This is to certify that the

dissertation entitled

Carbon Allocation and Secondary Metabolites in Abies grandis as Influenced by Nitrogen Fertilization and Seed Source

presented by

Rose-Marie Muzika

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Forestry

Kurt S. Pregitzer
Major professor

Date June 6, 1989

CARBON ALLOCATION AND SECONDARY METABOLITES IN *ABIES GRANDIS* AS INFLUENCED BY NITROGEN FERTILIZATION AND SEED SOURCE

By

Rose-Marie Muzika

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Forestry
Program in Ecology and Evolutionary Biology

1989

ABSTRACT

CARBON ALLOCATION AND SECONDARY METABOLITES IN *ABIES GRANDIS* AS INFLUENCED BY NITROGEN FERTILIZATION AND SEED SOURCE

By

Rose-Marie Muzika

Carbon allocation patterns were examined with respect to secondary metabolite production, growth and structure. Specifically, terpenoid and phenolic compounds in foliage were identified and quantified and height growth, biomass, and root to shoot ratio were determined. The overall objective of this research was to provide an understanding of allocation patterns as influenced by seed source of *Abies grandis* (Dougl.) Lindl., (grand fir). Changes in allocation with nitrogen fertilization were also examined.

Collection areas represented contrasts in physiography, vegetation, and soil types of five regions: western Montana, Cabinet Mts. of northern Idaho, Clearwater Mts. of north-central Idaho, East Cascades of west-central Washington, and the Olympic Mts. on the Olympic Peninsula of western Washington. These regional populations had at least two local populations which differed in site attributes.

Data were obtained from harvested one-year old seedlings grown under continuous light and three levels of nitrogen: *i)* control - no addition of nitrogen to nitrogen-deficient medium, *ii)* NH^+-NO_3^- to a final concentration of 22.4 g $N \cdot m^{-2}$, *iii)* NH^+-NO_3^- to a final concentration of 44.8 g $N \cdot m^{-2}$.

While physiographic features such as elevation and topography generally relate closely with frost hardiness, growth, or drought stress in many conifers of western North America, these do not seem to provide a selective force in the production of secondary chemicals in *Abies grandis*. There was, however, a strong correspondence between height growth, biomass and seed source.

Nitrogen significantly reduced the production of total phenolic compounds in most populations. The yield of terpenes was not affected; however, certain seedlings did respond to nitrogen by altering terpene metabolism within selected seed sources. Typically, nitrogen depressed production of beta-phellandrene, camphene, bornyl acetate and terpinolene, but only in some populations. Other terpenes were unaffected by nitrogen, but many demonstrated a significant interaction effect at the local level.

Phenolics were grouped into cinnamic acids, benzoic acids, and flavonoids, the latter typically the greatest with all treatments. Changes in these groups were not uniform with treatments, nor with population. Evidence for direct depression of individual phenolics with applied nitrogen was not apparent. Contrary to results of total phenolics and with current theory, however, two phenolic compounds, coniferyl alcohol and para-hydroxybenzoic acid, increased significantly with fertilization.

ACKNOWLEDGEMENTS

Although only one name appears as the author of this work, it represents the creation of numerous individuals, and to these people I extend my gratitude. My advisor and friend Kurt Pregitzer has served as mentor, motivator, and sparring partner, and has been a beacon of light for my endeavors. Other members of my committee, Drs. James Hanover, William Mattson, Donald Dickmann, Peter Murphy, and John Beaman, have been helpful in guiding my efforts and I truly appreciate their contributions.

The Idaho Forest Research Council and Dennis Joyce are responsible for a splendid *Abies grandis* seed collection. I thank Maria Pregitzer for field assistance and good food, and Dr. Carl Ramm for good advice, statistical and otherwise. Roy Prentice instructed me in the rearing of glass-house seedlings, and I remain appreciative of his efforts.

I extend much gratitude to Charles Campbell, Debora Gaudiello, Shyong Bao-Jen, and Dr. Andrew Smith, the most brilliant people on the planet, or at least M-13. My remarkable colleagues and friends, Dr. George Host, Diane Burbank, Daniel Herms and Brian Palik, have helped me immensely. I am indebted to my good friends who have endured with me: Carol Hyldahl, Michael Swisher, Ellen Prusok, and Jeffrey Deason.

For all their unrelenting love, I thank my wonderful family and for coping I thank Richard Hensh.

I am grateful for having known, albeit briefly, the late Dr. Jonathan W. Wright who began this journey with me. He sharpened my senses, enlightened me, always kept me thinking, and taught me the wonder of the genus *Abies*.

This dissertation is dedicated to my parents, without whose steadfast encouragement and devotion my aspirations would be weakened and without whose love my accomplishments would be meaningless.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The ways in which a plant uses nutrient resources and allocates carbon reflects the most opportune mode of existence in a given environment. For this reason, it seems unlikely that different species, particularly those growing in differing environments, would be similar in carbon allocation patterns. The potential for a plant to grow, reproduce, and endure a particular environment relies heavily on allocation of carbon resources. Ayala (1969) used the relationship of carbon allocation and environment as an expression of a measure of adaptedness to a given environment in that the ability of a population to transform materials into living matter, i.e. dry matter production per time and area defines adaptation.

Given the fact that a plant possesses a specific quantity of energy, resources, and ultimate products, the plant must partition these such that all conditions (plant fitness) are optimized. Obvious priorities of a plant are reproduction and survival. Both phenomena are strategy-dependent and differ among plants, but any amount of photosynthate and energy used for purposes that seem to be unrelated to growth, survival, and reproduction represent a portion of photosynthate unusable for vital processes. Other less apparent outlets of allocation represent products of secondary metabolic processes, storage products, intermediate products, or growth regulators, any of which may be vital for the plant. Breeding strategies incorporate the

optimization of carbon balance in plant populations subjected to spatial and temporal environmental fluctuations and constraints.

Primary metabolism fuels the plant with a diverse array of products such as amino acids, sugars, nucleotides, and acetyl CoA. Several primary metabolites, e.g. shikimic acid or mevalonic acid serve as origins or dominant intermediates in secondary metabolic pathways. Primary metabolites are universal and are intimately involved in all essential life processes, whereas secondary metabolites have a limited distribution, presumably important to the organism producing them, but generally non-essential for vital functions. Information about secondary metabolism in general, particularly the importance of secondary products, remains obscure. Nonetheless, these are produced on a relatively consistent basis within a species, and must therefore, present a cost to the plant.

Until recently most ecological and physiological research involving carbon balance of plants has examined the modes and operation of capturing and producing photosynthates. An increasing emphasis has been placed on ability of plants to partition photosynthate to serve vital functions.

Allocation patterns have largely been examined on a very gross level - e.g. reproductive versus non-reproductive, root to shoot production. The extent to which physiological responses and processes are governed by allocation remain generally unknown. Even production at the biochemical level has been on a broad scale, and alterations in particular pathways have not been closely examined. Only recently has production of chemicals been examined as an important avenue of plant allocation and as an adaptive response to resource availability.

The idea that environment greatly influences the balance of carbon and allocation patterns has been demonstrated several times, primarily with variations in morphology and growth. Stress factors such as moisture or nutrient deficiencies as well as the amelioration of resources has dramatic influence on structural carbon allocation. Few of these studies of environmentally-induced changes in carbon allocation, however, have examined physiological, let alone biochemical changes in allocation.

Certain differences in allocation patterns would be expected with different species, as well as differences over time and with environmental conditions. Both phylogenetic constraints in biochemistry and temporal environmental constraints determine allocation patterns. Bazzaz *et al.* (1987) suggest that the allocation issue may be examined at three levels: evolutionary, ecological, and physiological. The first level addresses plant fitness and presents the general issue of allocation to fecundity and reproductive effort versus the allocation to components of survival like growth and vigor. At the ecological level, the question relates to the investment in one function versus that of another. Studies at the ecological level seek to define the question of relevance of such products as secondary chemicals. The importance of the physiological level involves the partitioning of resources within the plant and consequences of resource gain and loss. Physiological responses to resource availability (or other factors important in controlling carbon balance) naturally accompany changes in allocation patterns. It is important to focus on the factors that regulate the observed changes in patterns of allocation under various circumstances.

Discussions concerning production of secondary metabolites as defense mechanisms have been emphasized since the first allusion to that

purpose of chemicals by Frankel (1959). Unfortunately, much of the research verifying the importance of defense chemicals has been correlative and unsubstantiated. Gulmon and Mooney (1986) have attached actual costs of production of these chemicals in terms of CO_2 fixed, hence quantifying these as a sink for plant resources. Defensive compounds notwithstanding, numerous other chemicals are produced by plants, most of which have functions which remain uncertain. Regardless of function, a 'cost' remains. This cost acts as an important factor in the economy of carbon allocation in a plant. Although often supplanted by more obvious carbon sinks, e.g. leaves, roots, stems, any amount of photosynthate used for production of compounds not directly related to primary metabolism represents an uncertain drain and unavailable resource for primary functions.

Analyses of secondary chemicals have traditionally aimed at examining changes in chemistry with a change in environment (perhaps subsequent nutritive quality), or to distinguish ecotypes based on secondary metabolic characteristics (chemotypes). The former tends to ignore the genetic component while the latter excludes [often] environmental effects. The intent of the present study was to assess both the influence of environment, (both natural and imposed) and seed source on carbon allocation.

Recent research has addressed the issue of carbon allocation specifically to secondary metabolites with the underlying assumption of a subsequent effect on the growth of a plant; yet studies have not closely examined tradeoffs in growth and production of chemicals. In other words, specific changes in carbon allocation patterns have not been identified.

The research of secondary chemicals has arisen from an interest in causes of insect or herbivore-plant interactions, primarily with the suggestion that these chemicals serve as defense. Pathologists and entomologists have approached the questions of production of secondary metabolites as: "under which conditions are plants more susceptible to predators and pathogens?" Much of the research involved the relation of nutrient status with production of chemicals. Prominent theories maintain that environment, especially conditions of resource availability dictate the type and amount of defenses formed (Coley *et al.* 1985). These defenses, subsumed under the category of secondary metabolites, represent a critical facet of carbon allocation. Explanations of secondary metabolite production presume that the chemistry is adaptive and that evolutionary constraints interact in that the environment in which a plant has adapted determines the types and amounts of secondary metabolites produced. Most of the theory is largely generalized and does not include discussions of intra- and interspecific variation or differences at population or family levels. Moreover, very little theory has been based on experimentation.

Coley (1987) suggests that allocation patterns are so closely regulated that growth rate explains interspecific variation in patterns of secondary chemical production. The "availability" hypothesis (Mattson 1980) maintains that an optimization in vital processes, (reproduction, growth and chemical defense) must occur, and that plants have evolved different allocation patterns to secondary chemical production because of constraints of reproduction and growth. This prevailing thought accents the idea that the production of N-based secondary chemicals would be enhanced under conditions where N supplies exceed that amount required for growth.

Conversely, non-nitrogenous compounds would be formed when carbon supplies are above adequate for growth, and N is presumably limiting.

Phenolic compounds form the most prominent classes of natural products in plant. These are very reactive products chemically and easily subjected to oxidation, substitution and coupling reactions. All have an aromatic ring with at least one hydroxyl and phenolic acids (*sensu stricta*) bear one phenolic hydroxyl group and a carboxy function. This class of compounds has often been the object of studies concerning herbivory or pathogen resistance, but environmental conditions affecting their production remain largely unknown. The production of phenolics, both as a group, and individual phenolic compounds will be examined in this research.

Terpenoids also form a prominent class of plant secondary compounds. These are formed via the mevalonic acid pathway from head to tail condensation of individual isoprene units, C_5H_8. Depending on the number of isoprene units in the compounds, this pathway may formulate monoterpenes ($C_{10}H_{16}$), sesquiterpenes, ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), or tri-, tetra- or polyterpenes. Most noted for the monoterpenoid essential oils, this group of compounds also includes gibberellins (diterpenes) or mixed terpenoids such as chlorophyll (Goodwin 1967.)

The question to which this research is directed is not if there exists any inherent value to production of chemicals, nor the genetics of secondary metabolism, nor the factors affecting the production of these chemicals universally. Rather, the purpose of this research is to enlighten and elucidate important components of the question of how a plant utilizes resources and allocates photosynthates. Moreover, the purpose is to understand the control

of seed source and the control of environment on carbon allocation within a species, with an emphasis on allocation to secondary chemicals.

This research aims to examine 'genetic' differentiation, but in a non-traditional manner. Rather than address differences in morphology, anatomy, or general chemical markers, genetic differentiation is examined as a physiological or ecological response. The underlying premise of this is that the maximization of fitness via allocation and carbon balance depends on genotype and that this dependence may be modified by environmental factors.

OBJECTIVES AND HYPOTHESES

The goal of this research was to understand the patterns of carbon allocation to growth, phenolics and terpenoids in grand fir (*Abies grandis*). Differences in carbon allocation patterns among populations, and differential responses of these populations to nitrogen fertilization were addressed. The research identifies alternative avenues for carbon utilization and how these change in relation to each other given a change in nitrogen availability.

The specific objectives of this study were:

1. to examine carbon allocation patterns in terms of growth and production of two groups of secondary metabolites: terpenoids and phenolics.

2. to understand patterns of allocation among various seed sources (regional populations), and to the extent possible, local populations within the regional populations.

3. to study the effect of nitrogen fertilization on allocation to structure and secondary metabolism.

4. to examine the response to N fertilization among the various populations.

5. to relate ecological field data to observed differences in biochemical and morphological responses of populations to determine if the selective forces that control phenotypic expression of a species operate at the community level in determining forest community attributes.

Hypothesis #1

No differences exist in carbon allocation patterns within the species, *Abies grandis*, or among regional or local populations of this species. Patterns in root and shoot biomass and in production of secondary metabolites are relatively constant within a species, all being strongly constrained by the genetic architecture of the species. Environmental factors will have little control over the balance of carbon.

Alternately, differences in regional and local environments and even individual half-sibling families will be manifested in variation in carbon allocation. Height growth, root biomass, shoot biomass, and allocation to terpenes and to phenolics will reflect differences in seed source origin.

Hypothesis #2

Fertilization with nitrogen will not produce a difference in allocation of resources to secondary metabolites. The production of terpenes and phenolics will be influenced minimally by nitrogen amendment, since nitrogen is not incorporated into the structure of the secondary compounds specifically examined.

Although allocation to structure has been shown to change with fertilization, changes in allocation to secondary metabolites has not been verified. Given the opportunity for increased growth with increased nitrogen fertilization, a disproportionate amount of carbon resources will be allocated to the growth process and little to production of secondary metabolites.

Hypothesis #3

Each population of *Abies grandis* (at all levels) will respond to N fertilization in the same manner. There will be no differences in the manner in which carbon is allocated for structural growth or secondary metabolites. Although population differentiation among tree species has been readily manifested in growth, morphology, or frost hardiness, differentiation cannot be demonstrated through carbon allocation patterns.

Alternately, the response to N will not be uniform for *Abies grandis*, and will be highly dependent on seed source. Allocation will directly reflect the habitat of origin, particularly nutrient status as well as other factors, such as aspect, elevation and slope, that serve as strong selective forces for population differentiation in western North America.

METHODS

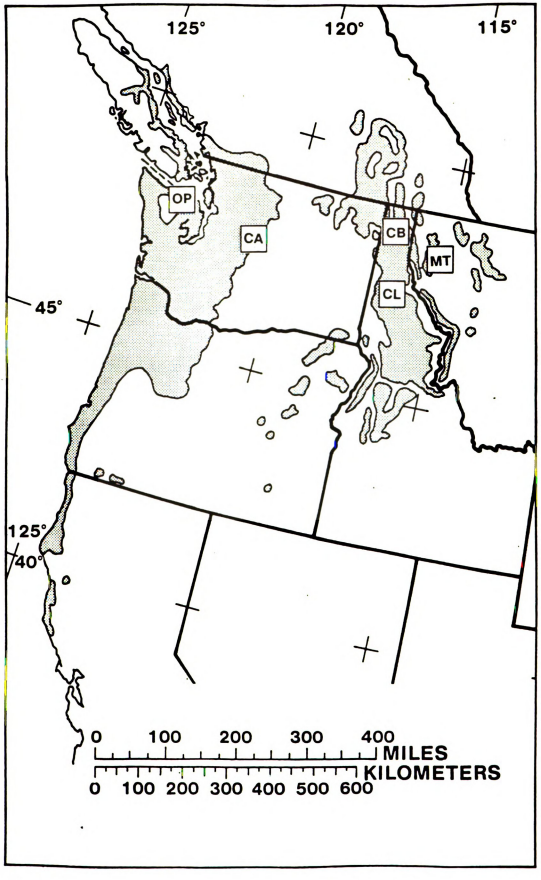
FIELD METHODS

Abies grandis is a mid-elevational species found from the Pacific coast of Vancouver Island on the west and northeast into southern British Columbia, south to Sonoma County in northern California along the coast, and east through Washington, Oregon, Idaho and into northwest Montana (Figure 1). It ranges from 51° to 39° N latitude and from 125° to 114° W longitude (Muller 1936). Altitudinally, *Abies grandis* is found from nearly sea level along the coast to an elevation of 1830 m in the Inland Empire (Figure 2). Best growth is attained at 900 m to 1500 m (Fowells 1964).

Abies grandis seed was collected from representative habitats and site conditions along an east-west longitudinal macroclimatic gradient. The regional populations are shown on Figure 1. Latitude was kept relatively constant in the sampling since it has been shown to be a strong selective force in population differentiation of conifers in western North America (Campbell 1979, Rehlfeldt 1978). Along this transect, growing period decreases and severity of summer drought increases as one moves eastward (Kincer 1941).

Broad regions were chosen to represent the diversity of climatic conditions within the range of this species. Within each regional population, pairs of local populations were sampled. The paired populations were separated by no more than 10 km but more than 500 feet in elevation, and they were collected from different aspects. Maps depicting distances between local populations are included in Figure C.1. These pairs represented differing ecological conditions: *i*) relatively warm-dry ecological conditions, at low elevations and south-facing slopes, and *ii*) cool-moist

Figure 1. Range map of *Abies grandis*, from Little (1971), with locations of regional populations (seed sources) indicated :
MT = Montana
CB = Cabinet Mts.
CL = Clearwater Mts.
CA = Cascade Mts
OP = Olympic Mts.


125°
120°
115°
OP
CA
CB
MT
CL
45°
125°
40°
0 100 200 300 400
MILES
0 100 200 300 400 500 600
KILOMETERS

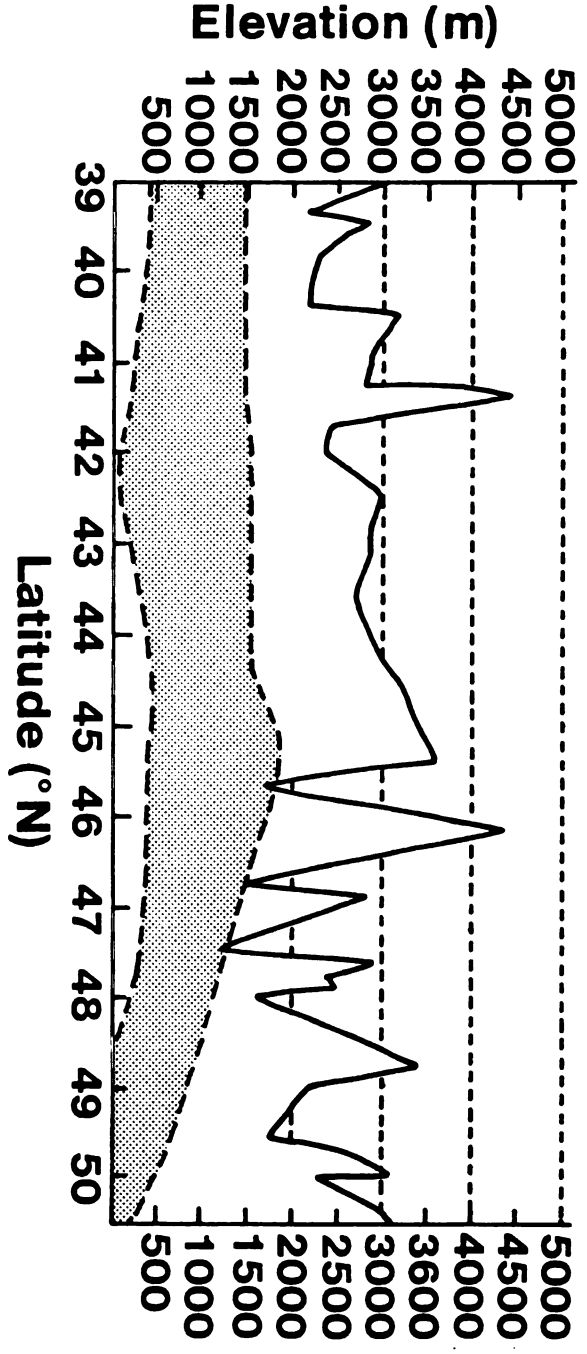


Figure 2. Elevational distribution of *Abies grandis*. Dotted area indicates distribution of grand fir, solid line indicates maximum elevation (Lui, 1971).

conditions at higher elevations, along north-facing slopes. General site information is included in Table 1, with more detailed information in Appendix C. Although seeds were procured from a total of eighteen local populations, germination and viability were low, thus some seed sources are not represented by seedlings and were not included in this study.

Seeds were collected during the late summer of 1983 from the upper crown of six open-pollinated trees at each of the local populations. Seed trees selected within a given population were separated by at least 50 m and were representative of a random sample of those phenotypes that were bearing cones.

Seeds from the individual trees were cleaned and stored separately. Seeds from each tree provided seedlings that were at least half-sibling families, with an unknown proportion of full-siblings.

Seed source location data were obtained in the summer of 1986 and 1987 by relocating seed collection sites. A representative plot center was chosen to correspond with location of seed collection, in an area as uniform as possible, and representative of all six trees. Canopy coverage of all species less than 1.5 m tall was estimated by cover classes used in habitat typing (Pfister *et al.* 1977), within a 1000 m^2 circular plot. Habitat typing nomenclature of Daubenmire and Daubenmire (1976) and Franklin and Dyrness (1973) was also used in identifying habitat types of northern Idaho and Washington. Near the plot center a soil pit was dug and soil and parent material were characterized. General topographic features of the surrounding area and the plot were noted. Within the 1000 m^2 each tree over 7.6 cm was measured to the nearest tenth cm. Taxonomic nomenclature

Table 1. Names, abbreviated reference, physiographic location and selected site information for grand fir seed collections.

		Elevation	Aspect	Slope
Flathead Range, western Montana				
MT 1 Hog Heaven	Mission Mts.	1444 m	SSW (220°)	22%
MT 2 Jewel Basin	Swan Mts.	969 m	WSW (244°)	3%
MT 3 Porcupine Creek	Swan Mts.	1297 m	ENE (80°)	40%
MT 4 Cilly Creek	Swan Mts.	1099 m	WSW (244°)	8%
Cabinet Mts., northern Idaho				
CB 5 Gleason Meadow	Selkirk Mts.	760 m	SSE (144°)	1%
CB 6 Galena Point	Selkirk Mts.	1314 m	SSW (200°)	32%
CB 7 Trestle Ridge	Cabinet Mts.	1440 m	NNW (295°)	42%
CB 8 Trout Creek	Cabinet Mts.	780 m	ENE (80°)	12%
Clearwater Mts., north-central Idaho				
CL 9 Moscow Mt.	Palouse Range	1036 m	WSW (258°)	35%
CL 10 Brown's Meadow	Palouse Range	902 m	NNE (20°)	27%
CL 11 Ove Creek	Clearwater Mts.	885 m	NNE (30°)	70%
CL 12 Riggear Peak	Clearwater Mts.	660 m	S (184°)	31%
Eastern Cascade Mts., Washington				
CA 13 Davis Creek	Wenatchee Mts.	982 m	NW (310°)	13%
CA 14 Teanaway Ridge	Wenatchee Mts.	1439 m	NE (50°)	28%
CA 15 Alder Creek	Entiat Mts.	687 m	S (175°)	5%
CA 16 Entiat Mt.	Entiat Mts.	959 m	NNE (36°)	45%
Olympic Mts., Olympic Peninsula, Washington				
OP 17 Dungeness	Olympic Mts.	510 m	E (95°)	13%
OP 18 Hamma Hamma	Olympic Mts.	242 m	S (194°)	16%

followed Hitchcock and Cronquist (1973). Vegetation data for all sites can be found in Table C.1. - C.4. of Appendix C.

Elevation was measured with a Terra Altimeter Model MT-5 calibrated using a nearby benchmark and corrected for temperature. Slope (percent) , and angle of the horizon above the horizontal (degrees) were measured with a clinometer, and aspect with a compass.

A composite soil sample for mineralizable nitrogen was taken from random areas within the 1000 m^2 plot . Mineralizable nitrogen was measured using a modified technique by Powers (1980). Five grams of sieved, air dried soil were added to 16 x 150 mm test tubes containing 12.5 ml of distilled water. Tubes were sealed and incubated for 14 days in darkness at 30 °C. Soil solution was centrifuged with 4.0 N KCl for one minute. Samples were filtered and diluted to 25 ml and analyzed with a Technicon autoanalyzer.

GREENHOUSE METHODS

Seeds were sown in 7.62 cm x 7.62 cm x 30 cm (tall) cardboard pvc-coated bands, housed in cartons that contained 36 bands per carton. Seeds were randomly sown with one family of half-siblings per band. The first sowing was done on 3 March 1986. The purpose of this experiment was to gather general information about growth and secondary metabolite variation in the species. The medium consisted of a mixture of perlite, peat, and vermiculite in the proportions of 1:2:1. These seedlings, as well as the seedlings in the successive studies were grown under continuous light and

accelerated growth conditions (Hanover 1980). Seedlings were harvested after one year.

A second sowing was completed in October 1986 for examination of treatment differences. The previously mentioned media and technique were used. After six months, the seedlings were divided into three groups. One group was fertilized with NH_4^+-NO_3^- as a 7.3 ppm solution such that final concentration was equivalent to 22.4 $g \cdot m^{-2}$. Another group was fertilized with a 14.6 ppm solution such that final concentration was equivalent to 44.8 $g \cdot m^{-2}$. Treatments were biweekly for a duration of five months (ten treatments) with 50 ml water each treatment. A third group of seedlings (control) was provided with equivalent volumes (50 ml) of deionized water each treatment time . The nitrogen level of the control soil mixture ranged from 110 to 130 ppm.

When seedlings were harvested, root and shoot were separated at the point exactly midway between the first lateral root and lowest branch. Shoot fresh weights, including foliage, were determined. Roots were dried to constant weight at 70 °C. A subsample of shoots was dried and regressions developed for estimating the dry weight of biomass.

LABORATORY METHODS

Terpenes

Following harvesting of seedlings needles were removed after freezing with liquid nitrogen. Two grams of needles from each seedling were distilled for 8 hr in a circular still. Hexane was used to extract the condensate. Menthone was used as the internal standard, and K-values were determined

prior to analysis of samples on the gas-chromatograph. Approximately 0.5 ul of the distillate was injected into a Varian Gas Chromatograph equipped with a 30 m DX-3 column. This capillary column is semi-polar with approximately 50% polyethylene glycol. The temperature programming was as follows: 8 min at initial temperature of 70 °C followed by an increase of 6°/min with a final temperature of 220 °C, held for 15 minutes.

Phenolics

Needles were removed from branches with liquid nitrogen. The needles were then ground with mortar and pestle in liquid nitrogen so that they remained frozen, and subsequently freeze dried. Approximately 50 mg of freeze dried material was weighed into a test tube. One ml of acetone was added and the test tubes were placed in a sonicator for 30 min, after which the acetone was transferred into another flask. The acetone extraction was repeated three times, twice with 50% acetone. Following the acetone extraction, the needles were filtered and the acetone was removed. Each sample was extracted three times with CH_2CL_2. The extract was taken to volume in a 50 ml volumetric flask. This solution was then partitioned for Folin-Ciocalteu total phenolic analysis and for individual phenolic analysis using HPLC.

Exactly 10 ml of this aqueous solution was placed in a 25 ml volumetric flask for Folin-Ciocalteu total phenolic analysis. The process is a modification of the Folin-Ciocalteu method by Singleton and Rossi (1965). Folin-Ciocalteu solution (3.75 ml) was added to the 10 ml aqueous extract. After 30 seconds, but before 8 minutes had passed, 1.25 ml of 20% Na_2HCO_3 solution was added, and the solution was brought to volume. After two

hours, absorbance was measured at 765 nm with a Perkin-Elmer UV lamba spectrophotometer. Total phenolics were expressed as gallic acid equivalents (mg/l) by comparison with calibration curves prepared from standard solutions.

The remaining 40 ml of the aqueous solution was hydrolyzed with cellulase. The reaction took place overnight, in the dark. Following hydrolysis, a drop of H_3PO_4 was added and the solution was filtered and extracted three times with 40 ml, 30 ml, and 20 ml of ethyl acetate. The ethyl acetate was removed and the residue dissolved in 1 ml of acetonitrile (CH_3CN). Compounds were identified using GC-FTIR and GC-MS. When samples were used for the GC-MS, the residue was dissolved in BSTFA as a derivitizing agent.

A Waters HPLC was used in conjunction with an automated gradient controller, Waters data module, and WISP 710B autosampler. A Lambda-Max Model 481 Spectrophotometer was used at a wavelength of 280 nm and 0.2 absorbance units. A 75 microliter sample was injected onto a C_{18}, 3 micron cartridge.

The HPLC gradient was as follows: 98% aqueous phase (1% TFA) and 2% ACN isocratically for two minutes. For the next 33 minutes the gradient proceeds linearly to 70% aqueous and 30% ACN. The gradient was linear for 10 minutes to 100%, and returned to 98% acetonitrile and remains stationary for 15 minutes. Acetonitrile was recycled, and equilibrated for 10 minutes each run.

A blank was run at the beginning of each day and a phenolic standard mix containing 22 common phenolics was injected after several runs during a day.

Phenolic constituents were not quantified *per se,* because of unsuccessful attempts with internal standards, i.e. finding a compound that does not co-elute with a phenolic present in the extract. The peak areas of compounds of interest were used as an estimate of quantity and individual phenolics were expressed as a percentage of total peak area.

NUMERICAL ANALYSIS

Terpene data from the gas chromatograph and phenolic data from the HPLC were transformed using logarithmic and arc sine transformations, respectively. Statistical analyses were performed using the reciprocal averaging option of DECORANA (Hill 1979) and SAS (SAS 1985).

Treatment and population differences were assessed with SAS general linear models for unbalanced design. Differences in means were determined with Tukey's multiple comparisons (HSD). Correspondence analysis was performed with SAS/Interactive Matrix Language. Non-parametric statistics were used for determining the effect of nitrogen fertilization on production of individual phenolics. Specifically, the Smirnov two sample test statistic (Conover 1971) was used for assessing variation from control levels.

RESULTS

SITE CHARACTERISTICS

Table 1 contains an abbreviated description of seed sources and pertinent information. This table contains codes by which the individual populations will be subsequently referred. The first two letters represent the regional populations and the numbers refer to successive east to west local populations. Additional detailed site information appears in Appendix C.

Soil characteristics and edaphic attributes were among the site variables measured for each local populations. Results of some of these analyses are found below (Table 2.).

Table 2. Soil properties from local seed sources. Mineralizable nitrogen values (ppm) represent composite soil samples to 10 cm.

Local Population	Min. N	Parent Material	Soil Depth
MT1	5.80	Fluvial materials	> 60 cm
MT2	3.43	Sandstone	> 60 cm
MT3	4.23	Limestone	60 cm
MT4	3.77	Ash and tephra	> 75 cm
CB5	3.24	Volcanic	> 100 cm
CB6	4.27	Granite	75 cm
CB7	3.61	Granite	> 75 cm
CB8	6.83	Granite	> 60 cm
CL9	13.10	Volcanic ash	> 50 cm
CL10	2.04	Volcanic ash	> 50 cm
CL11	6.79	Basalt	> 100 cm
CL12	6.88	Basalt	> 100 cm
CA13	2.86	Basalt	> 100 cm
CA14	3.02	Basalt	60 cm
CA15	0.97	Granodiorite	90 cm
CA16	5.36	Quartz diorite	50 cm
OP17	2.26	Volcanic ash	> 100 cm
OP18	4.81	Fluvial materials	> 100 cm

The intent of sampling the specific seed sources was to procure seeds from sources representing a variety of habitats. Theoretically, habitat differences would result in changes in community composition corresponding to underlying environmental gradients. To examine differences in community structure, ground flora was used as an indicator of site variability. Cover values of ground flora species for each site were used in the reciprocal averaging option of DECORANA (Hill 1979), detrended correspondence analysis, to investigate how the sites differentiate in an ordination.

The first axis of the Detrended Correspondence Analysis represents a geographic gradient, with the two Olympic Mts. populations separated quite distinctly (Figure 3). Other populations separate well along the second axis. The four Cascade populations are grouped low on the DCA axis 2. Montana populations remain at the high end of DCA axis 2.

SEEDLING STRUCTURAL FEATURES

Height Growth

Under controlled conditions, the Olympic Peninsula populations exceeded all other populations in height growth (Figure 4). Differences were not significant between the Olympic Mts. population and the Cascades, but the Olympic mean height was significantly different from the others.

There were no differences with treatment among regional populations, except with for the Olympic population which produced significantly taller seedlings at both levels of fertilization ($P < 0.001$). Each

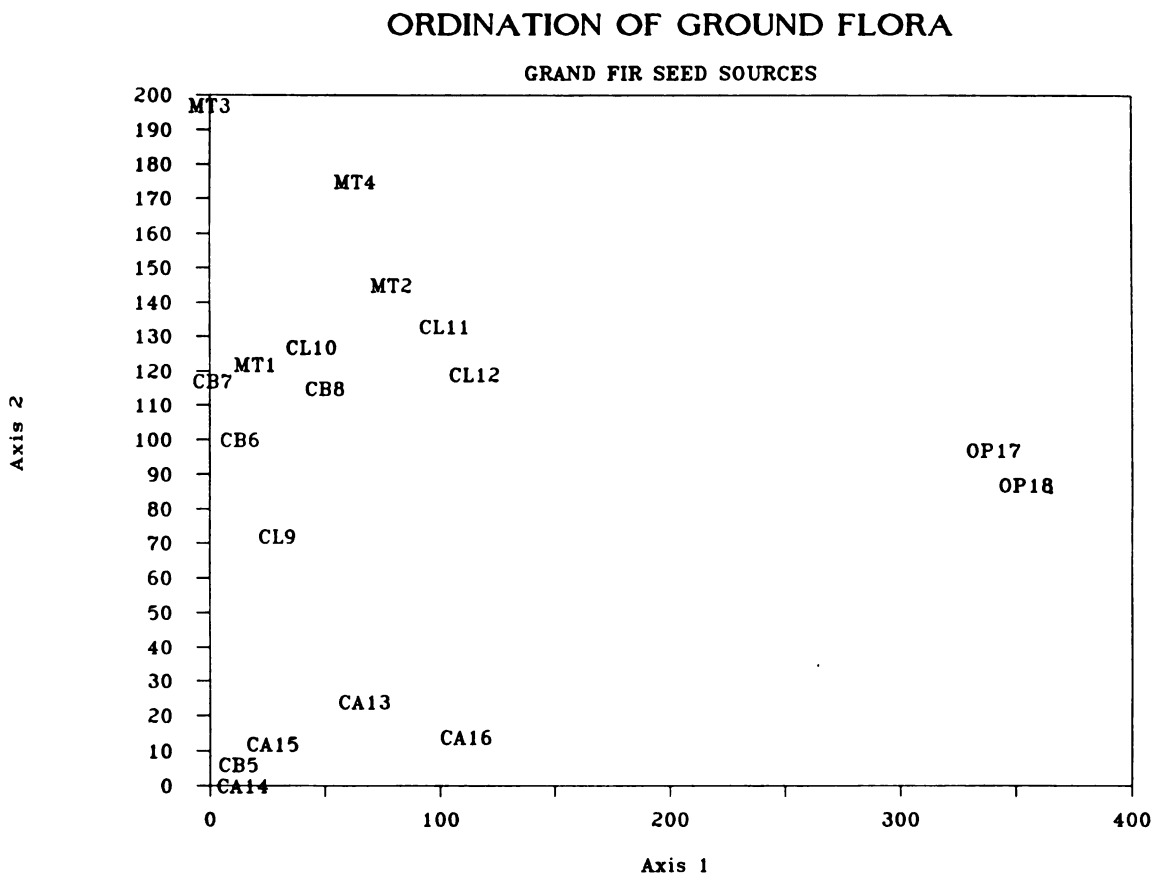


Figure 3. Ground flora ordination of local populations using DECORANA. Site notation follows Table 1.

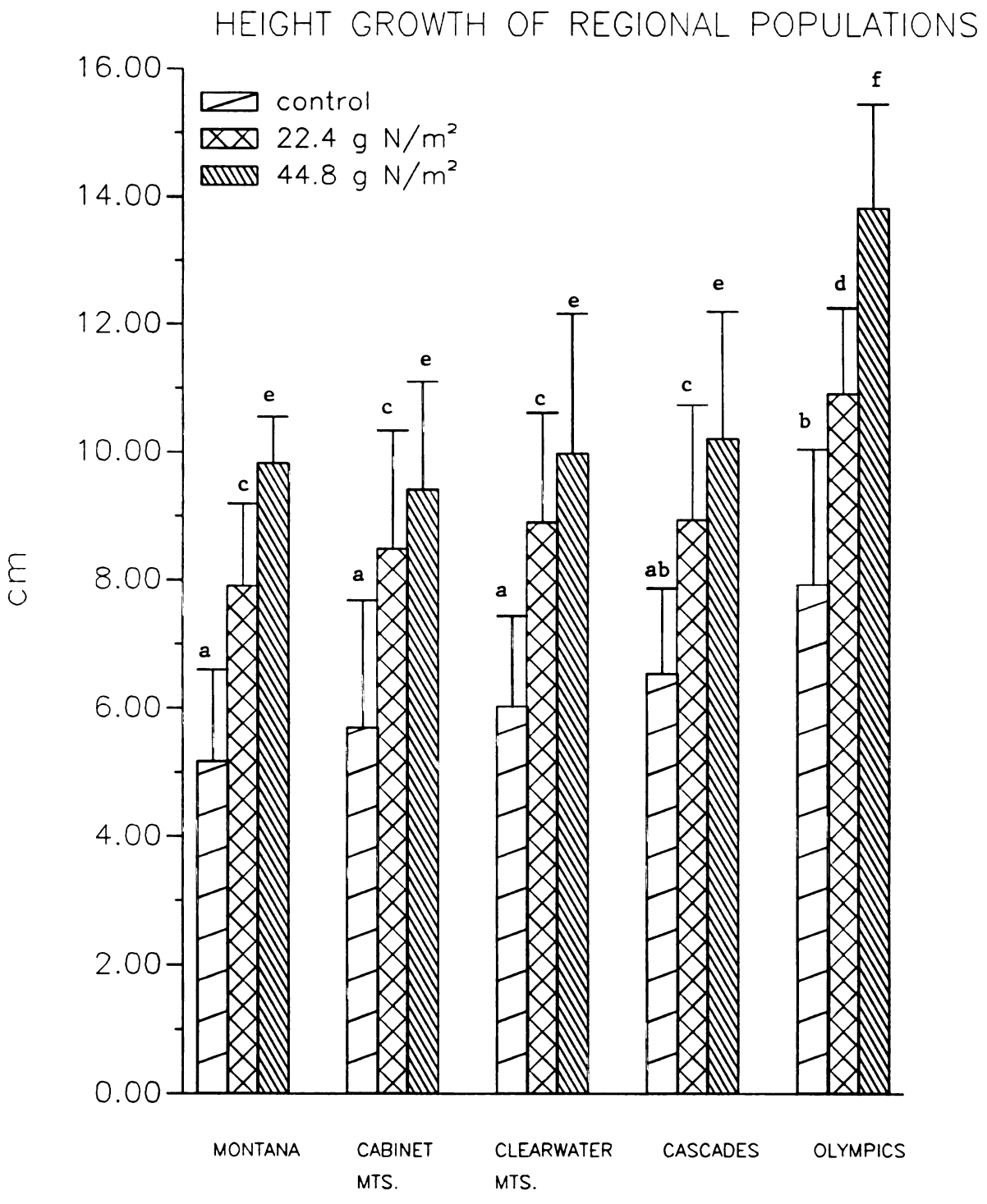


Figure 4. Average height growth of regional populations. Mean values with the same letter are not significantly different ($P < 0.05$).

treatment did result in a significant increase in height within each regional seed source ($P < 0.001$ in all cases).

To determine if intra-population (intra-seed source) variation existed, height growth of the local populations was examined using analysis of variance. Results are presented in Table 3. At the control level, local population Dungeness from the Olympic Peninsula differed significantly ($P < 0.05$) from two of the local populations of the Clearwater Mt. seed source - Brown's Meadow and Moscow Mt. Within the Clearwater Mt. seed source, however, both Brown's Meadow and Moscow Mt. were significantly shorter than Ove Creek ($P < 0.05$). There was also a differential response among these local populations. Moscow Mt. differed significantly from Brown's Meadow and Ove Creek ($P < 0.005$) at the highest nitrogen treatment. Clearwater Mts. represented the only regional population with intrapopulation variation of height growth. The differences among treatments within local populations corresponded to differences that arose among the regional populations.

Table 3. Difference in height growth (cm)(standard deviations) among local populations of one-year-old greenhouse grown seedlings. Mean values with the same letter are not significantly different ($P < 0.05$).

Populations	Treatment		
	control	22.4 g $N \cdot m^{-2}$	44.8 g $N \cdot m^{-2}$
MONTANA			
Porcupine Cr .	5.95(.212)^{a}	7.48(1.38)^{d}	9.15(0.07)^{e}
Cilly Cr.	4.63(1.76)^{a}	8.73(0.55)^{d}	10.5(0.73)^{e}
CABINET			
Trestle Ridge	5.28(1.22)^{a}	7.25(0.78)^{df}	8.85(1.63)^{e}
Trout Cr.	5.99(2.46)^{a}	8.72(1.94)^{df}	9.97(1.75)^{de}
CLEARWATER			
Moscow Mt.	5.45(1.31)^{ab}	8.72(1.93)^{df}	11.16(1.38)^{eg}
Brown's Mead.	5.58(1.13)^{ab}	8.19(1.41)^{d}	8.41(1.62)^{dh}
Ove Cr.	6.89(1.42)^{a}	9.53(1.68)^{df}	10.42(2.22)^{d}
CASCADES			
Davis Cr.	6.63(1.45)^{a}	9.67(2.17)^{df}	9.80(1.46)^{d}
Teanaway	6.22(1.53)^{a}	8.19(1.78)^{d}	10.53(1.54)^{e}
Alder Cr.	6.30(1.20)^{a}	9.33(1.36)^{def}	10.57(1.75)^{eg}
Entiat Mt.	6.67(1.27)^{a}	8.78(1.53)^{d}	10.29(1.47)^{d}
OLYMPICS			
Dungeness	7.92(2.11)^{ac}	10.9(1.35)^{f}	13.82(1.63)^{gh}

The combined model anova table (Table 4) indicates three sources of significant variation, the seed source, local population within seed source, and treatment ($P < 0.001$). The treatment x population term was not significant ($P < 0.05$) with either local populations within regional or with regional seed sources alone.

Table 4. Results of analysis of variance of height growth of seedlings as affected by nitrogen fertilization and seed source.

Source	df	SS	MS	F	P
Regional populations	4	254.966	63.742	23.78	< 0.0001
Local(Regional) populations	7	84.0	12.116	4.52	< 0.0001
Treatment	2	823.275	411.638	153.58	< 0.0001
Regional*Treat	8	32.504	4.063	1.52	*ns*
Loc(Reg)*Treat	14	39.916	2.851	1.06	*ns*
Error	266	719.934			

Table 5. Results of analysis of variance of root to shoot ratio of seedlings as affected by nitrogen fertilization and seed source.

Source	df	SS	MS	F	P
Regional populations	4	0.813	0.203	5.58	< 0.0003
Local(Regional) populations	8	0.339	0.042	1.16	*ns*
Treatment	2	4.620	2.310	63.36	< 0.0001
Regional*Treat	8	0.113	0.014	0.39	*ns*
Loc(Reg)*Treat	14	0.270	0.019	0.53	*ns*
Error	183	6.672			

Root to Shoot Ratio

Unlike height growth, root to shoot ratio (r:s) showed no significant differences among control values for regional seed sources. Addition of nitrogen, however, did depress the r:s, but to a lesser extent in the Cascade and Olympic populations than in the others. (Figure 5). Only in the Montana population did additional nitrogen (44.8 g $N \cdot m^{-2}$) further significantly decrease the r:s.

There were no differences among local populations in r:s control values. With addition of 22.4 g $N \cdot m^{-2}$ only local populations Brown's Meadow and Ove Creek differed from each other ($P < 0.005$). Both sites are in the Clearwater seed source.

The r:s anova table (Table 5) resembles the height growth analysis in that there are significant differences with regional and treatment effects, and no significant interaction. However, local populations within regional populations were not significantly different.

BIOMASS

Total biomass

Values for above and below ground biomass are presented in Table 6. Production of dry weight was the same for all populations, under controlled conditions. Each level of fertilization significantly increased biomass, and the Olympic Mts. population produced significantly greater biomass at the 44.8 g $N \cdot m^{-2}$ level than the other populations.

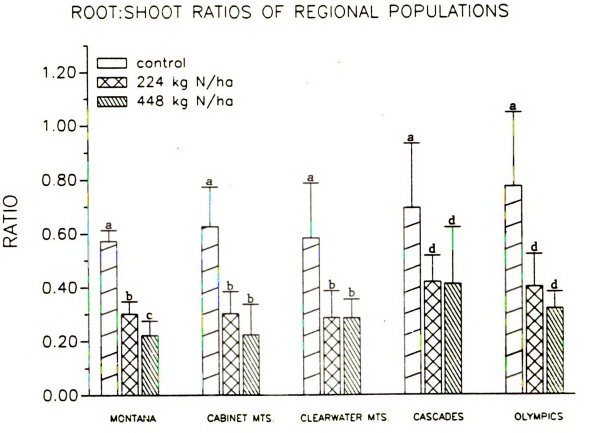


Figure 5. Average root to shoot ratios of regional populations. Mean values with the same letter are not significantly different ($P < 0.05$).

Table 6. Average total biomass (g dry wt) (standard deviations) of seedlings of regional populations of grand fir. Mean biomass values with the same letter are not significantly different ($P < 0.05$).

Populations	Treatment	
control	22.4 g $N \cdot m^{-2}$	44.8 g $N \cdot m^{-2}$
Montana		
.468(.139)[a]	.545(.060)[b]	.642(.124)[c]
Cabinet		
.394(.126)[a]	.516(.159)[ab]	.559(.141)[bc]
Clearwater		
.440(.134)[a]	.538(.157)[b]	.610(.157)[c]
Cascades		
.500(.168)[a]	.572(.136)[b]	.587(.179)[c]
Olympics		
.529(.160)[a]	.606(.178)[bc]	.741(.148)[d]

Shoot biomass

Shoot biomass did not differ significantly among regional populations under control conditions. Fertilization with 22.4 g $N \cdot m^{-2}$ significantly increased shoot biomass in all populations except Montana, although this population did increase with additional fertilization (Figure 6). Response was relatively uniform with all regional seed sources, except for the Cascade population which produced significantly less shoot biomass than the other populations at the highest nitrogen level.

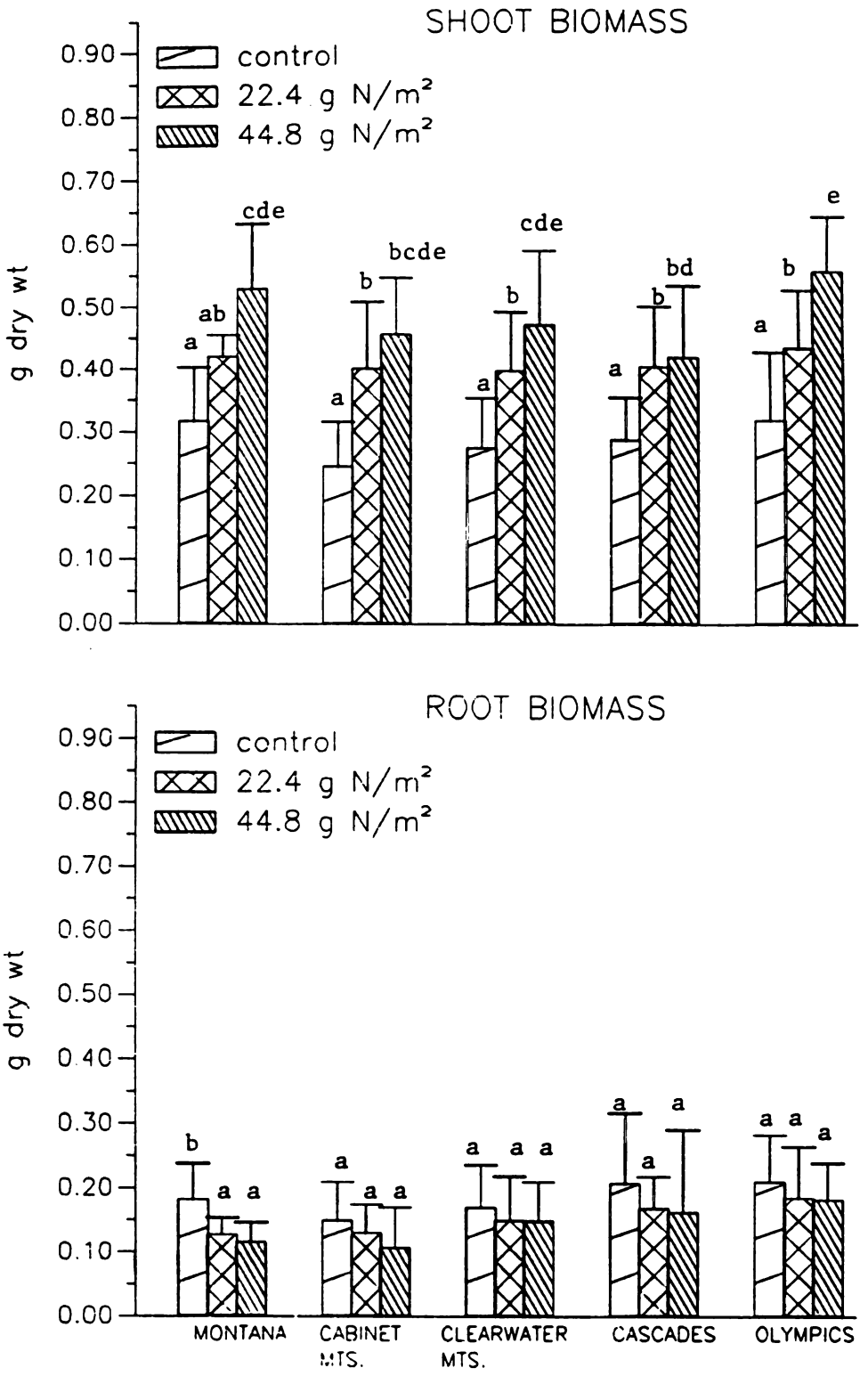


Figure 6. Average shoot biomass and root biomass of regional populations. Mean values with the same letter are not significantly different ($P < 0.05$).

Root biomass

Root biomass was uniform across the populations and with treatment (Figure 6). Root growth decreased with increasing nitrogen, but not significantly, except with the Montana population and then only between control and the 44.8 g $N \cdot m^{-2}$ level ($P < 0.05$).

There was intra-population variation, however, apparent when local populations were examined (Table 7). In the Clearwater Mts. alone Ove Creek (CL11) was significantly greater than both Brown's Meadow (CL10) and Moscow Mt. (CL9) at the control level. At the 22.4 g $N \cdot m^{-2}$ level, the same local populations differed. No differences were evident among the 44.8 g $N \cdot m^{-2}$ or among the Cascade local populations.

Local populations were examined to verify intra-population variability of all growth attributes (Table 7). Sufficient replicates for within population variation in all growth analyses were only available for the Cascade and Clearwater Mts. populations.

CHEMICAL FEATURES

Terpenes

Fourteen terpenes were extracted and identified in grand fir: twelve monoterpenes, one oxygenated monoterpene, bornyl acetate, and one sesquiterpene, cadinene. Figure 7 depicts some of the most common monoterpenes and their relative retention time. The most abundant of these was typically beta-pinene, followed by beta-phellandrene. These two, along with alpha-pinene and bornyl acetate usually accounted for 80 - 95% of total

Table 7. Differences in structural growth among local populations, and with treatment. Different letters in this table represent differences ($P < 0.05$) within each category.

C= control nitrogen level.

M=22.4 g $N \cdot m^{-2}$

H=44.8 g $N \cdot m^{-2}$

Local population	Height growth	Shoot biomass	Root biomass
	C M H	C M H	C M H
CB8	abc de d	ab c cd	- - -
CL9	a d f	a c d	a a a
CL10	a d f	ab c cd	a a a
CL11	bc de de	b c cd	b b ab
CA14	abc de f	ab abc abcd	ab ab ab
CA15	abc abcde f	ab abc abcd	ab ab ab
CA16	abc de f	ab c cd	ab ab ab
OP17	c e g	ab c d	ab ab ab

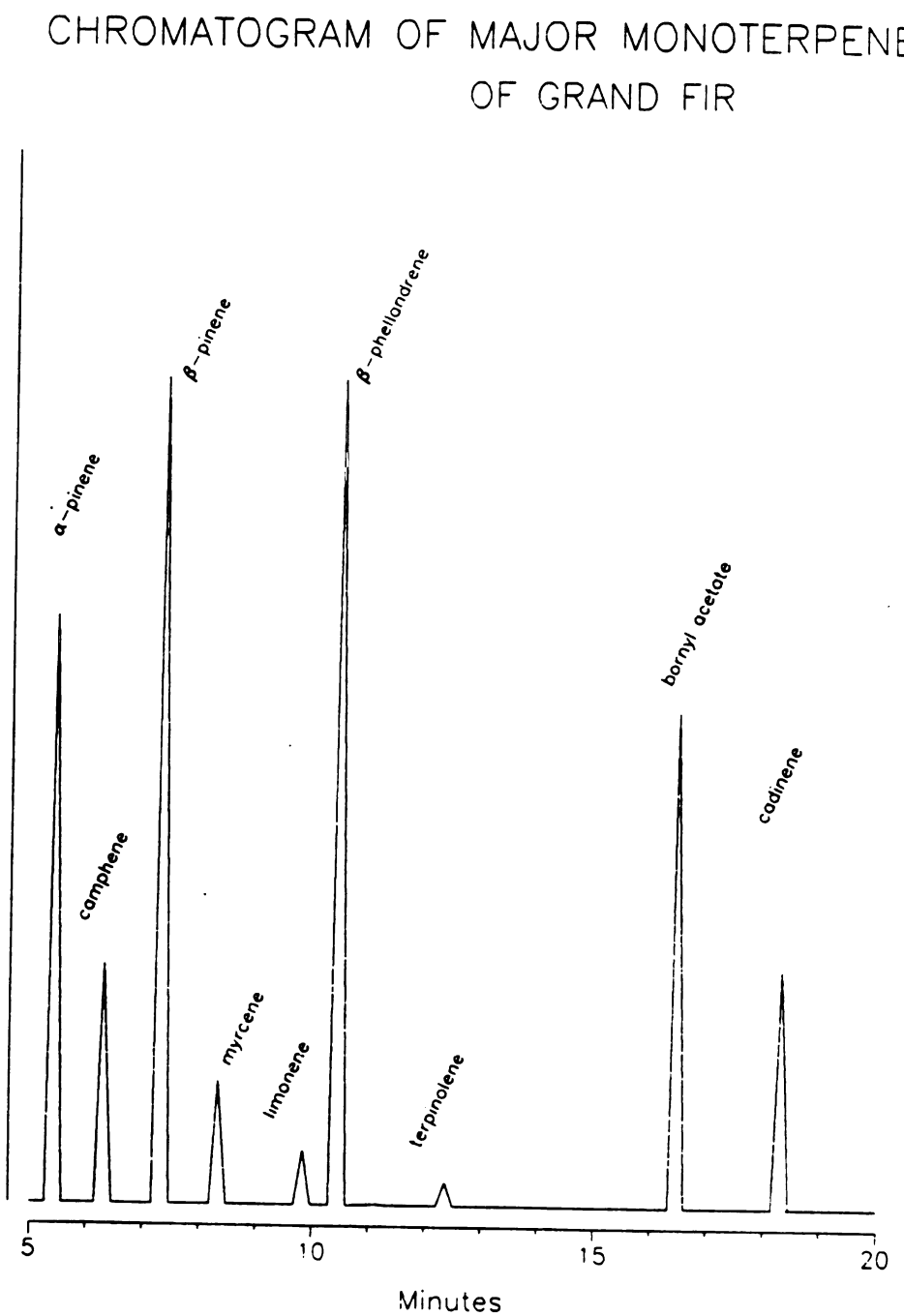


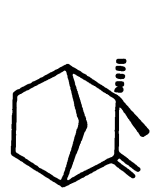
Figure 7. Representative chromatogram of some of the common monoterpenes of *Abies grandis*, indicating relative elution times on a DX-3 column. Height of peak corresponds to the relative amounts of each terpene.

quantity of terpene analyzed. Two terpenes, gamma terpinolene and camphor, occurred sporadically and in minute quantities, hence were not useful in data analysis. Structures of some of the monoterpenes appear in Figure 8.

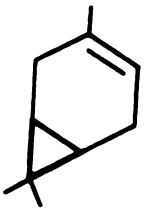
In the first study, which assessed the relative quantities and of each population for each terpene, differences among regional seed sources arose only in beta-phellandrene ($P < 0.005$), beta-pinene ($P < 0.005$), and tricyclene ($P < 0.05$). Table B.1 (Appendix B) lists values and standard deviations for regional and local populations. With beta-phellandrene, the Olympic Mts. population and the Cabinet Mts. differed; beta-pinene concentrations differed between Cabinet and Clearwater populations and the Cascades and Olympic populations. The Montana and Olympic populations differed in tricyclene concentration. In general, high intra-seed source variation exists.

The multivariate technique of correspondence analysis was used to determine the role and influence of terpenes in distinguishing seed sources. This represents the traditional mode of examining "chemotypes". The first two eigenvalues represent 80% of the total variation in the data set. The site scores and terpene scores are plotted in Figure 9. The terpene scores express the extent to which terpenes affect the analysis and site scores. The first axis is influenced by beta-phellandrene, myrcene, terpinolene, and others around these. The second axis is largely controlled by beta-pinene and bornyl acetate. Terpenes that aggregate around the 0,0 coordinates have little effect on the separation of sites with correspondence analysis.

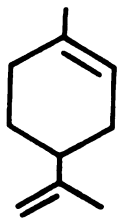
The first axis slightly resembles a geographic gradient, with the Olympic Peninsula population at one extreme of the first dimension. Sites of



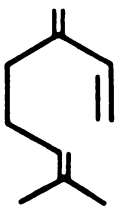
Camphene



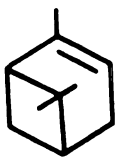
3-Carene



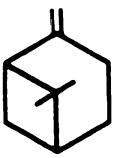
Limonene



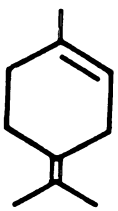
Myrcene



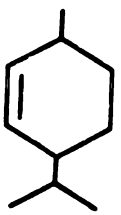
α-Pinene



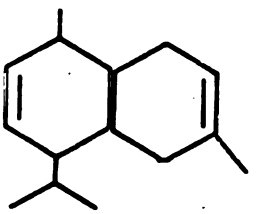
β-Pinene



Terpinolene



β-Phellandrene



Cadinene

Figure 8. Structures of some common monoterpenes extracted from the foliage of *Abies grandis*.

the lowest elevation (CL12 and OP17) are found at the high end of CA1, although other low elevation sites (CA15, CA16, and CB8) are found near the other extreme. Highest elevation sites (CB7, MT1 and CA14) fall out along CA2 suggesting that elevation may play an important role, albeit secondary, in terpene production. Local population CL11 clearly resides as an outlier, primarily because of an unusually high and consistent beta-pinene content. A striking result of the correspondence analysis is the large distance in ordination space between the paired local populations. For example, the average terpene composition of populations MT1 and MT2, separated by 27 km (Figure C.1.) is much greater than that of populations MT2 and CL9, separated by several hundred kilometers. In general, the local populations differed substantially, one compared to another, in their average terpene composition.

Comparison of studies

Results of this research represent data from two separate experiments. Correlations of amount of individual terpenes between the two studies appear in Table 8. Interpretation of results can only be considered valid if data are reproducible and techniques are consistent. The repeatability of techniques is reflected in coefficients of determination recorded in Table 8. In general, the two experiments produced consistently comparable quantities of monoterpenes in the control levels, but fertilization appeared to interfere with production of terpenes such that correlations were not as strong with treatments.

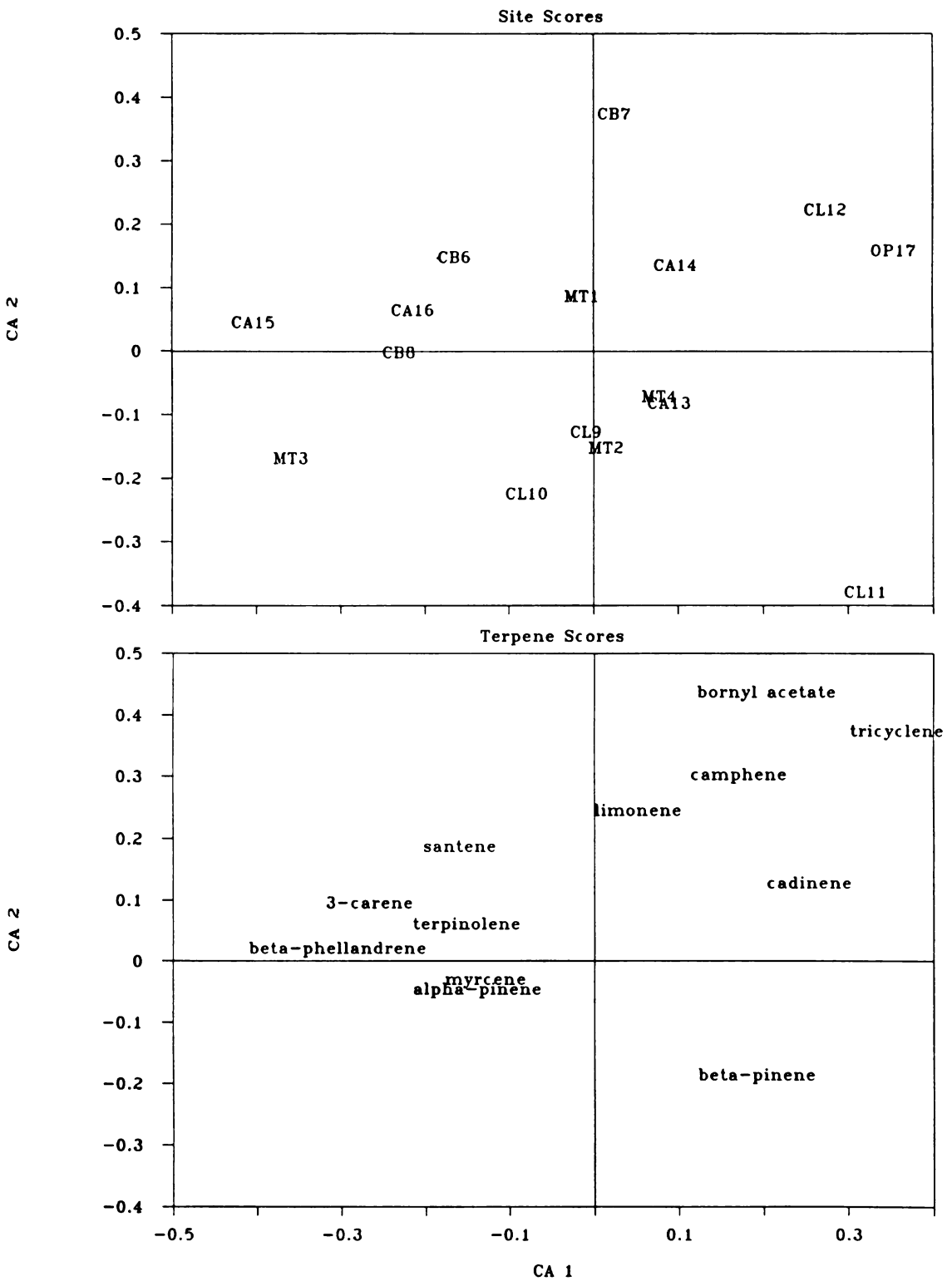


Figure 9. Site scores and terpene scores for correspondence analysis using terpenes. Notation follows Table 1.

Table 8. Coefficients of determination (r^2) for each terpene at each treatment level between replicated experiments. Significant correlations are represented by an asterisk ($P < 0.05$).

	TREATMENT		
	control	22.4 g $N \cdot m^{-2}$	44.8 g $N \cdot m^{-2}$
		r^2	
Terpene			
alpha-pinene	.989*	.827*	.983*
beta-phellandrene	.938*	.627*	.755*
beta-pinene	.760*	.945*	.340
bornyl acetate	.908*	.421	.960*
cadinene	.542*	.372*	.893*
camphene	.878*	.357	.538

Changes in Terpenes with Treatment

Table 9 presents the analysis of variance results for the effects and interactions of nitrogen on production of terpenes. With the control treatment, regional population differences were significant ($P < 0.01$) for camphene, bornyl acetate and cadinene, and 3-carene ($P < 0.001$). Differences in camphene and bornyl acetate occurred between the Cabinet Mountains and Clearwater Mountains and between the Cascades and the Clearwater Mountains. The Cabinet Mountains and Clearwater Mountains populations had significantly different quantities of cadinene, and differences in 3-carene distinguished the Olympic Mts. and Montana populations.

Table 9. Levels of significance as determined by analysis of variance.

* = 0.05 ** = 0.01 *** = 0.001

Source of Variation	alpha pinene	beta phellandrene	beta pinene	camphene	bornyl acetate	myrcene	limonene	cadinene	3-carene	terpinolene	tricyclene
Regional Populations	-	-	-	**	**	-	-	**	*	-	-
Local (Regional)	-	***	-	**	**	**	**	**	-	-	-
Treatment	-	**	-	*	*	-	-	-	-	***	
Regional x Treatment	-	-	-	*	*	-	-	-	-	-	-
Treatment x Local(Region)	-	***	-	**	*	***	**	***	-	***	-

Differences among local populations within regional populations were more prevalent. Beta-phellandrene ($P < 0.001$), camphene, bornyl acetate, myrcene, limonene, and cadinene ($P < 0.01$) differed among local populations within regional populations.

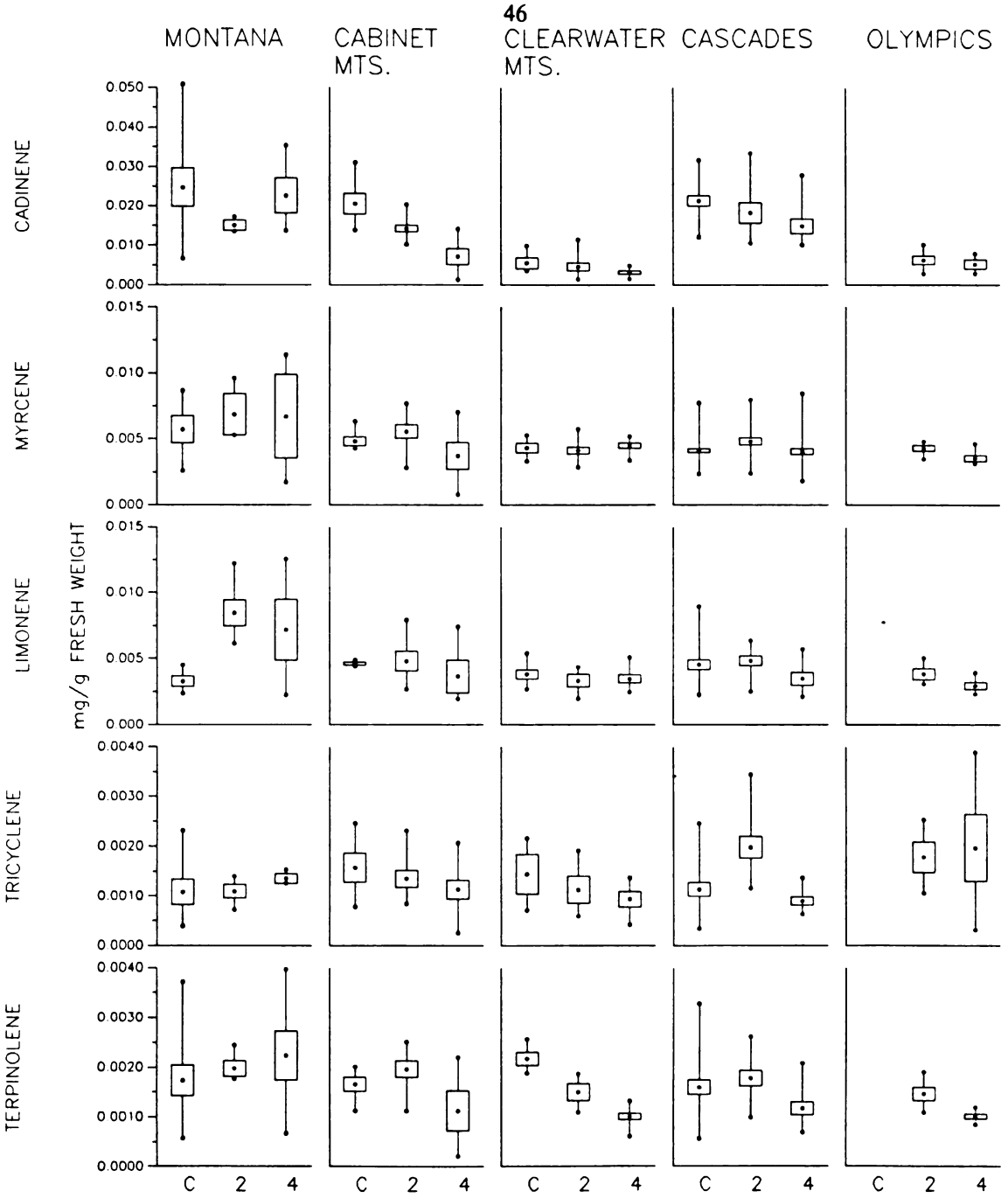
The effect of nitrogen fertilization on terpene production appears to be strictly tied to individual compounds and regional populations. (Figures 10 and 11). Treatment effects were apparent with the terpenes beta-phellandrene ($P < 0.01$), beta-pinene ($P < 0.01$), camphene ($P < 0.05$), bornyl acetate ($P < 0.05$), and terpinolene ($P < 0.001$). Differences were always between the control value and the 44.8 g $N \cdot m^{-2}$ treatment. Fertilization consistently depressed production of the terpenes except beta-pinene in Clearwater Mts. population, and hence fertilization effect was population specific. A decrease in production of beta-phellandrene occurred only in the Olympic Mts. populations while camphene decreased with fertilization in every population but the Olympic Mts. Bornyl acetate treatment differences were evident only in the Cascade and Clearwater Mts. The Clearwater and Olympic Mts. populations produced a significantly lower amount of terpinolene with additional nitrogen.

Two terpenes were seemingly unaffected by genetic or environmental influences. Alpha-pinene and beta-pinene did not differ among populations nor with treatment. Moreover, no significant interaction effect was apparent with these terpenes. Alpha-pinene and beta-pinene are two of the three most abundant terpenes. The lack of differences in quantities of the latter may be due to wide variation within samples and populations (Figure 10); however alpha-pinene consistency in production throughout populations and

Figure 10. Quantities of the five major terpenes in *Abies grandis* foliage for regional populations and changes with treatment. Boxes represent standard deviations about the mean, and the extent of the vertical line represents the maximum and minimum values. The legend refers to treatments where C = control, 2 = 22.4 g $N \cdot m^{-2}$ and 4 = 44.8 g $N \cdot m^{-2}$.

MONTANA
CABINET MTS.
CLEARWATER MTS.
CASCADES
OLYMPICS
β-PINENE
β-PHELLANDRENE
α-PINENE
BORNYL ACETATE
CAMPHENE
mg/g FRESH WEIGHT
C 2 4

Figure 11. Quantities of the five other major terpenes in *Abies grandis* foliage for regional populations and changes with treatment. Boxes represent standard deviations about the mean, and the extent of the vertical line represents the maximum and minimum values. The legend refers to treatments where C = control, 2 = 22.4 g $N \cdot m^{-2}$ and 4 = 44.8 g $N \cdot m^{-2}$.


MONTANA
CABINET MTS.
CLEARWATER MTS.
CASCADES
OLYMPICS
CADINENE
MYRCENE
LIMONENE
TRICYCLENE
TERPINOLENE
mg/g FRESH WEIGHT
0.050
0.040
0.030
0.020
0.010
0.000
0.015
0.010
0.005
0.000
0.0040
0.0030
0.0020
0.0010
0.0000
C
2
4

with treatments probably accounted for the lack of significance for that terpene.

Terpene yields

Table 10 indicates the total yield of terpenes for each regional population by treatment. The total yield of terpenes was derived by summing ten terpenes. There were no significant differences among means.

Table 10. Average yield of terpenes (mg/g fresh wt) (standard deviations) of seedlings of regional populations of grand fir. No statistical differences were apparent among geographic populations or treatments.

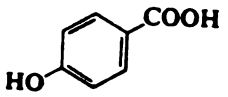
Populations	Treatment		
	control *[n]*	22.4 g $N \cdot m^{-2}$ *[n]*	44.8 g $N \cdot m^{-2}$ *[n]*
Montana	.239(.070) *[8]*	.551(.137) *[12]*	.409(.209) *[7]*
Cabinet	.334(.295) *[6]*	.292(.079) *[10]*	.287(.111) *[13]*
Clearwater	.239(.049) *[5]*	.290(.070) *[8]*	.287(.018) *[9]*
Cascades	.322(.176) *[11]*	.323(.100) *[8]*	.256(.128) *[13]*
Olympics	-	.296(.029) *[10]*	.417(.260) *[7]*

Phenolics

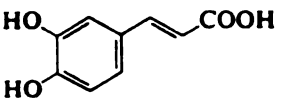
An array of simple phenols, phenolic acids, and flavonoids was extracted from grand fir foliage. Not all extracted components were positively identified, so only the known phenolics will be discussed. Structures of some common phenolics extracted from grand fir foliage and positively identified appear in Figure 12. Tables B.2 to B.6 (Appendix B) indicate the relative amounts of particular phenolics. Generally, the flavonols (a type of flavonoid) kaemferol, myricetin and quercetin were most abundant despite treatment effects and seed source, although data were widely variable.

The data were analyzed separately as three groups of phenolics: benzoic acids, cinnamic acids, and flavonoids. The compounds para-hydroxybenzoic acid, vanillic acid, gallic acid, protocatechuic acid, gentisic acid, and syringic acids were grouped as benzoic acids. The cinnamic acid group consists of cinnamic acid, caffeic acid, para-coumaric acid, ferulic acid, and sinapic acid. Myricetin, catechin, kaempferol, and quercitin represent the flavonoids.

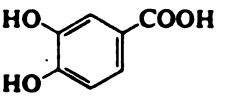
Figures 13 - 15 reveal changes in level of phenolic groups with treatment at each regional population. The benzoic acid group was the only one to manifest any differences in relative amounts among seed sources. Raw values appear in Appendix B, Table 3. The Cabinet Mts. seedlings had significantly greater amounts of benzoic acids than Montana, Cascades or Olympic Mts. populations. The difference in benzoic acids was primarily because of fluctuations in syringic acid (Table B.3). These groups did not change significantly with treatment, and there were no differences among any



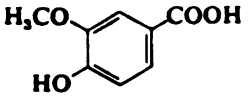
p-OH-Benzoic acid



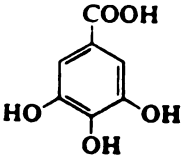
Caffeic acid



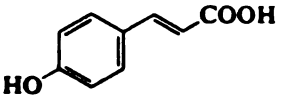
Protocatechuic acid



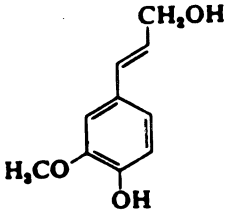
Vanillic acid



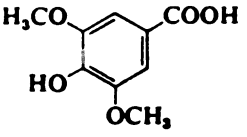
Gallic acid



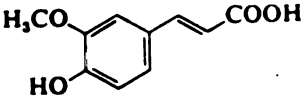
p-OH-Cinnamic acid (*p*-Coumaric acid)



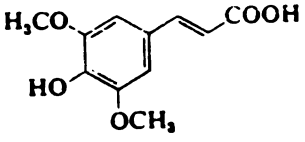
Coniferyl alcohol



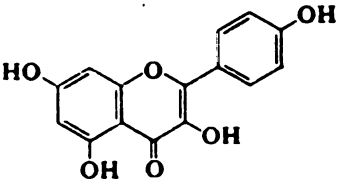
Syringic acid



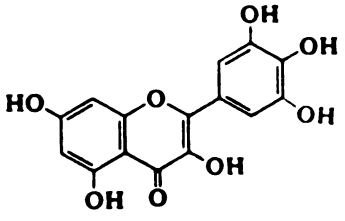
Ferulic acid



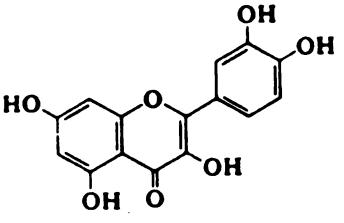
Sinapic acid



Kaempferol



Myricetin



Quercetin

Figure 12. Structures of some phenolic compounds extracted from the foliage of *Abies grandis*.

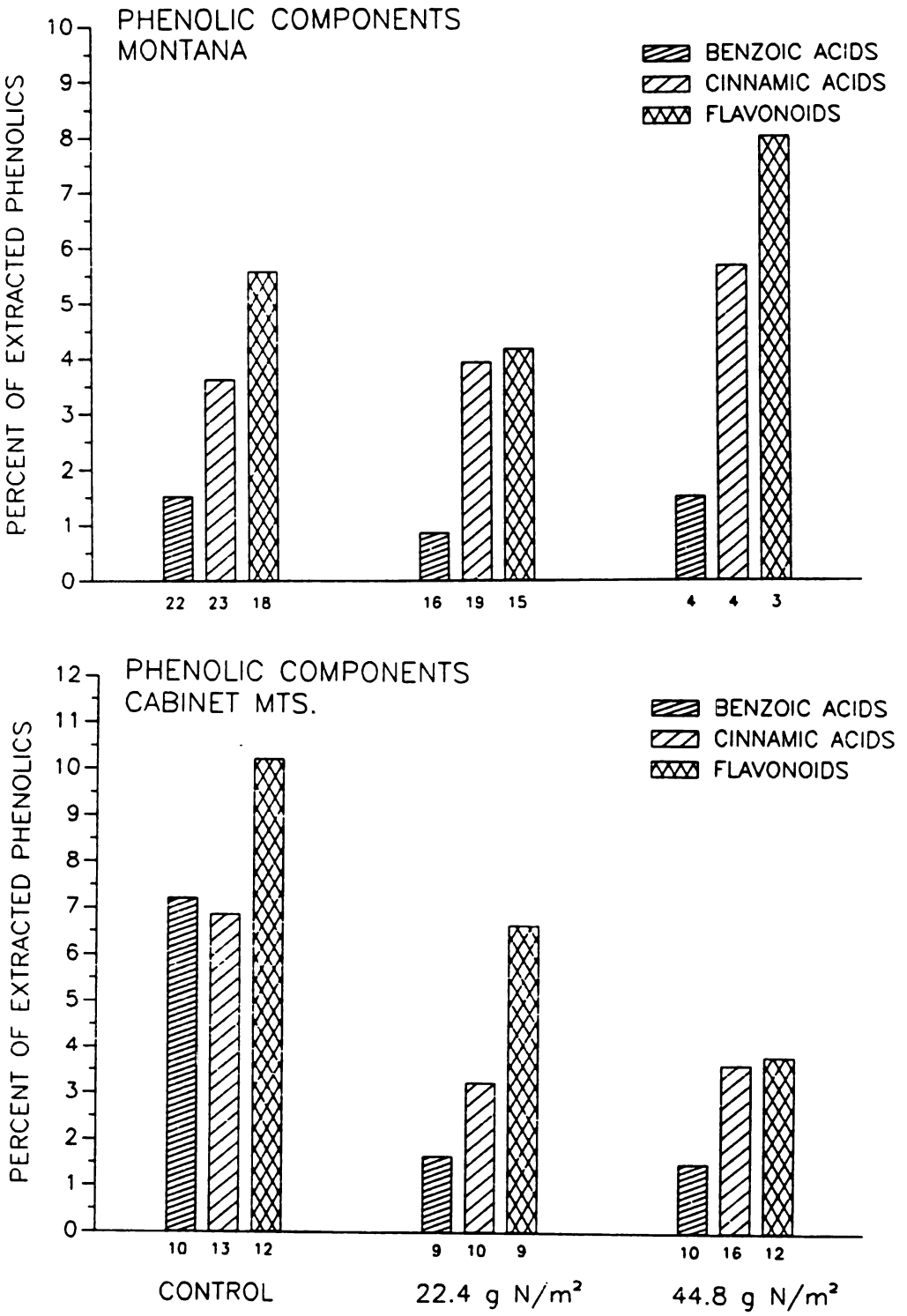


Figure 13. Changes in average percentage of total extracted phenolics by group and with treatments for Montana and Cabinet Mountains regional populations. Values below bar indicate sample size.

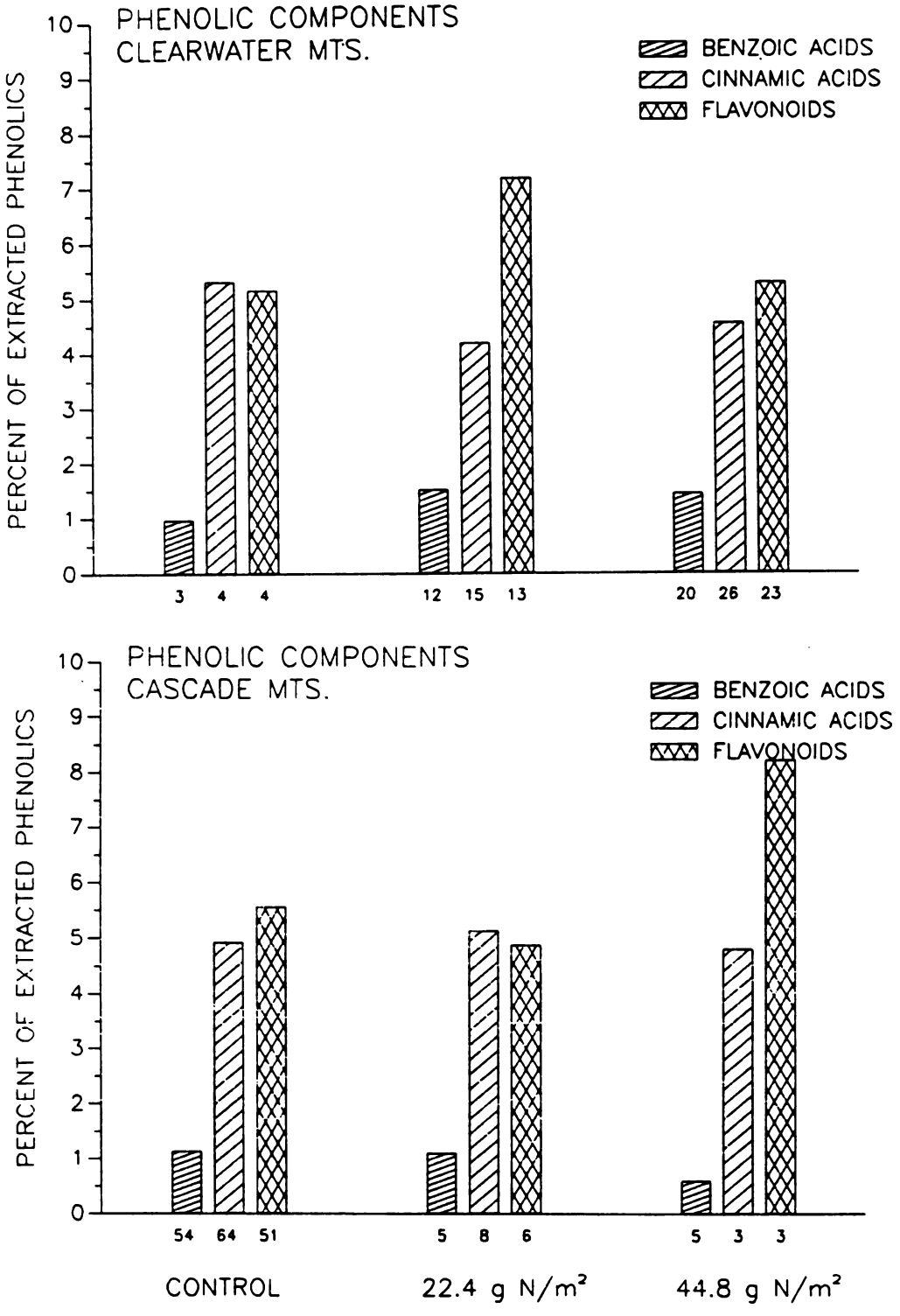


Figure 14. Changes in average percentage of total extracted phenolics by group and with treatments for Clearwater Mountains and the Cascades regional populations. Values below bar indicate sample size.

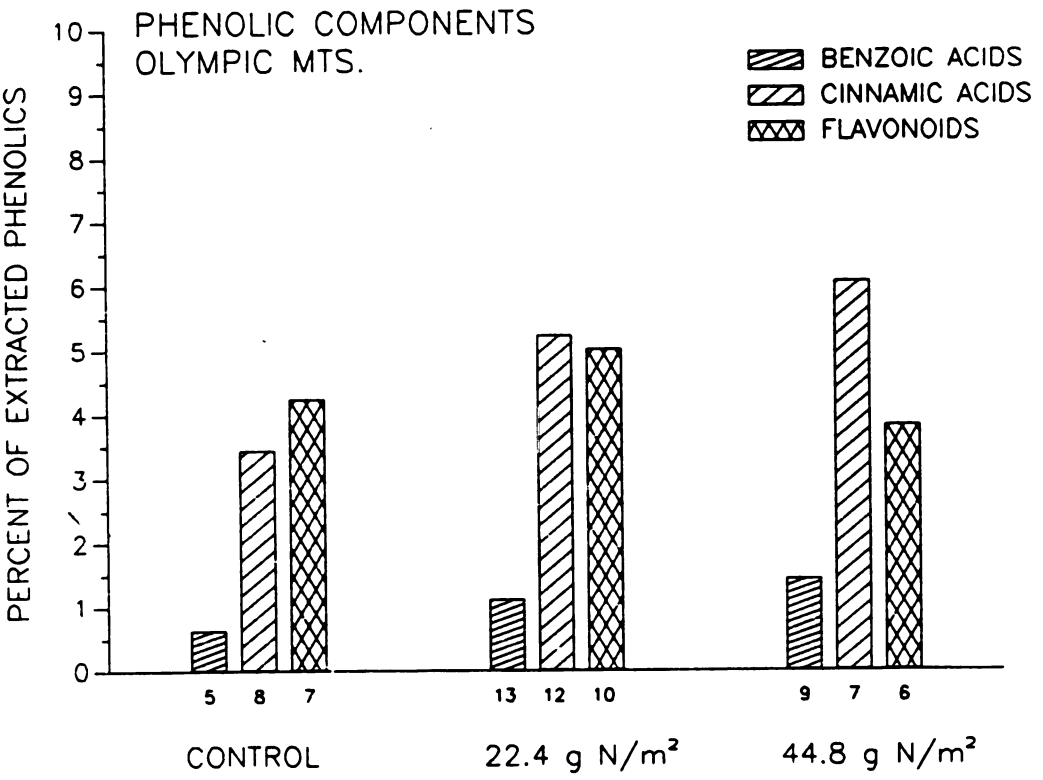


Figure 15. Changes in average percentage of total extracted phenolics by group and with treatments for the Olympic Mountains regional population. Values below bar indicate sample size.

populations with fertilization. Unlike terpenes, the analysis of variance revealed no significant interaction effect.

The effect of nitrogen fertilization on individual phenolics was examined graphically with empirical distribution functions (Figures 16 - 18). In these graphs, S_x represents the proportion of total samples, and the quantity is represented by the abscissa. Data in these graphs represent all populations grouped together by phenolic and treatment.

The Smirnov two sample test revealed that coniferyl alcohol and para-hydrobenzoic acid were the only phenolics to change with nitrogen fertilization. In both cases, fertilization with 44.8 g $N \cdot m^{-2}$ produced significantly greater amounts of these compounds compared with control levels.

Yield of phenolics

The concentration of total phenolics in the control treatment according to Folin-Ciocalteu was uniform across most of the seed sources. Differences among regional seed sources at the control level were apparent only between the Montana and Cascade populations ($P < 0.05$). Nitrogen fertilization caused a decrease in phenolic content, but not with all seed sources (Figure 19). In the Clearwater Mts., a reduction in phenolics occurred when seedlings were growing in the 44.8 g $N \cdot m^{-2}$ level ($P < 0.01$), but not with the intermediate fertilization. In the Cascade and Olympic populations, a decreased production of phenolics was evident with each level of fertilization. In the easternmost populations, Montana and Cabinet Mts.,

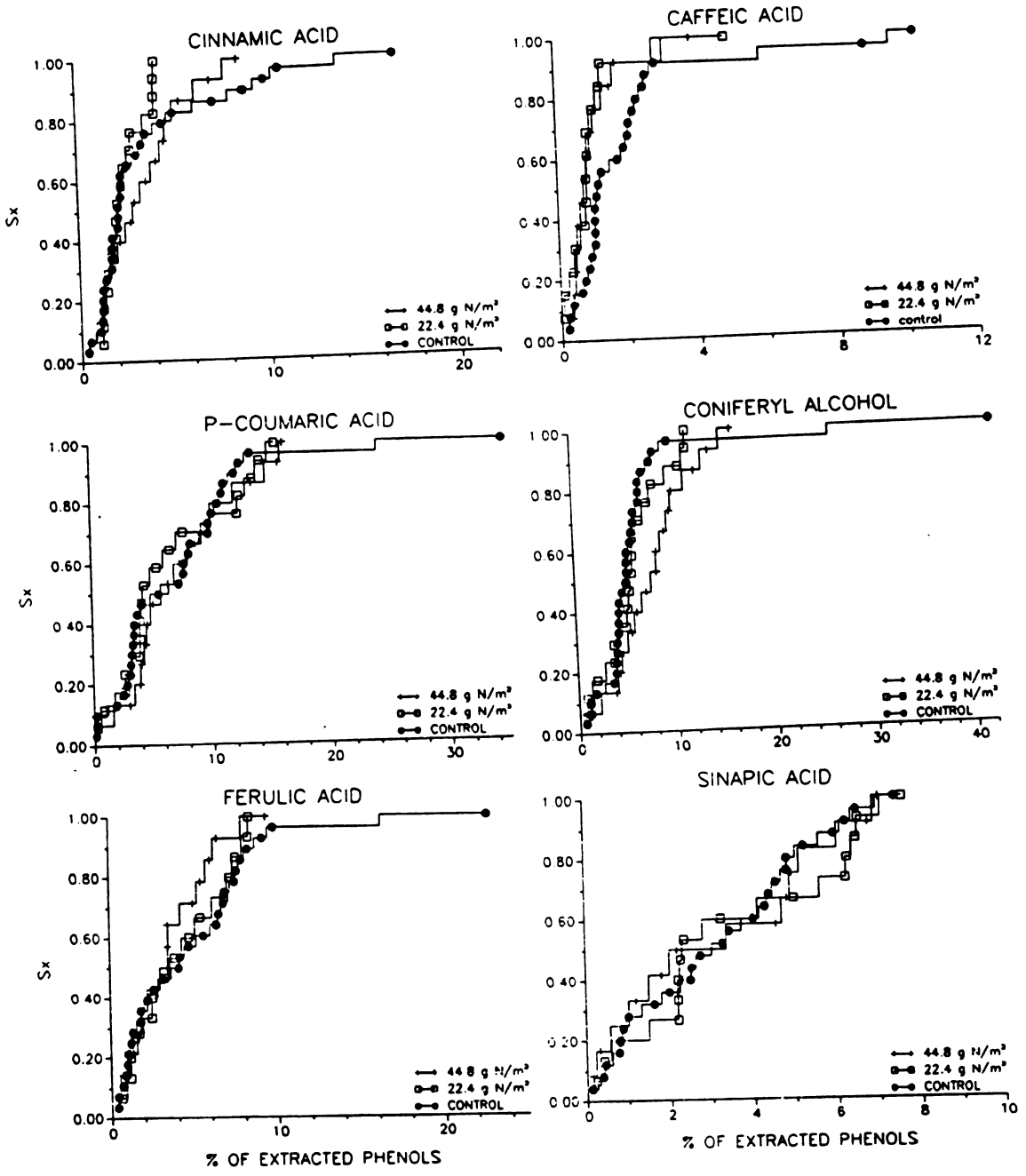


Figure 16. Individual empirical distributional functions showing changes in production of phenolics in the cinnamic acid group following fertilization.

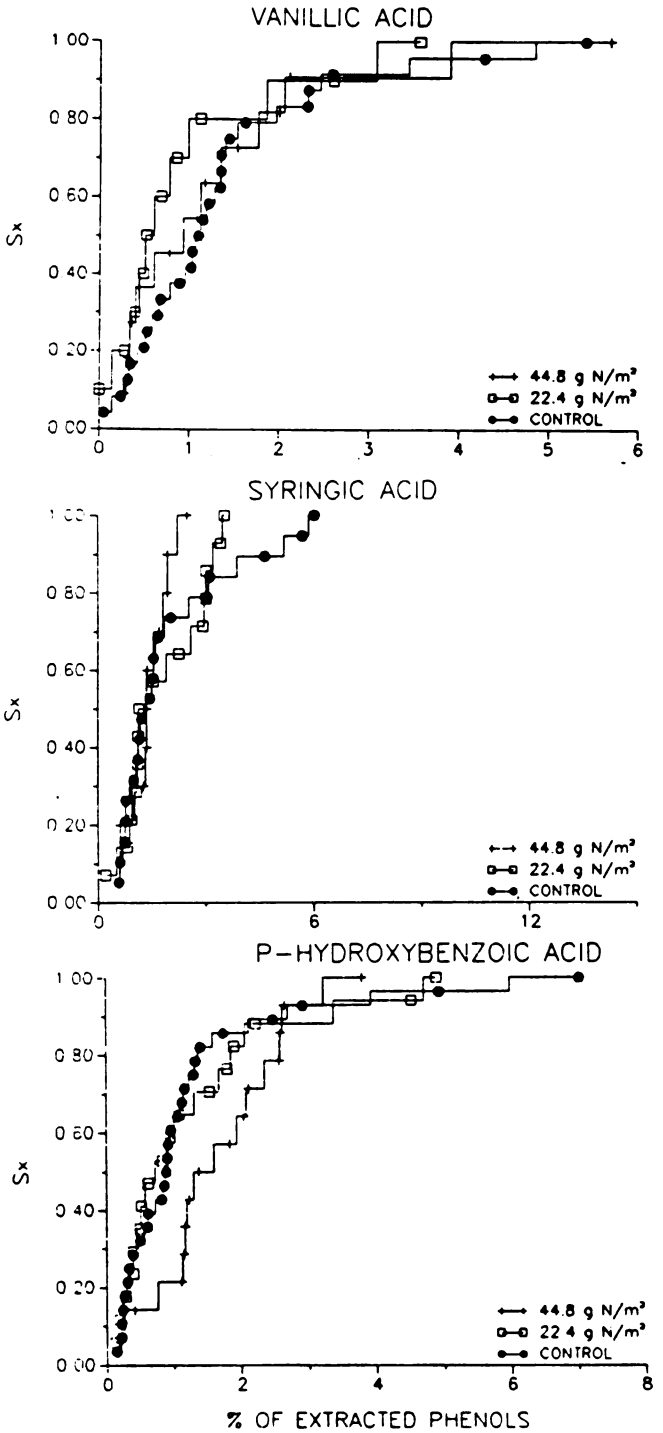


Figure 17. Individual empirical distributional functions showing changes in production of phenolics in the benzoic acid group following fertilization.

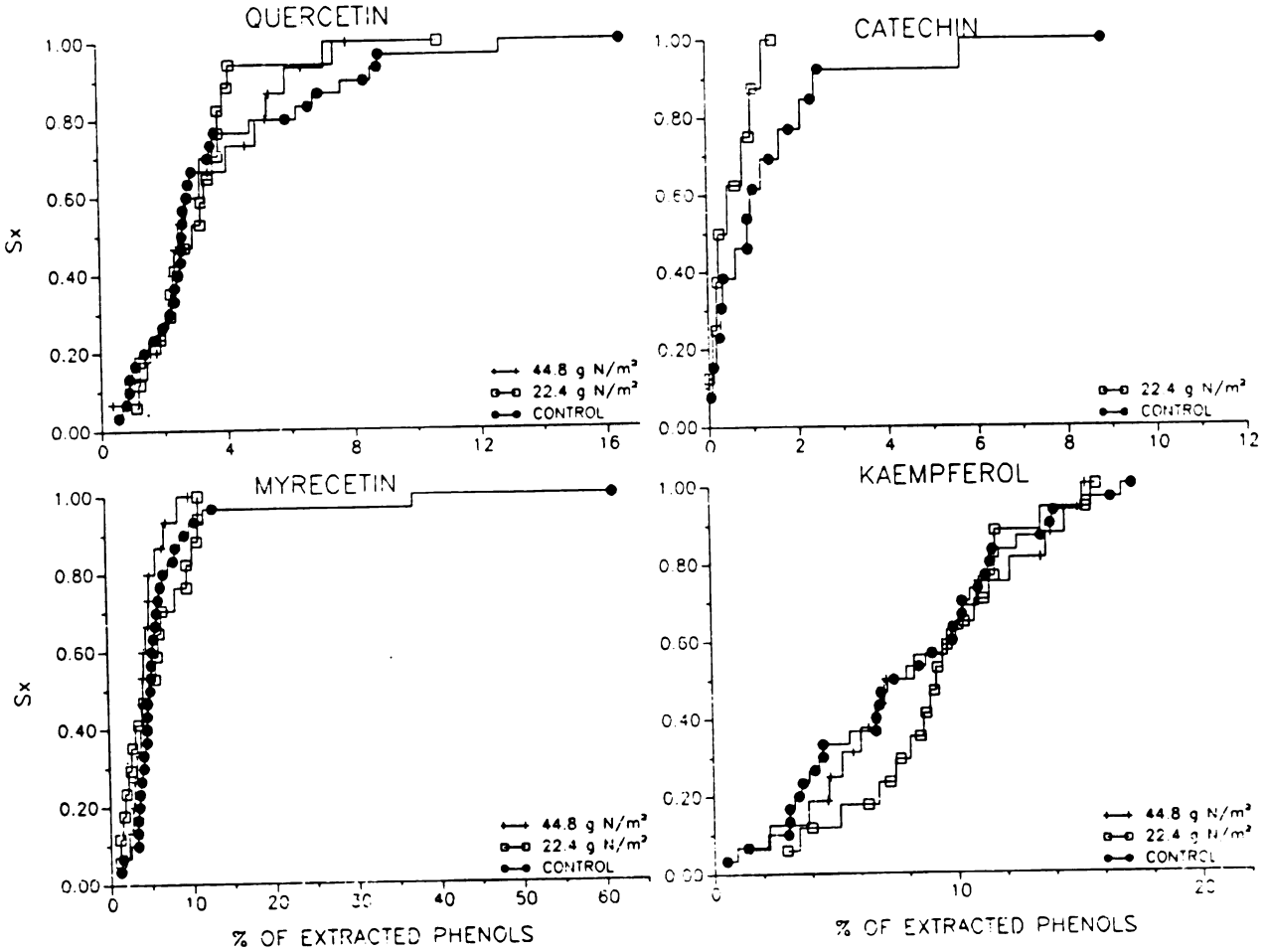


Figure 18. Individual empirical distributional functions showing changes in production of phenolics in the flavonoid group following fertilization.

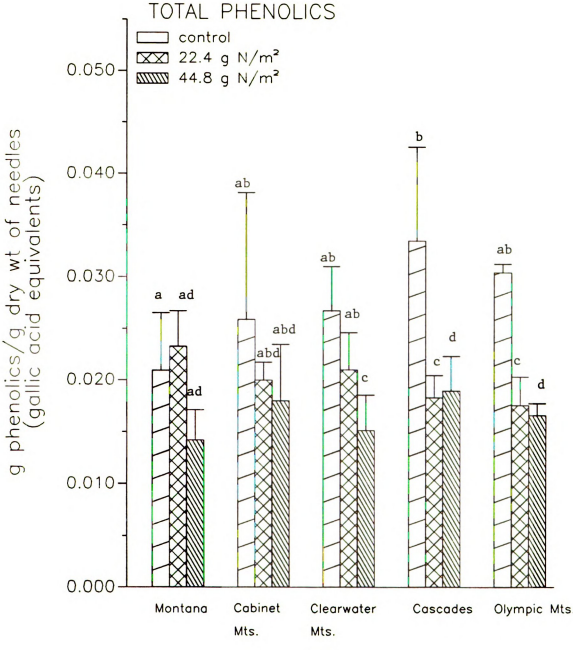


Figure 19. Total Folin-Ciocalteu phenolics and changes with treatment for the regional populations of *Abies grandis*. Mean values with the same letter are not significantly different ($P < 0.05$).

there was an apparent decrease in phenolics with fertilization, but the difference was not significant.

Although none of the correlations in the canonical analysis of phenolics, growth variables and site variables was significant (Table 11), the structure of the canonical variates provides some information about initial relationships. For example, the first canonical variate provides a contrast between elevation and latitude and mineralizable nitrogen, the latter which have only negative correlations. For example, elevation appears to be strongly related to biomass. Regional location, specifically latitude and longitude, provide the best relationship to total phenolic production, but in general the quantity of total phenols does not seem to be predictable from site variables. A single site variable does not explain chemical and growth parameters well.

Table 11. Structure correlation between environmental (site) variables and the first canonical variate of growth and phenolic data. Position refers to topographic position and nitrogen refers to mineralizable nitrogen.

	CANONICAL VARIATES			
	biomass	height	root:shoot	phenolics
elevation	0.7727	-0.3147	0.3668	-0.0063
habitat type	0.0483	0.6659	0.2683	-0.1491
aspect	0.2609	-0.0024	-0.1147	0.2172
slope	0.2192	0.1396	-0.2959	-0.0151
latitude	-0.4253	-0.3552	0.5262	0.1107
longitude	0.5910	0.0202	0.2950	0.1593
position	0.2006	0.0928	-0.2465	0.2204
nitrogen	-0.2388	0.2355	-0.0746	0.0834

DISCUSSION

Growth is a measure of resource gain from the environment, and is thus related to fitness. Similarly, other uses of resources, although not as directly measurable as growth, represent an element of plant fitness. A plant's teleological question might be: Should new growth be invested in *(i)* an increased capacity for resource acquisition, *(ii)* storage, *(iii)* the creation of new supporting tissues?

Bloom *et al.* (1985) equated allocation of resources in plants to predictions based on economic theory. For example, these authors maintain that plants adjust allocation so that their growth is equally limited by all resources. These physiological adjustments may be both short-term or long-term, i.e. genetically based. They suggest that an overall balance is necessary since each plant process is limited by the same balance of internal reserves.

In relation to quality of habitat, an exchange goes on, e.g. on infertile soil plants accumulate compounds that have low nutritive contents. Under low light conditions plants accumulate high nutrient concentration, primarily because these are not diluted with carbohydrate.

Plants characteristic of resource-rich environments generally are highly plastic in their allocation in response to environmental stress. In such situations, resource availability may be heterogeneous, both spatially and temporally, depending on competition. Plants from resource-poor environments are less plastic probably because resources are always limiting in both a spatial and temporal fashion. It seems that plants from relatively infertile sites have high root to shoot ratios that are fixed, and that exhibit little response to changes in the environment (Chapin 1980). Given a

possible change in the environment, the plant still may utilize resources effectively, but will probably do so by increasing storage reserves (Bloom *et al.* 1985).

Intraspecific variation in secondary chemistry production may be determined by a balance of resources, not absolute values (Bryant *et al.* 1983). This notion contrasts with the explanation of secondary chemical production as a function of resource availability and an adaptive solution to herbivory (Coley *et al.* 1985).

Attempts to manipulate the overall carbon budget of a plant have been made by Lincoln and Couvet (1989) using atmospheric CO_2 treatments. They found that with increases in CO_2, total amount of peppermint leaf sesqui- and monoterpenes increased; however, their proportion of leaf weight did not change, hence plant biochemical control seems to be crucial. The data support the notion that excess carbohydrates are not used to produce allelochemicals. When a phenotype changes allelochemicals, it is through regulation of biosynthetic pathways rather than as a result of carbon supply change.

TERPENES

Terpene variation

Considerable variation in terpene composition and quantity exists in some species with regard to seed source and environmental factors such as elevation. These studies describe genotypic differences, but environmentally induced differences remain uncertain. In studies of genetic stability and control of plant terpenoids, Crankshaw and Langenheim (1981) demonstrated little phenotypic plasticity in terpene composition in *Hymenaea* leaves. Over developmental time, however, they found differences among

individuals and populations as did Hall and Langenheim (1987) and Hanover and Furniss (1966), both in conifers. Langenheim *et al.* (1981) found little phenotypic plasticity in leaf resin composition of tropical legumes, although the quantity of resin (yield) increased with light intensity.

In the present study, correspondence analysis showed that substantial variation existed among the local populations in terpene composition (Figure 9). Relationships were stronger between individual terpenes and local populations than between terpenes and regional populations, since there was no discernible grouping of regional populations in the analysis.

Terpenes extracted and isolated in the present study were the same as found by von Rudloff (1976) in *Abies grandis*, which he characterized as a tricyclene - camphene - borneol - camphor type of conifer. The presence of substantial amounts of sesquiterpenes and a trace of santene distinguished *Abies grandis* terpenes from *Abies lasiocarpa* and *Abies amabilis*. He found beta-phellandrene and beta-pinene as the most variable terpenes and negatively correlated with each other. However, the occurrence of a high beta-pinene/low beta-phellandrene tree or vice versa, may be equally common in coastal or interior populations. Similarly, in the present study, beta-pinene and beta-phellandrene were influential terpenes in correspondence analysis. Beta-pinene accounted for separation along the first dimension, but it has differentiated populations from Montana, Clearwater Mts., and the Cascades. Similarly, beta-phellandrene distinguished local populations of Montana, Clearwater Mts., and the Cabinet Mts. Since proximity and opposition have meaning in correspondence analysis, beta-pinene and beta-phellandrene exert opposite influence on separation of populations of this study, as in von Rudloff 's.

Zavarin *et al.* (1977) found low variability of cortical essential oils in *Abies grandis* within and between populations of a more extensive, range-wide sampling. He found *Abies grandis* to be a chemically undifferentiated species with no distinctions between coastal and interior populations. Slight variations in terpenes did not distinguish population or seed sources. But, broadly, they associated 47° N latitude as the southernmost area of chemical homogeneity, south of which the percentages of components in the cortical oleoresin were not extremely predictable or consistent. The Clearwater Mts. populations of the present study are the only seed sources to occur south of 47°, but I found no greater inconsistencies within these groups than within other populations. I demonstrate what may be chemical differentiation because the Olympic Mts. populations differ significantly from many of the other groups. This finding corresponds to the Zavarin *et al.* (1977) delineation of 122° 15' longitude for chemical differentiation within grand fir. Sampling of the Olympic populations was minimal compared to other seed sources and small sample sizes could greatly influence results.

Over a wide latitudinal range extending into the *Abies grandis* - *Abies concolor* hybrid swarm, Houkal (1976) delineated intermediate populations. The distinctions were in terms of oxygenated monoterpenes increasing with more northern seed sources, with a decrease in simple hydrocarbons. Zavarin *et al.* (1977) found that a larger percentage of camphene in the cortical resin was characteristic of grand fir rather than white fir.

Total Terpene Yield

No strong relationship exists between total yield of terpenes and fertilization, and an obvious population x fertilization interaction occurs. The interaction precludes generalization about the effect of nitrogen, in that it makes no sense to refer to effect of nitrogen without considering the particular seed source. The ecological significance of the measure of total foliar terpenes is uncertain. Total foliar terpenes produced did not change the level of herbivory in a study by Lincoln and Couvet (1989). Caterpillars consumed more leaf tissue in elevated CO_2 conditions regardless of terpenes, thus questioning the defensive role of terpenes.

PHENOLICS

Total Phenolics

Phenolic compounds are formed via the shikimic acid pathway, the general scheme of which is outlined in Figure 20. This pathway gives rise to many compounds of special significance to woody plants, *vis.* lignin and tannin.

Janzen (1974) theorized that environmental influences in the tropics, particularly soil fertility, affect the production of secondary metabolites. Such phenotypic differences could ultimately result in genotypic variation. Nascimento and Langenheim (1986) found no differences in total phenols in a species growing on different soil types. Similarly, we found no differences among seed sources despite differences in soil and other site characteristics, except for the Montana and the Cascades populations. Sample size was insufficient to examine local population variation. Looking at one tree species, *Fagus sylvatica*, growing on different soils, Nicolai (1988) found phenolic content of freshly fallen leaves to be higher on nutrient poor sites.

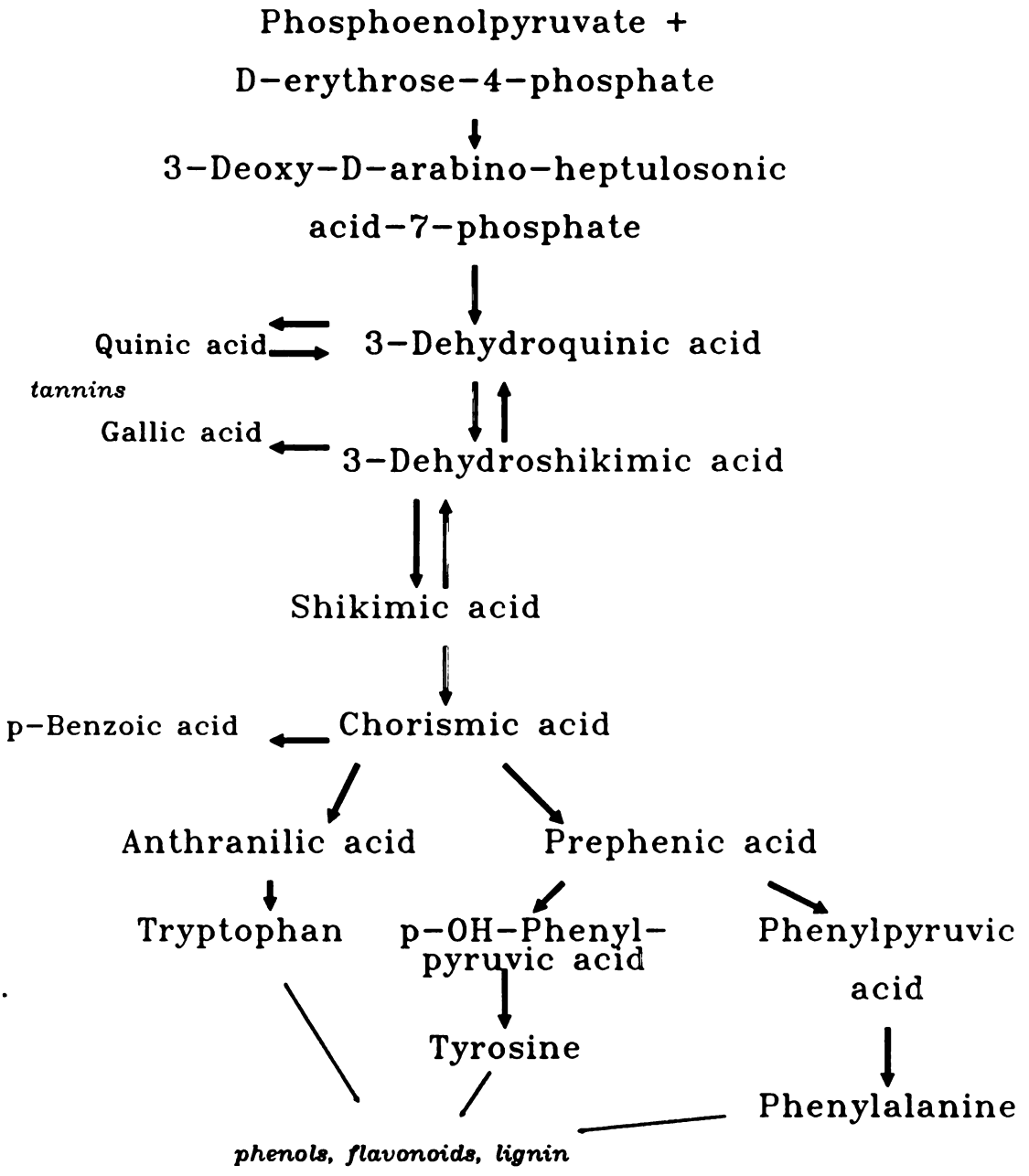


Figure 20. Basic outline of the Shikimic Acid Pathway.

Even after a year, the phenolic content of the litter layer was greater on the more nutrient poor site.

The current study demonstrated a strong depression of total phenolic compounds with nitrogen fertilization. Others have shown a decrease in phenolic content with nitrogen addition, particularly with cell cultures (Phillips and Henshaw 1977). Glyphis and Puttick (1989) found that soil N differences translated into leaf N differences and in polyphenol content in Mediterranean vegetation. Although phenolic content tended to be higher in nutrient-limited conditions, they found that fertilization with nitrogen had no effect on polyphenol content.

Both artificial and insect damage appear to increase levels of phenolics in birch foliage. Phenylalanine ammonia-lyase (PAL) increased with damage, and this enzyme is important as the first committed enzyme of phenylpropanoid metabolism in higher plants (Hanson and Havir 1981). This enzyme is crucial in production of phenolics by elimination of NH_3 from l-phenylalanine to produce trans-cinnamate. PAL could be inhibited to a greater extent than phenolics, probably because of the lower turnover rate of phenolics. Baldwin and Schultz (1983) suggest that phenolic synthesis occurs rapidly in response to damage of sugar maple and poplar and to nearby poplars, and that the 'communication' signal is phenolic; however, feeding behavior of generalist herbivores was unaffected by changes in phenolics (Hartley 1988).

The depression of total phenolic production with nitrogen fertilization in three populations would suggest an increase in other compounds, possibly protein constituents, since aromatic amino acids are synthesized in the shikimic acid pathway, as are phenolics (Torsall 1983). Kim *et al.* (1987)

found steady increases in amino acids except tryptophan in fertilized containerized seedlings of jack pine and black spruce. Carrow (1973) found similar trends in grand fir. The free amino acids are important for protein synthesis, while non-protein amino acids are toxic to many herbivores.

Inhibition of phenolics may occur at various points along the biosynthetic pathway. Katoh *et al.* (1989) suggest that tannin inhibition results later in the shikimate pathway, probably due to physiological disorder. The biosynthesis of phenolics in general may be considered to consist of various stages: (i) biosynthesis of glucose (ii) formation of six-membered alicyclic carboxylic acid and conversion into 3-dehydro-shikimic acid, i.e. the early shikimate pathway; (iii) conversion of 3-dehydroshikimic acid into aromatic compounds, i.e. the later shikimate pathway; (iv) further modification of aromatic intermediates, including hydroxylation of benzene rings. Gallic acid, important in formation of hydrolyzed tannins, could be produced by dehydration of 3-dehydro-shikimic acid, thus formation of all phenolics does not necessitate proceeding through amino acid synthesis entirely. However, these reactions, along with hydrolyzable tannin production, occur only in angiosperms, but they emphasize the fact that sequential production may not be consistent in the shikimic acid pathway.

Inhibition at stage (i) is unlikely because photosynthesis is usually accelerated with increasing nitrogen (Field and Mooney 1986). If phenolics are not produced, but glucose or some early product is increased, then that material is used in production of primary metabolites, which are then increased.

Preferential pathways or routes to phenolic synthesis differ depending on species (Torssell 1983). In some plants glucose may be the preferred

precursor to gallic acid, in others, phenylalanine might be more effective than glucose.

A decrease in production of phenol materials results from a decrease in activity of key enzymes such as PAL, or a reduction in substrate supply because of photosynthetic inhibition. Nutrient stress increases the supply of phenolic precursors, e.g. phenylalanine (Gershenzon 1984). A reduction of growth ostensibly leads to an increase of substrate. Delmoral (1972) indicates that when nitrogen is in short supply, aromatic amino acids may be deaminated so that the nitrogen may be used for other functions; the trans-cinnamic acid remaining from the deamination may then be used to build more complex phenolics. Hence when a plant is provided nitrogen, the production of amino acids and proteins proceeds at the expense of phenolics.

Grand fir foliage possesses a variety of phenolics, many of which are common in all plants. Both benzoic acids and cinnamic acids are present. Gentisic, syringic, para-hydroxybenzoic, protocatechuic, vanillic and gallic acids are examples of the first. Para-coumaric, caffeic, ferulic and sinapic acids are examples of the latter. Close relationships are apparent in that coniferyl alcohol, also found in grand fir and an important precursor to lignin, is derived from cinnamic acid. Quercetin, kaempferol, and myricetin are flavonoids with the widest distribution in nature, and are the most abundant phenolics isolated from the grand fir foliage. Catechin and gallocatechin are examples of flavans. The former serves as an intermediate in production of condensed tannins.

No previous research has examined fluctuation in particular phenolic components with changes in nutrient conditions. Phenolics are easily subject to oxidation, substitution and coupling reactions. Turnover and degradation

may occur quickly and unpredictably. Cinnamic acids or simple phenols such as catechol may be actively metabolized and polymerized or catabolized by the plant (Barz and Koster 1981). The seedlings in this study were grown under constant light and optimum conditions, thus phenolic production, metabolism and turnover was no doubt continuous. Flavonols such as kaempferol or quercetin may be actively metabolized. Such metabolism is most pronounced during times of intensive plant growth or differentiation (Wiermann 1981). Barz and Koster (1981) found accumulation and turnover of flavonols to cease when cell culture growth entered the stationary phase.

Interestingly, while the production of total phenolics decreased, the decrease was not apparent in the specific compounds identified. Individuals within the group may have changed, but based on the data, this seems unlikely. Other non-extracted phenolic components, e.g. tannins, may have accounted for an increased production under control (non-fertilized) conditions, and thus would not appear on the HPLC data. Specific phenolics decrease (Table A.2 to A.6), but for the most part, the variations in measurements are too great and may preclude possible significant differences.

Other measures of total phenolics such as all UV absorbing compounds have not provided much information regarding genetic control. Larsson *et al.* (1986) found dramatic differences in this assay within a clone and among treatments such as light intensity and nutrient level. Intraclonal variation among control levels was not discussed, however, leaving unanswered some aspects of genetic control, since clonal research represents an ideal opportunity to distinguish genotype versus environmental control of phenolic synthesis. Interesting still is the result that ramets did not respond

similarly to the treatments, suggesting a great deal of variation and minimal genetic control in the production of this group of compounds.

Other differences in amount of phenolics produced by tree species have been explained by environmental, physiological and age differences (Feeny 1970, Denno and McClure 1984). Young leaves may be more readily induced to produce phenolics, and also tend to have a higher phenolic concentration (Baldwin and Schultz 1983).

Phenolic array

Genetic control has not been verified with phenolics, despite the widespread use of flavonoids in biochemical systematics research. Anthocyanins appear to be the only group of phenolics for which genetic control has been established (Alston 1964). Groups or types of phenolics rather than individuals have been examined in relation to population differentiation.

It would seem that increased variation in individual phenolic compounds would have a genetic basis, while quantitative variation in this class of compounds would be due to environmentally induced shifts. Baldwin *et al.* (1986) state that the measure of total phenolics represents a poor marker for identifying geographic origin or seed source of *Acer saccharum*, primarily because of the confounding between-tree and seasonal variation.

HEIGHT GROWTH

In range-wide comparisons of inherent variability in height growth, Steinhoff (1986) compared coastal and interior seed sources for grand fir seedlings growing in N. Idaho. He found a fifty percent average superiority in height growth of coastal seedlings. Lines (1974) found significant

increases in height of coastal provenances over interior seed sources. Regression of height and latitude adjusted for elevation indicated a decrease of 3.6 cm per degree and accounts for 23% of the variation in height. Although the *Abies grandis* seedlings in the current study were only one year old and were growing under "optimal conditions", regression of seedling heights (Figure 21) showed 38% difference in height growth related to elevation alone. Regression of height growth with longitude of seed source produced a significant regression (r^2 = .98; P < 0.01).

In northern Idaho, regression of height on latitude alone accounted for nearly as much variation (10.5%) as did equations including habitat type (10.8%), elevation (11.8%) or all three variables (12.0%). In northern Idaho, Steinhoff (1986) found a decrease of 2.2 cm in height for every degree moved northward. Regression of height data and elevation of the current data (Figure 21) reveals a significant relationship (r^2= 87, P < 0.001). Since latitude was kept relatively constant in this study, it is not possible to assess the impact of that factor on the growth of these seed sources.

Seed sources from Oregon provide an interesting situation: height decreases from north to south, ostensibly from introgression of *Abies concolor* genes (Daniels 1969, Houkal 1976). Heights of interior seedlings in Oregon were approximately six percent less than the North Cascade seedlings.

Selection intensity of growing period appeared to increase with movement inland based on data by Rau and Weisgerber (1981) who found negative correlations with elevation of seed source and height growth of *Abies grandis*. The correlations were considerably weaker near the coast, but stronger in the eastern Cascades.

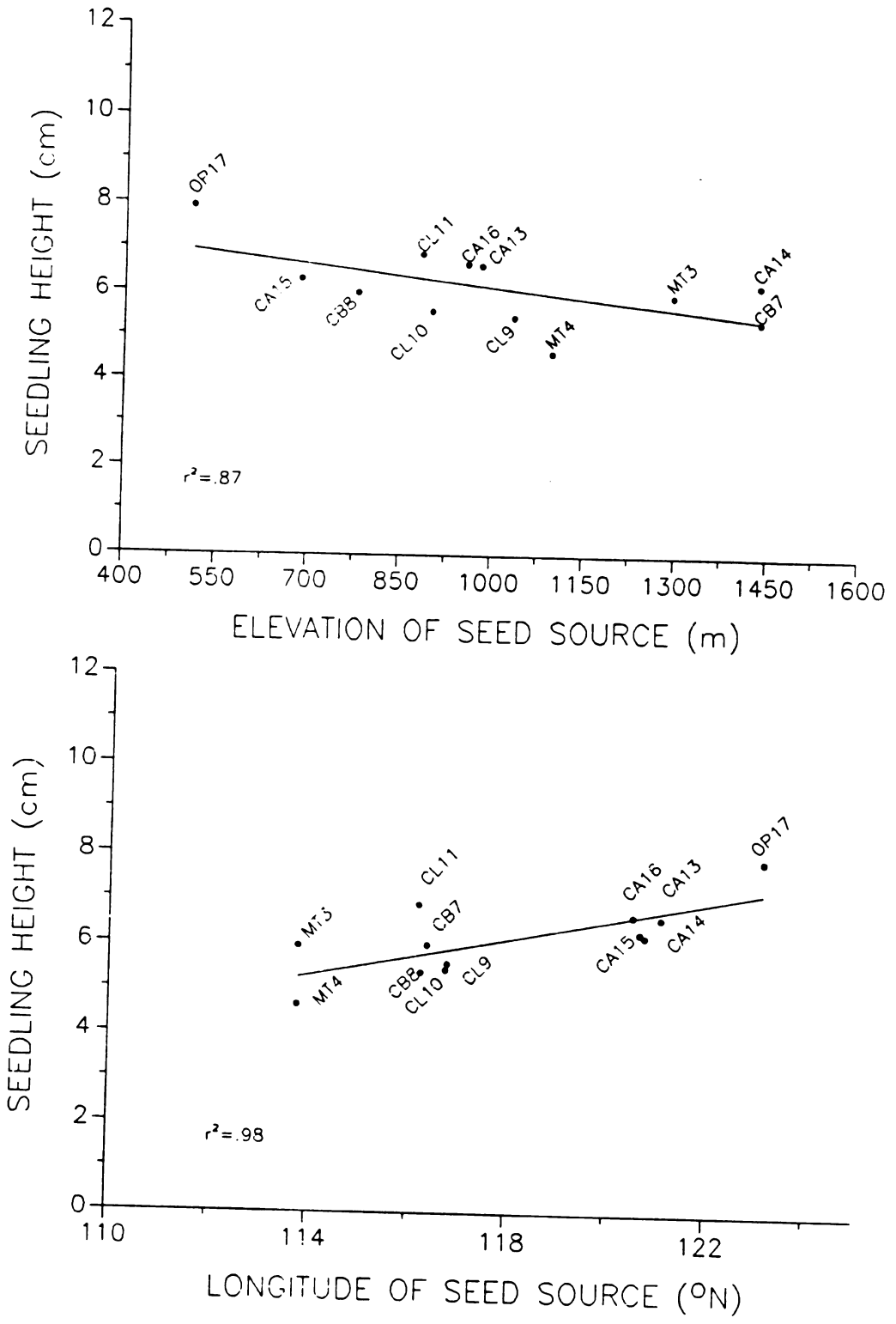


Figure 21. Regressions of height on elevation and on longitude of seed source.

Differences between families accounted for a greater percentage of total variation than differences between stands in Steinhoff's study. Overall, a latitude shift of 1.5° resulted in an average change of 5% in height and 10% in freezing injury to plants. Freezing injury of this species appears to be related to latitude of origin. No evidence was found in the Northern Idaho plantation of freezing death to foliage although temperatures reached -35°C. Results of survival in a plantation in Calhoun County, Michigan of grand fir seed sources used in this research indicate differential mortality (personal observation). Specifically, survival ranged from 48% (Olympic Mts.) to 64% (Cabinet Mts.), with 63% as average of all seed sources. The highest elevation site, Trestle Ridge (CB 7) in the Cabinet Mts. demonstrated the greatest survival (73%).

BIOSYNTHETIC ALTERATIONS

The relationship between pathways leading to terpenoid production and phenolic production is depicted in Figure 22. Clearly the precursor for both processes occurs early in the formulation of plant compounds. The details, however, are not so simple. For example, forms of terpenes have been shown to arise from independent systems. Bernard-Dagen (1988) found that monoterpenes are formed at the base of the needles, in the actively differentiating cells, while sesquiterpenes are formed in all parts of needles. Also, sesquiterpenes could be formed in light or dark, while monoterpenes are formed only under light conditions.

Generally, the conditions favorable for one pathway may suppress another pathway if there are common precursors, e.g. in the case of the shikimic acid pathway, amino acids and indole amino acids serve as common

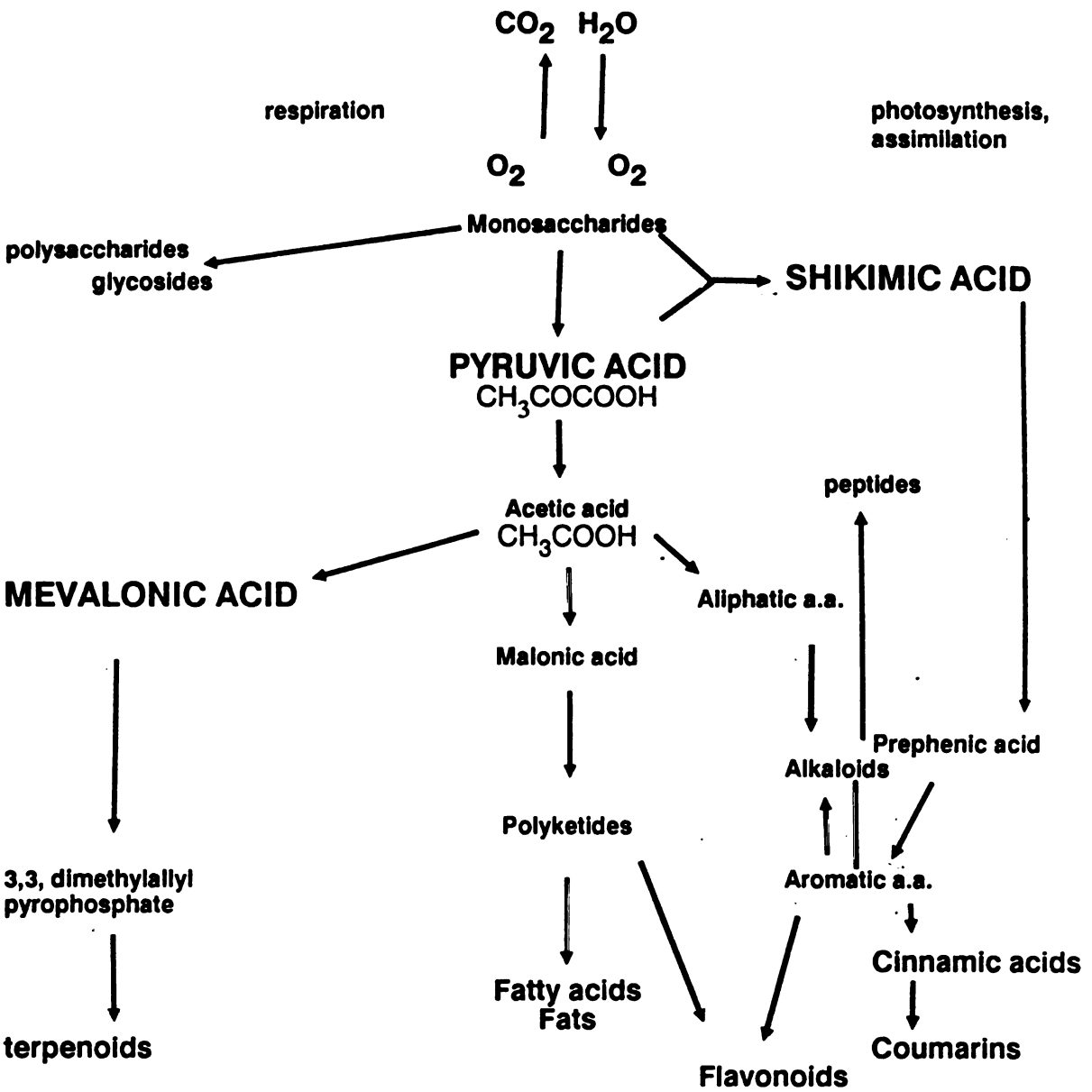


Figure 22. Fundamental pathways and relationship of primary and secondary metabolism. Modified from Torssell (1983).

precursors. It is obvious, however, that the mevalonic acid and shikimic acid pathways do not respond similarly.

Several factors have been shown to regulate phenolic production, and studies of plant cell cultures have provided evidence that for maximum production of most secondary metabolites, these cultures should be maintained under limiting conditions, particularly nitrogen and inorganic phosphate (Ibrahim 1987). A variety of external and internal biological functions regulate the production of secondary chemicals, and research suggests that phenolic products of the phenylpropanoid pathway may be even more sensitive to environmental stimuli than products of other biosynthetic pathways (Ibrahim 1987). The effect of carbon source on phenolic synthesis appears to be closely related to nitrate (Jessup and Fowler 1976), and Homeyer and Schultz (1988) found that the shikimate pathway is light dependent and affected by endproduct feedback.

Jessup and Fowler (1987) noted that an induction in nitrate assimilation leads to an increase in respiration and a shift from glycolysis to the pentose phosphate pathway. The pentose phosphate pathway provides part of the carbon skeleton of phenolic compounds as well as the NADPH required for their biosynthesis. Phosphate assimilation demonstrates a similar response (Schiel *et al.* 1984). Both the type and amount of N source affect the pattern and yield of phenolics formed. Fujita *et al.* (1981) found that shikonin derivatives (phenolics) in *Lithospermum* cells could only be achieved with NO_3^- as the sole N source and these phenolics were inhibited with NH_4^+.

In the present study, no individual compound, of those extracted, was reduced following fertilization. An increase with para-hydroxybenzoic acid

and coniferyl alcohol did occur following fertilization. Clearly the reduction in total phenolics reflects depression in production of phenolic compounds excluded in the extraction process.

Many compounds may be derived by mixed pathways. The phenolic compounds, quinones, provide an example of mixed biogenesis in that 7-methyl juglone comes from the polyketide pathway; but juglone, which is very similar, is derived strictly from shikimic acid. Several groups of metabolites have mixed biogenesis, i.e. an intermediate or metabolite from one pathway acts as a substrate for another metabolite from different pathway. Flavonoids are formed via the polyketide pathway (with three acetate groups), and a cinnamic acid from the shikimic acid route. Indole alkaloids come from shikimic acid and from a monoterpene (Torssell 1983). Because of the potential alteration of two pathways, one might expect that compounds of mixed biogenesis would be affected by changes in environment, particularly if these pathways are strongly environmentally controlled. The compounds of mixed biosynthesis isolated in the current study, i.e. flavonoids, did not reflect any changes with environment.

Although the depression of total phenolics with nitrogen fertilization has been clearly demonstrated in this study, the production of terpenes following fertilization was not predicted or explicable. Patterns of production of both groups of compounds, terpenes and phenolics, represent dissimilar modes of production (Figure 23). Total terpene yield was unchanged across treatments and seed source. Although differences were not always significant, trends were distinct with phenolic production, and, except for the Montana seed source, consistent depression of phenolic production was evident following fertilization. Nitrogen had no effect on

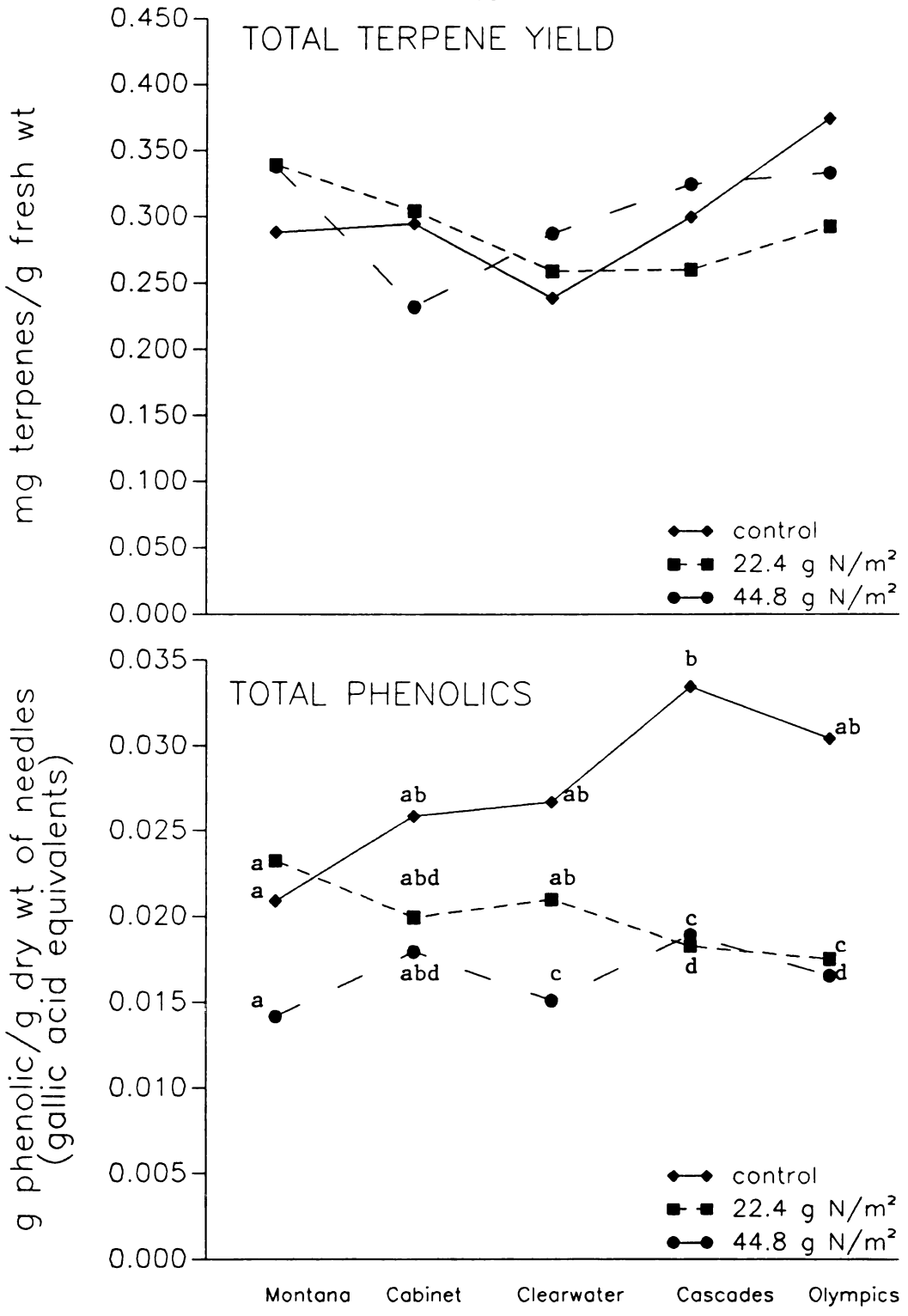


Figure 23. Variation in total terpene and phenolic yields following fertilization. No differences were apparent in total terpene yield. Mean values of total phenolics with the same letter are not significantly different ($P < 0.05$).

production of terpenes, probably because the immediate precursors of the mevalonic acid pathway are not common to other major groups of compounds. Although individual terpenes fluctuate with addition of nitrogen, no consistent trend is apparent, and the results suggest that intraconversion of terpenes occurs, since total yield remains the same.

Production of these two groups of compounds in terms of total phenols and yield of terpenes varied with seed source, and the relationship between them is depicted in Figures 24-26. Montana seedlings showed a dramatic decrease in phenolics at 44.8 g $N \cdot m^{-2}$. The decrease in phenolics was apparent at the 22.4 g $N \cdot m^{-2}$ level in the Cabinet Mts, one of the populations demonstrating a decrease in terpene yield with fertilization. Responses in the Clearwater Mts. populations reflect an inverse relationship in that an increase in total yield of terpenes occurred concomitantly with steady decline in phenolic production. Response in the Olympic Mts. and Cascade populations were surprisingly similar.

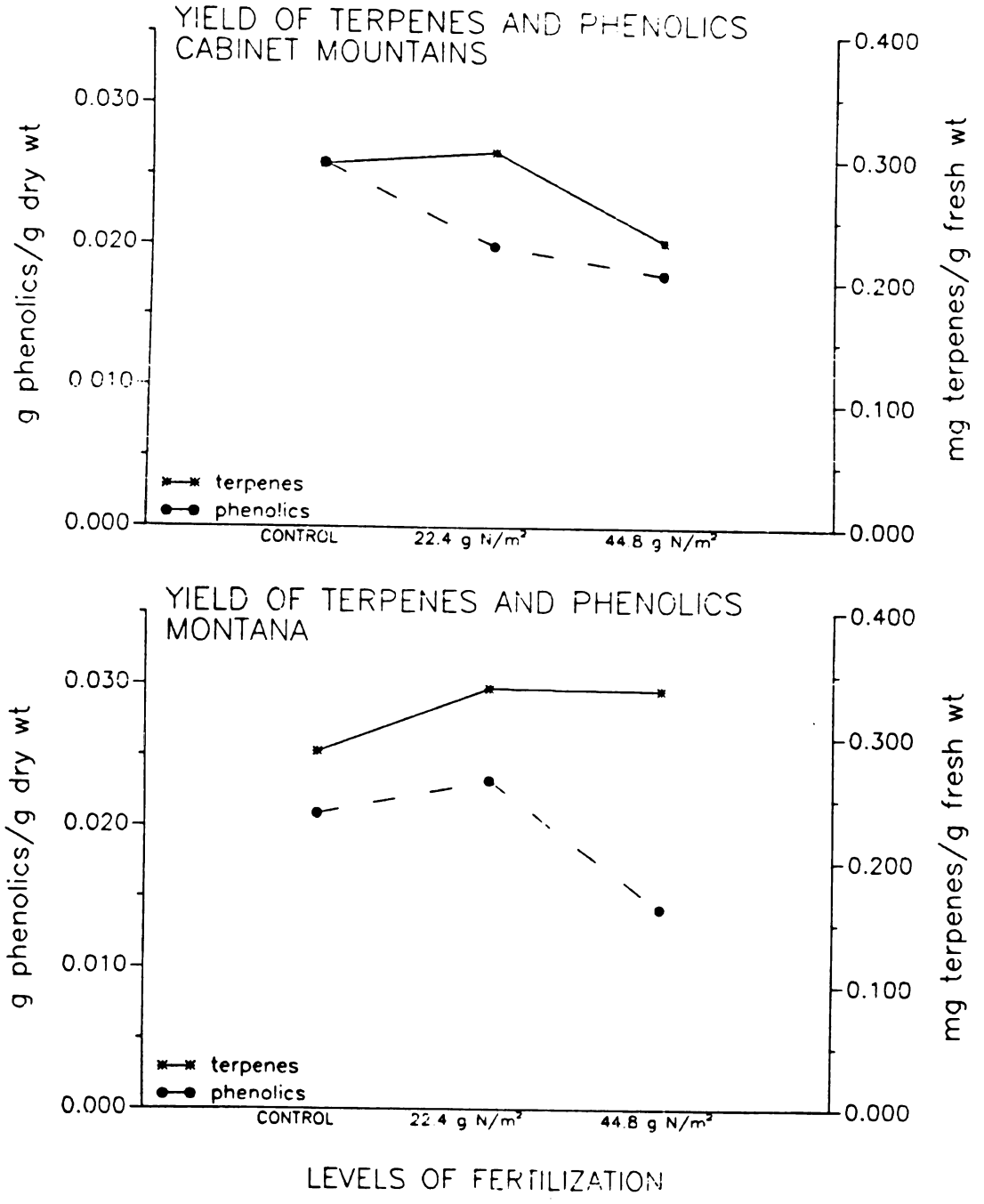


Figure 24. Variation in total phenolics and terpene yields with treatment for Montana and Cabinet Mountains regional populations.

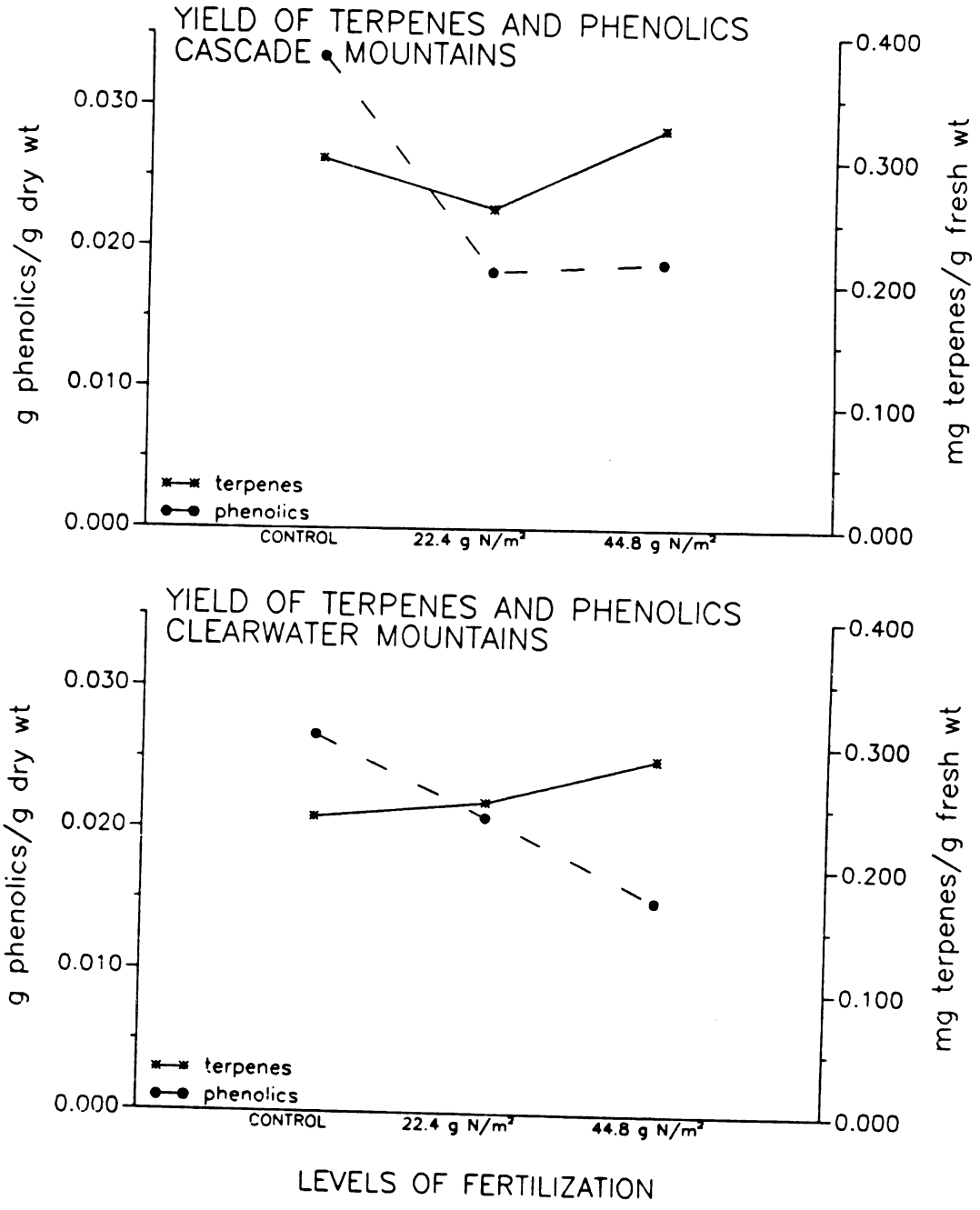


Figure 25. Variation in total phenolics and terpene yields with treatment for Clearwater Mountains and the Cascades regional populations.

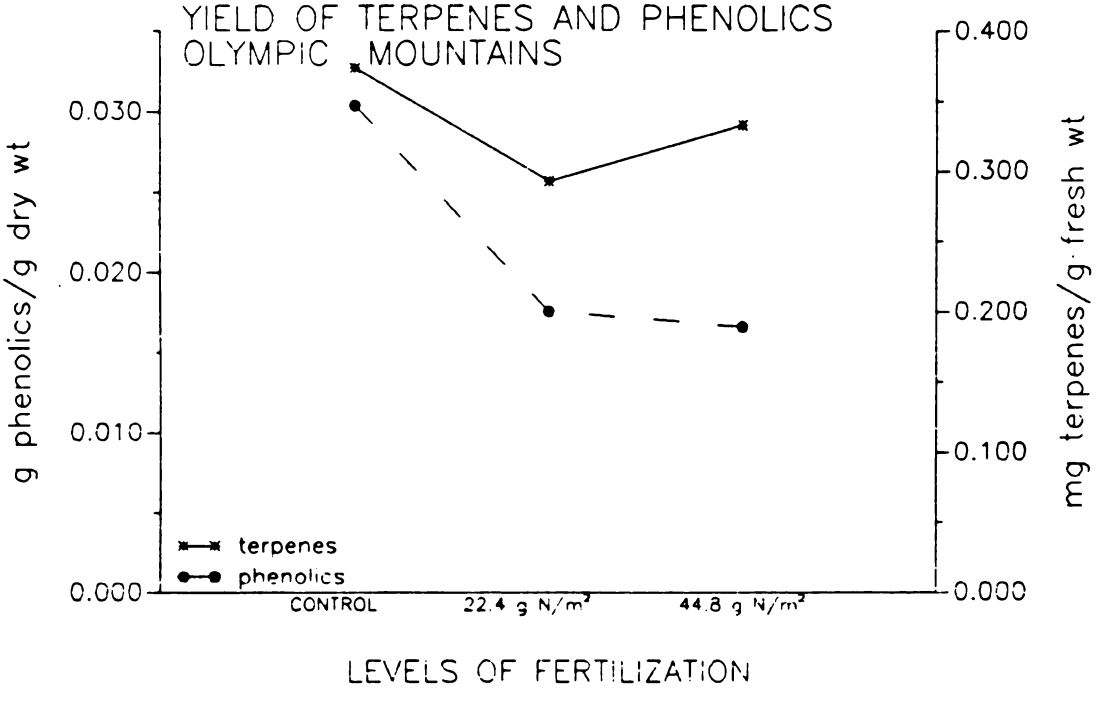


Figure 26. Variation in total phenolics and terpene yield with treatment for Olympic Mountains regional populations.

POPULATION DIFFERENTIATION AND GRAND FIR CHEMICAL / MORPHOLOGICAL CHARACTERISTICS

Namkoong *et al.*(1988) maintain that it is necessary to first define the environmental effect before one is capable of measuring a genetic response that expresses a genetic effect. It is important to remember that genetically caused variance and environmentally caused variance are not separate factors that generate phenotypes, rather both represent concomitant and simultaneous mechanisms and controls on production of phenotypes.

Houkal (1976) explained a great deal of variation in the *Abies grandis-Abies concolor* complex cortical monoterpenes with altitude, and many others have based ecotypic differentiation on terpene production in conifers (cf. Squillace 1976). In the current study, based on correspondence analysis, a strong trend with local populations arises. Regional populations, however, do not adhere to a generalized pattern based on terpene quantity. Trends relating to environmental gradients, e.g. elevation corresponding to morphological characteristics of seed sources, are also apparent in these populations of grand fir. Total height and the response of height to nitrogen fertilization distinguished regional populations (seed sources) well, setting the Olympic Mts. population apart from the others.

Most species of conifers exhibit a similar relationship between coastal and interior seed sources. *Abies grandis* exhibits considerable variation latitudinally, reflecting the length of growing season. Thus, major climatic differences represented by the more moist Olympic Mts. and cooler environments of higher elevation are apparent in the genotypes studied.

Populations demonstrating significant within-seed-source differences were in the Clearwater Mts., which happen to be the most southern

latitudinally. Differences in local populations were apparent in that the Ove Creek (CL 11) local population, the southernmost (Table 1), was significantly taller than the other local populations. Also the Clearwater Mts. present an interest setting environmentally. Rehfeldt (1983, 1986) found that *Larix occidentalis*, *Pseudotsuga menziesii*, and *Pinus ponderosa* seed sources from the Clearwater Mountains represent the seedlings with the most growth potential over the entire range of these species, primarily because this region has an unusually long growing season.

The elevational range of seed sources used in this study extends from 242 m to 1444 m, and encompasses the altitudinal distribution of the species (Figure 2). Variation in height growth and biomass have been demonstrated within a minimal elevational range of grand fir, but variation in secondary metabolites has not arisen from elevational changes. While Rehfeldt (1983) found variation in many characteristics including monoterpenes within 200 m in Douglas-fir, grand fir may exhibit moderate or nominal clinal variation with regard to chemical differentiation.

Since phenolic production was poorly related to seed source in this study, perhaps synthesis of these compounds is non-adaptive in relation to other traits, particularly morphological traits. Production of terpenes exhibited a strong local relationship, but not a discernible relationship at the regional level, thus demonstrating wide intra-population variation. The adaptive significance of secondary chemicals remains questionable, although much significance has been attached to certain compounds. Lack of a pattern corresponding to habitat conditions in my results supports this. Similarly, Townsend *et al.*(1972) found no relationship with latitude or

altitude in production of terpenes in *Pinus monticola*, and explained any variation that did exist in terms of genetic drift.

The principle of coherence as expressed by Clausen and Hiesey (1960) indicates that adaptive traits tend to be intercorrelated, and that the differentiation of species should be observed in correlated patterns of several traits. A commonly used example is the relationship between cold hardiness and growth potential. The control of production of secondary chemicals may be analogous to other traits in *Abies grandis* in that much intrapopulation variation exists. That is to say, *Abies grandis* may be a generalist. In examination of several coniferous species from western North America, Rehfeldt (1984) defined species as generalist or specialist depending on traits and clinal variation. Specialist species such as Douglas-fir or lodgepole pine have steep adaptive clines. Western larch (*Larix occidentalis*) and western white pine represent generalists with abundant intra-population variability and moderate, almost flat clines. Phenotypic plasticity represents an alternative strategy. A generalist can maintain genetic variability required for evolution and organize the genetic variability within the genetic system to achieve adaptation to heterogeneous environments. *Abies grandis* has been widely planted throughout the world and appears to adapt to a variety of environmental conditions. The adaptability probably reflects intrapopulation variation of the genetic architecture of this species.

SUMMARY

In terms of production of secondary metabolites, no strong association was found with environmental (site) variables. The random fluctuation in terpene and phenolic production and response in *Abies grandis* could be reflective of variation due to random genetic drift or demonstrate wide intrapopulation variation.

In reference to Hypothesis 1, carbon allocation patterns were generally similar among seed sources but height growth reflected increased growth potential with coastal genotypes.

Biosynthetic pathways do not all respond identically. Production of total phenolics was dramatically decreased with nitrogen fertilization, but total yield of terpenes was not affected. Individual phenolics and individual terpenes changed unpredictably, largely a function of seed source and specific compound.

Nitrogen fertilization produced noticeable differences in allocation to phenolics, which decreased while height growth and biomass increased. Terpenes as a group were unaffected by nitrogen fertilization. Hence, both the null and alternate hypotheses of Hypothesis 2 are true, depending on the class of compounds under consideration.

Most importantly, a significant treatment by local interaction occurred with terpenes, underscoring the genetic basis of terpene production and unpredictability of environmental effects. The significant interaction effect indicates that not all seed sources respond in the same manner to nitrogen fertilization at least regarding secondary metabolite, terpenes in particular (Hypothesis 3). Alternately, response in terms of carbon allocation to structure was uniform. No significant interaction was evident with phenolics.

The populations sampled do not represent significant divergence in production of monoterpenes and phenolics, but do with most growth characteristics.

Individual phenolic components have not been shown to account for the significant decrease in total phenolics occurring with nitrogen fertilization. Depression of total phenolics may be in part attributed to decrease in polyphenolic compounds which were not individually isolated.

Confounding factors rendering the results questionable include the conditions under which the seedlings were grown. Since light was constant and studies have shown light to be significant in production of monoterpenes (Bernard-Dagen 1988), production could have been inconsistent with field environmental conditions. Seedlings grew continuously. This also brings to mind the question of comparable results between glass-house grown seedlings and seedlings growing in a natural setting. The extent to which planting outside of native range affects phenotypic expression has always raised questions. Previous research has verified that nursery conditions mask genetic differences (Hermann and Lavender 1968).

With fertilization, the balance of carbon allocation within a seedling adjusts to produce primary structure, particularly shoot biomass at the expense of some secondary metabolites, namely phenolics, and probably non-amino acid constituents of the polyketide pathway. Conversely, secondary metabolites of the mevalonic acid pathway remain unaffected by fertilization.

APPENDIX A

LITERATURE REVIEW

LITERATURE REVIEW

GENERAL CARBON ALLOCATION

Variation in plant growth and differential growth of certain plant parts has been recognized since ancient times as evidenced by this quote from the agrarian Romans, Varro and Cato:

> ...in autumn and winter the roots develop more than does the leaf of the plant because they are nourished by the warmth of the roof of the earth, while the leaf above is cut down by the frosty air. (Harrison 1913)

An optimum balance of dry weight of shoot to root is species-specific and root to shoot ratio generally declines with size (Bray 1963) and age (Mooney 1972). Additionally the proportion of root to shoot may be ecologically meaningful regarding competition and succession (Monk 1966). But variation in plant allocation has ontogenetic, genetic and physiological controls.

Hunt and Lloyd (1987) maintain that innately fast-growing species are more root-oriented in their resource partitioning, and that such an innate trend held up in grasses despite nutrient stress and other environmentally-induced variation. Generally, growth-limitation in resources has been shown to create a change in the resource-partitioning of the plant.

Changes in plant allometry, growth and vigor with fertilization have been extensively documented. Differences at the species level and at higher levels are dramatic, e.g. variation in carbon acquisition and utilization between evergreen and deciduous plants (Mooney 1972). Changes in plant

chemistry as carbon sinks in allocation schemes have not been examined thoroughly.

Impetus in research to examine secondary chemicals has primarily derived from interest in these chemicals as 'defense' chemicals, referring to the possibility that they are deterrents or rank among a plant's array of protection against herbivores, insects, or pathogens. Ecological significance of these chemicals extends beyond the realm of defense. Many secondary chemicals serve as attractants for pollination, aggregating hormones, phytoalexins, or growth regulators (Harborne 1983). Many, but not all, appear to be allelochemics *sensu* Whittaker and Feeny (1971).

Research on carbon allocation in secondary chemistry has reflected apparent tradeoffs due to allocation patterns. Examples of compromises in growth have been explained by Coley (1987), who found a strong negative correlation between average annual growth rate and investment in defense in tropical trees. Further evidence of allocation tradeoffs have been found in negative correlations between the production of several monoterpenes and growth rate in *Pinus monticola* (Hanover 1966a, 1971).

Loehle (1988) examined strategic tradeoff in secondary chemicals he regarded as defensive compounds. The ultimate tradeoff was represented in terms of longevity, and he determined that trees with greater stemwood defenses were 2-3 times longer-lived than those with low defenses. The proximal tradeoff was viewed as a lowering of competitive ability in that the long-lived trees generally grew more slowly, and thus were apt to be overtopped or light-limited.

GENETIC CONTROL OF STRUCTURAL CARBON ALLOCATION

Generally, research has demonstrated that the effect of environment on control of carbon allocation is more predictable than the changes in allocation as a result of population differentiation; however genetic constraints on the relative size of plant parts do exist, but can be variable. The partitioning of biomass tends to be related to life history traits of particular plants, and Strauss and Ledig (1985) have demonstrated such a relationship using seed size, life span, competition tolerance and age at reproduction, with several members of the genus *Pinus*.

The root to shoot ratio is significantly controlled genetically such that dramatic treatments are required to change the root to shoot ratio with tree seedlings (Ledig and Perry 1965); but Crist and Stout (1929) emphasized inherent variability in the root to shoot ratio of annuals and biennials. Hermann and Lavender (1968) found differences in root and shoot dry weights of Douglas-fir populations from various elevations and different aspects. In general, the root to shoot ratio increased with increasing elevation of seed source. The mean dry weights of shoots and roots of seedlings from northern aspect parents were significantly greater than those of south aspect parents. The aspect differences were lessened (particularly for roots) with increases in elevation. Although Mullin (1985) found low heritability of root to shoot ratios among black spruce families, the families were significantly different in height growth and root collar diameters.

GENETIC CONTROL OF CARBON ALLOCATION TO SECONDARY CHEMISTRY

Changes in production of plant secondary chemistry may be quantitative, i.e. reflecting a change in absolute value, or qualitative which refers to the addition or substitution of compounds. Both types appear to be genetically controlled by a small number of loci (Chew and Rodman 1979). Control in two major groups of secondary chemicals, terpenes and phenolics, seems to differ; substantial evidence suggests that terpenes are under greater genetic control than phenolics.

Many studies have demonstrated the strong association of genotype with production of terpenes. Early in the century terpenes were considered to be controlled genetically when Baker and Smith (1920) distinguished species of *Eucalyptus* based on the volatile constituents. Subsequently, much research has focused on distinguishing populations based on terpenes, (chemotypes), particularly with conifers of western North America. Additionally, the genetics of terpene production has also gained prominence since the use of this class of secondary metabolites as taxonomic tools. Mirov (1961) summarized information on turpentine composition of 92 species in the genus *Pinus*, emphasizing the wide variability in terpenes. Forde (1964) decided that turpentine composition alone was insufficient in identifying hybrids of *Pinus attenuata* and *P. radiata*, but the major distinction between the turpentine composition in these two species is controlled by a single gene showing partial dominance. Hanover (1966a) contributed to understanding the genetic control of terpenoids by using clones of *Pinus monticola*. Genotypic control of monoterpenes has also been demonstrated by Esteban *et al.* (1976), who found lower intraclonal variation than interclonal variation

in Norway spruce. Monogenic control is apparent with certain monoterpenes, as in the case of 3-carene and terpinolene in Scots pine (Hiltunen 1976). The terpenes myrcene and beta-pinene have been shown to be controlled by two alleles at a single locus in *Pinus elliotti* (Squillace 1971) and limonene and beta-phellandrene have the same control in *Pinus taeda* (Squillace *et al.* 1980). Sesquiterpene inheritance has only been demonstrated in *Pinus pinaster* (Marpeau-Bezard *et al.* 1975).

Bernard-Dagen (1988) elucidated genetic and biochemical aspects of terpenoid control, particularly in *Pinus pinaster*, and found the monoterpenes 3-carene, terpinolene, myrcene, limonene and beta-phellandrene as well as caryophellene and longifolene are controlled by two alleles at the same locus.

Like terpenes, phenolics have been used in chemosystematic studies, therefore inferring a degree of genetic control. However, phenolics have not been used as extensively, nor as quantitatively. Flavonoids, predominantly, have been used as presence/absence markers, and many examples from angiosperm systematics exist. Stilbenes and proanthocyanidins also have been used taxonomically (Forrest 1975).

Hanover and Wilkinson (1970) distinguished four species of *Picea* and putative hybrids based on phenolic constituents. Research by Larsson *et al.* (1986) indicates that there is high variability within a clone, and limited genetic control for *Salix dasyclados* phenolics.

Fingerprinting of several genera of Pinaceae using flavonoids verified taxonomic relationships in the family previously established with morphological or immunological characteristics (Niemann and Van Genderen 1980). The utility of phenolics appear to vary among species, e.g. Niemann (1980) found quantitative differences in needle flavonoids of *Larix*

leptolepis, but Parker *et al.* (1983) found minimal variation in needle flavonoids of *Picea mariana*. Kaufmann *et al.* (1974) reported variation in a number of phenolic compounds in *Picea abies* needles from three geographically separated populations.

ENVIRONMENTAL CONTROL OF STRUCTURAL CARBON ALLOCATION

Since nitrogen is often the limiting nutrient in temperate forests, much research has been directed to understanding the physiological consequences of N stress as well as N fertilization. Nitrogen stress may alter relationships in growth among plant parts and compensate for partitioning, possibly at the expense of one aspect of growth such as relative growth rate (Robinson 1986).

The effect of N on structural components of tree species has been studied extensively, as has the effect on forest ecosystems. On a broad scale, the addition of N to forests of the Pacific Northwest resulted in hastening of canopy closure and an increase in leaf biomass (Keyes and Grier 1981, Johnson *et al.* 1982). When sites are nitrogen poor, N fertilization increased stem, branch and needle growth in Douglas-fir forests (Brix 1971, 1972, 1981, Ebell 1969, Mitchell 1980). Needles increased in dry weight, length, width, and surface area and the number of needles per shoot increased the second year; height growth increased by 30 to 50% (Ebell 1969).

Physiological processes are also altered with addition of nitrogen. Brix (1971) found that fertilization with the equivalent of 448 kg N/ha increased the photosynthetic capacity of new shoots. The enhanced photosynthetic rate was likely due to an increase in chlorophyll contents,

enhanced activity of carboxylating enzyme, increased conductances for CO_2 (Natr 1975, Mooney and Gulmon 1982), and improved utilization of carbohydrates (Sweet and Wareing 1966). Brix (1972) suggested that an improve tree water balance (i.e. lower tree water deficits) may occur during the day due to N fertilization and this would then have direct effects on stomatal conductances for CO_2.

According to Field and Mooney (1986) variation in leaf N can explain much of the variation in photosynthetic capacity across a wide range of plant communities. The best documented form of effect is that limits in RuBisCo enzyme by a deficiency of N limits photosynthesis. Of all forms of N in a leaf, 70%-80% represent proteins, 10% are in nucleic acids, 5%-10% form chlorophyll and lipoproteins, and the remainder are free amino acids (Field and Mooney 1986).

Biochemically, nitrogen fertilization has been shown to alter allocation within primary metabolic processes. Margolis and Waring (1986) found lower available polysaccharide and lower nonstructural carbohydrates, but higher free amino acids with nitrogen fertilization of Douglas-fir seedlings. Kim *et al.* (1987) fertilized containerized jack pine and black spruce seedlings with enhanced nitrogen concentrations and found that all amino acids except tryptophan increased. Carrow (1973) found that the ordinary appearance of phenylalanine at the beginning of the growing season was 'delayed by' all forms of N in fertilization in grand fir seedlings. An increase in tryptophan occurred however, with nitrogen fertilization. Both of these amino acids are aromatic and are produced in the shikimic acid biosynthetic pathway in which phenolic compounds are also produced.

ENVIRONMENTAL CONTROL OF CARBON ALLOCATION TO SECONDARY CHEMISTRY

The extent to which environmental factors control production of secondary chemicals has not been clearly elucidated, and depends on the type of secondary chemical in question. Far more research has been oriented toward understanding effects of stress (particularly moisture and nutrient) than the effects of nutrient enhancement. Powell and Adams (1973) found poor correlations between volatile oil yield and environmental variables such as temperature or moisture in herbaceous plants, and Hanover (1966c) found terpene composition to be quite stable with regard to environment. Four clones of *Pinus monticola* planted at various sites showed a similar terpene pattern. Smith (1964) noted a wide range of variation in *Pinus ponderosa*, both among trees and among sites, and concluded that there was no relationship to environmental conditions.

Lincoln and Langenheim (1978, 1981), studying *Satureja douglasii*, found the monoterpenoid composition to be genetically controlled. But, quantity of terpenoids has been correlated with light intensity (Lincoln and Langenheim 1978, 1979), temperature (Lincoln and Langenheim 1978), and moisture (Gershenzon *et al.* 1978).

Clark and Menary (1980) found an increased yield of peppermint essential oil associated with irrigation and nitrogen fertilization (100-300 kg·N·ha). Neither treatment alone, however, was effective in increasing yield. In nitrate limited environments, terpene yield increased with an accompanying increase in root growth (Mihaliak and Lincoln 1985). Lokar *et al.* (1985) found considerable variation in essential oil composition of *Artemisia alba*, depending on plant association and accompanying

environmental factors. Plants from areas of high precipitation and lower temperature produced a greater proportion of sesquiterpenes, in contrast to plants from a site with higher temperature and less precipitation which produced more oxygentated terpenes.

Based on evidence presented by Waring *et al.* (1985) and Bryant *et al.* (1983, 1985), growth is stimulated by nitrogen fertilization, but production of tannins and other compounds derived from shikimic acid is decreased. Bryant *et al.* (1985) suggest that dilution of phenolics is responsible for the apparent decrease. Jonasson *et al.* (1986) determined that temperature is an important factor in production of phenolics. With an increase in temperature, an accompanying increase in photosynthesis leads to an increase in total carbon fixed resulting in a dilution of nutrient concentrations, and hence an increase in secondary metabolites. These researchers also found consistent negative correlations between phenols of various types and N, P, and K.

In conifer species, Thorin and Nommik (1974) noted that the monoterpenes of *Pinus sylvestris* were entirely unaffected by fertilization. In contrast, Fretz (1976) found that daylength and fertilization influence individual monoterpenes in *Juniperus horizontalis*. Increased photoperiod produced an increase in alpha-pinene and limonene and decreased camphene, myrcene, and linalool. Fertilization with nitrogen increased only production of terpineol, and all other monoterpenes decreased or were not influenced by nitrogen.

Changes in cortical or xylem monoterpenes in moisture stressed trees has been suggested by Hodges and Lorio (1975), Gilmour (1977), Gollob (1980). Moisture stress also influences the production of foliar

monoterpenes of Douglas-fir (Cates 1983, Cates *et al.* 1983). According to summarized data provided by Gershenzon (1983), both water deficits and N deficiencies decrease production of terpenoids in trees, but in herbs and shrubs water stress increases terpenoid production and the influence of N deficiencies has not been determined.

Phenolic compounds are ubiquitous and possess several properties (e.g. antimicrobial activity) which implicate them in plant defense (Levin 1971, Zucker 1983), and Schopf (1986) has shown low molecular weight phenols to be inhibitory to herbivores. Even in ecosystem processes the occurrence of phenols, particularly polyphenols such as lignin, exert an influence on litter decomposition rates (Horner *et al.* 1988). Many environmental factors apparently control the synthesis of plant phenols, such as light (Langenheim et al. 1980, Mole et al. 1988), N levels (Margna 1977, Phillips and Henshaw 1977, Bryant *et al.* 1983), temperature and mineral nutrition (Wender 1970). Generally, a decrease in total yield occurs with diminishing light intensity, although light intensity does not affect composition of phenolics. In cell cultures, total phenolics increase substantially when medium becomes depleted of nitrogen or phosphorus (Westcott and Henshaw 1978, Knobloch and Berlin 1981). By adding nitrogen to the medium, growth is increased and phenolic production is suppressed (Phillips and Henshaw 1977). Other external stimuli such as wounding and interaction with microbes appear to influence phenolic biosynthesis (Jones 1984). Additionally, phenological changes have been shown to correspond with changes in phenolic content of plants (Beres 1984).

Mooney *et al.* (1983) maintain that polyphenols are the only effective deterrent to specialist herbivores. However, not all the phenolic compounds

formed are used by the plant as defensive compounds, nor do these all have any defined role. Certain biochemical attributes of these compounds, such as protein complexing ability of tannin, and their negative effects on digestibility and nutrient assimilation are critical in this regard (Levin 1971, Palo 1985). Watt (1989), however, found no evidence to suggest that the chemical composition of pine foliage had an effect on feeding.

Jacob and Rubery (1988) have defined a possible physiological role of flavonoids in modulating auxin transport. Flavonoids, specifically quercetin, apigenin, and kaemferol can inhibit auxin transport systems. Artzen *et al.* (1974) found the addition of quercetin or its glycoside enhanced the Hill reaction and photosynthetic phosphorylation. Addition of kaemferol caused an inhibition of cyclic or noncyclic photophosphorylation in isolated *Pisum* chloroplasts. The importance of their production and supposed tradeoff may be irrelevant to plant metabolism within a physiological context.

Ecologically, the role of secondary metabolites remains even more remote. Existence of structurally and functionally different chemicals in various species or even within closely related taxa may support the notion that plant species have evolved chemical adaptations manifested in different chemical solutions to a similar biological problem. An example is the occurrence of a group of phenolic compounds, flavanonols, in *Pinus* , but not in *Abies*.

Katoh *et al.* (1989) found a causal association between levels of foliar soluble sulfate (air pollution) and inhibition of the shikimate pathway. Consequently *Cryptomeria japonica* had decreased levels of foliar tannins and hence were susceptible to pathogens.

Theoretical proposals explaining changes in production of secondary metabolites with regard to environment have focused on both ecological and evolutionary aspects. Evolutionary discussions have concerned differences in amounts and type of defense chemicals and interspecific variation. The ecological perspective may be akin to examination of intraspecific variation in production of secondary metabolites. Interspecific differences have often been related to habitat differences. Much research has demonstrated that plants from resource-limited environments produce greater quantities of chemical defenses (Janzen 1974, Coley 1987). This relates to the growth rate hypothesis of Coley *et al.* (1985) which relies on the inverse relationship between growth rate and defense. The reasons for differences in growth rate are the same for differences in defense, i.e. species are adapted to a specific habitat.

While interspecific variation in secondary chemistry is explained by the adaptive solution, intraspecific variation is described by a more proximal theory. Bryant *et al.* (1983) derived the carbon/nutrient balance hypothesis which suggests that the balance of resources, not the absolute amounts are important in evaluating production of secondary chemicals. The production of specific types and amounts of chemicals represents a response to imbalance in the ratio of carbon to nutrients (particularly nitrogen). When a resource is present in amounts exceeding that necessary for growth, the remainder becomes substrate for chemical defense.

STUDIES OF THE GENUS *ABIES*

Many studies have examined the morphological characteristics of *Abies grandis*, particularly the *Abies grandis-Abies concolor* complex (Daniel 1969, Hamrick and Libby 1972, Houkal 1976). Lacaze and Tomassone (1967), using morphological and growth traits of grand fir seedlings planted in France, had developed a dividing line for the species. Ecotypes were distinguishable west of the crest of the Cascades, and north to south variation was noticeable for Cascade populations between 43° and 44° N latitude.

Terpene composition in the genus *Abies* has been investigated for determining relationships among seed sources and to provide a chemical understanding of the hybrid swarm, *A. grandis* and *A. concolor*. Houkal (1976) found a steady increase in the white fir "chemotype" with elevation. Hybrid individuals could be identified using terpene composition (Zavarin *et al.* 1975, 1977), based on proportion of camphene, in that grand fir produced much camphene with a minimal proportion of 3-carene. These authors maintained that grand fir was chemically constant from 47° N latitude northward, and that changes in populations occurred at 122°15' W longitude. Smedman *et al.* (1969) characterized grand fir as having considerably more sesquiterpenes than other members of the genus *Abies*. These studies used cortical oleoresin for terpene analysis. Only von Rudloff (1976) has examined the foliar terpenes of grand fir, but indicated that the twig oil and needle oil are strikingly similar in grand fir, unlike other firs. He recorded minor differences between coastal and interior populations with little within-tree and tree-to-tree variation.

Phenolics have only been examined in the wood component of grand fir. Not all the individual phenolic components were identified, but Puritch

(1977) found matairesinol, hydroxymatairesinol, conidendrin, and a phenolic glucoside in the heartwood, and many unknown glucosides in the sapwood. He also found a new phenolic glucoside in the sapwood correlated with aphid infestation. Kasper (1969) found pinitol, sesquoyitol, and an unknown compound with a ketone group in cold water extracts of grand fir. At least three classes of flavonoid were found in foliage of *Abies amabilis* by Parker *et al.* (1979).

APPENDIX B

CHEMICAL RESULTS

Table B.1 Mean values and standard deviations of terpenes of local population. Values are in mg/g fresh weight.

POPULATION	alpha pinene	beta phellandrene	beta pinene	bornyl acetate	cadinene
MONTANA	0.0545(0.016)	0.0868(0.042)	0.1345(0.097)	0.029(0.025)	0.0163(0.012)
Hog Heaven	0.0519(—)	0.0996(—)	0.1084(—)	0.0329(—)	0.0258(—)
Jewel Basin	0.0534(0.018)	0.0978(0.051)	0.1495(0.072)	0.0227(0.017	0.0157(0.012)
Porcupine	0.0529(0.009)	0.0905(0.030)	0.0846(0.059)	0.0171(0.007)	0.0167(—)
Cilly Creek	0.0565(0.021)	0.0766(0.036)	0.1582(0.129)	0.0380(0.034)	0.0155(0.013)
CABINET	0.0608(0.017)	0.1035(0.384)	0.0798(0.027)	0.0409(0.019)	0.0160(0.007)
Galena Point	0.0597(0.007)	0.1107(0.017)	0.0833(0.012)	0.0368(0.014)	0.0159(0.005)
Trestle Ridge	0.0504(0.014)	0.0713(0.028)	0.0668(0.0408)	0.0502(0.028)	0.0123(0.006)
Trout Creek	0.0966(—)	0.1710(—)	0.1050(—)	0.0291(—)	0.0281(—)
CLEARWATER	0.0527(0.0148)	0.0682(0.0458)	0.1819(0.1010)	0.0227(0.0218)	0.0193(0.015)
Moscow Mt.	0.0403(—)	0.0600(—)	0.0947(—)	0.0193(—)	0.0012(—)
Brown's Meadow	0.0530(0.010)	0.0942(0.079)	0.1547(0.013)	0.0079(0.003)	0.0138(0.007)
Ove Creek	0.0610(0.019)	0.0519(0.008)	0.2941(0.117)	0.0163(0.017)	0.0312(0.021)
Riggear Peak	0.0451(0.015)	0.0581(0.004)	0.1255(0.014)	0.0520(0.020)	0.0169(0.002)
CASCADES	0.0585(0.028)	0.1066(0.0310)	0.0632(0.0107)	0.0207(0.0096)	0.0113(0.009)
Teanaway	0.0279(—)	0.0731(—)	0.0733(—)	0.0223(—)	0.0248(—)
Alder Creek	0.0535(—)	0.1166(—)	0.0607(—)	0.0181(—)	0.0068(—)
Entiat Mt.	0.0763(0.029)	0.1185(0.037)	0.0594(0.014)	0.0212(0.016)	0.0069(0.007)
OLYMPICS	0.0467(0.0173)	0.0575(0.0399)	0.1511(0.0580)	0.0580(0.0492)	0.0363(0.024)
Dungeness	0.0467(0.017)	0.0575(0.0399)	0.1511(0.058)	0.0580(0.049)	0.0363(0.024)

Table B.1 (cont'd). Mean values and standard deviations of terpenes of local population. Values are in mg/g fresh weight.

POPULATION	camphene	limonene	myrcene	terpinolene	tricyclene
MONTANA	0.0157(0.012)	0.005(0.003)	0.005(0.002)	0.002(0.001)	0.0020(0.001)
Hog Heaven	0.0195(----)	0.0052(----)	0.0057(----)	0.0022(----)	0.0021(----)
Jewel Basin	0.0233(0.017)	0.0054(0.002)	0.0054(0.002)	0.0018(0.001)	0.0028(0.001)
Porcupine	0.0108(0.007)	0.0020(----)	0.0033(----)	0.0013(----)	0.0007(----)
Cilly Creek	0.0141(0.011)	0.0053(0.003)	0.0049(0.009)	0.0021(0.001)	0.0015(0.001)
CABINET	0.0197(0.009)	0.0059(0.003)	0.005(0.002)	0.0022(0.000)	0.0020(0.001)
Galena Point	0.0161(0.003)	0.0059(0.004)	0.0053(0.001)	0.0023(0.000)	0.0016(0.000)
Trestle Ridge	0.0252(0.013)	0.0055(0.003)	0.0038(0.001)	0.0019(0.001)	0.0026(0.001)
Trout Creek	0.0175(----)	0.0068(----)	0.0080(----)	0.0027(----)	0.0020(----)
CLEARWATER	0.0155(0.015)	0.0049(0.0024)	0.0052(0.002))	0.0017(0.001)	0.0020(0.002)
Moscow Mt.	0.0094(----)	0.0024(----)	0.0037(----)	0.0011(----)	0.0011(0.000)
Brown's Meadow	0.0081(0.003)	0.0043(0.002)	0.0058(0.003)	0.0018(0.001)	0.0015(0.000)
Ove Creek	0.0101(0.009)	0.0045(0.002)	0.0056(0.001)	0.0015(0.000)	0.0014(0.001)
Riggear Peak	0.0350(0.019)	0.0071(0.003)	0.0042(0.000)	0.0021(0.000)	0.0036(0.002)
CASCADES	0.0124(0.004)	0.0050(0.000)	0.0051(0.001)	0.0022(0.000)	0.0013(0.00)
Teanaway	0.0173(----)	0.0050(----)	0.0040(----)	0.0018(----)	0.0015(0.002)
Alder Creek	0.0119(----)	0.0046(----)	0.0054(----)	0.0021(----)	----------
Entiat Mt.	0.0107(0.002)	0.0054(----)	0.0055(0.001)	0.0023(0.000)	0.0012(0.000)
OLYMPICS	0.0263(0.015)	0.0090(0.003)	0.0043(0.002)	0.0017(0.001)	0.0085(0.015)
Dungeness	0.0263(0.015)	0.0090(0.003)	0.0043(0.002)	0.0017(0.001)	0.0085(0.015)

Table B.2. Mean percentage and standard deviations of major phenolic constituents in grand fir foliage with two levels of nitrogen fertilization.

MONTANA SEED SOURCE

Phenolic	CONTROL	22.4 g $N \cdot m^{-2}$	44.8 g $N \cdot m^{-2}$
Caffeic acid	0.845(0.823)	0.327(0.427)	----------
Catechin	0.880(1.395)	0.460(0.427)	----------
Cinnamic acid	3.587(3.580)	2.538(1.203)	4.710(-----)
Coniferyl alcohol	5.403(3.773)	3.132(2.809)	4.250(-----)
Ferulic acid	3.637(3.708)	3.758(2.845)	3.430(-----)
Gallic acid	0.735(0.630)	0.635(0.742)	0.660(-----)
Gentisic acid	1.910(-----)	0.340(0.224)	----------
Kaempferol	9.897(3.183)	9.950(1.320)	13.490(-----)
Myricetin	5.755(1.578)	2.365(1.431)	5.110(-----)
P-coumaric acid	4.463(4.736)	6.844(6.732)	9.720(-----)
P-hydroxybenzoic acid	2.128(2.595)	0.940(0.793)	2.570(-----)
Protocatechuic acid	0.660(-----)	----------	----------
Quercetin	3.388(2.789)	2.432(1.277)	5.410(-----)
Sinapic acid	2.596(2.074)	2.536(2.872)	4.830(-----)
Syringic acid	1.214(1.083)	1.345(1.138)	2.490(-----)
Vanillic acid	1.804(2.196)	0.700(0.594)	0.280(-----)
Vanillin	1.543(0.629)	0.497(0.215)	----------

Table B.3. Mean percentage and standard deviations of major phenolic constituents in grand fir foliage with two levels of nitrogen fertilization.

CABINET MOUNTAINS SEED SOURCE

Phenolic	CONTROL	22.4 g $N \cdot m^{-2}$	44.8 g $N \cdot m^{-2}$
Caffeic acid	3.288(4.711)	2.207(2.332)	1.570(1.587)
Catechin	4.860(5.614)	0.615(0.587)	0.705(0.247)
Cinnamic acid	6.713(8.718)	2.693(1.235)	3.275(2.764)
Coniferyl alcohol	14.508(18.40)	5.133(2.535)	6.147(2.238)
Ferulic acid	3.893(3.070)	1.177(0.001)	2.750(2.589)
Gallic acid	----------	0.190(-----)	----------
Gentisic acid	0.770(-----)	0.200(-----)	1.850(-----)
Kaempferol	8.860(5.709)	10.547(1.788)	4.912(3.465)
Myricetin	18.760(8.405)	9.683(2.627)	4.468(2.029)
P-coumaric acid	12.505(15.126)	5.853(5.635)	4.598(1.405)
P-hydroxybenzoic acid	2.280(2.347)	1.893(2.604)	1.703(0.499)
Protocatechuic acid	----------	----------	0.23(-----)
Quercetin	5.687(7.245)	3.773(0.012)	3.668(3.549)
Sinapic acid	2.447(1.981)	2.200(0.001)	3.965(3.417)
Syringic acid	15.632(20.347)	3.020(0.000)	1.533(0.355)
Vanillic acid	0.925(0.601)	2.600(-----)	1.585(0.587)
Vanillin	2.455(2.835)	0.790(0.000)	1.953(1.482)

Table B.4. Mean percentage and standard deviations of major phenolic constituents in grand fir foliage with two levels of nitrogen fertilization.

CLEARWATER MOUNTAINS SEED SOURCE

Phenolic	CONTROL	22.4 g $N \cdot m^{-2}$	44.8 g $N \cdot m^{-2}$
Caffeic acid	1.110(-----)	0.913(0.489)	1.018(0.547)
Catechin	1.020(-----)	1.480(-----)	0.915(0.558)
Cinnamic acid	2.230(-----)	2.880(1.490)	4.056(2.567)
Coniferyl alcohol	5.120(-----)	8.282(3.344)	9.146(4.999)
Ferulic acid	4.100(-----)	5.030(2.546)	3.168(2.233)
Gallic acid	----------	0.530(-----)	0.610(0.212)
Gentisic acid	----------	----------	1.395(1.393)
Kaempferol	9.830(-----)	10.590(5.913)	9.066(3.176)
Myricetin	6.860(-----)	7.298(3.820)	4.601(2.537)
P-coumaric acid	10.630(------)	4.862(1.886)	8.014(5.505)
P-hydroxybenzoic acid	0.820(-----)	1.482(2.036)	1.644(1.279)
Protocatechuic acid	----------	0.780(-----)	0.16(-----)
Quercetin	2.930(-----)	5.128(3.835)	3.471(1.573)
Sinapic acid	4.300(-----)	3.987(2.238)	2.447(2.223)
Syringic acid	1.550(-----)	2.057(1.298)	1.233(0.561)
Vanillic acid	0.530(-----)	1.590(1.708)	1.852(2.265)
Vanillin	2.090(-----)	3.505(2.779)	0.620(-----)

Table B.5. Mean percentage and standard deviations of major phenolic constituents in grand fir foliage with two levels of nitrogen fertilization.

Phenolic	CASCADES SEED SOURCE		
	CONTROL	22.4 g $N \cdot m^{-2}$	44.8 g $N \cdot m^{-2}$
Caffeic acid	2.169(2.020)	0.635(0.262)	0.310(-----)
Catechin	1.077(0.902)	0.630(-----)	----------
Cinnamic acid	3.030(2.925)	1.270(0.227)	1.000(-----)
Coniferyl alcohol	4.757(1.773)	5.545(0.488)	12.340(-----)
Ferulic acid	5.900(5.412)	4.910(4.935)	9.380(-----)
Gallic acid	1.701(0.956)	----------	0.280(-----)
Gentisic acid	0.713(0.364)	0.064(-----)	0.520(-----)
Kaempferol	7.746(4.739)	9.000(1.880)	15.050(-----)
Myricetin	5.562(3.124)	4.120(3.026)	7.270(-----)
P-coumaric acid	6.886(3.986)	9.910(7.863)	4.020(-----)
P-hydroxybenzoic acid	0.886(0.607)	0.800(0.396)	1.110(-----)
Protocatechuic acid	0.376(0.066)	----------	----------
Quercetin	3.347(2.314)	1.485(0.559)	2.390(-----)
Sinapic acid	3.670(2.441)	4.455(2.977)	----------
Syringic acid	1.523(0.703)	1.605(0.926)	0.620(-----)
Vanillic acid	1.443(1.004)	----------	0.440(-----)
Vanillin	1.554(0.816)	0.795(0.403)	1.720(-----)

Table B.6. Mean percentage and standard deviations of major phenolic constituents in grand fir foliage with two levels of nitrogen fertilization.

OLYMPIC MOUNTAINS SEED SOURCE

Phenolic	CONTROL	22.4 g N/m^2	448 g N/m^2
Caffeic acid	1.050(-----)	1.000(0.368)	0.575(0.163)
Catechin	0.890(-----)	----------	----------
Cinnamic acid	3.845(1.761)	2.040(0.524)	2.285(0.643)
Coniferyl alcohol	4.370(0.452)	8.160(3.123)	7.170(3.592)
Ferulic acid	2.175(1.393)	5.860(2.279)	4.225(1.082)
Gallic acid	----------	----------	------
Gentisic acid	0.230(-----)	0.820(0.608)	1.250(0.424)
Kaempferol	3.595(0.785)	6.290(3.320)	5.880(1.739)
Myricetin	4.140(0.721)	6.050(3.487)	3.550(0.707)
P-coumaric acid	5.210(2.927)	7.633(4.182)	12.435(5.268)
P-hydroxybenzoic acid	0.550(0.438)	1.620(0.707)	1.940(1.004)
Protocatechuic acid	----------	0.340(0.099)	0.490(-----)
Quercetin	4.955(5.494)	2.687(0.490)	2.015(0.389)
Sinapic acid	2.455(1.167)	5.373(2.773)	4.560(-----)
Syringic acid	1.110(-----)	1.797(1.416)	1.645(0.403)
Vanillic acid	0.680(-----)	0.583(0.244)	1.305(0.318)
Vanillin	1.270(-----)	3.420(-----)	----------

APPENDIX C

SITE DESCRIPTIONS

SITE DESCRIPTIONS

Below are general site descriptions and features of each local population of grand fir seed sources. Vegetation data for each site appears in succeeding tables of this appendix.

REGIONAL POPULATION: MISSION MTS., WESTERN MONTANA
Local population: Hog Heaven (MT1)

This site, the highest elevation of the Montana populations, is situated at 1444 m in the Mission Mts. The location is Township 26 North, Range 22 West, the east 1/2 of the southwest 1/4 of section 36. The overstory vegetation is predominatedly *Abies grandis* and some *Pseudotsuga menziesii*, with *Abies lasiocarpa* near the periphery, with total basal area of 38.81 $m^2 \cdot ha^{-1}$. The habitat type is *Abies grandis/Xerophyllum tenax*, and the area appeared somewhat disturbed by grazing.

The soil was quite shallow, extending only to 30 cm and was minimally developed. Parent material was of a fluvial origin.

REGIONAL POPULATION: SWAN MTS., WESTERN MONTANA
Local population: Jewel Basin (MT2)

Jewel Basin is located at 907 m, in a valley bottom in the Swan Mountains in Township 24 North, Range 19 West, in the northeast 1/4 of section 2. The habitat type is *Abies grandis/Clintonia uniflora*, the *Arnica nudicaulis* phase. The site represents a relatively disturbed evenaged *Abies grandis* stand, with reproduction limited to this species, *Acer glabrum*, and *Picea englemanii*. *Betula papyrifera* is also common, but only as an overstory

species. Total basal area at this site is 37.98 $m^2 \cdot ha^{-1}$. General site vigor is low; many trees appeared to be infected with fungus and were declining. Tree cores suggest that *Abies grandis* was released approximately 40 years ago when logging removed *Pinus monticola* and possibly *Larix occidentalis*.

The soil consists of a variety of rock types, primarily fluvial material in a calcareous matrix (pH > 7 in top 30 cm). Generally, there is very little soil development, and it, along with extreme stoniness (75% by volume) obscures soil horizons. The soil has low moisture-holding capacity, but the valley position may override soil deficiencies accounting for site quality. The parent material for this site is water-transported metamorphic and sandstone quartzite.

REGIONAL POPULATION: SWAN MTS., WESTERN MONTANA
Local population: Porcupine Cr. (MT3)

This site is located in the Swan Mts. of western Montana at Township 24 North, Range 18 West in the south 1/2 of the southeast 1/4 of section 15 and also in the north 1/2 of the Northeast 1/4 of section 22. The vegetation is characteristic of the *Clintonia uniflora* phase of *Thuja plicata/Clintonia uniflora* habitat type. It is midslope, on an extreme slope (40%) at 1297 m. The slope length is greater than 100 m. The site supports a total basal area of 46.35 $m^2 \cdot ha^{-1}$ with *Abies grandis* as the predominant species. Many large, dead *Pinus monticola* were present, mortality likely due to blister rust. The stand is even-aged, approximately 50 years old, and probably initiated following fire. *Taxus brevifolia* is abundant in the shrub layer and understory.

The soil is shallow with bedrock apparent at 60 cm. The parent material appeared to be limestone with massive, partially decomposed

limestone occurring at 12-60 cm. It is predominantly ash with textural differences and water-lain mudstone.

REGIONAL POPULATION: SWAN MTS., WESTERN MONTANA
Local population: Cilly Creek (MT4)

This site is located at 1099 m in the Swan Mts. The topographic position is a bench at the base of a mountain. The habitat type appears to be *Abies grandis/Clintonia uniflora*, in the *Aralia nudicaulis* phase. *Abies grandis* is the dominant overstory species, *Pseudotsuga menziesii* and *Acer glabrum* var. *douglasii* are the only other overstory species present; total basal area for the site is 35.8 $m^2 \cdot ha^{-1}$. The unevenaged stand consists of large old growth trees of *Abies grandis* and *Pseudotsuga menziesii*. *Abies grandis* dominates because of the removal of other species such as *Pseudotsuga menziesii* and *Larix occidentalis*, and *Thuja plicata*.

Volcanic ash and tephra cover the silt-loam surface soil layers. The soil is alkaline and a deep ash with no sign of bedrock to 75 cm. The top 60 cm had less than 5% rocks by volume, and the last few cm bore 30 - 45% rocks by volume. The location of this area is Township 24 N, Range 18 west, the south 1/2 of the southeast 1/4 of Section 16.

REGIONAL POPULATION: SELKIRK MTS., NORTHERN IDAHO
Local population: Gleason Meadow (CB5)

This site, at 760 m in the Selkirk Mts., had been high graded 8 - 10 years prior to sampling. *Tsuga heterophylla* and non-vigorous *Abies grandis* remained and the site probably represent the *Tsuga heterophylla/Pachistima myrsinites* habitat type. Total basal area was only 16.92 $m^2 \cdot ha^{-1}$.

Topographically, the site resembles an outwash plain with sand from glacial deposits, nestled into bedrock hills. Soil was mottled, fluvial, silty with some large sand fragments. It appeared to be the product of two different parent materials, one was likely fluvial, and the other, volcanic ash. The site is located at Township 58 north, Range 5 west, in the SE 1/4 of Section 3.

REGIONAL POPULATION: SELKIRK MTS., E. WASHINGTON
Local population: Galena Point (CB6)

Galena point is located in Township 34 north, Range 45 east in section 33 (N 1/2 of the NW 1/4) and in section 28 (S 1/2 of the SW 1/4). This unevenaged stand represent the *Tsuga heterophylla/Pachistima myrsinites* habitat type. The predominately *Abies grandis* stand supported 39.67 $m^2 \cdot ha^{-1}$ of basal area.

The parent material of the soil was granitic, and the soil was well developed.

REGIONAL POPULATION: CABINET MTS., NORTHERN IDAHO
Local population: Trestle Ridge (CB7)

Trestle Ridge is midslope of Trout peak at 1440 m elevation. The site is located at Township 58 north, Range 2 east, the southwest 1/4 of the northeast 1/4 of section 19. The vegetation is characteristic of the *Abies lasiocarpa/Xerophyllum tenax* habitat type. The slope is steep (42%) and the soil is not well developed and 20% - 25% rocks by volume. The parent material is granitic. *Abies lasiocarpa* dominates the unevenaged stand with strong support by *Larix occidentalis* and *Pinus contorta*. The vegetation appears to be in a transition zone between mid- to high elevation with vegetation representative of both. *Abies grandis* appears to be the major

replacing species, as the gaps of 45-75 years old are filling with these. Many of the large *Tsuga heterophylla* were at least 150 years old. Total basal area at the site is 31.92 $m^2 \cdot ha^{-1}$.

REGIONAL POPULATION: CABINET MTS., NORTHERN IDAHO
Local population: Trout Creek (CB8)

Trout Creek is located near Trestle Ridge, but at an elevation of 780 m, along a gently sloping ravine. The site occurs in Township 58 north, Range 1 east, in the south half of section 28. Regeneration of *Abies grandis* is well represented in this *Tsuga heterophylla/Pachistima myrsinites* habitat type with a total overstory basal area of 35 $m^2 \cdot ha^{-1}$. Windthrow damage is prevalent here, and recent cutting has taken place. The diverse overstory consists of *Thuja plicata, Tsuga heterophylla* and *Ts. mertensiana*, and *Betula papyrifera*. Understory vegetation is relatively sparse; *Rosa gymnocarpa* and *Pachistima myrsinites* represent the dominant shrubs and *Coptis occidentalis* and *Chimaphila umbellata* occur as widespread forbs.

Soil is deep, but not well developed. The parent material is granitic and samples consist of a variety of cobbles. The well-worn nature of the material indicates it had been transported great distances.

REGIONAL POPULATION: CLEARWATER MTS., N. IDAHO
Local Population: Moscow Mountain (CL9)

Moscow Mt. is located in the Palouse Range at 1039 m in Township 40 North, Range 3 West, in the south 1/2 of the southeast 1/4 of section 5. The vegetation characterizes an *Abies grandis/Clintonia uniflora* habitat type. This relatively steep (35%) midslope position supports 36.81 $m^2 \cdot ha^{-1}$ in aboveground basal area. This site is essentially a cedar (*Thuja plicata*) stand

with considerable openess and a diverse array of ground flora species. Evidence exists of substantial windthrow damage accompanied by previous logging. This area is also prone to heavy grazing.

The soil appeared to be deep with good development. Most of it was of ash origin and rock-free.

REGIONAL POPULATION: CLEARWATER MTS., N. IDAHO
Local Population: Brown's Meadow (CL10)

Thuja plicata dominates this stand, and the only *Abies grandis* present were roadside trees. This is a dense stand with minimal light penetration to the forest floor. The major form of disturbance appears to be grazing, and there is no evidence of recent logging. The topographic position is upper midslope near the ridge at 902 m in the Palouse Range. The site is situated in Township 40 North, Range 4 West, the northwest 1/4 of the northeast 1/4 of section 1. The habitat type of this site is *Thuja plicata/Athyrium felix-femina*. The total aboveground basal area represented the maximum of the interior populations: 53.26 $m^2 \cdot ha^{-1}$, the majority of this accounted for by *Thuja plicata*.

Soil on this site appeared ashy and volcanic in origin, deep with no rocks present. The proximity of this stand to Moscow Mt. would suggest the same parent material.

REGIONAL POPULATION: CLEARWATER MTS., N IDAHO
Local Population: Ove Creek (CL11)

Ove Creek is located in Township 38 North, Range 2 East, the east 1/2 of the northeast 1/4 of Section 33. The elevation is 885 m and the topographic position is midslope on an extreme slope of 70%. This is *Abies grandis/Pachistima myrsinites* habitat type, the stand consisting of *Abies*

grandis predominately with a varied understory. It typifies a moist, north-facing slope. *Pseudotsuga menziesii* and *Pinus monticola* were removed approximately twenty years ago, and current total basal area is 25.32 $m^2 \cdot ha^{-1}$. Soil is ashy and underlain with basalt.

REGIONAL POPULATION: CLEARWATER MTS., N IDAHO
Local Population: Riggear Peak (CL12)

This site was located in Township 37 North, Range 2 East and in section 8. Basal area here was least of all site (13.3 $m^2 \cdot ha^{-1}$), and was exclusively *Abies grandis*. This area was highly disturbed, particularly from grazing and was adjacent to an old field and orchard. The site was probably *Abies grandis/Calamagrostis rubescens* habitat type. Soil was derived from basalt parent material; the soil was heavy and dry with little development.

REGIONAL POPULATION: EAST CASCADES, WESTERN WASHINGTON
Local Population: Davis Creek (CA13)

This site is in the Wenatchee Range of the Cascades in Township 21 North, Range 14 East, in the northwest 1/4 of Section 23. The elevation is 982 m along a broad, gently sloping saddle or ridge. The canopy is closed and incident light is minimal; consequently, shrubs (primarily *Acer*) are not very tall or thick. Remnant *Pseudotsuga menziesii* stumps and an occasional large tree of this species outside the plot suggest that this species was logged from the area and *Abies grandis* are actively replacing them. *Thuja plicata* is regenerating by layering. Above-ground basal area in this stand is 47.68 $m^2 \cdot ha^{-1}$. The habitat type is *Abies grandis/Linnaea borealis*, the latter species covering some debris entirely.

The parent material of this soil is basalt. The soil is relatively well developed with some clay occurring beyond 30 cm. Distance to bedrock is beyond measure.

REGIONAL POPULATION: EAST CASCADES, WESTERN WASHINGTON
Local Population: Teanaway Ridge (CA14)

This highly disturbed site is located on an upper slope, near the ridgetop in the Wenatchee Mts. The area was logged and subject to intensive grazing by sheep, and some of the original parent trees were removed. It is located at 1439 m in an *Abies amabilis/Vaccinium membranaceum* association *sensu* Franklin and Dyrness (1973). It is dominated by *Abies amabilis* with a total aboveground basal area of 23.82 $m^2 \cdot ha^{-1}$. *Abies grandis* and *Abies amabilis* were the only reproduction. Understory and ground flora were sparse, presumably because of grazing, with *Berberis nervosa, Arnica latifolia* and *Vaccinium* spp. dominating.

The soil showed minimal development and was extremely rocky (40% by volume). The bedrock was shallow and consisted of heavily weathered basalt. The pH was 6.5 to 7 from the surface to 60 cm. The site is situated in Township 21 North, Range 17 East, Section 7.

REGIONAL POPULATIONS: EAST CASCADES, WESTERN WASHINGTON
Local Population: Alder Creek (CA15)

This site is located above a drainage, and the parent trees are found along the drainage on a gentle slope (5%). It is located in Township 27 North, Range 17 East, the west 1/2 of the north 1/4 of Section 12. The elevation is among one of the lowest for *Abies grandis*, 687 m in the Entiat

Mts. The habitat type is *Abies grandis/Vaccinium membranaceum* with a total above-ground basal area of 34.98 $m^2 \cdot ha^{-1}$ in an area that appeared to have been selectively logged.

The soil was deep, perhaps overlaying till. The soil consisted of tephra and ash from the surface to approximately 90 cm.

REGIONAL POPULATIONS: EAST CASCADES, WESTERN WASHINGTON
Local Population: Entiat Mountain (CA16)

The Entiat Mt. site had low basal area, 23.82 $m^2 \cdot ha^{-1}$, and appeared to be the driest of all sites, although the aspect (36°) is northeast. It is located midslope at 959 m, subject to grazing and irregular logging with some evidence of fire. As a result the stand is evenaged, composed primarily of *Abies grandis*, *Pseudotsuga menziesii*, and *Pinus ponderosa*. The soil is shallow with bedrock occurring at 50 cm. The bedrock material was a quartz matrix with mica particles, specifically metamorphic schist with much mica. Volcanic pumice clasts mixed in extremely weathered basalt and pyroclastic ignembrite were also present in rock samples. The understory of this *Abies grandis/Calamagrostis rubescens* habitat type was sparse with *Calamagrostis rubescens* accounting for 45-50% cover. The location is Township 25 North, Range 19 East, the west 1/2 of the northwest 1/4 of section 6.

REGIONAL POPULATION: OLYMPIC MOUNTAINS, OLYMPIC PENINSULA, WASHINGTON
Local Population: Dungeness (OP17)

This site was located in Township 29 North, Range 4 West, East 1/2 of Section 25 at an elevation of 520 m. This site supported by far the greatest above-ground basal area of all sampled areas: 81.54 $m^2 \cdot ha^{-1}$. This area was situated on a bench in midslope between two rather steep slopes. The plant

association is in the *Tsuga heterophylla* zone with relatively sparse understory and shrub layer. The soil was very deep consisting of volcanic ash and silt loam.

REGIONAL POPULATION: OLYMPIC MOUNTAINS, OLYMPIC PENINSULA, WASHINGTON
Local Population: Hamma Hamma (OP18)

This site was at the lowest elevation of all sites, at 242 m in Township 24 North, Range 4 West, the south 1/2 of Section 3. It is at a streamside, toeslope position, where *Abies grandis* is typically found near the coast. The overstory is an admixture of *Tsuga, Thuja, Pseudotsuga, Picea*, with *Abies grandis* as a rare and minimal component. However, *Abies grandis* is a strong member of the regenerating cohort of this recently logged area. Total above-ground basal area is 47.63 $m^2 \cdot ha^{-1}$. The plant association resembles the *Tsuga heterophylla* zone, according to Franklin and Dyrness (1973). The soil was directly impacted by proximity to the stream in that extremely deep humus was found to seven inches, overlaying a very stony soil.

Table C.1. Overstory vegetation of local populations. Basal area of tree species greater than 1.5 m tall and dbh greater than 9 cm.

	m^2/ha						
	MT1	MT2	MT3	MT4	CB5	CB6	CB7
Abies grandis	33.1	34.3	14.6	28.1	4.2	19.6	1.1
Abies lasiocarpa							5.3
Acer glabrum		0.3	0.1	1.1			
Betula papyrifera		1.3	0.4				
Larix occidentalis	13.5	0.1	5.8				8.7
Picea engelmannii		0.2	1.9			0.7	1.4
Pinus contorta	8.8						4.4
Pinus monticola		1.8	9.8			1.9	
Pseudotsuga menziesii			8.3		6.6		9.1
Taxus brevifolia			0.3				
Thuja plicata			5.1	4.2	15.2		
Tsuga heterophylla				8.5	2.1		2.1

Table C.1. Continued

	m^2/ha					
	CB8	CL9	CL10	CL11	CL12	CA13
Abies grandis	12.6	6.8	4.5	13.1	13.3	24.6
Abies lasiocarpa				1.8		
Acer glabrum				0.1		
Betula papyrifera	0.5				0.7	
Larix occidentalis	8.7	2.6				
Picea engelmannii	1.4					
Pinus contorta	4.4					
Pinus monticola						0.8
Pinus ponderosa						1.2
P. menziesii	0.4	3.7	13.6	11.4		17.0
Salix scouleriana				0.2		
Thuja plicata	8.4	30.0	33.5			3.9
Tsuga heterophylla	8.8					

Table C.1. Continued

	m^2/ha				
	CA14	CA15	CA16	OP17	OP18
Abies grandis	12.8	16.8	8.7	6.7	9.1
Abies amabilis	11.4				
Abies lasiocarpa	0.7				
Alnus rubra					0.3
Acer macrophyllum				6.7	7.8
Pinus albicaulis	0.6				
Pinus contorta		16.8			
Pinus monticola		0.2			
Pinus ponderosa			5.3		
P. menziesii	2.6	13.7	8.2	46.8	13.8
Thuja plicata		0.6		20.3	8.0
Tsuga heterophylla		0.1		1.0	8.5

Table C.2. Understory vegetation of local populations. Tree species less than one meter tall. Values represent relative cover classes where:

+ = present in stand, not in plot	3 = 25-50%
t = 0-1%	4 = 50-75%
1 = 1-5%	5 = 75-95%
2 = 5-25%	6 = 95-100%

	MT1	MT2	MT3	MT4	CB5	CB6	CB7	CB8	CL9
trees < 1m									
Abies grandis	t	t	t	t	2	t	t		2
Acer glabrum		t	t	2			t	t	
Betula papyrifera				t					
Larix occidentalis							t		
Picea engelmannii		t					t		
Pinus contorta					1				
Pinus monticola									t
Pinus ponderosa					t				
Pseudotsuga menziesii									t
Salix scouleriana							t		
Sorbus scopulina							t		
Thuja plicata			t	t	t	t	t	1	1
Tsuga heterophylla					3	2		t	

Table C.2b. Continued.

	CL10	CL11	CL12	CA13	CA14	CA15	CA16	OP17	OP18
trees < 1m									
Abies grandis	t	1	1	t	t	2	1	t	t
Acer glabrum			t				t		
Acer macrophyllum									t
Pinus contorta						1			
Pinus monticola		t		t	t	t			
Pinus ponderosa							t		
Prunus, sp.			1						
P. menziesii	t	t	+	t			t		
Salix scouleriana		t							
Thuja plicata	t	+		t		t			t
Tsuga heterophylla								t	

Table C.3. Composition and cover of shrub understory vegetation of local populations.

	MT1	MT2	MT3	MT4	CB5	CB6	CB7	CB8	CL9
shrubs									
Acer glabrum				2					t
Alnus incana					t				
Amelanchier alnifolia	t		t	1			t	t	
Ceanothus sanguineus									t
Ceanothus velutinus					1				
Holodiscus discolor		t		t			t		
Lonicera utahensis	t		t	t		t	t	t	
Menziesia ferruginea	t		1						
Pachistima myrsinites	t		1	t	t	1	2	2	t
Prunus virginiana		t							
Rhamnus alnifolia				t					
Ribes lacustre		t		t					
Ribes viscosissiimum								t	
Rosa gymnocarpa	1	1	t	1	t	t	2	2	t
Rubus idaeus				t					
Rubus parviflorus		t	t	2	1			1	
Salix scouleriana					t	t			t
Sorbus scopulina					t				
Spirea betulifolia	t	t	t	t	t				
Symphoricarpos albus		t		2				t	t
Taxus brevifolia			5	1					
Vaccinium caespitosum									
Vaccinium glob + memb	2	t	t		1	1	3	1	2

Vaccinium scop + myrt 2 t

Table C.3. Continued.

	CL10	CL11	CL12	CA13	CA14	CA15	CA16	OP17	OP18
shrubs									
Acer circinatum				2		1		t	
Acer glabrum	t	2	1						
Alnus incana				t					
Amelanchier alnifolia	t	1							
Gaultheria shallon								2	t
Holodiscus discolor		3	2				t		t
Lonicera utahensis		1							
Menziesia ferruginea	t								
Pachistima myrsinites			1	t	1	t			
Philadelphus lewisii		1							
Physocarpus malvaceous		1	1						
Prunus virginiana			1						
Rhamnus purshii			t						
Ribes montigenum					1				
Ribes viscosissiimum	t								
Rosa gymnocarpa	t	1	1	t	t	t	t		
Rosa nutkana							t		t
Rubus nivalis			t						
Rubus parviflorus		1	1			t			
Rubus ursinus								1	t
Salix scouleriana						t	1		
Spirea betulifolia	t	1	2	t		1	1		

Symphoricarpos albus	t		1		1		1					
Vaccinium caespitosum												
Vaccinium glob + memb			2				1		2			
Vaccinium myrtillus												t
Vaccinium parviflorum											t	
Vaccinium scop + myrt	t							1		t		
Vaccinium uliginosum											1	

Table C.4. Composition and cover values of subshrub, perennial forbs, ferns, and perennial graminoid understory vegetation of local populations.

	MT1	MT2	MT3	MT4	CB5	CB6	CB7	CB8	CL9
subshrubs									
Arctostaphylos uva-ursi	t				3				
Berberis repens	1	1	t	t	t				
Clematis columbiana				t					
Gaultheria ovatifolia				2				1	
Linnaea borealis	1	t	t	t	4	2		1	1
perennial forbs									
Adenocaulon bicolor	t	2	t	1	t	t		t	t
Anaphalis margaritacea					1				
Antennaria racemosa	t								
Aralia nudicaulis		2		3	t			1	
Asarum caudatum								t	
Balsamorhiza sagittata									
Chimaphila umbellata	t		t	t	t	t	1	2	1
Circaea alpina					t				
Cirsium					t	t			
Clintonia uniflora	t	t	1	1	1	1	t	1	1
Coptis occidentalis					t		t	2	1
Corallorhiza sp.						t	t		t
Cornus canadensis		1			t				t
Disporum hookeri	t	1	t	1				t	t
Epilobium angustifolium					t		t		

Fragaria vesca	t	t		t	t					
Fragaria virginiana	t				t			t		
Galium triflorum		t		t					t	
Goodyera oblongifolia	t	t	t	t		t	1	t	t	
Hieracium albertinum	t	t			t	t		t	t	
Listera caurina	t									
Mitella caulescens		2	t	t						
Mitella stauropetala		t								
Monotropa uniflora			t							
Osmorhiza chilensis	t	t		t				t		
Pedicularis racemosa					t			t		
Potentilla glandulosa				t						
Pterospora andromedea	t		t			t				
Pyrola asarifolia	t		t			t			t	
Pyrola secunda										t
Smilacina racemosa		t	t	t						
Smilacina stellata	t	t		t	t	t		t	t	
Solidago sp.							t			
Streptopus amplexifoli			t	t						
Thalictrum occidentale	t	t		t					t	
Tiarella trifoliata					t		t			
Trillium ovatum			t	t		t		t		
Veratrum californicum									t	
Vicia americana									t	
Viola orbiculata	t	t	t			t	t	t	t	
Viola sp.			t		t				t	
Xerophyllum tenax	5		t			2	4			

<u>ferns and allies</u>										
Athyrium felix-femina		t		1				t	t	
Gymnocarpium dryopteris		t								
Pteridium aquilinum								t		
<u>perennial graminoids</u>										
Bromus vulgaris	t	t			1	t		t	t	
Calamagrostis rubescens	t	t		t						
Carex sp.		t				1	t			t
Luzula parviflora						t				

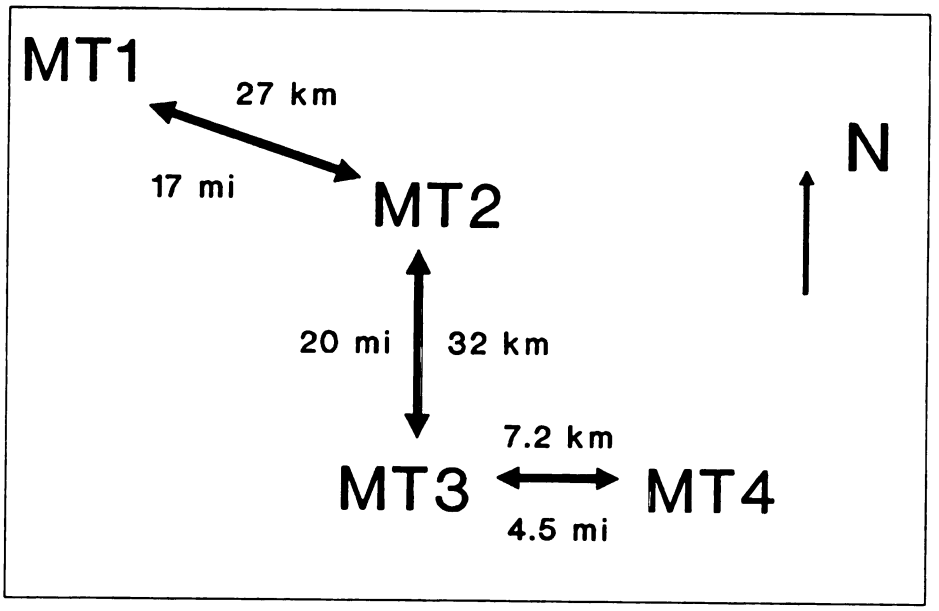
Table C.4. Continued.

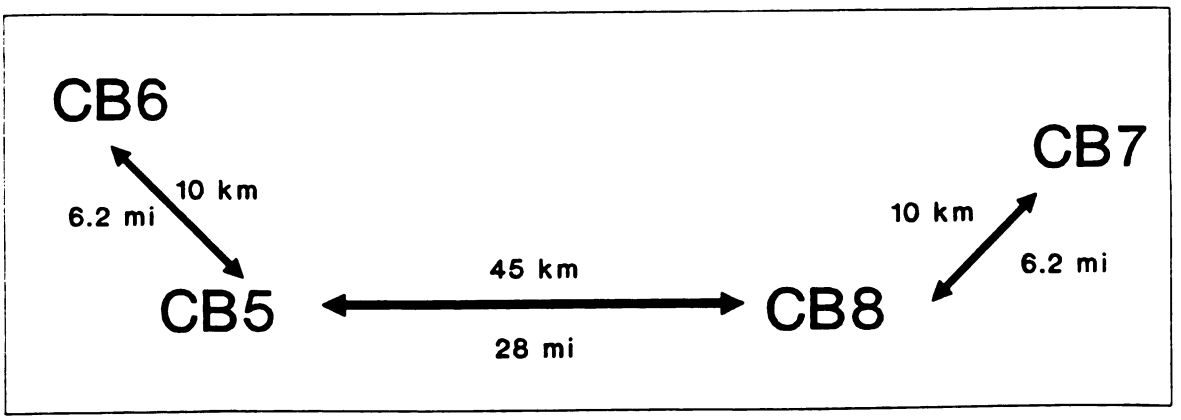
	CL10	CL11	CL12	CA13	CA14	CA15	CA16	OP17	OP18
subshrubs									
Arctostaphylos uva-ursi						t			
Berberis nervosa				1		t	2	2	2
Berberis repens					1		t		
Clematis columbiana	t								
Gaultheria ovatifolia					1				
Linnaea borealis	t	t	t	2	t	1		1	
perennial forbs									
Achlys triphylla				1	t			1	1
Adenocaulon bicolor	t	t	2	t					
Anaphalis margaritacea						t			
Antennaria luzuloides							t		
Apocynum androsaemifolia						t	t		
Arenaria macrophylla				t	t				
Arnica latifolia					1				
Asarum caudatum								1	t
Chimaphila umbellata	t			t	1	2	t		
Cichorium intybus			t						
Cirsium				2					
Clintonia uniflora	t	t	t	t		t	1		
Coptis occidentalis	t	1							
Corallorhiza sp.				t					
Disporum hookeri	t	1				1		1	
Fragaria vesca		t	t						

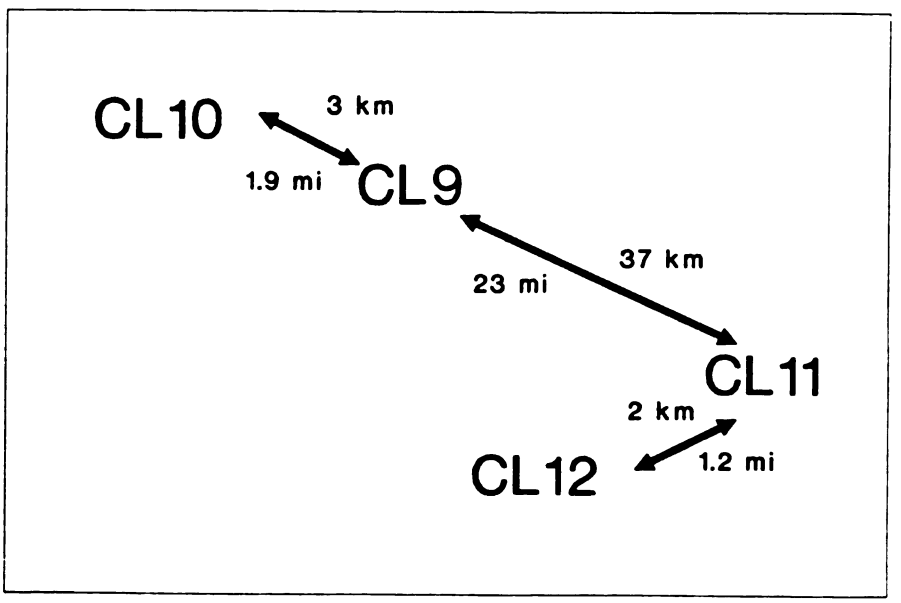
Fragaria virginiana		t	1	t				t	t
Galium triflorum	t	t	1	t				t	t
Goodyera oblongifolia	t	t		t		1		t	t
Hieracium albertinum		t	1		t	t	t		
Hypericum perforatum			1						
Lathyrus nevadensis		1			1				
Ligusticum apiifolium							t		
Lupinus sp.						t			
Mitella caulescens	t							1	
Mitella nuda					t				
Mitella pent.		t							
Myosotis sp.							t		
Osmorhiza chilensis		t			t			1	t
Pedicularis racemosa t									
Prenanthes alata								t	t
Prunella vulgaris			1						
Pterospora andromedea	t			t			t		
Pyrola asarifolia						1			
Pyrola picta				t	t	t			
Pyrola secunda		t			t	t	t		
Senecio						t			
Smilacina stellata	t	t	t	t					
Streptopus amplexifolius		1	1						
Thalictrum occidentale	t	t	t						
Tiarella trifoliata								t	
*Trautvetteria carolinien*sis	t								
Trientalis latifolia t	1		t				t		

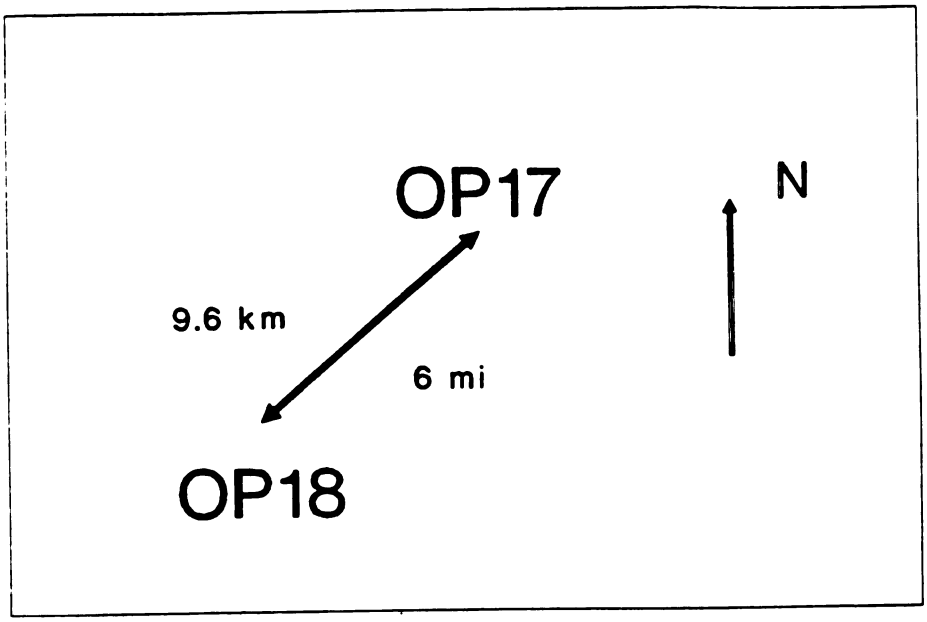
Trifolium repens			1									
Trillium ovatum	t			t	t							
Veratrum californicum	t	1										
Vicia americana	t											
Viola orbiculata	t	t	t									
Viola sp												t
ferns and allies												
Pteridium aquilinum		t		t								
Polysticum munitum		t	2						3	2		
Adiatum pedatum									t			
Blechnum spicant									t			
perennial graminoids												
Bromus vulgaris		t	2									
Calamagrostis rubescen		1	1				3					
Carex sp.		t	t	t		t	t					
Luzula parviflora					t							

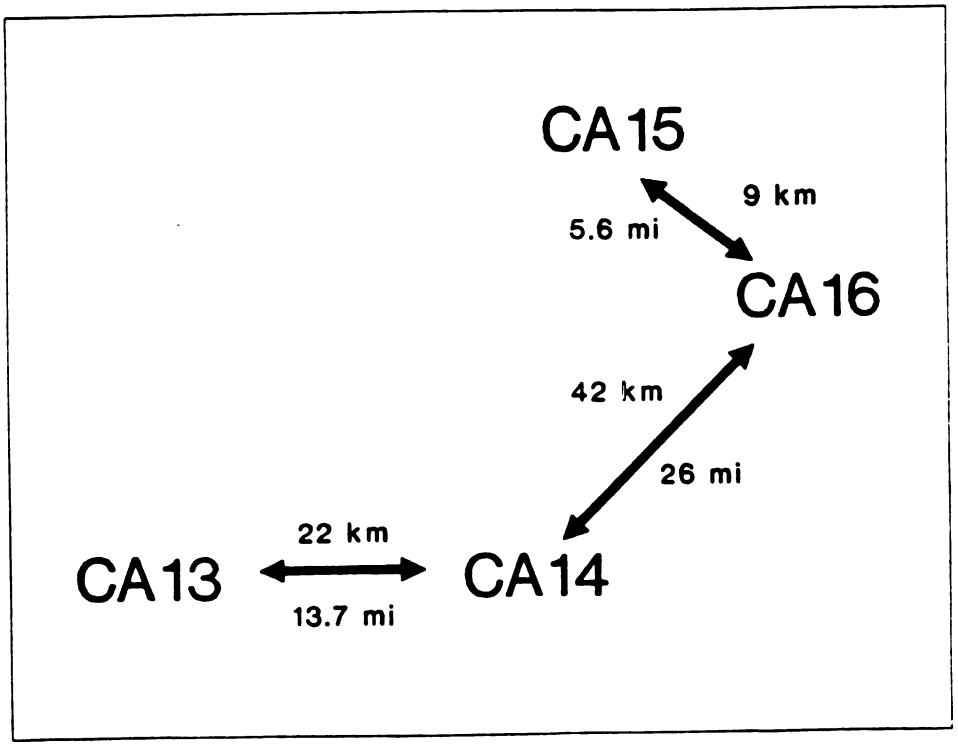
Figure C.1. Diagrammatic maps representing distances between local populations.


MT1
27 km
17 mi
MT2
N
20 mi
32 km
7.2 km
MT3
MT4
4.5 mi


CB6
10 km
6.2 mi
CB7
10 km
6.2 mi
45 km
CB5
CB8
28 mi


CL10
3 km
1.9 mi
CL9
37 km
23 mi
CL11
2 km
CL12
1.2 mi


OP17
N
9.6 km
6 mi
OP18


CA15
9 km
5.6 mi
CA16
42 km
26 mi
22 km
CA13
CA14
13.7 mi

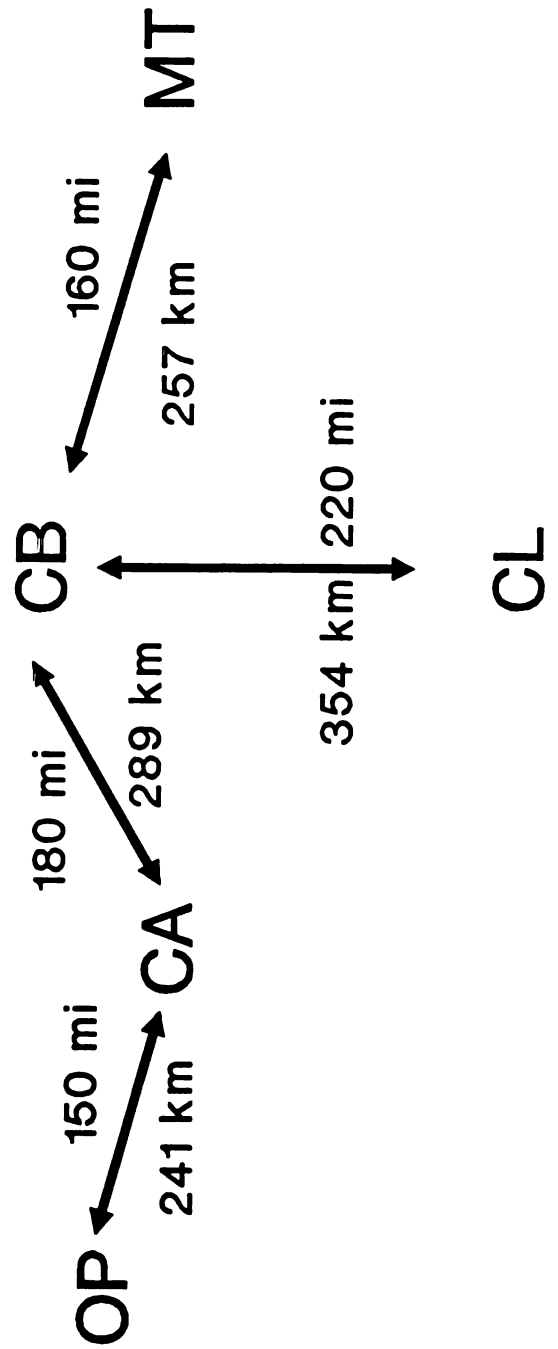


Figure C.2. Diagrammatic maps representing distances between regional seed sources.

BIBLIOGRAPHY

BIBLIOGRAPHY

Alston, R.E. 1964. The genetics of phenolic compounds. Pages 171-204 *in* J.B. Harborne, editor. The biochemistry of phenolic compounds. Academic Press, New York, New York.

Arntzen, C. J., S. V. Falkenthal, and S. Bobick. 1974. Inhibition of photophosphorylation by kaemferol. Plant Physiology 53: 304-306.

Aung, L.H. 1974. Root-shoot relationships. Pages 29-61 *in* E.W. Carson, editor. The plant root and its environment. University Press of Virginia, Charlottesville, Virginia.

Ayala, R. J. 1969. An evolutionary dilemma: fitness of genotypes versus fitness of populations. Canadian Journal of Genetics and Cytology 11: 439-456.

Baldwin, I.T. and J. C. Schultz. 1983. Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. Science 221: 277-279.

Baldwin, I.T., J.C Schultz, and D. Ward. 1987. Patterns and sources of leaf tannin variation in yellow birch (*Betula allegheniensis*) and sugar maple (*Acer saccharum*). Journal of Chemical Ecology 13: 1069-1078.

Baker, R.T. and H.G. Smith. 1920. Research on the eucalypts especially in regard to to their essential oils. Ed. 2, Sydney Techol. Mus. New South Wales Technical Education Service No. 13.

Barz, W. and J. Koster. 1981. Turnover and degradation of secondary (natural) products. Pages 35-84 *in* E.E. Conn, editors. The Biochemistry of Plants Volume 7. Secondary Plant Products. Academic Press, New York, New York.

Bazzaz, F.A., N.R. Chiariello and P.D. Coley, and L.F. Pitelka. 1987 . Allocating resources to reproduction and defense. BioScience 37: 58-67.

Beres, C. 1984. Phenol and non-structural carbohydrate contents in the leaves of *Quercus petraea*. Acta Botanica Hungarica 30: 461-467.

Bernard-Dagen, C. 1988. Biosynthesis of lower terpenoids: genetic and physiological controls in woody plants. Pages 329-351 *in* J.W. Hanover and D.E. Keathley, editors. Genetic manipulation of woody plants. Plenum Press, New York, New York.

Bloom, A.J., F.S. Chapin, and H.A. Mooney. 1985. Resource limitations in plants - an economic analogy. Annual Review of Ecology and Systematics 16: 363-392.

Boudet, A.M., A. Graziana, and R. Ranjeva. 1985. Recent advances in the regulation of the pre-aromatic pathway. Pages 135-159 *in* C.F. Van Sumere and P.J. Lea, editors. The biochemistry of plant phenolics. Annual Proceedings of the Phytochemical Society of Europe Volume 25, Clarendon Press, Oxford.

Bray, J.R. 1963. Root production and the estimation of net productivity. Canadian Journal of Botany 41: 65-72.

Bridges, J.R. 1987. Effects of terpenoid compounds on growth of symbiotic fungi associated with the southern pine beetle. Ecology and Epidemiology 77: 83-85.

Brix, H. 1971. Effects of nitrogen fertilization on photosynthesis and respiration in Douglas-fir. Forest Science 17: 407-414.

Brix, H. 1972. Nitrogen fertilization and water effects on photosynthesis and earlywood-latewood production in Douglas-fir. Canadian Journal of Forest Research 2:467-478.

Brix, H. 1981. Effects of thinning and nitrogen fertilization on branch and foliage production in Douglas-fir. Canadian Journal of Forest Research 11: 502-511.

Brix, H. and L.F. Ebell. 1969. Effects of nitrogen fertilization on growth, leaf area, and photosynthesis rate in Douglas-fir. Forest Science 15: 189-196.

Brooks, J.E., J.H. Borden, and H.D. Pierce. 1987. Foliar and cortical monoterpenes in Sitka spruce: potential indicators of resistance to the white pine weevil, *Pissodes strobi* Peck (Coleoptera:Curculionidae). Canadian Journal of Forest Research 17: 740-745.

Bryant, J.P., F.S. Chapin and D.R. Klein. 1983. Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40: 357-368.

Bryant, J.P., F.S. Chapin, P.B. Reichardt, T.P. Clausen. 1985. Adaptation to resource availability as a determinant of chemical defense strategies in woody plant. Pages 219-237 *in* T. Swain and G. Cooper-Driver, editors. Recent Advances in Phytochemistry Volume 19.

Bryant, J.P., T.P. Clausen, P.B. Reichardt, M.C. McCarthy, and R.A. Werner. 1987. Effect of nitrogen fertilization upon the secondary chemistry and nutrition value of quaking aspen (*Populus tremuloides* Michx.) leaves for the large aspen tortix (*Choristoneura conflictana* (Walker)). Oecologia(Berlin) 73: 513-517.

Campbell, R.K. 1979. Genecology of Douglas-fir in a watershed in the Oregon Cascades. Ecology 60: 1036-1050.

Campbell, R.K. and J.F. Franklin. 1981. A comparison of habitat type and elevation for seed-zone classification of Douglas-fir in western Oregon. Forest Science 27: 49-59.

Carrow, J.R. 1973. Free amino acids in grand fir needles and the effects of different forms of foliar-applied nitrogen. Canadian Journal of Forest Research 3: 465-471.

Cates, R.G., R.A. Redak, and C.B Henderson. 1983. Patterns in defensive natural product chemistry: Douglas-fir and Western spruce budworm interactions. Pages 3-20 *in* P.A. Hedin, editor. Plant resistance to insects, American Chemical Society Symposium Series 208. American Chemical Society, Washington D.C.

Cates, R.G., C.B. Henderson, and R.A. Redak. 1987. Responses of the western spruce budworm to varying levels of nitrogen and terpenes. Oecologia (Berlin) 73: 312-316.

Chapin, F.S. 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11: 233-260.

Charlwood, B.V. and D.V. Banthorpe. 1978. The biosynthesis of monoterpenes. Progresses in Phytochemistry 5: 65-125.

Chew, F.S. and J.E. Rodman. 1979. Plant resources for chemical defense. Pages 271-307 *in* G.A. Rosenthal and D.H. Janzen, editors. Herbivores: Their interaction with secondary plant metabolites. Academic Press, New York.

Chung, H.H. and R.L Barnes. 1977. Photosynthate allocation in *Pinus taeda*. I. Substrate requirements for synthesis of shoot biomass. Canadian Journal of Forest Research 7:106-111.

Clark, R.J. and R.C. Menary. 1980. The effect of irrigation and nitrogen on the yield and composition of peppermint oil *(Mentha piperita* L.). Australian Journal of Agricultural Research 31: 489-498.

Clausen, J. and W.M. Hiesey. 1960. The balance between coherence and variation in evolution. Proceedings of the National Academy of Science 46: 494-506.

Coley, P.D. 1987. Interspecific variation in plant anti-herbivore properties: the role of habitat quality and rate of disturbance. New Phytologist 106(Supplement): 251-263.

Coley, P.D. and J.P. Bryant, and Chapin F.S. 1985. Resource availability and plant antiherbivore defense. Science 230: 895-899.

Conover, W.J. 1971. Practical Nonparametric Statistics. John Wiley and Sons, Inc., New York.

Crankshaw, D.R. and J.H. Langenheim. 1981. Variation in terpenes and phenolics through leaf development in *Hymenaea* and its possible significance to herbivory. Biochemical Systematics and Ecology 9: 115-124.

Crist, J.W. and G.J. Stout. 1929. Relation between top and root size in herbaceous plants. Plant Physiology 4: 63-85.

Croteau, R. 1984. Biosynthesis and catabolism of monoterpenes. Pages 31-64 *in* W.D. Nes, G. Fuller, and L-S Tsai, editors. Isopentenoids in Plants. Marcel Dekker, New York, New York.

Daniels, J.D. 1969. Variation and intergradation in the grand fir - white fir complex. Ph.D. Dissertation, University of Idaho, Moscow, Idaho.

Delmoral, R. 1972. On the variability of chlorogenic acid concentration. Oecologia (Berlin) 9: 289-300.

Denno, R.F. and M.S. McClure. 1983. Variable plants and herbivores in natural and managed systems. Academic Press, New York, New York.

Daubenmire, D.R. and J.B. Daubenmire. 1976. Forest vegetation of Eastern Washington and Northern Idaho. Washington State University Technical Bulletin 60, Washington Agriculture Station, Pullman, Washington.

Esteban, I., F. Bergmann, H.-R. Gregorius, and O. Huhtinen. 1976. Composition and genetics of monoterpenes from cortical oleoresin of Norway spruce and their significance for clone identification. Silvae Genetica 25: 59-66.

Feeny, P.P. 1970. Seasonal change in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51: 565-581.

Field, C. and H.A. Mooney. 1986. The photosynthesis-nitrogen relationship in wild plants. Pages 25-55 *in* T.J. Givnish, editor. On the economy of plant form and function. Cambridge University Press, Cambridge, England.

Forde, M.B. 1964. Inheritance of turpentine composition in *Pinus attenuata* X *radiata* hybrids. New Zealand Journal of Botany 2: 53-59.

Forrest, G.I. 1975. Variation in polyphenol content within and between Sitka spruce provenances at different sites. Canadian Journal of Forest Research 5: 46-54.

Fraenkel, G.S. 1959. The raison d'etre of secondary plant substances. Science 129: 1466-1470.

Franklin, J.F. and C.T. Dyrness. 1973. Natural vegetation of Washington and Oregon. United States Department of Agriculture General Technical Report PNW-8. Pacific Northwest Forest and Range Experiment Station, Portland, Oregon.

Fretz, T.A. 1976. Effect of photoperiod and nitrogen on the composition of foliar monoterpenes of *Juniperus horizontalis* Moench. cv Plumosa. Journal of the American Society of Horticultural Science 101: 611-613.

Fowells, H.A. 1965. Silvics of forest trees of the Unites States. United States Department of Agriculture Forest Service Handbook No. 271. Unites States Government Printing Office, Washington, D.C.

Fujita, Y., Y. Hara, T. Ogino, and C. Suga. 1981. Production of shikonin derivatives by cell suspension cultures of *Lithospermum erythrorhizon*. I. Effects of nitrogen sources on production of shikonin derivatives. Plant Cell Reports 1: 59-60.

Gershenzon, J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. Pages 273-320 *in* B.N.Timmermann, C. Steelink, and F.A. Loewus, editors. Recent advances in Phytochemistry Volume 18.

Gershenzon, J., D.E. Lincoln, and J.H. Langenheim. 1978. The effect of moisture stress on monoterpenoid yield and composition in *Satureja douglasii*. Biochemical Systematics and Ecology 6: 33-43.

Gilmour, A.R. 1977. Effects of soil moisture stress on monoterpenes in loblolly pine. Journal of Chemical Ecology 3: 667-676.

Gittins, R. 1985. Canonical Analysis. Biomathematics Volume 12. Springer-Verlag, Berlin.

Glyphis, J.P. and G.M. Puttick. 1989. Phenolics, nutrition and insect herbivory in some garrigue and maquis plant species. Oecologia (Berlin) 78: 259-263.

Gollob, L. 1980. Monoterpenes composition in bark beetle resistant loblolly pine. Naturwissenschaften 67: 409-410.

Goodwin, T.W. 1967. The biological significance of terpenes in plants. Pages 1-23 *in* J.B. Pridham, editor. Terpenoids in plants. Academic Press, New York.

Gulmon, S.L. and H.A. Mooney. 1986. Costs of defense and their effects on plant productivity. Pages 681-698 *in* T.J. Givnish, editor. On the economy of plant form and function. Cambridge University Press, Cambridge, England.

Hall, G.D. and J.H. Langenheim. 1987. Geographic variation in leaf monoterpenes of *Sequoia sempervirens*. Biochemical Systematics and Ecology 15: 31-43.

Hamrick, J.L. and W.J. Libby. 1972. Variation and selection in western United States montane species. I. White fir. Silvae Genetica 21: 29-35.

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

Hanover, J.W. 1966b. Environmental variation in the monoterpenes of *Pinus monticola* Dougl. Phytochemistry 5: 713-717.

Hanover, J.W. 1971. Genetics of terpenes II. Genetic variances and interrelationships of monoterpene concentrations in *Pinus monticola*. Heredity 27: 237-245.

Hanover, J.W. 1980. Control of tree growth. BioScience 30: 756-762.

Hanover, J.W. and M.M. Furniss. 1966. Monoterpene concentration in Douglas-fir in relation to geographic location and resistance to attack by the Douglas-fir beetle. Pages 23-28 *in* The Second Forest Genetics Workshop Proceedings.

Hanover, J.W. and R.C. Wilkinson. 1970. Chemical evidence for introgressive hybridization in *Picea*. Silvae Genetica 19: 17-22.

Hanson, K.R. and E.A. Havir. 1981. Phenylalanine ammonia-lyase. Pages 575-626. *in* E.E Conn, editors. The Biochemistry of Plants Volume 7. Secondary Plant Products. Academic Press, New York, New York.

Harborne, J.B. 1983. Introduction to Ecological Biochemistry. Academic Press, New York.

Harborne, J.B. 1985. Phenolics and plant defence. Pages 393-408 *in* C.F. Van Sumere and P.J. Lea, editors. The biochemistry of plant phenolics. Annual Proceedings of the Phytochemical Society of Europe Volume 25. Clarendon Press, Oxford.

Harrison, F. 1913. Roman Farm Management: The Treatises of Cato and Varro. The Macmillan Company, New York.

Hartley, S.E. 1988. The inhibition of phenolic biosynthesis in damaged and undamaged birch foliage and its effect on insect herbivores. Oecologia (Berlin) 76: 65-70.

Haukioja, E, P. Niemela, and S. Siren. 1985. Foliage phenols and nitrogen in relation to growth, insect damage, and ability to recover after defoliation, in the mountain birch *Betula pubescens* ssp *tortuosa*. Oecologia (Berlin) 65: 214-222.

Hermann, R.K. and D.P. Lavender. 1968. Early growth of Douglas-fir from various altitutes and aspects in southern Oregon. Silvae Genetica 17: 143-151.

Hill, M.O. 1979. DECORANA - a FORTRAN program for detreneded correspondence analysis and reciprocal averaging. Section of Ecology and Systematics, Cornell University, Ithaca, New York.

Hiltunen, R. 1976. On variation, inheritance and chemical interrelationships of monoterpenes in scots pine (*Pinus sylvestris* L.). Annales Academiae Scientiarum Fennicae 1-54.

Hitchcock, C.L. and A. Cronquist. 1973. Flora of the Pacific Northwest, An Illustrated Manual. University of Washington Press, Seattle, Washington.

Hodges, J.D. and P.L. Lorio. 1975. Moisture stress and composition of xylem oleresin in loblolly pine. Forest Science 21: 283-290.

Homeyer, U. and G. Schultz. 1988. Activation by light of plastidic shikimate pathway in spinach. Plant Physiology and Biochemistry. 26: 365-370.

Horner, J.D., J.R. Gosz, and R.G. Cates. 1988. The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems. American Naturalist 132: 869-883.

Houkal, D.J. 1976. Terpene and morphological variation in the grand fir hybrid complex. Ph.D. Dissertation; University of Idaho, Moscow, Idaho.

Hunt, R. and P.S. Lloyd. 1987. Growth and partitioning. New Phytologist 106(Supplement): 235-249.

Ibrahim, R. K. 1987. Regulation of synthesis of phenolics. Pages 77-95 *in* I.K. Vasil, editor. Cell culture and somatic cell genetics of plants. Volume 4 Cell Culture in Phytochemistry. Academic Press, New York.

Iwasa, Y. and J. Roughgarden. 1984. Shoot/root balance of plants: Optimal growth of a system with many vegetative organs. Theoretical Population Biology 25: 78-105.

Jacob, M. and P.H. Rubery. 1988. Naturally occurring auxin transport regulators. Science 241: 346-349.

Janzen, D. H. 1974. Tropical blackwater rivers, animals, and mast fruiting by the Dipterocarpaceae. Biotropica 6: 69-103.

Jessup, W. and M.W. Fowler. 1976. Interrelationships between carbohydrate metabolism and nitrogen assimilation in cultured plant cells. I. Effects of glutamate and nitrate as alternative nitrogen sources on cell growth. Planta 132: 119-123.

Johnson, D.W., Cole, D.W., Bledsoe, C.S., Cromack, K., Edmonds, R.L., Gessel, S.P., Grier, C.C., Richards, B.N. and Vogt, K.A. 1982. Nutrient cycling in forests of the Pacific Northwest. Pages 87-112 *in* R.L. Edmonds, editor. Analysis of Coniferous Forest Ecosystems in the Western United States. Hutchinson Ross Publishing Company, Stroudsburg, Pennsylvania.

Jonasson, S., J.P. Bryant, F.S. Chapin, and M. Andersson. 1986. Plant phenols and nutrients in relation to variations in climate and rodent grazing. American Naturalist 128: 394-408.

Jones, D. H. 1984. Phenylalanine ammonia-lyase: regulation of its induction, and its role in plant development. Phytochemistry 23: 1349-1359.

Kasper, J.B. 1969. Organic compounds in cold-water extracts of grand fir heartwood. Canadian Journal of Botany 47: 551-55.

Katoh, T., M. Kasuya, S. Kagamimori, H. Kozuka, and S. Kawano. 1989. Effects of air pollution on tannin biosynthesis and predation damage in *Cryptomeria japonica*. Phytochemistry 28: 439-445.

Kaufmann, U., M. Wellendorf, and M. Hansen. 1974. Thin layer chromatography of fluorescent phenolic compounds in needles. Degree of genetic control in *Picea abies* L. Forest Tree Improvement 8: 1-32.

Keyes, M.R. and C.C. Grier. 1981. Above- and below-ground net production in 40-year-old Douglas fir stands on low and high productivity sites. Canadian Journal of Forest Research 11: 599-605.

Kim, Y.T., C. Glerum, J. Stoddart, and S.J. Colombo. 1987. Effect of fertilization on free amino acid concentrations in black spruce and jack pine containerized seedlings. Canadian Journal of Forest Research 17: 27-30.

Kincer, J.B. 1941. Climate and Weather Data for the United States. Pages 685-748 *in* Climate and Man, Yearbook of Agriculture, United State Department of Agriculture, Washington, District of Columbia.

Knobloch, K-H, and J. Berlin. 1981. Phosphate mediated regulation of cinnamoyl purtrescine biosynthesis in cell suspension cultures of *Nicotiana tabacum*. Planta Med. 42: 167-172.

Kozlowski, J. and R.G. Wiegert. 1986. Optimal allocation of energy to growth and reproduction. Theoretical Population Biology 29: 16-37.

Lacaze, J.F. and R. Tomassone. 1967. Contributions a l'etude de la variabiltye infraspecifique d'*Abies grandis* Lind. Characteristiques juveniles. Annales Sciences Forestieres 24: 277-325.

Langenheim, J.H., S.P. Arrhenius, and J.C. Nascimento. 1981. Relationship of light intensity to leaf resin composition and yield in the tropical leguminous genera *Hymenaea* and *Copaifera*. Biochemical Systematics and Ecology 9: 27-37.

Larsson, L., A. Wiren, L. Lundgren, and T.Ericsson. 1986. Effects of light and nutrient stress on leaf phenolic chemistry in *Salix dasyclados* and susceptibility to *Galerucello lineola* (Coleoptera) Oikos 47: 205-210.

Ledig, F.T. and T.O. Perry. 1965. Physiological genetics of the shoot-root ratio. Proceedings of Society of American Foresters.

Levin, D.A. 1971. Plant phenolics: an ecological perspective. American Naturalist 105: 157-181.

Lincoln, D.E. and D. Couvet. 1989. The effect of carbon supply on allocation to allelochemicals and caterpillar consumption of peppermint. Oecologia (Berlin) 78: 112-114.

Lincoln, D.E. and J.H. Langenheim. 1978. Effect of light and temperature on monoterpenoid yield and composition in *Satureja douglasii*. Biochemical Systematics and Ecology 6: 21-32.

Lines, R. 1974. A preliminary provenance trial with grand fir (*Abies grandis* Lindl.). Scottish Forestry 28: 85-98.

Little, E.L. 1971. Atlas of United States Trees Vol.1. United States Department of Agriculture Misc Pub. No.1146.

Liu, T.S. 1971. A monograph of the genus *Abies*. National Taiwan University Department of Forestry, Taipei, Taiwan.

Loehle, C. 1987. Constraints on tree breeding: growth tradeoffs, growth strategies, and defensive investments. Forest Science 33: 1089-1097.

Loehle, C. 1987. Tree life history strategies: the role of defenses. Canadian Journal of Forest Research 18: 209-222.

Loehle, C. 1988. Forest decline: endogenous dynamics, tree defenses, and the elimination of spurious correlation. Vegetatio 77: 65-78.

Lokar, L.C., V. Maurich, G. Mellerio, M. Moneghini and L. Poldini. 1985. Variation in terpene composition of Artemisia alba in relation to environmental conditions. Biochemical Systematics and Ecology 13 : 327-333.

Lundkvist, K. 1982. Patterns of adaptation in forest trees III. Genetic structure in natural and cultivated forest tree populations. Silva Fennica 16: 141-149.

Maddox, G.D. and N. Cappuccino. 1986. Genetic determination of plant susceptibility to an herbivorous insect depends on environmental context. Evolution 40: 863-866.

Margna, U. 1977. Control at the level of substrate supply - an alternative in the reglation of phenyl propanoid accumulation in plant cells. Phytochemistry 16: 419-426.

Margolis, H.A. and R.H. Waring. 1986. Carbon and nitrogen allocation patterns of Douglas-fir seedlings fertilized with nitrogen in autumn. I. Overwinter metabolism. Canadian Journal of Forest Research 16: 897-902.

Margolis, H.A. and R.H. Waring. 1986. Carbon and nitrogen allocation patterns of Douglas-fir seedlings fertilized with nitrogen in autumn. II. Field performance. Canadian Journal of Forest Research 16: 903-909.

Marpeau-Bezard, A., P. Baradat, and C. Bernard-Dagan. 1975. Les terpens du Pin maritime: Aspects biologiques et genetiques. IV Heredite de la teneur en deux sesquiterpenes: Le longifolene et le caryophyllene. Annales des Sciences Forestieres 32: 185-203.

Mattson, W.J. 1980. Herbivory in relation to plant nitrogen content. Annual Review of Ecology and Systematics 11: 119-161.

McClure, J.W. 1975. Physiology and functions of flavonoids. Pages 970-1055 *in* J.B. Harborne, T.J. Mabry, and H. Mabry, editors. The Flavonoids. Academic Press, New York, New York.

Mihaliak, C.A., D. Couvet, and D.E. Lincoln. 1987. Inhibition of feeding by a generalist insect due to increased volatile leaf terpenes under nitrate limiting conditions. Journal of Chemical Ecology 13: 2059-2067.

Mihaliak, C.A. and D.E. Lincoln. 1985. Growth pattern and carbon allocation to volatile leaf terpenes under nitrogen-limiting conditions. Oecologia (Berlin) 66: 423-426.

Miroz, N.T. 1961. Composition of gum turpentines of pines. United States Department of Agriculture Technical Bulletin 1239, 158 pp.

Mole, S., J.A.M. Ross, and P.G. Waterman. 1988. Light-induced variation in phenolic levels in foliage of rain-forest plants. I. Chemical changes. Journal of Chemical Ecology 14: 1-21.

Mole, S. and P.G. Waterman. 1988. Light-induced variation in phenolic levels in foliage of rain-forest plants. II. Potential significance to herbivores. Journal of Chemical Ecology 14: 23-34.

Mooney, H.A. 1972. The Carbon Balance of Plants. Annual Review of Ecology and Systematics 3: 315-346.

Mooney, H.A., S.L. Gulmon and N.D. Johnson. 1983. Physiological constraints on plant chemical defenses. Pages 21-36 *in* P. Hedin, editor. Plant resistance to insects. American Chemical Society Symposium Series 208. American Chemical Society, Washington D.C.

Monk, C. 1966. Ecological importance of root/shoot ratios. Bulletin of the Torrey Botanical Club. 93: 402-406.

Muller, K.M. 1936. *Abies grandis* und ihre Klimarassen. Milleilungen der Deutschen Dendrologschen Gesellschaft. 48: 82-132.

Mullin, T.J. 1985. Genotype-nitrogen interactions in full-sib seedlings of black spruce. Canadian Journal of Forest Research 15: 1031-1038.

Namkoong, G., H.C. Kang, and J.S. Brouard. 1988. Tree Breeding: Principles and Strategies. Spring-Verlag. New York.

Nascimento, J.C. and J.H. Langenheim. 1986. Leaf sesquiterpenes and phenolics in *Copaifera multijuga* on contrasting soil types in a central Amazonian rain forest. Biochemical Systematics and Ecology 14: 615-624.

Natr, L. 1975. Influence of mineral nutrition on photosynthesis and the use of assimilates. Pages 537-556 *in* J.P. Cooper, editor. Photosynthesis and productivity in different environments. Cambridge University Press.

Nicolai, V. 1988. Phenolic and mineral content of leaves influences decomposition in European forest ecosystems. Oecologia (Berlin) 75: 575-579.

Niemann, G.J. 1980. Phenolics from Larix needles. XVI. Inter- and intra-clonal variation in *Larix leptolepis*. Canadian Journal of Botany 58: 2313-2317.

Niemann, G.J. and H.H. van Genderen. 1980. Chemical relationships between Pinaceae. Biochemical Systematics and Ecology 8: 237-240.

Palo, R.T. 1984. Distribution of birch (*Betula* spp.), willow (*Salix* spp.), and poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense againsts herbivores. Journal of Chemical Ecology 10: 499-520.

Palo, R.T. 1985. Chemical defense in birch: inhibition of digestibility in ruminants by phenolic extracts. Oecologia (Berlin) 68: 10-14.

Palo, T.R., K. Sunnerhemi, and O. Theander. 1985. Seasonal vairation of phenols, crude protein and cell wall content of birch (*Betula pendula* Roth.) in relation to ruminant in vitro digestibility. Oecologia (Berlin) 65: 314-318.

Parker, W.H., J. Maze, and D.G. Mclachlan. 1979. Flavonoids of *Abies amabilis* needles. Phytochemistry 18: 508-510.

Parker, W.H., P. Knowles, F. Bennett, A. Gray, and T. Krickl. 1983. Habitat-dependent morphological and chemical variation in *Picea mariana* from northwestern Ontario. Canadian Journal of Botany 61: 1573-1579.

Pfister, R.D., B.L. Kovalchik, S.F. Arno, and R.C. Presby. 1977. Forest habitat types of Montana. USDA Forest Service General Technical Report INT-34.

Phillips R. and G.G. Henshaw. 1977. The regulation of synthesis of phenolics in stationary phase cell cultures of *Acer pseudoplatanus* L. Journal of Experimental Botany 28: 785-794.

Pollack, J.C. and B.P. Dancik. 1985. Monoterpene and morphological variation and hybridization of *Pinus contorta* and *Pinus banksiana* in Alberta. Canadian Journal of Botany 63: 200-222.

Powell, R.A. and R.P. Adams. 1973. Seasonal variation in the volatile terpenoids of *Juniperus scopularum* (Cupressaceae). American Journal of Botany 60: 1041-1050.

Powers, R.F. 1980. Mineralizable soil nitrogen as an index of nitrogen availability to forest trees. Soil Science Society of America Journal 44: 1314-1320.

Puritch, G.S. 1977. Distribution and phenolic composition of sapwood and heartwood in *Abies grandis* and effects of the balsam woolly aphid. Canadian Journal of Forest Research 7: 54-62.

Rau, H.M and H. Weisgerber. 1981. The provenance trial with *Abies grandis* in Hesse during the nursery stage. IUFRO Working Party S 2.0.14. Abies Provenances.

Redak, R.A. and R.G. Cates. 1984. Douglas-fir (*Pseudotsuga menziessi*)-spruce budworm (*Choristoneura occidentalis*) interactions: the effect of nutrition, chemical defenses, tissue phenology, and tree physical parameters on budworm success. Oecologia (Berlin) 62: 61-67.

Rehfledt, G.E. 1978. Genetic differentiation of Douglas-fir populations from the northern Rocky Mountains. Ecology 59: 1264-1270.

Rehfeldt, G.E. 1983. Ecological adaptation of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) populations. III. Central Idaho. Canadian Journal of Forest Research 13: 626-632.

Rehfeldt, G.E. 1984. Microevolution of conifers in the northern Rocky Mountains: a view from common gardens. Pages 132-146 *in* R.M. Lanner, editor. Proceedings of the Eighth North American Forest Biology Workshop.

Rehfeldt, G.E. 1986. Development and verification of models of freezing tolerance for Douglas-fir populations in the Inland Northwest. United States Department of Agriculture Forest Service Research Paper INT-369.

Rhoades, D.F. 1985. Offensive-defensive interactions between herbivores and plants: their relevance in herbivore population dynamics and ecological theory. American Naturalist 125:205-238.

Rhodes, M.J.C. 1985. The physiological significance of plant phenolic compounds. Pages 99-117 *in* C.F. Van Sumere and P.J. Lea, editors. The biochemistry of plant phenolics. Annual Proceedings of the Phytochemical Society of Europe Volume 25. Clarendon Press, Oxford.

Riberau-Gayon, P. 1972. Plant Phenolics. Oliver and Boyd, Edinburgh.

SAS Institute, Inc. 1985. SAS Users Guide: Statistics. Cary, North Carolina.

Schopf, R. 1986. The effect of secondary needle compounds on the development of phytophagous insects. Forest Ecology and Management 15: 55-64

Schiel, O., K. Jarchow-Redacker, G. Piehl, J. Lehmann, and J. Berlin. 1984. Increased formation of cinnamyl putrescines by fedbatch fermentation of cell suspension cultures of *Nicotiana tabacum*. Plant Cell Reports 3: 18-20.

Seigler, D. and P.W. Price. 1976. Secondary compounds in plants: primary functions. American Naturalist 110: 101-105.

Singleton, V.L. and J.A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144-158.

Smedman, L.A., K. Sanjberk, E. Zavarin, and T.R. Mon. 1969. Oxygenated monoterpenoids and sesquiterpenoid hydrocarbons of the cortical turpentine from different *Abies* species. Phytochemistry 8: 1471-1479.

Smith, R.H. 1964. Variation in the monoterpenes of *Pinus ponderosa* Laws. Science 143: 1337-1338.

Squillace, A.E. 1971. Inheritance of monoterpene composition in coritical oleoresin of slash pine. Forest Science 17:381-387.

Squillace, A.E. 1976. Analyses of monoterpenes of conifers by gas-liquid chromatography. Pages 120-157 *in* J.P. Miksche, editor. Modern methods in forest genetics. Springer-Verlag, New York.

Squillace, A.E., O.O. Wells, and D.L. Rockwood. 1980. Inheritance of monoterpene composition in cortical oleoresin of loblolly pine. Silvae Genetica 29: 141-152.

Steinhoff, R.J. 1978. Early growth of grand fir seedlings in northern Idaho. Pages 359-365 *in* Proceedings of the IUFRO joint meeting of working parties, Vol. 2: Lodgepole pine, sitka spruce, and *Abies* provenances. Ministry of Forests, Vancouver, British Columbia, Canada.

Steinhoff, R.J. 1986. Nursery performance of grand fir provenance collections in north Idaho. Pages 145-151 *in* A.M. Fletcher, editor. IUFRO *Abies grandis* provenance experiments. Forestry Commission Research and Development Paper 139, Forestry Commission, Orpington, Kent, England.

Strauss, S.H. and F.T. Ledig. 1985. Seedling architecture and life history evolution in pines. The American Naturalist 125: 702-715.

Swain, T. 1985. Plant phenolics: Past and future. Pages 453-467 *in* C.F. Van Sumere and P.J. Lea, editors. The biochemistry of plant phenolics. Annual Proceedings of the Phytochemical Society of Europe Volume 25. Clarendon Press, Oxford.

Tahvanainen, J., E. Helle, R. Julkenen-Tiitto, and A. Lavola. 1985. Phenolic compounds of willow bark as deterrents against feeding by mountain hare. Oecologia (Berlin) 65: 319-323.

Thorin, J. and H. Nommik. 1974. Monoterpene composition of cortical oleoresin from different clones of *Pinus sylvestris*. Phytochemistry 13: 1879-1882.

Torssell, K.B.G. 1983. Natural Product Chemistry. John Wiley and Sons, New York.

Townsend, A.M., J.W. Hanover, and B.V. Barnes. 1972. Altitudinal variation in photosynthesis, growth, and monoterpene composition of western white pine (*Pinus monticola* Dougl.) seedlings. Silvae Genetica 21: 133-139.

von Rudloff, E. 1976. Chemosystematic studies in the genus *Abies* II. Leaf oil analysis of grand fir. Canadian Journal of Botany 54: 1926-1931.

von Rudloff, E., M.S. Lapp, and F.C. Yeh. 1988. Chemosystematic study of *Thuja plicata*: multivariate analysis of leaf oil terpene composition. Biochemical Systematics and Ecology 16: 119-125.

Waring, R.H. and G.B. Pitman. 1985. Modifying lodgepole pine stands to change susceptibility to mountain pine beetle attack. Ecology 66: 88-97.

Waring, R.H., A.J.S. McDonald, S. Larsson, T. Ericsson, A. Wiren, E. Arwidsson, A. Ericsson, and T. Lohammar. 1985. Differences in chemical composition of plants grown at constant relative growth rates with stable mineral nutrition. Oecologia (Berlin) 66: 157-160.

Watt, A.D. 1989. The chemical composition of pine foliage in relation to the population dynamincs of the pine beauty moth, *Panolis flammea*, in Scotland. Oecologia (Berlin) 78: 251-28.

Wender, S.H. 1970. Effects of some environmental stress factors on certain phenolic compounds in tobacco. Recent Advances in Phytochemistry 3: 1-29.

Wescott, R.J. and G.G. Henshaw. 1976. Phenolic synthesis and phenylalanine ammonia-lyase activity in suspension cultures of *Acer pseudoplatanus* L. Planta 131: 67-73.

Whitham, T.G. and C.N. Slobodchikoff. 1981. Evolution by individuals, plant-herbivore interactions and mosaics of genetic variabilty. Oecologia (Berlin) 44: 287-297.

Whittaker, R.H. and P.P. Feeny. 1971. Allelochemic: chemical interactions between species. Science 171: 757-770.

Wiermann, R. 1981. Secondary plant products and cell and tissue differentiation. Pages 85-116. *in* E.E. Conn, editor. The Biochemistry of Plants Volume 7. Secondary Plant Products. Academic Press, New York, New York.

Wilkinson, R.C. 1985. Comparative white pine weevil attack susceptibility and cortical monoterpene composition of western and eastern white pine. Forest Science 31:39-42.

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

Zavarin, E., K. Snajberk and W.B. Critchfield. 1975. Geographic variability of monoterpenes from cortex of *Abies concolor*. Biochemical Systematics and Ecology 3:191-203.

Zavarin, E., K. Snajberk and W.B. Critchfield. 1977. Terpenoid chemosystematic studies of *Abies grandis*. Biochemical Systematics and Ecology 5:81-93.

Zucker, W.V. 1983. Tannins: Does structure determine function? An ecological perspective. The American Naturalist 121: 335-365.