xylem tissue. Habeck and Weaver (1969) analyzed the terpene composition of resin blisters of Engelmann and white spruce. Hanover and Wilkinson (1969) have analyzed the cortical oleoresins of blue spruce in their study of hybridization with white spruce. They found that 3-carene levels could be used to separate the species and hybrids. Recently, Reed and Hanover (1983) have completed a study of nine blue spruce populations distributed throughout the range of the species. They analyzed cortical oleoresins and found that the nine populations could be separated based on their monoterpene composition. Rottink and Hanover (1972) also found that blue spruce cultivars could be distinguished by the monoterpenes of their cortical oleoresins. In comparing the results of the previous studies it is apparent that differences (mostly quantitative) in monoterpene composition exist between blue and Engelmann spruce. Von Rudloff (1975a) suggested that these differences are large enough to suffice for studies of possible hybridization between the species.

The observation of an area of extensive range overlap of blue and Engelmann spruce in the Scotch Creek drainage of southwestern Colorado, encouraged the present author to investigate the possibility of natural hybridization between these species. Previous investigations concerning the detection of natural hybridization between blue and Engelmann spruce have been hampered by an inability to completely distinguish between pure forms of each species (Daubenmire, 1972; Taylor et al, 1975; Mitton and Andalora, 1981). It is clear that traits must be found which can be used to unequivocably separate pure forms of the two species before investigations concerning their natural hybridization can be undertaken.

The purpose of this study was to analyze the monoterpenes of the cortical oleoresin of blue and Engelmann spruce found at extreme ends (low and high elevations, respectively) of the Scotch Creek drainage to determine which monoterpenes may be useful in differentiating the two species. Once determined, the use of such terpenes will hopefully present a less ambiguous picture of the genetic relationship between blue and Engelmann spruce than previous investigations have afforded.

Materials and Methods

The study area has been described elsewhere (Schaefer and Hanover, 1983a) so will not be described in detail in this paper. The study area consisted of two collection zones at opposite ends of the Scotch Creek Drainage in the San Juan National Forest of Colorado. The lower (blue spruce) zone is at an elevation of 2590 m, and the upper (Engelmann spruce) zone is at 2970 m. The trees for this study were selected in the fall of 1981 and sampled in the spring of 1982. These were the same trees that were sampled for a morphological comparison of blue and Engelmann spruce (Schaefer and Hanover, 1983a). Cortical oleoresin samples were collected from 37 of the 38 blue spruce selected, while only 13 of 24 Engelmann spruce yielded sufficient oleoresin to warrant collection.

The sampling technique involved clipping several branches from the middle to upper third of the crown. Several two-year-old twigs were then cut from these branches. The oleoresin exuding from the cut surface of the two-year-old twigs was drawn into a calibrated microcapillary pipet. Each resin sample was then placed in an air-tight container and stored under refrigeration at 2°C until analysis by GLC. Before analysis the samples were diluted to 5% with pentane and, depending upon the sample size, various amounts of heptane were added as an internal standard. The samples were analyzed on a Varian 3700 GLC equipped with an SE-30 capillary column (50 m x .25 mm), and a flame ionization detector. One µl of sample was injected with a split ratio of 30:1. The sample was run isothermally at 70°C for ten minutes and then programmed to 250°C at 10°C/min.. The final temperature was maintained for 22 minutes. Integration was performed with a Hewlett Packard 3300 electronic integrator. Standard curves of the response of known concentrations of monoterpenes relative to the response of heptane were prepared for quantifying cortical monoterpenes. Peak identification was achieved by comparisons with the relative retention times of known standards. Results were expressed as percentages of the total oleoresin.

Statistical Procedures

Statistical analyses were performed on the transformed values of the arcsin of the square root of the individual percent of oleoresin values. Analysis of variance procedures were used to determine which monoterpenes might be of value in distinguishing between blue and Engelmann spruce. The monoterpenes that differed significantly between the species were then used in a discriminant function analysis. Discriminant analysis is a multivariate statistical technique that maximizes the differences between groups while minimizing the differences within groups, and has proved to be a useful tool in studies of species relationships among forest trees (Clifford and Binet, 1954; Mergen *et al*, 1965; Namkoong, 1966; Ledig *et al*, 1969; Dancik and Barnes, 1975; Flake *et al*, 1978; Kudray and Hanover, 1980). The first step in such studies is to define a discriminant function based on two or more reference populations. This function should completely separate the species if it is to be useful in the second step of classifying individuals of unknown species assignment.

Analysis of Progeny Terpenes

Cortical oleoresin samples were also collected from one progeny of each of the trees sampled in Colorado. The seed was collected in the fall of 1981 and sown in the greenhouse in early January, 1982. Seedlings were grown under accelerated-optimal-growth conditions (Hanover *et al*, 1976) for six months, and then placed outdoors in a shade house to induce dormancy. Oleoresin samples were collected in late September by making a small incision in the stem of the seedling. The exuding resin was drawn into calibrated micro-capillary pipets. Resin was collected from all 38 blue spruce progeny selected, and from all 24 of the Engelmann spruce progeny. Storage and analytical techniques were identical to those used with the resin from the parent trees.

Results and Discussion

Twenty-two compounds were detected in the cortical oleoresin of mature blue and Engelmann spruce. The means for each terpene and several unknown compounds are listed in Table 5. Only one qualitative difference between the species was detected. Unknown 18 did not occur in blue spruce, whereas small amounts were present in 10 of the 13 Engelmann spruce. Three unknown compounds of high molecular weight were detected, but these peaks were not well resolved and therefore were excluded from the analysis. Quantitative differences between the species were numerous, with most being statistically significant (Table 5). Blue spruce resin contained much more tricyclene, α-pinene, camphene and bornyl acetate than Engelmann spruce. Bornyl acetate was detected in all except one blue spruce, but in only 3 Engelmann spruce. Engelmann spruce resin contained substantially more 3-carene than blue spruce, as well as higher levels of the 4 unknown peaks 6, 12, 18 and 19. Except for unknown 6 these compounds occurred in fairly small quantities, however they are significant in that they were detected in very few blue spruce and

Table 5. The mean concentrations of 12 monoterpenes and 10 unknown compounds of blue and Engelmann spruce.

Compound	% of Oleoresin		Significance
	Blue	Engelmann	
Unknown 1	.03	.02	ns
Tricyclene	.14	.02	**
Unknown 3	.04	.11	**
α-pinene	17.08	4.84	**
Camphene	.41	.13	**
Unknown 6	.30	1.04	**
β-pinene	3.17	4.63	*
Myrcene	3.98	3.58	ns
Unknown 9	.05	.08	**
3-carene	7.25	11.10	**
α-terpinene	.05	.06	ns
Unknown 12	.01	.06	**
Limonene	5.92	6.16	ns
γ-terpinene	.08	.10	ns
Terpinolene	.92	1.46	**
Citronellol	.04	.08	ns
Bornyl acetate	.64	.01	**
Unknown 18	0.00	.04	**
Unknown 19	trace	.05	**
Unknown 20	†	†	
Unknown 21	†	†	
Unknown 22	†	†	
TOTAL	40.11	33.57	

†These peaks were not included in the analysis

*Significant at the .05 level of probability

**Significant at the .01 level of probability

nsNot statistically significant at either of the above probability levels in almost all of the Engelmann spruce. Engelmann spruce also had higher levels of unknown 3, β-pinene, unknown 9 and terpinolene.

The results of the present study of blue spruce are consistent with the findings of Reed and Hanover (1983) and Rottink and Hanover (1972) for the peaks these studies have in common with the present study. Many of the peaks detected in the present study were not reported by these authors. Tricyclene, α-terpinene, citronellol, bornyl acetate, and several unknowns were detected in this study but not by the previous authors, probably as a result of the greater sensitivity of capillary GLC compared to packed columns. Sabinene and β-phillandrene were not detected in the present study since the SE-30 column was not able to separate these compounds from those adjacent to them. If these two monoterpenes were present they would be included in the peak areas for β-pinene and limonene respectively. Von Rudloff (1975b) detected many of the same peaks as reported here in his analysis of blue spruce twigs. The concentrations for the individual components of the twig oil were somewhat higher than those of the present study, probably as a result of including both xylem and cortical resins in his analysis. Von Schantz and Juvonen (1966) also analyzed the monoterpenes of the twigs of blue spruce. Again, these results did not agree with those of the present study. Moore (1980) analyzed the volatile oils of blue spruce bark. The oils were extracted using a steam distillation procedure.

His results agree quite well with those of the present study. He did not report several peaks detected here, including tricyclene, α-terpinene, citronellol and several unknowns. These compounds were present in fairly small quantities which may not have been detectable with the packed columns used by Moore (1980).

The author is not aware of any studies of the cortical oleoresins of Engelmann spruce with which the results of this study can be compared. Von Schantz and Juvonen (1966) analyzed twig oils, but the inclusion of xylem resins in the analysis rendered comparisons with the present study futile. Habeck and Weaver (1969) collected resin from bark blisters in their investigation of natural hybridization in white and Engelmann spruce. Their results were very different from those of the present study. Hanover (1966b) found that the chemical composition of resin from bark blisters was quite different from resin collected from the cortical tissues of western white pine. Habeck and Weaver (1969) suggested that there were many problems associated with collecting resins from bark blisters.

The analysis of the cortical oleoresins of the open pollinated progeny of the mature trees in this study revealed no new compounds. One unknown compound included in the seedling analysis was detected in the mature trees, but was excluded from that analysis since very few trees contained this compound. The means for each terpene and several unknown compounds are listed in Table 6. Unknown 12, which

Table 6. The mean concentrations of 12 monoterpenes and 10 unknown compounds of blue and Engelmann spruce seedlings.

Compound	% of Oleoresin		Significance
	Blue	Engelmann	
Unknown 1	.07	.06	ns
Tricyclene	.10	.05	**
Unknown 3	.01	.02	ns
α-pinene	13.03	9.39	**
Camphene	.46	.35	**
Unknown 6	.31	.49	*
β-pinene	5.70	10.97	**
Myrcene	1.44	2.34	*
Unknown 9	.05	.08	**
3-carene	5.02	5.20	ns
α-terpinene	.01	.02	ns
Limonene	5.74	7.62	**
γ-terpinene	.05	.09	*
Terpinolene	.68	.94	**
Citronellol	.05	.11	**
Bornyl acetate	.83	.14	**
Unknown 18	0.00	.05	**
Unknown 23	trace	.06	**
Unknown 19	trace	.08	**
Unknown 20	†	†	
Unknown 21	†	†	
Unknown 22	†	†	
TOTAL	33.55	37.04	

†These peaks were not included in the study

*Significant at the .05 level of probability

**Significant at the .01 level of probability

nsNot statistically significant at either of the above probability levels was present in all of the mature Engelmann spruce, was not detected in any of the progeny. This compound was also not present in any of the blue spruce progeny. The quantitative differences between mature trees and seedlings were statistically significant for eight terpenes and 3 unknowns in blue spruce (Table 7) and seven terpenes and one unknown in Engelmann spruce (Table 8). Hanover (1971) noted a striking increase in β-pinene levels in western white pine progeny seedlings over those of the parents. A similar result is evident in the present study, especially in Engelmann spruce. Engelmann spruce averaged 11% while the parents averaged only 4.6% β-pinene. The difference in blue spruce is less striking with 5.7% and 3.2% in the progeny and parents respectively. Concentrations of α-pinene and bornyl acetate were also much higher in Engelmann spruce seedlings than in mature trees. Squillace (1971) found no effect of age on monoterpene composition for trees two to 20 years old. He noted that Fisher (1966) found significant changes in monoterpene composition occurring in slash pine seedlings five to 18 months old, but that after that the composition changed little for up to 20 years. Developmental effects on monoterpene composition in spruce have been studied by Von Rudloff (1972, 1975b, 1975c) by analyzing the volatile oils of expanding buds, shoots and needles from mature spruce. He has found that significant differences in monoterpene composition exist between newly expanding tissues and mature tissues. The present study also indicates that the monoterpene

Table 7. The mean concentrations of 12 monoterpenes and 11 unknown compounds of mature trees and the seedling progeny of blue spruce.

Compound	% of Oleoresin Parents	% of Oleoresin Progeny	Significance
Unknown 1	.03	.07	**
Tricyclene	.14	.10	**
Unknown 3	.04	.01	**
α-pinene	17.08	13.03	**
Camphene	.41	.46	ns
Unknown 6	.30	.31	ns
β-pinene	3.17	5.70	**
Myrcene	3.98	1.44	**
Unknown 9	.05	.05	ns
3-carene	7.25	5.02	**
α-terpinene	.05	.01	**
Unknown 12	.01	0.00	**
Limonene	5.92	5.74	ns
γ-terpinene	.08	.05	**
Terpinolene	.92	.68	**
Cintronellol	.04	.05	ns
Bornyl acetate	.64	.83	ns
Unknown 18	0.00	0.00	ns
Unknown 23	0.00	trace	ns
Unknown 19	trace	trace	ns
TOTAL	40.11	33.55	

**Significant at the .01 level of probability

nsNot significant at either the .01 or .05 level of probability

Table 8. The mean concentrations of 12 monoterpenes and 11 unknown compounds of mature trees and the seedling progeny of Engelmann spruce.

Compounds	% of Oleoresin Parents	 Progeny	Significance
Unknown 1	.02	.06	**
Tricyclene	.02	.05	**
Unknown 3	.11	.02	**
α-pinene	4.84	9.34	**
Camphene	.13	.35	**
Unknown 6	1.04	.49	**
β-pinene	4.63	10.97	**
Myrcene	3.58	2.34	*
Unknown 9	.08	.08	ns
3-carene	11.10	5.20	**
α-terpinene	.06	.02	**
Unknown 12	.06	0.00	**
Limonene	6.16	7.62	ns
γ-terpinene	.10	.09	**
Terpinolene	1.46	.94	**
Citronellol	.08	.11	**
Bornyl acetate	.01	.14	*
Unknown 18	.04	.05	ns
Unknown 23	0.00	.06	ns
Unknown 19	.05	.08	ns
TOTAL	33.57	37.04	

*Significant at the .05 level of probability

**Significant at the .01 level of probability

nsNot significant at either of the above probability levels composition of juvenile tissues (i.e., seedling cortical tissues) differs from that of mature tissues. These differences appear to be quantitative rather than qualitative. The age at which these differences subside in spruce is not known. This question deserves further inquiry.

As in the mature trees unknown 18 was not present in any blue spruce. It was present in 16 of the 24 Engelmann spruce seedlings, however. The levels of tricyclene, α-pinene, camphene and bornyl acetate were significantly higher in blue spruce than in Engelmann spruce, while the levels of unknown 6, β-pinene, myrcene, unknown 9, limonene, γ-terpinene, terpinolene, citronellol and unknowns 18, 19 and 23 were significantly higher in Engelmann spruce (Table 6). These species differences differ from those of the mature trees for six compounds. Species differences for unknown 3 and 3-carene were significant for mature trees, but not for seedlings. Species differences for concentrations of myrcene, limonene, γ-terpinene and citronellol differed signficantly in the seedlings but not in the mature trees.

Two discriminant analyses were performed, one using the data from mature trees and one using seedling data. All of the terpenes and unknowns which differed significantly between species were used in each analysis. Thirteen such compounds were used in the discriminant analysis of mature trees, and 14 coumpounds were used in the seedling analysis. The compounds used in the two analyses differed slightly.

Differentiation of Blue and Engelmann Spruce Using Discriminant Function Analysis

The discriminant analysis of the monoterpenes from the cortical oleoresin of mature blue and Engelmann spruces proved very useful in distinguishing between these species (Figure 11). The gap between the species distribution on this function covers 29% of the total range of the species on the function. Each species is distributed over 35.5% of the total range.

The discriminant analysis of the seedling monoterpenes did not distinguish between blue and Engelmann spruce as well as the function based on mature cortical monoterpenes (Figure 12a). One Engelmann spruce was misclassifed as a blue spruce while another occurred in a somewhat intermediate position. The parents of both of these trees appeared to be typical Engelmann spruce based on their scores on the previous discriminant function. These trees were also scored as typical Engelmann spruce in a morphological analysis of blue and Engelmann spruce (Schaefer and Hanover, 1983a). The fact that the discriminant function based on seedling monoterpenes misclassifies pure forms of one of the species is an indication that it may not be of much use in detecting natural hybrids between blue and Engelmann spruce.

Thirty seedlings (15 blue and 15 Engelmann) grown from seed collected from trees outside of the study area provided an independent data set to further test the ability of the seedling discriminant function in differentiating between

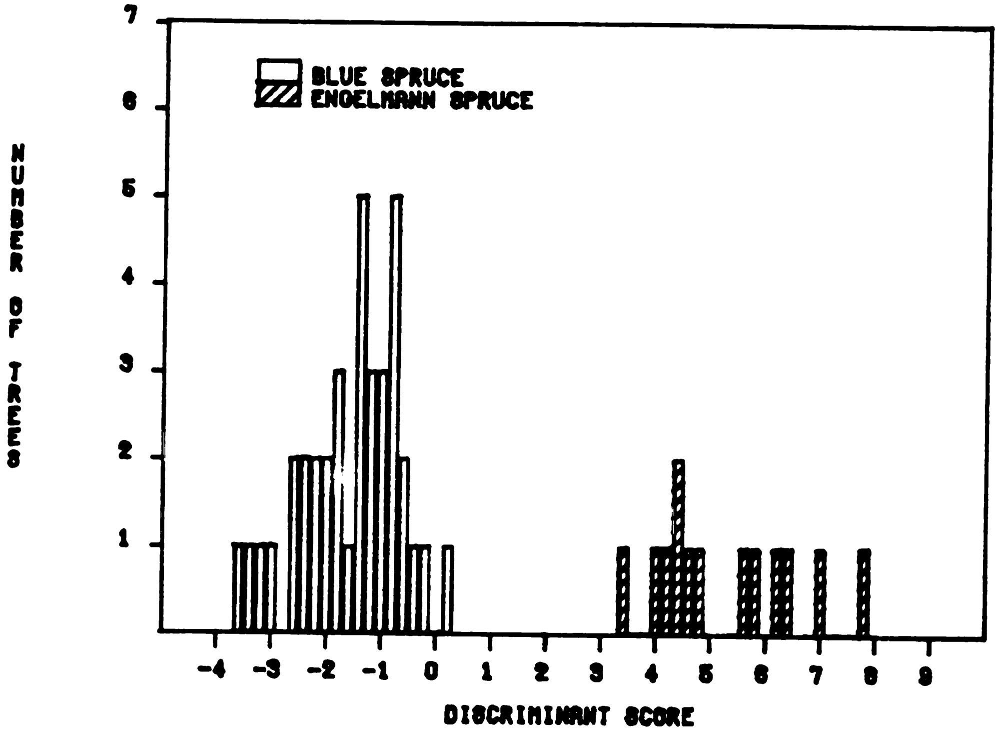

Figure 11. Discriminant analysis of blue and Engelmann spruce based on terpene concentrations in mature trees.

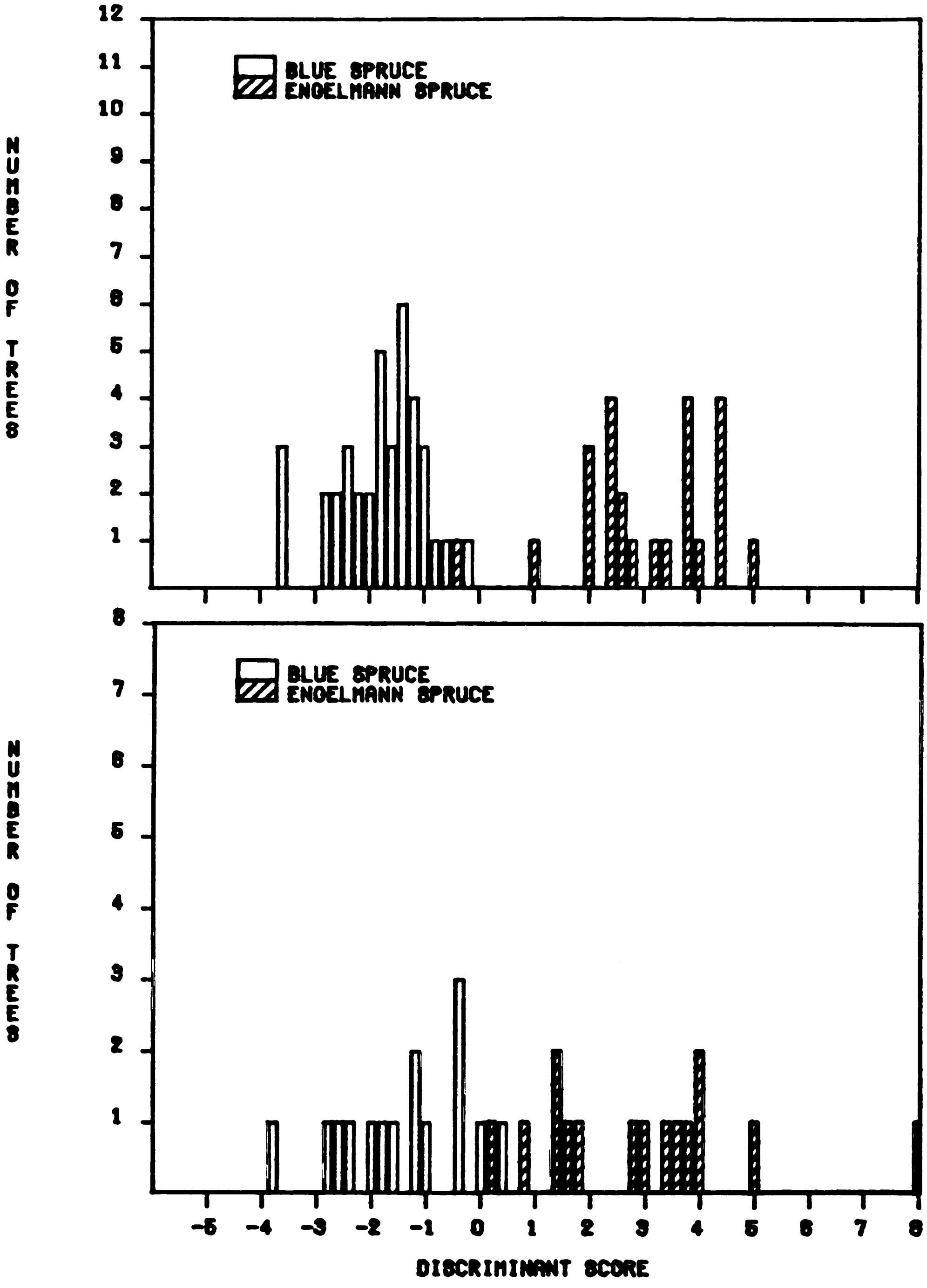

Figure 12. a) Discriminant analysis of blue and Engelmann spruce based on terpene concentrations in seedlings.
b) Classification of 15 blue and 15 Engelmann spruce seedlings grown from seed collected from outside of the Scotch Creek drainage based on terpene concentrations in seedlings.

blue and Engelmann spruce. The classification of these seedlings is shown in Figure 12b. None of the blue spruce were misclassified, however two of the Engelmann spruce were classified as blue spruce. There is no separation of blue and Engelmann spruce on this function. Again, this illustrates the limited ability of the seedling discriminant function to distinguish between blue and Engelmann spruce.

Conclusions

The results of the present study indicate that, as in many other species, the monoterpenes of blue and Engelmann spruce can play a significant role in taxonomic studies involving these species. Previous studies involving the relationship of blue and Engelmann spruce have generally concentrated on morphological characters, although Taylor *et al* (1975) included 3 leaf phenolics in their study and Mitton and Andalora (1981) considered one enzyme polymorphism in theirs. These researchers had difficulty in separating pure forms of each species with the traits that they investigated. The present results indicate that the cortical monoterpenes should be useful in clearing up ambiguities resulting from morphological analyses of blue and Engelmann spruce, as they clearly have significant diagnostic potential.

CHAPTER IV

EVIDENCE OF NATURAL HYBRIDIZATION BETWEEN BLUE AND ENGELMANN SPRUCE IN SOUTHWESTERN COLORADO

Abstract

Four discriminant functions based on morphological and chemical traits and a morphological index were used to classify 147 trees found in an area of extensive range overlap between blue and Engelmann spruce. Thirty-eight blue and 24 Engelmann spruce sampled from pure populations of each species were used as the reference populations. The results indicated that blue and Engelmann spruce do hybridize under natural conditions, although such hybridization is very limited in occurrence. Putative hybrids appear to resemble Engelmann spruce more strongly than blue spruce. Gene flow in the direction of Engelmann spruce appears to be favored over that in the direction of blue spruce.

Evidence of Natural Hybridization Between Blue and Engelmann Spruce in Southwestern Colorado

Introduction

There are nine extant species of North American spruces, six of which can be assigned to two groups based on morphological and chemical traits, and species crossability patterns. The three spruces which can not be assigned to a cohesive group are the relic species *Picea chihuahuana* Martinez, *Picea mexicana* Martinez and *Picea breweriana* S. Wats.. Each of these spruces occupies a very small range and is few in number. Brewer spruce is restricted to the higher elevations of the Siskiyou Mountains and neighboring coast ranges, in southern Oregon and northern California (Harlow and Harrar, 1969). *Picea chihuahuana* and *P. mexicana* occur in small disjunct populations in northwestern and northeastern Mexico, respectively (Gordon, 1968). Wright (1955) and others (Gordon, 1968, 1976; LaRoi and Dugle, 1968; Nienstaedt and Teich, 1972; Roche and Fowler, 1975) have grouped red (*P. rubens* Sarg.) and black (*P. mariana* (Mill) B.S.P.) spruce together in a northeast species complex, and Sitka (*P. sitchensis* (Berg.) Carr.), white (*P. glauca* Moensch), Engelmann (*P. engelmannii* Parry) and blue (*P. pungens* Engelm.) spruce in a northwest species complex. White and black spruce form the connecting link geographically between these complexes, however, there is not conclusive evidence of their crossing in nature (Parker and McLachlan, 1978). The spruces of the northwest complex are considered to have been derived from a single Asian progenitor which migrated across the Bering land bridge over 14 million years ago (Wright, 1955; Fowler, 1966; Hills and Ogilvie, 1970; Nienstaedt and Teich, 1972). Wright (1955) suggested that *Picea jezoensis* (Sieb. Zucc.) Carr. might be the link between the northwest species and those of Asia. More recent crossability studies by Fowler (1966), and the discovery of *Picea banksii* cones by Hills and Ogilvie (1970), indicate that white spruce (through *P. banksii*) should be assigned a central position in the evolution of the spruces of the northwest complex. Hills and Ogilvie suggested that white spruce is derived from the now extinct *P. banksii* and that the remaining species of the complex are derived from white spruce. Limited fossil evidence and knowledge of past geological events indicate that speciation of the northwest spruces was probably the result of climatic and topographic disturbances since the middle Oligocene, which culminated in the late Pliocene (Axelrod, 1958; Florin, 1963; MacGinitie, 1969; Wolfe, 1969; Axelrod and Bailey, 1976). The extant northwest spruces were probably all at least partially differentiated before Pleistocene glaciation.

Speciation of Blue and Engelmann Spruce

Blue and Engelmann spruce are considered to be very closely related species. Daubenmire (1972) and Taylor *et al*, (1975) have suggested that blue spruce is a recent derivation of Engelmann spruce. This idea was based on the much smaller geographic range and reduced variability in reproductive structures of blue spruce compared to Engelmann spruce. Daubenmire (1972) suggested that a mutation occurred which caused the progenitors of blue spruce to become immediately incompatible with Engelmann spruce, and which also caused these progenitors to have a different ecological amplitude than Engelmann spruce. Although in theory such a major mutation may have occurred, there is little evidence to support this idea. All of the spruces which have been examined cytogenetically have 2n=24 chromosomes which are very similar in appearance. Thus no change in chromosome number or major structural chromosomal change could be responsible for the speciation of blue and Engelmann spruces. Mikkola (1969) found that no single mechanism was responsible for interspecific incompatibility in nine spruce species, including white and Sitka spruce of the northwest spruce complex. Some degree of incompatibility was observed in all crosses, with embryogenesis reaching many different stages. The results of Kossuth and Fechner (1973) substantiate Mikkola's findings, as the former identified four principle stages of developmental irregularities in interspecific crosses of blue and Engelmann spruce. The occurrence of a sudden major change in compatibility is highly unlikely in light of these findings.

Blue spruce may have been derived from Engelmann spruce during the intense orogenic activity of the Pliocene or perhaps earlier times. Variants of Engelmann spruce which favored slightly warmer and drier habitats may have existed along the lower altitudinal margins in the more southerly portions of the range of this species, even before the occurrence of any massive uplifts. Under the generally equable climates characteristic of pre-Pliocene epochs there would be little to induce such variants to draw away from the main population. When significant uplifts did occur, and the climate began to cool, a larger array of contrasting habitats would become available, possibly enhancing species formation. Reproductive barriers would arise more or less by accident as a result of selection favoring either diverging group in its respective habitat. In the absence of extensive intermediate habitats such selection could result in two distinct taxa as evidenced by the presence of blue and Engelmann spruce today. Although this discussion is highly speculative, it does provide a reasonable alternative to proposals of sudden large mutations and "instant" speciation.

The Position of Blue Spruce in the Northwest Complex

Blue spruce is somewhat of an enigma among the spruces of the northwest complex. White spruce hybridizes with Sitka and Engelmann spruce in areas of range overlap, and Sitka and Engelmann spruce will cross under artificial conditions (Johnson, 1939; Richens, 1945; Little, 1953; Wright, 1955; Garman, 1957; Horton, 1959; Habeck, 1964; Weaver, 1965; Daubenmire, 1968, 1974; LaRoi and Dugle, 1968; Ogilvie and Von Rudloff, 1968; Habeck and Weaver, 1969; Roche, 1969; Hanover and Wilkinson, 1970; Wilkinson *et al*, 1971; Nienstaedt and Teich, 1972; Roche and Fowler, 1975; Fowler and Roche, 1977; Copes and Beckwith, 1977; Thompson and Kujit, 1976; Von Rudloff, 1977; Strong, 1978). Apparently blue spruce will not cross readily with any of these three species, either in nature or artificially. Limited success has been achieved in crosses with white spruce (Yablokov, 1960; Santamour, 1967; Hanover and Wilkinson, 1969; Kudray and Hanover, 1980), and some hybrid seed has been produced from crosses with Engelmann spruce (Fechner and Clark, 1969; Kossuth and Fechner, 1973). In its natural range blue spruce seems to have rather strict habitat preferences, yet it can be planted worldwide, over a much broader range of environments than any of the other northwestern spruces. Studies concerning the relationship of blue and Engelmann spruce indicate that relatively little is known regarding the position of blue spruce in the northwest spruce complex (Wright, 1955; Weaver, 1965; Habeck and Weaver, 1969; Daubenmire, 1972; Taylor _et_ _al_, 1975; Strong, 1978; Mitton and Andalora, 1981).

Investigations concerning the detection of natural hybridization between blue and Engelmann spruce have been hampered by an inability to completely separate reference individuals of each species. One might consider three possible reasons for this:

1) Blue and Engelmann spruce are so similar that no one trait or set of traits will consistently differentiate the species;

2) Diagnostic traits exist between these species, but were not used, or were used along with non-diagnostic traits;
3) The effects of differentiation of local populations were not taken into consideration.

The results of two recent studies by Schaefer and Hanover (1983a, 1983b) suggest that reasons two and three were probably responsible for the difficulties encountered by earlier investigators.

The discovery of an extensive area of range overlap between blue and Engelmann spruce in the Scotch Creek drainage of southwestern Colorado encouraged the present author to investigate the nature of the genetic relationship between these species. An approach taking into consideration items two and three above was adopted. The use of morphological and chemical traits to distinguish between reference individuals of blue and Engelmann spruce was reported by Schaefer and Hanover (1983a, 1983b). The reference populations were located at opposite ends (low and high elevations) of the Scotch Creek drainage. The purpose of this study was to describe the genetic relationship between blue and Engelmann spruce in the Scotch Creek drainage. This will be accomplished through application of the morphological index and discriminant analyses defined in two previous studies (Schaefer and Hanover, 1983a, 1983b) to a large number of spruce growing within the area of overlap (intermediate elevations) of the two species.

The Study Area

The Scotch Creek drainage is part of the Dolores River watershed in southwestern Colorado. It is located in Dolores County about three miles south of Rico (Figure 13a). The extreme upper and lower ends of this drainage were described in another paper (Schaefer and Hanover, 1983a) so will only be covered briefly here.

Elevation of the drainage is 2590 m at the lower end and rises to over 3050 m at the upper end (Figure 13c). The valley floor is narrow and has an east-west orientation with steep (up to 110%) slopes. There are many smaller tributaries which feed Scotch Creek from the north and south, adding to the overall topographical complexity of the drainage (Figure 13b).

Engelmann spruce descends to an elevation of about 2670 m in the study area, while blue spruce can be found at elevations of up to 2820 m. The area of overlap comprises about 4.8 road kilometers. Blue spruce is the predominate species at the lower end of the overlap zone, while Engelmann spruce predominates at the upper end. The mid-point of this zone is characterized by about equal numbers of both species occurring in a broad flat area surrounding the junction of Aspen Creek and Scotch Creek. The valley floor probably reaches its greatest width (100 m) in this area. The north-facing slopes rise from within a few meters of the creek and are quite steep. Blue spruce does not appear to grow anywhere on these slopes. The south-facing slopes are gentler

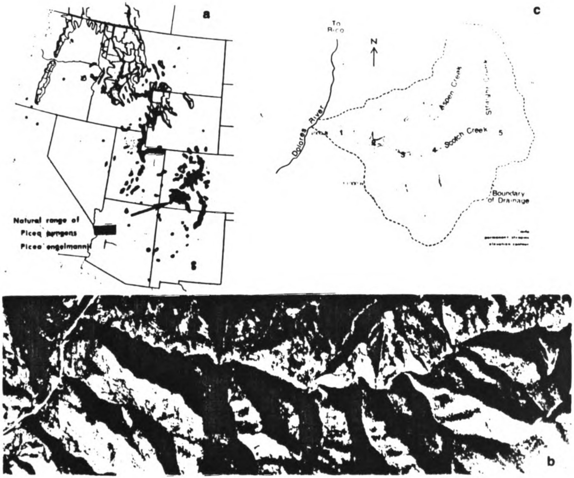

Figure 13. a) Location of the Scotch Creek drainage, b) topography of the drainage, c) diagram of the study area.

and rise much farther from the stream. Blue spruce predominates on these slopes with a few Engelmann spruce occurring near the bottom of the slope.

Three collection areas located at the upper, middle and lower ends of the zone of range overlap were delineated. Two collection areas were also outlined to sample the blue and Engelmann spruce reference populations at the lower and upper ends of the study area, respectively.

Materials and Methods

Except for the inclusion of collections from the area of range overlap the materials and methods of the present study are identical to those of two previous studies (Schaefer and Hanover, 1983a, 1983b). Only a brief description of these procedures will be related here.

It was anticipated that the first 50 trees in each of the five collection zones that met the fecundity requirement (>30 current year's cones) would be sampled. As the result of a relatively poor seed year this goal was not realized. In the fall of 1981 cone collections were made from 38 blue spruce in area one, 49 spruce in area two, 45 spruce in area three, 49 spruce in area four and from 24 Engelmann spruce in area five. During the collection of blue spruce cones in area one, four trees which resembled Engelmann spruce were discovered. Cones were collected from these trees and they were included in the present study. A total of 17 cone and cone scale traits were measured. These measurements were based on four cones or cone scales from each tree.

Seed and seedling data included the number of sound seeds/cone, 100 seed weight, percent germination, germination rapidity, seedling hypocotyl color, number of cotyledons and seedling height at seven months.

Foliage and cortical oleoresin collections were also made from each parental selection, and six morphological traits were scored in the field. Fourteen foliar traits were analyzed. Except for bud scale orientation all measurements were made on two-year-old twigs and needles. All needle measurements were based on four needles per tree from which an average value was determined.

Cortical oleoresins were collected from the base of two-year-old twigs. Fewer trees were represented in these samples since many did not yield sufficient oleoresin. A total of 172 trees were sampled; 40 from area one, 45 from area two, 38 from area three, 36 from area 4 and 13 from area five. Cortical oleoresins were also collected from 206 seedlings (one from each parental selection) and from 15 blue and 15 Engelmann spruce seedlings grown from seed collected from outside of the study area. The oleoresin was analyzed for monoterpene composition using a Varian 3700 GLC equipped with an SE-30 capillary column and an FID detector.

Statistical Approach

Quantitative traits that were previously (Schaefer and Hanover, 1983a, 1983b) determined to be diagnostic were used with Discriminant Function Analysis to differentiate reference individuals of blue and Engelmann spruce, while the qualitative traits were used in a morphological index (Table 9) (Schaefer and Hanover, 1983a). The reference individuals for both of these techniques were from collection zones one (blue spruce) and five (Engelmann spruce). Three discriminant functions were defined based on morphological traits and mature tree and seedling chemical traits. A fourth function combining mature tree morphological and chemical traits was defined in the present study. These functions and the morphological index were used to classify the individual trees from within the zone of range overlap between blue and Engelmann spruce.

Results and Discussion

The morphological index (Figure 14) classified all but two trees into two groups. These results suggest that there is little or no exchange of genetic material between blue and Engelmann spruce. However, these results disagree with those of the more objective discriminant analyses (Figures 15-18). This situation suggests that the morphological index of the present study may suffer from one or two deficiencies. The first pertains to the subjective nature of the measurements of the traits used in the index. There is room for observer bias in such measures which may have resulted in the determination of larger apparent differences between the species than were actually present. The second deficiency involves the imprecision with which these traits were measured.

Table 9. Quantitative and qualitative traits included in the Discriminant Analyses and Hybrid Index, respectively.

TRAITS	
Quantitative	Qualitative
# of stomatal lines	Twig pubescence
Free scale length	Branching habit
Needle sharpness	Scale apex Margin
	Bark texture
	Epicormic branching
	Twig color
	Scale apex shape

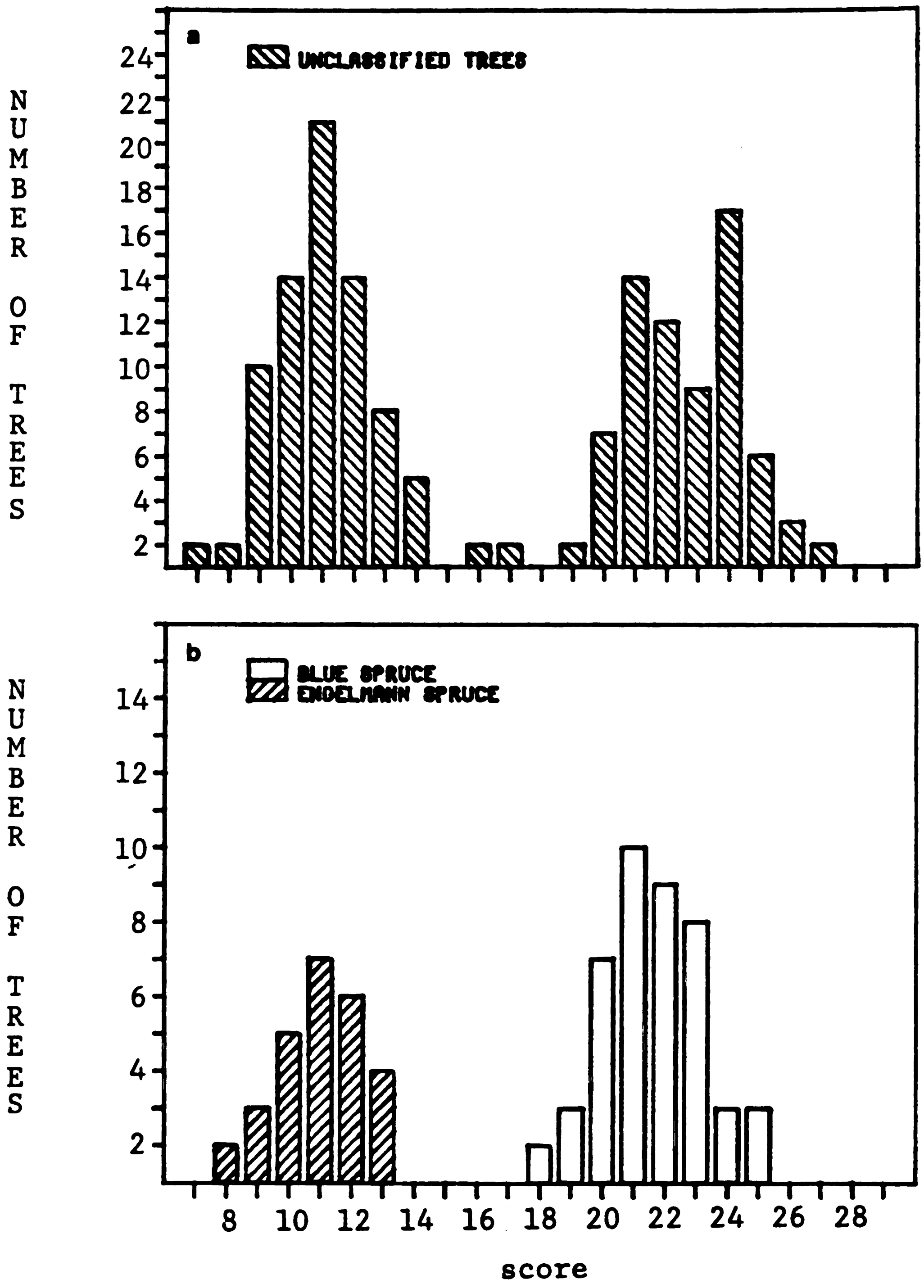

Figure 14. The distribution of a) unclassified individuals from the area of range overlap and b) reference individuals on the morphological index.

The measurement scales may not have consisted of a sufficient number of intervals to adequately represent the variation present in these traits. The morphological and chemical traits included in the discriminant analyses were all measured quantitatively, resulting in a minimum of measurement bias and an accurate representation of the variation present for these traits. Therefore, the results of the discriminant analyses are considered more reliable than those of the morphological index in the present study.

The discriminant function based on morphological traits was very effective in distinguishing between reference individuals of blue and Engelmann spruce (Figure 15a) (Schaefer and Hanover, 1983a). Ten trees appear to be of possible hybrid origin based on their scores on the discriminant function (Figure 15b). These trees appear to bear a closer relationship to Engelmann spruce than to blue spruce. The distribution of the intermediate trees is indicative of several backcrosses to Engelmann spruce and perhaps 1 or 2 F_1 hybrids. Possible introgression of blue spruce genes into the Engelmann spruce population is also suggested by the shift in the mean of the formerly unclassified Engelmann spruce group (Figure 15b).

The discriminant function based on the cortical monoterpenes of mature trees was also very effective in distinguishing between blue and Engelmann spruce (Figure 16a) (Schaefer and Hanover, 1983b). Thirteen cortical oleoresin components were included in the discriminant analysis

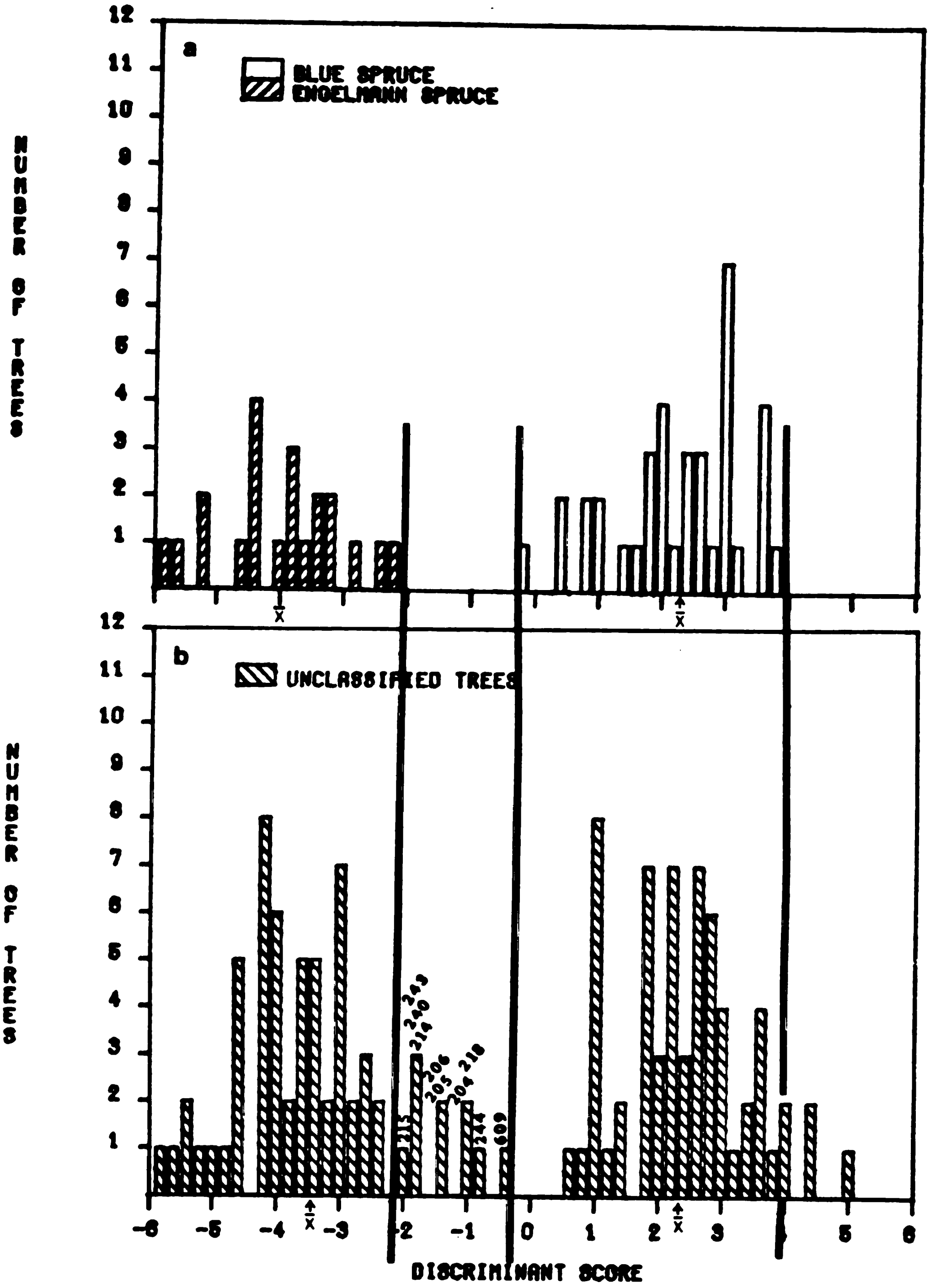

Figure 15. The distribution of a) reference individuals and b) unclassified individuals from the area of range overlap on the discriminant function based on morphological traits.

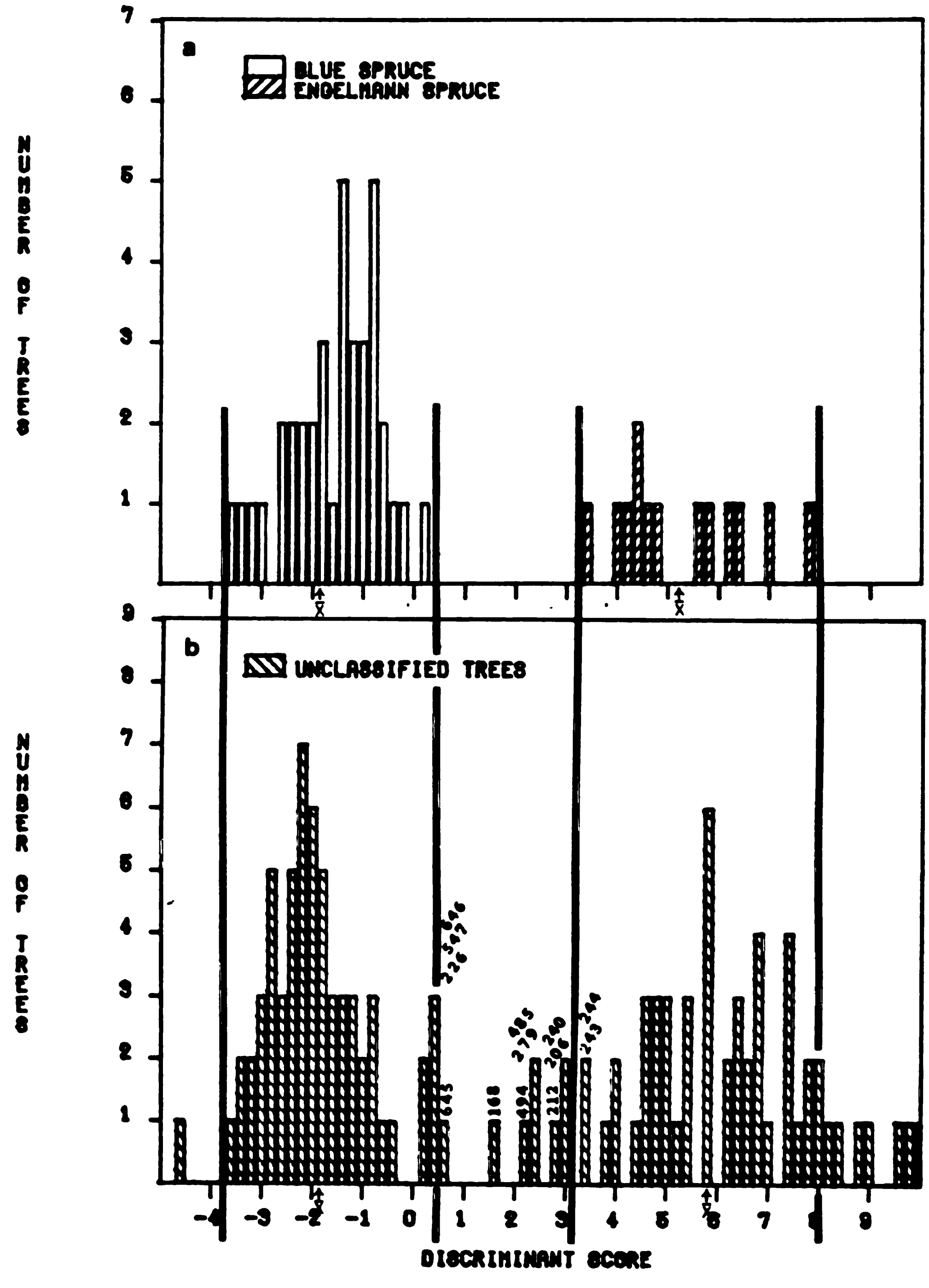

Figure 16. The distribution of a) reference individuals and b) unclassified individuals from the area of range overlap on the discriminant function based on mature tree cortical monoterpenes.

(Table 10). The distribution of unclassified trees on this function may be explained in part as a filling in of the Engelmann spruce distribution as the sample size is increased (Figure 16b). This result suggests that the 13 Engelmann spruce used as reference individuals may not have been sufficient to adequately represent the variation present in Engelmann spruce for the various monoterpenes. Four trees (#'s 206, 240, 243, 244) that were determined to be morphologically intermediate are located on the left shoulder of the Engelmann spruce distribution toward what might be considered an intermediate position. The remaining individuals in this portion of the Engelmann spruce distribution were all classified as Engelmann spruce by the morphological discriminant analysis.

Few of the trees in the study of Taylor et al, (1975) that were determined to be intermediate, were intermediate for both morphological and chemical traits. They suggested that introgression would result in various combinations of mixed traits since morphological traits appeared to segregate independently of chemical traits. In the present study correlations between morphological and chemical traits were very low.

Perhaps the best illustration of gene exchange between blue and Engelmann spruce is provided by the discriminant analysis based on the combined set of morphological and chemical traits (Figures 17a and 17b). The results are consistenet with those of the separate morphological and chemical

Table 10. The cortical oleoresin components of mature blue and Engelmann spruce included in the discriminant analyses.

Cortical oleoresin component
Tricyclene
Unknown 3
α-pinene
Camphene
Unknown 6
β-pinene
Unknown 9
3-carene
Unknown 12
Terpinolene
Bornyl acetate
Unknown 18
Unknown 19

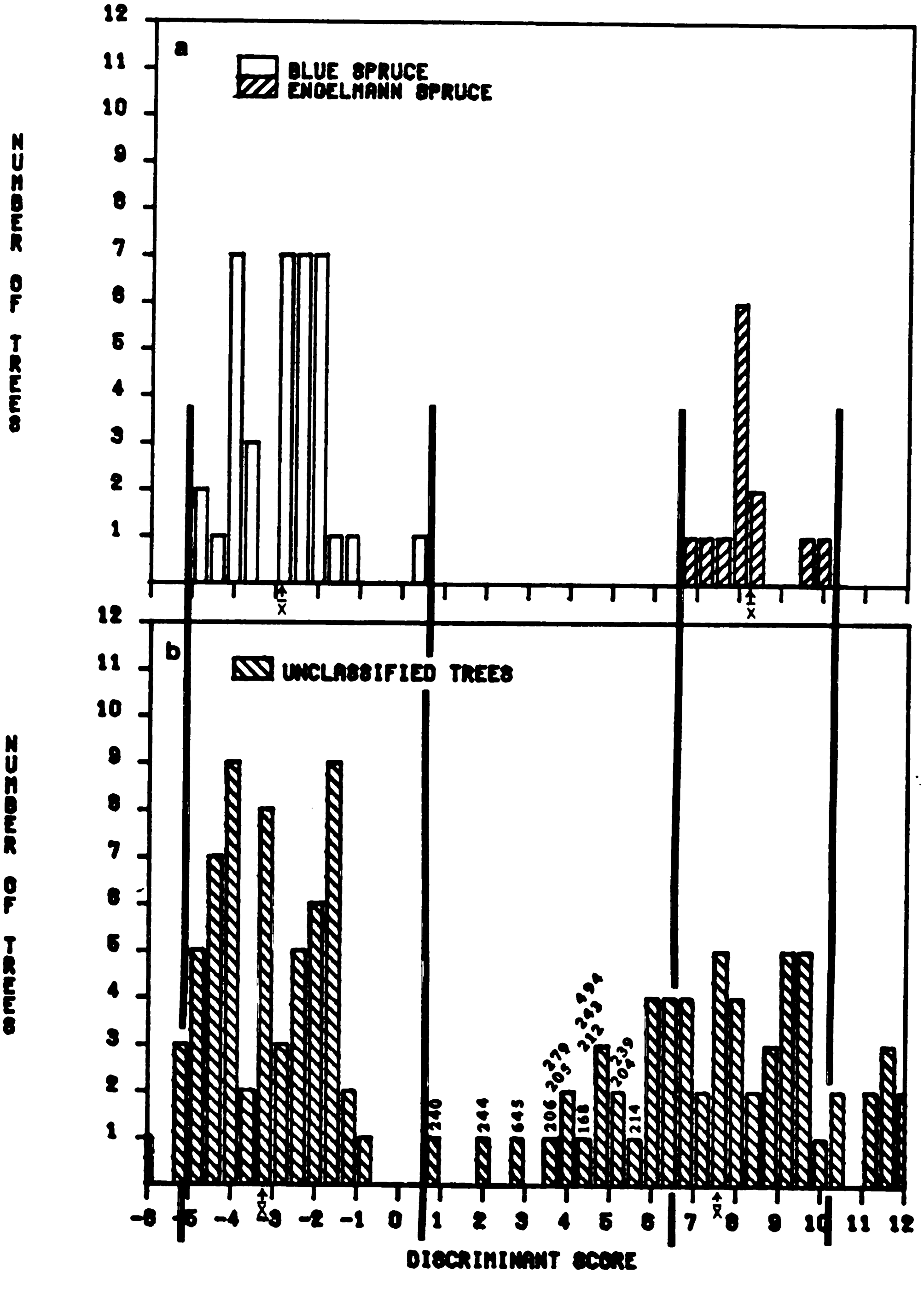

Figure 17. The distribution of a) reference individuals and b) unclassified individuals from the area of range overlap on the discriminant function based on morphological and chemical traits.

discriminant analyses. Again the distribution of the Engelmann spruce individuals from the area of range overlap may be explained in part by a filling in of the tails of the reference population's distribution as the sample size is increased. However, from Figure 17b it is clear that the shoulder to the left (towards the blue spruce distribution) of the Engelmann spruce mean is much longer than that to the right. The mean of the distribution of Engelmann spruce from the area of range overlap is also closer to blue spruce than is that of the Engelmann spruce reference population. Together, these observations suggest possible hybridization and introgression between blue and Engelmann spruce. The distribution suggests stronger gene flow toward Engelmann spruce than toward blue spruce. Upwards of 13 trees might be considered putative hybrids based on this analysis. Eleven of these trees were determined to be intermediate in either the morphological or possibly the chemical analyses (Table 11).

The putative hybrids detected were not distributed evenly within the study area. Three of the four trees discovered in collection area one that resembled Engelmann spruce appear to be putative hybrids. The fourth tree is an Engelmann spruce. Two trees in each of collection areas two and three appear to be of hybrid origin, while six trees in area 4 may be placed in this category. The presence of Engelmann spruce and putative hybrids in what was thought to be an entirely blue spruce population (collection area one) suggests the possibility that

Table 11. Classification of intermediate trees based on each of the three discriminant analyses for mature trees.

Tree #	Collection Zone	Analysis Morphological	Terpene	Morphological + Terpene
168	3	Engelmann	Intermediate	Intermediate
204	1	Intermediate	Engelmann	Intermediate
205	1	Intermediate	Engelmann	Intermediate
206	1	Intermediate	Engelmann	Intermediate
212	2	Engelmann	Engelmann	Intermediate
214	2	Intermediate	Engelmann	Intermediate
215	2	Intermediate	Engelmann	Engelmann
218	2	Intermediate	Engelmann	Engelmann
226	4	Blue	Intermediate	Blue
239	4	Engelmann	Engelmann	Intermediate
240	4	Intermediate	Engelmann	Intermediate
243	4	Intermediate	Engelmann	Intermediate
244	4	Intermediate	Engelmann	Intermediate
279	3	Engelmann	Intermediate	Intermediate
494	3	Engelmann	Intermediate	Intermediate
547	3	Blue	Intermediate	Blue
609	4	Intermediate	Engelmann	Engelmann
645	4	Blue	Intermediate	Intermediate
646	3	Blue	Intermediate	Blue

the blue spruce in this area may be contaminated with Engelmann spruce genes. This might explain why the distribution of the blue spruce from the zone of range overlap did not deviate from that of the blue spruce reference population.

Schaefer and Hanover (1983b) found that a discriminant function based solely on the monoterpenes of seedling cortical oleoresin was unable to distinguish between reference individuals of blue and Engelmann spruce (Figure 18a). They suggested that such a function would be of limited used in detecting natural hybridization between these species. The classification results (Figure 18b) are in sharp contrast to those of the previous discriminant analyses. The progeny analysis indicates that the species are not at all separated and that there is abundant gene flow between them. The weakness of the function in classifying reference individuals of each species severely restricts its usefulness in classifying trees of questionable specific assignment. The results of this analysis illustrate the importance of a particular function's diagnostic ability in leading to meaningful biological interpretations.

It is interesting to speculate on the influence of ecological factors on the distribution of blue, Engelmann and putative hybrid spruce within the study area. Blue spruce was found on south-facing slopes or along the valley bottom. Within the area of range overlap this species was never observed on north-facing slopes. Engelmann spruce was found on north-facing slopes, along the valley bottom, and to a limited

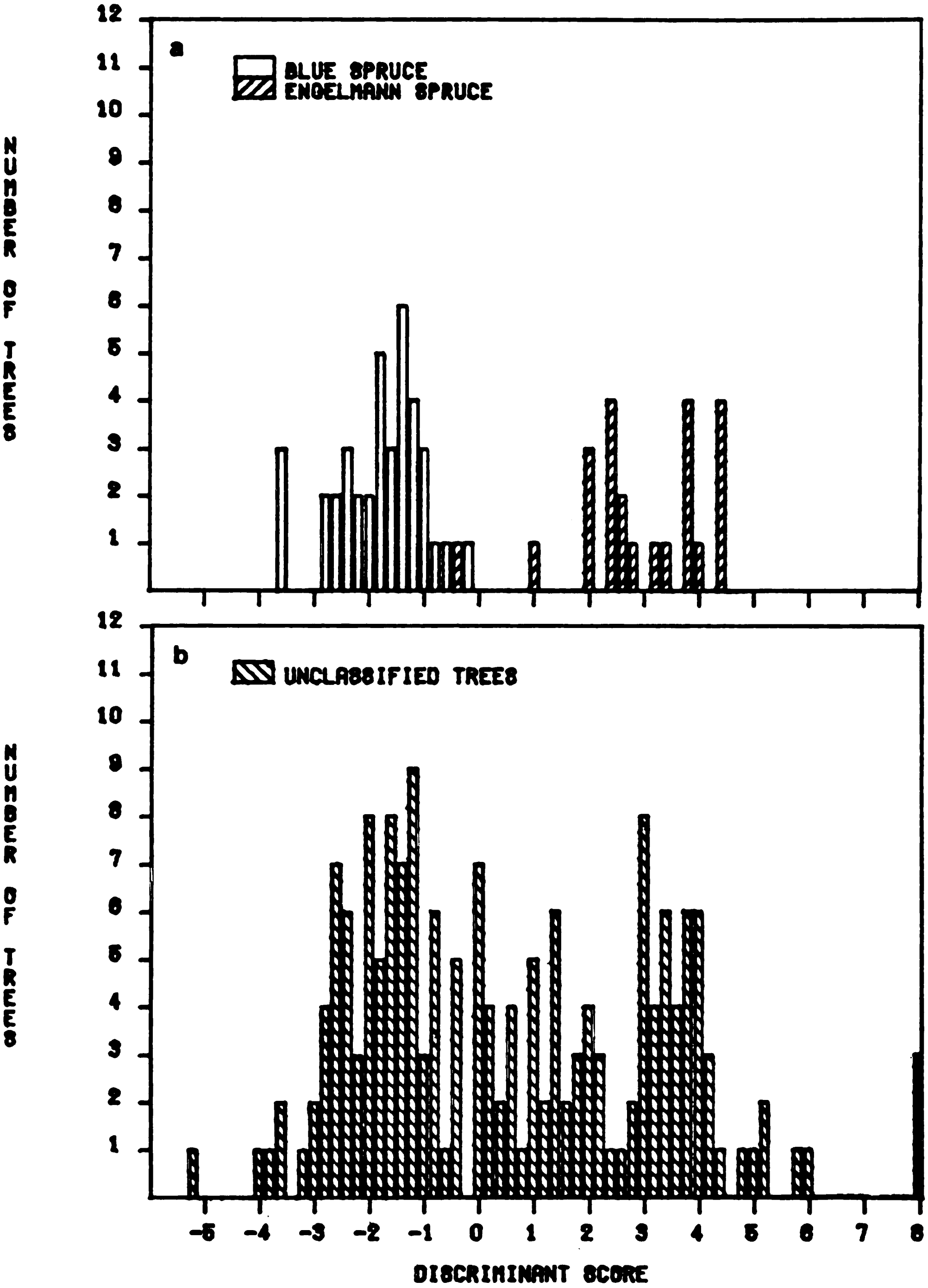

Figure 18. The distribution of a) reference individuals and b) unclassified individuals from the area of range overlap on the discriminant function based on seedling cortical monoterpenes.

extent at the lower edges of some south-facing slopes within the area of range overlap. The putative hybrids were located in the flat areas along the valley bottom in all but two cases. These two trees were growing on gentle (10%) south-facing slopes. The classical hybrid habitat apparently does not exist in the study area, since pure forms of both species grow in abundance throughout the areas where hybrids were found. It is interesting to note that the hybrids are generally restricted to habitats intermediate to the extreme habitats within the drainage.

Summary

Four discriminant functions based on morphological and chemical traits and a morphological index were used to classify 147 spruce from an area of extensive range overlap between blue and Engelmann spruce. The results of the two techniques did not agree. The results of the discriminant analyses were considered more reliable and therefore all of the conclusions were based on these results.

The results indicated that blue and Engelmann spruce do hybridize under natural conditions, although such hybridization is very limited in occurrence. The detection of putative hybrids was difficult based on monoterpenes alone. This was attributed to the small size (13 individuals) of the Engelmann spruce reference population. A combination of morphological and chemical traits was the most effective in detecting putative hybrids. The intermediate types appeared to exhibit a stronger affinity to Engelmann spruce than to blue spruce, suggesting that gene flow is favored from blue to Engelmann spruce.

CHAPTER V

Conclusions and Recommendations for Further Study

Several morphological and chemical traits serve to distinguish between blue and Engelmann spruce found in the Scotch Creek drainage. The ability of these traits to differentiate blue and Engelmann spruce from other portions of the range of each species has not been tested. The results of earlier investigations (Daubenmire, 1972; Taylor et al, 1975) suggest that the diagnostic value of the traits used in this study may be reduced somewhat if applied to trees from other areas. This question could be addressed in a study of range-wide intra- and interspecific variation in blue and Engelmann spruce. The author is not aware of any comprehensive studies of this nature.

Seedling cortical monoterpenes were of little use in distinguishing between blue and Engelmann spruce. Concentrations of several monoterpenes, especially β-pinene, were much higher in the seedlings than in mature representatives of each species. Hanover (1971) obtained similar results in western white pine. Squillace (1971) found that age effects on monoterpene composition ceased after two years in slash pine. The differences between mature and juvenile spruce monoterpene composition appear to be quantitative rather than qualitative. Investigations concerning the age at which these differences subside in spruce are needed.

The results of the three discriminant analyses based on mature trees indicate that blue and Engelmann spruce will hybridize under natural conditions, although such hybridization is very limited in occurrence. Taylor et al (1975) reached the same conclusion, while Fechner and Clark (1969) and Kossuth and Fechner (1973) showed that these species will cross artificially at low frequencies.

The distribution of the intermediate trees on the discriminant functions suggested the presence of possibly several F_1 hybrids and substantially more backcrosses to Engelmann spruce. Gene flow appeared to be directed more toward Engelmann spruce than toward blue spruce. The reasons for this are obscure. Reproductive barriers in spruce have been found to be generally prezygotic (Mikkola, 1969; Kossuth and Fechner, 1973). If blue spruce is unable to serve as a female parent in crosses with Engelmann spruce, as earlier studies have suggested, and one also assumes that blue spruce is unsuccessful as the female parent in crosses with the F_1 and future hybrids, the reason for gene flow in generally one direction may be partially explained. Considering the above assumptions blue spruce could only take part in crosses with the hybrid when blue spruce is the male parent. On the other hand, Engelmann spruce could take part as both a male and a female. Therefore there is a much greater potential for crosses to occur with Engelmann spruce than with blue spruce and gene flow would tend to be directed toward Engelmann spruce.

Whether this is sufficient to result in the distribution of hybrid types in the present study is questionable, and other factors may very well be involved.

A thorough knowledge of the crossability of blue and Engelmann spruce is necessary to confirm the status of the putative hybrids found in this study, and to provide greater insight regarding the pattern of gene exchange between these species. Only extremely limited compatibility studies have been attempted to date. Attempts should also be made to identify other traits which may be useful in distinguishing between blue and Engelmann spruce. Detailed cytogenetic studies and the use of enzyme polymorphisms could provide a strong line of evidence to support or refute the results of the present study. Ernst and Hanover (personal communication)[1] have initiated a crossability study involving many of the pure species and putative hybrid forms found within the Scotch Creek drainage. The use of isoenzymes to distinguish between parental forms and hybrid progeny is anticipated. Their results should provide answers to many of the questions raised in the present study.

---

[1] S. Ernst and J.W. Hanover, graduate assistant and Professor of Forest Genetics, respectively, Michigan State University.

LIST OF REFERENCES

LIST OF REFERENCES

Adams, R.P. 1982. A comparison of multivariate methods for the detection of hybridization. Taxon, 31(4):646-661.

Adams, R.P., E. von Rudloff, T.A. Zanoni, and L. Hogge. 1980. The terpenoids of an ancestral/advanced species pair of *Juniperus*. Biochem. Syst. Ecol. 8:35-37.

Adams, R.P., T.A. Zanoni, E. von Rudloff, and L. Hogge. 1981. The south-western USA and northern Mexico one-seeded junipers: Their volatile oils and evolution. Biochem. Syst. Ecol. 9(2/3):93-96.

Alexander, R.R. 1974. Silviculture of subalpine forests in the central and southern Rocky Mountains: The status of our knowledge. USDA For. Serv. Res. Pap. RM-121. 88 p.

Anderson, E.G. 1939. Recombination in species crosses. Genetics 24:668-698.

Anderson, E.G. 1949. Introgressive hybridization. 2nd ed. (1968). Hafner Publishing Co., New York and London.

Antonovics, J. and A.D. Bradshaw. 1970. Evolution in closely adjacent plant populations VIII. Clinal patterns at a mine boundary. Heredity 25:349-362.

Axelrod, D.I. 1958. Evolution of the madro-tertiary geoflora. Botanical Rev. 24:433-509.

———. 1968. Tertiary floras and topographic history of the Snake River Basin, Idaho. Geol. Soc. Amer. Bull. 79:713-734.

———, and H.P. Bailey. 1976. Tertiary vegetation, climate and altitude of the Rio Grande Depression, New Mexico-Colorado. Paleobiology 2:235-254.

Bailey, D.K., K. Snajberk, and E. Zavarin. 1982. On the question of natural hybridization between *Pinus discolor* and *Pinus cembroides*. Biochem. Syst. Ecol. 10(2):111-119.

Bongarten, B.C. 1978. Genetic and environmental variation in shoot growth and other traits of blue spruce (Picea pungens). Ph.D. thesis, Mich. State Univ., East Lansing, MI.

———, and J.W. Hanover. 1982. Hybridization among white, red, blue, and white x blue spruces. For. Sci. 28(1):129-134.

Buchert, G.P. 1981. Genetic variation of morphological and physiological traits in blue and Engelmann spruce. Ph.D. thesis, Mich. State Univ., East Lansing, MI.

Clifford, H.T. and F.E. Binet. 1954. A quantitative study of a presumed hybrid swarm between Eucalyptus elaeophora and E. goniocalyx. Aust. J. Bot. 2:325-336.

Colinvaux, P.A. 1967. Bering Land Bridge: Evidence of spruce in late Wisconsin times. Science 156:380-383.

Copes, D.L. and R.C. Beckwith. 1977. Isoenzyme identification of Picea glauca, P. sitchensis and P. lutzi popultions. Bot. Gaz. 138(4):512-521.

Dallimore, W. and A.B. Jackson (Revised by S.G. Harrison). 1967. A handbook of Coniferae and Ginkgoaceae. St. Martins Press, New York. 729 p.

Dancik, B.P. and B.V. Barnes. 1975. Multivariate analysis of hybrid populations. Naturaliste Can., 102:835-843.

Daubenmire, R. 1968. Some geographic variation in Picea sitchensis and their ecologic interpretation. Can. J. Bot. 46:787-798.

Daubenmire, R. 1972. On the relation between Picea pungens and Picea engelmannii in the Rocky Mountains. Can. J. Bot. 50:733-742.

———. 1974. Taxonomic and ecologic relationships between Picea glauca and Picea engelmannii. Can. J. Bot. 52:1545-1560.

———, and J.B. Daubenmire. 1968. Forest vegetation of eastern Washington and northern Idaho. Wash. Agric. Exp. Sta. Tech. Bull. 60. Pullman, Wash.

Eklundh, C. 1943. Artkorsningar inom sl. Abies, Pseudotsuga, Picea, Larix, Pinus och Chamaecyparis, tillhörande fam. Pinaceae. (Species crosses within the genera Abies, Pseudotsuga, Picea, Larix, Pinus and Chamaecyparis, belonging to the family Pinaceae.) Svensk Papp Tidn. 46:55-61, 101-105, 130-133. (Abstract in Forestry Abs. 5:95-96. 1943).

Erlich, P.R. and Raven. 1969. Differentiation of populations. Science 165:1228-1232.

Fechner, G.H. 1983. Silvics of native and naturalized trees of the United States and Puerto Rico: *Picea pungens* Engelm., blue spruce. In preparation.

Fechner, G.H. and R.W. Clark. 1969. Preliminary observations on hybridization of Rocky Mountain spruces. Proc. 11th Meeting, Comm. Forest Tree Breeding in Canada. Ste. Anne de Bellevue, Quebec. pp. 237-247.

Fisher, G.S. 1966. Variations in composition of turpentines from slash pines. (Abstr.) Amer. Chem. Soc. D-4, Winter Meeting, Phoenix, Ariz.

Flake, R.H. and B.L. Turner. 1973. Volatile constituents, especially terpenes, and their utility and potential as taxonomic characters in populational studies. IN/ Chemistry in Botanical Classification (Eds. G. Bendz, J. Santesson). pp. 123-128. Academic Press, New York.

Flake, R.H., E. von Rudloff and B.L. Turner. 1969. Quantitative study of clinal variation in *Juniperus virginiana* using terpenoid data. Proc. Nat. Acad. Sci. 64(2):487-494.

——, L. Urbatsch and B.L. Turner. 1978. Chemical documentation of allopatric introgression in *Juniperus*. Syst. Bot. 3:129-144.

Florin, R. 1963. The distribution of conifer and taxad genera in time and space. Acta Horti Bergiani, 20(4):122-312.

Forrest, G.I. 1980a. Geographic variation in the monoterpene content of sitka spruce cortical oleoresin. Can. J. For. Res. 10:458-463.

——. 1980b. Seasonal and spatial variation in cortical monoterpene composition of sitka spruce oleoresin. Can. J. For. Res. 10:452-457.

——. 1980c. Geographical variation in the monoterpenes of *Pinus contorta* oleoresin. Biochem. Syst. Ecol. 8:343-359.

——. 1980d. Variation in monoterpene composition of the shoot cortical oleoresin within and between trees of *Pinus contorta*. Biochem. Syst. Ecol. 8:337-341.


H
Ha
Ha
Ha
Ha

Fowells, H.A. 1965. Silvics of forest trees of the United States. USDA For. Serv. Agric. Hndbk. NO. 271. 762 p.

Fowler, D.P. 1966. A new spruce hybrid - *Picea schrenkiana* x *P. glauca*. Proc. 2nd Genetics Workshop, U.S. For. Serv. Res. Pap. NC-6:44-47.

———. 1983. The hybrid black x sitka spruce, implications to phylogeny of the genus *Picea*. Can. J. For. Res. 13:108-115.

———, and L. Roche. 1977. Genetics of Engelmann spruce. USDA For. Serv. Res. Pap. WO-30. 13 p.

Garman, E.H. 1957. The occurrence of spruce in the interior of British Columbia. Can. Dept. Lands and Forest, Tech. Publ. T49. 31 p.

Gordon. 1952. Spruce identification by twig characteristics. For. Chron. 28(3):43-45.

———. 1968. Ecology of *Picea chihuahuana* Martinez. Ecology 49:880-896.

———. 1976. The taxonomy and genetics of *Picea rubens* and its relationship to *Picea mariana*. Can. J. Bot. 9:781-813.

Haag, W.G. 1962. The Bering Strait Land Bridge. Sci. Amer. 206(1):112-123.

Habeck, J.R. 1964. A report of suspected hybridization among Engelmann spruce, white spruce and Colorado blue spruce in Western Montana. Proc. Mont. Acad. Sci. 24:5.

Habeck, J.R. and T.W. Weaver. 1969. A chemosystematic analysis of some hybrid spruce (*Picea*) populations in Montana. Can. J. Bot. 47:1565-1570.

Hamrick, J.L. and R.W. Allard. 1972. Microgeographical variation in allozyme frequencies in *Avena barbata*. Proc. Nat. Acad. Sci. 69(8):2100-2104.

Hanover, J.W. 1966a. Genetics of terpenes. 1. Gene control of monoterpene levels in *Pinus monticola* Dougl. Heredity, 21(1):73-84.

———. 1966b. Environmental variation in the monoterpenes of *Pinus monticola* Dougl. Phytochem. 5:713-717.

———. 1966c. Inheritance of 3-carene concentration in *Pinus monticola*. For. Sci. 12(4):447-450.

———. 1971. Genetics of terpenes II. Genetic variances and interrelationships of monoterpene concentrations in *Pinus monticola*. Heredity 27(2):237-245.

———. 1974. Biochemical analysis of tree speciation. Proc. 3rd North Amer. Biol. Workshop, Ft. Collins, Colo. Sept. 2-12. pp. 106-131.

———. 1975. Comparative physiology of eastern and western white pines: Oleoresin composition and viscosity. For. Sci. 22(3):214-221.

———, and R.C. Wilkinson. 1969. A new hybrid between blue spruce and white spruce. Can. J. Bot. 47(11):1693-1700.

———, and R.C. Wilkinson. 1970. Chemical evidence for introgressive hybridization in *Picea*. Silvae Genet. 19:17-22.

Hanover, J.W., E. Young, W.A. Lemmien, and M. Van Slooten. 1976. Accelerated-Optimal-Growth: A new concept in tree production. Mich. State Univ. Agric. Exper. Sta. Res. Rep. No. 317. 16 p.

Harlow, W.M. and E.S. Harrar. 1969. Textbook of Dendrology. McGraw-Hill, New York. 512 p.

Hills, L.V. and R.T. Ogilvie. 1970. *Picea banksii* n. sp. Beaufort formation (tertiary), northwestern Banks Island, Arctic Canada. Can. J. Bot. 48:457-464.

Hiltunen, R., P.M.A. Tigerstedt, S. Suvoneu, and J. Pohjola. 1975. Inheritance of 3-carene quantity in *Pinus sylvestris* L. Farm. Notisbl. 84:69-72.

Hopkins, D.M. 1959. Cenozoic history of the Bering Land Bridge. Science 129:1519-1528.

———. 1967. The Cenozoic history of Beringia - A synthesis. IN/ (D.M. Hopkins, ed.) The Bering Land Bridge. Stanford Univ. Press, Stanford, Calif. pp. 451-484.

Horton, K.W. 1959. Characteristics of subalpine spruce in Alberta. Forestry Branch, Canada Dept. Northern Affairs and National Resources. For. Res. Div. Tech. Note. No. 76. 20 p.

Hubbs, C.L., L.C. Hubbs and R.E. Johnson. 1943. Hybridization in nature between species of catostomid fishes. Contrib. Lab. Vertebr. Biol. Univ. Mich. 22:1-76.

Hunt, R.S. and E. von Rudloff. 1974. Chemosystematic studies in the genus *Abies*. I. Leaf and twig oil analysis of alpine and balsam firs. Can. J. Bot. 52:477-487.

Hunt, R.S. and E. von Rudloff. 1979. Chemosystematic studies in the genus *Abies*. IV. Introgression in *Abies lasiocarpa* and *Abies bifolia*. Taxon, 28(4):197-305.

Johnson, L.P.V. 1939. A descriptive list of natural and artificial interspecific hybrids in North American forest tree genera. Can. J. Res. 17(c):411-444.

------, and C.C. Heinberger. 1946. Preliminary report on interspecific hybridization in forest trees. Can. J. Res. 24(c):308-312.

Jones, J.R. 1974. Silviculture of southwestern mixed conifers and aspen: The status of our knowledge. USDA For. Serv. Res. Pap. RM-122. 43 p.

Jones, J.R. and N.T. Bernard. 1977. USDA For. Serv. Gen. Tech. Rep. RM-34. 11 p.

Kapp, R.O. 1970. Pollen analysis of pre-Wisconsin sediments from the Great Plains. IN/ (Dort, W., Jr. and J.K. Jones, Jr. eds.) Pleistocene and recent environments of the central Great Plains. Kansas Univ. Press, Kansas. pp. 143-155.

Klecka, W.R. 1976. Discriminant Analysis, pp. 434-367. IN/ N.H. Nie, C.H. Hull, J.G. Jenkins, K. Steinbrenner and H.H. Bent (eds.). Statistical Package for the Social Sciences. McGraw-Hill, N.Y.

Kossuth, S.V. and G.H. Fechner. 1973. Incompatibility between *Picea pungens* Engelm. and *Picea engelmannii* Parry. For. Sci. 19(1):50-60.

Kudray, G.M. and J.W. Hanover. 1980. A preliminary evaluation of the spartan spruce (*Picea glauca* x *P. pungens*). Res. Pap. NO. 405. Mich. State Univ. Agric. Exp. Sta., East Lansing, MI.

LaMotte, R.S. 1952. Catalogue of the Cenozoic plants of North American through 1950. Mem. Geol. Soc. Amer. New York 51. 381 p.

LaRoi, G.H. and J.H. Dugle. 1968. A systematic and genecological study of Picea glauca and P. engelmannii, using paper chromatograms of needle extracts. Can. J. Bot. 46:649-687.

Larsen, C. Syrach. 1934. Forest tree breeding. (Denmark) K. Vet. og Landbohøjskole Aarsskr. pp. 93-113.

———. 1937. The employment of species, types and individuals in forestry. (Denmark) K. Vet. og Landbohøjskole Aarsskr. pp. 69-222.

Larsen, J.A. 1965. The vegetation of the Ennadai Lake Area, N.W.T.: Studies in subarctic and arctic bioclimatology. Ecol. Monographs. 35:37-59.

Lawrence, L., R. Bartschot, E. Zavarin and J.R. Griffin. 1975. Natural hybridization of Cuppressus sargentii and C. macrabiana and the composition of the derived essential oils. Biochem. Syst. Ecol. 2:113-119.

Ledig, F.T., R.W. Wilson, J.W. Duffield and G. Maxwell. 1969. A discriminant analysis of introgression between Quercus prinus L. and Quercus alba L.. Bull. Torrey Botan. Clubs. 96(2):156-163.

Leopold, E.B. and H.D. MacGinitie. 1972. Development and affinities of tertiary floras in the Rocky Mountains. pp. 147-200. IN/ Graham, A., (ed.) Floristics and paleofloristics of Asia and Eastern North America. Elsevier Publ. Co., Amsterdam.

Lewis, H. 1966. Speciation in flowering plants. Science 152:167-171.

Li, H.L. 1953. Present distribution and habitats of the conifers and taxads. Evol. 7:245-261.

Little, E.L. 1953. A natural hybrid spruce in Alaska. J. For. 51:745-747.

Little, E.L., Jr. 1971. Atlas of United States trees. Volume 1. Conifers and important hardwoods. USDA For. Serv. Misc. Publ. NO. 1146.

———, and S.S. Pauley. 1958. A natural hybrid between black and white spruce in Minnesota. Am. Mid. Nat. 60(1):202-211.

Löve, D. 1959. The postglacial development of the flora of Manitoba: A discussion. Can. J. Bot. 37:547-585.

MacGinitie, H.D. 1953. Fossil plants of the florissant beds, Colorado. Carnegie Inst. of Wash. Publ. 599, 188 p.

———. 1962. The kilgore flora, a late miocene flora from northern Nebraska. Univ. Calif. Publ. Geol. Sci. 35:67-158.

———. 1969. The Eocene Green River flora of northwestern Colorado and northeastern Utah. Univ. Calif. Publ. Geol. Sci. 83:140 p.

Manley, S.M. 1971. Identification of red, black and hybrid spruces. Dept. of the Environ. Can. For. Serv. Publ. NO. 1301.

Manley, S.A.M. 1972. The occurrence of hybrid swarms of red and black spruces in central New Brunswick. Can. J. For. Res. 2(4):381-391.

Manley, S.A.M. and D.P. Fowler. 1969. Spruce budworm defoliation in relation to introgression in red and black spruce. For. Sci. 15(4):365-366.

Marco, H.F. 1931. Needle structure as an aid in distinguishing Colorado blue spruce from Engelmann spruce. Botan. Gazette 92:446-449.

McNeilly, T. 1968. Evolution in closely adjacent plant populations. III. Agrostis tenuis on a small copper mine. Heredity 23:99-108.

Mergen, F., D.T. Lester, G.M. Furnival, and J. Burley. 1965. Discriminant analysis of Eucalyptus cineria x Eucalyptus maculosa hybrids. Silvae Genet. 15:148-154.

Mikkola, L. 1969. Observations on interspecific sterility in Picea. Ann. Bot. Fenn. 6:285-339.

Mirov, N.T. 1948. The terpenes (In relation to the biology of the genus Pinus). Ann. Rev. Biochem. 17:521-540.

———, E. Zavarin, K. Snajberk and K. Costello. 1966. Further studies of turpentine composition of Pinus muricata in relation to its taxonomy. Phytochem 5:343-355.

Mitton, J.B. and R. Andalora. 1981. Genetic and morphological relationships between blue spruce, Picea pungens Engelm. and Engelmann spruce, Picea engelmannii Parry in the Colorado Front Range. Can. J. Bot. 59:2088-2094.

Moore, P.P. 1980. Developmental variation in volatile oil of blue spruce. Ph.D. dissertation, Forestry Dept., Mich. State Univ., East Lansing, MI.

Morgenstern, E.K. 1969. Winter drying of red spruce provenances related to introgressive hybridization with black spruce. Dept. of Fish. and For., Ottawa, Can., Bimonthly Research Notes 25(4):34-36.

Morgenstern, E.K., A.G. Corriveau and D.P. Fowler. 1981. A provenance test of red spruce in nine environments in eastern Canada. Can. J. For. Res. 11:124-131.

Morgenstern, E.K. and J.L. Farrar. 1964. Introgression hybridization in red spruce and black spruce. Univ. of Toronto, Fac. of Forestry Tech. Rept. NO. 4. 46 p.

Namkoong, G. 1966. Statistical analysis of introgression. Biometrics 22:488-502.

Nienstaedt, H. and A. Tiech. 1972. The genetics of white spruce. USDA For. Serv. Res. Pap. WO-15, 24 p.

Ogilvie, R.T. and E. von Rudloff. 1968. Chemosystematic studies in the genus Picea (Pinaceae). IV. The introgression of white and Engelmann spruce as found along the Bow River. Can. J. Bot. 46:901-908.

Parker, W.H. and D.G. McLachlan. 1978. Morphological variation in white and black spruce: Investigation of natural hybridization between Picea glauca and P. mariana. Can. J. Bot. 56:2512-2520.

Pauly, G. and E. von Rudloff. 1971. Chemosystematic studies in the genus Pinus: The leaf oil of Pinus contorta var. latifolia. Can. J. Bot. 49:1201-1210.

Reed, A.N.F. and J.W. Hanover. 1983. Geographic variation in cortical monoterpenes of blue spruce, Picea pungens. In preparation.

Reed, J.F. and G. Freytag. 1949. The resin sac pattern in the needles of Engelmann spruce and blue spruce from southwestern Wyoming. The Amer. Midland Natur. 41(1):233-236.

Richens, R.H. 1945. Forest tree breeding and genetics. Imperial Agricultural Bureau Joint Publication. NO. 8. Imperial Bureau of Plant Breeding and Genetics. Cambridge, England. 79 p.

Roche, L.A. 1969. A genecological study of the genus Picea in British Columbia. New Phytol. 68:505-554.

Roche, L.A. and D.P. Fowler. 1975 Genetics of sitka spruce. USDA For. Serv. Res. Pap. WO-26. 15 p.

Ronco, F. 1970. Influence of high light intensity on survival of planted Engelmann spruce. For. Sci. 16(3):331-338.

Rottink, B.A. and J.W. Hanover. 1972. Identification of blue spruce cultivars by analysis of cortical oleoresin monoterpenes. Phytochem. 11:3255-3257.

Santamour, F.S., Jr. 1965. Biochemical taxonomy. Morris Arbor. Bull. 16:31-33.

Santomour, F.S., Jr. 1967. Growth and characteristics of some hybrid spruces. Morris Arbor. Bull. 18:18-20.

Schaefer, P.R. and J.W. Hanover. 1983a. A morphological comparison of blue and Engelmann spruce in southwestern Colorado. In preparation.

Schaefer, P.R. and J.W. Hanover. 1983b. Monoterpene concentrations in cortical oleoresin of blue and Engelmann spruce. In preparation.

Schmidt, W.C., C.A. Wellner, and R.R. Alexander. 1973. Engelmann spruce-subalpine fir. IN/Silviculture systems for the major forest types of the United States. USDA For. Serv. Handbook. NO. 445:40-41.

Squillace, A.E. 1971. Inheritance of monoterpene composition in cortical oleoresin of slash pine. For. Sci. 17(3):381-387.

Squillace, A.E. 1976a. Biochemical genetics and selection: Composition of volatile terpenes. IUFRO, Joint Meeting on Advanced Generation Breeding, Bordeaux - June 14-18, pp. 167-178.

——. 1976b. Analysis of monoterpenes of conifers by gas-liquid chromatography. IN/ (J.P. Miksche, ed.) Modern methods in forest genetics. pp. 120-157.

——, O.O. Wells, and D.L. Rockwood. 1981. Inheritance of monoterpene composition in cortical oleoresin of loblolly pine. Silvae Gent.

Strong, W.L. 1978. Evidence for Picea pungens in north-central Montana and its significance. Can. J. Bot. 56:1118-1121.

Taylor, R.J., S. Williams, and R. Daubenmire. 1975. Interspecific relationships and the question of introgression between Picea engelmannii and Picea pungens. Can. J. Bot. 53:2547-2555.

Taylor, T.M.C. 1959. The taxonomic relationship between Picea glauca (Moench.) Voss and P. engelmannii Parry. Madrono 15:111-115.

Thaarup, P. 1945. Bastarden sitkagran x Hvidgran. (The hybrid sitka spruce x white spruce.) Dansk. Skovfor. Tidsskr. 30:381-384.

Thompson, L.S. and J. Kujit. 1976. Montane and subalpine plants of the Sweetgrass Hills, Montana, and their relation to early postglacial environments of the northern Great Plains. Can. Field Nat. 90:432-448.

Tobolski, J.J. and J.W. Hanover. 1971. Genetic variation in the monoterpenes of Scotch pine. For. Sci. 17(3):293-299.

Von Rudloff, E. 1962. Gas-liquid chromatography of terpenes. Part V. The volatile oils of the leaves of black, white and Colorado spruce. Tappi 45(3):181-184.

———. 1964. Gas-liquid chromatography of terpenes. Part X. The volatile oils of the leaves of sitka and Engelmann spruce. Can. J. Chem. 42:1057-1062.

———. 1966. Gas-liquid chromatography of terpenes - XIV. The chemical composition of the volatile oil of the leaves of Picea rubens Sarg. and chemotaxonomic correlations with other North American spruce species. Phytochem. 5:331-341.

———. 1967. Chemosystematic studies in the genus Picea (Pinaceae) I. Introduction. Can. J. Bot. 45:891-901.

———. 1972. Seasonal variation in the composition of the volatile oil of the leaves, buds, and twigs of white spruce (Picea glauca). Can. J. Bot. 50(7):1595-1603.

———. 1975a. Volatile leaf oil analysis in chemosystematic studies of North American conifers. Biochem. Syst. Ecol. 2:131-167.

———. 1975b. Seasonal variation of the terpenes of the leaves, buds, and twigs of blue spruce (*Picea pungens*). Can. J. Bot. 53:2978-2982.

———. 1975c. Seasonal variation in the terpenes of the foliage of black spruce. Phytochem. 14:1695-1699.

———. 1977. Variation in leaf oil terpene composition of sitka spruce. Phytochem. 17:127-130.

———, and M.J. Holst. 1968. Chemosystematic studies in the genus *Picea* (Pinacea) III. The leaf oil of a *Picea glauca* x *Picea mariana* hybrid (Rosendahl spruce). Can. J. Bot. 46:1-4.

Von Schantz, M. and S. Juvonen. 1966. Chemotaxonomische untersuchungen in der gattung *Picea*. Acta. Bot. Fenn. 73:1-51.

Watts, W.A. and H.E. Wright, Jr. 1966. Late Wisconsin pollen and seed analysis from the Nebraska Sandhills. Ecol. 47(2):202-210.

Weaver, T.W. III. 1965. Variation in the spruce complex of the northern Rocky Mountains, *Picea glauca*, *Picea pungens*, *Picea engelmannii*. Masters thesis, Univ. of Montana.

Wells, P.V. 1970. Vegetational history of the Great Plains: A post-glacial record of coniferous woodland in southeastern Wyoming. IN/ (W. Port, Jr. and J.K. Jones, Jr., eds.) Pleistocene and recent environments of the central Great Plains. Univ. Press Kansas, Manhattan. pp. 185-202.

Wilkinson, R.C. and J.W. Hanover. 1972. Geographical variation in the monoterpene composition of red spruce. Phytochem. 11:2007-2010.

Wilkinson, R.C., J.W. Hanover, J.W. Wright, and R.H. Flake. 1971. Genetic variation in the monoterpene composition of white spruce. For. Sci. 17(1):83-90.

Wolfe, J.A. 1969. Neogene floristic and vegetational history of the Pacific Northwest. Madrono 20:83-110.

Wolfe, J.A. 1972. An interpretation of Alaskan tertiary floras. pp. 201-233. IN/Graham, A. (ed.) Floristics and paleofloristics of Asia and Eastern North America, Elsevier Publ. Co., Amsterdam.

Wright, H.E. 1970. Vegetational history of the Central Plains. IN/ (W. Dort, Jr. and J.K. Jones, Jr., eds.) Pleistocene and recent environments of the central Great Plains. Univ. Press Kansas, Manhattan. pp. 157-183.

Wright, J.W. 1955. Species crossability in spruce in relation to distribution and taxonomy. For. Sci. 1(4):319-349.

Yablokov, A.S. 1960. Wide hybridization in silviculture and greenbelt work. Survey and Prospects. IN/ (N.V. Tsitsin, ed.) Wide hybridization of plants. Proc. of the Conf. on wide hybridization of plants and animals. IPST, Jerusalem, Israel. pp. 48-61.

Zavarin, E., W.B. Critchfield, and K. Snajberk. 1969. Turpentine composition of Pinus contorta x Pinus banksiana hybrids and hybrid derivatives. Can. J. Bot. 47:1443-1453.

Zavarin, E., W.B. Critchfield, and K. Snajberk. 1978. Geographic differentiation of monoterpenes from Abies procera and Abies magnifica. Biochem. Syst. Ecol. 6:267-278.

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

———. 1972. Geographical variability of monoterpenes from Abies balsamea and A. fraseri. Phytochem. 11:1407-1421.

Zavarin, E., K. Snajberk, and R. Derby. 1980. Terpenoid and morphological variability of Pinus quadrifolia and its natural hybridization with Pinus monophylla in northern Baja California and adjoining United States. Biochem. Syst. Ecol. 8:225-235.