



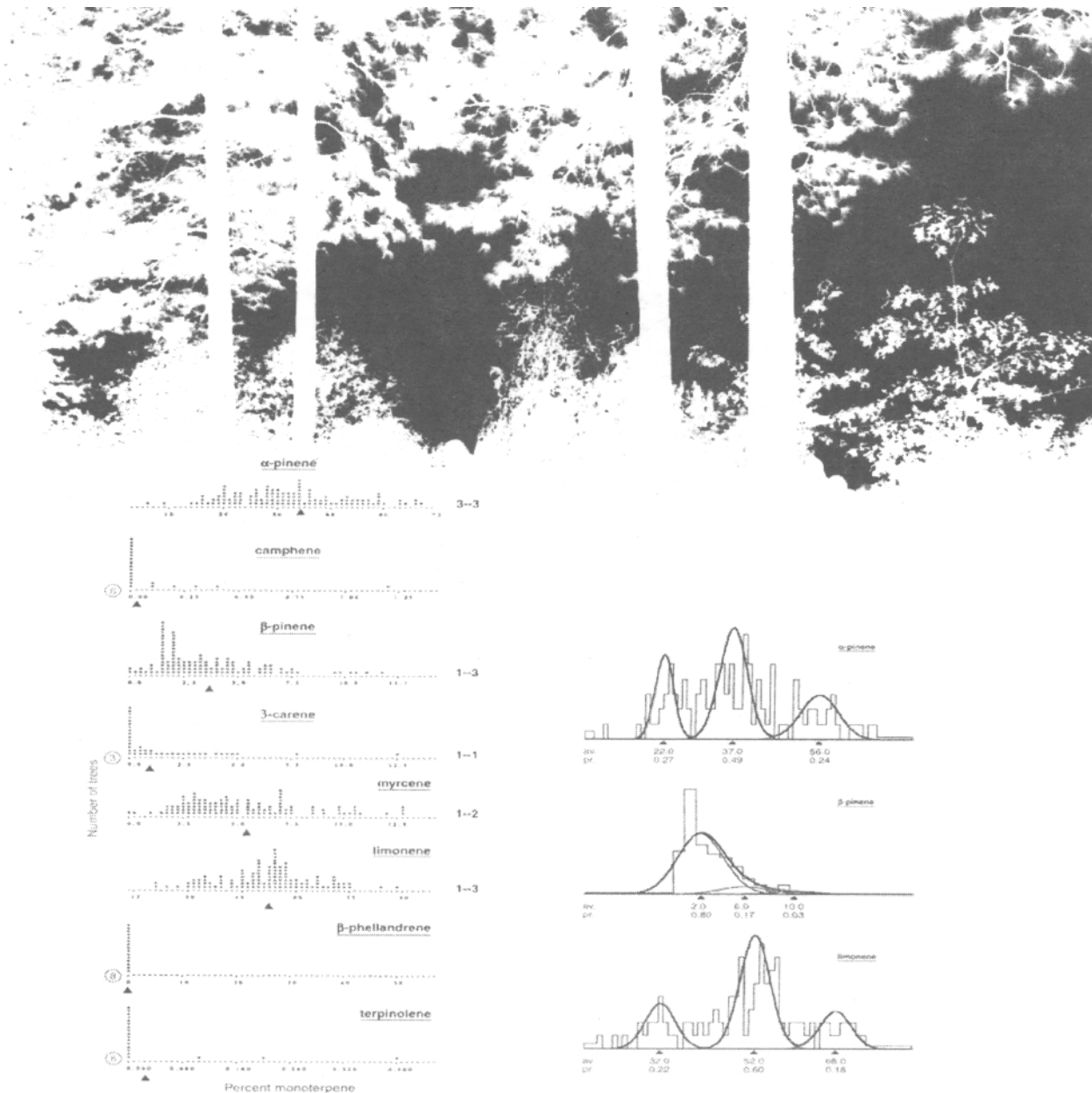
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Xylem Monoterpenes of Pines: Distribution, Variation, Genetics, Function

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Abstract

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Retrieval Terms: Pinus species, pine grafts, bark beetles, western pine beetles, gas liquid chromatography, California.

The monoterpenes of about 16,000 xylem resin samples of pine (*Pinus*) species and hybrids—largely from the western United States—were analyzed in this long-term study of the resistance of pines to attack by bark beetles (*Coleoptera: Scolytidae*), with special emphasis on resistance to the western pine beetle (*Dendroctonus brevicomis*). The samples were analyzed by gas liquid chromatography, and the results reported are expressed as normalized monoterpene composition. Optical isomers were not separated. The study covered (a) 19 pine species in California, (b) 86 pine hybrids and 26 parent species at the Pacific Southwest Research Station's Institute of Forest Genetics, (c) 21 half sibling populations of *Pinus ponderosa* Dougl. ex Laws., (d) 56 full sibling populations of *P. ponderosa*, (e) interspecific and intraspecific pine grafts, (f) effects of procedural parameters, and (g) bioassay of resistance to *Dendroctonus brevicomis*. The results suggested that (a) these monoterpenes and associated hydrocarbons were primarily controlled by genes in pine xylem—hepane, undecane, α -pinene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene—and evidence strongly suggested a pair of additive alleles for each monoterpene that competes with each other for a fixed amount of a five-carbon precursor; (b) all of these might also be produced by isomerization and possibly may be byproducts of biosynthesis of other monoterpenes; (c) the quantity of all these can be modified by several environmental factors often associated with forms of stress, and possibly by other minor genetic factors; and (d) these monoterpenes were not controlled by genes in pines but are probably the results of isomerization and byproducts of biosynthesis: α -thujene, nonane, camphene, γ -terpinene, ocimene, α -phellandrene, and terpinolene.

The Author:

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Cover Image: Chapter 6, Figure 6-2.

Xylem Monoterpenes of Pines: Distribution, Variation, Genetics, Function

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In Brief

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Retrieval Terms: *Pinus* species, pine grafts, bark beetles, western pine beetles, gas liquid chromatography, California.

The monoterpenes are an important group of chemicals occurring in many plant species. They are particularly common and abundant in conifers—especially the pines (*Pinus* species). They are important because they have been associated with plant defense against insect attack and disease infection. Quite the opposite, they might also act in attracting insects to plants directly or indirectly. They are used for plant classification. And commercially, they are used as solvents and can be building blocks in the synthesis of organic chemicals.

This report brings together both published and unpublished information about the distribution, variation, genetics, and function of the xylem monoterpenes of pines. The reported studies are largely derived from a long-term investigation of the host relationship of bark beetles (*Dendroctonus*) to the pines, with special emphasis on the resistance of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) to the attack of western pine beetles (*D. brevicomis*) and the function of xylem resin in this relationship. The focus is on this relationship rather than on the study of monoterpenes as such.

The monoterpenes of about 16,000 xylem resin samples of pine species and hybrids—largely from the western United States—were analyzed in this study of the resistance of pines to attack by bark beetles (*Coleoptera: Scolytidae*). The samples were analyzed by gas liquid chromatography, and the results reported are expressed as normalized monoterpene composition. Optical isomers were not separated. Results, by major study areas, were as follows:

Variation and distribution of the xylem monoterpenes in the 19 pines of California:

- The 19 species, which are found naturally in California, were sampled with a wide range of local and regional intensity.
- Ten species had considerable local or regional variation or both in composition and can be characterized by the incidence of types of composition: ponderosa pine (*P. ponderosa*), lodgepole pine (*P. contorta*), singleleaf pinyon pine (*P. monophylla*), bristlecone pine (*P. aristata*), bishop pine (*P. muricata*), Coulter pine (*P. coulteri*), washoe pine (*P. washoensis*), whitebark pine (*P. albicaulis*), sugar pine (*P. lambertiana*), western white pine (*P. monticola*). The last three, all soft pines, are extremely variable locally and regionally, and may have as many as 40 composition types in a stand of 100 trees.

- Three species have little or no variation and can be characterized fairly adequately by average composition: Jeffrey pine (*P. jeffreyi*), Digger pine (*P. sabiniana*), knobcone pine (*P. attenuata*).
- Six species have some variation, but the data currently available are insufficient to permit characterization by the type of composition: foxtail pine (*P. balfouriana*), Torrey pine (*P. torreyana*), Parry pine (*P. quadrifolia*), pinyon pine (*P. edulis*), limber pine (*P. flexilis*), and Monterey pine (*P. radiata*).
- In every adequately sampled species, the frequency distribution of one or more of their individual monoterpenes was multimodal, often trimodal, giving some support to the hypothesis that the genetic structure for each monoterpene is due primarily to the additive effect of two alleles at a single locus.

Xylem monoterpenes composition of 86 inter- and intra-specific pine hybrids and 26 parent species at the Station's Institute of Forest Genetics (Chapter 4):

- The 86 inter-and intra-specific pine hybrids and 26 parent species growing in the arboretum at the Pacific Southwest Research Station's Institute of Forest Genetics, near Placerville, California, are catalogued in Chapter 4.
- Most of the parent species are from North America, but a few are from Central America and Eurasia.
- Much of the data are from a study of *P. ponderosa*, *P. jeffreyi*, *P. coulteri*, and loblolly pine (*P. taeda*).
- The number of trees sampled for a given parent or hybrid was usually small, and only generalizations can be offered; and the actual parents of most hybrids were almost always unavailable for terpene analysis. In many cases, the pollen was from a mixture of trees; therefore, conclusions were almost always based on the composition of the parent as a species.
- The composition of hybrids was usually intermediate, in a general sense, between two parent species. The lack of close intermediacy, though, would appear to be attributed to an unequal contribution of alleles by the two parent trees in an additive allele system.
- A given monoterpene can be lost in the first backcross.
- Some hybrids had monoterpenes that had not been reported in the parents, suggesting that the parent species had not been adequately sampled.

Populations of 4- to 12-year-old half sibling families of P. ponderosa (Chapter 5):

- Chapter 5 reports population studies of 4- to 12-year-old half siblings of *P. ponderosa*, mostly nursery grown. These half-siblings were from parent trees in three widely separated geographical areas. Full siblings were derived from many of these same parent trees.
- Studies included about 1500, four to 12-year-old half siblings of 27 trees that were selected primarily on the basis of monoterpene composition.

Three of the ecotypic (monoterpene races) regions of *P. ponderosa* were represented: Tehachapi, Sierra Pacific, and Escalante, and results were based on 25 to 200 siblings per parent.

- Composition usually varied greatly in two or more major components.
- Average composition of a set of progeny tended to be broadly intermediate between the parent tree and the average of the local stand. Trees with similar composition produced progeny that were quite similar in composition.
- Limonene and myrcene content, however, were lower than expected when intermediacy prevailed, and α -pinene and β -pinene were higher. A juvenile effect is postulated which is more or less evident in all young growth. The effect of this juvenile factor is much the same as that shown by plants under stress. All young trees or young parts of older trees, therefore, may be under some level of stress—the stress of rapid growth.
- Nearly one-third of the half siblings of a high limonene parent could be classed as high limonene or potentially so.
- Some high limonene trees were found among the progeny of a parent which could be classed only as near-high limonene.
- A rare tree was found with ultra high limonene, i.e., with about 70 pct., though such trees have not been found in mature, natural stands.
- There were some differences between the three regions in the comparative ratios between parent and progeny of a given monoterpene, suggesting that the locality of a parent tree has an effect of the juvenile response or that there are minor genetic effects which are distinctive to a region.
- Two forms of stress—over-application of a phytocide and prolonged growth in a container—caused a sharp change in quantitative composition, usually evidenced by lower limonene and myrcene and higher α -pinene and β -pinene.

Results, in general, indicate that the quickest and cheapest way to obtain trees with a diverse array of composition is the use of wind pollinated seed from localities with much variation in individual tree composition.

Population studies of 4- to 12- year-old full sibling families of P. ponderosa (Ch.6):

- About 3,000 samples of 4- to 12-year-old full siblings of 56 sets of progeny from 7 females and 14 male trees were examined.
- Forty-two sets of progeny were analyzed for modal distribution of individual monoterpenes. In all cases, the actual number of modes derived from the analysis equalled or exceeded the number of modes expected from the assignment of additive alleles to the two parents; this supports the additive allele hypothesis for five monoterpenes in *P. ponderosa*: α -pinene, β -pinene, 3-carene, myrcene and limonene; myrcene, though, appears to be a rare allele.
- High limonene content trees always breed true, but the juvenile effect was clearly evident in the progeny.

- Other combinations of parents also produced high limonene progeny.
- Crosses of high limonene trees produced progeny which could be classed as ultra-high limonene—about 80 pct—a type of composition not yet found in natural stands. Many other types of composition were found among the progeny which have not yet been found in natural stands.
- The composition of a single set of progeny was quite variable, suggesting the effects of several non-genetic factors on the basic genetic structure.
- Differences between the progeny of a female with two males which were near and far away from the female were noticeable. These differences were evidenced in much the same way as stress; and it is postulated that there is a level of stress in the progeny of parents which are far apart, or possibly "off-site".
- The effects of stress were clearly evident in full siblings, and were often greatest in trees with high limonene concentration.
- Breeding on grafts produced the same array of progeny as breeding on the parent tree.
- Over an 8-year period there were no sharp shifts in the composition of a set of progeny in a given location; this indicates slow changes over many years: α -pinene and β -pinene decreasing, myrcene and limonene increasing, and 3-carene increasing or decreasing, depending on the composition. There probably will be an increase in β -phellandrene with age. These observations are based on sampling different sets of trees from the same population, and not on resampling the same set of trees.

There was no decrease in total seed or sound seed per cone in crossing parents about 500 miles apart; and progeny of such crosses are not growing nearly as well as indigenous progeny.

Inter- and intra-specific grafts (Chapter 7):

- Chapter 7 is a report on 40-year-old interspecific grafts of pine species and of 12-year-old intraspecific grafts of *P. ponderosa*.
- Forty years after grafting, the composition of the scion of inter-specific grafts—ponderosa /Jeffrey, Jeffrey/ ponderosa, Digger/ponderosa—was about the same as the parent species, but the composition of the root stock was being affected by the scion and the effect was proportional to the distance from the graft union.
- Measurements were made of 6- to 17-year-old intra-specific grafts of *P. ponderosa*.
- The qualitative and quantitative composition of the scion was radically different from the parent tree for about 10 years after grafting. The greatest differences were in grafts in which the composition of the root stock and the scion were most widely divergent. Three possible causes are postulated: (1) the severe stress of grafting, (2) a strong effect of the

root stock, (3) an expression of a minor genetic structure through the 0-0 set of alleles.

- Between 10 and 17 years after grafting, but primarily between 15 and 17, the composition of the scion suddenly and rapidly became much like that of the parent tree.
- After this sudden change, the scion began to affect the composition of the root stock much as in older inter-specific grafts noted above.

Effect of sampling and procedural parameters (Chapter 8):

- Trees over 40 years old had little or no difference in composition attributable to radial, or circumferential position of the sample. There was small and consistent variation attributable to vertical position of the sample in 40 to 60 years old *P. ponderosa*: α -pinene and β -pinene decreased slightly from top of the tree to the bottom, and myrcene and limonene increased slightly.
- Little or no change in composition took place during a 9-year interval between resampling the same large number of *P. ponderosa*.
- For *P. ponderosa* under 15 years old, there maybe significant quantitative shifts in the topmost two to four internodes.
- No effects on analysis could be attributed to variations in type of sample preparation or time of storage of whole resin samples. However, there were very large qualitative and quantitative differences between results in this report, and those results on which the monoterpene sample was derived from steam distillation of mill-ground sapwood.
- There was a marked difference between xylem and cortex resin monoterpene composition of *P. ponderosa*.

Bioassay of resistance to western pine beetle (Chapter 9):

- Eight to 12-year-old *P. ponderosa*, derived from the half and full siblings studies, were used; the procedure was forced attack on irreversibly stressed trees.
- Success of attack of *D. brevicomis* was inversely proportional to both the concentration of limonene and to the quantitative flow of xylem resin.
- In general, trees of the size tested—about 2 to 3 inches in diameter—were relatively resistant; but within this relatively resistant state, good comparative determinations were made.

Stress:

Six growth conditions, noted under previous headings, were deduced as stressful in *P. ponderosa* under 17 years of age: juvenile period of growth, rapid growth, great distance between parents, over-application of phytocides, prolonged root containment, and grafting. All six were evidenced by an increase in β -pinene and α -pinene, and by a decrease in myrcene and limonene.

General Introduction

Summary: Resins are complex mixtures of organic chemicals. They are found in many kinds of plants, commonly in conifers, and particularly in the pines. Resins or their components can protect the plant from insect attack and disease infection. On the other hand, they can also attract insects. Monoterpenes, constituents of pine resin that have an empirical formula of $C_{10}H_{16}$, are quite volatile and have been associated with both plant resistance and attraction. Monoterpenes have been used in plant classification, as organic solvents, and as base molecules in the commercial synthesis of organic compounds. Though strongly controlled by genes, monoterpenes are quantitatively quite variable in pines, probably due in a large part to the competition of sets of additive alleles for a fixed amount of C-5 precursor. Smaller quantitative variation appears to come from isomerization, from biosynthetic by-products, from several conditions of stress, and from age. This paper reports results of a study on the host relationship of pines to bark beetles.

This paper describes the xylem monoterpenes of pines: their distribution, variation, function, and genetics. Monoterpenes are quite volatile and generally give pines their characteristic fragrance. Though all monoterpenes have the same empirical formula, they vary in structure, primarily in the position of rings and double bonds. The overall evidence suggests (a) that the following monoterpenes and associated hydrocarbons found in pine xylem resin are primarily controlled by genes: heptane, undecane, α -pinene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene; (b) that the genetic mechanism is a pair of additive alleles at a single locus for each of these components; (c) that the above components might also be produced by isomerization and possibly may be by-products of the biosynthesis of other monoterpenes; (d) that the quantity of these monoterpenes can be modified by a number of environmental factors, often associated with forms of stress and possibly by other minor genetic factors; and (e) that the following are not controlled by genes but are probably the result of isomerization and by-products of biosynthesis: α -thujene, nonane, camphene, gamma-terpinene, ocimene, α -phellandrene, and terpinolene. These conclusions are based on gas-liquid chromatographic (GLC) analysis and other recorded measurements.

Xylem resin of pines is often and simply defined as a supersaturation of rosin (resin acids) in turpentine (terpenes). The word "resin" can be quite limited to products of conifers (Mirov 1961) or to a wide variety of plants (Langenheim 1990). The terpenes are volatile liquids and are largely monoterpenes with an empirical formula of $C_{10}H_{16}$; they generally comprise about 25 percent of the total weight of resin. The monoterpenes are defined as a class of terpenes containing two isoprene molecules. The remainder of the liquid fraction is chiefly sesquiterpenes ($C_{15}H_{24}$); the amount of the latter in whole resin ranges from about 0 to 20 percent. Thus, the aggregate of all terpenes in whole resin ranges from about 25 to 45 percent. The monoterpenes

are much more volatile than the sesquiterpenes. There are several resin systems in pines distinct from each other, including needle, bud, cone, cortex, root, as well as xylem. In xylem, resin is formed in the epithelial cells surrounding the resin ducts.

The general assumption has been that resin is an end product of metabolism and does not enter any other subsequent biosynthetic processes.

The natural function of resin in pine xylem has long been debated and discussed. A general hypothesis is that resin protects the tree from insect attack and disease infection. Although this hypothesis has usually been at a generalized level without specific documentation, there seems to be some evidence to support the protection hypothesis.

The close association of xylem resin of pines with the success or failure of bark beetle attack has been studied for a long time, starting with observations by A. D. Hopkins (1909), followed by early experimental work by Miller (1950), Callaham (1955), Vité and Wood (1961), and by experimental breeding and bioassay by Smith (1982b). Richmond (1985) showed that pine oil—a liquid aggregate of pine resin extracts with limonene as the primary constituent—could, when applied as a spray to the trunk, protect *P. contorta* from attack by the *Dendroctonus ponderosae*. One interesting and dramatic exception to the protective function is the very different role that resin plays in the operation of bark beetle pheromones. In these cases, one or more of the monoterpene constituents of *P. ponderosa* actually synergize the action of bark beetle pheromones to make them more potent attractants. Thus, the presence of the particular monoterpene in xylem resin helps to concentrate bark beetle attacks on a specific tree or to localized areas and helps to make the attacks more successful. There also is some evidence that monoterpenes may be used by bark beetles to make pheromones. Chapter 9 will give experimental evidence that resin can function in the resistance of pines to bark beetle.

Constituents of resin, particularly the monoterpenes, have been used in plant taxonomy, in the differentiation of species and varieties and in the verification of hybrids.

In addition to its natural functions, xylem resin has been used by humans for a variety of purposes for thousands of years. In much of the period of wooden ships, resin was used as the caulking material for joints; thus, the origin of the term "naval stores," i.e., materials used by the navy. Resin and its derivatives have served as a fuel, solvent, and source of fragrance. As a solvent it is quite commonly used in paints. Recently, there has been renewed interest in the use of turpentine as a general organic solvent. Hayes (1988) contends that turpentine might be substituted for many halogenated solvents that are so widely used in industry but which are often much more environmentally hazardous than a natural product such as turpentine. Monoterpenes can be used as base molecules in the synthesis of organic compounds, such as paclitaxel (Wender 1997 a, b). The current status of the commercial use of resin was reviewed by Zinkel (1981).

Early work on the identity of the xylem monoterpene in pines was summarized by Mirov (1961). That work simply reported the aggregate identity within a species; it was usually necessary to combine samples from several trees to obtain the amount of resin needed for the analyses used at that time. Thus, Mirov's work primarily reported that there was a wide range in monoterpene composition among some of the pine species throughout the world. Mirov (1961) gave some evidence for regional variation in a couple of species, but the report lacks evidence of local variation among trees of the same species.

The rapid analysis of small samples of resin became possible with the development of gas chromatography. This permitted the analyses of large

numbers of individual trees and the basis for determining local and regional varieties of the xylem monoterpenes of pines, and for determining the genetic basis for these characteristics. The results of some of these studies encouraged a theory on the co-evolution of pines and bark beetles (Mitton and Sturgeon 1982). I reviewed the book (Smith 1985) and cited several aspects of the relationship of western pine beetle *Dendroctonus brevicomis* to *P. ponderosa* that do not fit the requirements of the co-evolution hypothesis.

The following are 10 generalizations about the biosynthesis and genetics of xylem monoterpenes in pines:

1. A large body of evidence shows that the total amount of the aggregate of the monoterpenes in whole xylem resin is quite constant for the pines at about 25 percent (Bannister and others 1962, Mirov 1961, Zavarin and others 1969). This evidence is particularly strong for *P. ponderosa* (Smith 1977) with an average of about 25 percent monoterpenes in nearly 3,000 trees measured; and this 25 percent figure was true for trees throughout the range of the species. Therefore, the normalized system and the absolute quantitative system are strongly related. This assumption is strengthened by data given in Chapter 8 on sampling parameters which showed, in general, that there was little variation in normalized composition with differences in temporal and spatial sampling, and with variations in the preparation and analysis of samples from mature trees.
2. The best evidence is that there is a C-5 precursor for all monoterpenes—and probably for the sesqui- and di-terpenes as well (Sandermann 1962). Two molecules of C-5 precursor unite to form a single molecule of C-10 monoterpene. The addition of another C-5 unit forms a C-15 sesquiterpene. And the total amount of the monoterpenes appears to be quite constant.
3. A fixed amount of this C-5 precursor is probably produced—or there is a biosynthetic feedback regulatory mechanism which keeps the amount fairly constant—which the monoterpene alleles, through their respective enzymes, draw upon. This results in a *de facto* competition between the monoterpene alleles for the C-5 precursor. Thus the amount of a given monoterpene depends not only on its own genetic makeup but also on the genetic makeup of the other monoterpene alleles. For example, a tree with alleles for only α -pinene will have a monoterpene composition of nearly 100 percent α -pinene. But a tree with the same alleles for α -pinene and also with alleles for other monoterpenes will have a monoterpene composition with much less than 100 percent α -pinene.
4. The structure noted in (3) makes for theoretical quantitative intermediacy in the composition of progeny, but this intermediacy becomes less sharp in a system in which there are two or more different sets of alleles between the two parents. Thus, in a species like *P. ponderosa* where there is clear evidence of five major monoterpenes, the discrimination of alleles and loci becomes less clear and more difficult.
5. Five additional conditions can also reduce the sharpness of allelic discrimination:
 - (a) the ever-present variation in any biological system;
 - (b) the long periods during which xylem resin remains in place and subject to environmental and biological forces, including isomerization;

- (c) the variation, though small, in the sampling and analytical procedures;
 - (d) the possible presence of a minor genetic structure;
 - (e) the action of several forms of stress.
6. Empirical evidence, from an extensive study of the local and regional variation of the xylem monoterpenes of *P. ponderosa*, shows a tendency for each of the five major monoterpenes of *P. ponderosa* and other pines to have at times and under some circumstances a trimodal frequency distribution (Smith 1977). This trimodality suggests two additive alleles at a single locus for each gene. For a working hypothesis and for the discussion of the results, the values of 0 and 1 are assigned to these two alleles so that each gene can have one of three allelic structures (0-0, 0-1, or 1-1).
 7. To test the genetic hypothesis and get the best resolution, it would be advisable to work with many different types of parent trees and large numbers of progeny. Because of logistics and the limited nature and purpose of the study, the best parent trees and number of progeny could not be selected in the studies reported herein. That is, the basic goal of many of the studies was the production of trees with desirable types of composition and not the study of gene structure.
 8. The monoterpenes in xylem resin can be generally classed as major or minor depending on the amount present. Major components are α -pinene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, and the related hydrocarbons heptane and possibly undecane. In general, these occur at greater than 15 percent in at least some trees. Minor components occur at less than 15 percent.
 9. A discussion of the minor monoterpene components, which are nearly always less than 15 percent, might be helpful at this time, since they will be discussed only briefly within the text. The more common minor components are α -thujene, camphene, α -phellandrene, gamma-terpinene, ocimene, terpinolene.
 10. There are at least four explanations for the presence of small amounts of these minor monoterpenes: (a) isomerization, (b) biosynthetic by-products, (c) slight activity by the 0-0 set of alleles, and (d) a minor genetic effect, though there is no firm research evidence for such a structure. And the explanation may not be clearly resolved easily because the preparation and analytical procedures are not that well-tuned for such small amounts of materials. However, large numbers of samples may offset this inadequacy.
 - (a) **Isomerization:** This is a most likely explanation. All monoterpenes have the same empirical formula; they differ only in molecular configuration. The change from one isomer to another can take place easily and with little energy. An example of this is β -phellandrene. In analytical samples from trees with a large amount of β -phellandrene, the amount of β -phellandrene sometimes quickly decreases, even under refrigeration, with irregular shifts in the amounts of the remaining monoterpenes; and the sample usually changes to a more viscous state. The change to a more viscous state suggests

polymerization. In trees like *P. rigida* and *P. elliottii*, the amount of β -phellandrene can vary more than usual from one location to the other in the tree, suggesting isomerization. The effects of age may also be an indication of isomerization. Camphene, α -phellandrene, and gamma-terpinene are often lacking or in very small amounts in young trees, but they begin to be present in trace and small amounts as the tree ages. Myrcene and limonene also increase with tree age.

- (b) **Biosynthetic by-products:** There are at least two clear linkages in the monoterpenes of pines, 3-carene to terpinolene, and sabinene to terpinolene. The ratio of 3-carene to terpinolene is usually about 15:1 while sabinene to terpinolene ranges from 3:1 to about 1:1. In these linkages the ratios are large enough to measure; it might be possible for other linkages to exist, but the ratios are too small or variable. There do not seem to be alleles for terpinolene because it is never found except in relationship to 3-carene or sabinene. Therefore terpinolene might be called a biosynthetic by-product.
- (c) **Activity by the 0-0 set of alleles:** Trees like *P. jeffreyi* and *P. sabiniana*, which appear to have a (1-1) set of alleles for heptane, usually have trace to small amounts of several monoterpenes. Though these small amounts could be derived from isomerization of heptane by a pathway which is not yet known, it might be that (0-0) pairs of alleles for these monoterpenes are slightly active. In some backcrosses in hybrids, a monoterpene occurs in small amounts where one would expect either a moderate amount from a (0-1) set of alleles or none at all from a (0-0) set.
- (d) **A minor genetic effect:** There were some small differences between the sets of progeny of one female with two different males with the same monoterpene composition and with the same assignment of alleles. This difference could be attributed to a minor structure which has not yet been resolved. Such a structure could produce small to trace amounts of monoterpenes.

Additional introductory material, specific to a topic, will be given at the beginning of the appropriate chapter.

Review of literature was terminated in 1990, when the first draft of the full manuscript was completed, except for the articles by Wender (1997).

General Procedures

Summary: All analyses were of fresh resin extracted by tapping large trees and by mini-tapping or severing small trees. Nearly all samples were prepared for analysis by dilution in chromatographic pentane. Analysis of a sample was by gas-liquid chromatography, with a thermal conductivity detector, using a column of β , β' oxydipropionitrile. Optical isomers were not separated. Composition of individual trees was determined by normalization of peak areas for the monoterpene portion of whole resin. Composition of stands was best expressed by the characteristics of the frequency distribution of the amount of the individual monoterpenes. Stands or species that were quite variable in composition were subjected to a coding procedure that classified all trees into a limited number of monoterpene types of composition. The numbers of trees in each coded type were subjected to statistical analyses in order to compare stands or regions.

Much of this chapter consists of the general procedures for sample collection, preparation and analysis, and of data analysis carried out by the author. Additional procedures that are specific to a chapter will be given, when necessary, in a section at the beginning of that chapter. The procedures for cited work will not be given. Most were generally so similar to those given here that results were considered comparable. However, Mirov (1961), prior to the availability of GLC (gas liquid chromatography), often obtained his sample by open-faced collection similar to commercial naval stores practice. He usually aggregated the resin from several trees for analysis, and separated terpenes from resin acids by vacuum distillation. He separated the volatiles into individual components by fractional distillation. For limited sampling for his later work, Mirov did use closed-face collection by employing a 1-inch punch tap fitted with a 30-cc collection vial.

Collection and Preparation of Resin Sample

Some early sampling was done with the 1-inch punch tap noted above for Mirov. However, two procedures were used for nearly all sampling of living trees, a 0.56-inch diameter macro-tap and a 0.25-inch diameter micro-tap.

For a macro-tap, a hole was made through the bark and for about 0.25 inch into the xylem using a brace and 0.56-inch bit. Nearly all sampling was done within the basal 3 feet of the tree except for studies on the effect of height of sample. Often it was helpful to shave down the area of bark so that the entry hole could be more easily made. This shaved area of the bark also facilitated the location of tapped trees when the collection vials were retrieved. The hole had a slightly upward slant to facilitate the flow of resin into a vial. The hole was cleared of debris and the collection vial, a 5-cc shell, was inserted directly into the entry hole so that the lip of the vial was past the phloem and cambium and just into the xylem. Collection vials remained in place for 8 hours to 2 days depending on resin flow and the schedule of field activities. The short period

was more than adequate, in hot weather and at low elevation, when sampling good resin-producing trees like *P. ponderosa*, *P. lambertiana*, *P. monophylla*. The long period was needed in cold weather, at high elevation, and for poor resin-producing trees like *P. albicaulis*, *P. contorta*.

Collection vials were tightly corked and taken to the laboratory for sample preparation. Collected samples were usually put on ice if there was a long period—of several days—between collection and preparation.

For a micro-tap, a 0.25-inch diameter hole was drilled through the bark and phloem for about 0.25 inch into the xylem using a hand brace. The hole was slanted 30°-45° downward so that the bottom of the hole acted as a collecting reservoir for the xylem resin as it exuded from the xylem. The hole was cleared of debris. Resin was collected from the catchment hole by using a thin glass rod. A large drop of resin was picked up with the glass rod and placed in a 1-cc screw-cap sample vial and taken to the laboratory for sample preparation. Usually 20 to 30 vials were collected at one time and taken to the laboratory for immediate processing. This procedure was used only for trees that were within 30 minutes of a laboratory, though it could be used at any time with the use of a portable laboratory. Procedures for sampling severed trees will be given in Chapter 5.

Samples were prepared for analysis by adding an approximately equal volume of chromatographically pure pentane to the freshly collected resin sample. Nearly all prepared samples were held in 1-cc screw-cap vials. Pentane is the best solvent for the chromatographic column because it elutes from the column before any monoterpenes. For the micro-tap samples, the pentane was simply added to the vial in which the glass rod sample had been placed. These prepared samples were stored at 0° C except during analysis. A portable field laboratory was used for some of the sample preparation of the 19 pine species of California.

In some limited and early work (1963-65), the sample of gross resin was distilled in a small short-path still, such as a Hickman still, and the liquid fraction was used for analysis without the use of a solvent. No difference in analysis was found between pentane-prepared and still-prepared samples.

Chromatographic Analysis

All samples were analyzed for monoterpenes by gas-liquid chromatography with a thermal conductivity detector. The column was stainless steel, 1/8 inch by 6'-12' with a solid phase of 80/100 mesh chromosorb W/AW and a liquid phase of 5 percent β , β' oxydipropionitrile (Klouwen and ter Heide 1962). Operating temperatures were 63° C to 68° C on the injector, and 150° C to 160 ° C on the column and on the detector. The carrier was helium at 20 to 40 ml per minute.

The monoterpenes were not separated into optical isomers.

In the early years (1963-1980), the sample analysis was recorded by a continuously balancing strip recorder with a disc integrator to measure peak areas of each monoterpene and the total peak area for a sample or a tree; peak areas were then normalized. In the later years (1980-1989), a programmed electronic integrator was used with immediate calculation and display of normalized peak areas. Both devices gave identical or near-identical results with the same sample. Size of the injected sample varied from 0.2 to 1.0 μ l, and time of sample analysis varied from 8 to 15 minutes depending on operating parameters.

Identification of peaks was periodically checked by internal standards or by preparative recovery and reanalysis of individual peaks, but most peaks were identified by reference to previous samples or to mixtures of known

monoterpenes. Two alternate columns, apiezon and LAC, were sometimes used to check identity.

Some observations about the β , β' column are worth noting. Over the course of the work, numerous columns with the same specification were used, because the β , β' column usually performs adequately for only 3 to 6 months. Yet, all columns did not perform the same initially or subsequently. Some were unable to totally or adequately separate heptane from the pentane solvent. There also were two sets of monoterpenes which were not always clearly separated: 3-carene/sabinene, and limonene/myrcene/ β -phellandrene. Deterioration of a column showed up as a gradual decline in the degree of separation of those sets of components. Deterioration was usually irreversible; yet occasionally, a column would improve in response to a period of rest. Glass-wool plugs were placed at the far end of the injector nearest the column and between the injector and the column. These acted as traps for the solid portions of the resin sample to keep them from slowly migrating onto the column, thus lowering the separation capabilities of the column. Changing the glass-wool plugs—daily in the injector and weekly between the column and the injector—was found to lengthen the useful life of a column, and to slightly improve the separation of components.

Data Analysis

Individual trees: The amounts of components in the sample from each tree were always normalized. That is, each monoterpene was expressed as a percentage of the total monoterpenes in the sample. Normalized expression will be used throughout this report, both for the author's work and for cited work.

The use of normalization has been questioned by some researchers who prefer to express the xylem monoterpenes as an absolute quantity. However, I and others have considerable data to support normalization, as noted earlier in the Introduction. Additionally, I have made hundreds of measurements to determine any change in normalized composition due to time of sampling, position of sample in the mature tree, and differences in procedure. The results showed the normalized composition to be very constant; the results are reported in Chapter 8. Therefore, I believe normalization is valid and as appropriate as any other procedure for expressing monoterpene composition. Likewise, because the monoterpene portion of xylem resin is nearly always at about 25 percent, absolute concentration can be approximated by reducing the normalized amounts by 75 percent.

Sets of trees, basic statistics: Whenever there were two or more trees in a set, the basic statistics of mean, maximum, minimum, and standard deviation (S.D.) were calculated for each component. This was a standard procedure for most data, not so much for interpretation, but to maintain a standard base for all sets of data. These figures have limited interpretive value, particularly if there is considerable range in components; they could be misleading because of multimodal distributions.

Sets of trees, frequency distribution: Whenever there were sufficient numbers of trees in a set—usually greater than 30—the frequency distribution of each component was plotted. From this display of the data, one can see whether the distribution is a single mode or multimodal, and how the individual measurements are distributed among the modes. These displays have very good interpretive value. These also provided a way to present concisely a large volume of data and to show the range and abundance of the percentages of monoterpene components in a stand or species.

Sets of trees, composition types: Whenever there was a multimodal distribution for one or more monoterpenes within a plot, as shown by the

frequency distribution just discussed, the trees in the set were classified into types of composition. The limits of each mode were derived directly from the frequency distribution. In nearly all cases three modes were described, and these were given values of 0, 1, or 2 for the low, middle, and high mode. Then the composition of each tree was reduced to a coded composition by converting the actual composition to a single digit of 0, 1, or 2 according to the modal location of the actual value. This caused a reduction in the number of types of composition in the set of trees; these sets were quite suitable for statistical procedures. The number of trees in each coded type was determined, and the whole set of trees was normalized by composition types, i.e., the number of trees of each coded type was expressed as a percentage of all the trees in the set. A program (MacDonald and Green 1988) was sometimes used to refine and analyze the multimodal distribution. This program assists in determining the number of modes in each distribution and in defining their means and their proportions; it will be described more fully in the section on full siblings (Chapter 6).

I believe that in examining local and regional variation of stands of a species, there is excellent analytical and interpretive value in the characteristic of frequency distribution of individual components. I further believe that the most meaningful, concise, and accurate way to characterize a stand of trees or a species, where there is considerable variation, is by the frequency of types of composition, because all components are viewed as a unit in a tree. Any procedure that uses individual components, such as means and standard deviation, or some form of principal component analysis may not be as effective or meaningful because of normalization and the interdependence of the components. Frequency of composition types can be used only if a species has variation in one or more components. It cannot be used for trees that have little variation, like *P. jeffreyi*, *P. sabiniana*, and *P. attenuata*. The coded expression of composition types may also coincide to some degree with the genetic constitution of a stand or species. The composition types are derived from the frequency distributions.

Distribution and Variation of the Xylem Monoterpenes of the 19 Pine Species of California

Summary: The 19 pine species that are found naturally in California were sampled with a great range of local and regional intensity, 12 by the author and 7 by others. All trees were greater than 30 years old. Little or no variation in composition could be attributed to the time or place of sampling the tree. The major monoterpenes, usually greater than 15 percent in at least some trees, found among the 19 species are: α -pinene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene. The minor components, usually less than 15 percent in nearly all trees, are: camphene, γ -terpinene, terpinolene, tricyclene, ocimene, p -cymene. Three related hydrocarbons—heptane, nonane, and undecane—can also occur as minor components in some species. Heptane is a major component in two species. Ten species have considerable local and/or regional variation in composition. They can be characterized by the incidence of types of composition: *P. ponderosa*, *P. contorta*, *P. monophylla*, *P. aristata*, *P. muricata*, *P. coulteri*, *P. washoensis*, *P. albicaulis*, *P. lambertiana*, and *P. monticola*. Some of the soft pines are extremely variable locally and regionally and may have as many as 40 composition types in a stand of 100 trees. Numbers of composition types for a species ranged from 9 to 120. The *P. ponderosa* species was divided into seven regions and four transition zones on the basis of composition types. Three species, *P. jeffreyi*, *P. sabiniana*, *P. attenuata*, have little variation and can be characterized fairly adequately by average composition. The remaining six species have some variation, but the data currently available are insufficient to permit characterization of types of composition: *P. balfouriana*, *P. torreyana*, *P. quadrifolia*, *P. edulis*, *P. flexilis*, and *P. radiata*. In every adequately sampled variable species, the frequency distribution of one or more of the individual monoterpenes was multimodal, often trimodal, supporting the hypothesis that the genetic structure of each monoterpene is primarily two additive alleles at a single locus. Optical isomers were not separated.

This chapter reports the distribution and variation of the xylem monoterpenes of the 19 pine species native to California. Eighteen species were sampled in California, and some of these were sampled beyond California for the more widely distributed species in the western United States. Data for *P. edulis* were taken from a cited article in which all samples were from trees outside California.

Twenty-three pine species are found naturally in western United States (Critchfield and Little 1966), with 19 found in California (Griffin and Critchfield 1972). Information on their general distribution, to the extent of the research coverage in this report, has been compiled in *table 3-1*.

An alternate way of listing the 19 species is by taxonomic classification (*table 3-2*), but I will not follow this order. The four pine species in western United States not found in California are those whose principal distribution

Table 3-1—The 19 pine species of California, their generalized distribution in western United States(*), the degree of coverage in this report, and the principal scientists reporting on xylem monoterpenes for each species

Species	Generalized distribution			Coverage in this report ¹		Principal research ²
	Only Calif.	Mostly Calif.	Western U.S.	Local	Regional	
Pinus						
1. <i>P. albicaulis</i>			*a	1	3	S
2. <i>P. aristata</i>			*	2	1	Z
3. <i>P. attenuate</i>		*		1	2	S
4. <i>P. balfouriana</i>	*			1	1	Others
5. <i>P. contorta</i>			*a	1	2	S
6. <i>P. coulteri</i>		*b		2	1	S
7. <i>P. edulis</i>			*	3	1	Z
8. <i>P. flexilis</i>			*a	3	3	S
9. <i>P. jeffreyi</i>		*b		1	1	S
10. <i>P. lambertiana</i>		*b		1	1	S
11. <i>P. monophylla</i>			*	1	2	S/Z
12. <i>P. monticola</i>			*a	1	3	S/Z
13. <i>P. muricata</i>		*		1	2	Others
14. <i>P. ponderosa</i>			*ab	1	1	S
15. <i>P. quadrifolia</i>		*b		2	1	Z
16. <i>P. radiata</i> ³	*			1	1	Others
17. <i>P. sabiniana</i>				1	2	S
18. <i>P. torreyana</i>	*			1	1	Z
19. <i>P. washoensis</i>		*c		1	1	S

¹ 1 =extensive, 2 = fair, 3 = sparse.

² S = RH Smith; Z = Eugene Zavarin and associates;
Others = other than S or Z.

³ Recent work gives varietal status to stands on the islands off northern Baja California.
a: also in Canada;
b: also in Mexico;
c: also in Nevada.

Table 3-2—The taxonomic ordering of the 19 pine species of California within the genus *Pinus* as derived from Critchfield and Little (1966)

Subgenus	Section	Subsection	Species
Strobos	Strobos	Cembrae	<i>P. albicaulis</i>
		Strobi	<i>P. monticola</i>
	Parrya		<i>P. lambertiana</i>
			<i>P. flexilis</i>
		Cembroides	<i>P. edulis</i>
			<i>P. quadrifolia</i>
			<i>P. monophylla</i>
Pinus	Parrya	Balfourianae	<i>P. balfouriana</i>
			<i>P. aristata</i>
	Pinus	Ponderosae	<i>P. ponderosa</i>
			<i>P. washoensis</i>
	Pinus		<i>P. jeffreyi</i>
		Sabinianae	<i>P. sabiniana</i>
			<i>P. coulteri</i>
			<i>P. torreyana</i>
		Contortae	<i>P. contorta</i>
	Pinus	Oocarpae	<i>P. radiata</i>
			<i>P. attenuate</i>
			<i>P. muricata</i>

is in Mexico, with their northern limits extending into the other States bordering on Mexico. These four species are: *P. leiophylla*, *P. discolor*, *P. engelmannii*, and *P. strobiformis*.

The 19 pines will be covered in this report in alphabetical order (*table 3-1*). Briefly included will be some of the published work of the author and a large volume of unpublished data that have been collected over a period of about 15 years. Publications of others will be reviewed to the extent that some information will be given for each of the 19 species; however, review of published work will not be extensive.

Both the local and regional coverage varied greatly among the 19 species (*table 3-1*). Early in the study of variation in xylem resin monoterpenes, it became apparent that a better description of species with considerable variation would be secured with a larger sampling of local populations that would define the population by the frequency of types of composition rather than by average differences between individual components. Description by individual components seems to be the least adequate for species with variation in composition. For example, it is possible for two stands to be nearly alike for the average of the various components, yet they can be quite different in the characteristics of the frequency distribution of individual components and in the frequency of types of composition. To work with types of compositions, it is generally necessary to have 50 to 100 trees in a sample. Frequency distributions for modes were not statistically analyzed for the 19 species because of unknown parentage. Modal analysis was carried out for full siblings of *P. ponderosa* (see Chapter 6). Though the determination of composition types was based on criteria derived from the frequency distribution data, there may be a genetic basis for it as well. The information on the 19 species in this chapter is largely confined to xylem resin monoterpenes with little or no coverage of other attributes of the species. These other attributes have been studied for many of these species.

The procedures used for much of the work reported in this chapter are covered in Chapter 2. The GLC procedures for cited work varied, but they did not affect the results except in a few instances in which young seedlings were used instead of mature trees. Such exceptions will be noted as they occur in the text. An additional sampling procedure successfully used by others for sampling the tree was to take an increment core of the sapwood and then extract the xylem monoterpenes with solvents. This procedure was particularly useful where trees were difficult to reach, and, therefore, it was not necessary to make the second trip to collect the resin sample if a tapping procedure had been used. To repeat, optical isomers were not separated.

Only one sampling procedure seemed to produce results different from those described in this report. That procedure was to mill-grind fresh xylem and extract the volatiles by steam distillation (Anderson and others 1970).

There was no elaborate system for selecting trees to be sampled. The procedure was simply "the next tree." A "first tree" was chosen at the beginning of the stand, and a tap was made. The "next tree" was the nearest tree forward when standing at the tap. The distance and direction of the next tree were noted so that they could be easily located for sample retrieval. In general, all trees were greater than 8 inches in diameter, without obvious defects, and greater than 30 years old—most more than 40. Though the distance to the "nearest tree" was often more than 50 feet—a distance considered adequate for genetic research—there were many instances in which the distance was less than 50 feet.

#1. *Pinus albicaulis* Engelm. (whitebark pine)

P. albicaulis is the only North American pine in the subsection *Cembrae*, the nut pines, of the subgenus *Strobus*; the other four species in the subsection are Eurasian. *P. albicaulis* is found extensively in western United States and Canada

(fig. 3-1). It is largely limited to higher elevations, particularly in the southern portion of its range where it is often the pine found at the tree line. Mirov (1961) reported the monoterpene fraction of *P. albicaulis* to be near-pure 3-carene; he also reported unusually large amounts of sesquiterpenes, particularly *albicaulene*. The discussion that follows is adapted from Smith (1990). Sampling was limited to a very small portion of the species range.

Three stands of *P. albicaulis* were sampled for xylem resin as follows:

<i>Number of Trees Sampled</i>	<i>Local</i>	<i>National Forest</i>	<i>Mountain Range</i>
43	Mt. Rose, Nev.	Toiyabe	Sierra Nevada
62	Mt. Shasta, Calif.	Shasta-Trinity	Cascades
23	Paulina Mtns., Ore.	Deschutes	Cascades

This sampled portion of the tree's range is characterized by small, widely separated stands at high elevation. All trees were probably greater than 40 years old, though some were quite small.

Results and Discussion

The three *P. albicaulis* stands differed considerably in average composition of xylem monoterpenes and in the range of the components (table 3-3). However, no composition of any tree in the three stands resembled that which Mirov (1961) reported for the other four species of Cembrae—that is, high in α -pinene and β -pinene. The two Cascade stands differed much more from the Sierra Nevada plot than they differed from each other, but there were noticeable differences between the two Cascade plots. Although in a normalized analysis a change in one component can cause a change in another, the total monoterpene content of whole resin was fairly constant. Thus, the normalized system is a close approximation of the absolute amounts, and the differences observed can be considered real.

The major component in all trees at Mt. Rose was 3-carene, as Mirov (1961) had reported (table 3-3). 3-Carene was also a major component at Mt. Shasta and Paulina Mountains. As with other pines there was a strong, positive association of 3-carene and terpinolene. However, the ratio of the two components was quite different in other pines; in *P. albicaulis* the ratio of 3-carene to terpinolene was about 10:1. In most other pines, like *P. ponderosa*, it ranged from 20:1 to 15:1.

The Mt. Rose trees also had small to moderate amounts of α -pinene, β -pinene, myrcene, limonene, and β -phellandrene. These same components were in larger amounts in the Mt. Shasta and Paulina Mountains trees; β -phellandrene was generally a trace component in all trees.

The individual component averages of the Mt. Rose stand differed markedly from those of the Cascade stands. The averages of several components were about the same in the two Cascade stands, but the ranges were usually different. This could be caused by differences between the two stands in the size of the sample. That is, in a species so variable it may be necessary to have a large sample size—from my experience, 70 to 100 trees—to estimate the range of variation with confidence. The two Cascade stands differed markedly in the average and range of β -pinene and 3-carene (table 3-3). The differences between the two stands in sabinene was caused by one tree at Paulina with 23 percent sabinene. As in several other pines there was a strong positive relationship between sabinene and terpinolene, with nearly a 1:1 ratio—a ratio quite similar for the two components in *P. monophylla*, as will be shown later in this chapter.

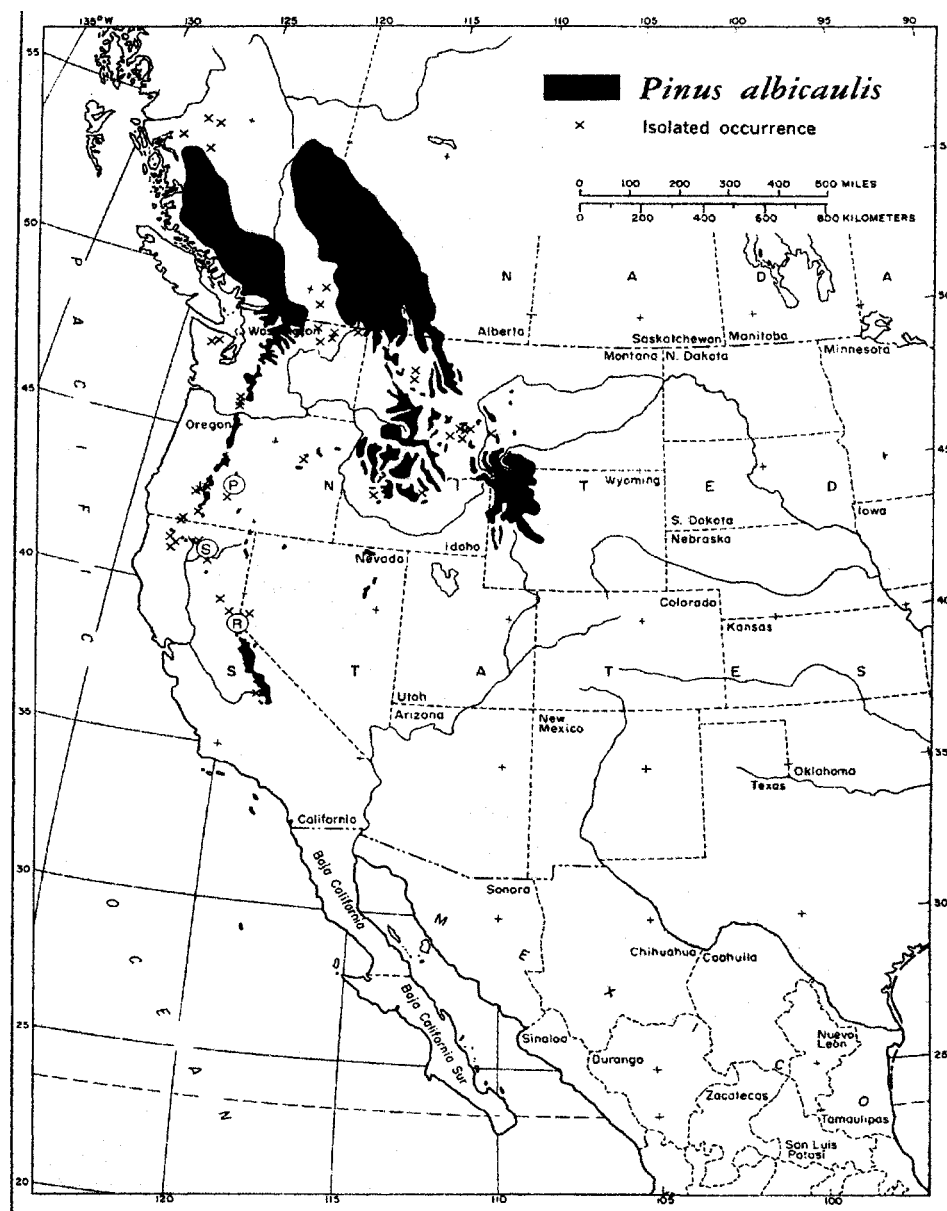


Figure 3-1—Location of three stands of *P. albicaulis* sampled for xylem resin monoterpenes: P = Paulina Mtns.; S = Mt. Shasta; R = Mt. Rose; distribution taken from Critchfield and Little (1966).

Table 3-3—Average ($\bar{x}$), maximum, and minimum xylem resin monoterpenes of *Pinus albicaulis* in three stands: Mt. Rose, Mt. Shasta, Paulina Mountains; n = number of trees in a stand; * = trace

Monoterpene	Mt. Rose n = 43		Mt. Shasta n = 62		Paulina Mts. n = 23	
	$\bar{x}$	Max-Min	$\bar{x}$	Max-Min	$\bar{x}$	Max-Min
	percent ¹					
α -Pinene	1.9	4 - 1	2.7	8 - 2	2.9	5 - 0
β -Pinene	0.3	1 - *	4.6	39 - *	11.4	20 - 0
3-Carene	83.2	94 - 70	54.0	82 - 40	45.1	68 - 8
Sabinene ²	0.0	0 - 0	0.0	0 - 0	1.0	23 - 0
Myrcene	6.1	18 - *	28.1	44 - 1	27.8	69 - 16
Limonene	1.0	5 - 0	3.6	11 - 0	3.4	14 - 0
β -Phellandrene	0.4	1 - 0	0.3	1 - 0	1.3	16 - 0
γ -Terpinene	*	0 - 0	*	0 - 0	*	0 - 0
Terpinolene	6.9	14 - 0	6.0	11 - 4	6.8	20 - 2

¹Normalized percent, * = trace.

²Found in one tree at Paulina Mtns.

Therefore, the same tree with a large amount of sabinene also had a large amount of terpinolene.

Three trees at Paulina are of note: the high-sabinene tree discussed above, a high-myrcene tree, and a high- β -phellandrene tree; this is the highest amount of myrcene I have found in any pine. The percentage composition of these three trees is as follows:

	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>sabinene</i>	<i>myrcene</i>	<i>limonene</i>	<i>β-phellandrene</i>	<i>terpinoline</i>
1.	2.6	11.3	8.1	23.0	25.3	8.9	tr.	20.0
2.	3.6	tr.	23.9	0.6	68.8	tr.	tr.	4.0
3.	4.6	21.1	21.2	tr.	26.0	6.8	16.1	4.1

Are these three trees really unusual, or are there similar trees in other parts of the species distribution?

The three stands differed markedly in the characteristics of the frequency distribution of four variable components: β -pinene, 3-carene, myrcene, and limonene (*fig. 3-2*); α -pinene was only moderately variable. The frequency distributions of the four variable components tend to be trimodal. When all three stands are viewed together, an approximation was made of the three modes and the frequency intervals that describe them (*table 3-4*).

The monoterpene composition types, obtained by converting the composition of each tree to a four-digit value (*table 3-4*), showed a wide range of 25 types (*table 3-5*). When the occurrence of these types was normalized for each stand, the large difference between the Sierra Nevada stand and the two Cascade stands was clearly evident (*fig. 3-3*), and the substantial difference between the two Cascade stands was also clearly evident. That is, the types of composition found in the two Cascade stands were somewhat similar, but their frequencies were quite different. For example, type 10 was by far the most common type at Mt. Rose, but that type did not occur at all in the two Cascade stands. Likewise types 1 to 5 were very common in the Shasta stand, were uncommon in the Deschutes stand, and did not occur at all in the Mt. Rose stand.

The coefficient of association between the stands was very low, at about 0.1, though one might question the use of this analysis because of the large differences in numbers of trees in the three stands. Even without this statistic, the differences between stands were evident, i.e., the two Cascade stands were different in the frequency of types, and the Sierra Nevada stand was clearly different from the two Cascades stands in both presence and frequency of compositional types as noted above. Likewise the numbers of composition types in the three stands were very different, with only four types at Mt. Rose, 13 types at Paulina, and 21 types at Mt. Shasta.

These differences in means, ranges, and in the kinds and frequency of composition types suggest that there are major genetic differences between the Cascade and Sierra Nevada stands—probably at the gene level—and minor genetic differences between the two Cascade stands—probably at the allele frequency level. The study was limited to a very small portion of the tree's range. It is apparent that with such large local variation, larger numbers of trees were needed at both the Mt. Rose and Paulina Mountains to improve the analyses. Obviously, too, a survey of the rest of the species distribution would be useful. There appears to be little relationship, based on xylem monoterpenes, between *P. albicaulis* and the other four pines of the subsection *Cembrae*. According to Mirov (1961), the monoterpenes in all four Eurasian species are largely α -pinene and β -pinene.

Table 3-4—Intervals in the frequency distribution of normalized percentages for the individual monoterpenes of *P. albicaulis* that were used to define the low (0), medium (1), and high (2) modes

Mode value	Code	Monoterpene			
		β -pinene	3-carene	myrcene	limonene
		----- percent ¹ -----			
Low	0	0 - 3	0 - 58	0 - 10	0
Medium	1	4 - 16	59 - 78	11 - 30	1 - 6
High	2	17 - 100	79 - 100	31 - 100	7 - 100

¹ Normalized percent.

Table 3-5—Twenty-five coded types of xylem monoterpene composition of *P. albicaulis*, their frequency in the 125-tree sample (N), and the average actual composition of these coded types; * = trace

Coded No.	Type ²	N	Actual composition monoterpene ¹									
			α -p	cam	β -p	car	sab	myr	lim	β -ph	γ -t	ter
			----- percent ³ -----									
1	0011	1	2.5	-	2.2	56.0	-	29.2	3.7	0.3	0.4	5.4
2	0012	4	1.7	*	0.3	54.2	-	28.3	9.4	*	0.5	5.6
3	0020	11	2.5	0.1	0.7	49.3	0.1	41.2	*	0.5	0.1	5.5
4	0021	14	2.4	*	1.5	51.8	*	34.7	3.4	0.3	0.1	6.4
5	0022	4	2.3	*	0.6	42.3	-	41.8	7.0	0.3	0.1	5.6
6	0110	9	1.8	0.1	0.4	74.3	-	13.8	0.2	0.5	-	8.9
7	0121	1	1.9	0.3	0.8	60.0	-	31.3	0.5	0.3	-	4.7
8	0111	3	1.7	*	0.5	63.4	-	23.0	3.5	0.2	0.2	7.5
9	0112	5	1.6	0.1	0.2	61.9	-	21.5	8.6	*	0.2	5.7
10	0200	6	2.4	0.3	0.4	84.7	-	4.6	0.2	0.3	0.1	6.8
11	0201	29	1.9	*	0.3	85.1	-	4.0	1.4	0.3	*	7.0
12	0210	2	1.5	*	0.3	79.5	-	12.6	0.3	0.2	-	5.5
13	1010	3	3.2	*	12.3	53.8	*	23.6	*	0.3	-	6.7
14	1011	4	2.3	0.1	10.8	49.7	*	23.0	4.9	1.0	*	7.6
15	1012 ⁴	6	2.6	*	11.2	43.2	3.8	21.0	9.9	0.3	-	8.4
16	1020	7	4.3	*	10.1	44.4	*	35.6	*	0.3	-	5.1
17	1021	1	2.5	*	5.4	41.1	*	41.9	4.5	0.6	-	3.9
18	1110	2	1.8	*	9.5	63.1	*	17.6	*	0.4	-	7.7
19	1101	3	2.5	*	8.1	67.2	-	9.6	4.8	0.7	-	6.7
20	1111	2	2.1	0.1	5.0	64.5	*	16.4	3.4	0.4	-	7.8
21	1112	1	2.7	*	6.6	60.7	-	16.7	6.7	0.3	-	6.5
22	2001	2	5.8	0.2	34.4	48.0	-	4.5	2.1	0.2	-	4.8
23	2010	3	4.2	*	19.3	47.0	*	22.2	*	0.4	-	6.1
24	2011	4	4.3	0.2	18.8	42.3	*	25.7	2.9	0.6	-	5.0
25	2012	1	4.9	*	21.1	26.2	*	26.0	6.8	16.1	-	4.1

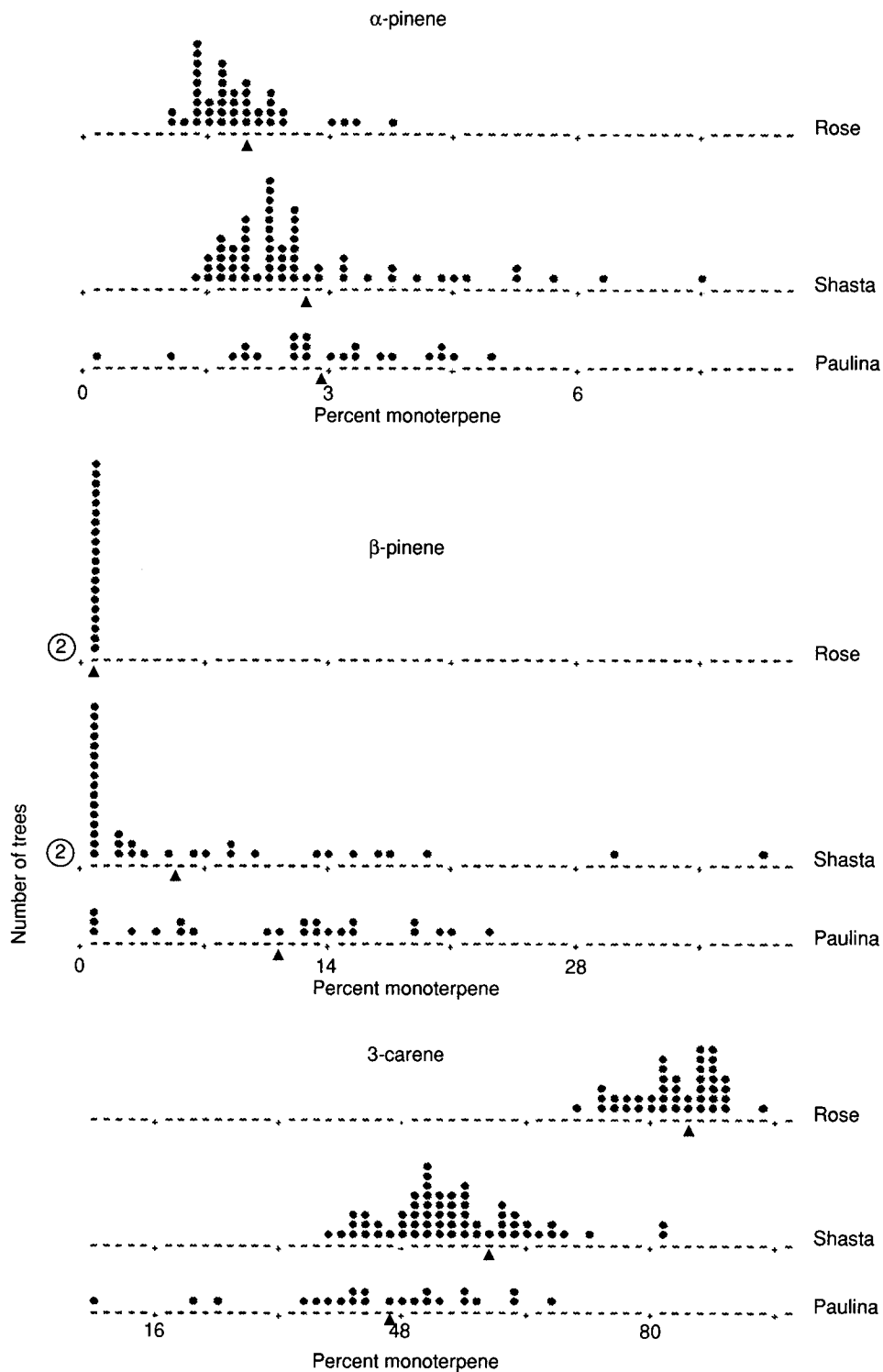
¹ α -pinene, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, γ -terpinene, terpinolene.

² From left to right: β -pinene, 3-carene, myrcene, limonene; see table 3-4 for values of code numbers.

³ Average for the normalized composition for the trees in each type.

⁴ One tree with 23 percent sabinene and 20 percent terpinolene.

Figure 3-2—Frequency distribution of the amount of the listed monoterpenes, in three stands of *P. albicaulis*, Mt. Rose, Mt. Shasta, Paulina Mountains, listed on the right; each dot is the measurement of one tree except where (n) indicates each represents *n* trees; ▲ is average of set.



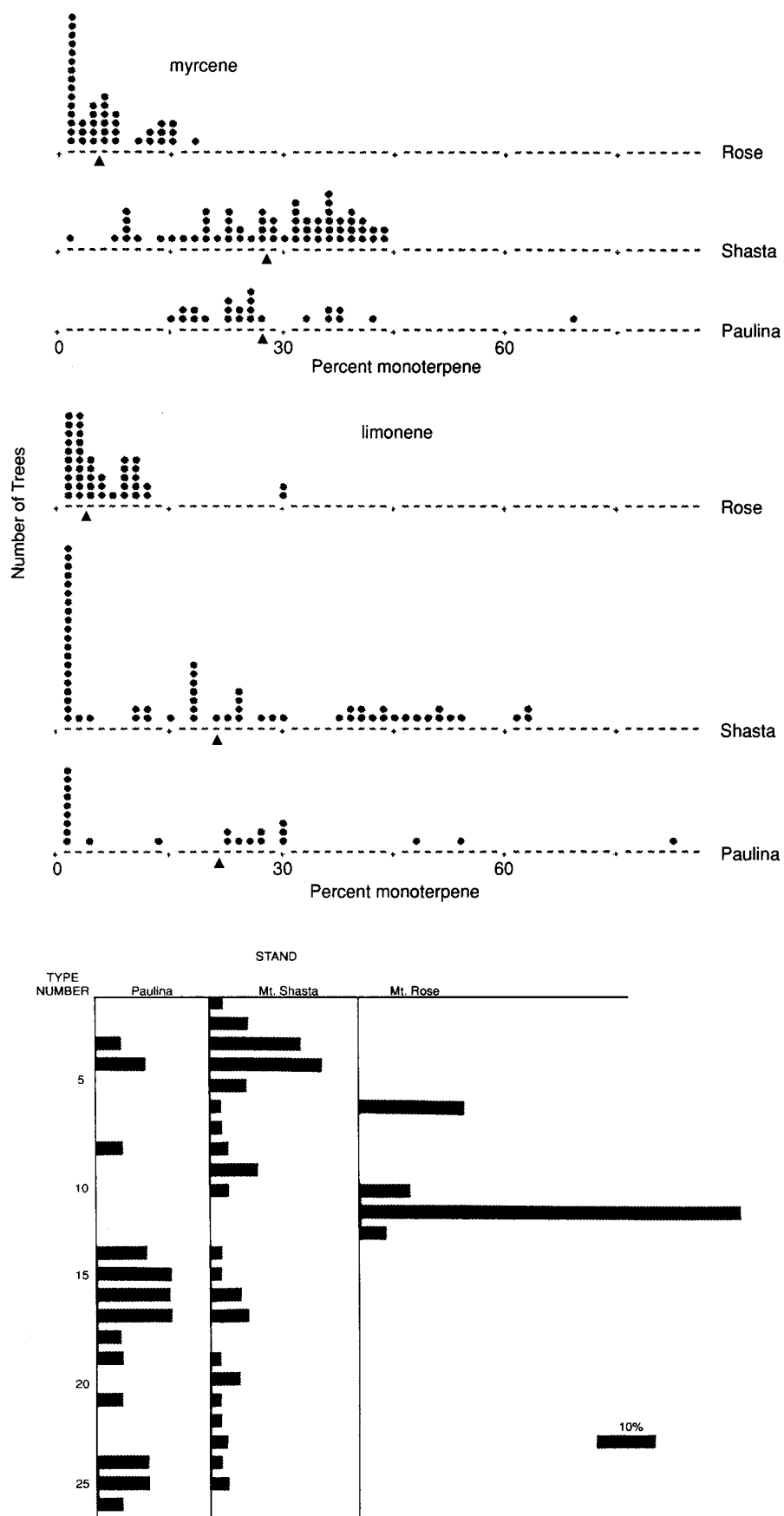


Figure 3-3—Frequency distribution of 26 types of monoterpene composition in three stands of *P. albicaulis* see table 3-5 for coded and actual composition of all types; occurrence normalized for each stand.

#2. *Pinus aristata* Engelm. (bristlecone pine)

P. aristata occurs in widely scattered, generally small stands of trees at high elevation in Colorado, Utah, Nevada, east central California, northern Arizona, and northern New Mexico (fig. 3-4). It is most notable for its long life; trees more than 4,000 years old have been found.

Mirov (1961) sampled trees in the White Mountains of east central California and found them to have about 96 percent α -pinene. At that time the species name was *Pinus aristata*. Zavarin and Snajberk (1973) and Zavarin and others (1976) sampled trees throughout the range of *P. aristata* and found two distinct monoterpene compositions. Trees in the western portion of the range—including Utah, Nevada, and California—were much like those in Mirov's report, generally having about 95 percent α -pinene and small to trace amounts of several other monoterpenes. Those in the eastern portion of the range—including Colorado and the northern parts of Arizona and New Mexico—were found to have only 1 to 5 percent α -pinene and about 75 to 85 percent 3-carene with small amounts of minor components (table 3-6). On the basis of this difference and several morphological differences as well, *Pinus aristata* was divided into two species: *Pinus aristata* in the east and *Pinus longaeva* in the west (Zavarin and others 1976). Because *P. longaeva* is not listed in Griffin and Critchfield (1972), this review will retain the name as *P. aristata*, though *P. longaeva* will be used with reference to recent publications.

Zavarin and others (1976) made a more comprehensive study and found some agreement with their previous work, but they also found some differences. Some stands within the geographic area assigned to *P. aristata* or *P. longaeva* had a monoterpene composition which differed markedly from that of either of the two species. Most notably, three stands were found well within the geographic area assigned to the western form, i.e., *P. longaeva*, that averaged approximately 46 percent α -pinene and 39 percent 3-carene plus minor components (table 3-6). These localities were at Spring Mountain, Nevada; Tamarack Canyon, California; and White Mountains, California (table 3-7). Likewise one stand at Humphreys Peak, Arizona, had an average composition of about 23 percent α -pinene and 43 percent 3-carene; this same stand had usual amounts of limonene (10 percent), β -phellandrene (9 percent), and β -pinene (9 percent) (table 3-6). These average values, however, mask considerable variation which is seen by a listing of individual trees (table 3-7).

Zavarin and others (1980, 1982) expanded their sampling in western Nevada and eastern California and found the earlier results to be generally correct, but they also found three California stands with striking differences. The area of Tamarack Canyon had trees typical of *P. longaeva*—with about 90 percent α -pinene—as well as trees which could be classed as hybrids—with about 58 percent α -pinene and 27 percent 3-carene. Much the same situation was found in a stand at Cerro Gordo in the Inyo Mountains of southeastern California. Further confusing the situation was the finding by Zavarin and others (1980, 1982) of stands at Sentinal Peak and Telescope Peak, both in the mountains west of Death Valley, California, that were completely typical of eastern *P. aristata* stands with about 75-85 percent 3-carene, 5-7 percent α -pinene, and trace to small amounts of minor components. There were generally 10 trees per stand in those reported studies.

It is difficult get a clear picture of separation of *P. longaeva* and *P. aristata* on the basis of xylem monoterpene composition by looking only at the aggregate data of these three reports. That is, there were stands deep in the range of *P. longaeva* that have the monoterpene composition of stands classified as *P. aristata*.

Zavarin and others (1976) proposed that the most likely explanation of the trees classed as *P. longaeva* / *P. aristata* was a hybridizing of the two at some time

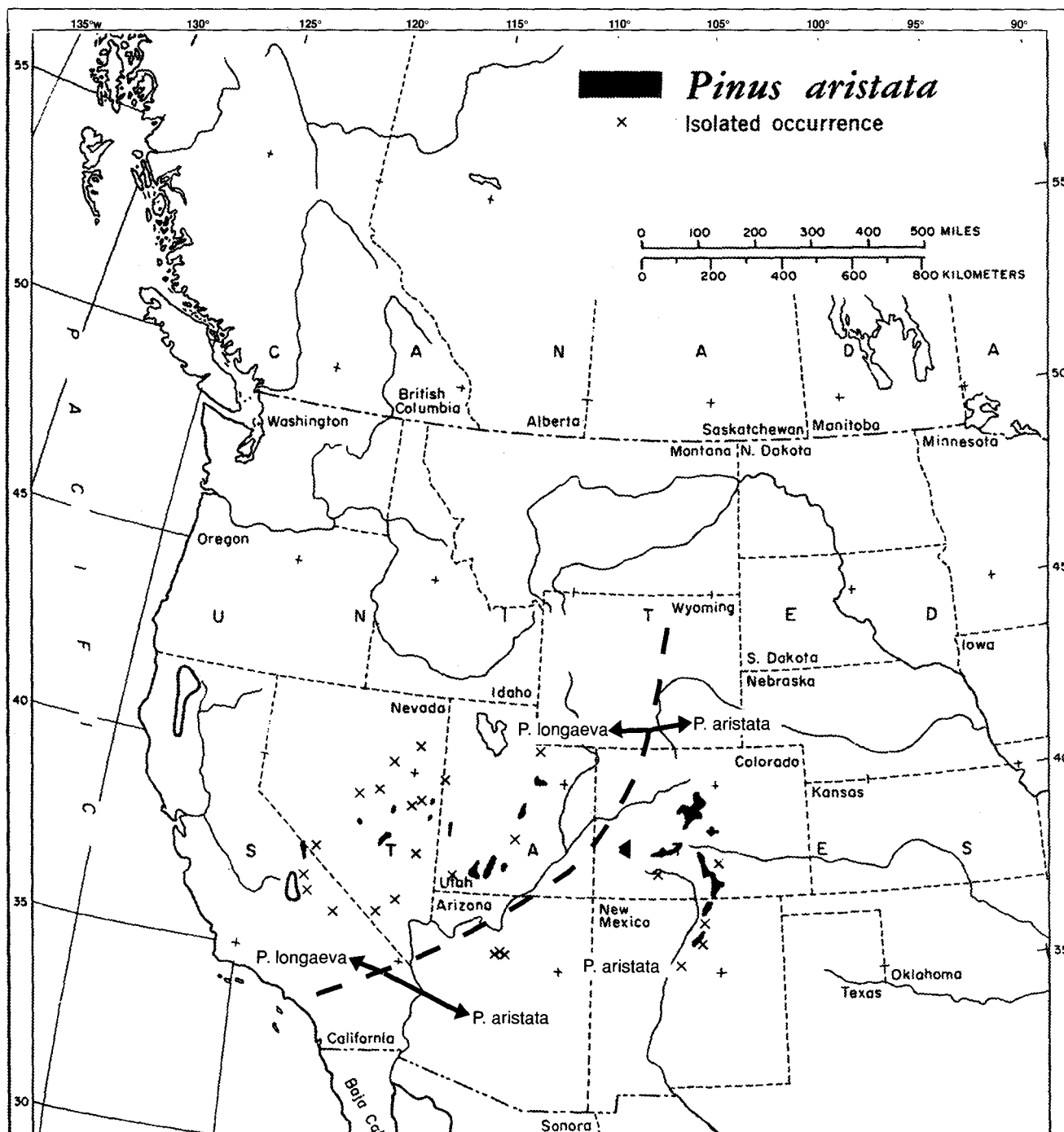


Figure 3-4—Distribution of *P. aristata* (from Critchfield and Little 1966) with the approximate separation into *P. aristata* and *P. longaeva* (dashed line) as adapted from Zavarin and others (1976).

Table 3-6—Average xylem monoterpene composition of stands of *P. aristata* (*aris*), *P. longaeva* (*long.*), and mixed stands as derived from Zavarin and others (1976). See Table 3-7 for composition of trees classified as hybrids of the two species

Stand Location	Species	Stands	Total trees	Monoterpenes ¹									
				α -p	cam	β -p	car	sab	myr	lim	β -ph	γ -t	ter
		Number		percent ²									
a	<i>long.</i>	8	108	94.0	1.5	2.0	**	0	0.4	1.5	0.5	**	**
b	<i>aris.</i>	4	43	6.0	0.1	6.0	73.1	0	2.5	4.5	3.5	0.3	3.5
c	mixed	3	12	46.0	1.1	5.0	39.1	0	2.0	4.5	1.5	0	1.3
d	<i>aris.</i>	3	34	4.9	0.1	3.5	71.5	0.7	1.5	7.9	4.0	0.3	3.7
e	mixed	1	4	23.0	**	9.0	43.0	0	1.2	10.5	9.0	**	4.0

¹ α -pinene, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, γ -terpinene, terpinolene.

² a: Duschene, Utah; Mt. Helen, Utah; Cedar City, Utah; Timber Mt., Nev; Spring Mt., Nev; White Mtns., Calif.; Tamarack Cn., Calif.; Onion Valley, Calif.

b: Conejos Rv., Colo; Leavenworth, Colo.; Lake Peak, N.M.; Sentinal Pk., Calif.

c: Spring Mtn., Calif.; Tamarack Cn., Calif.; White Mtns., Calif.

d: Fremont Pk., Ariz; Aggissiz Pk., Ariz.; Humphrey Pk., Ariz.

e: Humphrey Pk., Ariz.

³ li d **

Table 3-7—Xylem monoterpene composition of individual trees classed by Zavarin and others (1976) as possible hybrids of *P. aristata*-*P. longaeva*

Locality and State	Monoterpene ¹									
	α -p	cam	β -p	car	sab	myr	lim	β -ph	γ -ter	terp
	percent ²									
Spring Mts., NV	61.5	3.6	14.5	0	0.1	3.8	12.4	0.6	0	3.6
White Mt., CA	57.2	0.5	0.7	37.7	*	0.7	0.8	0.7	0.1	1.4
White Mt., CA	49.3	0.6	0.3	48.2	0	0	1.0	0	0	0
Tamarack Can., CA	37.6	0.3	0.5	58.4	0	0.4	0.2	0.5	0	0
Tamarack Can., CA	31.1	0.6	1.5	60.0	0	1.0	0.7	2.4	0	0
Humphreys Pk., AZ	32.5	0	4.1	48.1	*	1.1	7.9	3.7	*	2.6
Humphreys Pk., AZ	14.3	0.2	11.5	46.1	*	1.5	9.7	11.2	*	5.5
Humphreys Pk., AZ	19.8	0	14.5	26.2	*	1.3	13.3	18.3	*	6.1
Humphreys Pk., AZ	24.4	0	7.1	53.4	*	1.0	9.8	2.8	*	1.4
Lake Pk., NM	64.5	4.8	6.4	0	0	0	24.1	0	*	0

¹ Normalized: α -pinene, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, γ -terpinene, terpinolene.

² Normalized; * = trace.

in the past. However, an equally valid explanation was that the so-called hybrid was the older and parent tree of the three types. Zavarin and others (1976) showed evidence of heterozygosity of 3-carene; there was no discussion about the heterozygosity of α -pinene. If both α -pinene and 3-carene were heterozygous in the mixed stands, crossing and segregation could easily produce either high α -pinene or high 3-carene. Another factor to consider was the long period of time that stands have been isolated from one another so that there had been little opportunity for the constant mixing of alleles. It is an interesting condition, and we may not have the final picture of *P. longaeva* or *P. aristata*.

There was the question of tree age also. Nearly all studies of xylem monoterpenes of pines have been with trees less than 100 years old, although a few trees more than 100 years old were used in studies of *P. ponderosa*, *P. lambertiana*, and *P. jeffreyi*. I do not know of a study comparing young and very old *P. aristata*.

#3. *Pinus attenuata* Lemm. (knobcone pine)

P. attenuata ranges extensively in California, though a sizable portion of its range does extend northward into southern Oregon (fig. 3-5). It is one of the group of California closed cone pines, along with *P. muricata* and *P. radiata*. *P. attenuata* was used in early pine hybridization work at the Institute of Forest Genetics (IFG), in Placerville, Calif., in an attempt to combine its drought and cold tolerance with the growth characteristics of *P. radiata*. Mirov (1961) reported the xylem monoterpene composition of *P. attenuata* to be near-pure α -pinene.

Two stands were sampled in California for this study: 89 trees near Gold Run, Placer County, and 39 trees near Mt. Shasta, Siskiyou County (fig. 3-5). The average composition of both stands was nearly identical, 94 to 96 percent α -pinene with small to trace amounts of camphene, β -pinene, 3-carene, myrcene, β -phellandrene, and terpinolene (table 3-8). The standard deviations were extremely low, indicating low variability in the trees in both stands. That the two stands were nearly identical in monoterpenes, though about 200 miles apart, suggests that the minor components are products of biosynthesis or isomerization and are not caused by external environmental factors. The frequency distributions of individual monoterpenes were very similar for the two stands (fig. 3-6). There was little or no difference in composition between a basal tree sample and one at 20 feet (see table 8-1).

Bannister and others (1959) examined the resin from 16 *P. attenuata* that were from seed collected in California but grown in New Zealand. Nine of the trees were about 45 years old, and seven were 5 years old. The composition of the 45-year-old trees was very much like those of the trees in this study, though trace to small amounts of some of the components were not reported (table 3-8). The composition of the 5-year-old trees was slightly different with an average of 5.2 percent 3-carene. This is one of the few instances in which very young trees were sampled in the study of the 19 species. One would suspect either that the unusually high 3-carene was an age factor which will not persist, or that the young trees were progeny of a particular tree which was inclined to contain small amounts of 3-carene, as noted by the listing in table 3-9. Also, one tree at Mt. Shasta had about 4 percent 3-carene (table 3-9).

Anderson and others (1969) used cut logs from trees near Mt. Shasta and extracted the resin from the sapwood by chipping the xylem in a mill and extracting the volatiles by steam distillation of the chipped xylem. They found radically different monoterpene composition: 19 percent camphene, 49 percent 3-carene, 6 percent myrcene, 6 percent limonene, 17 percent β -phellandrene, and 8 percent unidentified. These results indicate that either composition is

Figure 3-5—Distribution of *Pinus attenuata* (from Griffin and Critchfield 1972); and the location of the Mt. Shasta (B) and Gold Run (A) stands; ① is location of *P. radiata*.

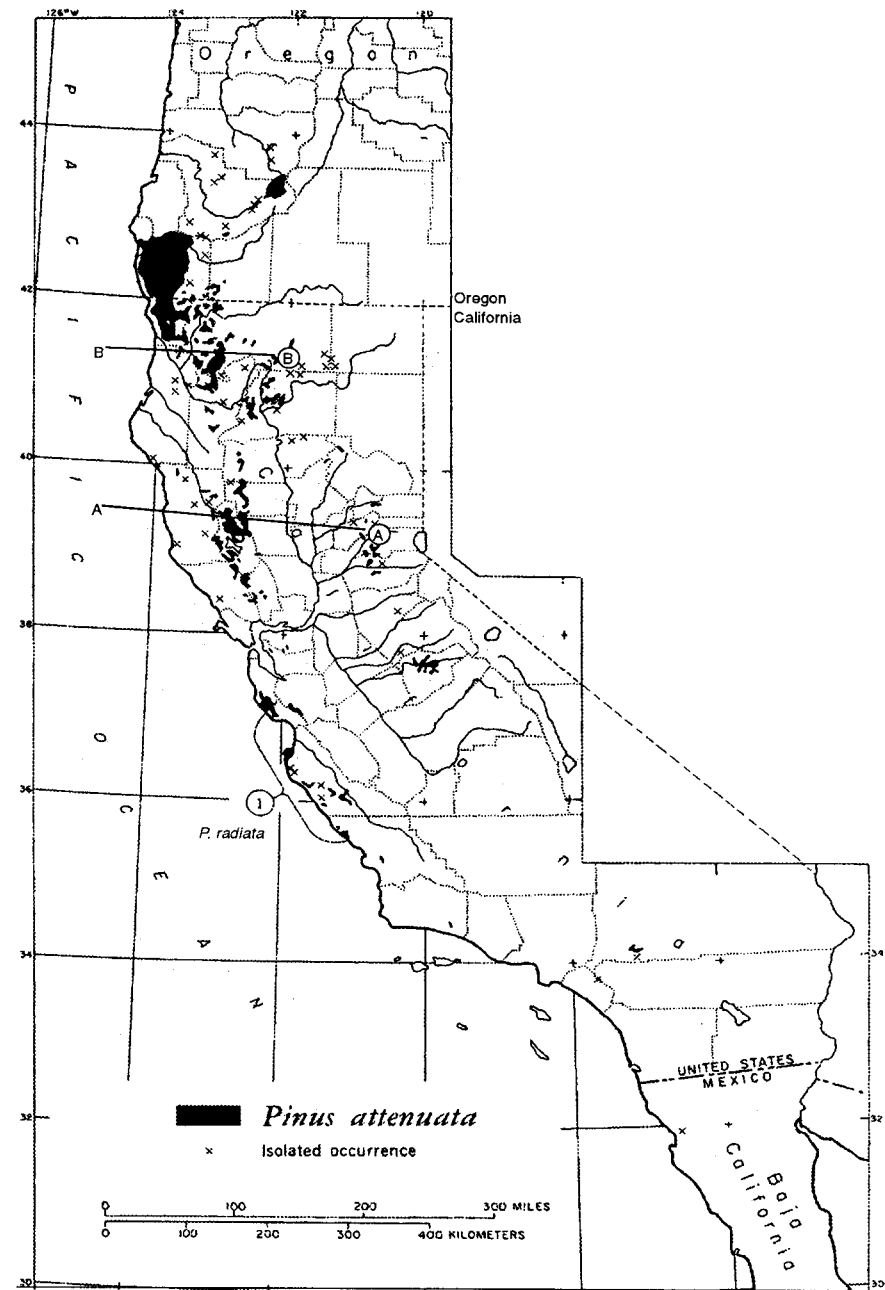


Table 3-8—Average xylem monoterpene composition of four stands of *P. attenuata*; Mt. Shasta and Gold Run trees were from this study; data from two stands in New Zealand trees were taken from Williams and Bannister (1962)

Source of trees	Age of trees	N	Monoterpene ¹							
			α -p	cam	β -p	β -car	myr	lim	β -ph	ter
	yr		percent ²							
Gold Run	20-40	89	96.1	0.1	1.8	0.2	0.8	0.5	0.6	*
Mt. Shasta	> 40	39	94.2	0.3	2.5	0.4	0.9	0.5	1.1	*
New Zealand ³	45	9	95.5	-	4.5	-	-	-	-	-
New Zealand ³	5	7	92.5	-	2.1	5.2	-	-	-	-

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.

² Normalized, * = trace, - = zero.

³ From seed collected in California.

Table 3-9—Xylem monoterpene composition of selected individual trees of *P. attenuata*

Source	Monoterpene ¹							
	α -p	cam	β -p	car	myr	lim	β -ph	ter
	percent ²							
Mt. Shasta	95.8	0.3	1.5	0.2	0.8	0.5	0.7	*
Mt. Shasta	91.5	0.3	2.0	4.3	0.6	0.1	0.8	0.8
Gold Run	97.1	*	1.0	0.2	0.8	0.4	0.3	-
Gold Run	94.3	0.1	2.5	*	1.0	0.8	1.3	-
Gold Run	94.8	*	1.7	1.8	0.7	0.4	0.6	-

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinoline.
² Normalized; * = trace, - = zero.

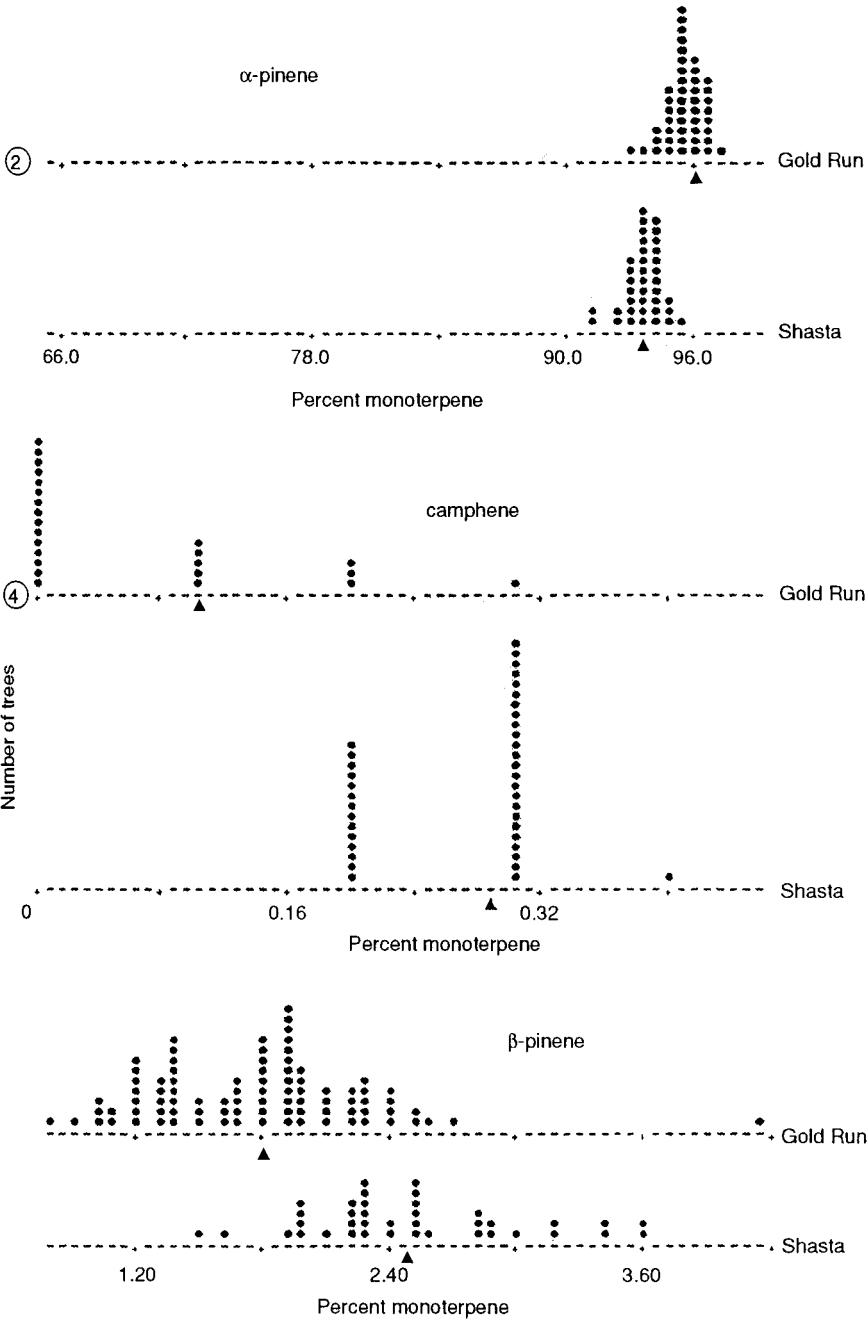
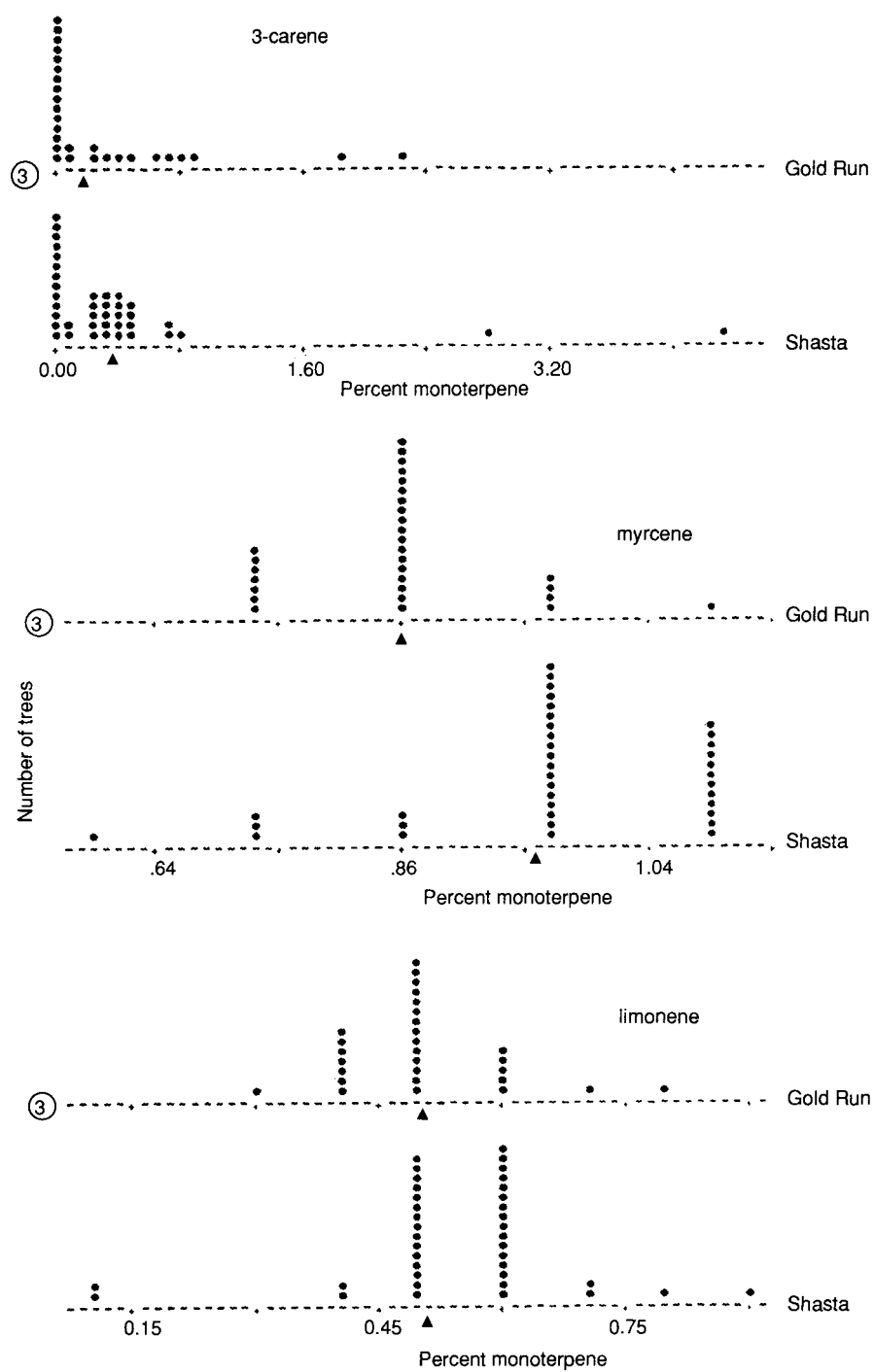
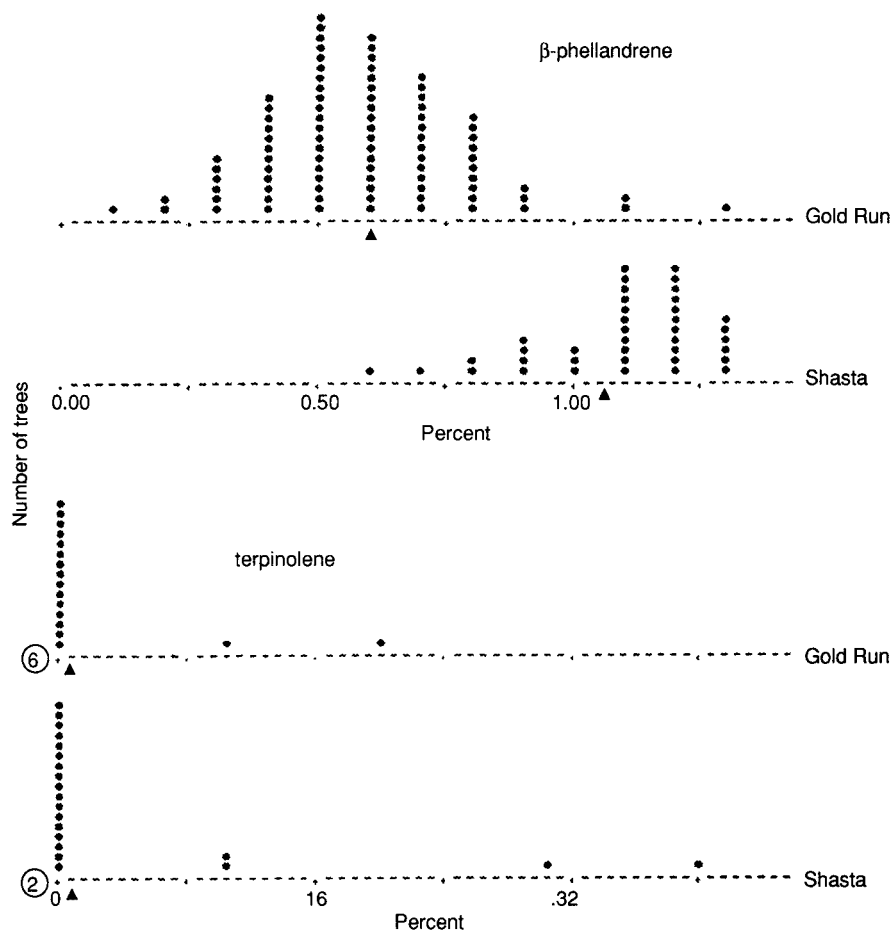


Figure 3-6—Frequency distribution of the amount of the noted monoterpenes of *Pinus attenuata* in two stands: Gold Run (A), Mt. Shasta (B), listed on the right; each dot is measurement of one tree except where (n) indicates each represents n trees; Δ is average of set.

(fig. 3-6 continues on next 2 pages).





changed in the tree as the resin is deposited deep in the sapwood or gross changes were caused by the extraction process. Studies with other pines showed little or no change in composition of annual growth rings.

#4. *Pinus balfouriana* Grev. and Balf. (foxtail pine)

P. balfouriana is limited to two high-elevation centers in California: a northern population in the Klamath Mountains, and a southern population in the southern Sierra Nevada, largely in Sequoia-Kings Canyon National Park (fig. 3-7). The two populations appear to be distinct in several morphological characteristics (Mastrogiuseppe and Mastrogiuseppe (1980). Mirov (1961) reported the xylem monoterpene composition of the northern source to be about 90 percent α -pinene, plus 2 percent β -pinene, and 2 percent limonene. He found the southern source to be about 98 percent α -pinene and without limonene. Mirov's northern sample was an aggregate from 70 trees and the southern sample from 20 trees.

More recently, the xylem monoterpene composition of the two populations was reported to be slightly different by Mastrogiuseppe and Mastrogiuseppe (1980) (table 3-10). Both populations were high in α -pinene, but the northern population had about 9 percent limonene whereas the southern population had only about 1 percent limonene (table 3-10). Numbers of trees sampled were not given by Mastrogiuseppe and Mastrogiuseppe, nor were ranges given for components. Thus, one cannot tell whether the 9 percent limonene in the northern source was the result of most trees with small amounts of limonene, or most trees without limonene and some trees with substantial amounts. If it was the latter condition, then one might assume that limonene might be controlled by a gene, and the two populations were genetically different. If it was the former condition, i.e., most trees with small amounts of limonene, then we might assume that the difference in limonene was due to isomerization or environmental factors and that the two sources were not different.

Snajberk and others (1979) sampled 30 trees each in the northern and the southern populations and found essentially the same difference as Mastrogiuseppe and Mastrogiuseppe (1980); i.e., the northern population had about 8 percent limonene while the southern had less than 1 percent. Snajberk and others (1979) reported, but did not substantiate, that there was considerable overlap in the frequency distribution of limonene in the two areas, and also reported a large S.D. Thus, it appeared to me (from the S.D.) that there was a possibility of the northern population having some trees with very small amounts of limonene and some with moderate amounts, possibly 10 to 15 percent. If so, the difference in limonene between the two populations could be genetic. A display of the frequency distributions of the components would have been helpful. Both papers did accord subspecies status to the two populations on the basis of monoterpenes and a number of morphological features.

The composition of the southern population is quite similar to *P. aristata* in that region (see species #2 of this chapter), which is sometimes mistaken for *P. balfouriana*. The two species are allopatric but grow near each other.

#5. *Pinus contorta* Dougl. ex Laud (lodgepole pine)

The species *P. contorta* is the most widely distributed pine in North America (fig. 3-8); worldwide, it is exceeded only by Scots pine (*P. sylvestris* L.), which is widely distributed across Eurasia. Ecologically, *P. contorta* is of prime significance because of the tremendous range and area it occupies. This species has several variable characteristics that have caused it to be variously subdivided into a number of varieties. The most recent classification (Critchfield 1980) proposed five races: Rocky Mountain-Intermountain (var. *latifolia*

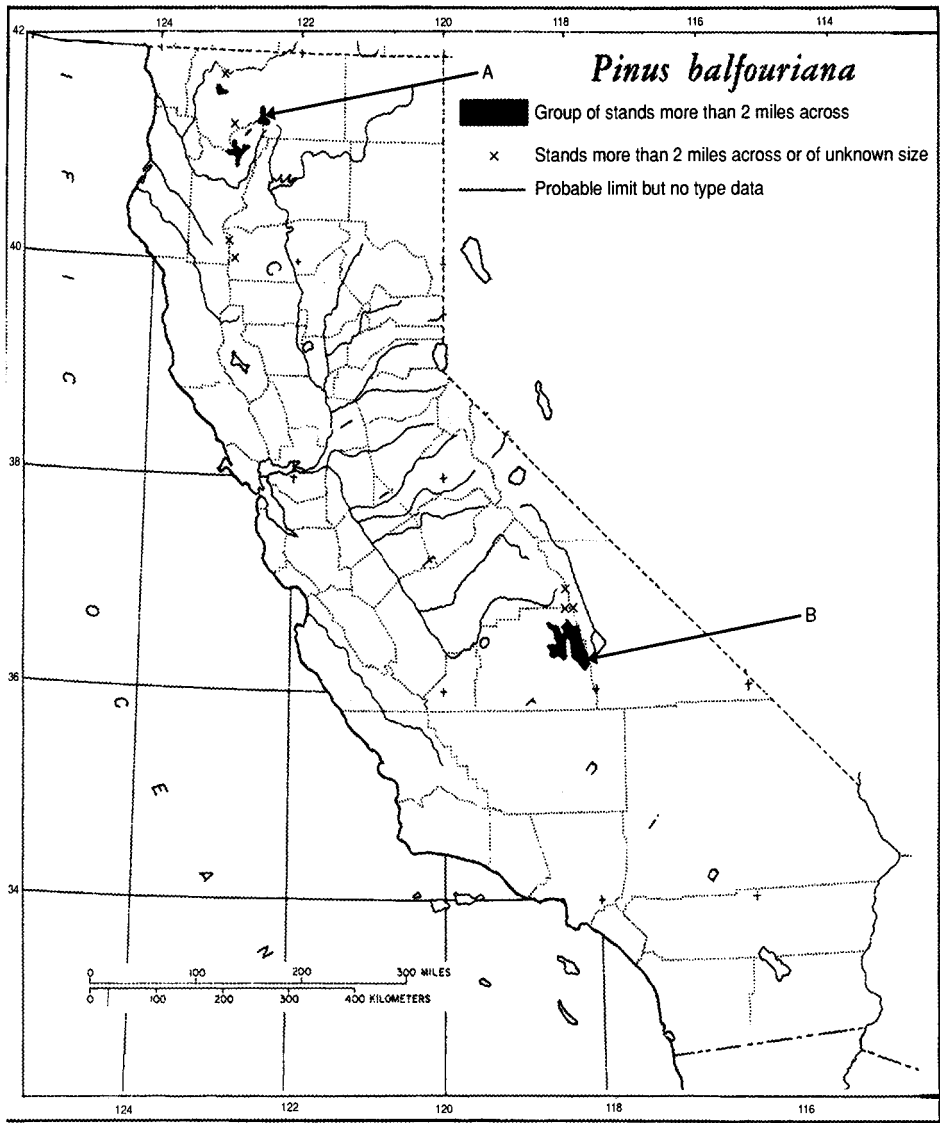


Figure 3-7—Distribution of *P. balfouriana* in California, taken from Griffin and Critchfield (1972); (A) is Klamath Mtns. population, (B) is Sierra Nevada population.

Table 3-10—Average xylem monoterpene composition of northern and southern populations of *P. balfouriana*; adapted from Mastrogiuseppe and Mastrogiuseppe (1980); number of trees sampled not given

Population	Monoterpene ¹								
	α -p	cam	β -p	3-car	myr	lira	β -ph	ter	p-cy
	-----percent ² -----								
Northern	85.4	1.0	2.2	6.2	0.6	9.3	0.2	0.9	0.2
Southern	94.8	1.0	1.9	0.1	0.4	1.2	0.1	0.2	0.4

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene, para-cymene.

² Normalized.

Figure 3-8—Distribution of *Pinus contorta* (taken from Critchfield and Little 1966) and the locations of sampled stands; see table 3-11 for specific location of stands.

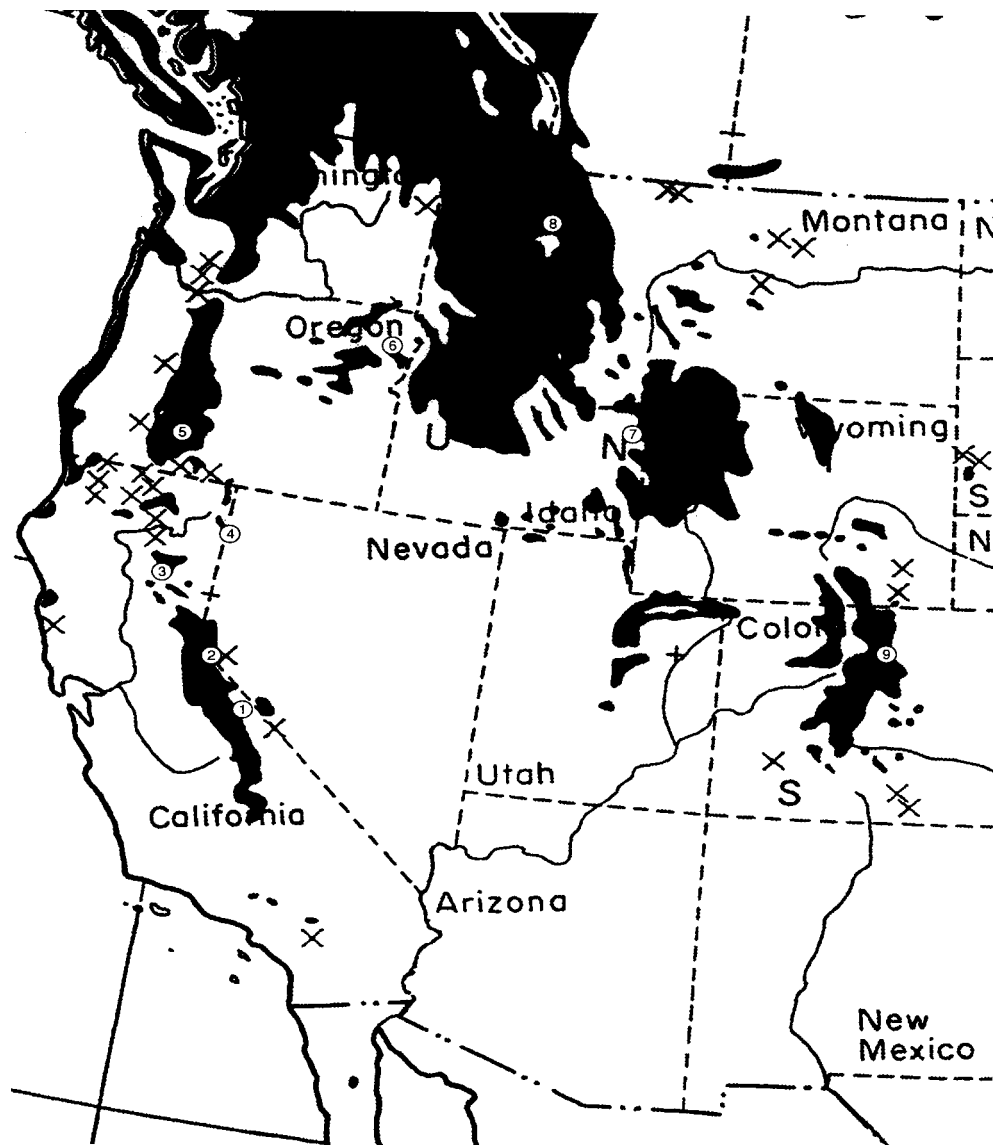


Table 3-11—Location of stands of *P. contorta*, number of trees tapped for resin, and number and percent of trees producing a sample of resin

Stand	National Forest	Locality	Trees tapped	Trees yielding resin	
			<i>number</i>	<i>number</i>	<i>percent</i>
1	Inyo, Calif.	Deadman Summit	100	87	87
2	Eldorado, Calif.	Ralston Peak	120	100	83
3	Lassen, Calif.	McCoy Flat	120	67	56
4	Modoc, Calif.	Homestead Flat	137	51	37
5	Winema, Oreg.	Buckeye Flat	140	85	61
6	Wallowa-Whitman, Oreg.	Granite Summit	56	37	66
7	Targhee, Idaho	Island Park	89	47	53
8	Flathead, Mont.	Hungry Horse	36	15	42
9	Arapaho, Colo.	Buffalo Peak	111	92	83
(Total)			909	581	(Av)64

(Av)=average

[Engelm.] Critchfield), Sierra-Cascade (var. *murrayana* [Balf.] Critchfield), Coastal (var. *contorta*), Mendocino County White Plains (var. *bolanderi* Parl. Vasey), and Del Norte County (not named).

One of the variable characteristics of *P. contorta* was the monoterpene composition of its xylem resin. Mirov (1961) reported the monoterpene fraction of the xylem resin from trees in the central Sierra Nevada to be composed almost entirely of β -phellandrene with small to trace amounts of other monoterpenes. I looked at trees in the same area and concurred with Mirov that β -phellandrene was the major monoterpene, averaging about 70 percent of the terpene fraction (Smith 1964c). I also found as much as 15 percent β -pinene and 3-carene in some trees, and rather consistent amounts—ranging from 3 to 7 percent—of α -pinene, sabinene, myrcene, and limonene in nearly all trees. In a study of *P. contorta* in different locations in the Sierra Nevada, little difference was found in average composition over the rather wide geographic region (Smith 1967b). However, a few trees were found with 3-carene greater than 20 percent. Little or no difference in composition was found between a basal tree sample and one at 20 feet (table 8-1). Zavarin and others (1969) sampled 12 trees in the Sierra Nevada and found that all but one fell within the range I reported in 1967. The one tree that was different had nearly equal amounts of 3-carene and β -phellandrene. Lotan and Joye (1970) analyzed the resin from 11 trees in southwest Montana and found a much different composition and much greater variation than that found in the Sierra Nevada. In the Montana trees, β -phellandrene ranged from 8 to 83 percent, β -pinene from 6 to 58 percent, 3-carene from 2 to almost 60 percent, and α -pinene from 4 to 50 percent.

Mirov (1956) reported that *P. contorta* and *P. banksiana* appeared to interbreed in a broad area in central Alberta, Canada, as indicated by the xylem monoterpene composition. Such trees show the monoterpene characteristics of both parents, i.e., fairly large amounts of β -pinene (*P. banksiana*) and β -phellandrene (*P. contorta*). Additional reports of introgression or hybridization of *P. contorta* and *P. banksiana* as indicated by monoterpene composition were made by Smith (1967a), Zavarin and others (1969), and Lotan and Joye (1970).

Cole and others (1981) extracted the monoterpenes from xylem of selected trees at three intervals during the most active portion of the growing season on the Cache National Forest in Utah. The proportional amount of the total monoterpenes varied somewhat, but not greatly, over the sampling period, but the normalized composition varied very little with about 14 percent β -pinene, 56 percent β -phellandrene, and 30 percent 3-carene plus myrcene and other minor components.

A Range-Wide Study

The following discussion of a range-wide study of the xylem monoterpenes is adapted from Smith (1983) with additional data. Nine nearly pure stands of *P. contorta* were sampled in five western states (fig. 3-8) (table 3-11). Stands #1 to #5 were in the region usually assigned to var. *murrayana*, and stands #6 to #9 in the region usually assigned to var. *latifolia*. No stands were in regions assigned to var. *contorta*, or var. *bolanderi*. The procedures used are those given in Chapter 2.

Quantity of Resin Flow. Resin was obtained from as many as 87 percent of the trees tapped in the Inyo stand, #1, and from as few as 37 percent of the trees tapped in the Modoc stand, #5 (table 3-11). The amount of resin that flows from each tap varied from more than 4 cc to 0 cc with many less than 0.1 cc. This variation in quantity of resin flow between trees was also found in a study of *P. ponderosa* (Smith 1977) and was studied or observed for other species of pine as well. The low flow in *P. contorta* might be attributed to cooler temperatures at the higher elevations; however, the flow of resin from *P. contorta* was much less

than that from *P. washoensis*. Forty *P. washoensis* were tapped at the same general location and at the same time as the *P. contorta* in the Modoc stand. Only 37 percent of the *P. contorta* yielded resin, whereas 98 percent of the *P. washoensis* yielded resin and the amount per tree ranged from 2 to 5 cc.

Monoterpene Components. The average for each monoterpene component in each of the nine stands showed appreciable variation between and within stands in three components: β -pinene, 3-carene, and β -phellandrene (table 3-12, fig. 3-9). All other components generally occurred at rather constant and relatively small amounts: α -pinene, 3 to 8 percent, with a few trees greater than 10 percent; camphene, less than 1 percent; sabinene, less than 2 percent; myrcene, 3 to 4 percent; α -phellandrene, less than 2 percent; and terpinolene, less than 1 percent. Limonene was almost always a minor component, ranging from 0 to 3 percent in most trees, but in a few trees it was 5 to 10 percent. There was a possible trace of α -thujene which elutes near the trailing edge of α -pinene. Lotan and Joye (1970) reported trace amounts of p-cymene, allo-ocimene, and γ -terpinene, but did not report sabinene and α -phellandrene. It is difficult to assess the value of minor components in determining differences within and between species and varieties. Such differences could be real or products of sampling and analysis. It is possible that the genetic structure for β -phellandrene is the same in all trees, very likely two additive alleles at a single locus. It is further possible that most of the variation is caused by differences in the genetic structure for β -pinene and 3-carene, by combinations of the two, and by other modifying factors such as isomerization.

Stands #1 to #5, in areas assigned to var. *murrayana*, were quite similar in average stand composition (table 3-12); but stand #9, in an area assigned to var. *latifolia*, was similar to stands #1 to #5 and to those described by Cole and others (1981). Therefore, average stand composition did not follow the variety assignment.

Stands #6 to #8, in areas assigned to var. *latifolia*, differed from each other appreciably in average stand composition; they also differed greatly from stands #1 to #5 and stand #9. Again, average composition did not parallel variety assignment. As one proceeds from stands #6 to #7 to #8, β -pinene increased and β -phellandrene decreased. No sharp gradations are discernible in stands #1 to #5.

Composition Types

The frequency distribution of β -pinene, 3-carene, and β -phellandrene (fig. 3-9), had a tendency to be trimodal, and three levels of the percentages were assigned (table 3-13). When actual composition was converted to coded composition, using prescribed intervals (table 3-13), 15 composition types were defined (table 3-14). The coefficients of association (table 3-15) were calculated from the frequency of composition types (fig. 3-10). These coefficients clearly fell into three groups.

- A southwestern type was composed of seven stands; five of these—Inyo, Eldorado, Lassen, Modoc, Winema—were in the Sierra Nevada-Cascade and were in the region assigned to var. *murrayana*. The other two stands that have a strong association with the southwestern stands—Wallowa-Whitman and Arapaho—were in quite different parts of the Rocky Mountains, a region usually assigned to var. *latifolia*. All seven stands were closely aligned by average composition (table 3-12) by types of composition (fig. 3-10), and by coefficients of association (table 3-15). The Wallowa-Whitman stand did appear to be slightly transitional between the southwestern and central type. It had strong coefficients of association with the Winema and Arapaho stands, but associations with the other southwestern stands were only moderate.

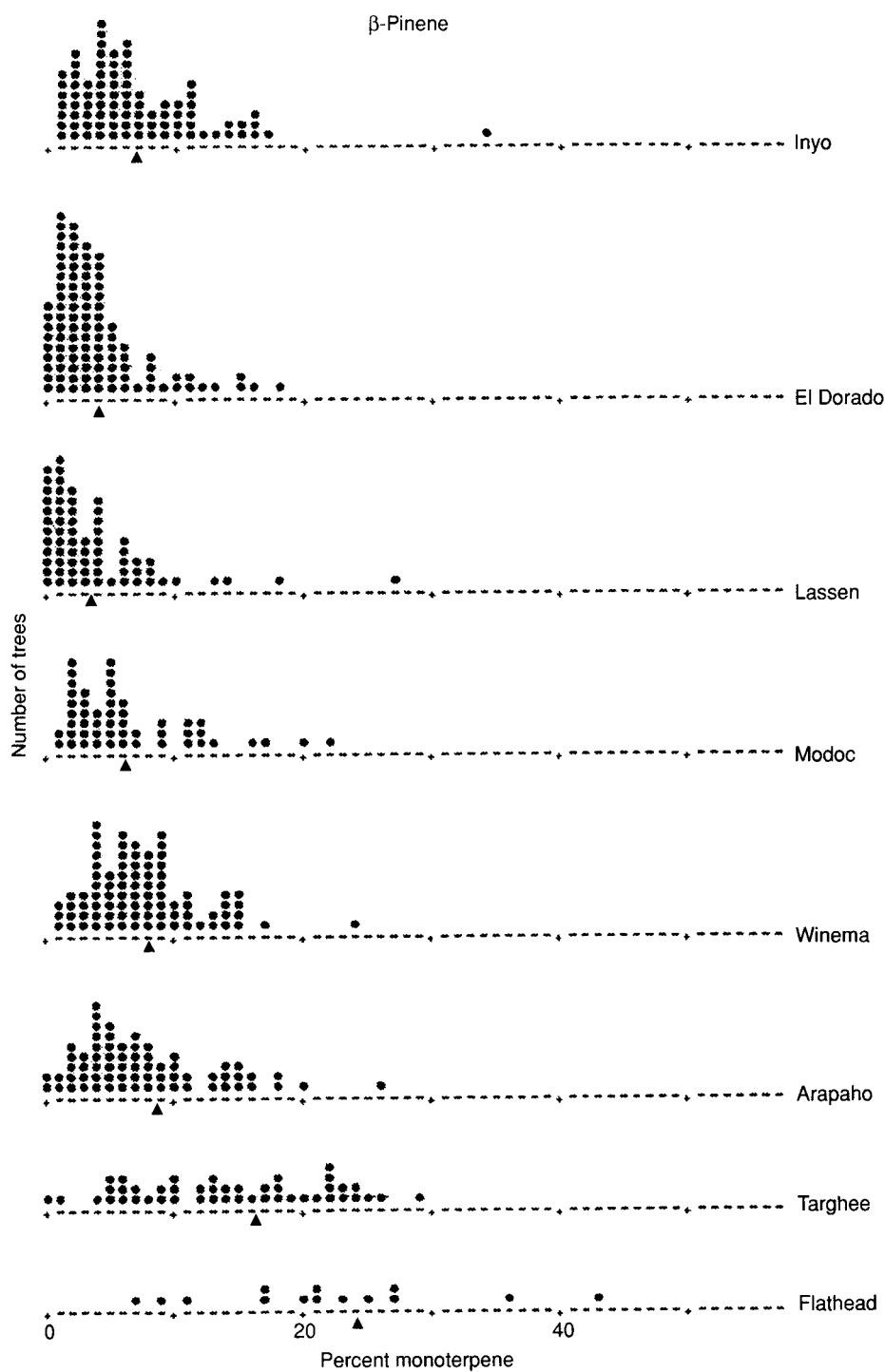
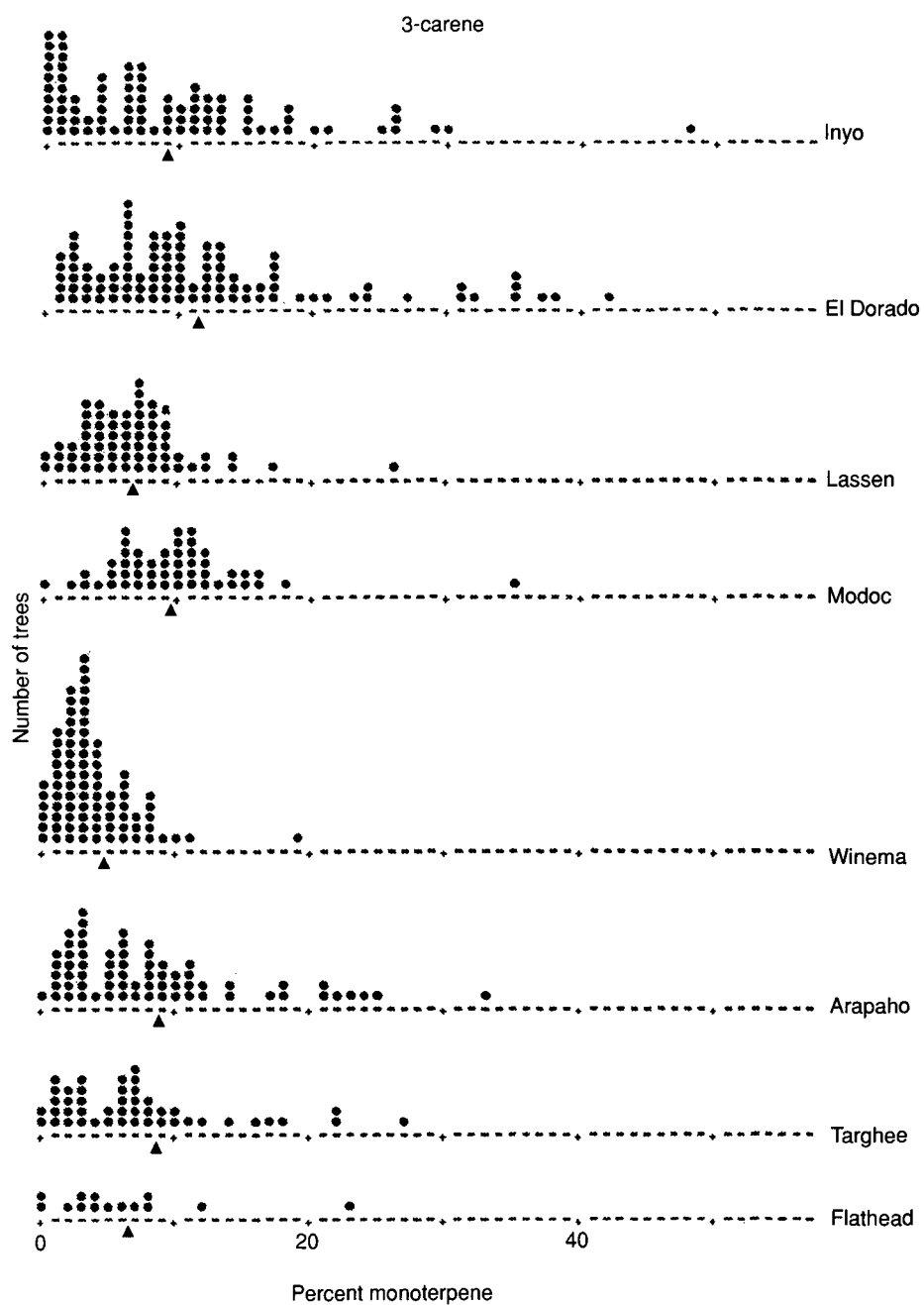


Figure 3-9—Frequency distribution of the amount of three xylem monoterpenes of *P. contorta* in stands in the eight listed National Forests; each dot is measurement of one tree; ▲ is average of set.

(fig. 3-9 continues on next 2 pages)



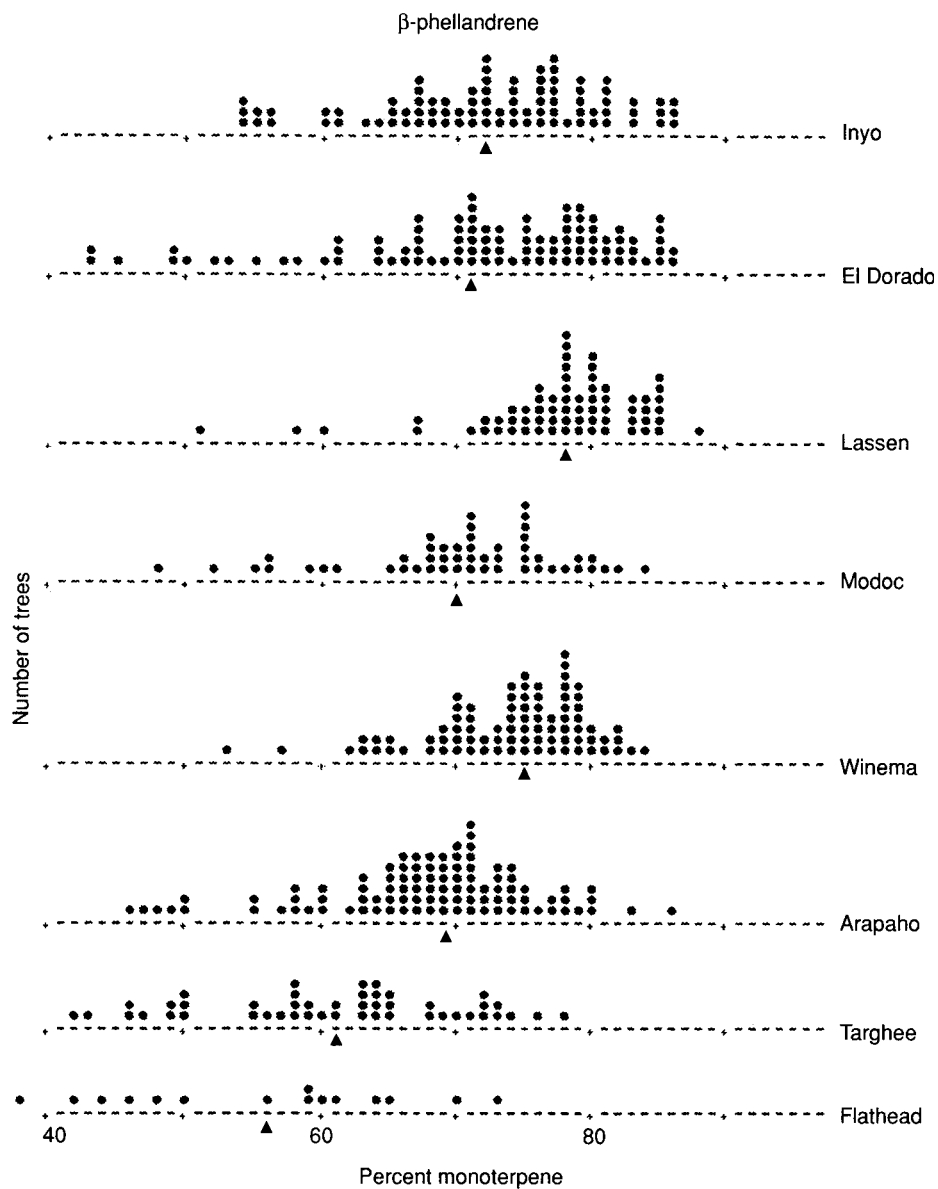


Figure 3-10—Distribution of 15 types of resin composition (numbered) in stands of *P. contorta* in the eight listed National Forests; distribution was normalized for each stand, see Table 3-14 for approximate composition.

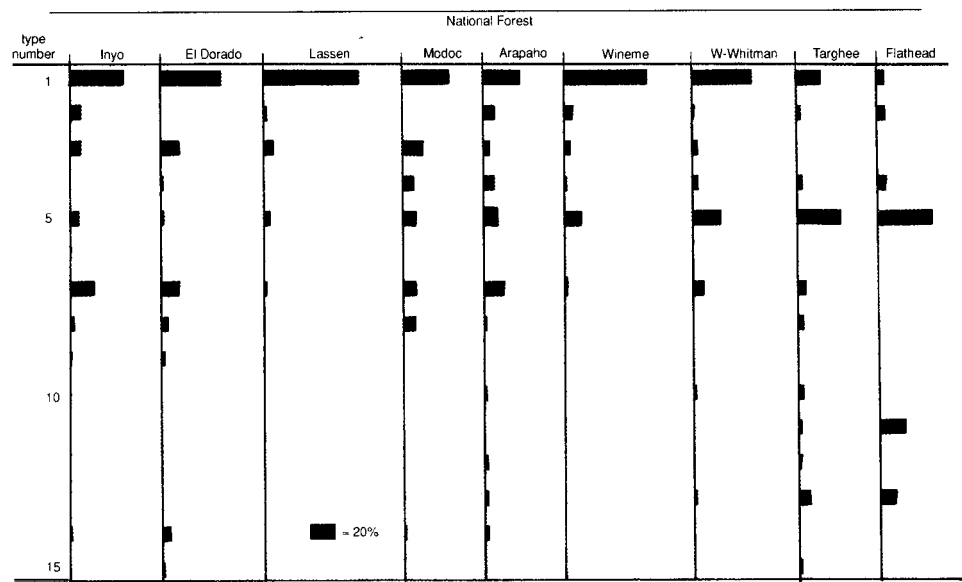


Table 3-12—Average monoterpene composition of stands of *P. contorta* on nine national forests; see Figure 3-8 and Table 3-11 for stand information

Stand Trees		National Forest ²	Monoterpene ¹							
			α -p	β -p	car	sab	myr	lim	β -ph	ter
----number----			-----percent ³ -----							
1	87	Inyo	5.2	6.8	8.8	1.6	3.7	1.5	71.9	*
2	100	Eld	4.7	3.9	11.7	2.2	3.6	1.4	71.1	*
3	67	Las	4.8	3.8	6.0	1.8	3.9	1.0	77.7	*
5	85	Win	5.7	7.5	4.6	2.2	3.6	1.2	75.1	*
4	51	Mod	4.7	6.3	9.5	1.5	3.8	2.1	70.3	*
9	92	Ara	5.6	8.7	9.7	2.5	1.9	1.6	69.2	0.3
6	37	W-W	5.9	10.7	7.4	1.7	3.4	1.5	66.3	*
7	47	Tar	6.5	19.8	8.7	1.2	3.3	2.9	61.0	*
8	15	Fla	7.2	22.4	6.6	1.6	3.1	1.4	56.7	*

¹ α -pinene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, terpinolene.
² Inyo, Eldorado, Lassen, Winema, Modoc, Arapho, Wallowa-Whitman, Targhee, Flathead.
³ Normalized; * = trace; also trace amounts of α -phellandrene, α -thujene, γ -terpinene in many trees.

Table 3-13—Percent intervals of individual monoterpenes of *P. contorta* used to define the low, medium, and high mode of the frequency distribution of three monoterpenes, taken from Smith (1983)

Component	Code value		
	0	1	2
-----Intervals in percent-----			
β -Pinene	0-10	10.1-30	30.1-100
3-Carene	0-10	10.1-30	30.1-100
β -Phellandrene	0-52	52.1-70	70.1-100

Table 3-14—Fifteen coded types of composition of *P. contorta* and their actual average composition, in nine stands in nine National Forests' 1 in western United States, partially adapted from Smith (1983)

Composition Coded		Composition ² Actual		
Number		----- percent ³ -----		
1	002	5	8	73
2	102	13	2	73
3	012	4	13	72
4	001	9	9	67
5	101	19	1	66
6	201	33	1	54
7	011	6	22	59
8	111	17	12	59
9	021	1	35	52
10	100	25	7	49
11	200	43	2	43
12	010	9	27	47
13	110	18	25	47
14	020	1	42	45
15	120	15	31	43

¹ Winema, Lassen, Modoc, Eldorado, Inyo, Wallowa-Whitman, Arapaho, Targhee, Flathead.

² From left to right: β -pinene, 3-carene, β -phellandrene; minor components omitted.

³ Normalized for each composition; minor components comprise the remainder of the 100 pct; see table 3-12 for approximate amounts. Normalized for each plot.

Table 3-15—Coefficients of association between stands of *P. contorta* based on types of monoterpene composition

Stand number ¹ and National Forest								
	Eld	Las	Mod	Win	W-W	Ara	Tar	Fh
1 Inyo	.81	.72	.58	.67	.67	.84	.03	.00
2 Eldorado		.83	.74	.69	.53	.69	.01	.00
3 Lassen			.66	.94	.68	.67	.01	.00
4 Modoc				.58	.49	.71	.03	.00
5 Winema					.81	.73	.05	.00
6 Wallowa-Whitman						.86	.22	.00
9 Arapaho							.38	.05
7 Targhee								.46
8 Flathead								

¹ See figure 3-8 for locations of stands.

stand was poorly associated with stands in the southwestern type and with the northeastern type, though it was associated more strongly with the northeastern type. The stand thus appeared to be transitional.

- A northeastern type was represented by only one stand, the Flathead, which was poorly associated with the Targhee stand, and was not associated at all with the southwestern stands. The Targhee and the Flathead stands had much of the same types of composition but differed in their frequencies.

The southwestern region was characterized by the 002 composition type. This type was also common in the central region, but it was not the common type in the northeastern region, though it did occur there, but not commonly. The 101 type was most common in the northeastern region, fairly common in the central region, and only moderately common in the southwestern region. The 110 type was fairly common in the northeastern region, rare in the central region, and absent in the southwestern region. The 012 type was fairly common in the southwestern and central regions but was absent in the northeastern region.

Summary

P. contorta was sampled for xylem monoterpenes in areas assigned to var. *murrayana* and var. *latifolia*; areas assigned to other varieties were not sampled. There was considerable local variation in all stands, particularly in α -pinene, β -pinene, 3-carene, and β -phellandrene. All stands from the region assigned to var. *murrayana* had considerable local variation. However, they were similar in average composition and were strongly associated by types of composition. Much like *P. ponderosa*, as will be shown later in this chapter, there was surprising strong association between stands where local variation was large and where one would expect greater differences. Stands from the region assigned to var. *latifolia* were not similar in average composition and were not strongly associated by types of composition. Stands in northern Montana and southeastern Idaho differed sharply from the var. *murrayana* stands both in average composition and in types of composition; but the two stands also differed sharply from each other. However, stands in northern Colorado and eastern Oregon were most strongly associated with the typical var. *murrayana* stands, though they tended to be slightly intermediate between the var. *murrayana* stands and the other two var. *latifolia* stands. Limonene was about 2 percent in all stands and had only small variation. If the increase in β -pinene was indicative of the influence of *P. banksiana* on *P. contorta*, then the influence of *P. banksiana* extends far beyond the zone of introgression in Alberta, Canada, as proposed by Mirov (1956).

#6. *Pinus coulteri* D. Don (Coulter pine)

P. coulteri is limited to the coastal mountains of California from Mt. Diablo in the north to the northwest portion of Baja California in the south (fig. 3-11). It often occurs with *P. jeffreyi* in southern California with which it can hybridize naturally; but natural hybrids are rare. Smith (1977) reported a possible hybrid of *P. coulteri* and *P. ponderosa* in the San Bernardino National Forest. *P. coulteri* and the natural hybrid of Jeffrey x Coulter were heavily used in the breeding program at the Institute of Forest Genetics (IFG) from about 1940 to 1970 because *P. coulteri* grows well under dry conditions and has a good growth rate. However, the tree generally has heavy limbs and sometimes poor commercial form. *P. coulteri* is one of the three big-cone pines in California; the other two are *P. sabiniana* and *P. torreyana*.

Mirov (1961) analyzed trees growing at IFG for terpenes and reported the xylem volatiles to be about 5 percent heptane, 33 percent α -pinene, 33 percent β -phellandrene, and 10 percent undecane. Smith (1967b) analyzed 20

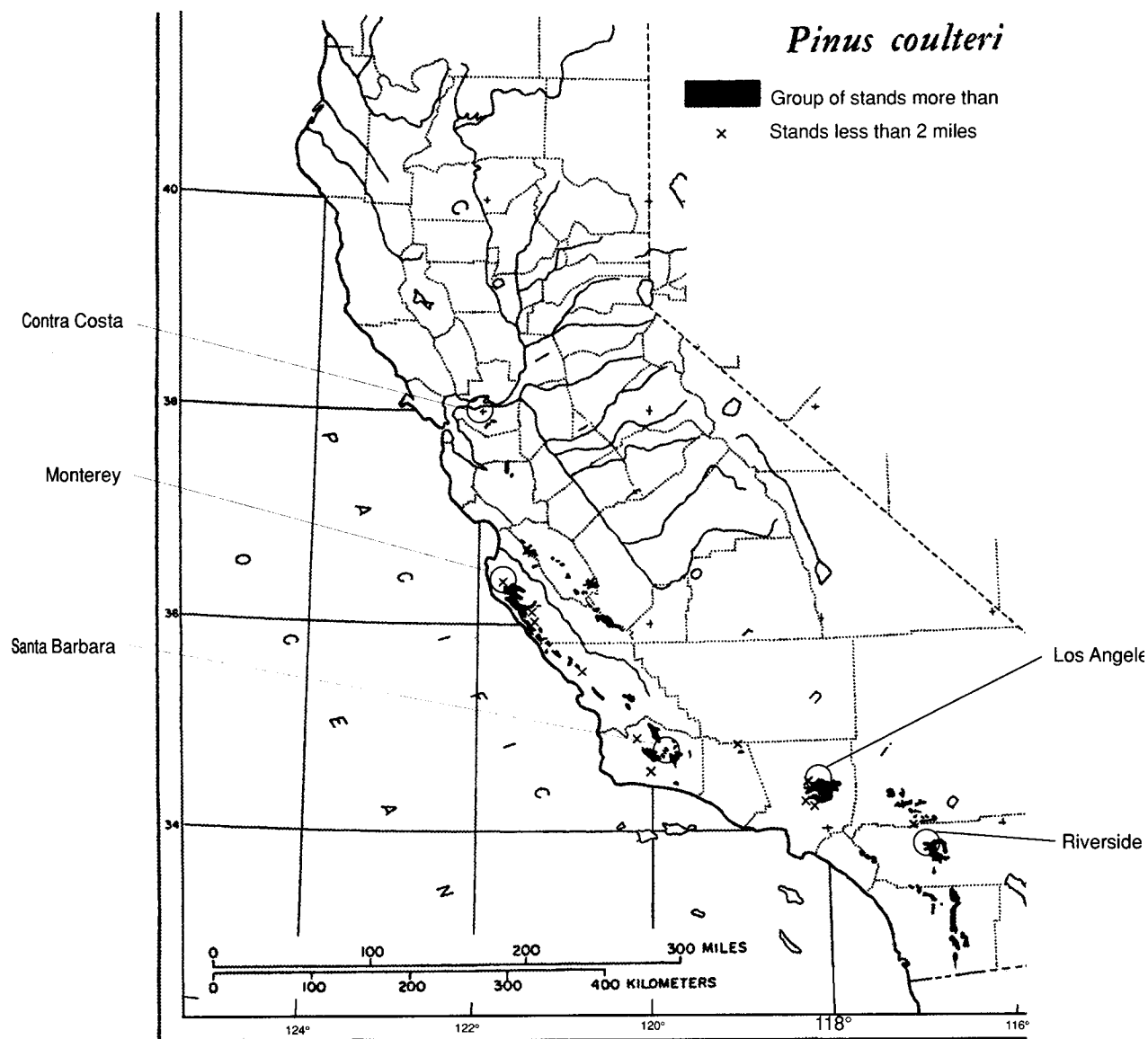


Figure 3-11—Distribution of *P. coulteri* in California (taken from Griffin and Critchfield 1972) and the county of location of stands used for sampling mature trees.

trees at IFG and found a considerable range in α -pinene, myrcene and β -phellandrene with measurable amounts of several other monoterpenes as well as heptane (table 3-16). Little or no difference in composition was found between a basal tree sample and one at 20 feet (see table 8-1).

More recently, I examined two sets of populations. One set was 64 trees, greater than 40 years old, in nine natural stands from throughout the range; the other set was 105 2-year-old seedlings, growing at IFG, from three sources: Mt. Diablo, Figueroa Mountain, and San Diego County. There were 35 trees from each of the three sources. This is one of the few times that very young trees are reported in the 19 pines of California.

The results of the analysis of the 64 trees from natural stands (table 3-17) showed an average composition fairly similar to the earlier results (Smith 1967b). The variation and the small numbers of trees at the nine locations prohibited any firm conclusion about local and regional characteristics. All localities had considerable variation in α -pinene, myrcene, and β -phellandrene, and there was considerable variation in composition among the trees (table 3-18).

The only obvious regional difference was generally higher levels of α -pinene and sabinene in the north along with lower β -phellandrene. However, larger sampling was needed. The frequency distribution of the aggregate of 64 trees showed large variation in several components and suggested a trimodal distribution for α -pinene, undecane, myrcene, limonene, and β -phellandrene (fig. 3-12). But the limited data did not permit an analysis of types of composition.

The composition of *P. coulteri* was distinctive in having large amounts of α -pinene, myrcene, and β -phellandrene; as noted earlier, the largest amounts of myrcene found in pine were found in *P. albicaulis*. I know of no other pine in the United States which had such a distinctive composition in its xylem resin as *P. coulteri*. It did, however, resemble some sources of *P. patula* in Mexico (see Chapter 4, #70). In some respects this composition might suggest that *P. coulteri* was a link between *P. ponderosa* and the big-cone pines.

The analysis of the 2-year-old seedlings gave quite different and surprising results (table 3-19). Although the averages of these three plots did not differ greatly in composition, they differed greatly from the composition of the old, naturally growing trees (tables 3-16 and 3-17). There was a very strong juvenile effect, because the amounts of several monoterpenes were quite different in young seedlings than in mature trees: heptane, nonane, undecane, camphene, sabinene, myrcene, limonene, and β -phellandrene were much higher in mature trees than in 2-year-old seedlings, while α -pinene, β -pinene, and 3-carene were much lower in mature trees. As will be shown later in Chapter 5, this same general condition was found at a much lesser degree when comparing young and old *P. ponderosa* and has been called the juvenile factor. One can only speculate that the increase in minor components like camphene was a reflection of cumulative isomerization with age. The large differences in major components like myrcene and β -phellandrene could be caused by either a strong juvenile effect or changes in the gene-controlled synthesis or both, though large-scale, long-term isomerization was a possibility.

The frequency distribution of the three nursery sets of 2-year-old seedlings indicated a trimodal distribution for α -pinene, myrcene, and β -phellandrene (fig. 3-13). Though this condition usually warranted coding and tree-type classification, as well as distribution, the presence of such a strong, and presumably temporary, juvenile effect would not yield meaningful results.

In summary, and based on limited local sampling, local populations of mature *P. coulteri* varied considerably in α -pinene, myrcene, and β -phellandrene. However, there was no strong suggestion of sharp regional

Table 3-16—Average ($\bar{X}$) xylem monoterpene composition of 20 *P. coulteri* growing at the Institute of Forest Genetics (IFG) [taken from Smith (1967b)], and composition of individual trees selected to illustrate the range of composition

Source of trees ²	N	Monoterpene ¹											
		hep	non	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
		-----percent ³ -----											
($\bar{X}$)	20	1.3	1.3	36.5	2.0	1.9	2.1	0.5	1.6	20.3	3.8	28.1	0.3
17	1	0.6	0.9	25.2	*	2.2	2.5	1.1	1.8	41.4	4.4	19.9	
17	1	5.9	3.2	32.4	*	5.7	1.9	0.3	0.3	16.2	3.0	29.4	0
18	1	0.8	1.1	51.0	*	1.9	1.9	0.2	5.3	5.7	4.8	26.0	1.3
18	1	1.9	1.7	33.2	3.8	0.2	1.7	0.5	7.6	15.9	2.6	26.8	4.0
19	1	0.6	1.2	29.9	3.6	0.9	2.1	0.6	0.9	16.5	9.0	34.7	

¹ Heptane, nonane, α -pinene, undecane, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, terpinolene.

² 17 = Los Angeles County; 18 = Mount Diablo; 19 = unknown; X = all sources.

³ Normalized; * = trace.

Table 3-17—Average xylem monoterpene composition of 9 stands of *P. coulteri* in California, the general average ($\bar{X}$) for all stands totalling 64 trees, and the maximum and minimum for the aggregate of all trees

County	Trees no.	Monoterpene ¹											
		hep	non	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
		-----percent 2-----											
Contra Costa	3	1.7	1.4	41.5	2.7	0.8	1.9	0.2	4.8	14.2	3.8	25.1	0.4
Monterey	4	*	1.3	29.5	*	5.4	0.9	0	1.2	20.0	5.8	34.6	0.8
Santa Barbara	5	1.8	1.1	37.2	*	4.4	1.1	*	0.4	18.7	5.1	30.0	*
Los Angeles	4	2.6	1.8	28.4	1.2	3.3	1.9	0.5	0.8	29.9	3.3	26.1	0
Riverside	15	*	1.3	23.1	*	5.2	*	0	0.5	20.6	6.6	41.3	0.8
San Diego	9	*	1.2	26.2	*	4.8	0.9	0	0.5	19.2	4.4	42.4	0.2
San Diego	7	1.1	1.4	40.0	3.2	1.0	2.2	0.4	1.3	18.0	3.4	27.2	0.5
Unknown	6	0.9	1.0	31.5	1.3	2.7	1.9	0.7	1.0	22.7	4.9	31.7	0
Several ³	11	*	1.0	28.7	0	4.5	1.4	0	1.4	22.0	5.5	34.4	1.0
($\bar{X}$)	(64)	0.6	1.2	29.8	1.7	1.4	1.4	0.2	1.0	20.7	5.0	34.8	0.4
Max		6	4	51	8	6	3	2	6	42	19	59	
Min		0	0	12	0	0	0	0	0	0	1	20	

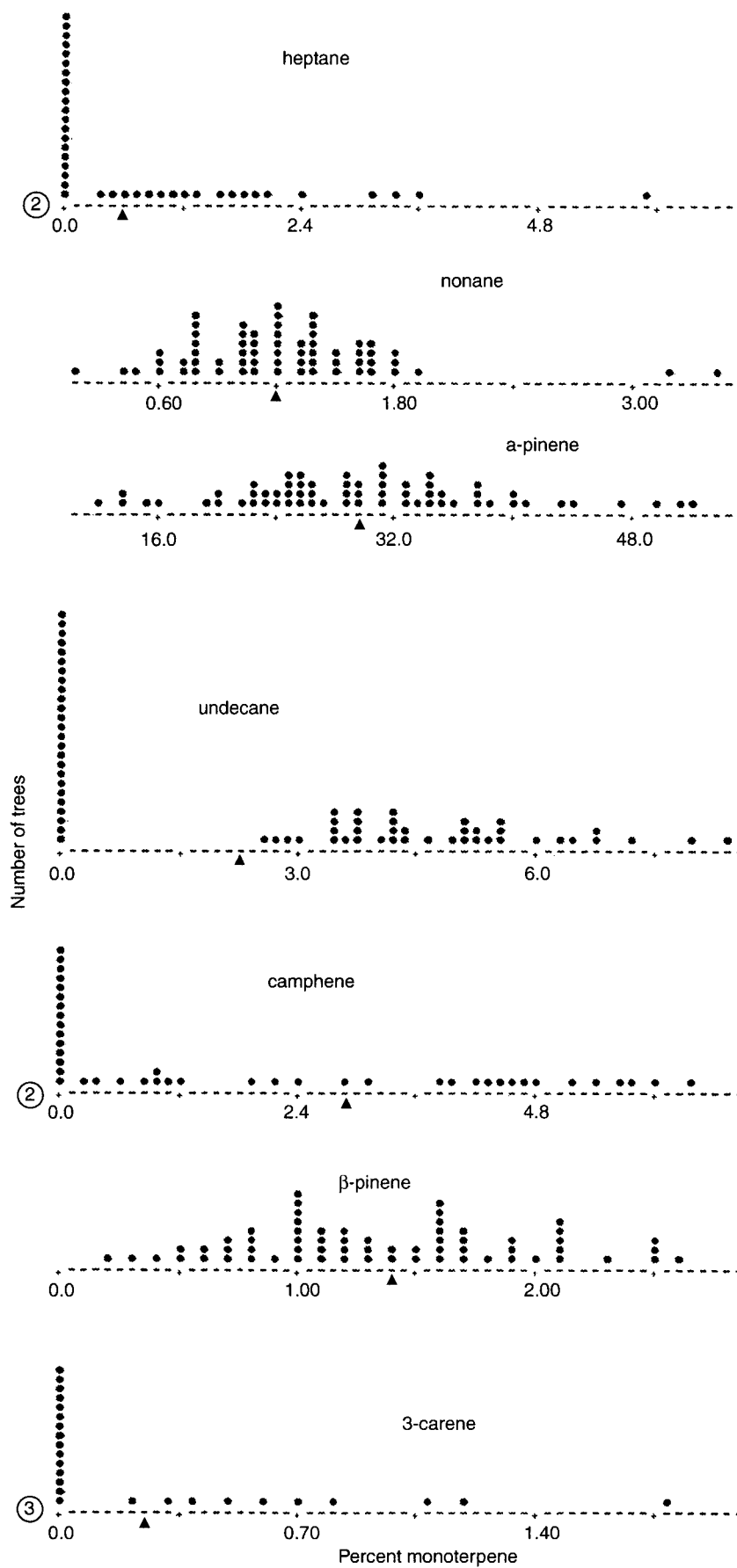
¹ Heptane, nonane, α -pinene, undecane, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, terpinolene.

² Normalized; * = trace.

³ These trees are growing in an Institute of Forest Genetics (IFG) arboretum from seed collected from unknown sources.

Figure 3-12—Frequency distribution of the amount of the noted monoterpenes in the aggregate of 64 mature trees in nine stands of *P. coulteri*; each dot is measurement of one tree except where (n) indicates each represents *n* trees; ▲ is average of set.

(continues on next page).



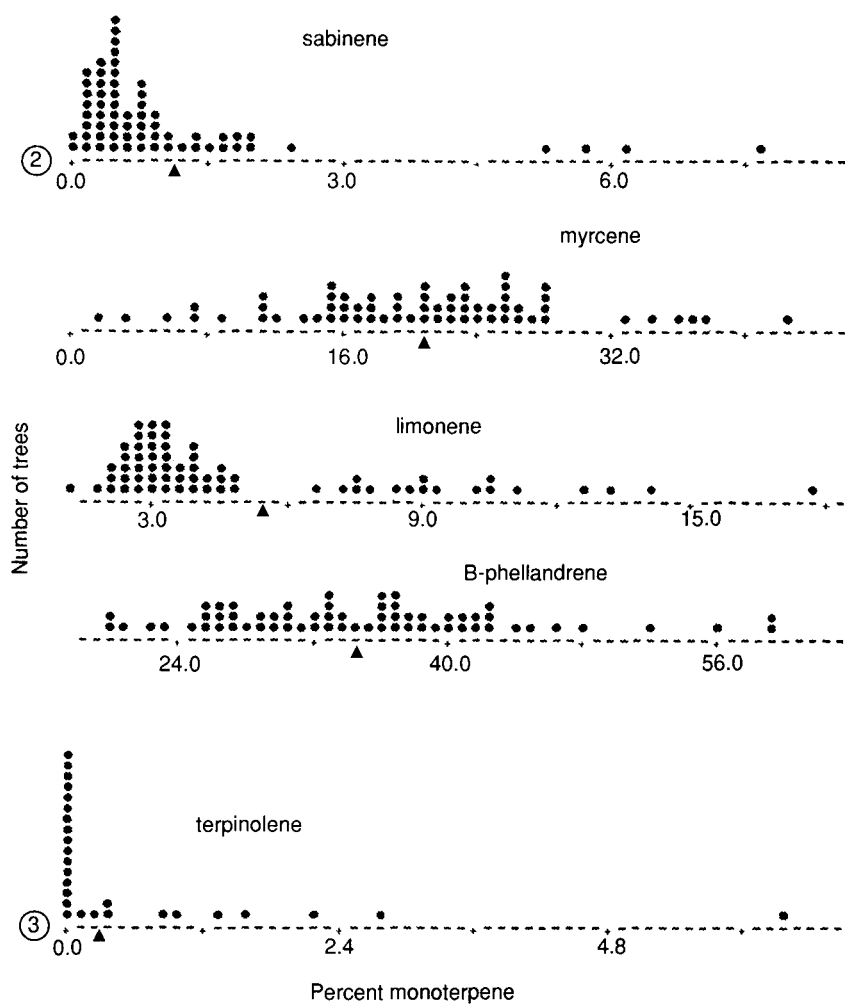


Table 3-18—Xylem monoterpene composition of individual *P. coulteri* selected to show the range in composition in California

County source	N	Monoterpene ¹											
		hep	mon	α -p	und	cam	β -p	3-car	sab	myr	lim	β -ph	ter
-----percent ² -----													
Contra Costa	1	0.8	1.1	51.0	-	1.9	1.9	0.2	5.3	5.7	4.8	26.0	1.3
Contra Costa	1	2.4	1.5	40.3	4.3	0.4	2.1	*	1.5	21.0	3.9	22.5	*
Los Angeles	1	0.6	1.3	26.8	-	4.4	1.7	0.3	0.5	37.9	3.4	22.8	-
San Diego	1	*	0.6	23.6	5.1	*	1.2	*	0.6	24.2	2.6	42.1	-
Contra Costa	1	*	0.8	40.7	4.4	*	1.6	*	2.4	6.9	4.5	36.4	-
Unknown	1	1.5	1.5	52.2	4.0	*	1.2	-	0.3	3.4	2.0	33.6	*
Unknown	1	*	1.7	11.8	5.1	*	0.6	-	0.7	17.3	2.8	59.2	*
Unknown	1	*	0.4	19.4	5.4	-	0.4	-	0.6	25.9	14.2	33.9	*
Unknown	1	*	1.9	15.5	4.4	-	0.5	-	0.7	33.0	3.0	40.0	*

¹ Heptane, nonane, α -pinene, undecane, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, terpinolene.

² Normalized; * = trace, - = zero.

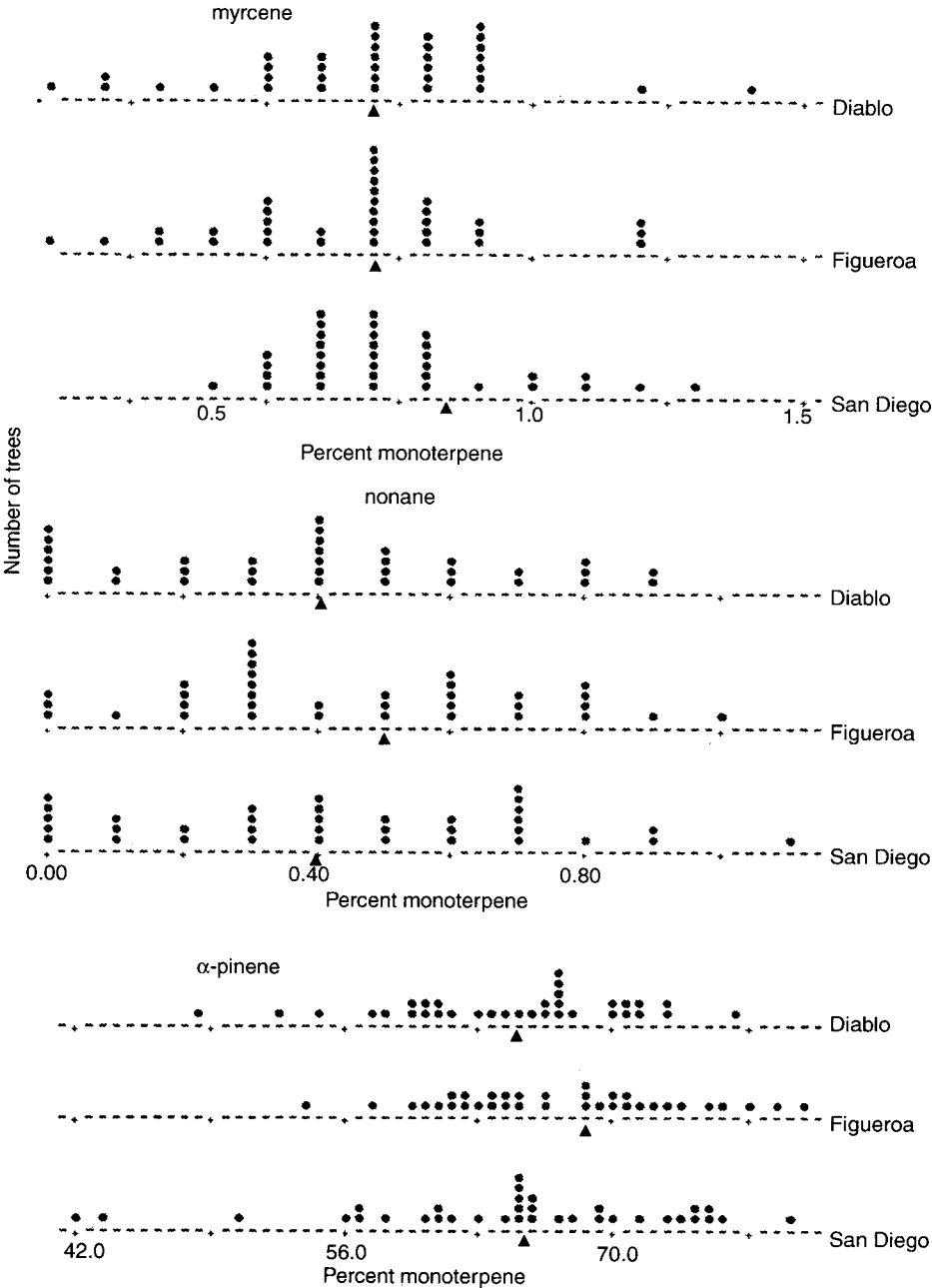
Table 3-19—Average xylem monoterpene composition of three sets of 2-year-old nursery stock of *P. coulteri*, of the aggregate of the three sets, and of the aggregate of 64 trees in nine older stands

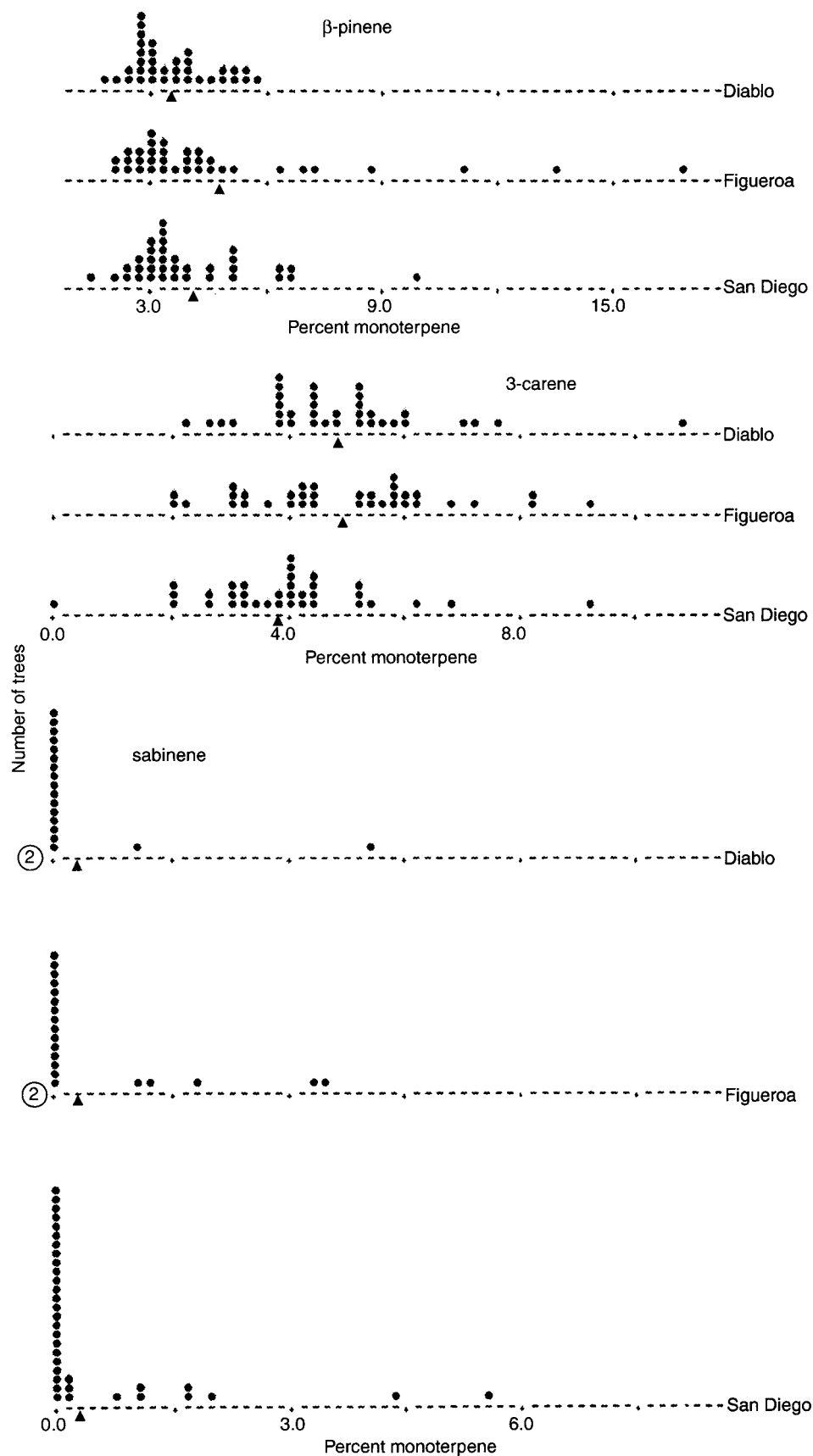
Source ²	N	Monoterpene ¹											
		hep	non	α-p	und	cam	β-p	car	sab	myr	lim	β-ph	ter
		percent ³											
1	35	*	0.4	64.7	0	0.2	3.5	4.8	0.4	0.7	0.8	24.1	0.3
2	35	*	0.5	67.3	0	0.1	4.7	4.9	0.3	0.7	0.8	20.4	0.4
3	35	*	0.4	64.8	0	0.1	3.9	3.9	0.5	0.8	1.1	24.0	0.3
4	105	*	0.4	65.6	0	0.1	4.1	4.5	0.4	0.7	0.9	22.8	0.4
5	64	1.6	1.2	29.8	1.7	1.4	1.4	0.2	1.0	20.7	5.0	34.8	0.4

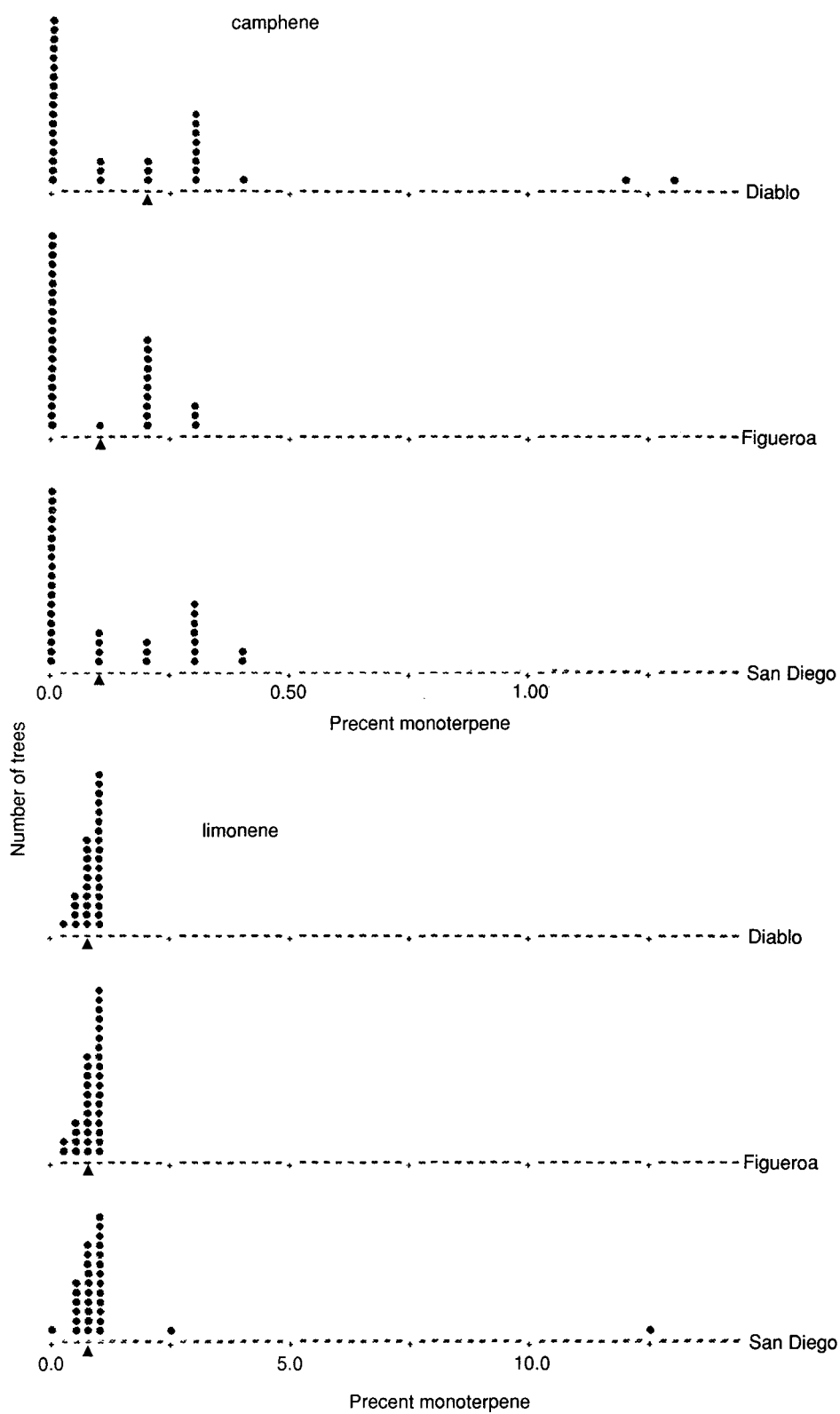
¹ Heptane, nonane, α-pinene, undecane, camphene, β-pinene, 3-carene, sabinene, myrcene, limonene, β-phellandrene, terpinolene.
² 1 = Mount Diablo, 2 = Figueroa Mtn., 3 = San Diego County, 4 = 1+2+3, 5 = average of 64 mature trees from nine stands
³ Normalized; * = trace..

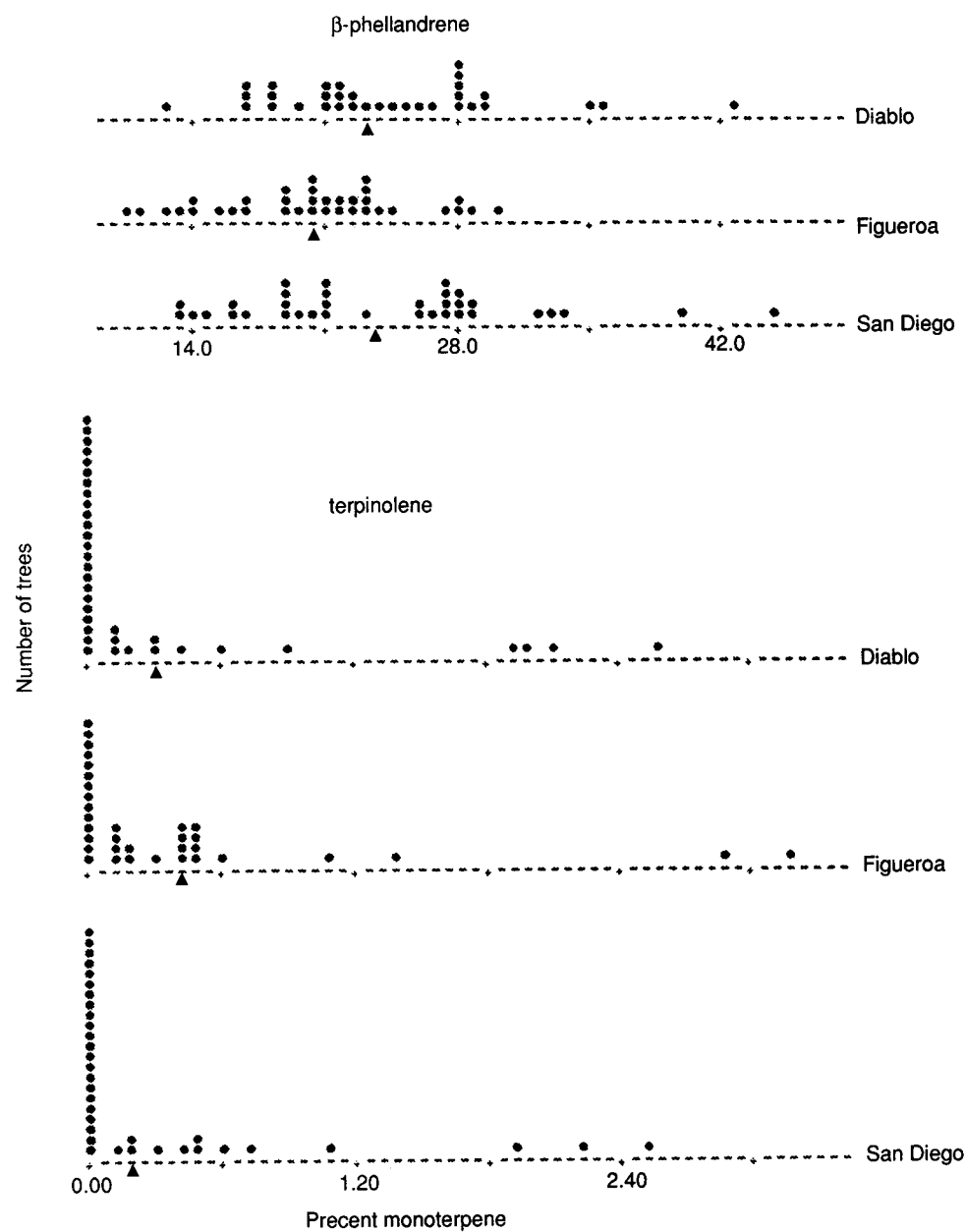
Figure 3-13—Frequency distribution of the amount of the noted monoterpenes in three sets of 2- year-old seedlings of *P. coulteri* of seed collected at the three locations listed on the right; each dot is measurement of one tree except where (n) indicates each represents n trees; ▲ is average of set.

(fig. 3-13 continues on next 3 pages).









- A central type was represented by only one stand, the Targhee. This differences. Examination of 2-year-old seedlings showed a very strong juvenile effect. However, all data suggested gene control of α -pinene, myrcene, and b-phellandrene. The small amounts of the other monoterpenes and the related hydrocarbons heptane and undecane were probably not controlled by genes but were products of isomerization or were by-products of biosynthesis. The monoterpene composition of *P. coulteri* was totally different from that of the other two big-cone pines, *P. torreyana* and *P. sabiniana*; both are discussed later in this chapter.

#7. *Pinus edulis* Engelm. (Colorado pinyon pine)

P. edulis is widely distributed in the southern Rocky Mountains, primarily in Utah, Colorado, Arizona, and New Mexico (fig. 3-14). In California, an isolated stand is located in the New York Mountains on the eastern edge of the Mojave Desert. The identity of this stand of *P. edulis* in California was uncertain for a long time, but quite recently it was determined to be *P. edulis* (Des Lauriers and Ikeda 1986). Des Lauriers and Ikeda found—both by number of needles per fascicle and number of resin canals—that the trees at the highest elevation in the New York Mountains are *P. edulis*; those at the lowest elevation are *P. monophylla*; and those at intermediate elevation could be called a hybrid of the two species. Des Lauriers and Ikeda did not analyze xylem monoterpenes of any of the three groups of trees. Thus, *P. edulis* was found in California, albeit in one isolated and restricted locality in the Mojave Desert.

Mirov (1961) reported the monoterpene portion of xylem resin of *P. edulis* to be 81 percent α -pinene, 4 percent myrcene, 11 percent 3-carene, 4 percent limonene, 4 percent terpinolene, 4 percent an unknown monoterpene, and a substantial amount of ethyl caprylate. He attributes part of the fragrance of pinyon resin to ethyl caprylate.

Snajberk and Zavarin (1975) analyzed the resin of trees from New Mexico and Utah and found the monoterpene composition to be very much like that described in Mirov's report. In addition, they reported small to trace amounts of camphene, sabinene, β -pinene, and ocimene.

More recently Zavarin and others (1989) made a more thorough study of the full range *P. edulis*. Eighteen stands of about 10 trees each were sampled in Utah, Colorado, New Mexico, Texas, and Arizona. Through the use of dendrograms, Zavarin and others (1989) divided *P. edulis* into two forms: a southern form in southeastern Arizona and southwestern New Mexico, called Apache, typified by plot #34 in Grant County, New Mexico (table 3-20), and a northern form in the major portion of the tree's range north of the southern form. The northern form is typified by stand #14 (table 3-20). Zavarin and others (1989) also listed the frequency distribution for some of the individual components.

From the range and average values (table 3-20) and from the frequency distribution of individual components, it seemed that a sample larger than 10 trees per stand was needed to characterize the monoterpene composition of *P. edulis*. When the frequencies of individual tree measurements were examined (Zavarin and others 1989), it appeared that camphene, α -pinene, and limonene were generally higher in the northern form, and sabinene and 3-carene were generally higher in the southern form; however, there was a very strong overlap in the occurrence of individual components in the northern and southern forms. It would be interesting to see what the frequency distribution would be for a 50- to 100-tree stand in each of the two areas of the northern and southern forms.

Judging by the multimodal frequency distribution and by the maximum and minimum values of individual components given by Zavarin and others (1989), at least three components seemed to be controlled by genes: α -pinene, 3-carene, and sabinene. Again, larger plots would help gain a better understanding of local variation.

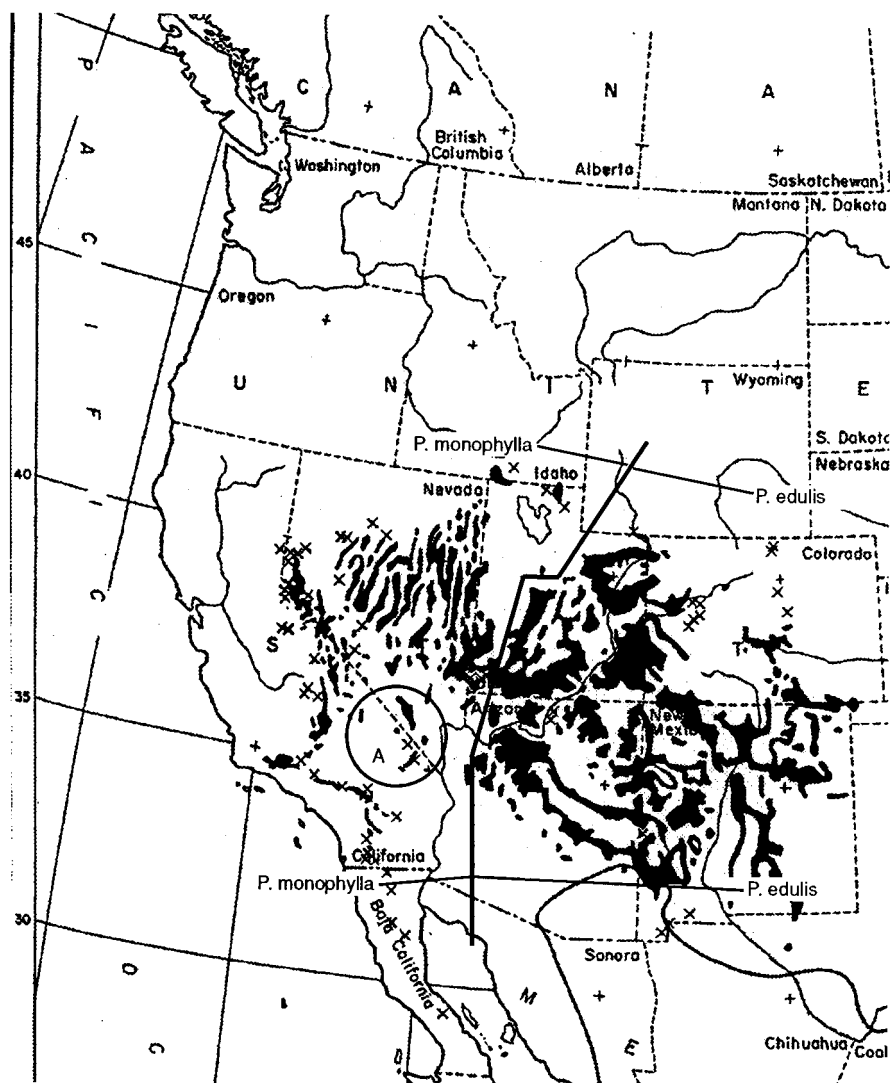


Figure 3-14—Distribution of *P. monophylla* and *P. edulis* (taken from Critchfield and Little 1966); (A) is location of *P. edulis* in California.

Table 3-20—Average xylem monoterpene composition of six stands of *P. edulis* selected from 18 stands reported by Zavarin and others (1989), and the maximum and minimum values of the more variable components; N = number of trees

Stand No.	Locality county/state	Monoterpene ¹												
		N	α -p	cam	β -p	car	sab	myr	lim	β -ph	γ -ter	oci	ter	cy
-----percent ² -----														
9	Navajo, AZ	3	80.8	5.3	2.3	5.1	0.2	1.1	2.5	0.5	*	0.2	0.1	1.2
14	Chafee, CO	10	75.9	0.8	0.8	6.1	0.3	1.2	5.2	0.3	5.7	0.1	2.7	1.0
20	Coconino, AZ	10	80.7	3.8	1.5	5.2	0.8	1.0	2.6	0.4	0.3	0.2	0.7	2.2
22	Culberson, TX	10	89.0	0.9	0.7	4.0	0.1	0.3	0.9	0.2	1.7	0.1	0.3	2.4
34	Grant, NM	10	69.8	1.0	1.2	6.3	12.2	1.9	1.0	0.4	0.4	0.1	3.4	1.6
38	Apache, AZ	10	65.5	0.9	0.7	17.3	0.2	3.0	2.6	0.8	1.6	1.5	5.2	0.8
χ^3		(188)	80.5	1.5	1.0	6.8	1.7	1.3	2.3	0.5	0.9	0.3	1.7	1.4
Min ⁴			30	0		0	20	0						
Max ⁴			94	9		36	28	8						

¹ α -pinene, camphene β -pinene, 3-carene sabinene, myrcene, limonene, β -phellandrene, γ -terpinene, ocimene, terpinolene, p-cymene includes all trees reported by Zavarin and others.

² Normalized; * = trace.

³ Averages for all trees; (188) includes all trees in Zavarin and others (1989).

⁴ As estimated from the frequency distributions of all trees given by Zavarin and others (1989).

In 1984, I sampled four trees growing at the Institute of Forest Genetics (IFG) from seed collected in southern New Mexico (*table 3-21*). The composition of three of the trees somewhat resembled that of trees reported from southern Arizona by Zavarin and others (1989), with about 65 percent α -pinene. However, the average of about 24 percent 3-carene reported in the trees at IFG was considerably higher than that reported by Zavarin and others (1989). The composition of the fourth tree was nearly pure α -pinene, a composition not reported by Zavarin and others (1989); from the frequency distribution such a composition probably occurs. Little or no difference in composition was found between a basal tree sample and one at 20 feet (see *table 8-1*). The results with *P. edulis* pointed to the need for a larger number of trees at a location.

#8. *Pinus flexilis* James (limber pine)

P. flexilis is widely distributed in western United States, primarily in the central and northern Rocky Mountains (*fig. 3-15*), though it is also found on scattered ranges in the Great Basin. Its distribution is fairly limited in California and generally restricted to higher elevations. Mirov (1961) found the xylem monoterpene to be nearly pure α -pinene in trees in the White Mountains of California.

I examined eight trees at the Institute of Forest Genetics (IFG) grown from seed collected from various locations. The composition varied so widely that the average composition was meaningless. Therefore, the composition of the individual trees was given (*table 3-22*). The sampling was far too small to make any reliable statements. However, α -pinene, β -pinene, 3-carene, sabinene, and myrcene were quite variable and occurred in substantial amounts in some trees. Additionally, some trees had small to trace amounts of nonane, camphene, undecane, limonene, β -phellandrene, ocimene, and terpinoline. The tree from Canada was sharply different from the trees from Idaho and Nevada. The Canadian tree was forked, and the composition of the two forks were nearly identical. The ratio of sabinene to terpinolene was about 3:1 in the seven trees that had measurable amounts of sabinene. This was a different ratio than that found in other pines reported in this chapter: *P. muricata* and *P. albicaulis* were about 1:1, *P. monophylla* was about 2:1. Little or no difference in monoterpene composition was found between a basal tree sample of *P. flexilis* and one at 20 feet (see *table 8-1*).

It is apparent that a much more intensive and extensive sampling of *P. flexilis* would be helpful and is needed before more confident statements can be made.

#9. *Pinus jeffreyi* Grev. and Balf. (Jeffrey pine)

P. jeffreyi is primarily distributed in California, though its northern limits extend into southern Oregon and its southern limits into Baja California (*fig. 3-16*). It is sometimes mistaken for *P. ponderosa*; commercially both are called western yellow pine. In the major portion of its range, *P. jeffreyi* generally grows at a higher elevation and on drier sites than *P. ponderosa*, though both species are found at the same elevation. Likewise, the elevational characteristics tend to be reversed in some places at the northern and southern limits of the range of *P. jeffreyi*. In northwestern California and southeastern Oregon, *P. jeffreyi* replaces *P. ponderosa* on abundant ultramaphic soils. Natural hybrids of *P. jeffreyi* and *P. ponderosa*, and of *P. jeffreyi* and *P. coulteri*, have been found, though more rarely than commonly believed. Cartwright (1988) sampled trees in six sympatric stands of *P. jeffreyi* and *P. ponderosa* and found that about 1 percent were possible hybrids. *P. jeffreyi* was used in much of the pine hybridization studies at IFG from about 1940 to 1970. The compositions of some of the hybrids that were developed are given in Chapter 4. Mirov (1961) reported that all studies of the xylem resin showed that the volatile portion of *P. jeffreyi* was nearly pure heptane.

Table 3-21—Xylem monoterpene composition of individual trees of *P. edulis* growing at Institute of Forest Genetics (IFG) from seed collected in New Mexico; the remeasurements for trees No. 2 and 4 were made in 1988, 4 years after the first measurement

Tree Number	Monoterpene ¹								
	α -p	cam	β -p	car	myr	lim	β -ph	oci	terp
	percent ²								
1	96.0	0.5	0.8	-	0.5	-	-	1.2	0.1
2	64.3	0.3	0.6	23.5	4.1	0.6	-	3.4	3.1
3	58.1	0.3	0.5	29.6	2.1	3.0	0.4	2.3	3.7
4	69.0	0.5	0.6	20.6	1.4	0.3	-	3.4	4.1
2	65.0	0.2	0.6	23.0	4.2	*	0.2	3.1	3.6
4	68.3	0.3	0.6	21.4	2.3	*	0.2	4.0	2.9

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellendrene, ocimene, terpinolene.
² Normalized, *=trace, -=zero.

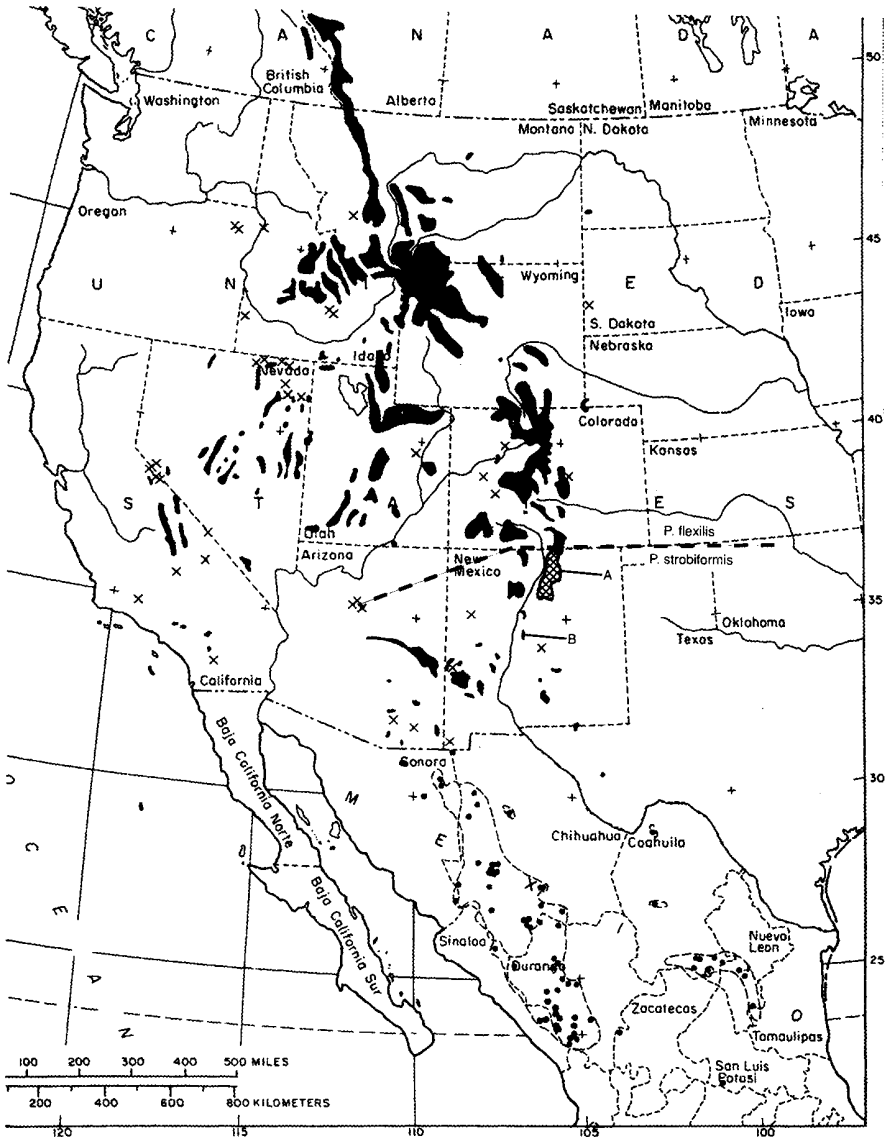


Figure 3-15—Distribution of *P. flexilis* and *P. strobiformis*; A & B are possible introgression (taken from Critchfield and Little 1966).

Table 3-22—Xylem monoterpene composition of eight *P. flexilis*, growing at the Institute of Forest Genetics from seed collected at the source

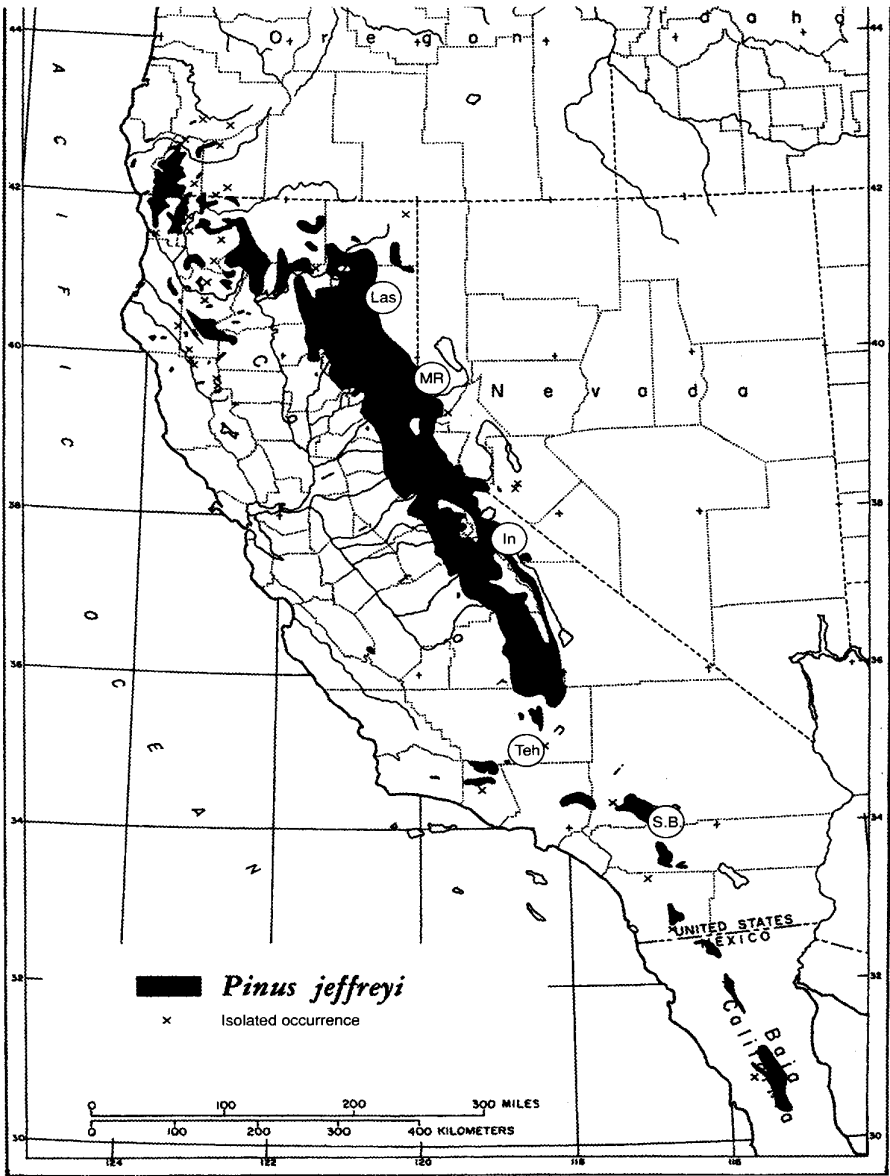
Source	Monoterpene ¹											
	non	α-p	cam	und	β-p	car	sab	myr	lim	β-ph	oci	ter
	percent ²											
Idaho	1.5	46.7	1.7	-	35.2	-	6.6	3.0	0.4	1.4	-	3.3
Idaho	0.1	64.7	0.4	-	17.3	-	5.5	7.6	*	0.9	1.0	2.5
Nevada	*	67.4	3.4	-	17.8	-	8.2	1.2	1.0	-	-	0.7
Nevada	1.0	58.5	0.9	-	14.0	-	16.1	2.3	0.2	1.0	0.1	5.8
Nevada	*	54.7	0.7	-	13.5	-	19.8	1.8	*	1.5	0.1	7.9
Canada ³	0.1	30.0	0.1	-	7.4	48.1	*	10.7	*	0.7	0.2	2.8
Canada ³	0.1	29.7	0.1	-	7.2	47.8	*	11.2	*	0.7	0.2	3.0
Nevada	*	79.3	1.6	-	11.2	-	4.5	1.2	0.5	0.4	.2	1.0
Nevada	1.5	71.0	1.3	-	10.3	-	8.1	2.0	0.8	0.8	-	4.0

¹ Nonane, α-pinene, camphene, undecane, β-pinene, 3-carene, sabinene, myrcene, limonene, β-phellandrene, ocimene, terpinolene.

² Normalized; * = trace, - = zero.

³ These two measurements are forks of the same tree from Canada.

Figure 3-16—Distribution of *P. jeffreyi* (taken from Critchfield and Little 1966), with location of six stands that were sampled.



An analysis was made of the volatile portion of the xylem resin of 128 trees growing at IFG from seed collected throughout the range of *P. jeffreyi* and from localities nearby on the Eldorado National Forest (Smith 1967b). The results (table 3-23) agreed, in general, with Mirov's report in that about 95 percent of the volatile portion was heptane with trace to small amounts of several monoterpenes. There were little or no differences in composition between a basal sample and one at 20 feet (see table 8-1). In addition to the 95 percent heptane, the three monoterpenes that occurred in the greatest amounts, but always less than 5 percent, were 3-carene, myrcene, and β -phellandrene. A stand of 20 *P. jeffreyi* that was growing in the South Warner Mountains was analyzed for the volatile portion of the xylem resin. Again, the results were similar to those in previous reports except that one tree had characteristics suggestive of a hybrid with *P. washoensis* and/or *P. ponderosa*; its composition was 17 percent heptane, 2 percent α -pinene, a trace of α -thujene, 4 percent β -pinene, 55 percent 3-carene, 6 percent myrcene, 11 percent limonene, and 3 percent terpinolene. The heptane and nonane showed *P. jeffreyi* parentage; the low α -pinene, high 3-carene, and trace of α -thujene suggested *P. washoensis* parentage; and the relative high limonene suggested *P. ponderosa* as a possible parent. Since all three parent trees grow fairly close together in this area, it is conceivable that all three species could be involved in the parentage.

I sampled five stands to more thoroughly represent the range of *P. jeffreyi* pine (fig. 3-16). The size of stands varied greatly.

The results again supported all the earlier reports: heptane was about 95 percent of the volatile portion of the xylem resin with trace to small amounts of several monoterpenes (table 3-24); about 8 percent 3-carene was found in one tree and 12 percent β -phellandrene in another. The frequency distribution of heptane, 3-carene, myrcene, and β -phellandrene showed some variation. There tended to be a greater percentage of heptane and a lower amount of the minor components in the northern stands; the amount of heptane was also less variable in the northern stands (fig. 3-17). The data were not applicable to the coding procedure and only a selection of individual trees is given to show the range of composition (table 3-25).

I concluded that *P. jeffreyi* had a (1-1) set of alleles for heptane, and there appeared to be no active alleles for any monoterpenes. The presence of trace to small amounts of several monoterpenes could have been caused by biosynthetic by-products or by environmental effects. However, the source of the biosynthetic by-products was not known because there were only very small amounts of the monoterpenes, and it was not likely that they would be derived from heptane.

As discussed elsewhere, it could be that *P. jeffreyi* does have a (0-0) set of alleles for the monoterpenes and that these (0-0) sets can be slightly active.

#10. *Pinus lambertiana* Dougl. (sugar pine)

P. lambertiana is the largest of the pines in size, sometimes reaching a diameter of 10 feet, with diameters of 6 feet quite common in over mature stands. It usually occurs as a component of the mixed conifer forest and is not often found in pure stands. Though its principal range is in California, it extends northward into southern Oregon and southward into northern Baja California (fig. 3-18). *P. lambertiana* reaches its maximum growth in the central Sierra Nevada.

Mirov (1961) reported a composition of 60 to 75 percent α -pinene, 5-10 percent β -pinene, and smaller amounts of β -phellandrene and other monoterpenes.

I sampled six stands, two in southern California and four in the Sierra Nevada/Cascades (fig. 3-18) with the standard macro-tap (see Chapter 2). The

Table 3-23—Average composition of the xylem volatiles of 128 *P. jeffreyi* and a selection of individual tree composition (taken from Smith 1967b)

Source	N	Monoterpene ¹											
		hep	non	α-p	und	cam	β-p	car	sab	myr	lim	β-ph	ter
		-----percent ² -----											
Mean	128	94.7	1.1	0.4	0.5	*	0.2	1.4	*	0.9	*	0.8	*
Eld ³	1	99.1	0.5	0.1	0.1	0	0.1	0.2	*	0	0	0	0
Eld	1	94.6	3.0	0.5	0.2	0	0	0.2	0	0	0	1.4	0
Eld	1	92.9	1.3	0.1	0.1	0	0.1	5.2	*	0.1	0	0	0
Eld	1	90.7	1.3	0.4	1.0	0	0	3.6	*	1.6	0	1.5	0
Mex ⁴	1	88.6	0.6	1.1	0.4	0	1.1	1.1	*	1.6	0.8	4.5	0
Mex	1	90.1	1.2	1.6	1.1	0	1.7	1.8	*	0.4	0.6	1.6	0
Eld	1	89.1	1.0	0.7	0.1	0	0.3	3.0	0.2	2.3	0.2	3.4	*

¹ Heptane, nonane, α-pinene, undecane, β-pinene, 3-carene, sabinene, myrcene, limonene, β-phellandrene, terpinolene.

² Normalized; * = trace.

³ Eld = trees growing on the Eldorado National Forest

⁴ Mex = trees growing at IFG from seed collected in Mexico.

Table 3-24—Average composition of the xylem resin volatiles in five stands of *P. jeffreyi*; and the average ($\bar{X}$) of all trees; n = number of trees

Source	n	Monoterpene ²							
		hep	non	α-p	β-p	car	myr	lim	β-ph
		-----percent ³ -----							
SB	66	94.7	0	0.3	0.7	0.1	0.8	0.2	2.5
TH	16	91.6	0.9	0.4	0.4	1.5	1.6	0.1	3.0
IN	36	95.3	0.2	0.4	0.2	0.4	0.4	0.1	2.3
MR	40	93.5	0.6	0.6	0.1	0.6	1.5	0.1	1.3
LA	103	98.0	0	0.3	0.5	0.1	0.5	0.1	0.1
($\bar{X}$)	(261)	95.7	0.2	0.4	0.5	0.2	0.8	0.1	1.4

¹ San Bernardino, Tehachapi, Inyo, Mt. Rose, Lassen.

² Heptane, nonane, α-pinene, β-pinene, 3-carene, myrcene, limonene, β-phellandrene.

³ Normalized.

($\bar{X}$) = average () = total

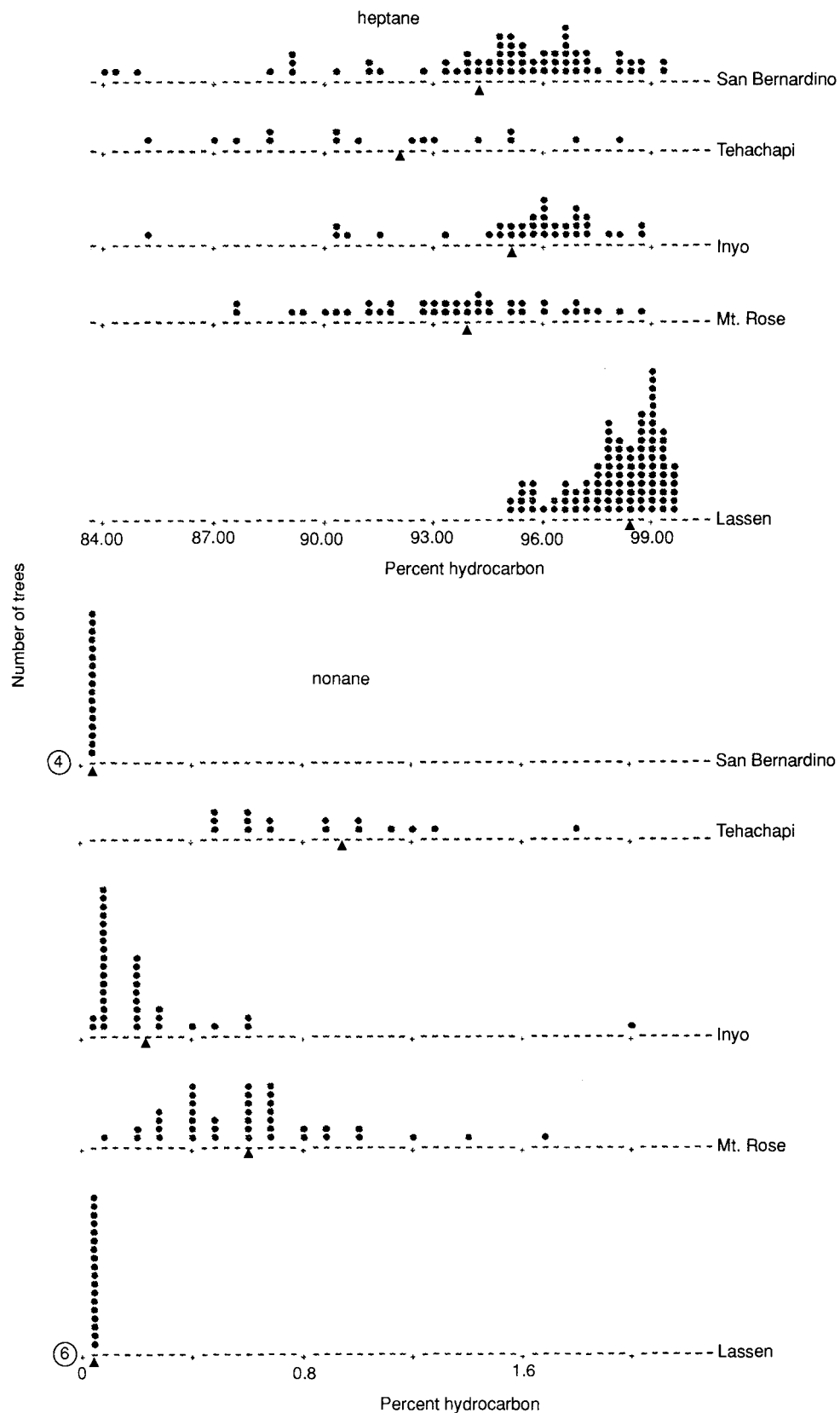
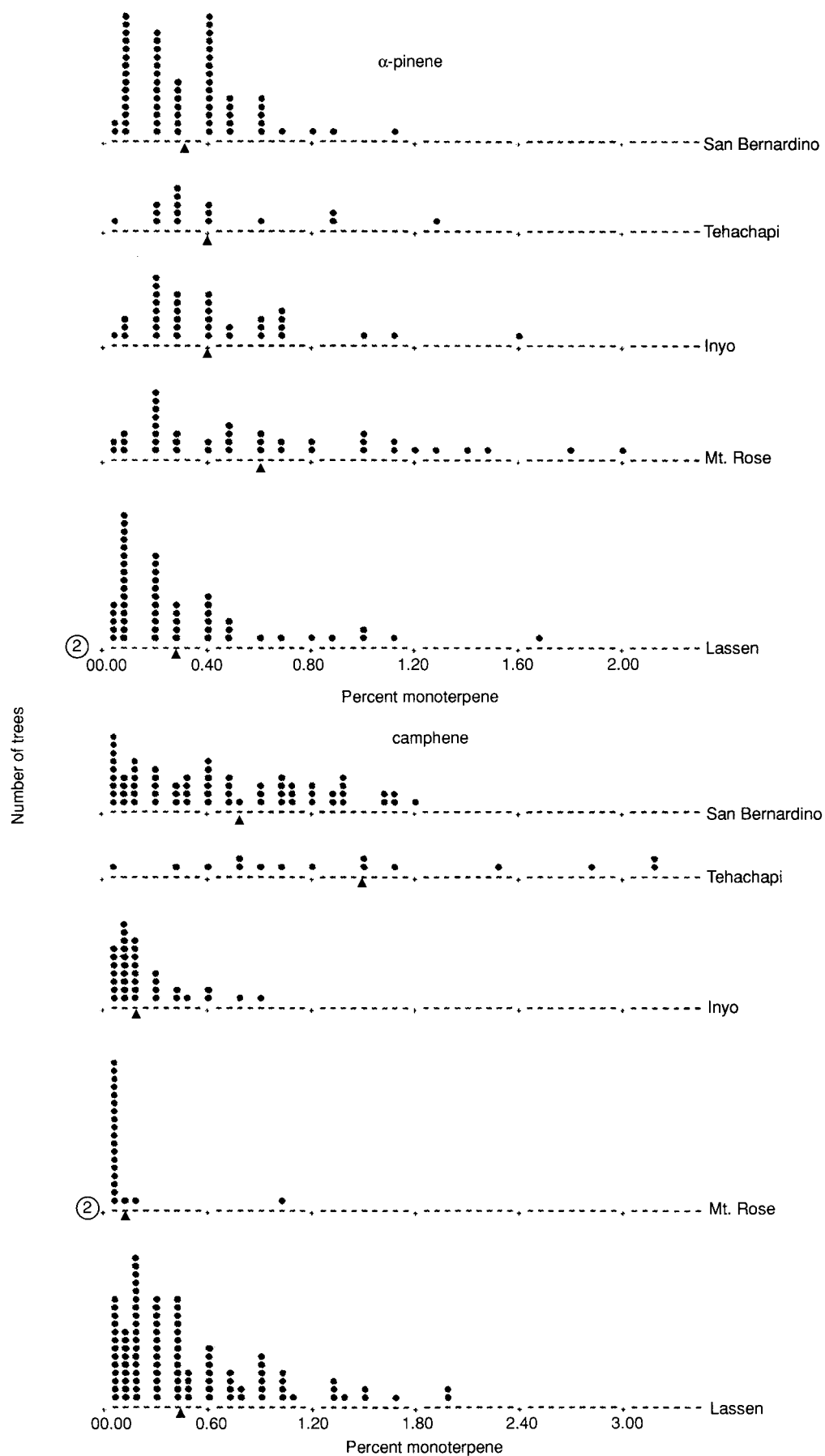
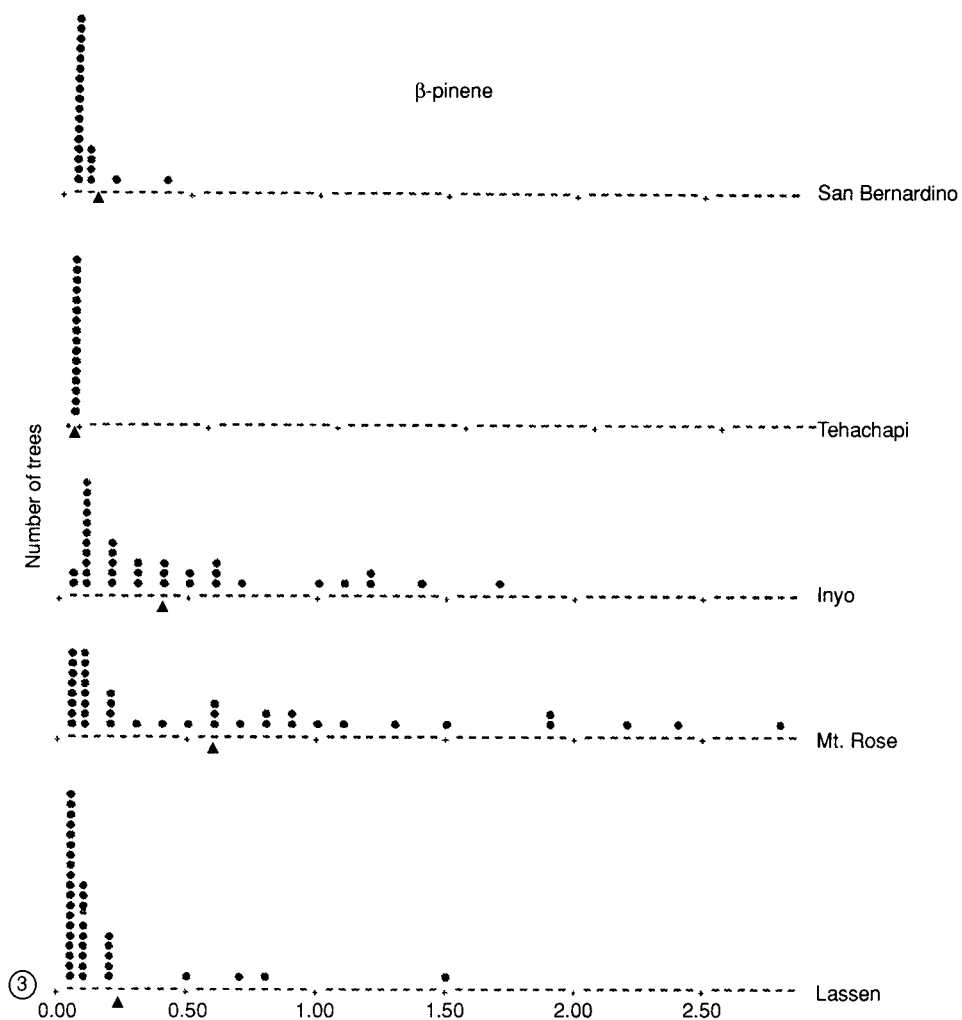
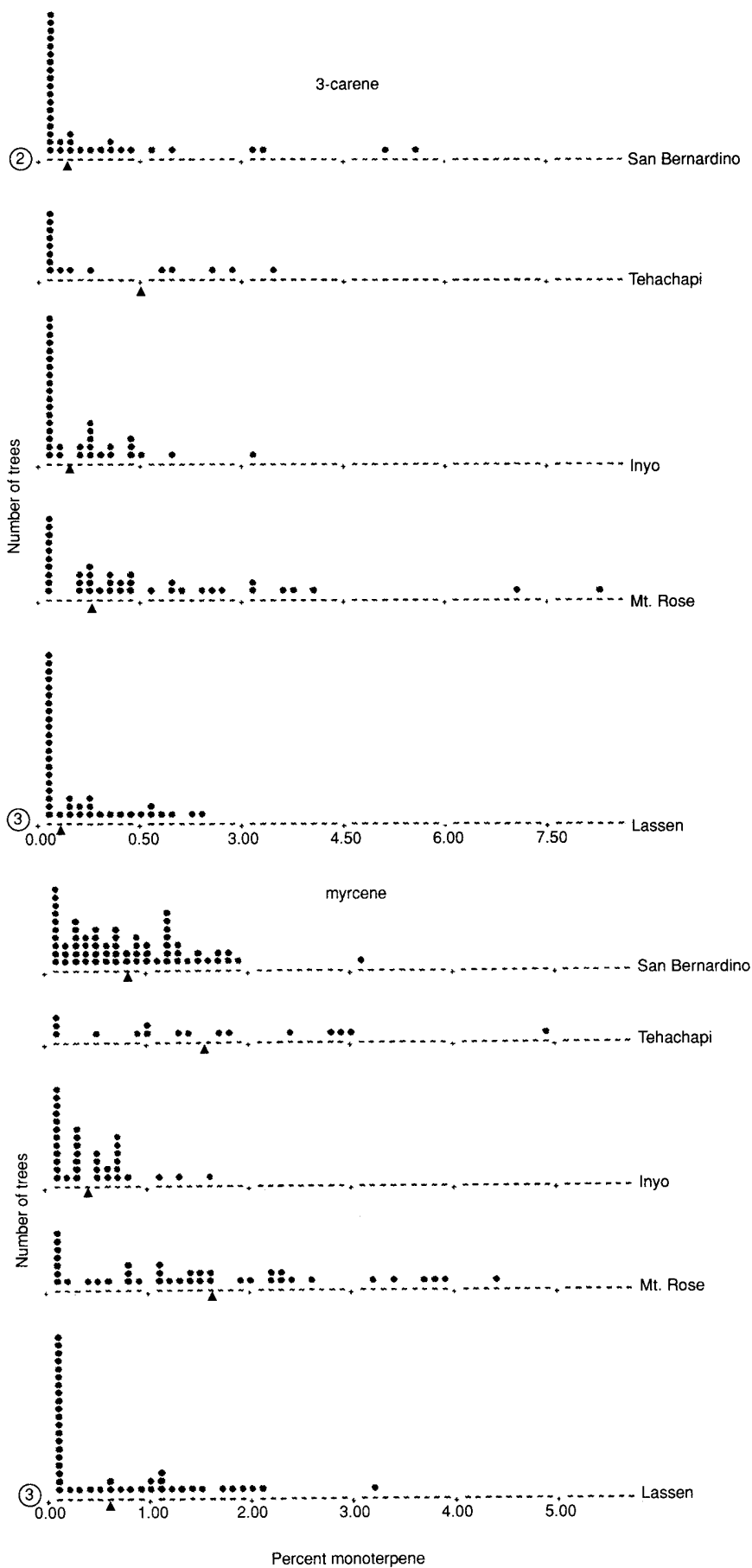


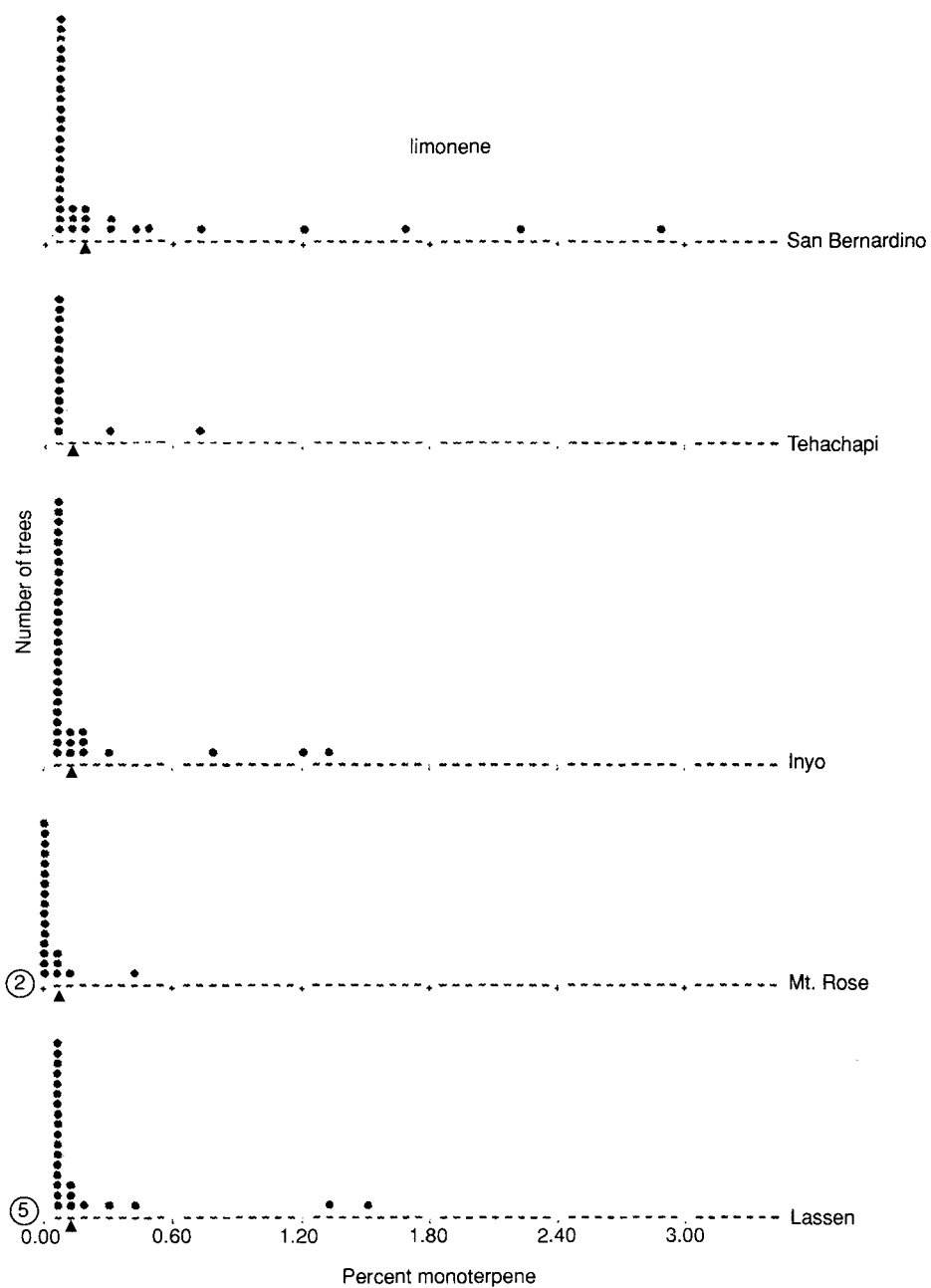
Figure 3-17—Frequency distribution of the amount of the noted volatile components of the five stands of *P. jeffreyi* listed on the right; each dot is measurement of one tree except where ④ indicates each represents *n* trees; ▲ is average of the set.

(fig. 3-17 continues on the next 5 pages).









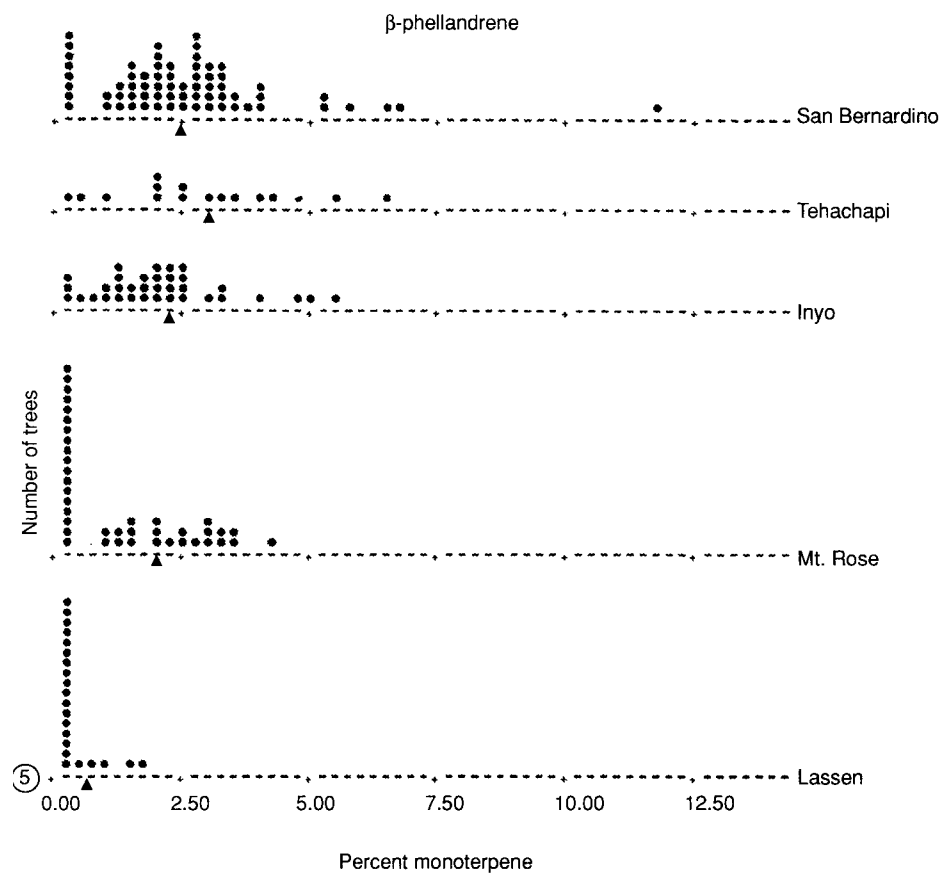


Table 3-25—Composition of xylem resin volatiles of selected *P. jeffreyi* illustrating variation in composition

Source ²	Monoterpene ¹								
	hep	non	α-p	cam	β-p	car	myr	urn	β-ph
-----percent ³ -----									
MR	94.6	3.0	0.5	0	0.1	0.2	0	0	1.5
MR	99.1	0.5	0.1	0	0.1	0.2	0	0	0
SB	90.3	1.8	0.9	2.8	0	0.3	0.5	0	3.4
SB	87.0	0.7	0.9	1.5	0	0	4.9	0	4.8
SB	87.5	1.3	0.3	3.2	0	3.5	0	0.3	3.2
SB	85.1	1.0	1.3	3.2	0	0.2	2.4	0	6.6
TH	84.8	*	0.5	1.1	0	3.4	1.9	2.9	5.3
TH	88.4	*	0.5	1.6	0	5.1	0.9	0	3.2
TH	84.3	*	0.9	0.4	0	2.0	0.5	0.1	11.7
TH	84.0	*	0.6	1.4	0.4	5.6	0.8	0.4	6.8
IN	85.3	0.2	1.6	0.2	1.2	1.0	0.7	*	9.9
MR	90.3	1.4	0.4	0.2	2.8	2.1	0.4	0	2.5
MR	87.7	0.1	2.0	0.1	0.1	8.2	0.2	0.1	0.3

¹ Heptane, nonane, α-pinene, camphene, β-pinene, 3-carene, myrcene, limonene, β-phellandrene.

² MR = Mt. Rose, SB = San Bernardino, TH = Tehachapi, IN = Inyo.

³ Normalized; * = trace

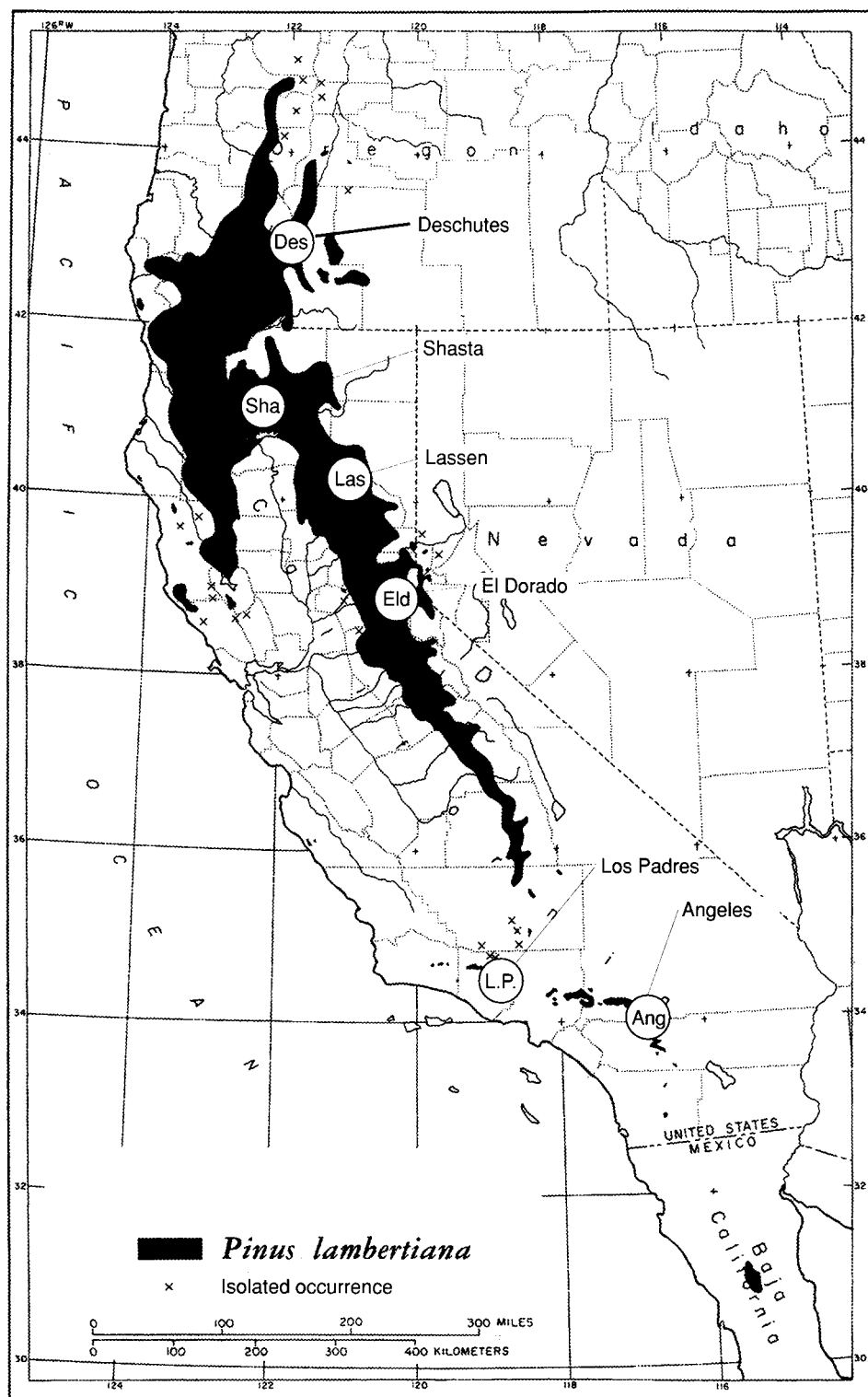


Figure 3-18—Distribution of *P. lambertiana*, taken from Critchfield and Little (1966), and the National Forest location of the six stands that were sampled for xylem monoterpene.

average composition values of the trees recorded for each location (*table 3-26*) were misleading because of the great variation as shown by the ranges and by the frequency distributions (*fig. 3-19*). It is quite clear from these averages and the frequency distributions that the two southern California stands were quite distinct from the Sierra Nevada/Cascade stands in having an average of about 10 percent undecane. The two southern California stands differed from each other in α -pinene, β -pinene, 3-carene, myrcene, and limonene. The 11.6 percent myrcene in the San Bernardino stand was quite misleading because of the three trees with greater than 45 percent myrcene. Without these three trees, its frequency distribution was not at all different from the other stands. There was little or no difference in composition between a basal sample and one at 20 feet (see *table 8-1*).

The monoterpene α -pinene was extremely variable in all stands; even the few trees in the southern California plots showed great variation. Proceeding southward from the Deschutes, the average amount of α -pinene decreased through the Shasta and Lassen stands. However, the Eldorado stand went the other way, with the average increasing. The Los Padres stand had the lowest average, whereas the San Bernardino stand was about as low as the lowest of the four northern stands.

Undecane was markedly different in the six stands; it was found in appreciable amounts in all trees in the Los Padres and San Bernardino stands, whereas it was absent from most trees in the four northern stands. When it did occur in the north, it was usually in small to trace amounts.

Camphene was lacking in most trees in all stands; it was present only in small to trace amounts when it was found.

β -Pinene, like α -pinene, was quite variable in all stands. However, the average amount in the four northern stands was about the same. The average amount in the Los Padres stand was significantly lower than that in all other stands.

3-Carene averaged about the same in the four northern stands except for the unusually large 22 percent in the Lassen stand; the San Bernardino stand was a bit lower, whereas the Los Padres stand was significantly lower. The ratio of 3-carene to terpinolene was in the range of 10:1 to 20:1, much like other pines.

Sabinene was absent from most trees but occurred in small amounts in some trees and in appreciable amounts of up to 20 percent in a few trees in the Lassen, Eldorado, and Southern California stands. The ratio of sabinene to terpinolene was in the range of 3:1 to 4:1 which is much like the ratio in *P. flexilis*. Both *P. lambertiana* and *P. flexilis* are in the *strobi* subsection of the subgenus *Strobes*. Thus, this ratio could be a marker for the subsection. The ratio of sabinene to terpinolene in *P. muricata* and *P. albicaulis* is about 1 to 1, and in *P. monophylla* is about 2 to 1; these three species are in different subsections, and none is in the *strobi* subsection of the genus *Pinus*. The varying ratio of sabinene to terpinolene is puzzling, since where as, the ratio of 3-carene to terpinolene was always about the same, at about 20 to 1 in all pines.

Myrcene averaged about the same in all stands; the higher average in the San Bernardino was caused by the three trees with greater than 45 percent.

Limonene averaged about the same in all stands. The higher average in the San Bernardino stand was caused by three trees with greater than 45 percent limonene content.

Beta-phellandrene averaged about 1 to 2 percent in all stands except in the Lassen where the average was slightly more than 3 percent. This greater average was caused by a general increase to about 6 percent in many trees and by the occurrence of several trees with greater than 9 percent.

Terpinolene, as in all pines analyzed so far, followed the previously described pattern of 3-carene.

Table 3-26—Average xylem monoterpene composition of stands of *P. lambertiana* in six national forests; *n* = number of trees

		Monoterpene ²									
National Forest ¹	n	α-p	und	cam	β-p	car	sab	myr	lim	β-ph	ter
percent ³											
Des	68	56.8	0.2	0.1	19.8	9.5	0.1	6.7	4.9	0.8	1.1
Sha	70	45.0	-	0.2	27.6	9.4	0.5	8.8	6.3	1.0	1.2
Las	108	31.8	1.0	0.2	21.8	22.1	2.3	9.1	6.1	3.1	2.2
Eld	109	51.4	1.3	0.1	25.2	8.1	2.5	6.3	1.1	2.2	1.4
L. P.	18	22.3	7.7	-	7.8	46.1	0.4	7.6	3.2	0.7	4.2
S.B.	31	34.1	10.7	0.2	19.4	12.8	0.5	11.6	8.9	0.8	0.9

¹ Deschutes, Shasta-Trinity, Lassen, Eldorado, Los Padres, San Bernardino.
² *α*-pinene, undecane, camphene, *β*-pinene, 3-carene, sabinene, myrcene, limonene, *β*-phellandrene, terpinolene.
³ Normalized; - = zero.

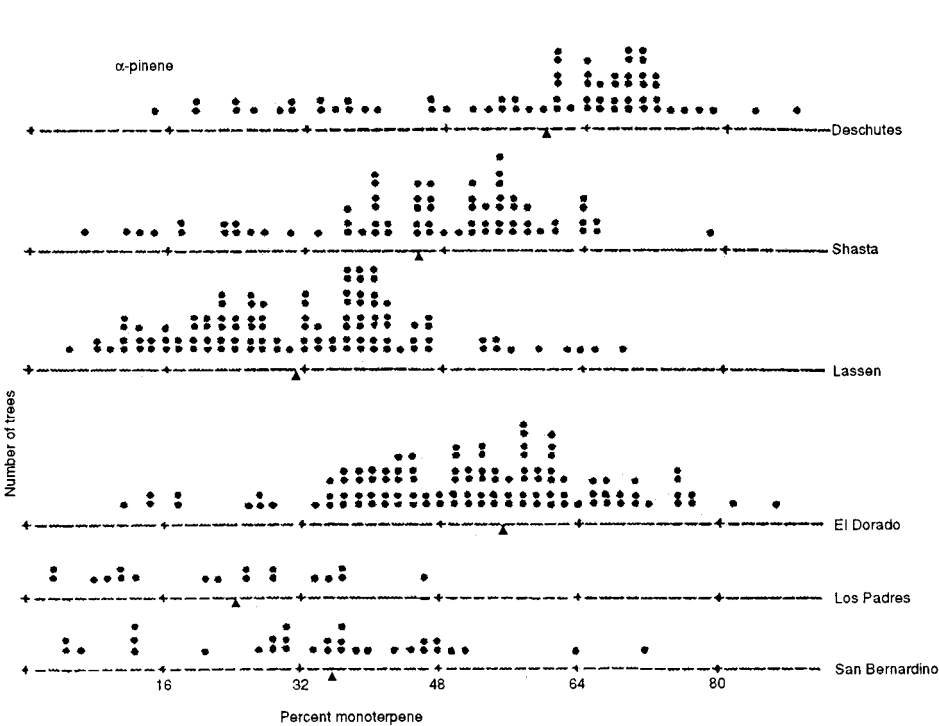
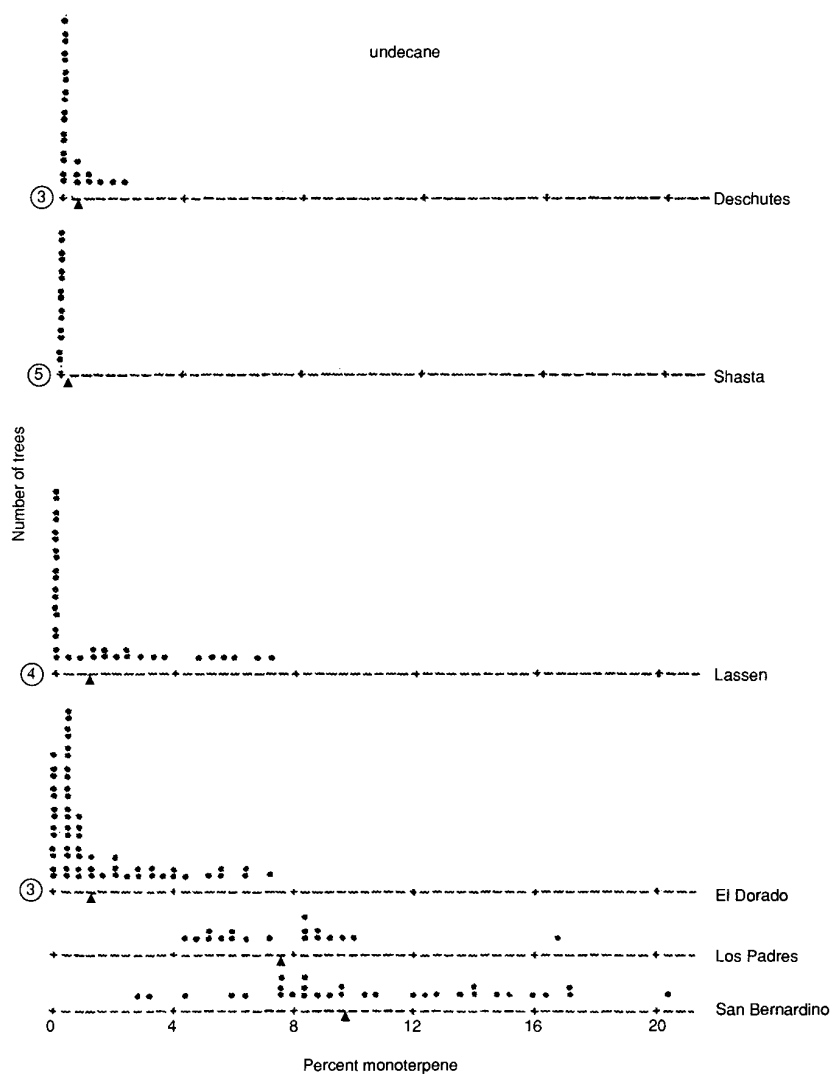
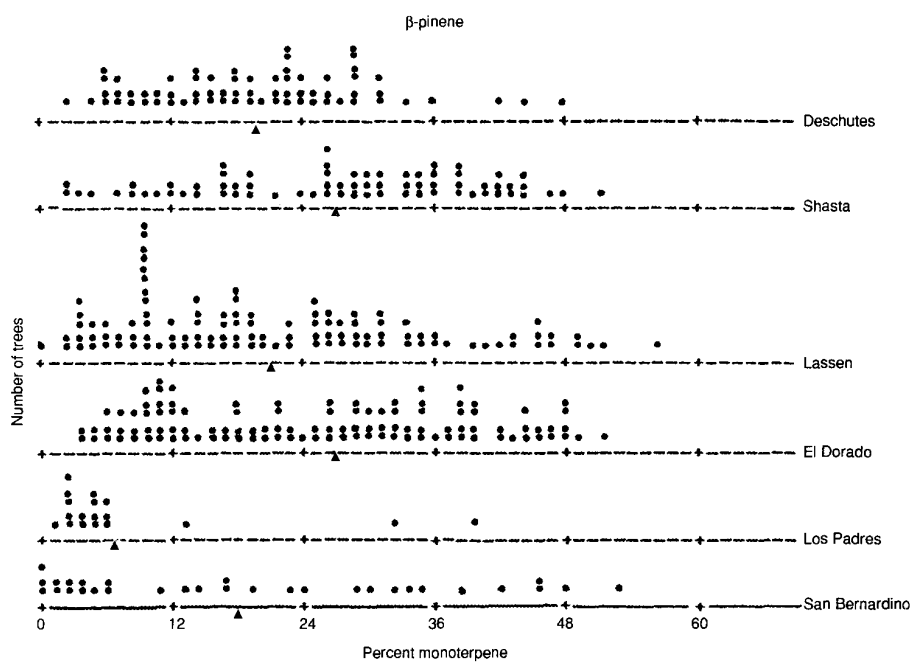
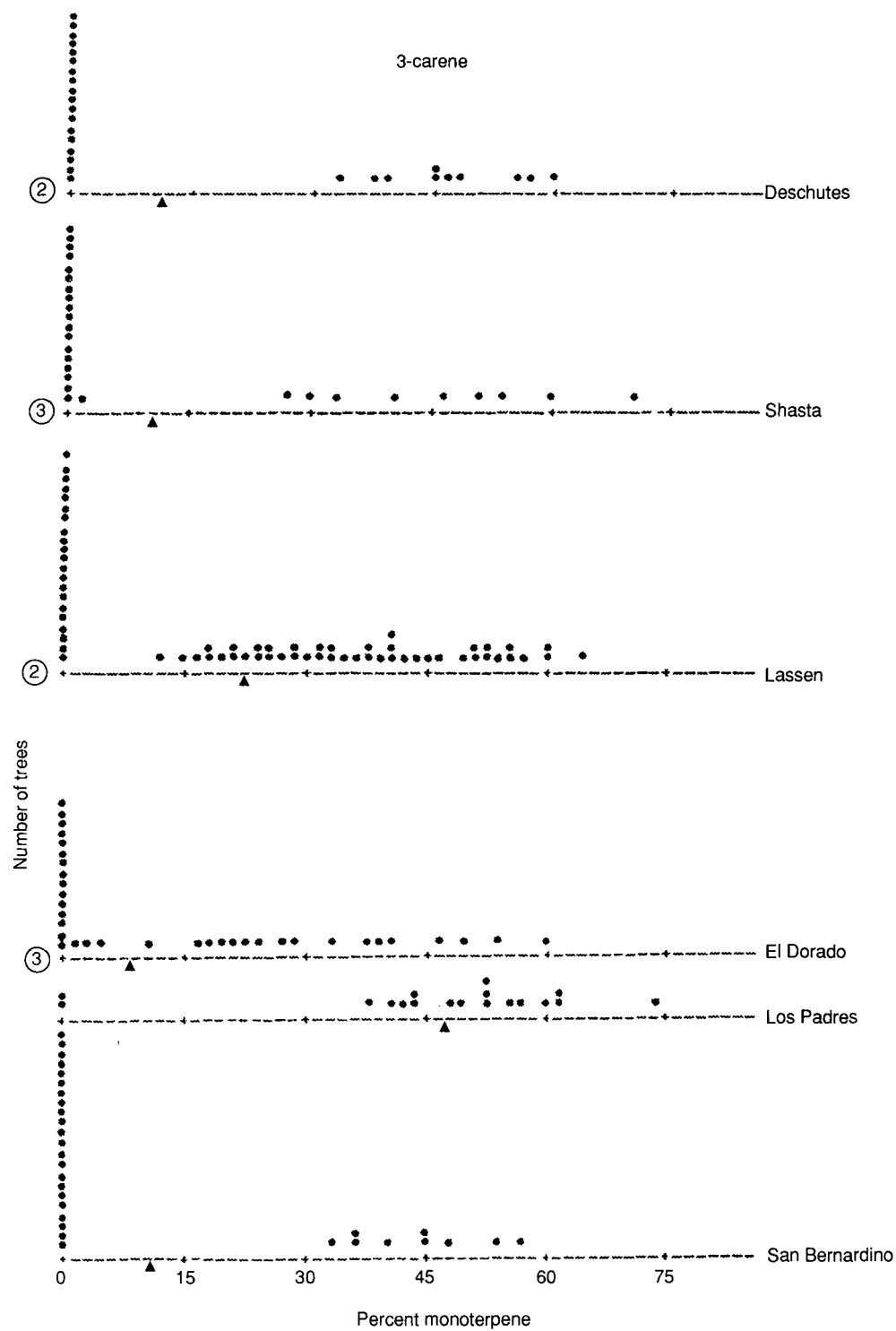
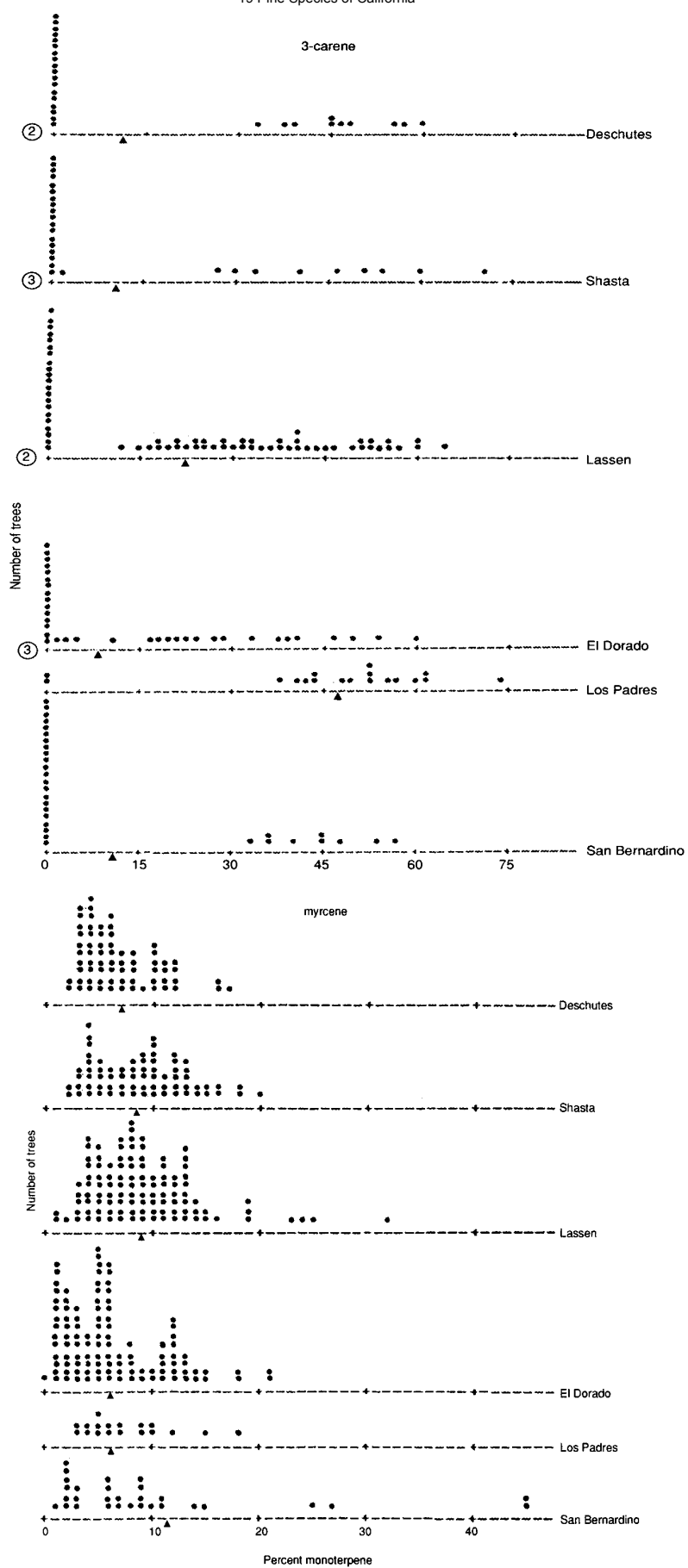


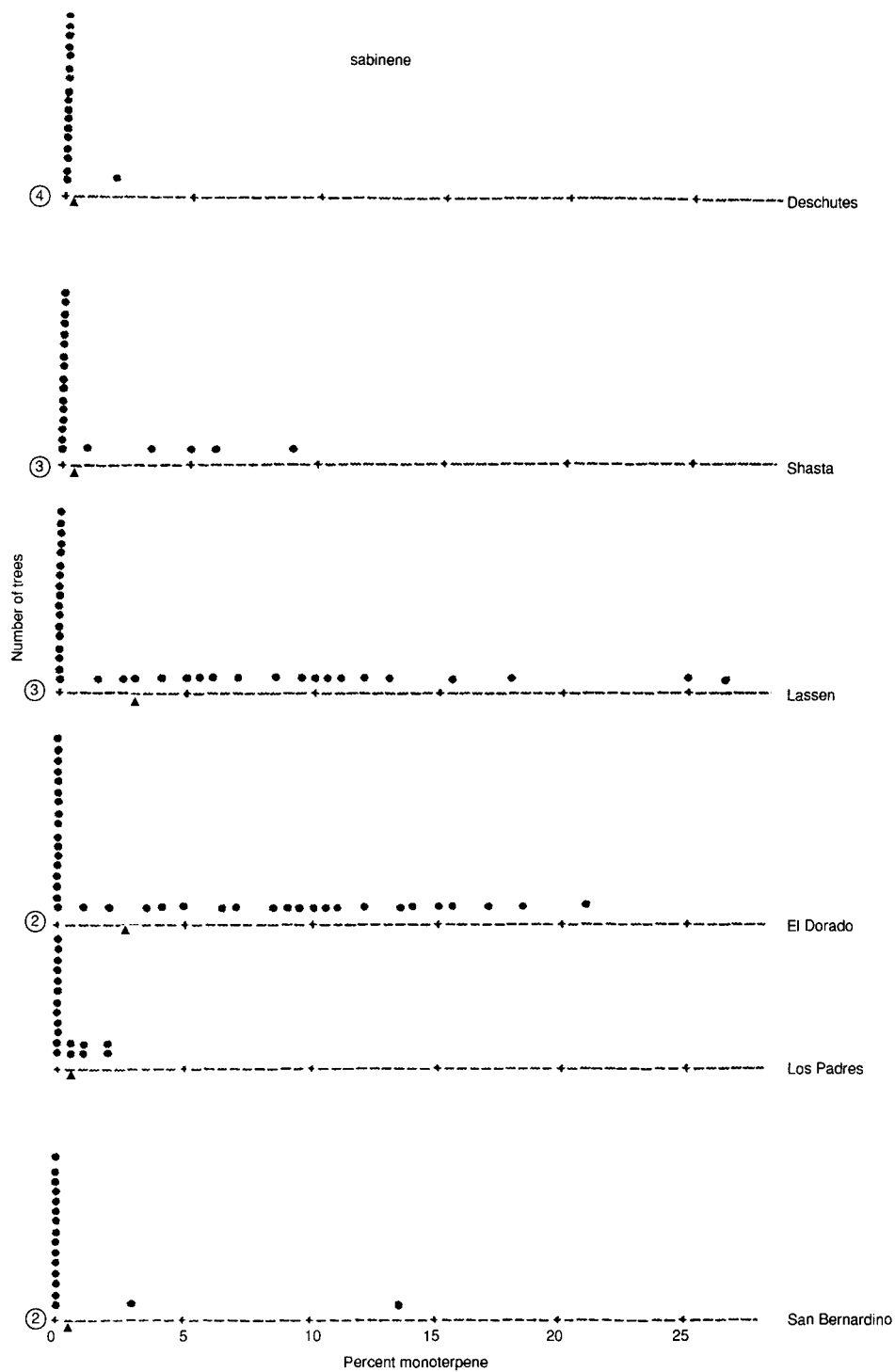
Figure 3-19—Frequency distribution of the amount of the noted monoterpenes found in the xylem resin of stands of *P. lambertiana*, in the six National Forests listed on the right; each dot is measurement of one tree except where (n) indicates each represents *n* trees; ▲ is average of set.

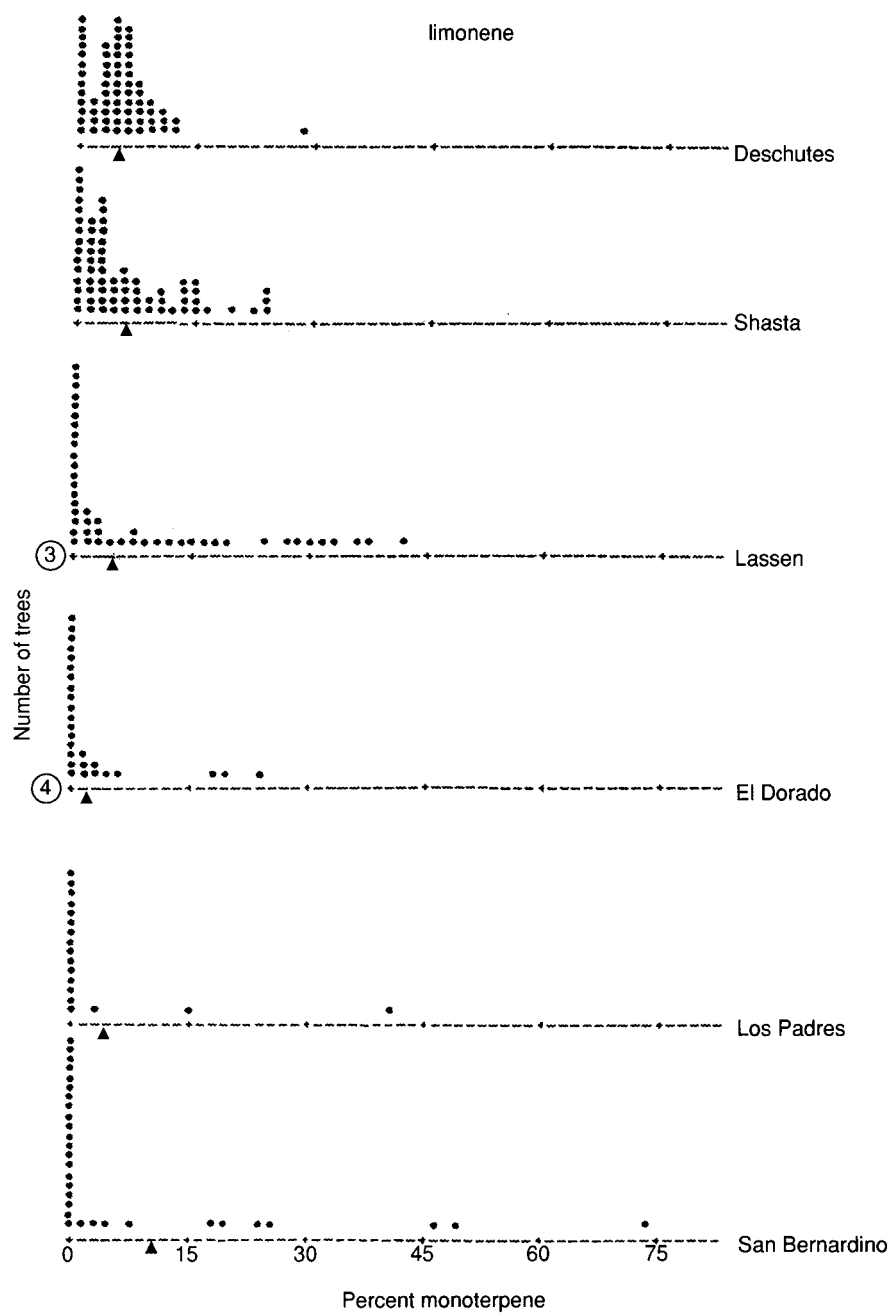
(fig. 3-19 continues on the next 7 pages).

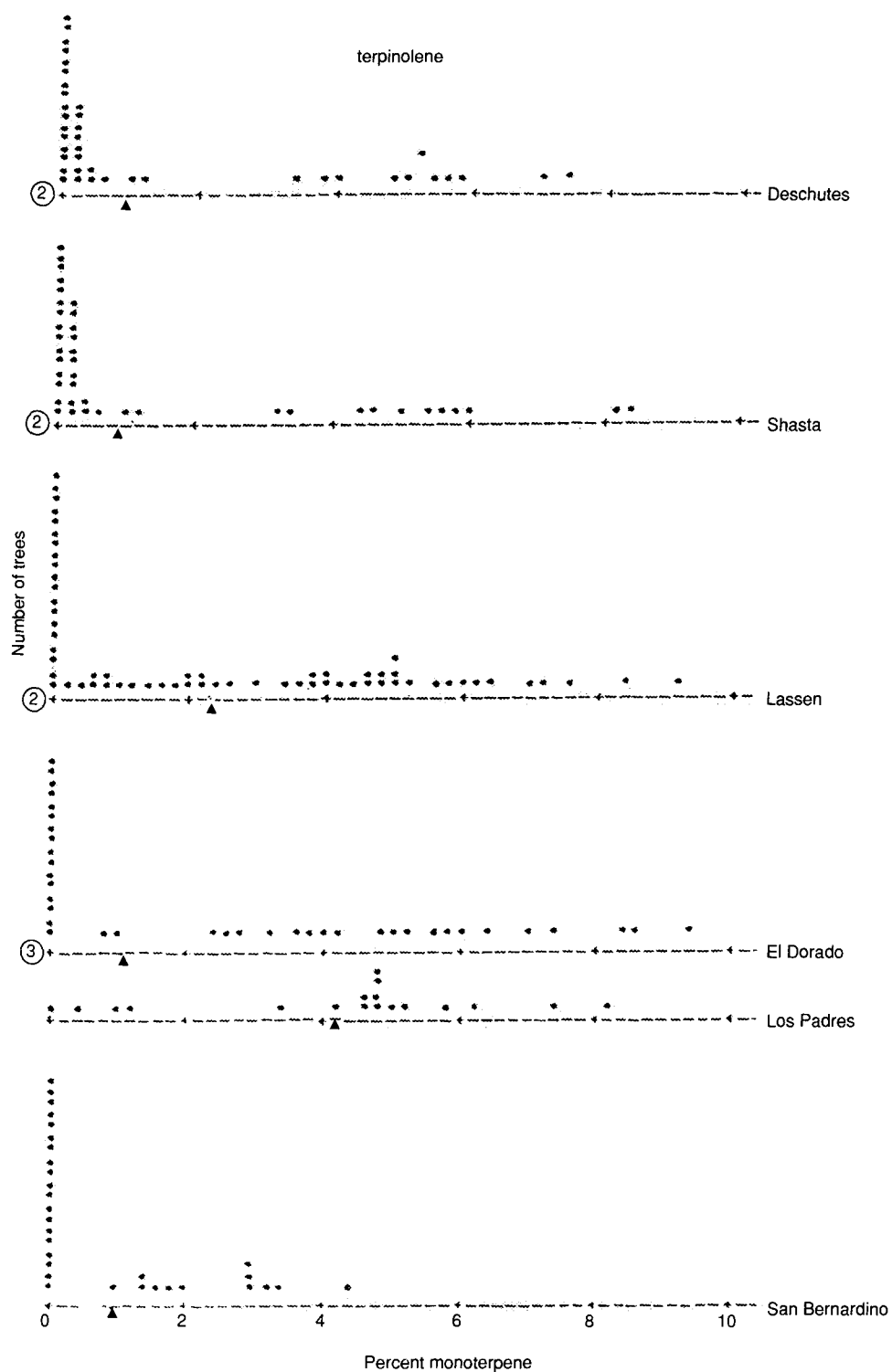


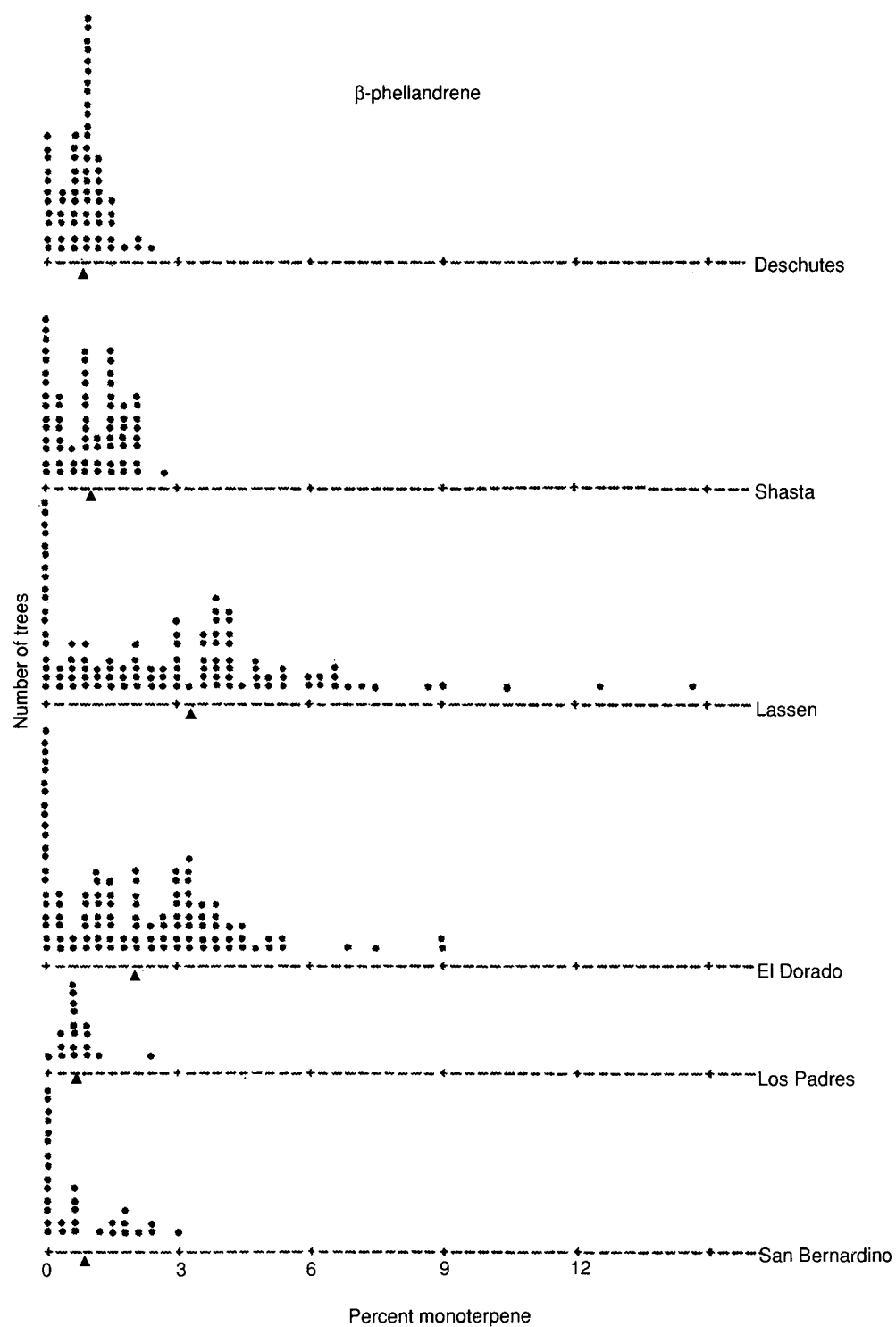












The frequency distributions of the individual components (*fig. 3-19*) showed the great variation within and between stands in nearly all components. There was reasonable evidence in the frequency data that five monoterpenes (α -pinene, β -pinene, 3-carene, myrcene, limonene) were under gene control and that all five frequency distributions were multimodal. The classification of sabinene was uncertain. It sometimes was found in amounts suggestive of gene control, but it was nearly absent in most trees. When the frequency distributions were viewed as a whole for the four northern stands, it was possible to define three modes each for α -pinene, β -pinene, 3-carene, myrcene, and limonene (*table 3-27*). The two small stands in southern California were not considered in the determination of modes because they were too small and have unusually large amounts of undecane.

When the coding procedure—using the criteria in *table 3-27*—was applied to the four northern stands (Deschutes, Shasta, Lassen, Eldorado), 65 types of composition were defined (*table 3-28*). Though sabinene occurred in the 5 to 15 percent range in several trees and greater than 20 percent in a few trees, it could not meet the coding criteria and was not included in the coding process. These 65 types of composition were very unevenly distributed among the four large northern stands (*fig. 3-20*). When the coefficient of association procedure was applied to the frequency of tree types in each stand, the results (*table 3-29*) clearly showed that the four northern stands were very poorly associated with each other. For example, the Deschutes stand had a large proportion of its composition types between types #55 and #65, whereas the other three stands had few or none of this type. The Shasta stand had a large proportion of its types between #37 and #45, the Lassen a large portion between #5 and #15, whereas the Eldorado had no grouping like the other three stands.

Ten trees were selected from the Los Padres and San Bernardino stands to illustrate some of the more common types of composition (*table 3-30*).

The grand mean for the 345 trees in the four northern stands was calculated (*table 3-28*). This was coded to 11011 type which was the fifth most common type of composition. Thus, the average for the species was the fifth most common type of composition. This illustrates the advisability of describing a stand, when variation is great, by the frequency of composition types and not by average composition.

In summary, the xylem monoterpene composition of *P. lambertiana* was locally and regionally quite variable in the Sierra Nevada/Cascade stands. Five monoterpenes— α -pinene, β -pinene, 3-carene, myrcene, and limonene—were quite variable and appear to be under gene control. Sabinene did not generally occur in amounts that were indicative of gene control except in a few trees in the Eldorado and Lassen stands. All four northern stands—Deschutes, Shasta, Lassen and Eldorado—had large within-stand variations and all four were very weakly associated with each other on the basis of the frequency of composition types. The Los Padres and San Bernardino stands were quite different from the four northern ones, primarily because of the presence of 8 percent to 10 percent undecane. The San Bernardino stand differed from the Los Padres stand by having larger amounts of limonene and myrcene. A stand of 100 trees may not be adequate in determining the range and frequency of types of composition; and larger stands are needed in Southern California.

#11. *Pinus monophylla* Torr. & Frem. (single leaf pinyon)

Pinyon pines are the typical conifer in the arid southwestern United States at lower elevations. Two species, *Pinus monophylla* and *P. edulis*, are by far the most common, occupying about 75,000 square miles (Lanner 1981). *P. monophylla* is generally found west of the Nevada-Utah state line, and *P. edulis* is found to the east of that line (*fig. 3-21*). Two other pinyons, *P. discolor* and

Table 3-27—Intervals of the percentages of the frequency distribution of individual monoterpenes of *P. lambertiana* used to define the low (0), medium (1), and high (2) modes for the five noted monoterpenes

Mode ¹	Monoterpene				
	α -pinene	β -pinene	3-carene	myrcene	limonene
	----- Intervals in percent -----				
0	0 - 30	0 - 20	0 - 15	0 - 2	0 - 2
1	31 - 63	21 - 40	16 - 34	3 - 9	3 - 2
2	64 - 100	41 - 100	35 - 100	10 - 100	13 - 100

¹These mode numbers were used in the coding procedure in table 3-28.

Figure 3-20—Distribution of the 65 types of xylem monoterpene composition in the four northern stands of *P. lambertiana*; distribution is normalized for each stand; see table 3-28 for data on types of composition.

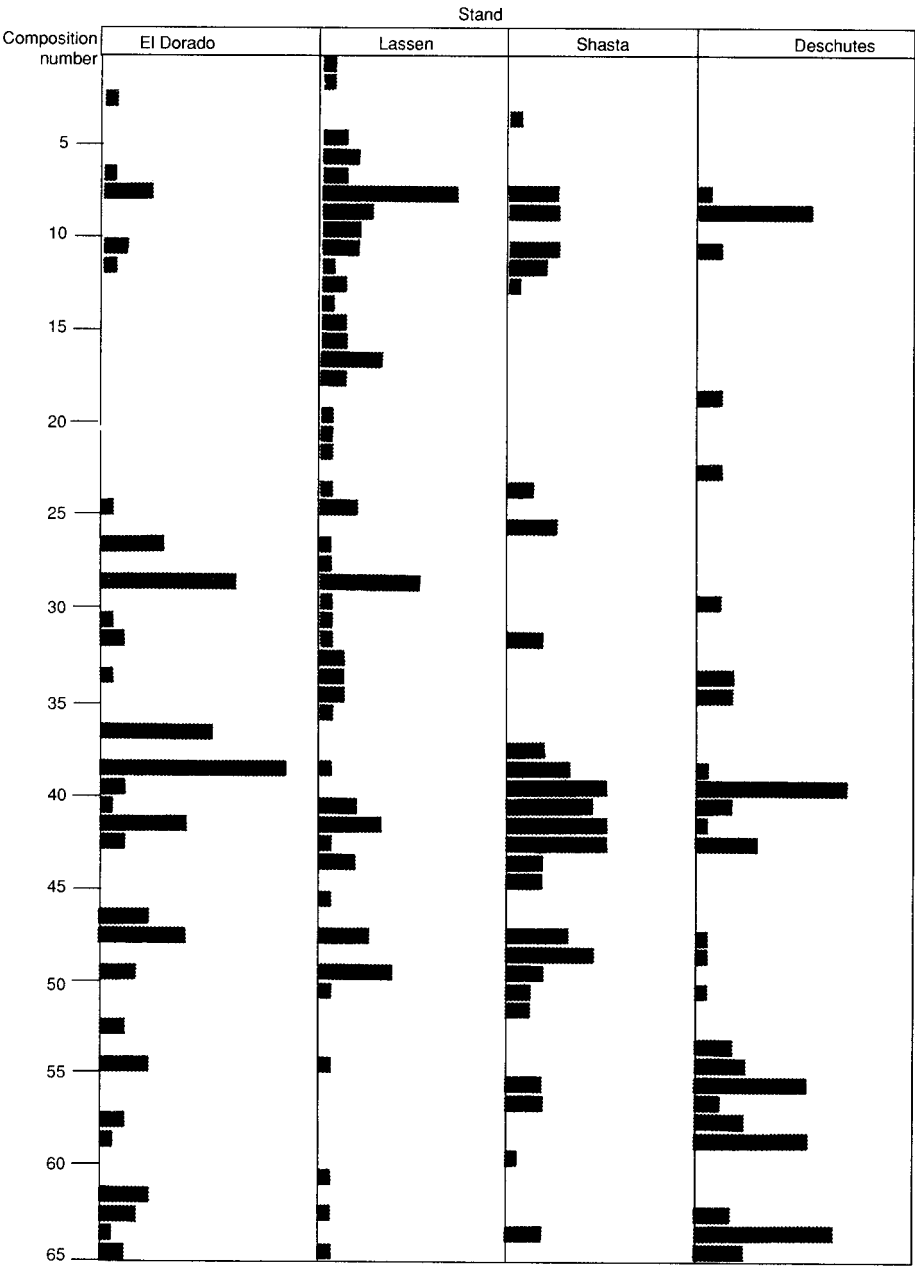


Table 3-28—The 65 coded types of xylem monoterpene composition of *P. lambertiana*, their numerical abundance (N) within the 345 trees in the four northern stands, and their actual average monoterpene composition

Actual monoterpene composition ¹											
Code											
N	type ²	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent ³ -----											
1	00 021	25.8	0.2	0.4	15.9	11.3	26.5	8.6	3.0	6.7	4.0
1	00 022	26.3	0	0	17.9	0	0	14.2	38.1	3.0	0
1	00 110	26.2	1.6	0	19.3	23.2	15.3	4.9	0	4.2	5.0
1	00 120	24.9	0	0.2	17.8	32.4	5.1	15.3	0	0.8	3.4
2	00 111	17.2	1.5	0.7	10.0	27.7	12.8	7.8	11.4	9.0	2.1
3	00 112	18.7	1.9	0.1	8.1	26.8	11.5	4.7	17.5	4.6	5.9
3	00 121	18.6	0.9	0.2	15.5	28.5	5.7	13.9	6.5	5.8	4.1
20	00 210	19.5	1.1	0.2	10.1	50.0	4.7	6.1	0.6	2.3	5.2
12	00 211	18.0	0.3	0.2	6.0	54.0	1.8	6.5	5.4	1.3	6.1
3	00 212	17.5	0.9	0	3.0	51.9	0	4.9	16.0	0.7	5.1
9	00 220	20.0	0.7	*	12.2	46.1	1.1	12.4	0.2	1.4	4.8
4	00 221	15.3	0.7	0.1	5.7	53.0	0	11.7	5.2	2.5	5.3
2	01 012	23.5	2.0	0	26.8	0	0	6.2	32.7	8.9	0
1	01 020	27.1	2.5	0.9	33.8	0	0	31.6	0	2.7	0
2	01 022	24.9	0.8	0.2	29.9	0	0	13.2	29.3	1.9	0
2	01 110	26.1	0.8	0	35.5	26.4	0	4.2	1.3	3.2	2.5
6	01 120	22.3	0	0.1	28.0	30.0	0.3	14.7	0.3	2.7	1.6
2	01 210	18.2	0.9	0.4	26.2	37.9	0	8.5	0.7	4.2	3.1
1	01 211	25.8	0	0	24.6	38.5	0	4.5	2.5	0.6	3.3
1	01 220	19.0	0	0.4	22.3	40.3	0	15.1	0	1.5	1.2
1	02 020	31.0	1.3	0	45.3	0	0	18.8	0	1.5	0
1	02 021	30.0	2.2	0	44.4	0	0	12.2	8.0	1.3	0
1	10 011	60.7	0	0	20.2	0	0	7.2	11.0	0	0.9
5	10 012	43.6	0.1	0.1	13.5	4.0	0.2	7.1	30.2	0.6	0.5
4	10 020	56.9	0.8	0	18.6	0	0	20.9	0.1	2.3	0
3	10 022	47.0	0	0.3	13.0	0	0	17.8	21.5	0.3	*
6	10 100	49.0	1.6	*	10.4	24.3	7.2	1.8	0.2	0.8	4.5
1	10 101	44.5	0	0.3	9.3	22.9	7.1	1.3	8.2	5.2	1.0
21	10 110	41.6	1.5	0.1	10.9	23.5	9.2	5.3	0.5	2.3	4.5
2	10 111	39.1	1.5	0.5	16.8	27.2	0	7.1	3.3	3.0	2.8
2	10 112	35.2	0.3	0.1	10.0	19.3	3.6	4.8	23.3	0	3.5
5	10 120	38.5	1.5	*	14.2	25.1	3.7	11.2	0.7	0.8	3.7
3	10 121	38.5	0.1	0.3	15.3	18.3	7.9	11.2	1.0	3.4	3.5
6	10 210	35.5	1.5	0.8	11.7	40.7	0	5.3	0.7	1.0	2.8

(continues on next page)

		Actual monoterpene composition ¹									
Code											
N	type ²	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
		-----percent ³ -----									
5	10 211	34.2	1.4	0.2	5.9	41.8	1.1	3.7	6.3	0.9	4.5
1	10 220	32.7	1.3	0	11.7	35.5	0	10.8	0.7	3.0	4.1
9	11 000	57.4	1.5	0.1	34.3	1.1	0.6	1.5	0.4	2.7	0.7
2	11 001	55.4	0	0.3	38.1	0	0	1.8	2.7	1.6	0.1
23	11 010	56.9	0.7	*	33.4	0.2	0	5.1	0.6	2.7	*
16	11 011	56.4	0.1	0.1	29.7	1.8	0.2	4.3	5.7	1.2	0.1
12	11 012	46.5	0.6	0.2	27.8	0	0.3	6.7	15.8	1.9	0.1
22	11 020	49.3	0.8	0.2	32.4	0	0.7	13.8	0.3	1.9	0.3
12	11 021	51.5	0.1	0.1	29.0	0	0.4	11.6	5.8	0.9	0.2
6	11 022	37.9	0.9	0.2	27.9	0	0	12.3	18.8	2.0	0.1
2	11 110	40.1	2.6	0.3	28.8	19.6	0	5.6	0.4	2.1	0.5
1	11 121	36.7	0	0.2	25.3	20.6	0	10.8	4.9	0.9	0.7
5	12 000	45.9	1.7	0	46.4	0	0	1.4	0.4	4.3	0
17	12 010	41.3	1.1	0	47.7	0.1	0	6.3	0.2	3.2	0.1
6	12 011	43.0	0	0.2	43.6	0.3	0	7.0	3.7	2.1	0.2
12	12 020	39.1	0.1	0.1	44.6	0.2	0	13.1	0.4	2.7	0
3	12 021	34.5	0	0.2	43.8	0	0	14.2	4.5	2.5	*
1	12 022	31.1	0	0	41.7	0	0	11.6	13.6	2.0	0
2	20 000	80.4	2.2	0.2	14.3	0.8	0	1.6	0	0.7	0
2	20 001	74.1	0.8	0.1	15.7	0	0	2.0	6.5	0.7	0.1
8	20 010	76.9	0.7	0	13.8	0	0	6.5	0.4	1.5	0.1
8	20 011	72.3	0.1	0.2	16.4	0	0	4.3	6.0	0.6	0.1
3	20 012	66.4	0.6	0	15.6	0	0	5.5	11.7	0	0.1
4	20 020	72.2	0.1	0.2	14.8	0	0	12.0	0	0.8	0
7	20 021	67.4	0.1	0.2	13.6	0	0	11.8	6.2	0.6	0.1
1	20 022	63.3	0	0.4	11.3	0	0	9.6	15.2	0	0.3
1	20 100	69.5	0	0.5	9.8	17.6	0	1.3	0.4	0.3	0.6
5	21 000	68.5	0.5	0	26.5	0	0	1.0	0.1	3.2	0
7	21 010	66.8	0.6	0.1	25.9	0	0	5.5	0.2	1.1	0
11	21 011	62.9	0.6	0.1	22.2	4.1	0	4.2	4.8	0.7	0.4
4	21 020	62.6	0	0.1	21.2	5.0	0	9.6	0.2	1.1	0.5
345	[11011]	45.2	0.7	0.1	23.6	12.9	1.6	7.7	4.4	2.0	1.6

¹ α -pinene, undecane, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, terpinolene.

² From left to right α -pinene, β -pinene, 3-carene, myrcene, limonene; see table 3-27 for values of coded numbers.

³ Normalized; * = trace.

Table 3-29—Coefficients of association of the four northern stands of *P. lambertiana*

National Forest	Eld	Las	Sha	Des
	-----coefficients-----			
Eldorado		.10	.01	.06
Lassen			.02	.04
Shasta				.03
Deschutes				

Table 3-30—Xylene monoterpene composition of 10 individual *P. lambertiana*, selected from a population of 49 trees, which illustrate the range in composition in the Los Padres and San Bernardino stands

Stand	Monoterpene ¹								
	α -p	und	β -p	car	sab	myr	lim	β -ph	ter
	-----percent ² -----								
Las Padres	3.8	8.5	3.4	73.3	*	4.0	*	0.6	6.1
	22.4	6.1	6.3	51.9	*	7.4	*	0.7	4.8
	3.1	8.4	1.5	43.0	-	3.2	40.0	*	1.0
	36.3	4.7	39.1	-	-	17.5	-	2.3	-
	7.2	16.9	5.7	53.2	0.5	11.5	*	0.7	4.6
San Bernardino	37.3	8.6	2.3	-	-	51.3	-	-	-
	64.6	12.6	16.0	-	-	2.9	0.7	1.6	1.6
	33.6	10.2	-	-	-	9.2	46.6	-	-
	4.1	14.0	1.2	-	-	7.1	73.3	-	-
	48.6	2.8	23.3	-	13.4	6.5	0.5	-	4.3

¹ α -pinene, undecane, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, terpinolene.

² Normalized; * = trace, - = zero.

(formerly, *P. cembroides*) and *P. quadrifolia*, are primarily found in Mexico, but their ranges just reach into the adjoining States of Texas, New Mexico, Arizona, and California.

In early work, Mirov (1961) reported that *P. monophylla* and *P. edulis* had quite similar monoterpene composition, i.e., a large percentage of α -pinene and much smaller amounts of several other monoterpenes. Zavarin and others (1971) examined the two species more closely using gas chromatography and concluded that the composition of the two species was too similar to be a strong diagnostic character for determining species of individual trees. They did find, however, some average differences between the two species, with limonene higher in *P. monophylla* and 3-carene higher in *P. edulis*. Zavarin and others (1971) also found some evidence of geographic differences in *P. monophylla* and showed some variation in some of the components. Zavarin and Snajberk (1980) extracted resin from the xylem of 11 species of pinyon and found large differences between some species, but, again, only small differences were found between *P. edulis* and *P. monophylla*. A study was subsequently made of *P. monophylla* and *P. quadrifolia* where their ranges overlap in southern California (Zavarin and others 1980). Both species were high in α -pinene; stand averages for 10 trees for *P. quadrifolia* ranged from 93 to 95 percent, and for *P. monophylla* ranged from 77 to 86 percent. *P. monophylla* had greater amounts of camphene, myrcene, and limonene than *P. quadrifolia*.

I sampled five stands that represented the trees' range (fig. 3-21, table 3-31). The results have been reported (Smith and Preisler 1988). Subsequent to that work I sampled a stand near Pine Mountain on the Los Padres National Forest. The following discussion was adapted from the two studies.

The six stands fairly well represented the range of *P. monophylla*, though no stand was located near *P. edulis*, along the eastern limits of *P. monophylla*, and no stand was located near *P. quadrifolia* along its southern limits (fig. 3-21).

A total of 10 monoterpenes were identified in the xylem resin of the 533 trees of *P. monophylla* sampled (table 3-32). Three of these— β -pinene, β -phellandrene, and camphene—occurred in trace amounts or less than 1 percent; two others—ocimene and terpinolene—comprised 1 to 8 percent of the total monoterpenes and have a unimodal distribution. The remaining five components— α -pinene, 3-carene, sabinene, myrcene, and limonene—ranged considerably in amounts and tended to have a trimodal distribution (fig. 3-22). Little or no difference in composition was found between a basal tree sample and one at 20 feet (see table 8-1).

A comparison of the different studies of *P. monophylla*, as noted above, showed fairly similar average composition (table 3-33), but the larger sample had a much greater range for all major components.

Among the six stands in the range-wide study, α -pinene averaged 84 to 85 percent in the two Nevada stands, Austin and Ely, while averaging 76 to 80 percent in the four California stands: Diamond Valley, Mammoth Lakes, Pine Mountain, and Wrightwood (table 3-32). 3-Carene averaged less than 1 percent in the Nevada stands while averaging 2 to 4 percent in the California stands. Sabinene averaged about 2 percent at Austin, Ely, and Diamond Valley; about 3 to 4 percent at Wrightwood and Pine Mountain; and about 2 percent at Mammoth Lakes. Limonene averaged about 3 percent at Austin. Myrcene averaged about 5 to 6 percent in all stands.

Measurements of 12 sets of forked trees with low forks, which were fairly common in single leaf pinyon, showed that the compositions of the two forks of a tree were almost identical; the compositions of the sets of forks were fairly representative of the stand.

From the frequency distribution of α -pinene, 3-carene, sabinene, myrcene, and limonene in five of the stands (fig. 3-22), three levels of

Figure 3-21—Distribution of *P. monophylla*, taken from Critchfield and Little (1966).

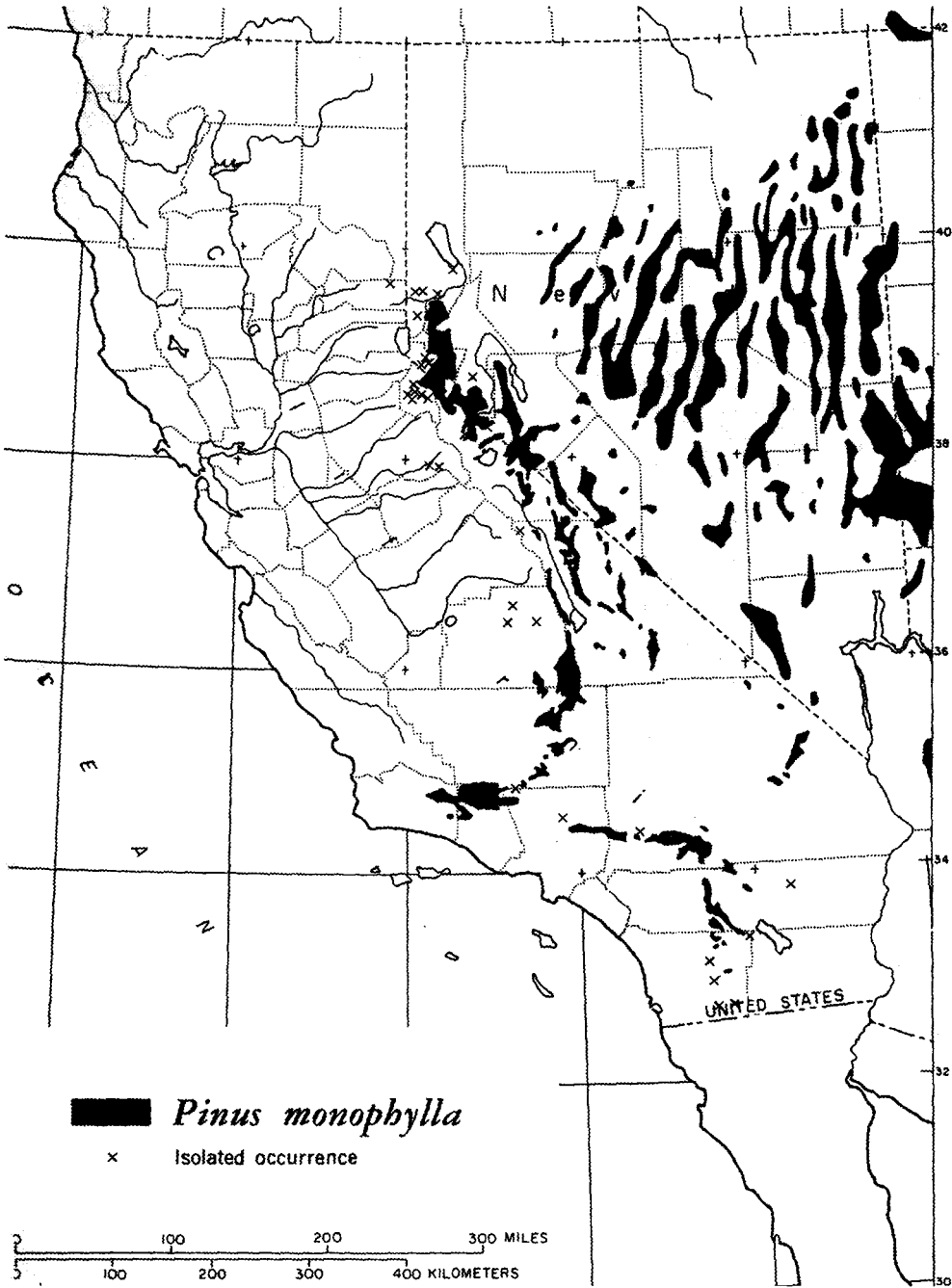


Table 3-31—Locations of six stands that were sampled for the monoterpene composition of xylem resin in *P. monophylla*

State locality and abbr.		Physiographic area	National Forest	Trees sampled
				no.
California				
Diamond Valley	DV	Carson Valley	Toiyabe	96
Mammoth Lake	ML	Owens Valley	Inyo	91
Wrightwood	WW	San Gabriel Mtns	Angeles	99
Pine Mountain	PM	Pine Mountain	Los Padres	47
Nevada				
Austin	AU	Toiyabe Range	Humboldt	100
Ely	EL	Ward Mountain	Humboldt	100

Table 3-32—Average monoterpene composition of the xylem resin in six stands of *P. monophylla*; see table 3-31 for location of stands; n = number of trees

Monoterpene	California				Nevada	
	DV ¹ n=96	ML n=91	PM n=47	WW n=99	AU n=100	EL n=100
	percent ²					
α -Pinene	79.8	77.4	76.4	80.3	85.0	84.3
Camphene	*	*	*	*	*	*
β -Pinene	0.4	0.4	0.7	0.4	0.0	0.0
3-Carene	4.0	2.2	2.9	3.0	0.4	0.4
Sabinene	2.1	5.8	4.9	3.1	1.8	2.1
Myrcene	6.4	5.1	4.9	4.5	5.1	5.3
Limonene	0.9	1.9	1.6	2.4	3.0	1.1
β -Phellandrene	*	*	0.2	*	*	*
Ocimene	3.5	3.2	4.5	3.6	2.3	3.6
Terpinolene	2.5	3.4	3.5	2.7	1.0	2.0

¹ Diamond Valley, Mammoth Lakes, Pine Mountain, Wrightwood, Austin, Ely.

² Normalized; * = trace.

Table 3-33—Comparison of the averages (x), and the ranges of the xylem monoterpenes of *P. monophylla*: from Zavarin and others (1971, 1980) and this report; n = number of trees sampled

Monoterpene	1971 n = 14		1980 n = 40 x ¹	1995 n = 533	
	x	range		x	range
-----percent ² -----					
α-Pinene	82.5	67 - 90.0	81.2	81.4	40 - 97
Camphene	0.5	0 - 1.0	2.2	*	*
β-Pinene	1.0	0 - 1.0	1.0	0.4	0 - 3
3-Carene	0.5	0 - 4.0	0.7	2.1	0 - 47
Sabinene	3.5	0 - 12.0	.0	3.0	0 - 24
Myrcene	6.0	3 - 13.0	6.3	5.3	0 - 22
Limonene	2.0	0 - 13.0	5.1	1.9	0 - 23
β-Phellandrene	*	0.1	1.2	*	*
Terpinolene	2.0	0 - 7.0	1.0	2.3	0 - 11
γ-Terpinene	.0	.0	*	.0	0
Ocimene	2.0	1 - 4.0	0.6	3.2	0 - 10
p-Cymene	.0	.0	0.5	.0	0
Total	100.0		99.8	99.6	

¹ No ranges given in Zavarin and others (1980).

² Normalized; * = trace

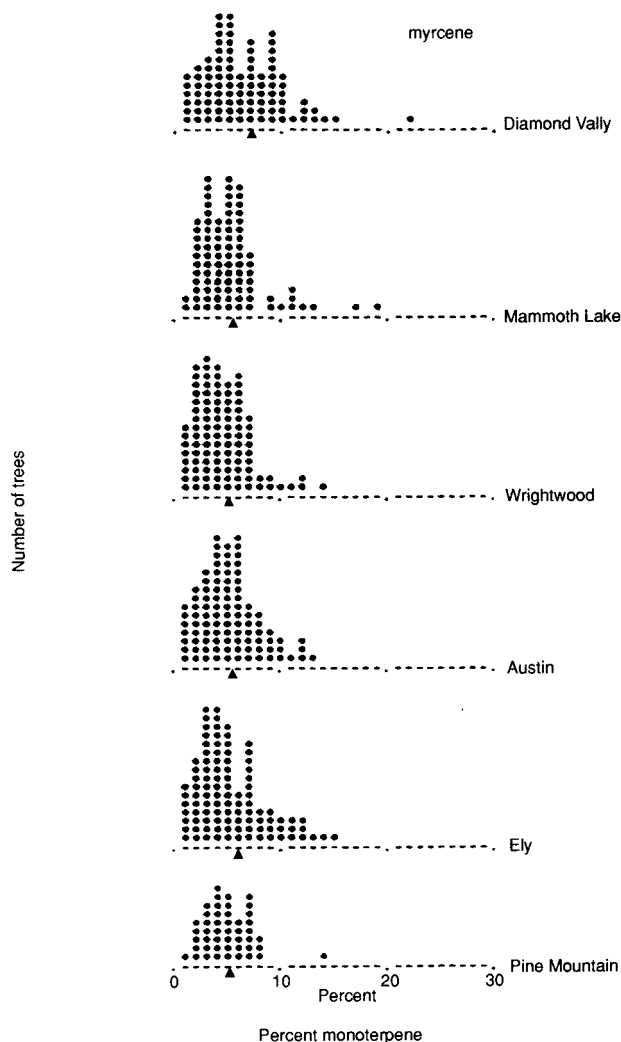
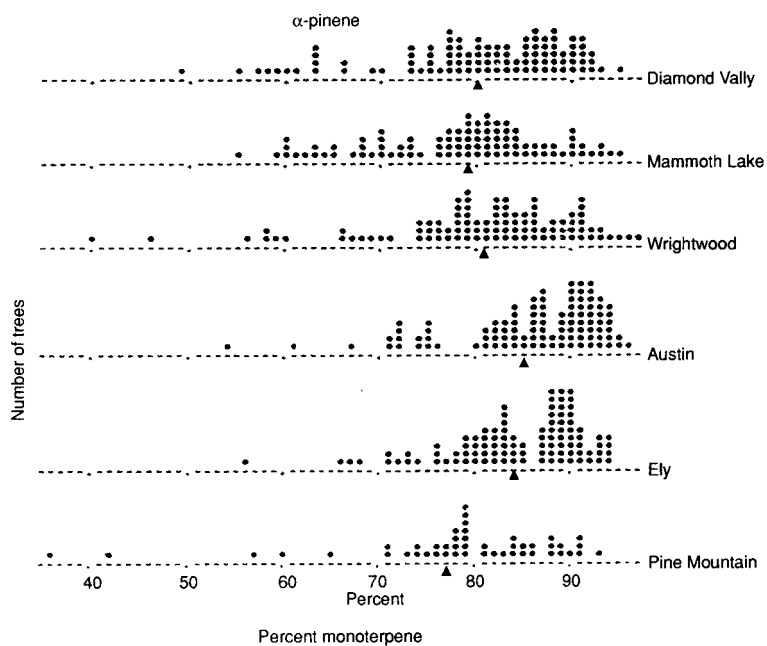
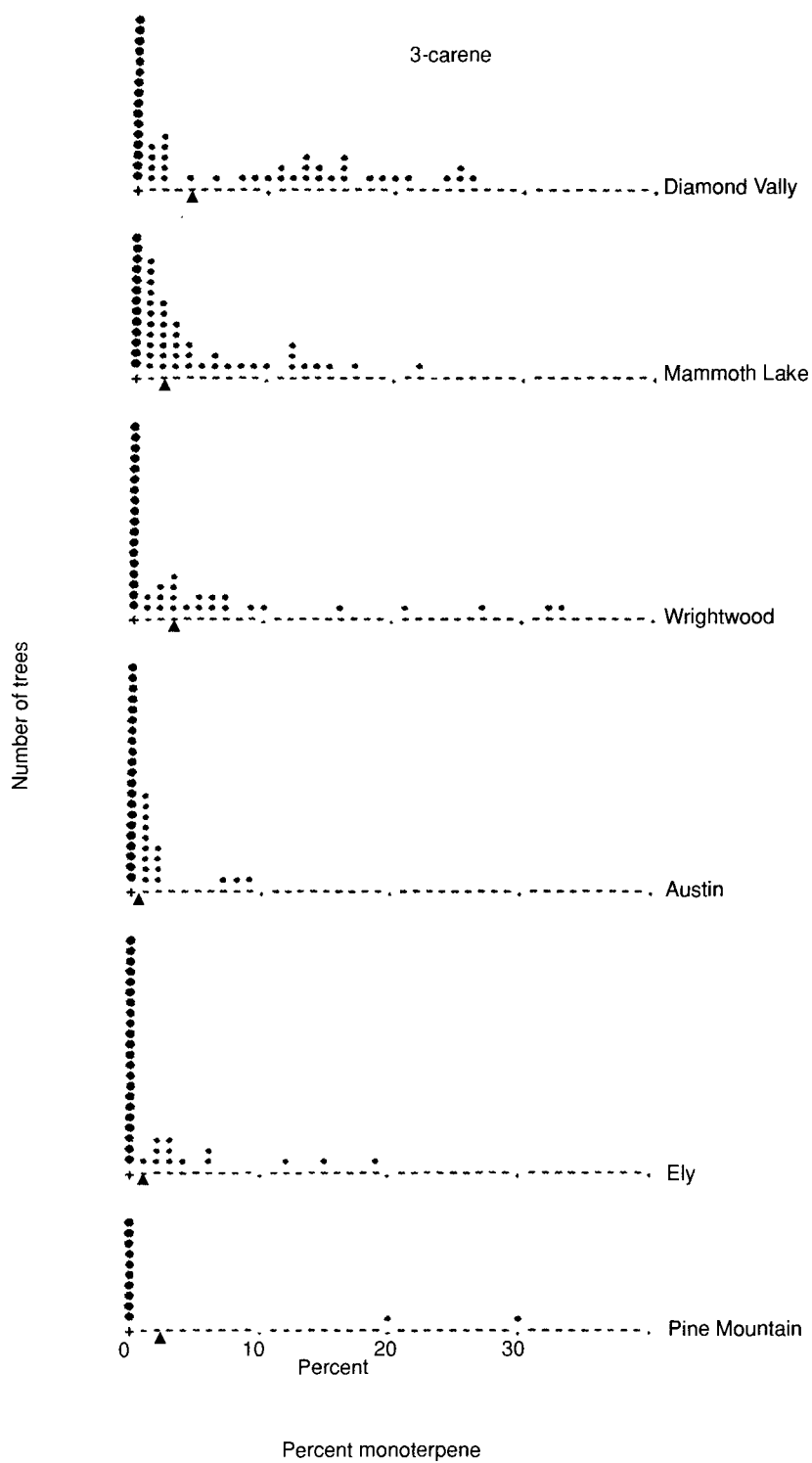
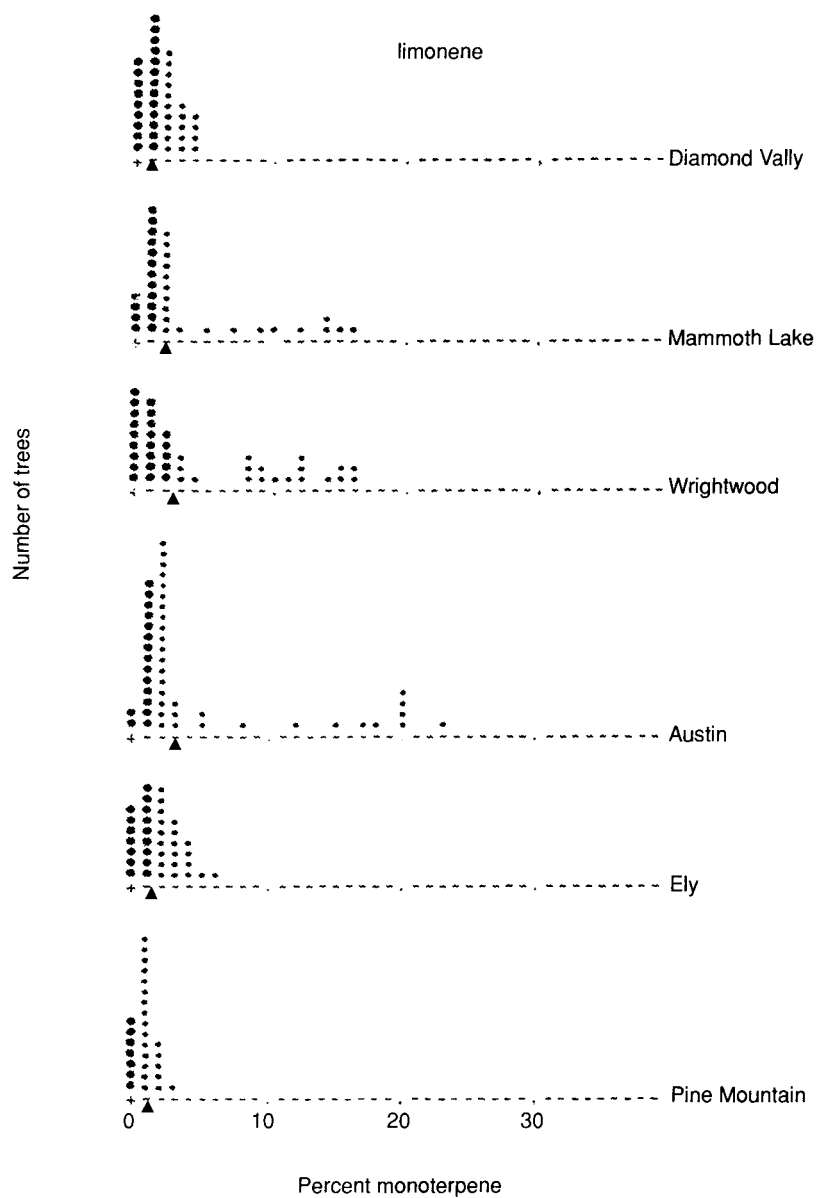
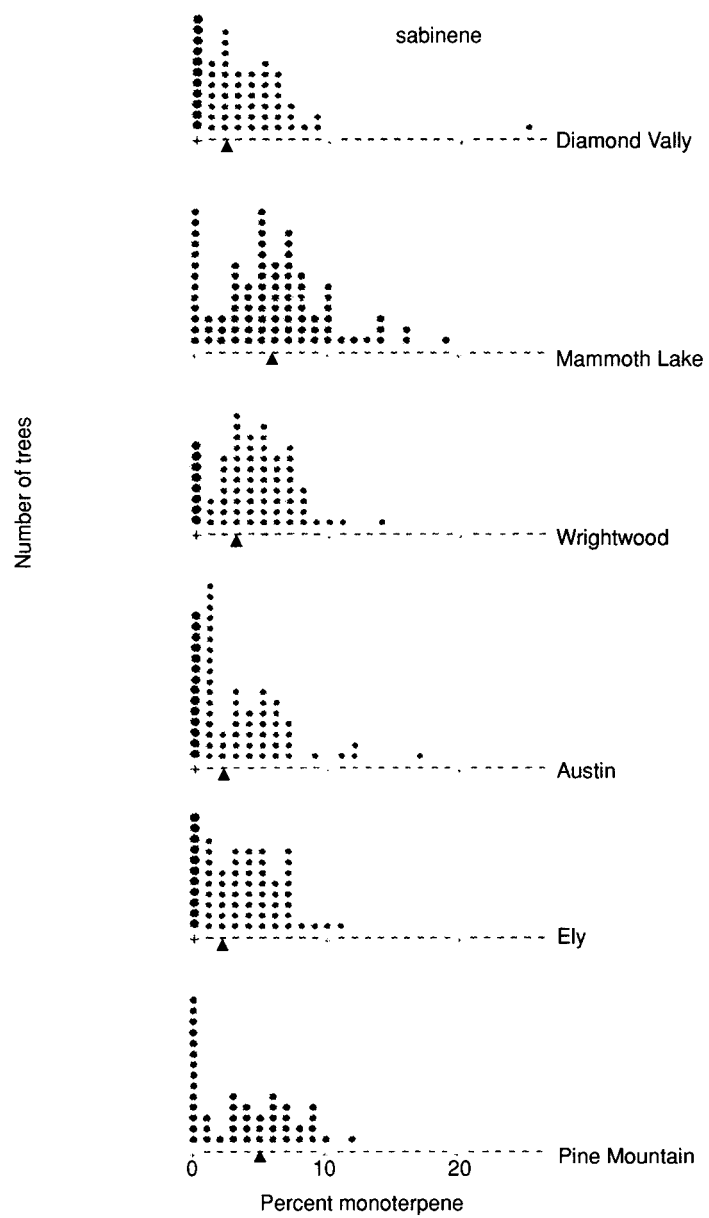


Figure 3-22—Frequency distribution of the amount of the noted monoterpenes in six stands of *P. monophylla*, listed on the right; each dot is measurement of one tree, except for sabinene, 3-carene, and limonene where each large dot indicates four trees and each small dot indicates one tree; ▲ is average of stand.

(fig. 3-22 continues on the next 4 pages).







occurrence were given code values of 0, 1, 2, (*table 3-34*). In *fig. 3-22*, each dot is one tree for α -pinene and myrcene, but for sabinene, limonene, and 3-carene each large dot is four trees and each small dot is one tree. The monoterpene composition for each tree was then expressed as a five-digit value in terms of the code values for α -pinene, 3-carene, sabinene, myrcene, and limonene. A total of 41 tree types of composition were identified; 28 occurred more than once, and 13 occurred only once (*table 3-35*). The individual trees varied from type 20000 to type 01211.

Five types of composition—20000, 10100, 20100, 10010, 10110—comprised about 70 percent of the trees (*fig. 3-23*). These five types were found in each of the five stands, but differed markedly in frequency. For example, type 20000 accounted for about 49 percent of the trees at Austin but only about 13 percent of the trees at Mammoth Lakes. Type 10100 accounted for about 38 percent of the trees at Mammoth Lakes but only about 9 percent of the trees at Diamond Valley. The types that did not occur in all stands varied markedly in presence and frequency. For example, type 00200 represented about 6 percent of the trees at Mammoth Lakes but was not found in the other stands.

The coefficient of association, based on the occurrence of types of composition, was calculated within and between stands; the within-stand calculation was based on a comparison of the first and second half of the stand in order of sampling.

All coefficients of association within stands were about 0.90; coefficients between stands were considerably lower, suggesting that the five stands were different (*fig. 3-24*). The coefficients were strongest between the three northern stands, Diamond Valley, Ely, and Austin. Surprisingly, two of the most distant stands, Ely and Wrightwood, had a comparatively strong association of 0.45; and two of the closest stands, Diamond Valley and Mammoth Lakes, had a comparatively weak association of 0.12. Could the 0.90 coefficient of association between the two halves of the stands be a measure of inherent local variation?

In some pine species, it is possible to determine the relation of 3-carene to terpinolene, and of sabinene to terpinolene as noted earlier for *P. albicaulis* and *P. lambertiana*. *P. monophylla* presented an opportunity to look at these two relations in the same species. There were 59 trees with only sabinene and terpinolene and nine trees with only 3-carene and terpinolene. There were many other trees with all three components; these could not be used for determining ratios. The results of the analysis of the ratios showed the ratio of 3-carene to terpinolene was about 6:1, and the sabinene-to-terpinolene ratio was between 2:1 and 1:1 (*fig. 3-25*). Both ratios were different from other pines. The ratio of 3-carene to terpinolene found in most pines was about 20:1, the ratio of sabinene-to-terpinolene varied from about 1:1 to 3:1.

The sampling of the species did not include areas where *P. monophylla* could hybridize with *P. edulis* in the east or with *P. quadrifolia* in the southwest. Lanner (1974) reported considerable morphological evidence of hybridization of *P. monophylla* with *P. edulis* where two species occur together in western Utah. Zavarin and others (1980) report on possible hybridization of *P. monophylla* with *P. quadrifolia* in southern California and Baja California.

Zavarin and Snajberk (1980) also proposed three subspecies for *P. monophylla* as follows:

1. Subsp. *monophylla* in the major portion of the range north and west of a line from Los Angeles County to about Lake Mead.
2. Subsp. *californium* in the area south of the demarcation noted in (1).
3. Subsp. *fallax* in the area of northeastern Arizona.

The scope of the work reported in this volume did not permit comment on this subdivision of *P. monophylla*; all stands that were sampled were within the

Table 3-34—Intervals that were used to define the low (0), medium (1), and high (2) modes in the frequency distribution of the individual monoterpenes of *P. monophylla*

Monoterpene	Code value		
	0	1	2
-----intervals in percent-----			
α-Pinene	0 - 70.4	70.5 - 84.4	84.5 - 100
3-Carene	0 - 4.4	4.5 - 11.4	11.5 - 100
Sabinene	0 - 2.4	2.5 - 10.4	10.5 - 100
Myrcene	0 - 7.4	7.5 - 16.4	16.5 - 100
Limonene	0 - 5.4	5.5 - 16.4	16.5 - 100

Figure 3-23—Occurrence of monoterpene composition types in five stands of *P. monophylla* at Austin (AU), Ely (EL), Diamond Valley (DV), Wrightwood (WR), Mammoth Lakes (ML); see table 3-35 for actual composition of coded types.

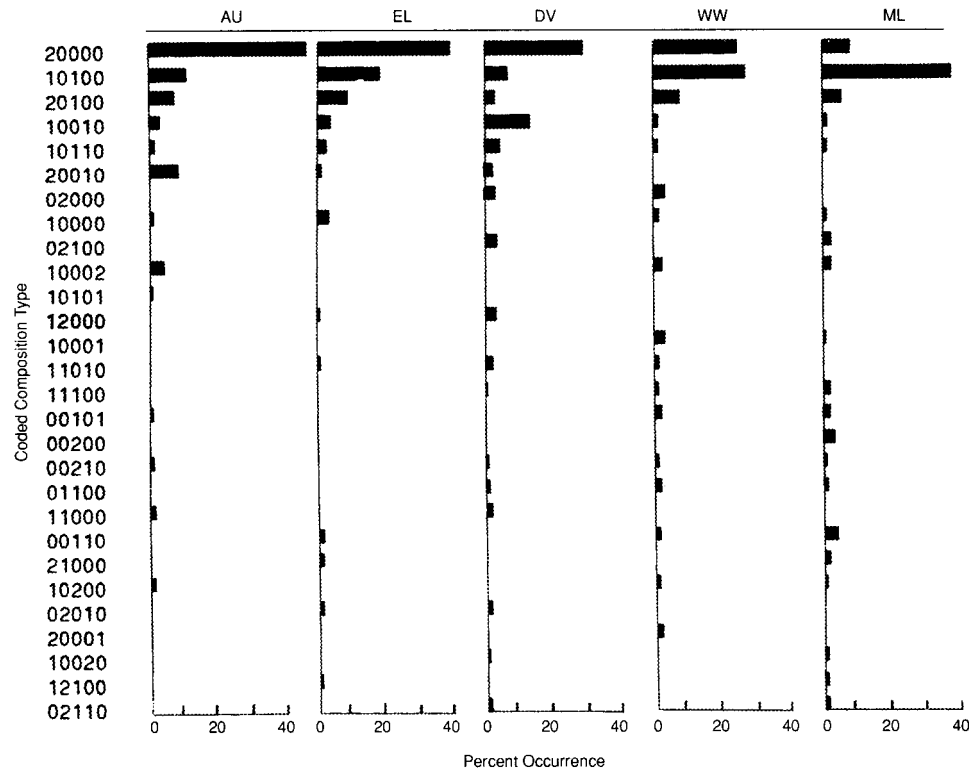


Table 3-35—Twenty-eight coded tree¹ types of composition found in *P. monophylla*, their actual composition, and their occurrence in the species; see figure 3-23 for occurrence of types in each stand.

Coded type ²					Composition Actual ^{2,3}						Occurrence in the species ⁴
α	3	s	m	ℓ	α -p	car	sab	myr	lim	O+T	
-----percent -----											-----percent -----
2	0	0	0	0	90.5	0.2	0.4	4.2	0.9	3.5	31.7
1	0	1	0	0	80.3	0.4	5.7	4.7	0.9	7.4	21.4
2	0	1	0	0	87.0	0.2	4.1	3.0	0.7	4.8	8.6
1	0	0	1	0	81.4	0.5	0.5	11.0	1.3	5.0	6.2
1	0	1	1	0	76.4	0.1	5.0	9.4	1.1	7.6	3.4
2	0	0	1	0	86.3	0.1	0.4	8.7	0.6	2.6	3.1
0	2	0	0	0	57.9	26.8	0.4	6.1	1.0	7.7	2.0
1	0	0	0	0	81.7	1.3	0.9	5.6	1.3	8.6	1.8
0	2	1	0	0	59.4	19.3	5.9	4.7	0.7	11.2	1.8
1	0	0	0	2	73.0	0.2	0.4	2.3	20.0	2.9	1.4
1	0	1	0	1	75.6	0.5	4.2	1.7	12.4	4.8	1.4
1	2	0	0	0	75.2	15.5	0.3	3.5	1.9	4.2	1.4
1	0	0	0	1	78.9	0.0	0.3	2.8	12.9	6.0	1.2
1	1	0	1	0	76.5	7.7	0.0	9.3	1.0	5.1	1.2
1	1	1	0	0	75.7	6.8	4.7	3.9	0.9	7.8	1.0
0	0	1	0	1	66.1	0.6	7.5	3.0	13.0	8.2	1.0
0	0	2	0	0	65.7	0.0	15.2	5.1	2.0	11.0	1.0
0	0	2	1	0	60.4	0.4	14.7	11.9	1.0	11.2	1.0
0	1	1	0	0	67.7	7.0	7.0	5.0	1.6	11.2	1.0
1	1	0	0	0	81.5	8.5	0.6	1.9	1.8	4.7	0.8
0	0	1	1	0	67.0	0.6	8.8	12.2	1.4	9.6	0.8
2	1	0	0	0	87.3	5.9	0.0	3.4	0.3	3.0	0.6
1	0	2	0	0	73.6	0.7	14.2	4.5	0.5	5.8	0.6
0	2	0	1	0	66.8	19.6	0.0	9.2	0.7	5.0	0.6
2	0	0	0	1	86.1	0.0	0.1	1.9	7.8	3.6	0.4
1	0	0	2	0	75.9	1.1	0.0	17.1	1.5	3.6	0.4
1	2	1	0	0	72.4	12.1	4.7	4.5	0.1	5.9	0.4
0	2	1	1	0	59.2	15.2	7.1	9.4	0.7	7.5	0.4

¹ 15 types (not tabled, occurred once: 10011, 10102, 10111, 10210, 11001, 00020, 01001, 01101, 01200, 02101, 02210, 00100, 00120, 00010, 01211, 00100, 00120, 00010).

² α -pinene, 3-carene, sabinene, myrcene, limonene, ocimene + terpinolene.

³ Normalized for each coded type.

⁴ Normalized for all trees in all stands.

Figure 3-24—Coefficient of association, r , indicated by numbers on lines, between stands of *P. monophylla* based on the occurrence of types of monoterpene composition; distribution of species was taken from Griffen and Critchfield (1972). AU = Austin, DV = Diamond Valley, EL = Ely, ML = Mammoth Lakes, WW = Wrightwood.

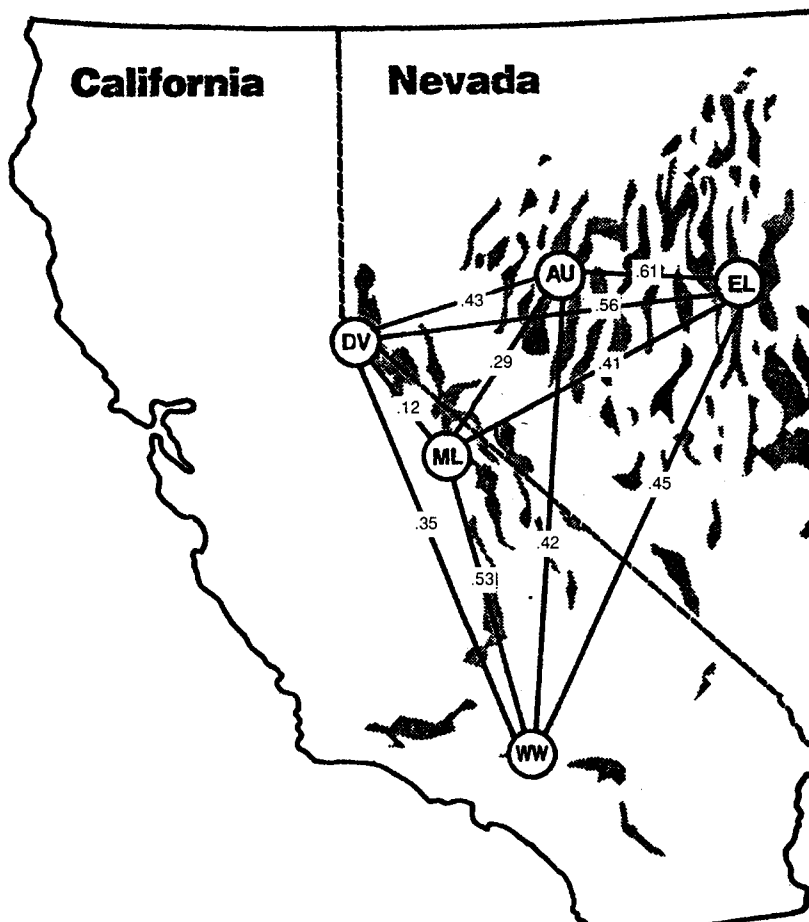
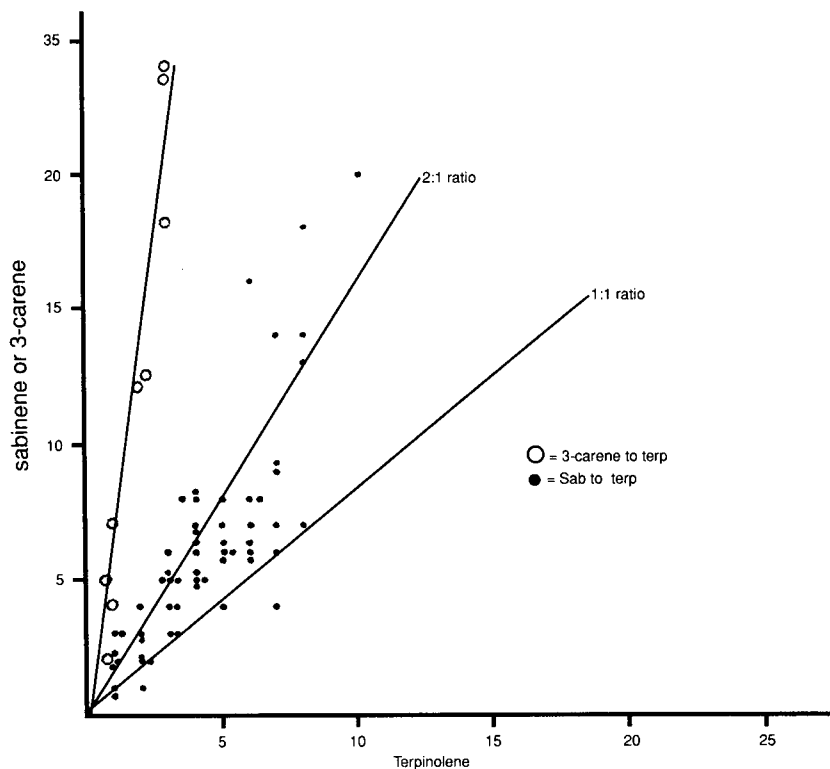


Figure 3-25—Correlation of the amounts of 3-carene/terpinolene ($\circ$) and of sabinene/terpinolene ($\bullet$) in the xylem resin of *P. monophylla*.



region assigned to subsp. *monophylla*. Likewise, it appeared that the taxonomy and relationships of *P. monophylla* and *P. edulis* cannot be adequately resolved on the basis of xylem monoterpenes. There were only small differences between the means of the two widespread pinyons, *P. monophylla* and *P. edulis*, and there was considerable overlap in the maximum and minimum of the individual monoterpenes, at least as could be determined from the S.D. values of *P. edulis* reported by Zavarin and others (1980).

Anderson and others (1970) found quite a different composition when extracting monoterpenes by steam distillation of mill-ground xylem: 46 percent α -pinene, 22 percent myrcene, 8 percent β -phellandrene, 7 percent limonene, 1 percent camphene, 2 percent β -pinene, 5 percent γ -terpinene and 4 percent terpinolene. Reports on other pines using steam distillation of mill-ground xylem usually differed from the composition reported by GLC analysis of fresh resin. Apparently steam distillation of xylem changed the composition of the monoterpenes. However, there was no change in composition with steam distillation of fresh resin followed by vacuum separation by Mirov (1961) or by short-path distillation of fresh resin at atmospheric pressure (see Chapter 8).

The genetic structure of α -pinene was likely the same in all *P. monophylla* reported in this chapter: a (1-1) set of additive alleles at a single locus and variation in composition were caused by differing alleles for 3-carene, sabinene, myrcene, and limonene. Thus the condition is much like that noted for β -phellandrene in *P. contorta* earlier in this chapter. Though the average composition for 3-carene, sabinene, myrcene, and limonene was low (table 3-32), well below 6 percent, there were individual trees with a large enough amount to be considered of genetic origin, albeit a rare allele (table 3-35, fig. 3-22).

#12. *Pinus monticola* Dougl. (western white pine)

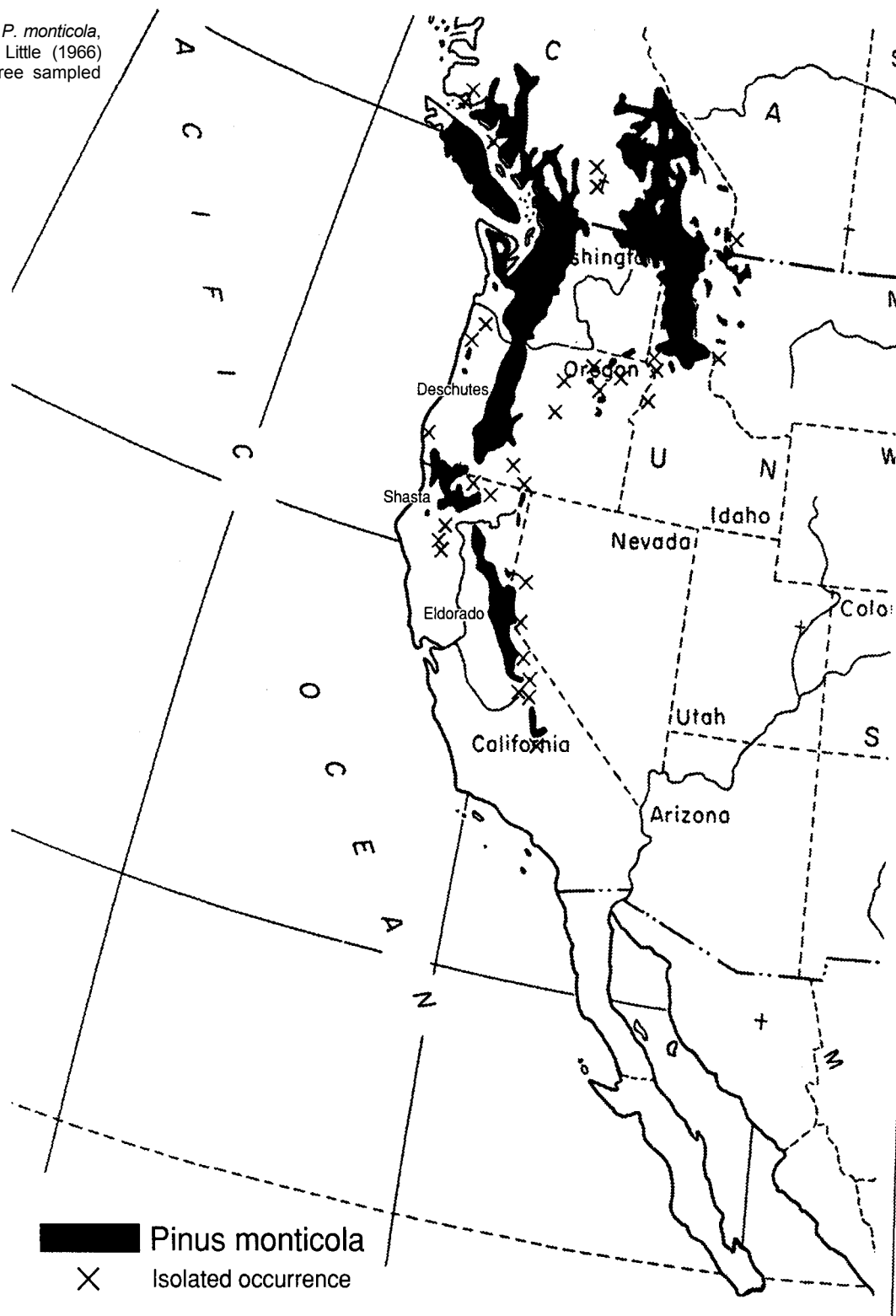
P. monticola is widely distributed in western North America, and its range can be viewed as two nearly disjunct sections. A Rocky Mountain section ranges from southeastern British Columbia southward to north central Idaho, northeastern Washington, and northwestern Montana with scattered stands in northeastern Oregon (fig. 3-26). A western section extends from southwestern British Columbia southward along the Cascades, Klamath Mountains, and the Sierra Nevada to central California.

Mirov (1961) sampled trees from the Warner Mountains and determined that the xylem resin was composed of about 60 percent α -pinene, 26 percent β -pinene, and 2 percent undecane. He later sampled trees from Priest River in northern Idaho and found the following results: 2 percent heptane, 32 percent α -pinene, 45 percent β -pinene, 7 percent limonene, 2 percent undecane, and 12 percent others as well as sesquiterpenes.

Hanover (1966) analyzed the cortex resin of 1- to 3-year-old tissue of older trees and compared the variability in α -pinene, β -pinene, myrcene, 3-carene, and limonene. He found a considerable range in composition between trees and little variation within trees.

Zavarin and others (1990) conducted an extensive sampling of the xylem monoterpenes of *P. monticola* in which they also found a large range in several

Figure 3-26—Distribution of *P. monticola*, taken from Critchfield and Little (1966) and the location of the three sampled stands.



of the components. They sampled 20 stands of about 10 trees each in a fairly comprehensive sampling of the tree's range.

I sampled three stands between 1983 and 1985 as follows (*fig. 3-26*):

Stand no.	National Forest	Locality	Number of trees
1	Eldorado	Echo Summit	104
2	Shasta-Trinity	Lake Siskiyou	60
3	Deschutes	Lookout Mountain	16

The results showed that the three localities differed markedly in average composition (*table 3-36*) and that there was a great range in the frequency distribution of the individual components (*fig. 3-27*). Though α -pinene occurred most abundantly, a large number of trees had considerable amounts of undecane, β -pinene, 3-carene, limonene, and β -phellandrene. There were also some trees with moderate amounts of sabinene and camphene. The ratio of sabinene to terpinolene was about 2:1, thus much like *P. lambertiana*. The averages reported by Zavarin and others (1990) for 10-tree plots near the three stands reported here were somewhat similar, but a 10-tree stand appeared to be inadequate for describing the composition of a species with such great variability. When all three stands were viewed together, a trimodal distribution can be defined and coded for α -pinene, β -pinene, 3-carene, myrcene, and limonene (*table 3-37*). Using the modal criteria in *table 3-37*, all trees were coded and the frequency of types of composition was determined (*table 3-38*). These showed a great range in composition types in the Eldorado stand, with a total of 38 types, Shasta had only seven types, and Deschutes nine types, though there were only 16 trees in the sample (*fig. 3-28*).

Of the 38 types in Eldorado, 18 occurred only once. No type was abundant in all three stands. For example, there were 33 trees in aggregate in types 12000, 12001, 12002, and 12011 in the Eldorado stand, while Shasta and Deschutes had only one tree with these same four types. Likewise, Shasta had 41 trees in aggregate in types 20000 and 20001, while Eldorado had only two. Two trees of the 10100 type had unusually large amounts of (3-phellandrene: one with 24.6 percent and one with 23.4 percent. One of the trees in type 12000 had 18.4 percent β -phellandrene, and one tree in type 02000 had 21.5 percent. The 21000 type was the only one that was fairly common in all three stands: 4 percent in Eldorado, 16 percent in Shasta, and 30 percent in Deschutes.

The coefficient of association for the Eldorado and Shasta stands was 0.0. No determination was made for the Deschutes stand because it had only 16 trees.

A comparison of my study (reported above) and that of Zavarin and others (1990) cannot be made with confidence because the latter reported only average composition and did not give kinds of individual tree composition nor frequency distribution characteristics. However, it is readily apparent that there was a great range in the amount of α -pinene, β -pinene, 3-carene, sabinene, myrcene, limonene, and β -phellandrene. There was considerable similarity in the ranges of the individual components found by Zavarin and others (1990) in 20 stands located throughout the tree's distribution, and in the ranges reported here (*table 3-39*) from three stands located in the southwest portion of the tree distribution. The evidence suggested the advisability of a more intensive and extensive study of the xylem monoterpenes of *P. monticola*.

Table 3-36—Average monoterpene composition of the trees in three stands of *P. monticola* in three National Forests; n = number of trees in stand

Component	National Forest		
	Deschutes	Shasta	Eldorado
	n=16	n=60 percent ¹	n=104
Nonane	1.6	1.1	1.5
α -Pinene	71.1	82.5	53.0
Undecane	2.0	3.0	5.6
β -Pinene	14.1	9.1	21.7
3-Carene	3.1	0	7.0
Sabinene	1.0	0.4	0.2
Myrcene	1.2	1.0	1.9
Limonene	1.6	2.3	6.4
β -Phellandrene	3.5	0.4	1.6
Terpinolene	0.7	0.2	0.7

¹ Normalized.

Table 3-37—Intervals of the frequency distribution of the normalized percentages that were used to define the low (0), medium (1), and high (2) modes of the five noted monoterpenes of *P. monticola*

Monoterpene	Mode		
	0	1	2
	intervals in percent ¹		
α -Pinene	0 - 44	45 - 62	63 - 100
β -Pinene	0 - 10	11 - 20	21 - 100
3-Carene	0 - 10	11 - 30	31 - 100
Myrcene	0 - 2	3 - 5	6 - 100
Limonene	0 - 2	2 - 10	11 - 100

¹ Normalized.

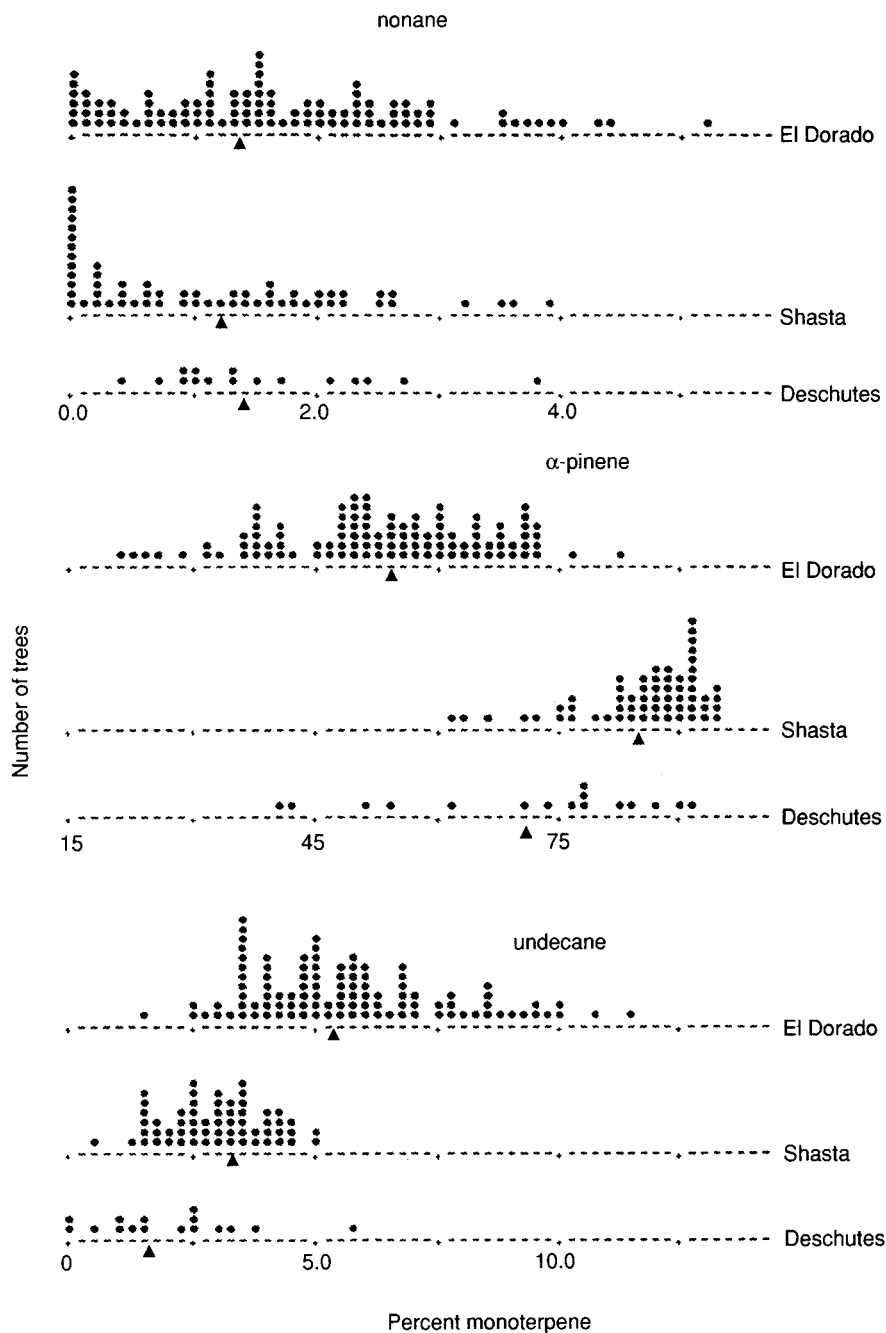
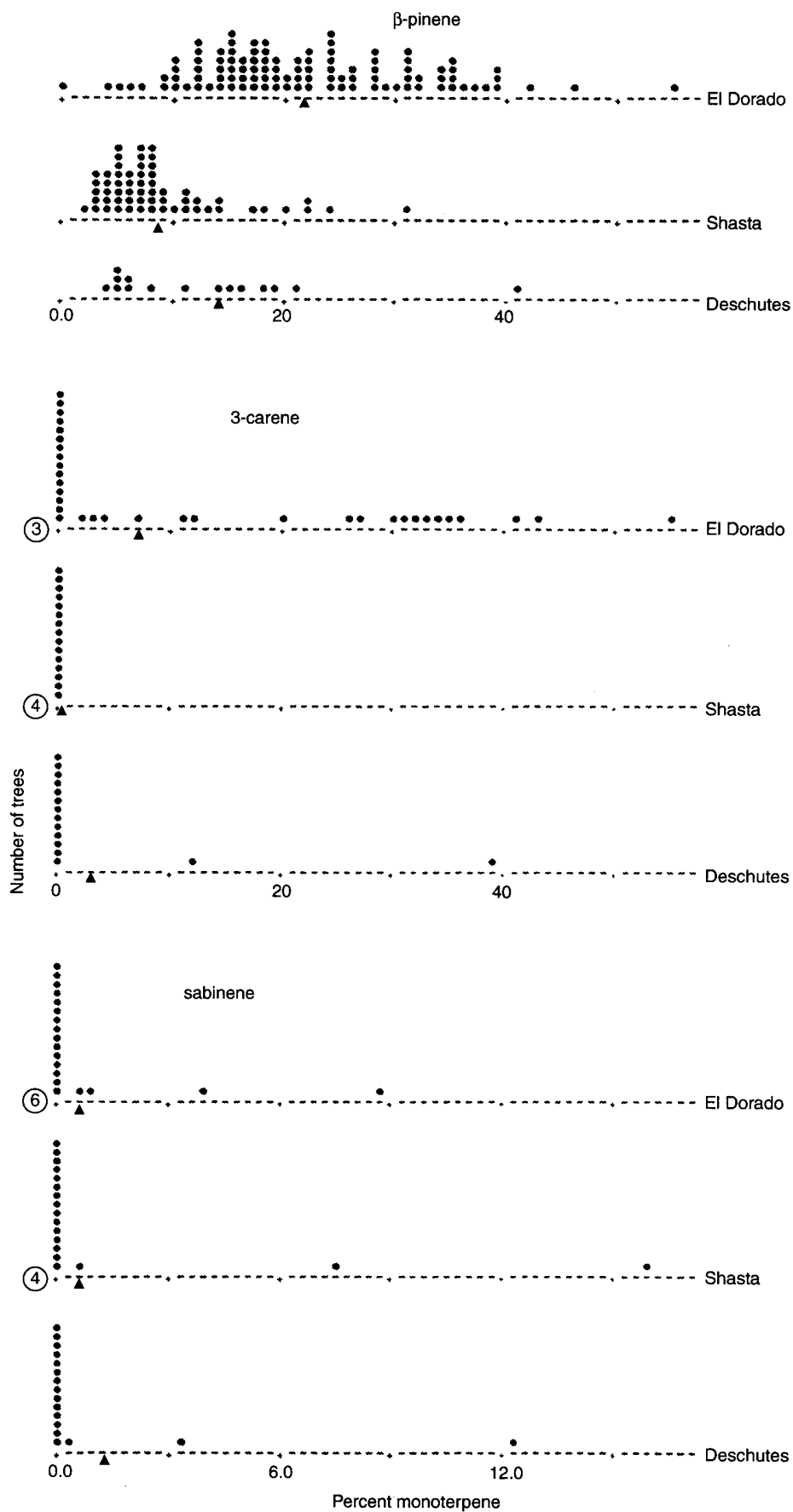
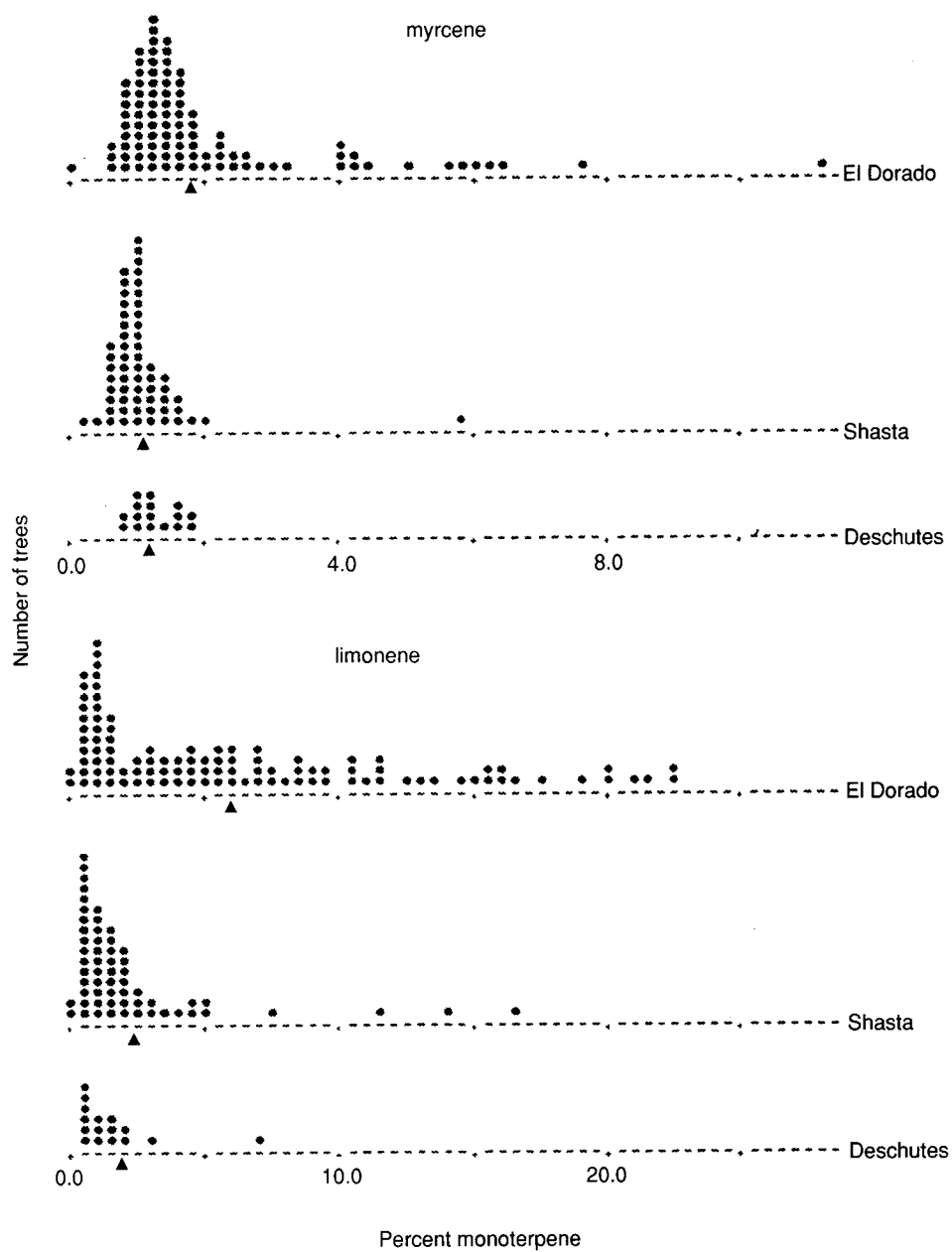
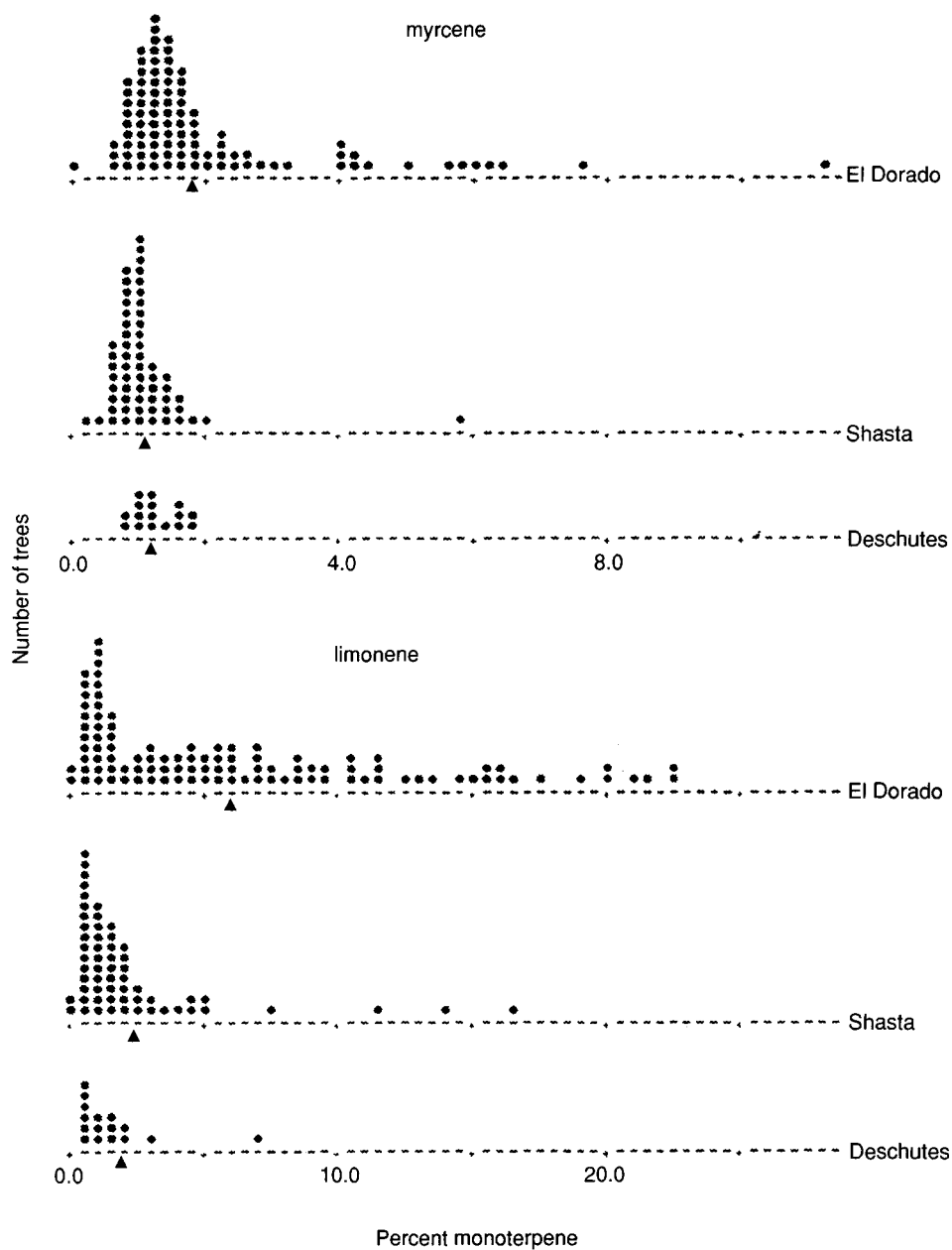


Figure 3-27—Frequency distribution of the amount of the 11 noted xylem monoterpenes and related hydrocarbons in stands of *P. monticola* in the three National Forests listed on the right; each dot is measurement of one tree except where $\textcircled{n}$ indicates each represents n trees; $\blacktriangle$ is average of stand.

(fig. 3-27 continues on the next 4 pages).







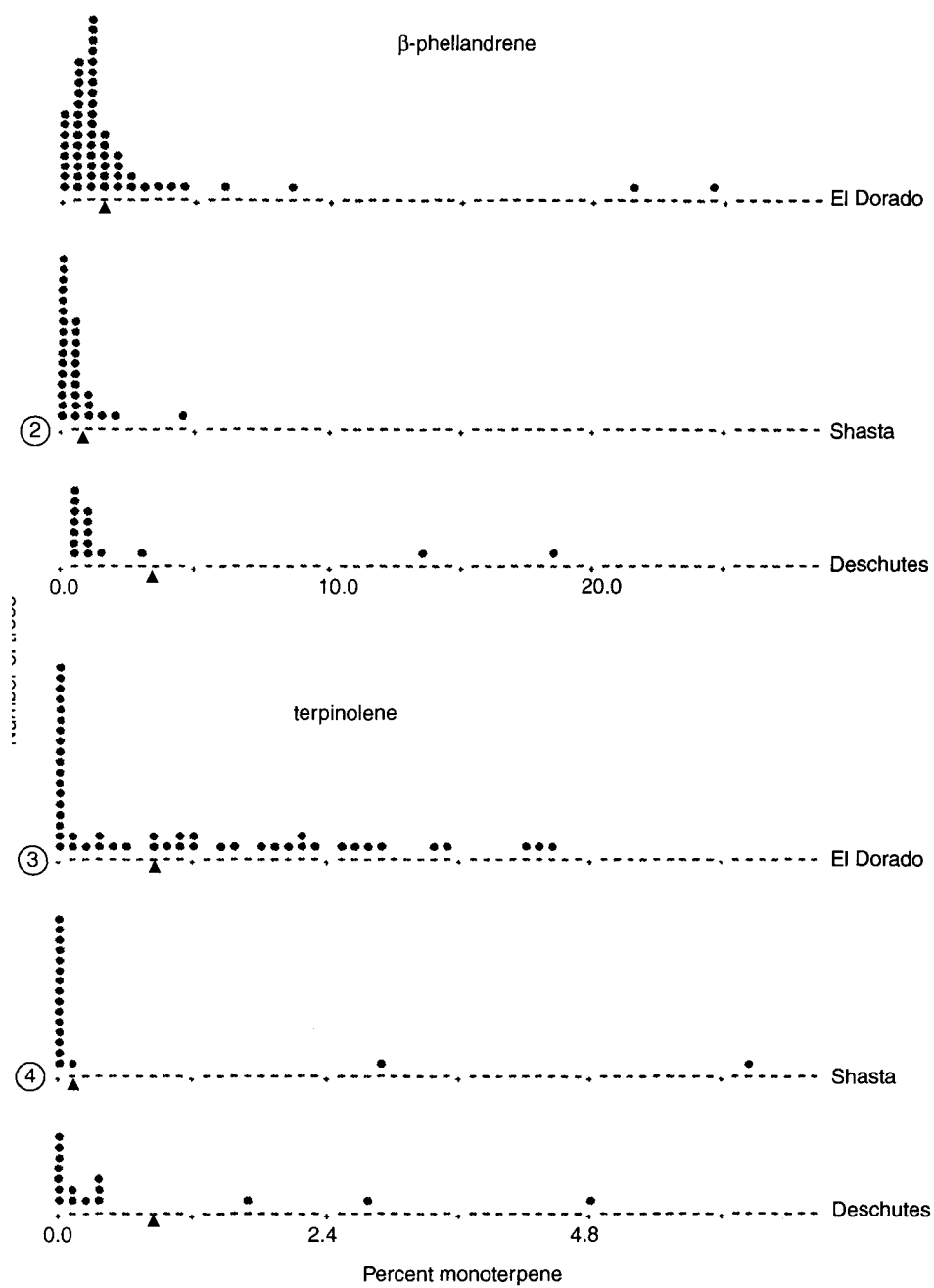


Table 3-38—Coded types of composition of *P. monticola*, their occurrence (*n*) in all trees, and their average actual composition

Code ¹	n	Monoterpenes ²									
		und	α -p	cam	β -p	car	sab	myr	lim	β -ph	ter
		-----percent ³ -----									
00200	1	0.9	42.3	1.5	7.9	38.7	0	1.7	0.6	3.2	2.8
00201	1	0.6	36.6	3.9	10.1	36.4	0	1.7	7.2	1.4	2.1
00212	1	2.0	28.3	5.1	6.0	33.8	0	2.5	16.6	1.5	4.2
01200	3	0.8	34.7	6.2	15.3	37.1	0	1.7	0.5	1.4	2.1
01201	5	1.7	36.5	5.7	11.9	34.3	0	1.6	4.6	1.4	2.2
01210	3	1.5	37.8	6.0	15.4	31.5	0	3.9	0.7	0.7	2.3
01211	1	5.2	26.0	9.3	15.5	35.5	0	2.6	3.0	0.8	2.0
02000	5	1.3	37.6	5.4	44.0	1.9	0.1	1.3	1.2	5.6	0.8
02001	1	4.3	37.2	11.6	34.6	0	0	1.3	9.1	0.5	1.2
02002	1	0.3	39.9	5.2	31.2	0	0	1.5	20.1	1.8	0
02021	1	2.2	41.4	10.7	34.6	0	0	5.8	4.6	0.6	0
02200	2	0.8	28.1	4.2	16.2	43.1	0	1.9	1.2	1.3	3.3
02201	2	0.6	27.1	2.6	24.8	36.0	0	1.9	4.2	1.1	1.8
10002	1	2.7	62.0	9.0	10.2	0	0	1.3	14.5	0	0.2
10022	1	1.4	60.2	3.9	9.9	0	0	5.6	17.7	0.4	0
10122	1	1.6	48.5	5.7	3.5	11.2	0	6.3	21.7	0	1.2
10200	1	2.6	56.6	5.4	5.4	26.7	0	1.2	0.3	0.4	1.1
11000	1	1.3	52.0	3.5	19.0	0	8.6	0.8	0.6	8.6	4.3
11001	4	2.0	61.0	5.3	17.0	0	3.1	1.0	6.5	2.0	1.2
11002	6	1.3	54.8	6.9	17.4	0.6	0	1.4	16.6	0.8	0.2
11021	1	2.0	58.5	5.5	15.8	0	0	6.0	7.1	4.0	0.5
12000	14	1.4	55.3	4.6	35.2	0	0.3	1.2	1.0	2.7	0.3
12001	10	1.7	52.3	5.3	29.6	1.0	0	1.3	6.9	1.5	0.3
12002	7	1.3	48.4	5.2	25.8	0.5	0.1	1.4	16.3	0.9	0.1
12011	4	2.8	51.8	8.3	27.9	0	0.2	3.7	5.0	0.8	0.4
12021	2	2.5	47.5	6.2	23.1	0	0.4	8.6	8.8	1.6	1.4
12101	1	0.0	53.7	3.0	23.8	12.4	0	1.3	5.3	0.4	0
20000	36	1.2	89.1	2.9	6.5	0	0.3	0.9	1.1	0.4	0.1
20001	13	1.2	84.3	2.8	6.3	0	1.3	1.2	4.4	0.1	0.5
20002	5	1.2	75.1	2.5	6.0	0	0.4	1.0	14.7	0	0.1
20020	1	0	97.9	1.7	5.7	0	0	5.7	0	0.7	0
21000	19	1.3	78.9	3.6	14.7	0	0	0.9	1.2	0.8	0.2
21001	8	2.5	66.8	6.9	15.8	0.5	0	1.2	5.6	0.6	0.1
21002	2	0.5	68.1	5.4	13.1	0	0	1.0	11.1	0.5	0.4
21010	1	1.3	66.8	4.7	18.4	0	0	4.1	1.4	0.8	2.2
21011	2	0.9	66.1	5.1	17.0	0	0	3.8	6.8	0.4	0
21120	1	2.7	68.4	4.7	14.8	0	0	7.5	1.0	0.9	0
22000	9	1.3	70.1	3.8	24.0	0	0	1.0	0.8	0.7	0
22001	1	1.1	64.3	2.8	22.1	0	0	2.2	6.7	0.9	0
22002	1	1.4	70.2	4.5	11.7	0	0	1.7	10.5	0	0

¹ From left to right: α -pinene, β -pinene, 3-carene, myrcene, limonene for code types.² Actual composition: undecane, α -pinene, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, terpinolene.³ Normalized.

Table 3-39—Maximum and minimum percentage for individual monoterpenes in the xylem resin of *P. monticola* report in two sources: (A) derived from this report, (B) derived from Zavarin and others (1990)

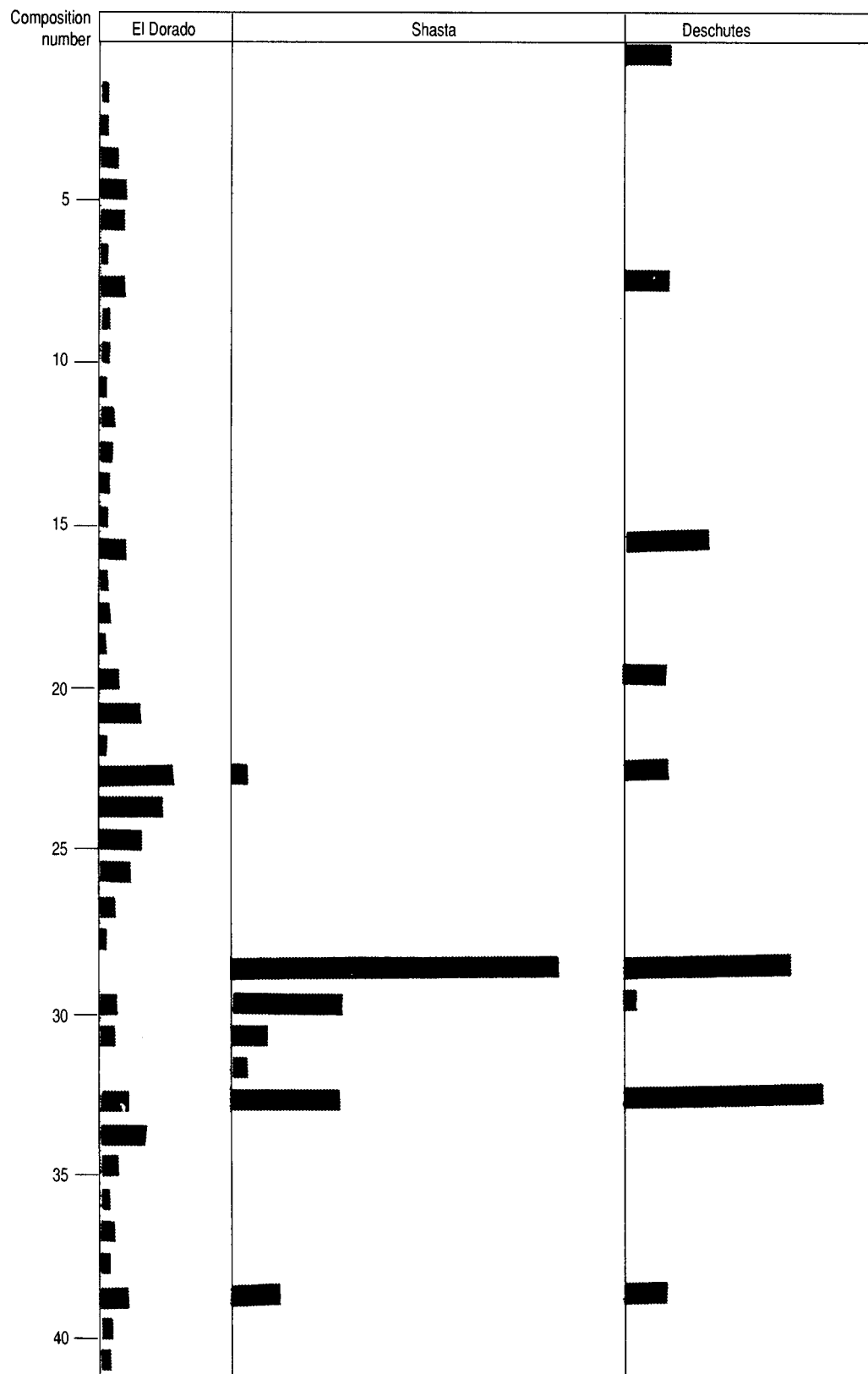
Monoterpene	A n=180 ¹		B n=191 ²	
	Maximum	Minimum	Maximum	Minimum
	-----percent ³ -----			
Nonane	5	0	0	0
α -Pinene	94	20	95	4
Undecane	12	0	46	0
β -Pinene	55	0	51	0
3-Carene	55	0	68	0
Sabinene	16	0	28	0
Myrcene	11	0	14	0
Limonene	23	0	68	0
β -Phellandrene	25	0	18	0
Terpinolene	6	0	0	0

¹ Three stands, southwest portion of range.

² Twenty stands, throughout the range.

³ Normalized.

Figure 3-28—Occurrence of 41 coded types of composition in three stands of *P. monticola*; frequency of types normalized for each stand; see table 3-38 for actual average composition of coded types.



#13. *Pinus muricata* D. Don. (Bishop pine)

P. muricata is essentially limited to California with its southern limits just extending into northern Baja California (fig. 3-29). It is a coastal species, generally found within just a few miles of the ocean; but it has been successfully planted at the Institute of Forest Genetics (IFG) in the Sierra Nevada foothills more than 100 hundred miles from the ocean. It has also been successfully planted in Australia and New Zealand.

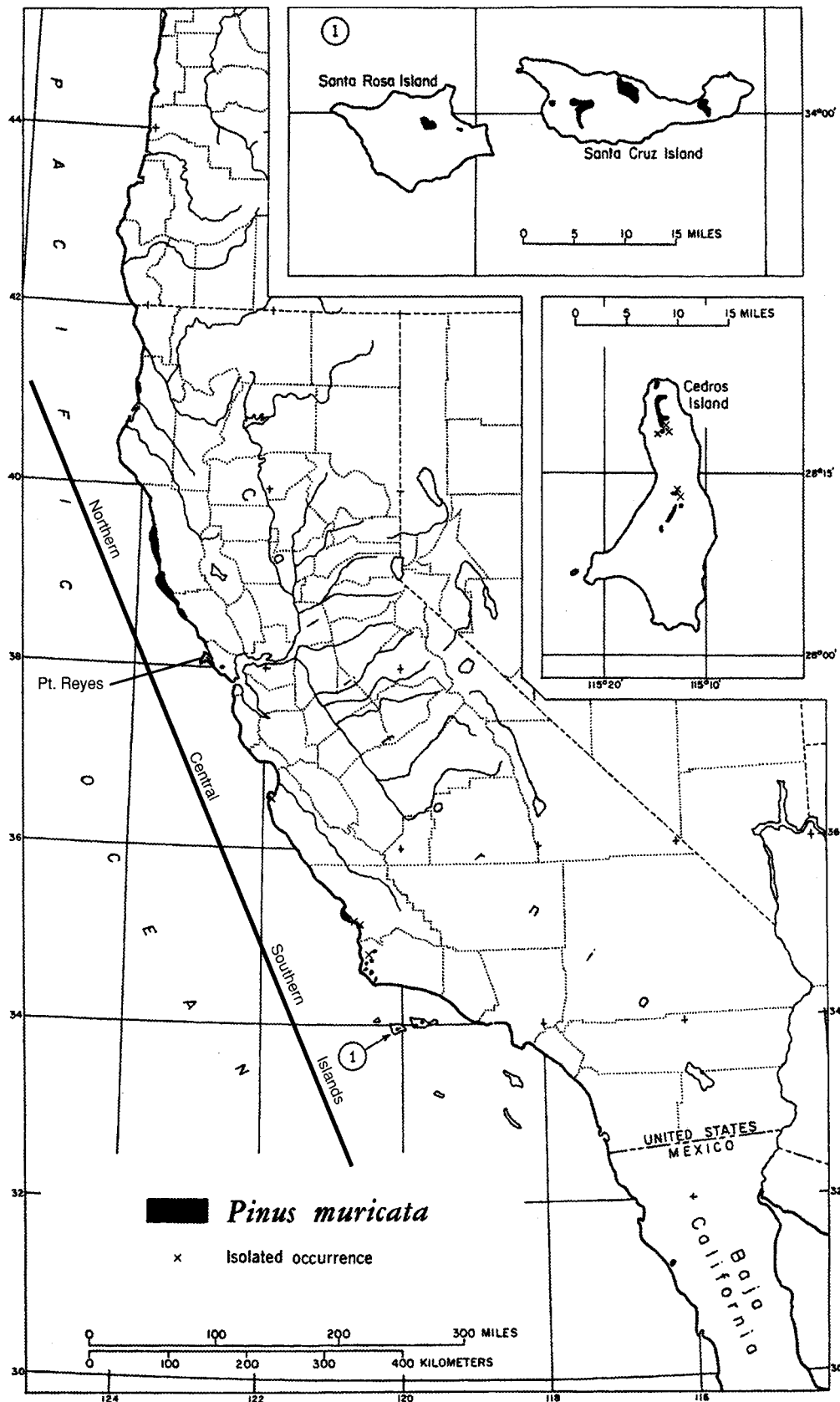
The study of the xylem monoterpenes of *P. muricata* is a most interesting story. In 1947, Mirov (1961) first examined resin from trees from the northern portion of the range that were growing at the Institute of Forest Genetics and found the xylem monoterpene portion to be near-pure α -pinene. Then Duffield (1951), finding sharp morphological differences between the northern, central, and southern stands, as well as those on the Channel Island, proposed four species or four varieties. Mirov (1961) then looked at trees growing at IFG which represented the four groups proposed by Duffield. Sample size was adequate only for optical rotation analysis, but the analysis showed that the four groups had large differences in optical rotation of the total turpentine sample, suggesting large differences in monoterpene composition.

Forde and Blight (1964), with the aid of gas chromatography, analyzed trees from the four locations for xylem monoterpenes (table 3-40) and found four types of stands much like those proposed by Duffield: (1) northern stands with large amounts of α -pinene; (2) central stands with large amounts of 3-carene; (3) southern stands with large amounts of sabinene and terpinolene; and (4) Channel Island stands with composition much like the southern stands but with some trees having considerable β -pinene. The Channel Island stands are sometimes called *P. remorata*, but Griffin and Critchfield (1972) do not list it as a separate species. Most of the trees sampled by Forde and Blight (1964) were growing in Australia from seed collected at the designated locations in California. Blight and McDonald (1963) were the first to report moderate to large amounts of sabinene in the xylem resin of a pine in *P. muricata*. Since then, sabinene has been reported in a number of pines.

Mirov and others (1966) sampled native stands more intensively and extensively. Their results confirmed the results of Forde and Blight (1964). Mirov and others (1996) also found evidence of a very narrow transitional stand between the northern and central regions near Annapolis (table 3-40). Their sampling of the Channel Islands showed much greater variation than Forde and Blight (1964). This variation could either have been caused by a larger sample, or their sampling included both Santa Cruz and Santa Rosa Islands. A substantial amount of β -pinene was found in the Channel Island trees, but Forde did not find β -pinene in trees on the Channel Islands. Mirov and others (1966) also reported moderate amounts of myrcene, limonene, and β -phellandrene which have not been reported in other publications.

I analyzed the resin from a stand of 106 trees at Pt. Reyes, which is in the central region as defined by Mirov and others (1966), in order to get a better understanding of local variation and of the kinds and frequency of types of composition in a small stand in the central region of *P. muricata*. The range of values for α -pinene and 3-carene was generally greater than that reported from smaller stands in the same geographic area by Forde and Blight (1964) and Mirov (1961) (table 3-40). Since Forde and Blight (1964) and Mirov and others (1966) did not give averages for their stands, a comparison cannot be made. Mirov and others (1966) found trees with as much as 95 percent α -pinene in the central region; neither Forde and Blight (1964) nor my sampling found α -pinene in excess of 88 percent. Little or no difference in composition was found between a basal tree sample and one at 20 feet (see table 8-1).

Figure 3-29—Distribution of *Pinus muricata* in California, taken from Griffin and Critchfield (1972); regional types indicated by solid line on the left.



The frequency of the individual components in the 106 tree stands suggested a trimodal distribution for both α -pinene and 3-carene (fig. 3-30). Estimates were made of the limits of the three modes for α -pinene and 3-carene (table 3-41). When the composition of the individual trees was coded according to the modal distribution criteria for α -pinene and 3-carene, six types of composition, out of the nine possible ones, were found in the 106 trees (table 3-42).

It is clear (table 3-42) that 89 percent of the trees were of three coded types: 0-2, 1-1, and 2-0, where the first number is α -pinene and the second is 3-carene. The other 11 percent were of three other types, 1-2, 2-1, and 0-1. It is possible that there were only the first three types, and the other three types were artifacts of the analytical and coding procedures, or were the result of the aggregate biological variation or minor effects.

It might be assumed that all the trees in this central region of *P. muricata* had a (1-1) set of alleles for 3-carene, and that the variation encountered was caused by varying sets of alleles for α -pinene. However, under such conditions one would expect equal amounts of α -pinene and 3-carene in some trees, where both have a (1-1) set of alleles. Such was not the case. The alternative was to consider nonequal competition for the C-5 precursor or a very strong environmental effect. It appears that the genetics of α -pinene and 3-carene might be different in *P. muricata* than in other pine, which is highly unlikely, or some other unknown factor is present.

Ten intraspecific hybrids of *P. muricata* growing at IFG were examined (table 3-43). The results did not fully agree with the regional subdivisions. The progeny of Inverness x Fort Ross, high 3-carene x high α -pinene, were as expected, with mixtures of α -pinene and 3-carene, but the progeny of Monterey x Fort Ross, high 3-carene x high α -pinene, were not as expected. One would again expect mixtures of α -pinene and 3-carene; instead the progeny are near-pure α -pinene. The Monterey parent could have been misidentified; or could have been a high α -pinene *P. radiata*, or a *P. attenuata* x *P. muricata* from the northern region could have been the female parent. Likewise three of the progeny of San Vicente x Monterey had no sabinene where one would expect considerable sabinene. There also may be much more local variation within the regions than had been reported. An experimental breeding program of *P. muricata* with specific types of composition would certainly be recommended.

Forde and Blight (1964) used steam distillation of the whole resin followed by GLC analysis for some of their samples, and results were very similar to samples prepared differently. This, of course, differed sharply from results by steam distillation of mill-ground xylem reported by Anderson and others (1969, 1970). Their results differed sharply from results obtained by using the sample preparation procedures reported in this volume and by others for *P. attenuata*, *P. monophylla*, and *P. quadrifolia*. As far as I can determine, the only difference between the two procedures was that Forde and Blight (1964) and Mirov (1961) used gross resin while Anderson and others (1969, 1970) used mill-ground xylem followed by steam distillation.

#14. *Pinus ponderosa* Dougl. ex Laws. (ponderosa pine)

P. ponderosa is one of the most valuable, widespread, and variable pines in the world. In western North America it extends from southern Canada into northern Mexico and from the Pacific Ocean to the Great Plains (fig. 3-31). Within that geographical definition it is noticeably absent from an area where one would expect it: western Wyoming, southeastern Idaho, and southwestern Montana. An illustration of the wide range of sites it occupies is the associated plants at extremes of its distribution: it can be associated with redwood on the west coast, with larch in the north, with Great Plains grasses in the east, and with cactus in the southwest. Details of its distribution, growth, habits, and

Table 3-40—Range of five monoterpenes in stands of *P. muricata* in California or originating from seed collected in California as reported by different researchers; n = number of trees

Regional Type	Locality ¹	By ²	n	Monoterpene ³				
				α -p	β -p-	car	sab	terp
				percent ⁴				
Northern	Trinidad Head to Fort Bragg	F	17	95 - 98	0	0	0	0
	2 mi. N. of Annapolis	M	38	91 - 99	0	0	0 -	40 - 1
Transition	Annapolis	M	10	2 - 98	0	0 - 84	0 - 4	0 - 10
Central	1 mi. S. of Annapolis	M	3	4 - 6	0	79 - 84	2 - 3	2 - 8
	Inverness to Monterey	F	14	1 - 12	0	74 - 88	0	7 - 10
	Stewarts Point to Monterey	M	32	1 - 20	0	86 - 95	0 - 1	0 - 7
	Pt. Reyes	S	106	2 - 36	*	57 - 88	0	5 - 8
Southern	San Luis Obispo to Lompoc	F	20	3 - 14	0	1 - 2	50 - 73	18 - 43
	Lompoc	M	20	4 - 13	0	1 - 2	44 - 74	8 - 31
	San Vicente ⁵	M	n ⁵	6 - 9	2 - 3	0	41 - 62	3 - 31
Channel Islands	Santa Cruz Island	F	7	23 - 32	0	1 - 2	32 - 38	27 - 40
	Santa Cruz Island	M	10	12 - 55	2 - 14	0 - 1	25 - 62	4 - 33
	Santa Rosa Island	M	15	12 - 60	2 - 20	0 - 1	9 - 62	9 - 12

¹ Locality of stand in California or source of seed in California when grown in New Zealand and reported by Forde (1964).

² Reported by: F = M. Forde; M = Mirov and others; S = Smith.

³ α -pinene, β -pinene, 3-carene, sabinene, terpinolene; most studies also report small to trace amounts of camphene, myrcene, limonene β -phellandrene γ -terpinene and α -thujene.

⁴ Normalized; * = trace.

⁵ Baja California, number of trees not given; also reported for this locality was 4 percent myrcene, 10 percent limonene, 15 percent β -phellandrene.

Table 3-41—Intervals of the frequency distribution of individual monoterpenes of *P. muricata* that were used to define the low (0), medium (1), and high (2) modes for α -pinene and 3-carene

Code value	Monoterpene	
	α -Pinene	3-Carene
	-----intervals in percent ¹ -----	
(0)	0 - 6	0 - 24
(1)	7 - 14	75 - 82
(2)	15 - 100	83 - 100

¹ Normalized.

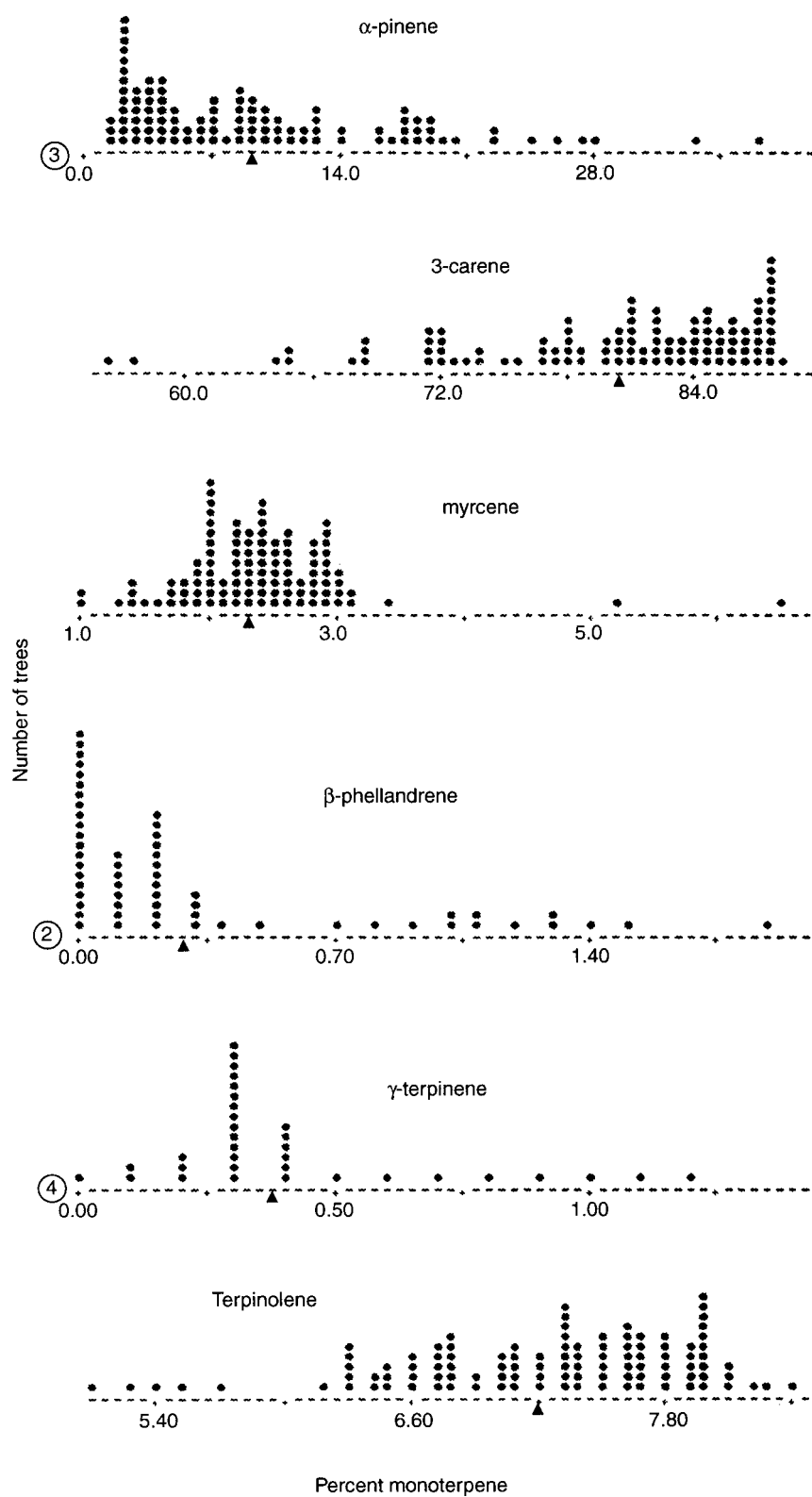


Figure 3-30—Frequency distribution of the amount of the listed xylem monoterpenes in 106 *P. muricata* in one stand at Pt. Reyes, California; each dot is measurement of one tree except where (n) indicates each represents *n* trees; ▲ is average of set.

Table 3-42—The six coded types of composition in a stand of *P. muricata* at Pt. Reyes California their frequency in 106 trees, and their actual average composition; see table 3-41 for mode definition

Code ¹	Value Frequency in 106 trees	Actual composition ²					
		α -p	3-car	myr	β -ph	γ -ter	terp
		-----percent ³ -----					
02	42	3	86	3	1	1	8
11	27	10	79	2	1	*	7
20	20	21	69	2	1	1	7
12	4	7	83	2	0	0	7
21	4	17	74	2	0	0	7
01	3	6	82	2	1	1	7

¹ Respectively: α -pinene, 3 carene; see table 3-41 for monoterpene values.

² α -pinene, 3-carene, myrcene, β -phellandrene, γ -terpinene, terpinolene.

³ Normalized; * = trace.

Table 3-43—Xylem monoterpene composition of 10 intraspecific hybrids of *P. muricata* growing in the arboretum at the Institute of Forest Genetics (IFG)

Source ¹	Monoterpene ²									
	α -p	cam	β -p	car	sab	myr	lim	β -ph	oci	ter
	-----percent ³ -----									
In x Fr	41.0	0.4	1.6	49.4	-	1.8	0.4	0.7	0.2	4.3
In x Fr	47.7	0.3	1.2	42.8	-	1.7	0.4	0.8	0.2	4.3
In x Fr	38.0	0.3	1.2	52.0	-	1.9	0.4	0.6	0.4	5.2
Mon x Fr	93.4	0.7	2.9	-	-	1.0	0.8	1.1	-	-
Mon x Fr	95.6	0.7	1.7	-	-	0.9	0.5	0.4	-	-
Mon x Fr	97.1	0.7	0.9	-	-	0.8	0.4	0.1	-	-
SV x In	53.6	0.4	1.7	38.4	-	1.5	0.4	0.7	0.2	3.2
SV x Mon	29.8	0.4	2.3	59.2	-	1.6	0.5	0.8	0.1	5.1
SV x Mon	2.9	0.2	0.3	83.6	-	2.4	0.5	0.6	0.7	9.0
SV x Lom	8.9	0.1	1.5	-	53.3	2.6	1.4	1.6	1.0	29.5

¹ In: Inverness (middle type), Fr: Fort Ross (northern type), Mon: Monterey (middle type), SV: San Vicente (southern type), Lompoc (southern type).

² α -pinene, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, ocimene, terpinolene.

³ Normalized; - =zero.

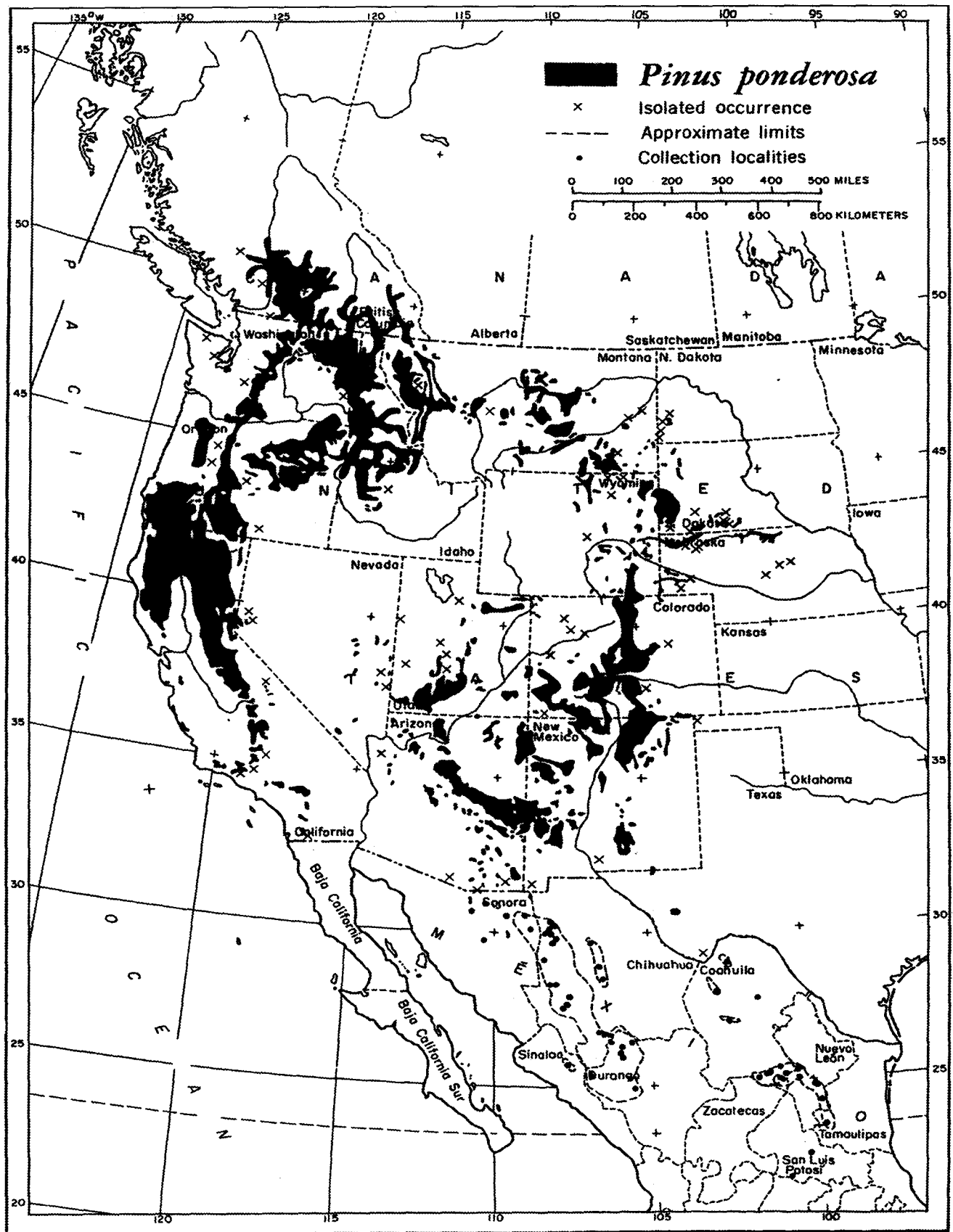


Figure 3-31—Distribution of *Pinus ponderosa*, as taken from Critchfield and Little (1966).

genetics are covered by Wang (1977) and Conkle and Critchfield (1987). The latter is strongly focused on genetic variation and the relationship of *P. ponderosa* to other pines.

Additional information on *P. ponderosa* xylem monoterpenes is given later in this volume on half-sibling populations (Chapter 5), on full-sibling populations (Chapter 6), on grafts (Chapter 7), on procedural measurements (Chapter 8) and on bioassay of resistance to attack of western pine beetle (Chapter 9). Included in Chapter 8 are several studies that showed little or no difference in composition of samples taken from various horizontal locations and over long intervals of time. There was a very small but consistent shift in composition from top to bottom of a mature tree: α -pinene and β -pinene decrease, and myrcene and limonene increase.

Traditionally, *P. ponderosa* has been divided into three varieties: var. *ponderosa* in the far West, var. *scopulorum* Engelm. in the northern and central Rocky Mountains and var. *arizonica* (Engelm.) Shaw in the southern Rockies (Wang 1977). Weidman (1939) proposed a different classification on the basis of climate. More recently, two classifications have been proposed on the basis of early growth characteristics (Squillace and Silen 1962; Wells 1964). All four classifications—traditional, climate, and two based on early growth—differ noticeably. One of the more variable characteristics of *P. ponderosa*—among many variable ones—is xylem monoterpenes. Mirov (1961) found strong evidence for regional differences by examining trees growing at the Institute of Forest Genetics (IFG) from seed collected throughout the range of the tree; there were large regional differences in α -pinene, β -pinene, and 3-carene. But Mirov's sampling was insufficient to define regional types. Smith (1964a) also found considerable variation in α -pinene, β -pinene, 3-carene, and limonene among local populations in Eldorado County, California. Smith and others (1969) examined a large number of trees growing at IFG from seed collected throughout the range and made the first attempt to define regional variation on the basis of average composition. Smith (1964a, 1964b, 1966c, 1968) found within-tree composition to be quite constant. Three studies have been made which describe parts or all of the regional variation. (1) Peloquin (1964) examined trees in southwestern Arizona and concluded that there is great variation and that there was strong evidence of hybridization between *P. ponderosa* and *P. engelmannii*. (2) Sturgeon (1979) examined a series of stands in northern California and southern Oregon, and the results solidify that part of the regional classification of *P. ponderosa* and also suggest some degree of relationship between the resin composition of the stand and the severity of depredation by western pine beetle. (3) Smith (1977) made a range-wide study with 65 stands of about 75 trees per stand and described five regional and four transitional ecotypes on the basis of stand averages (fig. 3-32). When the average stand values for the five major monoterpenes in each stand were mapped, they fell into natural groupings which suggest regional types of stands. All stands that differed significantly from each other by no more than one of the five major components were placed in the same region (Smith 1977). Stands differing by two or more components were placed in other regions. Five regions were defined by this procedure. Each of the five regions differed significantly from each other in at least two of the components, and often in all five. There were other stands outside of these regions that differed significantly from each other in two to five components and from the adjoining regions in four or five components; these were grouped geographically into transition zones. Thus, stands within a region were quite similar to each other, while stands in zones were neither strongly similar to each other nor to the stands in the adjoining regions.

A classification based on the whole composition of a tree was also proposed (Smith 1977) in which the monoterpene composition of each tree was coded on

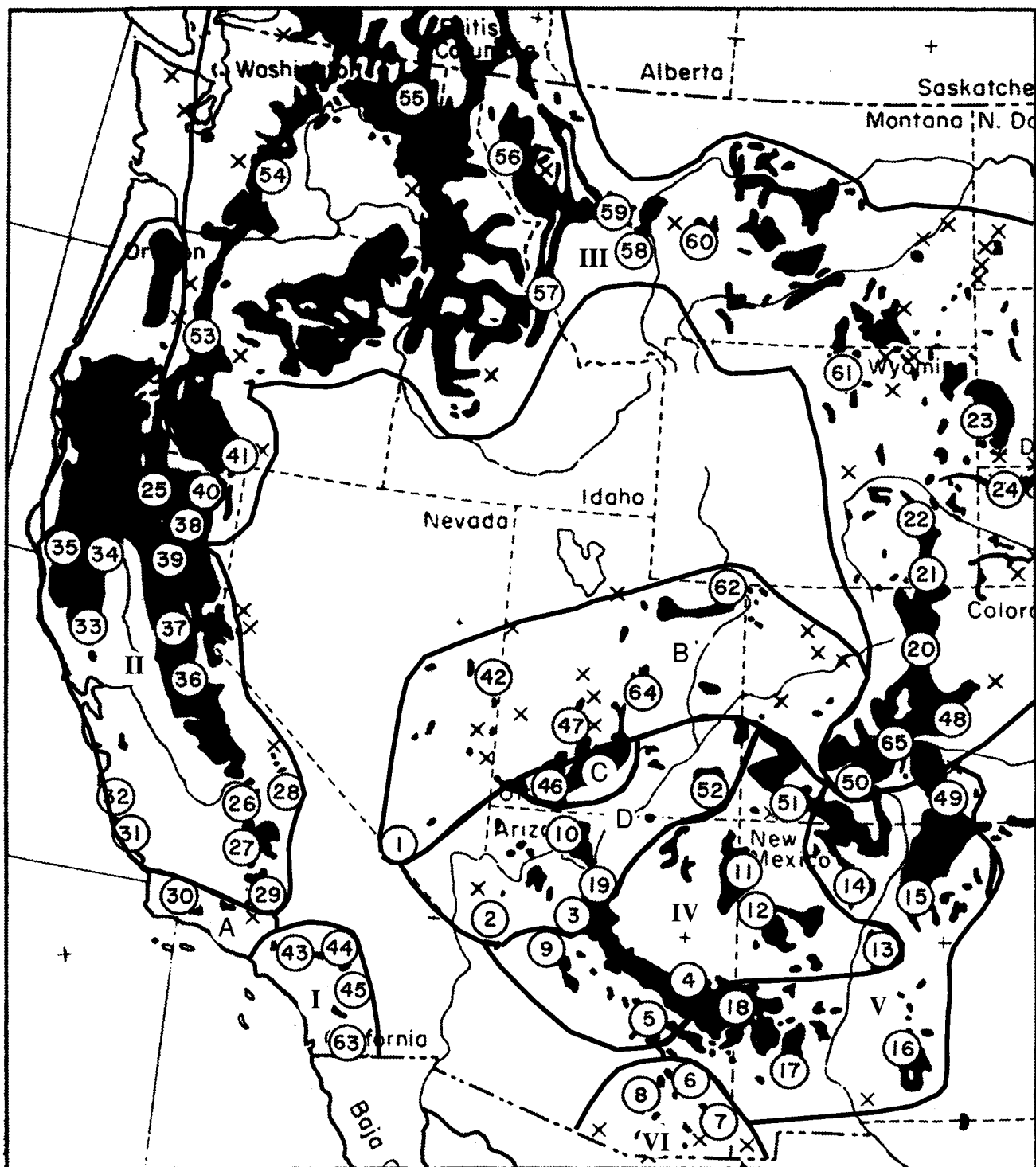


Figure 3-32—Regions and zones of *P. ponderosa* xylem monoterpene ecotypes in western United States based on correlation of frequency of occurrence of tree types of monoterpene composition in 65 stands, superimposed on the tree's distribution from Critchfield and Little (1966). Regions are: I, San Jacinto; II, Sierra Pacific; III, Cascade Northern; IV, Mogollon West; V, Mogollon East; VI, Coronado. Zones are: A, Tehachapi; B, Escalante North; C, Cedar Breaks; D, Escalante South.

the basis of modalities in the frequency distribution of each of the five major monoterpenes, enabling the composition of each tree to be expressed as a discrete five-digit number. This is called the coding procedure. Thus five variates, the five major components, were expressed as a single variate, the coded composition of the tree. Accordingly, each stand could then be characterized by the number of trees of each type of composition, and the distribution of the frequency of the types of composition conformed generally to the region and zone classification established by average monoterpene composition noted above, but there were some differences. At that time, however, no analysis was made of the frequency of these tree types by stands, nor was an analysis made of the intraregional structure other than that done on the basis of average composition. Coding provides a procedure for analyzing all major components simultaneously rather than on the basis of individual components one at a time or sequentially. Such a system should be superior to the analysis of stand averages in determining the overall regional structure and the uniformity or variation within regions. A multivariate procedure, such as the one used, would also be of value when an interrelationship between components is suspected.

A Regional Classification

The coding of 4,894 trees, using the coding criteria (*table 3-44*), defined 112 composition types. The 60 most common types comprised 97 percent of the trees in the study (*table 3-45*). These 60 types were used to determine the frequency of each type in each of the 65 stands; frequency was normalized to give the percentage of each type in the stand. Because nearly all stands had 70 to 80 trees, the normalized value of occurrence of coded types and the actual numbers of types of composition were quite similar. Early in the study, a 100-tree stand was considered, but the logistics of the field work would not accommodate such sampling, and an 80-tree stand was selected. In this Chapter the phrase "frequencies of tree types" refers to the normalized frequencies, i.e., to percentages.

The association between stands was calculated by using the frequencies of tree types in each stand as the variable. These were called coefficients of association and were used as a measure of the similarity in the distributions of types of trees in the various stands. The coefficients calculated in this manner were strongly influenced by the most frequent tree types. Any two stands in which the distribution of the most frequent types of trees was quite similar tended to be strongly correlated even if the distributions of the less frequent compositions were somewhat different. Hence, when an association was used to classify stands into regions, the classification method was such that stands with similar frequencies of the most common types of trees were usually classified together. This approach, therefore, provides some measure of magnitude, similarity, or association. A chi square value was calculated for all stand pairings. When the association was negative between two stands, the coefficient was simply designated as a negative and the association between the two stands was simply given a minus category without a numerical value.

Stands were geographically arrayed—using the classification proposed earlier (Smith 1977) as a guide—according to the strength of association, by the pattern of occurrence of types of composition, and by the association of each grouping with other groupings (*table 3-46*). Thus, some degree of flexibility had to be applied in determining regions and zones. When all 65 stands were aligned according to the value of association for all pairings, a structure was established (*fig. 3-32*), composed of six regions and four transition zones; this resembled, to some degree, the classification originally proposed (Smith 1977). This structure showed the average association between the stands of each of six groupings to be about 0.8 to 0.9 (*table 3-46*); these were called regions: (I) San Jacinto, (II) Sierra Pacific, (III) Cascade Northern, (IV) Mogollon West, (V)

Table 3-14—Intervals in the frequency distribution of individual monoterpenes of *P. ponderosa* that were used to define the low (0), medium (1), and high (2) modes for the five noted monoterpenes

Monoterpene	Code Value		
	0	1	2
	----- intervals in percent ¹ -----		
α -Pinene	0-17.4	17.5 - 64.4	64.5 - 100
β -Pinene	0 - 4.4	4.5 - 35.4	35.5 -100
3-Carene	0-15.4	15.5 - 35.4	35.5 -100
Myrcene	0 - 2.4	2.5 - 15.4	15.5 -100
Limonene	0 - 2.4	2.5 - 17.4	17.5 -100

¹ Normalized.

Table 3-45—The 60 most common composition types of *P. ponderosa* xylem resin: A = the numerical listing of the 60 types; B = coded type designation; the five numbers under B are respectively from left to right α -pinene, β -pinene, 3-carene, myrcene, limonene, each of the five values under C are average actual composition of the trees of coded type listed in column B and in the same order¹; N = numerical occurrence of each type in 4,731 trees classified. See figure 3-33 for the distribution of the types in each region and zone.

A	B	C ^{1,2}					N	A	B	C ^{1,2}					N	A	B	C ^{1,2}					N
		α	β	3	m	l				α	β	3	m	l				α	β	3	m	l	
----- percent ¹ -----							----- percent ¹ -----							----- percent ¹ -----									
1	02012	10	49	1	12	27	168	21	02211	7	38	38	8	6	21	41	11111	37	21	28	6	6	33
2	02022	9	45	*	19	25	88	22	02121	7	39	23	18	10	17	42	12000	46	50	1	1	1	19
3	02011	11	60	2	12	14	28	23	02110	10	47	29	11	1	31	43	20010	89	1	*	9	1	34
4	02021	11	55	*	19	13	25	24	02120	8	40	40	19	1	12	44	10201	40	*	48	2	6	162
5	01022	9	30	*	20	39	19	25	00211	6	1	69	10	10	136	45	10110	56	1	31	9	1	36
6	01112	7	28	27	11	24	119	26	00212	6	1	56	9	24	27	46	10111	53	1	29	6	8	99
7	01122	6	26	25	19	23	46	27	02112	7	38	23	10	20	11	47	10200	40	1	52	2	1	121
8	02020	13	62	*	23	1	9	28	01022	9	30	*	20	39	19	48	11101	41	20	28	2	7	22
9	02111	8	41	26	11	11	127	29	00220	4	1	71	19	*	53	49	20011	80	1	*	7	11	43
10	01211	6	21	51	10	9	853	30	00221	5	1	61	17	12	24	50	10101	57	*	30	2	9	59
11	01111	6	32	32	12	13	57	31	01201	5	17	63	2	8	79	51	20001	88	1	*	1	8	62
12	12011	36	49	1	6	7	20	32	11110	37	24	29	7	1	45	52	10102	46	*	26	2	22	8
13	01200	6	20	65	2	1	36	33	11200	30	16	48	2	1	29	53	10100	61	1	33	1	*	16
14	01212	5	17	44	10	21	112	34	00210	5	1	78	11	*	104	54	10112	39	*	28	5	25	9
15	01221	5	17	46	18	11	96	35	11210	29	14	45	7	1	73	55	12010	41	49	1	7	1	10
16	02210	8	39	41	8	1	45	36	11211	30	12	44	5	5	44	56	20002	74	1	*	2	23	9
17	12110	28	40	24	5	1	24	37	00201	8	*	77	2	8	15	57	12101	31	39	21	1	6	9
18	01121	7	29	28	20	14	50	38	10210	39	1	50	7	1	209	58	20100	67	*	27	1	*	10
19	01210	6	20	59	10	1	618	39	10211	36	1	49	5	6	160	59	12001	43	45	2	1	7	16
20	01220	5	17	55	18	*	116	40	11201	30	14	45	2	5	56	60	20000	96	1	*	1	1	141

¹ Small amounts of other components such as camphene β -phellandrene and terpinolene account for sum of normalized composition.

² α = α -pinene β = β -pinene 3 = 3-carene m = myrcene l = limonene.

¹ Normalized * = trace.

Mogollon East, and (VI) Coronado. The average association for the two other groupings was only 0.6 to 0.7; these were called transition zones: (B) Escalante North, and (D) Escalante South. Two stands, #30 and #46, could not be clearly aligned with any of the groups. These two were also called transition zones—(A) Tehachapi and (C) Cedar Breaks. Thus, by strength of coefficients-of-association grouping, six regions and four transition zones of ponderosa pine monoterpene ecotypes were established. The term "transition zone" was used for an area between regions, or other zones, where nearby stands were not strongly associated and where composition types resembled, somewhat, those of the regions and zones around the zone.

It was clear that all groupings differed from each other in either the incidence or frequency of several types of composition (*fig. 3-33*). Increasing the number of sample trees in a region or zone may increase the total number of types of composition in that region or zone; but the number of types in a 75-tree stand did not increase.

The following is a discussion of each region and zone and its association with other regions and zones (*table 3-46* and *figs. 3-32* and *3-33*). The order of presenting the regions and zones was a clockwise route from southern California northward to the Canadian border, then eastward to the Rockies, and then southward through the Rockies and Great Basin to southern Arizona. A similar transect gave the frequency distribution of the five major components— α -pinene, β -pinene, 3-carene, myrcene, limonene—in a representative stand from each region and zone (*fig. 3-34*). The average coefficient of association and the range will be given for each set of comparisons. This is shortened in the following discussion to "association."

Region I, San Jacinto, had four stands and had an average internal association for the four stands of 0.89 ranging from 0.87 to 0.97. San Jacinto was associated positively with Tehachapi, the coefficient of association averaging 0.54 and ranging from 0.49 to 0.57; and with Sierra Pacific averaging .05 and ranging negative to 0.17. San Jacinto was associated negatively with all other regions and zones. The most common type of composition, #1, was also found in Tehachapi, Sierra Pacific, and Escalante North.

Transition Zone A, Tehachapi, had only one stand and was given a hypothetical internal association of 1.00. It was associated positively with three regions: San Jacinto, as noted above; Sierra Pacific with an average association of 0.71 and ranging from 0.51 to 0.81; Cascade Northern with an average association of 0.26 and ranging from 0.10 to 0.44. Tehachapi was also associated positively with two transition zones: Escalante North with an average association of 0.36 and ranging from 0.25 to 0.50 and Cedar Breaks with an average of 0.03 and without a range of values because both were single stand zone associations. Tehachapi was associated negatively with all other regions and zones. Tehachapi had six fairly abundant coded types, three of which were common types in San Jacinto while three were common types in Sierra Pacific.

Region II, Sierra Pacific, had 14 stands with internal association of 0.88 ranging from 0.66 to 0.97. Sierra Pacific was associated positively with San Jacinto and Tehachapi as noted above. It was also positively associated with Cascade Northern, with an association of 0.44, ranging from 0.14 to 0.76; with the Escalante North with an average of 0.56 and ranging from 0.27 to 0.79; and with Cedar Breaks, with association of 0.11, ranging from 0.02 to 0.16. Sierra Pacific was negatively associated with all other regions and zones. The more common types of composition in Sierra Pacific were also commonly found in Tehachapi, Cascade Northern, and Escalante North. Some types common to Sierra Pacific and Escalante North were not common to Cascade Northern. Sturgeon (1979) suggested that the Sierra-Pacific region extended into the Siskiyou and Klamath Mountains of northern California, and into southern Oregon, west of the crest of the Cascades.

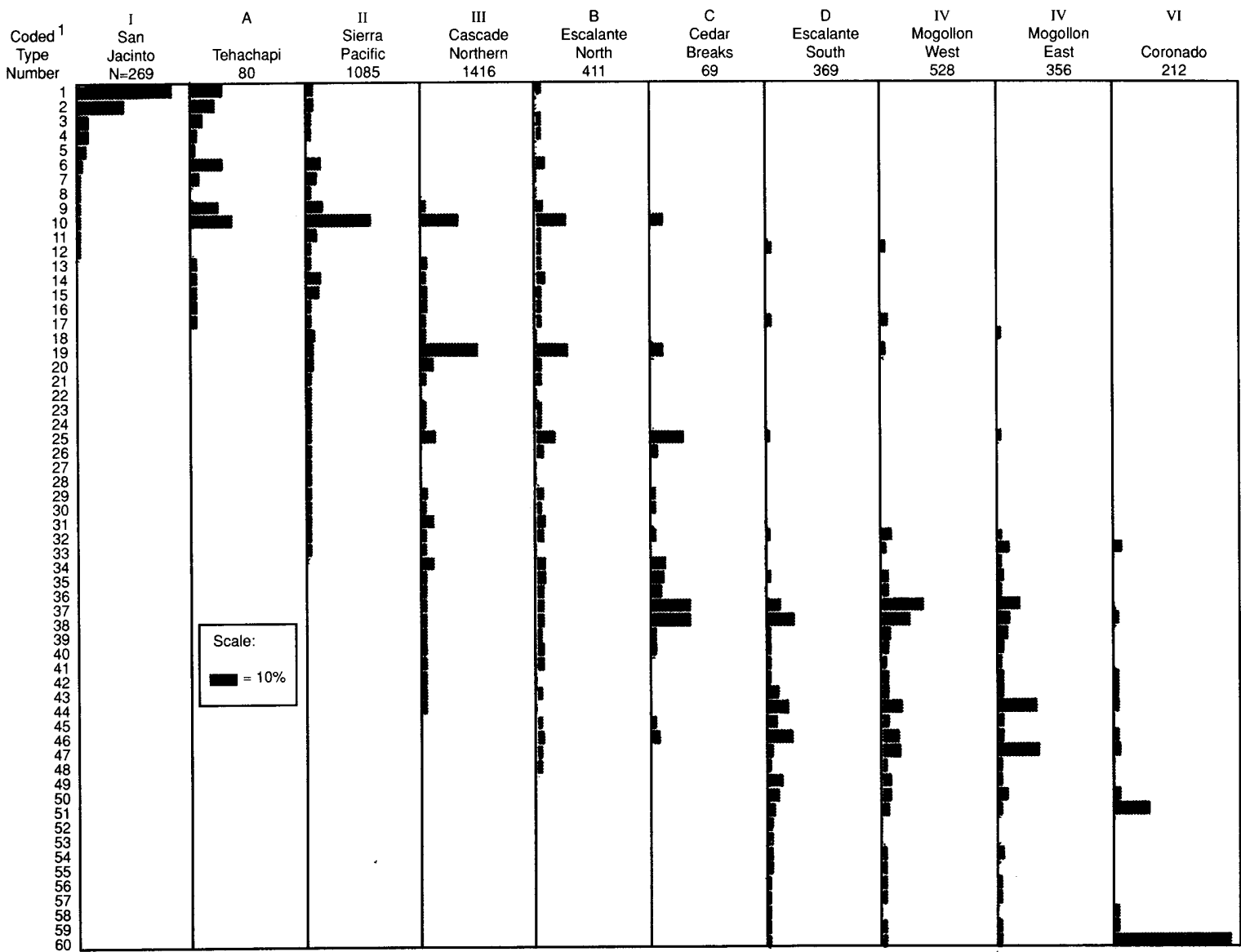


Figure 3-33—
 Occurrence of 60 types of monoterpene composition of *P. ponderosa* in the 10 regions and zones shown in figure 3-32. These types comprise 97 percent of the trees in the study. Occurrence, represented by bars, is normalized within each region or zone. Refer to table 3-45 for coded and actual composition.

¹See Table 3 for actual composition of coded types

	SJ	T	SP	CN	EN	C	ES	MW	ME	CO	Plot #
I. San Jacinto (S.J.)	9 9 9	1	0 1 1 0 1 2 2 - - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	43
	9 9 6	1	0 1 0 1 1 1 1 1 2 0 0 0 -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	44
	9 9 6	0	- 1 - 1 0 0 1 1 1 - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	45
	5	0	- 0 0 1 0 0 1 1 1 - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	63
A. Tehachapi	7 6 6 6 7 8 7 8 8 8 6 6 7 6	2	1 3 4 4 3 2 2 2 4 3 2 4 1 2 3 2 3 1	3 5 3 5 3 4 0	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	30
	9 9 9 9 9 9 9 8 9 9 9 9 9 9	4	2 6 7 7 5 4 4 4 7 5 5 7 3 4 6 4 7 3	4 8 5 3 5 7 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	25
	9 9 8 9 9 9 6 8 9 9 9 9 9	3	1 5 6 7 5 3 4 3 7 5 4 7 2 4 5 3 6 3	4 8 4 7 5 7 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	26
	9 8 9 9 9 7 8 9 9 9 9 9 9	4	2 5 7 7 5 4 4 4 7 5 5 7 2 4 5 7 4 4	4 8 4 8 5 7 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	27
	8 9 9 8 6 8 9 8 9 9 9 9 9	4	2 5 7 7 5 4 4 4 8 5 5 7 2 4 5 4 7 4	5 8 3 8 6 7 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	28
	9 9 9 7 9 8 8 8 9 8	4	2 5 6 6 4 3 4 3 6 4 4 6 2 4 6 3 6 3	4 8 3 7 4 6 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	29
	9 9 8 9 9 9 9 9 9 9	4	2 5 7 7 5 4 4 4 7 5 5 6 3 4 6 4 6 3	4 8 4 7 5 7 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	31
	9 7 9 9 9 9 9 9 9	4	2 5 7 7 5 4 4 4 7 5 4 7 2 4 6 4 7 4	5 8 4 8 5 7 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	32
	8 9 9 9 9 9 9 9 9	4	3 6 6 7 5 4 4 4 7 5 5 6 3 4 6 4 6 3	4 8 5 7 5 6 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	33
	9 9 7 7 7 7 7	3	2 4 4 5 4 3 3 3 5 3 3 4 2 3 4 3 4 1	3 5 4 4 3 4 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	34
	8 8 9 8	3	2 5 5 5 4 3 4 3 6 4 4 6 2 3 5 3 5 2	3 7 4 6 4 6 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	35
	9 9 9	3	2 5 6 6 5 3 4 3 7 5 4 6 2 4 5 3 6 2	4 7 5 7 5 6 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	36
	9 9 3	1	4 6 6 4 3 4 3 6 4 4 6 2 3 5 3 5 2	4 7 4 7 4 6 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	37
	9 3	1	5 6 6 4 3 4 3 7 4 4 6 2 3 5 3 6 3	4 8 4 7 5 7 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	39
	5 3 6 7 8 6 5 5 5 8 6 6 8 4 5 6 4 7 4	5	8 6 7 6 6 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	40
II. Sierra Pacific (SP)	9 9 9 8 9 9 9 9 7 6 9 8 9 9 9 9 8 8	9	7 6 7 7 3 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	20
	9 8 8 9 9 8 9 7 6 9 7 9 9 8 9 7 8	6	6 6 5 6 1 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	21
	9 9 9 9 9 9 9 7 9 9 9 9 9 9 9 8	8	8 7 8 7 4 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	22
	9 9 9 8 9 9 7 9 9 8 9 9 9 9 8	7	8 7 8 7 5 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	23
	9 9 8 8 9 7 8 9 8 8 9 9 9 7	7	8 7 8 7 5 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	24
	9 9 9 9 8 9 9 9 9 9 9 9 8	7	7 7 7 8 3 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	38
	8 9 9 8 9 8 9 9 9 9 9 8	6	7 7 7 7 3 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	41
	9 8 7 9 8 8 9 8 9 9 9 9	9	7 6 7 8 4 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	48
	8 9 8 9 9 9 9 9 9 8	7	7 7 7 8 3 3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	53
	8 9 9 7 9 8 8 9 7	6	8 6 8 8 6 3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	54
	8 9 7 9 7 7 8 7	6	6 5 8 9 7 5	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	55
	9 9 9 9 9 9 8	6	7 7 7 8 4 3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	56
	8 9 9 8 9 8	7	8 6 9 9 7 4	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	57
	9 8 9 8 8	6	6 6 6 7 2 3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	58
	9 9 9 8	7	7 6 7 9 4 4	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	59
	9 9 7	6	8 6 8 7 4 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	60
	8 9 7 7 7 7 3 2	8	7 7 7 7 3 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	61
	8 8 8 6 9 8 6 3	8	8 6 9 8 6 3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	65
	8 6 5 8 8 4 3	8	6 5 8 8 4 3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	50
	6 5 7 7 5 2	6	5 7 7 5 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	49
B. Escalante N(EN)	6 8 6 6 2	6	8 6 6 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	1
	5 5 3 1	5	5 3 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	42
	8 8 4	8	8 8 4	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	47
	5 6	5	6	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	62
	5	5	0 0 1 1 0 1 1 1 2 1 1 1 0 0 2 - 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	64
C. Cedar Breaks (C)	4 4 5 1 5 7 7 6 6 7 6 5 3 2 3 2 2 - 0	4	4 5 1 5 7 7 6 6 7 6 5 3 2 3 2 2 - 0	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	46
	7 7 2 7	7	7 2 7	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	2
	8 6 7	8	6 7	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	3
	6 8	6	8	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	10
	5 1 1 2 5 1 3 2	5	1 1 2 5 1 3 2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	19
	7 5 7 6 6 8 8	7	5 7 6 6 8 8	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	52
	9 8 8 9 8 9	9	8 8 9 8 9	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	4
	8 7 9 8 8	8	7 9 8 8	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	5
	7 7 9 8	7	7 9 8	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	9
IV Mogollon West (MW)	8 8 7	8	8 7	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	11
	8 8	8	8	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	12
	9 7 5 7 6 6	9	7 5 7 6 6	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	13
	7 6 8 6 6	7	6 8 6 6	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	51
	9 8 9 9	9	8 9 9	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	14
V Mogollon East (ME)	7 9 9	7	9 9	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	15
	8 8 1 2 1	8	8 1 2 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	16
	9 1 3 1	9	1 3 1	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	17
	0 2 0	0	2 0	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	18
	9 9	9	9	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	6
	9	9	9	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	7
	0	0	0	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	8
IV Coronado (C)				- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	

Table 3-46—Coefficients of association, with decimal point removed, among 65 mature stands of *P. ponderosa* and the delineation of six regions (I - VI) and four transition zones (A-D); stand numbers along right side are from Figure 3-31; 0 equals zero, (-) equals minus value; triangular shaded areas delineate stands within a region or zone, except for the two, single stands of the Tehachapi and Cedar Breaks transition zones which are fully outlined to show their association with all stands.

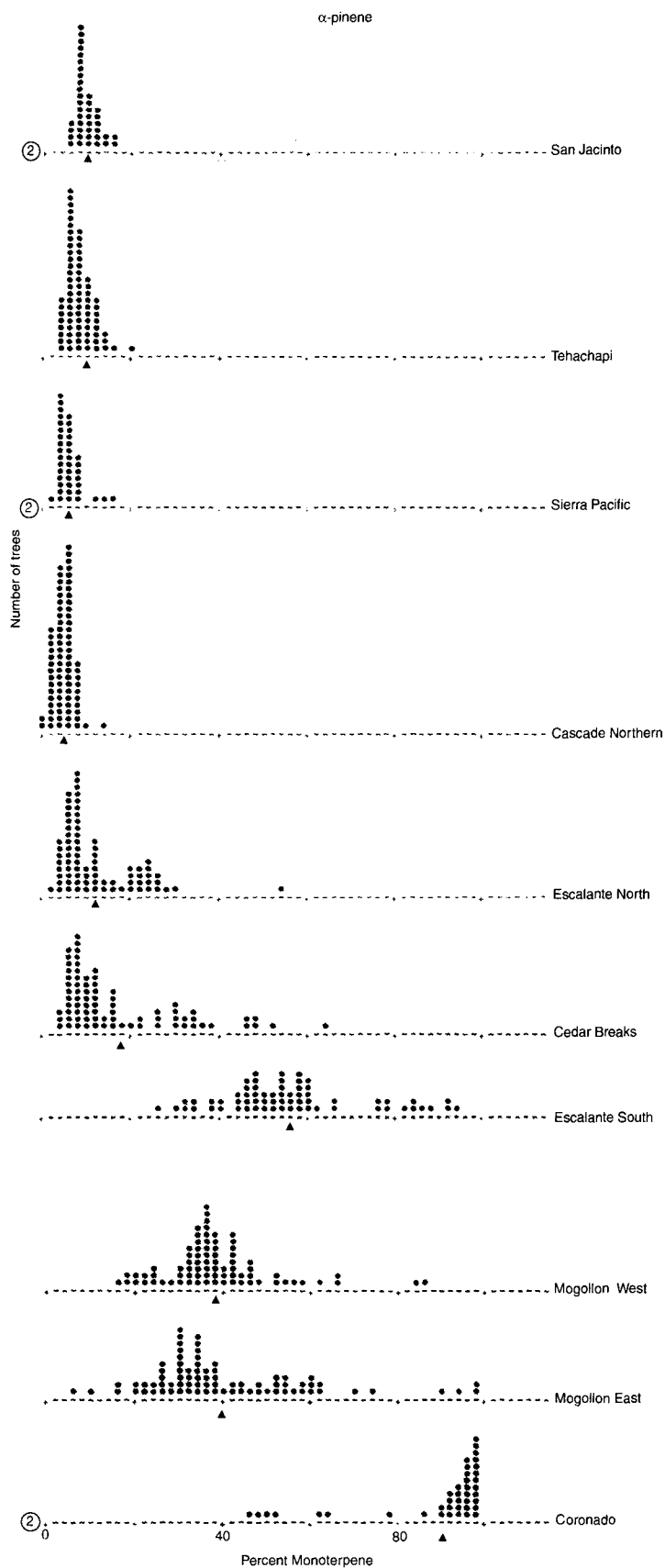
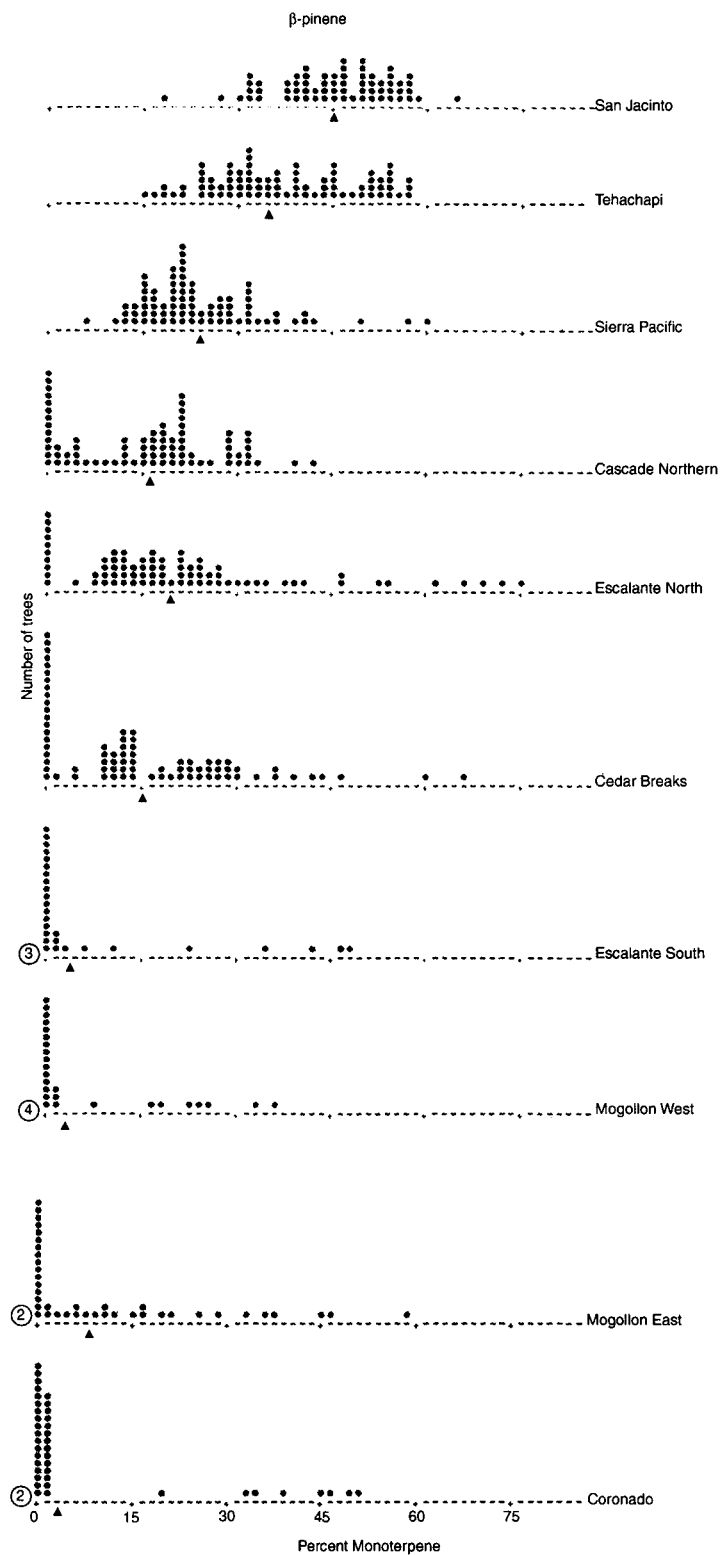
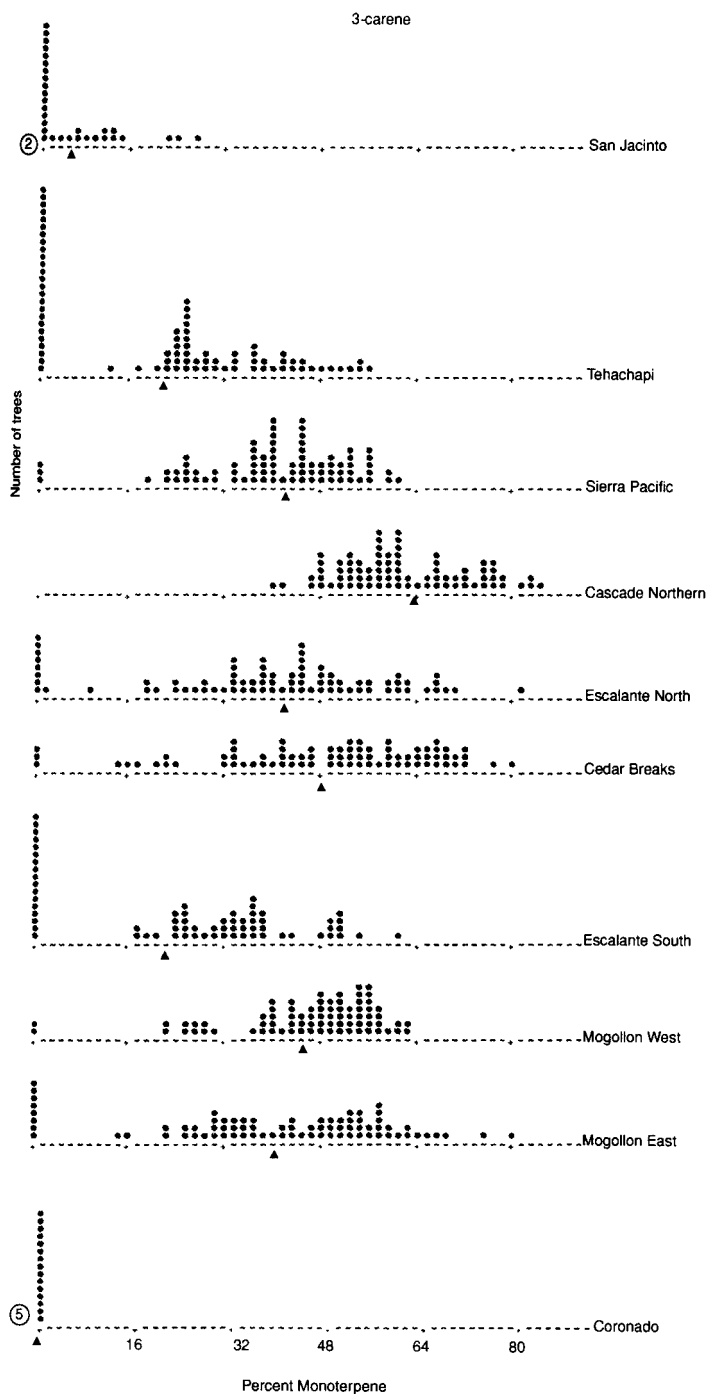
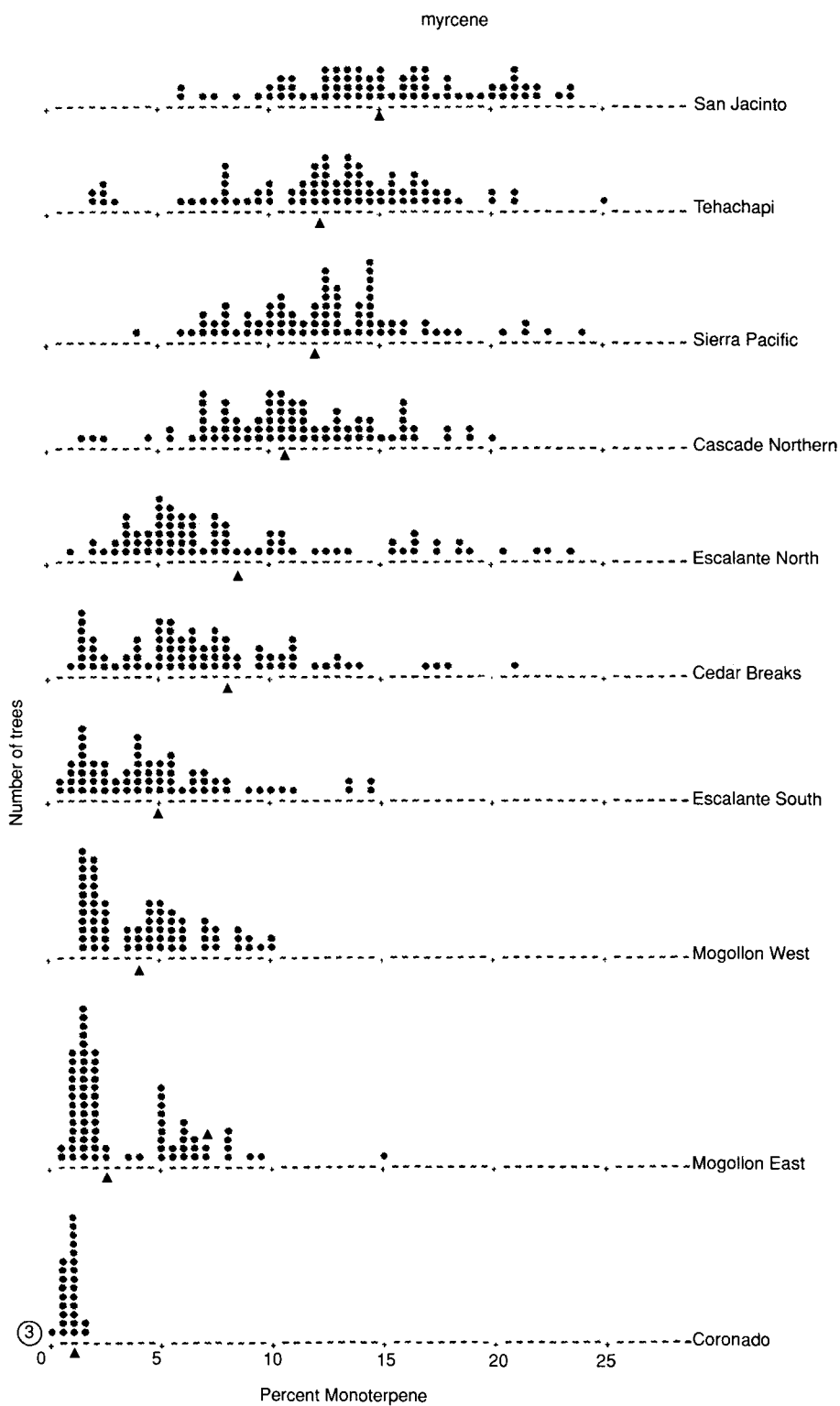


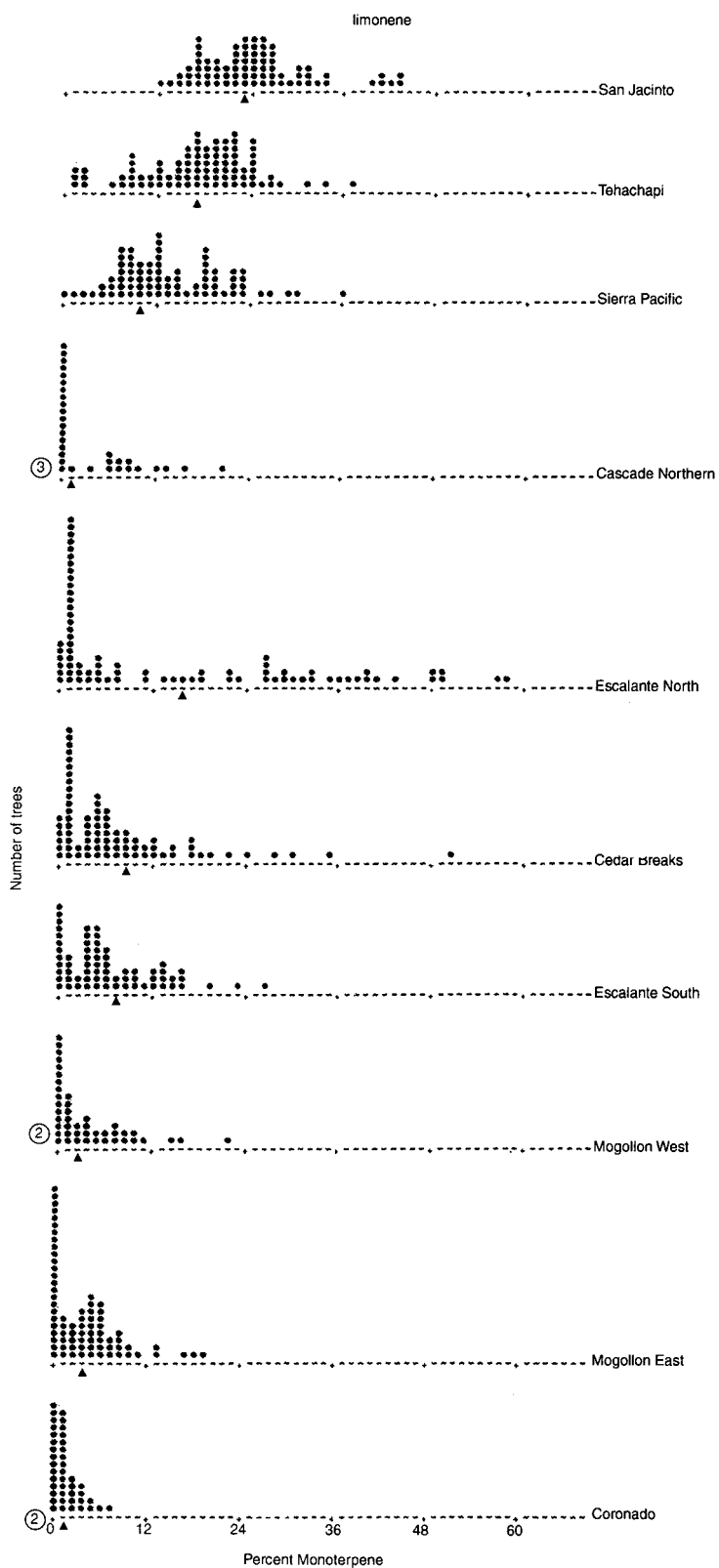
Figure 3-34—Frequency distribution of the amount of the noted monoterpenes in a typical stand of *P. ponderosa* in each of the six regions and four transition zones, listed on the right; each dot is measurement of one tree, except where (n) indicates each is *n* trees; ▲ is average of stand.

(fig 3-34 continues on the next 4 pages).









Region III, Cascade Northern, had 19 stands with internal association of 0.88, ranging from 0.68 to 0.98. Cascade Northern was associated positively with Sierra Pacific and Tehachapi as noted above. It was also associated positively with Escalante North, with an association of 0.65, ranging from 0.16 to 0.95; and with Cedar Breaks, with of 0.23, ranging from 0.20 to 0.25. Cascade Northern was negatively associated with all other regions and zones. The frequency of types of composition in Cascade Northern showed how it was linked with both Sierra Pacific to the west and south and to Escalante North to the south. Using other characteristics of *P. ponderosa*, others have found a significant shift at the Missouri River in Montana. No such shift was found in this study using xylem monoterpenes. The shift found by others should have occurred between stands 58 and 60. Though Cascade Northern was the largest geographical area, there was surprisingly excellent association among widely separated plots (table 3-45). For example, the association between stand #65 in southern Colorado and stand #41 in northern California was 0.80 (table 3-46).

Transition Zone B, Escalante North, had six stands, with an internal association of 0.63, ranging from 0.28 to 0.83. Escalante North was associated positively with Cascade Northern, Sierra Pacific, and Tehachapi as noted above. It was also associated positively with Cedar Breaks, with an average association of 0.32, ranging from 0.14 to 0.61; and with Mogollon West, with an association of 0.01, ranging from a negative association to 0.20. It was negatively associated with Escalante South, both Mogollon regions, and Coronado.

Escalante North had a great range in types of composition, tending to link it with all regions and zones (fig. 3-33). The separation of Escalante North and Escalante South into two transition zones is clearly evident from the frequency of types of composition (fig. 3-33), the characteristics of the frequency distribution of individual monoterpene components (fig. 3-34), and the differences in the coefficients of association with other regions and zones (table 3-46).

Transition Zone C, Cedar Breaks, had only one stand and therefore had no internal association but has been given a hypothetical one of 1.00. Cedar Breaks was associated positively with Escalante North, Cascade Northern, Sierra Pacific, and Tehachapi as noted above. It was also associated positively with Escalante South, with an average association of 0.41, ranging from 0.05 to 0.50; with Mogollon West, with an average association of 0.60, ranging from 0.50 to 0.69; and with Mogollon East, with an average association of 0.26, ranging from 0.18 to 0.35. Cedar Breaks was associated negatively with only two regions, Coronado and San Jacinto. It was tempting to place Cedar Breaks in either of the two Escalante zones. However, the occurrence of compositional types suggested that it should stand alone between the two zones. It would be interesting to see what types of composition would show up in a 200- to 300-tree sample in Cedar Breaks.

Transition Zone D, Escalante South, had five stands, with an internal association of 0.56, ranging from 0.22 to 0.84. Escalante South was associated positively with Cedar Breaks as noted above. It was also associated positively with Mogollon West, with an association of 0.57, ranging from 0.12 to 0.83; with Mogollon East, with an association of 0.43, ranging from .00 to 0.74; and with Coronado, with an association of 0.02, ranging from a negative association to 0.21. It was negatively associated with all other regions and zones.

The two Escalante zones and Cedar Breaks were the most interesting geographical areas of *P. ponderosa* because in aggregate, they had just about every type of composition found in *P. ponderosa* in a sample of only a little more than 800 trees. There is little doubt that more work should be done in this area, particularly in studies of large stands of several hundred trees each.

Region IV, Mogollon West had seven stands, with an internal association averaging 0.79 and ranging from 0.65 to 0.97. Mogollon West was associated

positively with Escalante South and Cedar Breaks as noted above. It was also associated positively with Mogollon East, with an association of 0.62, ranging from 0.38 to 0.78; and with Coronado, with an association of 0.05, ranging from a negative association to 0.25. It was associated negatively with all other regions and zones.

Region V, Mogollon East, had five stands, with an internal association averaging 0.87, and ranging from 0.72 to 0.94. Mogollon East was associated positively with Mogollon West, Escalante South, and Cedar Breaks as noted above. It was also associated positively with Coronado, with an association of 0.11, ranging from a negative association to 0.32. It was associated negatively with all other regions and zones.

The two Mogollon regions were the weakest regions with internal association of about 0.80. Yet they were associated with each other, with an association of only 0.6, and they differed markedly in the level with which they were associated with other regions and zone (*table 3-47*). They had much the same types of composition but differed in the frequencies (*fig. 3-33*).

Region VI, Coronado, had three stands, with an internal association averaging 0.9 and ranging from 0.86 to 0.94. It was associated positively with Mogollon East, Mogollon West, and Escalante South as noted above. It was associated negatively with all other regions and zones. It was clear that this region stands alone on the strength of a high percentage of 20000 coded types, i.e., near-pure α -pinene, but this same type of tree can be found in the two Mogollon regions and the Escalante South zone. Pelouin (1984) proposed an elevational transition zone between the Coronado region and Mogollon east region with the Mogollon region at the higher elevation.

In summary, each of the six regions had very strong internal coefficients of association of 0.8 to 0.9 between stands within that region, but the associations between stands of two different regions were, at best, 0.5 to 0.7, and many were negative. The two Mogollon regions were the weakest regions, with internal associations of about 0.8. However, if one were to combine the two Mogollon regions into one region, the internal association would drop to 0.7. Thus, though one might choose to combine the two Mogollon regions into a single, large region, I have chosen the two-region structure because the single large region would have too low an internal integrity. The difference between regions and zones was also clearly evident in the configuration of the frequency distribution of the individual monoterpenes in a typical stand of about 80 trees for each region and zone (*fig. 3-34*).

Two of the transition zones, Tehachapi and Cedar Breaks, had only one stand each. Therefore, they had no internal value for range and were given a hypothetical internal value for association of 1.00. The weakest groups were the Escalante North and South transition zones. One might be tempted to add them to adjoining regions and zones or to make one large transition zone. However, to do so would weaken the internal strength of the zones and would change the associations with other regions and zones. Given the present data, this assignment appears the most appropriate.

The structure based on the total composition of the tree as a single unit should be the strongest (*figs. 3-32 and 3-33, table 3-46*).

All regions had three to five characteristic types of monoterpene composition that were usually found less commonly in adjacent regions or zones (*fig. 3-33*). The four transition zones tended not to have their own characteristic types; instead, they usually had several types which were often more common in other, usually adjacent, regions or zones. In general, a strong association between stands was largely dependent on the high frequency of three to five common types of trees, and weakly dependent on the low frequency of 10 to 20 other types.

Four of the regions with an internal average association of 0.9 were quite different in the nature and extent of the distribution of *P. ponderosa*: San Jacinto, Sierra Pacific, Cascade Northern, and Coronado. Sierra Pacific and Cascade Northern extend over very great distances (600 to 1,500 miles). These regions were characterized by much greater continuity of stands, though within each there are isolated stands. Both Coronado and San Jacinto are geographically small regions, with a maximum extent of less than 150 miles. Both were also somewhat similar in having small stands with discontinuous distribution. This suggested that stands may have been more continuous in the recent past, and since then have become more isolated.

The Cedar Breaks zone occupied a singular and pivotal position in this geographic structure of *P. ponderosa*. It was associated positively with all regions and zones except San Jacinto and Coronado. Additionally, all regions and zones listed before it (I, II, III, A, B) had no positive associations with those listed after it (IV, V, VI, D).

Differences between all regions and zones were also clearly evident from the frequency distribution of the five major monoterpenes in a typical stand in each region and zone (fig. 3-34).

Though the areas classified as regions were fairly homogeneous, with very high internal correlations, they possess the capacity for very large shifts in monoterpene structure as shown by the wide range of monoterpene types in a given stand and by the large frequency distributions of several components. One would wonder about the forces which produce such stability and uniformity in the face of such a potential for differentiation. Stands in two of the regions may be an indication of possibilities that may be underway. Stand 35, located in the North Coast range of California of the Sierra Pacific region, has an association of 0.7 with three other stands in the region. This was much lower than other stands in the region. Is *P. ponderosa* in the coastal mountains beginning to differentiate from the rest of the region? Stand 55, located in the northern part of the Cascade Northern region, had an association of 0.7 or 0.6 with 10 other stands in the region; this was a much greater difference than any other stand. Is this an indication that another region of *P. ponderosa* extends northward into Canada? However, the sharp lines of division of the mode classification procedure plus small environmental effects on monoterpene composition could cause a slight shift in the classification of the type of some trees, causing an occasional stand to be less strongly associated with the other stands in the region. Stand size of 80 trees was expected to reduce the incidence of such inconsistencies.

Further study is needed in the northern end of the Sierra Pacific region and the northern end of the Coronado region to see if there is elevational transition with adjoining regions.

The two single-stand zones, Tehachapi and Cedar Breaks, appeared to offer the best opportunities for further study. Both areas were small and moderately to poorly associated with several regions or zones. The aggregate of the two Escalante zones also appeared to offer excellent opportunities for further study, but they were much more extensive than the two one-stand zones. The Escalante zones also presented the most difficulty in placement of stands, and their location could be modified by more intensive study of additional and larger stands. The distribution of *P. ponderosa* in the two Escalante zones tended to be more disjunct than in other areas.

Neither of the two classifications based on monoterpenes (Smith 1977), or as given in this volume, closely resembled those derived by other criteria. These criteria included the traditional three varietal classifications based primarily on morphology and geography (Wang 1977), that based on climate (Weidman 1939), or those based on early growth characteristics (Wells 1964)

(Squillace and Silen 1962). For example, the area assigned to traditional var. *ponderosa* included all or part of three regions and one transition monoterpene ecotype: San Jacinto, Sierra Pacific and Cascade Northern regions, and the Tehachapi transition zone. The agreement of monoterpene ecotypes with the other varietal classifications of *P. ponderosa* was equally as poor as with the traditional classification.

Conkle and Critchfield (1987) used all available criteria in describing the distribution of *P. ponderosa*. Adjustments were made in the basic three-variety structure to incorporate studies of mature morphology, seed and seedling characteristics, xylem resin, and cone color, as well as long-term intraspecific breeding studies.

Adjustments made in this report to the original classification did not change the conclusion in the earlier report that the distribution of certain species of *Dendroctonus* follows the ecotypes of this classification. Of particular note is that the northern limits of distribution of *D. adjunctus* Bland. falls in Escalante transition zones.

One of the objectives of the work with *P. ponderosa* was to locate trees high in limonene for study and breeding because early research showed that limonene reduced the success of western pine beetle attack. Four locations were found (table 3-48): Ranger Peak on Los Padres National Forest, Adin Summit and Blue Lake on Modoc National Forest, and Wheeler Peak on Humboldt National Forest (now part of the Great Basin National Park). The breeding work done with these trees is covered in Chapter 6. Their generalized formula was 20 percent α -pinene, 0 percent β -pinene, 0 percent 3-carene, 20 percent myrcene, and 60 percent limonene. The four areas do have similarities: (a) modest to high amount of limonene; (b) trees without β -pinene; and (c) trees without 3-carene. The few stands with high limonene had similar averages for limonene, but they differed enough in types of composition to be in different regions or zones.

Local Variation

In 1970, after finding the first high-limonene tree as discussed in the previous section, a study was made at Adin Summit (fig. 3-31, stand #40) to see if there were more high-limonene trees there, that could be used for breeding and bioassay research, and to determine the variation among five stands of differing size and location in that area.

Five stands were used for the study as follows:

1. The original stand #40: 78 trees;
2. A surrounding large stand: 366 trees;
3. A large stand about 1 mile west: 377 trees;
4. A large stand about 3 miles southwest: 641 trees;
5. A standard stand about 15 miles north: 94 trees.

The composition of all trees was coded according to specifications (table 3-44) the frequency of types of composition in each stand was determined (fig. 3-35), and stands were compared by coefficients of association.

Though the stands were quite similar, their coefficient of association were generally about 0.6 rather than the 0.7 to 0.8 that one would expect for stands in the Sierra Pacific region—the region in which the stands were located. However, the basic stand at Adin Summit may be right at the beginning of a transitional zone between the Sierra Pacific region and the Cascade Northern region. No such zone was found or reported earlier in this section. But somewhere in this general geographic area one might expect to find such a zone. Stand 5, located about 15 miles north of Adin Summit, had the poorest coefficients of association. This suggested that Stand #5 was approaching the Cascade Northern region. It would be possible to establish a transition zone about 15 miles north of Adin Summit, near stand #5. There are stands of *P. ponderosa* throughout the area,

Table 3-47—Average coefficient of association between Mogollon East and Mogollon West regions of *P. ponderosa* and associated regions and zones; bracketed (), values are average internal values for the stands within each of the two regions

Region or zone	Region	
	Mogollon East	Mogollon West
Coronado	0.11	0.02
Mogollon East	(0.87)	0.62
Mogollon West	0.62	(0.79)
Escalante South	0.43	0.57
Cedar Breaks	0.26	0.60
Escalante North	negative	0.01

Table 3-48—Average xylem monoterpene composition in four stands of *P. ponderosa* and of the composition of individual trees in them classed as high limonene, and the percent terpene in whole resin

Stand ¹ number	dbh inches	Trees sampled number	Monoterpene ²					Terpene in whole resin	
			α -p	β -p	car	myr	lim	ter	
			percent ³						percent
40	-	78	6.2	20.4	44.3	13.8	11.8	2.6	25
	26	1 ⁴	17.6	0.7	0	22.1	58.1	0	26
	7	1 ⁵	21.7	3.2	0	18.3	56.8	0	-
	9	1 ⁵	20.8	23.1	0	12.0	42.9	0	23
	28	1 ⁵	14.5	18.8	0	21.3	45.3	0	22
42	-	79	11.9	20.9	38.4	8.4	14.9	2.1	26
	17	1	25.6	0.4	0	16.1	57.9	0	22
	14	1	24.3	0.4	0.1	18.1	56.9	0	22
30A	-	36	6.0	29.0	25.1	18.0	21.0	1.0	24
	15	1	11.6	3.0	0.2	14.9	70.3	0	24
BL	-	40	8.8	21.7	44.8	11.6	9.8	2.7	-
	30	1	6.5	11.7	0	27.4	54.6	0	-

¹ See figure 3-31 for location of stands.

² α -pinene, β -pinene, 3-carene, myrcene, limonene, terpinolene.

³ Normalized, minor components comprise the remainder of 100 percent.

⁴ Found in original 78-tree stand.

⁵ Found when stand was enlarged to about 1600 trees.

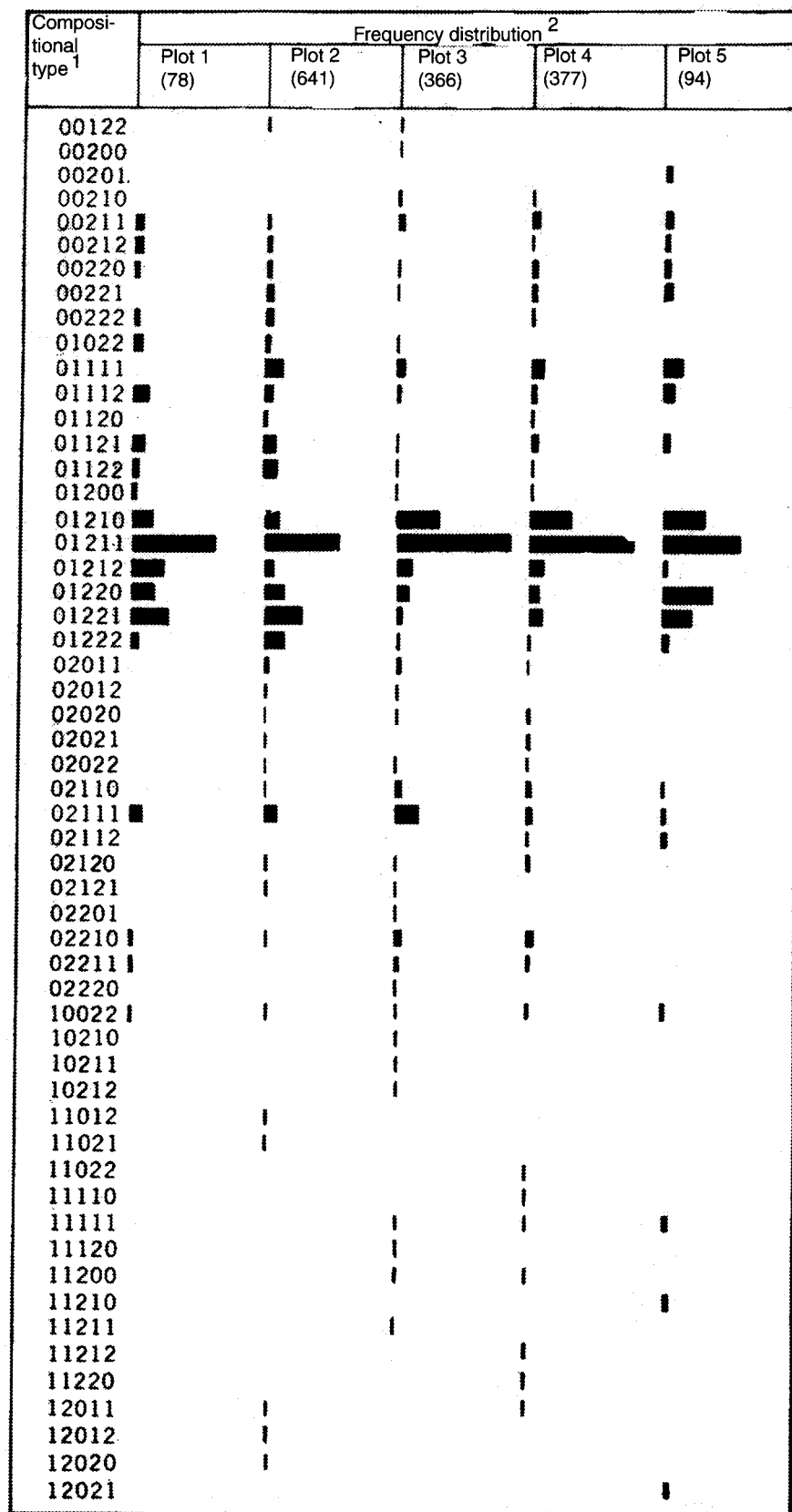
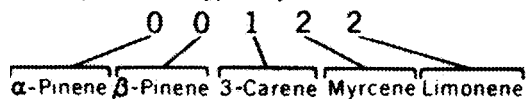
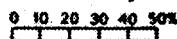


Figure 3-35—Normalized distribution of composition types of *P. ponderosa* xylem monoterpenes in five stands of different sizes near Adin Summit, Modoc National Forest; see *table 3-45* for actual composition of coded types. Occurrence is normalized for each stand.

¹Compositional type key:



²Frequency distribution key:



and it would be interesting to sample a series of stands running northeast of Adin Summit into southern Oregon.

One other small study is of note here in addition to what is given in Chapter 8. An elevational transect was made in the Central Sierra Nevada to see if there is any effect of elevation on composition. Twelve small stands of 10 to 20 trees each were sampled between Placerville and Lake Tahoe from 1800 feet elevation to 6500 feet, and a 181-tree stand at 3800 feet, about mid-elevation, was sampled for comparison. Although there was considerable local variation among the trees of a given stand, there was essentially little or no difference in average composition over the complete range of elevation. The following was the average percent composition, minor components omitted, for the extremes in elevation; N is number of trees:

Elevation	N	α -pinene	β -pinene	3-carene	myrcene	limonene
ft		-----percent-----				
1800	19	7	26	41	12	12
6500	13	6	25	39	14	13
all	163	6	29	36	10	14
3800	181	7	28	33	12	17

#15. *Pinus quadrifolia* Parl. (Parry pine)

P. quadrifolia is found in a few scattered stands in San Diego, Imperial, and Riverside Counties in southern California. However, the major portion of its range is in northern Baja California, Mexico, at lower elevations in the San Pedro Martir Mountains (fig. 3-36). It is another pinyon pine and grows on dry sites, much like *P. monophylla* and *P. edulis*.

Mirov (1961) analyzed the resin of trees in Riverside County and found the monoterpene composition to be nearly-pure α -pinene; there was also a substantial amount of sesquiterpene.

Zavarin and others (1980) looked more extensively and concurred with Mirov that the monoterpene composition was near-pure α -pinene. Zavarin and others (1980) also found small to trace amounts of tricyclene, camphene, β -pinene, 3-carene, limonene, myrcene, β -phellandrene, terpinolene, γ -terpinene, ocimene, and p-cymene.

Zavarin and others (1980) studied the possible introgression of *P. quadrifolia* and *P. monophylla* to get additional data on variation of Parry pine. They found little or no variation throughout the range (table 3-49); the average amount of α -pinene was essentially 94 percent in all stands with the S.D. ranging from only 2.1 to 2.6. No other monoterpene averaged more than 3 percent; most averaged less than 1 percent. Zavarin and others (1980) also found a stand with monoterpene composition and morphological characteristics suggestive of introgression between *P. quadrifolia* and *P. monophylla*. It would seem that monoterpene evidence of introgression would have to be found in the minor components because both species—*P. quadrifolia* and *P. monophylla*—were very high in α -pinene. The average α -pinene for *P. quadrifolia* was greater than 90 percent while the average for *P. monophylla* was slightly more than 80 percent. But many individual trees of *P. monophylla* had more than 90 percent α -pinene. Thus, one might be inclined to separate the two pines as species on the basis of α -pinene because they were somewhat different. Many *P. monophylla* trees can have appreciable amounts of limonene, 3-carene, myrcene, β -phellandrene, and ocimene; these might be useful in separating the two species.

Figure 3-36—Distribution of *P. quadrifolia* in southern California, taken from Little and Critchfield (1966).

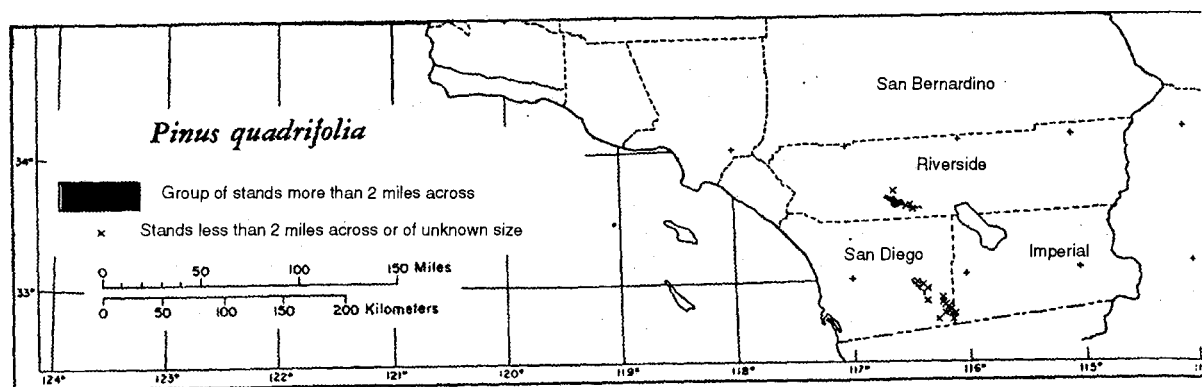


Table 3-49—Average xylem monoterpene composition of four natural stands of *P. quadrifolia* adapted from Zavarin and others (1980) and of one plantation stand at IFG; n = number of trees in the sample

Monoterpene component	Stand location				
	California	Baja California			
	Laguna Mtns	Rumorosa	Pine Solo	Mikes Peak	IFG ¹
	-----percent ² -----				
Tricyclene	*	*	*	*	-
α -Pinene	94.9	93.2	94.9	94.0	84.7
Camphene	1.1	1.3	0.7	1.1	0.4
β -Pinene	0.7	0.4	0.4	0.6	0.8
3-Carene	0.3	0.2	*	0.1	1.6
Myrcene	0.8	0.7	1.1	1.2	4.1
Limonene	0.9	2.7	1.5	1.8	0.9
β -Phellandrene	0.1	*	*	*	2.2
γ -Terpinene	-	0.1	*	0.2	*
Ocimene	1.0	0.8	0.5	0.5	5.3
p-Cymene	0.1	0.1	0.3	0.5	-
Terpinolene	0.2	0.5	0.1	0.1	0.9
n	20	10	10	10	6

¹From seed collected in San Diego Co.; also had a trace of sabinene.

²Normalized; * = trace, - = zero.

Six trees growing at the Institute of Forest Genetics (IFG) from seed collected in San Diego County (*table 3-50*) were much lower in α -pinene than reported by Zavarin; some of these individual trees had much greater 3-carene (9.3 percent), ocimene (6.5 percent), and myrcene (6.6 percent) than had been previously reported for *P. quadrifolia*. Although the differences between the set of 10 trees grown at IFG and the four sets reported by Zavarin and others (1980) were small, they were consistent enough to suggest an environmental effect. Both myrcene and β -phellandrene were different in the two sets. These two components have been suspected of responding to environmental factors in other instances. One might conclude that all *P. quadrifolia* have a (1-1) set of alleles for α -pinene and that all other monoterpenes were derived from isomerization or biosynthetic by-products. Therefore, if introgression with *P. monophylla* was evidenced by the minor monoterpenes that appear to be nongenetic, one would have to assume that isomerization of monoterpenes was under some type of inherited control, or was related to the basic composition that is under strong genetic control.

Little or no difference in composition was found between a basal tree sample and one at 20 feet (see *table 8-1*).

#16. *Pinus radiata* D. Don (Monterey pine)

The natural range of *P. radiata* is limited to three clearly separated localities along the coast of California (*fig. 3-37*). *P. radiata* has been widely planted in California and around the world, particularly in Australia, Chile, South Africa, and New Zealand. As noted earlier, *P. radiata* and *P. attenuata* were extensively used in pine hybridization research from about 1940 to 1960 at the Institute of Forest Genetics (IFG) at Placerville. There is some uncertainty about the varietal status of *P. radiata* in Baja California. Griffin and Critchfield (1972) mentioned it but did not include it in the distribution. Millar (1986) gave varietal status to the species in Baja California, in fact claiming that it consisted of two varieties. I am following Griffin and Critchfield and will not include the southern island varieties; also, there were no terpene data on the island varieties.

Mirov (1961) reported four early results of studies of the xylem monoterpene composition to be various combinations of α -pinene (α -p) and β -pinene (β -p) as follows:

<i>Study</i>	α -p	β -p	<i>Reported by</i>	<i>Location of trees</i>
	---- percent -----			
1	34	64	Barrud	none given
2	24	75	McCombs	New Zealand
3	41	56	Arbuzou	Russia
4	75	22	Mirov	IFG

None of these reports showed any significant amount of monoterpenes other than α -pinene and β -pinene, but the range in these two components was large.

The first detailed study by gas chromatography was by Bannister and others (1962) who sampled five populations: the three natural populations in California and two plantation populations in New Zealand. Size of sample ranged from 27 to 53 trees. Their results showed α -pinene varying from about 20 to 50 percent and β -pinene varying from about 42 to 80 percent, with the amounts nearly complements. They also reported trace amounts of camphene, limonene and β -phellandrene. Though no individual tree data were given, their diagram of the relationship of α -pinene and β -pinene suggested a trimodal

Table 3-50—Xylem monoterpene composition of six trees of *P. quadrifolia* grown in an arboretum at the Institute of Forest Genetics (IFG) from seed collected in San Diego Counts,

No.	Monoterpene ¹									
	α -p	cam	β -p	car	sab	myr	lim	β -ph	oci	ter
-----percent ² -----										
1	76.7	0.4	0.6	9.3	-	3.1	0.5	0.1	6.2	2.6
2	86.8	0.3	0.6	-	-	6.6	-	-	5.0	0.4
3	81.3	0.4	0.9	-	0.6	3.9	2.6	4.4	5.5	0.3
4	88.1	0.5	0.8	-	-	1.9	0.4	0.9	6.2	1.2
5a ³	90.8	0.4	1.1	-	-	2.3	0.5	2.0	2.2	0.5
5b	88.1	0.4	1.0	-	-	2.8	0.8	1.3	5.5	0.1
6a	84.6	0.5	0.6	-	-	6.5	0.7	-	6.5	0.6
6b	86.8	0.5	0.6	-	-	5.9	0.5	-	5.5	0.2

¹ α -pinene, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellandrene, ocimene, terpinolene.

² Normalized; - = zero.

³ a and b are samples of the same tree taken 5 years apart.

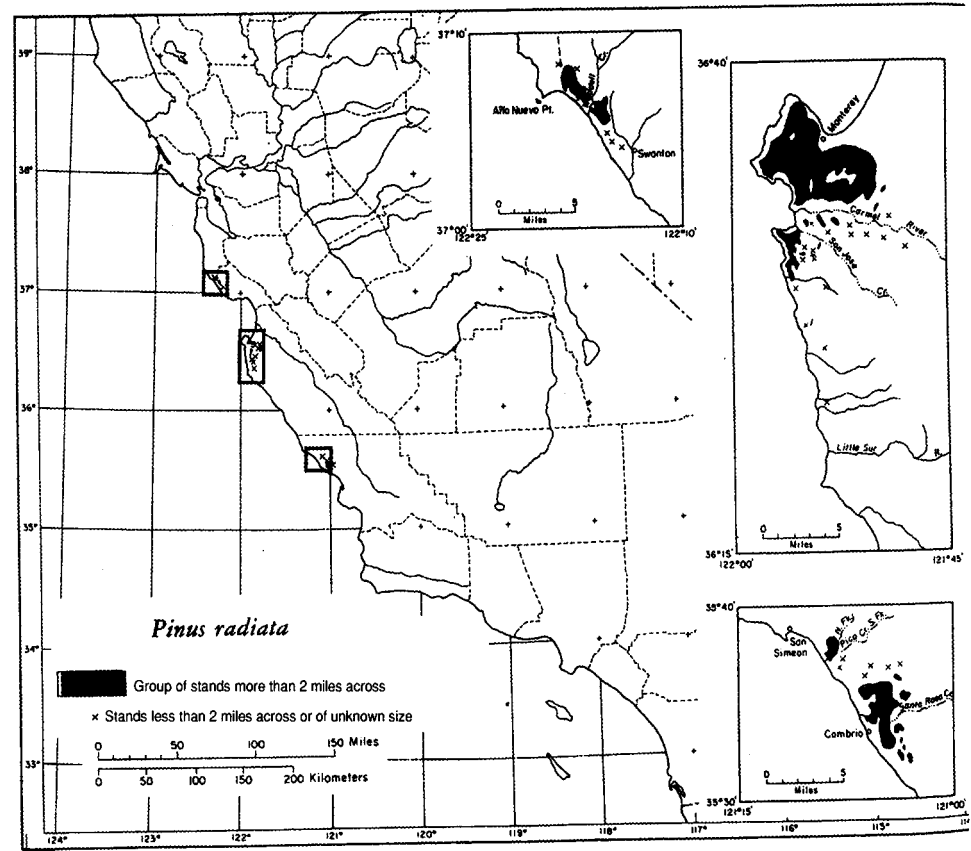


Figure 3-37—The natural distribution of *P. radiata* in California, taken from Critchfield and Little (1966); stands on islands off the coast of Baja California are not included.

distribution of the two components (Bannister and others 1962); the data suggested modes for α -pinene at 20 to 30 percent, 30 to 45 percent, and 45 to 60 percent, with respective percentage ratios of 30:46:24 for the low, medium, and high modes. Low mode for β -pinene was 40-55 percent, mid β -pinene was 56-70 percent, and high β -pinene was 71-80 percent. Alpha-pinene was the complement of β -pinene. Bannister and others (1962) in one of the early studies of within-tree variation, showed no change in composition with time and within-tree position. Likewise, I could find little or no difference in composition between a basal tree sample and one at 20 feet. On the basis of averages, there were no sharp differences among the three California sources.

Forde (1964), in investigating the inheritance of *P. radiata* x *P. attenuata* hybrids, showed β -pinene in 37 *P. radiata* to range from 25 to 50 percent; the distribution appeared to be a single mode. Forde concluded that *P. radiata* was heterozygous for monoterpenes.

Another detailed study by Blight and others (1964) examined 240 trees in eight localities including the three natural stands in California and five plantations in New Zealand. They reported average stand values with a narrow 23 to 35 percent α -pinene and 64 to 76 percent β -pinene, with about 1 to 2 percent minor components. No listing of individual trees nor the frequency distributions of individual components were given, but the small standard deviations could suggest that most of the individual trees were very close to the average.

Valenzuela and others (1966) examined five trees planted in Chile. Their results differed somewhat from the previous report, showing much higher α -pinene (38 percent), small amounts of limonene (4 percent) in some trees, and a significant amount of sabinene (6 percent) in three trees. Could they have been working with trees with some content of *P. muricata*?

Strauss and Critchfield (1982) sampled 308 hybrids of *P. radiata* x *P. attenuata* (2-4 years old) for xylem monoterpenes and subjected the results to various statistical analyses. They concluded that β -pinene—found in *P. radiata* but not in *P. attenuata*—was controlled by a single dominant gene. However, they had difficulty in discriminating between a single-gene and a two-gene modal. From their frequency distribution the recessive condition resulted in a mode extending from 0 to 12 percent and the dominant mode extending from 12 to 90 percent. I have not yet found a mode of this magnitude, 12 to 90 percent, in any pine. The data by Bannister and others (1962), as noted earlier, showed a different pattern from which one might conclude a trimodal distribution. The current assessment of the genetic structure of α -pinene and β -pinene in *P. radiata* is still not clear. However, one might speculate on a (0-1) pair of alleles for α -pinene and a (1-1) pair for β -pinene. This would explain the trimodal distribution given by Bannister and others (1962). The mode proportions of 30:46:24 were close to the theoretical one of 25:50:25 for the low, middle, and high modes. The fact that the low mode was about 25 percent rather than near zero could be explained by isomerization and by some activity by the (0-0) set of alleles. The modes for β -pinene were always greater than those for α -pinene but still trimodal depending on the set of alleles for α -pinene, (0-0), (0-1), or (1-1). Some of the reported studies used young and old trees. Thus some of the inconsistencies could be caused by the presence of a juvenile factor as was discussed earlier in this Chapter for *P. ponderosa* and for *P. coulteri*.

In summary, the xylem monoterpene composition of *P. radiata* was generally reported as 20-45 percent α -pinene and complementary amounts of β -pinene, i.e., 55-80 percent. The frequency distribution of the two components in one report tended to be trimodal, suggesting two additive alleles; in another report the distribution looked monomodal. Both reports were based on 35 to 40 trees, which may not be enough. An examination of planted trees in Chile showed as much as 5.5 percent limonene, 13.5 percent sabinene, and 2 percent

myrcene in some trees. The presence of these unusual amounts of these latter three components is puzzling. It might be that some of the seed was *P. muricata* or a natural hybrid of *P. radiata* and *P. muricata*, because the southern variety of *P. muricata* can have a large amount of sabinene.

#17. *Pinus sabiniana* Dougl. (Digger pine)

Pinus sabiniana is limited to California (fig. 3-38). The greater part of its distribution is the lower elevation of foothills surrounding the Central Valley. But there also are stands in certain portions of the Coast Ranges, the Siskiyou Mountains, and an outlying stand on the Hat Creek Rim in eastern Shasta County. *P. sabiniana* along with *P. coulteri* and *P. torreyana* comprise the big cone pines. Mirov (1961) reported that all early analyses found greater than 95 percent heptane in the xylem resin volatiles of *P. sabiniana*.

I analyzed trees in two stands in the counties of Sacramento and Shasta for a total of 99 trees (fig. 3-38); both stands were a mixture of young and old trees but all were greater than 40 years old. Nearly all trees in both stands had more than 96 percent heptane ranging from 93.6 percent to 99.8 percent (table 3-51). The only monoterpene in any amount was camphene, with an average of about 0.5 percent in the Shasta stand. Nonane averaged about one percent, with a high of more than five percent. Nonane, like heptane, is a hydrocarbon that is sometimes associated with monoterpenes. The two stands differed only in the average amount of heptane, with about 99 percent in the southern source and about 98 percent in the northern source (table 3-51). The most notable difference between the two stands was the frequency distribution of nonane (fig. 3-39). The northern stand seemed to have a bimodal distribution tightly clustered between 0.5 and 2.5 percent with a few values at 0 percent; the southern stand had a large cluster at 0 percent nonane with a few values scattered all the way to about 6 percent. The data were insufficient for determining whether the difference in nonane was environmental or genetic.

P. sabiniana and *P. jeffreyi* are very similar in having about 95 percent heptane plus small to trace amounts of several monoterpenes. However, they differ in that *P. sabiniana* does not have myrcene or β -phellandrene, which are two of the more common trace components in *P. jeffreyi*.

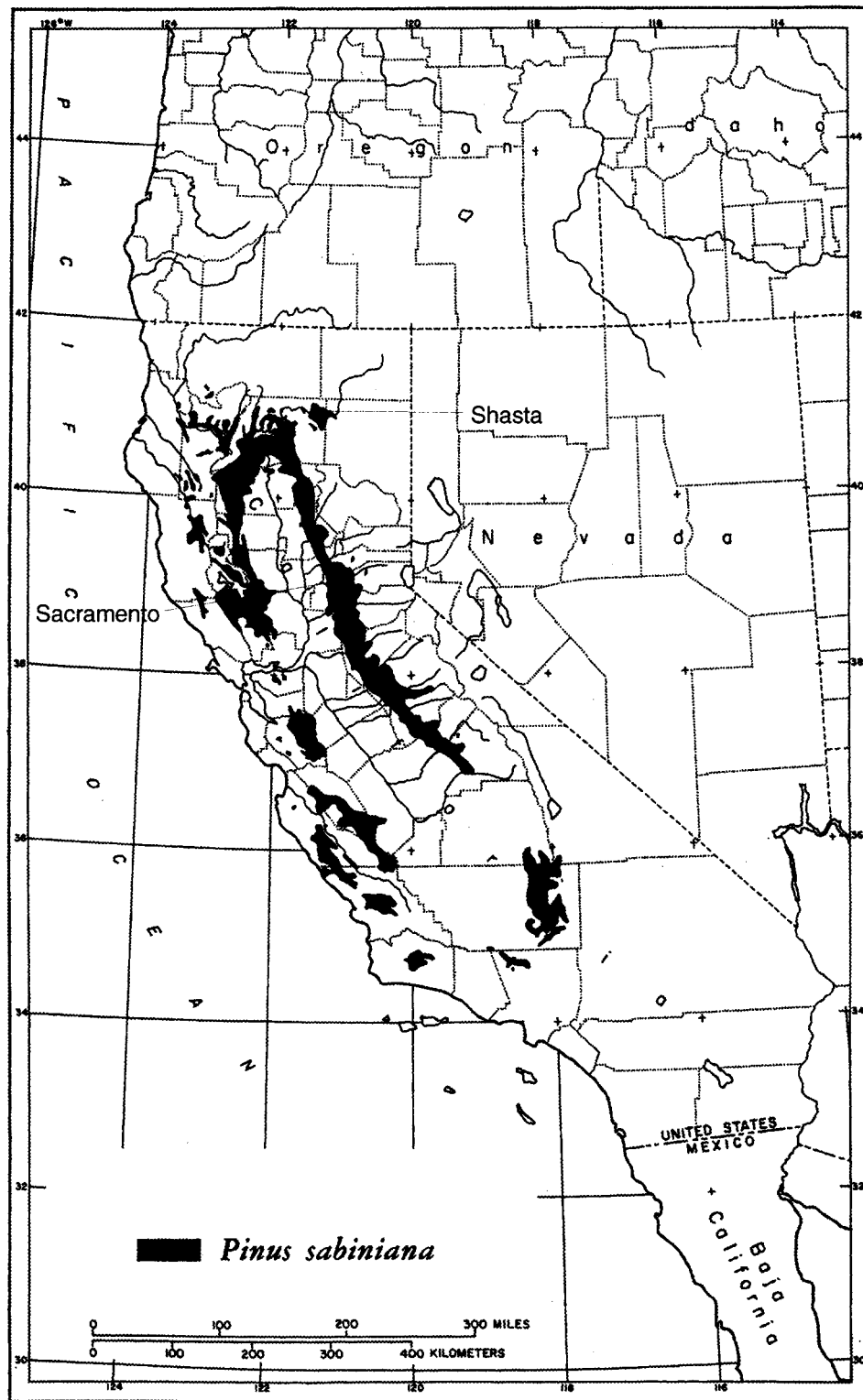
#18. *Pinus torreyana* Parry (Torrey pine)

P. torreyana is a rare pine found at only two locations in southern California. One stand of less than 6000 trees occupies a small strip of coastal land north of San Diego (fig. 3-40). A second stand is found on the Channel Island of Santa Rosa, 175 miles to the northwest; this population is confined to an area of about 800 acres and probably has fewer trees than the mainland stand. Zavarin and others (1967) analyzed both stands—13 mainland trees and five island trees—for xylem monoterpenes (table 3-52).

No data on individual trees were given by Zavarin and others (1967) but it is apparent that the two stands differ in the amount of β -phellandrene and limonene. Four other sets of trees growing at the Institute of Forest Genetics (IFG) from seed collected from the San Diego source showed nearly the same composition as Zavarin's San Diego trees (table 3-52). There was less heptane in the IFG-grown trees; the remaining components were nearly the same in the natural trees or the trees planted at the IFG, despite a large age difference.

P. torreyana is not only rare in numbers and distribution, but is also unusual in apparently having a (1-1) set of alleles for limonene. It would be helpful to have a larger sample from both localities and to have the frequency distribution of the individual components for evaluating β -phellandrene as a characteristic separating the two populations. If the average β -phellandrene (8 percent) in the

Figure 3-38—Distribution of *Pinus sabiniana*, taken from Griffin and Critchfield (1972), and the location of the two stands that were sampled



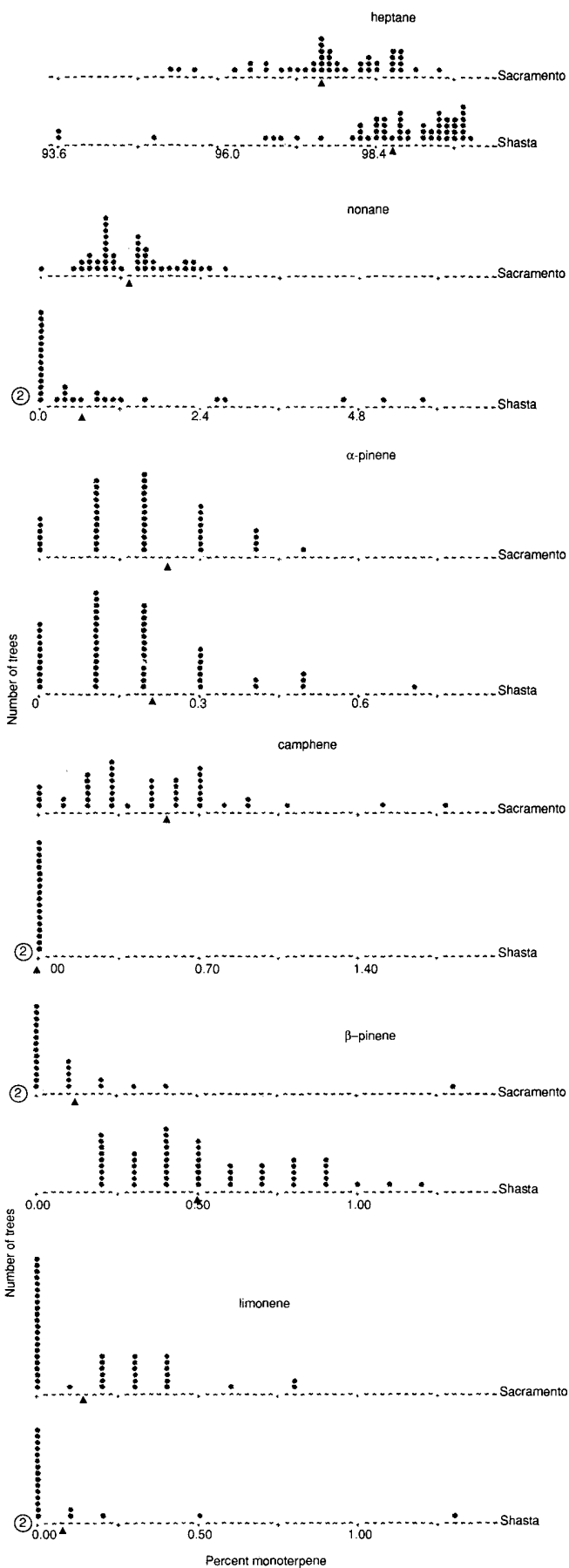


Figure 3-39—Frequency distribution of the amount of the noted monoterpenes and volatile components of the xylem resin in two stands of *P. sabiniana*: Shasta County and Sacramento County, listed on the right each dot is measurement of one tree except where (Ⓝ) indicates each represents *n* trees; ▲ is average of stand.

Figure 3-40—Distribution of *P. torreyana* in California, taken from Critchfield and Little (1966).

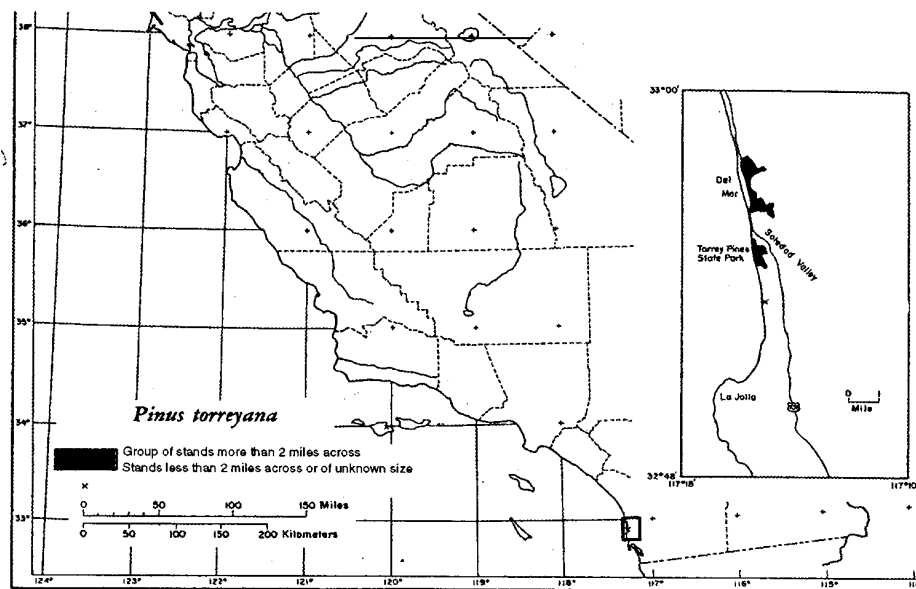


Table 3-51—Average ($\bar{X}$) standard deviation (S.D.) and range for the xylem monoterpenes in two stands of *P. sabiniana* in California: Sacramento and Shasta County; n = number of trees in stand

Monoterpene	Sacramento (n=54)				Shasta (n=44)			
	($\bar{X}$)	S.D.	min	max	($\bar{X}$)	S.D.	min	max
	---- percent ----				---- percent ----			
Heptane	98.6	1.4	93.6	99.8	97.7	0.9	95.3	99.4
Nonane	0.6	1.3	0	5.7	1.3	0.6	0	2.8
α -Pinene	0.2	0.2	0	0.7	0.2	0.1	0	0.5
Camphene	0	0	0	0	0.5	0.4	0	1.8
β -Pinene	0.5	0.3	0.2	1.2	0.1	0.2	0	1.3
Limonene	0.1	0.2	0	1.3	0.2	0.2	0	0.8

¹ Normalized.

Table 3-52—Average monoterpene composition of six sets of *P. torreyana*

Component	Set					
	I	II	III	IV	V	VI
	----- percent l -----					
Heptane	3.6	2.2	*	*	*	*
Nonane	2.4	1.7	2.4	1.7	0.3	1.6
α -Pinene	2.8	2.7	2.5	2.2	2.2	2.4
Camphene	-	-	*	*	0.4	0.2
Undecane	7.4	6.1	7.5	8.1	8.3	8.2
β -Pinene	*	0.2	*	*	*	*
Myrcene	1.7	2.9	2.9	2.9	2.8	2.6
Limonene	73.4	84.2	84.7	85.2	84.8	84.8
β -Phellandrene	8.7	*	-	-	-	-
Terpinolene	0.1	0.5	*	0.3	0.7	0.4
Set	N	Source of tree l		A	Source of Data	
I	7	Natural stand; Santa Rosa Island		?	Zavarin and others (1967)	
II	13	Natural stand; San Diego		?	Zavarin and others (1967)	
III	6	IFG arboretum; San Diego, seed		40	This report	
IV	6	IFG arboretum; San Diego, seed		45	Same trees as III	
V	8	IFG nursery; San Diego, seed		10	This report	
VI	10	IFG nursery; San Diego, seed		13	This report	

¹ Normalized; * = trace, - = zero.

Santa Rosa Island population was caused by some trees with moderate amounts and some trees with none, then one might suspect that there was an allele for β -phellandrene making the island population genetically different from the mainland population where β -phellandrene was absent. If, however, the β -phellandrene in the Santa Rosa Island population was caused by a small amount in nearly all trees, then one would suspect an environmental difference. This situation is very similar to the two populations of *P. balfouriana* where limonene was the component in question. These differences could also be caused by a minor genetic effect which has not yet been explained. However, this uncertainty points out the great advantage of frequency distribution analysis over the use of $\bar{x}$, S.D., S.E., etc.

The composition of the trees growing at IFG, from seed collected from the San Diego stand, was almost identical to that of the natural population near San Diego; this was not the case for *P. quadrifolia* whose population at IFG from seed from San Diego differed somewhat from the natural population near San Diego. Also, the composition of 6- to 10-year-old seedlings from San Diego was essentially the same as that of mature trees.

P. torreyana is grouped with *P. sabiniana* and *P. coulteri* as the big cone pines. The monoterpenes of this group of pines show little or no relationship. *P. sabiniana* is near-pure heptane; *P. torreyana* is near-pure limonene; and *P. coulteri* is quite variable in three monoterpenes, α -pinene, myrcene, and β -phellandrene.

#19. *Pinus washoensis* Mason and Stockwell (Washoe pine)

P. washoensis is another rare pine, though it may not be as rare as was once thought. It was found and described in 1938 as a small isolated stand on the southern slope of Mt. Rose, Nevada, which is in the central Sierra Nevada northeast of Lake Tahoe (Mason and Stockwell (1945) (fig. 3-41). Two other stands have been described, one on Bald Mountain about 20 miles northwest of Mount Rose, the other near Patterson Meadow in the South Warner Mountains about 150 miles north of Mount Rose (fig. 3-41). However, quite recently several other stands have been located in Oregon and as far north as British Columbia that appear to be *P. washoensis* (Haller 1961). Currently, there is much uncertainty about the identity and distribution of *P. washoensis*.

Mirov (1961) reported the xylem monoterpenes to be primarily varying mixtures of 3-carene and β -pinene, plus small to trace amounts of α -pinene.

In 1963, I sampled 28 trees of the Mount Rose population at the location of the type tree of Mason and Stockwell. The results (table 3-53) were generally similar to Mirov's, but there were differences as well: 3-carene was always high but still ranged widely from 49 to 70 percent; β -pinene ranged from about 1 to 33 percent; myrcene averaged 14 percent and ranged from 6 to 21 percent.

I also reported on an investigation of trees at the Patterson Meadow population, as well as nearby populations which could be *P. washoensis* (Smith 1971). The results strongly suggest that the population at Patterson Meadow may be much larger, extending 7 to 10 miles across the southern end of the South Warners, south of Eagle Peak. However, populations of pines farther north through Cedar Pass did not resemble *P. washoensis* but were probably part of the vast Cascade Northern monoterpene type of *P. ponderosa* noted earlier in this chapter.

In the South Warner study a tree was found at a lower elevation whose monoterpene composition resembled a cross of *P. jeffreyi* with *P. washoensis* and *P. ponderosa*. The composition of the tree was 17 percent heptane, 2 percent nonane, 2 percent α -pinene, a trace of α -thujene, 4 percent β -pinene, 55 percent 3-carene, 6 percent myrcene, 11 percent limonene, and 3 percent terpinolene.

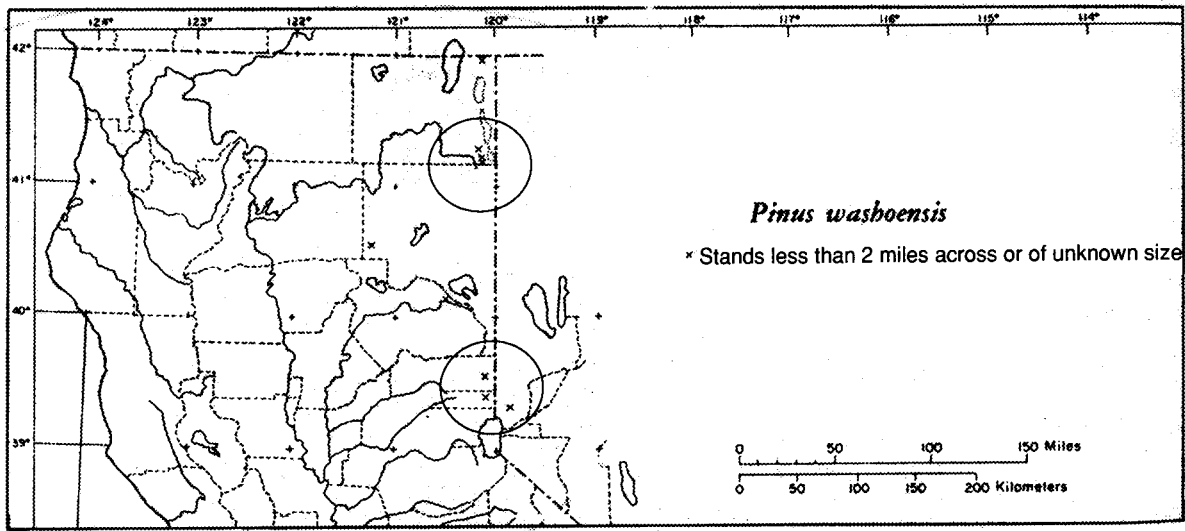


Figure 3-41—Distribution of *P. washoensis* in northeastern California and northwestern Nevada indicated by x circled, as taken from Griffin and Critchfield (1972).

Table 3-53—Average xylem monoterpene composition of 28 *P. washoensis* of the Mt. Rose population and composition of four trees selected to illustrate differences; taken from Smith (1967b)

N	Monoterpene ¹							
	α -p	β -p	car	myr	lim	β -ph	γ -ter	terp
	-----percent ² -----							
28	5.0	9.8	65.6	13.9	1.7	1.1	0.2	2.8
1	6.2	6.4	63.4	10.4	10.2	1.1	0	2.2
1	4.5	22.6	59.9	8.9	0.7	1.2	*	2.3
1	3.6	0.4	70.4	20.5	1.2	1.2	0.3	3.7
1	7.0	33.1	49.9	6.6	0.6	0.6	0	2.1

¹ α -pinene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, γ -terpinene, terpinoline.

² Normalized; * = trace.

A. more recent study was also made of the Mt. Rose population of *P. washoensis* and the South Warner population. The averages of the individual stands in each area and for all trees in the area showed the two stands to be quite similar (table 3-54), yet they had some small differences. The frequency distribution of the individual components in each stand clearly showed the similarities (fig. 3-42).

Little or no difference was found in the composition of *P. washoensis* between a basal tree sample and one at 20 feet (see table 8-1).

The relationship of *P. washoensis* and *P. ponderosa* has been discussed by Critchfield (1984) using several characteristics including cone morphology, crossability, and xylem monoterpenes. To further the study of this relationship I compared *P. washoensis* from the South Warner stand and the Mt. Rose stand with nearby and distant sources of *P. ponderosa* (taken from #14 of this chapter). Eight stands were used (table 3-55); the smaller *P. washoensis* stands were aggregated in order to have about 40 to 60 trees in each stand.

The coding criteria and procedures described for *P. ponderosa* (table 3-44) were used to code the composition of all trees (table 3-56). The frequency distribution of types of trees in each stand was determined (fig. 3-43). Stands were compared by coefficient of association (table 3-56).

The coefficients of association (table 3-56) showed that the two sources of *P. washoensis*, Mt. Rose and South Warners, had strong coefficients with each other, though the Mahogany Ridge stand, farthest west from the Patterson Meadow stand, was the least strongly associated of the *P. washoensis* sources. The latter stands were poorly associated with *P. ponderosa* from three sources in the Sierra Pacific: Adin, Modoc National Forest; Pacific, Eldorado National Forest; and Blue Lake, Modoc National Forest. The Mahogany Ridge stand of *P. washoensis* had a coefficient of association of only 0.21 with the *P. ponderosa* stand at Blue Lake, only 2 to 3 airline miles away, but had a coefficient of 0.85 with the *P. ponderosa* stand at Camas Creek, about 50 airline miles to the north. However, the Blue Lake stand of *P. ponderosa* was still more strongly associated with the Mahogany Ridge stand *P. washoensis* than are other *P. ponderosa* stands of the Sierra Pacific Region. Thus one might conclude that the Blue Lake stand was not pure *P. ponderosa* and was not strongly associated with stands in either the Sierra Pacific or Cascade Regions (fig. 3-43, table 3-56). It was possible that the *P. washoensis* stand at Mahogany Ridge and the *P. ponderosa* stand at Blue Lake were affecting each other through either or both cross breeding and seed dispersal. The Blue Lake stand of *P. ponderosa* may be a transitional type of *P. ponderosa*. There was speculation that such a transition zone, between Sierra Pacific and Cascade Regions, might be found in northeastern California and southern Oregon. The *P. ponderosa* stand at Camas Creek in the North Warner, which was classified as a Cascade Northern stand of *P. ponderosa*, had strong associations with all sources of *P. washoensis*. As Critchfield noted, it appeared that *P. washoensis* is closely related to the Cascade Northern race (resin) of *P. ponderosa* as defined earlier in this chapter.

The Blue Lake stand contains *P. ponderosa* BL-6047, which was a high-limonene tree used in the latter stages of the breeding studies (see Chapter 6).

Table 3-54—Average monoterpene composition of 11 plots of *P. washoensis* in two stands (Mt. Rose and South Warners) and the average of each stand

Stand	Plot	N	Monoterpene ¹							
			α -p	β -p	car	my	lim	β -ph	γ -ter	terp
Mt. Rose			-----percent ² -----							
	1	23	4.9	9.9	65.7	14.0	1.3	1.1	0.2	2.9
	2	21	4.2	8.3	67.5	12.3	1.9	0.7	0.2	4.9
	3	19	4.9	12.2	66.2	9.7	0.6	1.0	0.4	5.1
	4	10	3.9	10.5	65.8	13.1	0.5	1.0	0.4	4.9
	5	10	5.1	16.1	59.3	10.7	2.3	1.3	0.3	4.9
	6	12	5.1	15.4	60.0	12.3	2.8	*	*	4.0
	Total:	95 ³								
	Average:		4.6	10.8	65.5	12.1	1.3	1.0	0.3	4.4
South Warners			-----percent ² -----							
	7	21	5.6	9.4	63.9	13.3	2.6	0.7	0.1	4.3
	8	39	6.9	18.6	57.5	10.1	2.7	0.7	0.1	3.2
	9	20	6.1	13.2	59.9	13.9	1.6	0.8	0.2	4.0
	10	21	6.0	12.3	64.6	11.4	0.8	0.9	0.1	3.7
	11	23	6.8	12.6	66.3	8.9	0.8	0.6	0.2	3.5
		Total:	124 ³							
	Average:		6.4	14.0	61.8	11.3	1.8	0.8	0.1	3.7

¹ α -pinene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, γ -terpinene, terpinolene.² Normalized; * = trace.³ Total number of trees.

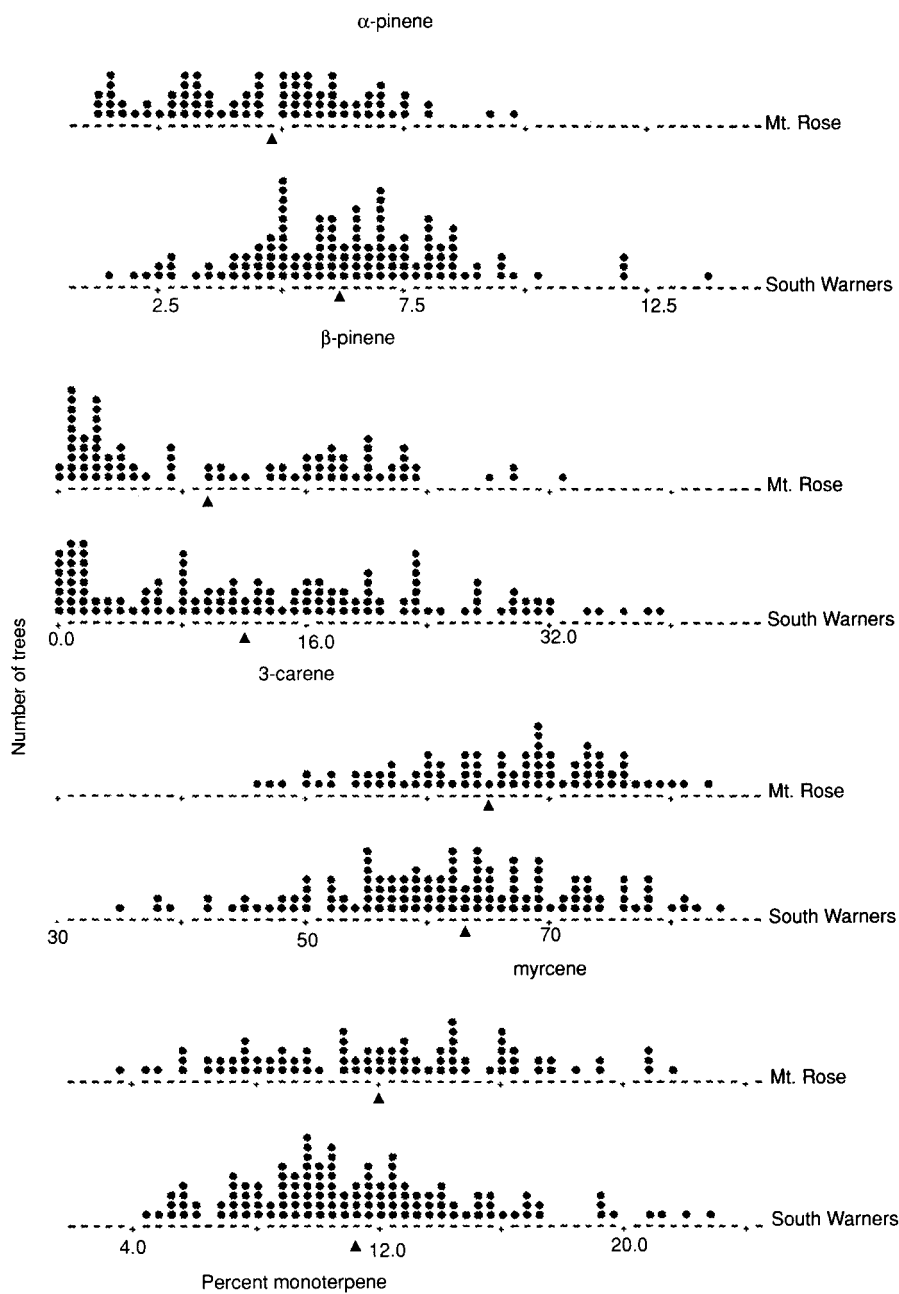


Figure 3-42—Frequency distribution of the amount of the noted monoterpenes in two stands of *P. washoensis*; Mt. Rose and South Warners; each dot is measurement of one tree except where $\bar{n}$ indicates each represents n trees; $\blacktriangle$ is stand average.

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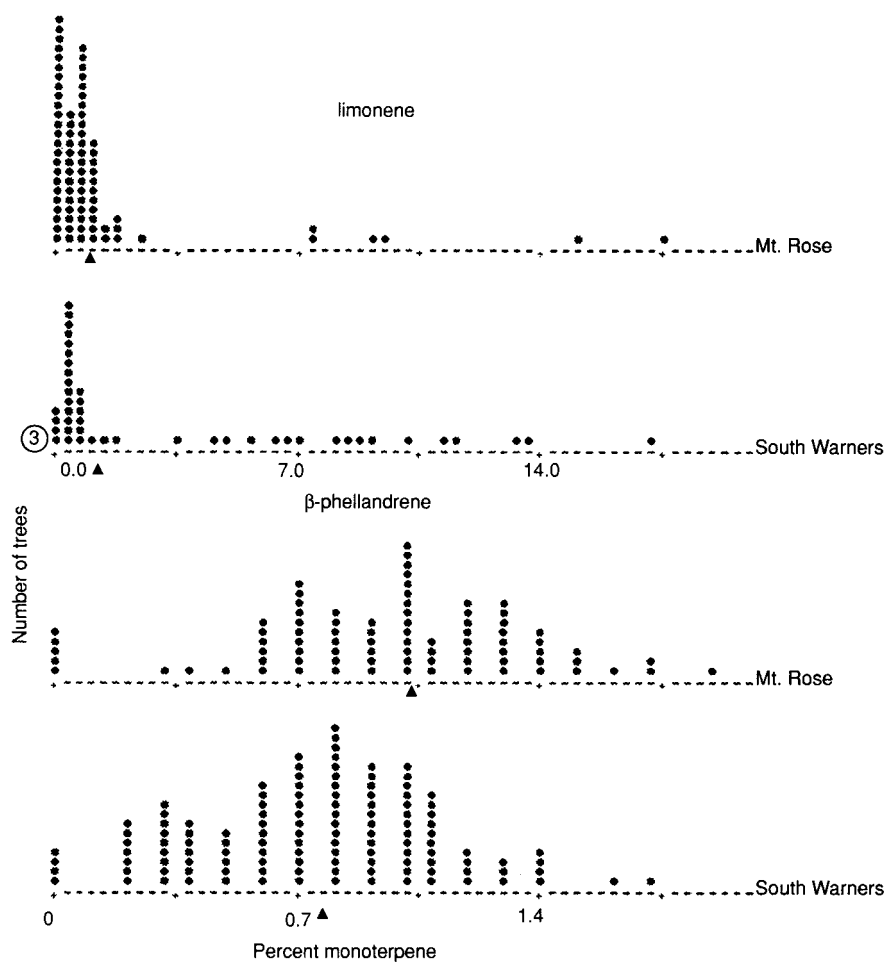


Table 3-55— Coded types and actual average composition in 2 stands of *P. washoensis* and in 5 stands of *P. ponderosa*; see figure 3-43 for frequency of occurrences of types in the stands

Type #	Coded type ¹					Actual composition ¹				
	α	β	car	my	li	α	β	car	my	li
	number					percent ²				
1	0	0	2	1	0	5	1	78	11	1
2	0	0	2	1	1	6	1	69	10	10
3	0	0	2	1	2	6	1	56	9	24
4	0	0	2	2	0	5	1	71	19	*
5	0	0	2	2	1	5	1	61	17	11
6	0	0	2	2	2	6	2	50	22	23
7	0	1	1	1	1	8	32	32	12	13
8	0	1	1	1	2	7	27	27	11	24
9	0	1	1	2	1	7	29	28	20	14
10	0	1	1	2	2	6	26	25	19	23
11	0	1	0	2	2	13	29	1	21	32
12	0	1	2	0	0	6	20	66	2	1
13	0	1	2	1	0	6	20	59	10	1
14	0	1	2	1	1	6	21	51	10	9
15	0	1	2	1	2	5	17	44	10	21
16	0	1	2	2	0	5	17	55	18	1
17	0	1	2	2	1	5	17	46	18	11
18	0	1	2	2	2	4	13	43	21	22
19	0	2	0	1	2	10	49	1	12	27
20	0	2	0	2	2	9	45	*	19	25
21	0	2	1	1	1	8	41	27	11	11
22	0	2	1	2	1	7	37	26	16	10
23	0	2	1	2	2	4	40	20	17	19
24	0	2	2	1	0	5	45	38	10	1
25	0	2	2	1	1	7	38	38	8	6
26	1	0	0	2	2	26	2	0	18	47
27	1	0	2	1	1	36	1	49	5	6
28	1	1	0	2	2	24	20	1	19	36
29	1	2	1	1	0	28	40	23	5	1

¹ α -pinene, β -pinene, 3-carene, myrcene, limonene; minor components omitted: camphene, β -phellandrene, terpinolene; see table 3-53 for amounts of minor components.

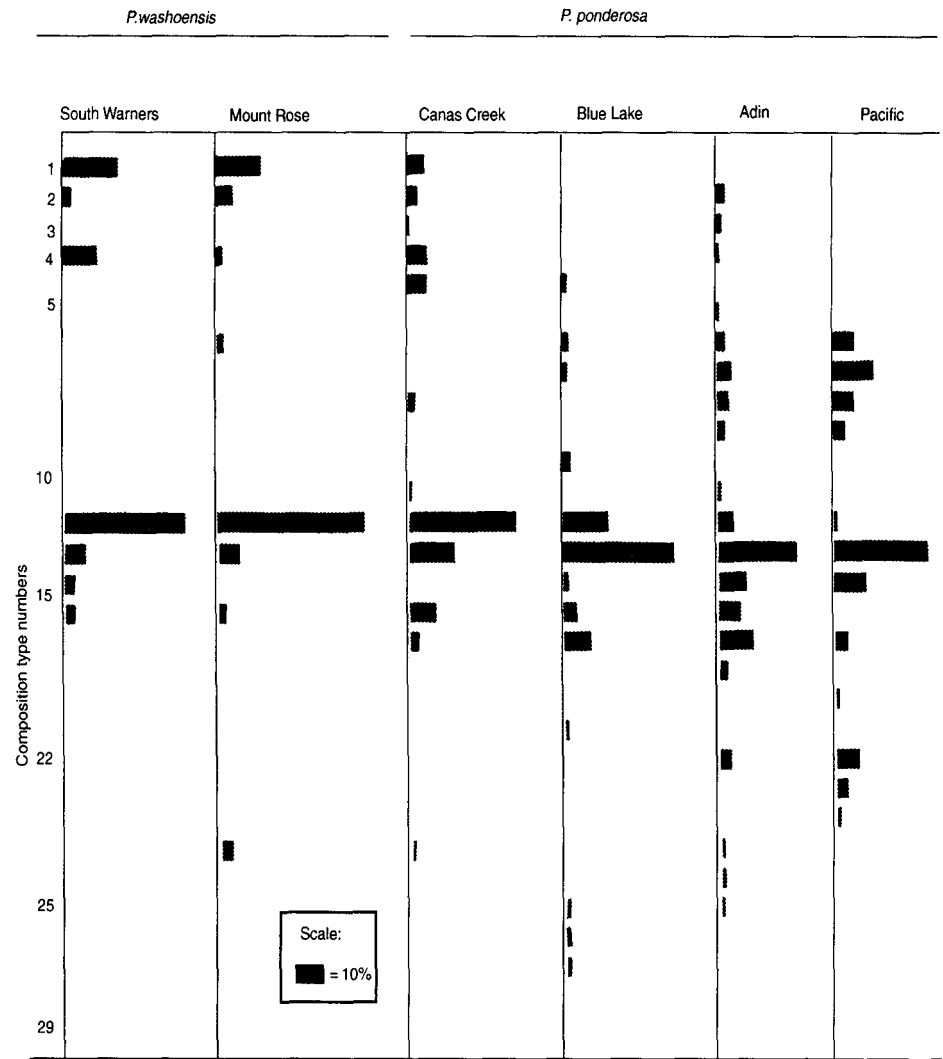
² Normalized; *=trace.

Table 3-56—Coefficient of association between stands of *P. washoensis* and *P. ponderosa* on the basis of the frequency of types of monoterpene composition

Location ¹		Species						
		----- <i>P. washoensis</i> -----			----- <i>P. ponderosa</i> -----			
		M. Rig	MR-1	MR-2	Cam-41	BL	Ad-40	Pac-37
1	Pat-Hom	.84	.70	.73	.85	.21	.07	.00
2	M. Rig		.69	.86	.77	.03	.00	.06
3	MR-1			.71	.70	.04	.01	.05
4	MR-2				.71	.07	.01	.02
5	Cam-41					.31	.13	.01
6	BL						.80	.58
7	Ad-40							.71
8	Pac-37							

¹ Patterson-Homestead (1), Mahogany Ridge (2), Mt. Rose (3,4), Camas Creek-41 (5), Blue Lake (6), Adin-40 (7), Pacific-37 (8).

Figure 3-43—Distribution of types of composition in two stands of *P. washoensis* and four stands of *P. ponderosa*; distribution was normalized for each stand.



Monoterpene Composition of 88 Hybrids and 24 Parent Species at the Institute of Forest Genetics

Summary: This chapter catalogs the xylem monoterpene composition of 88 intra- and interspecific pine hybrids and 24 parent species growing in the arboretum at the Institute of Forest Genetics, near Placerville, California. Most of the parent species were from North America, but a few were from Central America and Eurasia. Much of the information is about *P. ponderosa*, *P. jeffreyi*, *P. coulteri*, and *P. taeda*. The number of trees sampled for a given parent or hybrid was usually small, and only generalized statements can be made about monoterpenes; likewise, the actual parents of most hybrids were almost always unavailable for terpene analysis. In many cases the pollen was a mixture of trees; therefore, conclusions were almost always based on the composition of the parent as a species. The composition of hybrids was usually intermediate, in a general sense, between the two parent species; the lack of close intermediacy would appear to be attributed to an unequal contribution of alleles by the two parent trees in an additive allele system. A given monoterpene can be lost in the first backcross. Some hybrids had monoterpenes that had not been reported in the parents, suggesting that the parent species had not been adequately sampled.

The xylem monoterpene composition was determined for many of the intra- and interspecific pine hybrids that were developed at the East Arboretum of the USDA Forest Service's Institute of Forest Genetics (IFG), near Placerville, California, from about 1935 to about 1965. Therefore, all trees were older than 20 years at the time of this report, and many were older than 40 years. In all but a few cases, the number of trees available was not sufficient to determine the extent of variation in the composition of an individual cross. This report is fairly extensive and representative of the trees available in the East Arboretum at IFG, but it is not at all complete. The breeding procedures used to develop the hybrids were described by Cumming and Righter (1948). Included in the sampling was the composition of some of the parent tree species growing in the same arboretum. Much of the pollen used in the breeding was a mix of a number of trees of that species or hybrid. The basic objective of much of the early breeding was to determine crossability of species and hybrids as well as the appearance and characteristics of the progeny in general. It was thought that the use of a pollen mix would lower the likelihood of failure because of individual tree incompatibility. However, the use of pollen mix did reduce the inference that can be made about the progeny. The report in this volume touches upon one of the objectives of the breeding program: the characteristics of the progeny. All the trees reported were still growing at IFG, nearly all in the East Arboretum. Included in this report were some of the data of an earlier report from the same arboretum (Smith 1967a); however, these trees were

removed in a 1966 thinning of the arboretum. Where appropriate, reference will be to Mirov's (1961) report, and to other reports in order to provide information about parent species. I found Mirov's report to be correct in identifying components that were present. However, in some instances he failed to report the presence of components—often occurring in small to moderate amounts—which have been found since his work. This could be attributed to lack of extensive sampling or to the lack of more sensitive analytical procedures. Nevertheless, there was general agreement in the qualitative composition of this report and that of other reports. There were instances of quantitative differences, but that was to be expected considering the general variation found in the monoterpene composition of many pines and the action of modifying factors.

The presence of small to trace amounts of several terpenes, usually called minor components, in many species and hybrids requires some comment. One might speculate that these were by-products of the synthesis of the major monoterpenes in a given species or hybrid, or that they were products of isomerization of the major monoterpenes, or that there was a secondary genetic mechanism. Likewise, the major components were found in small amounts in many trees, even in species in which they were major components. And thus there was the possibility that the amount of a given component could be produced by both a major and minor mechanism. Only two clear linkages of major with minor components was found: the relationship of terpinolene to 3-carene with a ratio varying from 1 to 20 to about 1 to 10 depending on the species, and the relationship of terpinolene to sabinene with a ratio varying from 1:1 to 1:2, depending on the species.

The classification and nomenclature of pines used in this section will be that given by Critchfield and Little (1966). Changes made since the time the breeding was done will be noted in the sections dealing with the particular species. However, there still was uncertainty about the varietal status of some pines, notably *P. ponderosa*, *P. contorta*, *P. halepensis*, *P. pinaster*, *P. montezumae*. The taxonomic discussions will be few and brief since the report's objective was to present the xylem monoterpene characteristics of the species and hybrids. A botanical description of many of the hybrids was given by Little and Righter (1965); these will be noted within the text where appropriate without comment.

Trees were sampled by the 9/16-inch macro-tap and 5-cc vial or by mini-tap with the tap hole acting as the resin collector, between 1981 and 1988. Sampling and analysis were described under "Procedures" in Chapter 2. Both pentane and acetone preparations were made for trees where the presence of heptane was suspected.

The monoterpene compositions of the individual parent species or hybrid used in a cross were not available; therefore, in all cases the report was based on generalized composition of the parent trees used in the crosses.

Some General Statements About Monoterpenes

The following generalized statements can be made from an examination of all the listings.

- The gene structure for xylem monoterpenes might be either two additive alleles at a single locus, polygenic, or a dominant/recessive pair of alleles. The evidence seemed strongest for additive alleles. There was some evidence that under certain circumstances, not yet understood, the (0-0) pair of alleles might have a small degree of activity rather than zero activity.

- Trace or small amounts of monoterpenes can be present although the gene for that monoterpene may be absent; this may be caused by isomerization from other monoterpenes or by-products of biosynthesis.
- The evidence suggested that the following monoterpenes or associated hydrocarbons were directly controlled in xylem resin of pines by genes: heptane, nonane, α -pinene, β -pinene, 3-carene, sabinene, myrcene, limonene, and β -phellandrene, whereas the following appeared to be produced by isomerization, or by some other mechanism as yet not understood, or by direct association with a gene-controlled monoterpene: nonane, undecane, camphene, α -phellandrene, γ -terpinene, ocimene, terpinolene, α -thujene.
- Because there appeared to be a fixed amount of a five-carbon precursor for the biosynthesis of monoterpenes, there was, in effect, "competition" among the monoterpene genes for this fixed amount. And there may be unequal competition for the precursor.
- The expression of one gene was dependent on both its allelic structure as well as that of the competing genes. For example, a (1-1) pair of alleles for α -pinene will produce a monoterpene composition of nearly all α -pinene if all other pairs of alleles for other monoterpenes were (0-0). But, if there was a (1-1) or (0-1) pair of alleles for one or more of the other monoterpenes, the composition will not be nearly all α -pinene. Instead, it will be divided up among the components that have (1-1) or (0-1) alleles or possibly even (0-0) in some instances. If additive alleles were the mechanism, then interspecific progeny might not be intermediate in composition because one parent could have more alleles than the other. Thus, with competition for a fixed amount of precursor, the monoterpene composition of the progeny would be more like one parent than the other.

Eighty-eight intra- and interspecific crosses and 24 parent species were listed in this chapter. The most common hybrids were those of *P. ponderosa* and *P. jeffreyi*, and this reflected the emphasis of the breeding programs at IFG from about 1935 to 1965. The listing was alphabetical by the female parent or the female parent of the hybrid when used as a female. There have been a few taxonomic changes since the breeding was done; these will be noted in the text.

Explanation of Format

For each species or cross, the number of progeny analyzed and their average normalized percent composition will be listed first below the lined heading. This will be followed by individual listing of the composition of one or more of the trees that were selected from the total number to show the variation in composition of individual trees. Below these individually listed trees may be given the composition listed by Smith (1967a) of the same species or hybrid, or by Mirov (1961) of one or more of the parent species involved in the cross. Mirov's sample was usually obtained by aggregating the resin from several trees; therefore, no number of trees was given when citing his report. Where appropriate, the data of Chapter 3 will be referenced.

The "Source" column designates the origin of the data for each row as follows:

TR = This Report

M = Mirov (1961)

S = Smith (1967a)

All other references will be footnoted in the customary way.

The number of trees, "n," follows "Source."

All trees are listed in the conventional way with the female parent preceding the "X" and male parent following it. Hybrids are designated by a period between two species names and with an underline covering both trees.

Asterisks (**) indicate hybrids that were described by Little and Righter (1965).

Abbreviations of monoterpenes are as follows:

hep	= heptane	β -p	= β -pinene	lim	= limonene
non	= nonane	car	= 3-carene	β -ph	= β -phellandrene
and	= undecane	sab	= sabinene	γ -ter	= gamma-terpinene
α -p	= α -pinene	myr	= myrcene	oci	= ocimene
cam	= camphene	α -ph	= α -phellandrene	ter	= terpinolene
α -th	= α -thujene	*	= trace amount	-	= zero amount

Percentages are given in digits, * indicates trace, - indicates zero. Where the value space is blank, no value was available from the source.

Most cited reports gave the number of trees sampled and normalized composition, but there were a few cases where this was not done.

#1. *Pinus attenuata*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph
-----percent: normalized -----								
TR	5	96.4	0.4	1.0	0.6	1.3	0.2	0.2
TR	1	97.2	-	1.9	-	0.5	0.2	0.1
TR	1	92.2	0.2	1.1	2.6	3.5	0.1	0.2
M		98.0	-	-	-	-	-	-

The above format is explained in the introduction to this chapter.

This species is largely limited to California, though its distribution does extend northward into southern Oregon. Five trees were sampled, and all had the same composition, nearly pure α -pinene, with small amounts of camphene, β -pinene, 3-carene, myrcene, limonene, and β -phellandrene. Only α -pinene and myrcene had amounts greater than 1 percent. This composition agreed with all previous work (Mirov 1961, Williams and Bannister 1962) and with a large sample study in this report (see Chapter 3, #3). It can easily be concluded that *P. attenuata* has a (1-1) set of additive alleles for α -pinene. It might also be concluded that the small amounts of the other terpenes were products of isomerization or biosynthetic by products and reflect the natural variation in this property.

#2. *P. attenuata* x *P. radiata***

Source	n	α -p	cam	β -p	car	myr	lim	β -ph
----- percent: normalized -----								
TR	7	63.9	-	35.2	0.1	0.5	0.2	0.4
TR	1	68.8	-	30.7	-	0.4	0.1	0.1
TR	1	56.9	-	42.4	-	0.3	0.2	0.3
S	1	59.0	2.0	38.0	-	1.0	1.0	1.0

The above format is explained in the introduction to this chapter.

This hybrid was considered one of the early successes of pine hybridization at IFG. It presumably combined the growth properties of *P. radiata* with the cold and drought tolerance of *P. attenuata*. The hybrid was expected to be suited for the lower elevations of the Sierra Nevada.

Seven trees were sampled, and the average was 64 percent α -pinene and 35 percent β -pinene with small amounts of camphene, 3-carene, myrcene, limonene, β -phellandrene. The variation among the seven trees was not great, with α -pinene ranging from 69 to 57 percent, and β -pinene from 42 to 31 percent. The range of these two components was much greater in a sample of 300 4-year-old trees (Strauss and Critchfield 1982). However, they were using the composition of very young trees, and the age of xylem sampled was, apparently, 3 to 4 years. In that study (Strauss and Critchfield 1982), it was concluded that β -pinene was controlled by a dominant/recessive mechanism, as was also concluded earlier (Forde 1964). Neither report gave the range in composition of individual trees which would be helpful in assessing the relationship of α -pinene to β -pinene and in determining the modal characteristics of the frequency distribution.

#3. *P. attenuata* x *P. muricata***

Source	n	α -p	cam	β -p	car	sab	myr	lim	β -ph	ter
----- percent: normal -----										
TR	3	63.9	0.3	3.2	-	21.5	1.0	0.3	*	9.8
TR	1	40.6	0.1	5.7	-	35.3	1.9	0.4	*	16.0
TR	1	83.7	0.6	2.1	-	7.9	0.1	0.6	0.1	5.1
TR	1	67.3	0.3	1.8	-	21.2	1.0	*	*	8.4
S	1	44.0	1.0	3.0	*	34.0	1.0	1.0	1.0	16.0

¹Listed as *P. remorata* when breeding was done.

The above format is explained in the introduction to this chapter.

The male parent was designated as *P. remorata* at the time the breeding was done. However, that species is now considered part of *P. muricata*. The three trees sampled had a wide range in composition, reflecting the great variation in the male parent (Forde and Blight 1964, Mirov and others 1966, Chapter 3 of this volume, #13). With a wider source of male parents, one would expect even a greater variety in composition. The composition of two of the trees suggests that the male parent was high in sabinene, which is characteristic of the sources of *P. muricata* in Southern California. Three of the trees also showed the general 2:1 relationship of sabinene to terpinolene.

#4. *Pinus brutia*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----										
TR	3	55.1	0.3	20.1	19.8	1.7	0.2	0.9	*	1.8
TR	1	72.1	0.4	17.5	7.1	1.4	*	1.1	-	0.2
TR	1	40.5	0.2	28.5	25.9	1.6	-	0.8	-	2.4
M		62.0		17.0	13.0					2.0

The above format is explained in the introduction to this chapter.

This was designated as *P. halepensis* var. *brutia* when breeding was done, but recent isozyme research established *P. brutia* as a separate species (Conkle and others 1988). The composition of these three trees somewhat resembled those reported under item #5 below, *P. brutia* x *P. halepensis*, although they tended to more closely resemble *P. halepensis* x wind. They did not resemble the *P. halepensis* reported by Mirov and others (1966) who reported *P. halepensis* with about 95 percent α -pinene, *P. brutia* with about 62 percent α -pinene, and the remaining 30 percent about equally divided between β -pinene and 3-carene. (Also see *P. halepensis*, #32 below.)

#5. *P. brutia* x *P. halepensis*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----									
TR	5	79.8	0.3	5.1	8.1	0.7	4.3	0.6	0.9
TR	1	78.7	0.6	11.6	-	1.5	6.0	1.6	-
TR	1	95.8	0.2	0.8	-	*	3.0	-	0.1
TR	1	47.6	*	0.5	40.	1.0	5.9	*	4.3

The above format is explained in the introduction to this chapter.

These progeny were reciprocals of item #33 below, and the results were quite similar both in average amounts and in the variable kinds of composition. Both *P. brutia* (#4) and *P. halepensis* (#32) were generally low or lacking limonene, although one of the *P. halepensis* listed for #32 had 7 percent limonene. From the above data, one might speculate that limonene was higher than reported for some *P. halepensis*.

#6. *Pinus contorta* var *contorta* x wind

Source	n	α -p	cam	β -p	car	sab	α -ph	lim	β -ph	ter	
-----percent: normalized-----											
S	1	9.0	1.0	4.0	5.0	2.0	1.0	2.2	2.0	73.0	1.0
M	-	5.0	-	-	-	-	-	-	-	95.0	-

The above format is explained in the introduction to this chapter.

Only one tree was reported and that was from a previous publication (Smith 1967a). Mirov (1961) reported this variety as about 5 percent α -pinene and 95 percent β -phellandrene. The composition for the one tree listed above was well within the range of that reported in a range-wide study of *P. contorta* (Smith 1985). For more information on this species, see Chapter 3.

#7. *P. contorta* var. *murrayana* x *P. contorta* var. *contorta*

Source	n	hep	α -p	cam	β -p	car	sab	α -ph	myr	lim	β -ph	ter
-----percent: normalized-----												
S	1	*	6.0	1.0	8.0	2.0	2.0	1.0	3.0	2.0	76.0	*
S	1	*	8.0	1.0	4.0	18.	2.0	1.0	3.0	2.0	59.0	1.0

The above format is explained in the introduction to this chapter.

These two trees, half-siblings and reported earlier (Smith 1967a), fell well within the expected range of compositions. There was a noticeable difference between the two half-siblings in both 3-carene and β -phellandrene.

#8. *P. contorta* x *P. contorta* var. *murrayana*

Source	n	α -p	cam	β -p	car	sab	α -ph	myr	lim	β -ph	ter
-----percent: normalized-----											
TR	1	7.7	0.5	15.4	37.5	0.5	-	1.5	2.3	34.6	*

The above format is explained in the introduction to this chapter.

The composition of the one tree was well within the expected: high in β -phellandrene and 3-carene, moderate amounts of β -pinene and α -pinene, and small to trace amounts of camphene, sabinene, myrcene, limonene, and terpinolene. The absence of terpinoline in this one tree was puzzling because one would expect it to be between 1.5 and 2.0 percent since it was almost always at a ratio of 1:15 to 1:20 with 3-carene.

#9. *P. contorta* var. *murrayana* x *P. contorta* var. *bolanderi*

Source	n	α -p	cam	β -p	car	sab	α -ph	myr	lim	β -ph	ter
-----percent: normalized-----											
TR	10	5.8	0.6	5.4	9.3	1.2	0.3	2.6	0.8	74.0	0.2
TR	1	7.1	2.8	13.4	2.5	*	-	*	*	74.2	-
TR	1	5.0	0.1	1.7	-	1.3	0.5	2.8	1.3	87.3	*
TR	1	3.5	-	0.4	30.9	5.2	-	1.1	-	57.6	1.4
TR	1	6.6	0.5	6.2	3.9	*	*	3.7	78.6	78.6	0.4

The above format is explained in the introduction to this chapter.

The intraspecific hybrids of these two varieties of *P. contorta* showed considerable variation in β -pinene, 3-carene, sabinene, and β -phellandrene. However, the range in composition suggested that the var. *murrayana* and var. *bolanderi* cannot be separated on the basis of monoterpene composition.

#10. *P. contorta* var. *murrayana* x *P. banksiana***

Source	n	α -p	cam	β -p	car	sab	α -ph	myr	lim	β -ph	ter
-----percent: normalized-----											
TR	9	58.0	0.1	20.2	1.6	0.2	*	0.9		18.1	*
TR	1	91.3	-	7.8	-	-	-	1.0	-	-	-
TR	1	49.6	0.2	25.1	2.9	*	*	1.2	0.4	20.6	*
TR	1	65.8	0.4	13.5	-	-	-	1.0	-	19.3	-
S	1	55.0	1.0	23.0	2.0	*	*	2.0	1.0	18.0	-

The above format is explained in the introduction to this chapter.

The nine trees reported here showed a general intermediacy between the two parents. Mirov (1956), reporting an examination of trees in Alberta, Canada, where the two species *P. contorta* and *P. banksiana* were sympatric, showed that many trees had resin properties intermediate between the two parent species. Intermediate composition was reported for hybrids of controlled pollination (Zavarin and others 1969). This latter study also gave some evidence that the genes for the two species acted independently of each other. The one tree without β -phellandrene and with 91 percent α -pinene was completely off scale for any progeny of the two species and might be considered a rogue. A more comprehensive treatment of hybridization of *P. contorta* and *P. banksiana* was given by Zavarin and others (1969). In that report Zavarin and others were puzzled by the lack of β -phellandrene in some of the hybrids. They may have been working with young hybrids, and it will be shown in other parts of this volume (see *P. coulteri*, Chapter 3) that the production of β -phellandrene may be greatly suppressed in young trees and that the amount of β -phellandrene increased with age of the tree. Mirov (1961) listed *P. banksiana* with 85 percent α -pinene and 10 percent β -pinene.

#11. *P. contorta* var. *murrayana*. *banksiana* x *P. banksiana*

Source	n	α -p	cam	β -p	car	sab	α -ph	myr	lim	β -ph
-----percent: normalized -----										
TR	1	81.9	3.0	15.0	-	-	-	-	-	-

The above format is explained in the introduction to this chapter.

This backcross was nearly all α -pinene and β -pinene. The lack of β -phellandrene can be explained by starting with a (1-1) set of alleles for β -phellandrene in the *P. contorta* parent, a reduction to a (0-1) set in the F-1 generation, and a further reduction to a (0-0) set in this listed tree. These results supported either the dominant/recessive alleles or an additive set of alleles for β -phellandrene. However, Smith (1983) found strong evidence for additive alleles for β -phellandrene in a range-wide study of *P. contorta*.

#12. *P. contorta* var. *murrayana*.*banksiana* x *P. contorta* var. *murrayana*.*banksiana*

Source	n	α -p	cam	β -p	car	sab	α -ph	myr	lim	β -ph	ter
-----percent: normalized -----											
TR	9	56.4	0.3	20.6	1.4	0.1	0.1	1.5	0.6	19.0	0.2
TR	1	34.1	0.1	14.1	4.1	-	-	2.4	0.4	44.9	0.1
TR	1	11.9	0.2	54.9	*	-	-	1.6	0.6	30.3	0.3
TR	1	85.4	*	10.5	1.7	-	-	1.1	0.4	0.7	0.2
TR	1	57.8	1.0	19.8	0.2	0.2	0.3	1.4	*	19.0	*

The above format is explained in the introduction to this chapter.

These backcrosses had a wide range of compositions as illustrated by the individually listed trees. Though Mirov (1961) reports only 10 percent α -pinene in *P. banksiana*, Zavarin and others (1969) reported a considerable range from about 40 percent to 90 percent and reported β -pinene ranging from about 20 percent to about 50 percent. These values were derived from data given as normalized composition of total resin. Therefore, the variation and range in these nine trees were expected when the variation of the two parent species was considered. Among these F2 generation progeny one can see *P. banksiana*-like progeny with high α -pinene and moderate β -pinene and *P. contorta*-like progeny with high β -phellandrene.

#13. *Pinus coulteri* x wind

Source	n	hep	non	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized-----													
S	1	*	*	53.0	2.0	2.0	2.0	*	2.0	2.0	1.0	36.0	1.0
S	1	1.0	1.0	43.0	4.0	*	3.0	*	*	15.0	5.0	28.0	*
M	-	5.0	-	33.0	10.0	-	-	-	-	-	-	33.0	*

The above format is explained in the introduction to this chapter.

The first two trees listed were reported earlier (Smith 1967a), and their composition was well within that expected. This species was reported here because it was used in a number of crosses involving *P. ponderosa* and *P. jeffreyi*. (See Chapter 3, #6 of this volume for additional data on *P. coulteri*.) Nearly all of the hybridization work done with *P. coulteri* was with the male tree. When mature, the very large, heavy, and heavily armed cone of *P. coulteri* would be difficult to handle, although this should be no problem with very young conelets at the time of pollination.

#14. *Pinus echinata*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized-----							
TR	11	65.1	0.1	31.8	1.5	1.1	0.6
TR	1	75.5	-	23.5	0.5	0.2	2.0
TR	1	38.2	*	59.6	0.9	0.5	0.9
TR	1	60.0	0.3	37.6	1.1	*	1.1
TR	1	89.3	0.5	8.5	1.2	0.4	0.1
M	1	85.0	-	11.0	-	-	-

The above format is explained in the introduction to this chapter.

The composition of this species was essentially varying percentages of α -pinene and β -pinene, and Mirov (1961) reported the same. The wide range in both components suggested that there can be (0-0), (0-1), or (1-1) sets of additive alleles. Coyne and Keith (1972) report a wide range in both α -pinene, 20 to 90 percent, and β -pinene, 10 to 70 percent. Their average composition was 48 percent α -pinene, 48 percent β -pinene and 1 percent camphene, myrcene, limonene, and β -phellandrene. Neither the α -pinene nor β -pinene was absent from any trees; this suggested the isomerization of one from the other when the first had a (0-0) set of alleles. It could also suggest that the (0-0) additive set or the (r-r) set, if one considered the dominant/recessive structure, had some degree of activity.

#15. *P. echinata* x *P. taeda***

<i>Source</i>	<i>n</i>	<i>α-p</i>	<i>cam</i>	<i>β-p</i>	<i>myr</i>	<i>lim</i>	<i>β-ph</i>
-----percent: normalized -----							
TR	38	64.5	0.2	30.9	2.2	1.4	0.6
TR	1	62.5	0.1	30.7	0.9	4.9	0.9
TR	1	76.9	-	16.4	5.7	0.9	0.1
TR	1	45.0	0.4	51.3	0.8	1.8	0.8
TR	1	89.3	0.5	8.5	1.2	0.4	0.1
TR	1	96.8	-	2.9	0.1	0.2	*
S	1	65.2	1.1	30.1	0.7	2.5	0.4

The above format is explained in the introduction to this chapter.

This was one of the more common hybrids in the arboretum. Both α -pinene and β -pinene tend to have a trimodal distribution, although the percent of α -pinene was about twice that of β -pinene. The one tree reported earlier (Smith 1967a) had just about the same composition as the 38 trees reported here. Although each species had less than 20 percent β -pinene, some of the progeny were greater than 20 percent. This suggested that both species had a (0-1) set of additive alleles for β -pinene. But if this were so, some trees with high β -pinene, i.e, (1-1) set, would be expected. Coyne and Keith (1972) showed enough limonene to suggest control of gene level—more than 25 percent in both parents—that one would expect a few progeny with fair amounts of limonene, but this did not occur. The range in the composition of parent trees used may have been too small for a confident statement of monoterpenes in this report.

#16. *P. echinata.taeda* x *P. taeda*

<i>Source</i>	<i>n</i>	<i>α-p</i>	<i>cam</i>	<i>β-p</i>	<i>myr</i>	<i>lim</i>	<i>β-ph</i>
-----percent: normalized -----							
TR	2	65.0	0.4	29.8	4.3	-	0.5

The above format is explained in the introduction to this chapter.

These two backcrosses had almost the same composition as the hybrid parent in #15 above.

#17. *P. echinata* x *P. echinata.elliottii*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized-----							
TR	8	56.7	0.4	32.4	0.8	2.2	7.6
TR	1	37.7	0.3	38.2	1.1	1.1	21.5
TR	1	56.5	0.3	39.7	0.8	1.6	1.0
TR	1	61.0	0.4	30.3	*	4.5	3.7

The above format is explained in the introduction to this chapter.

These eight backcrosses showed the predominance of α -pinene and β -pinene, but did not show the variation for either component that one might expect from the variation found in the previous listing, *P. echinata*. There was a wide range in β -phellandrene, a component usually in moderate and variable amounts in *P. elliottii*.

#18. *P. echinata.taeda* x *P. echinata.elliottii*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized-----							
TR	2	47.5	0.3	33.5	9.2	*	9.5
TR	1	54.0	0.2	29.9	7.3	*	8.7

The above format is explained in the introduction to this chapter.

Although only two trees were sampled, they expressed all three parents: α -pinene and β -pinene for *P. taeda* and *P. echinata*, myrcene for *P. taeda* and *P. elliottii*, and β -phellandrene for *P. elliottii*.

#19. *P. echinata.taeda* x *P. rigida.taeda*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized-----							
TR	3	60.8	0.2	27.9	6.4	*	4.6
TR	1	36.6	0.1	39.7	10.7	-	12.8
TR	1	77.5	0.3	18.3	3.5	-	0.5

The above format is explained in the introduction to this chapter.

The three trees sampled were quite variable in four components: α -pinene, 37 to 78 percent; β -pinene, 18 to 40 percent; myrcene, 4 to 11 percent; and β -phellandrene, from trace to 13 percent, suggesting that one or more of the three parents could have a (0-1) set of additive alleles for one or more of the four variable components.

#20. *Pinus elliotii* var. *elliotii*

Source	n	α -p	cam	β -P	myr	lim	β -ph
-----percent: normalized-----							
TR	5	73.0	0.5	17.0	1.2	0.5	7.9
TR	1	71.0	0.3	27.5	0.8	-	0.5
TR	1	77.4	0.5	9.2	1.6	0.5	10.8
TR	1	71.3	0.6	11.2	1.4	0.7	14.7
M		75.0	-	21.0	-	-	-

The above format is explained in the introduction to this chapter.

The five trees sampled showed very little variability in α -pinene but sizable variability in β -pinene and β -phellandrene, suggesting a (1-1) set of additive alleles for α -pinene and a (0-1) set for both β -pinene and β -phellandrene. The average of the five trees was very similar to Mirov's (1961) report. At one time *P. caribaea* was a name given to what is now *P. elliotii* var. *elliotii*.

#21. *Pinus engelmannii*

Source	n	α -p	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized-----										
TR	7	96.4	0.1	0.7	-	-	0.9	1.5	*	0.2
TR	1	98.3	0.2	0.7	-	-	0.4	0.4	-	-
TR	1	93.8	*	0.9	-	-	1.0	2.2	-	1.9
TR	1	93.6	0.2	0.1	-	-	0.8	5.4	-	-
M	-	80.0	-	2.0	-	-	-	2.0	-	-
S	1	95.0	2.0	2.0	-	-	1.0	*	*	-

The above format is explained in the introduction to this chapter.

These seven trees were nearly identical in composition. All seven were simply designated as *P. engelmannii* while item #22, which follows, was designated as *P. engelmannii* x w. There should be no difference if one assumes that the "wind" (w) was in a natural stand. *P. engelmannii* appeared to have a (1-1) set of alleles for α -pinene.

#22. *P. engelmannii* x *wind*

Source	n	α -p	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized-----										
TR	8	74.2	0.1	9.8	8.5	-	3.5	2.5	0.7	0.7
TR	1	97.7	0.3	0.6	-	-	0.7	0.6	0.1	-
TR	1	28.5	0.1	19.3	36.9	-	10.4	*	1.0	3.8
TR	1	44.8	0.1	43.8	-	-	9.9	*	1.3	-

The above format is explained in the introduction to this chapter.

This set of trees and the previous set, #21, were designated differently in the arboretum, and the separate designations are retained here. Also, some of the trees were labelled *P. apachea* which was a former name for *P. engelmannii*. Some botanists believe this tree is a southern form of *P. ponderosa*, and that it greatly resembles the southwestern form *P. ponderosa* var. *arizonica* in southern Arizona, particularly the Coronado ecotype (See Chapter 3, #14). The composition of the eight trees, reported as item #22 and labelled *P. engelmannii* x *wind*, showed large variability in α -pinene, β -pinene, and myrcene. One might suspect that the "wind" parents of this set might have included *P. ponderosa* or *P. ponderosa* var. *arizonica*. Both earlier reports of *P. engelmannii* (Mirov 1961, Smith 1967a) showed that the monoterpene portion was nearly all α -pinene. Therefore, some of the wind parents were not, more than likely, *P. engelmannii*.

#23. *P. engelmannii* x *P. ponderosa* var. *scopulorum***

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----									
S	1	17	*	1	73	2	1	1	4

The above format is explained in the introduction to this chapter.

This one tree, reported earlier (Smith 1967a), showed nearly all characteristics of var. *scopulorum* with high 3-carene and only moderate α -pinene. The ratio of 3-carene to terpinolene is close to the expected, between 20:1 and 15:1.

#24. *P. engelmannii* x *P. ponderosa* var. *arizonica***

Source	n	α -p	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized-----										
TR	5	87.9	0.1	9.9	-	-	0.7	0.7	0.3	
TR	1	98.0	0.1	0.4	-	-	0.6	0.3	0.2	-
TR	1	51.6	0.1	46.3	-	-	0.5	0.1	1.1	-

The above format is explained in the introduction to this chapter.

These hybrids closely resembled the composition of the previous listing, #22, of *P. engelmannii* x *wind*; the five progeny resembled either *P. engelmannii* with near-pure α -pinene or *P. ponderosa* var. *arizonica* with considerable amounts of β -pinene.

#25. *P. engelmannii* x *P. ponderosa*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----										
TR	3	30.9	0.1	12.2	34.2	6.5	12.9	0.3	*	2.7
TR	1	31.0	*	23.3	30.2	4.2	8.3	*	*	2.9
TR	1	32.0	*	2.5	39.0	8.5	15.1	*		2.8

The above format is explained in the introduction to this chapter.

The three hybrids showed the effect of both parents: α -pinene of *P. engelmannii* and β -pinene, 3-carene, and limonene of *P. ponderosa*. The composition of the three trees suggested that intermediacy of composition between two parents will hold only so long as two have equal numbers of active gene loci. Here, *P. engelmannii* probably had only one active gene locus, α -pinene; *P. ponderosa* had at least three: β -pinene, 3-carene, and limonene. Therefore, the progeny were not intermediate for α -pinene, at about 45 percent, but were more characteristic of α -pinene in *P. ponderosa*.

#26. *P. engelmannii* x *P. jeffreyi*.*ponderosa*

Source	n	hep	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----										
TR	6	0.5	49.8	0.1	16.0	21.5	5.8	3.7	1.2	1.4
TR	1	1.0	39.9	*	32.4	20.0	6.0	*	0.8	0.9
TR	1	*	82.1	*	1.1	9.4	6.6	-	0.5	*
TR	1	*	33.5	0.1	0.6	42.5	5.6	14.4	-	3.3

The above format is explained in the introduction to this chapter.

All trees had only a small but measurable amount of heptane. Thus, the effect of *P. jeffreyi* had been nearly lost in only two generations; yet one might ask why there were still measurable amounts of heptane. That is, one would expect either 0 percent or 10 to 20 percent. Two of three trees, listed individually, essentially resembled a cross of *P. engelmannii* with *P. ponderosa*, as in item #25.

#27. *Pinus flexilis*

Source	n	non	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized-----												
TR	2		68.8	3.3	*	19.4	4.5	1.4	0.5	0.8	1.6	
TR	1		71.7	5.3	*	11.4	7.4	1.5	0.3	0.4	2.1	
M	-		80.0	-	-	-	-	-	-	-	-	-
S	1	4.0	28.0	8.0	*	5.0	35.0	13.0	2.0	1.0	1.0	6.0

The above format is explained in the introduction to this chapter.

This species was far more variable than *P. griffithii* (formerly *P. excelsa*) used as the male parent in #28, with a large amount of α -pinene and a moderate amount of β -pinene, sabinene, and undecane. Smith (1967a) reported one tree with 28 percent α -pinene, 35 percent 3-carene and 13 percent sabinene with 6 percent terpinolene and trace to small amounts of nonane, undecane, β -pinene, limonene, and β -phellandrene. Additional data on *P. flexilis*, Chapter 3, #8, showed a great range in its composition.

#28. *P. flexilis* x *P. griffithii***

Source	n	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized-----											
TR	3	41.9		2.9	*	49.1		1.6	0.5	0.3	3.7
S	1	85.0	8.0	*	2.0	3.0	1.0	*	*		1.0

The above format is explained in the introduction to this chapter.

The male parent was labelled *P. excelsa*; it is now considered *P. griffithii* (see #30 below). The composition of the three progeny did not show the intermediacy of α -pinene. Both parents were high in α -pinene, but when 3-carene was a component of the *P. flexilis* parent, the progeny had only moderate amounts of α -pinene.

#29. *Pinus greggii* x wind

Source	n	α -p	com	β -p	car	α -ph	myr	lim	β -ph	ter
-----percent: normalized-----										
TR	5	6.9	0.4	0.7	7.2	0.9	1.2	13.2	69.5	0.3
TR	1	9.4	0.6	0.8	0.5	1.0	1.6	2.6	83.4	-
TR	1	5.9	0.2	0.5	12.6	0.5	*	22.5	57.6	0.2
TR	1	5.5	0.3	0.5	22.6	1.3	2.5	1.3	64.5	0.4
M	-	large			large				some	
1		7.3		1.4		0.8		32.4	45.3	

M No values given, just quantitative terms.

¹ Lockhart (1990a)

The above format is explained in the introduction to this chapter.

The five trees examined were quite variable in 3-carene, limonene, and β -phellandrene. Mirov (1961) was able to find only large amounts of α -pinene and β -pinene, but was unable to resolve the remainder of his analytical sample, though he speculated on β -phellandrene and limonene. As will be frequently noted, many of the pines of Mexico were found to be quite variable in xylem monoterpene composition (Smith 1982a). Lockhart (1990a) found a large amount of either limonene or β -phellandrene or both in nearly all trees in Nuevo Leon, Mexico, and found considerable range in composition throughout Mexico. Number of trees was not given, but large ranges in limonene and β -phellandrene were reported.

#30. *P. griffithii* x wind

Source	n	α -p	cam	β -p	sab	myr	lim	β -ph	ter
TR	2	-----percent: normalized-----							
		85.6	2.1	7.2	1.8	1.4	0.8	0.3	1.0
TR	1	84.9	4.2	2.6	3.5	1.8	0.7	0.4	2.0
M	-	88.0	-	-	-	-	-	-	-

The above format is explained in the introduction to this chapter.

The composition of the two trees, labelled *P. excelsa* in the arboretum, was high α -pinene with small or trace amounts of camphene, β -pinene, sabinene, myrcene, limonene, β -phellandrene, and terpinolene; these small amounts were not found in the next item, #31, suggesting that the wind parents of these two trees were quite different from the *P. griffithii* male parent of #31. The 2:1 ratio of sabinene to terpinolene was consistent with other pines.

#31. *P. griffithii* x *P. griffithii*

Source	n	α -p	cam	β -p	myr	lim
		-----percent: normalized-----				
TR	3	98.8	0.2	0.4	0.3	0.3

The above format is explained in the introduction to this chapter.

All three trees were nearly identical with greater than 98 percent α -pinene and small to trace amounts of other monoterpenes. This suggests that some *P. griffithii* had a (1-1) set of alleles for α -pinene with no active alleles for any other monoterpene. The difference between #31 and #30 may be biosynthetic, and not genetic.

#32. *Pinus halepensis* x *wind*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----									
TR	15	67.4	0.5	11.5	15.1	1.4	1.4	0.6	1.8
TR	6	76.6	0.2	7.0	11.7	1.5	0.6	0.5	1.0
TR	1	40.8	0.3	22.1	23.8	2.6	-	1.0	4.3
TR	1	81.6	0.5	7.4	7.6	2.0	-	0.9	*
TR	1	59.0	*	1.6	32.6	1.2	3.7	-	1.9
TR	1	98.4	*	0.5	0.2	0.8	-	-	-
M		97.0	-	-	-	2.0	-	-	-
¹	472	82.3	-	1.9	2.5	7.2	0.4	3.4	-

¹Schiller and Grunwald 1987.

The above format is explained in the introduction to this chapter.

The first two sets of trees, numbering 15 and 6 and growing at IFG, were examined at different times. Both sets were quite similar in average composition and different than that reported by Mirov and others (1966) who found three widely separated sources to be about the same with approximately 95 percent α -pinene; only four such trees were found among the 21 examined here. The other 17 had appreciable amounts of either β -pinene or 3-carene or both, which was more characteristic of *P. brutia*. Although 21 trees were hardly sufficient for an analysis of frequency distribution, there was a tendency for a multimodal distribution for α -pinene, β -pinene, and 3-carene. A recent paper by Conkle and others (1988) gave data from isozyme analysis to fix *P. brutia* as a separate species from *P. halepensis*. Conkle and others base their conclusions partly on the prior work of Mirov and others (1966). It would appear that the species and varietal status of *P. brutia* and *P. halepensis* is not fully resolved. For this report *P. brutia* will be considered a separate species.

Quite different results were also reported by Schiller and Grunwald (1987) who made a range-wide study of 472 7-year-old trees. By using a canonical discriminate analysis on individual monoterpenes, Schiller and Grunwald (1987) made a first-level division of *P. halepensis* into three geographic areas: N. Africa, Greece, and Spain; they also made second- and third-level divisions. Some support for the results of the canonical procedure was given by an analysis of types of composition, although the procedure for determining types of composition was not clear to me.

#33. *P. halepensis* x *P. brutia*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----									
TR	5	80.4	0.2	2.9	11.3	1.1	2.4	0.5	1.3
TR	1	58.1	0.3	4.5	24.3	2.0	7.3	1.1	2.4
TR	1	97.0	0.2	1.3		-	0.6	0.7	0.1

The above format is explained in the introduction to this chapter.

The composition of the five trees examined had considerable variation in α -pinene, 3-carene, and limonene. This agreed with the report by Mirov and

others (1966) who reported the composition of *P. halepensis* to be nearly pure α -pinene, but that of *P. brutia* (see #4 above) to be quite variable in α -pinene, β -pinene, and 3-carene. The five trees were quite similar in composition to the reciprocal trees in item #5 above.

#34. *Pinus jeffreyi*

Source	n	hep	non	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized-----													
TR	18	97.5	*	0.3		0.1	0.2	0.2	*	0.5	*	1.1	-
M	1	96.0											

The above format is explained in the introduction to this chapter.

These 18 trees were much like those reported elsewhere (Mirov 1961, Smith 1971) and in Chapter 3 of this volume. Simply stated, the volatile portion of *P. Jeffrey* xylem resin was nearly all n-heptane, and there is little, if any, local or regional variation. It is difficult to explain the small amounts of monoterpenes. There were no appreciable amounts of monoterpenes from which isomers could be formed unless such a mechanism existed for heptane. See Chapter 3 of this volume for more detailed data on *P. jeffreyi*.

#35. *P. jeffreyi* x *P. coulteri***

Source	n	hep	non	α -p	cam	β -p	car	sab	α -ph	myr	lim	β -ph
-----percent: normalized-----												
TR	13	66.9	0.6	14.1	1.4	0.6	0.3	0.1	0.2	4.4	1.3	10.1
TR	1	76.6	0.1	13.2	1.7	0.4	0.2	-	-	1.4	0.6	5.4
TR	1	50.7	1.9	11.6	1.9	0.1	-	0.2	-	5.7	10.5	17.5
TR	1	58.5	1.3	23.9	1.3	0.9	*	*	0.4	3.9	1.5	8.3
TR	1	94.2	0.5	1.1	-	-	0.2	-	-	1.4	-	2.8

The above format is explained in the introduction to this chapter.

The average n-heptane, 67 percent, for these 13 trees was much higher than expected, even if intermediacy, about 45 percent, was postulated. The same reason given for this phenomenon for the cross with *P. montezumae* may apply here also; i.e., *P. coulteri* might have an active site for n-heptane, since *P. coulteri* often had small to trace amounts (see #13 above). The one tree with nearly pure heptane, 94 percent, could be rationalized as a result of self-pollination. The remaining 12 trees showed the effect of both parents. One would expect greater α -pinene and β -phellandrene, since *P. coulteri* brings more alleles to the cross, but this may be in the normal range of variation.

#36. *P. jeffreyi* x *P. ponderosa***

Source	n	hep	non	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----											
TR	7	38.3	0.2	7.8	0.6	30.9	15.5	3.1	1.0	2.1	0.2
TR	1	52.8	0.4	4.0	0.3	16.2	25.2	0.5	-	-	*
TR	1	34.2	0.3	8.2	1.2	34.0	3.3	4.6	1.5	12.7	*
S	1	17.9	1.3	14.9	0	23.3	27.0	3.0	0.9	7.5	1.1

^sSmith (1967a) also records 3.2 percent undecane.

The above format is explained in the introduction to this chapter.

The average composition for these seven trees showed the expected characteristics of both parents, but the amount of n-heptane was often much lower than the expected 45 percent, if intermediacy was postulated. This could have been caused by *P. ponderosa* having more active alleles for monoterpenes. (Thus, *P. jeffreyi* alleles do not have equal access to the terpene precursor.) The one tree with 12.7 percent β -phellandrene was a bit puzzling since neither parent tree had a significant amount of β -phellandrene. Again, this might indicate the action of a minor genetic system. The tree listed for Smith (1966b) was used in testing the resistance of the hybrid to western pine beetle attack.

#37. *P. jeffreyi* x *P. ponderosa* var. *scopulorum***

Source	n	hep	non	α -p	cam	β -p	car	myr	lim	β -ph	y-ter	ter
-----percent: normalized-----												
TR	5	37.7	0.7	9.1	0.1	5.0	41.8	1.1	1.6	0.3	0.2	2.5
TR	1	33.5	0.7	19.6	0.1	11.8	29.9	0.9	0.9	0.4	0.1	2.0
TR	1	50.4	0.6	0.9	*	0.7	40.8	0.9	1.6	0.2	0.2	3.6
S	1	29.0	1.0	4.0	1.0	1.0	57.0	2.0	1.0	1.0	*	4.0

The above format is explained in the introduction to this chapter.

These five progeny resembled those in the previous item #36. Again, the amount of n-heptane was generally lower than the expected 45 percent, if intermediacy was postulated, and one might speculate that *P. ponderosa* var. *scopulorum* had more sites that compete for the terpene precursor. The composition of one tree closely resembled that of a previous report (Smith 1967b).

#38. *P. jeffreyi* x *P. washoensis***

Source	n	hep	non	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----											
TR	3	48.6	0.9	6.0	0.4	5.1	26.1	5.8	0.1	5.7	1.3
TR	1	35.7	0.3	7.9	0.4	1.6	42.4	9.8	*	0.1	1.9
TR	1	15.3	2.2	9.2	1.0	13.6	35.4	6.3	0.4	14.5	2.0
TR	1	94.8	0.2	0.9	-	*	0.4	1.3	-	2.4	-
S	1	32.0	-	2.0	-	2.0	45.0	12.0	1.0	1.0	4.0

The above format is explained in the introduction to this chapter.

The average amount of n-heptane in the three progeny was misleading as shown by the three individually listed trees. The 95 percent heptane in one of the individually listed trees strongly suggests that this tree was *P. jeffreyi*, probably caused by selfing, and not a hybrid. By discounting the 95 percent tree, the average for heptane is far below the postulated intermediacy of about 45 percent. Thus, crosses with *P. washoensis* were, as expected, similar to those with *P. ponderosa* var. *scopulorum* as noted in the previous item. The one tree with greater than expected amount of β -phellandrene really looked like a hybrid of *P. jeffreyi* with *P. coulteri*.

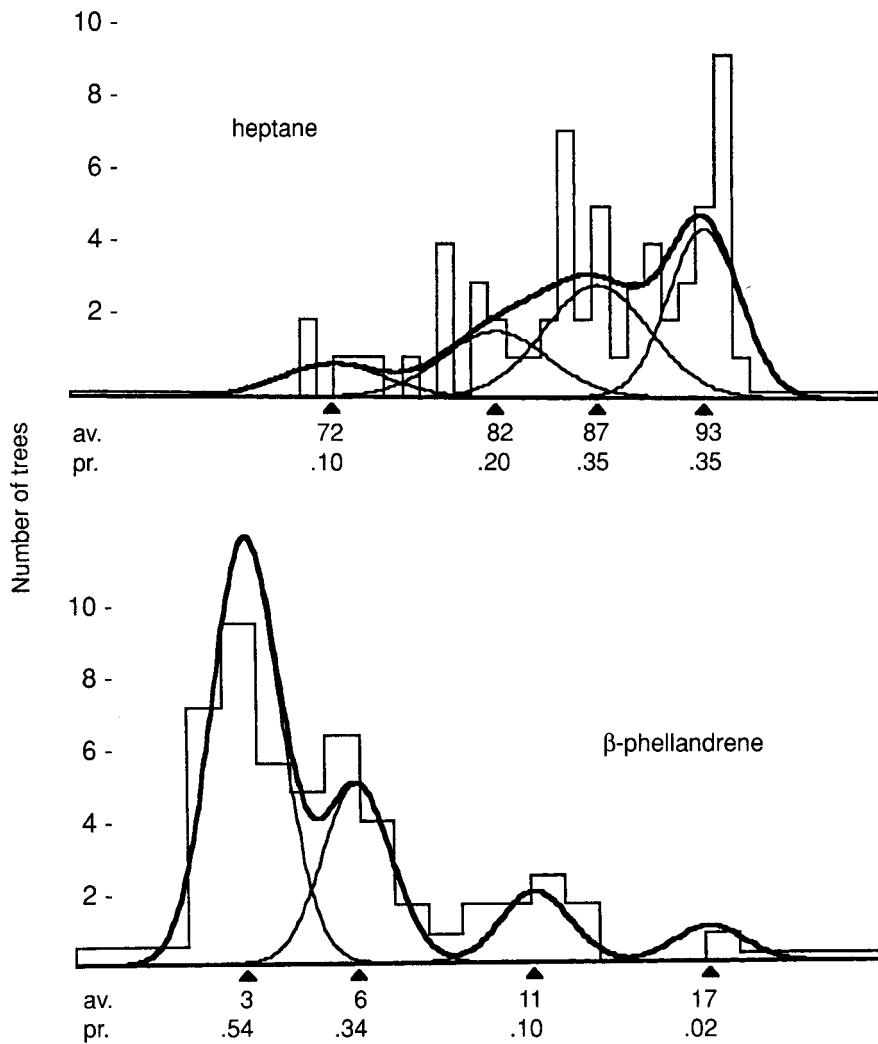
#39. *P. jeffreyi* x *P. montezumae***

Source	n	hep	non	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----											
TR	1	77.7	6.3	4.5	0.2	8.5	7.2	0.8	-	0.1	0.5

The above format is explained in the introduction to this chapter.

Only one tree was examined for this cross, and the composition was not as expected, if intermediacy was postulated; i.e., one would expect much lower heptane, about 45 percent, and much greater α -pinene, about 45 percent, in this hybrid. Even if the *P. montezumae* parent had only active alleles for α -pinene, n-heptane should not have been so high. An interesting possibility is that some varieties of *P. montezumae* might have moderate amounts of n-heptane. Could the *P. montezumae* parent have had an allele for heptane? Smith (1982a) found some variation in *P. montezumae* and its varieties, and high α -pinene and mixtures of α -pinene and β -pinene. *P. montezumae* almost always had a small amount of nonane, about 2 to 5 percent (Smith 1982a), and this seems to be carried through to the F-1 generation, which will be noted for the *P. ponderosa* x *P. montezumae* hybrid (see #78 below). One might use this as possible evidence of a minor genetic system as well as a marker for *P. montezumae*.

Figure 4-1—Frequency distribution (histogram) of heptane and β -phellandrene in 62 progeny of *P. jeffreyi* x *P. jeffreyi.coulteri*, and the modal analysis of the distribution; individual mode average (av.) (▲) given as a full number in percent of composition, mode proportion (pr.) given as a decimal based on a sum of 1.0; analysis based on normal distribution; heavy line is best fit of the histogram; thin line is best fit of the modes.



#40. *P. jeffreyi* x *P. jeffreyi.coulteri*

Source	n	hep	non	α -p	und	cam	β -p	car	sab	α -ph	myr	lim	β -ph
-----percent: normalized -----													
TR	62	86.0	0.5	3.8	-	0.9	0.2	0.3	0.1	*	2.3	0.5	5.7
TR	1	87.2	0.1	3.5	-	0.6	0.1	0.9	0.2	-	2.1	*	5.4
TR	1	77.6	0.3	5.3	-	1.1	0.1	*	0.1	-	3.0	0.2	12.3
TR	1	70.0	1.3	8.5	-	1.1	0.5	0.3	0.6	-	4.4	0.9	12.7
TR	1	65.2	0.1	9.1	-	1.0	0.2	0.4	0.3	*	4.8	0.7	18.3
TR	1	81.3	0.8	10.0	-	0.7	0.5	-	0.1	*	0.2	0.5	5.9
TR	1	95.4	0.1	1.1	-	0.2	0.3	0.6	0.3	-	0.2	1.9	*
S	1	72.0	1.0	12.0	*	*	1.0	1.0	*	-	2.0	1.0	11.0
S	1	93.0	*	1.0	*	2.0	*	*	*	-	1.0	*	3.0
S	1	85.0	1.0	1.0	-	1.0	*	*	*	-	6.0	*	5.0
S	1	83.0	*	5.0	-	1.0	3.0	1.0	*	-	3.0	*	4.0

^SSmith (1967a), full siblings.

The above format is explained in the introduction to this chapter.

Heptane was generally high, as expected. There was considerable range in the amount of heptane, α -pinene, and β -phellandrene. Myrcene, attributable to *P. coulteri*, had only a modest range and was lower than expected. All four components tended to have a multimodal frequency distribution as illustrated by heptane and β -pinene (fig. 4-1), suggesting additive alleles for heptane and β -phellandrene. Again, as for other hybrids, the one tree with near-pure heptane can be rationalized by accidental selfing of *P. jeffreyi*. Since the frequency distribution of both heptane and the monoterpenes tended to be multimodal, it might be concluded that they both draw on the same precursor.

#41. *P. jeffreyi.coulteri* x wind

Source	n	hep	non	α -p	cam	β -p	car	sab	myr	lim	β -ph
-----percent: normalized -----											
TR	2	72.9	0.4	8.9	1.4	0.3	*	0.2	3.5	1.5	10.7
TR	1	94.6	0.3	0.5	1.7	-	0.3	-	0.4	-	2.1
TR	1	82.3	0.5	4.2	1.1	*	-	0.2	2.7	0.6	8.4
TR	1	75.3	1.5	3.8	4.1	0.1	-	0.1	0.3	0.6	14.2
TR	1	64.5	0.7	12.0	1.3	0.1	-	0.2	5.5	0.9	15.0
TR	1	43.5	0.1	27.2	0.9	0.5	-	0.1	6.8	6.8	14.1
TR	1	17.9	2.8	27.3	2.8	-	-	0.6	14.3	5.9	28.4

The above format is explained in the introduction to this chapter.

The amount of β -phellandrene was quite similar to *P. jeffreyi* x *coulteri*. In fact, all trees were fairly similar to those of *P. jeffreyi* x *coulteri* (see #35 above). One really cannot be sure of the source of the wind pollen. Since both sets of trees (this item and #40 above) are quite similar, the wind pollen could have been *P. jeffreyi.coulteri*. The considerable variation in composition was attributed to *P. coulteri*.

#42. *P. jeffreyi*.*ponderosa* x *wind*

Source	n	hep	non	α -p	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----												
TR	3	3.7	0.1	4.4	0.1	16.5	42.7	13.5	13.9	1.0	0.1	2.7
TR	1	2.0	-	2.6	0.2	5.0	51.6	7.8	28.7	-	0.1	2.9
TR	1	4.2	*	4.2	-	21.0	41.6	13.3	11.0	1.6	0.2	2.9

The above format is explained in the introduction to this chapter.

It was clear that "wind" was most likely *P. ponderosa*, since all trees had nearly the composition of *P. ponderosa*. The presence of measurable amounts of n-heptane again suggests additive alleles; if there was a dominant/recessive structure, one would expect either no heptane (two recessive) or an appreciable amount (dominant-recessive). The one tree with about 29 percent limonene was interesting because high limonene was rarely found in *P. ponderosa* (see Chapter 5). Here again, as in several other instances, the presence of a small amount of heptane raised the question of its source because one would expect either zero percent or an appreciable amount of about 15 to 20 percent. Could these small amounts be derived from low activity by a (0-0) set of additive alleles? Or is there a minor genetic system?

#43. *P. jeffreyi*.*ponderosa* x *P. engelmannii*

Source	n	hep	non	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----											
TR	3	3.5	0.2	69.7	0.2	9.6	3.1	7.5	5.0	0.5	0.5
TR	1	0.2	0.6	83.1	0.3	2.4	2.3	11.1	-	-	-
TR	1	9.4	*	79.9	*	0.6	2.0	7.5	-	-	0.5

The above format is explained in the introduction to this chapter.

The composition of the three trees that had predominantly characteristics of *P. engelmannii* was a bit puzzling; the α -pinene was so much higher than expected and β -pinene, 3-carene, and n-heptane were so much lower than expected. Although one might rationally explain the condition by having a (0-0) pair of additive alleles for all the *P. ponderosa* genes— β -pinene, 3-carene, myrcene, and limonene—in the *P. ponderosa* parent of the *P. jeffreyi* x *P. ponderosa* hybrid and a (1-1) pair of additive alleles for n-heptane in the *P. jeffreyi* parent, it seems unusual that the condition occurred for all four genes at the same time. There was no such tree in a total of 12 progeny of crosses of *P. jeffreyi* with *P. ponderosa* and with *P. ponderosa* var. *scopulorum*, #36 and #37 above. The reciprocal of this cross, #26, was not too similar in composition to #43.

#44. *P. jeffreyi*.*ponderosa* x *P. jeffreyi*

Source	n	hep	non	α -p	β -p	car	myr	lim	β -ph	
-----percent: normalized -----										
TR	8	78.4	1.3	1.9	0.8	8.0	4.2	2.6	0.1	2.8
TR	1	81.1	0.3	2.5	0.8	11.7	2.2	1.4	-	*
TR	1	72.3	2.6	1.8	1.9	0.4	3.4	3.9	0.5	13.1
TR	1	61.3	1.1	2.1	0.5	10.2	22.2	2.5	-	-
TR	1	73.1	1.0	3.0	*	11.2	0.4	6.6	-	4.0
S		95.0	*	3.0	*	*	1.0	1.0	*	*

The above format is explained in the introduction to this chapter.

The average composition of the eight trees showed, as expected, the strong characteristics of *P. jeffreyi*; yet there were still some effects of *P. ponderosa* with 8 percent β -pinene, 4 percent 3-carene, and 3 percent each for myrcene and β -phellandrene. This again suggested that the cross produced some (0-0) alleles for β -pinene and 3-carene, whereas, if it was a double recessive, there would be no β -pinene or 3-carene. The 13 percent β -phellandrene was unusual because neither parent species had more than 1 or 2 percent generally. However, a rare *P. jeffreyi* with 12 percent β -phellandrene was reported for that species in Chapter 3; maybe such a tree was the male parent. Or there could have been an aggregate of several of the minor effects on monoterpene composition.

#45. *P. jeffreyi*.*ponderosa* x *P. ponderosa*

Source	n	hep	non	α -p	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized -----												
TR	8	3.9	0.1	7.6	*	10.9	45.8	*	13.4	15.7	0.4	1.8
TR	1	0.4	-	9.8	-	1.7	59.0	*	8.2	19.1	*	2.0
TR	1	2.1	-	10.0	0.1	20.5	33.3	-	15.8	14.7	*	2.1
TR	1	23.0	1.2	7.5	*	15.8	29.7	*	12.7	9.8	*	0.6
TR	1	1.0	-	6.0	-	10.4	49.7	*	11.8	17.6	1.7	0.8
S	1	*		8.0	1.0	21.0	47.0	-	6.0	13.0	2.0	2.0
S	1	4.0	1.0	10.0	*	21.0	39.0	-	7.0	15.0	1.0	3.0

The above format is explained in the introduction to this chapter.

In this cross the characteristics of *P. jeffreyi* were essentially lost because only one of the trees had an appreciable amount of n-heptane. All seven other trees were essentially like *P. ponderosa*. The two trees listed from Smith (1967a) were full siblings and show a strong similarity to the eight trees of this report. The relationship of biosynthesis between monoterpenes and heptane was not clear. No reciprocals of this cross were in the arboretum.

#46. [*P. jeffreyi* x (*jeffreyi.coulteri*)] x *P. ponderosa*

Source	n	hep	non	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized-----													
TR	6	11.8	0.3	7.0		0.2	17.5	31.0		6.4	11.1	13.3	1.4
TR	1	1.0	0.3	8.6		0.3	18.3	31.0		11.8	8.0	19.8	2.2
TR	1	23.4	0.6	7.3		0.1	18.3	20.7		6.5	9.8	12.4	0.9
TR	1	10.0	0.3	8.6		0.3	16.3	27.0		11.8	7.0	16.8	2.2
TR	1	14.7	0.6	6.2		-	-	22.9		6.7	24.2	22.4	2.6
S	1	*	*	11.0	2.0	*	27.0	26.0	*	3.0	14.0	16.0	4.0

The above format is explained in the introduction to this chapter.

The female parent was a naturally occurring backcross of *P. jeffreyi.coulteri* to *P. jeffreyi* (Zobel 1952). The average of the six trees showed characteristics of all three parents: heptane of *P. jeffreyi*; β -pinene, 3-carene, of *P. ponderosa*; β -phellandrene of *P. coulteri*; and limonene and myrcene of both *P. coulteri* and *P. ponderosa*. The one tree examined in 1967 had only a trace of heptane, but only one tree with only a trace of heptane was found in this six-tree sample.

All trees of this cross had a very poor and very slow flow of resin, a characteristic not unusual for many other interspecific crosses. On the other hand, the flow of resin from all three parent species was usually very good.

#47. [*P. jeffreyi* x *jeffreyi. coulteri*] x *P. jeffreyi.ponderosa*

Source	n	hep	non	α -p	und	cam	β -p	car	sab	myr	lim	β -ph
-----percent: normalized-----												
TR	10	45.0	1.1	10.1		0.3	9.1	3.1	*	8.5	2.2	20.3
TR	1	78.4	1.7	5.7		0.3	0.6	-		7.0	*	6.2
TR	1	39.2	0.1	3.9		0.4	*		*	11.6	*	45.1
TR	1	98.6	0.1	0.4		*	0.2	-		0.3	-	0.4
TR	1	25.3	2.7	6.0		*	11.4	24.8	*	9.8	6.1	12.6
S	1	20.0	1.0	12.0	1.0	-	40.0	5.0	*	2.0	8.0	12.0

The above format is explained in the introduction to this chapter.

The female was the same type as #46 above. The 10 trees examined had a great range in heptane, α -pinene, β -pinene, 3-carene, myrcene, limonene, and β -phellandrene, thus reflecting all three parent species. There were some unusual compositions for individual trees; for example, one with 78 percent heptane looked like *P. jeffreyi* x *Jeffreyi.coulteri*, another with 16 percent myrcene and 51 percent β -phellandrene looked like *P. coulteri*, and another with 14 percent β -pinene, 54 percent 3-carene, and 6 percent limonene looked somewhat like *P. ponderosa*. The small amount of heptane in some trees seemed puzzling, because both hybrid parents contain *P. jeffreyi*, but a (0-1) set of additive alleles for heptane in both parents was possible and could explain the small amount of heptane in some of these progeny.

#48. *Pinus longifolia*

Source	n	α -p	cam	β -p	car	sab	myr	lim	β -ph	γ -ter	ter
-----percent: normalized -----											
TR	2	15.8	0.1	9.7	64.2	*	3.3	0.6	0.7	0.4	5.4
TR	1	19.2	*	15.9	54.6	-	3.7	1.1	0.9	0.3	4.2
S	1	18.0	-	18.0	56.0	-	3.0	1.0	1.0	*	4.0
M	1	24.0		10.0	38.0	-	-	-	-	-	-

The above format is explained in the introduction to this chapter.

Only two trees were examined, and the average composition as well as the individual compositions were reasonably similar to that reported earlier (Mirov 1961, Smith 1967a). This species is now known as *P. roxburghii*.

#49. *Pinus monticola*

Source	n	hep	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized -----												
TR	12		53.6	0.7		22.1	7.7	5.7	3.1	1.5	3.6	1.8
TR	1		18.8	0.4		36.8	-	18.8	1.0	0.9	17.3	6.0
TR	1		77.8	*		15.6	-	-	5.8	0.5	0.2	-
TR	1		30.9	0.1		40.2	-	-	11.4	10.0	1.8	-
TR	1		19.1	*		23.9	48.8	-	4.6	*	1.0	2.6
TR	1		65.8	1.3		7.0	-	15.7	1.0	0.6	4.1	4.6
M			60.0	2.0		26.0	-	-	-	-	-	-

The above format is explained in the introduction to this chapter.

The average composition of 12 trees examined showed this species to be another of the α -pinene, β -pinene types, as also shown by Mirov (1961). However, the individual trees had a wide range in several other components. The proportions of 3-carene and sabinene to terpinolene were illustrated in the five individual trees listed: the two without both components had no terpinolene; the one with 3-carene and no sabinene had about a 20:1 ratio of 3-carene to terpinolene; and the ones with sabinene and without 3-carene had a ratio of about 3:1 of sabinene to terpinolene. See Chapter 3, #12, for more data on *P. monticola*.

#50. *P. monticola* x *P. monticola*

Source	n	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----												
TR	8	61.7	2.4		22.2	*	4.8	2.6	4.3	1.2	0.1	0.8
TR	1	49.7	3.1		25.2	*	2.3	6.6	10.1	1.3	0.9	0.9
TR	1	80.0	3.4		8.0	-	-	2.9	3.4	2.7	-	-
S	1	81.0	-	4.0	12.0	-	-	1.0	1.0	*	-	-

The above format is explained in the introduction to this chapter.

The composition of these eight trees closely resembled those of the previous item #49. The only difference between them was that #49 was wind-pollinated seed, whereas item #50 was control-pollinated.

#51. *P. monticola* x wind

Source	n	α -p	and	β -p	car	sab	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----											
TR	22	55.8	3.6	28.7	5.4	*	1.2	3.9	0.9	*	0.2
TR	1	55.7	2.1	31.0	*	-	0.6	9.8	0.8	-	-
TR	1	50.0	2.1	15.0	30.0	-	0.7	0.4	1.0	-	0.9
TR	1	92.4	-	6.6	-	-	0.7	0.1	0.2	-	-
TR	1	44.4	11.1	5.6	33.3	-	5.6	-	-	-	*

The above format is explained in the introduction to this chapter.

These 22 trees were designated as wind pollinated and were similar to items #49 and 50 above, *P. monticola* and *P. monticola* x *P. monticola*. The designation depends on where and how the parents were when they were pollinated. It was interesting that no tree in this set of 22 had a measurable amount of sabinene, yet in a much smaller sample of 11 trees, #50 above, two trees had considerable amounts of sabinene. The data on natural stands of *P. monticola* (Chapter 3, #12) showed large local and regional variation in α -pinene, β -pinene, 3-carene, and myrcene, and there was even appreciable variation in minor components: undecane, β -phellandrene, sabinene, and nonane.

#52. *P. monticola* x *P. strobus***

Source	n	α -p	and	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized -----											
TR	41	60.6	1.4	*	31.8	2.8	0.4	1.1	1.2	0.6	0.4
TR	1	54.6	*	*	36.9	3.1	-	4.3	0.4	0.7	-
TR	1	66.7	0.2	*	28.7	-	1.6	0.3	0.1	0.1	2.4
TR	1	86.6	*	*	12.9	-	-	0.2	0.3	*	-
TR	1	72.7	3.5	*	7.8	*	1.7	5.2	8.0	0.7	0.5
TR	1	56.3	3.0	*	7.0	27.1	-	2.2	2.1	0.2	2.2
TR	1	71.3	1.6		23.5	2.8	-	0.7	0.5	0.3	0.3
TR	1	41.4	1.9		50.4	-	-	0.7	3.9	1.6	-
S	1	52.0	-	3.0	43.0	-	-	1.0	*	*	-
S	1	52.0	1.0	1.0	41.0	-	-	1.0	1.0	1.0	-
M		73.0	-	-0	15.0						

The above format is explained in the introduction to this chapter.

Mirov (1961) reported 75 percent α -pinene and 15 percent β -pinene for *P. strobus*. The average of these 41 trees was just about intermediate between *P. monticola* and *P. strobus*, in a general sense. This hybrid also showed additional characteristics of *P. monticola*: 3-carene, sabinene, and limonene. There was a tendency for a trimodal distribution of both α -pinene and β -pinene in this hybrid, suggesting additive alleles.

#53. *P. monticola* x *P. peuce***

Source	n	non	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized -----										
S	1	*	67.0	2.0	28.0	-	1.0	1.0	1.0	-

The above format is explained in the introduction to this chapter.

This one hybrid was approximately intermediate to both parents. Mirov (1961) reports *P. peuce* with 50 percent α -pinene, 6 percent β -pinene, 2 percent myrcene, and 1 percent terpinolene.

#54. *P. monticola* x *P. griffithii*

Source	n	non	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized -----												
TR	19		81.7	1.6	-	14.3	0.9	-	0.5	0.3	0.2	*
TR	1		97.8	0.3	-	0.4	-	-	0.8	0.6	-	-
TR	1		74.5	1.6	-	23.8	-	-	*	0.1	*	-
TR	1		34.4	0.4	-	54.6	8.6	-	1.0	0.2	0.3	-
S	1		61.0	5.0	*	31.0	4.0	-	*	*	*	*

The above format is explained in the introduction to this chapter.

The average composition of these 19 trees was much higher in α -pinene than *P. monticola* x *P. strobus*, #52, and this was an expression of the higher α -pinene in *P. griffithii*. Mirov (1961) reported 90 percent α -pinene in *P. griffithii*, whereas in #30 and #31 (above) α -pinene was 85 percent and 98 percent. None of these 19 trees had any sabinene, and only one had a measurable amount of 3-carene. Apparently none of the female *P. monticola* had any sabinene or 3-carene; both components are fairly common in *P. monticola* (see Chapter 3). It appeared that individual trees of both parent species had a (1-1) set of additive alleles for α -pinene.

#55. *P. monticola* x *P. peuce. strobus*

Source	n	non	α -p	cam	β -p	car	myr	lim	β -ph
-----percent: normalized -----									
S	1	*	52.0	3.0	42.0	1.0	*	*	1.0

The above format is explained in the introduction to this chapter.

This single 3-way hybrid had about the expected composition of largely α -pinene and β -pinene with small or trace amounts of other monoterpenes.

#56. *Pinus muricata*

Source	n	α -p	α -th	cam	β -p	sab	myr	lim	β -ph	γ -ter	ter
-----percent: normalized -----											
TR	9	35.0	1.0	0.3	2.4	37.1	1.1	0.4	1.0	0.4	21.2
TR	1	5.2	3.0	1.0	1.4	56.7	*		0.7	0.3	31.8
TR	1	95.0	0.2	-	2.7	-	1.1	-	0.9	-	-
TR	1	43.1	*	0.2	3.7	29.3	1.3	*	1.8	0.5	20.1
M	-	98.0	-	-	-	-	-	-	-	-	-

The above format is explained in the introduction to this chapter.

The composition of this species was given because it was used with *P. attenuata* in item #3 above. These few trees again showed the great variation of composition in *P. muricata*. See Chapter 3, #13, for more data on *P. muricata*.

#57. *Pinus nigra* x *P. nigra*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized -----							
TR	5	86.9	0.6	2.0	1.0	9.3	0.2
TR	1	92.7	0.5	1.8	1.0	3.4	0.7
TR	1	74.6	0.3	1.4	2.0	21.6	
M	1	94.0	-				-

The above format is explained in the introduction to this chapter.

The average composition of these five trees, like all others of *P. nigra* and its varieties, was about 90 percent α -pinene and small to trace amounts of other monoterpenes. However, one tree had more than 22 percent limonene and 75 percent α -pinene, which was unusual. The only other hybrid with *P. nigra* that contained modest amounts of limonene was *P. nigra* x *P. resinosa*, item #67 below. Did this modest amount of limonene, listed here, reflect variation in *P. nigra* or was it a rogue and likely the progeny of *P. nigra* x *P. resinosa*? Hybrid #64 could be such a tree. The results suggest that *P. nigra* and its varieties have a (1-1) set of additive alleles for α -pinene.

#58. *P. nigra* var. *calabrica* x wind

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized -----							
TR	2	94.4	0.5	2.9	0.8	0.7	0.6

The above format is explained in the introduction to this chapter.

Only two trees of *P. nigra* var. *calabrica* were sampled, and both were nearly pure α -pinene at 94 percent; β -pinene was about 3 percent, and the remainder was small amounts of camphene, myrcene, limonene, and β -phellandrene.

P. calabrica was once a separate species, but it is now considered a variety of *P. nigra*. The varietal status of *P. nigra* probably is not fully resolved.

#S9. *P. nigra* var. *calabrica* x *P. nigra*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized -----							
TR	3	92.2	0.7	2.2	1.3	3.0	0.7
S	1	92.0	3.0	3.0	1.0	1.0	-

The above format is explained in the introduction to this chapter.

All three trees were quite similar. Smith (1967a) found much the same except for small differences in the minor components.

#60. *P. nigra.calabrica* x wind

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized-----							
TR	1	94.6	0.9	2.0	1.0	1.3	0.2

The above format is explained in the introduction to this chapter.

The composition of this one tree was about 95 percent α -pinene with small to trace amounts of other monoterpenes. As will be shown in the subsequent various intra specific hybrids and by item #58 (var. *calabrica*), all varieties of *P. nigra* had about the same composition, except as noted for one tree in item #57 above.

#61. *P. nigra* var. *calabrica* x *P. var. calabrica*

Source	n	α -p	cam	β -p	sab	myr	lim	β -ph
-----percent: normalized-----								
TR	4	94.9	0.8	1.4	*	0.9	1.9	0.2
S	1	94.0	1.0	3.0	*	1.0	1.0	*
S	1	92.0	3.0	3.0	*	1.0	1.0	*

The above format is explained in the introduction to this chapter.

The composition of the four trees was essentially like others of *P. nigra* and its intraspecific hybrids, with 92 to 95 percent α -pinene and small to trace amounts of other monoterpenes.

#62. *P. nigra* var. *calabrica* x *P. longifolia*

Source	n	α -p	cam	β -p	car	sab	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----											
TR	3	96.8	*	2.7	*	*	0.1	0.3	-	0.4	-
TR	1	96.4	*	3.4	-	-	0.1	0.1	-	-	-
TR	1	98.6	-	1.4	-	-	-	-	-	-	-

The above format is explained in the introduction to this chapter.

All three trees sampled had about the same composition, about 97 percent α -pinene, 3 percent β -pinene, and trace amounts of camphene, myrcene, and limonene. To achieve this composition, the male parent, *P. longifolia*, must have been near-pure α -pinene; however, because the *P. longifolia* reported earlier, #48, were high 3-carene and not high α -pinene, one might suspect that the three trees sampled were products of selfing of *P. calabrica*. The alternative was that there were *P. longifolia* with near-pure α -pinene without 3-carene, but such has not been reported.

#63. *P. nigra* var. *calabrica* x *P. thunbergiana*

Source	n	α -p	cam	β -p	myr	lim	β -ph
TR	5	-----percent: normalized-----					
		91.7	0.8	3.5	1.1	1.3	1.6
TR	1	93.3	0.8	3.9	1.0		0.9
TR	1	88.1	0.8	3.6	1.3	2.3	4.0

The above format is explained in the introduction to this chapter.

The composition of these five hybrids was nearly identical to those of other intervarietal crosses of *P. nigra*. The one tree with 4 percent β -phellandrene suggested that *P. thunbergiana* might have a (0-1) or (1-1) set of additive alleles for β -phellandrene. Mirov (1961) lists *P. thunbergiana* as 83 percent α -pinene and 10 percent β -pinene, but no β -phellandrene. As noted earlier, the 4 percent β -phellandrene could result from the aggregate of a number of small minor effects. Or the sampling of natural trees of *P. thunbergiana* was inadequate. *P. thunbergiana* was once known as *thunbergii*.

#64. *P. nigra* x *P. resinosa***

Source	n	α -p	cam	β -p	myr	lim	β -ph
		-----percent: normalized-----					
TR	4	78.0	0.5	10.3	0.6	8.7	2.0
TR	1	73.4	0.4	17.3	0.8	6.3	1.8
TR	1	80.6	0.5	2.3	0.1	14.5	2.1

The above format is explained in the introduction to this chapter.

These four hybrids suggested that *P. resinosa* had genes for α -pinene, β -pinene, and limonene. Mirov (1961) reported 92 percent α -pinene and 3 percent β -phellandrene. The one tree with 14.5 percent limonene resembles one listed as *P. nigra* x *P. nigra* in #57.

#65. *P. nigra* var. *cebenensis* x *wind*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph
		-----percent: normalized-----						
TR	11	95.6	0.7	1.4	*	0.9	1.0	0.4

The above format is explained in the introduction to this chapter.

These 11 trees were also varieties of *P. nigra*. Their average composition was nearly identical to varieties of *P. nigra* and of *P. nigra* var. *calabrica*. This limited analysis of intraspecific hybrids of *P. nigra* suggested that all varieties were near-pure α -pinene.

#66. *P. nigra* var. *cebenensis* x *wind*

<i>Source</i>	<i>n</i>	α - <i>p</i>	<i>cam</i>	β - <i>p</i>	<i>car</i>	<i>myr</i>	<i>lim</i>	β - <i>ph</i>
-----percent: normalized-----								
TR	2	94.8	0.8	1.4	0.2	0.9	1.3	0.5

The above format is explained in the introduction to this chapter.

The two trees were about 95 percent α -pinene with small or trace amounts of camphene, β -pinene, myrcene, limonene, and β -phellandrene. Thus, they were the same as other varieties of *P. nigra*.

#67. *P. nigra* var. *cebenensis* x *P. nigra*

<i>Source</i>	<i>n</i>	α - <i>p</i>	<i>cam</i>	β - <i>p</i>	<i>myr</i>	<i>lim</i>	β - <i>ph</i>
-----percent: normalized-----							
TR	5	94.8	0.8	1.7	0.9	1.6	0.3
S	1	92.0	2.0	2.0	1.0	2.0	*

The above format is explained in the introduction to this chapter.

P. nigra has had at times several varieties, *cebenensis* being one of them. These five trees are thus intraspecific hybrids. Their average composition was essentially 95 percent α -pinene, with small to trace amounts of several other monoterpenes.

#68. *P. nigra* var. *cebenensis* x *P. nigra* var. *cebenensis*

<i>Source</i>	<i>n</i>	α - <i>p</i>	<i>cam</i>	β - <i>p</i>	<i>myr</i>	<i>lim</i>	β - <i>ph</i>
-----percent: normalized-----							
TR	4	93.2	1.6	2.3	1.	1.8	0.2
S	1	95.0	2.0	2.0	1.0	1.0	-

The above format is explained in the introduction to this chapter.

The composition of these four intraspecific hybrids plus that of the one tree reported earlier were nearly the same, about 94 percent α -pinene and small to trace amounts of five other monoterpenes.

#69. *Pinus patula* x *wind*

Source	n	α -p	cam	β -p	car	α -ph	myr	lim	β -ph	ter
-----percent: normalized-----										
TR	1	6.7	0.5	0.5	0.6	1.0	1.5	2.1	87.0	0.2
M		5.0	-	-	-	-	-	-	75.0	-
¹	1	15.0	-	2.0	18.0	-	-	-	37.0	1.5

¹Lockhart (1990b) (Lockhart's analyses also included 13 percent sesquiterpene)

The above format is explained in the introduction to this chapter.

This one tree was similar to that reported by Mirov (1961) who found 5 percent α -pinene, 75 percent β -phellandrene, and 10-15 percent sesquiterpenes. *P. patula* apparently had a (1-1) set of additive alleles for β -phellandrene. Lockhart (1990b) found a wide range in composition in Central America stands, including high β -phellandrene like the ones above. Average values given are derived from a diagram and not a table. Lockhart's data showed the number of trees with a large amount of a given component and gave individual tree composition in bar graph form for only 21 trees. Nevertheless, trees in every Central American country seemed to have a different composition: either high α -pinene, or high 3-carene, or high limonene, or high β -phellandrene, or combinations of these four. From the data given by Lockhart, *P. patula* was extremely variable like *P. muricata*, *P. ponderosa*, and *P. lambertiana*.

#70. *P. patula* x *P. greggii***

Source	n	α -p	cam	β -p	car	α -ph	myr	lim	β -ph	ter
-----percent: normalized-----										
TR	13	35.8	0.2	0.5	17.2	0.2	16.9	0.7	27.4	0.8
TR	1	22.8	-	0.6	15.3	*	40.5	*	19.5	0.1
TR	1	46.3	0.4	0.4	14.2	0.3	1.2	1.1	34.8	1.2

The above format is explained in the introduction to this chapter.

These 13 trees were an interesting study in that six of the trees were very similar in composition and are illustrated by the first single tree listed, whereas the other seven were quite different than the first set and were like the second tree listed. Thus, the two distinct types of composition are quite different from each other. Mirov (1961) was not clear on *P. greggii* (see item #33 above). He simply lists a large amount of α -pinene and β -pinene. A large portion of his sample was unresolved, although he does speculate that the unresolved portion might be β -phellandrene and/or limonene. Neither *P. patula* nor *P. greggii* (#69 and #29 above) listed in this report had a large amount of α -pinene, yet all hybrids listed here had a moderate amount of α -pinene. Therefore, the sampling of both species was not complete until Lockhart (1990b) found a large amount of α -pinene in *P. patula*.

#71. *Pinus pinaster* x wind

Source	n	α -p	cam	β -p	myr	lim	β -ph	sab
-----percent: normalized-----								
TR	2	52.5	0.4	41.7	0.5	4.0	1.0	-
M	-	63.0	-	27.0	-	-	-	-
¹		85.0	*	11.0	-	2.0	-	2.0

¹Monteoliva and others 1969.

The above format is explained in the introduction to this chapter.

These two trees had near-equal amounts of α -pinene and β -pinene and small or trace amounts of camphene, myrcene, limonene, and β -phellandrene. Monteoliva and others (1969), in an intensive and extensive study reported 65 to 87 percent α -pinene, 0 to 3 percent camphene, 11 to 20 percent β -pinene, 2 to 5 percent sabinene, and 1 to 8 percent limonene. They did not list terpinolene, although one would expect it because of the amounts of sabinene. Neither sabinene nor terpinolene was found in the two listed trees. The composition of *P. pinaster* was quite variable.

#72. *P. pinaster* x *P. pinaster*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized-----							
TR	2	79.5	0.2	18.2	0.6	1.2	0.4
TR	1	95.9	0.1	2.8	*	1.2	*
TR	1	63.1	0.2	33.6	1.1	1.2	0.8

The above format is explained in the introduction to this chapter.

These two indicate a wide range in β -pinene and α -pinene, which was not shown in item #71. The range of these two trees was more like that given by Monteoliva and others (1969). However, sabinene, limonene, and 3-carene still did not occur in appreciable amounts, as one might expect from the report by Monteoliva and others (1969). One might speculate that *P. pinaster* was variable in monoterpenes and that the limited breeding failed to use trees with moderate amounts of camphene, sabinene, and limonene as noted by Monteoliva and others (1969). Another possibility might be that *P. pinaster* was variable in composition but that the limited breeding failed to use trees with moderate camphene, sabinene, and limonene.

#73. *Pinus ponderosa*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized -----										
TR	151	7.6	0.1	31.6	36.7	10.0	11.9	1.0		1.7
TR	1	0.1	-	36.9	30.0	12.4	9.0	-	-	1.5
TR	1	7.8	-	18.0	48.0	13.3	5.9	1.0	-	5.1
TR	1	10.3	-	52.5	-	21.4	14.0	1.9	-	-
TR	1	7.0	0.1	33.5	25.6	5.7	24.5	2.3	-	1.3
TR	1	10.6	*	55.1	-	12.7	21.5	*	-	-
TR	1	9.7	0.1	22.7	56.5	9.2	0.6	*	-	1.2
TR	1	5.1	0.6	9.0	48.3	12.0	21.0	1.3	-	2.8
TR	1	37.0	0.1	32.8	-	5.0	21.2	2.4	-	-
TR	1	15.6	-	36.5	24.1	20.6	*	1.6	-	1.5
S	1	5.0	-	15.0	51.0	9.0	16.0	1.0	*	3.0
S	1	6.0		23.0	43.0	5.0	20.0	1.0	*	2.0

The above format is explained in the introduction to this chapter.

The average of these 151 trees and the selection of individual compositions were listed because the trees are in the arboretum and readily available for study. The central Sierra Nevada was the source of most of them; those listed were fairly representative of trees found in the Sierra Pacific region. Five other sections of this report give a more thorough treatment of *P. ponderosa*, and its varieties in Chapters 3, 5, 6, 7, and 8. In all the 20 species or hybrids that follow where *P. ponderosa* was given without a variety designation, it was most likely *P. ponderosa* var. *ponderosa*; the other two varieties were var. *scopulorum* and var. *arizonica*.

#74. *P. ponderosa* x *P. ponderosa* var. *scopulorum*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized -----									
TR	45	14.7	0.2	22.4	45.0	9.4	9.3	0.8	1.7
TR	1	28.2	*	31.5	25.7	6.8	5.9	1.2	0.5
TR	1	16.4	*	15.3	52.6	6.1	8.2	-	1.2
TR	1	6.8	-	25.6	45.7	7.1	12.0	*	2.9
TR	1	5.8	0.9	15.9	56.9	17.1	*	0.7	2.5
S	1	5.0	*	27.0	50.0	7.0	7.0	1.0	4.0
S	1	28.0	1.0	33.0	29.0	3.0	7.0	1.0	2.0
S	1	32.0	1.0	26.0	26.0	6.0	6.0	2.0	2.0
S	1	35.0	1.0	28.0	24.0	2.0	8.0	1.0	1.0

^sSmith (1967a) full siblings.

The above format is explained in the introduction to this chapter.

The average composition of the 45 trees, as well as the range of the individually listed trees, was very much like that of the reciprocal cross (item #83). The earlier report (Smith 1967a) listed individual trees with much less 3-

carene and somewhat higher α -pinene. Both α -pinene and β -pinene tended toward a trimodal frequency distribution as illustrated by α -pinene (fig. 4-2). *P. ponderosa* var. *scopulorum*, as traditionally described, did not come out clearly as a definitive variety based on xylem monoterpenes; instead, types of composition found in the region assigned to var. *scopulorum* were found in regions assigned to the other varieties, and vice versa.

#75. *P. ponderosa* x *P. ponderosa* var. *arizonica***

Source	n	α -p	cam	β -p	car	sab	myr	lim	β - ph	γ -ter	ter
-----percent: normalized-----											
TR	24	30.1	0.2	25.4	28.1	0.7	5.9	7.0	0.7	*	1.7
TR	1	19.3	-	27.8	30.7	-	9.0	11.6	*	-	1.7
TR	1	56.5	0.5	36.8	-	-	1.4	3.8	1.0	-	-
TR	1	47.1	0.2	6.1	35.3	-	7.8	*	0.7	0.1	2.9
TR	1	23.6	0.4	37.4	-	16.8	3.2	5.6	1.2	-	11.8

The above format is explained in the introduction to this chapter.

The average of these 24 trees did not closely resemble the previous item #74, though some of the individual trees were quite similar, thus suggesting a difference between var. *arizonica* and var. *scopulorum*. The much greater α -pinene and lesser β -pinene, limonene, and 3-carene than in the previous intraspecific hybrid were as expected. Peloquin (1984) suggested that var. *arizonica* and *P. engelmannii* were introgressive in southern Arizona. Many individual trees of var. *arizonica* and *P. engelmannii* had the same composition. As with var. *scopulorum*, var. *arizonica* as traditionally described did not come out clearly as a definitive var. of *P. ponderosa*. This result was based on xylem monoterpene composition. The results for var. *scopulorum* were very similar for the same reason.

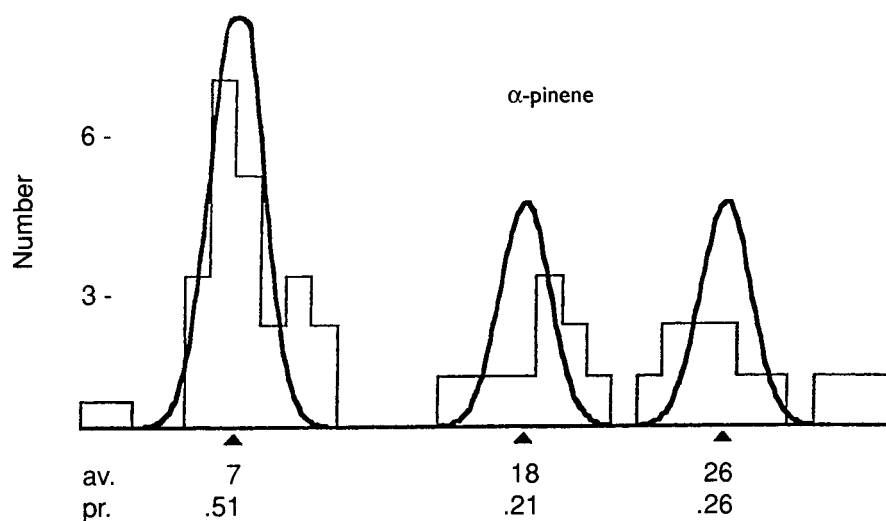


Figure 4-2—Frequency distribution (histogram) of α -pinene in 45 progeny of *P. ponderosa* x *P. ponderosa* var. *scopulorum*, and the modal analysis of the distribution; individual mode average (av.) (▲) given as a full number in percent of composition, mode proportion (pr.) as a decimal based on a sum of 1.0; analysis based on normal distribution; heavy line is best fit of the histogram; thin line is best fit of the modes.

#76. *P. ponderosa* x *P. engelmannii***

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
----- percent: normalized -----									
TR	58	32.9	0.2	19.4	26.7	6.7	11.7	0.7	1.5
TR	1	36.3	-	13.2	30.5	5.3	12.3	*	2.5
TR	1	19.1	0.3	24.2	35.3	3.7	14.5	1.5	1.5
TR	1	34.4	-	21.6	30.6	11.4	*	-	1.8
TR	1	47.4	-	19.1	-	8.0	25.0	-	0.4
TR	1	60.2	*	24.7	-	9.9	5.5	*	-
TR	1	8.8	0.6	33.2	42.4	6.0	6.2	*	2.6
TR	1	40.9	*	2.5	32.3	6.7	15.4	-	2.1
^s	1	40.0	*	14.0	36.0	4.0	4.0	1.0	2.0
^s	1	40.0	*	18.0	25.0	4.0	10.0	1.0	1.0
^s	1	29.0	*	28.0	29.0	4.0	5.0	1.0	2.0
^s	1	32.0	1.0	17.0	34.0	6.0	7.0	1.0	3.0

^sSmith (1967a) half-siblings

The above format is explained in the introduction to this chapter.

These 58 trees showed the effect of both parent species with high α -pinene from *P. engelmannii*, and β -pinene, 3-carene, myrcene, and limonene from *P. ponderosa*. The average composition was very similar to the previous hybrid (#75 above); this was to be expected since var. *arizonica* and *P. engelmannii* had similar compositions. As suggested earlier for item #21 above, *P. engelmannii* apparently had a (1-1) set of additive alleles for α -pinene because there was always an intermediate amount of α -pinene, mostly between 20 percent and 50 percent, in the hybrids. Two trees were the exception, having about 8 percent as illustrated by one of the listed trees (8.8 percent). The lack of high α -pinene in any of the hybrids suggested: (1) that the *P. ponderosa* parent had a (0-0) set of additive alleles for α -pinene and, therefore, there was no (1-1) set of alleles for α -pinene in the 58 hybrids; or (2) that the presence of alleles for three to four other components competing for the precursor, such as β -pinene, 3-carene, and limonene, would prevent the full expression of the (1-1) set of alleles for α -pinene. This is highly unlikely.

There was a distinct tendency for the frequency distribution of the major monoterpenes to be trimodal, as illustrated by α -pinene and β -pinene (fig. 4-3), providing more evidence for an additive alleles hypothesis.

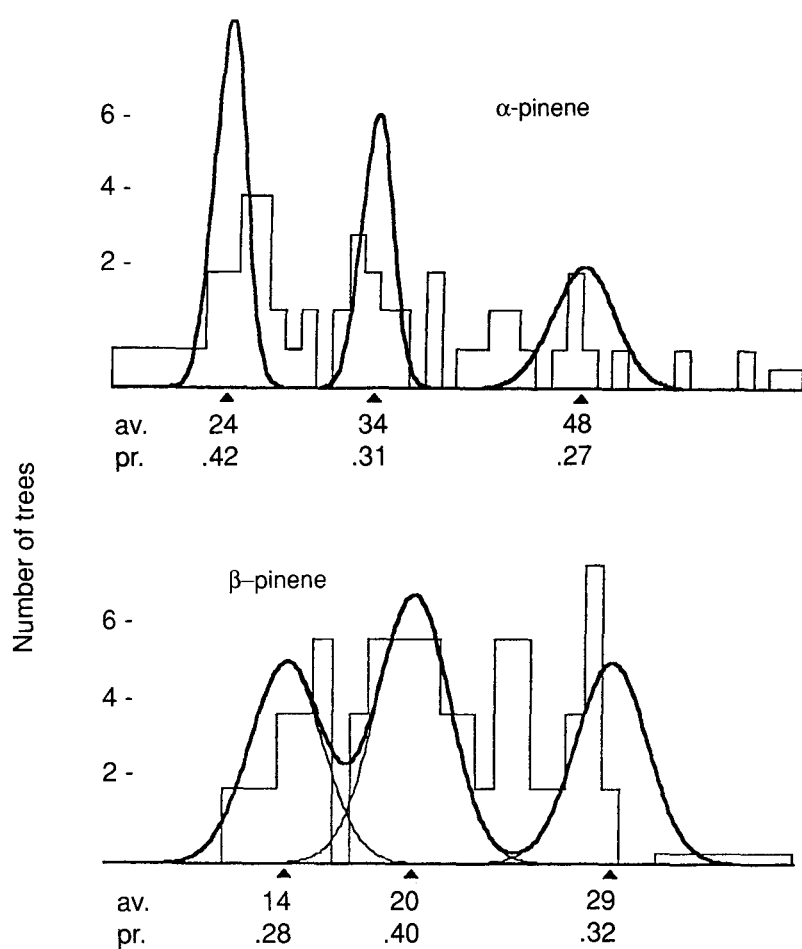


Figure 4-3—Frequency distribution (histogram) of α -pinene and β -pinene in 52 progeny of *P. ponderosa* x *P. engelmannii*, and the modal of the distribution; x = percent, y = number; individual mode average (av.) (▲) given as a full number in percent of composition, mode proportion as a decimal based on a sum of 1.0; analysis based on normal distribution; heavy line is best fit of the histogram, thin line is best fit of the modes.

#77. *P. ponderosa* x *P. washoensis*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----									
TR	2	10.2	0.1	29.6	38.7	18.6	1.6	*	1.5
S	1	11.0	-	40.0	26.0	11.0	9.0	1.0	2.0

The above format is explained in the introduction to this chapter.

These two trees were about as expected, because the composition of some individual *P. ponderosa* was somewhat the same as that of *P. washoensis*. Critchfield (1984) gives evidence that *P. washoensis* was very closely related to *P. ponderosa*, particularly var. *scopulorum*. Haller (1961) suggested that *P. washoensis* was more widely distributed than originally thought. The average composition was quite similar to the reciprocal cross, item #112 below (see Chapter 3, #19, for more data on *P. washoensis*).

#78. *P. ponderosa* x *P. montezumae***

Source	n	hep	non	α -p	und	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----												
TR	29	0.5	3.7	25.8	1.2	0.1	15.3	39.1	7.3	4.0	0.7	* 2.1
TR	1	*	3.5	33.2	*	*	9.6	40.2	6.2	6.9	0.3	0.1
TR	1	*	4.3	22.0	5.1	-	20.9	34.5	10.8	-	0.6	2.1
TR	1	-	3.0	21.4	-	0.8	2.3	47.9	16.0	3.9	1.2	* 3.3
TR	1	-	4.5	47.1	-	-	0.2	49.0	2.9	*	0.2	* 1.0
TR	1	13.6	3.2	11.3	-	*	13.0	47.1	4.8	3.7	0.6	* 2.4
TR	1	5.0	5.0	41.0	3.0	-	26.0	-	4.0	16.0	1.0	-

The above format is explained in the introduction to this chapter.

Both *P. ponderosa* and *P. montezumae* had considerable variation in composition and in range of monoterpenes (Smith 1977, Smith 1982a), and these 29 hybrids show this to be so. (Of course, the extent of the variation depended on the individual trees used in the breeding.) These 29 trees had a considerable range in several components plus having small amounts of heptane, nonane, and undecane in some trees; these latter three components were from *P. montezumae* and were typical of the species. Any time *P. montezumae* was involved in a cross (see #31 above, *P. jeffreyi* x *P. montezumae*), the progeny always had from 2 to 5 percent nonane; possibly this is evidence that a minor genetic structure might be active for the xylem monoterpenes in pines.

#79. *P. ponderosa* x *P. jeffreyi.coulteri*

Source	n	hep	non	α -p	und	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized -----													

S	1	69.0	2.0	13.0	2.0	*	2.0	1.0	1.0	3.0	*	*	-
S	1	66.0	2.0	15.0	*	1.0	1.0	1.0	1.0	4.0	1.0	1.0	-
S	1	80.0	1.0	8.0	1.0	1.0	1.0	1.0	*	3.0	1.0	1.0	-

The above format is explained in the introduction to this chapter.

All three were full siblings, and there was a small range in their composition, probably reflecting the range of natural variation. The average composition is much like that of #41 above, *P. jeffreyi.coulteri* x wind.

#80. *P. ponderosa* var. *scopulorum* x wind

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized -----									

TR	4	5.8	0.1	18.2	55.1	7.0	10.	0.3	2.7
TR	1	7.6	*	28.9	30.6	9.0	22.2	*	1.6
TR	1	5.8	0.5	0.4	86.0	2.9	*	1.0	3.3

The above format is explained in the introduction to this chapter.

The four trees should be like those of #82, below, but the average composition was quite different, suggesting that var. *scopulorum* is quite variable. The four trees above are about what would be expected: high 3-carene, low α -pinene, and moderate amounts of β -pinene, myrcene, and limonene.

#81. *P. ponderosa* var. *scopulorum* x *P. ponderosa* var. *scopulorum*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized -----									

TR	4	16.6	0.1	25.2	44.9	3.5	5.9	0.6	3.2
TR	1	5.8	0.3	20.3	63.3	4.1	0.4	1.6	4.2
TR	1	46.8	*	16.6	29.0	2.1	2.9	0.7	1.9

The above format is explained in the introduction to this chapter.

The distribution of *P. ponderosa* var. *scopulorum* was generally considered to be northern and central Rocky Mountains. Yet it was not at all clear where the line was drawn between this variety and the other two varieties of *P. ponderosa*. The *P. ponderosa* trees from the Rocky Mountains in Chapter 3 can be described as high 3-carene and low α -pinene. Thus, the average of the four trees listed above, with much higher α -pinene than would be expected, suggested that the parent trees were from the southern Rocky Mountains where var. *scopulorum* merges with the high α -pinene containing var. *arizonica*. Of the two individually listed trees, the first was about as expected of an intervarietal cross, low in α -pinene. The second individually listed tree was more like one expected from a cross of var. *scopulorum* with var. *arizonica*, high in α -pinene.

#82. *P. ponderosa* var. *scopulorum* x *P. ponderosa*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----										
TR	6	18.1	0.1	21.3	39.0	8.0	10.0	0.6	0.1	2.7
TR	1	5.1	-	23.8	50.5	4.8	12.4	-	-	3.3
TR	1	25.3	0.3	16.4	40.5	11.4	2.0	1.1	*	3.0
S	1	32.0	1.0	7.0	35.0	11.0	12.0	1.0	*	2.0

The above format is explained in the introduction to this chapter.

The average and individual listings were nearly identical to the reciprocal cross given in item #75 above.

#83. *P. ponderosa* var. *scopulorum* x *P. ponderosa* var. *arizonica*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----										
TR	2	15.5	0.1	47.2	28.2	4.4	1.6	1.2	0.1	1.8

The above format is explained in the introduction to this chapter.

The average of the two trees was quite low for α -pinene, if one assumes that var. *arizonica* was high α -pinene. Beta-pinene was usually high in both trees, considering the low β -pinene in both varieties; but a combination of two (0-1) alleles, i.e., one from each parent, could have produced the large amount of β -pinene by a (1-1) set of alleles in the progeny; however, this would have to be coupled with a combination of two sets of (0-1) or (0-0) alleles for α -pinene, resulting in a(0-0) or (0-1) set for α -pinene in some of the progeny.

#84. *P. ponderosa* var. *scopulorum* x *P. engelmannii*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----										
TR	8	28.4	*	6.0	52.7	2.8	4.8	0.7	0.4	4.0
TR	1	13.3	*	1.3	61.7	4.1	15.5	-	-	3.6
TR	1	52.7	*	7.9	33.1	2.0	1.0	0.5	0.1	2.7
TR	1	4.1	*	18.3	67.2	4.7	*	1.6	0.4	3.6
S	1	54.0	2.0	13.0	26.0	2.0	1.0	*	*	2.0
S	1	51.0	1.0	15.0	28.0	1.0	1.0	1.0	*	2.0

^SThese trees were full siblings.

The above format is explained in the introduction to this chapter.

The average composition of the eight trees was not what one would expect. The α -pinene was far below the intermediate level of about 45 percent, probably because var. *scopulorum* brings more alleles to the hybrid than does *P. engelmannii*. The latter probably had a (1-1) set of alleles for α -pinene and therefore at least one allele in the progeny. That is, it could be possible that the first listed tree (13.3 percent (α -pinene) had two alleles for 3-carene and one allele each for α -pinene and limonene. The range of composition was quite large, as shown by the three individually listed trees. An earlier report (Smith 1967a) showed two full siblings to have almost identical compositions.

#85. *P. ponderosa* var. *scopulorum* x *P. montezumae***

Source	n	non	α -p	und	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----												
TR	4	3.5	14.7	5.5	0.5	9.0	53.6	5.1	2.9	1.1	0.1	4.3
TR	1	3.5	9.0	5.7	0.9	13.1	51.9	7.0	4.6	1.5	0.1	2.9
TR	1	4.4	11.6	5.4	*	3.0	64.8	3.9	1.5	1.4	0.1	4.0

The above format is explained in the introduction to this chapter.

The α -pinene was much lower than the expected, 45 percent, for a hybrid involving *P. montezumae*; nevertheless, the male parent was *P. montezumae* because of the nonane in the four progeny. The 2 to 4 percent nonane had been a marker in other crosses with *P. montezumae*. This further suggested that there must be *P. montezumae* with low α -pinene and high 3-carene also present; other components in the resin composition resembled var. *scopulorum*.

#86. *P. ponderosa* var. *arizonica*

Source	n	α -p	cam	β -p	car	sab	myr	lim	β -ph	ter
-----percent: normalized-----										
TR	14	90.3	0.2	4.9	0.1	1.5	0.7	0.5	0.2	1.0
TR	1	95.8	0.3	0.7	-	1.2	0.7	0.3	*	1.0
TR	1	70.1	0.5	1.8	-	14.1	1.4	0.3	0.2	11.2
TR	1	40.3	-	55.7	-	-	1.0	1.0	2.0	1.2
M	-	34.0	-	14.0	3.0	-	4.0	-	-	-

The above format is explained in the introduction to this chapter.

Though this variety of *P. ponderosa* had been established for a longtime, the limits of distribution are not at all clear. Mirov (1961) gave it species status, as *P. arizonica*, whereas Critchfield and Little (1966) gave it variety status, and that will be used here. However, studies by Peloquin (1984) and Smith (1977) and data in Chapter 3 of this volume pose some questions about its distribution. In Chapter 3, the area considered var. *arizonica* has been divided into three regions and one transition zone on the basis of types of monoterpene composition. The average of the 14 trees reported here showed var. *arizonica* to have a (1-1) set of additive alleles for α -pinene. Yet, two of the trees listed had either a large amount of β -pinene or sabinene and terpinolene; these were some of the variations reported in Chapter 3 of this volume. Mirov (1961) reported about 60 percent α -pinene, 20 percent β -pinene, and 5 percent 3-carene for var. *arizonica*. The ratio of sabinene to terpinolene was nearly 1:1. I also found small amounts of sabinene in some trees in the region assigned to var. *arizonica* (Smith 1977).

#87. *P. ponderosa* var. *arizonica* x wind

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----									
TR	9	91.2	0.3	7.3	*	0.7	0.8	0.3	-
TR	1	95.2	0.3	0.9	-	0.7	2.6	0.3	-
TR	1	54.9	0.2	42.6	-	0.8	0.8	0.9	-
TR	1	88.5	0.2	9.3	-	0.6	1.5	*	-

The above format is explained in the introduction to this chapter.

These nine trees differed from the previous hybrid (#86) only in being designated as wind pollinated. The average for the nine trees was very much like that of #86 above, and the individually listed trees were also similar.

#88. *P. ponderosa* var. *arizonica* x *P. ponderosa*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----									
TR	8	20.0	*	25.1	44.9	5.7	1.5	0.6	2.2
TR	1	21.5	*	20.6	47.1	7.1	0.5	0.7	2.4
TR	1	15.4	*	23.0	48.4	4.8	5.2	0.3	3.0
S	1	28.0	*	22.0	39.0	3.0	4.0	1.0	2.0

The above format is explained in the introduction to this chapter.

The compositions of these eight intraspecific hybrids—all full siblings—were about as expected from such a cross, and the average was generally similar to the reciprocal, #75 above. The α -pinene in these trees above was far less than intermediate, probably because *P. ponderosa* brings more than one competing gene to the combination. Similar results were reported earlier by Smith (1967a).

#89. *P. ponderosa* var. *arizonica* x *P. engelmannii*

Source	n	α -p	β -p	car	myr	lim	β -ph
-----percent: normalized-----							
TR	1	96.9	1.4	-	1.7	*	*

The above format is explained in the introduction to this chapter.

Both var. *arizonica* and *P. engelmannii* can be classed as having a (1-1) set of additive alleles for α -pinene. Thus, this hybrid was exactly what one would expect.

#90. *P. ponderosa*.*engelmannii* x *P. engelmannii*

Source	n	non	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----										
TR	5	0.7	73.7	0.3	14.4	5.1	2.4	3.0	0.4	0.2
TR	1	-	69.5	0.3	29.2	-	0.2	0.2	0.7	0.1
TR	1	-	55.2	0.2	0.6	25.4	6.1	11.7	-	0.8
TR	1	3.5	90.6	*	2.0	-	3.8	-	-	-

The above format is explained in the introduction to this chapter.

The average composition of the five trees showed much higher α -pinene than the female parent type (see #76 above), again suggesting a (1-1) set of additive alleles in the *P. englemannii* male parent. The range of composition was to be expected considering the range of composition in hybrid #76 above.

#91. *P. ponderosa.engelmannii* x *P. jeffreyi.ponderosa*

Source	n	hep	non	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized -----											
TR	3	8.7	0.1	33.1	0.1	12.9	21.7	3.6	17.8	0.3	1.7
TR	1	26.1	0.2	37.1		2.4	3.3	5.5	25.3	-	-
TR	1	*	-		-		32.4	1.0	7.8	1.0	2.6

The above format is explained in the introduction to this chapter.

The high α -pinene characteristic of *P. engelmannii* had been lost in this backcross. The α -pinene of *P. engelmannii* could have been reduced to a (0-1) set in the first cross with *P. ponderosa*, and further reduced to a (0-0) set in the backcross with the second *P. ponderosa*. Or, the addition of alleles from *P. jeffreyi* and *P. ponderosa* could have lowered the amount of α -pinene.

#92. *P. ponderosa.engelmannii* x *P. ponderosa*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized -----										
	1	4.7	*	35.8	40.4	*	12.3	1.9	*	4.4

The above format is explained in the introduction to this chapter.

The high α -pinene characteristic of *P. engelmannii* has been lost in this backcross. The α -pinene of *P. engelmannii* could have been reduced to a (0-1) set of alleles in the first cross with *P. ponderosa*, and further reduced to a (0-0) set in the backcross with the second *P. ponderosa*.

#93. *P. ponderosa.engelmannii* x *P. montezumae*

Source	n	non	α -p	und	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized -----												
TR	6	3.5	43.7	0.9	0.1	13.6	27.3	3.5	4.9	0.7	*	1.3
TR	1	2.5	20.1	*	-	14.5	50.1	7.0	0.1	0.9	*	2.6
TR	1	1.6	76.5	*	0.1	15.0	-	*	5.7	1.0		-
TR	1	3.2	89.0	2.2	0.3	2.1	-	0.8	1.5	0.6		-

The above format is explained in the introduction to this chapter.

As noted earlier, whenever *P. montezumae* was involved in a cross there will be 2 to 5 percent nonane in the progeny. These six trees all had 2 to 5 percent nonane. The range in the remainder of the components was great, as one would expect with crosses of *P. montezumae* and *P. ponderosa*. That there was a consistent presence of nonane in low amounts in *P. montezumae* and its crosses leads one to suspect a minor genetic structure in pines for monoterpenes or related hydrocarbons.

#94. *Pinus rigida* x wind

Source	n	α -p	cam	β -p	car	myr	lim	β -ph
-----percent: normalized-----								
TR	9	39.6	0.2	33.0	*	1.0	1.0	24.3
TR	1	26.2	0.2	0.4	-	1.2	1.1	70.9
TR	1	21.1	0.3	41.8	-	1.7	1.0	34.2
TR	1	92.9	-	0.8	-	0.1	*	6.0
TR	1	35.9	0.3	42.8	-	0.2	1.8	18.7
TR	1	53.5	0.1	43.4	-	0.5	1.9	0.7
M		65.0	-	20.0	-	-	-	10.0

The above format is explained in the introduction to this chapter.

The five individually listed trees showed great range in *P. rigida* in α -pinene, β -pinene, and β -phellandrene, and even with only nine trees as a basis one can discern three levels for α -pinene and phellandrene: low, medium, high. Mirov (1961) listed high α -pinene and β -pinene and a small amount of limonene but did not list β -phellandrene. Additional data on *P. rigida* are given in Chapter 8.

#95. *P. rigida* x *P. taeda***

Source	n	α -p	cam	β -p	car	myr	lim	β -ph
-----percent: normalized-----								
S	1	75.0	2.0	9.0	-	4.0	2.0	9.0

The above format is explained in the introduction to this chapter.

The measurements for this one hybrid was about what might be expected with α -pinene and β -pinene from both parents and β -phellandrene from *P. rigida*.

#96. *P. rigida.taeda* x *P. taeda*

Source	n	α -p	cam	β -p	car	myr	lim	β -ph
----- percent: normalized -----								
TR	10	58.7	1.0	27.2	0.1	2.6	1.5	8.7
TR	1	77.8	-	6.1	0.8	1.4	0.7	13.3
TR	1	49.0	*	33.2	-	2.4	0.3	15.1
TR	1	60.1	8.9	12.3	-	0.6	0.4	17.7
TR	1	61.3	*	36.6	*	1.2	0.5	0.4

The above format is explained in the introduction to this chapter.

The effect of both parents was reflected in the average composition and in most of the individually listed trees. The one tree with only a trace of β -phellandrene suggested that this component had a (0-1) set of additive alleles in *P. rigida*. The one tree with 9 percent camphene was well above the level in either parent. Camphene was probably nongenetic in origin, or might be attributed to isomerization; or the production of camphene could also be a response to some external stimulus.

#97. *P. rigida.taeda* x *P. clausa*

Source n	α -p	cam	β -P	car	myr	lim	β -ph
----- percent: normalized -----							
TR	82.5	0.6	2.0	-	1.0	4.5	9.1

The above format is explained in the introduction to this chapter.

The low β -pinene in this three-way hybrid was not what might be expected. Mirov (1961) reported *P. clausa* as high β -pinene, and both *P. rigida* and *P. taeda* (previously discussed) had small to moderate amounts of β -pinene. However, if all three had a (0-1) set of additive alleles for β -pinene, it would be possible to have very low β -pinene in the progeny.

#98. *P. rigida.taeda* x *P. echinata.taeda*

Source	n	α -p	cam	β -P	myr	lim	β -ph
----- percent: normalized -----							
TR	3	71.2	0.2	25.1	2.1	0.7	0.5
TR	1	63.5	0.2	32.6	2.9	-	0.6
TR	1	82.5	0.1	14.3	2.7	-	0.1

The above format is explained in the introduction to this chapter.

In the four-way cross, the three progeny seemed more like *P. taeda* or *P. echinata* than *P. rigida*. The *P. rigida* parent could have been without β -phellandrene, as noted under #94 above, or β -phellandrene could have been lost in the cross with *P. taeda*.

#99. *Pinus strobiformis*

Source	n	hep	α -p	und	cam	β -p	car	sab	my	lim	β -ph
-----percent: normalized -----											
TR	6	-	71.3	-	0.6	22.3	-	2.8	1.3	0.3	0.3
TR	1	-	68.6	-	1.1	17.5	-	6.5	0.8	0.8	1.3
TR	1	-	92.6	-	-	1.0	-	1.5	4.9	-	-
TR	1	-	62.2	-	1.0	36.1	-	-	-	0.5	0.1
M	1	5	75.0	3	-	-	5	-	-	-	-
S	1	-	69.0	-	2.0	10.0	-	17.0	-	-	-

The above format is explained in the introduction to this chapter.

Mirov (1961) reported this species as *P. reflexa*, and it was often used as a synonym for *P. strobiformis*. Mirov postulated that on the basis of monoterpenes, *P. strobiformis* (i.e., *reflexa*) was not closely related to *P. flexilis*, but Smith (1967a) showed considerable similarity, particularly in the presence of moderate amounts of sabinene. Likewise, Critchfield and Little (1966) reported that *P. strobiformis* and *P. flexilis* introgress where they were sympatric in northern New Mexico and southern Colorado. The lack of terpinolene in the last listed tree is puzzling because there was a substantial amount of sabinene. Could this species be different from other pines and not have the sabinene-terpinolene linkage?

#100. *P. strobus* x *P. monticola*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized -----							
TR	7	58.3	0.5	39.5	0.7	0.4	0.5
TR	1	64.3	2.3	31.7	0.8	0.5	0.5
TR	1	47.8	1.2	47.8	1.3	0.7	1.4
S	1	48.0	1.0	48.0	1.0	1.0	1.0

The above format is explained in the introduction to this chapter.

The average composition of the seven trees, the composition of the individually listed trees, and that of the tree reported earlier (Smith 1967a) all show only small variations, yet, one would expect much more. There were several differences between this cross and its reciprocal, #52 above, i.e., there was no undecane, sabinene, or 3-carene in this set of crosses. However, the great variation in the monoterpenes of *P. monticola* (See #12 of Chapter 3) could explain such differences; it depended on the particular *P. monticola* used.

#101. *P. strobus* x *P. griffithii***

Source	n	α -p	cam	β -p	car	myr	lim	β -ph
-----percent: normalized-----								
TR	7	71.2	0.8	26.6	-	0.5	0.5	0.4
TR	1	71.7	0.3	26.9	-	0.5	0.3	0.2
TR	1	80.0	1.8	16.6	*	0.4	0.5	0.4
S	1	57.0	5.0	36.0	-	1.0	*	*

The above format is explained in the introduction to this chapter.

Mirov (1961) reported that *P. griffithii* (as *P. excelsa*) was nearly 90 percent α -pinene. Thus, again, intermediacy was not reached because of the introduction of other competing genes into the biosynthetic system—in this case, β -pinene.

#102. *Pinus taeda* x wind

Source	n	α -p	cam	β -P	myr	lim	β -ph
TR	7	-----percent: normalized-----					
		79.0	0.1	18.0	2.3	0.4	0.3
TR	1	75.0	-	21.9	2.6	0.4	0.1
TR	1	94.3	*	2.8	2.5	0.3	0.1
M		71.0		22.0		*	*
¹	374	60.0	1.0	30.0	3.0	-	-

¹Coyne and Keith (1972)

The above format is explained in the introduction to this chapter.

The average composition for the nine trees was much like that given by Mirov (1961) and not appreciably different from that given by Coyne and Keith (1972), who analyzed 374 trees. The range, shown by the two individually listed trees, was well within the results given by Coyne and Keith (1972).

#103. *P. taeda* x *P. taeda*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized-----							
TR	1	70.7	0.5	26.8	1.0	-	1.0

The above format is explained in the introduction to this chapter.

This one tree was quite similar to those listed for *P. taeda* x wind above, #102.

#104. *P. taeda* x *P. echinata*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized-----							
TR	1	61.8	0.3	33.5	3.7	*	0.8

The above format is explained in the introduction to this chapter.

The composition of this one tree was almost identical to the average composition of 38 reciprocal crosses in item #15 above. In more than 40 *P. taeda* x *P. echinata* hybrids listed in this report, not one had more than a small amount of limonene; yet Coyne and Keith (1972) reported moderate amounts of limonene, up to about 20 percent, in a few trees of both species. Apparently, the selection of the parent trees used in the breeding did not include trees with this characteristic. From the amounts of limonene listed by Coyne and Keith (1972) one might expect a (0-1) set of additive alleles in a few trees of each species.

#105. *P. taeda* x *P. rigida*

Source	n	α -p	cam	β -p	α -ph	myr	lim	β -ph
-----percent: normalized-----								
TR	3	49.6	0.4	35.5	0.1	1.1	0.4	12.9
TR	1	44.5	0.4	38.4	0.1	1.4	*	15.1
TR	1	45.5	0.3	45.2	0.1	1.0	0.3	7.6

The above format is explained in the introduction to this chapter.

This hybrid was about as expected with a large amount of α -pinene and β -pinene, and with a moderate amount of β -phellandrene. The differences between this set of progeny and those of the reciprocal, #95 above, could be explained by the additive allele hypothesis.

#106. *P. taeda.elliottii* var. *elliottii* x *P. elliottii* var. *elliottii*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized-----							
TR	1	52.2	*	19.7	17.5	*	10.3

The above format is explained in the introduction to this chapter.

This one backcross retained all the general characteristics of both species: α -pinene and β -pinene of *P. taeda*, and myrcene and β -phellandrene of *P. elliottii* var. *elliottii*.

#107. *P. taeda* x *P. echinata.taeda*

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized -----							
TR	3	71.9	0.3	23.5	3.4	-	0.3

The above format is explained in the introduction to this chapter.

The composition of the three trees was about the same as *P. taeda* x *P. echinata* in #104 above.

#108. *Pinus virginiana* x wind

Source	n	α -p	cam	β -P	myr	lim	β -ph
-----percent: normalized -----							
TR	6	53.4	0.3	44.0	0.9	0.2	1.3
TR	1	33.5	0.4	62.2	0.9	1.1	1.9
TR	1	21.2	0.2	76.2	0.9	*	1.5
M	-	98.0	-	-	-	-	-

The above format is explained in the introduction to this chapter.

The average composition was quite unlike that given by Mirov (1961). In fact, none of the six trees was at all like that of Mirov (1961) who reported *P. virginiana* as near-pure α -pinene. The difference between the two reports could have been caused by the small sample size.

#109. *Pinus virginiana* x *P. clausa***

Source	n	α -p	cam	β -p	myr	lim	β -ph
-----percent: normalized -----							
TR	5	26.4	0.4	69.8	0.9	0.8	1.8
TR	1	33.5	0.4	62.2	0.9	1.1	1.9
TR	1	20.6	0.1	74.0	1.1	1.7	2.5

The above format is explained in the introduction to this chapter.

Mirov (1961) listed *P. clausa* with about the highest level of camphene, about 10 percent, in any pine. Yet, nothing more than a trace of camphene could be found in any of the five hybrids. Mirov (1961) also listed *P. clausa* with 10 percent α -pinene and 75 percent β -pinene. The small number of sampled trees of the hybrids could explain this; or camphene may not be of genetic origin but may be a product of isomerization, and this isomerization had not yet taken place in these hybrids at IFG.

#110. *P. washoensis* x wind

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----									
TR	5	7.7	0.8	29.2	41.4	12.7	5.3	0.5	2.2
TR	1	8.5	0.2	18.3	55.6	13.7	0.7	0.1	3.0
TR	1	6.2	0.1	31.2	38.1	11.7	10.6		2.2
S	1	17.0	*	27.0	37.0	12.0	4.0	2.0	2.0
S	1	9.0	-	31.0	44.0	7.0	5.0	1.0	3.0

The above format is explained in the introduction to this chapter.

The average and range of the five trees was about as expected, with high 3-carene, moderate β -pinene, and smaller amounts of α -pinene, myrcene, and limonene. The ratio of 3-carene to terpinolene ranged from 15:1 to 20:1, which was expected. These trees were also similar to those described in previous reports (Smith 1967a, Smith 1971). See Chapter 3, #19, for additional information on *P. washoensis*. The ratio of 3-carene to terpinolene varied from 10:1 to 20:1.

#111. *P. washoensis* x *P. ponderosa***

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	ter
-----percent: normalized-----									
TR	15	8.4	0.2	27.5	41.4	13.4	6.1	0.2	1.8
TR	1	3.9	0.1	14.2	56.2	12.8	9.4	-	3.3
TR	1	6.8	0.2	37.7	33.0	19.4	0.8	-	2.1
TR	1	9.5	0.2	22.2	35.3	13.2	17.7	*	1.8

The above format is explained in the introduction to this chapter.

The average composition of these 15 trees was well within the expected composition. They really are no different than *P. ponderosa* and were very similar to the reciprocal, #77 above.

#112. *P. washoensis* x *P. ponderosa* var. *scopulorum***

Source	n	α -p	cam	β -p	car	myr	lim	β -ph	γ -ter	ter
-----percent: normalized-----										
TR	2	5.9	0.1	33.9	44.6	9.7	2.0	1.7	0.4	2.8

The above format is explained in the introduction to this chapter.

These two were quite similar to some of those listed in the previous item #111.

Population Studies of the Monoterpene Composition of 4- to 12-Year-Old Half Siblings of *P. ponderosa*

Summary: The study included about 1,500 half-siblings, 4 to 12 years old, of 21 *P. ponderosa* that were selected primarily on the basis of monoterpene composition. Three of the ecotypic regions (monoterpene races) of *P. ponderosa* were represented, and results were based on 25 to 200 siblings per parent. Composition of individual trees of a set of half-siblings usually varied greatly in two or more major components. Average composition of a set of progeny tended to be broadly intermediate between the parent tree and the average of the local stand. Parents with similar composition produced progeny with quite similar arrays of compositions and frequency distribution characteristics. However, limonene and myrcene were lower than the calculated intermediate amounts; α -pinene and β -pinene were higher. A juvenile effect is postulated that was more or less evident in all young growth. The effect of this juvenile factor was much the same as that shown by plants under stress. Therefore, all young trees or young parts of older trees may be under some level of stress—the stress of rapid growth. Nearly one third of the half-siblings of a high limonene parent could be classed as high limonene or potentially so. Some high-limonene trees were found among the progeny of a parent that could be classed only as near-high limonene. A rare tree was found with ultra-high limonene, though such trees have not been found in mature, natural stands.

There were some differences between the three geographic regions in the comparative ratios between parent and progeny for a given monoterpene, suggesting that the locality of a parent tree may have an effect on the juvenile response or that there were minor genetic effects that are distinctive to a region, or that there was an effect on a young tree grown far from its seed source. Two forms of stress—over-application of a phytocide and prolonged growth in a restrictive container—caused a sharp change in quantitative composition, usually evidenced by lower levels of limonene and myrcene and higher levels α -pinene and β -pinene. Results, in general, indicated that the quickest and cheapest way to obtain trees with a diverse array of compositions was the use of wind-pollinated seed from localities with much variation in individual tree composition.

Research on young half-sibling populations served three purposes: (1) to better understand natural populations; (2) to provide a readily available supply of trees with a diverse array of monoterpene compositions for research on host resistance, and for studies of monoterpene genetics; and (3) to guide the selection of parent trees for breeding programs and to make preliminary allele assignments for tree breeding studies.

One of the conclusions of the extensive study of natural stands of *P. ponderosa* (Smith 1977) (see Chapter 3) was that there was evidence of two additive alleles at a single locus for each of the five major monoterpenes of

P. ponderosa: α -pinene, β -pinene, 3-carene, myrcene, and limonene. Parallel research strongly indicated that the different components of *P. ponderosa* xylem monoterpene differed in their toxicity to western pine beetle, a serious pest of *P. ponderosa* (Smith 1965). Then, field experiments demonstrated that lowered success of beetle attack could be related to the quantity of resin that exudes from a wound and to the quality of monoterpenes; beetle attack was less successful in the trees with the greatest flow of resin and the greatest amount of limonene (Smith 1975).

These early studies showed the need for and desirability of breeding and population studies of the xylem monoterpenes of *P. ponderosa*. The types of composition obtained by various combinations of parent trees were of primary concern. Studies were conducted for this purpose from 1970 to 1980. Trees located by the range-wide study of *P. ponderosa* (see Chapter 3) were the source of the plant material. Parent trees were selected for the primary objective of getting different compositions, particularly high limonene, and therefore may not have been best suited for genetic studies. Nevertheless, the selection of parent trees seems to have been fortuitously adequate for genetic studies. Of course, in all breeding studies of *P. ponderosa*, cone production is often a strong factor in tree selection; the selected trees had to have cones the year of selection.

The first bioassay of resistance to western pine beetle (*Dendroctonus brevicomis*), using trees developed from the seed collection and controlled pollination work, was conducted in 1982 but the results were inconclusive because the trees were too small—less than 2 inches in diameter—and most beetle attacks were abnormal. The next bioassay was conducted in 1987. By that time the trees were 8 to 12 years old and 2-3 inches in diameter. That size is still far below the minimum diameter that is attacked by western pine beetle, under natural conditions, 6 to 8 inches in diameter. Nevertheless, the tests were conducted. The results, as reported in Chapter 9, clearly showed that the degree of success of beetle attack was inversely related to limonene content and also inversely related to the quantity of resin exudation—although exudation rate does not seem to apply too strongly in very small trees (see Chapter 9). However, the results also clearly indicated that trees of this size, about 3 inches in diameter, were not well suited for this type of bioassay. More confident bioassay should be made when the trees are 6 to 8 inches in diameter. However, small trees seemed to be suitable for beetle bioassay on a comparative basis, and this could greatly shorten the time to evaluate results of breeding and plant selection.

It would be helpful to introduce the subject of stress here because it became quite clear as population studies of very young trees progressed, that various forms of stress, or presumed stress, could markedly change the quantitative xylem monoterpene composition of trees less than 15 years old. Hodges and Lorio (1975) found no change in the xylem monoterpene composition of mature *P. taeda* that were put under experimental stress. They did find small changes in the composition of the resin acid. During the course of the studies reported, five sources of stress were found that markedly affected composition in very young trees: prolonged growth in restrictive containers, over application of a phytocide, great distance between parent trees, juvenile growth, and severe wounding. The first four sources of stress will be discussed within the various sections of this chapter; severe wounding will be discussed in Chapter 7 on grafts. In all cases, no matter what the type or source, stress was manifested by an increase in α -pinene and β -pinene and sometimes 3-carene, and by a decrease in myrcene and limonene. In a recent comprehensive review of stress in human cells, Welch (1993) found that the reaction of the cell was always the same: increased production of the same group of chemicals, no matter what the mode or source of stress. Thus, there was a basic similarity between plants and animals in response to stress.

Two other factors might be involved separately or in conjunction with the five noted above. First, at times and under certain conditions not fully understood, it appeared that the type of composition itself may be an important factor in stress and juvenile reaction, and β -pinene may be the component involved. This was a possible factor in the Ranger Peak progeny in particular and in the Wheeler Peak progeny to a lesser degree. Second, there were indications that the growth of the seedling well outside its seed zone may affect the level of activity of the previous factors.

Selection of Parent Trees

Parent trees for sibling studies were selected from two sources: trees previously noted in the extensive study of the regional and local variation of *P. ponderosa* xylem monoterpene (see Chapter 3, #14 of this volume), and a basic breeding tree, located at the Institute of Forest Genetics (IFG) near Placerville, California (fig. 5-1).

The following sets of trees provided wind-pollinated seed—their compositions will be given in the appropriate section (fig. 5-1):

1. four trees in the Ranger Peak locality on the Los Padres National Forest;
2. 13 trees in the Wheeler Peak locality on the Humboldt National Forest (the location is now in the Great Basin National Park);
3. two trees at the Adin Summit locality on the Modoc National Forest;
4. one tree in the Camino Arboretum at IFG; this tree is a graft of one of the trees at Adin Summit.
5. one tree on the Eldorado National Forest near IFG.

Cones from all the trees were taken to IFG, and seeds were extracted and stored by the standard procedures used for all work with pines (Cumming and Righter 1948).

Nursery and Plantation Practices

Seeds were stratified for at least 30 days before sowing in early spring. Nursery beds were 5 ft. by 98 ft. In the 1971-1979 nurseries, seeds were spotted every 6 in. in rows that were 6 in. apart. In the 1980-1982 nurseries, a 1-ft by 1-ft spacing was used and found to be much better for this type of work. The seeds in one row were always of the same source. The seeds for a given source in a nursery were usually in a single block of adjacent rows. Beds were irrigated for only the first 3 or 4 years.

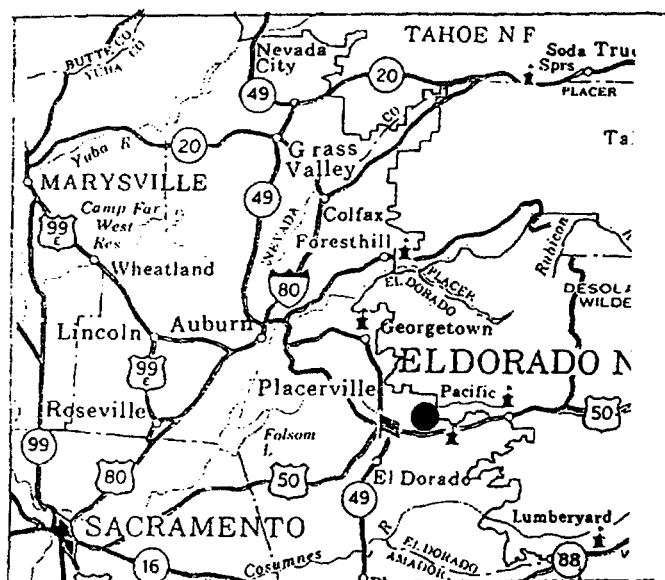
The beds were usually thinned first at 4 to 6 years by removing alternate plants or alternate rows. A second thinning was made at 6 to 8 years. Subsequent thinnings were made to provide the best spacing for the residual trees or to favor those trees which were considered most suitable for bioassay studies with western pine beetle. One bed was thinned more frequently to more accurately measure the possible effects of time of growth on composition.

The trees were grown and examined in four types of areas at IFG as follows.

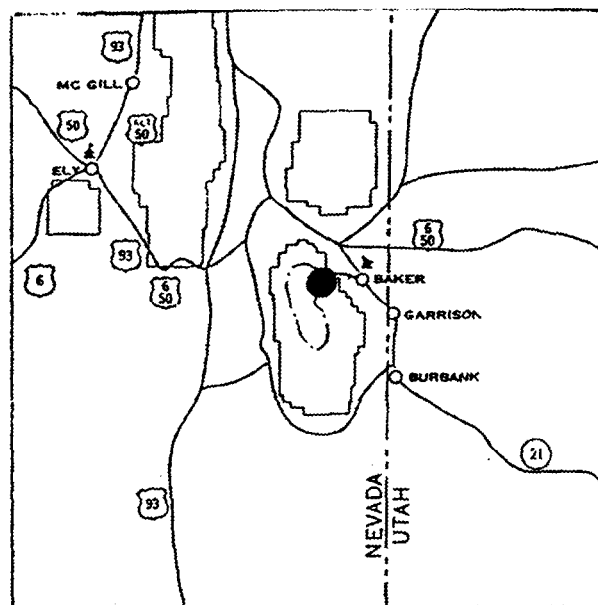
1. Nursery: Seeds were planted and trees maintained in the nursery bed until examination. Nurseries were started each year from 1971 to 1982 except 1973 and 1980.

2. Plantation: One-year seedlings from the nursery bed were transplanted to a 6-ft by 6-ft plantation with nine half-siblings in a block as bare root stock in 1978. The plantation was irrigated for 3 years.

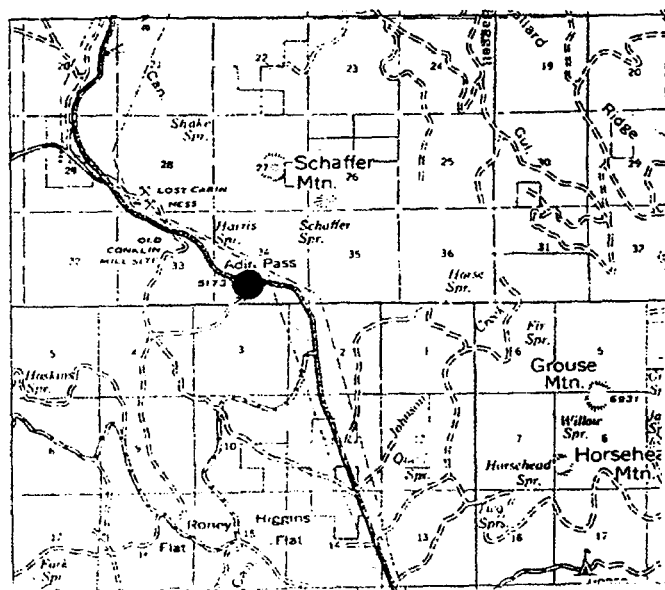
3. Containers: Seeds were planted in a container and were maintained there until trees were upscaled to larger containers. Two sets of containers were used. One set was started in 1978 and upscaled each year for 7 years from 1-in



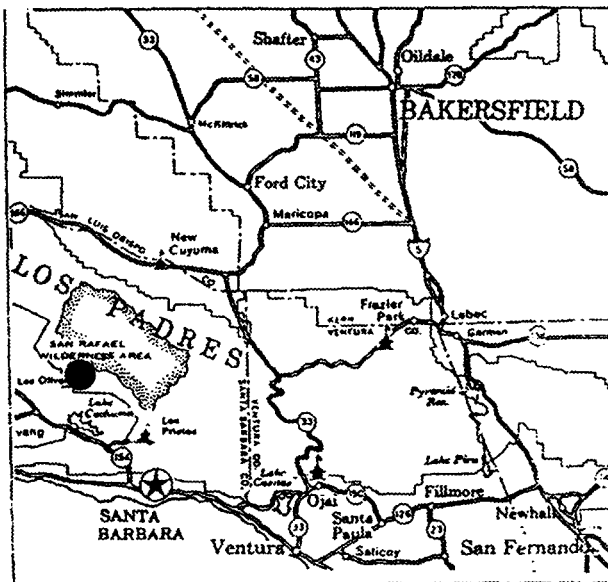
Institute of Forest Genetics



Wheeler Peak, Great Basin N.P.



Adin Summit, Modoc N.F.



Ranger Peak, Los Padres N.F.

Figure 5-1—Locations in California and Nevada used in the study of half-sibling populations of *P. ponderosa* (●).

by 6-in containers to 6-in by 18-in containers. The trees were then grown for two more years in these large containers. The other set was started in 1983 and upscaled for 3 years to 2-in by 10-in containers when they were examined. Containers were irrigated during all the growing season for all years.

4. Container/Plantation: Seeds were planted in containers and upscaled as described above. The trees were transplanted to a plantation. Two sets of containers were used. One set was upscaled to 3-in by 6-in containers at the fourth year and then outplanted. The other set was upscaled 5 years to 6-in by 18-in containers, held for 2 more years in these containers and then outplanted.

The plantation and the container/plantation were treated with a phytocide early in spring of the first 3 years after planting. The second-year treatment of the phytocide was too heavy, and many trees were killed; the trees that survived showed reduced growth for a year or two. It is likely that the whole plantation was under moderate to severe stress for at least a year with the intensity of the stress apparently irregularly scattered throughout the plantation. By the time of the first sampling in 1987, virtually all trees were growing very well with internode length of 18 in. to 24 in. However, there may have been some residual effect of stress.

Sampling for Resin

All trees were sampled for xylem resin either by severing the tree when the nursery bed was thinned or by making a basal micro-tap as described in Chapter 2.

Obtaining a sample of resin by the severing process was as follows. The tree was cut off about 6 in above the ground; this was usually within the first internode. The cut surface was scraped or cut clean with a razor blade. By waiting to do the scraping until a small amount of resin had exuded to the surface, the resin of the tree was acting as a *de facto* cleaning agent. If cortex resin was present, a ring of cortex about 0.25 in wide was cut from the top circumference of the small stump. This was done to prevent the mixing of xylem resin with the cortex resin. In general, trees less than 6 years old still have resin in the cortex at the base of the tree. Older trees usually do not have cortex resin in the basal internode, but this can vary by 2 to 4 years depending on rate of growth. In general, there will be no cortex resin where the bark has developed fissures and plates. Two to 4 hours or more after cutting and cleaning, sufficient resin for a sample would have exuded to the surface. A thin glass rod was moved across the surface to "pick up" a drop of resin. The rod with the drop of resin on the end was placed in a 2-cc screw-cap vial. When 10 to 20 such vials had been obtained, they were taken to the laboratory.

In the laboratory, an approximately equal volume of pentane (chromatographic grade) was added to each vial. The contents were stirred until the resin had been dissolved from the glass rod. The glass rod was removed, and the vial was tightly capped. The time between lifting the sample from the cut surface and the capping vial was usually less than 1 hour. Vials containing the prepared sample were held at 0°C until ready for analysis 2 to 8 months later.

Two variants of the severing procedure were used to study resin composition between annual rings and between internodes. For annual ring studies, the glass rod was carefully moved along a specific annual ring, and the sample for each ring of the tree was kept separate. For internodal studies the topmost internode was severed, and the resin sample taken as noted above. Then the next internode was severed, and so on down the tree. In one instance trees were sampled vertically by cutting a live limb off each whorl. This cutting of live branches was used most effectively on the edge trees of a nursery bed or on young plantation trees.

Trees that were to remain in the nursery bed or plantation were sampled by the basal micro-tap procedure described in Chapter 2.

It needs to be stressed that during the analysis of the first set of half-siblings from Ranger Peak in 1976, it became clear that the composition of these 4- to 6-year-old seedlings could be unstable or in a state of flux and might not be that of the tree when it matures. Therefore subsequent studies were designed to ascertain the magnitude of this condition and to determine when the monoterpene composition of young trees could be used for various types of analyses. GLC analysis was as described in Chapter 2 of this report.

To repeat, all measurements reported in this chapter were of different trees of the same seed source; i.e., there were no successive measurements of the same trees. Chapter 8 does have data on successive measurements of the same tree in the study that resampled young trees.

Tehachapi Transition Zone: Ranger Peak

Seeds were collected in 1970 from four trees located between Figueroa Mountain and Ranger Peak on the Los Padres National Forest (*fig. 5-1*). This was the location of an 80-tree stand classed as the Tehachapi Transition Zone for *P. ponderosa* (see Chapter 3, *P. ponderosa*, stand #30). An additional 40-tree stand was sampled in the same general area about a mile away. No high-limonene trees were found in the 80-tree stand, but one was found in the additional 40-tree stand. Trees were selected for cone collection on the basis of xylem monoterpene composition. The composition of the four female trees was listed (*table 5-1*). Tree RP-B37 was high in limonene and essentially without β -pinene and 3-carene; Trees RP-B41 and RP-B42 had moderate amounts of β -pinene, 3-carene, and limonene; and Tree RP-2374 was high β -pinene and without 3-carene but with moderate limonene. All four trees had moderate amounts of α -pinene and myrcene. Though these four trees were selected on the basis of monoterpene composition, they were fairly representative of the stand except for the high-limonene tree.

Seeds were planted in the 1971 nursery. The bed was thinned in 1976 and again in 1978; the remaining few trees were cut in 1981. Thus all analyses were of different trees and not successive measurements of the same tree. Number of trees sampled proved to be a bit small.

It was evident (*table 5-1*, *figs. 5-2* and *fig. 5-3*) that β -pinene and 3-carene were much higher than expected in the progeny and myrcene and limonene much lower than expected, when the composition of the parents and of the stand average, and the source of pollen, were considered. It was also readily apparent (*figs. 5-2* and *5-3*) that the frequency distributions of the major components were multimodal. Though there was some fluctuation in composition between sampling periods, this pattern of higher β -pinene and 3-carene, and of lower myrcene and limonene, held throughout the sampling period. However, the individual components were comparatively higher or lower with respect to the female parent (*table 5-1*). That is, for example, the progeny of RP-B37 were much lower than expected in limonene; yet compared to the progeny of the other three trees, RP-B37 was still highest in limonene, and almost by direct ratio. This general tendency held for other major components. The term 'juvenile factor' was applied to this condition in young *P. ponderosa*. The presence and effect of the juvenile factor in this set of half-sibling progeny at Ranger Peak influenced much of the sampling in the subsequent research on young *P. ponderosa*. There may be some evidence that limonene and myrcene will increase with age and β -pinene and 3-carene will decrease (*table 5-2*); larger samples could have improved the interpretation, particularly for the older trees.

Table 5-1—Xylem monoterpene composition of the four parent *P. (P)*, *ponderosa* at Ranger Peak the average composition of their wind-pollinated progeny (*F*) aggregated for 5 to 10 years of nursery growth, and the average composition of the surrounding 80-tree stand, the four parent trees, and the 195 aggregated progeny. *N* = number of trees; *x* = averages.

Tree	Class of tree	N	Monoterpene ¹							
			α -p	cam	β -p	car	my	lim	β -ph	ter
			-----percent ² -----							
RP-B37	P	1	11.9	**	2.6	0	15.7	69.7	**	0
	F	39	14.2	**	46.5	18.7	2.5	16.8	0.5	0.6
RP-B41	P	1	6.2	0.1	27.2	21.1	16.8	21.8	0.6	0.4
	F	51	11.1	0.2	46.7	26.8	1.7	9.7	0.4	1.1
RP-B42	P	1	6.5	**	39.2	23.2	9.9	22.9	1.3	2.0
	F	40	11.6	0.3	55.2	17.9	1.7	11.7	0.7	0.6
RP-2374	P	1	12.0	**	56.7	0	13.3	16.5	1.2	0
	F	65	14.5	0.4	55.0	18.3	1.8	8.3	0.4	0
X	P	4	9.2	0.1	30.2	11.1	13.9	32.7	0.8	**
X	F	195	13.0	0.3	51.8	20.5	1.9	11.0	0.5	0.7
X	Stand	80	8.2	**	36.7	25.2	13.1	17.3	**	1.5

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.²Normalized, ** = trace.**Table 5-2**—Average composition of all half-siblings from the aggregate of the four trees at Ranger Peak (see table 5-1) for three periods of nursery growth. *N* = number of trees.

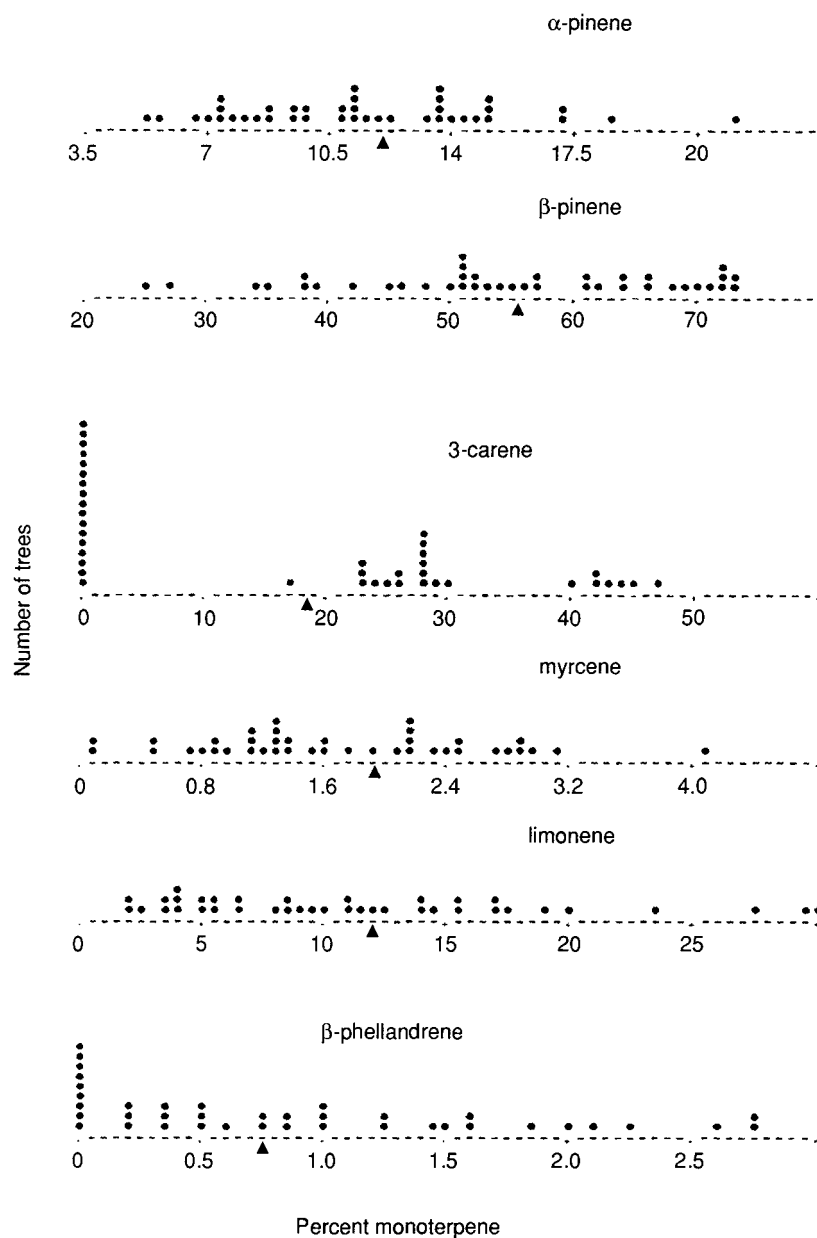
Age years	N	Monoterpene ¹							
		α -p	cam	β -p	car	myr	lim	β -ph	ter
		-----percent ² -----							
5	126	14.7	0.1	51.7	23.8	1.2	6.7	0.5	0.8
7	58	9.4	0.6	51.2	14.6	3.1	19.9	0.4	0.7
10	11	11.3	**	54.9	14.3	3.3	14.7	1.0	0.4

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.²Normalized, ** = trace.

B-42

Figure 5-2—Frequency distribution of the amount of noted monoterpenes in the 5- to 10-year-old progeny of *P. ponderosa* RP-B37 and RP-B42; each dot is measurement of one tree; [▲] is average of set.

(continues on next page).



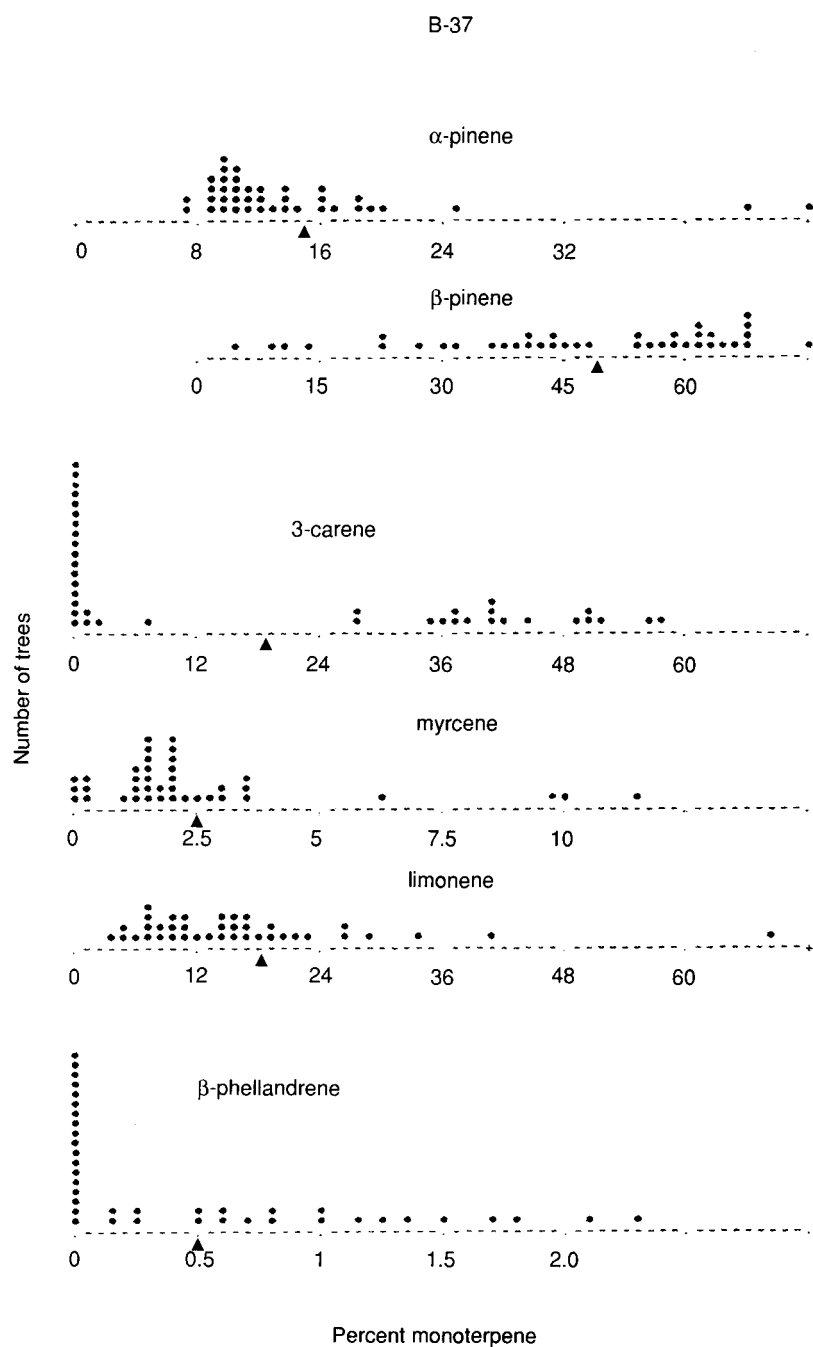
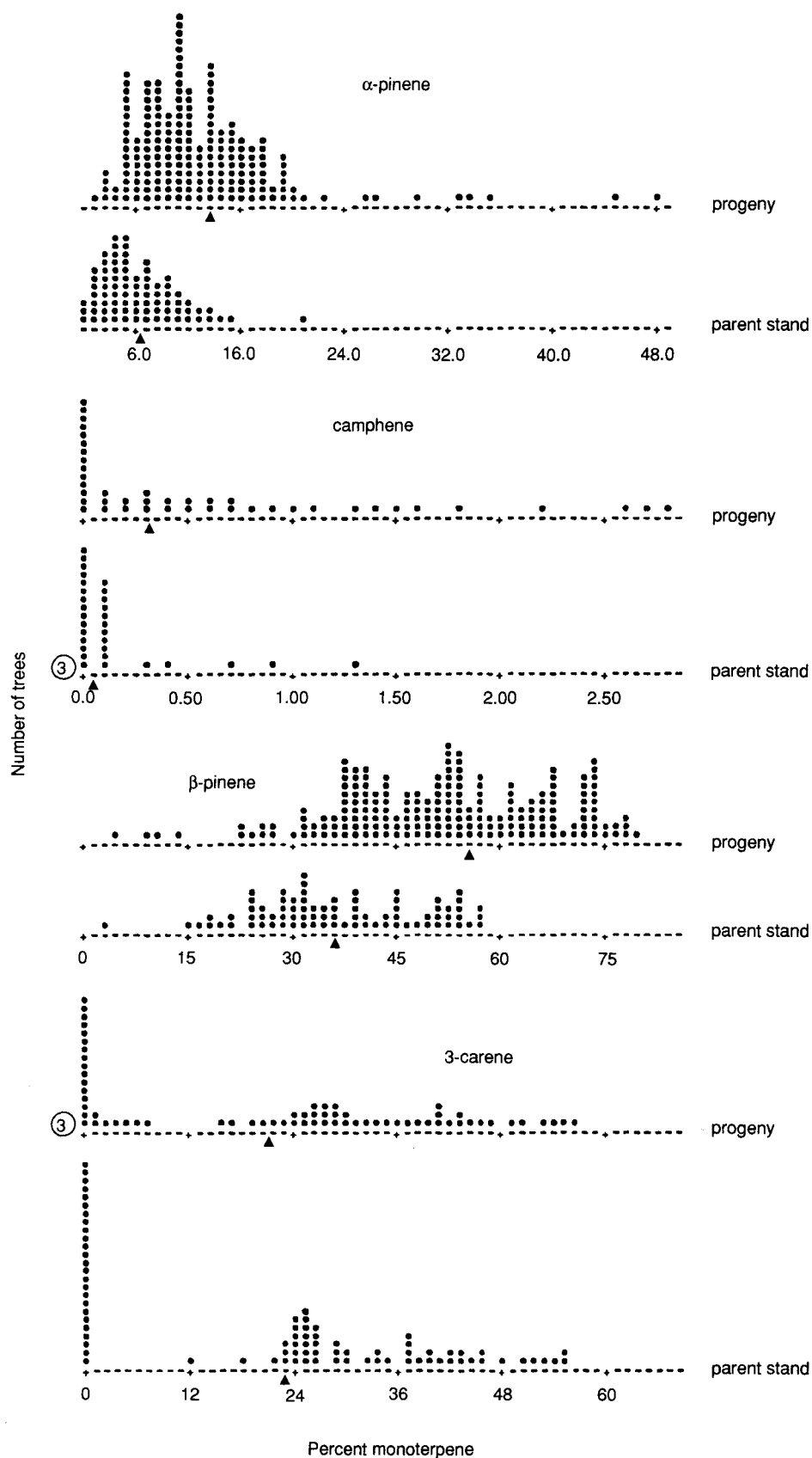
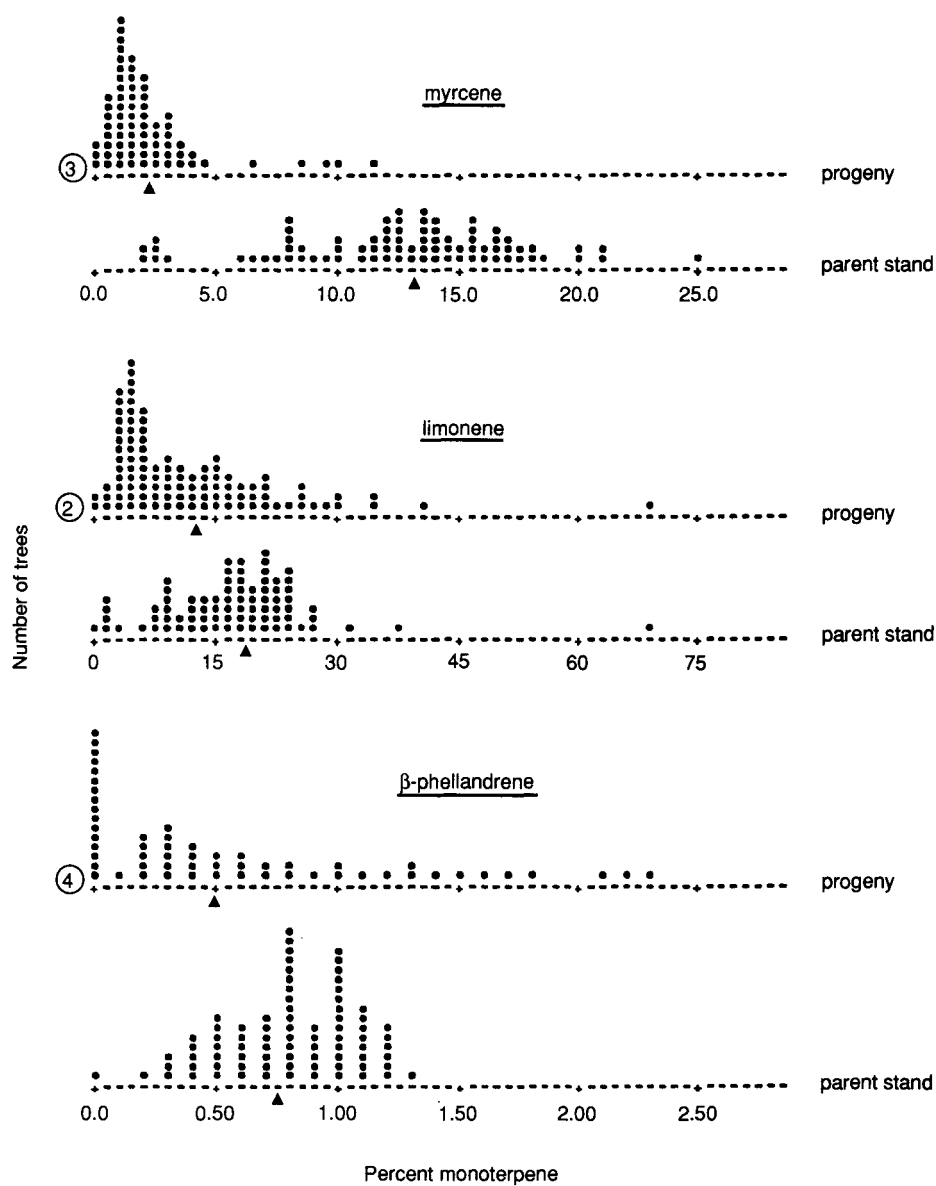


Figure 5-3—Comparative frequency distribution of the amount of the noted monoterpenes of the mature 80-tree stand of *P. ponderosa* at Ranger Peak and of the aggregate 5- to 10-year-old progeny of four female parent trees: RP-B37, RP-B41, RP-842, RP-2374; each dot is measurement of one tree, except where (n) indicates each is n trees; [▲] is average of set.

(continues on the next page)





This might be an appropriate place to discuss myrcene because it was classed as a major monoterpene, yet at most times it seems to be more like a minor one. Myrcene was always present in *P. ponderosa* throughout its entire range. It was nearly always present in small amounts, ranging from about 2 to 20 percent but mostly 5 to 15 percent, but in a rare tree it can be greater than 25 percent. Because both myrcene and limonene seemed to increase very slowly with age, one might speculate that myrcene was linked to limonene positively, in much the same way that terpinolene is linked to 3-carene and sabinene. But myrcene was found in trees that essentially lack limonene, whereas terpinolene was never present when 3-carene and sabinene were absent. Thus myrcene may be linked to limonene, may be produced in small amounts by isomerization or biosynthetic by-products, and, as will be shown later in the discussion of full siblings, was found as a rare allele. No tree with an unusually large amount of myrcene was found in the Ranger Peak progeny, despite having a high stand average, and it appeared that alleles for myrcene were lacking, though a rare (0-1) set would be expected at least. Thus, the large amount of myrcene in the Southern California mountains appeared to be caused by its positive linkage to limonene or to isomerization. One parent tree at Adin Summit, that was used in controlled breeding, had about 30 percent myrcene and was assigned a (0-1) set of alleles; the frequency distribution of the progeny of this tree very easily supported the assignment of an (0-1) set of alleles for myrcene to that tree; the progeny of this tree will be discussed in Chapter 6 of this volume. In the range-wide study of *P. ponderosa*, reported in Chapter 3 of this volume, one tree was found with about 50 percent myrcene. One might speculate that this tree had a (1-1) set of alleles for myrcene and was the product of the combination of two rare (0-1) alleles. The positive linkage of limonene and myrcene is a fascinating biological condition in that limonene was the more toxic of the monoterpenes to western pine beetle, and therefore enhanced tree survival, whereas myrcene synergized the attractive pheromone of the beetle and therefore enhanced beetle survival. This phenomenon was difficult to reconcile with the theory of coevolution; i.e., two chemicals were linked in some genetic way, yet one seemed to favor tree survival and the other seemed to hasten its death.

The primary objective of the study was to obtain a large selection of trees with a diverse array of monoterpene compositions for host resistance and resin studies. Ten such types of composition were found among the progeny of the four parent trees (*fig. 5-4*). One type of particular note was #1 which was classed as an ultra-high limonene, between 70 percent and 90 percent; this type was found, but not commonly, in the progeny of RP-B37, the high-limonene parent tree. An ultra-high limonene tree had not been found in natural populations of *P. ponderosa*.

Progeny classed as high limonene were produced by trees RP-B42 and RP-2374, two trees not classed as high limonene. Because the progeny that were classed as high limonene had no β -pinene also, one might assume a (0-1) set of alleles for β -pinene in the two parents.

The results of the study at Ranger Peak were not strongly encouraging and may have been weakened by inadequate sampling in the older sets of trees. Though confident analysis and conclusions could not be made, the results were quite valuable in determining the design and conduct of subsequent studies of half- and full siblings.

An early attempt to bioassay resistance was made, using these small trees, and the results were inconclusive (see Chapter 9). However, the experience did provide better guidance in the conduct of the other breeding and culturing studies. Tree RP-B37 was not used in controlled breeding, but it would have been interesting and helpful to do so because it had much lower α -pinene than

any of the other high limonene trees that were used, as reported in the section on full siblings. By its composition one would postulate that RP-B37 had no alleles for β -pinene or 3-carene, and might not have alleles for α -pinene and myrcene.

Sierra Pacific Region: Adin-Placerville

Three trees are discussed in this section: AS-3139, AS-3187, IFG-P15 (*table 5-3*); trees AS-3139 and AS-3187 were at Adin Summit, IFG-P15 on the Eldorado National Forest (NF) (*fig. 5-1*). Tree AS-3139 was also a graft growing at the Institute of Forest Genetics; seeds were collected from it at Adin and from the graft at IFG. AS-3139 was a high limonene tree and was used in the breeding programs. IFG-P15 was growing on the Eldorado NF near IFG; it was included for comparison of progeny. AS-3187 was also growing at Adin Summit and was used in controlled breeding programs. Progeny of AS-3139 were grown in the nursery and in the plantation; the progeny of IFG-P15 and AS-3187 were grown only in the nursery.

Again, as with the progeny from Ranger Peak, the composition was not what was expected (*table 5-3*) considering the composition of the female parent and the composition of the surrounding stand, the source of pollen. This is attributed to the juvenile factor. But the compositions of the progeny were still relatively comparable, as noted for the Ranger Park progeny; i.e., the progeny of AS-3139 were highest in myrcene and limonene, and those of AS-3187 were highest in β -pinene and 3-carene.

There were some shifts in the composition of nursery-grown progeny of AS-3139 and IFG-P15 over the early years of nursery growth (*table 5-4*), but no strong trends were evident, as was somewhat evident in the Ranger Peak progeny. There were, however, small but consistent increases in myrcene. Could this suggest that myrcene was isomerized from limonene or related to it in some way? The largest shifts in myrcene were in the progeny of AS-3139, the high-limonene tree. The aggregate frequency distributions strongly suggest multimodal distributions for α -pinene, β -pinene, 3-carene, myrcene, and limonene (*fig. 5-5*). There were large differences between the nursery-grown seedlings and the plantation-grown seedlings of AS-3139 (*fig. 5-5*). The largest difference was in α -pinene; the nursery-grown stock averaged 13.9 percent, and the plantation-grown stock averaged 25.5 percent. Limonene was the converse with 27.3 percent in the nursery and 15.3 percent in the plantation (*table 5-4*). Beta-pinene was much higher in the plantation, and 3-carene much lower.

The frequency distribution of the individual monoterpenes clearly showed the great differences between the nursery-grown and plantation-grown plants (*fig. 5-5*). The over application of a phytocide during the second and third growing seasons in the plantation was considered to have caused very severe stress to all trees in the plantation. This was the first evidence to suggest that stress could affect quantitative composition. Many trees were killed and those that lived had greatly reduced terminal growth for 2 years or more. However, by the time the trees were sampled in 1988 at 10 years, most trees were growing normally as indicated by the 18 in and 24 in. in terminal growth. Thus, there was a residual effect of stress.

There was no appreciable difference between the progeny of AS-3139 from the Adin tree or from the Placerville graft. This might be expected because both localities are in the Sierra Pacific Region of *P. ponderosa* and, therefore, have about the same general pollen background for xylem monoterpenes.

It is of particular note that for the progeny of AS-3139 the 27 percent average for limonene was considerably higher than the 14 percent for the progeny of WP-3298 and WP-3352, the two high-limonene trees at Wheeler

Figure 5-4—Ten types of monoterpene composition in the 5- to 10-year-old progeny of the four noted female *P. ponderosa* parents at Ranger Peak, the occurrence of the 10 types in the four sets of progeny: ([●]) = occurs commonly, ([○]) = occurs not commonly.

NOTE: footnotes for all of the tables and figures on the bottom of the page.

Type number	Monoterpene ¹					Parent Tree			
	α-p	β-p	Car	my	lim	RP-B-37	RP-B-41	RP-B-42	RP-2374
	Pct ²								
1	16	10	2	3	69	○			
2	4	0	39	4	50	○		○	○
3	45	9	2	3	40	○			
4	8	1	54	9	27	○			
5	10	58	0	4	25		○	○	
6	9	20	49	5	15		○	●	●
7	14	73	0	1	11		●		●
8	10	39	41	1	9	●	●		
9	13	57	24	1	4	●	●	●	●
10	15	75	0	6	2	●		●	●

α-pinene, β-pinene, 3-carene, myrcene, limonene

Table 5-3—Xylem monoterpene composition of the three parent trees *P. ponderosa* (*P*) at Adin-Placerville, the average composition of their wind-pollinated progeny (*F*) aggregated for 4 to 12 years of nursery growth, and the average composition of an 80-tree stand at Adin and at Placerville

Tree number	Class	Trees	Age	Monoterpene ¹							
				α -p	cam	β -p	car	my	lim	β -ph	ter
		<i>number</i>	<i>years</i>	<i>percent</i> ²							
AS-3139	P	1	40+	17.8	0	0.7	0	22.1	52.9	0.7	0
	F	69	4-12	13.9	0.2	21.9	28.1	7.0	27.3	0.1	1.6
AS-3187	P	1	40+	9.2	0.1	22.7	22.7	14.3	28.5	0.9	1.0
	F	10	4-12	8.7	0.1	25.8	45.4	5.3	12.0	0	2.7
IFG-P-15	P	---	---	---	---	no	data	---	---	---	---
	F	66	4-12	7.3	0.1	36.4	36.5	4.1	13.1	0.3	2.0
Adin	stand	80	40+	6.0	0	20.5	44.5	14.5	12.5	0.5	2.0
Placerville	stand	80	40+	6.0	0	29.5	34.5	13.0	15.5	**	2.0

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.
² Normalized, ** = trace.

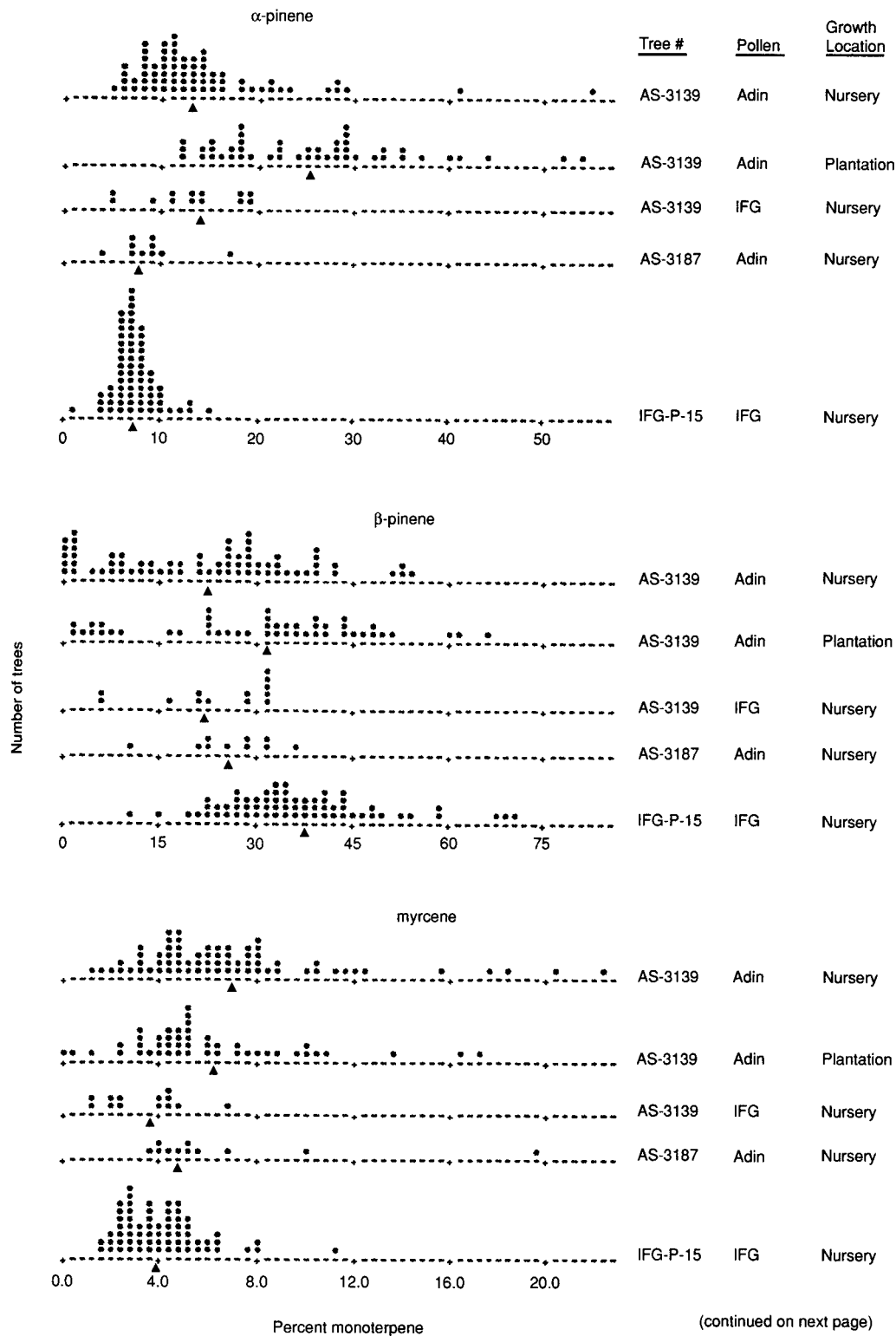


Figure 5-5—Frequency distribution of the amount of the noted monoterpenes of the progeny of the *P. ponderosa* listed on the right, with two different sources of pollen (Adin, IFG) and two places of growth (nursery, plantation); each dot is measurement of one tree; [▲] is average of set.

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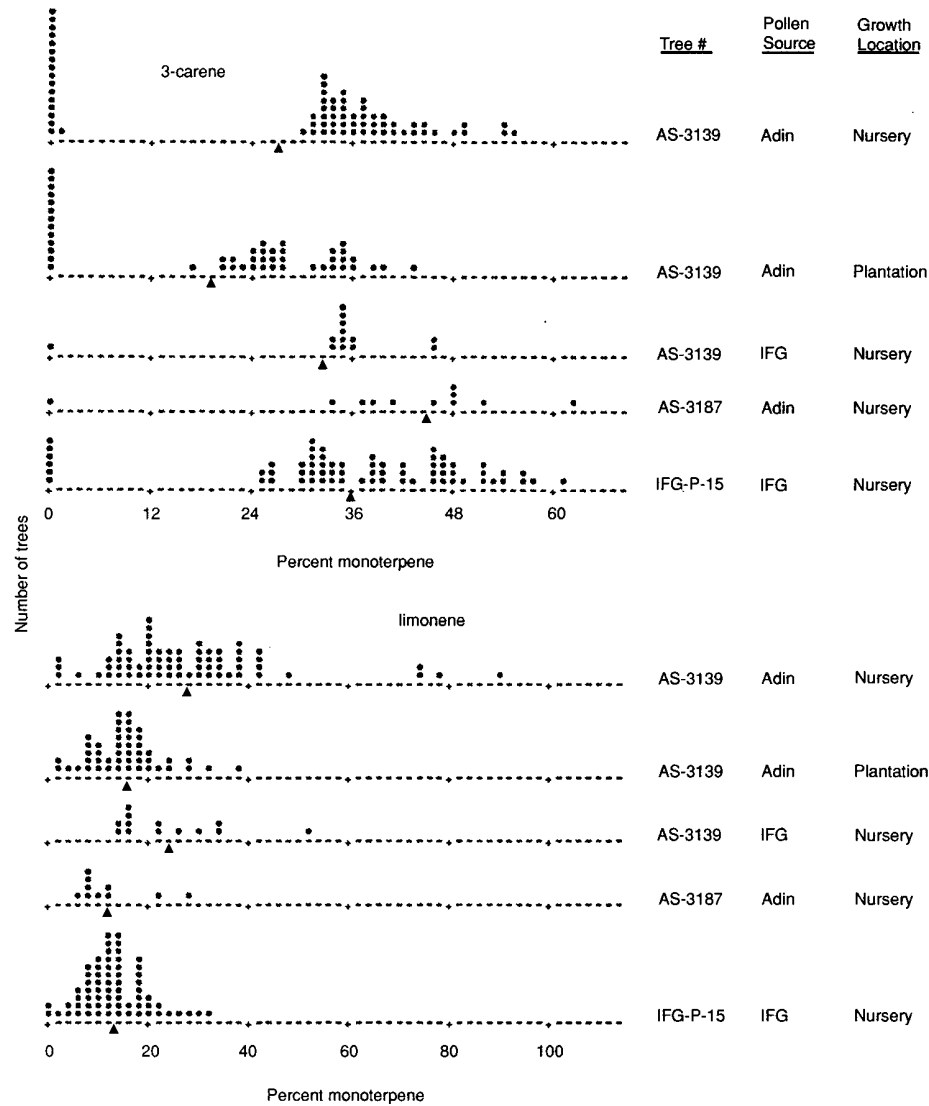


Table 5-4—Average monoterpene composition of the wind-pollinated progeny of AS-3139 and IFG-P15 for 4-12 years of growth in the nursery. *N* = number of trees, *x*=average.

Trees	Years growth	N	Monoterpenes ¹							
			α-p	cam	β-p	car	myr	lim	β-ph	ter
			<i>percent</i> ²							
AS-3139	5	6	12.9	**	28.0	17.5	3.2	37.4	0.6	0.3
	9	17	16.8	0.7	18.7	32.0	6.1	24.3	0.1	1.7
	10	18	13.9	0.1	21.6	24.7	7.8	30.4	**	1.4
	10	13	14.2	0.1	28.0	25.9	5.5	24.8	**	1.4
	11	15	10.6	**	19.0	33.8	9.5	25.0	**	2.1
X		(Σ69)	13.9	0.2	21.9	28.1	7.0	27.3	0.1	1.6
IFG-PI5	5	14	7.3	0.5	38.8	34.3	3.2	13.4	0.5	2.1
	9	13	8.4	**	38.4	35.6	3.5	11.4	0.5	2.0
	10	7	7.0	0.2	37.9	30.6	3.8	19.2	**	1.4
	10	21	7.3	0.1	36.2	37.7	4.4	12.2	0.2	2.6
	11	II	6.3	0.1	30.6	41.8	5.3	13.1	0.2	2.6
X		(Σ66)	7.3	0.1	36.4	36.5	4.1	13.1	0.3	2.0

¹ α-pinene, camphene, β-pinene, 3-carene, myrcene, limonene, β-phellandrene, terpinolene.

² Normalized, ** = trace.

Peak that will be discussed in the next section on the Escalante North Transition Zone. The limonene background in pollen, 12 percent for Adin and 14 percent for Wheeler, would lead one to expect a greater amount of limonene in the progeny from Wheeler than from Adin. I would postulate that this could again be an expression of stress in the Wheeler progeny growing at IFG far out of their Wheeler Peak provenance, and even out of their resin ecotype region, whereas the progeny of AS-3139 were growing in their resin ecotype region, though about 250 miles away. In fact all three trees located in the Sierra Pacific had progeny with greater limonene than the progeny of comparable trees at Wheeler Peak. Likewise, the progeny of AS-3139 were much higher in limonene than the progeny of RP-B37, the high limonene tree at Ranger Peak, 27 percent vs. 17 percent.

There were only 10 nursery-grown progeny of AS-3187. As expected, the average composition for nearly all monoterpenes was quite different from that of the progeny of AS-3139 (*table 5-3*), and the frequency distributions were also quite different (*fig. 5-5*). There were insufficient trees to determine if there were changes over time. It was obvious that the differences noted above were a reflection of the differences between the two parents, because one can assume that the background pollen was about the same for both trees.

The results with the progeny from Adin and IFG parent trees were far more encouraging than results with the Ranger Peak trees. There was a marked improvement in sampling numbers, and composition did not change with age enough to preclude confident analysis and conclusions. The average composition and the frequency distribution of individual monoterpenes of the progeny of AS-3139 differed markedly from that of AS-3187 and IFG-P15, which shows the difference in the female parents.

Twelve types of composition were found among the progeny of the three parent trees (*fig. 5-6*). These types clearly showed the difference between the female parents. The two types of ultra-high limonene (between 70 percent and 90 percent), #1 and #2 (*fig. 5-6*), were found only in the progeny of AS-3139. Such ultra-high compositions have not been found in natural stands. The limonene in the progeny of AS-3187 and IFG-P15 was well below 30 percent. Comparisons of the progeny of AS-3139 and those of the other two high limonene trees at Wheeler Peak, WP-3298 and WP-3352, will be made at the end of the section on Wheeler Peak.

Escalante North Transition Zone: Wheeler Peak

Seeds were collected from 13 trees at Wheeler Peak in 1971 (*fig. 5-7, table 5-5*) and planted in several nurseries starting in 1972. Wheeler Peak was in the Escalante North Transition Zone of *P. ponderosa* (see Chapter 3). Some seedlings of the two high-limonene parents, trees WP-3298 and WP-3352, were also transplanted to the 1978 plantation. Resin samples from all trees were obtained by plant severing or by micro-tap in the nursery, and by micro-tap in the plantation (see Chapter 2 and introduction to Chapter 6 for a description of this procedure).

Where possible, sets of the same nursery-grown half-siblings were compared for each period of growth and then aggregated into three or four age classes to get greater numbers per class. The aggregated classes gave a much better definition of the frequency distribution and averages.

In all cases, all trees in all sets are different, though taken from the same basic lot of seed; i.e., there were no successive measurements of the same trees. We were encouraged that, in general, the results with these 13 sets of progeny did not show as strong a juvenile effect as was found in the Ranger Peak progeny, though there was still some effect. I am inclined to believe that the

Figure 5-6—Twelve types of monoterpene composition in the progeny of the three noted parent *P. ponderosa*, aggregated for 4 to 12 years of nursery growth, and the occurrence of the types in each set of progeny: ([●]) = occurs commonly, ([○]) = occurs not commonly.

Type #	Monoterpene ¹					Parent Tree		
	α-p	β-P	car	my	lim	AS-3139	AS-3187	IFG-P15
			Pct ²					
1	7	0	0	2	90	○		
2	16	10	2	3	69	○		
3	4	0	39	4	50	●		
4	32	6	1	15	47	○		
5	14	39	0	5	42	○		
6	8	1	54	9	27	●		
7	10	58	0	4	25	○		○
8	7	34	34	4	20	●	●	○
9	1	ii	51	2	20			●
10	9	20	49	5	15		○	○
11	10	39	41	1	9		○	●
12	54	33	0	1	3	○		

¹: α-pinene, β-pinene, 3-carene, myrcene, limonene

²: Normalized

Table 5-5—Monoterpene composition of 13 parent trees at Wheeler Peak and of the average composition of the surrounding 80-tree stand (WP)

Tree Number	Monoterpene ¹							
	α-p	cam	β-p	car	my	lim	β-ph	ter
	percent ²							
WP-3292	12.8	0.1	75.7	0.1	8.5	1.3	1.7	0.1
WP-3293	7.1	0.1	10.8	67.0	9.6	0.8	0.4	4.0
WP-3294	4.6	0	5.0	55.8	5.0	26.1	0.7	2.8
WP-3298	25.6	0.1	0.4	0	16.1	57.9	0	0
WP-3299	6.4	0	0.2	43.0	7.9	40.4	0.8	1.1
WP-3301	6.5	0	0.6	68.8	18.2	0.6	0.6	4.6
WP-3312	4.8	0	7.3	33.8	3.5	48.0	1.0	1.7
WP-3318	3.6	0.1	10.4	18.4	3.0	40.6	0.8	0.7
WP-3320	12.0	0	0.3	60.7	5.6	26.6	0.4	2.7
WP-3359	24.3	0.1	31.3	42.5	10.6	0.6	1.0	2.6
WP-3352	22.4	0.2	0.4	0.1	13.1	56.9	0	0
WP-B475	7.0	0.2	62.7	0	12.5	1.4	0.8	0
WP-B477	6.0	0.1	16.4	63.2	9.4	0	0.6	3.5
X WP	6.9	0.1	29.0	34.0	13.0	15.0	1.0	2.0

¹ α-pinene, camphene, β-pinene, 3-carene, myrcene, limonene, β-phellandrene, terpinolene.

² Normalized.

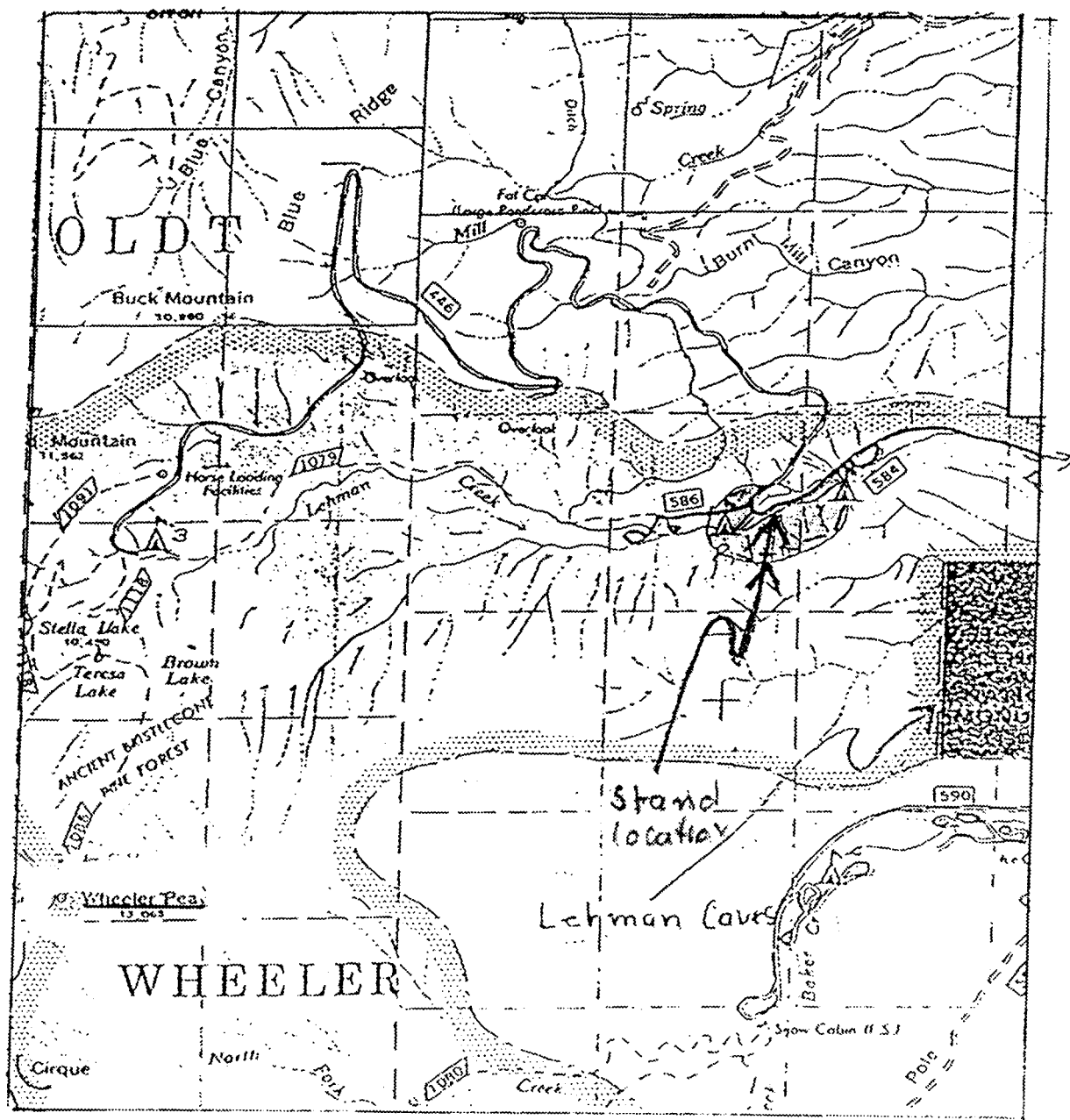


Figure 5-7—Location of the Wheeler Peak stand of *P. ponderosa* in eastern Nevada; see figure 5-1 for general location.

juvenile effect may be strongest in the types of composition found in the Tehachapi Transition Zone at Ranger Peak.

The following discussion will compare the compositions and frequency distributions of individual components of pairs of parents with similar and with dissimilar monoterpene compositions, will compare the progeny of groups of parents, and will compare the types of composition found among the progeny of all trees in the study. The average composition of the aggregate nursery-grown progeny of each of the 13 parents was listed (*table 5-6*). In some of the sets of comparison in the rest of this section on Wheeler Peak, the base line scale may not be the same for both trees for a given component. In most cases, the scales will be the same.

Comparison of Progeny of Parents with Similar Composition Trees WP-3298 and WP-3352

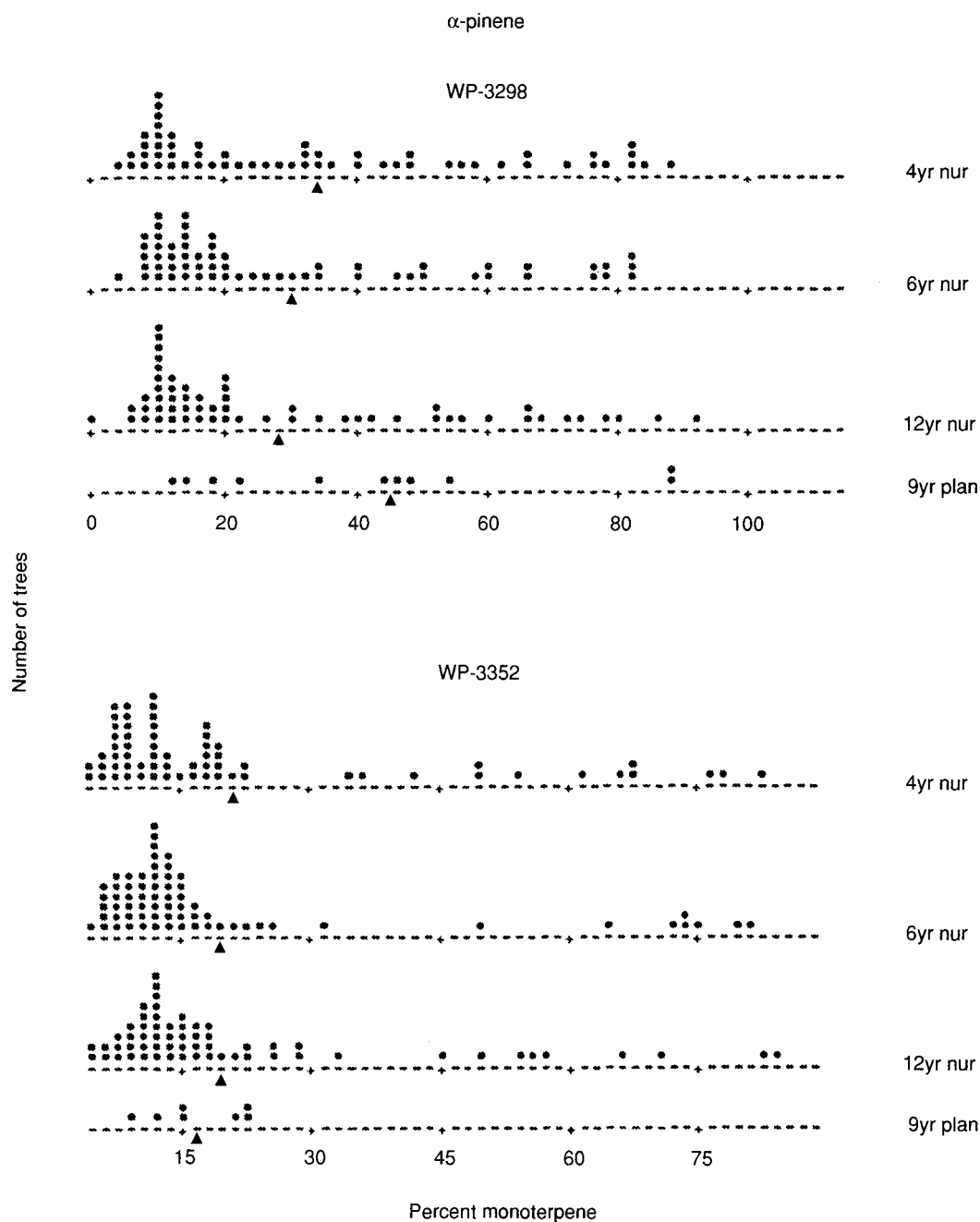
Both trees were classed as high limonene, nearly 60 percent, and essentially without β -pinene and 3-carene (*table 5-5*); neither tree served as a female in controlled breeding, but both served as males. Ten sets of nursery-grown progeny ranging in age from 4 to 12 years and one set of 9-year-old plantation trees were examined. The average composition varied somewhat for each nursery period as illustrated by the progeny of WP-3352, but there was no clearly defined trend (*table 5-7*); small sample size may have caused some of the apparent variation. This general pattern prevailed for the progeny in all sets. When the data were aggregated into three age classes for each set of progeny, variation with time became very small and there was no discernible trend in either set of progeny (*fig. 5-8*). There was no sharp tendency for α -pinene or β -pinene to decrease. The multimodal configuration of the frequency distributions of the two major monoterpenes was clearly evident.

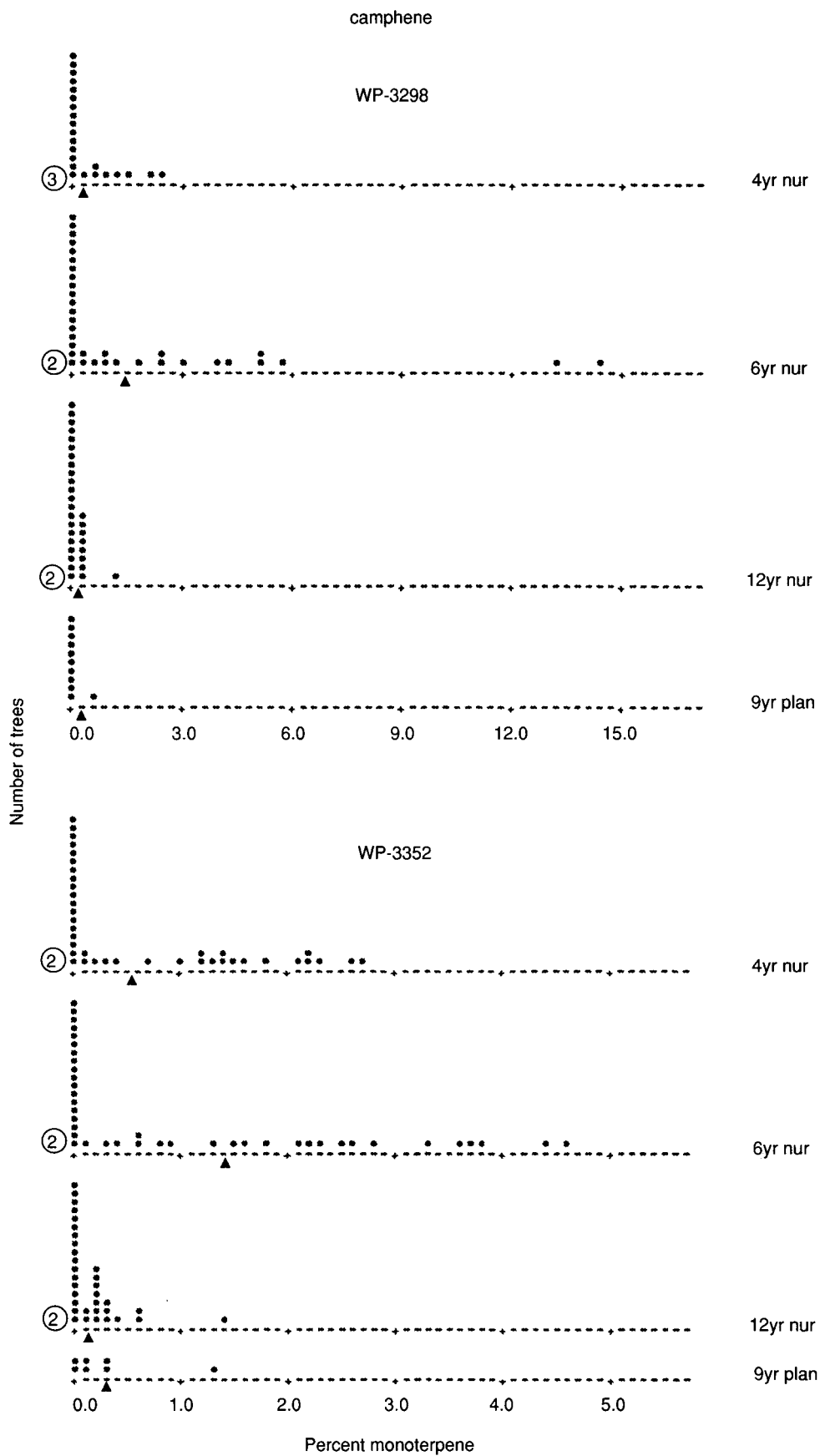
The set of plantation plants was lower in limonene and 3-carene and higher in α -pinene and β -pinene, and this may have been caused by the phytocide-related stress (*fig. 5-8*). The data from plantation trees were not used in further analyses. The effect of the phytocide-related stress was not as great as in the Adin/IFG progeny; and I attribute this to the presence of additional stress in all the Wheeler progeny caused by growing out of the seed provenance. Therefore, the added stress of the phytocide might not have been as great. Some of the fluctuation in the results could have been attributed to the large range in sample number.

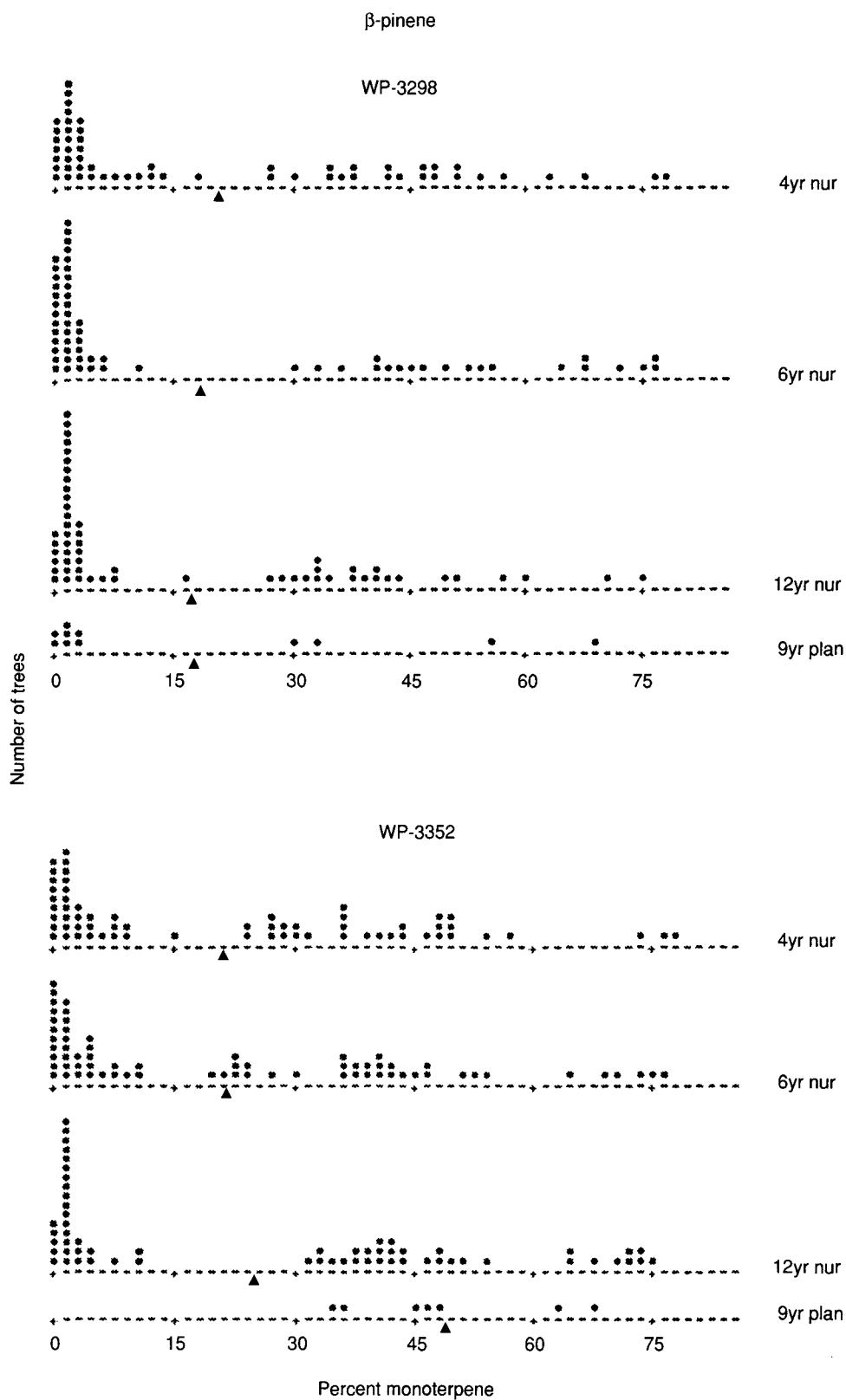
When all nurseries were aggregated into one set, omitting plantation trees, so that larger numbers of measurements were in each frequency distribution, the similar characteristics of the distributions of the progeny of the two parent trees were much clearer (*fig. 5-9*). Five of the monoterpenes had a multimodal distribution and a large range as follows (*fig. 5-9*): α -pinene, 1 to 85 percent; β -pinene and 3-carene, 0 to 75 percent; myrcene, 1 to 35 percent; limonene, 0 to 60 percent. The large number of values in the low mode of limonene, 0 to 6 percent, was puzzling. Because the female parents were high in limonene and assigned a (1-1) set of alleles, at least a moderate amount of limonene would have been expected in all progeny. It will be shown in the next section on full siblings (Chapter 6) that the progeny of high limonene x high limonene never had low limonene as expected; however, β -pinene and 3-carene were essentially lacking in such trees. Therefore, it was possible that limonene was not yet fully expressed in young progeny because of the excessive juvenile activity of the other monoterpenes, particularly β -pinene and 3-carene. There was a tendency for the frequency of low limonene trees to decrease with age (*fig. 5-8*) to give added support to the possibility that limonene was not fully expressed in young trees, but does so with age. Because α -pinene was much higher than expected in young trees, it was possible that α -pinene outcompetes limonene for the C-5 precursor in young trees.

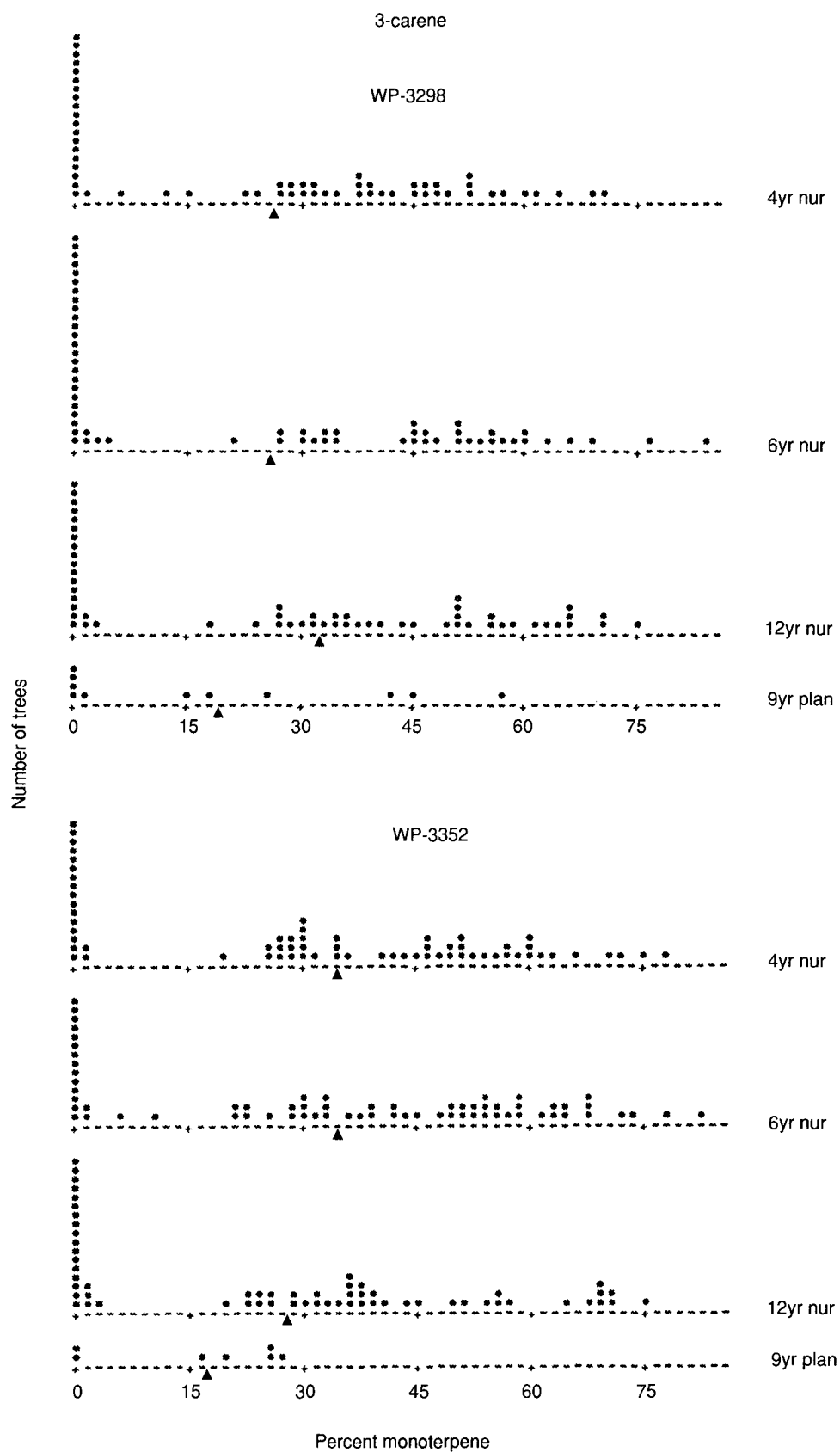
Figure 5-8—Frequency distribution of the amount of the noted monoterpenes of the wind-pollinated progeny of two *P. ponderosa* with similar composition, WP-3298 and WP-3352, with three ages of nursery growth (nur) and one of plantation growth (plan), as listed on the right; each dot is measurement of one tree; except where $\textcircled{n}$ indicates each represents n trees; [▲] is average of set.

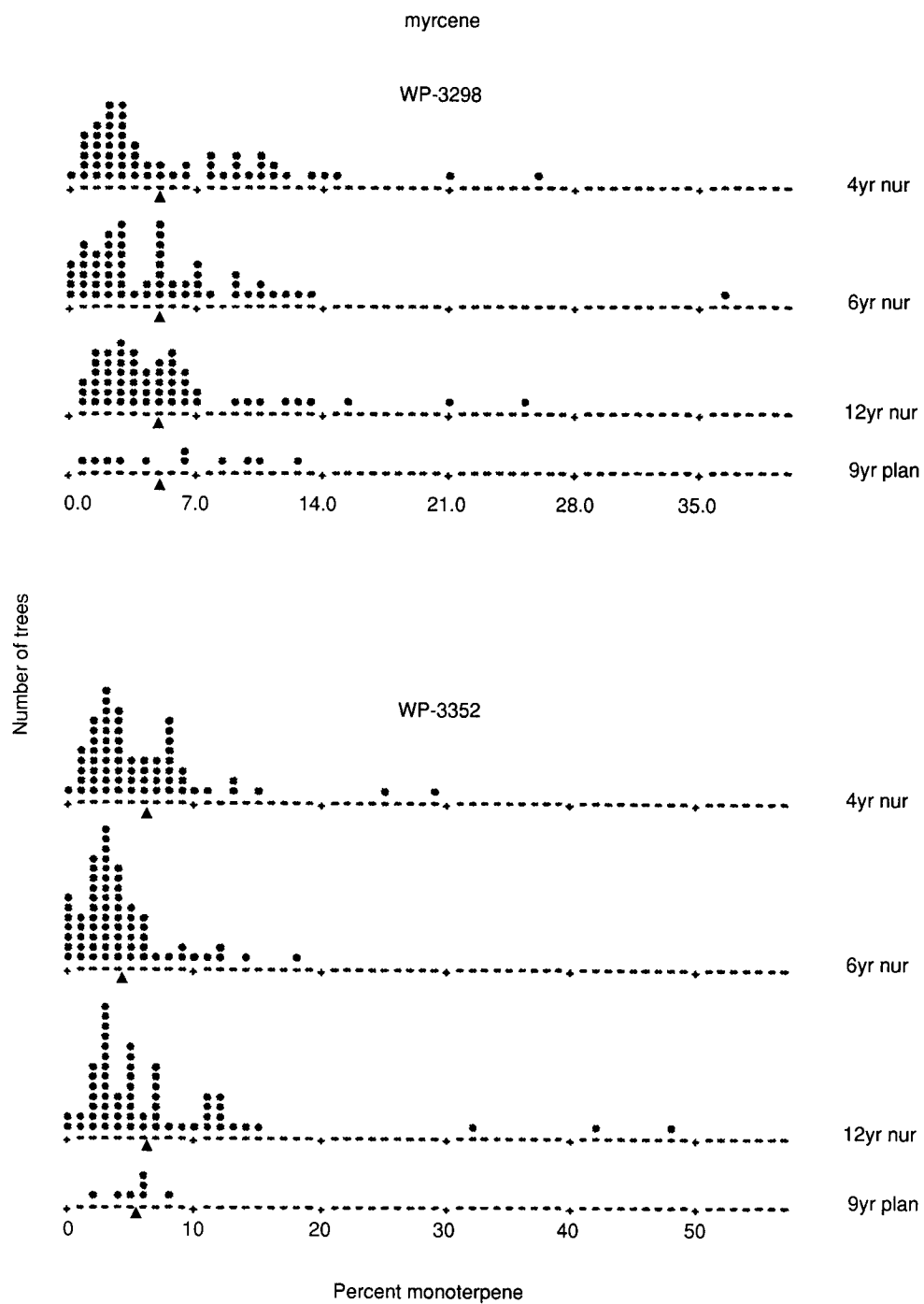
(fig 5-8 continues on next 6 pages).

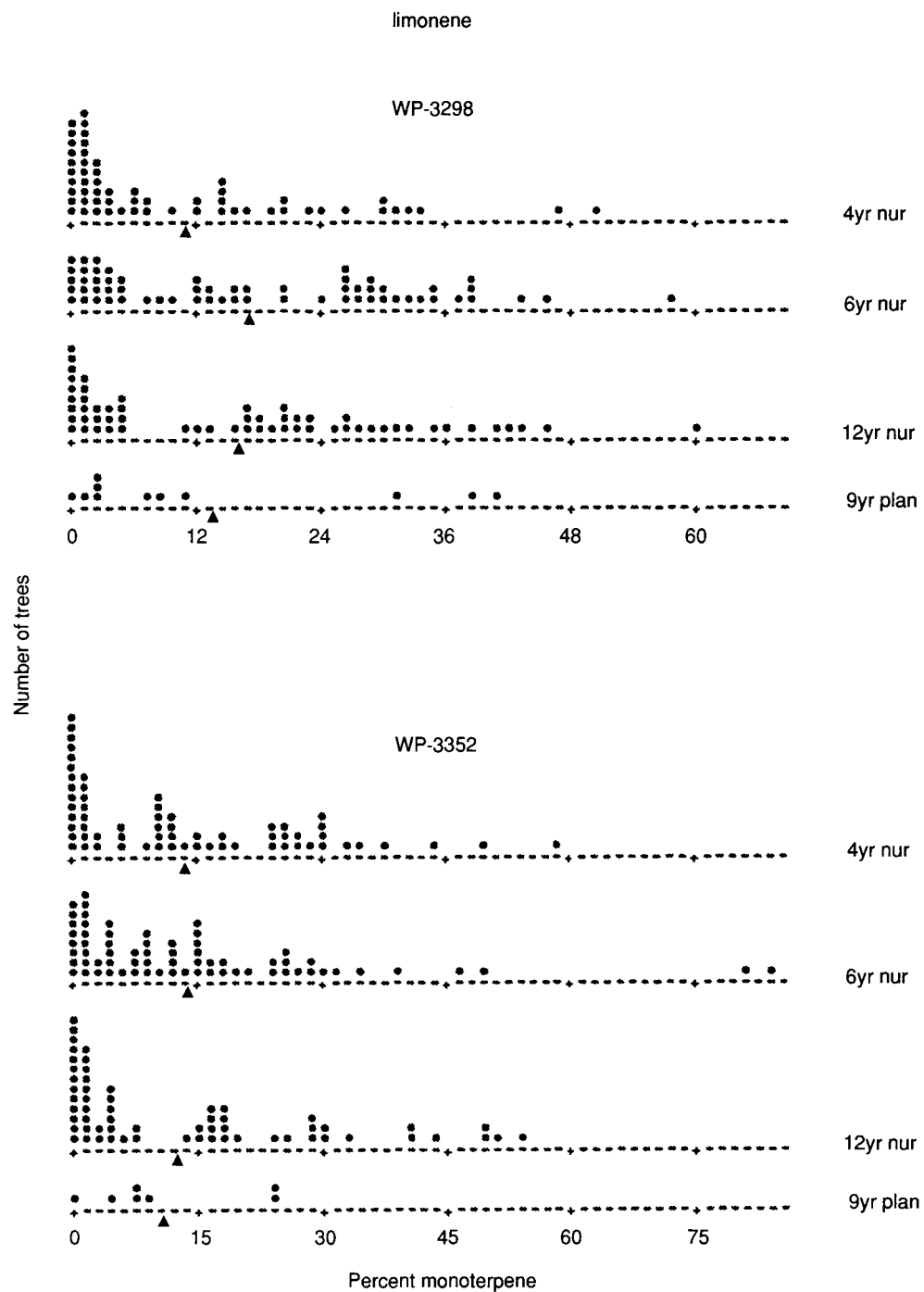












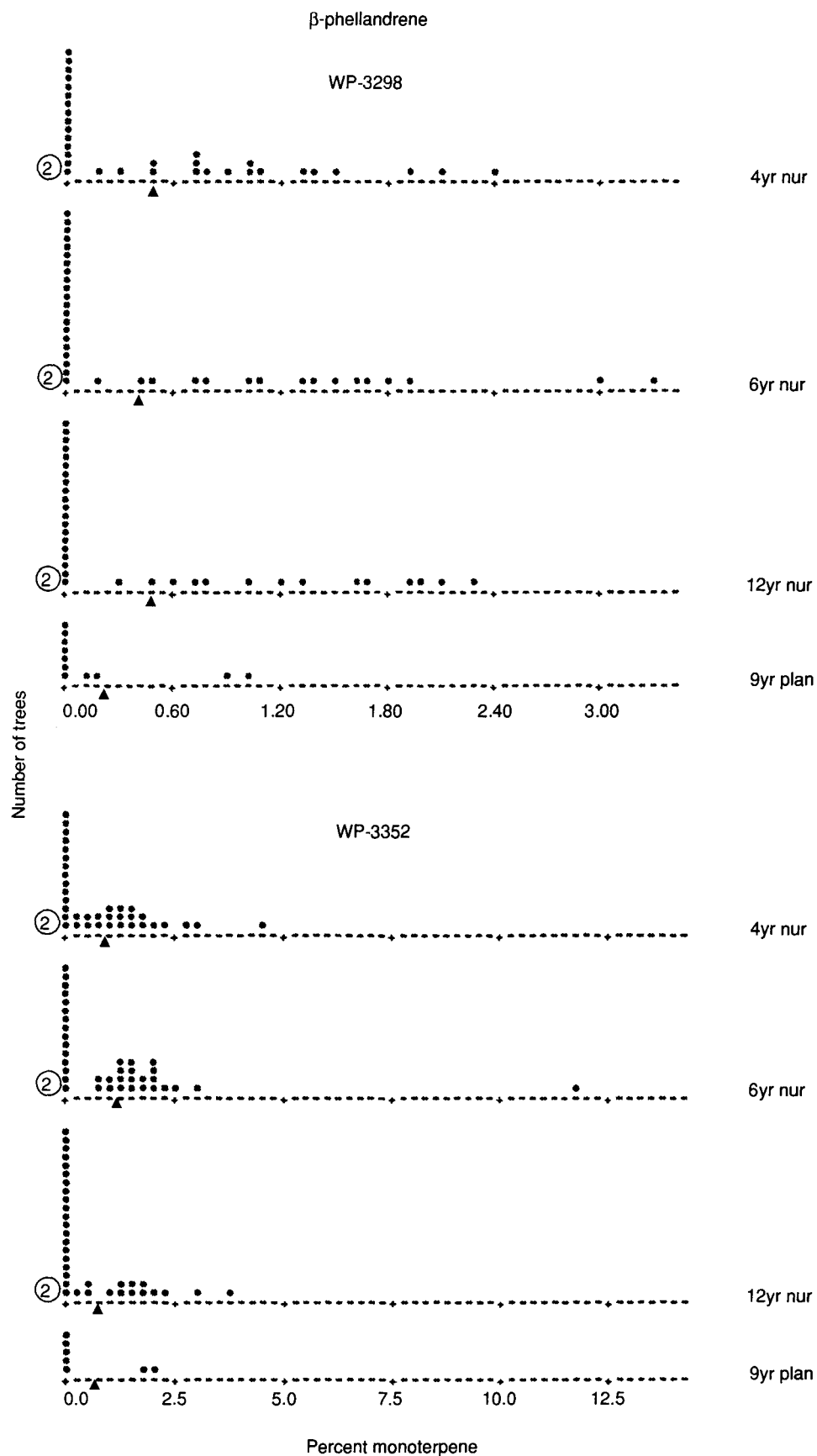
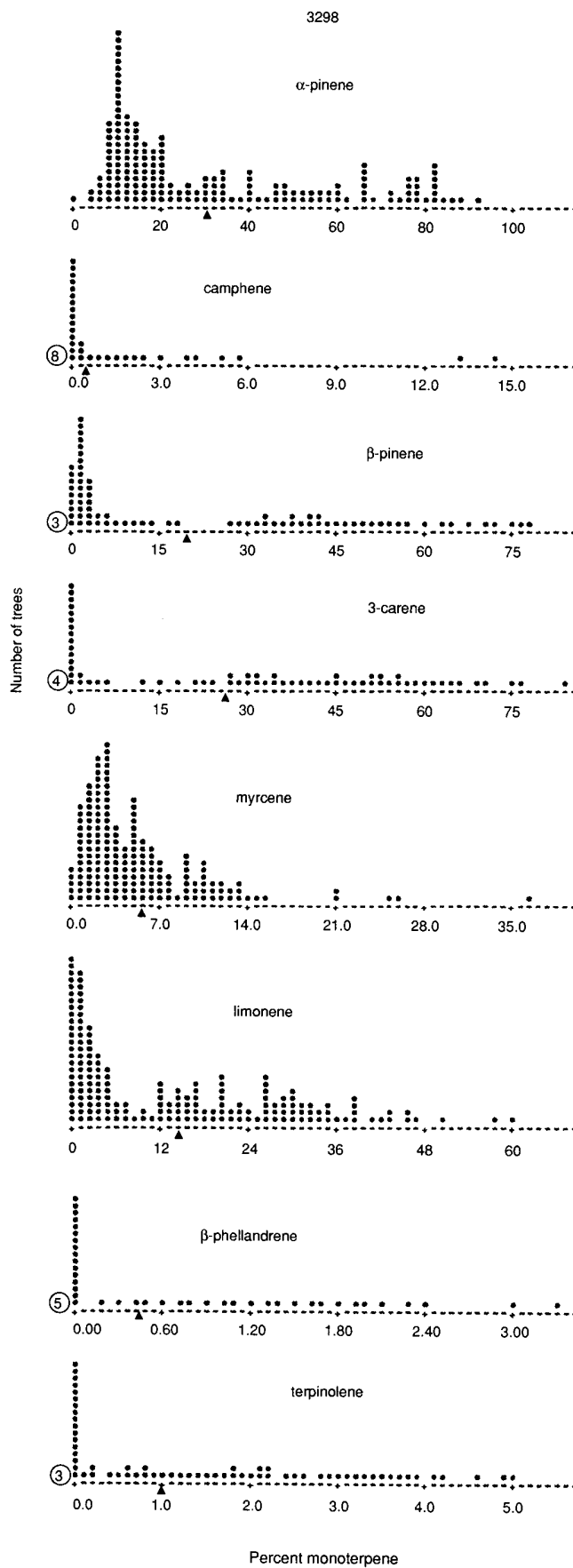


Figure 5-9—Frequency distribution of the amount of the noted monoterpenes in the progeny of two *P. ponderosa* with similar composition, WP-3298 and WP-3352, aggregated for 4 to 12 years of nursery growth; each dot is measurement of one tree except where (n) indicates each is *n* trees; [▲] is average of set.



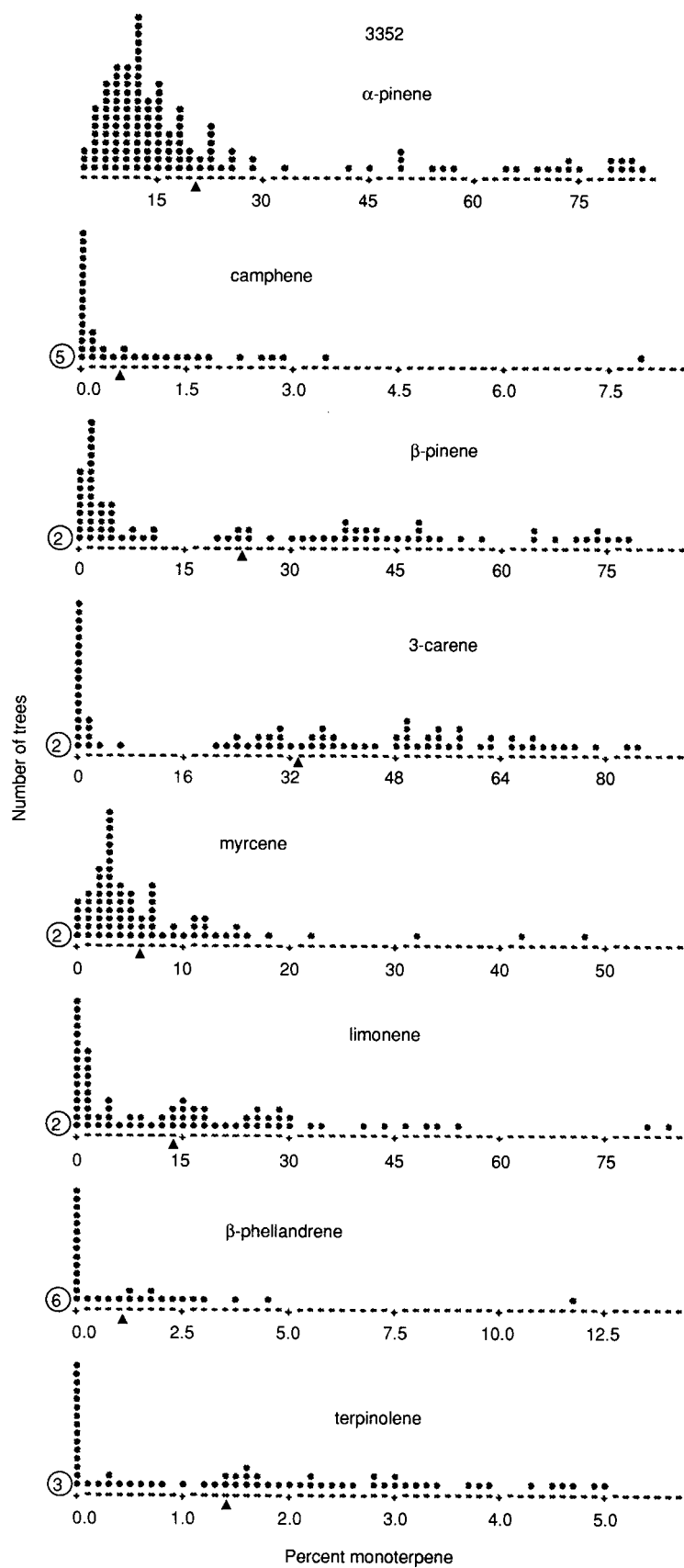


Table 5-6—Average composition of the wind-pollinated progeny of the 13 listed *P. ponderosa* at Wheeler Peak aggregated for 4 to 12 years of nursery growth; see table 5-7 for composition of female parent; * indicates a relatively high amount of the component in the female parent.

[illegible]¹ α-pinene, camphene, β-pinene, 3-carene, myrcene, limonene, β-phellandrene, terpinolene.²Normalized.

Table 5-7—Average xylem monoterpene composition of 10 sets of progeny of tree WP-3298 at Wheeler Peak with varying age of nursery growth at IFG when sampled, and one set of 9-year-old plantation-grown trees; and composition of the total sample and of the female parent (P). N = number of trees; x=average or sum().

Source ¹ and age			Monoterpenes ²							
			N	α -p	cam	β -p	car	myr	lint	β -ph
			<i>percent</i> ³							
Nur-	4	41	35.1	0.3	19.1	26.2	6.2	11.5	0.5	1.1
Nur-	5	11	31.1	*	24.1	33.9	3.2	5.3	0.5	1.6
Nur-	5	6	32.2	0.6	28.0	22.5	5.7	9.4	0.6	0.8
Nur-	6	26	27.8	3.1	22.1	27.3	5.1	12.5	0.8	1.0
Nur-	6	35	31.5	*	16.4	25.1	4.8	20.6	0.2	0.8
Nur-	9	8	40.3	0.1	19.3	16.3	4.5	17.5	0.5	0.9
Nur-	9	7	26.2	*	11.8	34.0	4.7	21.9	0.6	1.9
Nur-	10	15	32.3	0.1	9.4	41.8	5.0	8.9	0.1	2.4
Nur-	11	24	26.4	0.1	18.9	29.6	5.8	17.1	0.5 ⁴	1.6
Nur-	12	4	18.6	*	37.8	15.7	5.7	20.1	0.4	1.6
Plan-	9	11	42.6	0.1	18.0	18.6	5.9	13.2	0.2	1.2
X		(188)	31.8	0.2	18.9	27.4	5.3	14.3	0.4	1.3
P ⁴		1	25.6	0.1	0.1	0	16.1	57.9	0	0.1

¹Nur = nursery; Plan = plantation.

² α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.³Normalized; * = trace.⁴Composition of female parent, WP-3298.

Camphene was unusually high in the 6-year set of trees with an average of about 3 percent and with a few individual measurements between 4 and 6 percent. The trend to this unusually high level of camphene may have begun in the 4th- and 5th-year sets (*fig. 5-8, table 5-7*). Then, after the 6th year, camphene returned to the usual trace amount: 0.1 to 0.2 percent. This phenomenon, called the camphene anomaly, was apparent in all Wheeler Peak progeny. There was a lesser but noticeable trend toward higher camphene in the 7-year set of Ranger Peak progeny and the 5th and 9th year sets of Adin/IFG progeny. But this phenomenon was not apparent in any of the sets of full siblings, which will be discussed in Chapter 6. I postulate three explanations for this unusual phenomenon: (1) It could be a very transient and juvenile anomaly of *P. ponderosa* seedlings which may or may not be expressed. (2) It could be some other transient chemical that chromatographs as camphene. Though it checked out as camphene by internal standards, one cannot exclude some other chemical with the chromatographic properties of camphene. (3) It could be either of the two above but is expressed irregularly in different nursery beds or to other culturing practices. This apparent but small anomaly did not appreciably affect the measurements of the five major monoterpenes nor any of the analyses or conclusions.

Though there was general similarity between the progeny of WP-3352 and WP-3298 (*fig. 5-9*), as one might expect, some differences suggest that the two parent trees are not completely alike in monoterpene genetics or characteristics, or they respond slightly differently to environmental conditions. This difference was most clearly shown in α -pinene, which is much higher in the progeny of WP-3298 than in those of WP-3352, and β -pinene which was much lower in the progeny of WP-3298 than in WP-3352; but this difference could simply be the natural variation in the distribution of pollen. It is my assessment that the difference between the progeny of the two trees in the plantation was caused by differential overdosing of a phytocide. It was not possible to attribute the effect of a phytocide to individual trees. Some minor genetic structure might also cause such differences between the two sets of progeny, but such a minor structure has not been clearly shown or explained.

One might also speculate that the progeny of both WP-3298 and WP-3352 were growing far outside their seed and "resin" zones, and were under some degree of stress. Reaction to this stress was a modification of monoterpene composition and to the general stability of the system, and fluctuations will probably be more pronounced and variable in an unstable system.

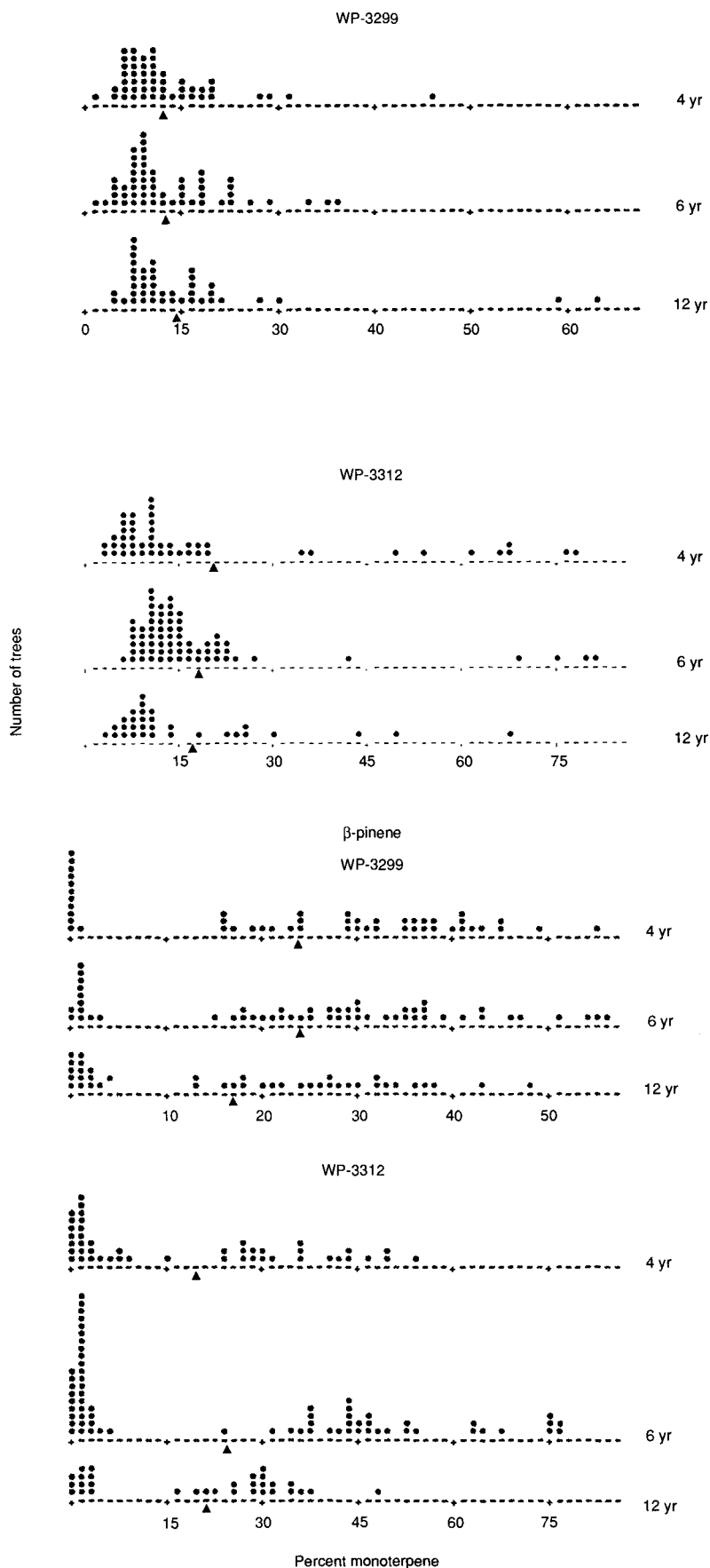
Types of composition will be discussed in a section at the end of this section on Wheeler Peak.

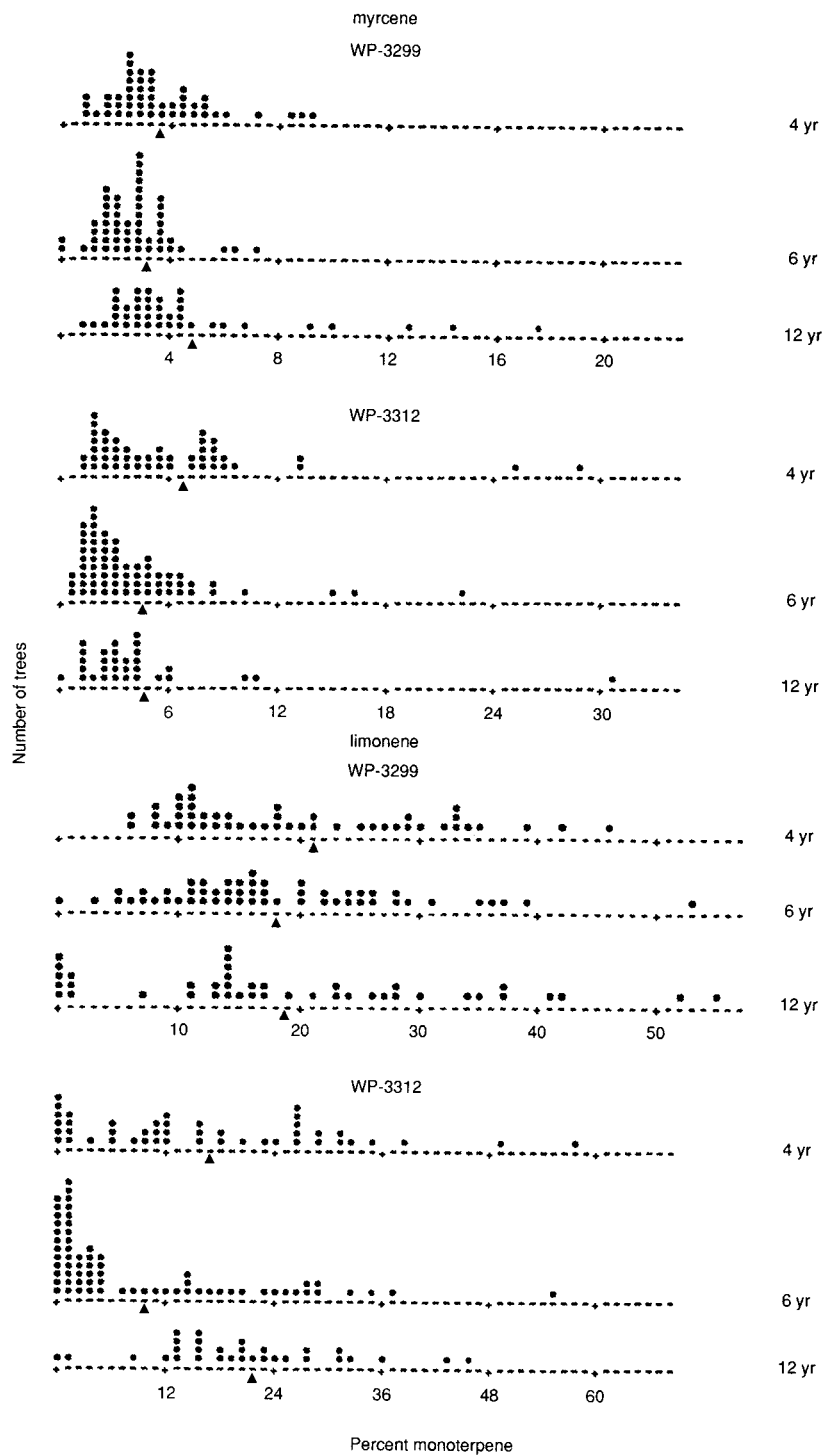
From these results the structure of alleles for both trees was tentatively placed at (0-1), (0-0), (0-0), (0-0), and (1-1), respectively, for α -pinene, β -pinene, 3-carene, myrcene, and limonene. The assignment of alleles will be developed further in Chapter 6

Trees WP-3299 and WP-3312

Each tree was near-high limonene, 40 to 45 percent, without β -pinene, but with high 3-carene, 34 to 43 percent (*table 5-5*). Six sets of nursery-grown trees were examined with ages varying from 4 to 12 years; there were no plantation sets. The average composition of the progeny varied slightly from set to set; but when the data were aggregated into three age classes with larger numbers, variation was greatly reduced (*fig. 5-10*), though β -pinene and limonene varied much more than in the progeny of the previous two trees, WP-3298 and WP-3352. The amount of α -pinene was considerably lower than in the progeny of the two previous trees, reflecting a much lower α -pinene in the female parents, and suggesting a (0-0) set of alleles for α -pinene in both trees: WP-3299 and WP-3312. The level of limonene was nearly the same in the progeny of all four

Figure 5-10—Frequency distribution of the amount of the noted monoterpenes of the progeny of two *P. ponderosa* with similar composition, WP-3299 and WP-3312, at three ages of nursery growth, listed on the right; each dot is measurement of one tree; [▲] is average of set.





trees, WP-3298, WP-3352, WP-3299, and WP-3312, despite a much lower limonene level in the WP-3299 and WP-3312 parents. It would appear that the genetic level of limonene was the same in all four parent trees, but the competitive presence of at least one allele for 3-carene in WP-3299 and WP-3312 resulted in a lesser amount of limonene in the WP-3299 and WP-3312 parents. The absence of a mode near zero for 3-carene in the progeny of WP-3299 suggested a (1-1) set of alleles in the parent tree; that is, all progeny of WP-3299 would have at least one allele for 3-carene and therefore no mode near zero. The progeny of WP-3312 had a mode for 3-carene near zero, though it was quite small, and the ratio of the size of the modes did not rule out a (0-1) set of alleles in WP-3312. Again, each age set is a different set of trees from the same seed lot.

Again, as with the progeny of trees WP-3298 and WP-3352, there was considerable range in the frequency distribution of components, but the same generalized pattern seemed to prevail when the samples were aggregated into three time sets (*fig. 5-10*), and still further into one set of all ages (*fig. 5-11*). There were some small differences; the progeny of WP-3299 had a smaller mode near 0 percent for limonene and 3-carene. The camphene anomaly was also present in the 6-year set of progeny; this was discussed under WP-3298 and WP-3352. The multimodal character of the distributions of the five major components was clearly evident. The large mode for limonene near zero could suggest a (0-1) set of alleles for limonene in the female parents. But the breeding studies reported in the next chapter clearly show that both trees have a (1-1) set. As noted before, the mode near zero was probably caused by the juvenile factor.

Trees WP-3320 and WP-3294

These two trees were somewhat similar to WP-3299 and WP-3312, except that WP-3294 and WP-3320 were lower in limonene, *ca.* 27 vs. 40 percent, and higher in 3-carene, *ca.* 58 vs. 36 percent (*table 5-5*). A total of 80-85 nursery-grown progeny of each tree was examined; the averages for the two sets were very similar. Quite different from the progeny of the previous two sets of progeny, the average amounts of α -pinene, β -pinene and limonene in the progeny of WP-3320 varied much less over time (*fig. 5-12*). There was some slight shifting over time in the progeny of WP-3294 (*table 5-8*).

The frequency distributions of both sets of progeny had about the same characteristics when all trees were aggregated into one class (*fig. 5-13*). There was one small puzzling difference between the two distributions. The progeny of WP-3320 had no mode near zero for 3-carene, a condition one would expect with an assignment of a (1-1) set of alleles for the 56 percent in the parent tree. On the other hand, three of the progeny of WP-3294 were at zero where one would expect none, because again one would be inclined to assign a (1-1) set of alleles for the 61 percent. If, however, a (0-1) set is assigned to WP-3320, a much more substantial mode than the one found would be expected at 0. The 0 percent for three of the progeny of WP-3320 could be caused by: (1) rogues, (2) some procedural error, (3) or another effect of the juvenile factor. But the evidence still seemed to justify assigning the parents a (1-1) set of alleles for 3-carene. The camphene anomaly was present in the progeny of both parents. The frequency distribution of limonene suggested a (0-1) set of alleles in each parent. Neither tree was used in controlled breeding, which could have resolved the question. The frequency distributions for β -pinene were nearly identical for the progeny of both trees with a large mode near zero and 2 to 3 other modes at about 18 percent and more than 45 percent. One would have to assign a (0-0) set of alleles for β -pinene in both parent trees; and the zero mode supported this. The larger-than-expected values for the remainder of the distribution was attributed to the juvenile factor. Apart from these rather small differences, the frequency distributions were quite similar.

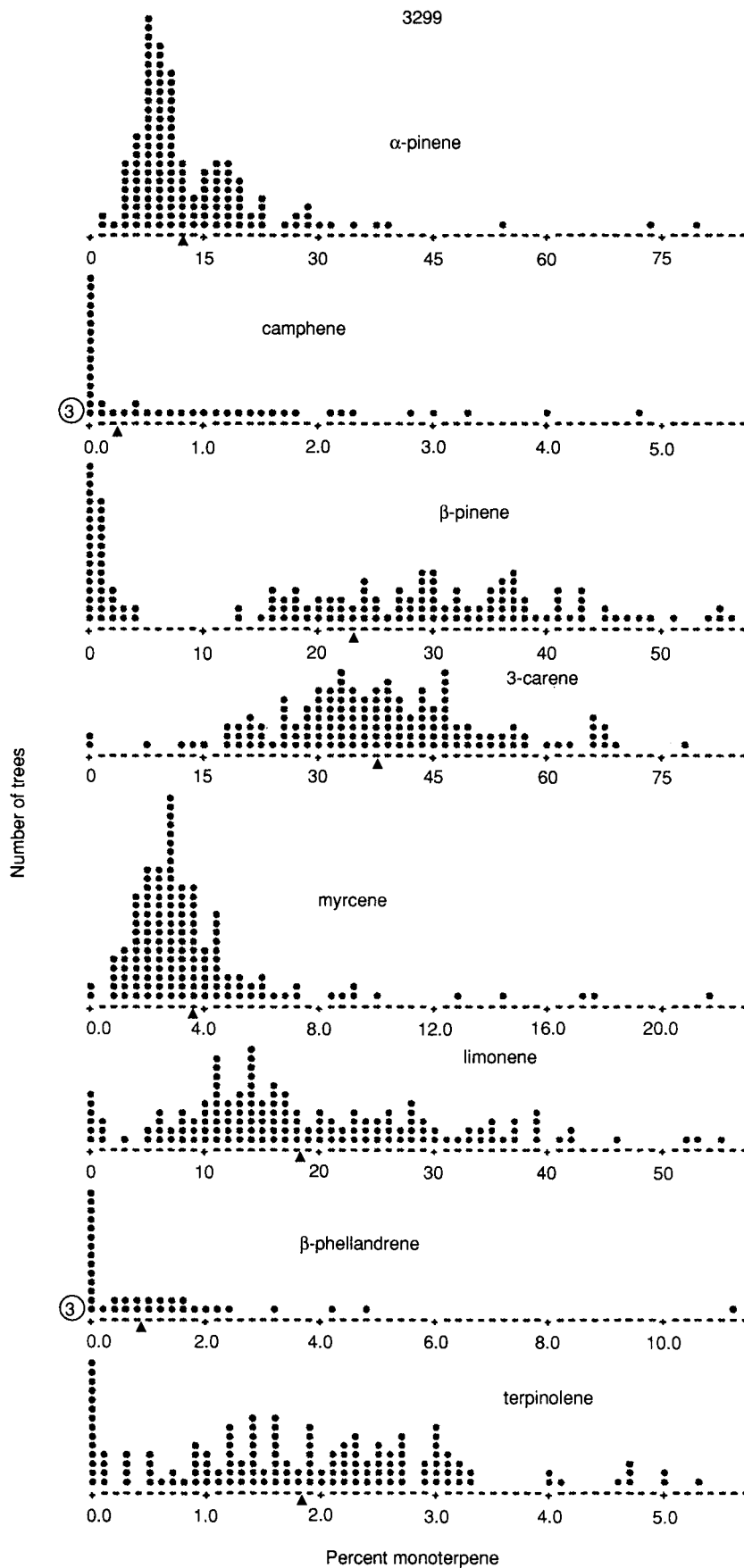
Table 5-8—Average xylem monoterpene composition of wind-pollinated progeny of tree WP-3294 for 4-12 years of nursery growth at IFG; and average composition of total sample and of the female parent (P). N = number of trees.

Years in nursery	N	Monoterpene ¹							
		α -p	cam	β -p	car	my	lim	β -ph	ter
		percent ²							
4	7	11.1	0.2	26.1	44.1	4.5	10.4	0.8	2.3
6	29	12.3	0.1	29.4	37.6	2.9	14.8	0.5	1.9
9	8	10.8	0.1	49.7	33.7	2.8	1.9	0.3	0.7
11	27	17.3	0.2	19.1	41.8	5.7	12.8	0.6	2.3
12	8	8.7	0.1	25.2	47.8	4.8	9.6	0.8	3.0
X	(Σ 79)	13.4	0.1	27.4	40.2	4.2	11.9	0.6	2.1
p ³	1	4.6	0	5.0	55.8	5.0	26.1	0.7	2.8

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.²Normalized.³Composition of female parent, WP-3294.

Figure 5-11—Comparative frequency distribution of the amount of the noted monoterpenes in 4- to 12-year-old half-sibling progeny of two *P. ponderosa*, WP-3299 and WP-3312, aggregated for 4 to 12 years of nursery growth; each dot is measurement of one tree; where (n) indicates each is *n* trees; [▲] is average of set.

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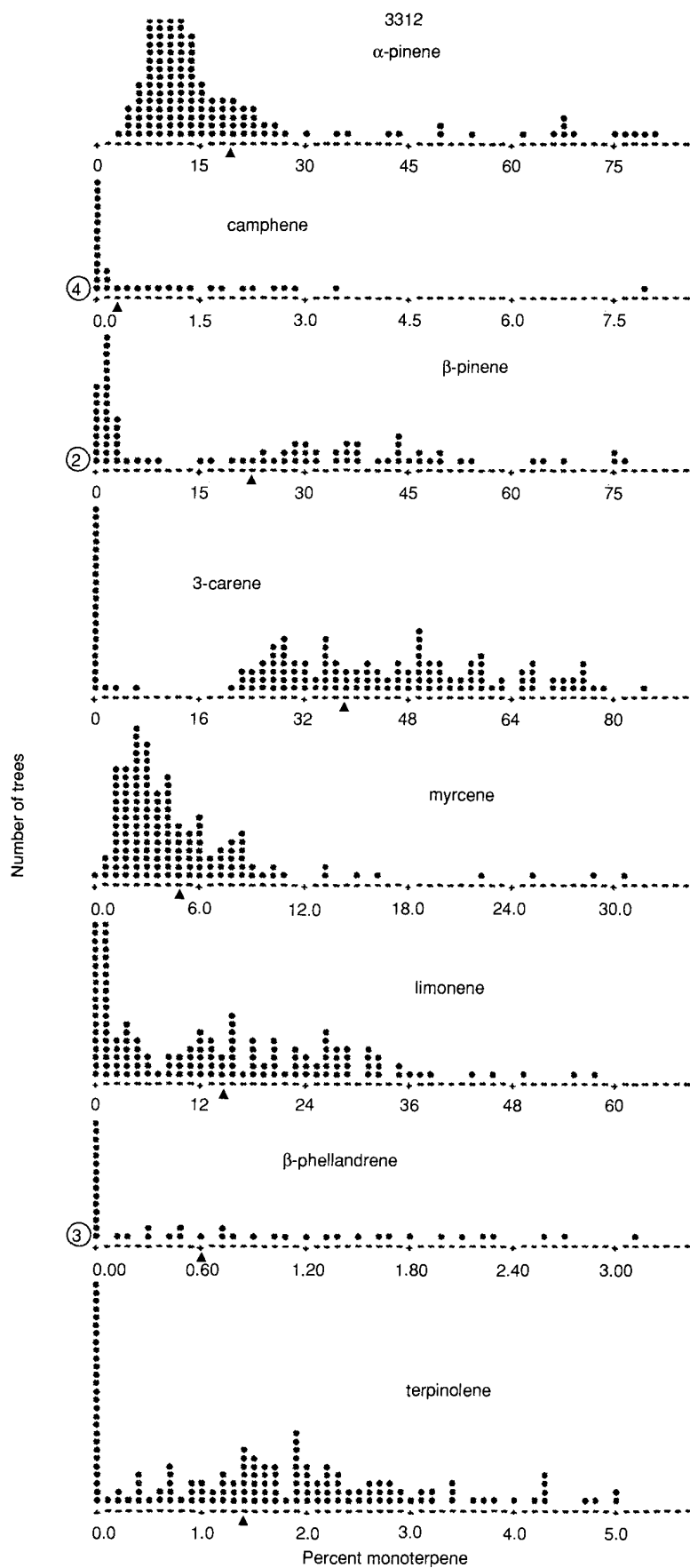
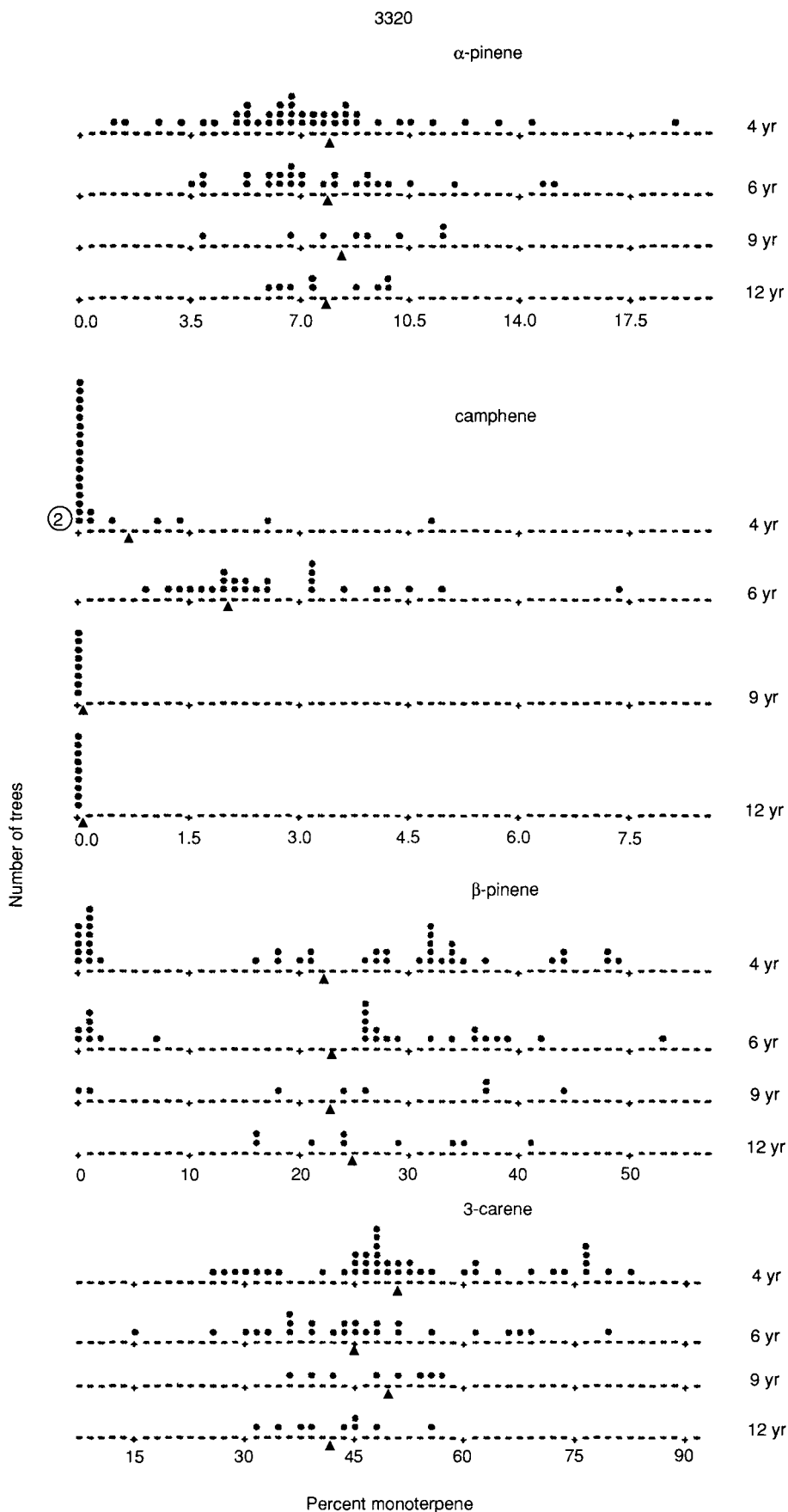
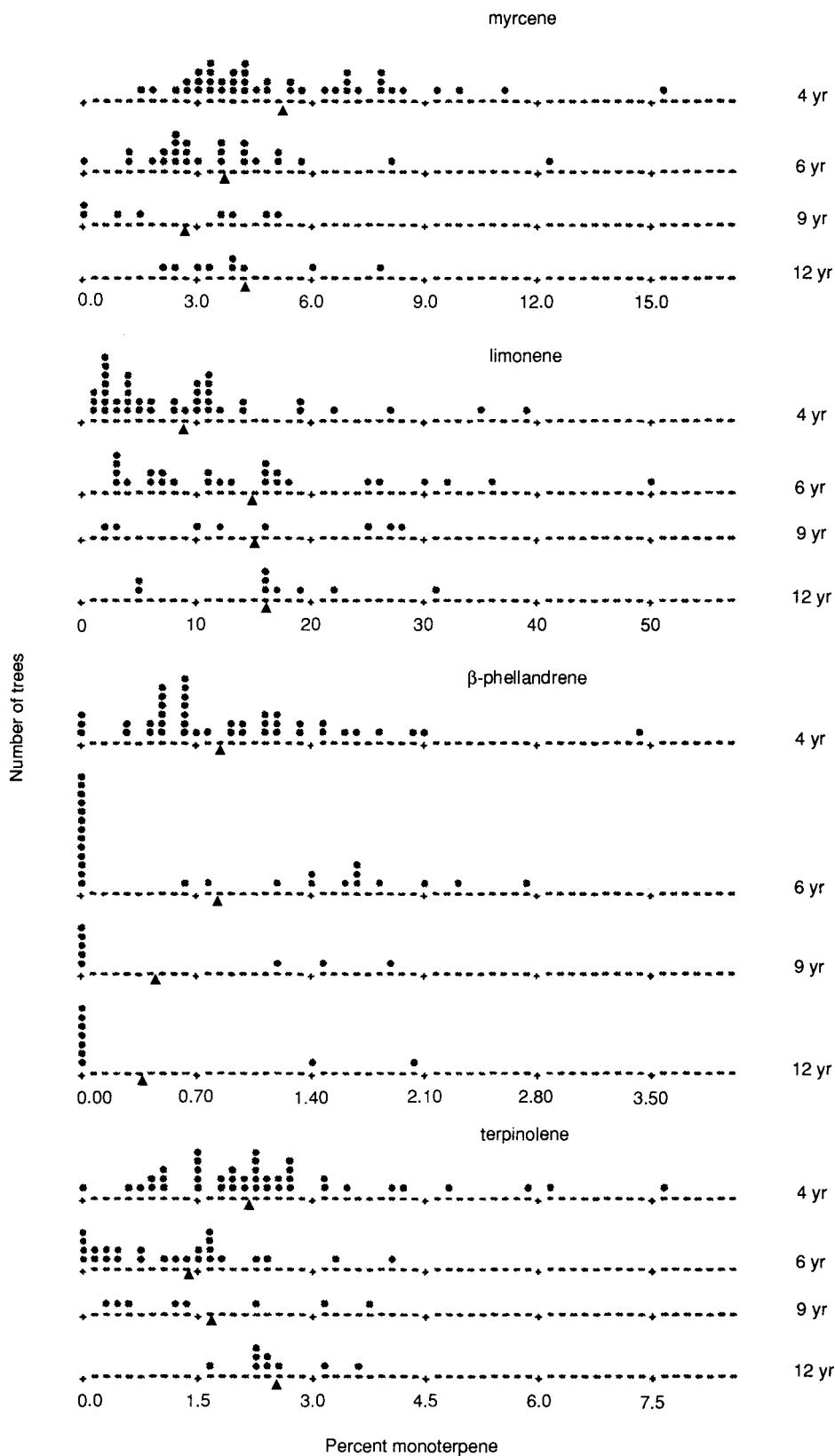


Figure 5-12—Frequency distribution of the amount of the noted monoterpenes of the progeny of *P. ponderosa* WP-3320 with four periods of nursery growth, listed on the right; each dot is measurement of one tree except where (n) indicates each is *n* trees; [▲] is average of set.

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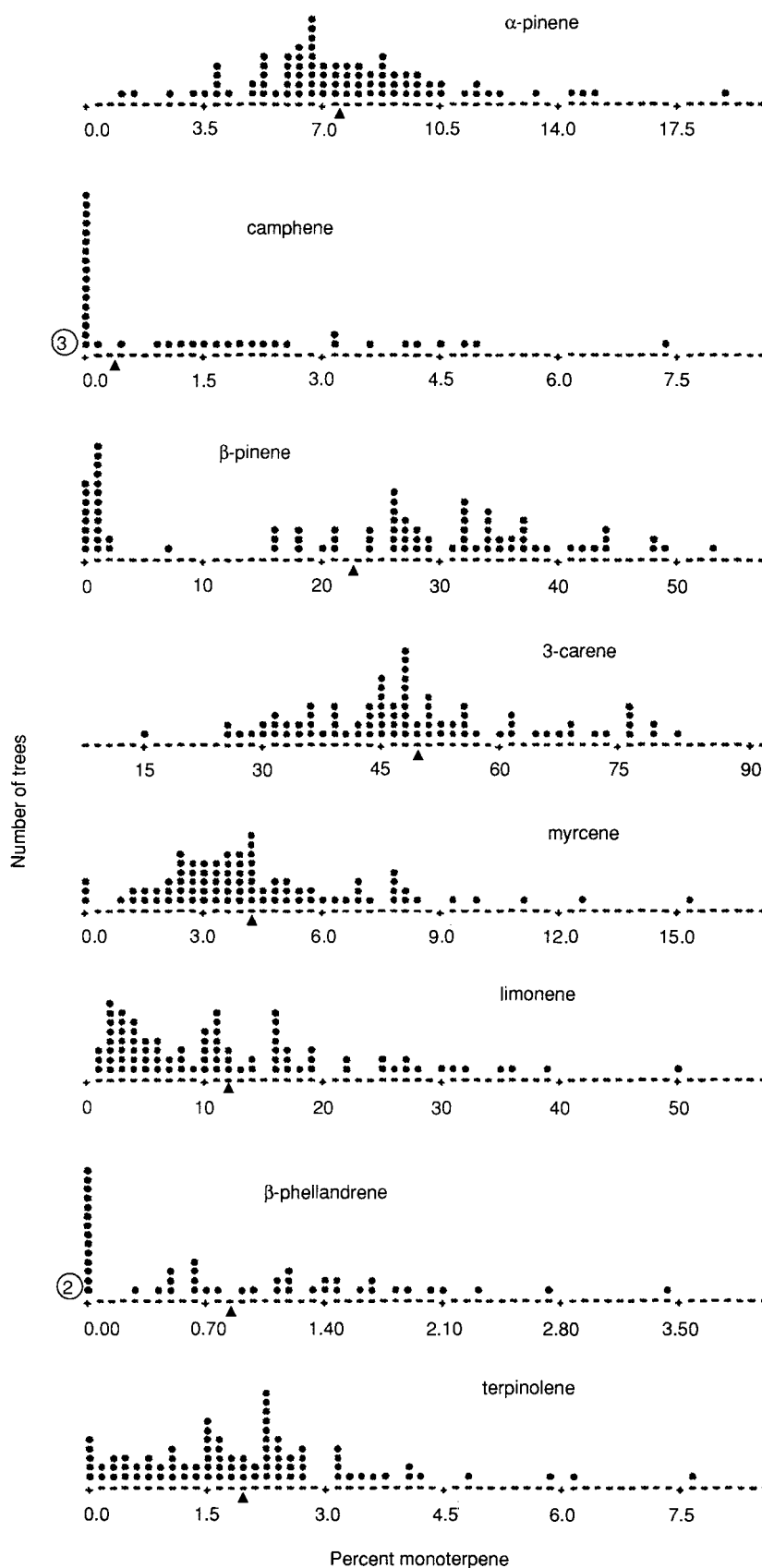


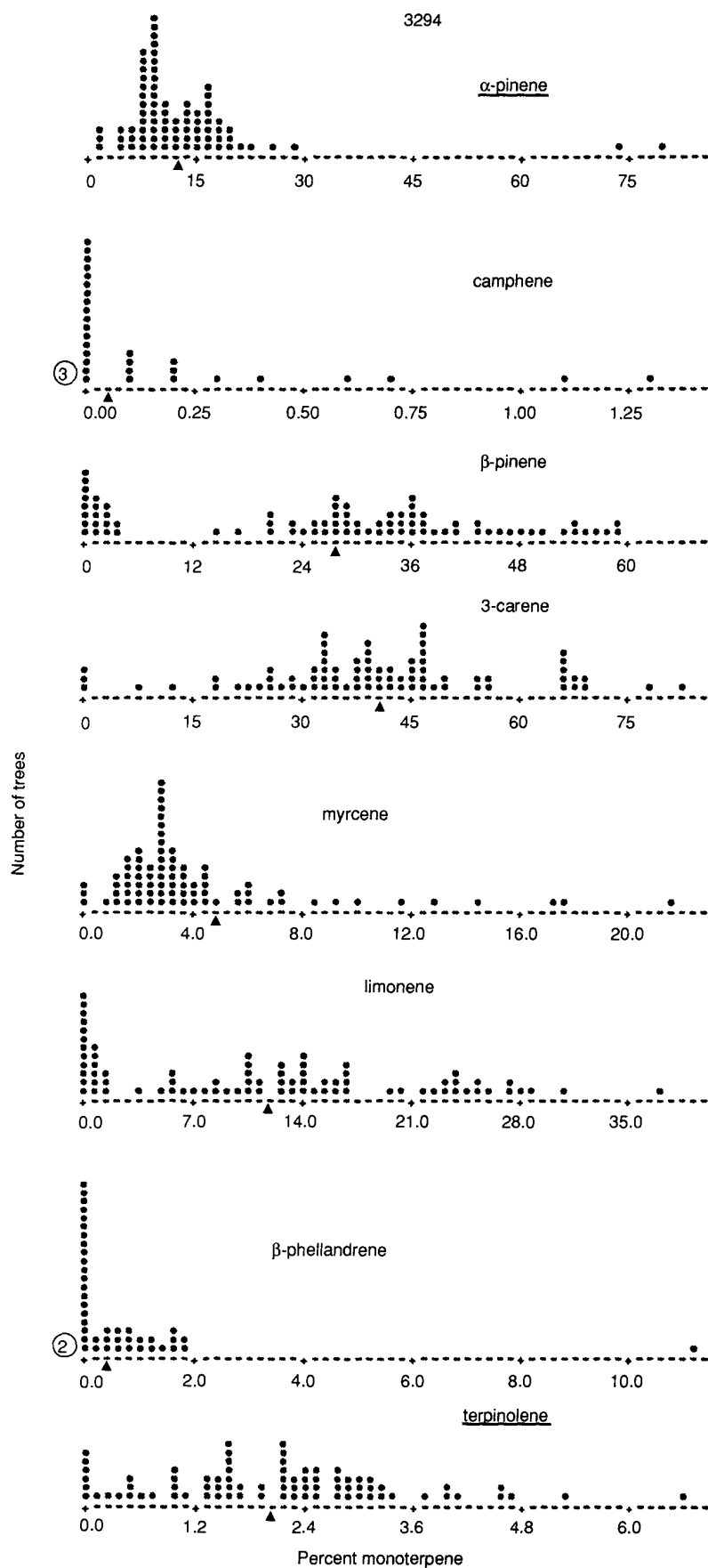
3320



3320

Figure 5-13—Comparative frequency distribution of the amount of the noted monoterpenes of the aggregated 4- to 12-year-old progeny of two *P. ponderosa* with similar composition, WP-3320 and WP-3394; each dot is measurement of one tree except where (n) indicates each is *n* trees; [▲] is average of set.





Trees WP-3293 and WP-B477

The parent trees had nearly identical compositions (*table 5-5*): very high 3-carene at about 65 percent with small amounts of α -pinene, β -pinene, and myrcene, and with very small amounts of limonene. The averages for the two sets were nearly identical. There were insufficient data for a clear assessment of time of growth of nursery-grown progeny, but the evidence with other sets of progeny suggested that they could be aggregated into one set (*fig. 5-14*). There were zero or near-zero modes for α -pinene, β -pinene, myrcene, and limonene, and there was no zero mode for 3-carene with all the values above 20 percent which would be expected from a (1-1) set of alleles. All limonene in both sets of progeny was well below 15 percent, with about three quarters of the values near zero. Some values above 20 percent would have been expected for limonene when the pollen background was considered; that is, one would have expected an allele for limonene in some of the progeny. Again this could be an effect of the juvenile factor; i.e., in young trees the alleles for limonene did not function, functioned incompletely, or were outcompeted by β -pinene or 3-carene. The data suggested a (0-1) set of alleles for β -pinene and limonene, and a (0-0) set for α -pinene and myrcene, in addition to the (1-1) set of alleles for 3-carene in both parents. Neither parent was used in controlled breeding to check out these assignments.

**Comparison of Progeny of Parents With Dissimilar Composition
Trees WP-3301 and WP-B475**

The two trees differed, respectively, as follows for WP-3301 and WP-B475: β -pinene, 1 percent vs. 65 percent; 3-carene, 69 percent vs. 0 percent; α -pinene, 7 percent vs. 22 percent (*table 5-5*). Both parent trees were very low in limonene at about 1 percent. There was a bit more shifting overall in composition over time, but this may be a reflection of inadequate sampling for some periods (*fig. 5-15*).

The difference between the two parents was clearly shown by the average composition of their progeny and by the characteristics of the frequency distributions of β -pinene and 3-carene (*table 5-6, fig. 5-16*). For β -pinene the progeny of WP-3301 had a large mode near zero with a few scattered measurements up to about 75 percent and an overall average of about 23 percent. For β -pinene, the progeny of WP-B475 had just a few measurements near zero with three distinct modes between about 39 percent to about 80 percent with an overall average of 44 percent. For 3-carene the reverse was true. The progeny of WP-3301 had a mode at 0 percent and three modes between 15 percent to 85 percent and an average of 45 percent, whereas the progeny of WP-B475 had a mode at 0 percent with a few scattered measurements between 12 percent and 60 percent and an average of 15 percent. Both sets had the camphene anomaly at 6 years. The presence of a small zero mode for 3-carene in the progeny of WP-3301, where none would be expected, and for β -pinene in the progeny of WP-B475 might be explained by the action of the juvenile factor or by isomerization. There could be a trend in the age series of limonene (*fig. 5-15*) toward a smaller number of trees at or near 0 percent with increased age, though the sample numbers were small. This could mean that the juvenile effect is decreasing with a resulting increase in limonene.

The characteristics of the frequency distribution for limonene were quite similar for both the time of growth sets (*fig. 5-15*) and for the aggregate sets (*fig. 5-16*): one mode at or near 0 percent with other measurements extending from about 8 percent to about 25 percent but with a couple of measurements near 40 percent. With a (0-0) set of alleles for limonene in the parents, one would expect a mode at or near 0 percent and a second mode around 20 and 30 percent, reflecting some alleles for limonene in the wind pollen source. The failure of the second mode to be larger was attributed to the juvenile effect. This same

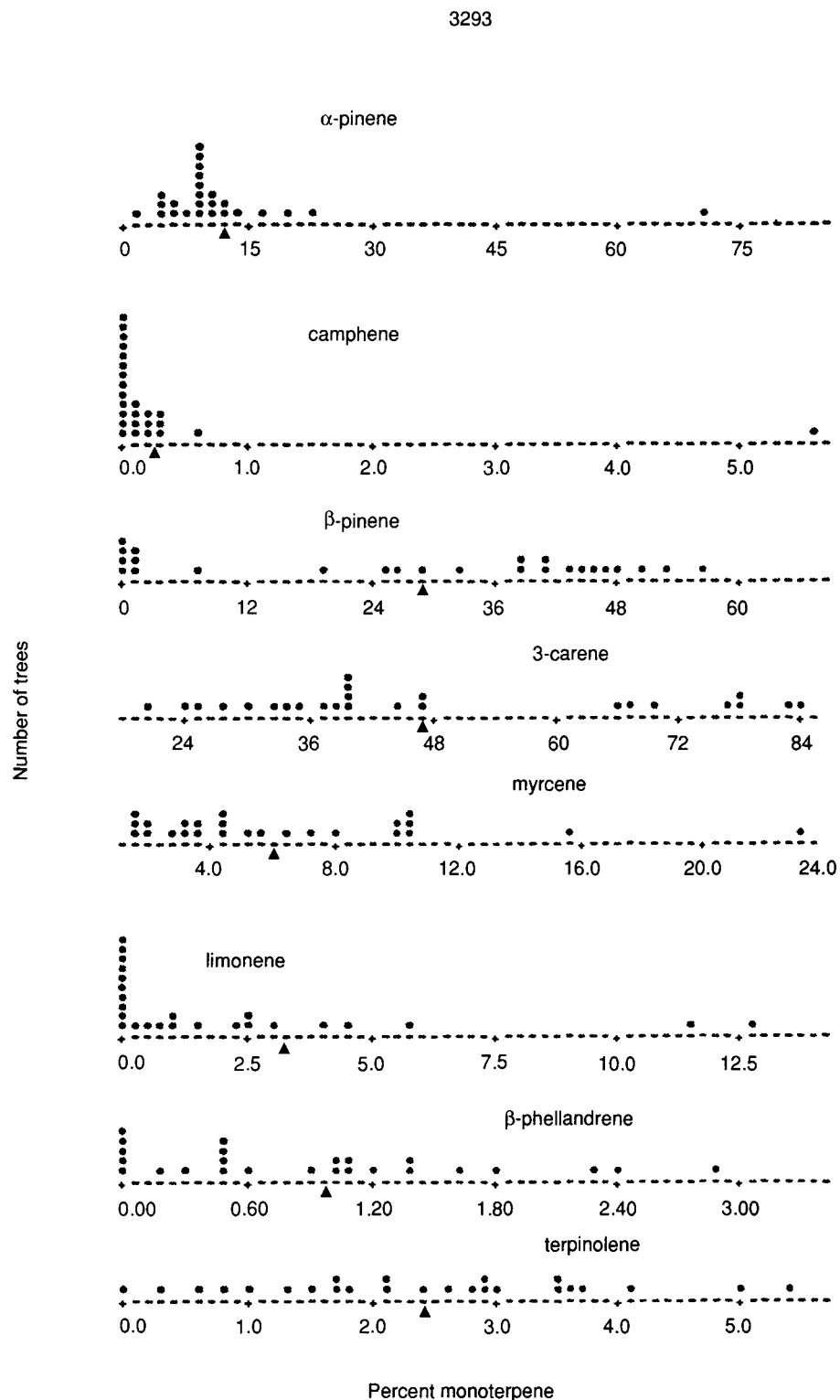
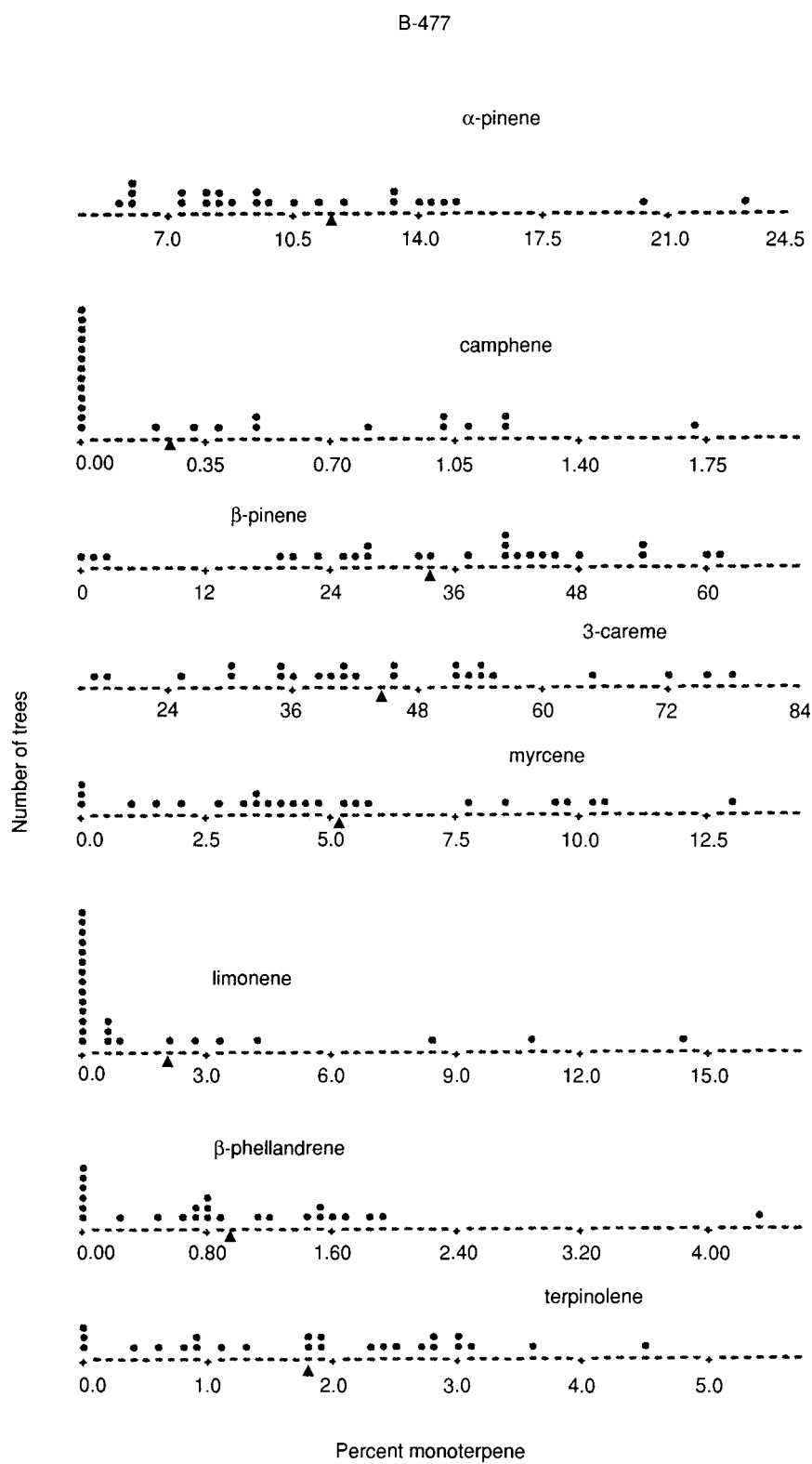


Figure 5-14—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of two *P. ponderosa* with similar composition, WP-3293 and WP-B477, aggregated for 4 to 11 years of nursery growth and 4 years of container growth; each dot is measurement of one tree; [s] is average of set.



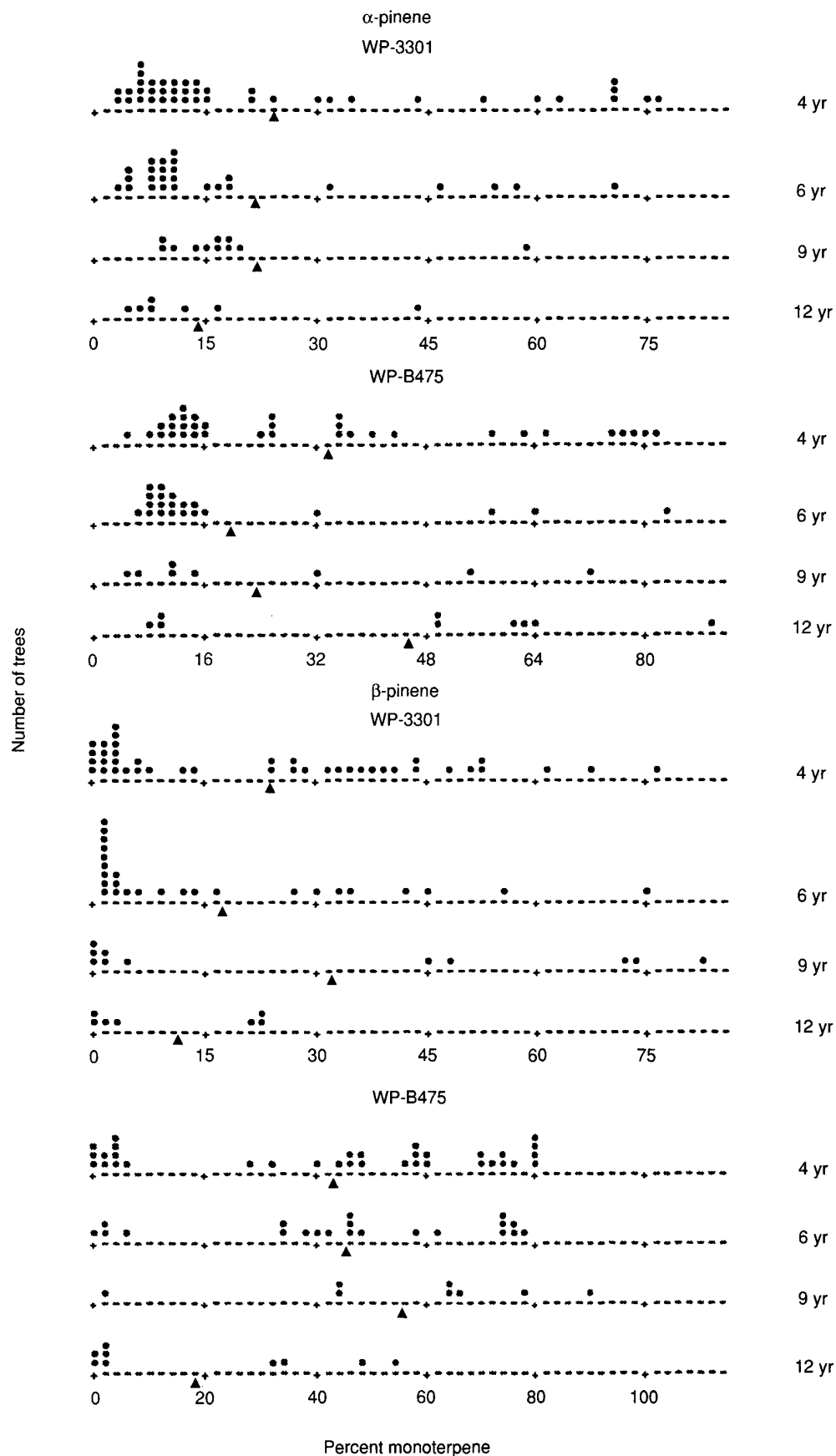
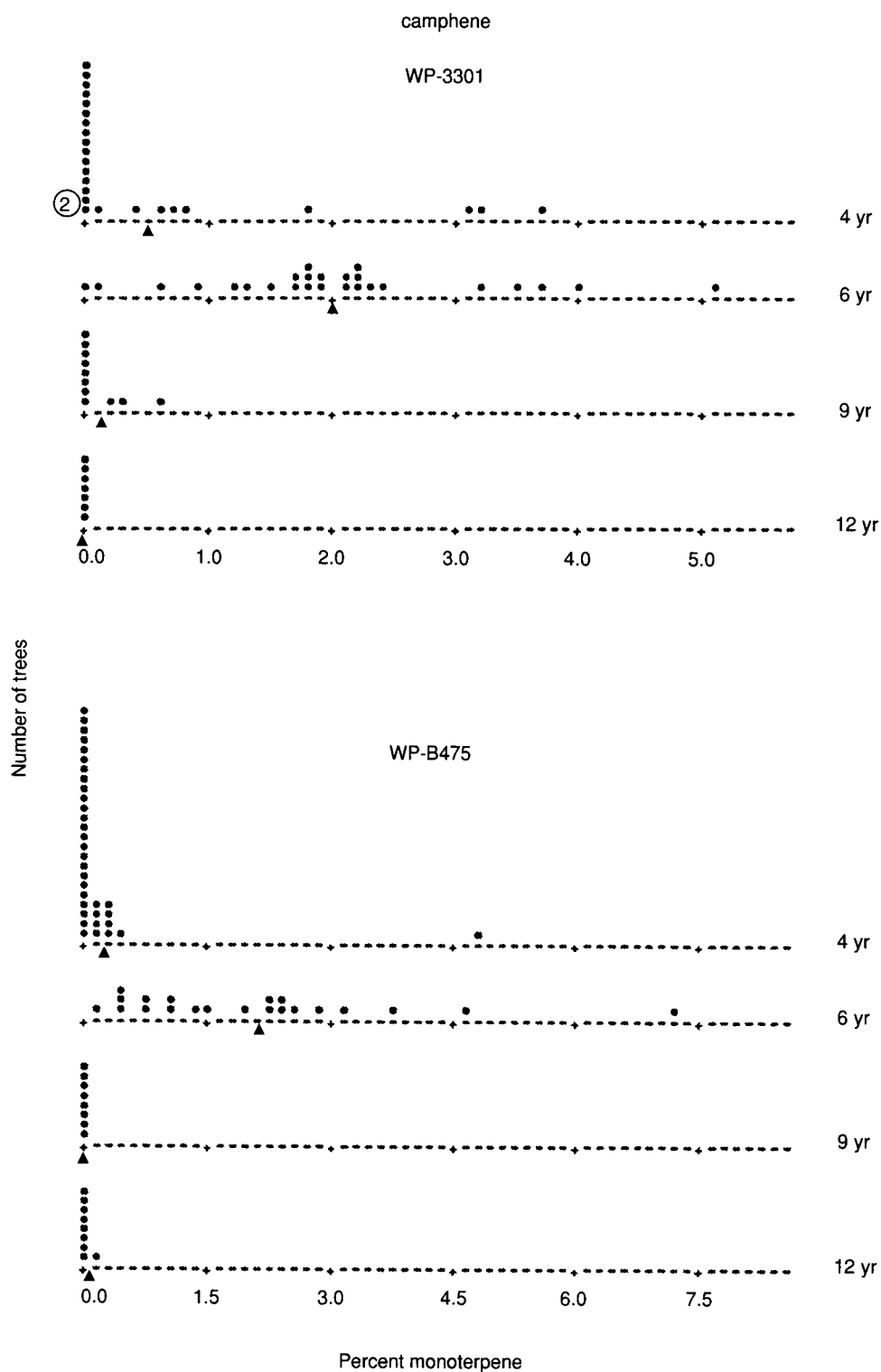
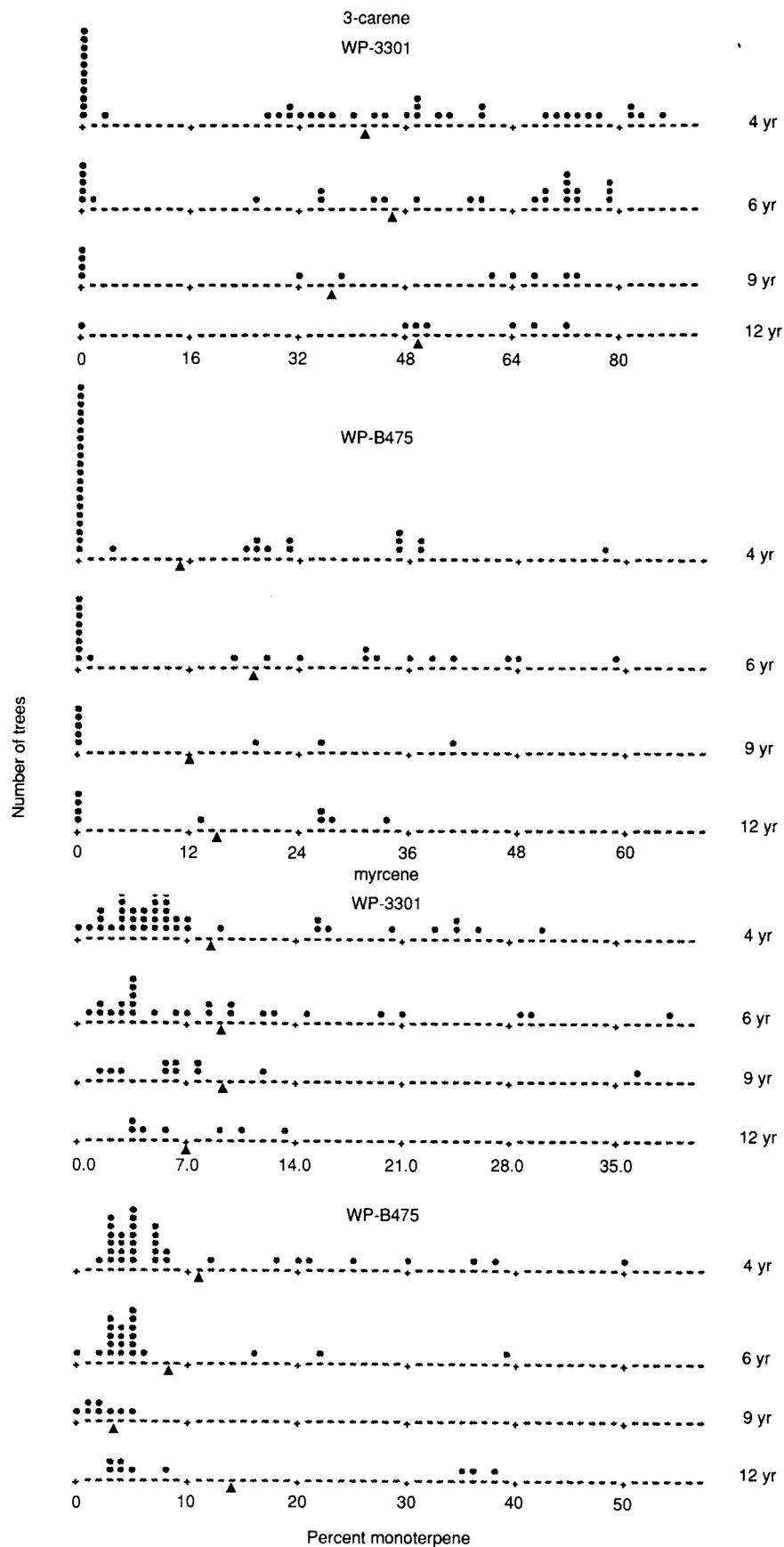
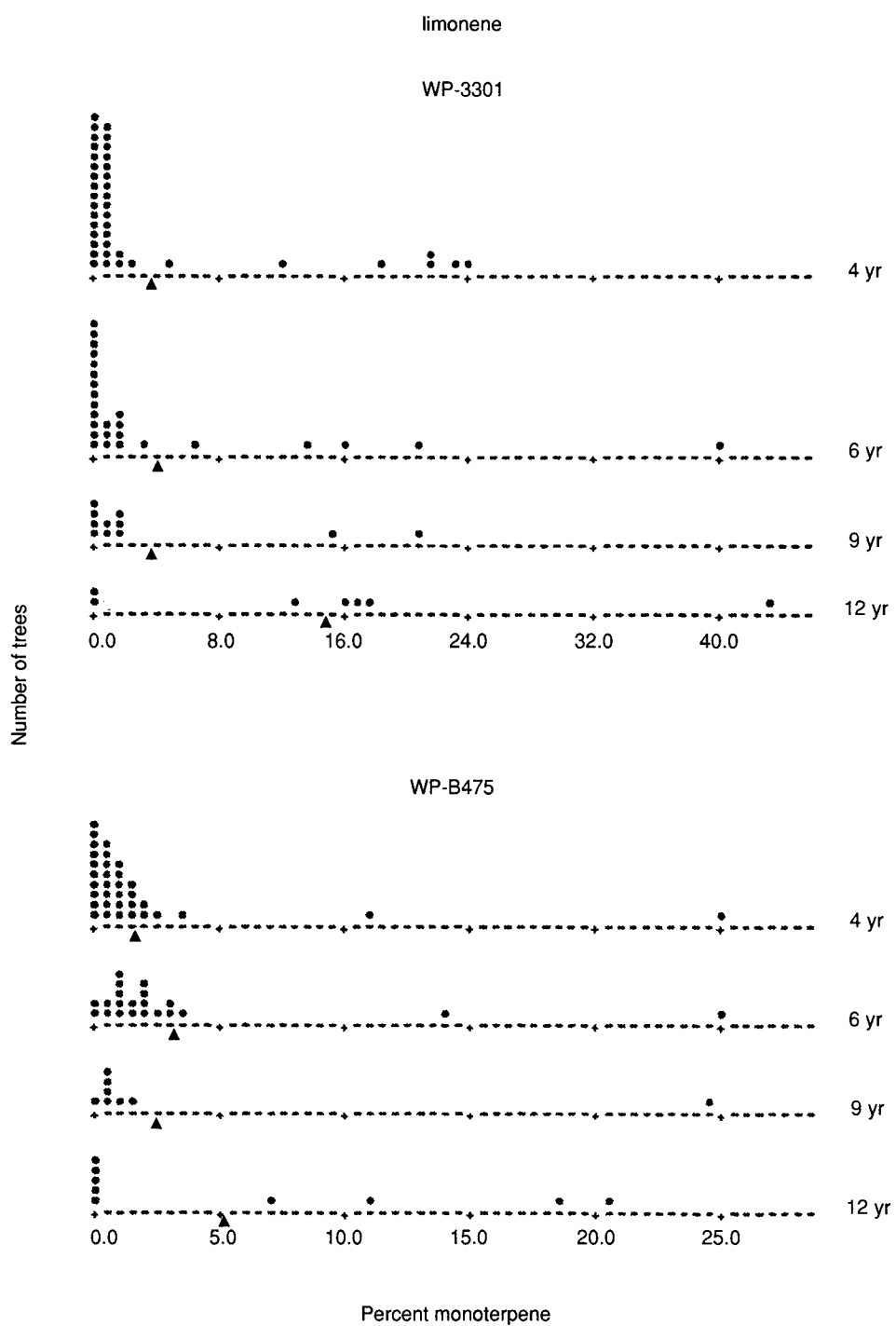


Figure 5-15—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of two *P. ponderosa* with dissimilar composition, WP-3301 and WP-B475, for the four periods of nursery growth listed on the right; each dot is measurement of one tree; [$\blacktriangle$] is average of set.







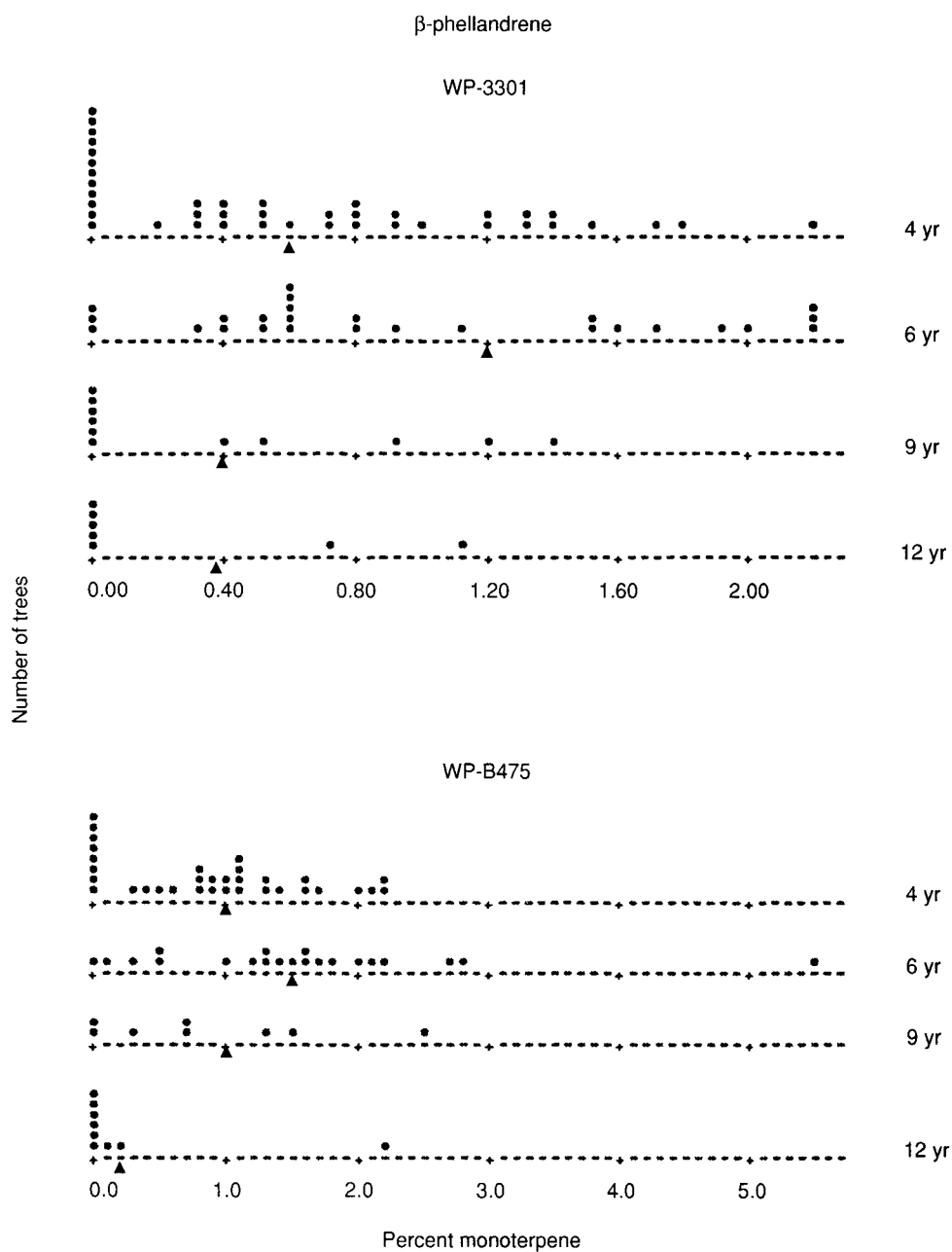
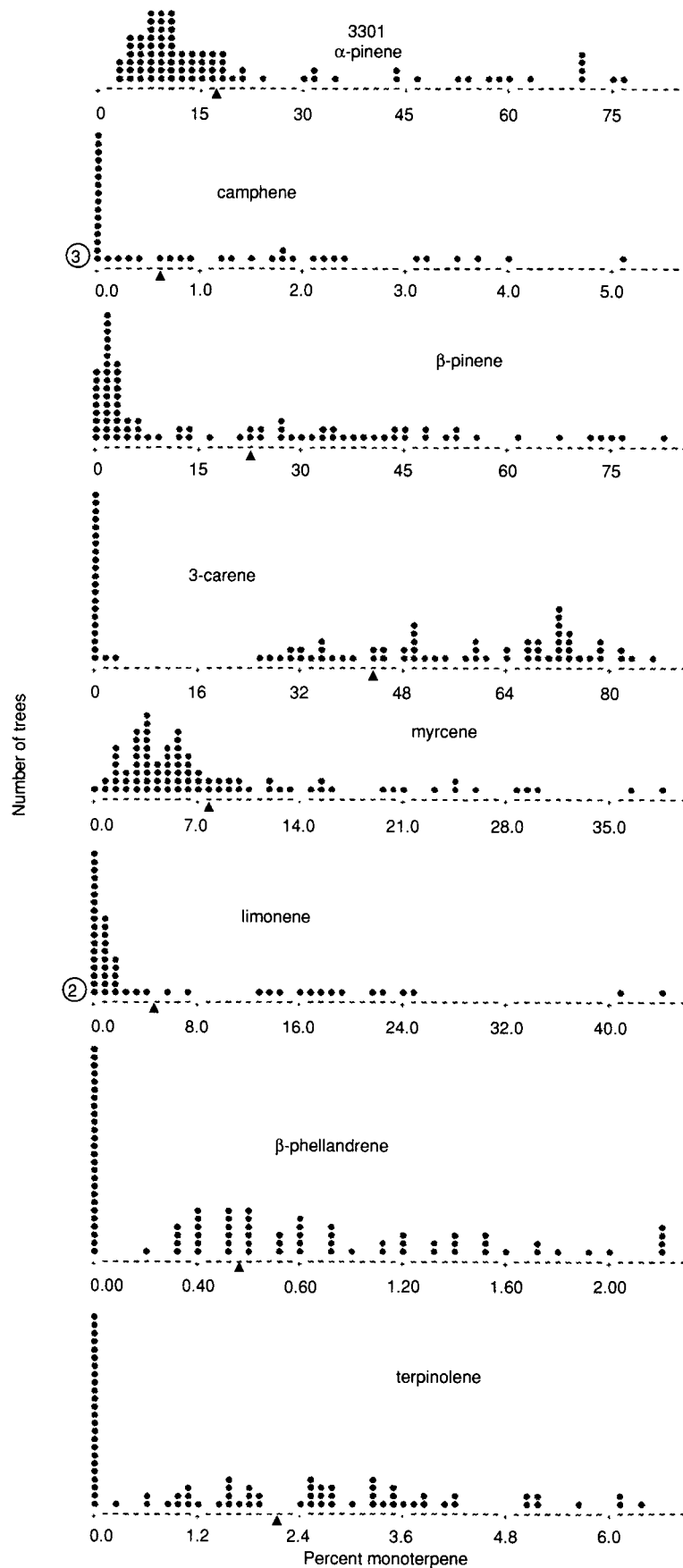
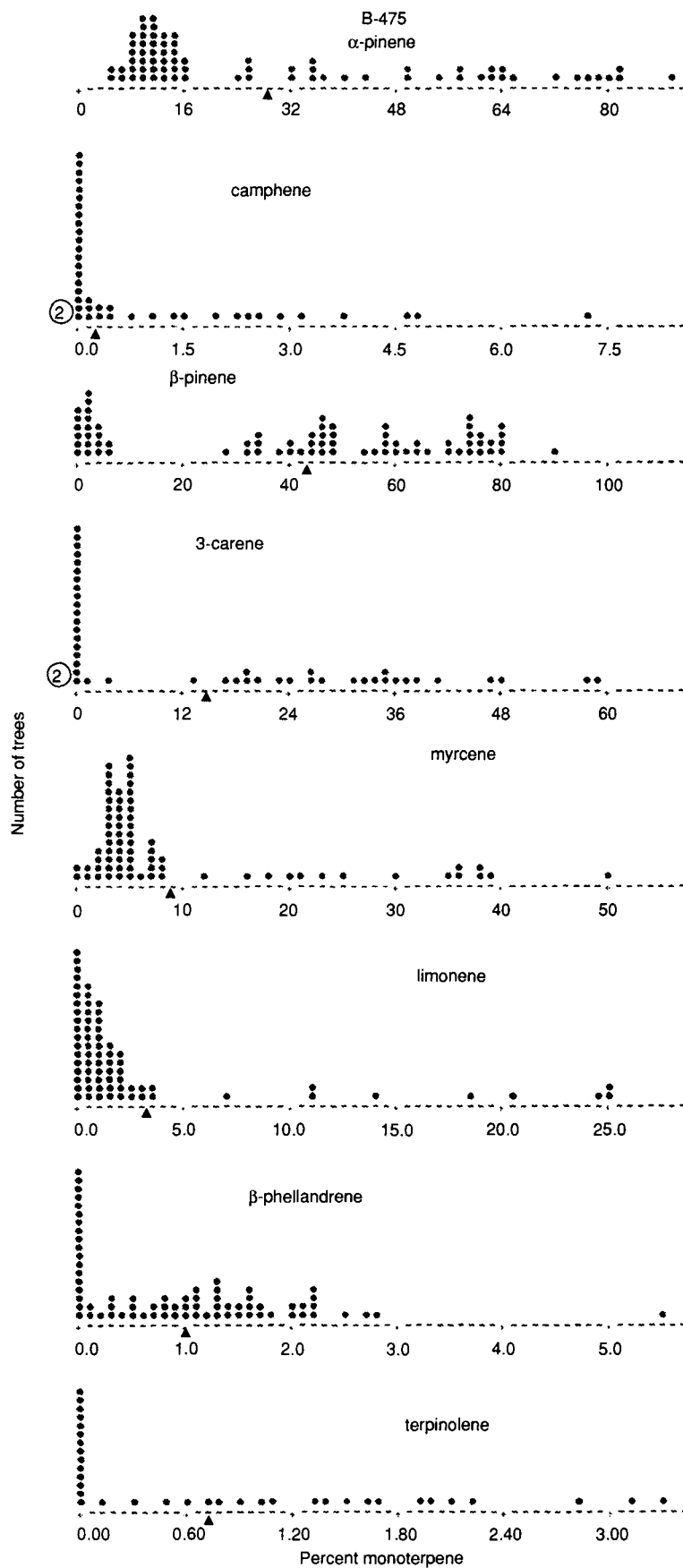


Figure 5-16—Frequency distribution of the amount of the noted monoterpenes in the progeny of two *P. ponderosa* with dissimilar composition, WP-3301 and WP-8475, aggregated for 4 to 12 years of nursery growth; each dot is measurement of one tree; where (n) indicates each is n trees; [▲] is average of set.





condition, i.e., lack of an expected mode greater than 20 percent for limonene, was found for other sets of progeny. There was a possible indication in the age series (*fig. 5-15*) that the 12-year-old trees may be starting to overcome this juvenile effect.

Trees WP-3359 and WP-3318

The two parents differed appreciably in all major components as follows respectively for WP-3359 and WP-3318: α -pinene, 13 vs. 27 percent; β -pinene, 31 vs. 10 percent; 3-carene, 42 vs. 18 percent; limonene, 1 vs. 40 percent (*table 5-5*). The composition of the progeny paralleled this pattern (*table 5-6*). There was some shifting in composition over time in the progeny of WP-3359 (*fig. 5-17*), but this could be attributed to the small number of samples at the later years. The two sets of progeny also differed in the characteristics of the frequency distributions of the aggregate populations for α -pinene, β -pinene, 3-carene, and limonene (*fig. 5-18*).

The progeny of WP-3359 in the age sets (*fig. 5-17*) showed the same trend for limonene at 12 years (as noted earlier for the progeny of WP-3301 and WP-B475) for a decrease in the number of trees with near-0 percent and an increase in larger values from 10 percent to 50 percent. This again suggested a weakening of the juvenile effect at about 12 years.

Trees WP-3292 and WP-3293

The two trees differed sharply in β -pinene, 76 vs. 11 percent, and in 3-carene, 0 vs. 76 percent (*table 5-5*). Both trees were very low in limonene. These differences are reflected in the composition of their progeny (*table 5-6*). There were insufficient data to determine composition shift over time in the progeny. As expected, the frequency distributions were very different for β -pinene and 3-carene (*fig. 5-19*). The progeny of WP-3292 had no mode for β -pinene near zero and had two or three modes between 25 and 80 percent; WP-3293 had a mode at zero with a scattering of measurements up to about 55 percent. For 3-carene the pattern was reversed; WP-3293 had a mode of zero with two or three modes between 20 and 80 percent. The frequency distributions for limonene were similar, a mode at zero and a scattering of values up to about 15 percent. Again, the lack of measurements above 20 percent for limonene was attributed to the juvenile effect. This condition had been noted previously for other sets of progeny.

Multiple-Tree Comparisons

Trees WP-3298, WP-3352, AS-3139

These three trees, classed as high limonene, had about the same composition. One could postulate that they had the same assigned structure of alleles: (0-1), (0-0), (0,0), (0-0), and (1-1), respectively, for α -pinene, β -pinene, 3-carene, myrcene, and limonene. However, WP-3298 and WP-3352 were growing at Wheeler Peak in the Escalante North Transition Zone with background pollen of 15 percent limonene, while AS-3139 was at Adin Summit in the Sierra Pacific Region with background pollen of 13 percent limonene, suggesting that alleles for limonene were a bit more abundant at Wheeler than at Adin. Comparison of the progeny of the three trees could be helpful in understanding the background pollen as well as the effect of the place of growth of the progeny and the geographic location of the parent.

There was no large shift in any component with the age of nursery growth (as noted previously in the discussion for each set of progeny), at least not of the magnitude found in the progeny of the Ranger Peak parents. This could be a unique characteristic of the Ranger Peak progeny, or it could be caused by the location of the type of parent trees; i.e., Ranger Peak parents had large amounts of β -pinene and low or no 3-carene. However, it was clear that the progeny of

the Wheeler Peak trees were suitable for comparative analysis at ages 4 to 12 years, though there was still some juvenile effect.

A comparison of the frequency distributions of the components of the progeny of the three trees follows (fig. 5-20).

α -pinene: The progeny of WP-3298 and WP-3352 were quite similar. The distribution was multimodal with one large mode at about 15 percent and two or more modes between 20 percent and 80 percent. The progeny of AS-3139 were somewhat different, with one very large mode at about 15 percent and a very small mode at about 20 percent. This suggested that there were many more trees at Wheeler than at Adin, with a (0-1) or (1-1) set of alleles for α -pinene. That is, there were more alleles for α -pinene in the pollen at Wheeler Peak.

Camphene: Trees WP-3298 and WP-3352 were very similar in amount and distribution of camphene and both differ slightly from AS-3139 in the distribution pattern, suggesting a minor genetic difference in camphene between the two Wheeler trees and AS-3139.

β -pinene: All three distributions were somewhat similar with one large mode near 2 percent and two or more modes between 2 and 75 percent. This suggested that the alleles for β -pinene were about the same in both localities. However, since there can be only one allele for β -pinene in the progeny—none of the parents had an allele for β -pinene—the 0-1 set for β -pinene can produce a large amount of β -pinene when α -pinene and limonene were low because of the lack of competition for the precursor, and because the juvenile effect seems to be expressed more strongly by β -pinene.

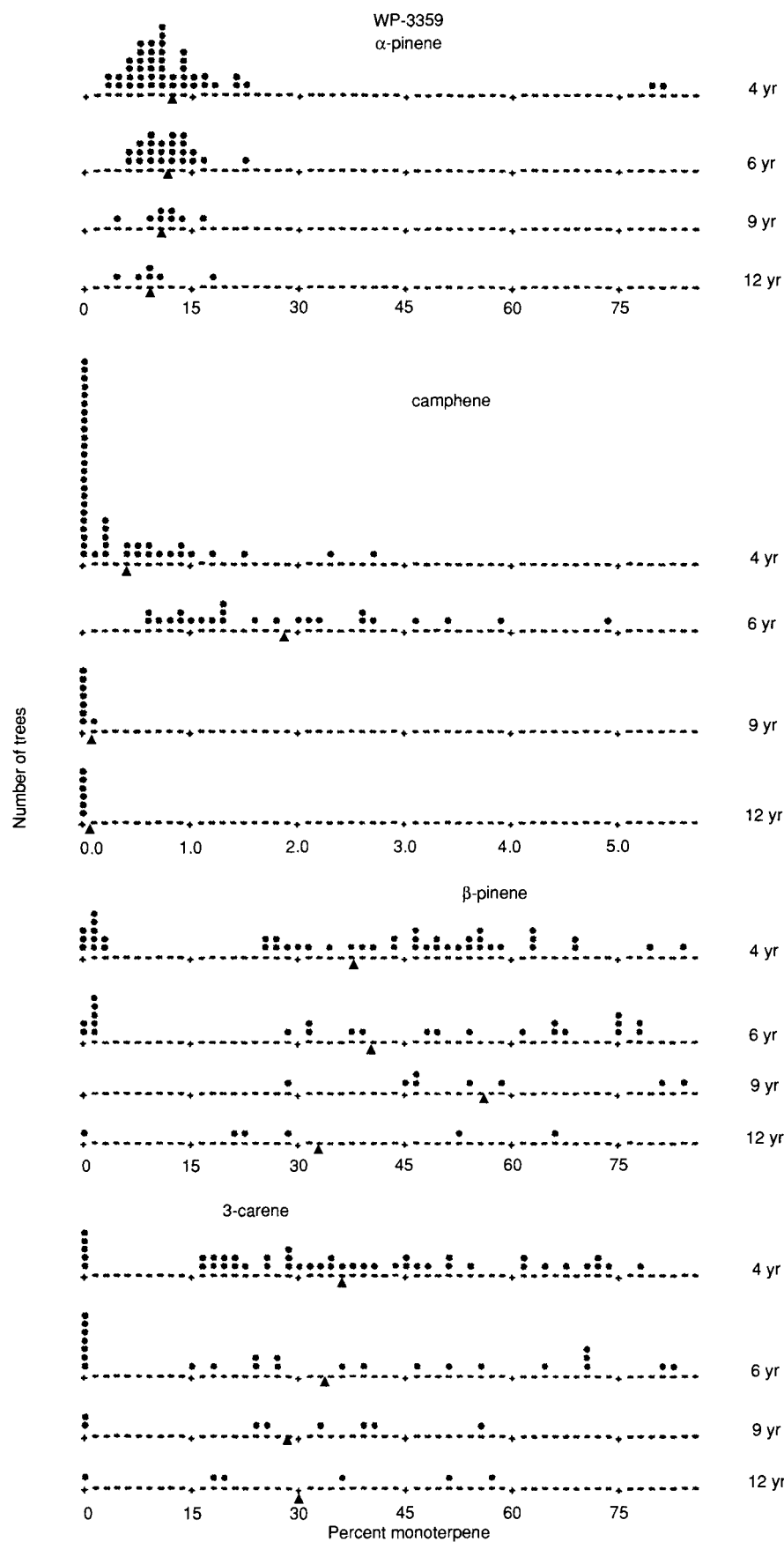
3-carene: The distribution of 3-carene was quite different between the two places of growth of the trees. Since there was no 3-carene in any of the three parent trees, one would expect a distribution like that of the progeny of AS-3139, one mode at or near 0 percent and a second mode near 30 percent. The distribution for the progeny of WP-3298 and WP-3352 had the expected mode at or near 0 percent and possibly two broad modes between 15 percent and 75 percent. There could be a difference in the allele structure between the two localities, though not likely, or there was a difference in the response of the seedlings to the place of growth: progeny of AS-3139 were growing "in" their resin region while the progeny of WP-3298 and WP-3352 were growing "out" of their region. I would postulate that it was the place of growth of the seedlings. Also, there could be a difference in a minor genetic structure between Wheeler and Adin, but such a structure has not yet been found.

Myrcene: The distributions for myrcene were quite similar for all three trees with one large mode at about 5 percent and a much smaller one at about 15 percent. This suggested that alleles for myrcene were present but rare at both localities. An allele for myrcene did not show up in any of the controlled breeding with the Wheeler Peak trees.

Limonene: The distributions of the progeny of the two Wheeler trees were quite similar but somewhat different from that of the Adin progeny. It could well be that when there was an allele for α -pinene in young trees, such as was likely in the progeny of the two Wheeler trees, the activity of the limonene alleles was greatly depressed because of the juvenile factor. This condition could account for the difference between the progeny of the two Wheeler trees and the progeny of the Actin tree in the frequency distribution of limonene. That is, the greater frequency of alleles for α -pinene at Wheeler Peak caused the differences between Wheeler Peak and Adin Summit progeny in both α -pinene and limonene, with greater α -pinene and lesser limonene at Wheeler Peak. There could also be an effect of locality of growth and differential response to the juvenile effect, or of a minor genetic structure.

The progeny of these three trees were sharply different from that of the

Figure 5-17—Frequency distribution of the amount of the noted monoterpenes in the progeny *P. ponderosa* WP-3359, with four ages of nursery growth listed on the right; each dot is measurement of one tree, [▲] is average of set.



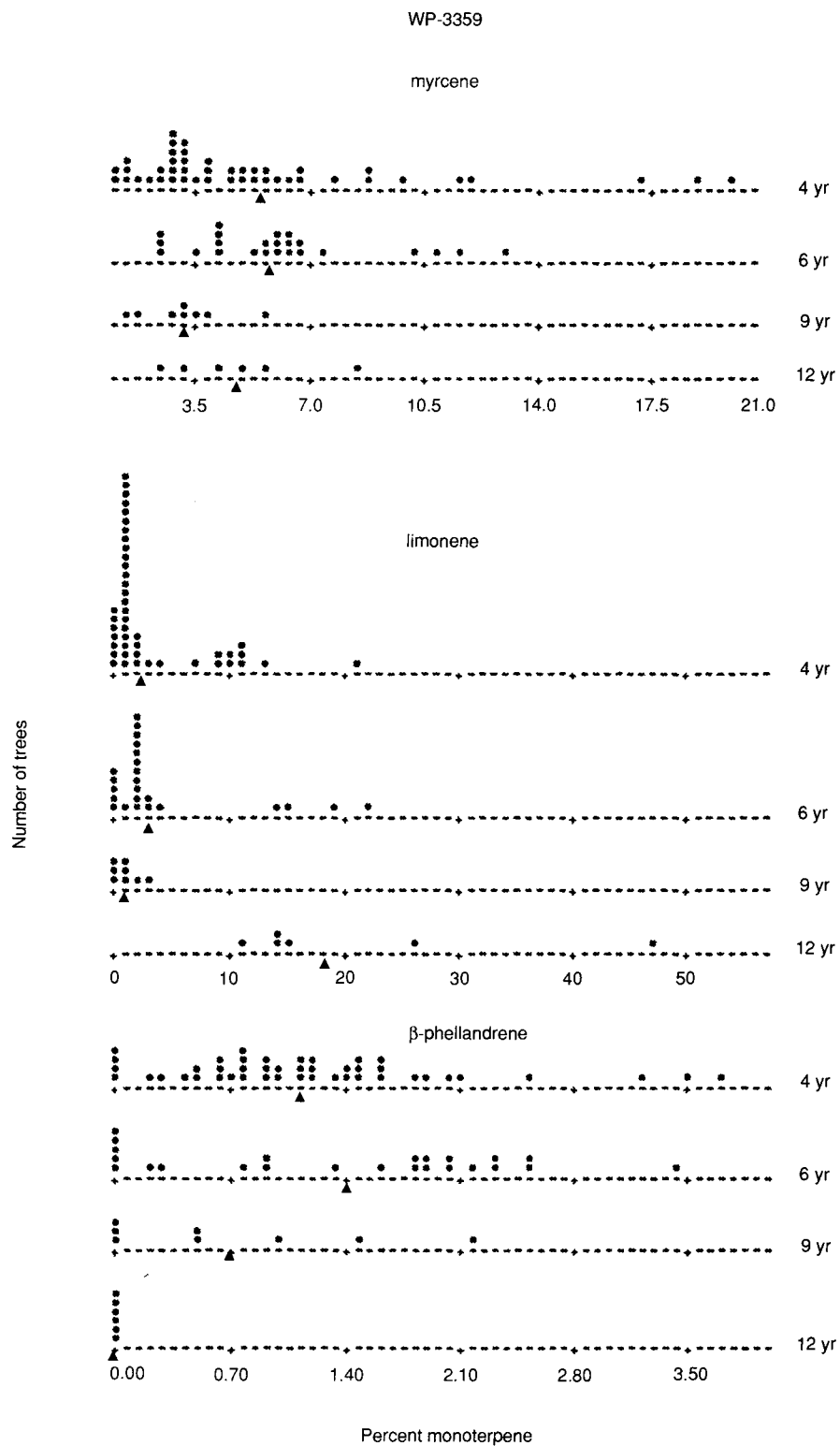
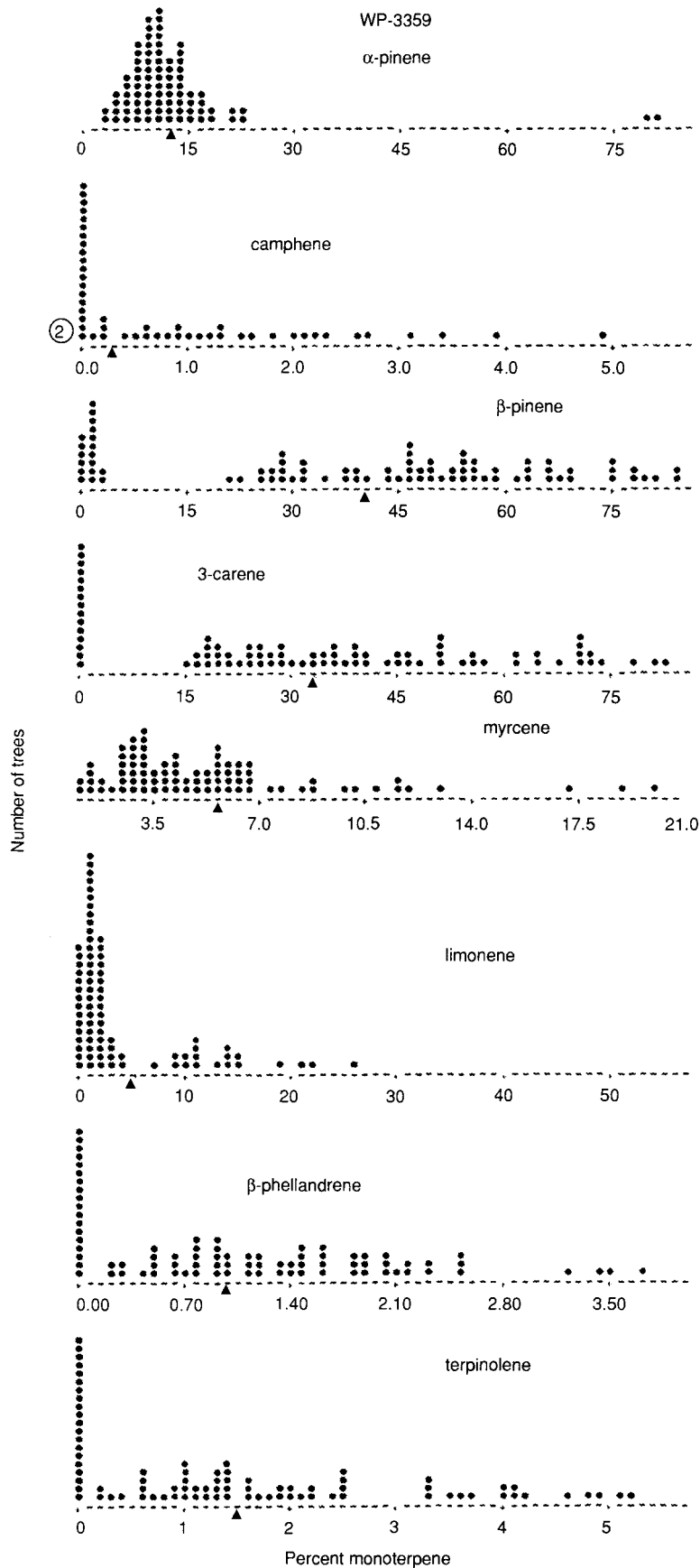


Figure 5-18—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of two *P. ponderosa* with dissimilar composition, WP-3359 and WP-3318; each dot is measurement of one tree; [▲] is average of set.



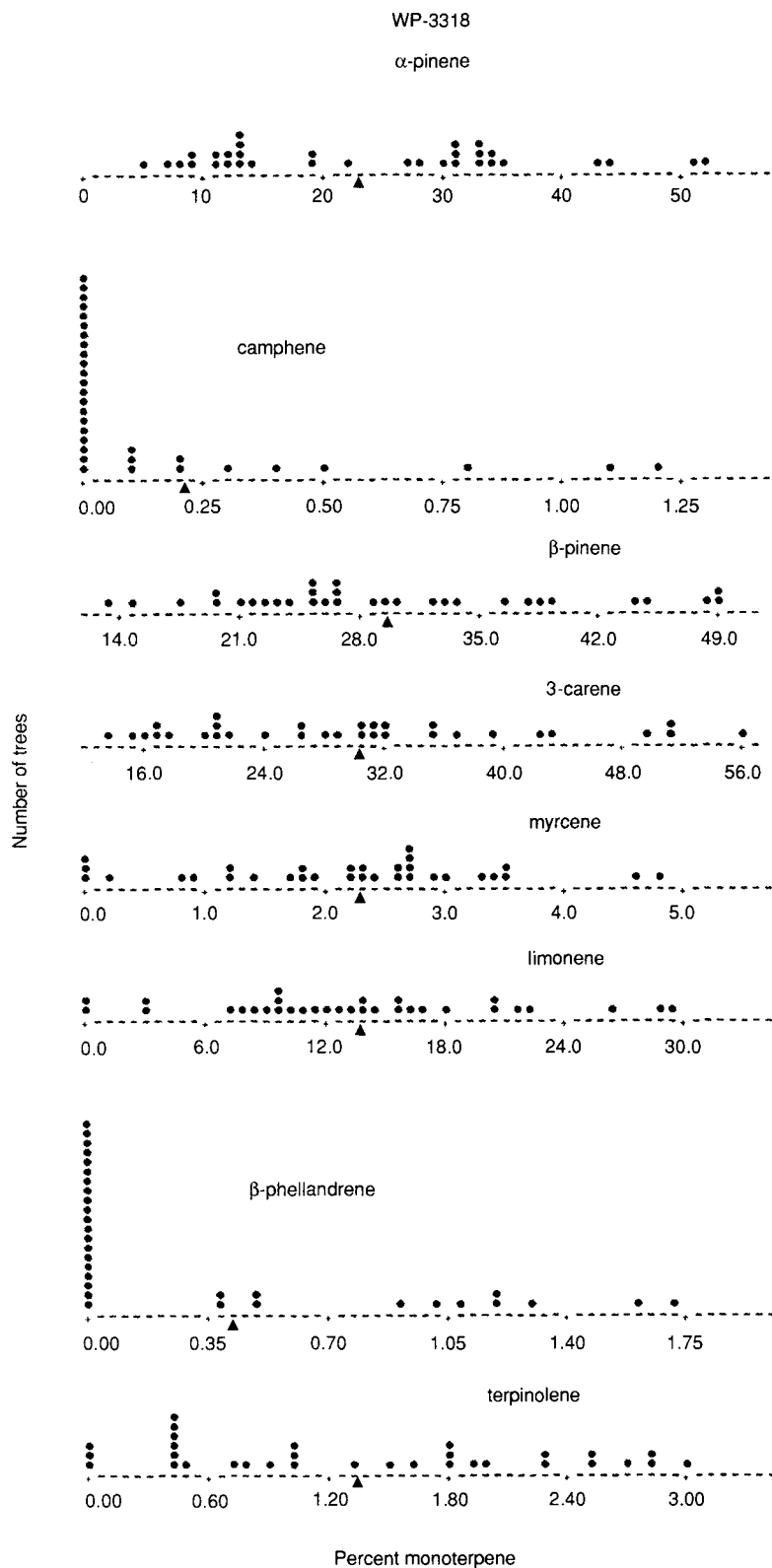
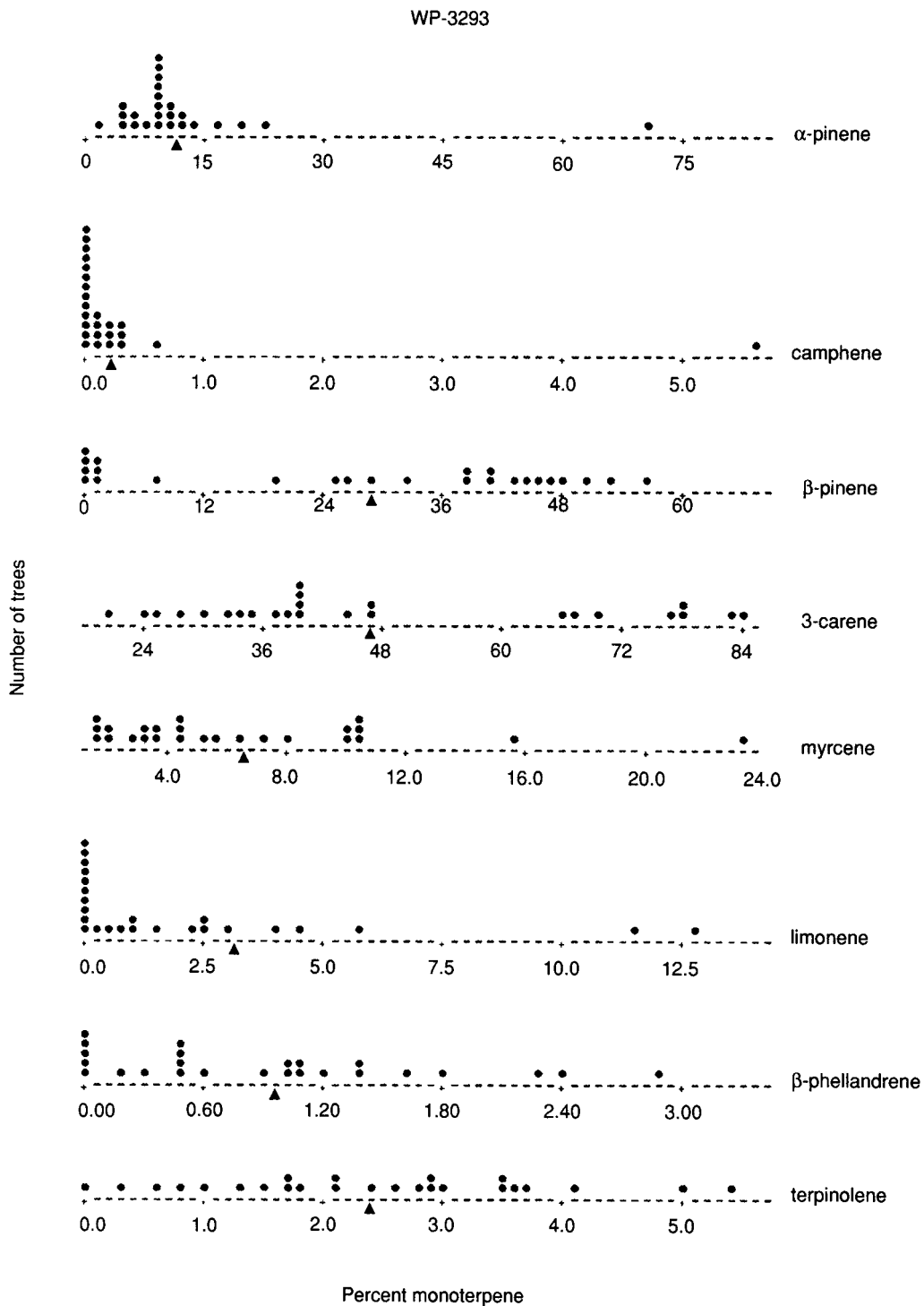


Figure 5-19—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of two *P. ponderosa* with dissimilar composition, WP-3293 and WP-3292; each dot is measurement of one tree; except where $(\bar{n})$ indicates each is n trees; [$\blacktriangle$] is average of set.

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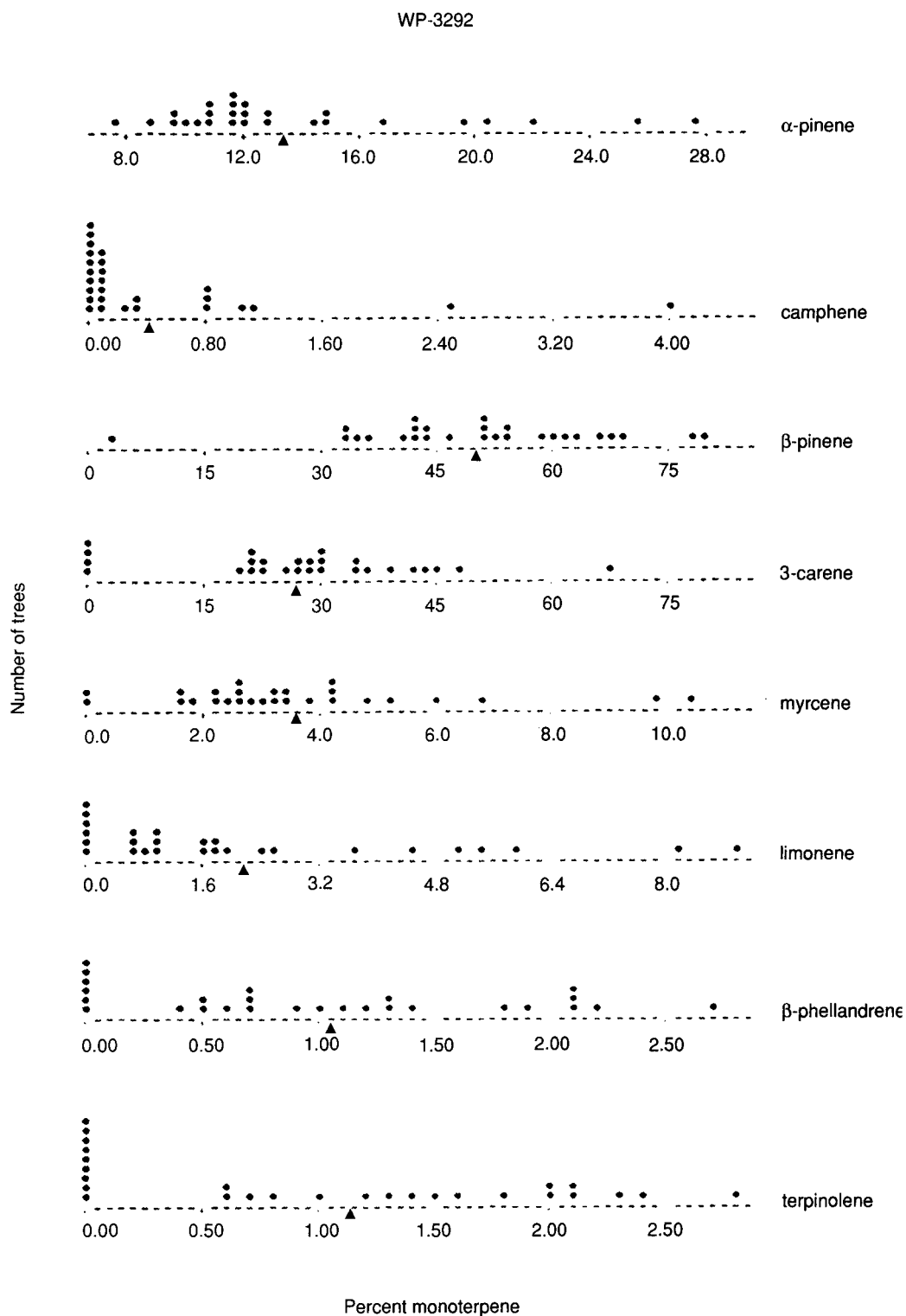
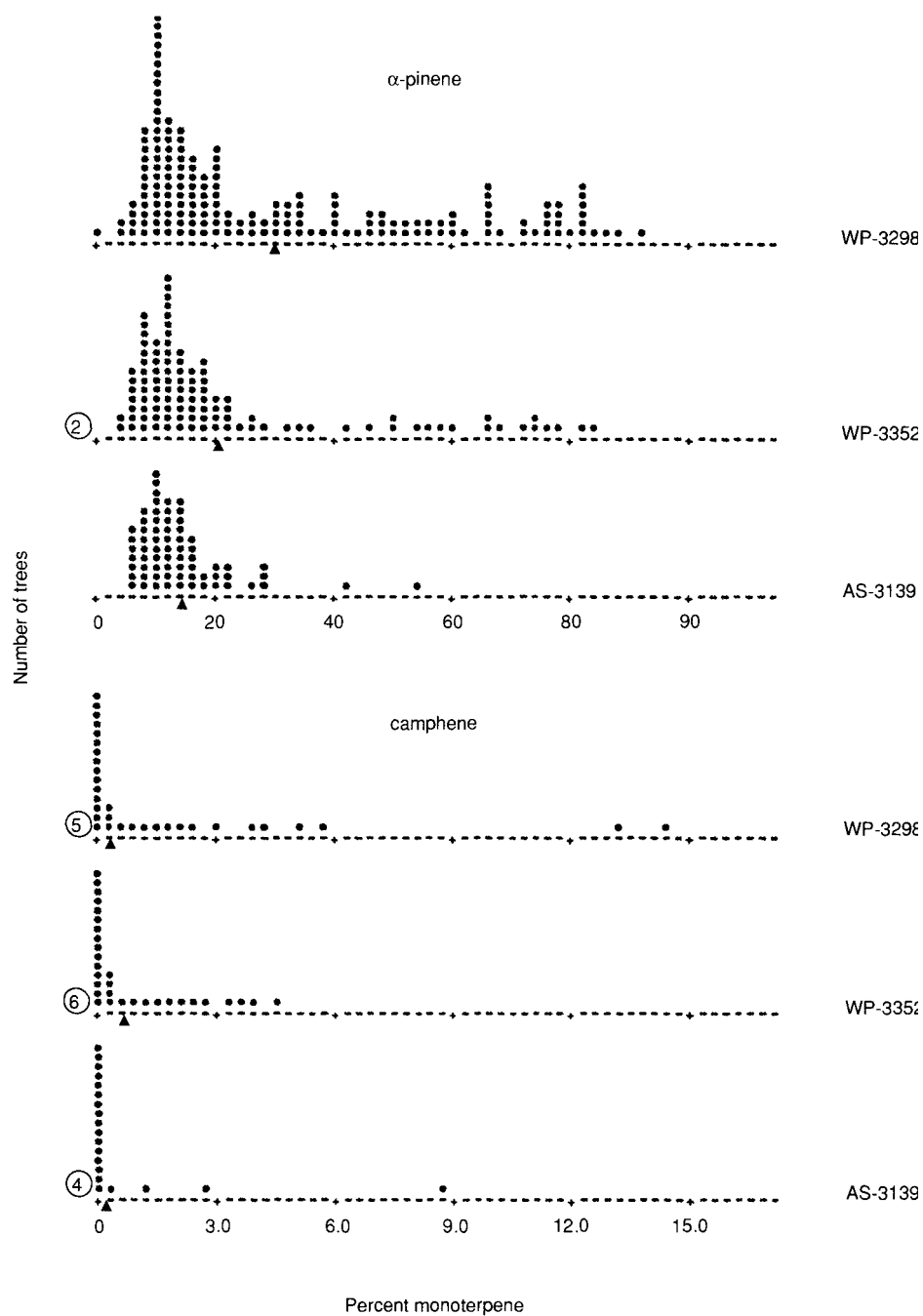
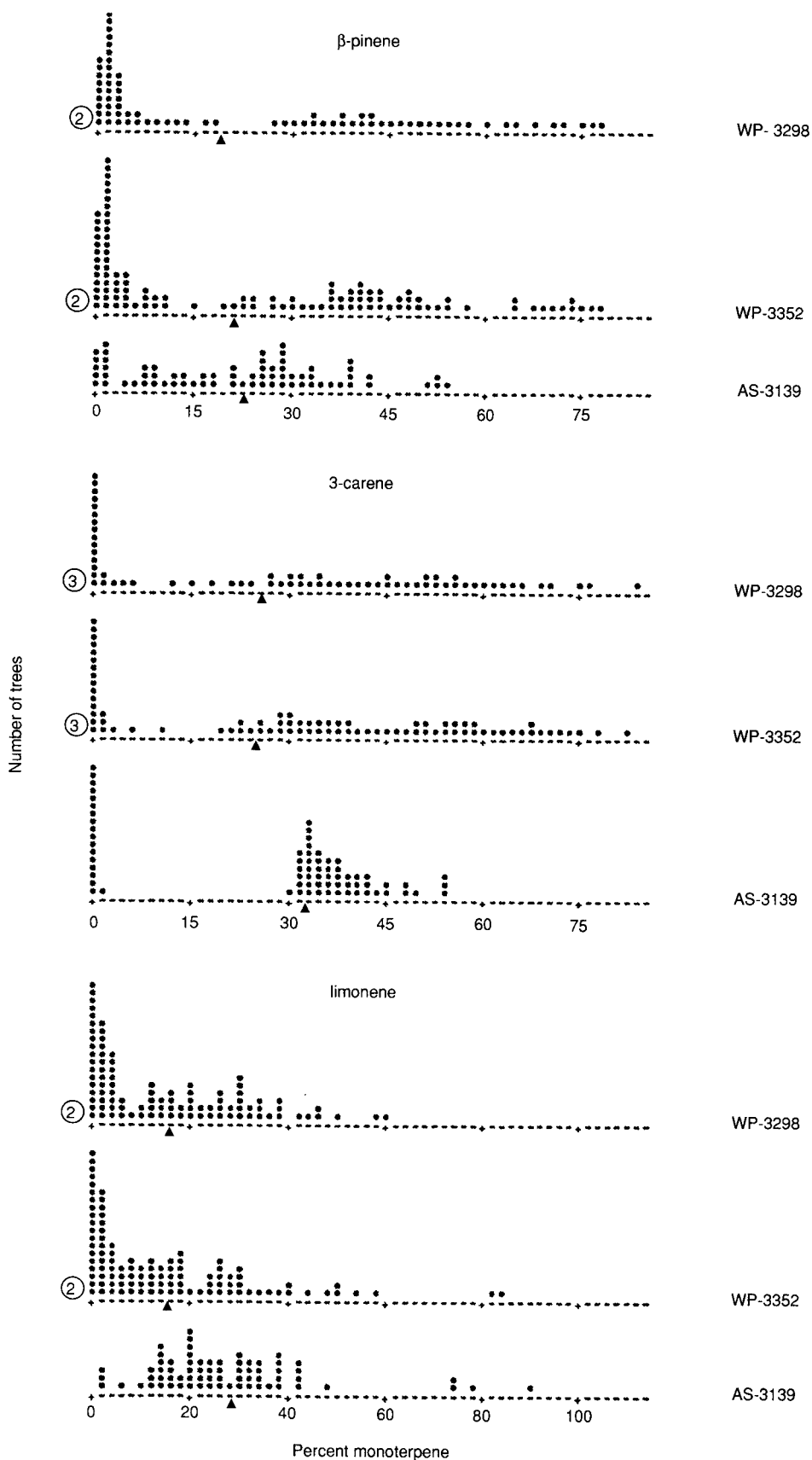
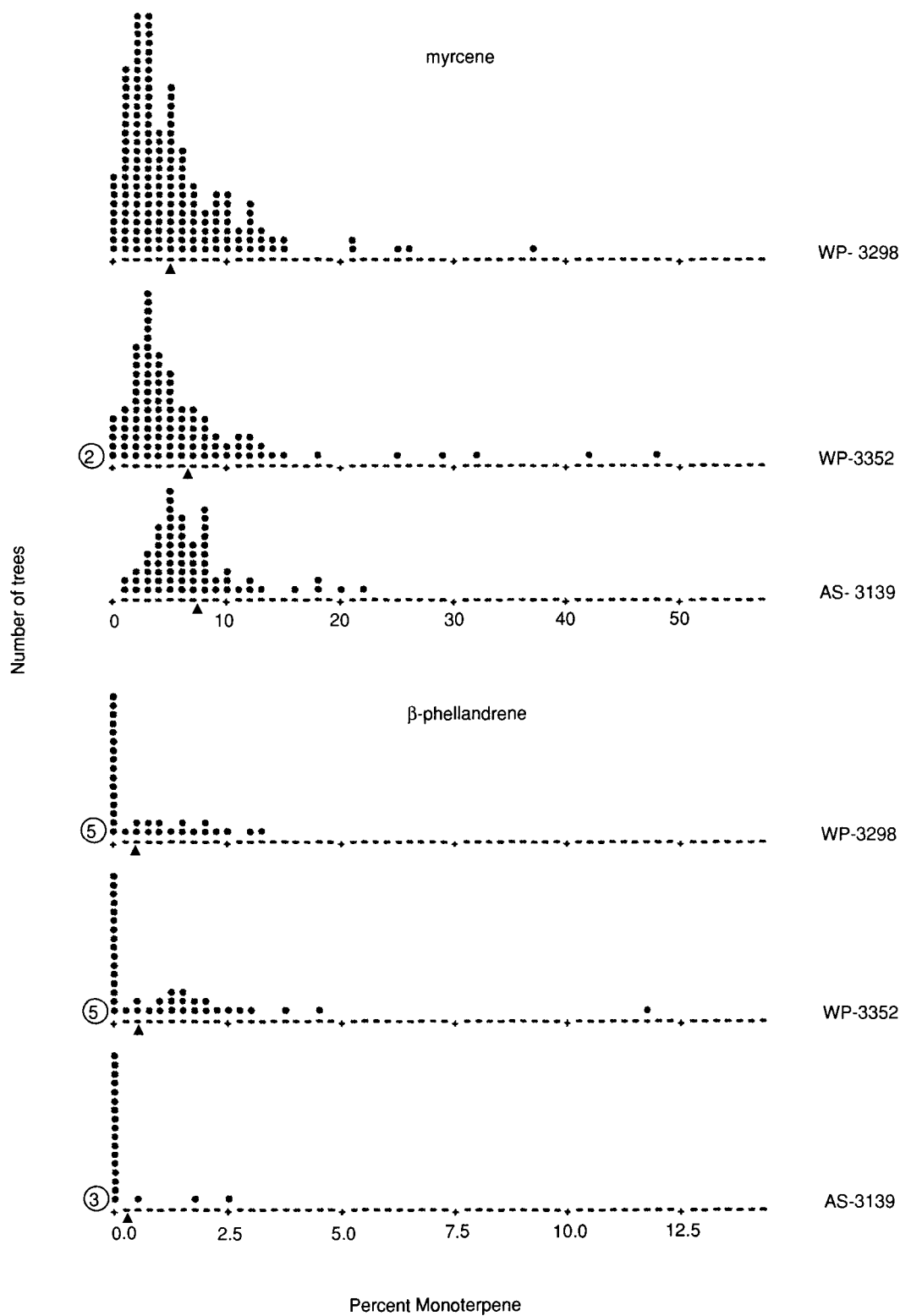


Figure 5-20—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of three high limonene *P. ponderosa*, WP-3298, WP-3352, and AS-3139; each dot is measurement of one tree except where (n) indicates each is n trees; [▲] is average of set.

(fig. 5-20 continues on next 3 pages)







progeny of RP-B37, the high-limonene tree at Ranger Peak, in the amount and distribution of limonene. Despite the fact that Ranger Peak had background pollen of about 22 percent limonene, the progeny of RP-B37 was only 17 percent limonene, with very few values above 20 percent. This could be caused by the greater frequency of alleles of β -pinene at Ranger Peak plus the apparent greater effect by β -pinene on the juvenile condition of the young progeny. However, there was an ultra-high limonene progeny of RP-B37.

Trees WP-3298, WP-3352, WP-3299, WP-3312

All four trees growing at Wheeler Peak were classed as high or near-high limonene—and each was assumed to have a (1-1) set of alleles for limonene—and all had wind-pollinated progeny that were high limonene or potential high limonene. They differed in that WP-3299 and 3312 had substantial 3-carene. The characteristics of the frequency distributions of the individual components of the progeny were generally what would be expected from the parental composition and the background pollen (*fig. 5-21*). However, there were some differences that suggested a differential effect of the juvenile factor, which depends on composition, on the effect of growth of a tree outside its ecosystem, and possibly on a minor genetic system. Not surprisingly the two high-limonene trees, WP-3298 and WP-3352, had the most progeny of the high or potentially high-limonene type.

For comparative analysis of the relationship of limonene to the frequency distribution of the other monoterpenes, three generalized types of composition were considered potentially high limonene: (1) those with greater than 20 percent limonene, (2) those with less than 20 percent β -pinene plus 3-carene, and (3) those meeting both requirements (*table 5-9*). The data for the progeny of the four trees were separated into the three groups on the basis of the conditions just stated. The average composition for the progeny in each of the three groups for each of the four parent trees was very similar, and their frequency distributions were also very similar (*fig. 5-19*).

The following is a discussion of some aspect of these three generalized types of composition.

(1) When limonene was greater than 20 percent (*fig. 5-21a*)

The frequency distributions for all components were somewhat similar for the progeny of all four trees when limonene was greater than 20 percent.

α -Pinene: Average α -pinene varied somewhat among the four sets of progeny, but the configurations of the distributions are similar. Each distribution had a mode at about 10 percent, another between 15 and 20 percent, and a third one near 40 percent. It was speculated that the first mode was caused by isomerization or by some activity by the (0-0) set of alleles.

β -Pinene: All four sets of progeny had a large mode at about 2 percent, probably caused by isomerization. Trees WP-3298, WP-3352, and WP-3312 had a few scattered values greater than 10 percent while WP-3299 had two distinct modes between 15 and 40 percent despite the lack of β -pinene in the parent tree. Because these were wind-pollinated progeny and the parent tree had essentially no β -pinene, it was difficult to say why the progeny of WP-3299 were so different from the other three trees.

3-Carene: Here again WP-3298, WP-3352, and WP-3312 were quite similar with one mode at about 1 percent and two other modes further up the percent scale. But WP-3329 had no mode below 10 percent although it did have two modes between 24 and 60 percent; this suggested a (1-1) set of alleles for 3-carene in WP-3299.

Myrcene: There was a general similarity among the four sets of progeny with a large mode near 3 percent and two much smaller modes further along the percent scale. Thus, none of the four trees had an allele for myrcene, and

Table 5-9—Average xylem monoterpene composition of three classes of the wind-pollinated progeny of four trees at Wheeler Peak. *N* = number of trees.

Tree	Class of compositor	N	Monoterpene ¹							
			α -p	cam	β -p	car	my	lim	β -ph	ter
			<i>percent</i> ²							
3298	3	50	29.9	0.5	3.9	24.1	5.9	34.7	0.1	1.0
3352		52	20.5	0.2	5.7	31.8	5.7	34.2	0.4	1.2
3299		52	11.3	0.6	12.6	37.8	3.4	31.9	1.9	1.6
3312		32	17.8	0.5	8.0	36.5	3.9	31.4	0.2	1.6
3298	4	54	64.5	0.6	3.6	0.7	8.6	21.9	0.1	0.0
3352		35	56.5	0.1	4.5	0.6	12.2	25.4	0.6	0.1
3299		2	76.4	0.0	3.7	0.2	0.0	19.4	0.2	0.0
3312		15	64.0	0.3	2.7	0.4	12.6	19.6	0.2	0.0
3298	5	24	49.4	0.7	2.4	1.3	8.3	37.8	0.1	0.1
3352		17	40.7	0.1	3.7	0.7	8.7	45.7	0.3	*
3299		0								
3312		6	46.5	0.5	2.3	0.5	6.6	43.6	0.2	0

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.²Normalized; * = trace.³When limonene was equal to or greater than 20 percent.⁴When β -pinene and 3-carene was less than 20 percent.⁵When conditions of both ³ and ⁴ were met.

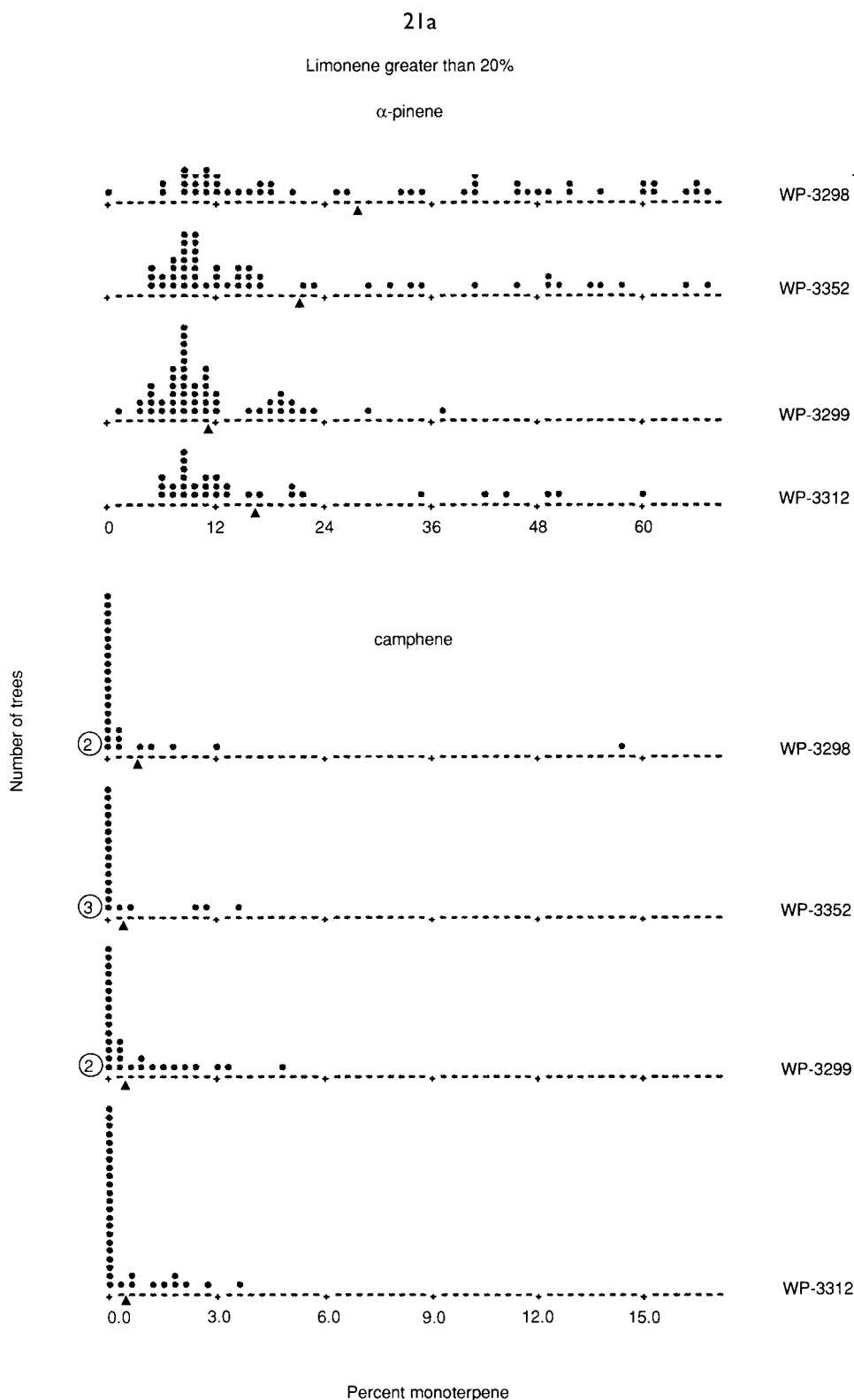
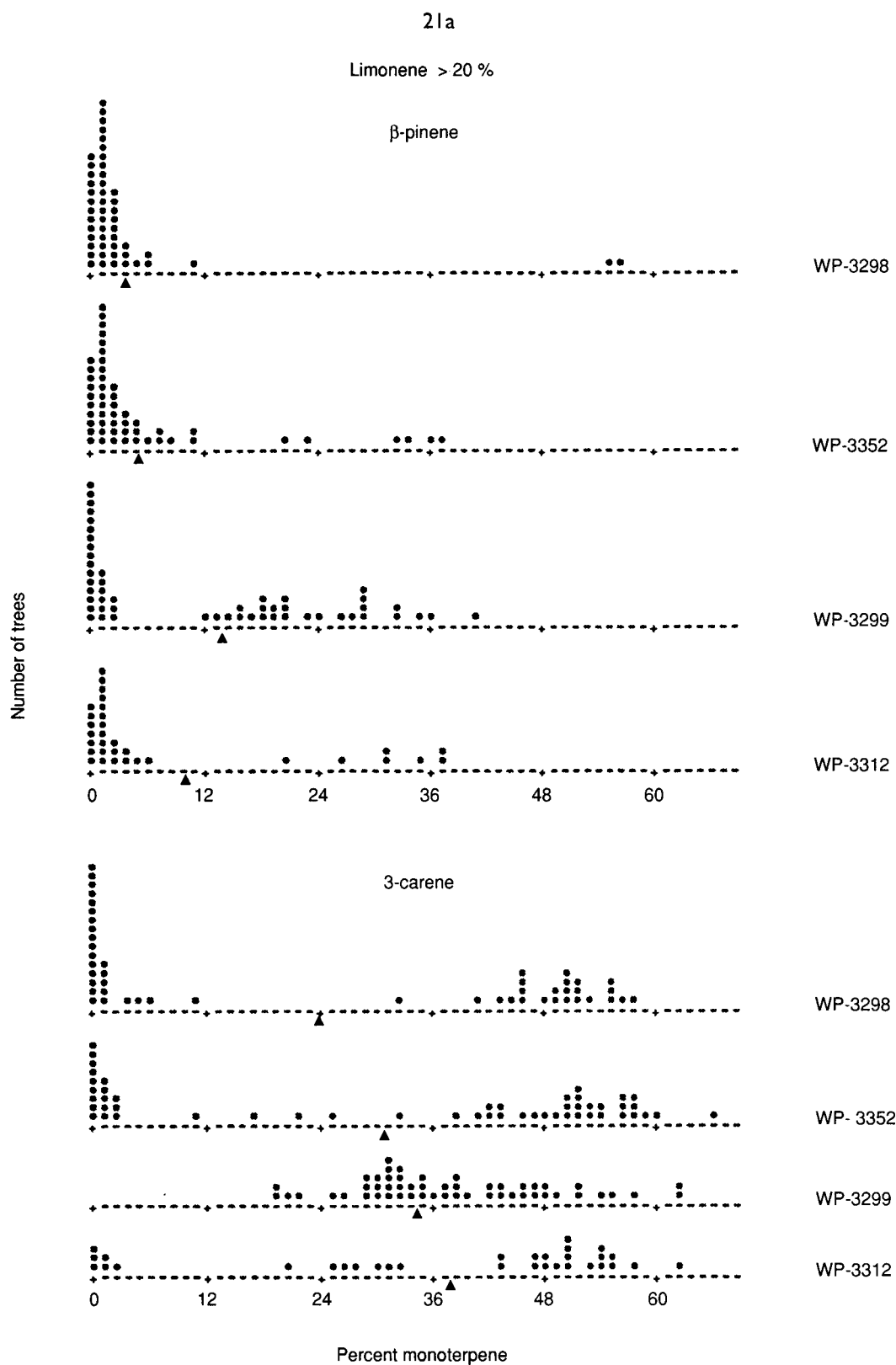
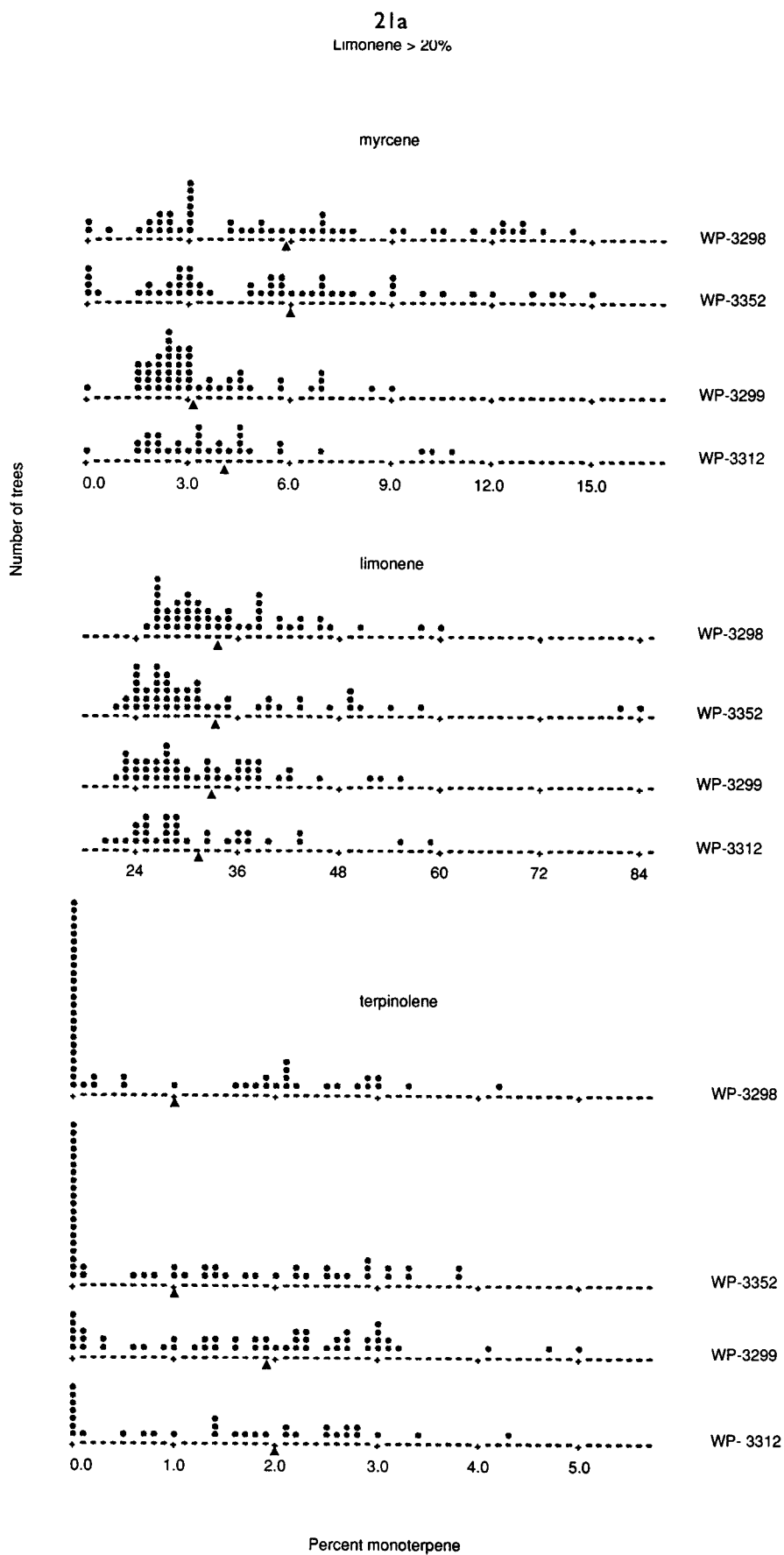


Figure 5-21—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of four *P. ponderosa* that were suspected of having a (1-1) set of alleles for limonene, WP-3298, WP-3352, WP-3299, and WP-3312, when progeny were grouped into classes: (a) greater than 20 percent limonene (21 a), (b) β -pinene plus 3-carene less than 20 percent (21 b), and (c) progeny meeting both conditions (21c); each dot is measurement of one tree, except where (n) indicates each is *n* trees. [▲] is average of set.

(fig. 5-21 continues through page 269)





they simply reflected the general level of pollen, or the same level of isomerization or production of biosynthetic by-products.

Limonene: Here again, all four were similar, with a large mode between 24 and 35 percent, which almost tended to be bimodal, and another mode above 35 percent. The smaller mode further along the scale could have been caused by these trees beginning to overcome the juvenile effect and had reached the start of "mature" status of monoterpene synthesis. Likewise, it can be speculated that all the trees in this classification could shift to the highest mode in future years.

(2) When β -pinene plus 3-carene was less than 20 percent (fig. 5-21b):

When this condition was met, all four sets went to high α -pinene. This was assumed to be caused by the juvenile factor. However, WP-3299 and WP-3312 had very few progeny in this class; i.e., β -pinene plus 3-carene was less than 20 percent. Because each parent had, apparently, alleles of β -pinene, 3-carene, or both, the progeny will usually have considerable amount of both. The general distributions were somewhat similar for β -pinene and 3-carene. The distributions for myrcene and limonene were quite similar for all four sets of progeny when the unequal numbers in the sets were considered. Limonene had a multimodal distribution, but the number of modes is not clearly defined.

(3) When limonene was greater than 20 percent and β -pinene plus 3-carene was less than 20 percent (fig. 5-21c):

The lack of more trees in this class limited conclusions. However, the tendency for high α -pinene was considered to be caused by the juvenile effect. In general, though, the distributions were somewhat similar.

Trees WP-3292, WP-3293, WP-3294, WP-3318, WP-B477

There were fewer than 35 progeny for each of these five trees, and they will be treated as one diverse unit to contrast the females. The frequency distributions of individual monoterpenes were developed (fig. 5-22) along with the average of the set, the composition of the female parent, and the stand average at Wheeler Peak to represent the composition of the background pollen. The following observations were made about the average composition with respect to the female parent and the stand average (table 5-10):

α -pinene was usually a bit higher in the progeny than in the female parent though it was usually close to the stand average. The progeny of WP-3318 were an exception to this, but this reflects the much higher α -pinene in WP-3318 than in the stand.

β -pinene was almost always higher in the progeny than in the female parent or the stand, except for WP-3293 which was 76 percent β -pinene in the female, a very high amount.

3-carene was either higher or lower in the progeny than in the stand, probably because of irregular response to the juvenile effect.

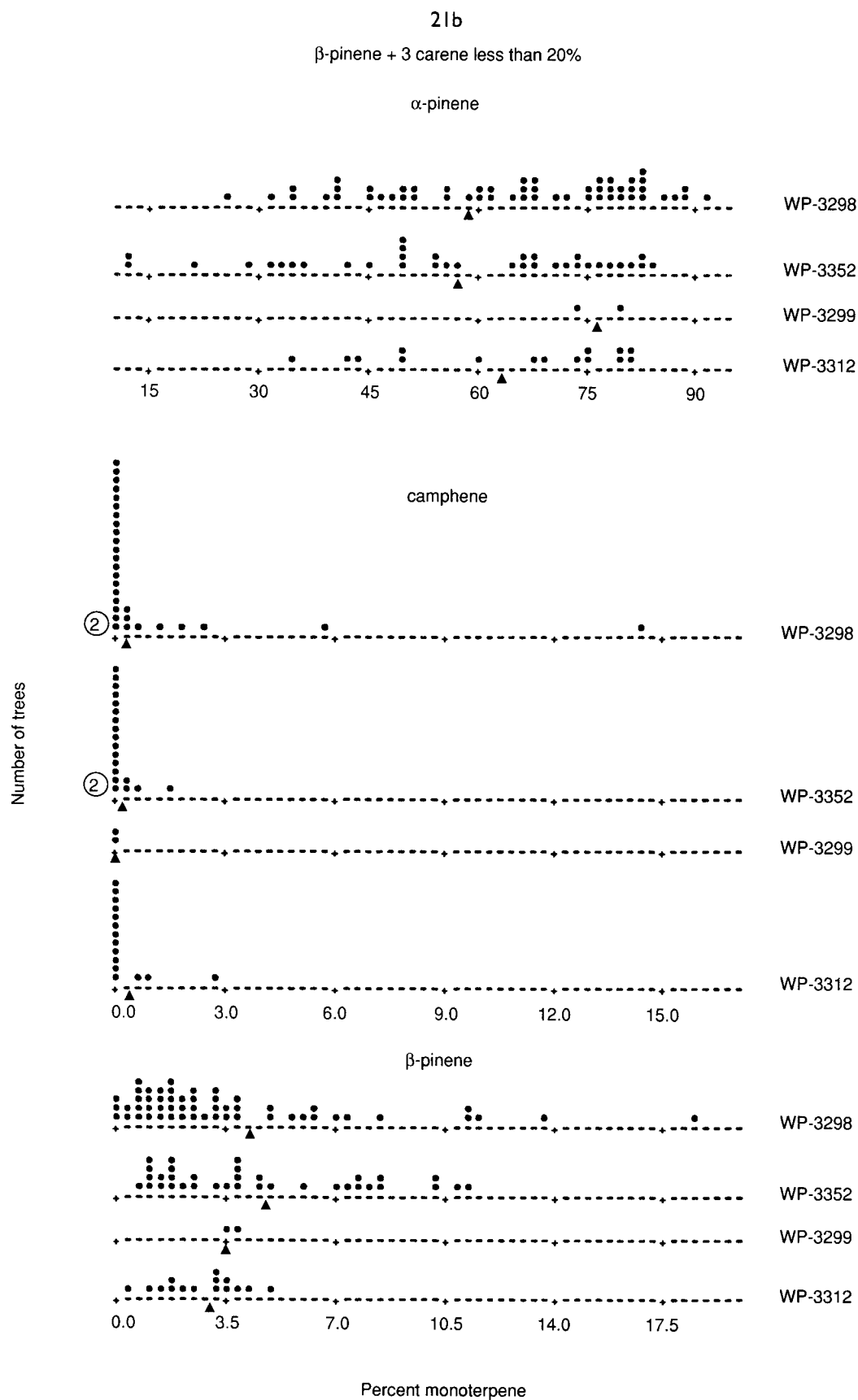
Myrcene was always lower in the progeny than in the female or the stand.

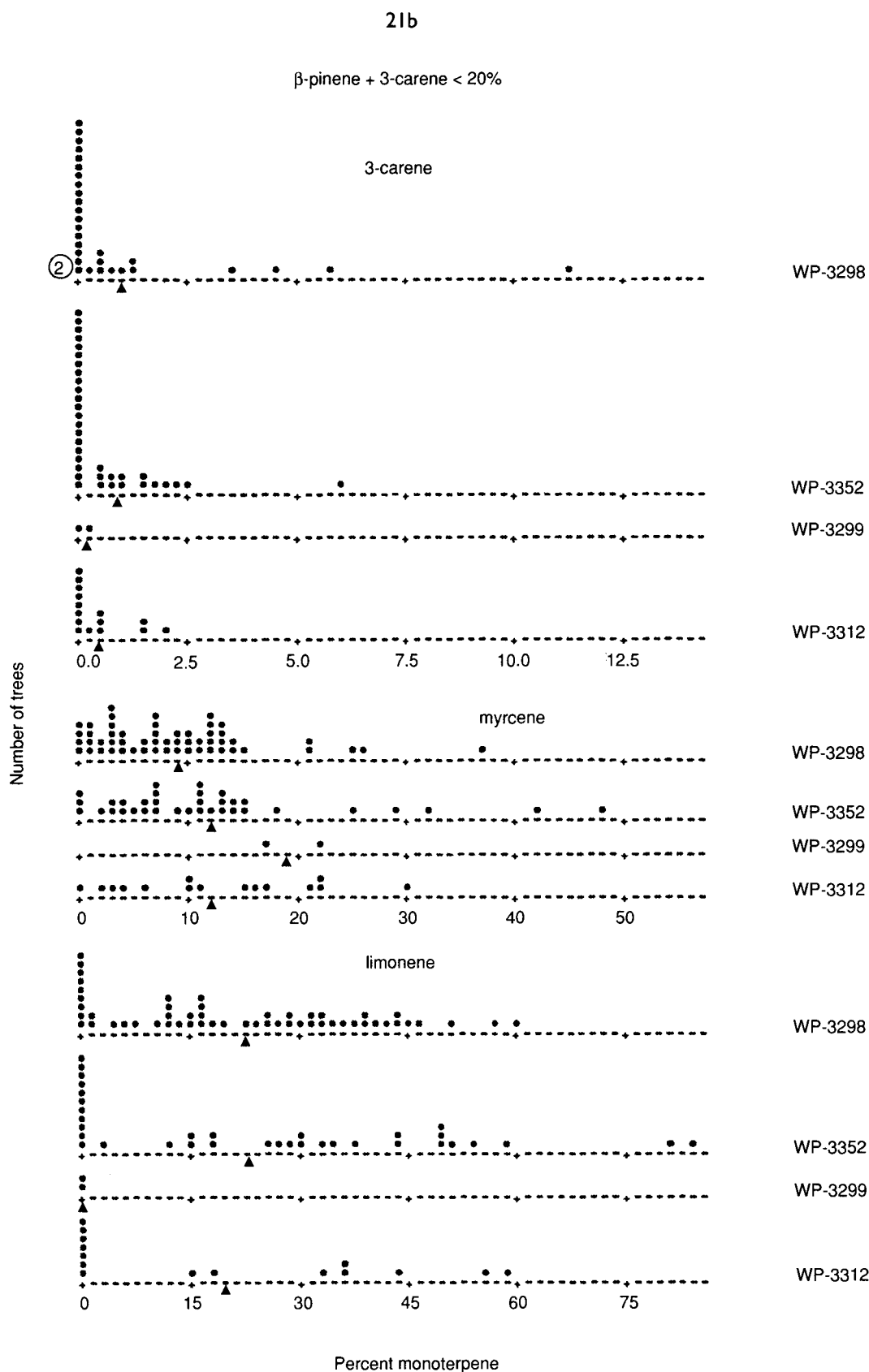
Limonene was always lower in the progeny than in the female or the stand.

The aggregate of these observations showed that the juvenile effect results in greater β -pinene and less myrcene and limonene in progeny, that 3-carene did not seem to be affected by the juvenile factor, and that α -pinene probably increased in the progeny.

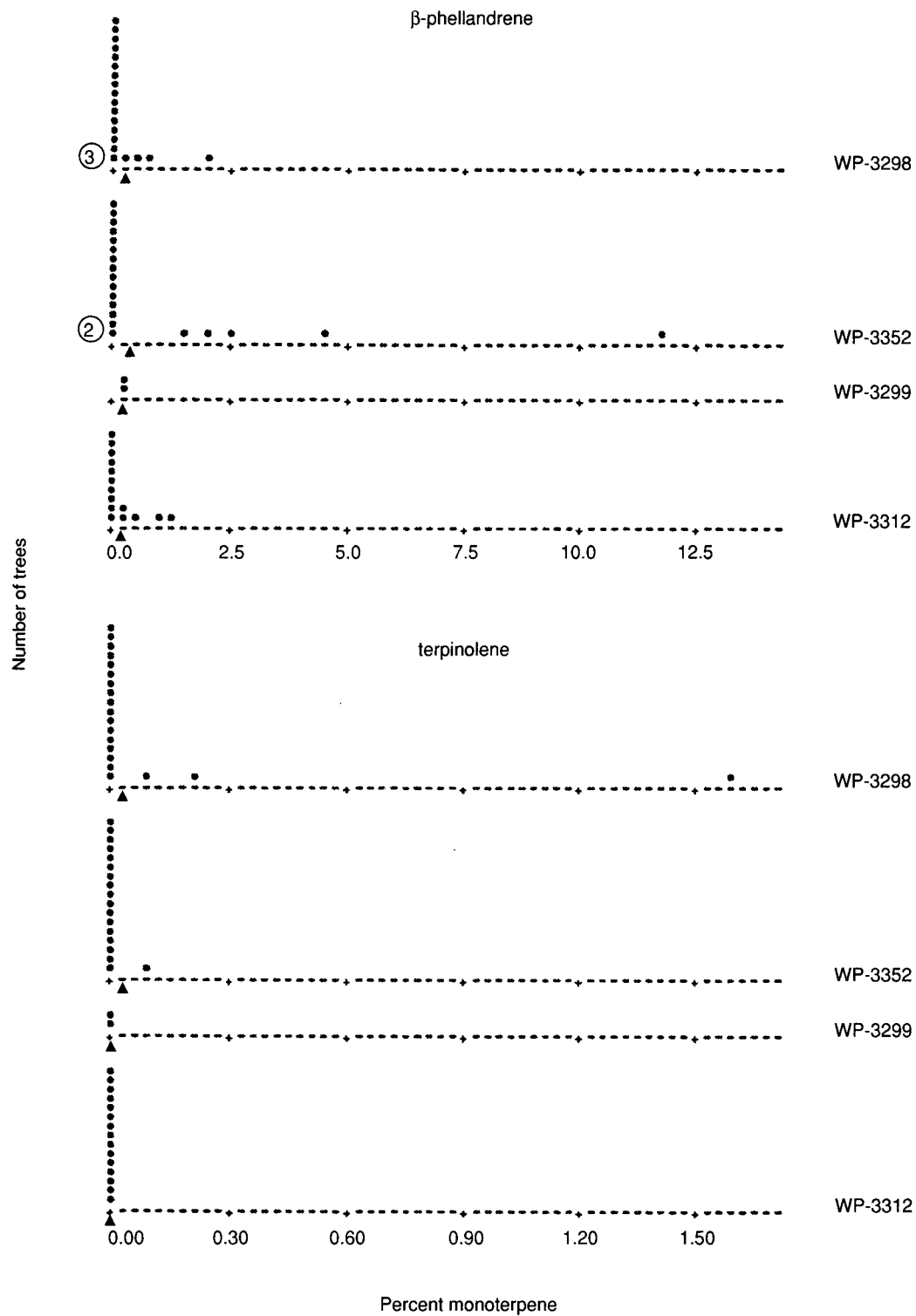
Trees WP-3298, WP-3352, WP-3299, WP-3312, WP-B475, WP-3301, WP-3320, WP-3359

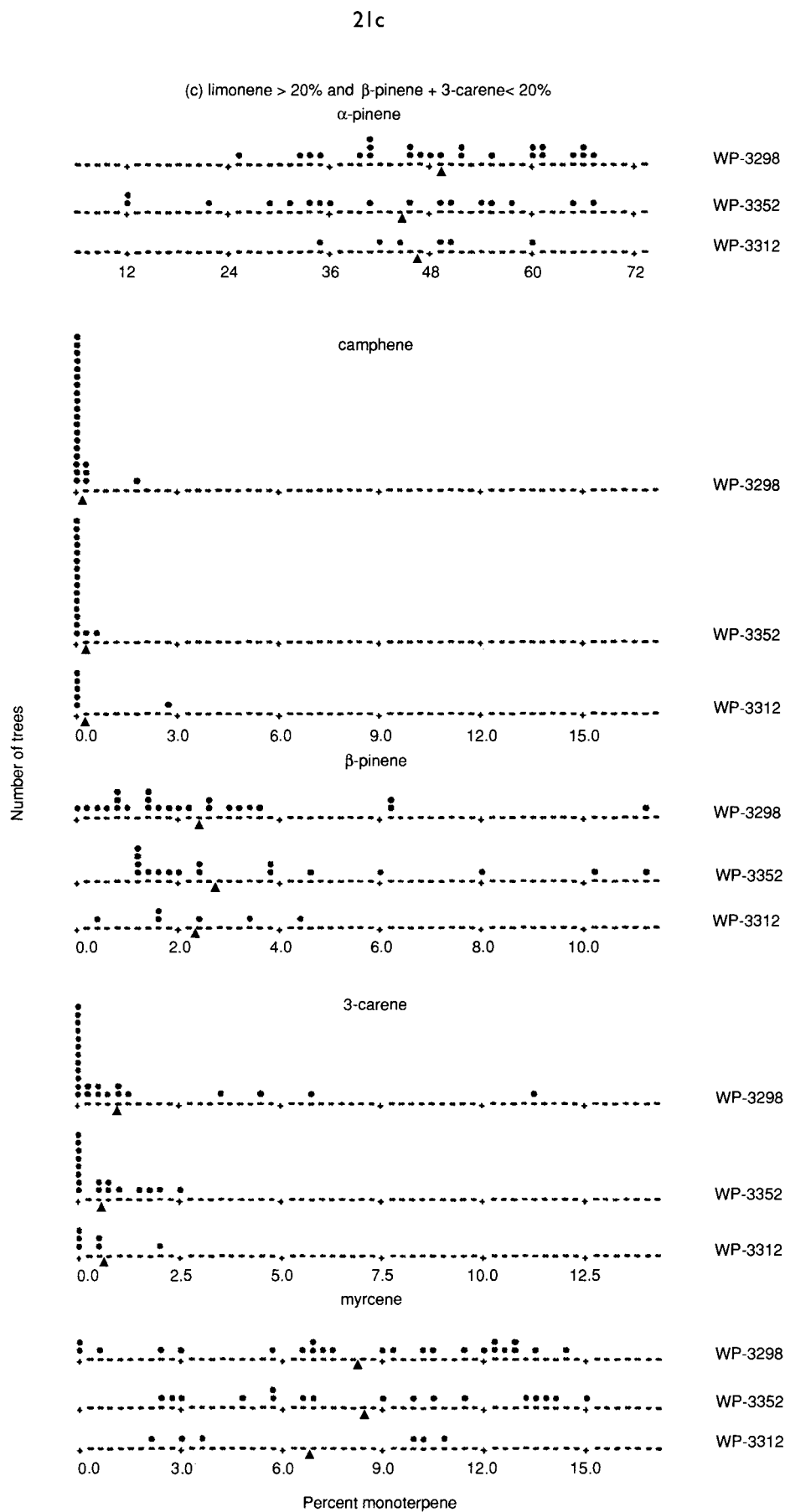
All eight trees had a total sample size of more than 70 progeny per parent tree, which was, therefore, sufficient for comparison of frequency distribution of each of the eight components (fig. 5-23). The very large range in the amount of the major components— α -pinene, β -pinene, 3-carene, myrcene, limonene—





21b

 β -pinene + 3-carene < 20%



21c

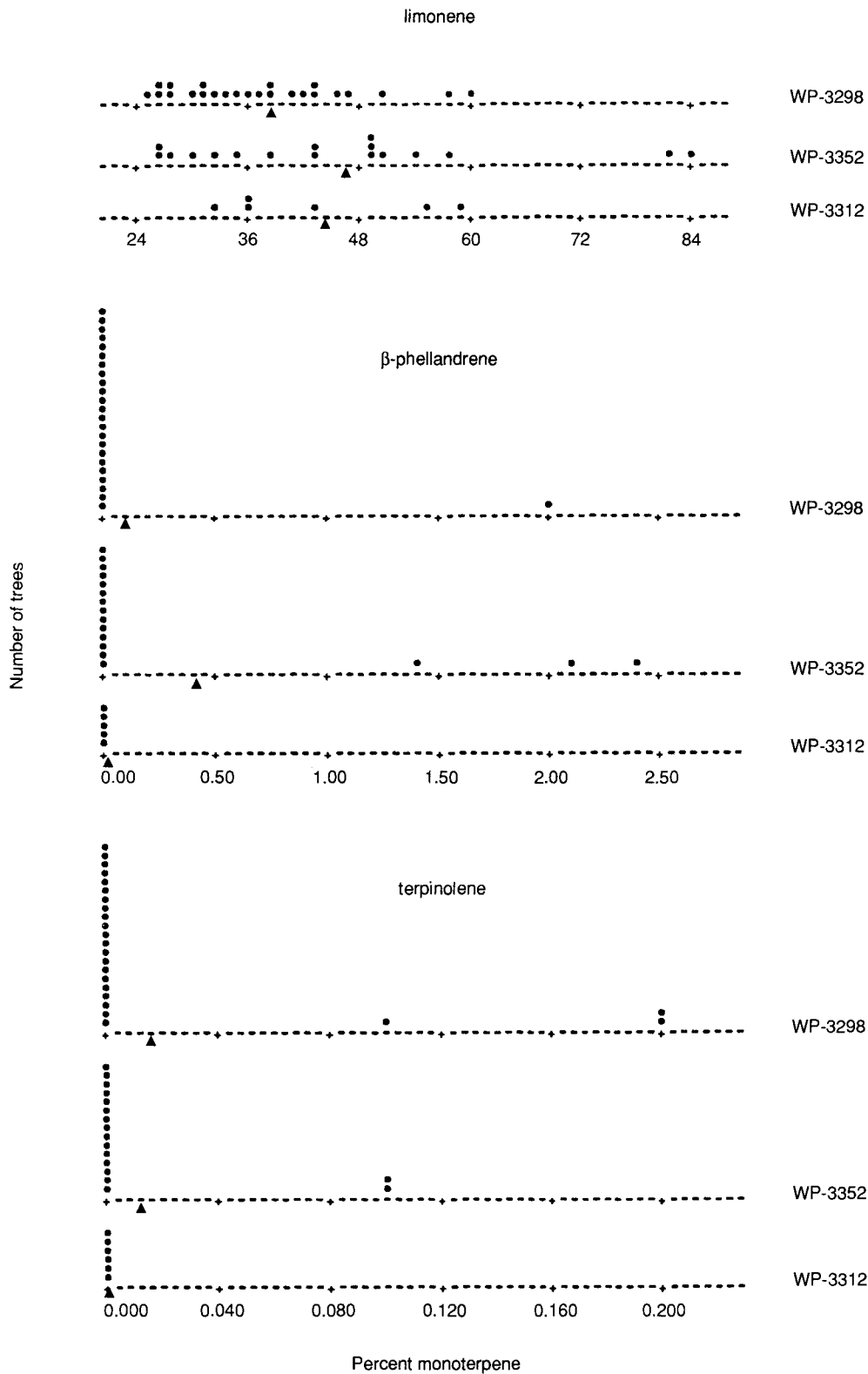
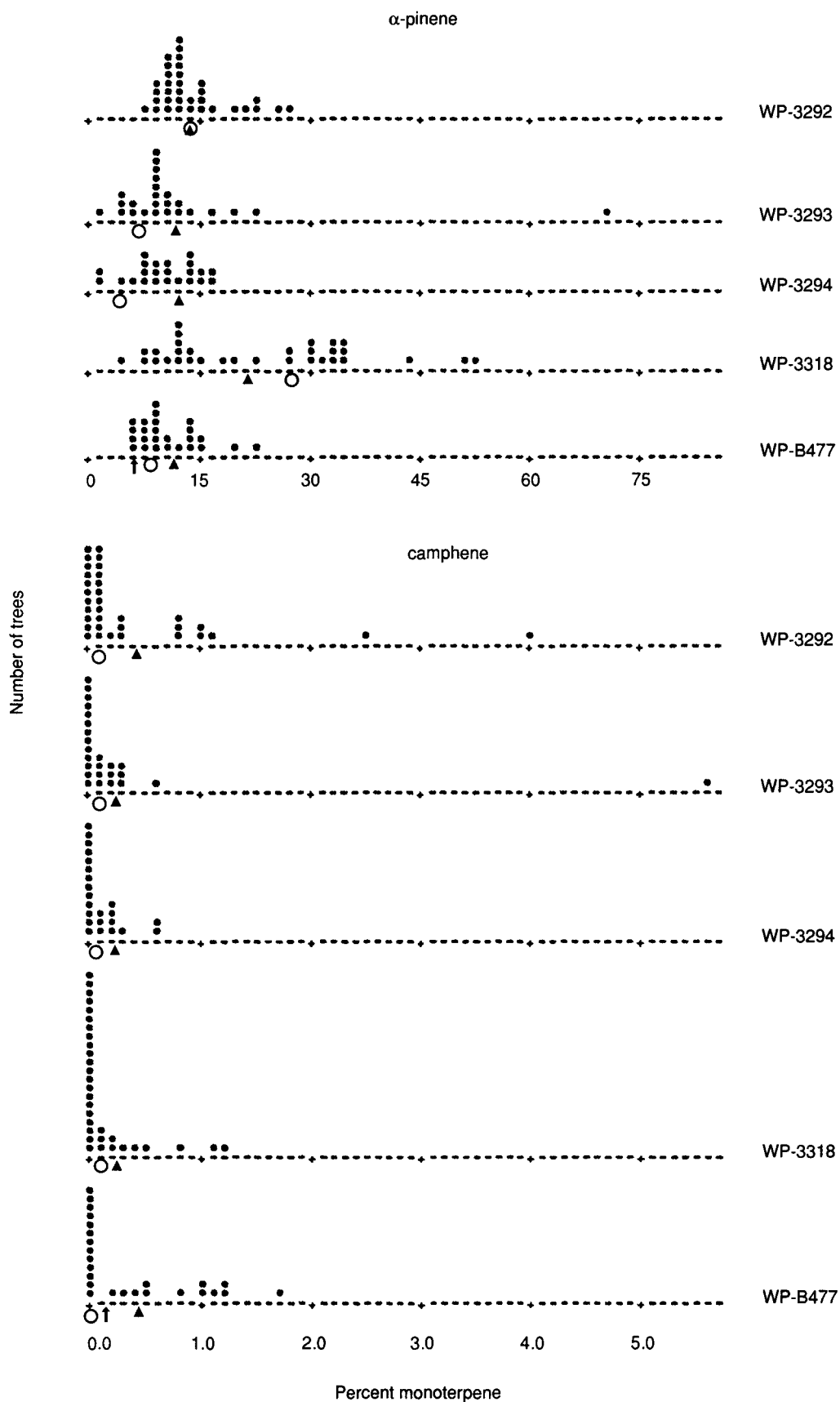
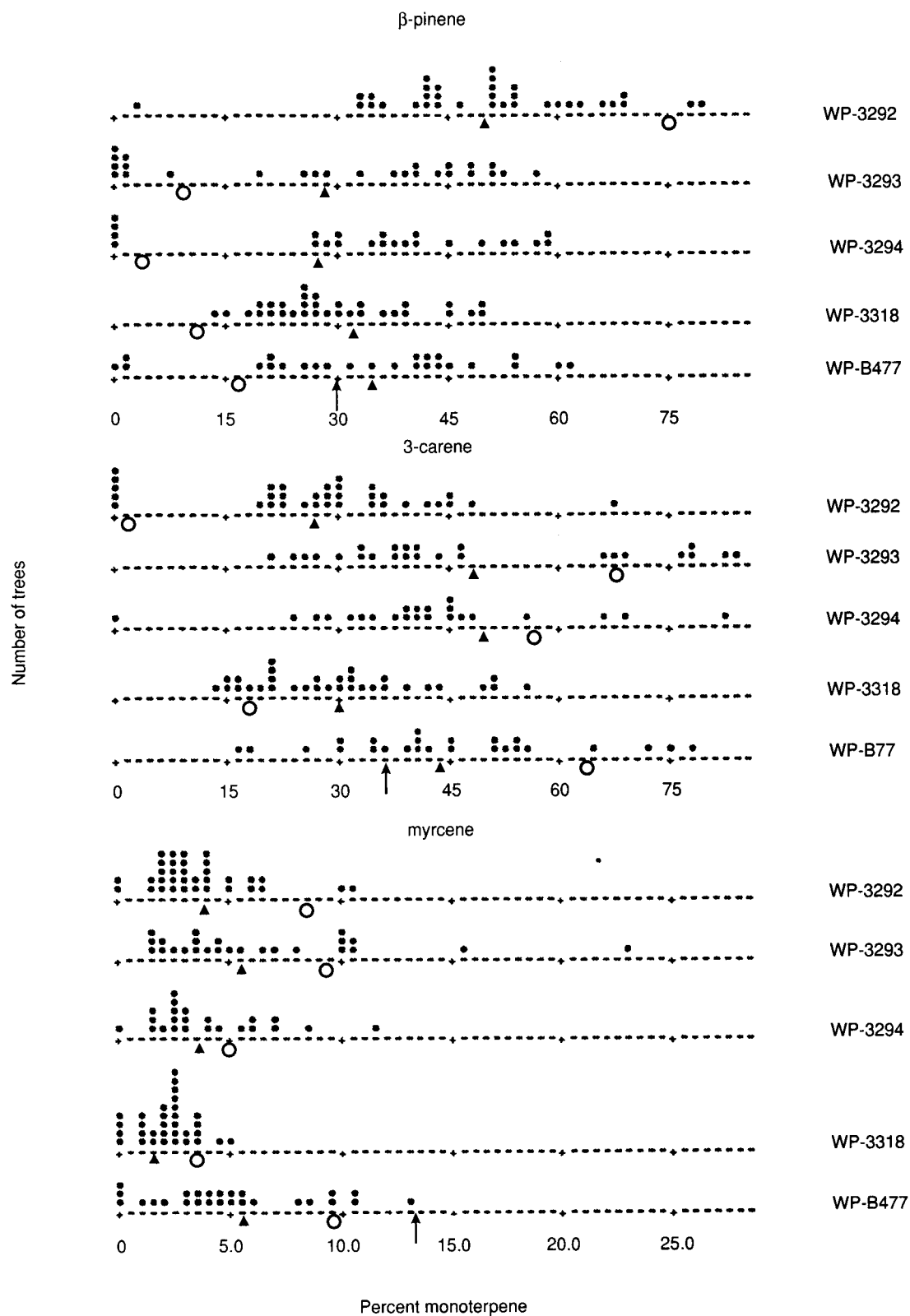
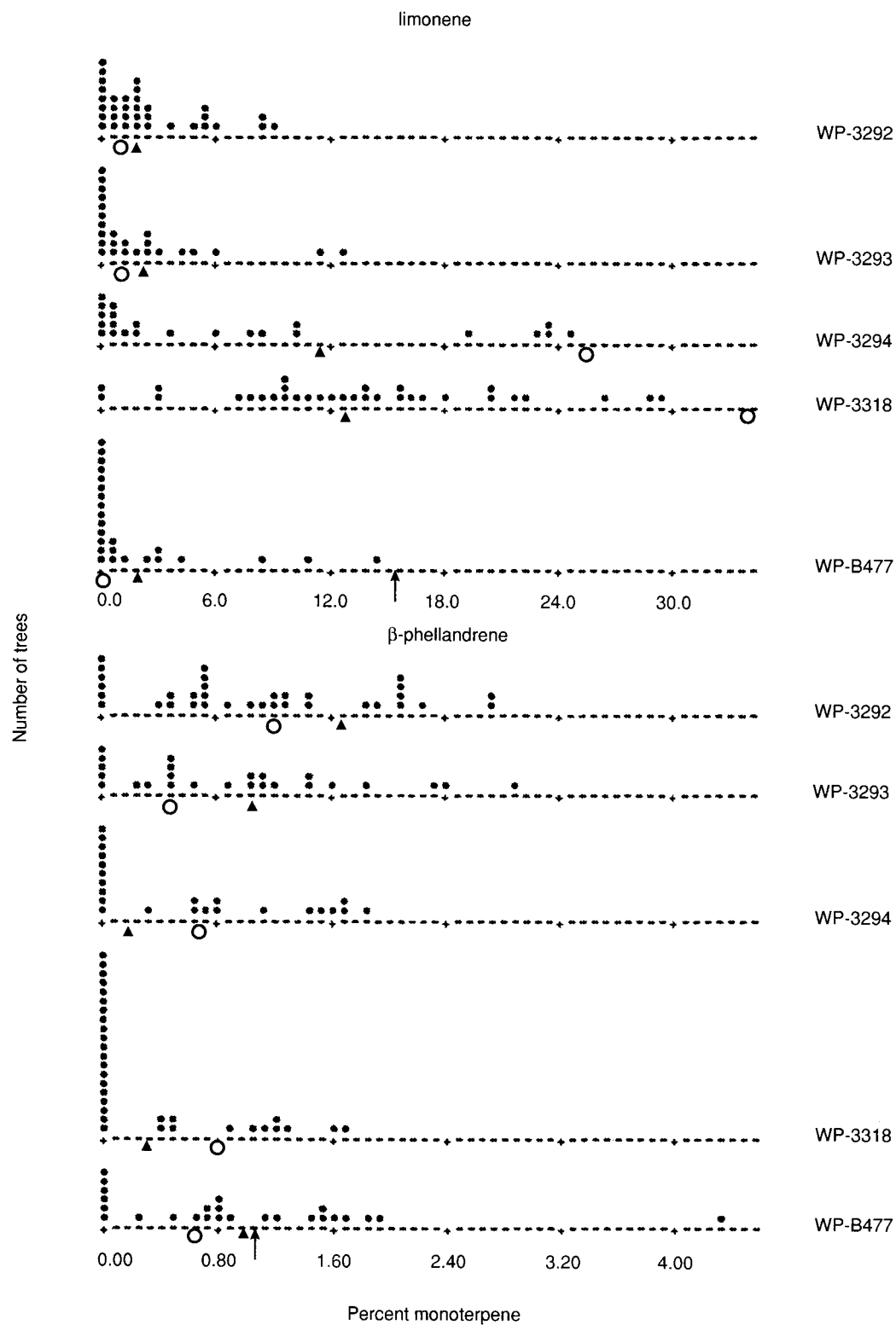
(c) limonene > 20% and β -pinene + 3-carene < 20%

Figure 5-22—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* WP-3292, WP-3293, WP-3294, W P-33 18, W P-B477; each dot is measurement of one tree; [Δ] is average of progeny set; $\circ$ = composition of parent tree; $\downarrow$ is 80-tree stand average.







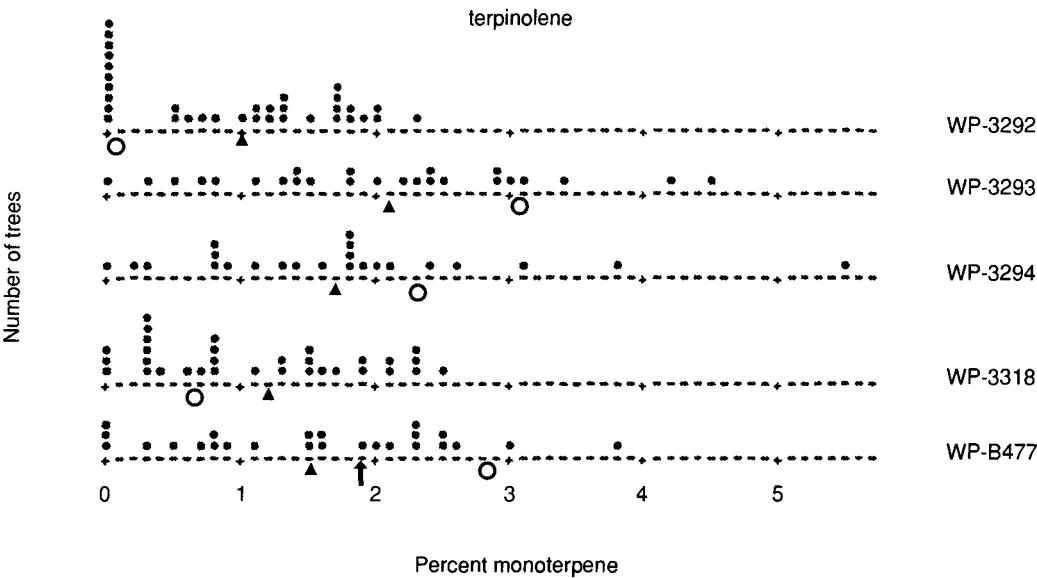


Table 5-10—Differences in the average amount of five major monoterpenes¹ between progeny and female parent and between progeny and stand average at Wheeler Peak

Progeny of	Direction ² of amount in progeny from amount in:									
	female for					stand for				
	α -p	β -p	car	myr	lim	α -p	β -p	car	myr	lim
WP-3292 ³	o	+	+	-	+	+	+	-	-	-
WP-3293	+	+	-	-	+	+	o	+	-	-
WP-3294	+	+	+	-	-	+	o	+	-	-
WP-3318	-	+	+	-	-	+	+	-	-	-
WP-B477	+	+	o	-	+	+	+	+	-	-

¹ α -pinene, β -pinene, 3-carene, myrcene, limonene.
² - = progeny less than female or stand.
+ = progeny greater than female or stand.
o = progeny about same as female or stand.
³ very low limonene.s

was readily apparent. A strong tendency for multimodal distributions, generally trimodal, was discernible in many distributions; this supported the hypotheses of two additive alleles at a single locus for each major monoterpene. The narrow mode between 0 and 2 to 3 percent for β -pinene, 3-carene, myrcene, and limonene was presumed to be caused by isomerization or biosynthetic by-products, by a minor genetic structure, or by a slight bit of activity by the (0-0) set of alleles. The larger near-0 percent mode for α -pinene could be a mix of the above or activity by the (0-0) set of alleles. There was a strong tendency for α -pinene to have a double mode between 0 to 20 percent which suggested that all the mechanisms noted above may operate. Therefore, in general, the differences in the composition of the female parent was reflected in the composition and frequency distribution of its wind-pollinated progeny and the general background pollen. However, it was readily apparent that the level of myrcene and limonene in progeny was well below the level of the parent tree, that the level of α -pinene and β -pinene was generally well above the parent except where the level in the parent tree indicated a (1-1) set of alleles in the parent, and that the level of 3-carene was about at the level of the parent or slightly below. This condition was presumed to be caused by the juvenile factor or the stress of growing far beyond its seed source and resin zone, or both. With age, these levels may slowly move toward the expected ones. Nevertheless, these data were adequate for comparative purposes. It was also readily apparent that the level of one component could affect the level of one or more of the other components.

Types of Composition

Thirty-seven types of composition were found among the progeny of the 13 parents (fig 5-24). The primary objective of the study was to produce trees with a large and diverse array of compositions; this was clearly accomplished. Types with large amounts of limonene were, as expected, found among the progeny of parents classed as high or near-high limonene. The ultra-high type was expected and two were found; both were progeny of WP-3352. These two were of note because they were very much like the ultra-high types found in the progeny from Ranger Peak (fig. 5-4) and Adin/Placerville (fig. 5-6). There were no ultra-high types among the progeny of WP-3298, when such was expected. This ultra-high type also resembled the composition of *P. torreyana* (see Chapter 3, #18) except that *P. torreyana* had small amounts of nonane and undecane. There were several types of composition that had not yet been found in natural stands. Were these types valid, or artifacts of the effects of stress and of the juvenile factor, or are they valid and will not survive the environmental pressures by maturity? The types of composition also show the very large range in the amount of the five major components: α -pinene 3 to 89 percent, β -pinene 0 to 91 percent, 3-carene 0 to 86 percent, myrcene 1 to 50 percent, limonene 0 to 86 percent.

Discussion: Half-Siblings *P. ponderosa*

Young progeny from Ranger Peak, Adin Summit, and Wheeler Peak had a somewhat different composition than would be expected from the composition of the female parent and the general background pollen. This difference was primarily attributed to a juvenile effect that was expressed by an increase in β -pinene and α -pinene and a decrease in myrcene and limonene with 3-carene either unaffected or inconsistent. Likewise, the juvenile effect was most evident in stands where β -pinene or α -pinene or both were high, much as in the stand at Ranger Peak. Because of the changing juvenile effect, half-siblings from Ranger Peak were not as useful as anticipated in understanding monoterpenes, but the results were quite helpful in guiding studies of progeny from Wheeler Peak and Adin/Placerville.

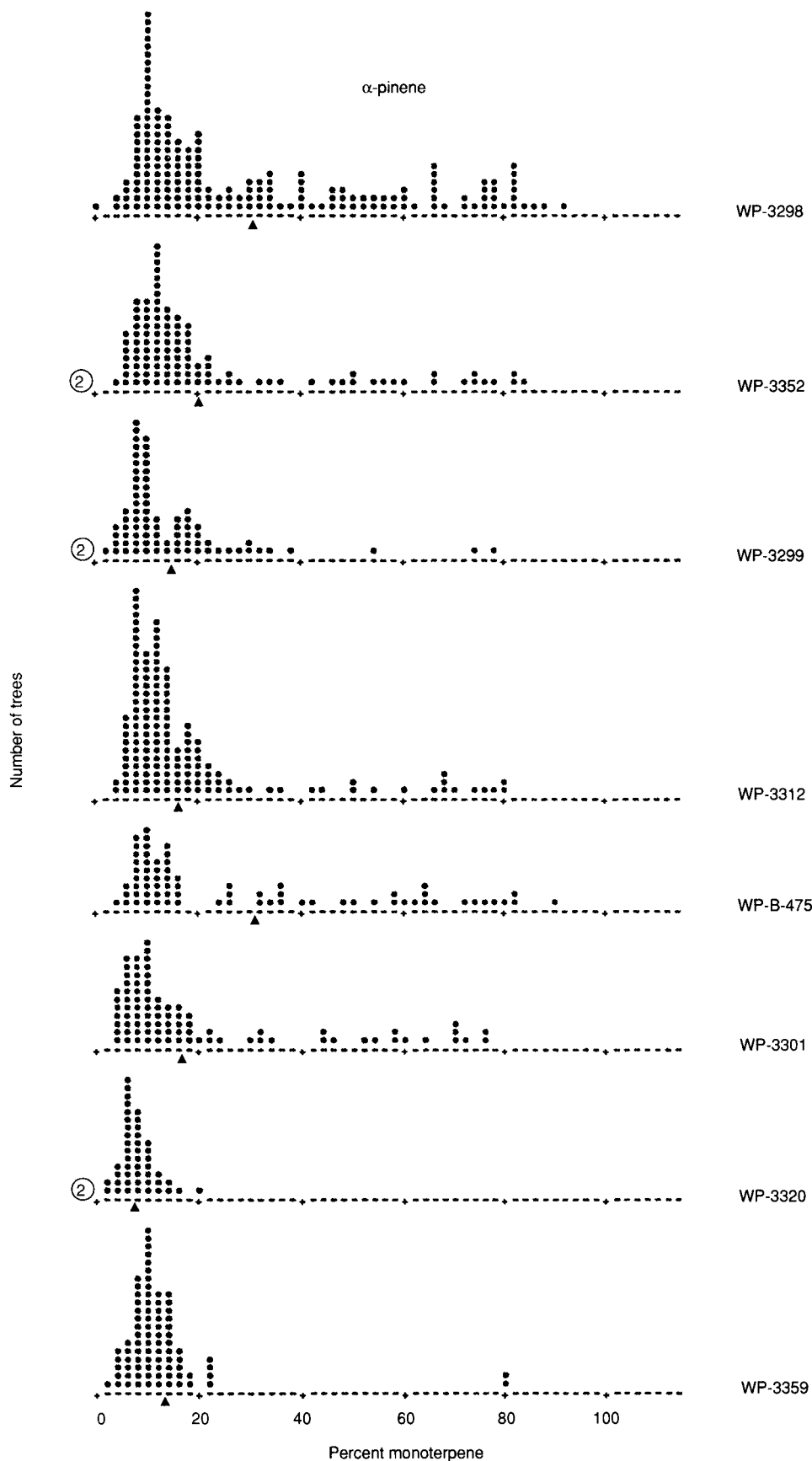
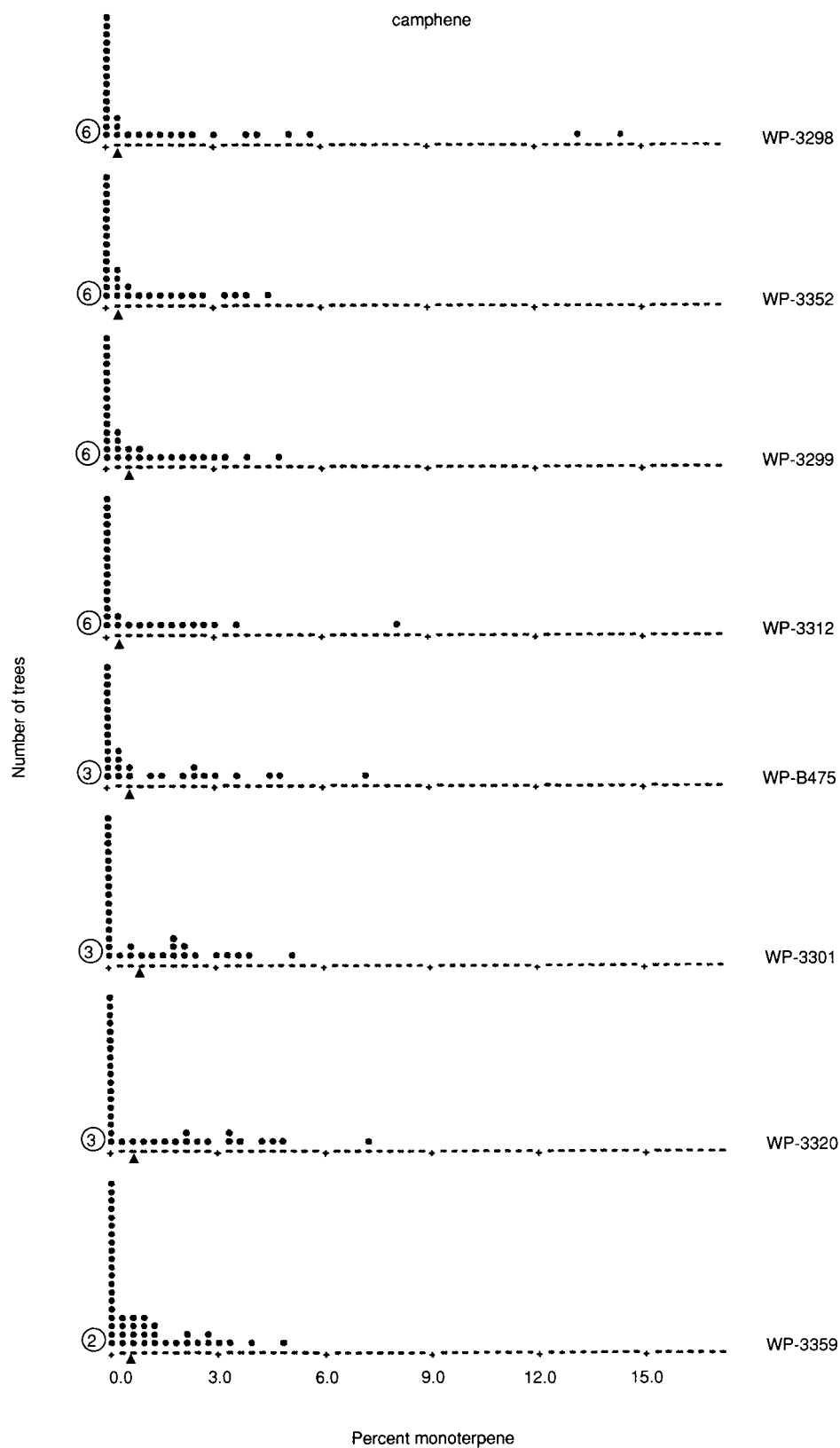
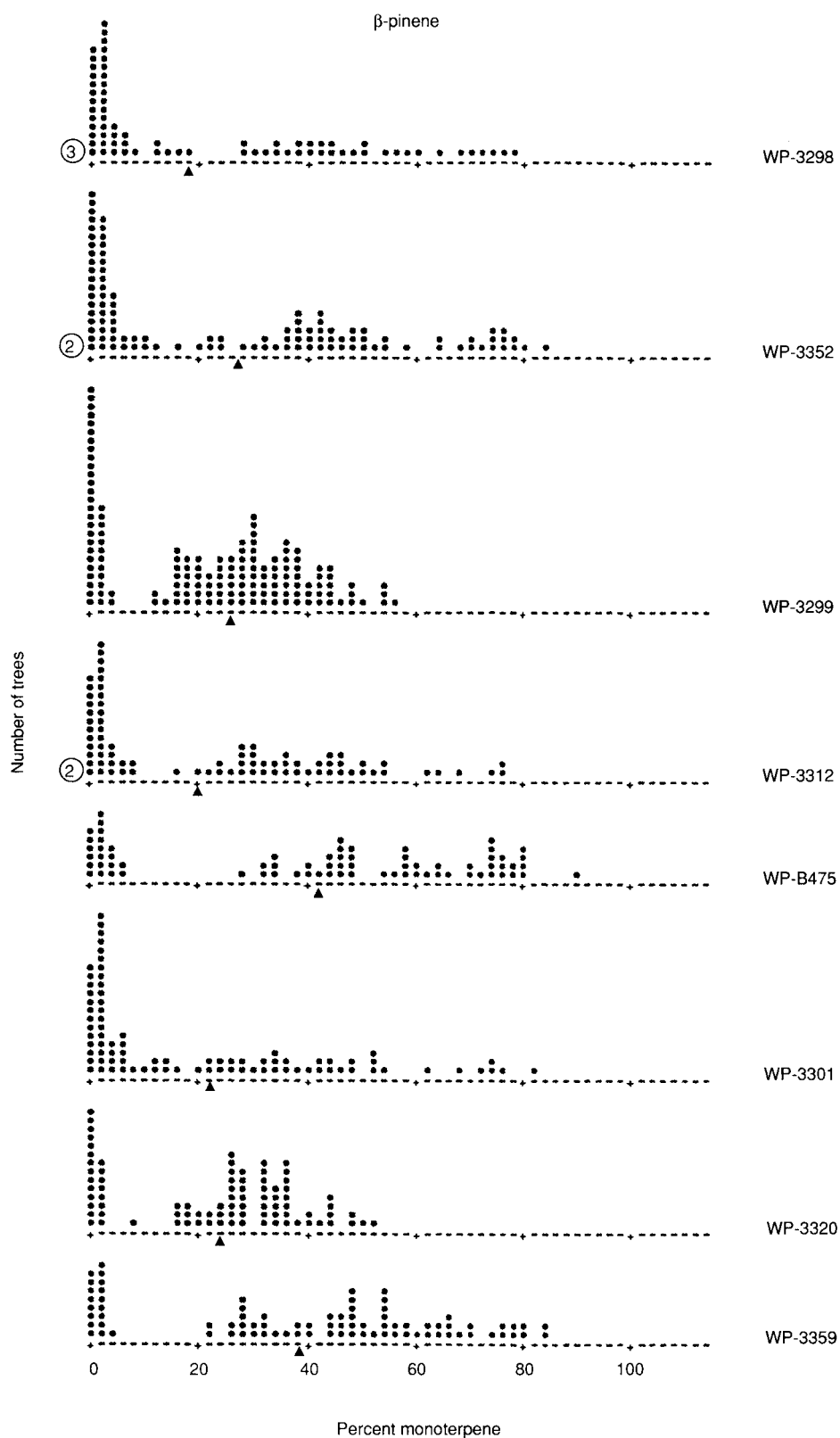
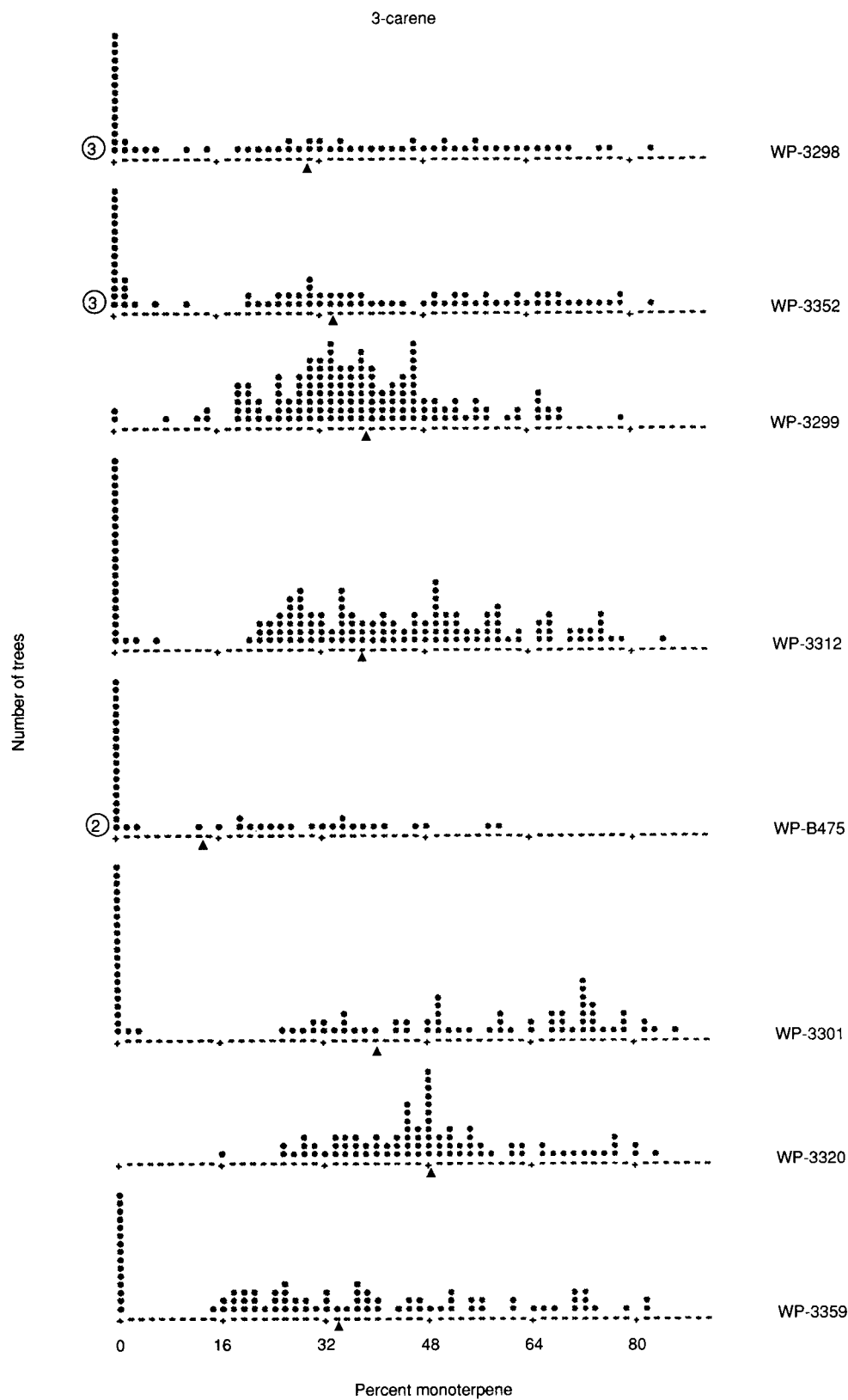


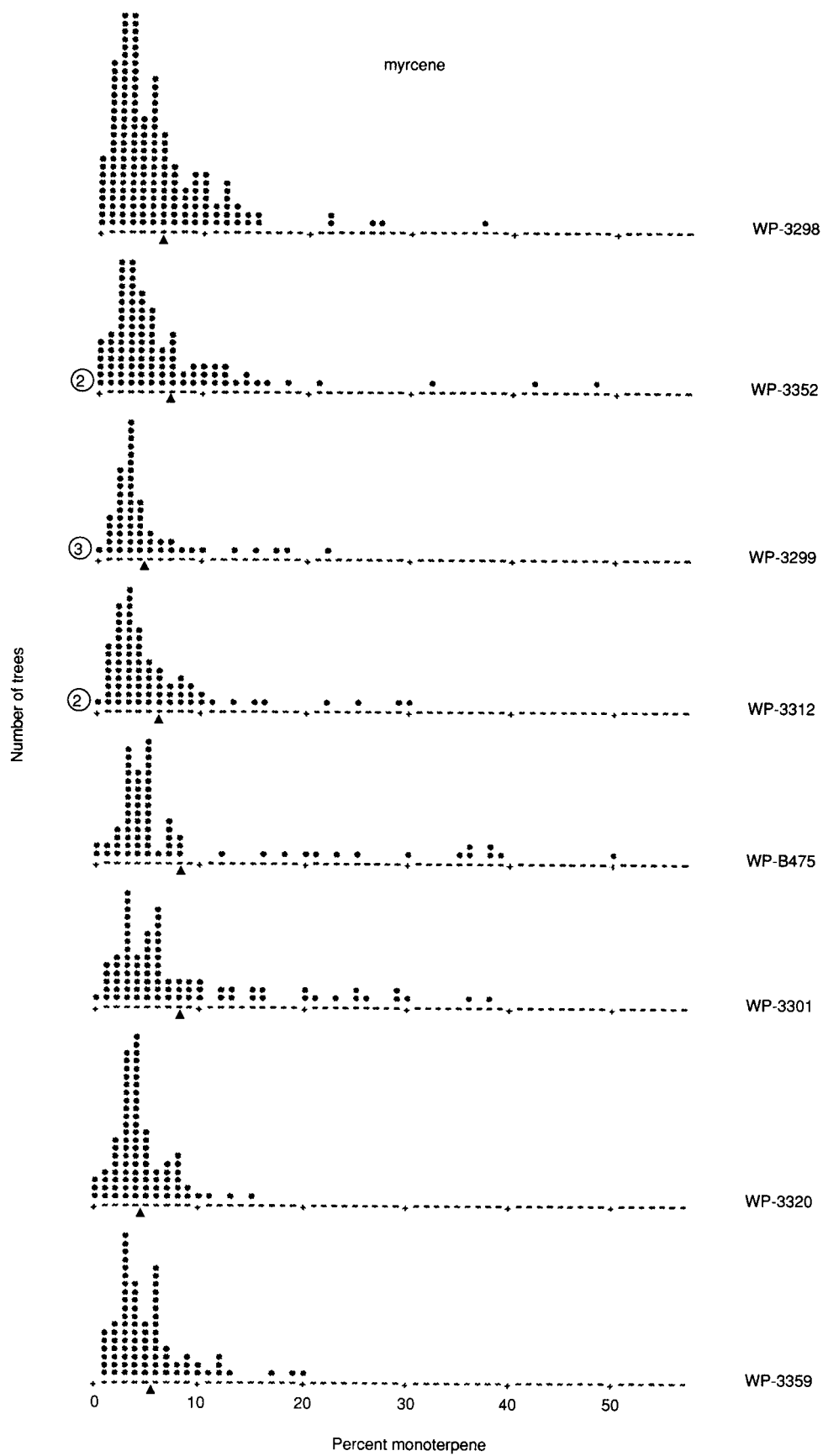
Figure 5-23—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of the eight female parent *P. ponderosa* listed on the right; each dot is $n(\bar{n})$ surement of one tree except where indicates each is n trees; [▲] is average of set; ↓ is 80-tree stand average.

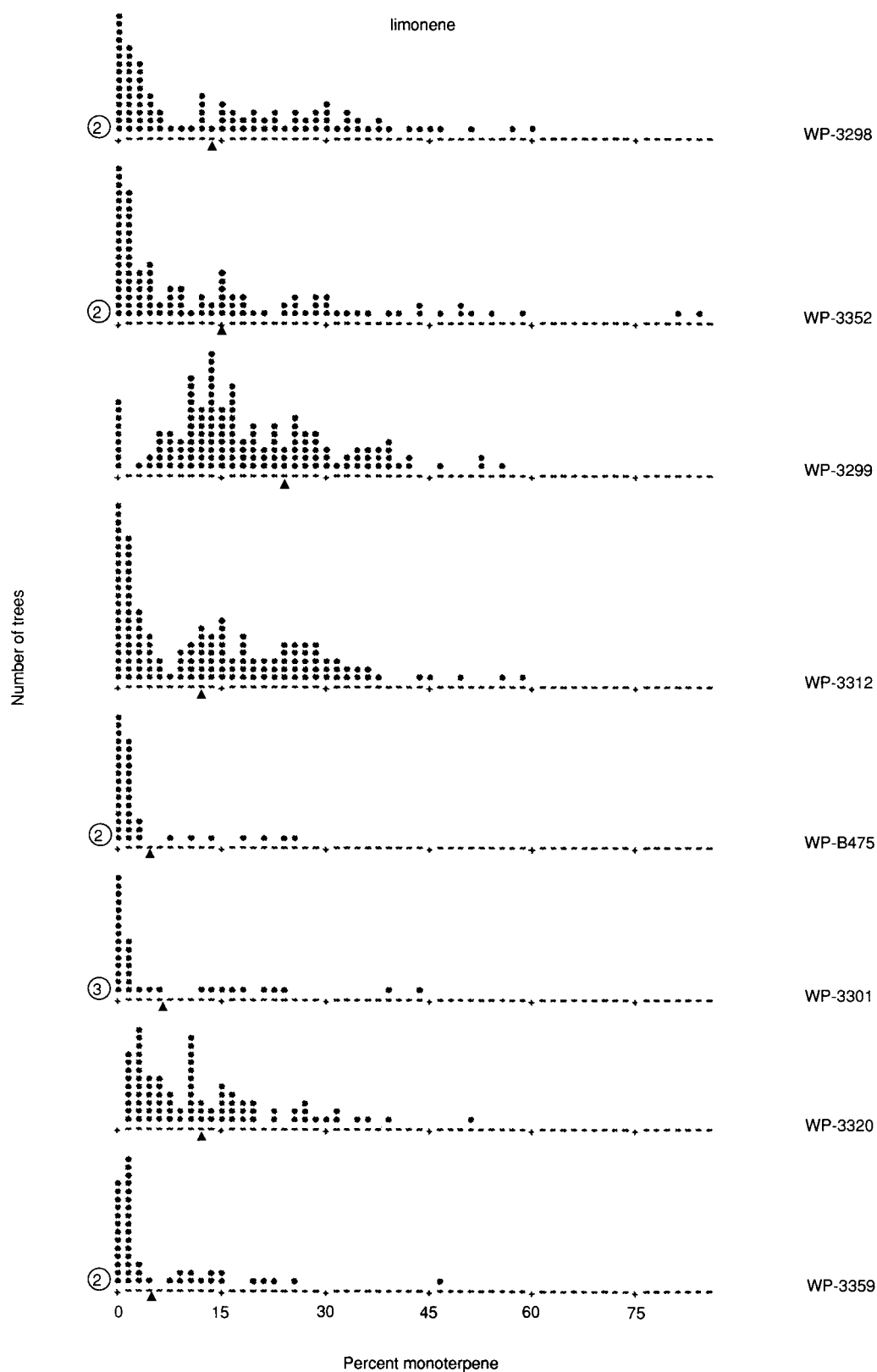
(fig. 5-23 continues on the next 7 pages)

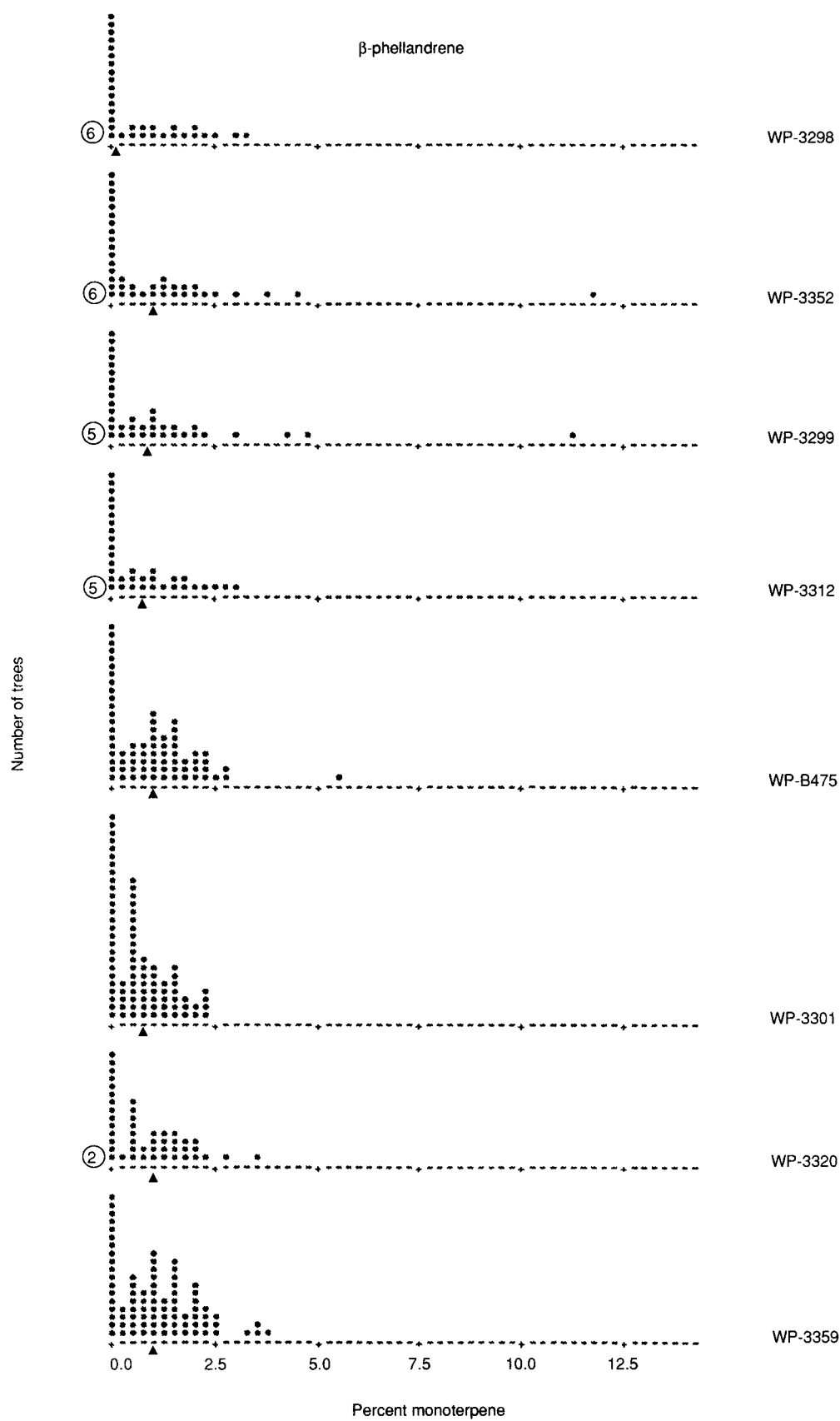












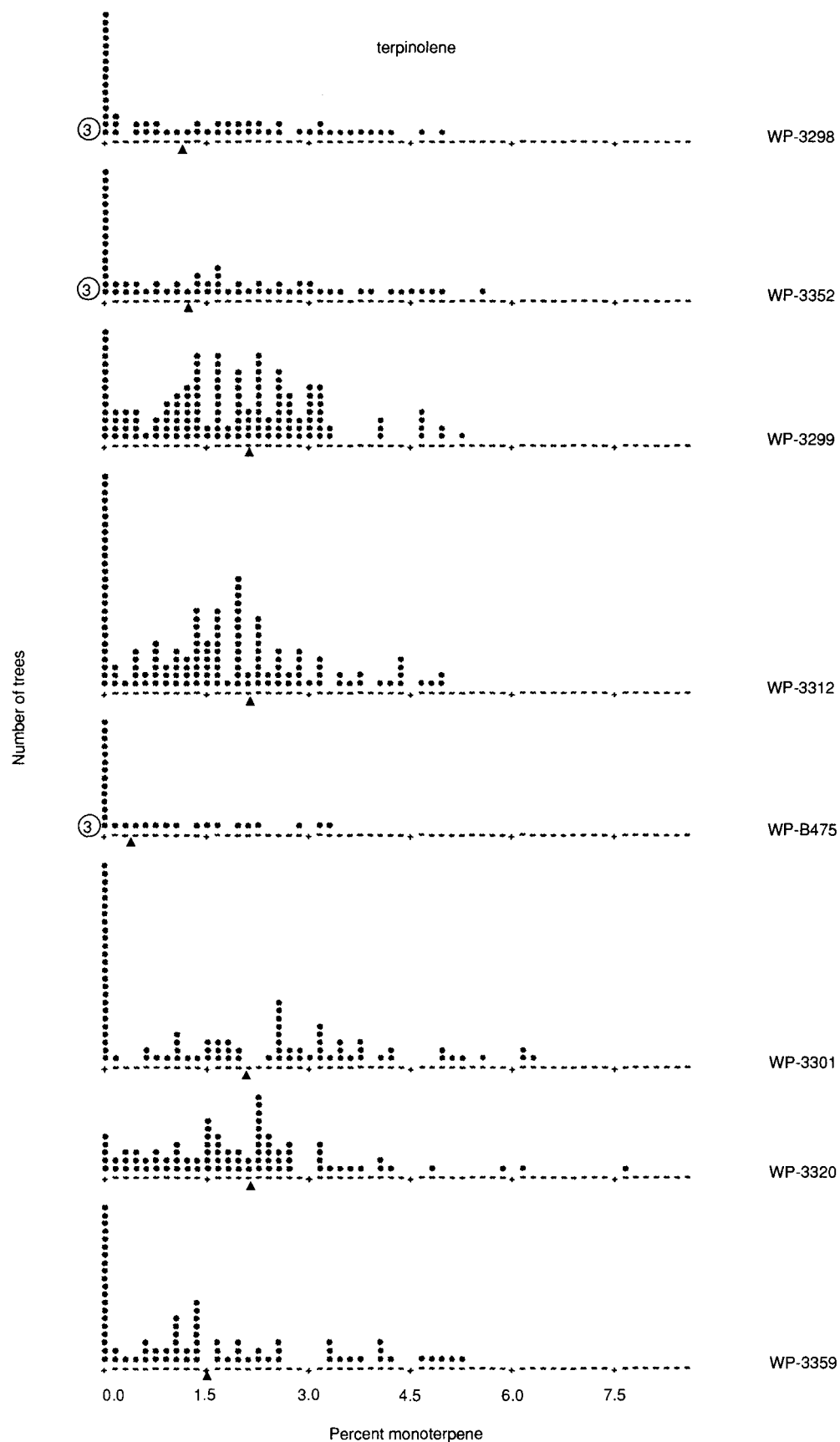


Figure 5-24—Thirty-seven types of monoterpene composition in the progeny of the 13 female parent *P. ponderosa* at Wheeler Peak, their average composition, minor components omitted, and their relative occurrence in each half-sibling family; [●] occurs commonly; [○] occurs not commonly.

Type #	α -p	Monoterpene ¹				3298	3352	3299	3312	3474	3301	3320	3359	3292	3293	3294	3318	β -477
		β -p	car	my	lim													
1	11	0	0	3	83		○											
2	35	2	1	4	59	○	○		○									
3	4	0	39	4	50			○	○			○						
4	32	6	1	15	47	○	○	○										
5	32	14	1	13	40						○							
6	7	21	31	3	37			○										
7	56	2	1	10	30	●	●											
8	8	1	54	9	27	●	●		○	○	○							
9	71	2	0	0	25				○	○	○					○		
10	9	59	0	6	25											○		
11	7	34	34	4	20	●	●	●	●									
12	20	8	45	4	19	●	●						○					
13	25	47	8	3	16			●									●	
14	9	20	49	5	15												○	
15	14	73	0	11	11													
16	8	1	61	15	11							○						
17	11	44	28	4	11								●				●	
18	10	39	41	1	9											●		●
19	6	32	48	3	7							●	●	●				
20	89	0	0	4	7	●	●			○								
21	20	45	47	1	7			●							○			
22	13	57	24	1	4	●	●			●		●						
23	17	73	0	6	2				●		○		○					
24	39	32	22	4	1					●							●	
25	4	51	20	25	1													
26	23	28	41	4	1								●					●
27	10	0	76	9	0				●		●	○	○	●	●	○		●
28	57	4	0	33	0					○								
29	35	58	0	5	0			●		●	●			●				
30	7	91	0	1	0					●								
31	34	2	60	4	0	●	●				●							
32	3	11	86	1	0			●				●						
33	46	13	1	38	0						○							
34	80	0	0	20	0	●	●	○			●		○					
35	70	0	26	4	0										○			
36	52	22	26	0	0												○	
37	43	6	0	50	0					○								

¹ α -pinene, β -pinene, 3-carene, myrcene, limonene

The juvenile effect was present in the progeny of trees from Adin Summit and Wheeler Peak, but did not seem as strong as at Ranger Peaks. There was little change in composition during 12 years of nursery or container growth. Therefore, the data on these trees was considered adequate for statistical analyses. There was some slight evidence that the juvenile effect was weakening at 12 years.

The primary objectives of the study were to produce a diverse array of compositions of monoterpenes for studies of host resistance and for monoterpene studies; the results clearly showed that this objective was achieved. It was also quite clear that the quickest and most economical procedure to obtain such trees, including high limonene trees, would be to establish a plantation of wind-pollinated progeny of high- and near-high limonene parent trees from an area with a wide range of compositions in the native stand, such as Wheeler Peak or Adin Summit. For efficiency of land space, such a plantation could be planted at a 3-ft by 3-ft spacing, sampled for resin at 8 or 10 years, and then thinned to about a 6-ft by 6-ft spacing to obtain a residual stand with desired compositions. A controlled breeding program, as will be shown later in the discussion of full siblings, can achieve the same objectives with greater predictability but requires longer time and at a much greater cost. The chance of getting ultra-high limonene is much greater with controlled breeding.

The variety and range of the composition types found in the study and of the range of the frequency distribution of individual components were much greater in the aggregate of the progeny than in the natural stand, and the question might be raised about such difference, particularly when one considered the small number of parent trees in the study (and these were chosen largely on the basis of high and low limonene). Two answers come to mind. (1) The compositions were a juvenile state of the progeny population, and before maturity the individual tree compositions will slowly change with a resulting decrease in the variety of compositions. (2) Many types, by themselves or in conjunction with related tree characteristics, will not survive.

There were some indications again that the amount of β -pinene could be a major factor for the variation and instability in the composition of young seedlings, either by itself or in conjunction with other conditions.

There was considerable evidence in these studies of 4- to 12-year-old half-siblings of *P. ponderosa* that the quality and quantity of the xylem monoterpenes were under strong gene control. The composition of the half-sibling progeny of parents with similar gene composition was very similar in both averages and in the configuration of the frequency distribution of individual components; the composition of half-sibling progeny of parents with dissimilar composition was different, but predictably so, considering the parent composition and the stand average composition that was the presumed composition of the pollen parents. However, several conditions can modify monoterpene composition as follows: (1) stress from the over application of a phytocide, (2) stress of prolonged growth in restrictive containers, (3) the juvenile period of growth, (4) stress of rapid growth, (5) the actual composition of the tree itself, particularly large amounts of α -pinene and β -pinene, (6) interactive competition among components for a fixed amount of biosynthetic precursor, (7) isomerization, (8) biosynthetic by-products, (9) growing far from the seed source, (10) and the possibility of a minor genetic structure. The possible action of these conditions separately or in concert dictates the use of a large number of samples and the maintenance of studies for extended periods.

The multimodal characteristics of the frequency distributions of individual components taken as a whole strongly suggested that the primary genetic structure for monoterpenes in *P. ponderosa* xylem resin is a pair of additive alleles at a single locus for each of the five major monoterpenes: α -pinene, β -

pinene, 3-carene, myrcene, and limonene. However, the amount of these major monoterpenes can be modified considerably by the juvenile effect and by stress factors noted above. And there may be a minor genetic structure that might be associated with the geographic source of the tree, but such a structure has not been resolved, if it does exist. Also, these major components might possibly be produced in small quantities by either isomerization or biosynthetic by-products. Minor components were also probably produced largely by the same two factors. Myrcene was considered a major component because it did occur at a sufficient percentage, though infrequently; it usually occurred in minor component amounts.

When a parent was grown in two locations more than 200 miles apart but in the same resin ecotype regions, the compositions of the two sets of progeny, i.e., one set from the parent at each location, were nearly identical.

The compositions of the half-sibling progeny from two different high limonene parents, having very similar composition and growing in two different resin ecotype regions, were still different enough to raise the questions of the differential effect of the two localities, the possibility of a minor genetic system, or the growth of one set of progeny more than 400 miles from its source and in a different ecotype region.

The incidence of an unusually large amount of camphene (2 to 4 percent), considered a minor component, in the 6th year of growth in the progeny of most trees, was considered an anomaly. This anomaly occurred in different calendar years of the study and in various nursery plantings but always during the 6th year of growth. It was possible that the anomaly may not be camphene but some unknown, transient chemical which chromatographs as camphene, though all the GLC evidence points to camphene. This anomaly was not large enough to measurably change any of the major monoterpenes or their analysis.

A type of composition called ultra-high limonene was found among the progeny of the three high-limonene trees used in the study, RP-B37 at Ranger Peak, AS-3139 at Adin Summit, and WP-3352 at Wheeler Peak. This type of composition was not at all common in the study and had not yet been found in natural stands. Whether any exist is not known; and "if not, why not," were unanswered questions. Types with high limonene, as expected, were found among the progeny of high limonene parents, including the three noted above as well as in the progeny of WP-3298, WP-3312 and WP-3299.

No marked change in quantitative composition was found with increase in age from 4 to 12 years. Thus the data were suitable for analyses and inference on a comparative basis but not on an absolute basis. (It appears that the age phenomenon found in the Ranger Peak progeny may be restricted to that locality or the types of composition found in that locality.) However, a juvenile effect was present in the Wheeler progeny, but this did not affect analysis. It is postulated that there will be a slow change over many years to the composition of the mature tree. This juvenile effect seemed to be manifested in an increase in α -pinene and β -pinene and a decrease in myrcene and limonene. These same changes seemed to be associated with various forms of stress. Thus one might postulate that all young pines are under some degree of stress which might be called the stress of rapid growth.

Monoterpene Composition of Populations of 4- to 11 -Year-Old Full Siblings of *P. ponderosa*

Summary: Fifty-six sets of full-sibling populations of *P. ponderosa* were derived from combinations of seven females and 14 males. Forty-two sets of progeny had sufficient numbers for modal analysis of the frequency distribution of individual monoterpenes. In all cases the actual number of modes derived from the analysis equalled or exceeded the number of modes expected, based on the assignment of additive alleles to the two parents. In about the remaining half of the analyzed distributions the actual number of modes was the same as the expected. This result supported the additive allele hypothesis for the five major monoterpenes in *P. ponderosa* - α -pinene, β -pinene, 3-carene, myrcene, and limonene; myrcene, though, appears to be a rare allele. Where both parents were assigned a (0-1) set of additive alleles for a given monoterpene, the ratio of the size of the low-middle-high modes was generally about 1:2:1, the predicted ratio; this was additional support to the hypothesis and to the assignment of alleles.

Fifty-eight types of monoterpene composition were found in the full array of 56 families. High limonene trees always bred true, but a juvenile effect was clearly evident in the progeny and was evidenced by an increase in α -pinene and β -pinene and a decrease in limonene and myrcene. Other combinations of parents also produced high limonene progeny. Some progeny could be classed as ultra-high limonene (80 to 90 percent), a type of composition not yet found in natural stands. Other types of composition were found among the progeny which have not yet been found in natural stands. The large variation of composition of a single set of progeny suggested the effects of several nongenetic factors on the basic genetic structure, as well as the effects of interactions within the basic genetic structure for the synthesis of monoterpenes.

There was a noticeable difference between the progeny of a female with two males, one that was near and one that was far from the female. This was evidenced in much the same way as the effects of stress, and it was postulated that there was a level of stress in the progeny of parents which were far apart, or "off-site." The effects of several forms of stress were clearly evident in full siblings, and were often greatest in high limonene trees, and where parents were far apart.

Breeding on grafts produced the same array of progeny as breeding on the parent tree of the graft. Over an 8-year period there were no sharp shifts in the composition of sets of the same progeny in a given location, indicating that changes will be slow over many years: α -pinene and β -pinene decreasing, myrcene and limonene increasing, and 3-carene increasing or decreasing depending on the composition. An increase in camphene and β -phellandrene with age was postulated.

All observations were based on sampling different sets of trees from the same seed lot and not on resampling the same set of trees. There was no incompatibility between the various parent trees. Reciprocal crosses showed equal contribution by male and female.

The assumption that the quality and quantity of xylem resin monoterpenes of *P. ponderosa* were strongly fixed by a genetic mechanism gradually worked its

way into the literature. This assumption appeared to have been strengthened by its repeated use in the literature, often without much supporting research evidence. The assumption probably gained much support when Mirov (1961) reported very little change in the composition of *P. ponderosa* xylem monoterpenes over short periods of 3 to 5 years. In that same report, Mirov noted that the composition of pine hybrids was, generally, intermediate to the parents and that the composition of trees planted far from the seed source was similar to the trees at the source. More recently, composition was shown to be very constant in mature *P. ponderosa* with respect to radial, horizontal, and longitudinal position in the tree and with time (see Chapter 8 this volume).

Controlled breeding of seven female *P. ponderosa* with from 5 to 12 pollen sources each was conducted. The composition of all trees was listed, and a preliminary assignment was made of the alleles for the five major monoterpenes in each tree based on the monoterpene composition (*table 6-1*). The basic objectives of the breeding were: (a) to determine if a supply of high limonene trees could be conveniently established at the USDA Forest Service's Institute of Forest Genetics, Placerville, Calif., for testing the effects of limonene on the success or failure of the attacks of *D. brevicornis* on *P. ponderosa*, and (b) to develop a research supply of trees with a diverse array of xylem monoterpene compositions at IFG for elucidating the genetics and variation of monoterpene composition. The discussion was organized around the seven female trees.

A wide selection of parent tree compositions seemed desirable for the breeding study because the action of a number of competing or interacting alleles and the effects of several nongenetic factors caused increased variation in composition. A good selection of parents, though heavily slanted to trees with higher limonene, was used; on hindsight, an even wider selection would have been desirable. However, the trees used were the best that could be obtained under the operational and fiscal constraints of the study.

Parent trees at Adin Summit were resampled at different locations on the tree and at different times in order to determine if the compositions were constant through time and space, or if the unusual compositions were spurious and invalid as others had claimed.

Breeding was carried out from 1972 to 1980. Trees that served as females were located at Adin Summit and at IFG; and grafts of Adin Summit trees, now growing at IFG, also served as female trees (*fig. 5-1*). Twenty or more grafts were attempted for several of the key trees at Wheeler Peak, the source of many of the pollen parents, but none survived. This failure prevented the desirable reciprocal crosses. The first efforts to collect pollen from the Wheeler Peak trees failed in 1970 because of improper timing. It was later found that the time and characteristics of catkin development were quite different at Wheeler Peak in eastern Nevada than on the western slopes of the Sierra Nevada in California. But a good supply was obtained in 1971 for use in 1972, and afterwards, when conelets were first available at Adin Summit. No pollen was collected from the high limonene tree at Ranger Peak (see Chapter 5), and unfortunately no breeding was done with this tree.

Adin Summit was an interesting and valuable study area. It had two high limonene trees, four trees with a large amount of limonene—but not enough to be classed as high limonene—and one tree with only an average amount of limonene. One of the high limonene trees was found in the original 80-tree stand used in the regional study of *P. ponderosa*. Two additional high limonene trees were found in a later sampling of 1600 trees in the area. Thus, on the average, there was one high limonene tree per about 500 in the area; yet, quite fortuitously, one such tree was found in the original 80-tree sample. Several near-high limonene trees were also found in the later sampling of 1,600 trees at Adin Summit.

The procedure for controlled pollination was that generally employed by IFG for all pines and is briefly described as follows (Anonymous 1948,

Table 6-1—Xylem monoterpene composition of the 16 listed *P. ponderosa* used as parents in the controlled pollination program, their diameter in inches, and the preliminary assignment of additive alleles to them for the major components

Tree no.	Monoterpene ¹									Assigned alleles ²				
	α -p	cam	β -p	car	myr	lim	β -ph	ter	Diam	α -p	β -p	car	myr	lim
	-----percent ³ -----									in.	-----number-----			
AS-3139	18	0	1	0	22	59	1	0	25	1	0	0	0	2
AS-3178	3	0	1	55	18	19	*	2	15	0	0	2	0	1
AS-3187	9	*	23	23	14	29	1	2	15	0	1	1	0	1
AS-3194	7	0	6	33	13	39	*	2	14	0	0	1	0	1
AS-5789	23	*	10	0	29	37	*	0	14	1	0	0	1	2
AS-A78	22	0	3	0	18	57	0	0	6	1	0	0	0	2
AS-A95	19	0	21	0	15	45	0	0	12	1	1	0	0	2
AS-A295	13	*	3	32	19	32	*	1	15	1	0	1	0	1
WP-3298	26	*	*	0	16	58	0	0	18	1	0	0	0	2
WP-3299	6	0	*	43	8	40	1	2	16	0	0	1	0	2
WP-3312	5	0	7	34	4	48	1	1	12	0	0	2	0	2
WP-3318	27	*	10	18	3	40	0	1	14	1	0	1	0	1
WP-3347	4	*	17	25	3	48	1	1	18	0	1	2	0	2
WP-3352	24	*	*	0	18	57	0	0	14	1	0	0	0	2
BL-6074	7	*	11	0	27	56	0	0	24	0	1	0	0	2
IFG-4B63	10	*	54	0	11	23	2	0	30	0	2	0	0	1

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, G3-phellandrene, terpinolene.² α -pinene, β -pinene, 3-carene, myrcene, limonene.³Normalized; * = trace.

Cumming and Righter 1948). Shoots were bagged with standard filter paper bags with a glassene window in early spring as soon as buds showed evidence of female flowers: in early- to mid-April at IFG and early- to mid-May at Adin. When the female conelets reached the receptive stage, each bag was given a shot of 1.0 cc of the designated pollen using a hypodermic syringe. The puncture hole made by the syringe was sealed with tape. Pollen was injected from early- to mid-June at Adin and mid- to late-May at IFG. Because the flowers on a single tree do not develop at the same rate, it was often necessary to work with a single tree for several days, and to work with a group of trees for a week or two. The uncertain spring weather at both locations, particularly at Adin Summit, and the normal biological variation of flowering also tended to stretch out the development period of the female conelets.

Pollination bags were removed, when conelet scales had been closed for about 3 weeks, to allow the shoot to grow freely, and an insecticide was applied when the bags were removed.

Early the next spring, cloth cone bags were placed over the shoots bearing the control pollinated conelets, both to protect the cone and to catch seed if seed drop was earlier than anticipated. From much experience by IFG personnel, it was found that cone bags cause little or no decrease in cone or shoot development. It should be noted that cones of *P. ponderosa* require two growing seasons to mature.

Cones were collected between mid-August and mid-September and were taken to IFG where seeds were extracted and stored at about 1 °C. Cones from five trees at Adin were recorded for seed production to determine the effect of the bagging and possible incompatibility between Adin and Wheeler trees which were about 400 miles apart. Seed stratification, establishment of nurseries and plantations, acquisition of resin samples, and GLC analysis are described in Chapter 5.

Resin samples were obtained by severing or mini-tap as described in Chapter 5.

GLC analyses were made of the monoterpene composition of 4- to 11-year-old progeny. Though the resin composition of these young trees may not have been quite that of a mature tree, the data appeared to be suitable for analysis on a comparative basis, and the conclusions drawn on the data were considered valid. Averages for compositions will be given, but it was believed that an analysis of the frequency distribution was much more appropriate, valid, and meaningful. Optical isomers were not separated.

Generally, 25 or more samples, i.e., progeny, were needed for good resolution of frequency distribution, though more confident interpretation was made with a greater number.

Normalized data of monoterpene composition for each set of progeny were first analyzed for averages and frequency distribution for each subset of age and place of growth. Subsets were aggregated if there was little or no difference due to age (and usually there was not). They were not aggregated by place of growth because there usually were substantial differences. On the other hand, the composition of 4-year-old container trees was nearly always similar to that of nursery-grown trees, as shown by averages and by the characteristics of the frequency distributions. Therefore, the data for 4-year-old container trees were used alone or were aggregated with the data for nursery-grown trees for further analysis. If place of growth caused marked difference in composition, only nursery-grown trees were aggregated for further analysis. Data on other places of growth—container, container/plantation, or plantation—were carried no further. (The 7- to 9-year-old container trees had a much different watering and culturing schedule than the 4-year-old container trees.) The aggregated nursery data were reanalyzed for basic statistics and frequency distributions.

It is important to emphasize again that measurements at different ages of nursery growth were not of the same set of trees but were of other sets of trees that were grown from the same seed lot.

A modal analysis (Macdonald and Green 1988) was made of all multimodal frequency distributions generally having 25 or more measurements. In the case of sets with fewer than 25, a few analyses were also made if there appeared to be a clear separation of modes. This analysis produced the means and proportions of the modes in a distribution. In several cases, modal analysis was not performed where the modes were very clearly separated. However, in most of these distributions the modes were not sharply and clearly separated. The number of modes derived from the analysis was compared to the number that was expected from the assignment of alleles to the two parent trees on the basis of their monoterpene composition. These data on the number of modes were used for genetic interpretation. Though this analysis also provided the proportion of the measurements in each mode, proportions were not decisive values for genetic interpretation but were used as corroborating evidence. Most sets of progeny had fewer than 50 trees, and with so many factors other than the basic alleles acting on a limited quantity of the C-5 precursor, it was possible that a shifting of just a few measurements from one mode to the other could make large changes in the relative proportions in the modes. Even with these uncertainties, many proportions were quite close to the expected. Mode proportions could be improved by using the expected number of modes rather than the number actually found in the calculation, which often produced more modes than expected because of the interaction of several modifying factors on the monoterpene system. The modal analysis also developed a chi square, and this was usually less than the degrees of freedom; therefore, the analyses were considered reasonable and the conclusions valid. When families were too small for modal analysis, the means and frequency distributions were given because they contributed to the interpretation. In several cases, when there was a clear separation of a mode near zero from one or more modes further along the percent scale, data for the mode near zero were not used in the modal analysis, but they were included in the modal count. This was particularly so for 3-carene.

A hypothesis is proposed, with supporting evidence presented, that the primary and dominant structure for determining the monoterpene composition of *P. ponderosa* xylem resin is two additive alleles at a single locus for each of five monoterpenes: α -pinene, β -pinene, myrcene, limonene, and 3-carene. The evidence that will be discussed in this section was quite strong for the stated hypothesis, and there was considerable supporting evidence in the studies of half-siblings (see Chapter 5) and in the survey of local and regional variation of the 19 pine species of California (see Chapter 3). There also might be one or more secondary and minor genetic mechanisms that can cause small shifts in the level of a given component; these secondary mechanisms, if they exist, have yet to be resolved. There were a number of factors, in addition to the basic genetic structure, that could also affect the amount of a major component as follows: age of tree (called the juvenile effect), isomerization, biosynthetic by-products, geographic source of tree, interaction of monoterpenes, and several forms of stress such as prolonged container growth, over application of a phytocide, and grafting. These several factors, as well as the possibility of a minor genetic structure, were secondary and their effects usually quite small. In aggregate, however, they could appreciably lower the resolution of the primary structure and deviations from the expected values. Four other monoterpenes—camphene, β -phellandrene, γ -terpinene, and terpinoline—were often present in small to trace amounts. They appeared to be products of isomerization or biosynthetic by-products, and were considered minor components. Of course, there could be a separate genetic mechanism for some of the minor components. These minor components were included in the

normalization, were not used in modal analysis, but were sometimes included in displays of frequency distributions.

Each of the five major monoterpenes can, therefore, have one of three sets of additive alleles: (0-0), (0-1), or (1-1). And each tree can have one of the three sets for each major monoterpene.

During the course of the work on full siblings another source of variation became apparent which seemed to be related to the distance between parents. In effect, the composition of progeny of parents which were far apart tended to have the characteristics of trees under some degree of stress, generally higher in α -pinene and β -pinene and lower in limonene and myrcene.

It will be shown that, within the constraints noted above, the quality and quantity of resin compositions of progeny were quite predictable when based on the additive allele assignment to the parents based on their resin composition.

For each family where modal analysis was used, the following will be given: (a) the frequency distribution of each monoterpene, (b) the modal analysis of each multimodal distribution where there were adequate sample numbers; (c) the means and proportions of the analyzed modes, (d) the number of modes expected from the assignment of alleles to the parent trees and the number of modes found by the analysis, and (e) the types of composition which were found in each family. In some cases an analysis was not made for distributions that were clearly two or three modes.

The results of the modal analysis were interpreted as follows. If the number of modes derived from the modal analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees, the additive allele hypothesis was satisfied. It was presumed that modes in excess of the expected number were caused by the interactions between monoterpene alleles for the limited amount of precursor and by the action of several modifying factors noted above.

The individual tree compositions in each family were classed into types similar to, but not fully the same as, that used for half-siblings in Chapter 5. Types of composition were selected which represented those in a family, in the families of a female, and in all the families in the study. It was not possible to quantify the occurrence of these types because of the wide range in family size. However, an estimate was made of whether each type occurred commonly or occurred but not commonly.

Crosses in Chapter 6 will be discussed by the families of the seven female parents with subheadings of pollen sources, the male parent, for each separate family.

Each parent tree was identified by a combination of numbers and letters. The first two letters showed the tree's location as follows: AS = Adin Summit, Modoc National Forest, WP = Wheeler Peak, Great Basin National Park, RP = Ranger Peak, Los Padres National Forest, BL = Blue Lake, Modoc National Forest, IFG = Institute of Forest Genetics, Placerville, Calif. (*fig. 5-1*).

Throughout this chapter, as was shown in Chapter 5, the composition of young trees was not fully what was expected from the combinations of the compositions of the parent trees. This effect was called the juvenile effect; it was manifested by greater α -pinene and β -pinene than expected and by lesser myrcene and limonene than expected. As noted earlier, this same effect was caused by factors associated with stress. However, though stress and the juvenile factor caused average composition values to deviate from the expected, the number of modes was unchanged. In effect, these modifying factors moved the modes up or down the percent scale depending on the mix of components. Thus the strong support for the additive allele hypothesis was the number of modes in the distribution of a component. Mode proportions gave additional support.

Crosses of *P. ponderosa* AS-3139 with 10 Pollen Sources

Tree AS-3139, the first high limonene tree that was found, was at Adin Summit (AS) on the Modoc National Forest (*fig. 5-1, table 6-1*). It was about 20 inches in diameter with good growth and good form. It was also successfully grafted at IFG, and three grafted trees are now growing in the Camino Arboretum. The reanalysis of Tree AS-3139, as well as the other parent trees at Adin Summit, showed their compositions to be essentially the same with changes in time and location of sample on the tree (*table 6-2*). This refuted the claim of others that the compositions of these trees were spurious measurements. The breeding studies reported in this chapter also showed the compositions to be valid and not spurious.

Pollen from 10 trees was used on Tree AS-3139; two of these pollen sources were used on the grafts of Tree AS-3139 at IFG (*table 6-3*).

The average composition of sets of full-sibling progeny between Tree AS-3139 and 10 male parents was recorded (*table 6-3*).

The measurements of the five trees for compatibility and for seed production were as follows (Trees WP-3298 and WP-3352 are at Wheeler Peak, about 400 miles from Tree AS-3139; Trees Adin 4 and 5 were other wind-pollinated trees, at Adin Summit, that were selected for only this one cone study):

	<i>AS-3139</i> <i>x</i> <i>WP-3298</i>	<i>AS-3139</i> <i>x</i> <i>WP-3352</i>	<i>AS-3139</i> <i>x</i> <i>wind</i>	<i>Adin-4</i> <i>x</i> <i>wind</i>	<i>Adin-5</i> <i>x</i> <i>wind</i>
Cones protected	yes	yes	no	no	no
Number of cones used	10	14	12	42	13
Number of sound seed/cone	78	69	46	45	48
Milligrams/seed	44	47	45	40	48

It was apparent that there was no noticeable incompatibility between the tree at Adin, AS-3139, and those at Wheeler, WP-3298 and WP-3352; that seed yield was greatly increased by protecting the first- and second-year conelets, and that there was no clear difference in seed weight because of parent source or treatment. Weight of seed was about average for *P. ponderosa*.

Progeny of *P. ponderosa* AS-3139 x WP-3298.

Tree WP-3298 was classed as a high limonene and was growing at Wheeler Peak (*table 6-1*); its monoterpene composition was essentially the same as that of Tree AS-3139 growing at Adin Summit, with about 58 percent limonene. Thus, this was a cross of two high limonene trees.

Normalized percent compositions of parents and progeny were as follows:

<i>Trees and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>1-pinene</i>	<i>3-carene</i>	<i>myrcene limonene</i>	
AS-3139 female	1	18	1	0	22	59
WP-3298 male	1	26	0	0	16	58
F-1 progeny	115	39	4	1	5	52

Resin of eight sets of nursery-grown progeny, ranging from 4 to 11 years old, were analyzed for monoterpene composition along with one set each of 9-year-old container-grown progeny, and one set of 9-year-old plantation-grown progeny (*fig. 6-1*). There were only rather small shifts, or no shifts, in the average of any of the individual monoterpenes in the eight age-sets of nursery

Table 6-2—Monoterpene composition of *P. ponderosa*, that were used in the breeding study, at different locations of sampling, and in different years; all trees were growing at or near Adin Summit, except "g" which were grafts at Institute of Forest Genetics.

Tree	Year	Sampling		α -p	Monoterpene ¹			
		Aspect	Height		β -p	3-car	myr	lim
			<i>feet</i>		<i>percent</i> ²			
AS-3139	1968	S	4	18.0	1.0	0	20.0	60.0
	1969	S	13	18.0	0.5	0	22.1	58.5
	1969	W	20	18.7	0.6	0	22.8	57.4
	1969	E	20	18.7	0.1	0	23.7	56.5
	1985	S	4	18.5	1.0	0	21.5	59.6
	1985	W	4	18.3	1.0	0	23.0	57.4
	1985	N	4	18.0	0.9	0	23.4	57.3
AS-3139g	1989	N	1	27.4	4.1	3.9	15.8	49.0
	1989	N	8	27.3	7.7	9.2	12.8	42.0
AS-5789	1968	S	4	22.0	9.7	0	29.2	38.1
	1985	S	4	22.9	11.5	0	28.7	36.8
	1985	E	4	22.1	10.4	0	30.2	37.2
	1985	W	4	24.3	11.4	0	28.8	35.5
AS-3187	1968	S	4	9.0	23.0	23.0	14.0	19.0
	1985	S	4	9.7	23.3	24.6	13.3	23.7
	1985	E	4	9.6	22.6	24.7	13.0	28.2
	1985	W	4	9.7	23.3	24.5	13.6	27.5
AS-3178	1968	S	4	3.0	1.0	55.0	18.0	19.0
	1985	S	4	3.0	0.5	48.6	23.2	21.2
	1985	W	4	3.0	0.5	49.7	21.6	21.9
AS-A95	1968	S	4	22.0	23.0	0	10.0	45.0
	1985	S	4	18.8	21.0	0	14.8	45.4
	1985	W	4	20.2	20.5	0	13.9	45.2
AS-A95g	1989	S	1	33.4	27.5	3.5	10.5	25.0
	1989	S	8	42.1	27.5	0	7.6	23.8
BL-6074	1972	E	4	7.4	10.5	0	27.3	54.7
	1985	E	4	7.2	10.2	0	27.2	55.2
	1985	W	4	7.0	10.4	0	26.9	55.6
AS-3194	1968	S	4	9.1	11.9	33.6	10.9	32.3
	1985	E	4	9.0	11.5	32.6	12.2	32.4
	1985	W	4	7.4	7.5	34.6	14.2	42.9
	1985	N	4	8.4	8.4	35.4	12.5	32.8

¹ α -pinene, β -pinene, 3-carene, myrcene, limonene.²Normalized; minor components omitted.

Table 6-3—Average monoterpene composition of progeny of Tree AS-3139 with 10 different males grown for varying periods of time (years) under different conditions: nur = nursery, con = container, copl = container/plantation, plan = plantation, N = number of trees.

Male	Growth location	Age	N	Monoterpene composition of progeny ¹							
				α -p	cam	β -p	car	my	lira	β -ph	terp
		yr		-----percent ² -----							
WP-3298	nur	4-11	115	39.4	* ²	3.6	0.9	5.2	51.9	0	*
WP-3298	con	9	3	58.7	0.2	4.8	0.9	4.1	31.3	0	0
WP-3298	copl	9	4	63.7	0.1	4.3	0	5.2	26.7	0	0
WP-3298	plan	9	22	57.3	0.1	4.0	0.1	8.0	30.6	0	0
WP-3352	nur	4-11	60	30.7	*	2.2	0.3	6.3	60.5	0	0
WP-3352	con	9	3	33.7	0.1	4.0	0.2	6.2	55.6	0	0
WP-3352	plan	9	21	44.5	0.1	3.7	0.1	8.1	43.5	0	0
WP-3299	nur	4-11	29	17.7	0.1	2.5	25.4	5.1	47.7	0	2.5
WP-3299	con	9	5	22.1	0.1	5.0	32.1	3.8	34.8	0	2.0
WP-3299	copl	9	8	37.0	0.1	6.9	11.3	7.5	36.5	0	0.8
AS-A95 ³	con	4	49	25.4	0	24.3	2.0	2.4	45.4	*	0.1
BL-6074	con	4	38	23.4	0	15.2	2.9	3.0	55.3	0	*
WP-3347	nur	4-11	33	8.7	0.1	30.3	28.4	3.7	26.8	0	1.7
WP-3347	con	9	8	9.3	0.1	38.5	30.5	2.6	17.3	0.5	1.5
WP-3347	copl	9	2	8.1	0.1	29.8	17.4	5.0	37.5	0	2.2
WP-3312	nur	4-11	32	10.7	0.1	14.4	34.1	2.9	36.3	0	1.6
WP-3312	con	9	9	10.0	0	12.0	43.0	2.3	27.3	0.1	3.0
WP-3312	copl	9	7	12.3	0.1	26.0	28.4	3.4	27.4	0.1	2.1
AS-5789	nur	4-11	35	27.0	*	15.5	0.1	10.9	46.5	0	0
AS-5789	con	9	9	32.6	0.2	36.8	0.1	8.2	21.3	0.8	0
AS-5789	copl	9	7	41.4	0.1	28.9	5.1	8.8	19.0	0.2	0.6
AS-3194	nur	4-11	27	13.3	*	15.6	18.9	3.6	47.3	0	1.3
AS-3194	con	9	8	24.5	*	10.3	25.5	2.2	35.3	0	2.0
AS-3194	copl	9	5	53.5	0.1	9.8	4.3	3.7	28.1	0	0.5
AS-3178	nur	4-11	33	9.3	0.1	2.9	41.7	7.6	35.9	0	2.5
AS-3178	con	9	4	11.1	0	1.7	46.0	9.5	28.8	0	2.9
AS-3178	copl	9	8	23.4	*	2.7	40.1	11.2	19.5	0	3.0

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.²Normalized, * = trace.³Grafts at IFG were pollen sources.

trees, ranging from 4 to 11 years. All of these trees could be classed as ultra-high, high, or potentially high limonene; that is, essentially lacking β -pinene and 3-carene. The composition of container trees and of the plantation trees differed sharply from that of the nursery trees in α -pinene and limonene (table 6-3). The amount of myrcene and β -pinene was apparently too small to show the effect of place of growth. To repeat, each set of trees was a different set derived from the same seed lot with different ages or location of growth. It is hypothesized that stress, from either prolonged container growth or overdose of a phytocide, was the cause of the difference between the nursery-grown trees and trees grown at other locations. Thus, the analysis of the progeny of Trees AS-3139 x WP-3298 was confined to the aggregate of nursery trees which average 52 percent limonene, 39 percent α -pinene, and small amounts of other monoterpenes (table 6-3).

The average amount of α -pinene was fairly constant at about 35 to 40 percent for varying periods of nursery growth of 4 to 11 years (fig. 6-1), but the range of individual values was quite large, generally between 15 and 60 percent. This general range prevailed at all growth periods, even with small number of trees. The average α -pinene for trees grown in containers or plantations for 9 years was much greater at about 57 percent (table 6-3). This large difference was attributed to the stress of prolonged root containment in containers and to the over application of a phytocide in the plantation. Though the averages were fairly constant over time in the nursery-grown sets of trees, and though the individual tree values ranged widely even in small families (fig. 6-1), the pattern of the frequency distributions was about the same for each age class.

The caption for Figure 6-2 is the basic caption for the following 42 figures with modal analysis.

Figure 6-2: On the left; frequency distribution of the amount of each of the noted monoterpenes in 4 to 11 year old progeny of P. ponderosa AS-3139 x WP-3298 each dot is measurement of one tree except where a circled number indicates each is that number; ▲ is average of set. On the right; modal analysis of the distribution of the noted monoterpenes; heavy line is best fit of the histogram; thin line is best fit of the modes; (▲) is average position of each mode; beneath it is the average (av) percent of each mode in full numbers and proportion (pr) of each mode base on a sum of 1.0 for all modes. In the middle; the expected (ex) and actual (ac) number of modes for the 5 major monoterpenes, expected was derived from assignment of alleles to parents, actual derived from modal analysis. Minor components not included in modal analysis. In using this caption, replace the two parents above with the two parents in the figure before you.

The average amounts of camphene, β -phellandrene, and terpinolene were near zero in all trees, and were not included in the calculation (fig. 6-2). The average amount of β -pinene and 3-carene was very low, confirming the assignment of a (0-0) set of alleles for both components in both parents. What little amount there was of these five components was likely due to isomerization or biosynthetic by-products. The amount of β -pinene, though quite low at about 4 percent, ranged more widely than the other four components, suggesting that β -pinene could be the key component in the juvenile factor, as discussed in the introduction to this chapter.

The average amount of myrcene increased gradually in the nursery-grown trees from about 4 percent at 4 years to about 8 percent at 11 years (fig. 6-1). The small number of samples (three) for the container-grown trees probably explains the lower myrcene than expected. The general increase in myrcene

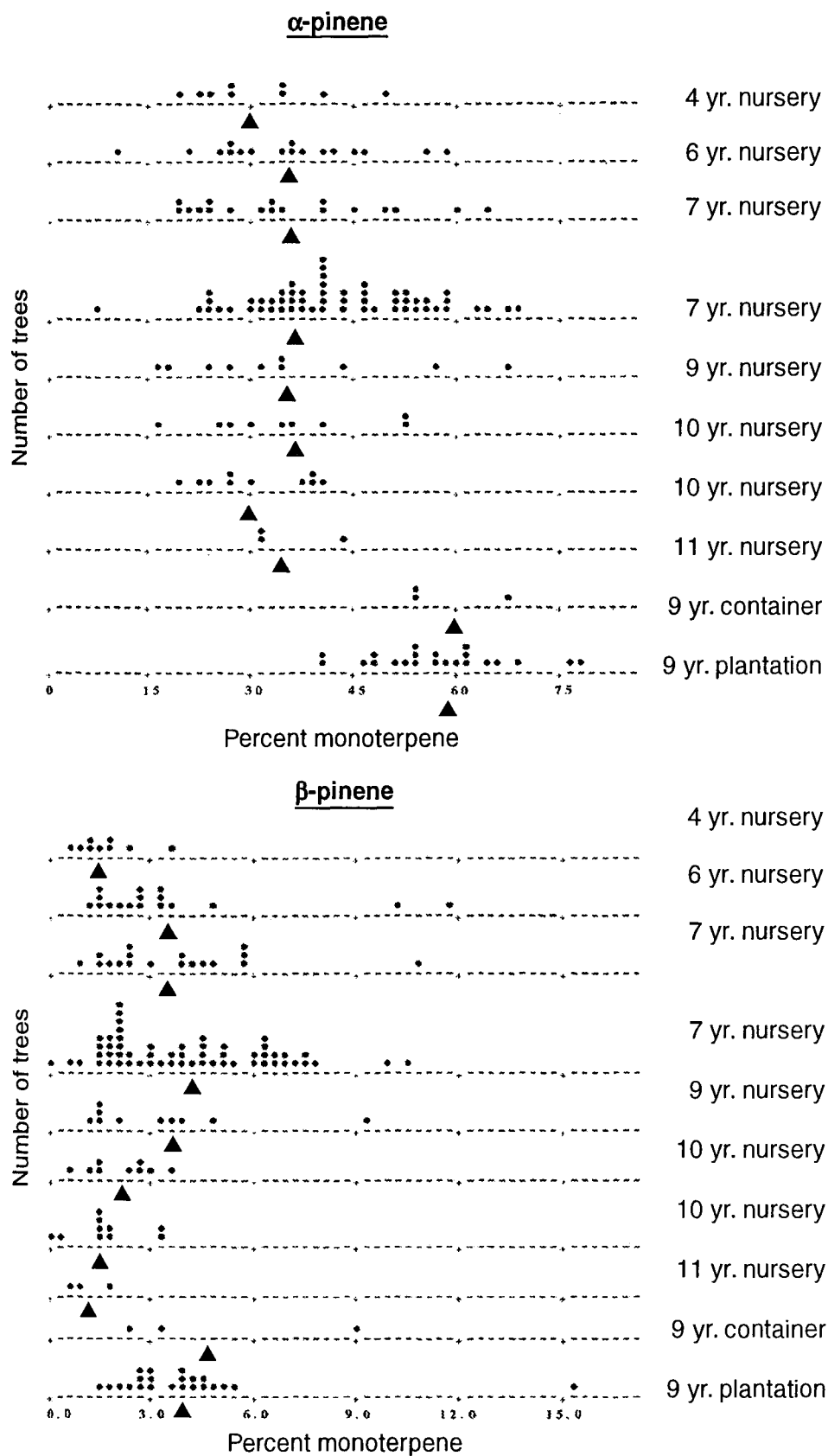


Figure 6-1—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-3139 x WP-3298 for eight periods of growth (years) in the nursery (nur) and for one period of container (con) and plantation (plan) growth, as listed on the right; each dot is measurement of one tree; [▲] is average of set.

(continued on next page)

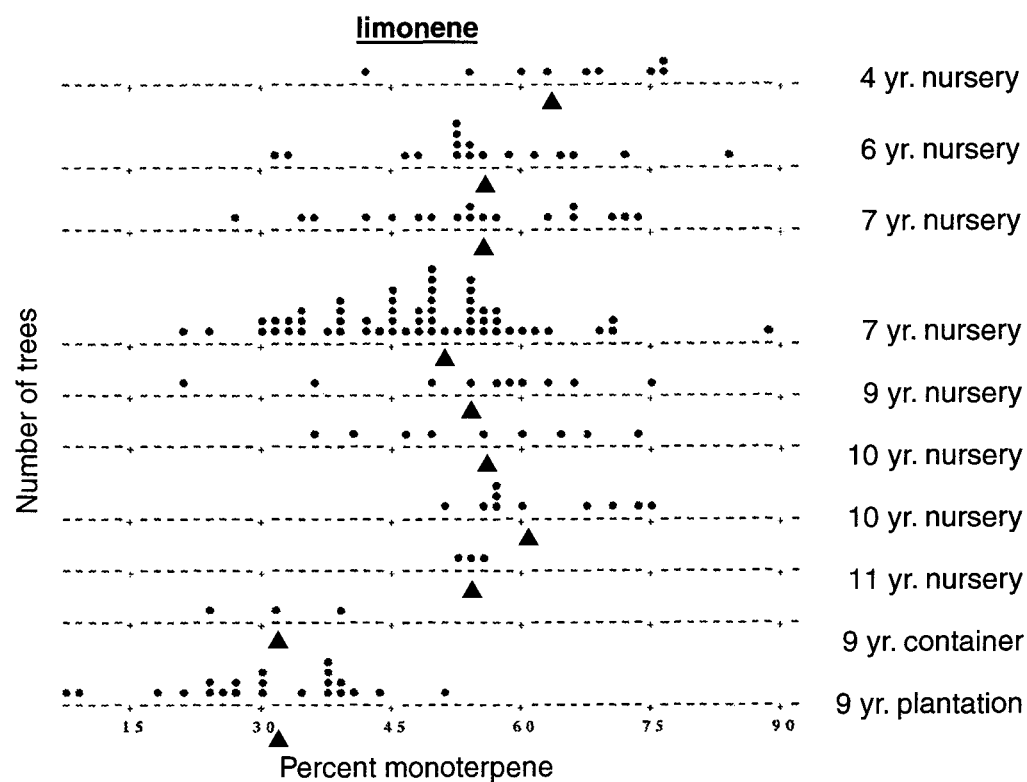
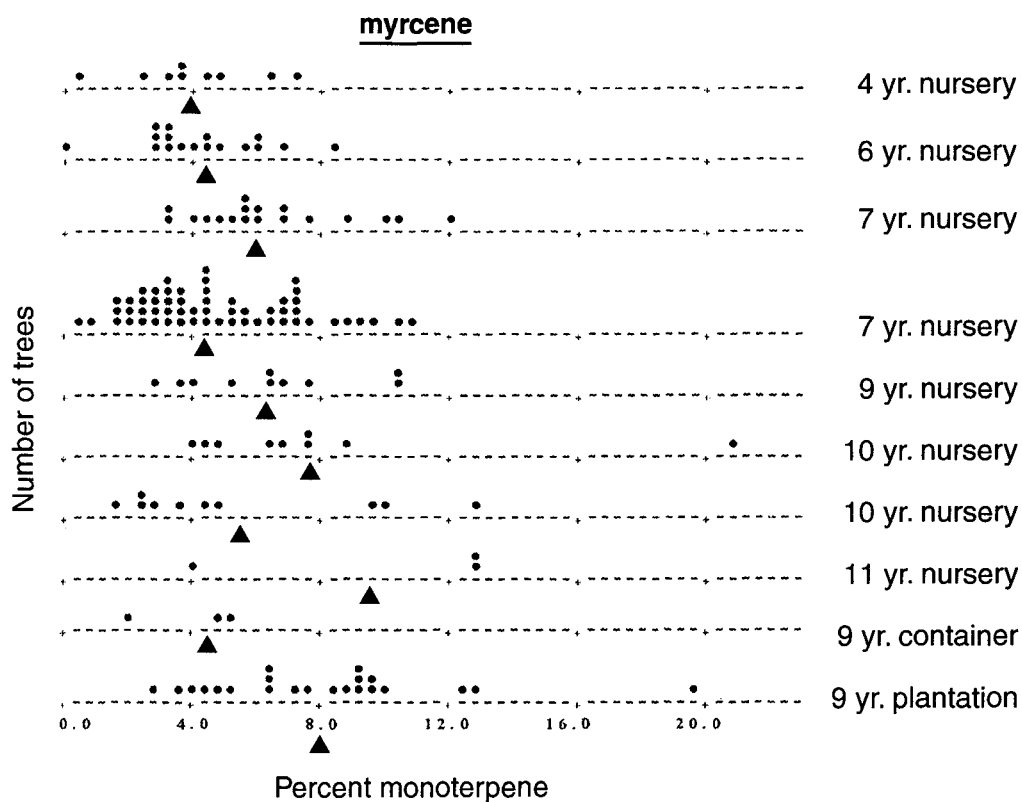


Figure 6-2—See full description on page 296.

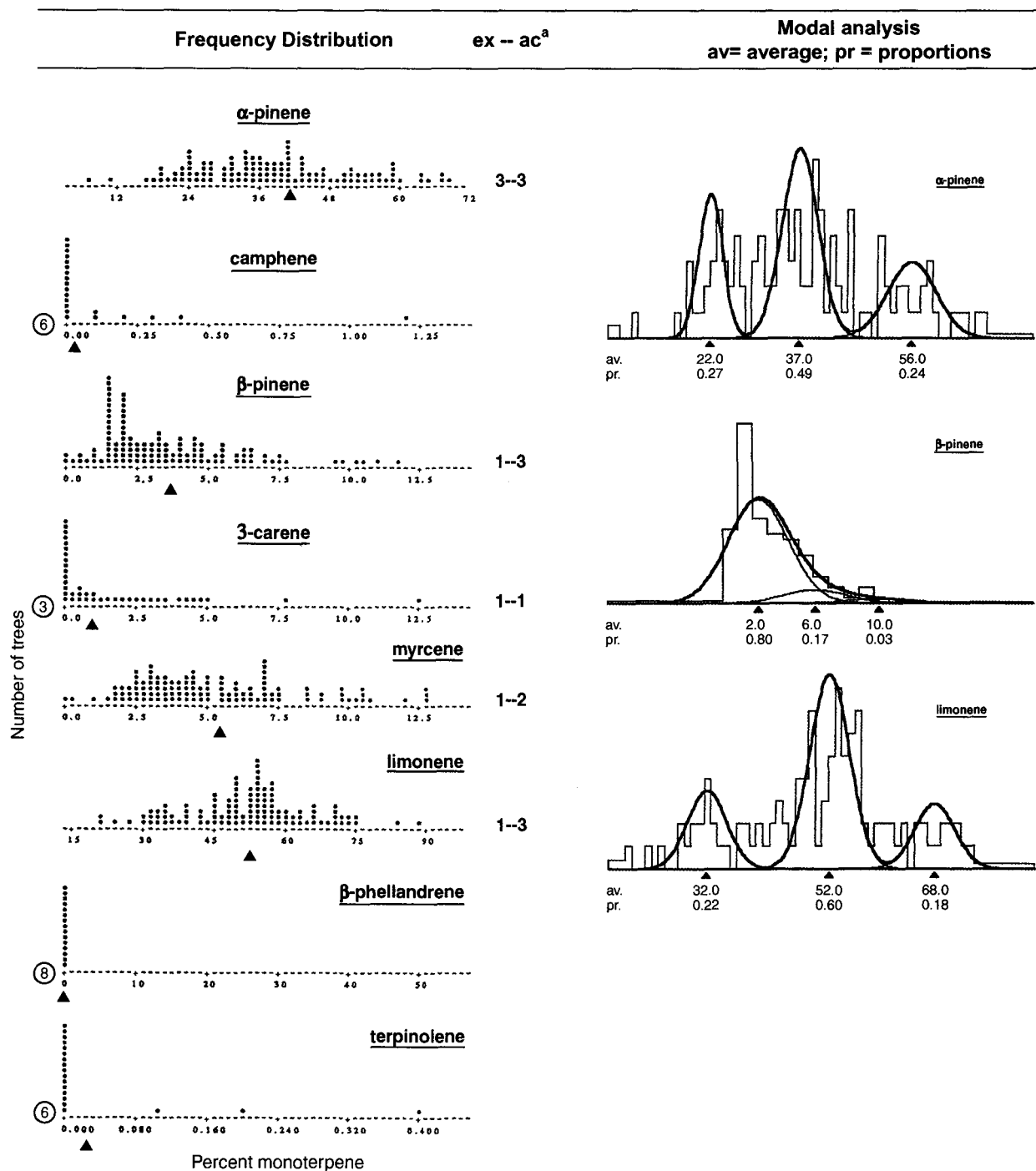
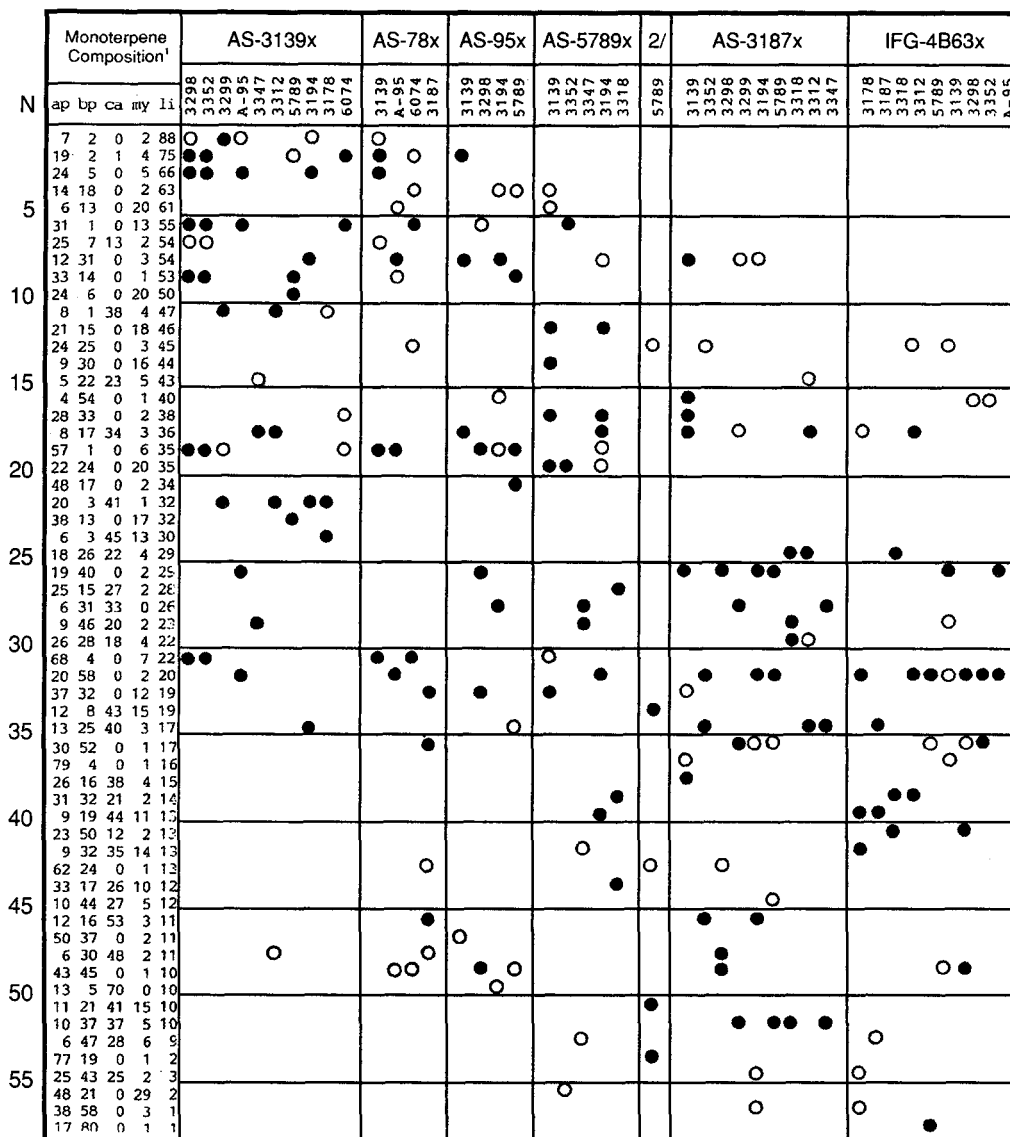
^aex = expected, ac = actual

Figure 6-3—Fifty eight types of monoterpene composition, and their occurrence, in 42 full sibling families of seven female *P. ponderosa* analyzed and discussed in this chapter; ● = occurs commonly, ○ = occurs but not commonly; The seven females are listed horizontally across the top with the males listed vertically under them. See Table 6-2 for prefix letters of male parents.



1) α -pinene, β -pinene, 3-carene, myrcene, limonene; minor components omitted.
2) AS-A295.

Table 6-4—Average monoterpene composition of three sets of nursery-grown progeny of *P. ponderosa* AS-3139 x WP-3298 grouped by the three classes of limonene: low = 0-39 pct., mid = 40-59 pct., high = 60-100 pct. N = number of trees.

Class of limonene	N	Monoterpene ¹							
		α -p	cam	β -p	car	my	lim	β -ph	ter
		-----percent ² -----							
low	22	58.4	*	3.9	0.2	5.1	32.4	0	*
mid	67	38.0	*	3.8	1.1	5.4	51.6	0	*
high	26	22.6	*	2.6	0.9	4.8	69.1	0	*

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.

²Normalized; * = trace.

suggested a juvenile phenomenon.

The average amount of limonene in sets of nursery trees was fairly constant at about 50 to 60 percent, though individual measurements ranged widely from about 5 percent to almost 90 percent (*fig. 6-2*). The average for the container-grown and plantation-grown trees, however, was much lower at about 30 percent. Thus, it appears that under stress the amount of limonene decreased and α -pinene increased.

The analysis of the frequency distributions showed that the number of modes actually derived by the analyses equalled or exceeded the number expected from the assignment of additive alleles to the parent trees (*fig. 6-2*). This supports the additive allele hypothesis. Mode proportions of α -pinene and limonene were close to the expected under the conditions of the study. The proportions of 27:49:24 for α -pinene were very close to the expected of 25:50:25; the 22:60:18 proportions for limonene were a reflection of the modes of α -pinene. These modes and proportions support the assignment of a (0-1) set of alleles to α -pinene in both parents, and a (1-1) set for limonene in both parents.

When all progeny were grouped by types of composition and were then categorized by relative abundance, it was readily apparent that nearly all progeny fell into the higher percentage classes of limonene (*fig. 6-3*).

For further analysis, all 115 progeny were placed in three classes of limonene as follows: (a) ultra-high limonene, greater than 60 percent (*fig. 6-3*, #1,2,3), (b) high limonene, 40 to 60 percent (*fig. 6-3*, #6,7,9), or (c) potentially high limonene, less than 40 percent but essentially lacking β -pinene and 3-carene (*fig. 6-3*, #19,31, *table 6-4*). However, α -pinene was present and appeared to have a (0-1) set of alleles in both parents. Therefore, some progeny had a (1-1) set of alleles for α -pinene; in such cases limonene will not be high. Likewise, some progeny had a (0-0) set of alleles for α -pinene; in such cases limonene will be ultra high. And the data on individual tree compositions supported this assumption (*fig. 6-3*). The modality of the distributions of limonene was also determined by varying allele composition of α -pinene. That is, the (0-1) set of alleles should produce progeny with a (0-0) set, a (0-1), or a (1-1) set of alleles for α -pinene with proportions of 1:2:1, respectively. Limonene was always (1-1) in all progeny, but the amount of it depended on the set of alleles for α -pinene that was competing for the fixed amount of precursor.

The results clearly showed the inverse relationship of limonene with α -pinene for the high and low mode, and the direct relationship with the mid-mode (*fig. 6-4*). The allelic structure of β -pinene, 3-carene, and myrcene was (0-0) in both parents and in all progeny. The question arises about the small amounts that do occur in the progeny. Three alternatives are proposed and there may be others: (a) isomerization from α -pinene and limonene, (b) a by-product of terpene synthesis, (c) a slight bit of activity by the (0-0) set of alleles under certain conditions, such as the juvenile state.

Though the composition of the progeny will very likely change over long periods of time, it is possible that some progeny will still have limonene in excess of 80 percent and thus be classed as ultra-high limonene; trees with such a composition have not been found in natural stands. Type #1 (*fig. 6-3*) closely resembles *P. torreyana* (see Chapter 3).

It was difficult to explain the average of 22 percent for the low mode of α -pinene that presumably was controlled by a (0-0) set of alleles except as an effect of the juvenile factor. It might be possible to attribute some of this to isomerization, but not all of it. One might speculate that the (0-0) set of alleles had some level of activity, particularly with no competing alleles, except limonene, and during the juvenile period of growth. This same factor could account for the shifting of the percent scale much higher than could be expected from equal access to the C-5 precursor. However, a few trees of the ultra-high limonene type (*fig. 6-3*, #1) had very little α -pinene; this was the composition

that was expected with a (0-0) set of alleles for α -pinene and a (1-1) set for limonene in the progeny. The results suggested, therefore, that the juvenile effect was quite variable or was dependent on some other variable factor.

Progeny of *P. ponderosa* AS-3139 x WP-3352

Tree WP-3352 was another high limonene tree, also growing at Wheeler Peak (WP). Its composition was nearly identical to that of both Tree AS-3139 and Tree WP-3298 (*table 6-1*).

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3139 female	1	18	1	0	22	59
WP-3352 male	1	24	0	0	18	57
F-1 progeny	60	31	2	tr	6	61

There was little change in the average composition of nursery trees over the 4 to 11 years of growth. Therefore, all nursery trees were aggregated into one set of 60 trees for analysis. The average monoterpene composition of nursery-grown progeny was fairly similar to that of the progeny of Trees AS-3139 x WP-3298 (*table 6-3*), though α -pinene was bit lower and limonene a bit higher; the frequency distributions were also quite similar (*fig. 6-5*). The effects of a phytocide on plantation-grown trees and of root containment on container-grown trees were clearly evident, though the differences were not as great in the progeny of Trees AS-3139 x WP-3352 as they were in the progeny of Trees AS-3139 x WP-3298. Nevertheless, only the data on the nursery trees were analyzed further.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees (*fig. 6-5*). The three modes for α -pinene support the assignment of a (0-1) set of alleles to both parents. Mode proportions were not as good as with the previous set of progeny.

With many fewer measurements, the trimodality of the distribution of α -pinene and limonene was not as easily discernible as it was with the progeny of Trees AS-3139 x WP-3298. However, modal separations were about the same for both α -pinene at 28 and 38 percent and limonene at 45 and 62 percent. The lack of 1:2:1 proportions for the three modes could be attributed to the small sample size and the combined effect of several modifying factors noted above. The types of composition in this family were essentially the same as those found in the progeny of Trees AS-3139 x WP-3298 (*fig. 6-3*).

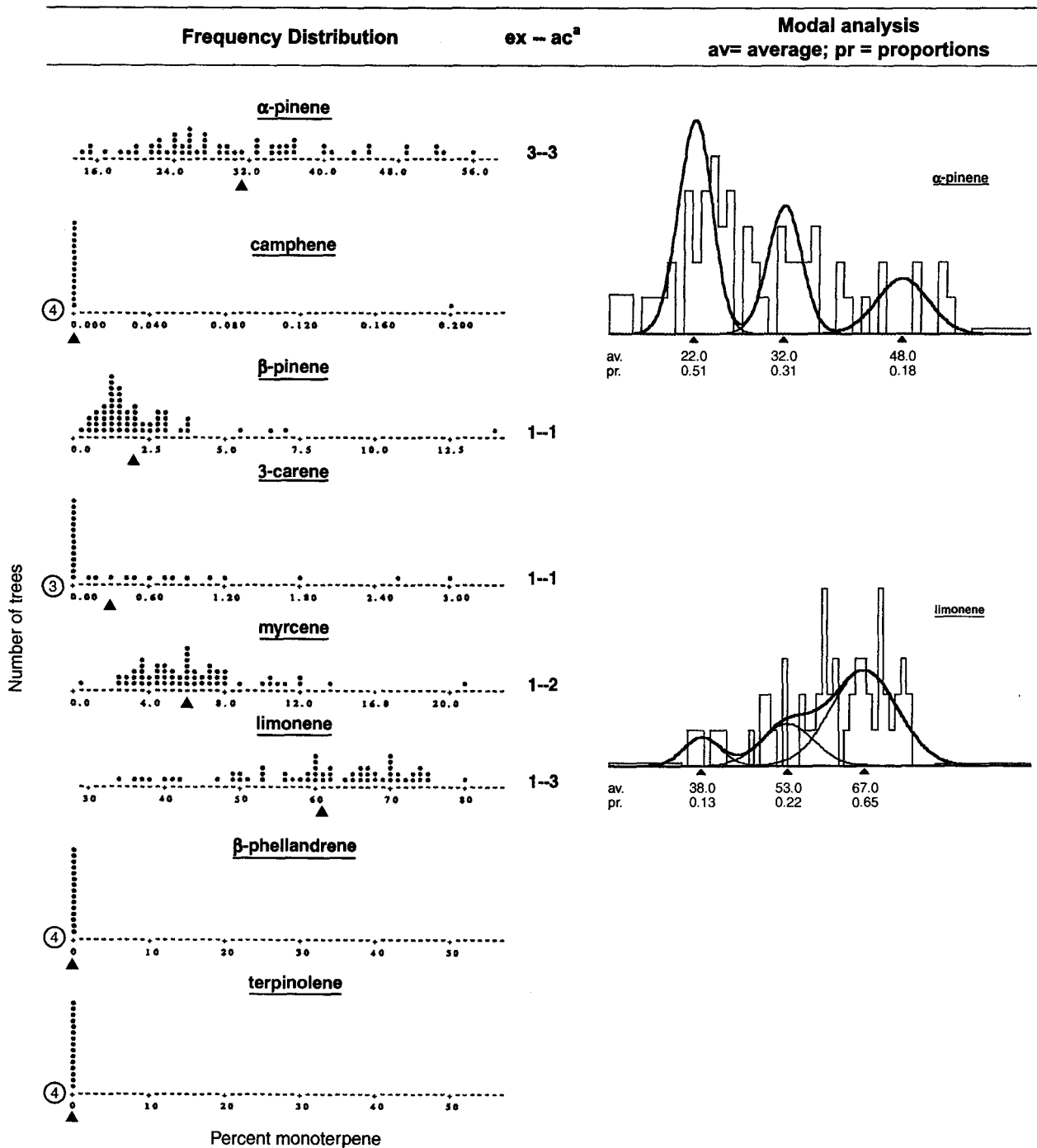
Progeny of *P. ponderosa* AS-3139 x WP-3299

Tree WP-3299 was also located at Wheeler Peak (WP). It was near-high limonene, but with high 3-carene. Normalized percent compositions of the parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3139 female	1	18	1	0	22	59
WP-3299 male	1	6	0	43	8	40
F-1 progeny	29	18	3	25	5	48

The nursery-grown progeny of the cross were nearly as high in limonene (*table 6-3*), about 48 percent, as in the two previous crosses (Tree AS-3139 x WP-

Figure 6-5—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3139 x WP-3352; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

3298 and x WP-3352), but α -pinene was much lower at 18 percent, supporting the assignment of a (0-0) set of alleles to α -pinene in Tree WP-3299. It was also observed that 3-carene in this family was higher at 25 percent, supporting the assignment of a (0-1) set of alleles for 3-carene in Tree WP-3299 (*table 6-1*). The composition of the container and container/plantation trees in this family was sufficiently different to exclude them from further analysis; only the nursery-grown progeny were used for modal analysis. The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-6*). This supports the additive allele hypothesis. Mode proportions were very good for 3-carene where a 50-50 ratio was expected.

Though Tree WP-3299 had less limonene than the two previous males, it was still high limonene with a (1-1) set of alleles, and its progeny with Tree AS-3139 clearly showed this by averages and by types of composition (*fig. 6-3*). In fact, the ultra-high type #1 (*fig. 6-3*) was more common in the progeny of WP-3299 than it was in the progeny of WP-3298 or WP-3352. This suggested that the juvenile effect was not as strong with 3-carene as it was with α -pinene.

Progeny of *P. ponderosa* AS-3139 x AS-A95

Tree AS-A95 was located at Adin Summit and was classed as near-high limonene but with β -pinene.

Normalized percent compositions of the parents and progeny were as follows:

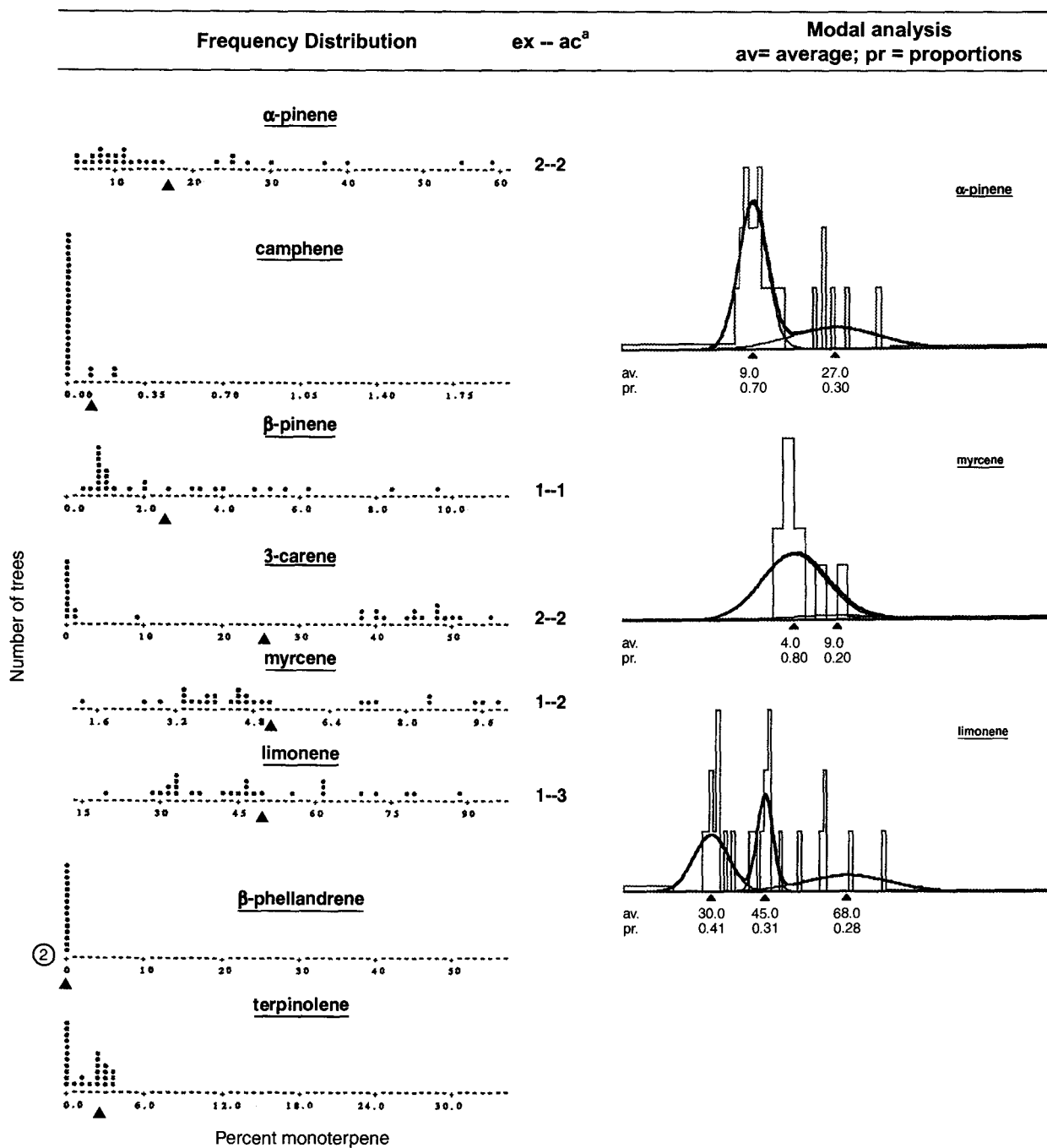
<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3139 female	1	18	1	0	22	59
AS-A95 male	1	19	21	0	15	45
F-1 progeny	49	25	24	2	2	45

Tree AS-A95 differed from the previous near-high limonene tree, Tree WP-3299, in having 0 percent 3-carene but having 21 percent β -pinene (*table 6-1*). Thus Trees AS-A95 and WP-3299 were reversed with respect to β -pinene and 3-carene. The composition of AS-A95 was quite constant over time and sampling locations (*table 6-2*). Breeding was done on grafted Tree AS-3139 in the IFG Arboretum with pollen from grafted AS-A95 also in the Arboretum. All progeny were grown in 4-year containers. The average composition of 49 trees showed the difference between Trees WP-3299 and AS-A95 when Tree AS-3139 was the female parent (*table 6-3*); the progeny of Trees AS-3139 x AS-A95 were higher in α -pinene and β -pinene, but nearly lacking in 3-carene, while 3-carene was much higher in the progeny of AS-3139 x WP-3299. Limonene was about the same for both families.

The analysis of the frequency distributions showed that the number of modes actually found by the analyses equalled or exceeded the number expected from the assignment of alleles to the parent trees (*fig. 6-7*). Mode proportions were quite close to the expected ones. By combining to get two modes for 3-pinene, proportions were 51:49. The proportions for three modes for α -pinene were 29:33:38.

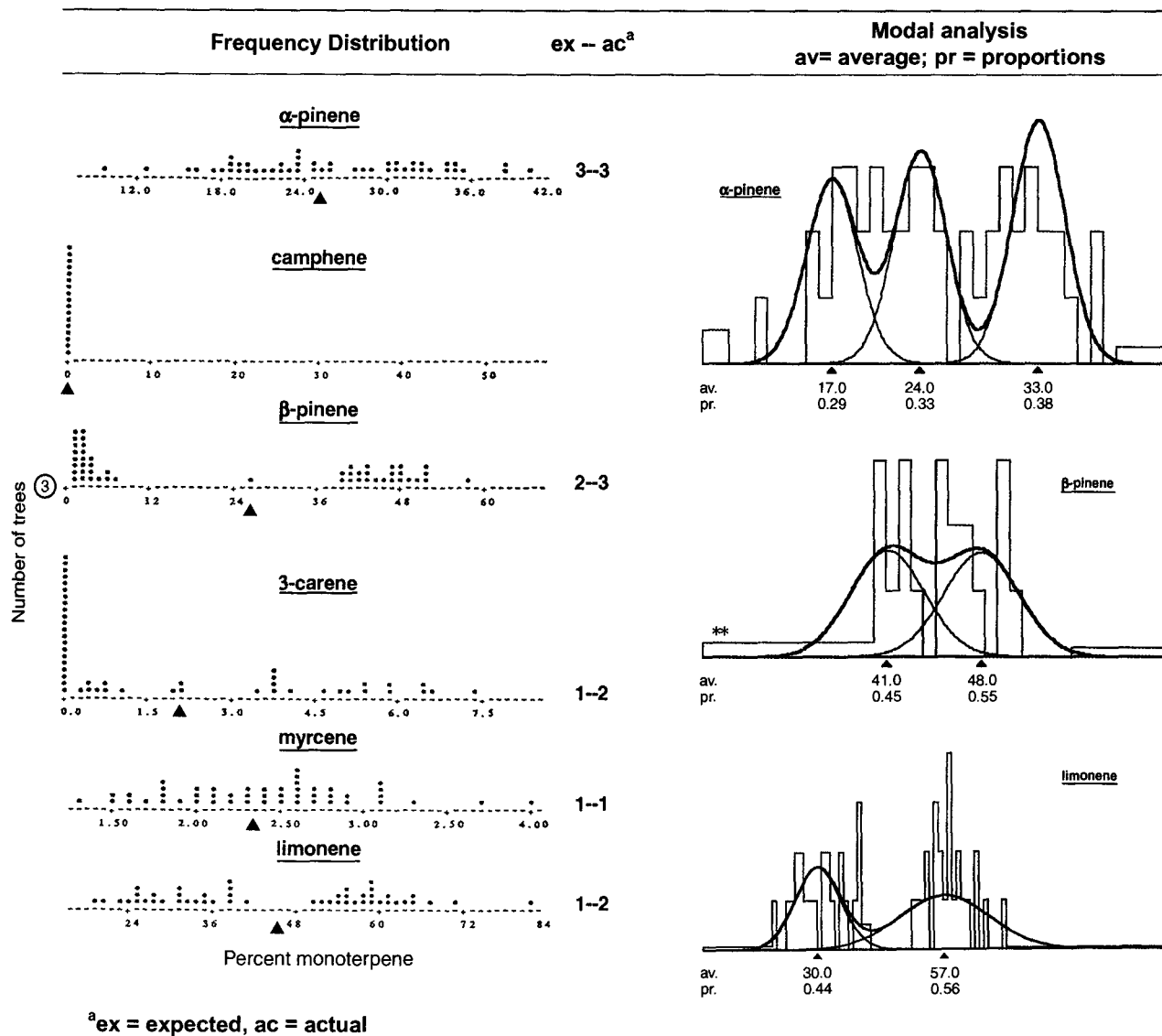
Ten trees out of 49 with greater than 60 percent limonene—and actually greater than 80 percent—were classed as ultra-high limonene while 15 others with greater than 40 percent were classed as high limonene (*fig. 6-3*). Thus two near-high limonene trees, WP-3299 (the previous tree), and AS-A95 (with different composition) can produce progeny with ultra-high limonene when crossed with a tree of high limonene composition, AS-3139. Another common type, and one that was expected, was #26 (*fig. 6-3*) with a large amount of β -

Figure 6-6—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3139 x WP-3299; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

Figure 6-7—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3139 x AS-A95; see caption of figure 6-2 for explanation of the format below; **= mode near zero was not used in mode analysis, but was included in the mode count.



pinene. The near-equal amount of β -pinene in the two modes supported the assignment of a (0-1) set of additive alleles for β -pinene in Tree AS-A95; i.e., a (0-0) set in Tree AS-3139 and a (0-1) set in Tree AS-A95 should give equal numbers of (0-0) and (0-1) in the progeny. The bimodal character of the high mode of β -pinene was attributed to the interaction with other alleles.

Progeny of P. ponderosa AS-3139 x WP-3347

Tree WP-3347, also growing at Wheeler Peak, was considered a near-high limonene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3139 female	1	18	1	0	22	59
WP-3347 male	1	4	17	25	3	48
F-1 progeny	33	9	30	28	4	27

Tree WP-3347 had modest amounts of both β -pinene and 3-carene (*table 6-1*); an assignment of a (0-1) set of alleles for both components was given on this basis. However, the breeding results indicated a (1-1) set for 3-carene in Tree WP-3347. And again these differences were reflected in the progeny of Trees AS-3139 x WP-3347 which averaged about 30 percent each for β -pinene and 3-carene; however, limonene was considerably lower at about 25 percent (*table 6-3*), reflecting the competition of an additional allele. Thirty-three nursery-grown trees were used for modal analyses.

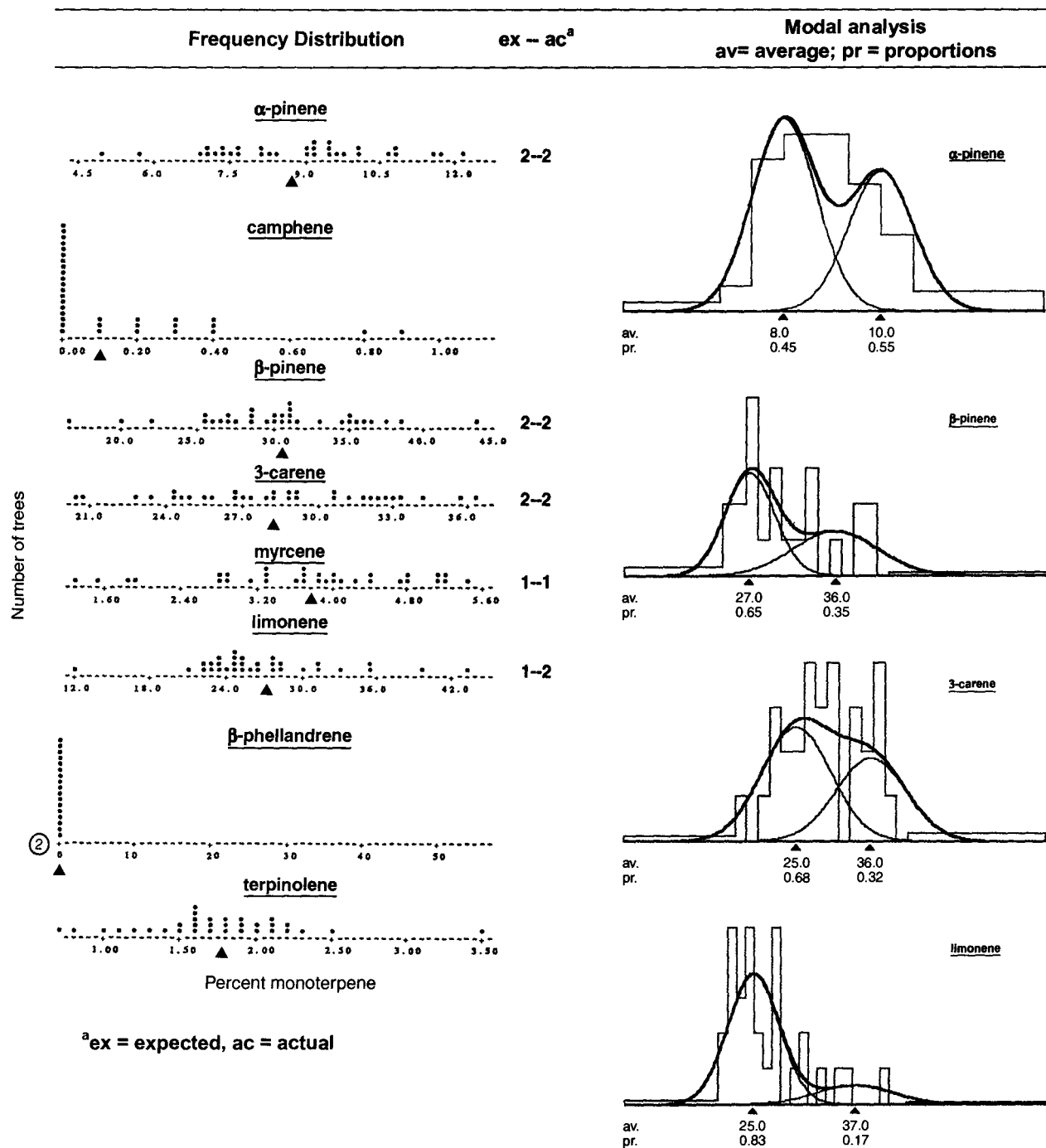
The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees (*fig. 6-8*). Mode proportions for α -pinene were 45:55, thus quite close to the expected 50:50.

If one considered other genetic structures, the progeny of this cross did not show a dominant-recessive structure at all for β -pinene or 3-carene. If dominant-recessive were to prevail, one would expect one mode at 0 and one in the mid range of the distribution. Instead, a two-mode characteristic was shown for both β -pinene and 3-carene with both modes in the mid range of the distribution. This result would be expected for an assignment of (0-1) set of alleles for β -pinene and 3-carene (*fig. 6-8*).

There were high limonene progeny but none of the ultra-high type; one monoterpene composition, type #18, was common; the other, type #15, was not common (*fig. 6-3*).

The lack of a mode at or near zero for 3-carene did not support the assignment of a (0-1) set of alleles to Tree WP-3347, as one might be inclined to do on the basis of 25 percent 3-carene in the male. Thus, a (1-1) set was assigned here and later with females AS-5789 and AS-3187. When each of these latter two female was crossed with WP-3347, there was no mode at or near zero; this confirms the assignment of the (1-1) set of alleles to WP-3347 for 3-carene. The 25 percent 3-carene in Tree WP-3347 must be right at the dividing point between one and two alleles. Also, there are three other competing alleles in WP-3347 so that the amount of 3-carene, even with two alleles, would be lower than usual.

Figure 6-8—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3139 x WP-3347; see caption of figure 6-2 for explanation of the format below.



Progeny of *P. ponderosa* AS-3139 x WP-3312

Tree WP-3312, growing at Wheeler Peak, was another near-high limonene tree.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3139 female	1	18	1	0	22	59
WP-331.2 male	1	5	7	34	4	48
F-1 progeny	32	11	14	34	3	36

Thus, Tree WP-3312 was somewhat like Tree WP-3299. Thirty-two nursery progeny were analyzed with the above percentages. (*table 6-3*)

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees (*fig. 6-9*). Mode proportions of α -pinene were not as close to the expected as they were for other sets of progeny, probably because of the three measurements greater than 18 percent that drew the average of the higher mode to 15 percent rather than 12 percent.

The presence of 3-carene in all progeny confirmed a (1-1) set of alleles in the male parent, and the lack of or only moderate amounts of β -pinene in all progeny confirms a (0-0) set of alleles for β -pinene in the male parent. Six progeny had 40 percent or more limonene and were classed as high limonene (*fig. 6-3*), but different from most high limonene trees, they all also had moderate amounts of 3-carene, as would be expected.

Progeny of *P. ponderosa* AS-3139 x AS-5 789

Tree AS-5789 was another near-high limonene tree, 37 percent, growing near Adin Summit. Its composition was quite constant over time (*table 6-2*).

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3139 female	1	18	1	0	22	59
AS-5789 male	1	23	10	0	29	37
F-1 progeny	35	27	16	0	11	47

The 29 percent myrcene was of note because Tree AS-5789 was the only tree with such a large amount, and it was given a (0-1) set of alleles for myrcene. Thirty-five nursery-grown progeny were analyzed (*table 6-3*). Again, the few container and container/plantation trees had a sharp decrease in limonene with increase in α -pinene and β -pinene; these trees were not used for modal analysis.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees (*fig. 6-10*). This supports the additive allele hypothesis and the assignment of alleles to the parent trees. Mode proportions for α -pinene were only moderately good at 43:40:17. By combining the two higher modes of myrcene, proportions of 37:63 were achieved.

One tree had 73 percent limonene (*fig. 6-10*) and was classed as ultra-high

Figure 6-9—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3139 x WP-3312; see caption of figure 6-2 for explanation of the format below:
**= mode near zero was not used in modal analysis, but was included in the mode count.

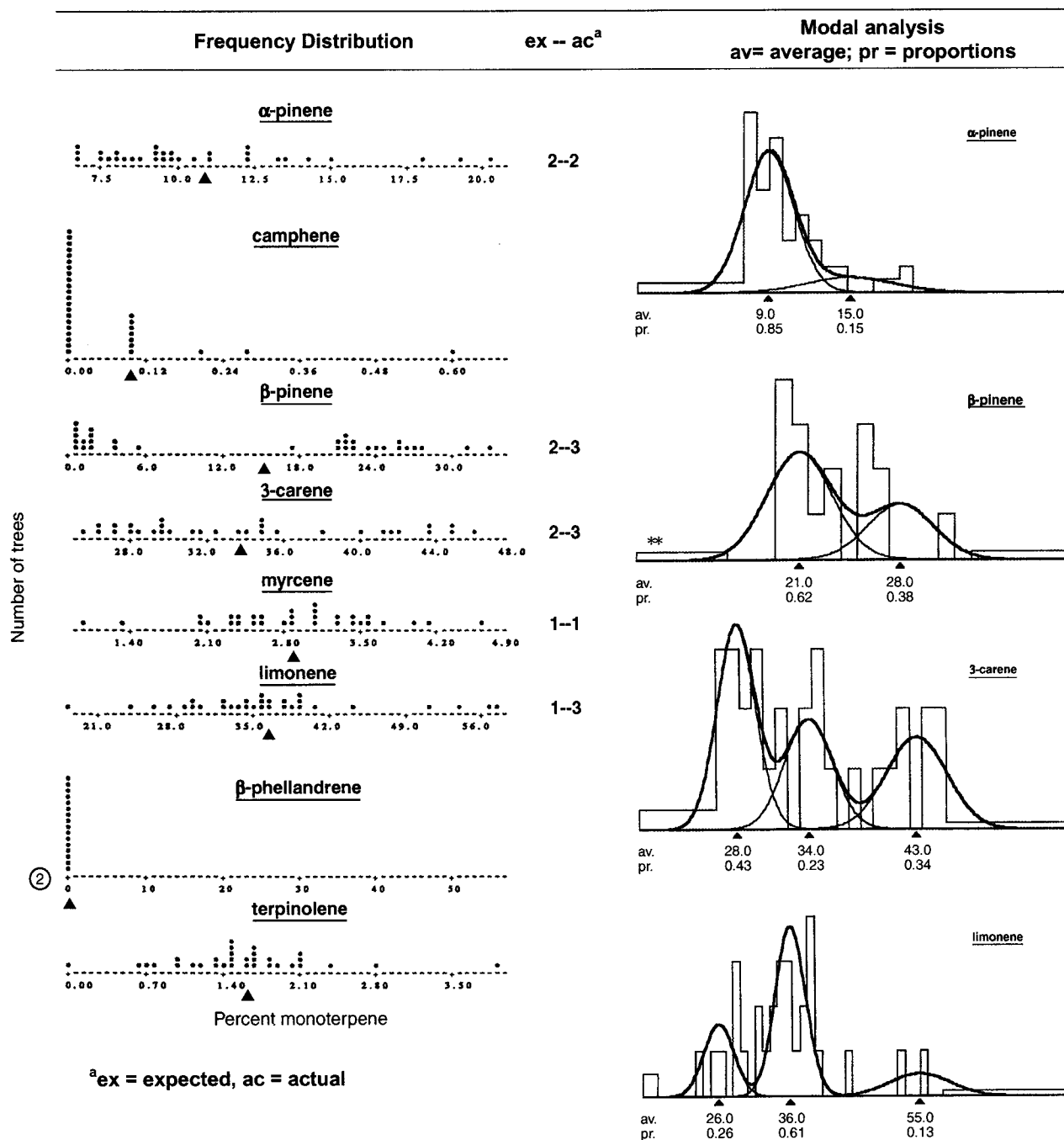
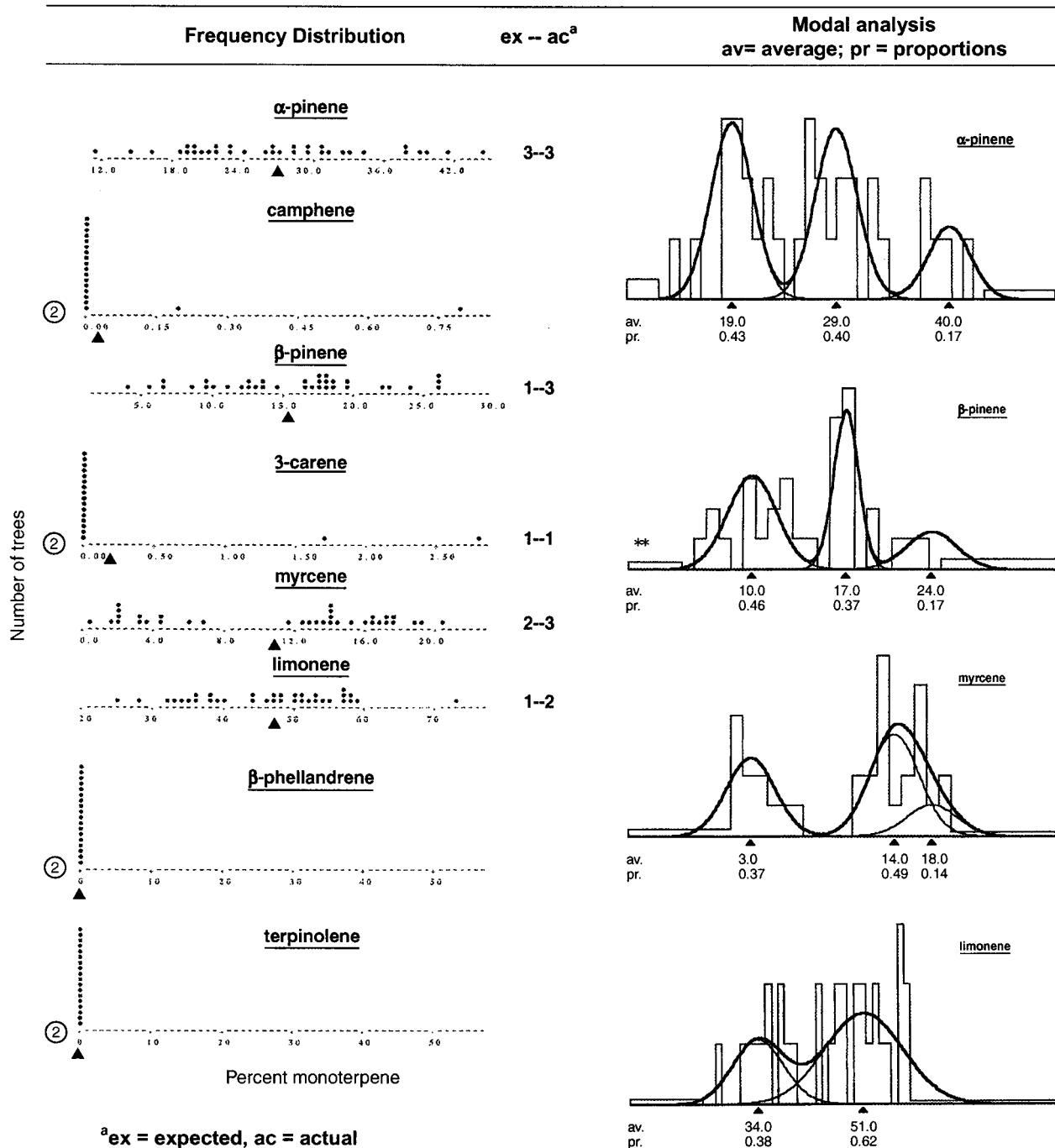


Figure 6-10—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3139 x AS-5789; see caption of figure 6-2 for explanation of the format below.



(fig. 6-3). Twenty-two trees had more than 40 percent limonene and were classed as high limonene. The higher percentage of myrcene in the progeny of Trees AS-3139 x AS-5789 differed from other progeny of near-high limonene males, and this was reflective of the 29 percent myrcene in Tree AS-5789. Both the high average for myrcene and the two clearly separated modes in the frequency distribution supported the assignment of a (0-1) set of alleles for myrcene in Tree AS-5789. The use of Tree AS-5789 as a female will be covered later and will add support to the assignment of a (0-1) set of alleles for myrcene. The reciprocal cross, Trees AS-5789 x AS-3139, had somewhat the same types of composition, but did not have an ultra-high type of limonene which was found in Trees AS-3139 x AS-5789, though not commonly. The two sets of reciprocals will be discussed in the section describing Tree AS-5789 as the female.

Progeny of *P. ponderosa* AS-3139 x AS-3194

Tree AS-3194, also growing at Adin Summit (AS), was another near-high limonene tree with 33 percent 3-carene (table 6-1). Its composition was quite constant over time and sample locations (table 6-2).

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3139 female	1	18	1	0	22	59
WP-3194 male	1	7	6	33	13	39
F-1 progeny	27	13	16	19	4	47

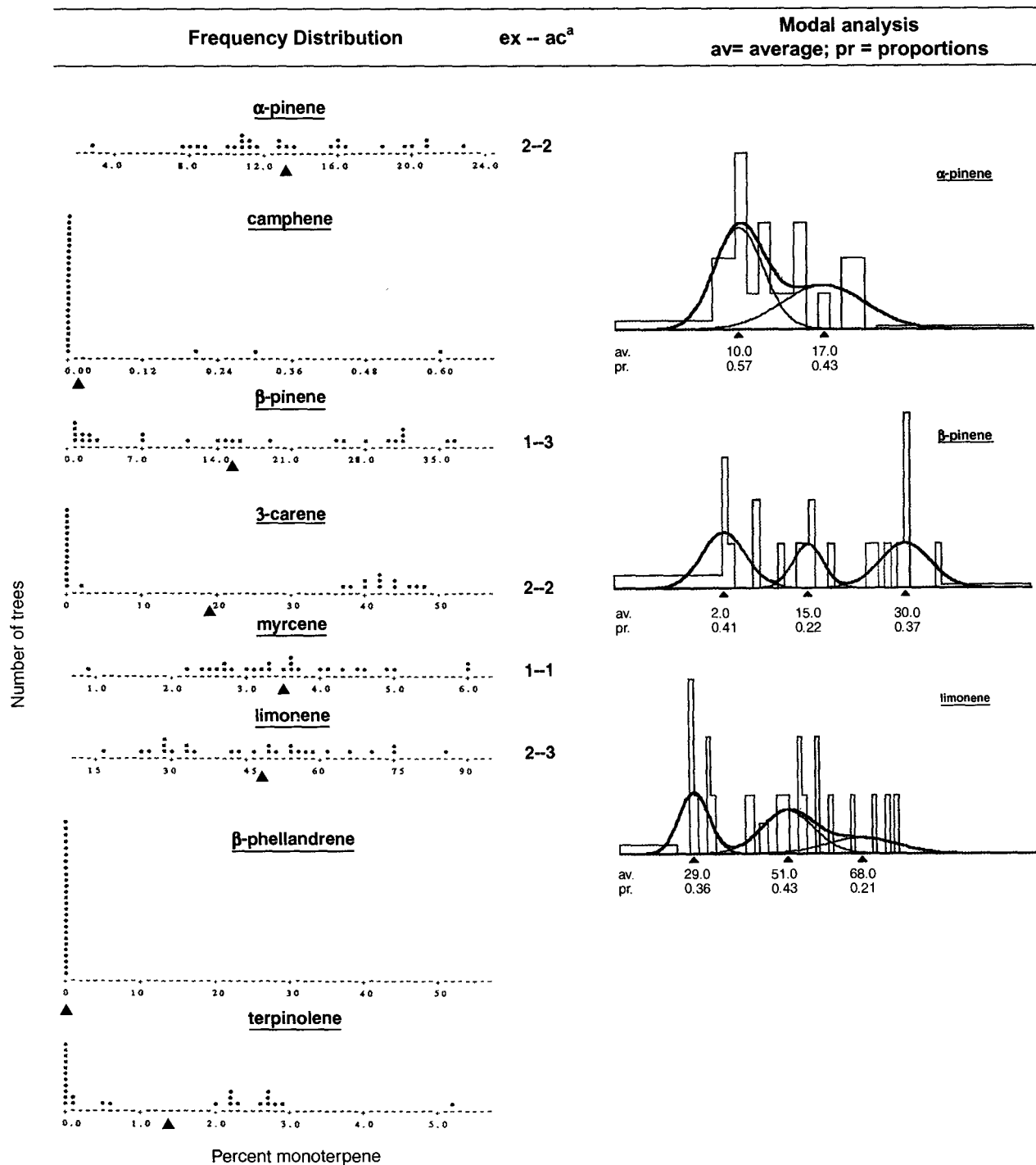
Again, the effects of growing in containers and container/plantation were clearly evident (table 6-3) with limonene much lower and α -pinene and 3-carene much higher in container and container/plantation than in the nursery-grown trees. The frequency distributions of these latter two sets of progeny were not used in the analysis of modes, and only nursery progeny were analyzed further.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees (fig. 6-11). This supported the additive allele hypothesis and the assignment of alleles to the parent trees. The mode proportions were only moderately close to the expected; the small number of trees in the sample could be the reason. However, with the small numbers, the three modes each for α -pinene and β -pinene and the two modes for limonene were clearly evident. Better mode proportions could be achieved by reducing the distribution to the expected number of modes.

Both ultra-high and high limonene trees were common in this cross between trees growing near each other (fig. 6-3); and the numbers of ultra-high and high limonene trees were much greater than when Tree AS-3139 was crossed with similar males which were a great distance away, such as Trees WP-3299, WP-3312, and WP-3347. This again suggested that progeny of parents separated by great distances may be under some level of stress, and stress was usually indicated by lower limonene and myrcene and by higher α -pinene and β -pinene. Tree AS-3194 was somewhat similar to Tree WP-3299—both were near-high limonene with moderate 3-carene—and the frequency distributions of 3-carene, myrcene, and limonene were somewhat similar for the sets of progeny, though Tree AS-3194 was within 3 miles of Tree AS-3139, whereas Tree WP-3299 was more than 400 hundred miles from Tree AS-3139.

Tree AS-3194 had excellent growth characteristics and could be a good candidate in other breeding studies.

Figure 6-11—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3139 x AS-3194; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

Progeny of *P. ponderosa* AS-3139 x AS-3178

Tree WP-3178 was selected for breeding because it did not have high or near-high limonene, but did have a very high 3-carene (*table 6-1*). Its composition was quite constant over time (*table 6-2*).

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3139 female	1	18	1	0	22	59
AS-3178 male	1	3	1	55	18	19
F-1 progeny	33	9	3	42	8	36

Thirty-three progeny were analyzed (*table 6-3*). Though there was a decrease in limonene in container trees to 29 percent and in container/plantation trees to 20 percent (*table 6-3*), there was little or no shift in 3-carene or β -pinene. And there was an increase in α -pinene. However, only the nursery-grown progeny were analyzed further.

The analysis of the frequency distributions (*fig. 6-12*) showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees. This supports the additive allele hypothesis and the assignment of alleles to the parent trees. Mode proportions were relatively close to the expected (*fig. 6-12*), especially if the expected number of modes was used.

There were no ultra-high limonene progeny (*fig. 6-3*). All progeny were high in 3-carene—supporting the assignment of a (1-1) set of alleles for 3-carene to Tree AS-3178; yet nine of them could be classed as high limonene by having more than 40 percent. Such trees, with an aggregate sum of about 80 percent for limonene plus 3-carene, would be excellent candidates for bioassay with western pine beetle because 3-carene is the second-most toxic monoterpene to the beetle.

Progeny of *P. ponderosa* AS-3139 x BL-6074

Tree BL-6074 was classed as a high limonene tree, 57 percent, on the strength of breeding studies. It was growing near Blue Lake (BL) in the South Warners of the Modoc National Forest, and was probably greater than 100 years old. Its composition was quite constant over time (*table 6-2*).

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3139 female	1	18	1	0	22	59
BL-6074 male	1	7	11	0	27	57
F-1 progeny	38	23	15	3	3	55

Different from the other three high limonene trees (AS-3139, WP-3298, WP-3352), BL-6074 had a small amount of α -pinene and moderate β -pinene, but a large amount of myrcene (*table 6-1*). Progeny were grown only in 4-year containers (*table 6-3*).

The analysis of the frequency distributions showed that the number of modes actually found in the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-13*). This supports the

Figure 6-12—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3139 x AS-3178; see caption of figure 6-2 for explanation of the format below.

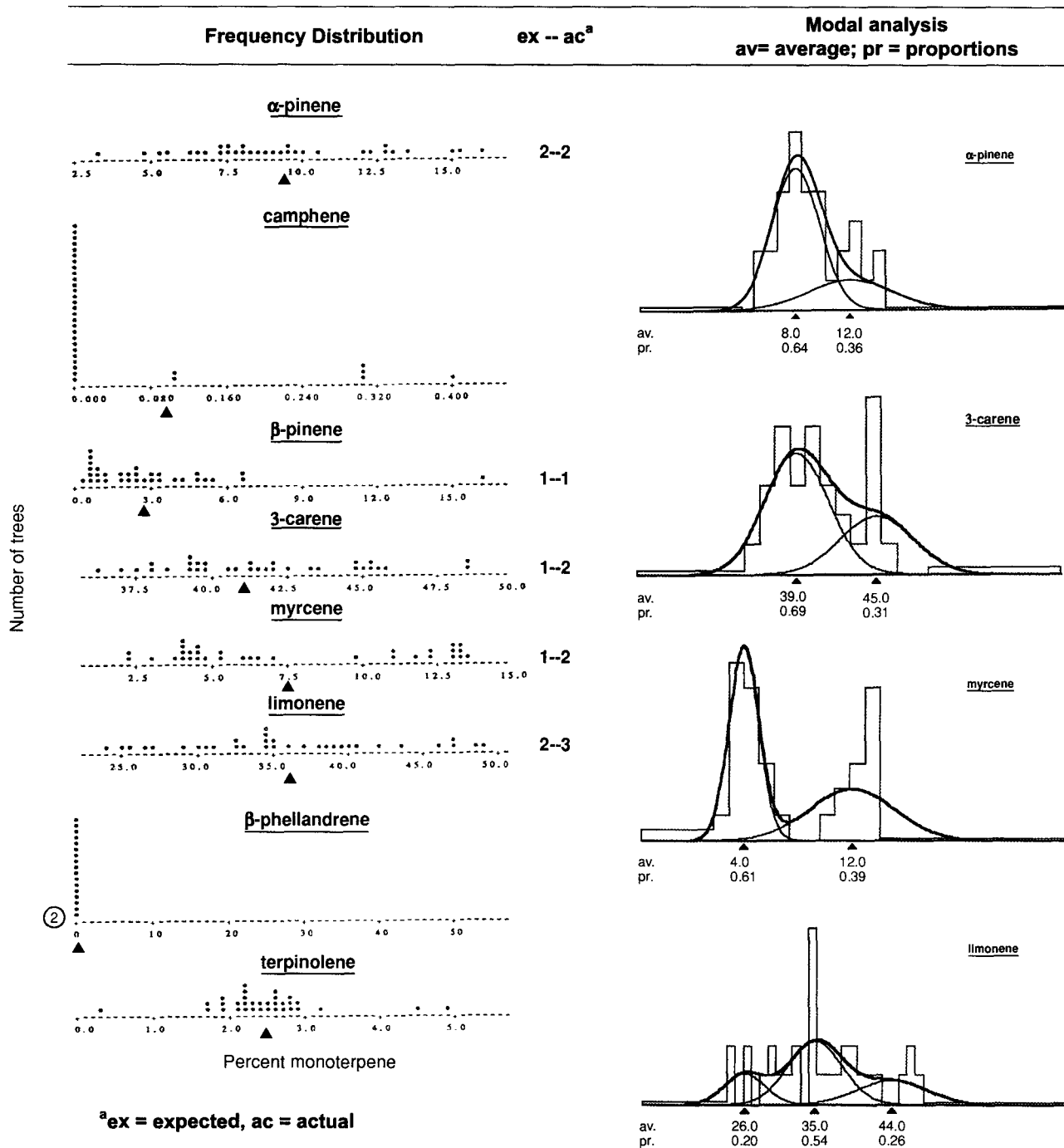
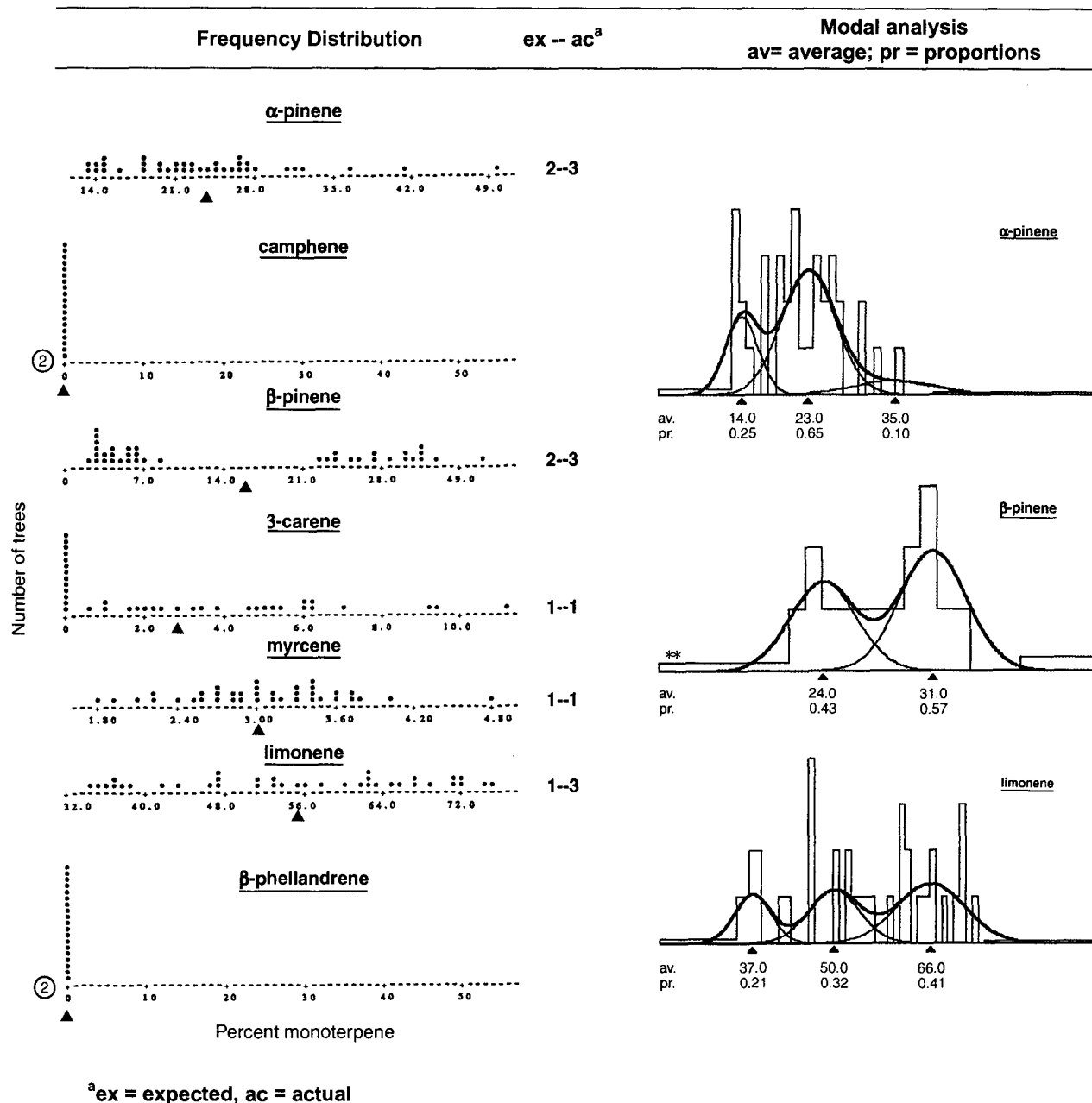


Figure 6-13—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3139 x BL-6074; see caption of figure 6-2 for explanation of the format below; **= mode between zero and seven percent was not used in modal analysis, but was included in the mode count.



additive allele hypothesis and the assignment of alleles to the parent trees. Mode proportions were relatively close to the expected when the effect of the modifying factors was considered. If the three modes for β -pinene were reduced to the expected two modes, the resulting two modes would have proportions of 55:45. Of the 38 progeny, 17 were ultra-high limonene, greater than 60 percent, and 14 others were high limonene, greater than 40 percent (*fig. 6-3*). Thus, there was a greater percentage of ultra-high limonene trees in this family than in any other family of Tree AS-3139. Though Tree BL-6074 did not qualify as high limonene because of the presence of β -pinene, it does qualify because of the large number of ultra-high limonene progeny it produced when crossed with Tree AS-3139.

Thus, BL-6074 was really high limonene, and therefore was assigned a (1-1) set of alleles for limonene, but it was of a different kind of high limonene. It would have been of much value to have used Tree BL-6074 as a female parent with several high and near-high limonene males. The nearness of Tree BL-6074 to Tree AS-3139, like Trees AS-A95 and AS-3194, apparently increased the level of limonene and myrcene, and decreased the level of α -pinene and β -pinene, thus increasing the number of high and ultra-high limonene progeny. The lower level of myrcene in this set of progeny than in the progeny of Trees AS-3139 x AS-5789 suggested that Tree BL-6074 has a (0-0) set of alleles for myrcene, though myrcene is only 2 percent less than in Tree AS-5789. The high level of myrcene in Tree BL-6074 might be due in part to old age. One might be tempted to assign a (0-1) set of alleles for myrcene since there was a suggestion of two modes in the frequency distribution, separated at about 3.2 percent, but the modal analysis could not separate the distribution into two modes. However, the small number of trees in the sample and the low average make such an assignment uncertain. It will be reported later in this chapter, in the discussion of the progeny of Trees AS-A78 x BL-6074, that two modes were found for myrcene. Thus there was mixed proof of an allele for myrcene in Tree BL-6074.

Discussion: Full Siblings of *P. ponderosa* AS-3139

There were little or no changes in composition with increase in age of nursery growth between 4 and 11 years; therefore, the aggregate data on such trees were suitable for analyses. However, there was a tendency for myrcene to increase slightly with age, but this increase was considered too small to affect the analyses. There were marked differences between nursery trees and those grown in containers for prolonged periods, in a plantation or in a container/plantation. The data from the latter three conditions were not used for further analysis. Stress was postulated as one of the causes of the differences in composition at different locations of growth.

The actual number of modes in the frequency distributions, as derived by modal analysis, equalled or exceeded the expected number of modes, as derived from the assignment of additive alleles to the five major monoterpenes; this supported the additive allele hypothesis and the assignment to Tree AS-3139 of sets of alleles of (0-1), (0-0), (0-0), (0-0), and (1-1), respectively, for α -pinene, β -pinene, 3-carene, myrcene, and limonene. Though mode proportions were not always close to the expected, they were reasonably close when all the effects of the modifying factors were considered. Reducing the modes to the expected number improved the proportions, thus adding support to the hypothesis. The results with Tree AS-3139 also confirm the assignment of alleles to the male parents.

Tree AS-3139 produced ultra-high and high limonene progeny when crossed with high limonene and near-high limonene males, but the number of these types of composition was greater with nearby males than with distant males. Likewise, the average limonene and the averages of the modes of the

frequency distribution were higher with nearby males. This lower limonene in the progeny with distant males is much like that which occurs with forms of stress. Therefore, it is postulated that progeny of parents, which are far apart, grew under some degree of stress.

One pollen source, Tree AS-5789, was assigned a (0-1) set of alleles for myrcene, and the amount of myrcene in progeny and the location of the modes in the frequency distribution substantiated this assignment. AS-5789 was the only one used in the whole population studies of *P. ponderosa* with an allele for myrcene. In all other crosses myrcene was probably derived from a nongene source.

The results of the crosses with near-high limonene males were particularly encouraging in the number of ultra-high and high limonene progeny, because near-high limonene trees were much more abundant for breeding than high limonene trees. Reciprocal crosses with Trees AS-A95 and BL-6074 would have been very helpful.

Data on the frequency distribution of the five major monoterpenes were brought together so that each could be viewed with the same base line for all families (*fig. 6-14*). Comparative frequency distributions of the individual monoterpenes of all 10 sets of progeny clearly showed that the position of the modes can shift depending on the types and number of the other alleles and on presence of forms of stress (*fig. 6-14*). However, the number of modes was fixed by the basic structure of the aggregate system. In some cases, the modes have been compressed because of the change in the scale of the base line.

Tree AS-3139 at Adin Summit, its grafts at IFG, and many of its progeny at IFG could be used for further study. Likewise, Trees AS-A95, BL-6074, AS-A78, and AS-3194 would be good subjects for breeding studies.

Crosses of *P. ponderosa* AS-A78 with Five Pollen Sources

Tree AS-A78, another high limonene tree (*table 6-1*), was growing near Tree AS-3139; it was found when the original stand at Adin Summit was expanded from 80 to 390 trees. It was used and sacrificed in a subsequent resistance bioassay field test with western pine beetle (Smith 1982b). However, six successful grafts of this tree are now growing in the IFG Arboretum near Placerville; all breeding was done on these grafts. All progeny were grown in a 7- to 9-year-old nursery or in 4-year containers. Chapter 7 contains data on the monoterpene composition of the grafts. Different from Tree AS-3139, AS-A78 was a small tree, only 7 inches in diameter, growing in a small group of pole-size trees. By its monoterpene composition, one would suspect that one of its parents was Tree AS-3139, a much older tree, or it might have derived from selfing of Tree AS-3139. Five pollen sources were used with Tree AS-A78, and the average composition of the progeny was obtained for six sets, two sets for Tree WP-3298 (*table 6-5*).

Progeny of *P. ponderosa* AS-A78 x AS-3139

Tree AS-3139 was also high in limonene and was the female parent in the previous set of progeny (*table 6-1*).

Normalized percent compositions of parents and progeny were as follows:

Tree and type	Number	α -pinene	β -pinene	3-carene	myrcene	limonene
AS-A78 female	1	22	3	0	18	57
AS-3139 male	1	18	1	0	22	59
F-1 progeny	61	31	4		5	60

Sixty-one nursery-grown progeny were examined, all essentially lacked β -pinene and 3-carene, as expected, and nearly all were classed as ultra-high or

Table 6-5—Average monoterpene composition of the progeny of *P. ponderosa* AS-A78 with five males. *N* = number of trees.

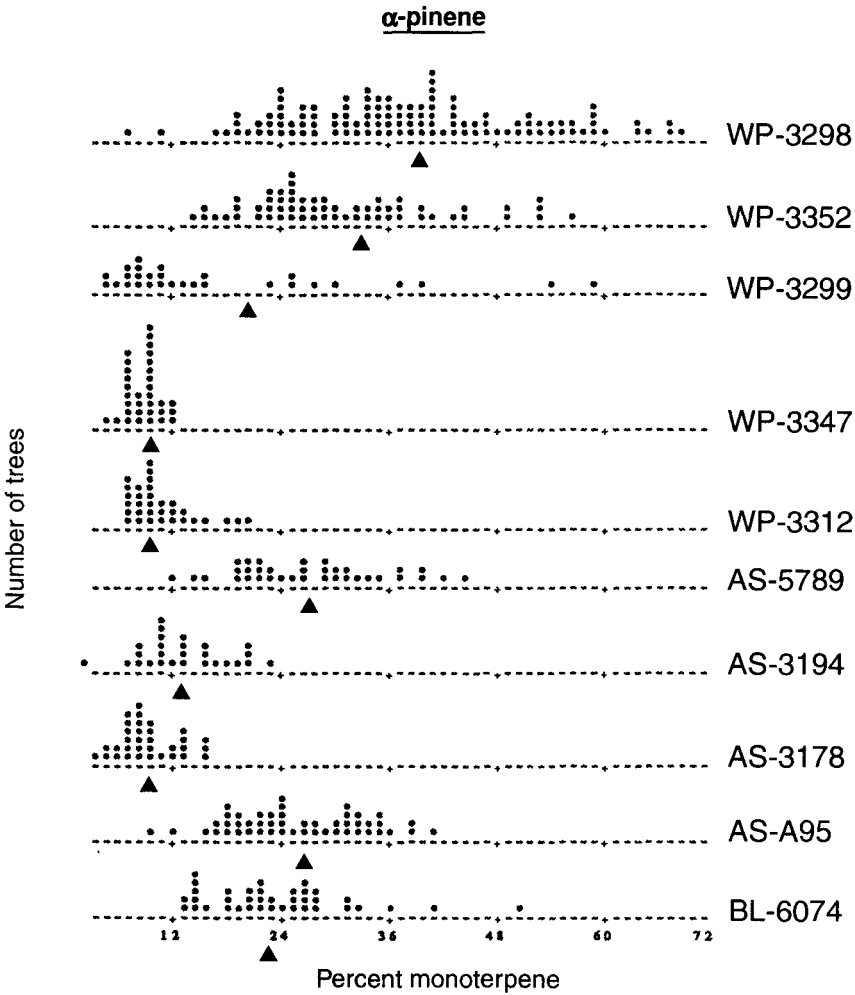
Male	Place of growth	Age	N	Monoterpene composition of progeny ¹							
				α -p	cam	β -p	car	myr	lim	β -ph	ter
		<i>yrs</i>						<i>percent</i> ²			
AS-3139	Nursery	7-9	61	31.2	*	3.9	0.1	4.5	60.3	0	*
WP-3298	Nursery	7-9	10	63.2	0	2.4	*	4.1	30.4	0	0
WP-3298	Container	4	4	52.1	0	10.9	0.3	3.1	33.7	0	0
AS-A95	Container	4	29	32.5	0	28.9	2.3	2.0	34.1	0	0.1
BL-6074	Container	4	25	26.0	0	29.4	1.3	3.0	40.1	0	0.2
AS-3187	Container	4	18	24.3	0	34.8	20.3	2.3	17.2	0.1	1.1

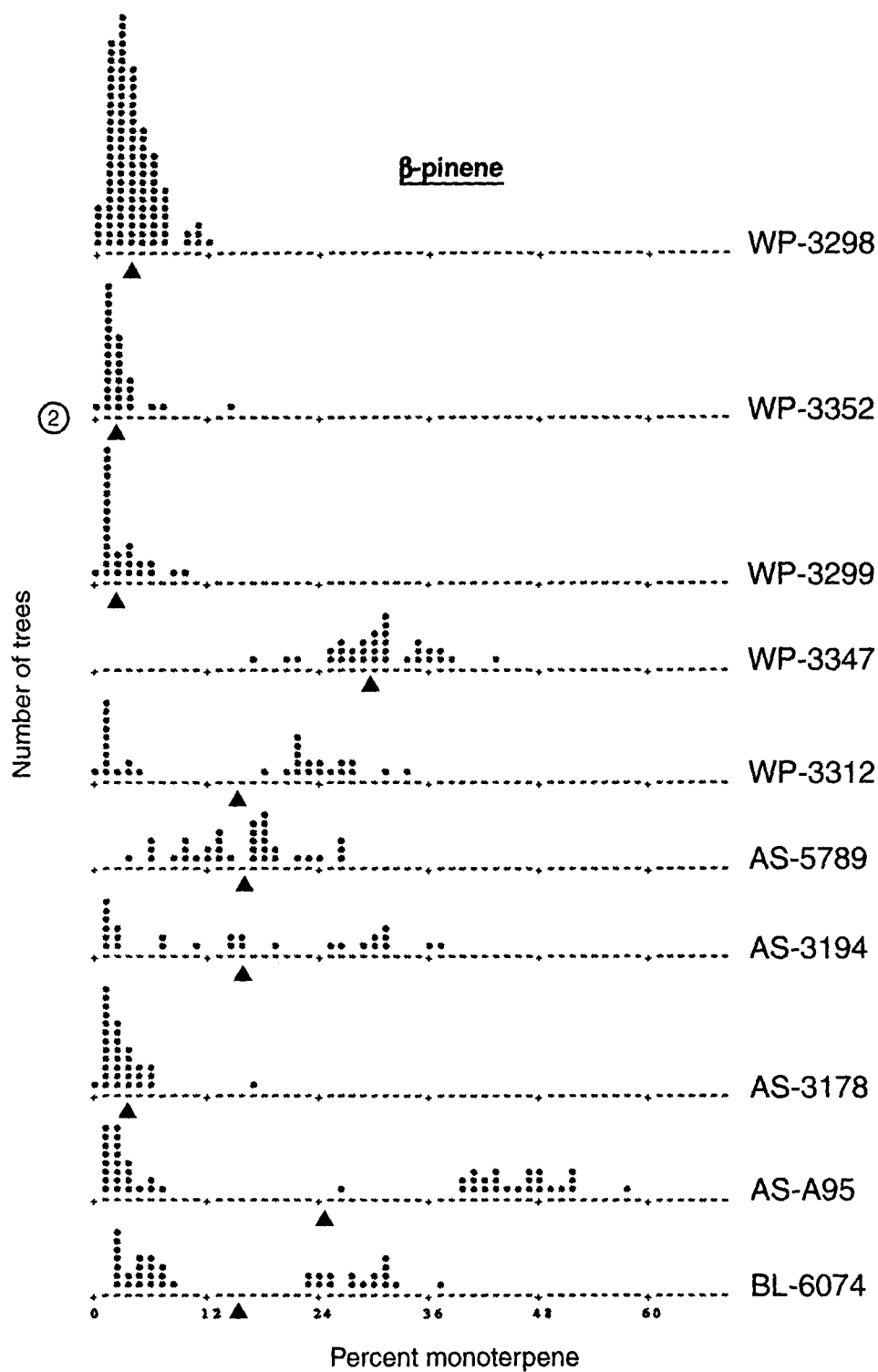
¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.

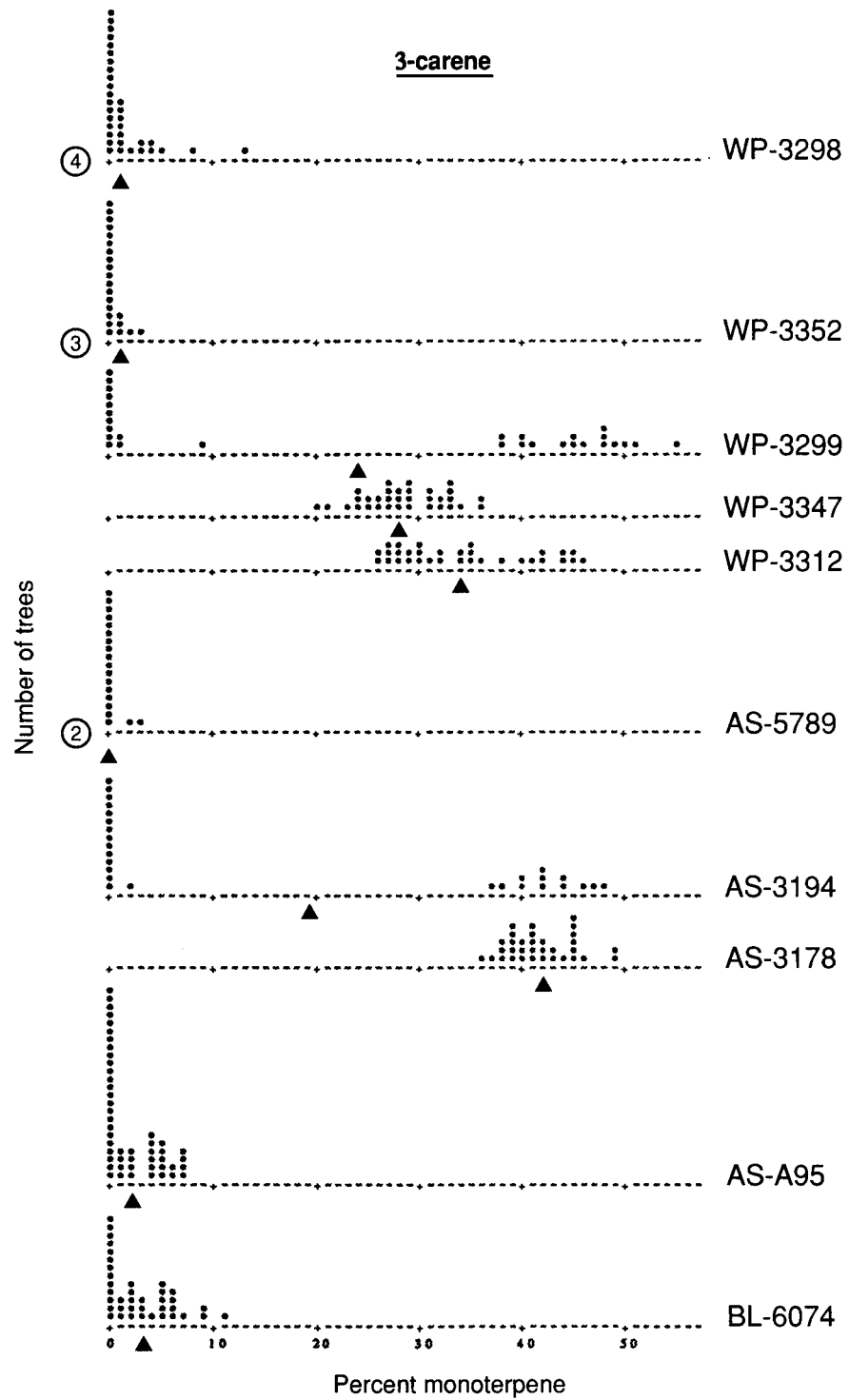
²Normalized; * = trace.

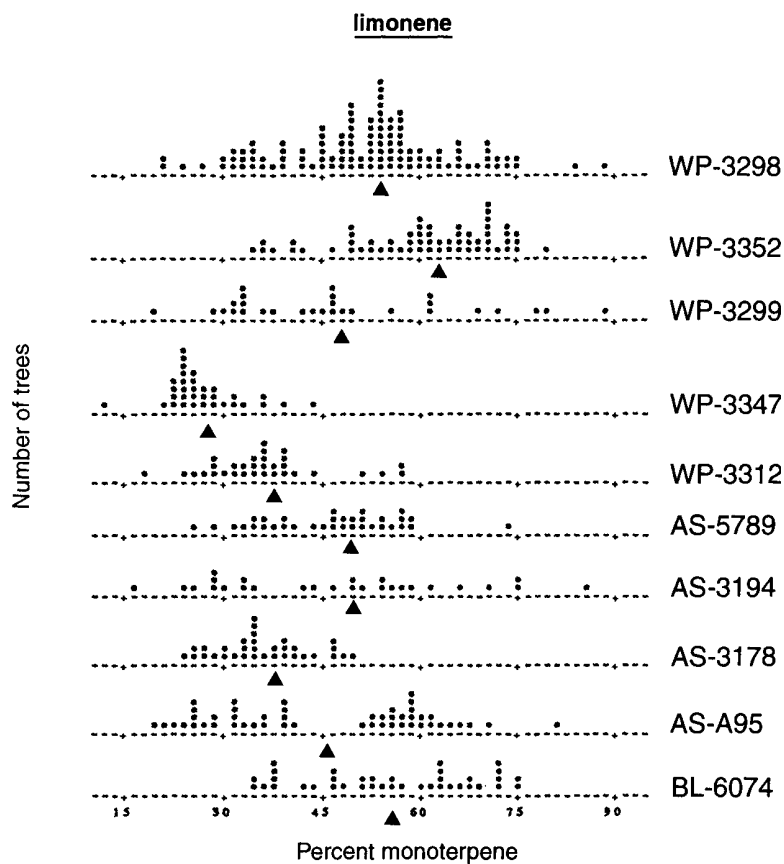
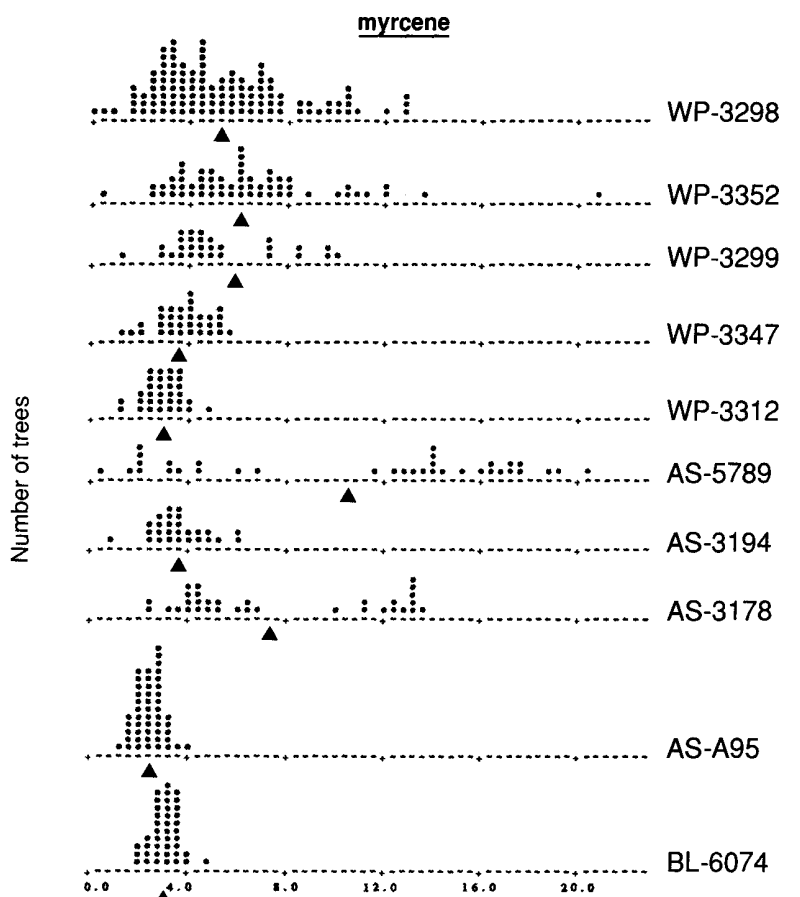
Figure 6-14—Comparative frequency distribution of the five major monoterpenes in the progeny of *P. ponderosa* AS-3139 when crossed with 10 different males listed on the right; each dot is measurement of one tree, except where (n) indicates each is n trees; [▲] is average of set.

(continues on next 3 pages)









high limonene with the complementary amount occurring as α -pinene (*table 6-5*). One would have expected all progeny to be at least high in limonene, but as noted earlier with other such crosses, such as Trees AS-3139 x WP-3298, some progeny had only moderate limonene with a complement of high α -pinene. A (0-1) set of alleles for α -pinene in both parents would explain this presence of low, moderate, or high α -pinene; this, coupled with a (1-1) set of alleles for limonene in both parents and competition for a fixed amount of precursor, would explain the presence of moderate, high, and ultra-high limonene.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-15*). This supports the additive allele hypothesis and the assignment of alleles to the parent trees. The trimodality of α -pinene and limonene was clearly evident and was strong evidence for additive alleles. Mode proportions for α -pinene were reasonably close to the expected when the effects of the modifying factors were considered, as noted previously. Proportions of 25:50:25 for α -pinene were expected; 31:38:31 were achieved by the analysis.

Three trees had greater than 80 percent limonene (*fig. 6-3*), and nearly 90 percent, suggesting that near-pure limonene *P. ponderosa* might be produced by breeding, though no such trees have been found in natural stands. The types of composition were much like those of the progeny of Trees AS-3139 x WP-3298 and AS-3139 x WP-3352; all three parents were classed as high limonene. Each of the three averages of the modes for limonene, at 36 percent, 60 percent, and 74 percent, were higher in the progeny of Trees AS-A78 x AS-3139 than in those of AS-3139 x WP-3298, at 32 percent, 52 percent, 68 percent. This is pointed out to illustrate the postulated effect of distance between parents; Trees AS-A78 and AS-3139 were in close proximity whereas Tree WP-3298 was about 400 miles away. Also, the general characteristics of the frequency distributions of the progeny of Trees AS-3139 x WP-3298 and AS-A78 x AS-3139 were quite similar, with the modes simply occupying different positions along the percent baseline.

Progeny of *P. ponderosa* AS-A78 x WP-3298

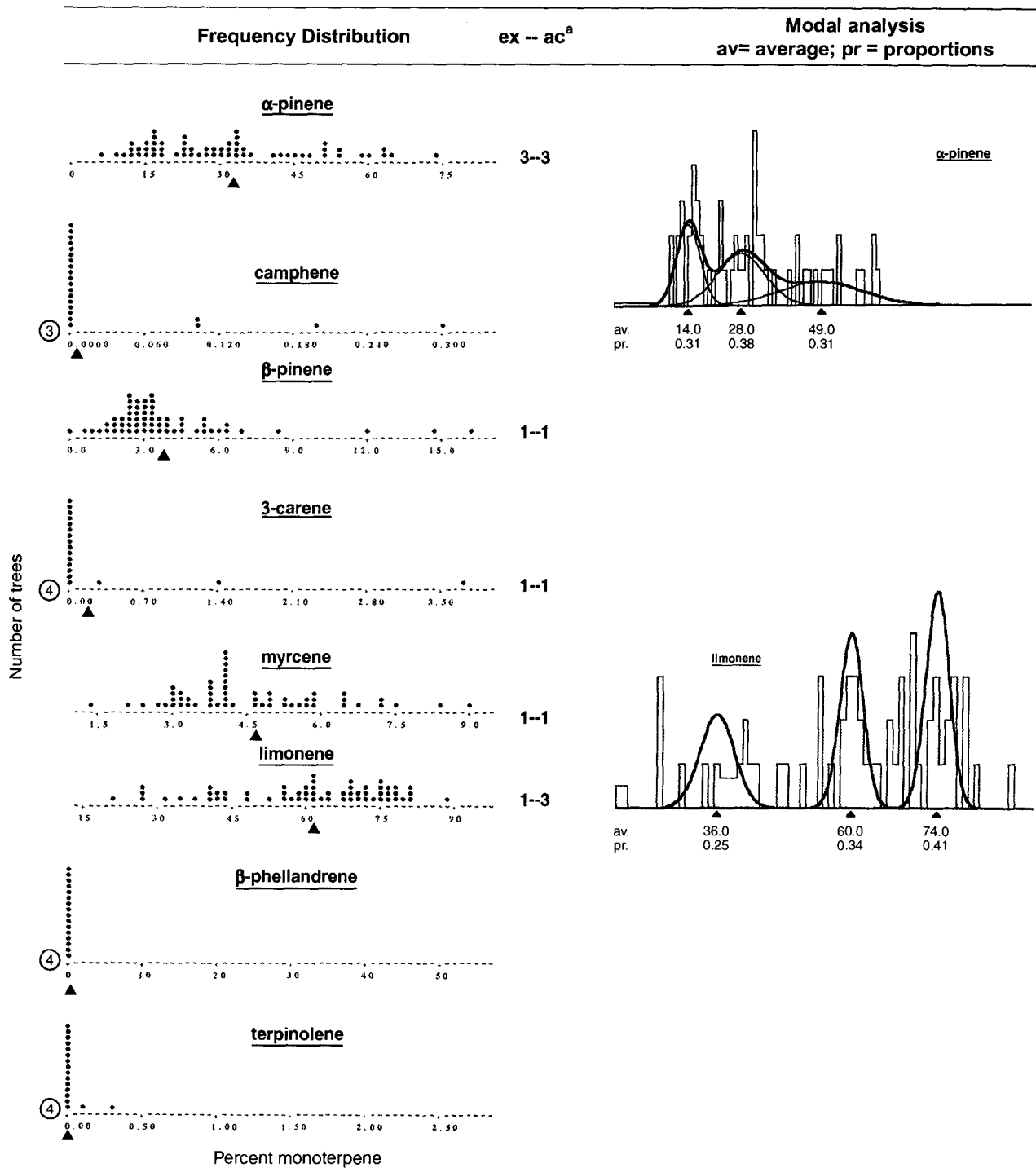
This is another cross of two high limonene trees. However, Tree WP-3298 was growing at Wheeler Peak, whereas Tree AS-3139, the previous male used with Tree AS-A78, was growing at Adin Summit.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A78 female	1	22	3	0	18	57
WP-3298 male	1	26	0	0	16	59
F-1 progeny	14	63	2		4	30

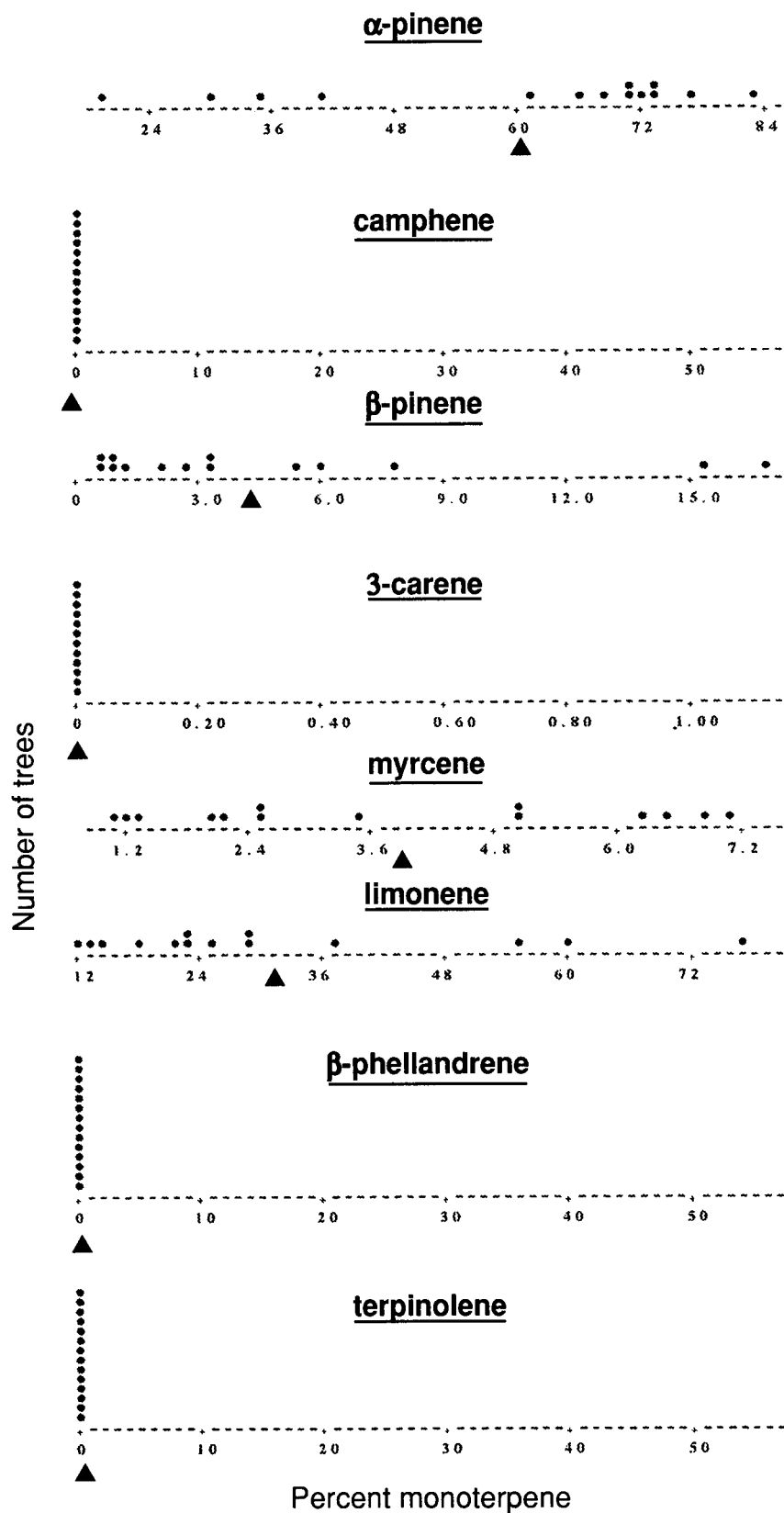
Quite different from the progeny of Trees AS-A78 x WP-3139, the progeny of Trees AS-A78 x AS-3298 were generally high α -pinene with limonene in complementary amounts (*table 6-5*). Nursery and container data were aggregated. Though a 14-tree sample was probably too small for a confident statement, the lack of more high limonene trees in the progeny of this cross suggested that the effect of distance between parents was quite pronounced. I suspect that with aging, the composition will shift toward greater limonene, because 3-carene and β -pinene are lacking or nearly lacking in all progeny (*table 6-5*). There was the possibility that the progeny of Trees AS-A78 x WP-3298 were under stress because their composition does resemble container-grown plants. Nevertheless, two of the progeny had greater than 60 percent limonene, and one had nearly 80 percent (*fig. 6-16*). Alternative explanations for the lower-than-expected limonene in this family were the

Figure 6-15—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-A78 x AS-3139; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

Figure 6-16—Frequency distribution of the amount of the noted monoterpenes in the 4- to 9-year-old progeny of *P. ponderosa* AS-A78 x WP-3298; each dot is measurement of one tree; [▲] is average of set.



juvenile effect, the distance between parents, or there were minor genetic differences between the two pollen sources. Trees AS-3139 and WP-3298 were in different ecological zones for resin. There were only four container-grown trees, and these resemble the nursery-grown ones, but the sample was too small for comment. The 14-tree sample was too small for modal analysis (*fig. 6-16*); however even these few trees had a wide range in α -pinene, from about 20 percent to 80 percent, and limonene, from 0 percent to 80 percent.

Progeny of *P. ponderosa* of AS-A78 x AS-A95

Tree AS-A95 was growing at Adin Summit and within 0.10 mile of Tree AS-A78, but all the breeding was done on grafts in the Camino Arboretum near Placerville.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A78 female	1	22	3	0	18	57
AS-A95 male	1	19	21	0	15	45
F-1 progeny	29	33	29	2	3	34

Tree AS-A95 was near-high limonene, 45 percent, but it does have 21 percent β -pinene. Twenty-nine 4-year-old container-grown progeny were examined, and they reflected the composition of both parents, as expected (*table 6-5*). The amount of α -pinene was quite variable from about 12 percent to near 75 percent (*fig. 6-17*), and the amount of limonene ranged about as widely, from 10 to 73 percent. As with some other crosses of two trees without 3-carene, there were small amounts of 3-carene in some progeny, ranging from 1 percent to about 10 percent. One might speculate on isomerization or low activity by the (0-0) set of alleles as the cause of the low amount of 3-carene.

The analysis of the frequency distributions showed that the number of modes found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-17*). This supports the additive allele hypothesis. The trimodality of α -pinene reflected the (0-1) set of alleles in each parent. Because both parents had a (1-1) set of alleles for limonene, the multimodal distribution of limonene was caused by the interaction of the three different sets of alleles for α -pinene, (0-0), (0-1), (1-1). Mode proportions were reasonably close to the expected when all the factors were considered. Mode proportions for α -pinene were 43:33:24; the two expected modes for β -pinene had proportions of 47:53.

There were both ultra-high and high limonene trees, as expected (*fig. 6-3*). The abundance of these types and the high average of limonene reflected the closeness of the parents.

Progeny of *P. ponderosa* AS-A78 x BL-6074

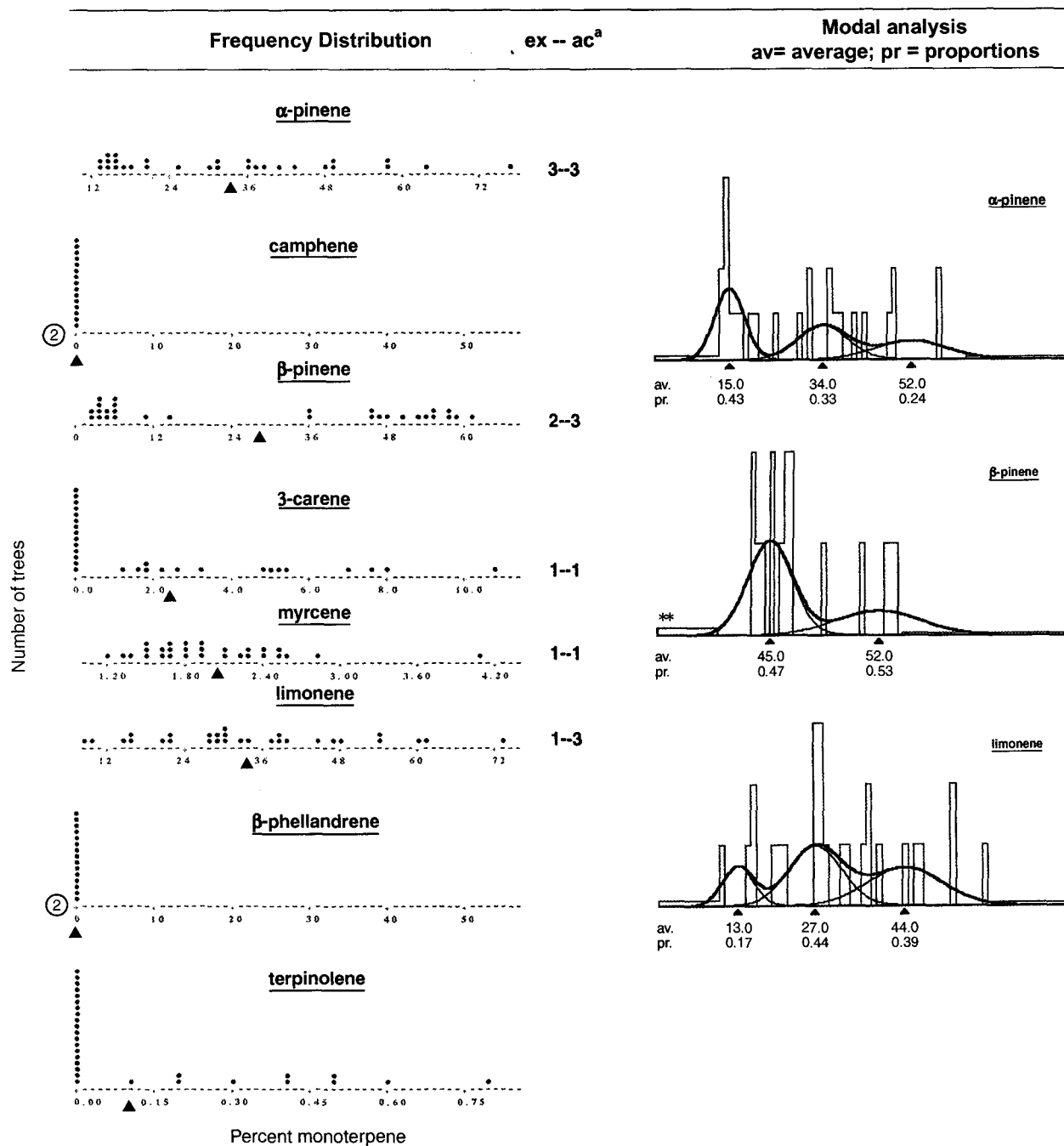
Tree BL-6074 was located near Blue Lake in the South Warners. It was found late in the work and was not used as much as other trees. Tree BL-6074 was classed as high limonene, but it was different from other trees of this class, in that it had a low amount of α -pinene and β -pinene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A78 female	1	22	3	0	18	57
BL-6074 male	1	7	11	0	27	57
F-1 progeny	25	26	29	1	3	40

The progeny of Trees AS-A78 x BL-6074 and those of Trees AS-A78 x AS-A95 were much alike in average composition and in frequency distributions (*table 6-5*, *figs. 6-17 and 6-18*).

Figure 6-17—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-A78 x AS-A95; see caption of figure 6-2 for explanation of the format below; **= mode between zero and 12 percent was not used in modal analysis, but was included in the mode count.



The analysis of the frequency distribution showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-18*). This supported the additive allele hypothesis and the assignment of alleles to the parent trees. Mode proportions were only moderately close to the expected, probably because of the excess number of modes. Myrcene appeared to have two modes, suggesting a (0-1) set of alleles for BL-6074. However, the general level of myrcene was so low that this assignment could be questioned. This was also noted for the progeny of Trees AS-3139 x BL-6074. The work on Tree BL-6074 was insufficient for making a confident assessment of the structure of its alleles for myrcene, and the (0-0) assignment was conditionally retained. The (0-1) assignment of alleles to β -pinene was shown by the two clearly separated modes with about equal proportions.

Both ultra-high and high limonene types of composition were found (*Fig. 6-3*); ultra-high types were not common; high types were common. Both the general level of limonene and the abundance of ultra and high types supported the classification of BL-6074 as a high limonene tree and the assignment of a (1-1) set of alleles.

AS-A78 x AS-3187

Tree AS-3187 was near Adin Summit. It was also heavily used as a female parent. Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A78 female	1	22	3	0	18	57
AS-3187 male	1	9	23	23	14	29
F-1 progeny	18	24	35	20	2	17

Tree AS-3187 was the only one used with Tree AS-A78 that had an assigned allele for 3-carene, and about one-half the progeny had 3-carene. Its composition was quite constant with time and location of sampling (*table 6-2*). And with additive alleles, two modes of equal proportions would be expected (*fig. 6-19*). Modal analysis was made, though there were only 18 trees in the set (*table 6-5*).

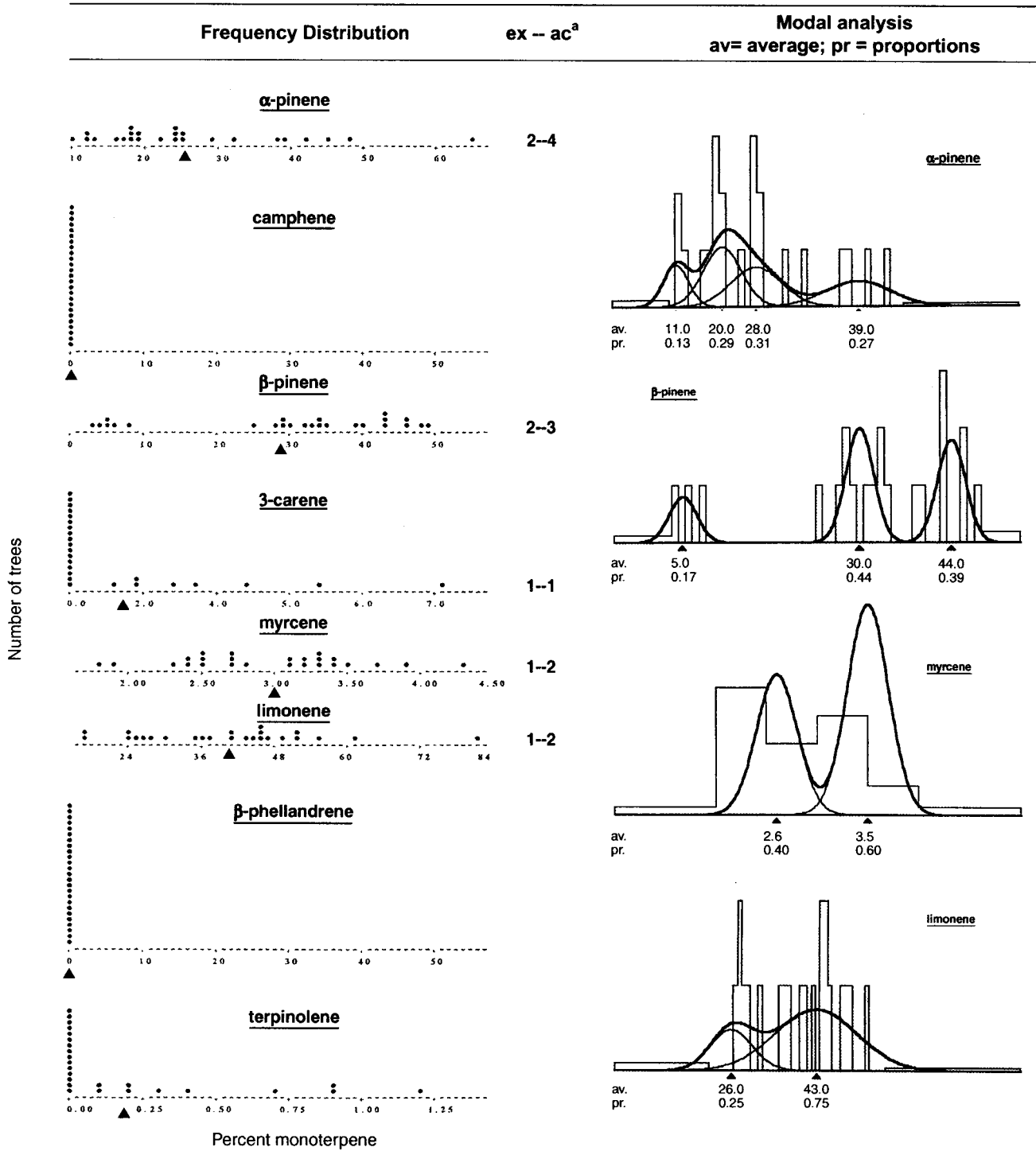
The analysis of the frequency distributions (*fig. 6-19*) showed that the number of modes found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents. There were two modes for 3-carene, as expected, and the proportions of 56: 44 were very close to the expected. By combining to get the expected two modes for α -pinene, proportions were 47:53; combining to get the expected two modes for limonene achieved mode proportions of 39:63.

Though a few high limonene trees were expected, none was found (*fig. 6-3*). With both α -pinene and β -pinene present in Tree AS-3187, the juvenile effect must have had added strength, thus lowering the amount of limonene more than usual. There were no potential high limonene trees. Whenever 3-carene or β -pinene was low or lacking, the composition went to α -pinene and not to limonene.

Discussion: Full Siblings of Tree AS-A78

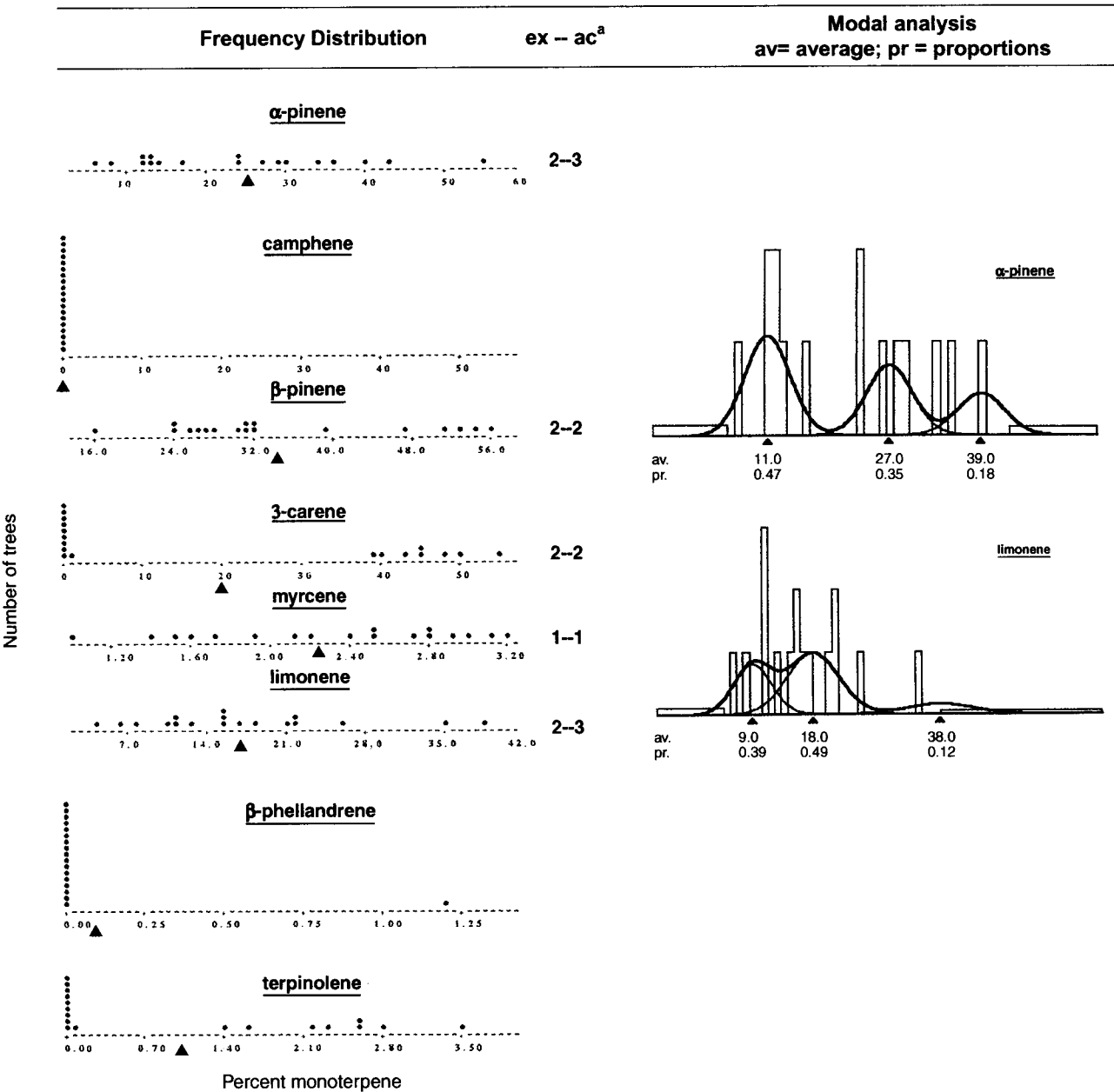
In all cases, the actual number of modes in the frequency distribution of the individual monoterpenes, derived by modal analysis, equalled or exceeded the expected number of modes, as derived from the assignment of alleles to the parents. This supported the additive allele hypothesis and the assignment of alleles to Tree AS-A78 and the male parents. Mode proportions were reasonably close to the expected under the conditions of several modifying factors; this was additional support for the hypothesis.

Figure 6-18—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-A78 x BL-6074; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

Figure 6-19—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-A78 x AS-3187; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

Tree AS-A78 produced ultra-high and high limonene progeny when crossed with high limonene males. However, the incidence of such trees was greater with nearby males than distant males. This is further evidence that there is some level of stress in the progeny of parents that are separated by great distances.

The number of modes in a frequency distribution was primarily fixed by the alleles of the parent trees, but where these modes were located along the percent scale was affected by the other alleles and by a number of other factors such as age, stress, and possibly by a minor genetic structure (*fig. 6-20*).

Several grafts of Tree AS-A78 are available for further research at IFG.

Crosses of *P. ponderosa* AS-A95 X Six Pollen Sources

Tree AS-A95 was classed as high limonene, 45 percent (*table 6-1*) with no 3-carene and moderate amounts of α -pinene (19 percent) and myrcene (15 percent); however, it had moderate β -pinene (21 percent), whereas other high limonene trees had little or no β -pinene. Its composition was essentially constant over time and with sample location (*table 6-2*). Tree AS-A95 was 12 inches in diameter and growing at Adin Summit, and it had excellent form and growth. However, the breeding was done on two grafts growing in the Camino Arboretum near Placerville. Composition of the graft was given in Chapter 7. Pollen of six trees was used (*table 6-6*).

Progeny of *P. ponderosa* AS-A95 x AS-3139

This was a cross of two high limonene trees; the natural trees were growing within 100 meters of each other.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A95 female	1	19	21	0	15	45
AS-3139 male	1	18	1	0	22	59
F-1 progeny	26	20	14	*	5	60

Tree AS-A95 was classed as a high limonene tree though it had only 45 percent limonene rather than 60 percent; this was caused by the presence of 21 percent β -pinene, giving β -pinene an assignment of (0-1) set of alleles. The breeding showed that it did have a (1-1) set of alleles for limonene and a (0-1) set for β -pinene. The average percent composition of 26 nursery-grown progeny for limonene was 60 percent, one of highest of all the sets of progeny (*table 6-6*). Container trees were much lower in limonene, much higher in β -pinene, and moderately higher in α -pinene (*table 6-6, fig. 6-21*). Therefore, only the data on the nursery sets were analyzed further. The two sets of reciprocal crosses—AS-3139 x AS-A95 and AS-A95 x AS-3139, which were discussed under AS-3139 as female—had similar compositions, respectively, for the five major monoterpenes: for the progeny of Trees AS-A95 x AS-3139 it was 20:14:0:5:59, and for the progeny of Trees AS-3139 x AS-A95 it was 25:24:2:2:45. The characteristics of the frequency distributions were quite similar also (*figs. 6-7 and 6-22*).

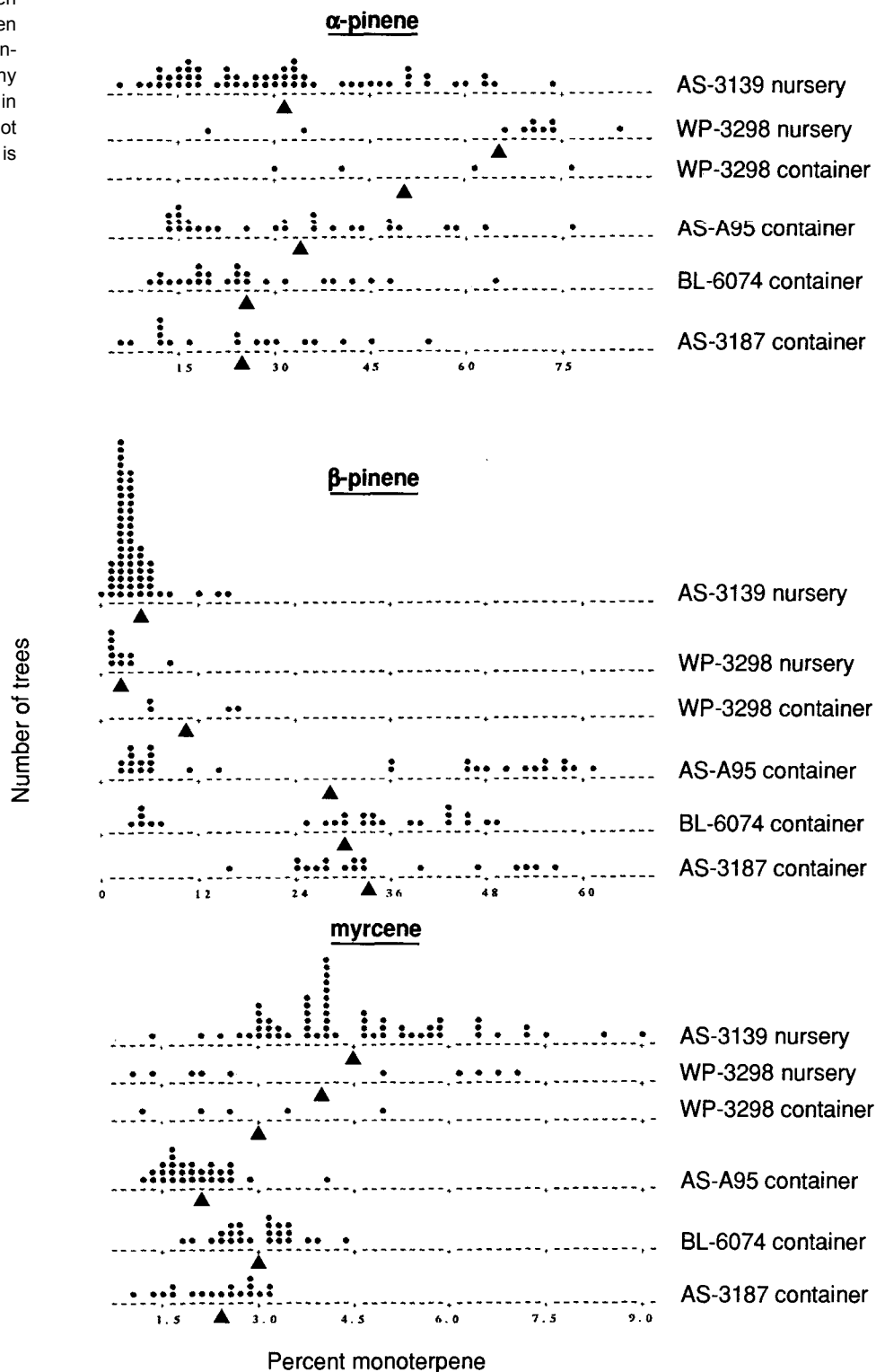
The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees (*fig. 6-22*). Mode proportions were not as close to the expected as was found in most other families. This could have been caused by the action of the modifying factors or the small size of the sample. However, the proportions of 58:42 for β -pinene were quite close to the expected 50:50. The three modes for α -pinene at 56:26:18 were not too good, probably because of the small size of the sample and the several modifying factors.

Table 6-6—Average monoterpene composition of the progeny of *P. ponderosa* AS-A95 with six males when grown in three locations. *N* = number of trees.

Male parent	Growth ¹ place	Age	N	Monoterpene composition of progeny ²							
				α -p	cam	β -p	car	myr	lim	β -ph	ter
		yr		percent ³							
AS-3139	nur	6-7	26	20.4	*	14.2	0.3	5.2	59.8	0	0
AS-3139	con	9	8	26.8	0.1	31.5	0.9	2.4	38.3	0	0.1
AS-3139	copl	9	4	12.5	*	39.6	22.0	4.5	20.4	0	1.0
WP-3298	nur	6-7	34	40.5	0.1	27.6	0.2	4.7	26.9	0	*
WP-3298	con	9	7	46.6	0.2	35.9	0.9	2.3	13.3	0.7	*
WP-3298	copl	9	4	52.3	0.1	12.4	0	5.9	29.3	0	0
WP-3352	nur	6-7	9	33.6	0.1	28.0	0.3	4.1	33.1	0	0
WP-3352	con	9	7	32.9	0.1	34.9	2.6	2.0	27.0	0.3	0.2
WP-3352	copl	9	8	47.4	0.1	23.0	0.2	3.3	25.9	0	0
AS-3194	nur	6-7	27	13.4	0.1	24.0	22.9	2.0	35.7	*	1.6
AS-3194	con	9	5	17.2	0.1	27.5	18.9	2.1	32.8	0	1.4
AS-3194	copl	9	4	46.1	0	23.8	9.1	2.7	17.0	0	1.1
AS-5789	nur	6-7	33	36.8	0.1	27.7	0	7.7	27.7	0	0
AS-5789	con	9	8	37.8	0.2	38.0	1.0	5.3	17.2	0.4	0.1
AS-5789	con	9	8	28.4	*	51.7	1.0	2.8	15.3	0.8	0.1
AS-5789	copl	9	9	50.9	0.1	34.9	0	4.7	8.8	0.5	0
BL-6074	con	4	7	21.4	*	42.1	2.9	2.4	30.1	0.8	0

¹nur = nursery; con = container; copl = container-plantation.² α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.³Normalized; * = trace.

Figure 6-20—Comparative frequency distribution of the amount of the four noted monoterpenes of the progeny of *P. ponderosa* AS-A78 when crossed with five different pollen sources in a nursery (nur) or container (con), listed on the right; progeny of Trees AS-A78 x WP-3298 grown in both nursery and container; each dot is measurement of one tree; [▲] is average of set.



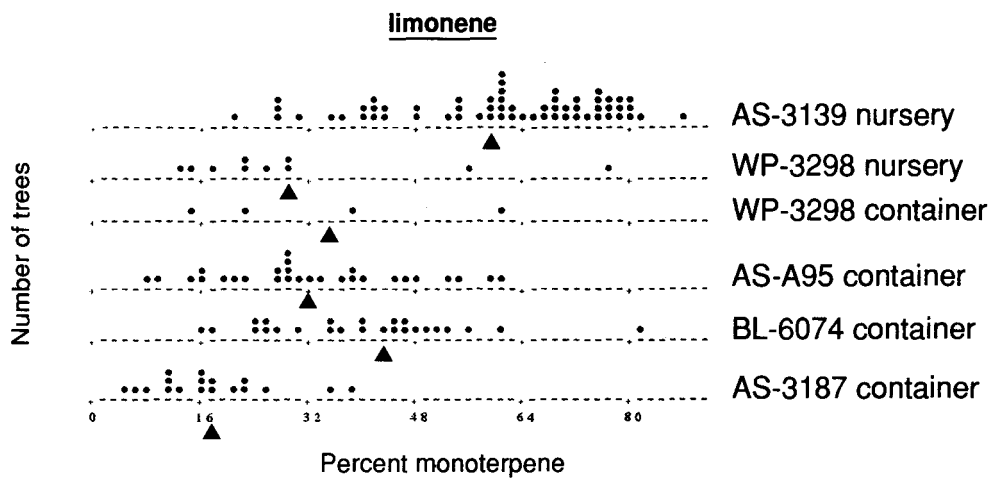
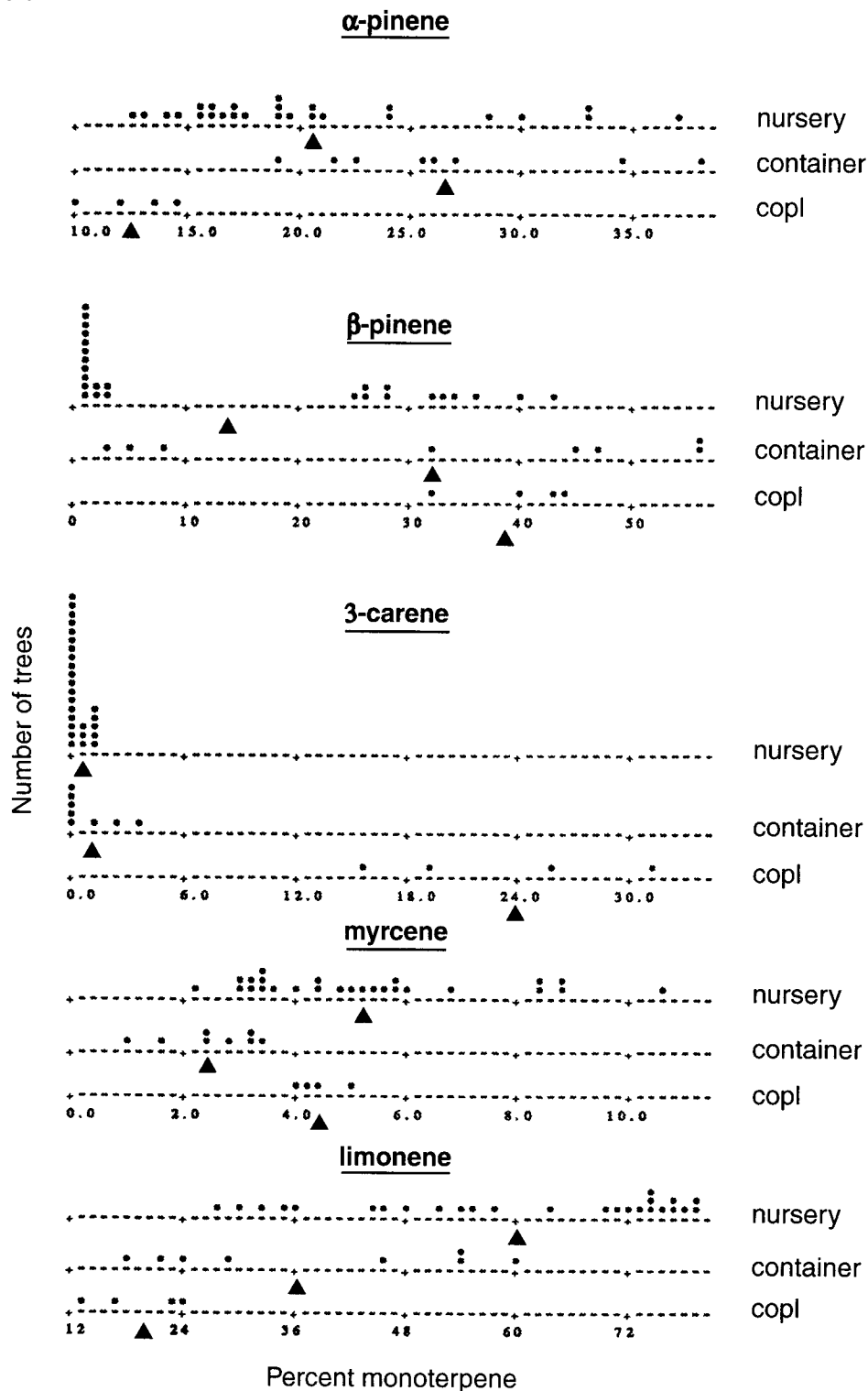


Figure 6-21—Comparative frequency distribution of the five major monoterpenes in the progeny of *P. ponderosa* AS-A95 x AS-3139 at three places of growth listed on the right: nursery (nur), container (con), container/plantation (copl); each dot is measurement of one tree; [▲] is average of set.



Ultra-high limonene progeny were common (*fig. 6-3*). Thus, as noted, earlier Tree AS-A95 performed like a high limonene tree. The closeness of the parents, about 100 yards, might also be a factor in the higher amount of limonene.

The frequency distribution of limonene in nursery-grown trees was of particular note in that it had such a strong mode between 70 and 80 percent (*fig. 622*). This was the strongest mode achieved in a cross of two high limonene trees.

Types of composition for the two reciprocal crosses were similar (*fig. 6-3*): types 1, 3, 6 for Tree AS-3139 x AS-A95 and types 2 and 8 for Trees AS-A95 x 3139.

Progeny of *P. ponderosa* AS-A95 x WP-3298

This was another cross of two high limonene trees; however, Tree WP-3298 was growing at Wheeler Peak, nearly 400 miles to the east in a different resin ecotype region of *P. ponderosa* (*table 6-1*).

Normalized percent composition of parents and progeny was as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A95 female	1	19	21	0	15	45
WP-3928 male	1	26	*	0	16	58
F-1 progeny	34	41	28	*	5	27

The composition of container and container-plantation trees differed from nursery trees (*table 6-6*). Container trees had greater α -pinene and β -pinene and considerably less limonene; container/plantation trees were also much higher in α -pinene but were much lower in β -pinene and slightly higher in limonene, but the four container/plantation trees may not have been sufficient for a conclusion. However, only nursery data were analyzed further.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees (*fig. 6-23*). Mode proportions were moderately close to the expected when the modifying factors were considered, though the proportions of 45:35:20 for the three modes of α -pinene, and 62:38 for two modes of β -pinene were fairly good.

Like the previous set of progeny, the frequency distribution of individual components had multimodal distribution for α -pinene, β -pinene, myrcene, and limonene (*fig. 6-23*); this was attributed to an 0-1 set of alleles for α -pinene in both trees, i.e., the same conditions noted for Trees AS-A95 x AS-3139.

There were many fewer high limonene trees and no ultra-high ones (*fig. 6-3*), probably because of the effect of the factor of distance between the parents.

Progeny of *P. ponderosa* AS-A95 x WP-3352

This cross, like the previous one, was another cross of two high limonene trees separated by about 400 miles (*table 6-1*). However, because there were only nine nursery trees, interpretation was limited.

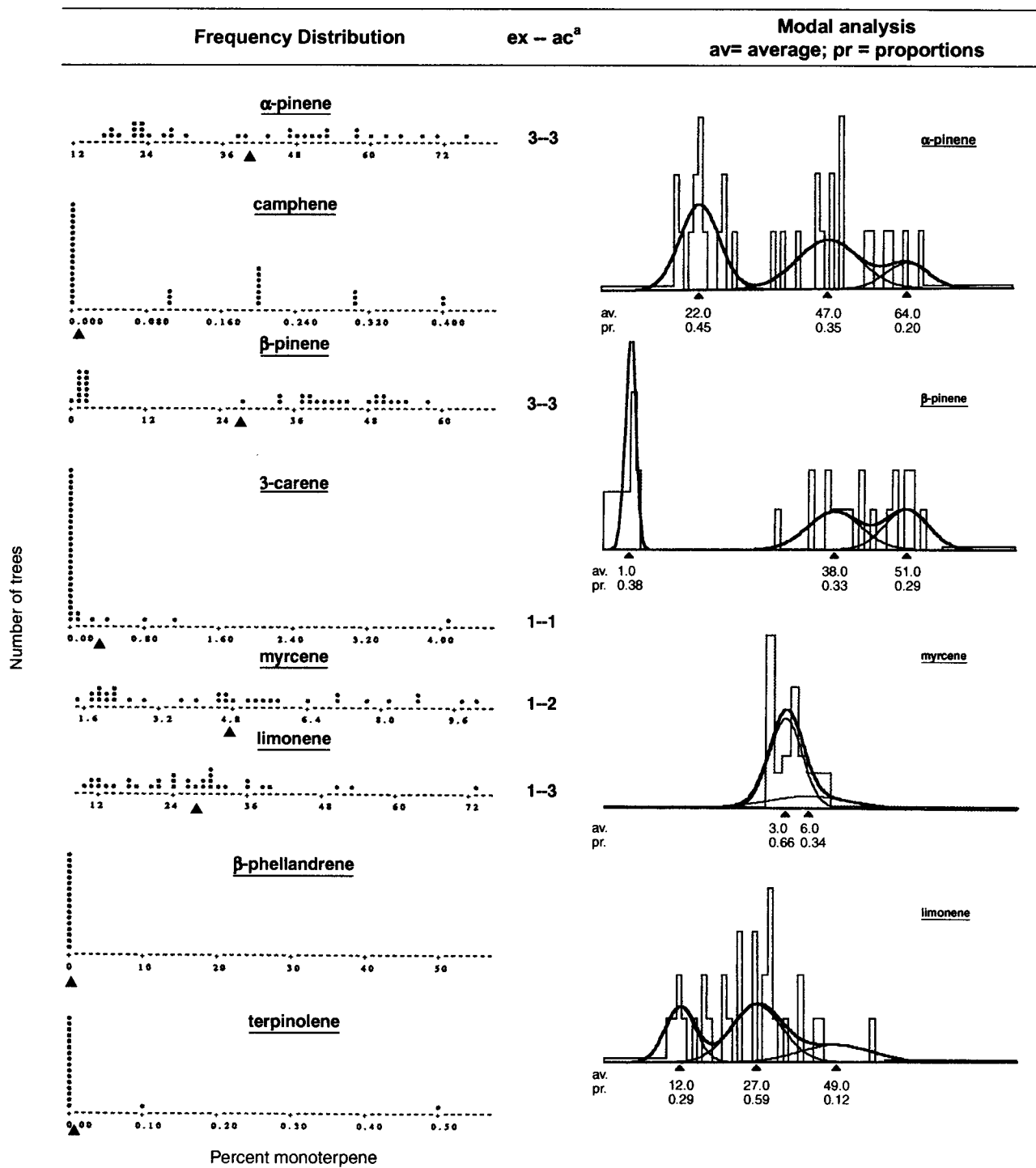
Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A95 female	1	19	21	0	15	45
WP-3352 male	1	24	*	*	18	57
F-1 progeny	9	34	28	*	4	33

The average composition of nursery, container, and container/plantation trees was somewhat similar to those progeny of Trees AS-A95 x WP-3298

^aex = expected, ac = actual

Figure 6-23—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-A95 x WP-3298; see caption of figure 6-2 for explanation of the format below.



grown in the same conditions (*table 6-6*). Thus, the progeny of both sets, Trees AS-A95 x WP-3352 and AS-A95 x WP-3298, showed the strong effect of the distance between parents. This was not the case for the progeny of Trees AS-A95 x AS-3139, where the two parents were near to each other. Though based on only nine trees, the frequency distributions showed considerable range in α -pinene, β -pinene, and limonene (*fig. 6-24*). No modal analyses were made of the distributions.

Though there were only nine trees, two of them were ultra-high limonene—clearly evident on the frequency distribution (*fig. 6-24*). This was unusual because there was only one such value in the progeny of Trees AS-A95 x WP-3298, a much larger family. Tree WP-3298 was growing in the same locality as Tree WP-3352. Thus, the factor of the distance between parent trees has more to it than has been proposed. It could easily be that the aggregate of factors such as stress, juvenile effect, etc., could either accentuate or dampen the effect of the distance between parents. Or, the occurrence of the two ultra-high limonene trees in the progeny of Trees AS-A95 x WP-3352 could simply be an expression of the large natural variation that was found throughout the breeding study.

Progeny of *P. ponderosa* AS-A95 x AS-3194

Tree AS-3194 was a 12-inch-diameter tree growing near Adin Summit with good growth and form. It was classed as near-high limonene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A95 female	1	19	21	0	15	45
AS-3194 male	1	7	6	33	13	39
F-1 progeny	27	13	24	23	2	36

Thus, this was a cross of a high limonene tree and a near-high limonene tree, growing near each other, with differing amounts of α -pinene, β -pinene, and 3-carene (*table 6-6*). The few container and container/plantation trees were not included in subsequent analyses.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-25*). Mode proportions were not very close to the expected. The proportions for α -pinene were apparently skewed by the one unusual measurement of more than 60 percent. Without this one measurement, the proportions were quite close to 50:50. By combining modes to get the expected two, proportions for β -pinene were 40:60. The zero mode for myrcene is quite unusual because there is almost always a measurable amount of it in all trees.

Both ultra-high and high limonene progeny were produced by these nearby parents, both near-high limonene (*fig. 6-3*). This could be a real advantage in a breeding program because near-high trees were much more common in natural stands than high limonene trees. This was another case in which the proximity of the two parents resulted in a higher level of limonene.

Tree AS-3194 was used as a male with five females (Trees AS-3139, AS-A95, AS-5789, AS-3187, IFG-4B63), and, though it was assigned a (0-1) set of alleles for limonene on the basis of 39 percent, there were indications that a (1-1) might be an equally suitable choice. There was considerable similarity in average limonene and in the characteristics of the frequency distributions of sets of progeny when Tree AS-3194 was the male. However, the limonene of the progeny never reached the average of progeny of crosses of two high limonene

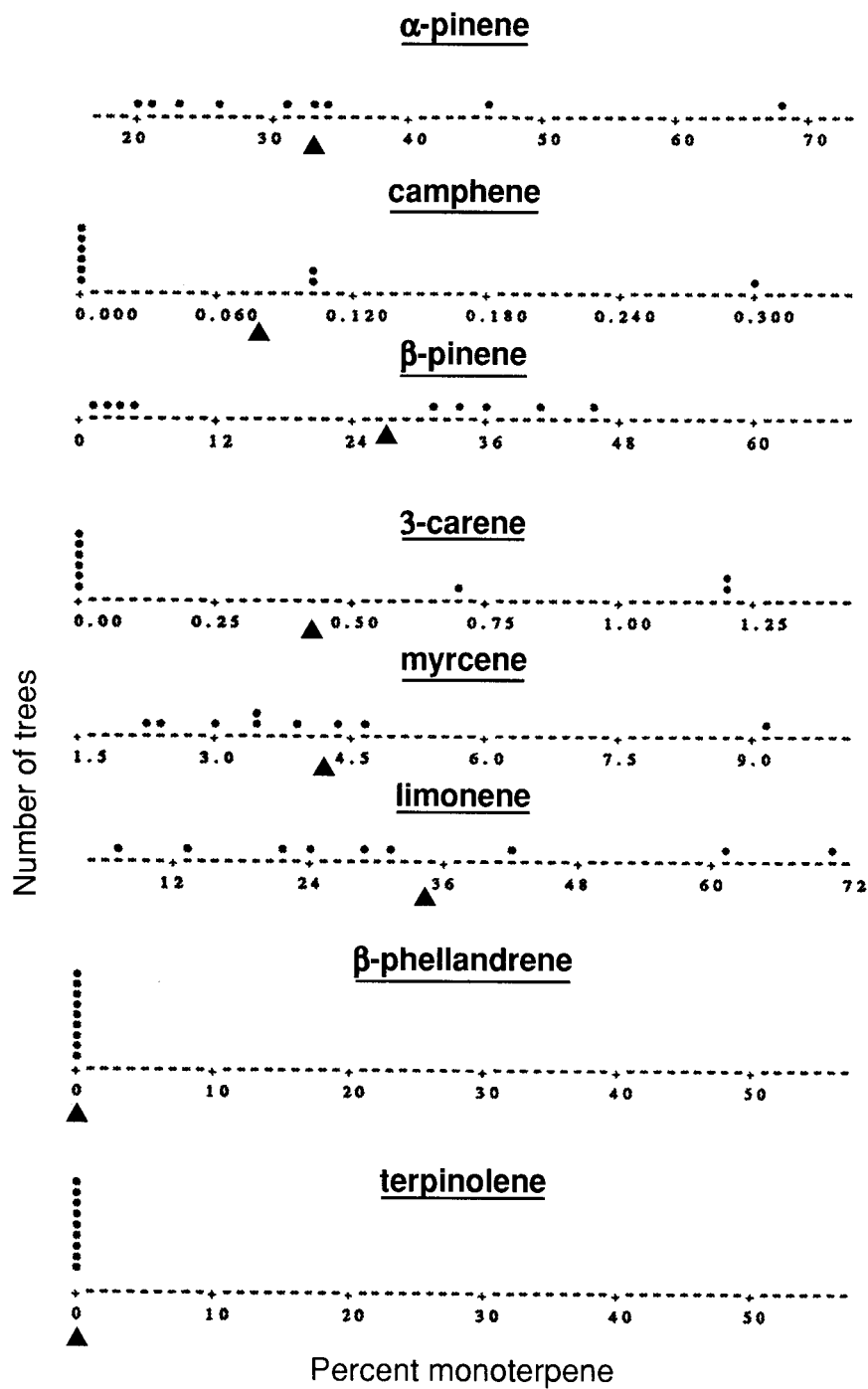
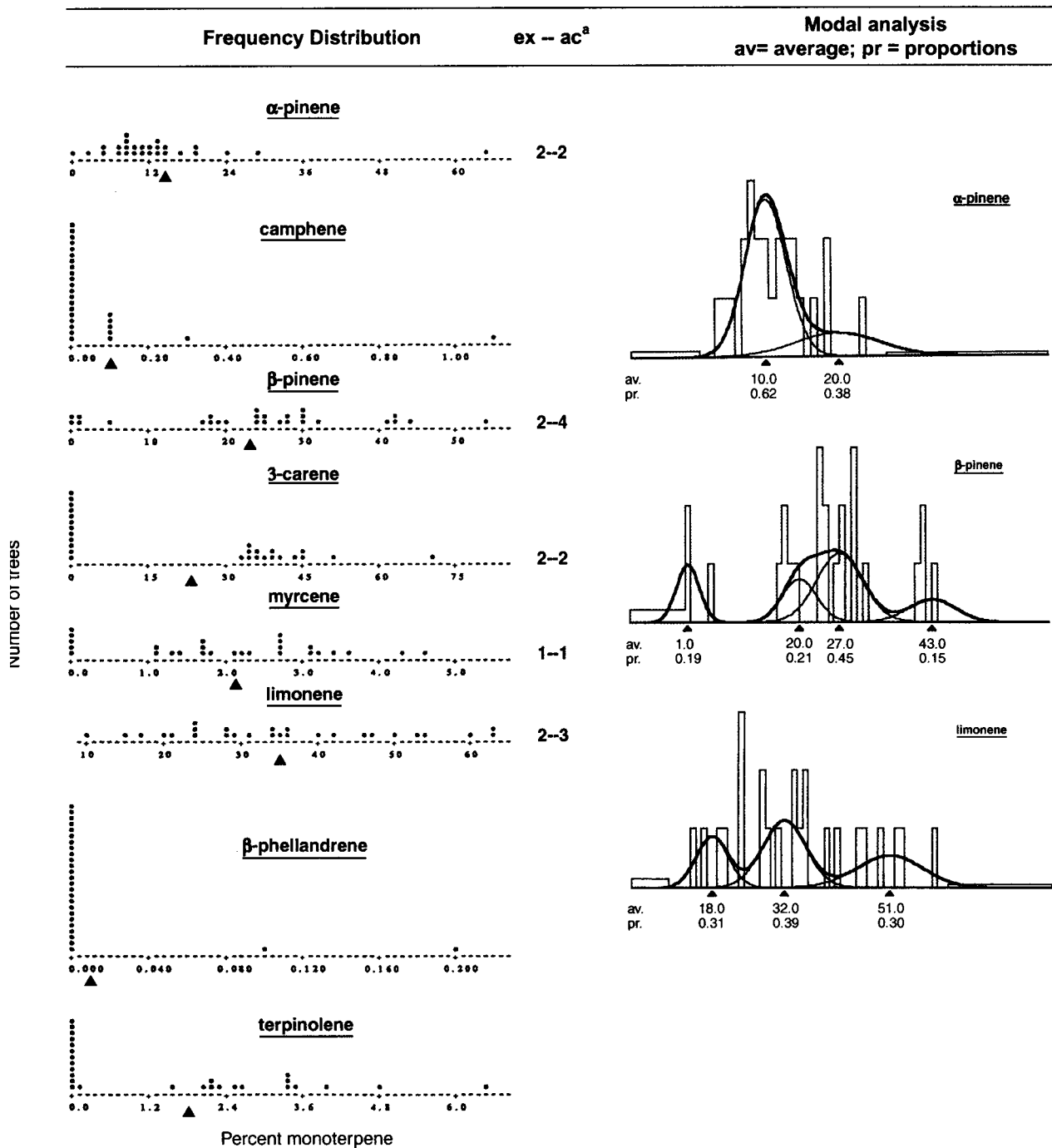


Figure 6-24—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-A95 x WP-3352; each dot is measurement of one tree; [▲] is average of set.

Figure 6-25—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-A95 x AS-3194; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

parents, probably because Tree AS-3194 had an allele for 3-carene, but individual progeny could be classed as ultra-high limonene (*fig. 6-3*). Using Tree AS-3194 as a female would have been very helpful.

Progeny of *P. ponderosa* AS-A95 x AS-5 789

Tree AS-5789, another near-high limonene tree, differed markedly from the previous male tree, AS-3194, in all major components except limonene (*table 6-1*).

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A95 female	1	19	21	0	15	45
AS-5789 male	1	23	10	0	29	37
F-1 progeny	33	37	28	0	8	27

These differences, between Trees AS-3194 and AS-5789 as pollen source, were generally reflected in average composition (*table 6-6*) and in the frequency distribution of individual components (*fig. 6-26*). The comparative average compositions of the five major components for progeny of Trees AS-A95 x AS-5789 vs. AS-A95 x AS-3194 were α -pinene, 37 vs. 13 percent; β -pinene, 28 vs. 24 percent; 3-carene, 0 vs. 23 percent; myrcene, 8 vs. 2 percent; limonene, 27 vs. 36 percent. Container and container/plantation trees were not included in the analyses because their compositions were quite different from those of the nursery-grown trees.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-26*). Mode proportions were fairly close to the expected. Proportions of 50:50 were expected for myrcene and were achieved. As in previous families with Tree AS-5789 as male or female, there were two clearly separated modes for myrcene with about equal proportions; this supports the assignment of a (0-1) set of alleles. If the two low modes for β -pinene were combined to get the expected two modes, the resulting proportions were 47:53. The proportions for α -pinene of 13:43:44 were considered moderately good in the presence of the several modifying factors and of the small sample.

Again, ultra-high limonene progeny were produced by high and near-high limonene parents, though not commonly (*fig. 6-3*); high limonene trees were common.

Progeny of *P. ponderosa* AS-A95 x BL-6074

Tree BL-6074 was high in limonene with low α -pinene and β -pinene (*table 6-1*).

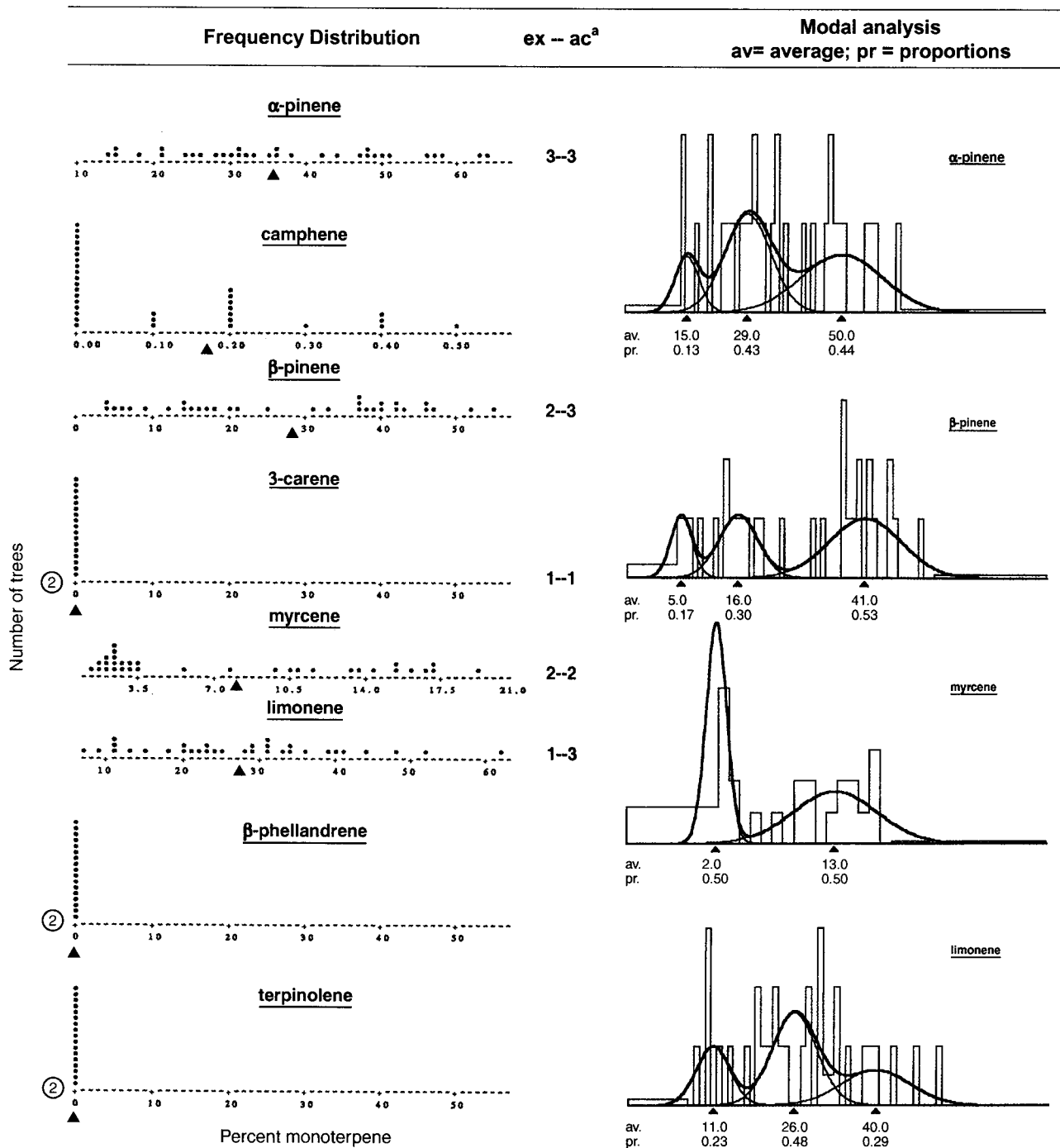
Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A95 female	1	19	21	0	15	45
AS-6074 male	1	7	11	0	27	57
F-1 progeny	7	21	42	3	2	30

There were only seven trees in the family, and this was considered too few for interpretation of frequency distribution, but it was apparent that there were large ranges in the amounts of α -pinene, β -pinene, 3-carene, and limonene (*table 6-6*) (*fig. 6-27*).

There was one ultra-high limonene tree among the seven progeny (not tabled).

Figure 6-26—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-A95 x AS-5789; see caption of figure 6-2 for explanation of the format below.



Discussion: Full Siblings of Tree AS-A95

The number of actual modes in the frequency distribution of individual monoterpenes—as derived from modal analysis—equalled or exceeded the expected number of modes—as derived from the assignment of additive alleles; this supported the additive allele hypothesis and generally supported the assignment of alleles to Tree AS-A95 and the pollen parents. Mode proportions were generally fairly close to the expected; this was additional support to the hypothesis.

In most families there was a marked difference between the composition of nursery trees and those in containers or a container/plantation. This difference was evidenced by greater limonene in the nursery trees and by greater α -pinene and β -pinene in the container or container/plantation trees. The number of samples in the containers and container/plantation was usually too small for confident assessment. Nevertheless, only nursery data were used in further analysis.

The average composition of the five sets of progeny of Tree AS-A95 was about as expected from the assignment of alleles to the parents and the action of several modifying factors.

There was an unusually large number of ultra-high limonene progeny in crosses with high limonene trees. The assignment of a (1-1) set of alleles to limonene in Tree AS-A95 seemed correct, and it did perform as a high limonene tree. It was a high limonene tree with one allele (0-1) each for α -pinene and β -pinene.

It is interesting that no ultra-high limonene trees were found in a 1600-tree sample at Adin Summit, yet many of them were produced by crosses of trees in the area, such as Trees AS-3139, AS-A78, and AS-A95. Have trees of this type failed to survive, or is this just a juvenile characteristic?

Crosses of *P. ponderosa* AS-5789 with Five Sources of Pollen

Tree AS-5789, growing near Adin Summit, was 12 inches in diameter and had good growth and form. It was classed as near-high limonene. Composition was quite constant over time and location of the sample on the tree (*table 6-2*). It had moderately abundant cone crop during the years of breeding, and comparatively larger families were obtained. It was also an easy and comfortable tree for climbing and breeding. It was crossed with five male trees, and progeny were grown in the nursery, in containers, and in a container/plantation (*table 6-7*). The progeny of this female were of particular interest because of the assignment of a (0-1) set of alleles for myrcene.

Progeny of *P. ponderosa* AS-5789 x AS-3139

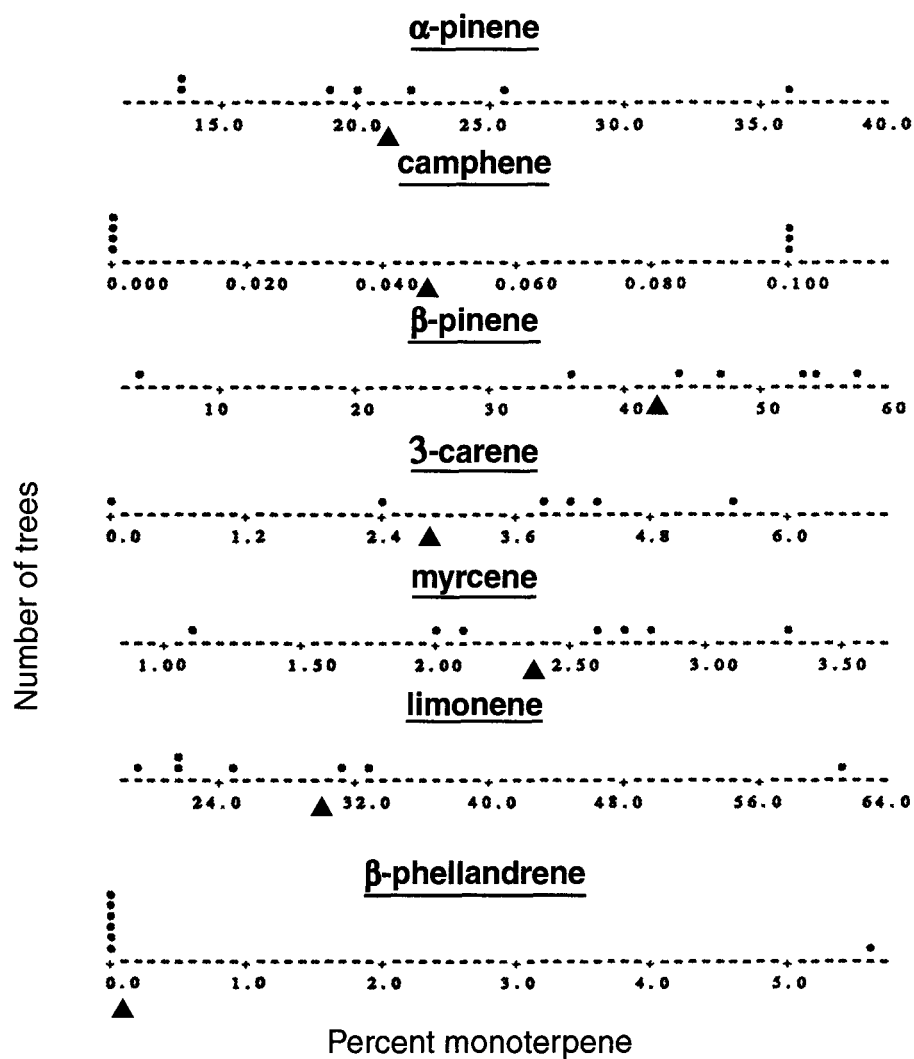
This was a cross of a near-high with a high limonene tree that were growing near each other.

Normalized percent compositions of parents and progeny were as follows:

Tree and type	Number	α -pinene	β -pinene	3-carene	myrcene	limonene
AS-5789 female	1	23	10	0	29	37
AS-3139 male	1	18	1	0	22	59
F-1 progeny	145	32	18	*	10	39

There was a sharp decrease in limonene in both container-grown trees and in container/plantation trees. Therefore only nursery data were used in further analysis.

Figure 6-27—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-A95 x BL-6074; each dot is measurement of one tree; [▲] is average of set.



The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees (*fig. 6-28*). A better assessment of the modal frequency distribution was possible because of the large number of samples (*fig. 6-28*). These analyses showed three to four readily discernible modes for four components. As expected, 3-carene was essentially zero. Mode proportions were close to the expected, and could be improved by reducing the modes to the expected number. If the two higher modes of myrcene were combined, to get the expected two modes in the distribution, mode proportions were 53:47. The two distinct modes for myrcene supported the assignment of the (0-1) set of alleles to Tree AS-5789. The upper mode was split into two by the modal analysis, probably because of the action of the modifying factors and the interactions of the basic monoterpene synthetic system. This two-mode distribution was achieved for all families of Tree AS-5789 to further support the assignment of (0-1) alleles to myrcene.

Ultra-high limonene progeny and high limonene progeny were produced by this cross of a near-high limonene with a high limonene tree (*fig. 6-3*). There were two ultra-high limonene and several high limonene trees. The large sample enabled a better assessment of types of composition. The incidence of ultra-high and high limonene trees was about the same for the reciprocal of this pair of trees, AS-3139 x AS-5789, as was the average of components. The two sets of reciprocals—even with small number of samples—were quite similar in response to the stress of container growth and growth in a container/plantation (*table 6-8*). Thus, monoterpene genes are not sex linked.

Progeny of *P. ponderosa* AS-5789 x WP-3347

This was a cross of two near-high limonene trees that were about 400 miles apart and that differed in other components.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-5789 female	1	23	10	0	29	37
WP-3347 male	1	4	17	25	3	48
F-1 progeny	71	7	39	29	6	17

Seventy-one progeny were examined (*table 6-7*). Container growth caused a decrease in limonene and an increase in α -pinene; container/plantation growth caused a slight increase in limonene and a large decrease in 3-carene. Again, the small number of trees for container and container/plantation trees may not warrant any strong conclusions. The container and container/plantation trees were not included in the modal analysis. The frequency distributions were large for α -pinene, β -pinene, 3-carene, myrcene, but unusually small for limonene (*fig. 6-29*). The three modes of limonene did not have the usual spread of more than 50 to 60 percent; instead it was only about 30 percent.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-29*). Mode proportions were reasonably close to the expected for myrcene, 45:55, and for β -pinene, 63:37, if two modes were used. These results support the additive allele hypothesis and the assignment of alleles to the parent trees.

There were no high limonene trees nor potentially high ones, though such would be expected (*fig. 6-3*). All trees had more than 20 percent β -pinene and 20 percent 3-carene. There should have been some trees lacking in β -pinene or

Table 6-7—Average xylem monoterpene composition of the progeny of tree *P. ponderosa* AS-5789 when crossed with five different males and when grown under three different conditions. *N* = number of trees.

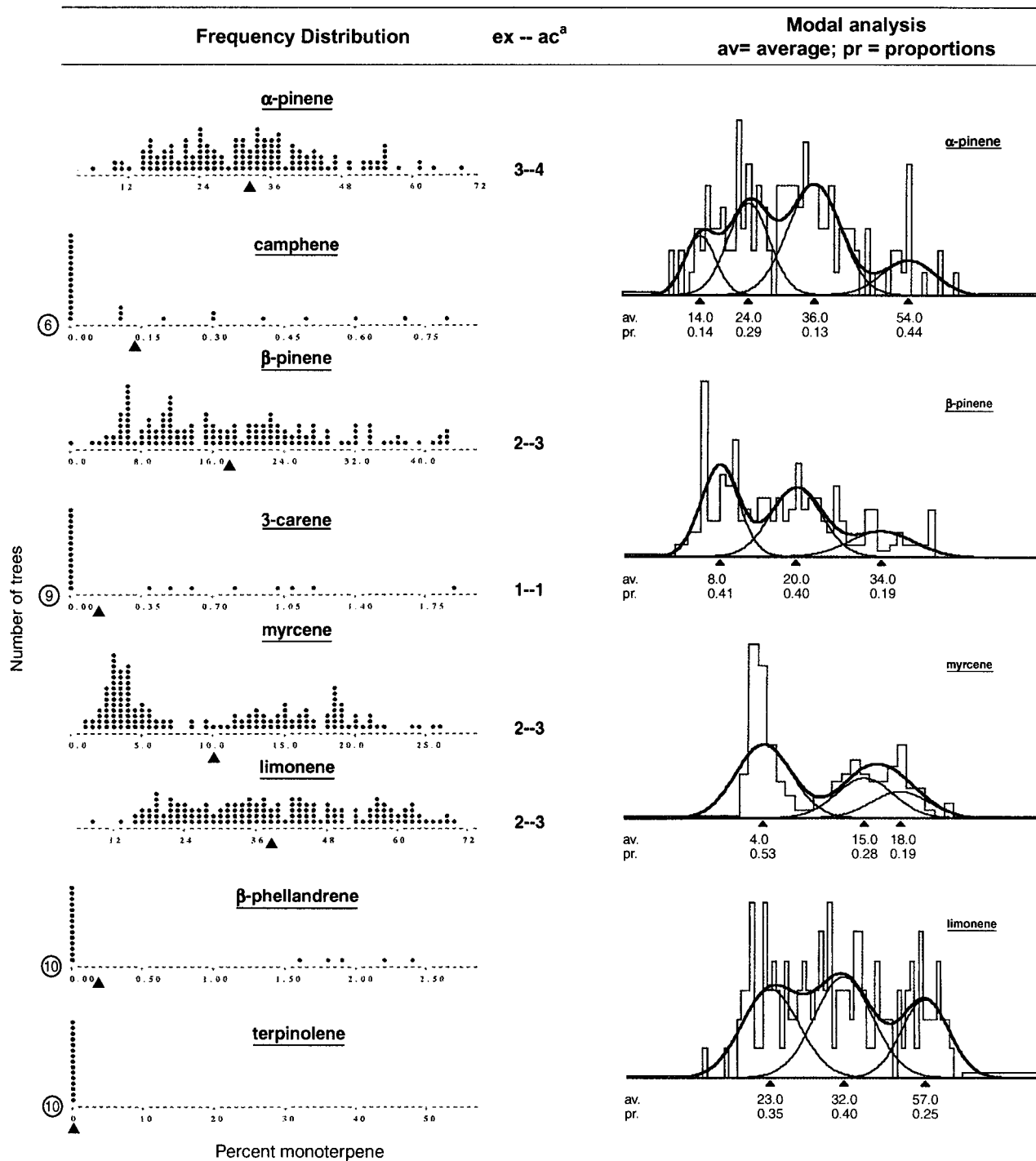
Male parent	Growth place ¹	N	Monoterpene composition of progeny ²							
			α -p	cam	β -p	car	myr	lim	β -ph	ter
			percent ³							
AS-3139	nur	145	32.2	0.1	18.2	0.1	10.0	39.3	0.1	0
AS-3139	con	16	25.2	0.1	34.7	9.5	5.6	23.9	0.3	0.5
AS-3139	copl	17	43.9	0.1	29.2	0	7.6	18.7	0.4	0
WP-3347	nur	71	7.1	0.2	38.6	29.4	5.6	17.2	0.4	1.5
WP-3347	con	7	8.0	0.1	45.5	28.3	3.0	12.1	0.9	1.8
WP-3347	copl	8	9.7	*	44.8	19.4	4.9	19.9	0	1.3
AS-3194	nur	75	16.5	0.1	27.0	25.2	7.7	21.7	0	1.0
AS-3194	con	7	8.2	0	36.3	39.8	6.6	5.3	1.3	2.6
AS-3194	copl	5	41.1	0.1	32.0	12.7	5.7	6.8	0.6	1.0
WP-3318	nur	68	21.8	0.2	26.6	23.6	4.0	15.6	0.2	1.3
WP-3318	con	9	29.3	0.2	32.0	23.7	3.0	10.3	0.4	1.2
WP-3318	copl	8	30.6	0.2	35.1	14.7	3.5	14.6	0.4	0.9
WP-3352	nur	9	32.1	*	28.0	0	11.3	28.1	*	0

¹nur = nursery 6 to 8 years; con = 9-year container; copl = 9-year container/plantation.² α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.³Normalized; * = trace.**Table 6-8**—Average monoterpene composition of sets of reciprocal crosses: (A) AS-3139 x AS-5789, and (B) AS-5789 x AS-3139 grown in 3 locations. *N*=number of trees.

Cross	Place of growth	N	Monoterpene ¹							
			α -p	cam	β -p	car	myr	lim	β -ph	ter
			<i>percent</i> ²							
A	Nursery	35	27.0	*	15.5	0.1	10.9	46.5	0	0
B	Nursery	145	32.2	0.1	18.2	0.1	10.1	39.3	0.1	0
A	Container	9	32.6	0.2	36.8	0.1	8.2	21.3	0.8	0
B	Container	16	25.2	0.1	34.7	9.5	5.6	23.9	0.3	0.5
A	Copl ₃	7	41.4	0.1	28.9	5.1	8.8	19.0	0.2	0.6
B	Copl	17	43.9	0.1	29.2	0	7.6	18.7	0.4	0

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.²Normalized; * = trace.³Container Plantation.

Figure 6-28—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-5789 x AS-3139 ; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

3-carene, or both; in such trees, limonene would have been high, but there were no such trees. I attributed this to the great distance between parents; this caused a type of stress that was reflected in greater β -pinene and 3-carene. The absence of a mode for 3-carene at or near zero supports the assignment of a (1-1) set of alleles for 3-carene to WP-3347.

Progeny of *P. ponderosa* AS-5789 x AS-3194

Both trees, growing near Adin Summit, were classed as near-high limonene, with 37 percent and 39 percent, respectively (*table 6-1*).

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-5789 female	1	23	10	0	29	37
AS-3194 male	1	7	6	33	13	39
F-1 progeny	75	17	27	25	8	22

Seven trees, grown in containers for 7 years, had a sharp reduction in limonene and increases in β -pinene and 3-carene, but there was a surprising decrease in α -pinene (*table 6-7*). The magnitude of the reduction in limonene was so great that one might speculate that the alleles for limonene did not function at all in either containers or in a container/plantation because of the stress of root containment and/or the over application of a phytocide. There was a sharp decrease in α -pinene in containers and a large increase in container/plantation. The effects of stress, therefore, seemed to be both large and unpredictable. Thus, only data for nursery plants were used in further analysis.

The analysis of the frequency distributions showed that the number of modes actually found in the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-30*). Mode proportions were fairly close to the expected. If the upper two modes of α -pinene were combined, proportions of 47:53 resulted; the two existing proportions for myrcene were 39:61. The proximity of the parents was reflected in a relatively high average for limonene and for the incidence of high limonene trees. These results support the additive allele hypothesis and the assignment of alleles to the parent trees. There were no ultra-high limonene trees, though such was possible, as shown by other crosses of near-high limonene trees (*fig. 6-3*). When both β -pinene and 3-carene were low or lacking, there was an increase in α -pinene rather than limonene. It seemed that in a few instances, at least, the increase would have been in limonene. This could be an effect of the juvenile factor, and, in time, trees like types #9 and #20 (*fig. 6-3*) might become ultra-high limonene trees.

This cross also illustrates the effect on composition by the interaction of the monoterpene synthesis system. Though parents were relatively high in limonene, the limonene composition was relatively low in the progeny because each parent brought another allele into the family to compete with limonene for the limited amount of the precursor.

Figure 6-29—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-5789 x W P-3347; see caption of figure 6-2 for explanation of the format below.

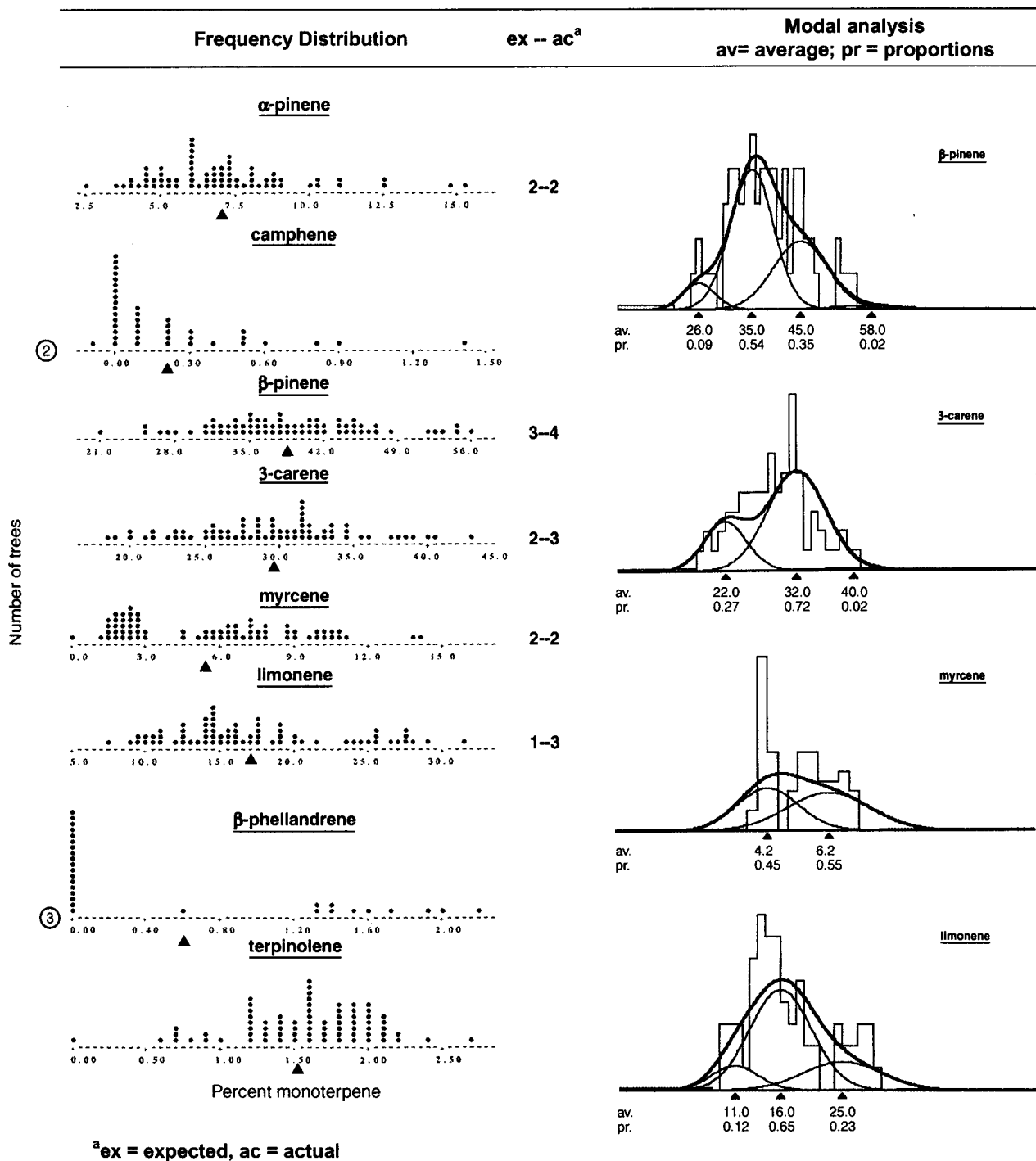
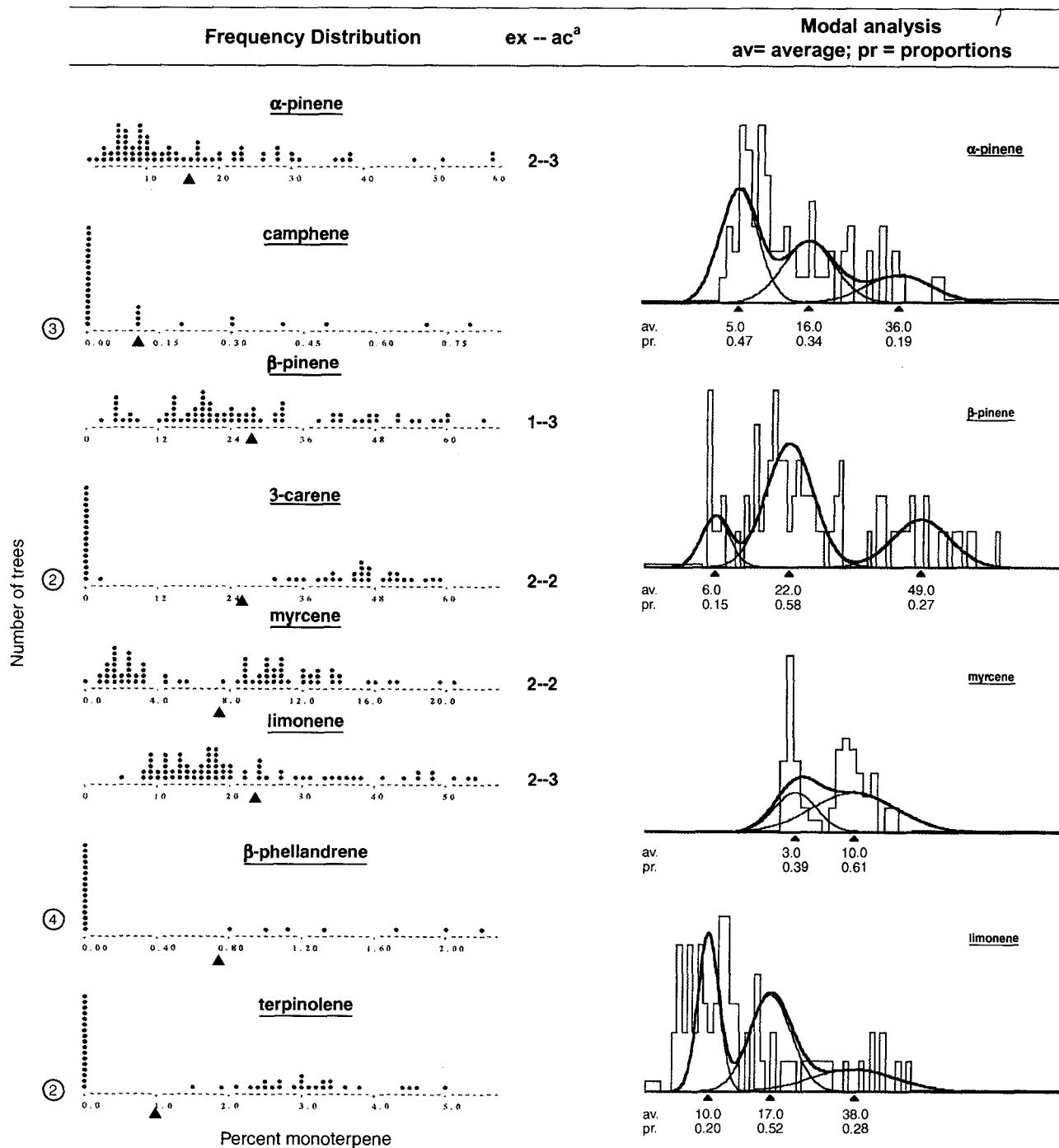


Figure 6-30—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-5789 x AS-3 194; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

Progeny of *P. ponderosa* AS-5798 x WP-3318

Tree WP-3318, like AS-5789, was classed as near-high limonene, but the two trees differed in the other components.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-5789 female	1	23	10	0	29	37
WP-3318 male	1	27	10	18	3	40
F-1 progeny	68	22	27	24	4	16

Thus, Tree WP-3318 had 18 percent 3-carene and 3 percent myrcene while Tree AS-5789 had 0 percent 3-carene and 29 percent myrcene (*table 6-1*). Trees grown in containers or in the container-plantation had lower limonene, higher α -pinene and β -pinene, but about the same amount of 3-carene (*table 6-7*). Therefore, only data on nursery trees were used in further analysis.

The analysis of the frequency distributions showed that the number of modes found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-31*). Mode proportions were fairly close to the expected: 47:53 for both 3-carene and myrcene. Mode proportions for α -pinene and limonene were not very close to the expected. The low amount of limonene in the progeny and the lack of the expected high limonene trees were attributed to the great distance between the parent trees.

The lack of a mode near zero for 3-carene would appear to be an anomaly. That is, with a (0-0) set of alleles for Tree AS-5789 and a (0-1) set for Tree WP-3318, one would have expected a moderate sized mode at or near 0 percent from a (0-0) set in the progeny. The lack of such results could be caused by some activity by the (0-0) set in the progeny due to the juvenile effect, or the incorrect assignment of a (0-1) in the male parent. However, other results in the study, which supported the (0-1) assignment, strongly suggested the juvenile effect as the cause and not an anomaly. This anomaly with WP-3183 as a male occurred in two other families.

Progeny of *P. ponderosa* AS-5789 x WP-3352

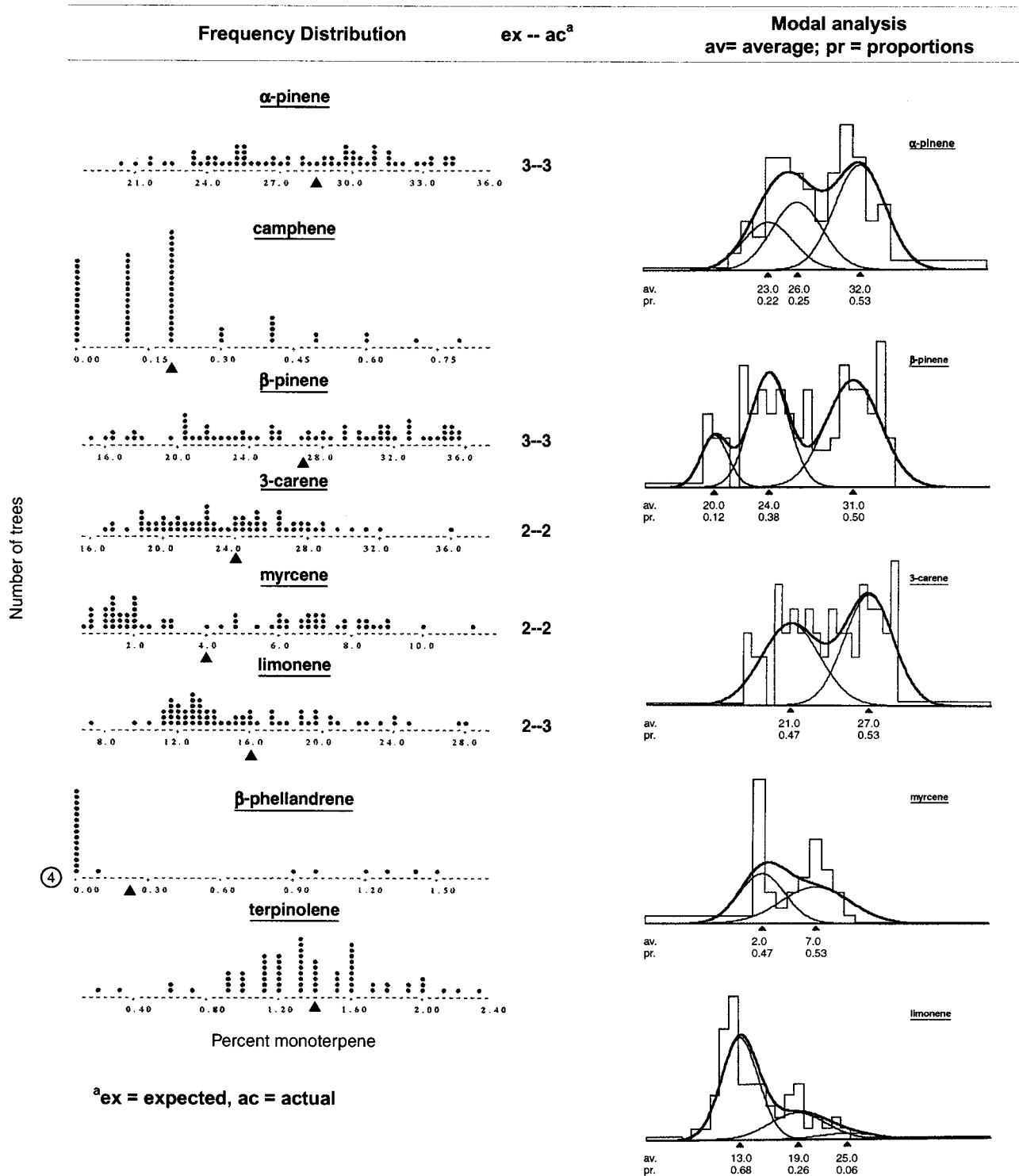
This was another cross of near-high limonene with high limonene, as in the previous cross; however the parents were about 400 miles apart.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-5789 female	1	23	10	0	29	37
WP-3352 male	1	24	*	*	18	57
F-1 progeny	19	32	28	*	11	28

Nineteen progeny were sampled and the average composition was somewhat different from the previous set, Trees AS-5789 x AS-3139 (*table 6-7*). This was attributed to the factor of the distance between parents—Tree AS-3139 was a couple of miles from Tree AS-5789, while Tree WP-3352 was about 400 miles away. The number of trees examined was insufficient for a good interpretation of frequency distributions (*fig. 6-32*). However, much like the progeny of Trees AS-5789 x AS-3139, the range of frequencies was large for α -pinene, β -pinene, myrcene, and limonene. Even with the small numbers, three modes for α -pinene and two for myrcene were easily discernable in this very small family (*fig. 6-32*). There was considerable similarity between this family

Figure 6-31—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-5789 x WP-3318; see caption for figure 6-2 for explanation of the format below.



and the family of Trees AS-5789 x AS-3139 in both average composition and in the characteristics of the frequency distributions, when the factor of distance between parents is considered. Both male parents—WP-3352 and AS-3139—were high limonene.

There were no ultra high limonene progeny (not tabled), again attributed to stress caused by the great distance between parent. Though high limonene trees were relatively common, they were not nearly as common as in the progeny of the previous cross of Trees AS-5789 x AS-3139.

Discussion: Full Siblings of Tree AS-5789

There were large differences in composition between nursery trees and trees grown in containers or in a container/plantation. This was a reflection of stress in the containers and container/plantation, and by the additional stress of the over application of a phytocide. This effect was evidenced by nursery trees having greater limonene and lesser α -pinene and β -pinene. Therefore only data on nursery trees were analyzed.

The actual number of modes in the frequency distribution of individual monoterpenes—as derived from modal analysis—equalled or exceeded the expected number—as derived from the assignment of alleles; this supported the additive allele hypothesis. Mode proportions were usually close to the expected to provide additional support to the hypothesis.

The data for all crosses supported the assignment to Tree AS-5789 of (0-1), (0-1), (0-0), (0-1), and (1-1) sets of additive alleles, respectively, for α -pinene, β -pinene, 3-carene, myrcene, limonene. Thus, though Tree AS-5789 was assigned a (1-1) set of alleles for limonene, the presence of three other alleles prevented it from functioning as a typical high limonene tree.

The average for limonene and the number of high and ultra-high limonene trees was always higher in comparable crosses when the two parents were in proximity than when they were far apart (fig. 6-3). This difference was expressed much like that attributable to stress. It is postulated that there was a level of stress in progeny of the parents that were far apart. However, progeny of both near and distant parents responded similarly to the additional stress of container growth and over application of phytocide, which was further evidence that the difference between near and distant parentage was stress or stress-like.

When the frequency distributions of the individual monoterpenes were compared for the families of Tree AS-5789, it was clear that the averages and position of the modes of multimodal distributions varied according to the other alleles in the cross, though the number of modes did not change (fig. 6-33). Also, that portion of figure 6-33 for myrcene illustrated its bimodal characteristic for all the families of Tree AS-5789. Such crosses always resulted in two modes of equal proportions, thus supporting the assignment of a set of (0-1) for myrcene in AS-5789. However, it also showed that myrcene was a rare allele.

Tree AS-5789 had ultra-high limonene progeny when crossed with a high limonene male. This again showed that it had a (1-1) set of alleles for limonene.

Reciprocal crosses were generally similar in average composition, in the occurrence of types of composition, and in the general characteristics of the frequency distributions. Likewise, both sets of reciprocals responded very similarly to the same location of growth.

Tree AS-5789 was pollinated with two males. One of them was within 2 miles of Tree AS-5789, and one was about 400 miles from Tree AS-5789. The ratios of limonene in the progeny vs. that in the male were calculated (table 6-9). These ratios again show that limonene in progeny of nearby parents was much greater than that in progeny of parents that were far apart. In general, the amount in nearby progeny was about twice as much. This was again an example of the effect of the distance between parents.

Figure 6-32—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-5789 x WP-3352; each dot is measurement of one tree; [▲] is average of set.

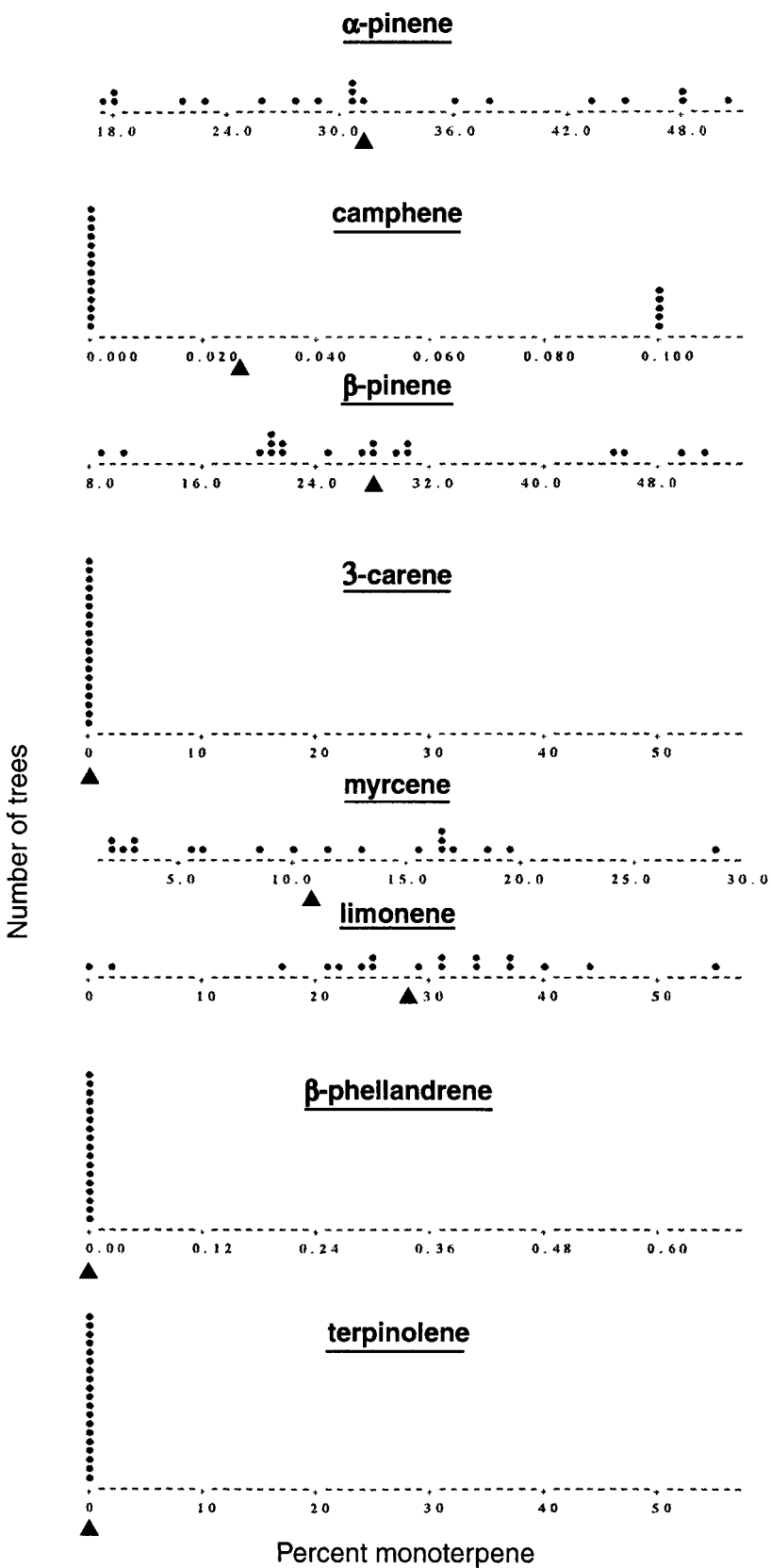
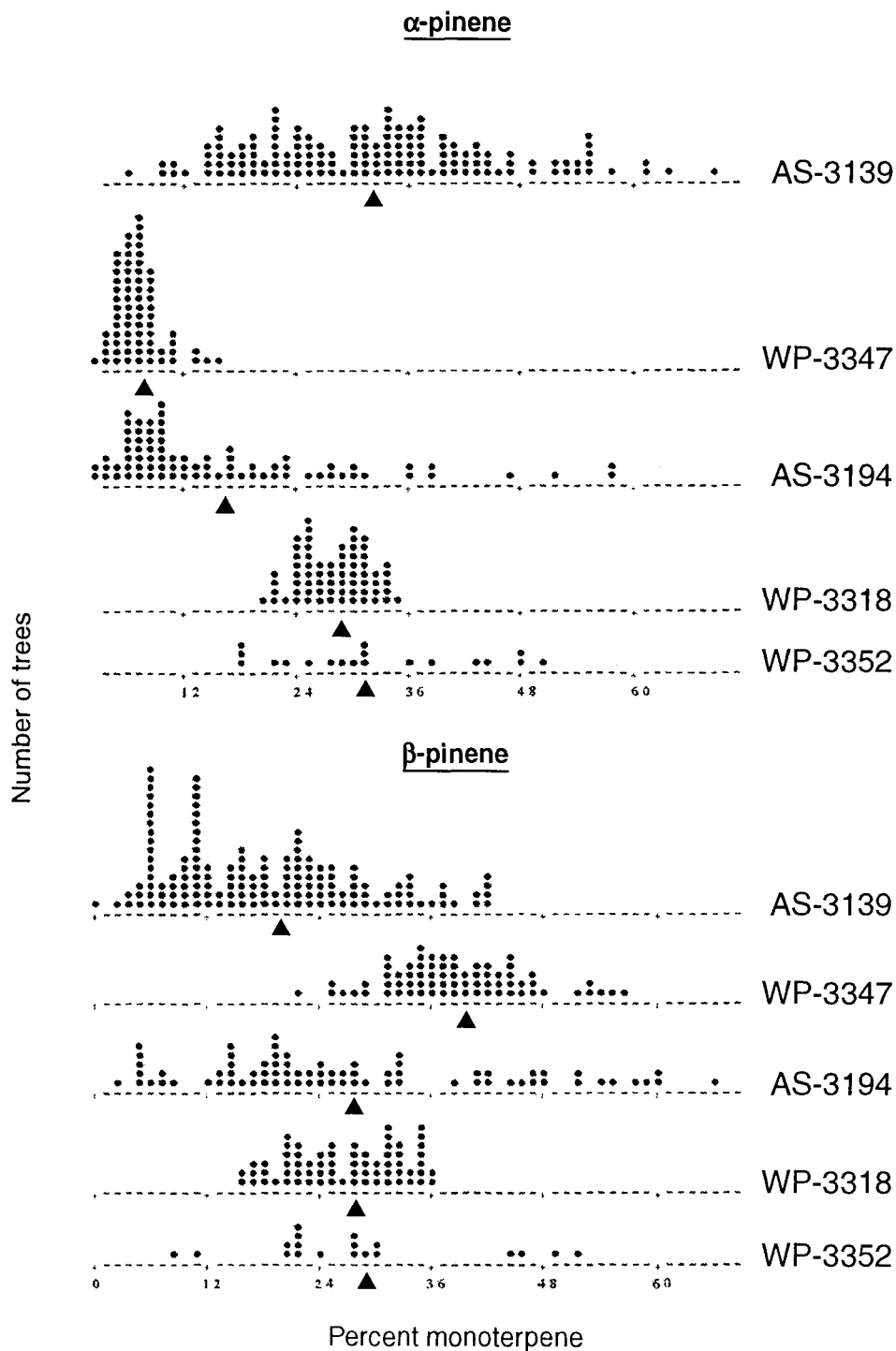
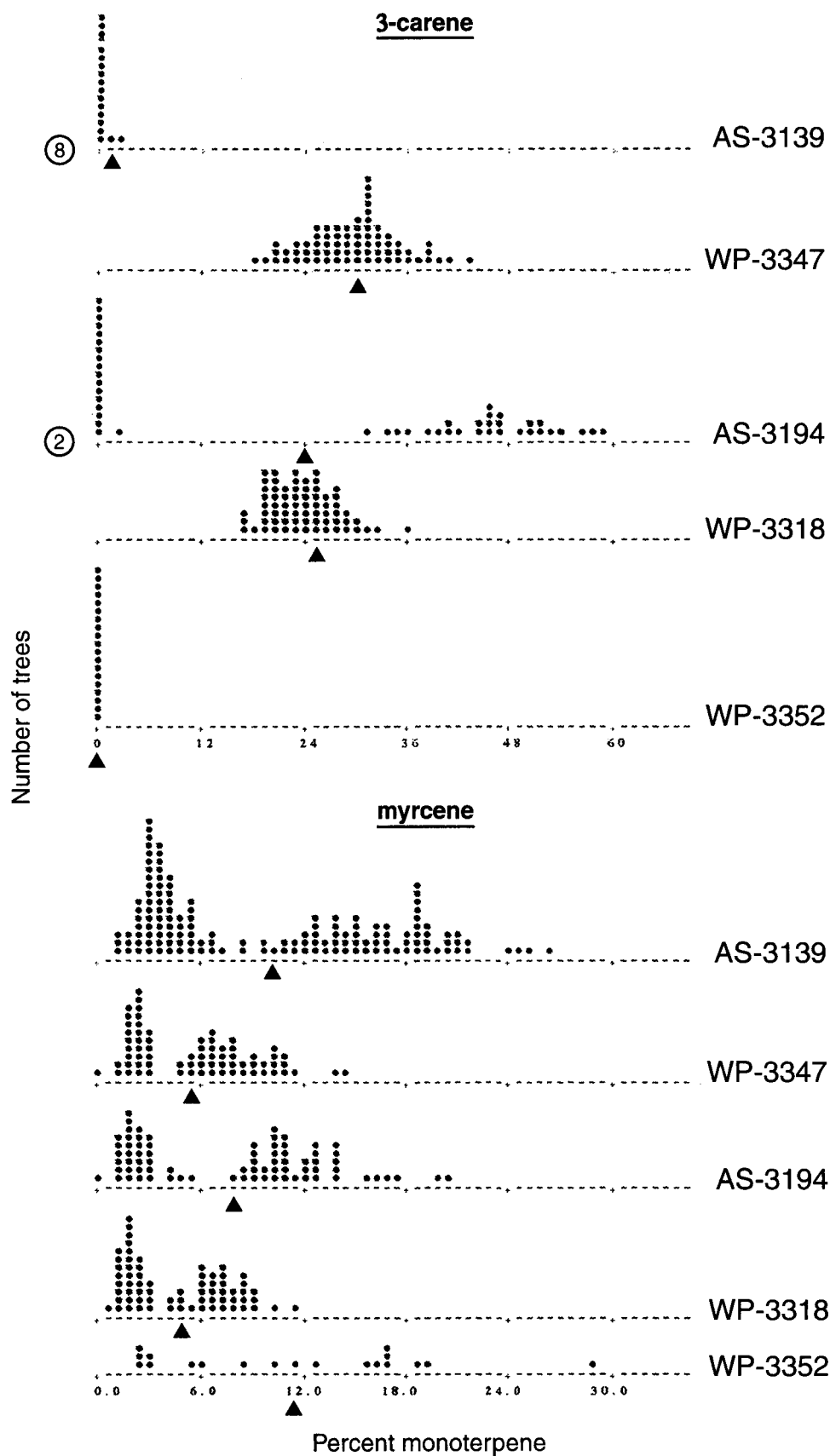


Figure 6-33—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-5789 when crossed with the five males listed on the right; each dot is measurement of one tree except where $\bar{n}$ indicates each is n trees; [$\blacktriangle$] is average of set.

(fig 6-33 continues on next 3 pages)





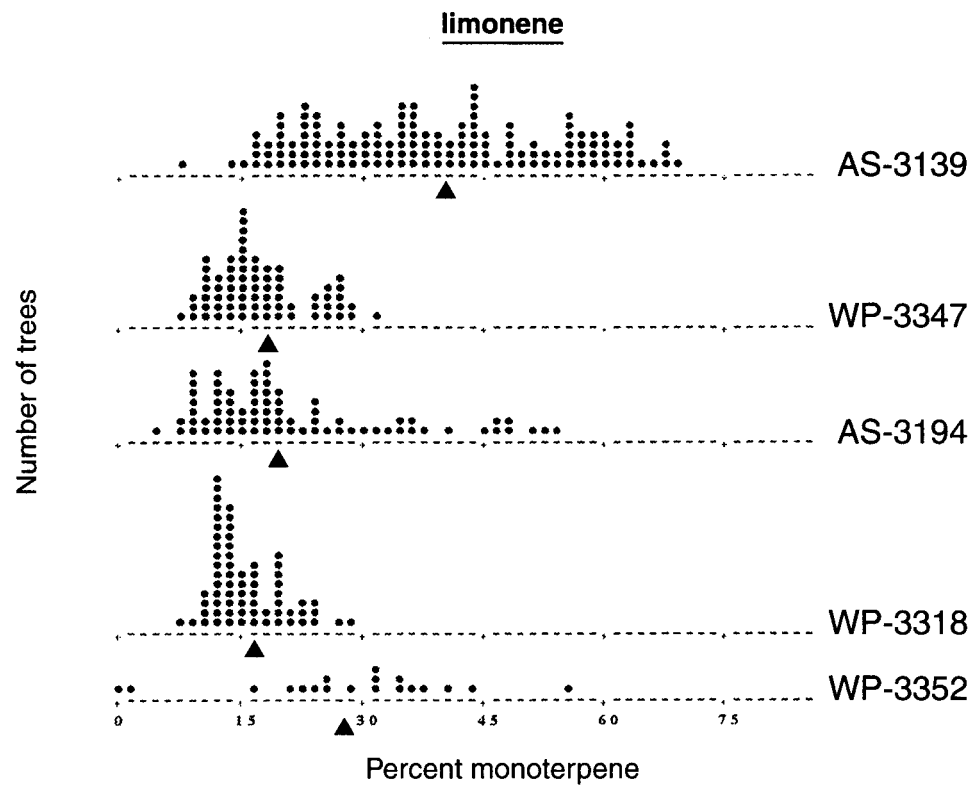


Table 6-9—Ratio of limonene in male parent tree to average limonene in four sets of progeny of AS-5789: two sets with nearby male (AS-3139, AS-3194), two sets with distant males (WP-3347, WP-3318)

Male tree	Distance: female-male	Limonene in:		Ratio for limonene: progeny to male
		male	progeny	
	<i>miles</i>		<i>pct¹</i>	
AS-3139	3	59	39	1:1.5
AS-3194	1	39	22	1:1.7
WP-3347	400	48	17	1:2.8
WP-3318	400	40	16	1:2.5

¹Normalized.

With the larger number of trees in the families of Tree AS-5789, mode assessment, analysis, and interpretation were made with greater confidence. But the conclusions reached from the results with the families of Tree AS-5789 were essentially the same as those made with much smaller families of other females. Thus, the results with Tree AS-5789 support the conclusions made with much smaller families. Though the larger families provide more confidence, they may not be all that much better in making correct conclusions.

Crosses of *P. ponderosa* AS-A295 X Three Pollen Sources

Tree AS-A295 was growing at Adin Summit (fig. 6-1). But the breeding was done on grafts in the Arboretum at IFG. Tree AS-A295 was selected for breeding because it had reasonable amounts of all the major components except β -pinene.

Progeny of *P. ponderosa* AS-A295 x AS-5789

Normalized percent compositions of parents and progeny were as follows:

Tree and type	Number	α -pinene	β -pinene	3-carene	myrcene	limonene
AS-A295 female	1	13	3	32	19	32
AS-5789 male	1	23	10	0	29	37
F-1 progeny	34	35	25	15	7	16

The average composition of 34 nursery-grown progeny was much higher than expected in α -pinene and β -pinene, and much lower than expected in limonene and myrcene (table 6-10). Container and container/plantation trees were even much higher in α -pinene or β -pinene and much lower in limonene: this was attributable to stress. Thus, only nursery data were used for further analysis.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (fig. 6-34). Mode proportions were very close to the expected. The three expected modes for α -pinene were 21:51:28 which is very close to the expected 25:50:25. Mode proportions of 30:70 were achieved for 3-carene by combining modes to get the expected two. The two modes for myrcene were 57:43, which was quite close to the expected, and proportions of 35:65 were obtained for limonene by combining modes.

Four trees had more than 40 percent limonene—as would be expected from a male lacking 3-carene and a female lacking β -pinene—and could be classed as high limonene (fig. 6-3), but there was a moderate amount of β -pinene in all of them. Trees without 3-carene and with small amounts of 3-pinene were high in α -pinene. These departures from the expected were probably due to the juvenile effect.

Progeny of *P. ponderosa* AS-A295 x AS-A95

Tree AS-A95 was much like Tree AS-5789, the previous tree, in having near-high limonene.

Percent normalized compositions of parents and progeny were as follows:

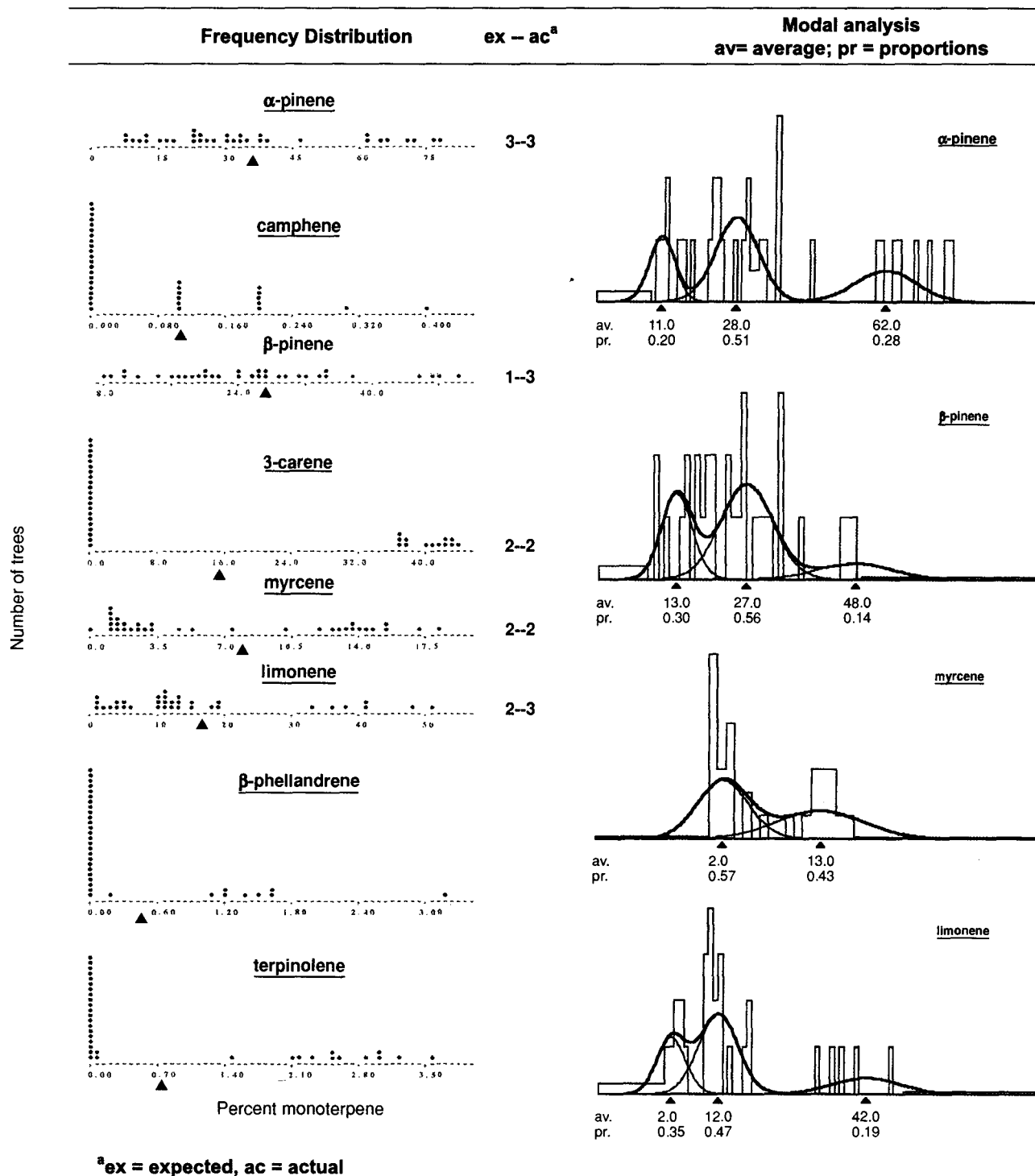
Tree and type	Number	α -pinene	β -pinene	3-carene	myrcene	limonene
AS-A295 female	1	13	3	32	19	32
AS-A95 male	1	19	21	0	15	45
F-1 progeny	8	26	44	9	3	17

The average composition of eight 4-year-old container trees was somewhat similar to progeny of Trees AS-A295 x AS-5789 (table 6-10). Some high limonene

Table 6-10—Average monoterpene composition of the progeny of AS-A295 with three males, grown in different locations, for varying periods of time. *N* - number of trees.

Male parent	N	Place ¹ of growth	Monoterpene composition of progeny ²							
			α -p	cam	β -p	car	myr	lim	β -ph	ter
			<i>percent</i> ³							
AS-5789	34	nur	34.8	0.1	25.4	15.3	7.2	15.9	0.4	0.9
AS-5789	6	con	24.4	0.1	39.6	24.4	4.8	3.9	0.8	1.9
AS-5789	4	copl	51.4	*	26.3	9.6	7.1	4.2	0.6	0.8
AS-A95	8	con-4	25.9	0.1	43.9	9.3	2.6	16.6	0.7	0.9
WP-3298	10	con-4	42.6	0	20.7	21.2	2.4	11.7	0.2	1.3

Figure 6-34—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-A295 x AS-5789; see caption of figure 6-2 for explanation of the format below.



trees were expected, but none was found (*fig. 6-3*); the 8-tree sample was too small to express the full range of composition types. The small sample also precluded an analysis of the frequency distributions (*fig. 6-35*). Even with so few trees, there was a large range in amounts of the major monoterpenes.

Progeny of *P. ponderosa* AS-A295 x WP-3298

Tree WP-3298 was high in limonene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-A295 female	1	13	3	32	19	32
WP-3298 male	1	26	*	0	16	58
F-1 progeny	10	43	21	21	2	12

Only 10 4-year progeny, which were grown in containers, were examined. The average composition was much higher in α -pinene, 43 percent, and much lower in limonene, 12 percent, than would be expected from additive alleles (*table 6-10*). Limonene was so low that one might suspect that one allele for it had not yet become functional. There were no high limonene progeny, though some were expected (*fig. 6-3*). The sample size was too small for an analysis of the frequency distributions (*fig. 6-36*); however, the distributional spread of four of the components was large, even with a small sample size. The expected two modes of α -pinene were discernible with proportions of 60:40.

Here, as with the progeny of Trees AS-A295 x AS-A95, the lack of high limonene progeny might be attributed to the small number of samples, or it might be an expression of the great distance between the two parents.

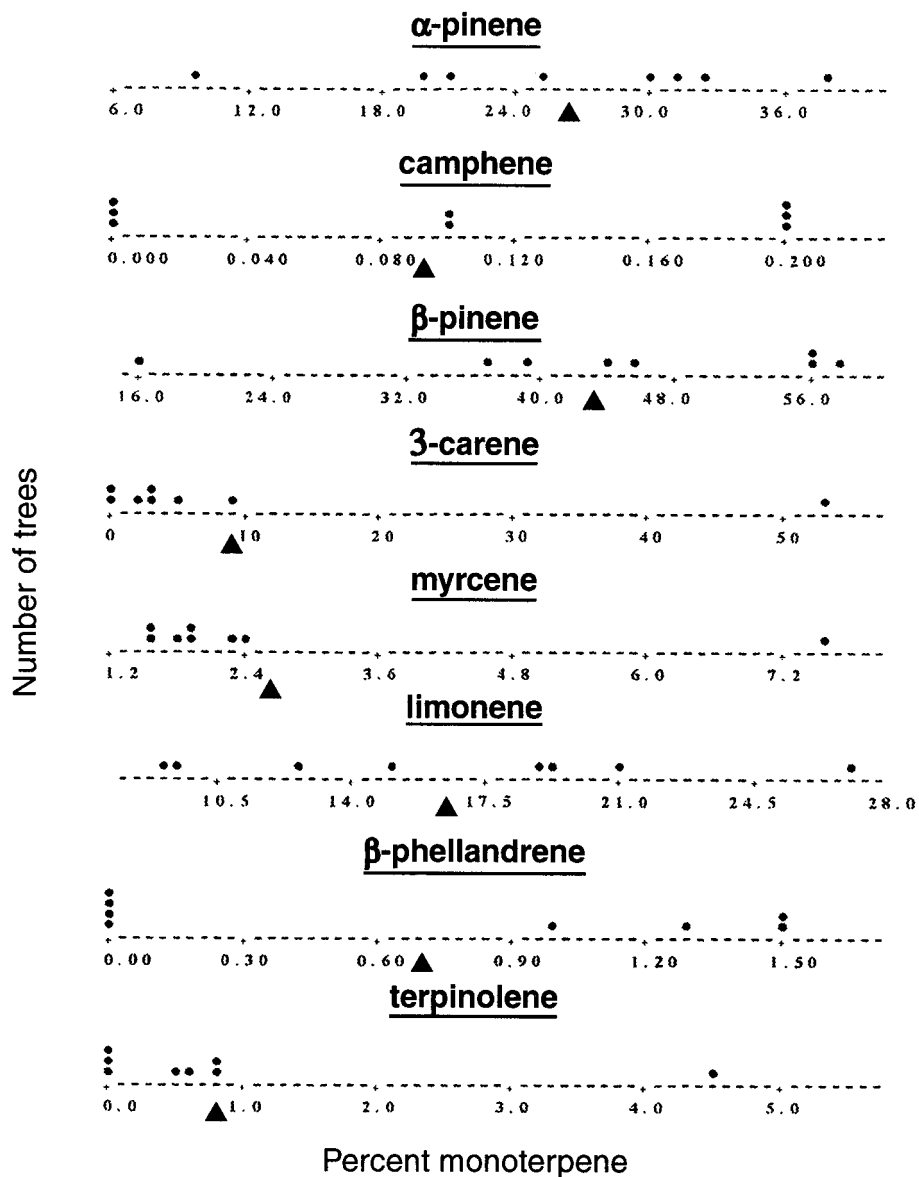
Discussion: Full Siblings of Tree AS-A295

For the one set of progeny large enough for analysis, the actual number of modes in the frequency distribution of individual monoterpenes—as derived from modal analysis—equalled or exceeded the expected number of modes—as derived from the assignment of additive alleles; this supported the additive allele hypothesis and the assignment of alleles to Tree AS-A295 and to the male parent. Though classed only as moderate high limonene, there were progeny, when crossed with another near-high male, Tree AS-5789, that were high in limonene. Breeding with Tree AS-A295 was too limited to permit additional discussion.

Crosses of *P. ponderosa* AS-3187 With 10 Pollen Sources

Tree AS-3187 had moderate amounts of β -pinene, 23 percent; 3-carene, 23 percent; myrcene, 14 percent; limonene, 29 percent (*table 6-1*). It was growing near Adin Summit, had good growth, but was rather limby. Its composition was quite constant over time and with location of growth (*table 6-2*). It had good cone crops during the period of breeding. As noted previously, it was a "comfortable" tree for breeding studies.

Figure 6-35—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-A295 x AS-A95; each dot is measurement of one tree; [▲] is average of set.



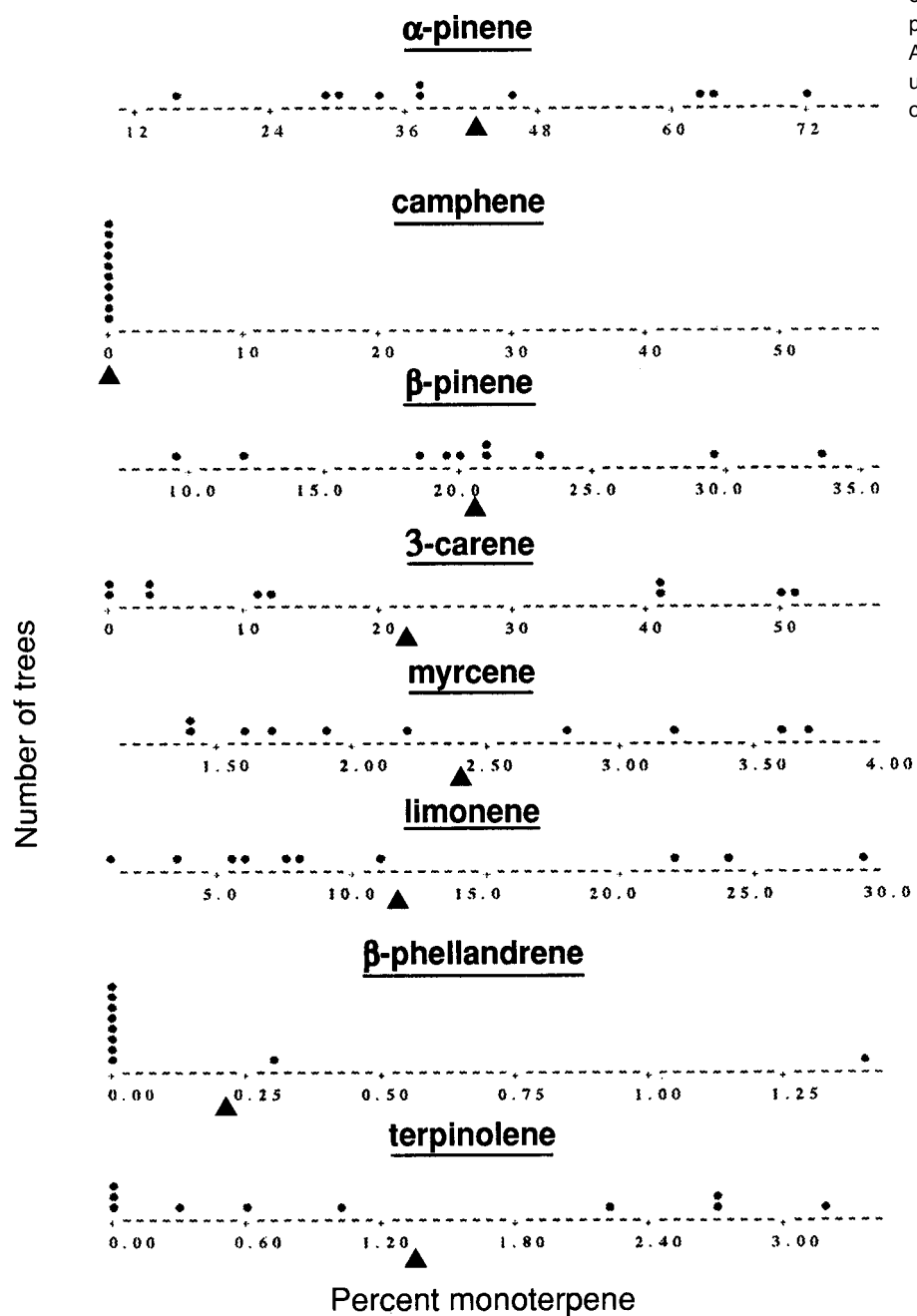


Figure 6-36—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-A295 x WP-3298; each dot is measurement of one tree; [▲] is average of set.

Ten sources of pollen were used in controlled breeding with AS-3187, and progeny were grown in the nursery, containers, and in a container/plantation for 6 to 9 years (*table 6-11*).

Progeny of AS-3187 x AS-3139

Tree AS-3139 was a high limonene tree growing near Tree AS-3187 at Adin Summit.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3187 female	1	9	23	23	14	29
AS-3139 male	1	18	1	0	22	59
F-1 progeny	88	17	29	17	4	32

There were 88 nursery-grown trees and only five container and container/plantation trees (*table 6-11*); therefore only the 88 nursery trees were used for further analysis.

There were no large shifts in average composition between 6 years and 11 years (*table 6-12*), or in the configuration of frequency distributions (*fig. 6-37*). Therefore, all nursery trees were aggregated for averages, frequency distributions, and for modal analysis.

The analysis of the frequency distributions showed that the number of modes equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-38*). Results with mode proportions were mixed. The two proportions for α -pinene, 76:24, probably were skewed because of the one measurement above 75 percent which drew the second mode too far up scale and shifted several measurements to the lower mode. By combining modes for β -pinene, proportions were 33:67; the proportions for the two modes for 3-carene were 42:58. By combining modes for limonene, proportions were 44:56.

High limonene trees were common, but there were no ultra-high ones because a low percent 3-carene was never linked with low β -pinene, a requirement of ultra-high limonene (*fig. 6-3*). There were 48 trees with 0 percent 3-carene and eight with less than 15 percent β -pinene, but there were no trees with both requirements. If this was a product of the juvenile factor, a few ultra-high limonene trees may develop as the trees mature.

Progeny of *P. ponderosa* AS-3187 x WP-3352

Tree WP-3352 was a high limonene tree at Wheeler Peak with a composition essentially the same as that of the previous male, Tree AS-3139.

Normalized percent compositions for parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3187 female	1	9	23	23	14	29
WP-3352 male	1	24			18	57
F-1 progeny	29	16	39	20	4	20

The average composition of the progeny differed from the progeny of Trees AS-3187 x AS-3139, the previous male (*table 6-11*); β -pinene was much higher, 39 percent, and limonene was much lower, 20 percent, than the corresponding averages for the progeny of Trees AS-3187 x AS-3139 that were 29 and 32 percent, respectively. The data for all nursery trees were aggregated for analysis, though there was an appreciable difference in 3-carene for the two periods of nursery growth (*table 6-12*). There was no difference in the location

Table 6-11—Average monoterpene composition of progeny of *P. ponderosa* AS-3187 when crossed with 10 different sources of pollen and grown in three locations: nursery (nur), container (con), container/plantation (copl). *N* = number of trees.

Male	Location of growth	Monoterpene composition of progeny ¹								
		N	α -p	cam	β -p	car	myr	lim	β -ph	ter
		<i>percent</i> ²								
WP-3352	nur	29	15.9	*	38.8	20.3	3.9	20.0	*	1.1
WP-3347	nur	77	7.7	0.1	34.1	31.1	2.8	21.5	*	1.8
WP-3347	con	16	8.2	*	38.5	32.3	2.4	16.6	0.3	1.6
WP-3347	copl	10	12.2	0.1	38.0	23.0	4.3	20.1	0	1.6
WP-3298	nur	52	22.4	*	37.3	19.4	3.2	16.1	0.1	1.0
WP-3298	con	8	32.7	0.2	33.7	20.6	2.7	10.0	0.5	1.0
WP-3298	copl	9	33.2	0.1	30.0	20.4	4.8	9.8	0.2	1.4
AS-3194	nur	89	13.2	*	31.1	29.5	3.1	21.2	0.1	1.7
AS-3194	con	8	8.4	*	34.3	39.0	1.9	14.4	0.4	1.6
AS-3194	copl	1	26.8	0.1	31.6	23.1	3.9	12.8	*	1.7
AS-3139	nur	88	16.6	0.1	28.7	17.0	4.3	32.2	*	1.1
AS-3139	con	1	21.4	0.2	36.9	0	4.4	36.9	*	*
AS-3139	copl	4	29.9	0.1	36.5	6.4	5.3	21.3	0	0.6
AS-5789	nur	34	17.5	*	42.9	15.4	6.5	16.6	0.1	0.8
AS-5789	con	8	16.1	0.1	54.5	8.7	6.1	12.5	1.4	0.6
AS-5789	copl	6	31.6	0.1	33.5	21.1	5.2	7.0	0.2	1.2
WP-3299	nur	33	13.5	*	28.0	25.9	4.9	26.2	0	1.6
WP-3299	con	5	14.7	0.4	30.5	34.9	3.6	12.8	0.7	2.2
WP-3312	nur	34	12.3	*	20.6	35.0	3.7	26.4	0	1.9
WP-3312	con	7	12.8	*	19.6	40.2	2.3	22.7	0	2.1
WP-3312	copl	8	17.3	*	26.9	23.1	3.9	27.0	0.2	1.6
WP-3318	nur	34	16.3	*	29.3	28.7	3.4	20.7	0	1.7
WP-3318	con	8	8.5	*	42.0	28.7	2.4	16.8	0.3	1.2
WP-3318	copl	9	23.7	0.1	33.5	17.0	3.4	20.8	0.2	1.2
AS-3178	nur	18	9.7	0	18.8	46.1	8.0	14.6	0	2.7
AS-3178	con	8	9.0	0.1	22.5	41.4	6.7	17.2	0.4	2.5
AS-3178	copl	9	19.0	*	21.2	36.1	6.1	14.6	0.1	2.7

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.² Normalized; * = trace.

Table 6-12—Average monoterpene composition of progeny of *P. ponderosa* AS-3187 when crossed with five different males with two ages (years) of nursery growth and, for aggregates (x). *N* = number of trees.

Male parent	Age	N	Monoterpene composition of progeny ¹							
			α -p	cam	β -p	car	myr	lim	β -ph	ter
			<i>percent</i> ²							
WP-3352	6	19	15.6	*	42.2	14.6	4.0	22.7	*	0.8
	11	10	16.4	0	32.2	31.1	3.6	14.8	0	1.9
	x	(=29)	15.9	*	38.8	20.3	3.9	20.0	*	1.1
WP-3347	6	34	7.1	0.2	33.7	33.2	2.3	21.6	0.1	1.8
	11	43	8.0	0.1	36.2	28.9	3.1	22.1	0	1.7
	x	(=77)	7.7	0.1	34.9	31.1	2.8	21.5	*	1.8
WP-3298	6	43	22.1	*	37.8	19.4	3.4	16.3	0.1	1.0
	11	9	23.9	*	34.8	19.8	5.0	15.4	0	1.1
	x	(=52)	22.4	*	37.3	19.4	3.7	16.1	0.1	1.0
AS-3194	6	43	12.4	0.1	32.9	27.4	2.9	22.6	0.2	1.5
	11	46	14.0	*	29.3	31.5	3.4	19.9	0	1.8
	x	(=89)	13.2	*	31.1	29.5	3.1	21.2	0.1	1.7
AS-3139	6	42	16.1	0.1	31.4	15.4	3.3	32.6	0.1	0.9
	11	46	17.1	0.1	26.2	18.4	5.2	31.9	0	1.2
	x	(=88)	16.6	0.1	28.7	17.0	4.3	32.2	*	1.8

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.²Normalized; * = trace.

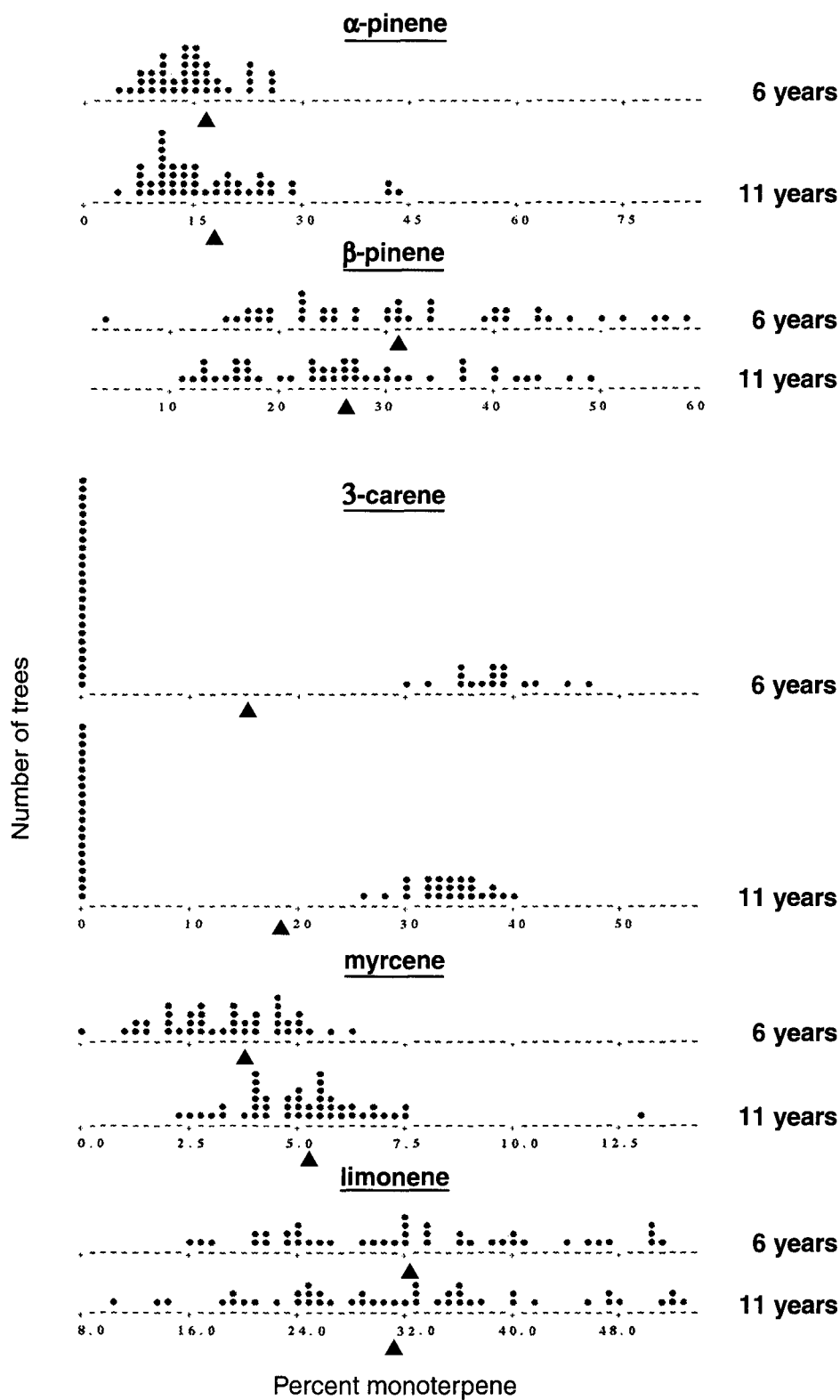
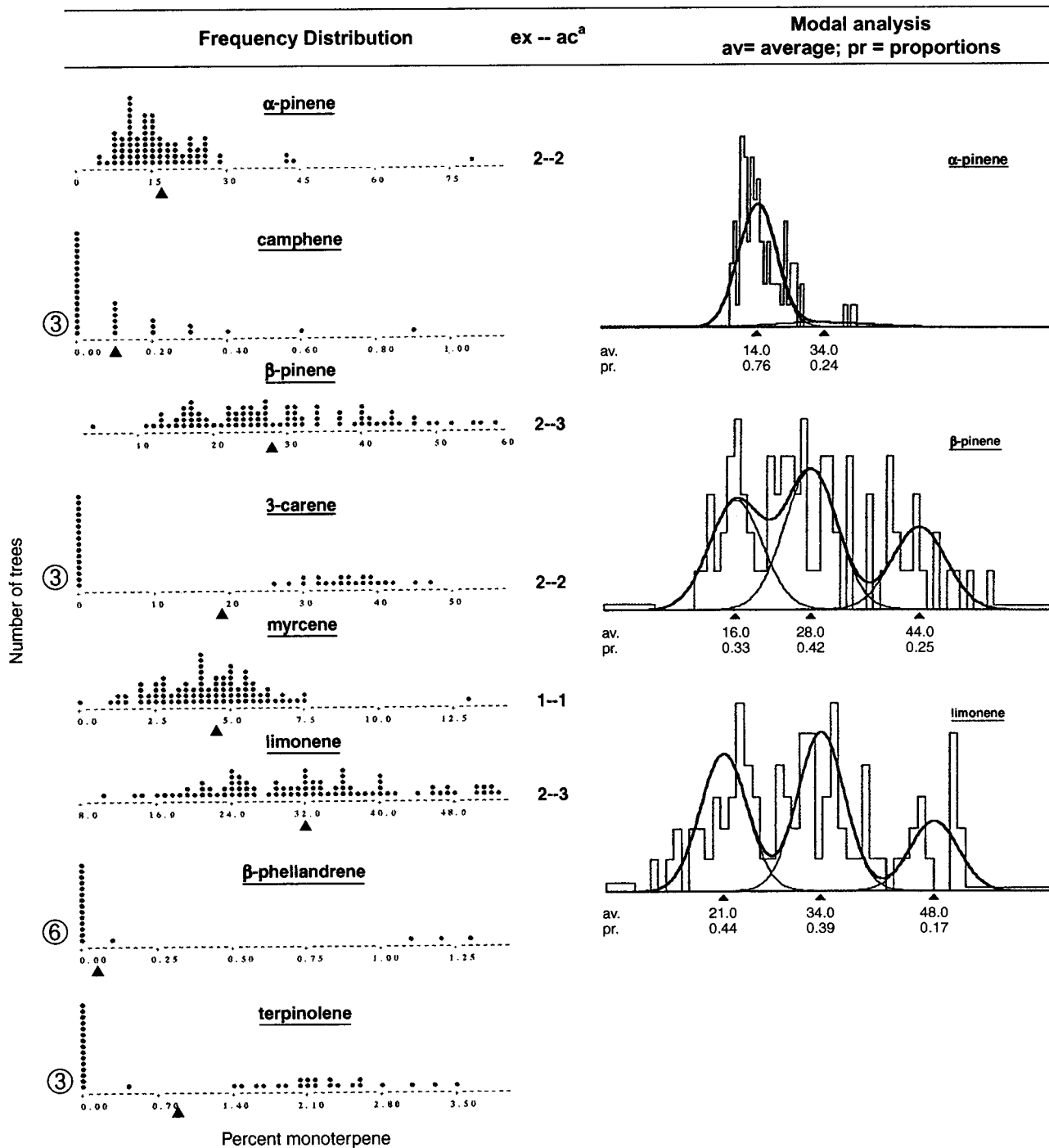


Figure 6-37—Comparative frequency distribution of the noted monoterpenes in the progeny of *P. ponderosa* AS-3187 x AS-3139 for two periods (years) of nursery growth listed on the right; each dot is measurement of one tree; [▲] is average of set.

Figure 6-38—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x AS-3139; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

of the modes because of the large separation of the two modes of 3-carene (*fig. 6-39*). The characteristics of the frequency distributions of the progeny of Trees AS-3187 x WP-3352 were quite similar to those of the progeny of Trees AS-3187 x AS-3139 except that the percent scale shifted.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-39*). Mode proportions were reasonably close to the expected for β -pinene and limonene, but poor for α -pinene. The distribution of α -pinene was skewed far to the right because of two values greater than 26 percent; this caused part of the mode between 18 and 20 percent to be included in the low mode, therefore causing poor mode proportions. Without the two extreme values above 26 percent, the two modes would be separated at about 16 percent, and mode proportions would be nearly equal. By combining modes for β -pinene to get the expected two modes, mode proportions were 63:37. Combining modes for limonene resulted in proportions of 46:54.

Only one tree had more than 40 percent limonene and could be classed as high limonene; it had 36 percent β -pinene, making it different from other high limonene trees (*fig. 6-3*). The whole set of progeny was low in limonene; this could be attributed to the juvenile factor, which caused greater amounts of α -pinene, β -pinene, and 3-carene, and to the great distance between parents which appeared to cause the same kinds of shifts—increase in β -pinene and α -pinene, and decrease in myrcene and limonene. These kinds of shifts were usually attributed to stress.

Progeny of *P. ponderosa* AS-3187 x WP-3298

The composition of the progeny when crossing Tree AS-3187 with Tree WP-3298, another high limonene tree at Wheeler Peak (*table 6-1*), was nearly identical to the composition of the previous cross of Trees AS-3187 x WP-3352.

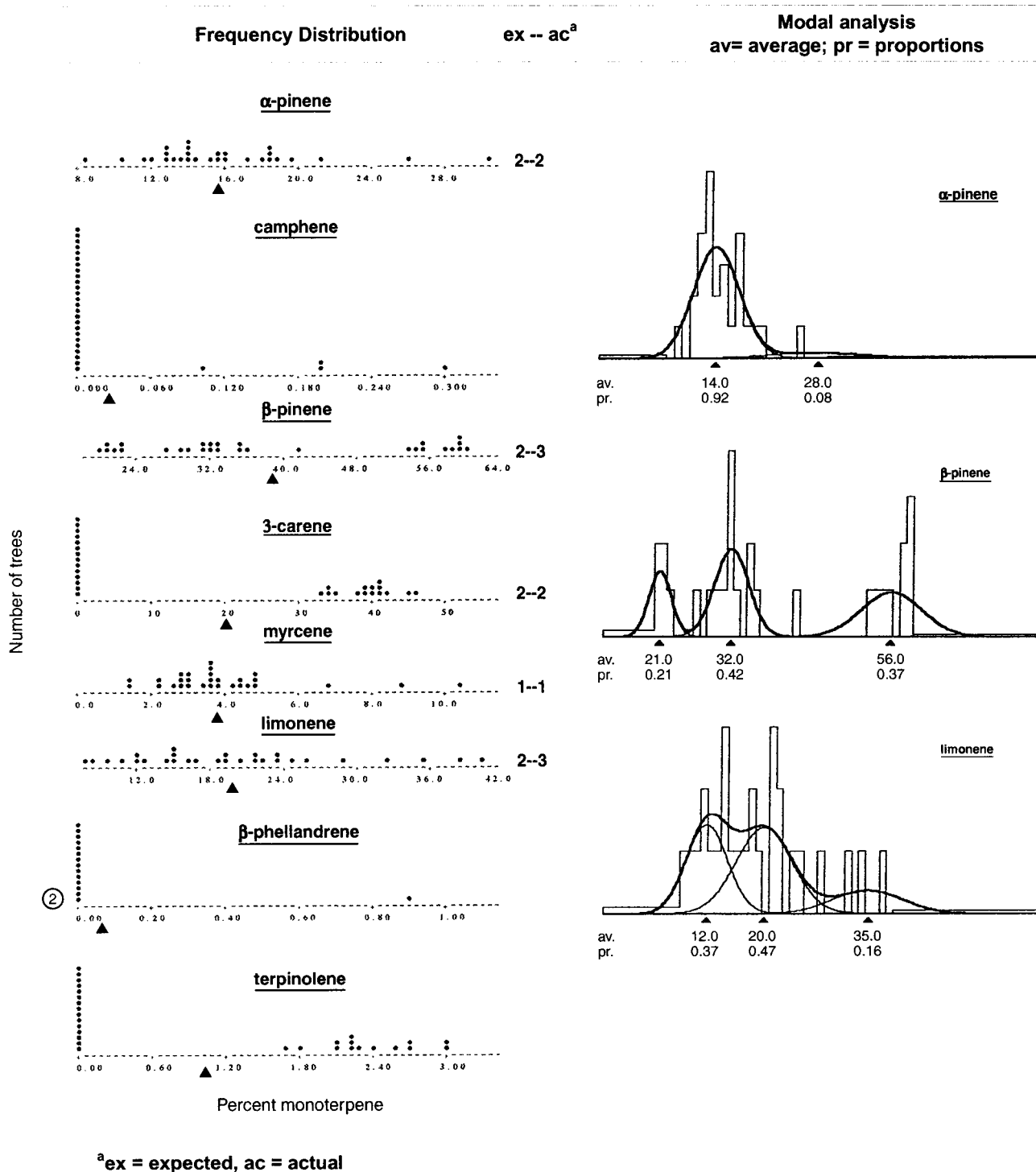
Normalized compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3187 female	1	9	23	23	14	29
WP-3298 male	1	26		0	16	58
F-1 progeny	52	22	37	19	3	16

Though the average compositions of the two sets of progeny, Trees AS-3187 x WP-3298 and AS-3187 x WP-3352, were very similar, as expected (*table 6-11*), there were some small differences in the characteristic of the frequency distributions. The frequency distributions of the progeny of Trees AS-3187 x WP-3298 were very similar for two periods of nursery growth, though the number of trees for the 7- to 11-year period was probably too small for reliable interpretation (*fig. 6-40, table 6-12*); only data on the aggregated nursery-grown trees were analyzed further.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-41*). Mode proportions were fairly close to the expected. When combining modes for α -pinene, β -pinene, and 3-carene, and limonene, proportions were 47:53 for α -pinene, 36:64 for β -pinene, 52:48 for 3-carene, and 53:47 for limonene.

Figure 6-39—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x WP-3352; see caption of figure 6-2 for explanation of the format below.



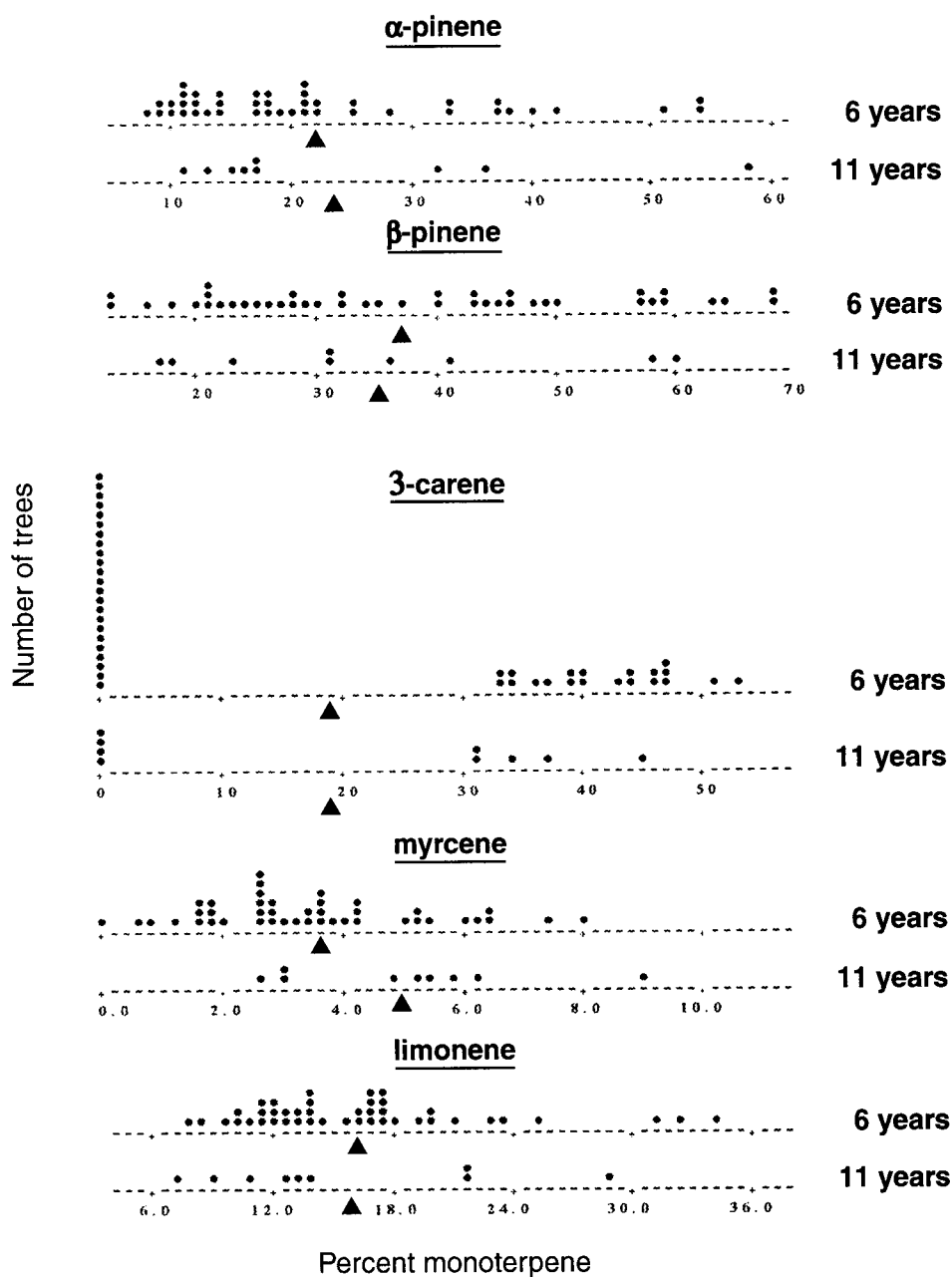
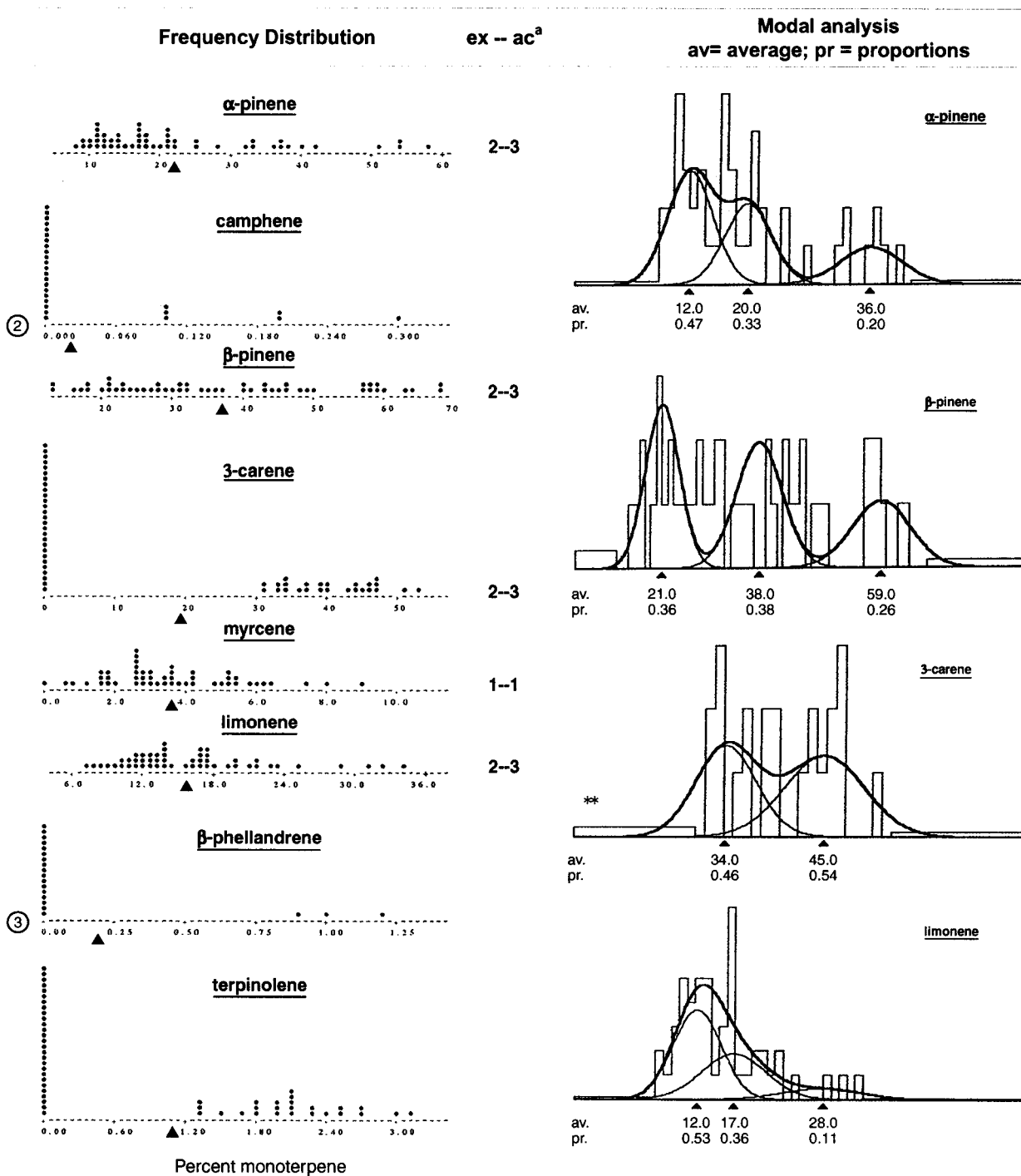


Figure 6-40—Comparative frequency distribution of the noted monoterpenes in the progeny of *P. ponderosa* AS-3187 x WP-3298 for two periods (years) of nursery growth listed on the right each dot is measurement of one tree; [▲] is average of set.

Figure 6-41—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x WP-3298; see caption of figure 6-2 for explanation of the format below; ** = mode at zero was not used in the modal analysis, but was included in the mode count.



There were no high limonene trees, though a few were expected, and again the parental distance factor could have been the cause, as noted earlier for the progeny of Trees AS-3187 x WP-3352 (*fig. 6-3*).

Progeny of *P. ponderosa* AS-3187 x WP-3299

Tree WP-3299 was another near-high limonene tree.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3187 female	1	9	23	23	14	29
WP-3299 male	1	6	*	43	8	40
F-1 progeny	33	14	28	26	5	26

The average composition of 33 nursery trees generally failed to show intermediacy, with limonene and myrcene lower, and α -pinene and β -pinene higher; 3-carene was about at the intermediate level (*table 6-11*). Only nursery-grown trees were used for further analysis.

The analysis of the frequency distributions showed that the number of modes found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-42*). Mode proportions were reasonably close to the expected. By combining modes to the expected number, mode proportions for β -pinene were 48:52, and for limonene the proportions were 55:45. The proportions of 33:30:27 for 3-carene were not too strong, but probably acceptable under the conditions of the various modifying factors.

Though there were high limonene trees, they were not as common as expected of a cross of two such trees (*fig. 6-3*). Again, it was postulated that the parental distance factor caused a level of stress in the progeny, expressed by lower limonene and higher α -pinene and β -pinene.

Progeny of *P. ponderosa* AS-3187 x AS-3194

These two trees were growing within 50 yards of each other near Adin Summit. Tree AS-3194 was classed as a near-high limonene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3187 female	1	9	23	23	14	29
AS-3194 male	1	7	6	33	13	39
F-1 progeny	89	13	31	30	3	21

The average for α -pinene and β -pinene was much higher than expected, and myrcene and limonene were much lower than expected in the progeny (*table 6-11*). The composition of the nursery trees differed from container and container/plantation trees; therefore, only nursery data were analyzed further. The characteristics of the frequency distributions of the two ages of nursery growth were quite similar (*table 6-12, fig. 6-43*), and the two sets were aggregated for further analysis. The larger population seemed to improve the resolution of modes and mode proportions.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-44*). Mode

Modal analysis

av= average; pr = proportions



Figure 6-43—Comparative frequency distribution of the noted monoterpenes in the progeny of *P. ponderosa* AS-3187 x AS-3194 for two periods (years) of nursery growth, listed on right; each dot is measurement of one tree; [▲] is average of set.

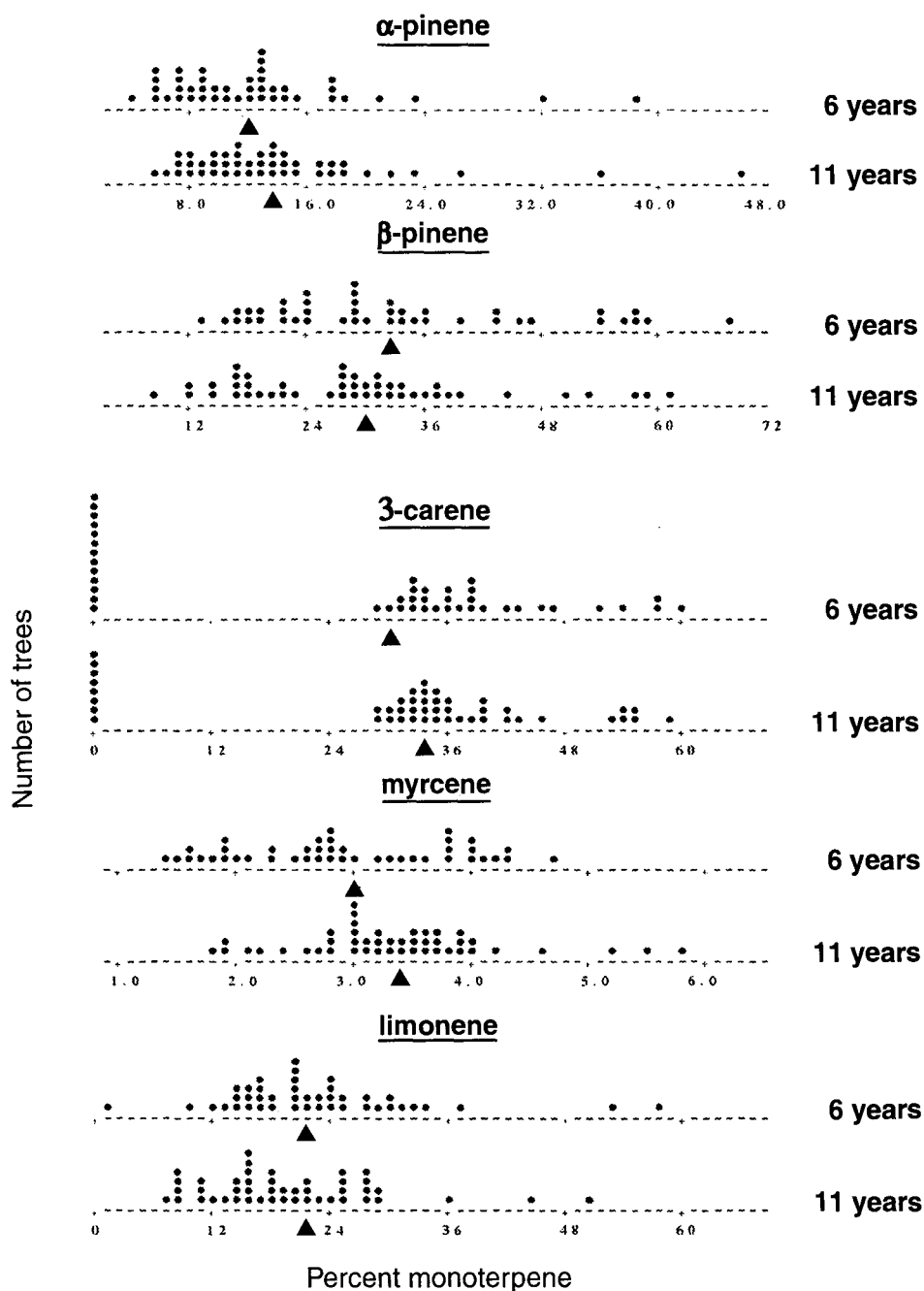
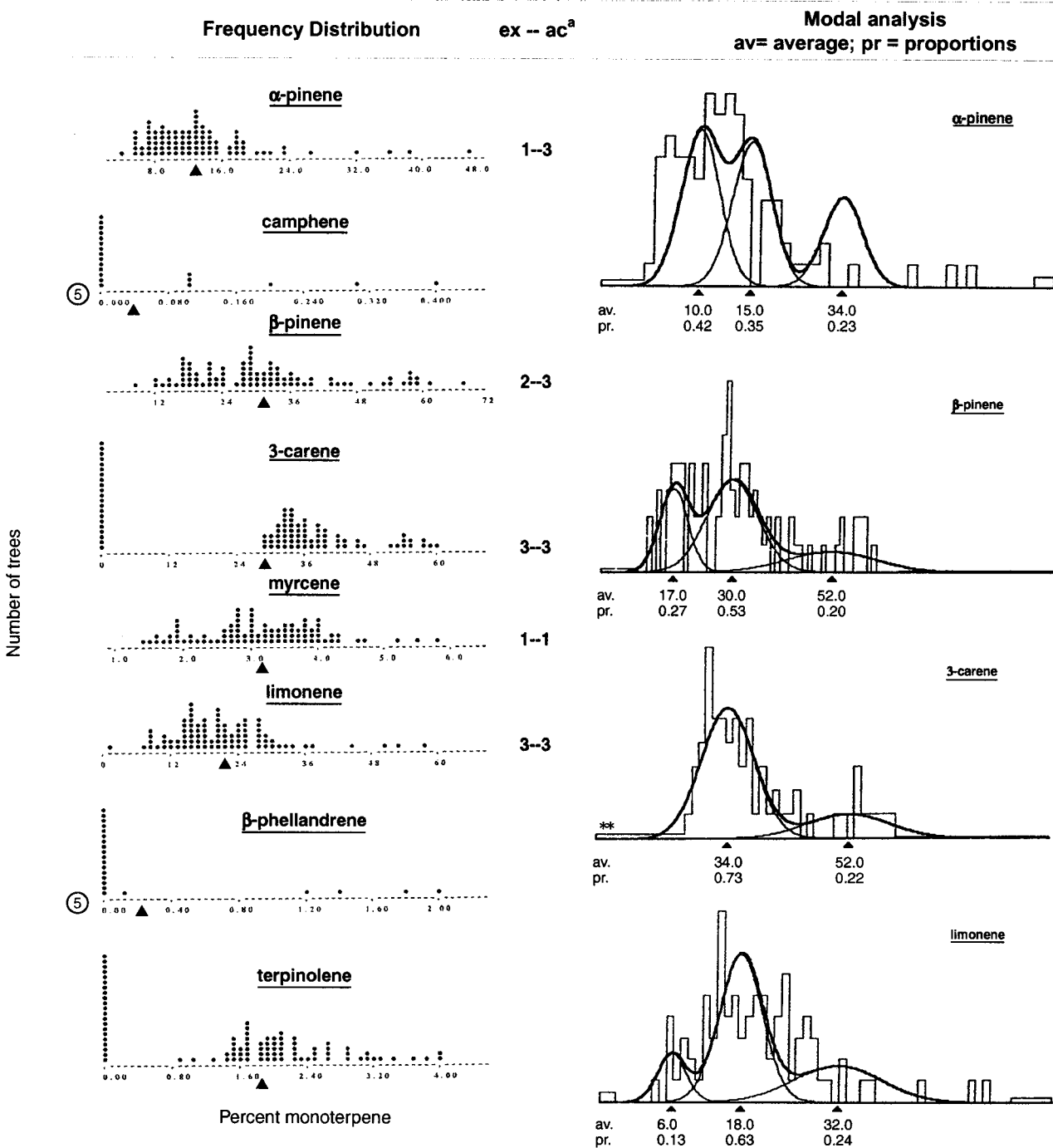


Figure 6-44—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x AS-3194; see caption of figure 6-2 for explanation of the format below; ** = mode at zero was not used in the modal analysis, but was included in the mode count.



^aex = expected, ac = actual

proportions were reasonably close to the expected when the possible effects of the modifying factors were considered. The proportions for the three modes of 3-carene were very good at 23:54:23, but the proportions for limonene were not too good at 13:63:24. The proportions probably were still acceptable under the conditions of the several modifying factors.

Neither parent was high limonene; yet there were a few high limonene progeny (*fig. 6-3*). One progeny had limonene near 60 percent, and if the juvenile effect lessens with age, this tree could become ultra-high limonene, because it lacked β -pinene.

Progeny of *P. ponderosa* AS-3187 x AS-5789

Both parents were growing near Adin Summit (*fig. 5-1*). Tree AS-5789 was classed as near-high limonene.

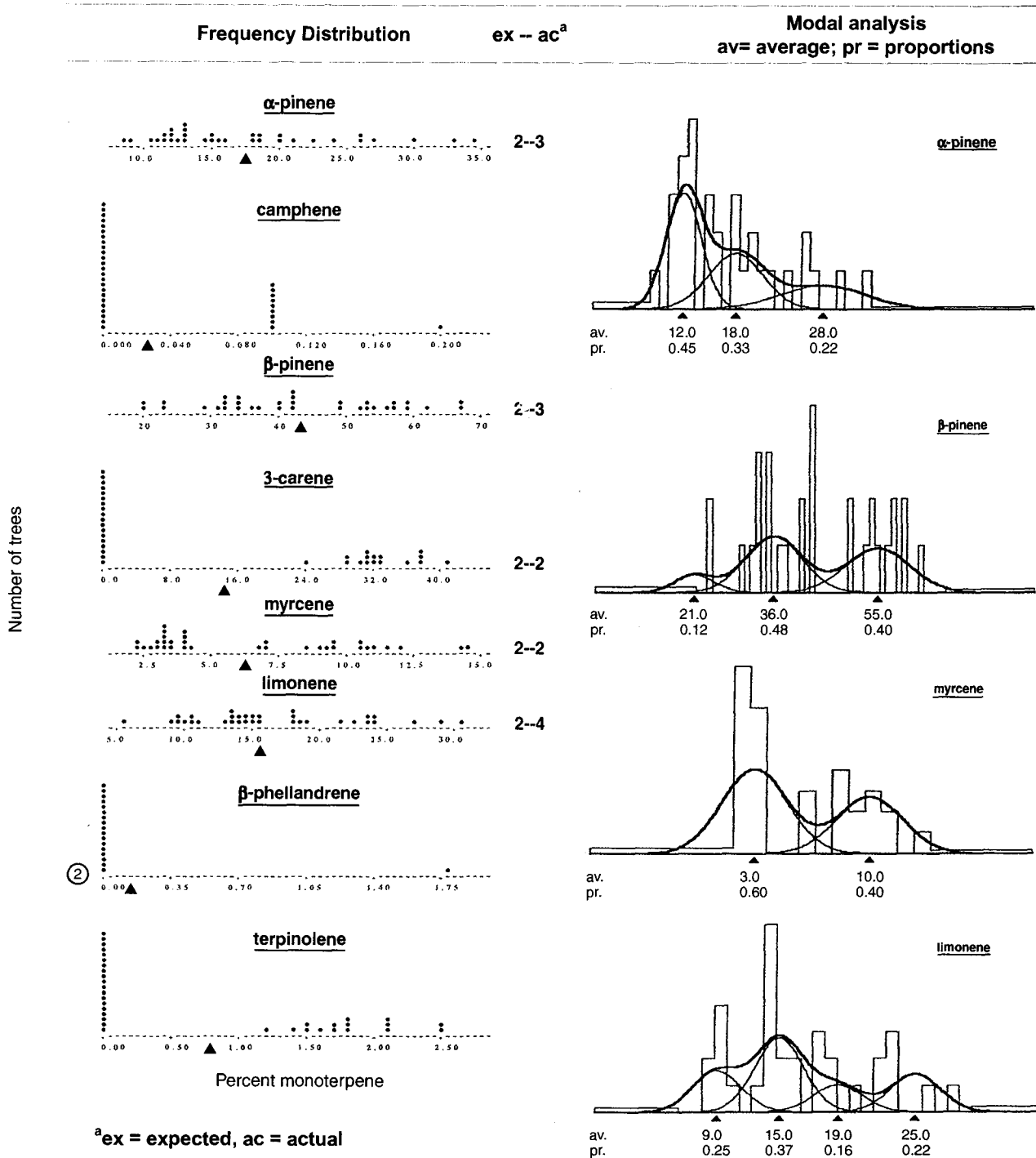
Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3187 female	1	9	23	23	14	29
AS-5789 male	1	23	10	0	29	37
F-1 progeny	34	18	43	15	7	17

Tree AS-5789 had the highest percentage of myrcene of any tree used in the controlled breeding program and was assigned a (0-1) set of alleles. The average composition of 34 nursery-grown trees (*table 6-11*) was much higher than the intermediate level for β -pinene and 3-carene, and much lower than the intermediate level for limonene and myrcene; the difference was attributed to the juvenile factor. The average for α -pinene was about as expected for intermediacy. The effects of being grown in containers and in a container/plantation on trees were mixed; limonene was greatly depressed, especially in container/plantation trees; α -pinene was depressed slightly in container trees but was greatly increased in container/plantation trees; both β -pinene and 3-carene increased and decreased differently in container trees and in the container/plantation trees. This inconsistency of expression in container trees and in the container/plantation trees was probably caused by variation in the dosage of phytocide and the effects of prolonged root containment in the container-grown trees. Thus, only the 34 nursery-grown trees were used in further analysis.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-45*). Mode proportions were fairly close to the expected. The proportions for limonene were 62:38 by combining the frequency distribution to the expected two modes. When combining modes of the frequency distribution to the expected two modes, proportions for α -pinene and β -pinene were 45:55 and 60:40, respectively. Proportions for myrcene were 60:40. There were two clearly separated modes for myrcene (*fig. 6-45*), adding support to the assignment of a set of (0-1) alleles to myrcene in Tree AS-5789. This characteristic of Tree AS-5789 prevailed when the tree was male or female—as noted earlier when Tree AS-5789 was the female parent.

Figure 6-45—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x AS-5789; see caption of figure 6-2 for explanation of the format below.



Most trees were high in β -pinene, and this could have been caused by the juvenile effect and by the presence of β -pinene in both parents (*fig. 6-3*). I would expect β -pinene to decrease with age and limonene to increase, though it is not likely that any will become high limonene.

Progeny of *P. ponderosa* AS-3187 x WP-3318

Tree WP-3318 was another near-high limonene tree (40 percent) growing at Wheeler Peak, with moderate α -pinene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3187 female	1	9	23	23	14	29
WP-3318 male	1	27	10	18	3	40
F-1 progeny	34	16	29	29	3	21

The average composition of 34 nursery progeny was not intermediate to the parents for any component (*table 6-11*). There were some differences in 9-year container and container/plantation trees; therefore, only the data on nursery trees were analyzed further.

The analysis of the frequency distributions showed that the number of modes found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents, except for 3-carene (*fig. 6-46*). The modal characteristics of 3-carene did not fit the expected and would have to be considered an anomaly. This possible anomaly was discussed in the section "Progeny of *P. ponderosa* AS-5789 x WP-3318." Thus the two anomalies occurred when Tree WP-3318 was a male. The third case of a possible anomaly, which will be reported later, also involved Tree WP-3318 as the male parent. Mode proportions were not as close to the expected ones as was found in most other families, probably because of the small sample number and the effects of the modifying factors. By combining modes of the frequency distribution to get the expected number of modes, proportions of 39:61 and 51:49 were achieved for α -pinene and β -pinene, respectively. The 3-mode proportions for 3-carene and limonene were only moderately close to the expected.

There were no high limonene progeny, though a few were expected (*fig. 6-3*). However, the parental distance factor probably accounted for this lack of high limonene.

Progeny of *P. ponderosa* AS-3187 x WP-3312

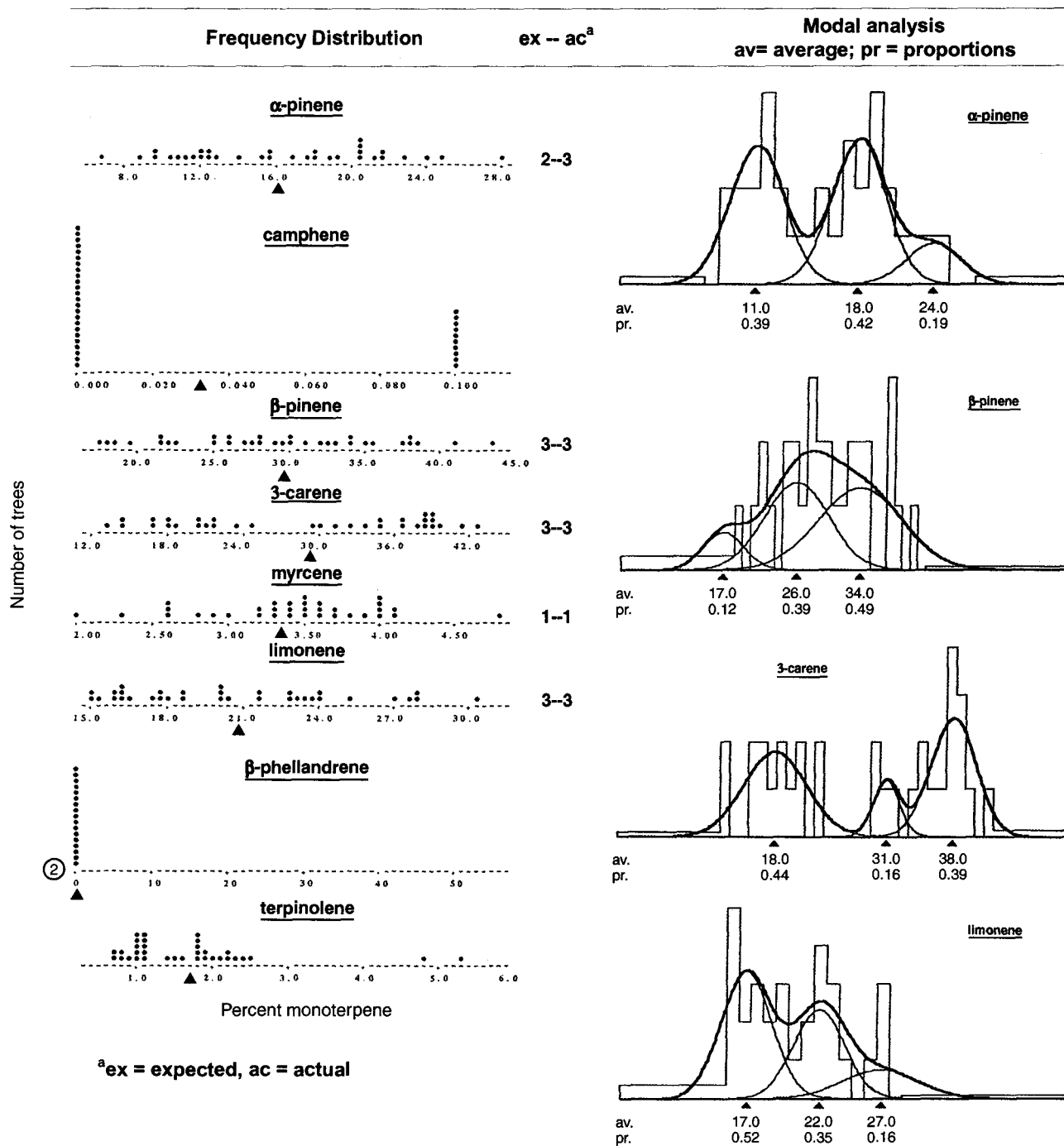
Tree WP-3312 was also classed as near-high limonene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3187 female	1	9	23	23	14	29
WP-3312 male	1	5	7	34	4	48
F-1 progeny	34	12	21	35	4	26

The average composition of 34 nursery-grown trees was not intermediate to the two parent trees (*table 6-11*); α -pinene and β -pinene were much higher than expected, limonene and myrcene were much lower, and 3-carene was about as expected. Again, these differences were attributed to the juvenile and

Figure 6-46—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x WP-3318; see caption of figure 6-2 for explanation of the format below.



the parental distance factors. Results with container and container/plantation trees were variable. Container trees were higher in 3-carene and lower in limonene (*table 6-11*); α -pinene did not show the usual increase in containers. Only data on nursery-grown trees were included in further analyses.

The analysis of the frequency distributions showed that the number of modes found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-47*). Mode proportions were very close to the expected when mode numbers were reduced to the expected. By combining modes of the analysis to get the expected number, proportions were 48:52 for β -pinene, 52:48 for 3-carene, and 50:50 for limonene.

There were two high limonene trees in contrast to none in other sets of progeny between Tree AS-3187 and other distant males (*fig. 6-3*). Therefore, the speculation that there could be such trees in the other sets of progeny between Tree AS-3187 and distant near-high limonene males seems valid.

Progeny of *P. ponderosa* AS-3187 x AS-3178

Tree AS-3178 had only moderate limonene, and was about 50 yards from Tree AS-3187.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3187 female	1	9	23	23	14	29
AS-3178 male	1	3	1	55	18	19
F-1 progeny	18	10	19	46	8	15

Though intermediacy was not found in the average composition of 18 nursery progeny, it was not all that far from it (*table 6-11*). There were some differences in the composition of container and of container/plantation trees. Only the nursery-grown trees were used further. But the small number of samples precludes an analysis of frequency distribution (*fig. 6-48*), and types of composition were not listed in a table.

There were no high limonene progeny. Though some were expected, they would have shown that such high limonene was possible in the progeny of two moderate-limonene trees growing in the same locality. There may not have been enough progeny for a high limonene progeny to occur.

Progeny of *P. ponderosa* AS-3187 x WP-3347

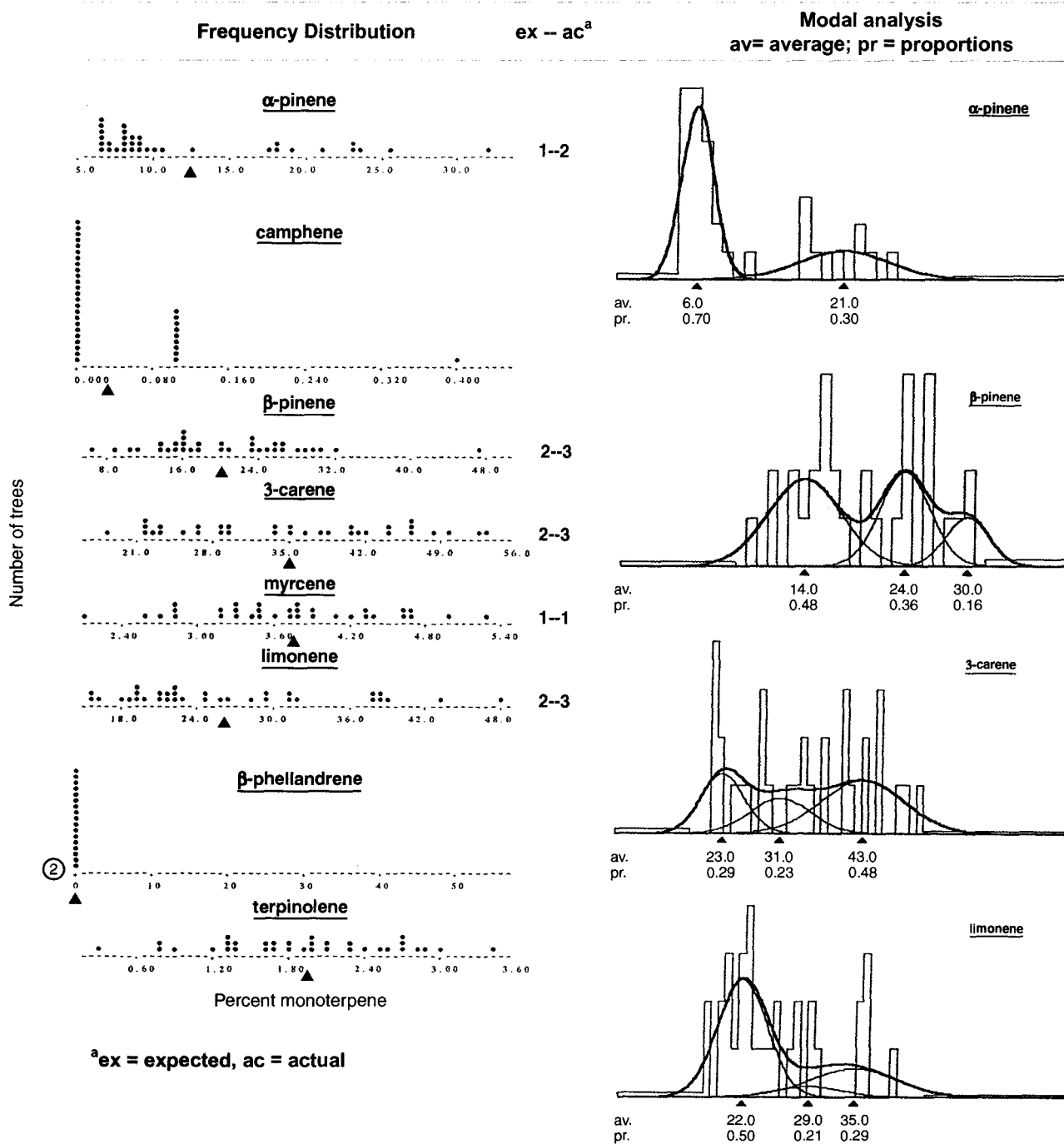
Tree WP-3347, growing at Wheeler Peak, was classed as near-high limonene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
AS-3187 female	1	9	23	23	14	29
WP-3347 male	1	4	17	25	3	48
F-1 progeny	77	8	34	31	3	22

The average composition of the progeny, as with other sets of progeny between Tree AS-3187 and males located at Wheeler Peak about 400 miles away, was higher than expected in β -pinene and 3-carene and lower in myrcene and limonene (*table 6-11*). Neither the average composition nor the characteristics of the frequency distributions seemed to be affected by age of

Figure 6-47—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x WP-3312; see caption of figure 6-2 for explanation of the format below.



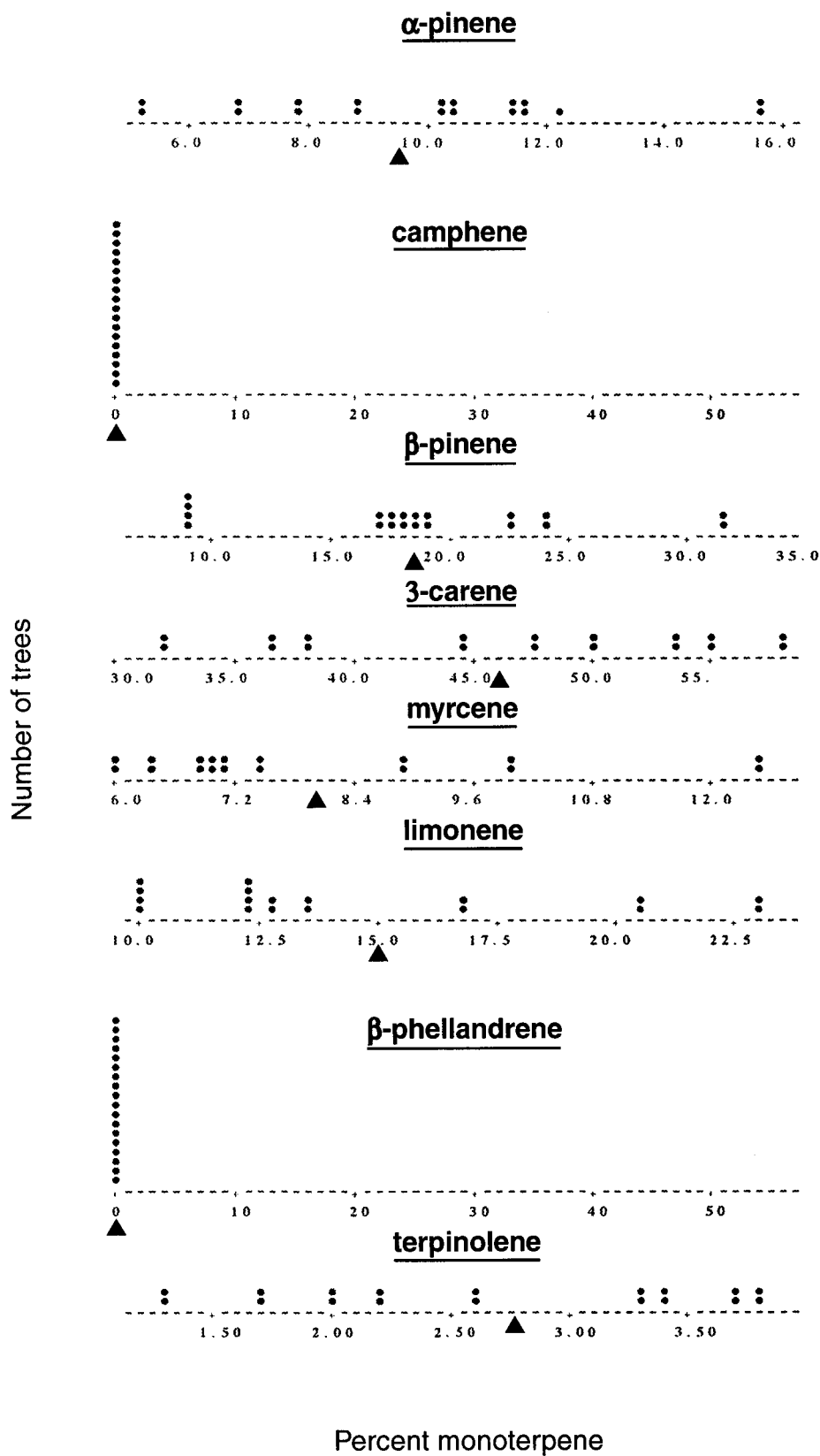
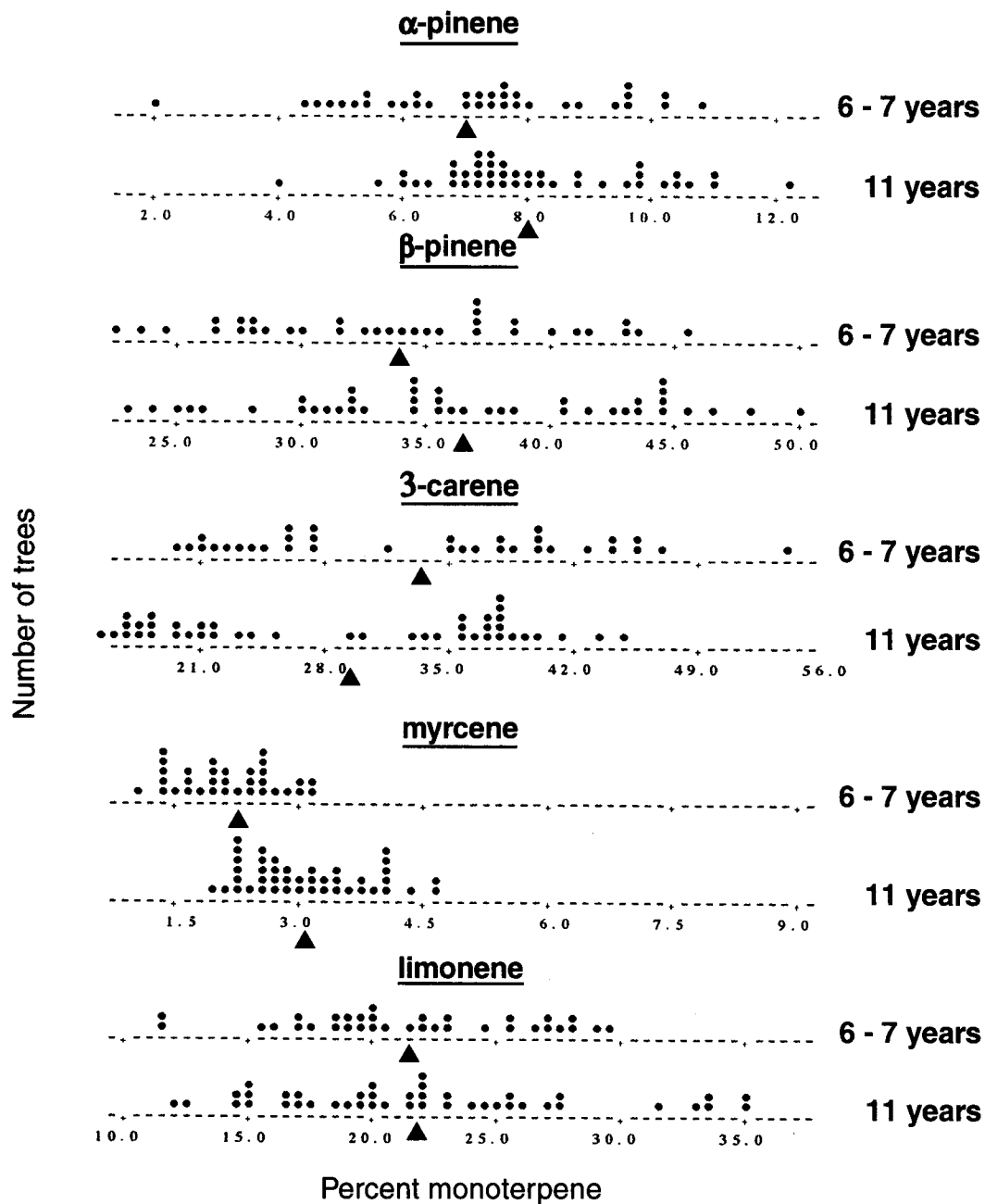


Figure 6-48—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-3187 x AS-3178; each dot is measurement of one tree; [▲] is average of set.

Figure 6-49—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-3187 x WP-3347 for two periods of nursery growth (years), listed on the right; each dot is measurement of one tree; [▲] is average of set.



nursery growth (*table 6-12*, *fig. 6-49*), though an age span of only 4 to 5 years may not be too meaningful. There were some rather small differences between nursery trees and container trees, particularly in 3-carene (*table 6-12*). However, the two sources were not aggregated for further analysis.

The analysis of the frequency distributions showed that the number of modes found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-50*). By combining modes to get the expected number, proportions were 33:67 for β -pinene and 45:55 for 3-carene. Proportions for limonene were poor even by combining modes.

None of the 98 progeny had more than 35 percent limonene (*figs. 6-3, 6-49*). One might speculate that because there were alleles for both β -pinene and 3-carene in both parents along with alleles for limonene, the progeny would tend to have alleles for all three components in most or all trees. Thus, through competition for a limited amount of precursor, limonene would tend to be less than 40 percent. That limonene was generally less than β -pinene and 3-carene may be caused by the juvenile effect or by limonene being unable to compete equally with β -pinene and 3-carene when they were both present during the juvenile period.

Discussion: Full Siblings of Tree AS-3187

The composition of nursery trees generally differed from that of container and container/plantation trees; therefore, only data on nursery trees were analyzed further. There was generally little or no difference due to age of growth in the nursery; therefore all nursery data were aggregated for further analysis. However, the differences due to place of growth were greatest for female trees with higher limonene. That is, limonene seemed to be more adversely affected by the juvenile factor than were other monoterpenes. Though the juvenile and parental distance factors had an appreciable effect on composition, the effect was relative and simply moved the modes to other positions on the percent scale; the number of modes was not changed, but their positions were.

The actual number of modes, as derived from modal analysis, with one exception, always equalled or exceeded the expected number, as derived from the assignment of additive alleles to the parents; this supported the additive allele hypothesis. The aggregate data also reaffirm the assignment of alleles to tree AS-3187 (*table 6-1*). Most mode proportions were reasonably close to the expected when using the expected number, providing additional support to the hypothesis.

No ultra-high limonene trees were found in the progeny of Tree AS-3187 (*fig. 6-3*), and high limonene progeny were not at all common, though high limonene males were used.

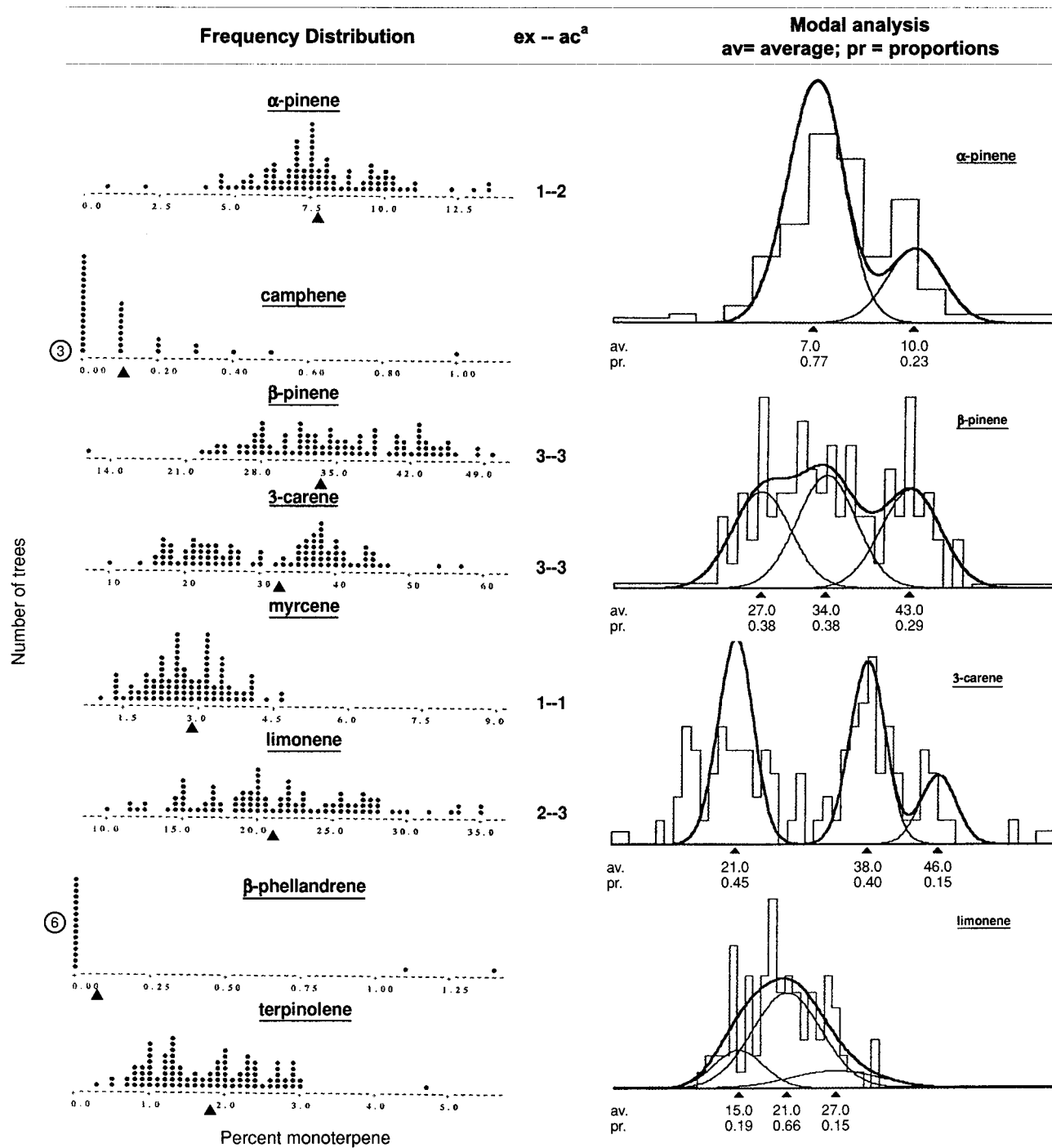
The average for limonene and the number of high limonene trees were much higher in sets of progeny with nearby males than with distant males. Again, a stress factor and the distance between parents were postulated as the causes.

Tree 3187 was an excellent tree for the breeding studies, and the results with its progeny added much to the conclusions. It was also a desirable tree because of its good cone crops, easy breeding, and ease with which it could be climbed.

Crosses of *P. ponderosa* IFG-4B63 with 12 Pollen Sources

Tree IFG-4B63 was growing at the Institute of Forest Genetics (*fig. 5-1*). It had been used for much breeding and other studies of *P. ponderosa* at IFG. Its monoterpene composition (*table 6-1*) was characterized as having 0 percent 3-carene; having a large amount of β -pinene, 55 percent; and having smaller amounts of α -pinene, 9 percent; myrcene, 12 percent; and limonene, 22 percent.

Figure 6-50—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x WP-3347; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

Twelve different sources of pollen were used with it (*table 6-13*). Though there was considerable range in the percent myrcene in the male parents, there was little range in the various sets of progeny. It was assumed that the juvenile effect caused a reduction of the full effect of myrcene and possibly limonene. The average composition of 5- to 7-year-old nursery trees was nearly the same as 4-year-old container trees; therefore, nursery and 4-year container trees were aggregated, but 9-year container and container/plantation trees were not aggregated because they differed somewhat and there were so few of them.

Progeny with moderate to high limonene had been expected in crosses with Tree IFG-4B63, particularly males with high or near-high limonene. But these progeny did not occur, though there were a few individual trees with 45 to 50 percent limonene. This could again point to the possibility of 13-pinene, which was very high in Tree IFG-4B63, being a strong factor in the juvenile effect. But just how this factor works was not clear. That is, does it operate by "turning down" limonene and myrcene, or by "turning up" α -pinene and β -pinene. However, the results with IFG-4B63 were satisfactory. Though the frequency distribution was moved up or down the percent scale, the modal structure generally remained unchanged.

Progeny of *P. ponderosa* IFG-4B63 x AS-3178

Tree AS-3178 was the reverse of Tree IFG-4B63 with respect to β -pinene and 3-carene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
AS-3178 male	1	3	1	55	18	19
F-1 progeny	70	11	45	22	5	17

There were only slight differences between data on nursery and 4-year-old trees; therefore they were aggregated. The average composition of the 70 trees was expected to have about equal amounts of β -pinene and 3-carene, because IFG-4B63 had two alleles for β -pinene and AS-3178 had two alleles for 3-carene. Instead, the average amounts in the progeny were 45 percent β -pinene and 21 percent 3-carene. This suggests, again, that β -pinene was the strong element in the juvenile factor. This was evident in all the progeny of IFG-4B63.

The analysis of the frequency distributions showed that the number of modes actually found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parent trees (*fig. 6-51*). This supports the additive allele hypothesis. By combining modes to get the expected number, mode proportions were 48:52 for β -pinene, and 39:61 for 3-carene.

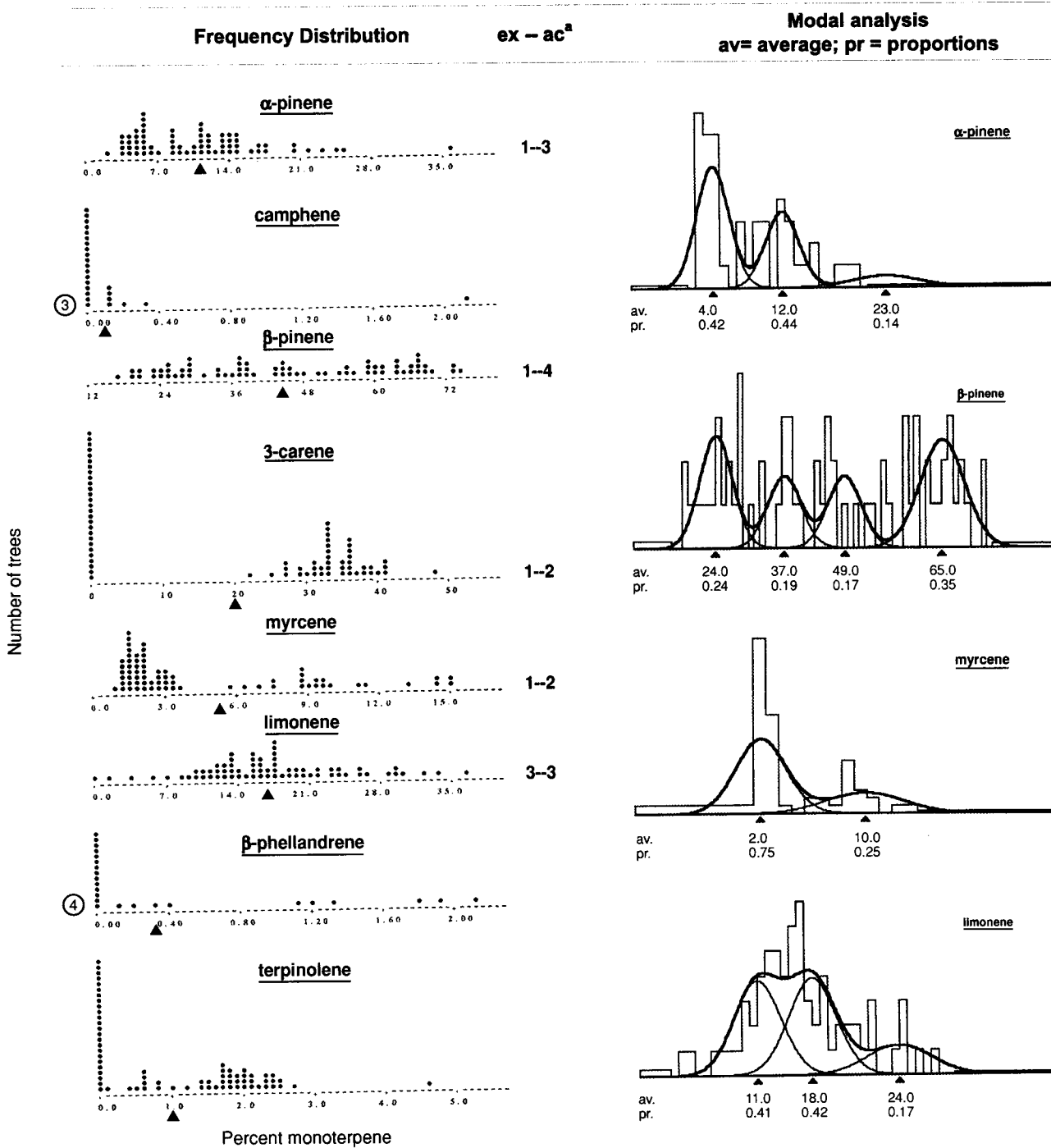
The frequency distribution of 3-carene had two modes, one at 0 and one at about 30 percent, where one mode was expected; i.e., with a (1-1) set of alleles for 3-carene in AS-3178, all progeny should have at least one allele for 3-carene. The one mode at 30 percent was expected, but not the mode at 0. I attribute this to the juvenile factor which seemed unusually strong when β -pinene was a large percent, and the percent of β -pinene was high in all progeny (*fig. 6-51, table 6-13*). This condition was also found in the progeny of IFG-4B63 x AS-3312 (discussed later in this section). And here too there was a large percent of β -pinene. However, when there were two alleles for 3-carene, and 3-pinene was absent or very small, there was no mode at 0 for 3-carene (See AS-3139 x 3178, AS-3187 x AS-3178, AS-3139 x WP-3347, AS-3139 x WP-3312). AS-3178 was the male parent in two of the examples, and in each of these two, the absence of a 0 mode for 3-carene supports the assignment of a (1-1) set of alleles for 3-carene

Table 6-13—Average xylem monoterpene of progeny of *P. ponderosa* IFG-4B63 when used with 12 sources of pollen and grown under different conditions. *N* = number of trees.

Male parent	N	Place of growth ²	Monoterpene composition of progeny ¹							
			α -p	cam	β -p	car	myr	lim	β -ph	ter
			<i>percent</i> ³							
AS-3178	51	nur	10.6	0.1	42.1	22.4	5.6	17.8	0.2	0.9
AS-3178	19	con-4	11.7	0	53.3	17.0	1.6	15.1	0.3	1.0
AS-3194	12	nur	13.0	*	48.6	22.5	1.4	13.0	0.4	1.1
AS-3194	11	con-4	10.4	0	52.5	14.6	1.7	19.9	0	1.0
AS-3187	23	nur	7.1	*	31.9	36.3	7.1	15.7	0	1.9
AS-3187	27	con-4	7.3	0	34.2	39.5	5.2	12.3	0.1	2.4
WP-3318	24	nur	21.0	0.1	39.2	18.1	2.4	18.3	0	0.9
WP-3318	8	con-9	21.8	0.2	43.0	19.8	1.7	11.7	1.0	1.0
WP-3318	7	con-4	21.2	0	43.5	20.2	1.3	12.8	0	1.0
WP-3318	3	copl	23.4	0.1	48.7	10.6	1.8	14.9	0	0.6
WP-3312	74	nur	13.3	*	40.0	18.8	3.3	23.4	0.2	0.8
WP-3312	9	con-4	14.0	0.1	34.7	30.0	1.7	18.2	0	1.2
WP-3299	8	nur	12.7	0	41.6	19.4	3.3	22.1	0	0.9
WP-3299	1	con-4	9.7	0	36.5	33.2	6.2	10.4	1.6	1.8
AS-5759	14	nur	18.0	0	66.5	0	4.0	11.3	0.1	0
AS-5789	16	con-4	16.8	0.1	67.3	0	4.6	9.5	1.0	0.8
AS-A95	26	nur	18.4	0.1	52.6	0	1.9	27.0	0	0
AS-A95	27	con-4	16.1	0	55.8	2.9	1.5	23.3	0	0.1
AS-3139	26	nur	19.6	0	49.7	2.6	2.6	28.0	0	0
AS-3139	21	con-4	16.3	0	49.0	5.5	2.0	27.0	0	0.3
WP-3298	23	nur	30.4	0	56.0	0	2.5	11.0	0	0
WP-3298	6	con-9	31.8	0.2	51.9	0.8	2.7	11.5	0	1.2
WP-3298	5	copl	45.4	0.1	44.2	0	3.2	7.1	0	0
WP-3298	25	con-4	28.0	0.1	55.0	3.1	2.1	10.0	1.4	0.2
BL-6074	2	nur	18.6	0.1	49.5	0	3.1	28.6	0	0
WP-3352	60	nur	18.1	0.1	60.8	0	2.3	18.1	0.4	0
WP-3352	8	con-9	19.4	0.1	63.2	1.4	1.9	12.7	1.3	0.1

¹ α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, terpinolene.²nur = nursery; con = container; copl = container-plantation.³Normalized; * = trace.

Fig. 6-51—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* IFG-4B63 x AS-3178; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

in AS-3178. It might be that, in the juvenile period, an allele for β -pinene prevents the action of an allele for 3-carene. Very little is known about the effects of the juvenile factor and just how it operates.

Progeny of *P. ponderosa* IFG-4B63 x AS-3194

Tree AS-3194 was classed as a near-high limonene tree.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
AS-3194 male	1	7	6	33	13	39
F-1 progeny	23	12	50	19	2	17

Though the data for nursery and 4-year container trees differed a bit (table 6-13), they were aggregated for averages and for frequency distribution display. The small sample number ruled out modal analysis. The averages for the progeny of Trees IFG-4B63 x AS-3178 and IFG-4B63 x AS-3194 were quite similar. Even with a small sample, a bimodal distribution was discernible for β -pinene and 3-carene (fig. 6-52).

Progeny of *P. ponderosa* IFG-4B63 x AS-3187

Tree AS-3187 had moderate amounts of β -pinene, 3-carene, myrcene, and limonene, and a small amount of α -pinene (table 6-1).

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
AS-3187 male	1	9	23	23	14	29
F-1 progeny	50	7	33	38	6	14

There was little difference between nursery and 4-year container data; therefore, they were aggregated. The average for 50 progeny (table 6-13) showed the usual juvenile effects in the reduced amounts of myrcene and limonene and the increased amount of β -pinene. However, the amount of 3-carene, 38 percent, was surprising because 3-carene was absent in Tree IFG-4B63 and present in only a moderate amount in Tree AS-3187. Most notable about the frequency distributions was their unusually small range (fig. 6-53). The combination of these two parents seemed to be different from most other combinations of parents, in the narrow range of the frequency distribution of individual components. There could have been a cancelling out of the factors that can cause variation. Though the ranges in the distributions were usually small, compared to other sets of progeny, they were still suitable for modal analysis. This narrow range in frequency distributions seemed to be more common in the progeny of Tree IFG-4B63 than in the progeny of other females.

The analysis of the frequency distributions showed that the number of modes found by the analysis equalled or exceeded the number expected based on the assignment of alleles to the parents (fig. 6-53). This supports the additive allele hypothesis. Mode proportions were very close to the expected: for α -pinene, 55:45; β -pinene, 44:56; and 3-carene, 64:36. The 3-mode proportions for limonene were not very close to the expected, probably because of the aggregate effect of the modifying factors.

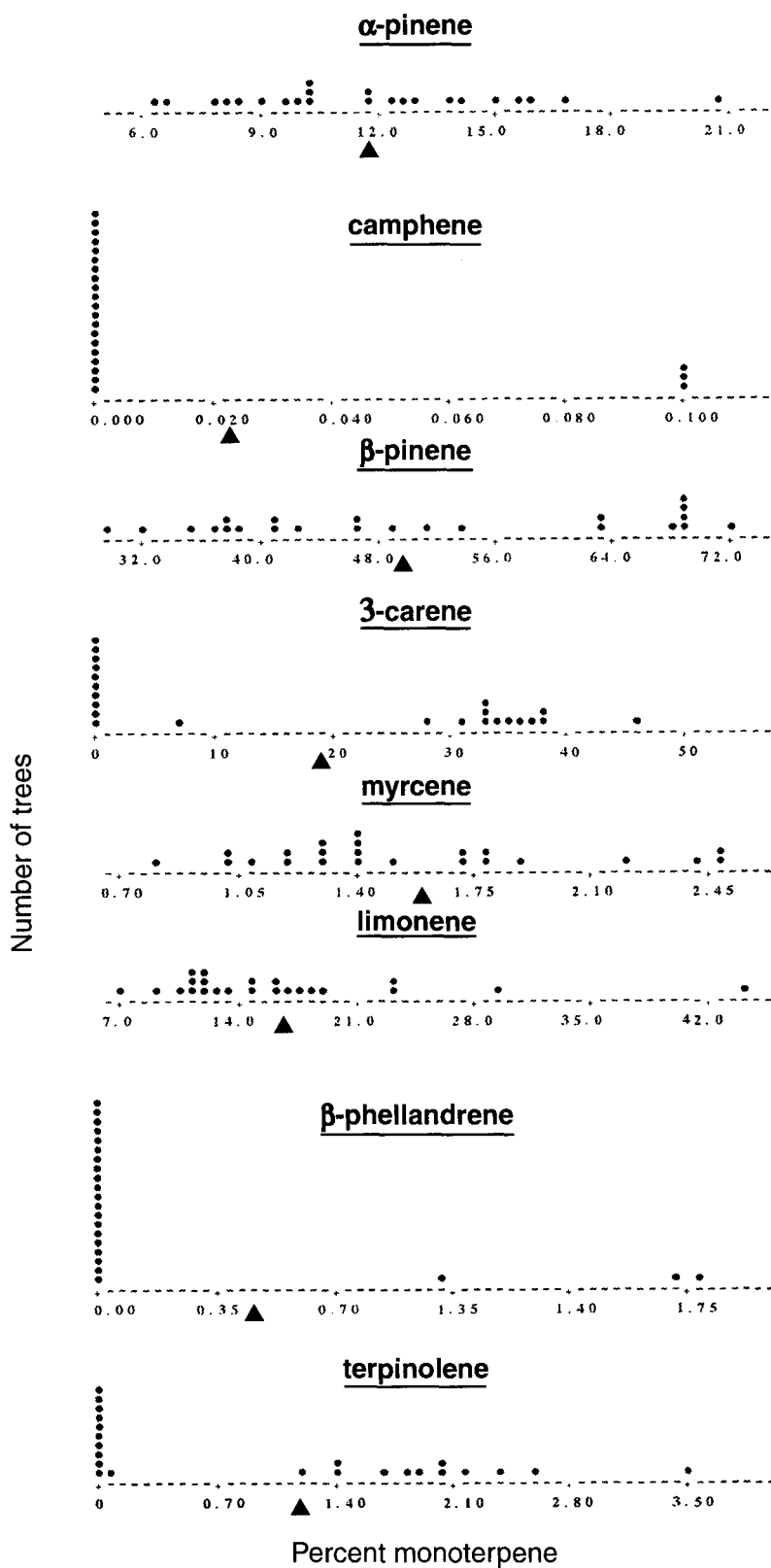
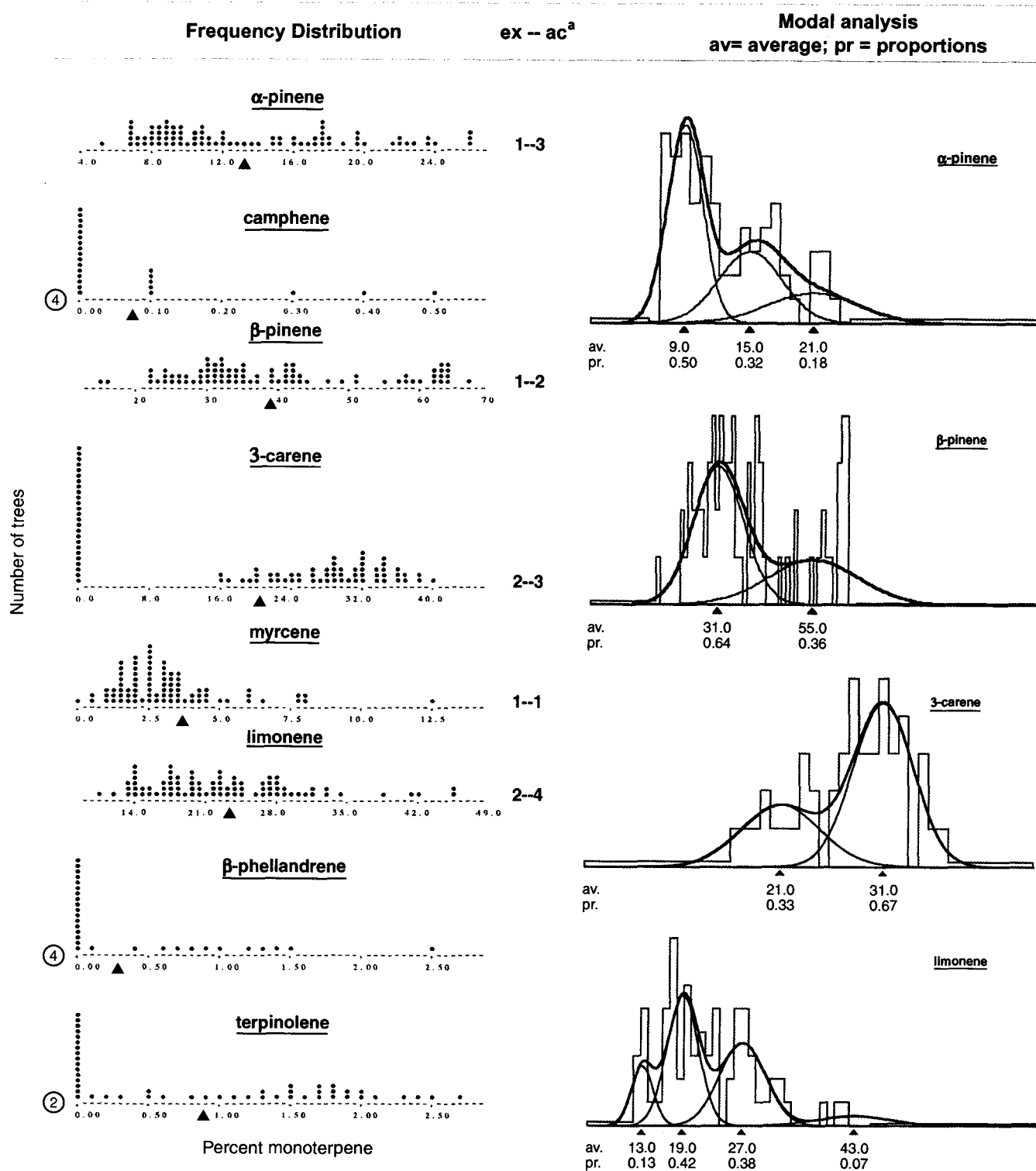


Figure 6-52—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* IFG-4B63 x AS-3194; each dot is measurement of one tree; [▲] is average of set.

Figure 6-53—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* IFG-4863 x WP-3187; see caption of figure 6-2 for explanation of the format below.



The lack of potentially high limonene trees was not surprising with the composition of the two parent trees (*fig. 6-3*).

Progeny of *P. ponderosa* IFG-4B63 x WP-3318

Tree WP-3318 was near-high limonene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
WP-3318 male	1	27	10	18	3	40
F-1 progeny	31	21	40	18	2	18

There was little difference between nursery and 4-year container data (*table 6-13*); therefore they were aggregated for further analysis. The 9-year container and container/plantation data did differ, somewhat, and were not included in further analysis.

The analysis of the frequency distributions showed that the number of modes found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-54*). Mode proportions were somewhat close to the expected, when all conditions were considered. The proportions for α -pinene at 44:56 and for 3-carene at 33:67 were fairly close to the expected. By combining to get the expected two modes for β -pinene, proportions were 41:59. The three modes for limonene were 24:60:16 and quite close to the expected.

None of the progeny had a mode at or near 0 percent for 3-carene where one would be expected. This was the third case of an anomaly with 3-carene as discussed previously for the progeny of Trees AS-5789 x WP-3318 and AS-3187 x WP-3318. All three cases involved Tree WP-3318 as a male which was assigned a (0-1) set of alleles for 3-carene based on the 18 percent 3-carene in its composition. Could there have been a mistake in the processing of Tree WP-3318? Or was there something unusual about this tree? High 3-carene and high β -pinene were the most common types of composition (*fig. 6-3*.)

Progeny of *P. ponderosa* IFG-4B63 x WP-3312

The composition of Tree WP 3312 was similar to that of Tree AS-3194 except Tree WP-3312 had somewhat higher limonene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
WP-3312 male	1	5	7	34	4	48
F-1 progeny	83	13	40	20	3	23

There were small differences between nursery and 4-year container data; however, it was not considered too large, and the two sets of data were aggregated for further analysis (*table 6-13*). The average composition of 83 nursery and container progeny was higher in limonene than the progeny of Trees IFG-B63 x AS-3194; the former was 23 percent vs. 13 percent for the latter.

The analysis of the frequency distributions showed that the number of modes derived by the analysis equalled or exceeded the number expected from

Modal analysis
av= average; pr = proportions



the assignment of alleles to the parents (*fig. 6-55*). Mode proportions were only moderately close to the expected. Combining for the expected two modes for 3-carene, proportions were 30:70, and combining for the expected two modes for limonene, proportions were 55:45.

The absence of a mode at or near 0 percent for 3-carene supported the assignment of a (1-1) set of alleles to Tree WP-3312.

Again, as in other sets of progeny of Tree IFG-4B63, the ranges in the frequency distributions were relatively narrow. However, the modal configuration was suitable for good analysis. Potentially high limonene trees were expected and were present, but were not common (*fig. 6-3*).

Progeny of *P. ponderosa* IFG-4B63 x WP-3299

Tree WP-3299 was near-high limonene with a large amount of 3-carene.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
WP-3299 male	1	6		43	8	40
F-1 progeny	8	13	42	19	3	22

There was only one 4-year container tree, and its composition was quite different from that of nursery trees; therefore, the container tree was not added to the nursery trees. Only eight progeny were sampled, and the average composition was higher in β -pinene and lower in 3-carene than expected (*table 6-13*), i.e., near-equal amounts would be expected. This again pointed to a juvenile increase in β -pinene. This same result was found in the progeny of Trees IFG-4B63 x WP-3178. With only eight samples, little can be said about the frequency distributions except that the range was quite large even with so few samples, and that the multimodal characteristics were beginning to be evident for 3-pinene, 3-carene, and limonene (*fig. 6-56*).

Types of composition were not put in a table, but trees with and without 3-carene were expected and found.

Progeny of *P. ponderosa* IFG-4B63 x AS-5789

Tree AS-5789, like Tree IFG-4B63, had no 3-carene, but had near-high limonene.

Normalized percent compositions for parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
AS-5789 male	1	23	10	0	29	37
F-1 progeny	30	18	67	0	4	10

Data on nursery and 4-year containers data were aggregated. The average of the aggregated 30 nursery and container trees was not as expected (*table 6-13*) when compared with the previous sets of progeny. Limonene was only about half as much as expected, and myrcene was much lower than expected. The average for limonene was 10 percent, while 20 percent was expected. It could be that one of the alleles for limonene had not yet become functional in the progeny of this particular combination of parents. However, if this were so, one would expect a reduction in the number of modes. Because this was not so,

Figure 6-55—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* IFG-4863 x WP-3312; see caption of figure 6-2 for explanation of the format below; **= mode at zero not used in modal analysis, but was included in mode count.

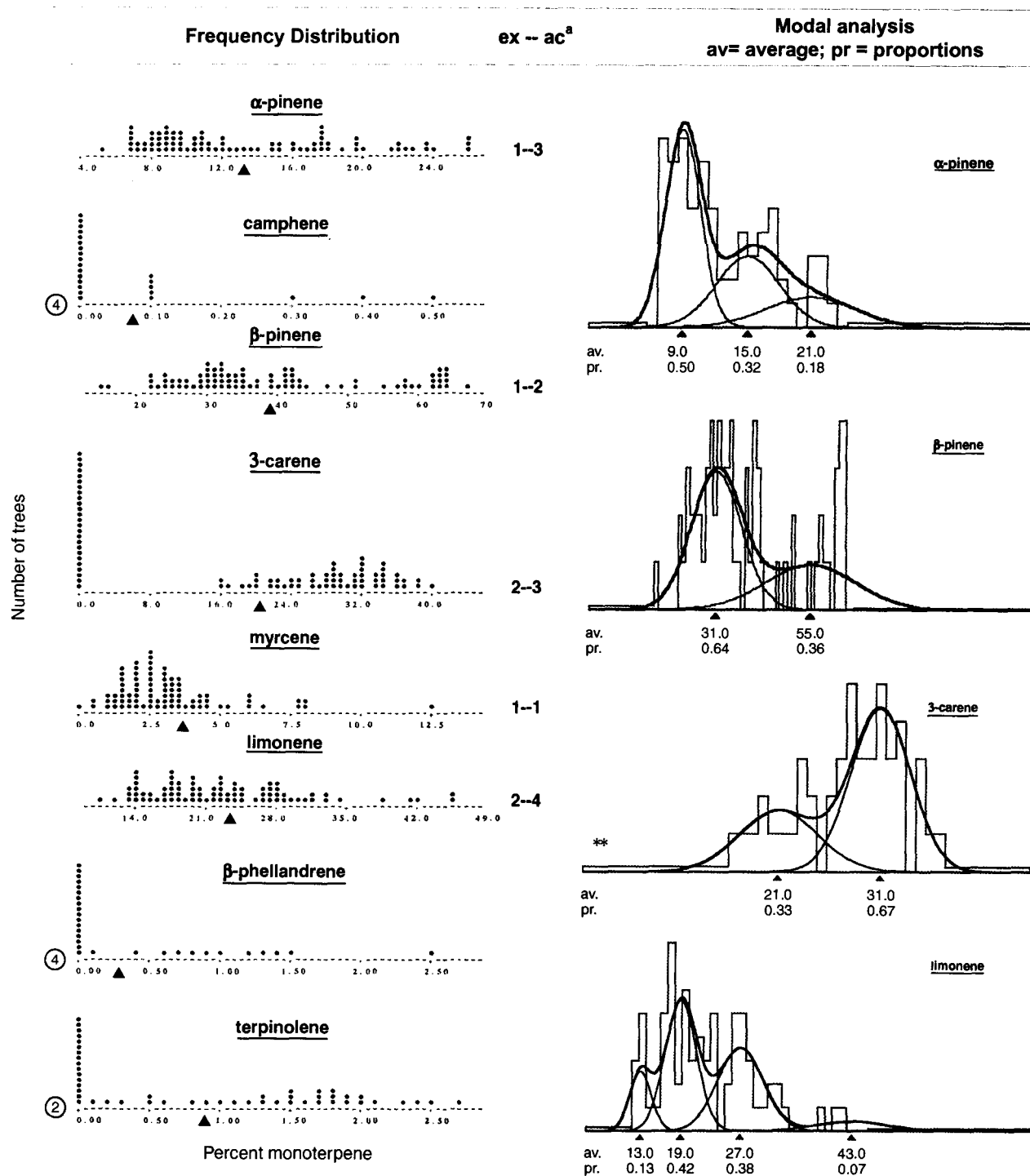
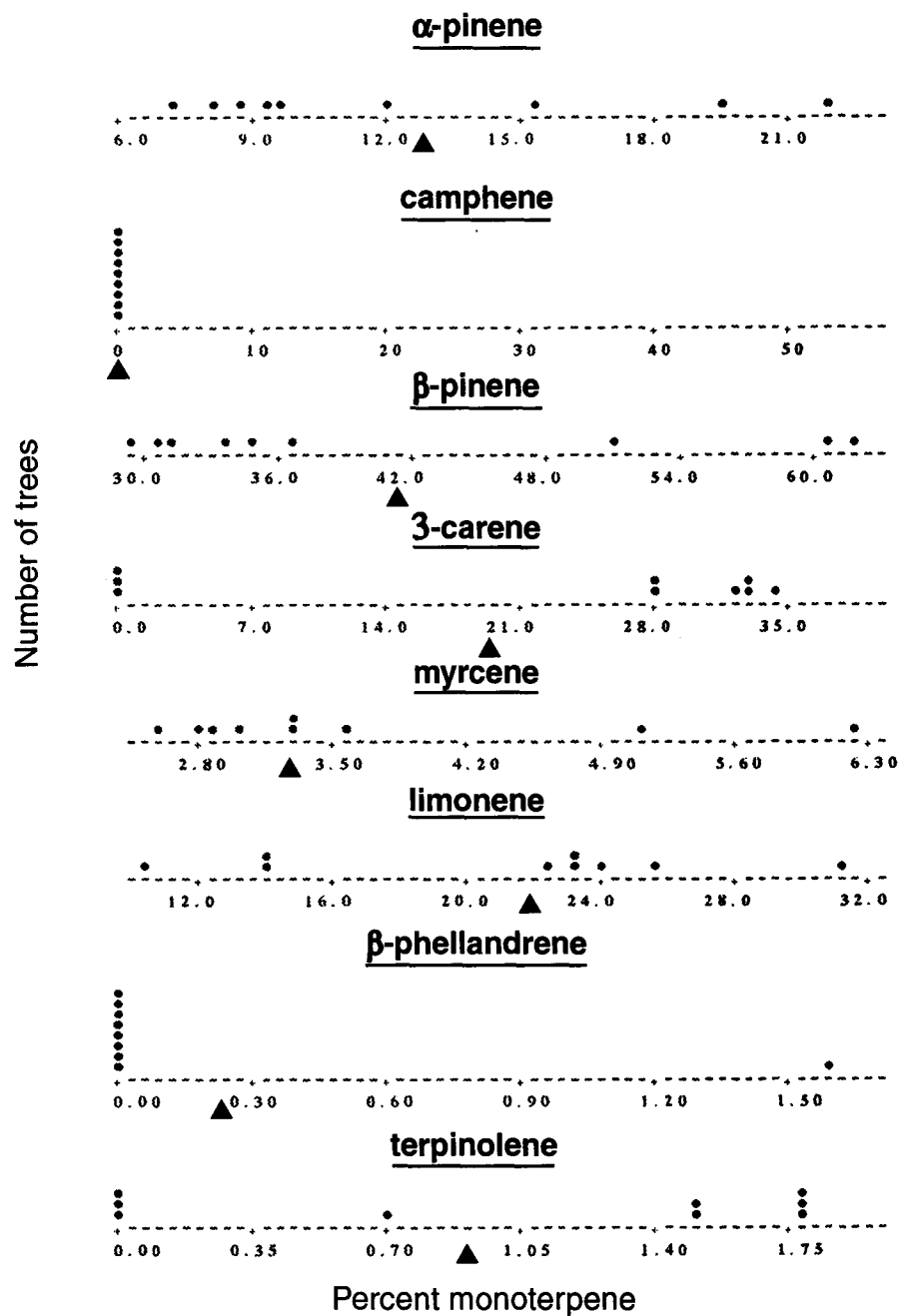


Figure 6-56—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* IFG-4863 x WP-3299; each dot is measurement of one tree; [▲] is average of set.



it appeared that any allele for limonene was affected. The composition of the progeny does reflect the high β -pinene and the absence of 3-carene, which was expected. The two modes for β -pinene support the assignment of a (1-1) set of alleles to Tree IFG-4B63 and a set of (0-1) alleles to Tree AS-5789. Myrcene had two modes of equal proportions which again supports the assignment of a (0-1) set of alleles to Tree AS-5789, as shown in other families where this tree was male or female.

The analysis of the frequency distributions showed that the number of modes derived by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-57*). Mode proportions were reasonably close to the expected. By combining modes for α -pinene and limonene, proportions of 39:61 and 40:60, respectively, were found.

It appeared that nearly all trees went to β -pinene rather than to limonene and myrcene (*fig. 6-3*). This could have been caused by the juvenile effect. As expected, all trees lacked 3-carene. There were not even small amounts; this suggested that 3-carene was not produced by isomerization in this parental combination. It will be shown shortly that the progeny of Trees IFG-4B63 x WP-3298 (both trees had no 3-carene) did have small amounts of 3-carene. This was suggestive of isomerization, but this is an uncommon occurrence.

Progeny of *P. ponderosa* IFG-4863 x AS-A95

Tree AS-A95 was near-high limonene. It was discussed earlier in this chapter as a male and as a female.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
AS-A95 male	1	19	21	0	15	45
F-1 progeny	53	17	54	2	2	25

The data for nursery and 4-year container plants were similar and were aggregated for further analysis (*table 6-13*). The average composition of 53 nursery and container progeny was much higher in limonene, 25 percent, than the progeny of crosses with Trees WP-3312 and WP-3298, all having higher limonene than Tree AS-A95. This could have been an expression of parents in different ecological (resin) zones. Trees IFG-4B63 and AS-A95 are about 150 miles apart, but both are in the Sierra Pacific Region (see Chapter 3 of this report). The other two trees were more than 400 miles from Tree IFG-4B63 and are in the Escalante North Transition Zone.

The progeny of Trees IFG-4B63 x AS-A95 had little or no 3-carene, as expected (*fig. 6-58*). There were, however, nine trees with about 8 percent 3-carene, and all were in the 4-year container group. This was much like the transient time-of-growth anomaly found for camphene in Chapter 5.

The analysis of the frequency distributions showed that the number of modes derived by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-58*). Mode proportions were reasonably close to the expected if modes were combined to get the expected number. This resulted in proportions of 35:65 for α -pinene, 45:55 for β -pinene, and 39:61 for limonene.

Figure 6-57—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* IFG-4B63 x AS-5789; see caption of figure 6-2 for explanation of the format below.

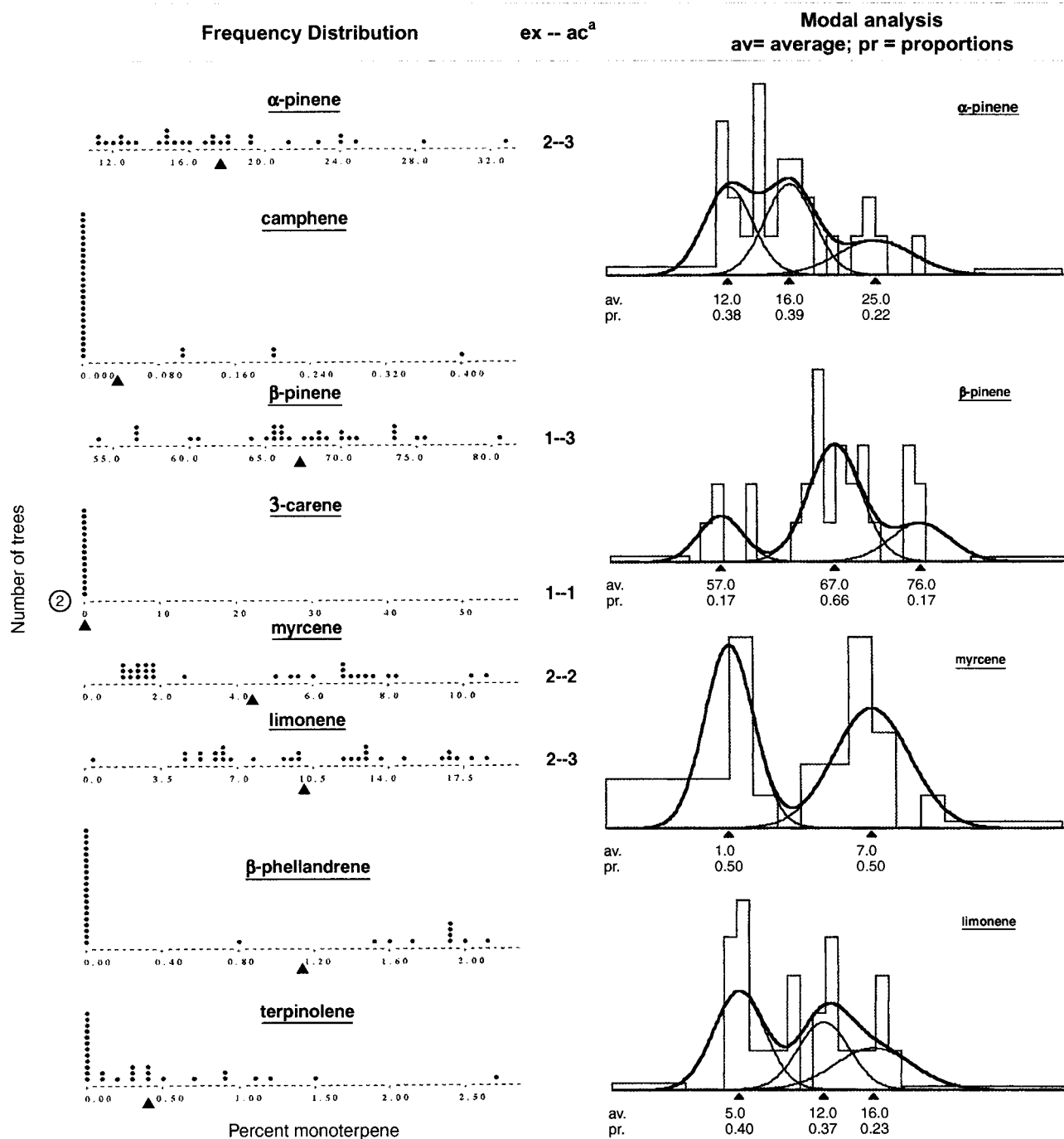
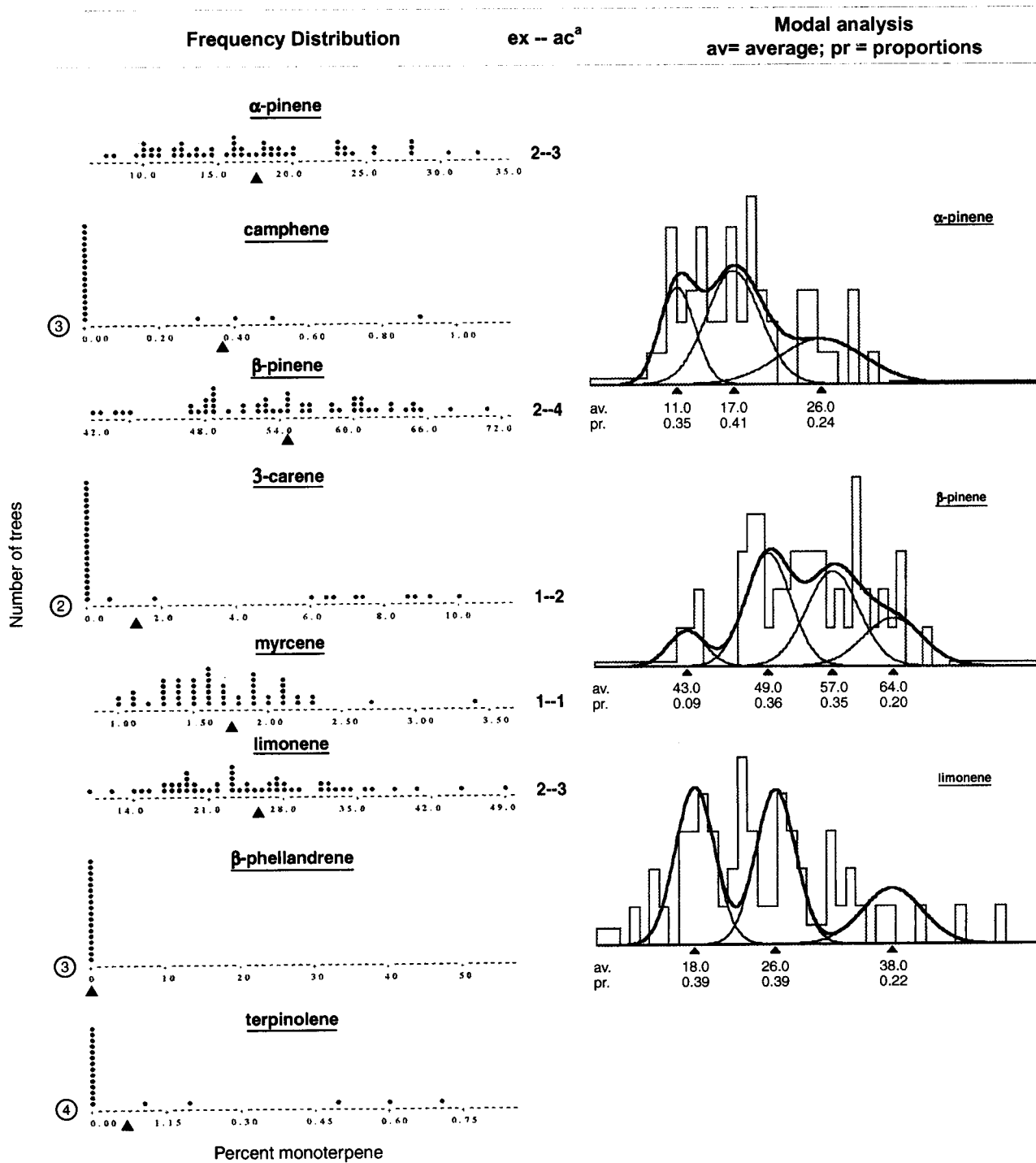


Figure 6-58—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* IFG-4B63 x AS-A95; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

Though there were only two high limonene trees, this was more than in the sets of progeny with a distant male tree with much more limonene than Tree AS-A95 (*fig. 6-3*). Again, the distance between parents was a possible reason.

Progeny of *P. ponderosa* IFG-4B63 x AS-3139

Tree AS-3139 was high limonene with essentially neither β -pinene or 3-carene.

Normalized percent compositions for parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
AS-3139 male	1	18	1	0	22	59
F-1 progeny	47	18	49	3	2	28

Data for nursery and 4-year container trees were quite similar (*table 6-13*) and were aggregated for further analysis; 9-year container and container/plantation progeny were quite different and were not used for further analysis.

The analysis of the frequency distributions showed that the number of modes derived by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-59*). Mode proportions were not as close to the expected as in other families. The proportions for α -pinene were strongly affected by one measurement at 42 percent. The combined modes for limonene had proportions of 78:22, which was marginally good. Again, as with the progeny of Tree AS-A95 above, limonene was much greater when parents were in the same ecological (resin) zone than when they were in different zones.

High limonene progeny were expected but none were found, although the level of limonene was better than most other crosses (*fig. 6-3*). Most of the composition went to β -pinene, possibly because of the juvenile factor. More trees may change to higher limonene as the trees age.

Progeny of *P. ponderosa* IFG-4B63 x WP-3298

Tree WP-3298 was another high limonene tree with a composition almost identical to that of Tree AS-3139. However, it was growing at Wheeler Peak, more than 400 miles to the east. It was reported earlier in this chapter as a pollen source with other females.

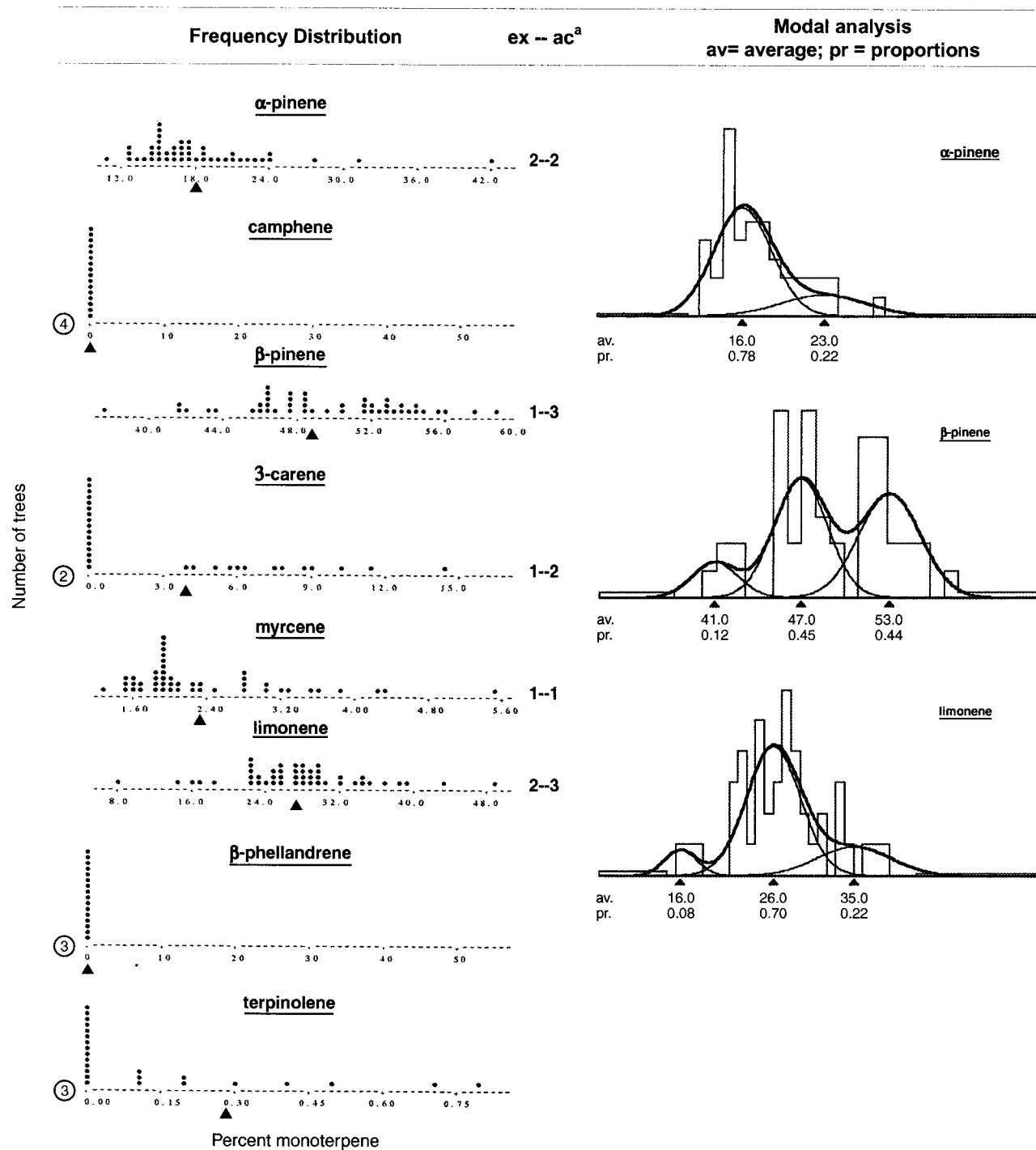
Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
WP-3298 male	1	26		0	16	58
F-1 progeny	48	29	56	2	2	11

Data on nursery and 4-year container trees were nearly identical and were aggregated for further analysis (*table 6-13*); the data for 9-year-old container and container/plantation trees were not aggregated with the nursery data. The average composition of the progeny of Trees IFG-4B63 x WP-3298 was quite different from the progeny of Trees IFG-4B63 x AS-3139; both males were high in limonene and had about the same composition for all other components. The progeny with WP-3298 were quite low in limonene, 11 percent, and were quite high in α -pinene, 29 percent, whereas the progeny with Tree AS-3139 averaged about 28 percent in limonene (*table 6-13*). The factor of distance between parents, and/or the juvenile effect, or the location of the parents in different ecological (resin) regions could explain this difference.

The analysis of the frequency distributions showed that the number of

Figure 6-59—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* IFG-4B63 x AS-3139; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

modes found by the analysis equalled or exceeded the number expected from the assignment of alleles to the parents (*fig. 6-60*). Mode proportions were comparatively good: 43:57 for α -pinene, and 48:52 for limonene by combining modes to get the expected number.

There were no high limonene trees, and only a rare potential one (*fig. 6-3*). Here again the composition went to higher α -pinene or β -pinene when 3-carene was lacking or very low. Though most trees had no 3-carene, as expected, there were several trees with about 5 to 10 percent; this could have been caused by isomerization, or by some minor genetic structure.

Progeny of *P. ponderosa* IFG-4B63 x WP-3352

Tree WP-3352 was another high limonene tree with nearly the exact composition of Trees WP-3298 and AS-3139, the two previous males used on Tree IFG-4B63 (*table 6-1*). WP-3352 was reported in this chapter as a pollen source with other females.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
WP-3352 male	1	24	*	*	18	57
F-1 progeny	60	18	61	0	2	18

The average composition of sixty nursery and container progeny was (*table 6-13*) very similar to the previous family where WP-3298 was the male parent. The progeny with Tree WP-3298 had the highest average β -pinene of the two, 66 percent, despite having only one allele for β -pinene, which it got from the female. Average limonene in this family, with WP-3352 as the male, was 18 percent; limonene was only 11 percent in the family with WP-3298 as the male. The frequency distributions of the progeny of Tree IFG-4B63 x WP-3352 differed only slightly from the previous set of progeny in mode configuration (*fig. 6-61*), though the percent scales were somewhat different.

The analysis of the frequency distributions showed that the number of modes derived by the analysis equalled or exceeded the number expected, based on the assignment of alleles to the parents (*fig. 6-61*). Mode proportions were fairly good. By combining to get the expected two modes for α -pinene and limonene, proportions were 46:53 and 37:63, respectively.

There were no high limonene trees, nor any potential ones (*fig-6-3*). If the juvenile factor was the cause of the lack of such trees, some may develop as the trees mature.

Progeny of *P. ponderosa* IFG-4B63 x BL-6074

Tree BL-6074 was high limonene, 57 percent, with a small amount of α -pinene and β -pinene (*table 6-1*). It was reported earlier in this chapter as a pollen source with other females.

Normalized percent compositions of parents and progeny were as follows:

<i>Tree and type</i>	<i>Number</i>	<i>α-pinene</i>	<i>β-pinene</i>	<i>3-carene</i>	<i>myrcene</i>	<i>limonene</i>
IFG-4B63 female	1	10	54	0	11	23
BL-6074 male	1	7	11	0	27	56
F-1 progeny	2	18	50	0	3	29

Though there were only two trees (*table 6-13*), the results still strongly show that progeny of parents with about the same composition, from different

Figure 6-60—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* IFG 4B63 x WP-3298; see caption of figure 6-2 for explanation of the format below.

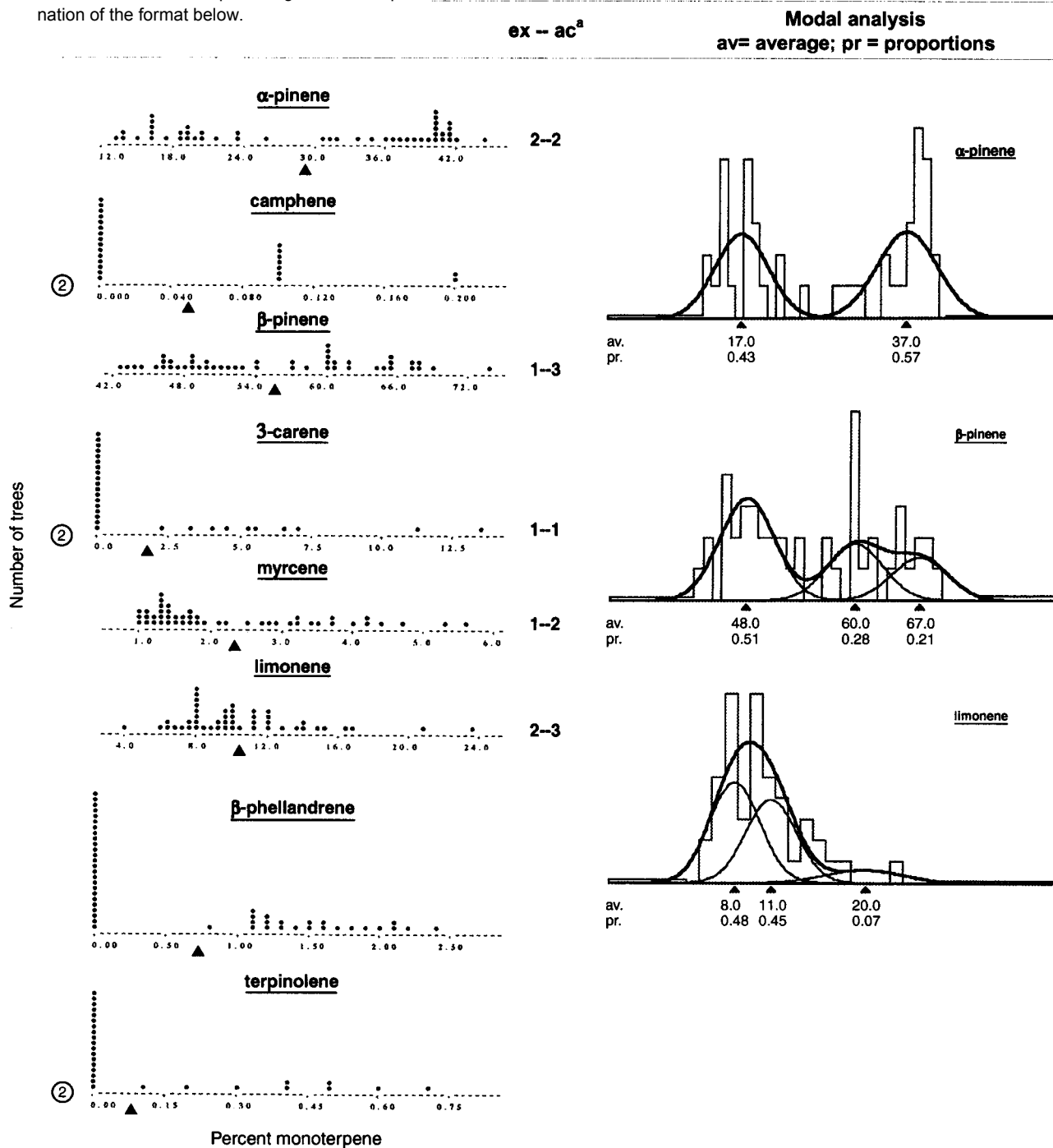
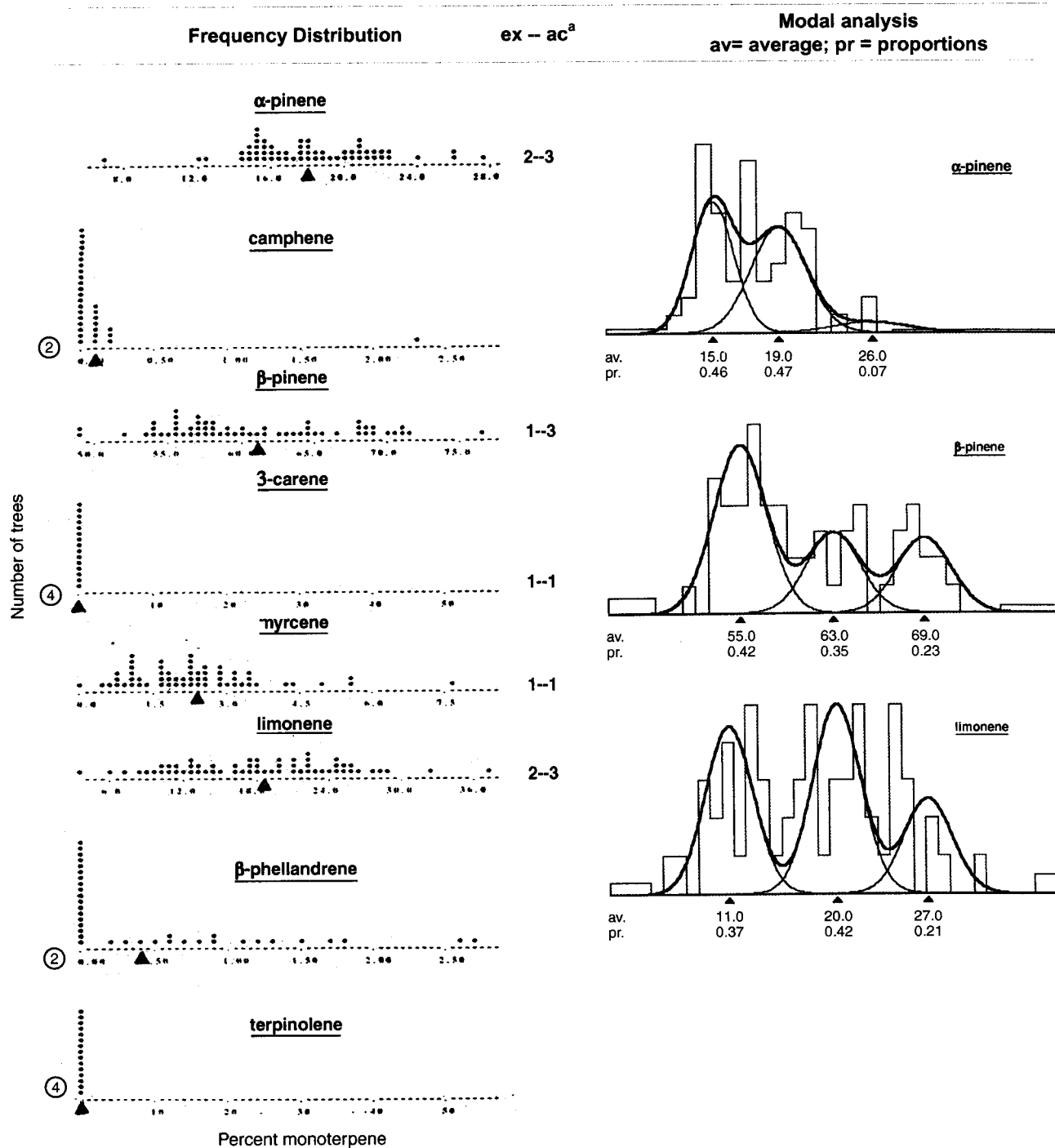


Figure 6-61—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* IFG-4B63 x WP-3352; see caption of figure 6-2 for explanation of the format below.



^aex = expected, ac = actual

ecological regions, may have progeny with somewhat different composition than progeny of parents in the same ecological region when both sets of parents have the same primary structure of alleles. This difference in composition could be due to a stress factor of early growth and to parents who are from different ecological regions. The average for limonene was 28 percent in the progeny of IFG-4B63 with three different high limonene males in the Sierra Pacific Region: Trees AS-3139, AS-A95, and BL-6074 (*table 6-13*). On the other hand, limonene averaged only 11 to 18 percent in the progeny with high limonene males from the Escalante North transition zone, Trees WP-3298 and WP-3352 (*table 6-13*). Again, this points to the effect of distance between parents.

Discussion: Full Siblings of Tree IFG-4B63

The compositions of nursery and 4-year container trees of the families of IFG-4B63 were usually about the same and were usually aggregated for analysis. The compositions of 9-year container and 9-year container/plantation trees nearly always differed from that of nursery trees and were not aggregated with the nursery and 4-year container trees for further analysis.

The actual number of modes, as derived from modal analysis, always equalled or exceeded the expected number, as derived from the assignment of additive alleles to the parent trees; this supported the additive allele hypothesis. In general, the data also confirm the assignment of alleles to Tree IFG-4B63 and to the males used with it. Mode proportions were generally close to the expected when the various modifying factors were considered; this provided additional support to the hypothesis. Mode proportions were usually improved by using the expected number of modes rather than the number actually derived from the modal analysis.

There were no ultra-high limonene trees among the progeny and very few high limonene ones. What few there were showed a tendency to be more common in sets of progeny with nearby males, again supporting the postulate that there is greater stress in progeny of more distant parents.

A higher average limonene had been expected in the progeny with high limonene males because Tree IFG-4B63 had no 3-carene and a moderate amount of limonene. Instead, the composition of most progeny was much greater than expected in α -pinene and β -pinene. This might be caused by the juvenile effect, particularly with large amounts of β -pinene present in all progeny; the average for limonene could increase with age. This was the only parent with two alleles for β -pinene, and the large amount of β -pinene in all progeny supports the assumption that β -pinene is a very strong element in the juvenile factor. Though this had a strong effect on composition, it apparently did not change the basic modal characteristics; instead, the modes were shifted strongly to the lower end of the percent scale.

The effect of parental distance on the level of limonene was clearly shown in sets of progeny with Tree IFG-4B63. The progeny with high limonene males in the same ecological region—Trees AS-3139, AS-A95, BL-6074—averaged 25 to 30 percent, with individual trees as high as 50 percent. The progeny with high limonene males from another ecological region—Trees WP-3298 and WP-3352—averaged about 14 percent, with individual trees less than 36 percent.

The aggregate data support the assignment of sets of alleles of (0-0), (1-1), (0-0), (0-0), (0-1), respectively, for α -pinene, β -pinene, 3-carene, myrcene, limonene to Tree IFG-4B63.

Discussion: Full Siblings of *P. ponderosa*

The breeding program of seven females with 4 to 14 sources of pollen produced 56 full sibling families; 42 of these families were large enough for modal analysis. Both male and female were selected primarily on xylem monoterpene composition and particularly on varying percentages of limonene. The monoterpene composition of these young progeny was close to the predicted one based on the assignment of additive alleles to the parent trees for the five major monoterpenes— α -pinene, β -pinene, 3-carene, myrcene, limonene—, but within the constraints of several modifying factors or conditions as follows:

- forms of stress
- a juvenile factor which was particularly strong in progeny with large amounts of α -pinene and β -pinene;
- a parental distance effect which really seemed to be somewhat related to parents being in different ecological (resin) zones as well as on the linear distance;
- seed growing outside the provenance of its parents;
- some degree of isomerization and biosynthetic by-products;
- competition within the genetic structure for a fixed amount of C5 precursor, resulting in a type of *de facto* complex gene; and
- the possibility of a secondary, and minor, genetic structure, as yet unidentified.

Both the parental distance effect and the juvenile effect were manifested in the same way: increase in α -pinene and β -pinene and decrease in myrcene and limonene. These same shifts were also associated with various forms of stress, such as prolonged root containment and over-application of a phytocide. Could it be that juvenile growth was "stressful" and that there was a degree of stress in the progeny of parents that were located at a great distance? The question of stress and its effect would be interesting and valuable research.

These modifying factors changed to some degree the quantitative composition, but the basic modal characteristics of the frequency distributions of individual components were not changed. Thus, the interpretive value of the data was considered good and valid.

The juvenile factor was a large one in the breeding study, and there was little evidence that its effect was weakening over the first eleven years of growth. The one possible exception was some evidence that the quantity of myrcene was increasing slightly over the 11-year period. The effect of the juvenile factor seemed to be dependent of the composition itself, being stronger in progeny with sizeable amounts of α -pinene and/or β -pinene.

Numbers of progeny in 42 families were considered adequate for modal analysis of the frequency distribution of the individual monoterpenes. In every family so analyzed, the number of modes derived by the analysis equalled or exceeded the number expected based on the assignment of additive alleles to the parents. This was strong support for the additive allele hypothesis and for the preliminary assignment of alleles to parent trees. The number of modes in excess of the expected number was attributed to the interaction within the biosynthetic structure of the monoterpenes and the several modifying factors noted above. Some 200 frequency distributions were analyzed in the 42 families; in about 100 of them the actual number of modes was the same as the expected number; in the remaining 100, the actual number was greater than the expected number. Mode proportions were generally close to the expected, considering the effects of conditions and factors just noted.

There were 32 cases where each parent was assigned a (0-1) set of alleles for the same component — either α -pinene, β -pinene, 3-carene, or limonene. In nearly all 32 cases, the modal separation was judged good to very good and

suitable for calculation of mode proportions. In these cases — of an (0-1) set of additive alleles for each parent — the modal proportions should be 25-50-25 for the low, middle and high mode. In many cases there was a marked improvement in mode proportions by using the expected number of modes instead of the ones derived from the modal analysis.

The following is a summary listing of the average mode proportions for each monoterpene when both parents were assigned a (0-1) set of additive alleles for that component—and when proportion of 25-50-25 are expected:

Component	Number of crosses with alleles of (0-1) x (0-1)	Average mode proportions		
		low	middle	high
α -pinene	12	34	39	27
β -pinene	10	23	44	33
3-carene	5	36	36	28
limonene	5	32	48	20

These proportions were considered quite good in these young trees considering the effects of all the above factors and conditions.

An examination was made of 73 crosses of parents with a combination of (0-0) x (0-1) alleles for a given component, where there should be two modes of equal proportions. Where there were more than two modes, adjacent modes were combined to get the expected two modes. The following is a listing of that examination, showing the component, the number of cases of such a cross, the percent of such cases where the proportions were better than 35-65, and those better than 40-60.

Component	Number of cases	Proportions better than 35-65 or 65-35	Proportions better than 40-60 or 60-40
		----- percent -----	
α -pinene	21	71	48
β -pinene	18	83	72
3-carene	16	94	56
myrcene	8	100	75
limonene	10	74	37

Thus both the number of modes and their proportions supported the additive allele hypothesis. The data do not support two alternative hypotheses: dominant-recessive alleles or multiple genes. Myrcene was not included in the three-mode examination because only one tree, AS-5789, had an assignment of (0-1) set of alleles; all other parents had a (0-0) set. This tree was used as female and male, and mode proportions averaged 53-47 and 46-54 respectively. Thus myrcene, though a rare allele, fitted the additive allele hypothesis along with the other four major components noted above.

One might hypothesize a multiple gene structure for the individual monoterpenes because of the large range in their frequency distribution in a stand. This study showed that several factors (noted above) can cause quantitative changes. All these factors in aggregate could cause a substantial increase in variation. However, there really was a *de facto* multiple gene effect because of the competition among the sets of additive alleles of the five major genes for a fixed amount of precursor; the whole gene structure was itself a

multiple gene. And there may be additional variation because of unique interactions of certain components or combinations of components. However data in this report strongly supported the additive allele hypothesis for each monoterpene, and explained the large variation.

One apparent anomaly was found with 3-carene: the absence of a distinct mode at zero percent when one would be expected with additive alleles; i.e. combinations of a (0-1) and (0-0) set of alleles; with such a combination there should be a (0-0) set in the progeny, resulting in a mode at or near 0 percent and an (0-1) set resulting in a mode well along the percent scale. In 28 families identified with this condition, (0-1)x(0-0), the zero mode for 3-carene was present in 25 families, as expected, but was not present in three families all of the same male. Thus it was a rare condition, and it might be explained by the juvenile factor, or by some degree of activity by the (0-0) set of alleles under certain circumstances that have not been identified for this particular male parent. In 21 families, both parents were assigned an (0-0) set of alleles for a given component; and in all families, there was only a single mode at 0 percent, as expected.

There were at least 58 types of individual tree composition among the more than 2,000 progeny of full sibling families; this was expected, given the range of parental variation, the action of additive alleles, and the presence of several modifying factors. Likewise, these types of composition were easily predictable on the basis of the assignment of additive alleles to the parents. This large array of types accomplished the objective of the study: to produce a large supply of trees with a diverse array of compositions at IFG for further study and experimentation on host relationship to bark beetles and on the genetics of monoterpenes. The types of composition ranged from 7-2-0-2-88 percent to 17-80-0-1-1 percent respectively for α -pinene, β -pinene, 3-carene, myrcene, limonene. The range of compositions was very large (*fig. 6-3*). Nearly all the 58 types were found in the range-wide study of *P. ponderosa* as reported in Chapter 3. However, one type classed as ultra-high limonene— 7-2-0-2-88 percent noted above— was found in the breeding study but was not found in the range-wide study; and such a composition was expected. Why such trees have not been found in natural stands is an interesting question. Is such a composition only a juvenile characteristic that changes as the tree matures? Do such trees fail to survive? Has the search of natural stands been inadequate? The breeding study found this type of composition to be common among the progeny of high limonene x high limonene trees; but it was present but not common with other combinations of parents. Thus, the rarity of high limonene trees could explain the lack of ultra-high limonene in natural stands. For example only one tree in about 500 was high limonene at Adin Summit; and there were two in about 80 at Wheeler Peak.

Several parent trees were found which could be of considerable value in the study of the variation in tree characteristics, further studies of monoterpenes, other genetic studies, such as host resistance, and plant adaptation to name a few. These trees were AS-3139, AS-A95, AS-3194, and AS-5789 at Adin Summit, Tree BL-6074 near Blue Lake, and several trees at Wheeler Peak. Additionally there are two plantations of about 300 young progeny at IFG as well as the 14 grafts of the Adin Summit trees which are now growing at IFG. The plantations contain most of the types of composition found in the study. More work with tree BL-6074 would have been of considerable value; it was the only high limonene tree with low α -pinene. This tree was found in a 40-tree stand that had the average limonene of Sierra Pacific Region. The stand also had a tree with about 40 percent limonene.

The breeding study found that there was little or no incompatibility between Wheeler Peak trees in eastern Nevada and Adin Summit trees in

northeastern California, though the progeny of trees between the two areas seemed to be under some degree of stress. The study also found that the progeny of grafts had about the same average composition and about the same types of composition as the progeny of the parent tree when crossed with the same male or female tree.

Analysis of the composition of the parent trees at Adin Summit strongly showed that the compositions of these selected trees were, essentially, constant over time and for different locations on the tree. Some scientists have called these compositions spurious and invalid. The reanalysis and the large body of results on half and full sibling populations in this report clearly proves this view to be incorrect.

Grafted Pines

Summary: Thirty years after grafting, the monoterpene composition of the scion of interspecific grafts-*P. ponderosa*-on-*P. jeffreyi*, *P. jeffreyi*-on-*P. ponderosa*, *P. sabiniana*-on-*P. ponderosa*, *P. torreyana*-on-*P. ponderosa*-was about the same as that of the parent species. However, the composition of the root stock was being affected by the scion, and the effect was proportional to the distance from the graft union. On the other hand, the qualitative and quantitative composition of the scions of 6- to 15-year-old intraspecific grafts of *P. ponderosa* were radically different from those of the parent tree for about 10 years after grafting. The greatest differences were in grafts in which the compositions of the root stock and the scion were most widely divergent. Three possible causes are postulated: (1) the severe stress of grafting, (2) a strong effect of the root stock, and (3) an expression of a minor genetic structure through the (0-0) set of alleles. Between 10 and 15 years after grafting, the composition of the scion suddenly and rapidly became much like that of the parent tree. After this sudden change, the scion began to affect the composition of the root stock much as in older interspecific grafts noted above.

The composition of xylem monoterpenes of grafted pines was discussed separately because published information was limited, and rather unexpected results were found. In general, success in grafting pines in the past has been variable and often poor.

Measurements of two sets of grafts were given. One set consisted of four interspecific grafts; the other set consisted of 14 intraspecific grafts of *P. ponderosa*. The interspecific grafts were made by Mirov in about 1940 using approach grafting of rooted trees. When grafting was judged successful, the root stock was cut off above the union, and the scion below the union.

The intraspecific grafts were made in 1971 with four of the *P. ponderosa* pines that were used in the *P. ponderosa* breeding program (see Chapter 6); 14 were successful. Bottle grafts were made for 12 of these trees, and approach grafts were made for the remaining two. All scions were cuttings from parent trees at Adin Summit. All root stocks were 2-year-old potted trees of unknown parents from the central Sierra Nevada. Graft unions were made in the first internode of the root stock. Root stock branches below the union were removed after the first year of grafting. Both root and scion were allowed to grow in the two remaining grafts, thereby producing two forked trees. All trees were maintained in pots in a greenhouse for two seasons and then outplanted in an arboretum.

The interspecific grafts were sampled by macro taps at about 30 years after grafting when the trees were 6-8 inches in diameter; the intraspecifics were sampled by mini taps in the basal internode of the scion at 10, 15 or 16, and 17 years (see Chapter 2 for procedures). At that time the scions were 2 inches to 3 inches in diameter at the basal internode. By waiting until 10 years after intraspecific grafting, it was assumed that any period of juvenile instability in xylem monoterpene composition would have passed and generally stable

monoterpene conditions would exist. The xylem monoterpene composition of the scions was not determined before the 10-year sampling, but the composition of the parent trees was known (*table 7-1*). In studies of side branch resin of young *P. ponderosa*, the resin composition was found to be the same as or nearly the same as the xylem resin of the main stem (see Chapter 8). Thus the assumption was made that the monoterpene composition of the scion was the same as that of the parent tree.

These same intraspecific grafts were used in a breeding program in 1978 and 1979. The evidence from their progeny shows that the scions' reproductive tissue was the same as the parent tissue. The scions produced sexually reproductive tissue in less than 6 years after grafting.

I believe that there were at least five conditions in grafts that were different from normal growth conditions, and that might alter quantitative monoterpene composition of xylem resin to some degree. I would call these the grafting effects.

1. *Wounding* of root stock and scion could alter some of the processes that result in monoterpene production and deposition in both tissues; this might be particularly true over long periods of months and years as the wounds heal.

2. *Juvenile instability* had been shown for very young tissue of *P. ponderosa* (see Chapter 8); the scion tissue was 1-year-old shoots.

3. There was severe *stress* in both scion and root stock for at least 2 years and possibly for several more until the union is fully functioning and the scion vegetative growth was fully expressed.

4. There could have been *antagonism*, or a type of *rejection response*, when joining tissues from two different trees. These four growth conditions, singly or combined, could produce the large changes that were found in the production and deposition of monoterpenes in the scions.

5. Inter-tissue exchange could result in the movement of biosynthetic materials or signals. It will be shown later in this chapter that the scion has an effect on the composition of the rootstock; the opposite might take place, i.e., root to scion, under the unusual conditions of grafting.

Interspecific Grafts

The results of the analysis of the xylem resin of interspecific grafts were taken from Smith (1982a) (*table 7-1*).

The composition of the scion portion of the *P. jeffreyi*-on-*P. ponderosa* graft was clearly only *P. jeffreyi* at all heights sampled above the graft union. There were no characteristics of *P. ponderosa* xylem monoterpenes in the scion. The composition of the *P. ponderosa* root stock xylem one foot below the graft union was still largely that of *P. jeffreyi*. However, there were small and measurable amounts of β -pinenes, 3-carene, and myrcene which were characteristic of *P. ponderosa*. The amounts of β -pinene, 3-carene, and myrcene increased at 5 feet below the union, and there were measurable amounts of α -pinene and limonene. Thus, the effect of the scion was decreasing with increasing distance below the graft union. Measurements of the *P. sabiniana*-on-*P. ponderosa* graft were very similar to the *P. jeffreyi*-on-*P. ponderosa* graft: above the graft union, the *P. sabiniana*-on-*P. ponderosa* scion was typical of *P. sabiniana*; below the union the characteristics of *P. ponderosa* increased with distance below the union.

The composition of the *P. ponderosa*-on-*P. jeffreyi* graft was quite different than those of the previous two grafts. The composition was only *P. ponderosa* at all points above and below the graft union. The composition of the *P. torreyana*-on-*P. ponderosa* graft was all *P. torreyana* above the graft union. No measurement could be made below the graft union because it was essentially at the ground line.

The presence of scion characteristics in the root stock pointed to the

Table 7-1—Monoterpene composition of scion and root stock of 30-year-old interspecific pine grafts: P. jeffreyi-on-P. ponderosa (J/P), P. ponderosa-on-P. jeffreyi (P/J), P. sabiniana-on-P. ponderosa (S/P), P. torreyana-on-P. ponderosa (T/P); --- = graft union (GU) at about 7 feet (7GU), except for T/P which was near the ground line.

Graft	Scion or root	Feet above ground	Monoterpene ¹									
			hep	α -p	cam	β -p	car	myr	lim	β -ph	γ -ter	terp
			percent ²									
J/P	Scion	16	98	*	*	*	*	2	*	-	-	-
	Scion	10	98	*	*	*	*	2	*	*	-	-
	G.U. ₃	7 ---										
	Root	6	94	*	*	2	2	2	*	*	*	*
	Root	2	70	2	*	10	12	4	2	*	*	*
P/J	Scion	20	-	8	-	29	50	7	3	1	*	2
	Scion	16	-	8	-	29	49	7	3	*	*	3
	Scion	10	-	8	-	29	49	7	3	1	*	3
	G.U.	7 ---										
	Root	6	-	8	-	29	50	7	3	1	*	3
	Root	2	8			29	49	7	4	*	*	3
S/P	Scion	15	99	*	1	-	-	-	-	-	-	-
	Scion	8	99	*	1	-	-	-	-	-	-	-
	G.U.	7 ---										
	Root	6	97	*	1	1	1	*	*	-	-	-
	Root	2	81	1	1	4	8	2	3	-	-	-
T/P	Scion	6	-	3	-	10	2	4	81	-	-	-
	Scion	2	3	-		9	-	4	85	-	-	-
	G.U.	0 ---										

¹heptane, α -pinene, camphene, β -pinene, 3-carene, myrcene, limonene, β -phellandrene, γ -terpinene, terpinolene.

²Normalized; * = trace, - = zero.

³Graft Union.

downward movement across the graft union of: (a) terpene-specific precursors, (b) biosynthetic signals, and/or (c) monoterpenes. The general assumption is that resin itself does not move except at wounds, though one cannot rule out slow diffusion over long periods of time. We do not know enough about monoterpene biosynthesis to evaluate the other two possibilities.

Intraspecific grafts of *P. ponderosa*

More than 50 grafts were made of trees at Adin Summit; but only 14 survived (*table 7-2*). Attempts to graft scions from Wheeler Peak trees were not successful. Most of the Adin grafts that died did so during the first season, but several died after outplanting. The xylem monoterpene composition of the root stock was not known. However, they were seedlings from central Sierra Nevada parents and, I assumed, had a generalized composition of 10:35:35:10:10, respectively, for α -pinene, β -pinene, 3-carene, myrcene, limonene. The composition of the root stock forks of the two forked grafts supports this assumption (*fig. 7-1*). (One had a composition of 9:19:49:7:12 for the five major components; the other was 8:36:40:6:7.)

The analysis of the composition by years and internodes will be covered for the 14 grafts derived from each of the four parent trees as noted (*table 7-2*) and for the two root stocks of the scions of Tree AS-A78 that were allowed to grow as forked tree. Two of the four parent trees of the scions—Trees AS-A78 and AS-3139—were without β -pinene and 3-carene. Therefore, they were high in limonene, myrcene, and α -pinene. The other two were classed as moderately high limonene: AS-A95 had moderate β -pinene but lacked 3-carene, Tree AS-A295 had moderate 3-carene but lacked β -pinene. Thus, each of the four parent trees lacked either or both β -pinene and 3-carene.

Results

Ten years after grafting, the composition of nearly all scions was markedly different from that of the parent stock (*fig. 7-1*). All four sets of scions had 15 to 20 or 20 to 30 percent β -pinene and 25 to 40 percent 3-carene despite the fact that parent Trees AS-3139 and AS-A78 essentially lacked both components, and that parent Trees AS-A95 had no 3-carene, and AS-A295 had no β -pinene. Between 10 and 15 years after grafting, a pattern of change appeared: β -pinene and 3-carene decreased, and α -pinene, myrcene, and limonene increased. The magnitude of the change was not large, but it was consistent for nearly all 14 scions and for all components. In general, this change seemed to start with a decrease in β -pinene and 3-carene. Then, between 15 and 17 years, this change was large and clearly obvious, and the composition of all scions moved rapidly toward that of the parent stock (*fig. 7-1*).

Many studies, some reported in Chapter 8, were made to determine the validity of basal sampling of *P. ponderosa* for xylem monoterpene composition. Three studies are of note: (a) 75 trees, more than 40 years old, and with a great range in variation of composition, were re-sampled after 9 years; (b) 72 trees 6 years old were re-sampled after 2 years; (c) 340 trees 8 - 11 years old were re-sampled after about 4 years. All studies showed no change or very little changes in composition between the two sampling periods. Thus one can assume from these studies that there was little or no change in the composition of the basal sample of *P. ponderosa* over periods of 5-9 years.

The following discussion is based on the sets of scions of the four parent trees. Generally the changes took place throughout the tree (*fig. 7-2*) and not just in the basal sample. Also, of the 14 scions, two made the large change prior to the 10th year, three between 10 and 15 years, and nine between 15 and 17 years.

***P. ponderosa* AS-A 78**

There were eight grafts of this parent. The root stock had not been cut off from

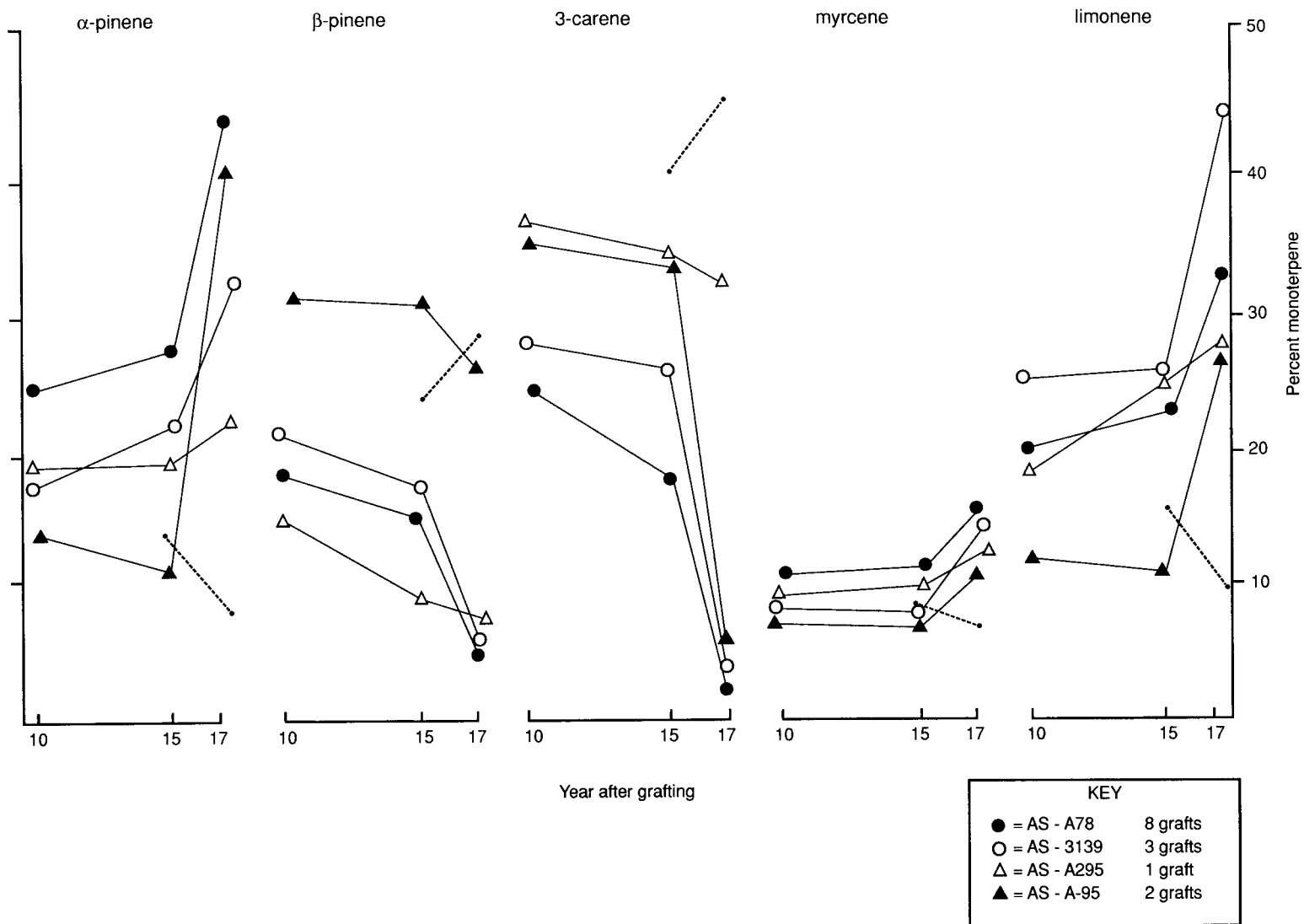


Figure 7-1—Trends in the average percentage of 5 monoterpenes in the basal internode of the scions of 4 grafted *P. ponderosa*, as noted in the key, at 10, 15, 17 years after grafting, and of the internode above the union of the 2 root stocks of the forked tree grafts, indicated by dashed line; see Table 7-2 for composition of the parents of the scions.

two of them. Thus these two were a type of forked tree.

At 10 years, the composition of seven of the scions was quite similar to each other but sharply different from that of the parent tree (*table 7-2, fig. 7-1*). The parent had essentially no β -pinene or 3-carene; the scions had an average of 18 percent and 24 percent, respectively. The amount of myrcene and limonene was much lower in the scions than in the parent, and there was not much variation in composition among the scions. The amount of α -pinene was about the same in both scion and parent. The composition of the 8th scion was somewhat similar to that of the parent; apparently it had changed before the 10th year.

By 15 years, a trend seemed to be developing in the seven scions noted above: α -pinene, myrcene, and limonene were increasing slightly while β -pinene and 3-carene were clearly decreasing.

At 17 years, all trends in all scions were pronounced, as β -pinene and 3-carene decreased to only 2 percent and 5 percent from the previous 18 and 24 percent, respectively. Limonene and α -pinene increased to nearly 40 percent and myrcene to about 15 percent. With these changes the compositions were starting to resemble that of the parent stock. Two scions still attached to the root stock responded in a similar manner and were averaged with the others. The composition above the union of the two attached root stocks did not show these sharp changes for the resampling between 15 and 17 years. In fact, the small changes, as recorded, were in the opposite direction of the changes in the first internode of the scions just a few inches away. The compositions of the internodes above the basal scions were essentially the same as the basal ones (*fig. 7-2*). Thus the changes appeared to be taking place throughout the whole scion for all seven scions (*fig. 7-2*).

The composition of the root stock at 17 years just below the graft union was nearly identical to that of the basal internode of the scion just above the union. This suggests that, like the 30-year-old interspecific grafts discussed above, the scion apparently was now starting to affect the root stock. No measurements were made of the root stock prior to 17 years, but on the basis of prior studies, I was quite certain that it was not like that of the scion. Of course, the measurements of the basal internode of the scion and root stock were only a few inches apart.

P. ponderosa AS-3139

There were three grafts of this parent tree, and all had the root stock cut off above the union. Like Tree AS-A78, Tree AS-3139 had very little or no β -pinene or 3-carene. And again like Tree AS-A78, all scions averaged about 20 percent β -pinene and 28 percent 3-carene at the 10-year sampling. At 15 years, all scions showed the same trends as Tree AS-A78: less β -pinene and 3-carene, and greater α -pinene, myrcene, and limonene. And again, the changes between 10 and 15 years were not large, though they were consistent for all scions. Between 15 and 17 years, the direction of all trends remained the same, but the magnitude was much greater (*fig. 7-1*). Thus at 17 years, the composition of the scions was shifting rapidly toward that of the parent stock. The composition of the scions farther up the tree was essentially the same as that of the basal internode of the scion. This strongly suggests that the changes were taking place throughout the whole scion (*fig. 7-2*). Again, like Tree AS-A78, the changes took place in all scions at about the same time.

P. ponderosa AS-A295

There were two scions of this parent tree, and both had the rootstock removed above the union to produce single stems. Tree AS-A295 had moderately high limonene, no β -pinene, but moderate 3-carene. At 10 years, the composition of

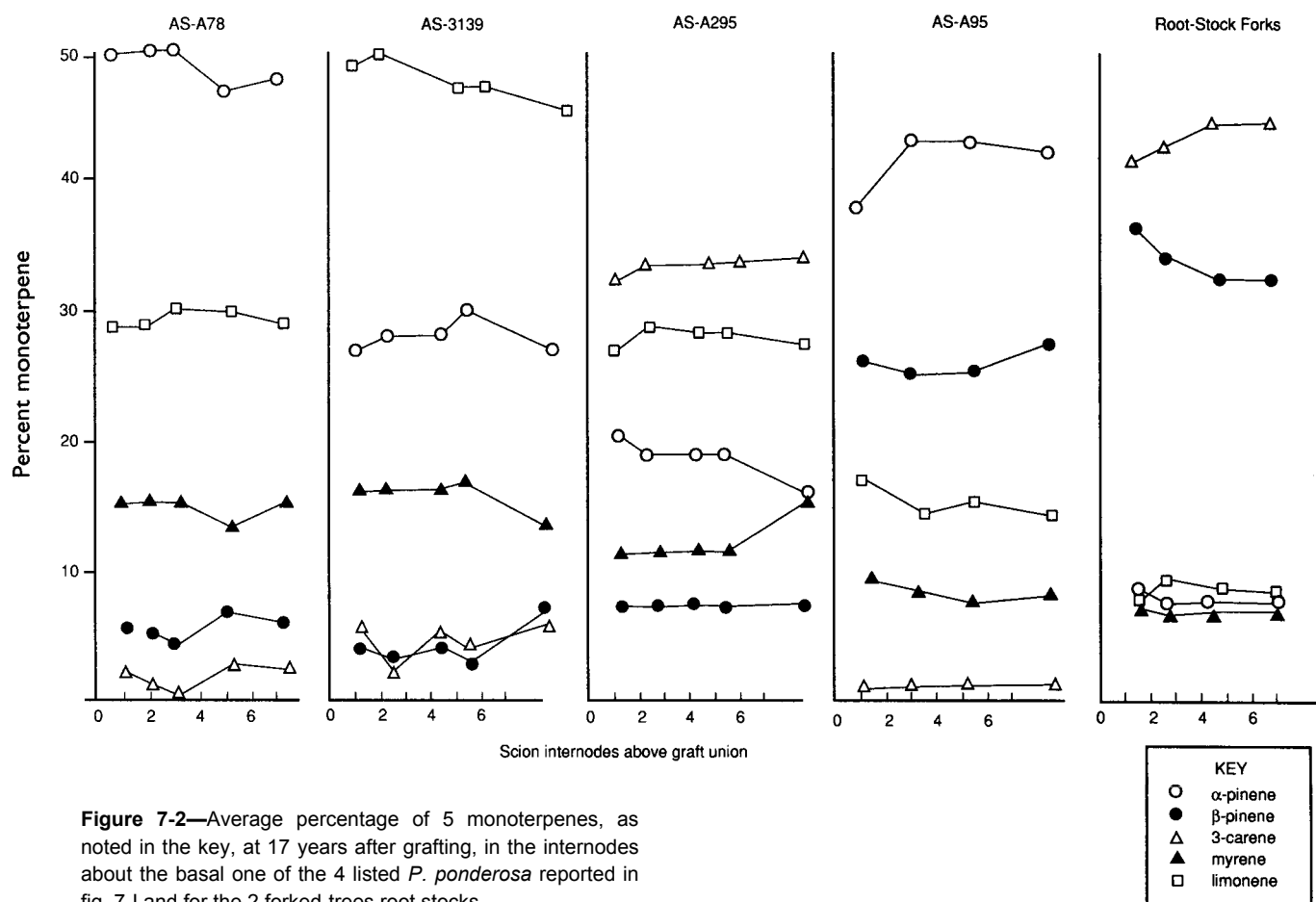
Table 7-2—Monoterpene composition of the xylem resin of the four parent trees that were the source of scions for the intraspecific grafts of *P. ponderosa*¹

Tree designation	Number of grafts	Approximate composition of parents ²						Tree diameter	Class of tree
		α -p	β -p	car	myr	lira	ter		
		<i>percent</i> ³						<i>in</i>	
AS-A78	8	20	-	-	20	60		8	suppressed
AS-3139	3	18	-	-	22	60	-	20	dominant
AS-A295	2	15	1	32	19	32	2	12	co-dominant
AS-A95	1	22	23	-	10	35	-	10	co-dominant

¹ All grafts are now growing at the Camino Arboretum of IFG. Trees AS-3139 and AS-A95 are still at Adin Summit; Trees AS-A78 and AS-A295 have been removed.

² α -pinene, β -pinene, 3-carene, myrcene, limonene, terpinoline.

³ Normalized.



one scion was somewhat like that of the parent tree (*table 7-2, fig. 7-1*). The second scion had about 18 percent β -pinene, while the parent tree had only 1 percent. Between 10 and 15 years, the same general pattern of increases and decreases prevailed for this second scion as in the previous sets of scions (*fig. 7-1*). Between 15 and 17 years the trends continued in the same direction in the second scion but at a much greater magnitude for α -pinene, β -pinene, myrcene, and limonene. However, this trend did not prevail for 3-carene which changed only slightly; 3-carene was present in the parent stock. Here again, the composition of the scion rapidly shifted toward that of the parent tree. As with the previous sets, the composition of the monoterpenes was fairly constant throughout the tree at 17 years (*fig. 7-2*). Also, at 17 years, the composition of the root stock was nearly identical to that of the basal internode of the scion. Thus, the scion appeared to be affecting the root stock.

P. ponderosa AS-A95

There was only one successful graft of this tree. With the parent lacking 3-carene but having 23 percent β -pinene, it was, therefore, the reverse of Tree AS-A295 that lacks β -pinene but had 32 percent 3-carene. The composition of the one scion at 10 years did not look like the parent because it contained 35 percent 3-carene (*fig. 7-1*). By 15 years, a decrease in 3-carene was perceptible, while at 17 years it had dropped to 8 percent from the 34 percent at 15 years. There were slight increases in α -pinene, myrcene, and limonene at 15 years with large increases by 17 years. There was only a slight decrease in β -pinene, but the amount was still somewhat near that in the parent tree.

Root Stock

Two sets of root stock were sampled for comparison with the composition of the scions: one set consisting of the 12 root stocks of the single-stemmed cleft grafts just discussed, and the other set consisting of the two root stocks that had been allowed to grow to form a type of forked tree with the attached scion. The 12 single-stemmed root stocks were sampled only at 17 years. The composition of all 12 closely resembled that of the respective basal internode of the scion just above the union (*fig. 7-3*), strongly suggesting that the scion was affecting the root stock. The composition of the forked root stock was determined above and below the graft union. Above the union the composition was much like that of a Central Sierra Nevada tree: high in β -pinene and 3-carene and moderate in α -pinene, myrcene, and limonene; below the union the composition was much like that of Tree AS-A78: little or no β -pinene and large amounts of α -pinene, myrcene, and limonene. Thus composition of the root stock tissue was different above and below the graft union.

Discussion: Intraspecific Grafts

The 14 intraspecific grafts of ponderosa pine could be considered interracial because of the great difference in qualitative and quantitative monoterpene composition between the scion and root stock. The differences were in the order of magnitude noted for the interspecific grafts.

At 10 years after grafting, the qualitative and quantitative composition of 12 of the scions was very different from the parent tree. Then between 10 and 15 years, the composition in all scions began to shift toward that of the parent tree. These changes greatly accelerated between the 15th and 17th year so that the composition of the scion nearly resembled that of the parent tree. Thus, these changes held for all 12 scions regardless of the composition. Within one set of clones of one scion source there were some small differences in the year of these changes. I have not found such changes in unaltered trees, and I know of no other such reports.

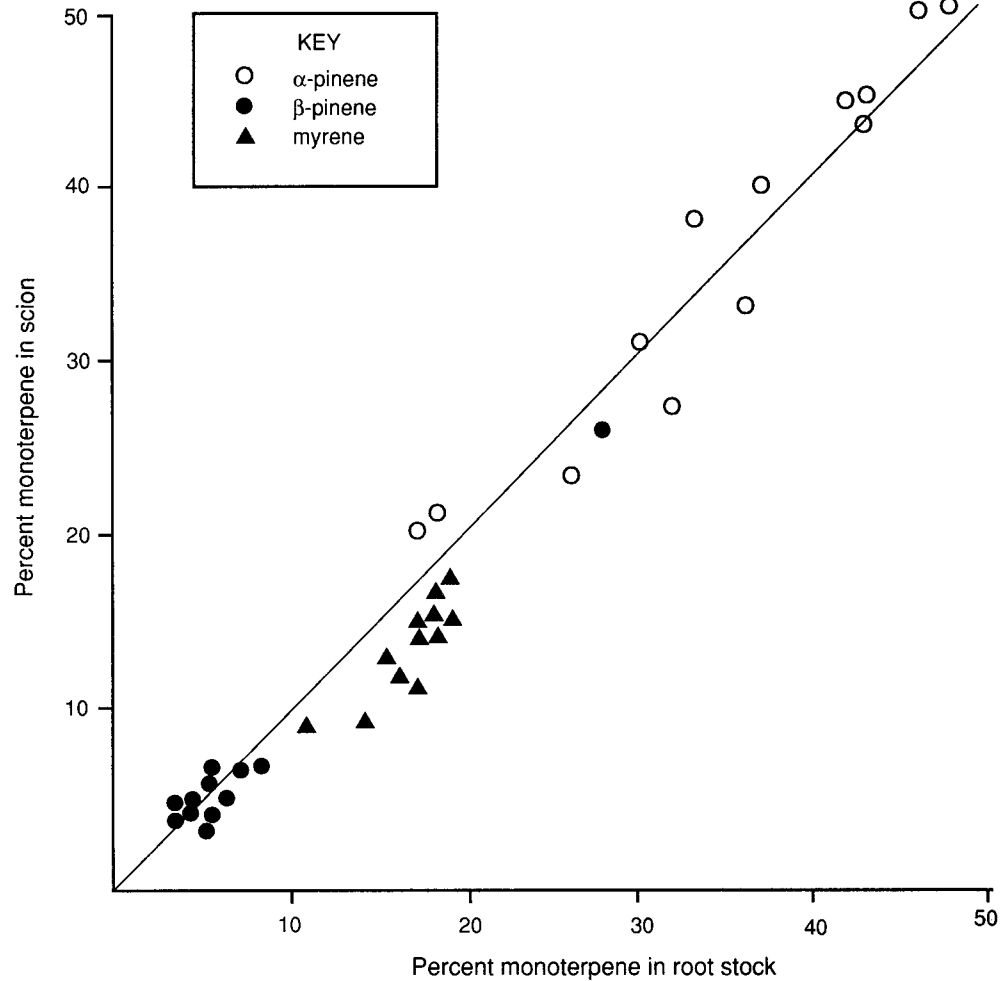


Figure 7-3—Regression of the amount of monoterpene, at 17 years after grafting, in the basal internode of the scion with the amount in the root stocks; limonene and 3-carene not plotted but fall within the ranges of the plotted components.

Following are some of the factors or conditions that might cause or contribute to the changes.

(a) After grafting, the scion does not have the expected monoterpene composition; the composition it does have suggests that a combination of postulated grafting effects places a very severe stress on the scion, or that the root stock has a very strong effect on the scion. The shifts in composition resembled that found for other forms of stress.

(b) The scion also is juvenile tissue under severe stress, and there could be a juvenile effect as well as a root stock effect.

(c) Both root and scion might also respond to severe wounding.

(d) An interaction of root stock effect and juvenile scion effect varied with time and possibly with the degree of divergence of the composition of the two different sources of tissues.

(e) Usually between 15 and 17 years after grafting, infrequently at about 10 years, there was a dramatic change as the scion appeared to start to function normally, resulting in a monoterpene composition that began to resemble that of the parent stock.

(f) Seventeen years after grafting, the scion stock appeared to be strongly affecting the composition of the root stock within 6 inches of the graft union; this is similar to the effect noted in the 30-year-old interspecific grafts noted earlier.

(g) Because the effect of scion on root in interspecific grafts was progressive and proportional to distance from the graft union, it might be assumed that the effect of root on scion was similar. But, in general, the effect on the composition of intraspecific grafts was not proportional to distance. Therefore, the effect observed in the scions of intraspecific grafts of *P. ponderosa* was an interaction of root effect and juvenile instability of the scion; an alternative is that there were unknown effects. There were limited indications in a few trees of distinct and separate root and scion effects; thus, an interaction seems likely.

(h) In intraspecific grafts, β -pinene and 3-carene decreased, and α -pinene increased sharply, whereas myrcene and limonene increased in large or moderate amounts.

(i) Though the vegetative tissue of the scions went through these changes, the reproductive structure was unaffected and, breeding was predictable, as noted in Chapter 6 on full siblings.

(j) A grafting study could be devised to elucidate some of the uncertainty in this discussion of intraspecific grafts of ponderosa pine. The study would use root and scion stock of different and similar monoterpene composition, would use different types of grafting and post grafting treatments (particularly to provide greater length of root stock), and would require annual monitoring of monoterpene composition and of the phloem tissue by electrophoresis. Approach grafts of already-established trees in the 1979 nursery could be made between trees of very different resin composition.

The grafting effect did not appear to be fully the same as the juvenile effect noted for young ponderosa pine in Chapters 5 and 6, but the juvenile effect may be part of the grafting phenomenon. In the juvenile effect, the expected components were present but not in the expected amounts, and the changes are slow. In the grafting effect, unexpected components were present in large quantities, and the changes were very sudden, very rapid, and very large. However, the juvenile effect may be involved as part of the grafting effect as suggested by some difference between parent and scion.

There is another remotely plausible explanation for these great changes in grafts for which I have no evidence. Could there be activity by the (0-0) set of alleles? Such monoterpenes as 3-carene and/or β -pinene were lacking in all

scions, and the breeding work reported in Chapter 6 validates an assignment of the (0-0) set of alleles to these two components in these trees. In all scions, where there was an (0-0) set of alleles for β -pinene and/or 3-carene, there initially were substantial amounts of these two components. Could the (0-0) set of alleles have a small amount of activity under normal conditions, as has been proposed in the report of young half- and full-siblings of *P. ponderosa*? Could the (0-0) set have much greater activity under the severe conditions of the stress of grafting? One of the responses to stress reported earlier was an increase in α -pinene, β -pinene, and sometimes 3-carene. If such a mechanism operates, it might also be the minor genetic structure proposed earlier in this report.

Measuring the Effects of Sampling and Operational Parameters: Regression, Trends Lines, Tabulation

Summary: Questions have been raised about the validity of a sample of xylem resin. When and where was the sample taken from the tree, how was it prepared for analysis, and how was it analyzed and stored? These questions were particularly cogent in the early years of gas chromatography when very small samples were used and much attention was given to individual tree composition. Much of Mirov's work (1961) was with large samples which were often secured by aggregating resin from several trees. Thus, the early work was not too helpful in addressing these concerns.

Much of the early work reported in this publication was with trees greater than 40 years old. Much later, as research included studies of siblings and half siblings, attention shifted to trees less than 15 years old. Results of the study with grafted pines were covered separately in Chapter 7 because of the unusual results.

With questions on validity not adequately answered, studies were made primarily in the early years of the research to determine the effects of (1) variation in temporal and spatial sampling and of (2) operational parameters and sample preparation and analysis. The results of all studies clearly show that there was little or no variation in monoterpene composition due to any of the variables examined in trees greater than 40 years old except for a very small increase from top to bottom of *P. Ponderosa* in myrcene and limonene and a decrease in β -pinene and α -pinene. The results of 33 studies were given in seven reports (Smith R.H., 1964b, 1966 a and b, 1967h, 1968, 1977, 1982a).

The basic objective of the 17 studies reported below, generally, was to see how well the plotted data were distributed with reference to the theoretical regression of 1.0 or how well they fitted a trend line.

Three procedures were used to analyze and display the data of these studies: simple regression, trend lines, and tabulation.

Simple regression was applied to all but a few studies when the objectives were:

- (1) to compare the composition of a given tree at different locations on the tree at a given sampling time;
- (2) to sample the same locations on the tree at different times;
- (3) to compare two different operational procedures using the same resin sample. Therefore, each plotted point was the intersection of the two measurements being compared. In all cases the reference line was the 1.0 regression and not the regression line of the data.

Trend Lines were applied to the vertical sampling of a tree at several points along the trunk. Straight lines were drawn that represented the trend lines.

Tabulation was used to record data not applicable to either of the above procedures, or to summarize large amounts of data.

Interpretive text was included only in the headings or captions. Thus, there was no separate text per se. Each study illustration in the chapter, or the single table, given below has a caption that will be a brief discussion of the illustration.

The report was divided into two sections: (a) sampling parameters and (b) operational parameters.

The basic GLC procedures are given in Chapter 2.

Table 8-1 and Study. 8-1. Height of sample: The composition of one tree each for 11 pine species at the Institute of Forest Genetics was compared at two sampling heights: (a) 3 feet (b) 20 feet. All trees were more than 40 years old. The data as listed below show little or no difference in normalized composition between the two heights even though there was wide range in the composition among the 11 species.

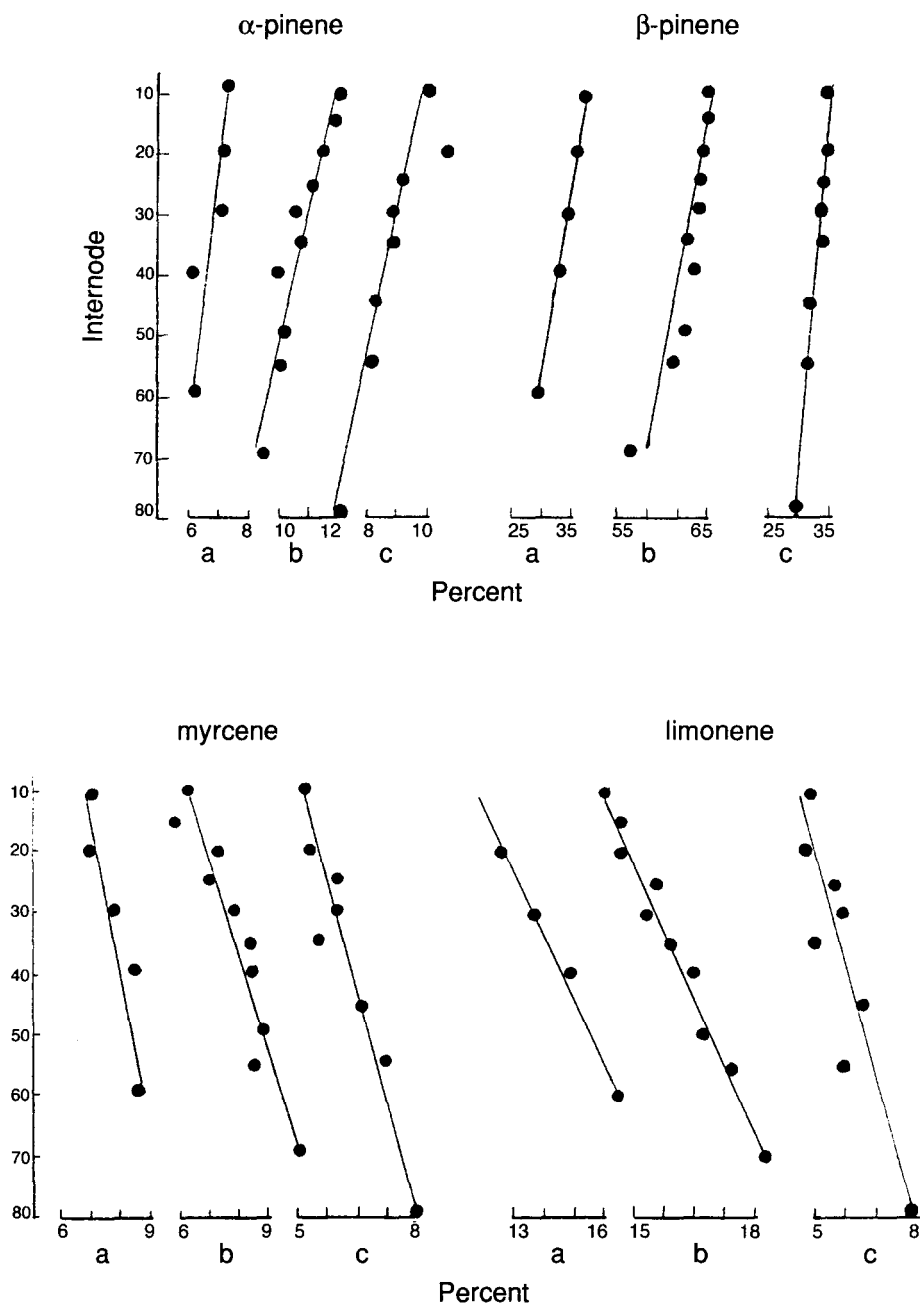
Pinus		Monoterpene ¹									
		α -p	cam	β -p	car	sab	myr	lim	β -ph	oci	ter
		pct ²									
<u>attenuata</u>	a ³	94.5	0.3	2.7	0	0	0.9	0.6	0.9	0	0.1
	a'	94.4	0.4	2.8	0	0	0.9	0.6	0.9	0	0.1
	b	94.3	0.4	2.8	0	0	0.9	0.6	0.9	0	0.1
	b'	94.2	0.3	2.8	0	0	1.0	0.6	1.1	0	0
<u>contorta</u>	a	4.6	0.3	11.5	25.9	0	3.9	0.7	51.2	0	1.8
	b	5.6	0.2	12.5	25.7	0	3.6	0.6	53.7	0	1.0
<u>coulteri</u>	a	25.1	0	3.8	0.5	0	23.5	14.4	32.7	0	0
	b	23.7	0	3.9	0.6	0	23.4	14.7	33.7	0	0
<u>edulis</u>	a	79.1	**	0.4	15.5	0	1.5	0	0	2.0	1.5
	b	76.2	**	0.4	18.6	0	0.9	0.1	0	2.0	1.6
<u>flexilis</u>	a	46.5	**	27.9	16.7	0	2.5	0.7	1.2	0	4
	b	49.5	1.0	30.8	11.2	0	2.1	0.7	1.2	0	3.5
<u>lambertiana</u>	a	64.9	0.1	24.6	0	0	9.9	0	0.4	0	**
	b	66.3	0.2	23.3	0	0	9.8	0	0.5	0	**
<u>monophylla</u>	a	71.1	**	0.6	0	10.4	3.0	4.2	0	4.7	6.0
	b	70.8	**	0.7	0	10.0	2.9	3.7	0	4.7	7.2
<u>muricata</u>	a	3.3	0	0.3	86.0	0	1.2	**	0.3	**	8.4
	b	4.5	0	0.3	84.8	0	1.3	**	0.5	**	8.1
<u>quadrifolia</u>	a	82.2	0.1	0.1	7.9	0	3.0	0	0	5.4	0.8
	b	82.9	0.1	0.5	8.2	0	3.0	0	0	4.8	0.9
<u>radiata</u>	a	24.4	0.2	73.8	**	0	0.6	0.4	0.7	0	0
	b	23.3	0.1	75.5	**	0	0.5	0.4	0.4	0	0
<u>washoensis</u>	a	10.2	0	44.7	25.8	0	11.1	4.0	1.4	0	1.4
	b	10.1	0	40.8	28.3	0	13.6	4.0	1.9	0	1.3

¹ α -pinene, camphene, β -pinene, 3-carene, sabinene, myrcene, limonene, β -phellendrene, ocimene, terpinolene

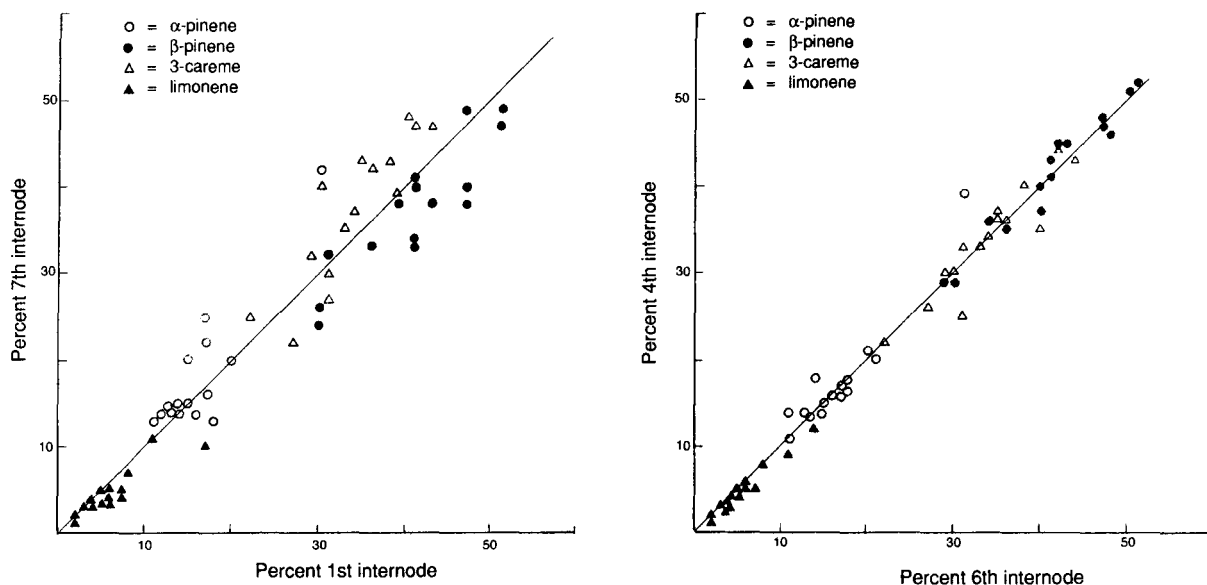
² Normalized. ** = trace

³ a' and b' are forks of a and b.

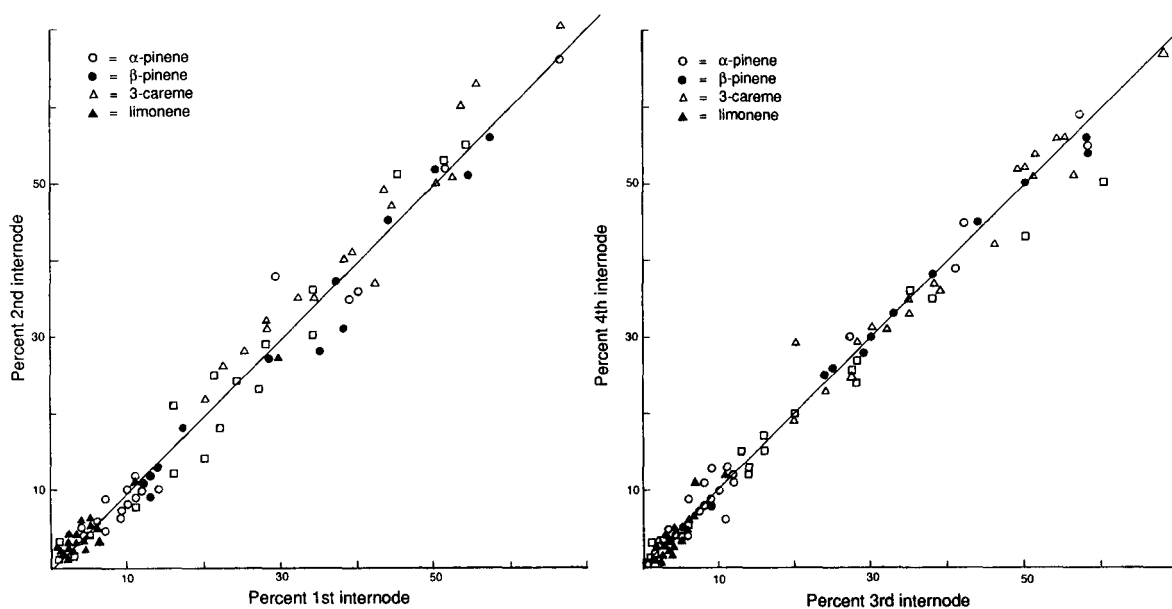
Study 8-2. Height of sample: Trend lines were drawn for the amount of the individual monoterpenes in the internodes of three *P. ponderosa* (a,b,c) 60 to 80 years old; internodes were counted from the top of the tree to the base, thus were essentially tree age at a given internode; each trend line has a separate percent base; trees were standing when sampled. All three trees were very similar in the slope and relative magnitude of the increase or decrease from 2-5% in the trend of the amount of four major monoterpenes. It should be pointed out that, though the sampling covered 20 to 50 years of internodes, the actual xylem layer sampled was essentially the same age, about 1 to 3 years for all samples. It is obvious that α -pinene and β -pinene decreased from top to base while myrcene and limonene increased.



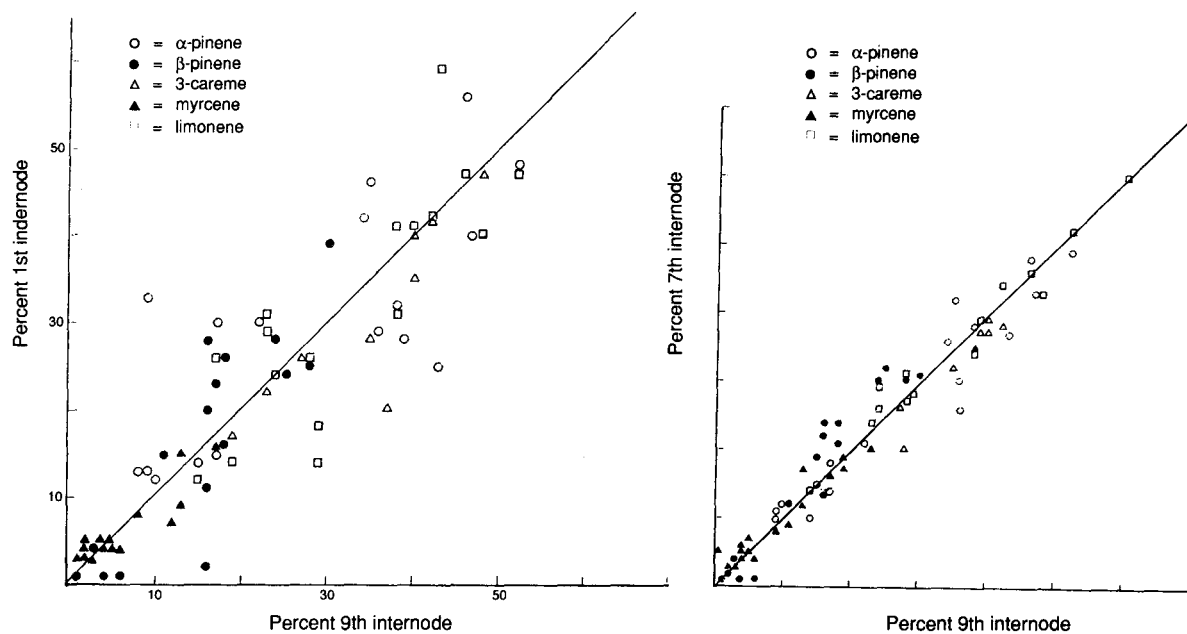
Study 8-3. Height of sample: Comparisons were made of the monoterpenes (in percent) between pairs of internodes in a population of 7-year old *P. ponderosa*. All samples were obtained by severing the tree at the internodes; internodes were counted from top of tree to base which was equivalent to tree age at a given internode. Diagonal line in regression of 1.0. Regression improved with age of internode.



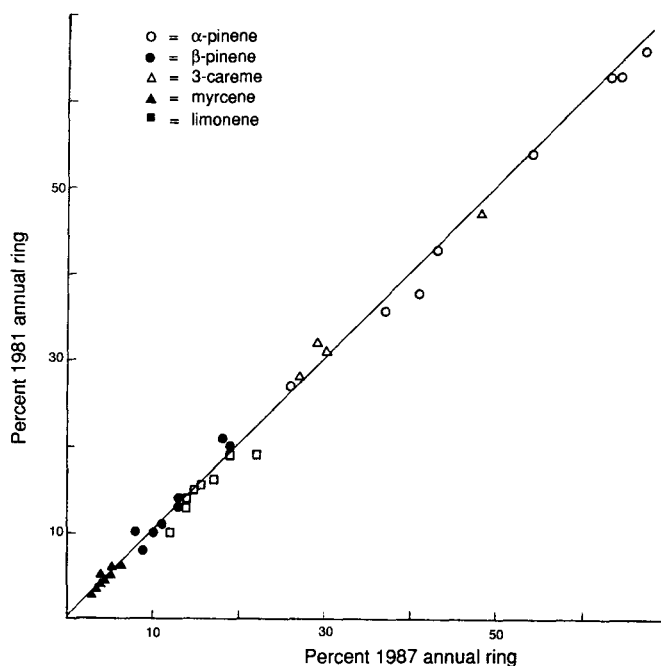
Study 8-4. Height of sample: Internode comparisons were made of the amount of the individual monoterpenes with different internode source of resin of 4-year old *P. ponderosa*; internodes were counted from the top to base of the tree, which is equivalent to tree age at an internode; diagonal line is regression of 1.0. Again, regression improved with age of internode.



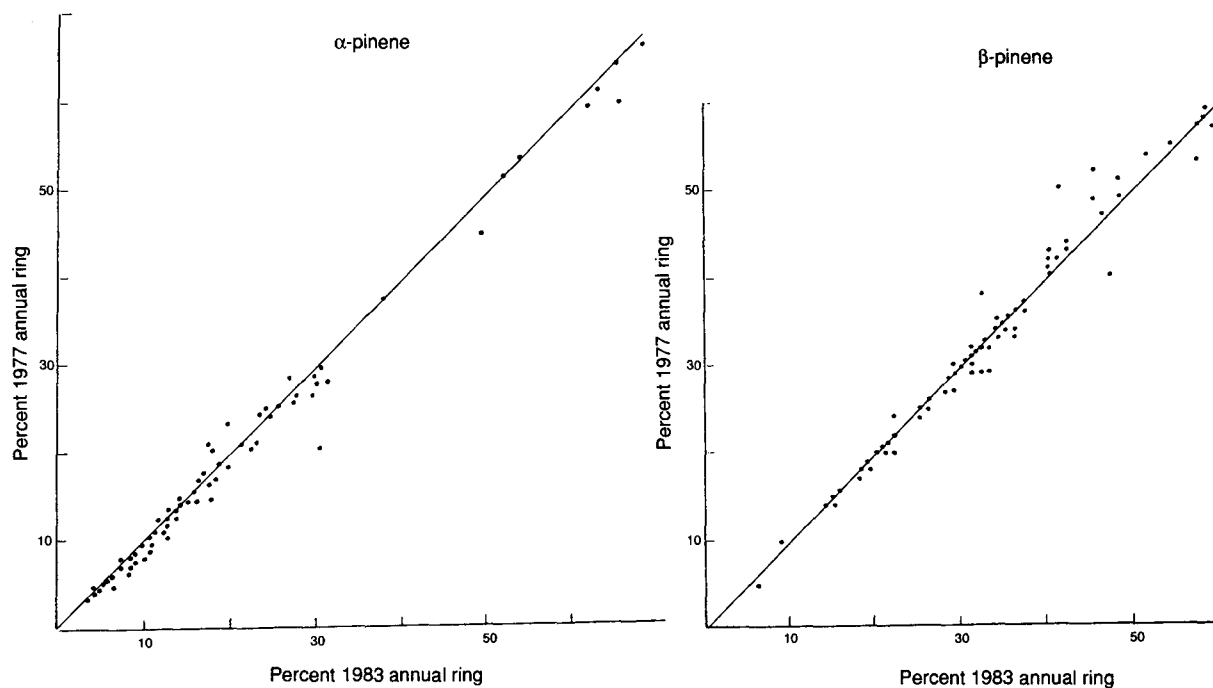
Study 8-5. Height of sample: Internodal comparison of the monoterpenes of pairs of internodes of a 9-year old full sibling population of *P. ponderosa*. Samples obtained by severing trees at internodes; internodes counted from top of tree to base. Again, regression improved with age of internode. Regression of 1st and 9th internodes were particularly poor. The results with these studies of internodes suggest that measurements of the composition of the first three internodes may not be valid, except for comparisons.



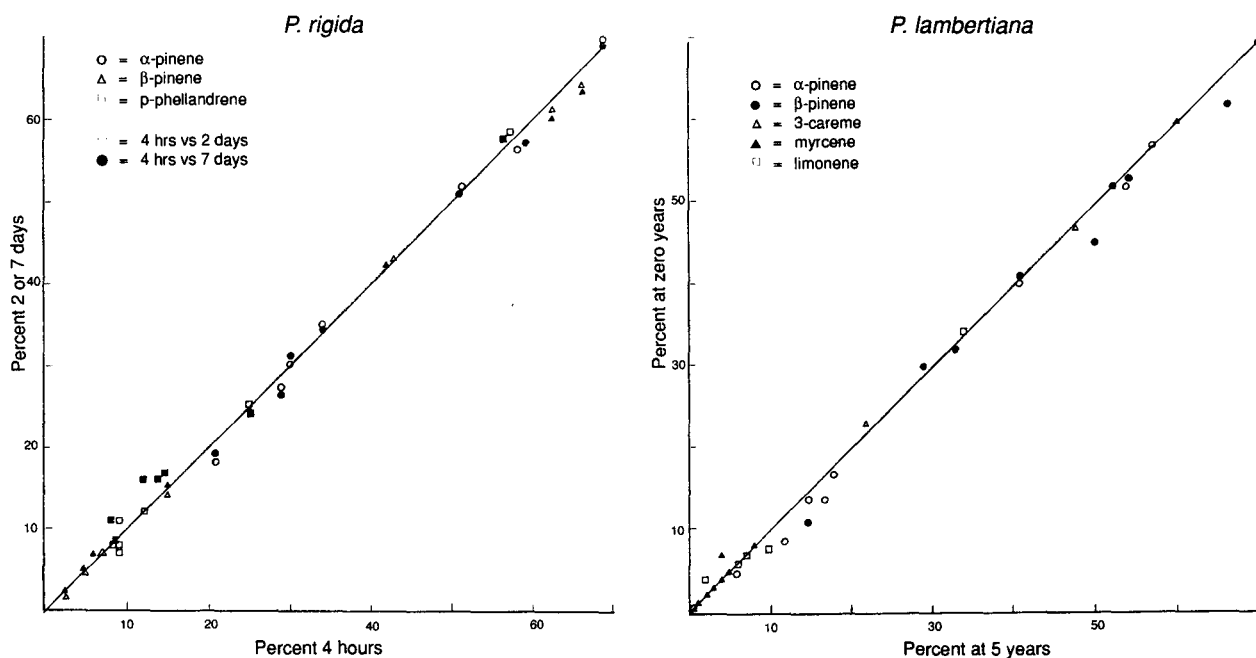
Study 8-6. Annual ring: Comparisons were made of the monoterpene composition of the 1981 and 1987 annual ring of the nursery-grown full siblings of 6-year old *P. ponderosa* IFG-4B63 x AS-5789; diagonal line is slope of 1.0. Clearly, all points were on the 1.0 diagonal or within 2 percent of it.



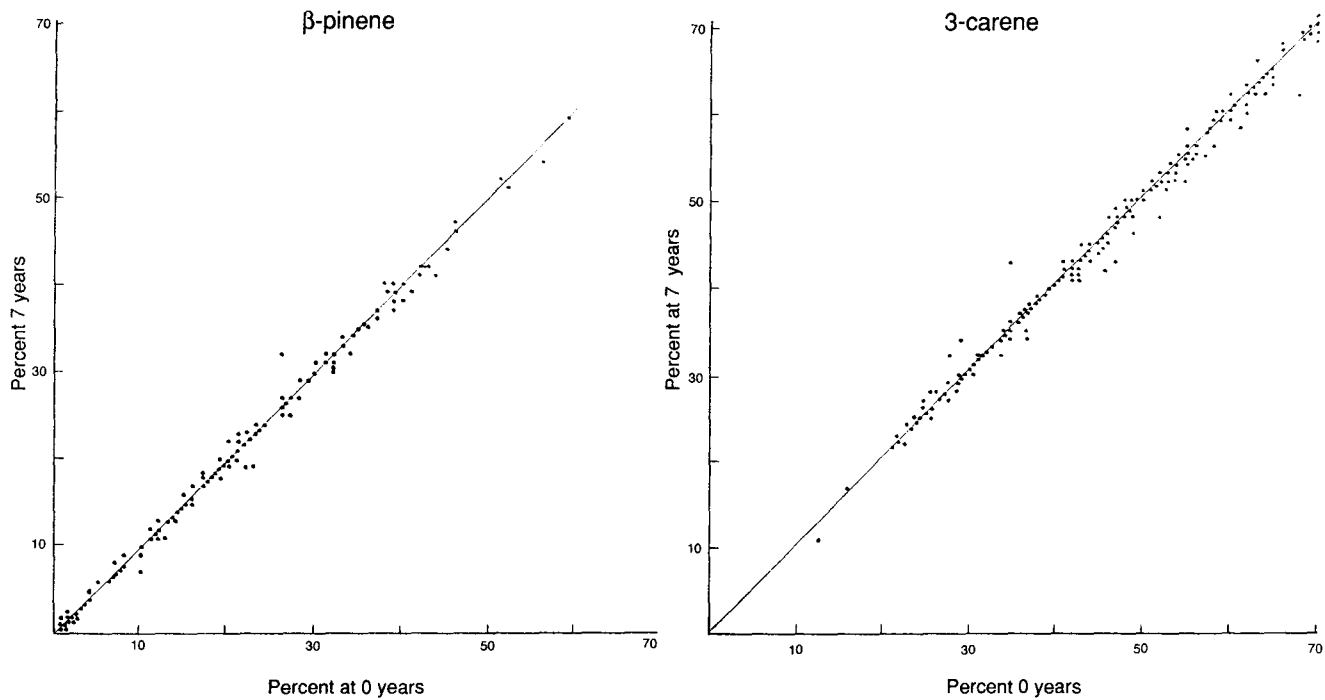
Study 8-7. Annual ring: Comparison were made of the monoterpene composition of the 1977 and 1983 annual ring at the basal internode of 72 6-year old *P. ponderosa*. Diagonal line is regression of 1.0. Over all there was very little difference, if any at all, between the composition of the 1977 and 1983 annual ring of the basal internode of these young trees; the results with myrcene and limonene were very similar.



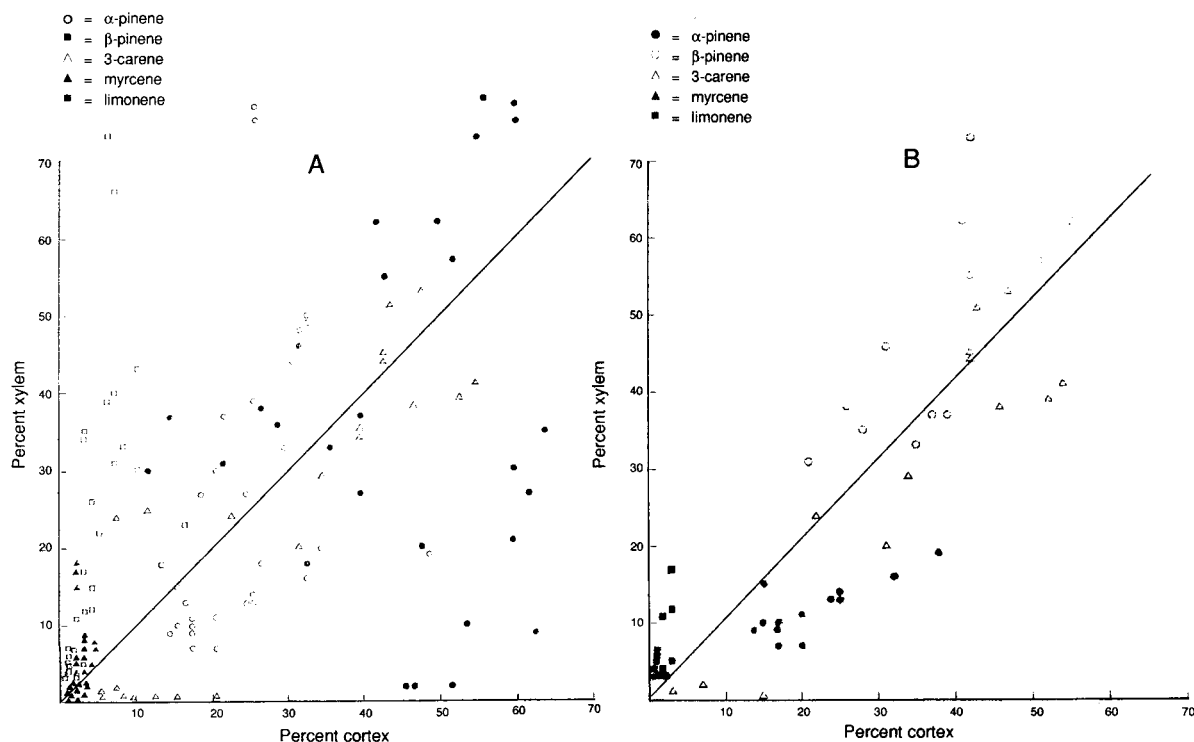
Study 8-8. Time of resin flow and sample storage: Comparisons were made of the monoterpene composition with differing time of resin flow and time of storage of prepared sample. Resin of seven *P. rigida* more than 40 years old was obtained at 4 hours, 2 days, and 7 days; and for 5 years of storage of prepared samples of *P. lambertiana*. Diagonal line is regression of 1.0. Data were regressed for pairings of time of flow or storage. There was, essentially, no difference in composition of three noted monoterpenes with time of flow of resin or for time of storage for five monoterpenes in prepared samples.



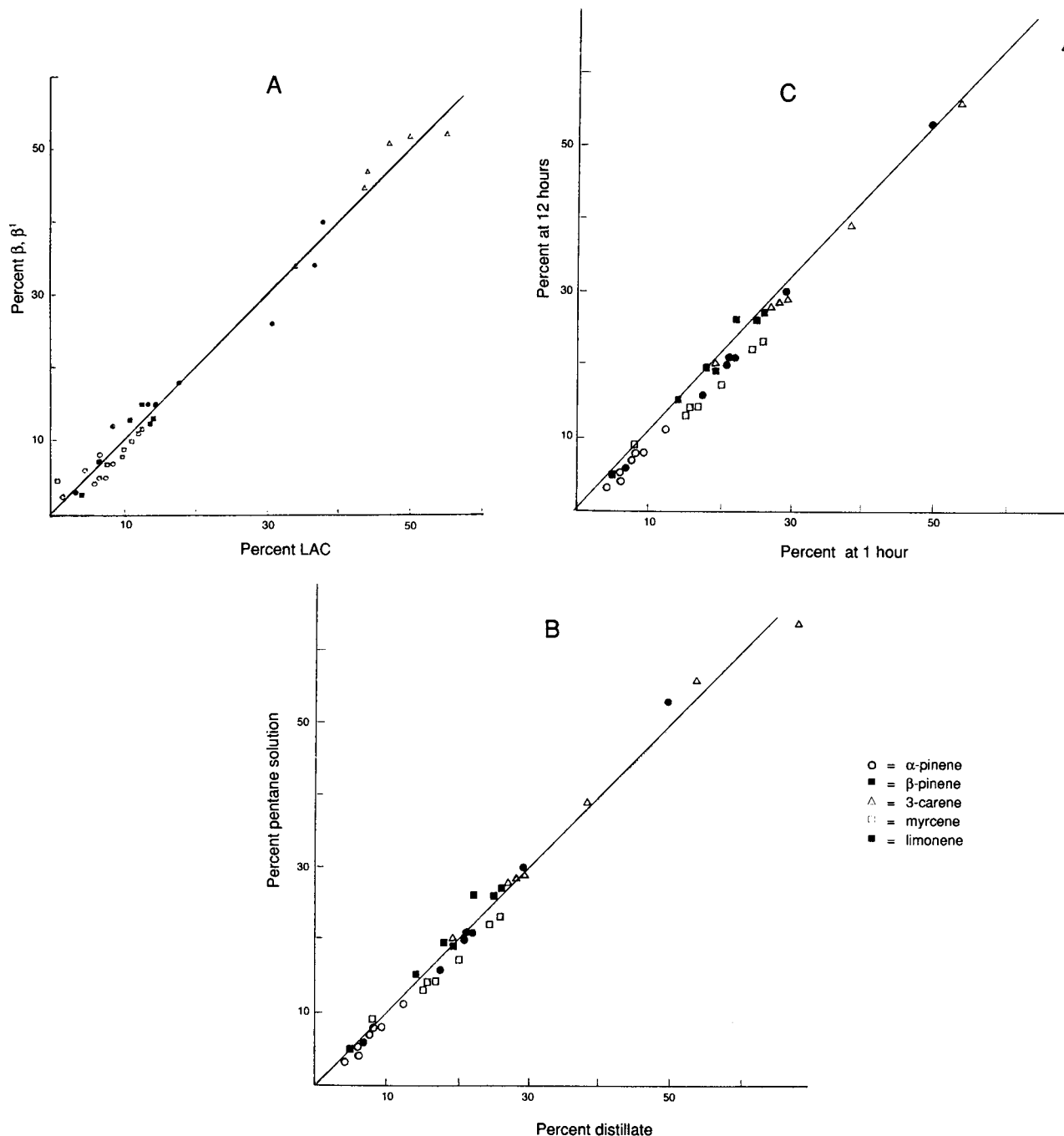
Study 8-9. Time of storage--Effect of storage time of pentane-prepared samples of 226 *P. ponderosa* was determined. Diagonal is regression of 1.0. A regression was made of the first analysis and the analysis after 7 years storage at 0 c. There was little, if any, change during storage. The data for myrcene and limonene were nearly identical to β -pinene and 3-carene.



Study 8-10. Source of resin: Correlations were made of the amount of monoterpenes with source of resin of *P. ponderosa*: cortex vs. xylem using (A) nursery-grown full siblings of Adin x Wheeler, and of (B) nursery-grown half siblings from Ranger Peak, diagonal line is slope of 1.0. Clearly, there was very poor relation between the components in xylem and cortex. The set from Ranger Peak was not as poorly related as the set from Adin/Wheeler full siblings. This suggested that composition itself can be a factor in the differences.



Study 8-11. GLC column, time of resin flow, analytical preparation: Three studies were made, all using correlation, of the following operational procedures: (A) the packing of the chromatographic column (LAC vs. β β' oxydipropionitrile), (B) the type of preparation of the sample (pentane vs. short path distillation), (C) and time of flow of resin (one hour vs. 12 hours). All studies used different samples of *P. ponderosa* greater than 40 years old. Diagonal is slope of 1.0. It was clear that none of the variables had a noticeable effect on the measurements.



The Function of Xylem Monoterpenes of Young *P. ponderosa* during Western Pine Beetle Attack (*Coleoptera: Scolytidae*): A Bioassay

Summary: A series of laboratory and field experiments were carried out to develop a working model for the resistance of *P. ponderosa* to western pine beetle (*Dendroctonus brevicomis*). The procedure was to force beetle attacks on irreversibly stressed 8- to 12-year-old trees, derived from the half- and full-sibling studies, as reported in Chapters 5 and 6. Assessment was by adult gallery length at 2 weeks. An inverse association was found between the amount of beetle boring and both the amount of resin flow and the percentage of limonene in trees that were 7.5-10 cm in diameter. Trees less than 5.0 cm in diameter did show the beetle-success/percent-limonene association but did not show the beetle-success/resin-flow association, probably because the trees were too small for testing.

A working model of the resistance of *P. ponderosa* to western pine beetle (WPB) (*Dendroctonus brevicomis* LeConte) was developed after a series of laboratory and field experiments (Smith 1975). The factors in the model were the quantity and quality of the xylem resin of *P. ponderosa*, and the quantity and quality of western pine beetle (WPB). The experiments measured three of the parameters of the model; beetle quality was not measured but was added to the equation simply on the presumption that both beetle and resin should have a qualitative property. A candidate resistant type of tree was found in a range-wide study of the variation and distribution of the xylem monoterpenes of *P. ponderosa* (Smith 1977). This chapter reports on a bioassay of young progeny that were derived from the studies of wind and controlled pollination of those selected trees (see Chapters 5 and 6).

The concept of host resistance did not appear in the early years of research and control of WPB on *P. ponderosa*. The first two decades, from about 1905 to 1925, were chiefly devoted to large-scale direct control programs to reduce beetle populations. It was assumed that there would be a proportionate reduction in the subsequent level of tree mortality. The results of the early programs were good, except they were quite variable, and usually temporary. In general, the results were not as effective as anticipated or hoped for (Craighead 1925, Craighead and others 1931; Miller 1926). About 1930, a concept started to emerge that could account for the variable and temporary results of direct control. This concept might be called host susceptibility and suitability. J.M. Miller, in a letter written in 1924, formalized the concept as follows, "The only answer [to variable and temporary results] seems to be through methods of stand management which will reduce or eliminate in the stand those trees

which are especially susceptible to western pine beetle attack." Many years later, Keen (1936) added the factor of population dynamics to the host susceptibility concept as follows, "Probably the most important factor in building up (bark) beetle populations is an abundance of suitable breeding material.... The beetles prefer certain trees that are in a susceptible condition for attack."

The concept of susceptibility launched a research effort to identify those tree characteristics which could be most strongly and consistently associated with western pine beetle attack and success. Much of that research was covered by Miller and Keen (1960). Shortly after the concept of host susceptibility was stated, and even before preliminary basic research results were available, initial attempts were made to apply forms of stand management to reduce subsequent losses due to WPB. Those initial efforts with considerable modification and adjustment were enormously successful and led to at least three landmark concepts: (a) the California Pine Rating System and its several derivations (Salman and Bongberg 1942, Smith and others 1981, Wickman and Eaton 1962; (b) the Keen Tree Vigor Classification (Keen 1936); and (c) stand hazard classification (Johnson 1949). These concepts applied primarily to *P. ponderosa* and WPB, though *P. jeffreyi* and the Jeffrey pine beetle (JPB) were added later. Thus, the concept of host susceptibility—that certain trees in a stand were more susceptible than others to western pine beetle attack—was shown to be valid. The removal of these high-risk trees greatly reduced subsequent losses for many years. The concept of susceptibility paid excellent dividends in both research and control. The success of this program seems to have been the impetus and justification for many of the subsequent studies of the effect of the host on the dynamics of both bark beetles and defoliators.

Though much of the early work emphasized susceptibility, there was an underlying assumption that host resistance might also be important. The abundance of susceptible and suitable trees fostered epidemics or maintained endemics, whereas resistant trees deterred epidemics. Hopkins (1902) noted that until the flow of resin was overcome, the mountain pine beetle (MPB) (*Dendroctonus ponderosae* Hopkins) was unable to extend galleries rapidly or to oviposit. Thus from early research onward, xylem resin was a suspected resistance factor, but little was done because of the preoccupation with susceptibility.

Miller (1950) was the first to consider the causes of resistance of *P. ponderosa* to WPB. Miller first turned to the host relationship of pine hybrids being developed at that time by the Institute of Forest Genetics. He was particularly intrigued by host x non-host hybrids. That is, what was the relationship of WPB to a hybrid of *P. ponderosa* x *P. jeffreyi*, a host x non-host hybrid? Miller (1950) conceded that it might be 20 years before these trees could be properly and reliably tested, because WPB typically did not attack trees under 6 inches in diameter.

Then in the early 1950's, Callaham (1955) studied quantitative characteristics of the xylem resin of *P. ponderosa* of different risk categories as defined by the California Pine Risk Rating System (Salman and Bongberg 1942). Callaham (1955) found that the duration of resin flow was greater in trees classed as lower risk, i.e., those trees least likely to be attacked and killed. Vite' and Wood (1961) subsequently found that *P. ponderosa* with high oleoresin exudation pressures (o.e.p.) were less frequently killed by mountain pine beetle than trees with low o.e.p. Results of both studies—resin flow and o.e.p.—could best be expressed as probabilities. The probabilities were greater that trees with a longer period of resin flow and higher o.e.p. were less likely to be attacked and killed than trees with shorter flow and lower o.e.p. In both studies some trees with the assumed resistant feature(s) were killed, and some with the assumed susceptibility features survived. One might logically conclude that

other unknown factor or factors had an appreciable effect on resistance.

The work started by Miller was continued with the emphasis on properties of xylem resin. Smith (1965) undertook laboratory studies with western mountain, and Jeffrey pine beetles. He concluded, in general, that: (a) beetles were able to tolerate the resin of host species of pine better than the resin of non-host species; (b) resin of hybrids of host and non-host was intermediate in its effect on beetle tolerances; and (c) there was a difference in the tolerance that WPBs have to the individual monoterpene components of their host resin, *P. ponderosa*. Extensive analysis of native *P. ponderosa* pine revealed high levels of local variation among trees and significant regional differences among stands in the monoterpene composition of *P. ponderosa* (Smith 1977) (see Chapter 3, #14).

In forced-attack studies on *P. ponderosa*—no-choice testing on irreversibly stressed trees of suitable size for beetle attack—WPB was found to be far less successful in constructing galleries and ovipositing in trees with high-limonene content than in trees with low limonene content; the trees used in these studies were 15-20 cm in diameter. Quantitative flow of resin was also important, with success of attack inversely related to flow (Smith 1966b). These studies were the basis of this model: resistance is a function of [beetle quantity x beetle quality] - [resin quantity x resin quality] (Smith 1975).

A tree selection and breeding program of *P. ponderosa*, as reported in Chapters 5 and 6, was carried out from 1972 to 1978. The program used trees selected for monoterpene composition to produce a readily available supply of trees having a large array of monoterpene compositions suitable for resistance bioassay and for chemical and genetic studies. By 1987, the progeny derived from the controlled breeding, along with trees from wind-pollinated seed of some of the parents used in the controlled breeding, were 10 to 12 years old and thought to be suitable for resistance testing, but the diameter of these trees at 7.5 to 10 cm was still far below the presumed size for western pine beetle attack.

This section briefly reports on early results in the evaluation of the resistance of these trees and on the degree of agreement of the results with the resistance formula that was developed from experiments with much larger and older trees. Favorable results could substantiate the early results on resistance, decrease the waiting time for evaluation of trees, more strongly link resistance with bioassay and with chemical assay, and, by using small trees, increase the replication and lower the cost for this type of testing.

Material and Methods

Selection of Parent Trees

Trees for the experiments were the products of the long-term selection and breeding program. Selection of the parent trees was difficult and uncertain. *P. ponderosa* and most other forest trees have not been fully cultured, and the effects of stand and site conditions on the desired characteristic are not at all well understood. Nevertheless, test trees were selected on the basis of the monoterpene composition of the xylem resin (Smith 1982b). Wind-pollinated seeds were collected from parent trees in 1970 and 1971 (see Chapter 5). Controlled breeding was carried out from 1973 to 1979 (see Chapter 6) using standard pine breeding procedures (Anonymous 1948, Cumming and Righter 1948). Seeds were planted and trees were cultured by standard nursery practices at the Institute of Forest Genetics (IFG) near Placerville, California (see Chapter 6 for breeding and culturing procedures).

Four sets of 9- to 12-year-old trees were selected, one set each from four different nurseries: 1974, 1975, 1976, 1979 (*table 9-1*). Each set had 10 to 16 trees that were selected on the basis of either high- or low-limonene content of the xylem resin. An effort was made to have comparable tree diameters for the two

Table 9-1—Average xylem monoterpene composition of four sets of *P. ponderosa* classed as high and low limonene used in forced-attack bioassay of resistance to western pine beetle

Year of nursery ¹	Age of trees ² <i>yr</i>	Average Monoterpene Composition						
		Trees	Replicate	α -pinene	β -pinene	3-carene	myrcene	limonene
		<i>number</i>		<i>percent</i> ³				
1974	14	5	14	12	11	20	8	48
		5	13	12	44	30	8	1
1975	13	7	21	29	1	0	4	52
		6	19	6	33	42	13	1
1976	12	6	23	31	2	0	7	59
		5	18	5	28	47	13	2
1979	9	10	10	24	5	0	9	64
		6	6	32	33	7	6	14

¹1974, 1975, 1976 had a regular sequence of 3 to 4 replicates per tree; 1979 had only the base section.²(x) = age of trees in years.³Normalized; small amounts of camphene β -phellandrene and terpinolene make up the remainder of 100 percent.

subsets, i.e., high and low limonene. However, despite a good number of trees to select from, there still was a small range in diameters of 7.5-10.0 cm in the 1975 and 1976 sets, and 5.0-10.0 cm in the 1974 set. The trees in the 1979 set were about 5.0 cm in diameter. Thus all trees in the four sets were well below the size of 15 cm considered a minimum size for western pine beetle.

Very little is known about the effects of phloem thickness on gallery construction. All research on western pine beetle has been on large trees where phloem thickness was not considered an important factor in attack and development. Phloem thickness was about 2 mm in the test trees, was probably marginal for gallery construction in the experiment, and may have been a confounding factor in the 1979 set of trees.

Bioassay: The procedure for preparing the young trees for tests was quite similar to that used on larger trees (Smith 1966b), except that trees in this report were less than 10 cm in diameter and were growing in a nursery. This procedure forced a group of beetles to attack a small, caged, circumferential section of a tree whose crown had just been cut off. The procedure, in brief, was as follows: The basal 1 to 2 m of the tree were caged with four contiguous but separated sleeve cages. The length of each caged section was selected to give about 300 to 450 cm² of bark area inside the cage. Caging material was 20 mesh plastic screening. Bands of polyurethane foam about 2.5 cm thick and about 10 cm wide were used to hold the screening away from the bark and to separate the sections of the tree (fig. 9-1). The bark under these bands was shaved smooth to presumably make an escape-proof fit; bark within the caged portion remained untouched. The beetle needs roughened bark to attack. Roughening of *P. ponderosa* bark starts at about 4-7 years of age.

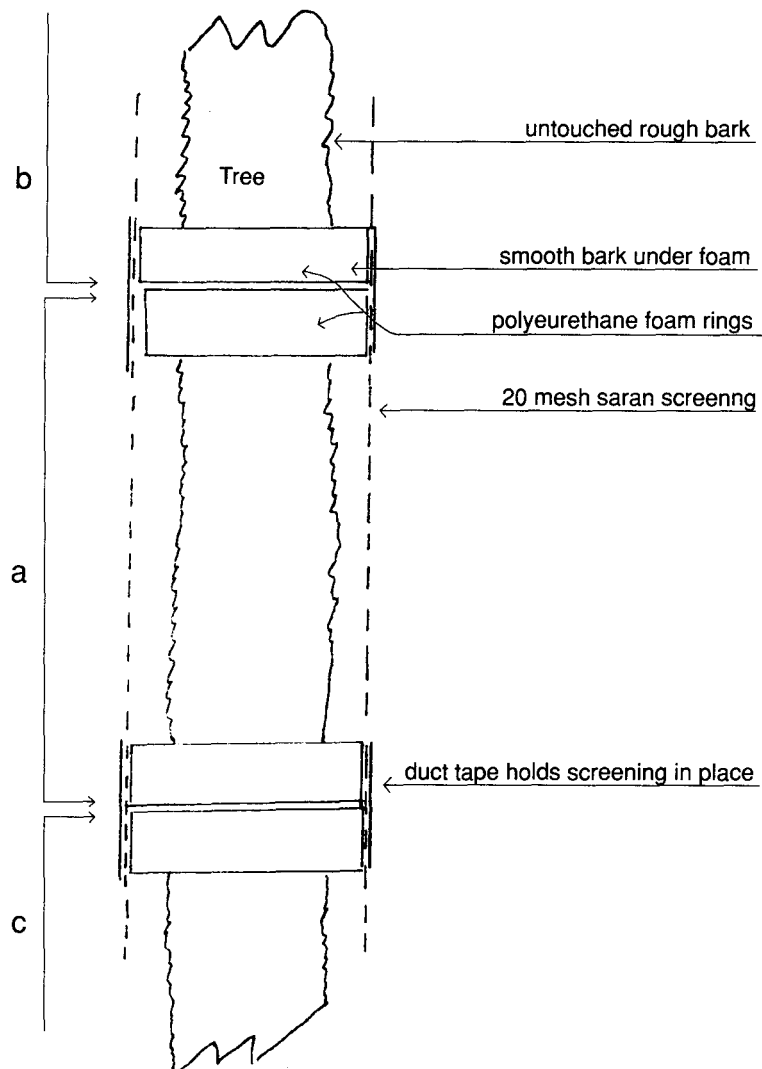
Beetles used in the tests were reared from infested bark taken from trees on the Sierra and Eldorado National Forests in California. The bark was gathered in early spring when the over-wintering brood was in the late larval stage. The brood bark was held in a cold room at 0 °C. As beetles were needed, bark was placed in a roofed, screened outdoor insectary at IFG. Beetles were collected in the evening in cooled petri dishes, 20 beetles per dish, held at about 5 °C during collection, and held at 0 °C overnight. These beetles had emerged over the previous 24-hour period. Beetles were sexed under cooled conditions the day after collection. Only females were used and were randomly apportioned to the replicates in a test; the number of beetles was proportional to bark area of the section being tested.

When a set of trees was ready to be tested, the trunk of each tree was cut off just above the caged portion early in the morning. The term "irreversibly stressed trees" was applied to trees treated in this way. Six hours later, the resin that had exuded to the cut surface along the outer half inch was weighed as a measure of resin flow. Twelve hours after cutting, i.e., early evening, female beetles were introduced into the top caged section of each tree at the rate of about one female per 30 cm². Thus each sleeve cage was charged with 7 to 10 females. An umbrella-like structure was placed on the top of the cut section to provide shade for the top section.

Two weeks later the sequence was repeated. The caging was removed from the top section, which was the section that had been under attack and was cut off for subsequent examination of beetle performance. Resin flow on the new cut surface was measured at 6 hours, and the new top caged section was charged with beetles early in the evening. Thus, three to four replicates were obtained from each tree. Each replicate of a tree had the same resin monoterpene composition (quality), but a different flow of resin (quantity). That is, the rate of flow of resin usually changed from section to section, but the monoterpene composition of all the sections of a given tree remained about the same.

Each section was examined within 4 hours after it was cut from the stem of

Figure 9-1—Schematic diagram of a longitudinal section of a sleeve-caged portion of *P. ponderosa* (a) and the portion of the section above (b) and below (c) the section.



the tree. The bark was carefully shaved off down to the xylem, and all attacks that reached the phloem were recorded and measured for gallery length, the measure of beetle success. If there was no evidence of any attacks on a section, it was discarded from the test. The lack of any evidence of attack in a section was rare; such cases could have been caused by the escape of all beetles. The necrosis associated with the beetle gallery was also measured. Though there was some difference in the extent of this necrotic area, there seemed to be a fairly strong positive relationship between gallery size and the size of the associated necrotic area.

Data Analysis: The average length of gallery for each caged section of each tree was calculated and paired with the value for the quantitative flow of resin in the outer 0.6 cm of the top cut of that section. The average gallery length for a caged section was based on 3 to 12 galleries, and this was considered a replicate. The resin flow value was based on only a single measurement. The data for each of the four sets of trees were analyzed separately. A regression was made between average gallery length and resin flow for each class of limonene. A one-way analysis of variance was made for the gallery lengths of high- and low-limonene trees and for resin flow from high- and low-limonene trees for each set of trees. A modified t-test was the basis for judging two populations of replicates to be different.

Results and Discussion

The results, though positive, were not as indicative as expected. This seems to be attributable to the small size of the trees; i.e., all trees were relatively resistant to beetle attack because of their small size. However, within this condition of "relative resistance" it was possible to compare two parameters, resin flow and resin quality, while holding beetle quantity constant. The primary reason for the experiments at this time was to see if bioassay could be made of small trees, thus reducing the waiting time for trees to reach suitable size for conventional testing. This hypothesis should be kept in mind as the results are discussed.

The regression data for the trees in the 1975 and 1976 nurseries show that adult gallery length was inversely related to resin flow (*fig. 9-2*), and that the average adult gallery length was significantly shorter in the high-limonene set of trees for all four experiments (*table 9-2*). This supported the resistance formula (Smith 1975). However, there were individual values for high and low limonene within the one standard deviation band for the opposite set. For example, in the 1975 nursery data, the value for two high-limonene replicates fell within the limits of one standard deviation of the low-limonene set of trees, and two low-limonene replicates fell within the limit of one standard deviation of the high-limonene set of tests. Obviously, variation was large and the number of replicates that were used was necessary.

The regression data for the 1974 nursery were inconsistent in that they showed a direct relation between resin flow and gallery length for only the low-limonene trees, but an indirect relationship—greater flow with shorter galleries—for the high-limonene trees, suggesting that success of attack (gallery length) was still inversely related to the percentage of limonene (*fig. 9-2*). There is a significant grouping of the high-limonene replicates toward the lower range of gallery length and a grouping of the low-limonene replicates toward the higher range of gallery length. In this experiment, the low-limonene trees were generally larger in diameter than the high-limonene trees.

The results of the 1974 nursery must be viewed with some question because of the temperature problems and because the trees within a set were quite variable in size. The umbrella-like structure did not seem fully adequate. It did screen out direct sunlight from the caged section, but it did not shade the

Table 9-2—Average adult gallery length (x) and resin flow (y) for four sets of replicates of high- and low-limonene *P. ponderosa* less than 3 inches in diameter

Nursery	Limonene class	Replicate	Gallery length		Resin flow	
			X	S.D. ²	y ²	S.D. ²
		number	mm		cc	
1974	high	14	4.1* ¹	3.0	4.0	2.1
	low	13	12.2	8.3	5.3	4.3
1975	high	21	6.7*	3.6	1.9*	1.4
	low	19	10.8	4.3	4.2	2.9
1976	high	23	5.7*	3.6	3.2	2.5
	low	16	12.0	8.9	5.2	4.8
1979	high	10	2.7*	2.3	1.8	1.8
	low	6	11.6	7.9	3.1	2.9

¹*Significantly lower at 5 pct level under the modified t-test.²S.D. = standard deviation.

section from the heat reflected from the open ground of the nursery. The 1974 nursery bed had been opened up to light too much by the previous season's work in attempting to use attacks by individual females. The residual trees in the 1975 and 1976 beds (as will be described below) provided more shade than did the residual trees in the 1974 bed. Additionally, as a result of the experience with the 1974 bed, cloth shading was erected along the east, south, and west sides of the 1975 and 1976 nurseries. Thus, in a way, the 1974 nursery was a learning process. Local temperature control was not a problem in the prior work (Smith 1966b, 1969) with larger trees in natural stands because the sections were not close to the ground, and the natural stand provided general shading. A series of temperature readings showed that the temperature under the umbrella and the caged section was about 5 °C higher than at an official weather station about 300 ft away. On the other hand, the temperature at the weather station was about 5 °C higher than in the shade of a well-stocked ponderosa pine stand about 150 ft away.

The results with the 1975 and 1976 nurseries were strong support for the working model—the differences between low and high limonene were significant (*table 9-2*), and the correlation of decreased gallery length with resin quantity was evident (*fig. 9-2*).

The regression data of the 1979 set of replicates were the reverse of the 1975 and 1976 data; the correlation of gallery length with resin flow was direct rather than inverse. Yet gallery length for the high-limonene replicates was significantly shorter than the low-limonene replicates. This experiment suggests quite strongly that this tree size, less than 5.0 cm in diameter, was not suitable for this type of testing. Also, phloem thickness may have been a confounding factor in the 1979 set.

For all experiments, average gallery length was 4.8 mm for high-limonene trees and 11.7 mm for low-limonene trees (*table 9-2*). Though resin flow was always greater in the low-limonene set, it was at the significant level only in the 1975 nursery. Thus, it appeared that limonene content may be more effective than resin flow in reducing success of beetle attack in small trees.

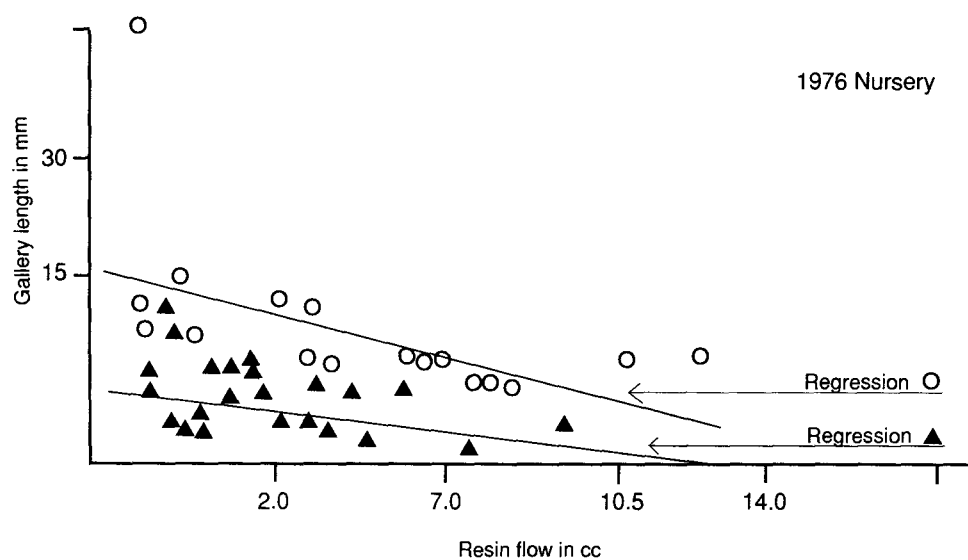
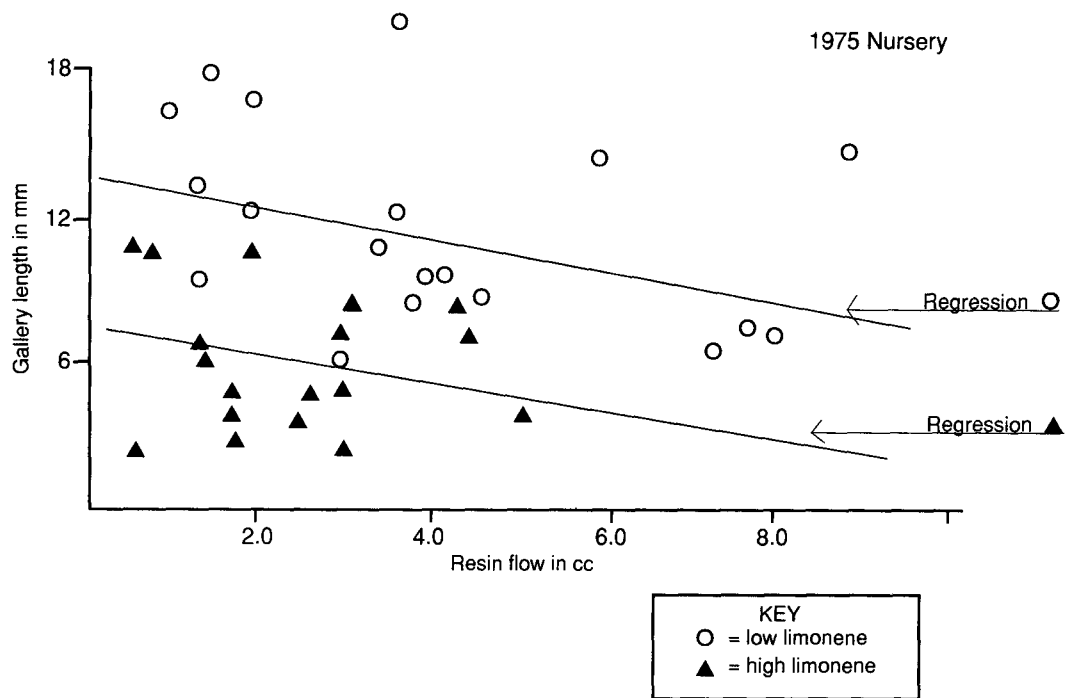
Conclusions

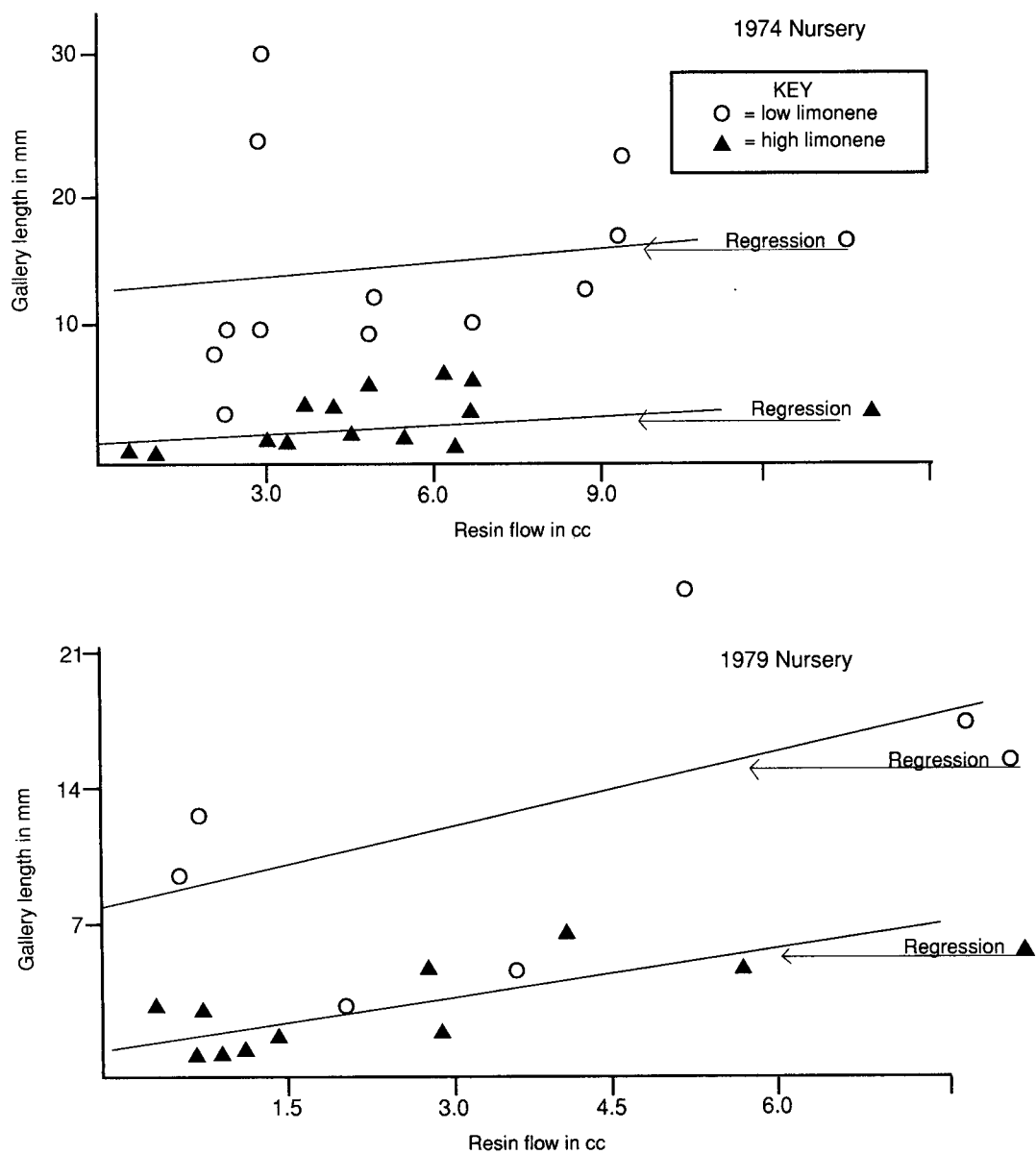
From the results and my observations of all previous work with trees greater than 15 cm in diameter and of the trees used for this report, it appeared that trees with a diameter less than 7.5 cm were not suitable, that trees with diameters greater than 7.5 cm but less than 12.5 cm are adequate for comparative testing, and that trees with diameters greater than 15 cm will probably give both comparative and absolute classification of the resistance of trees.

It was quite clear from all the data on gallery length that the type of trees used in the experiments was generally resistant to western pine beetle attack, and experiments on such trees can be only comparative and not absolute. From extensive previous work with forced attacks on larger trees, I would have expected gallery length to be well above 100 mm in sections of low limonene with low flow of resin for the period of the test. Yet in the experiments reported here, gallery length for low-limonene and low-resin flow ranged from 10 to 20 mm. One can speculate that only one or more of the following factors could have caused this phenomenon: small diameter, thin phloem, thin bark, the high temperature of the micro-environment of the nursery. The large variation in gallery length, even within a single replicate, might also be explained by these same factors.

In the earlier work with larger trees using the irreversible stress procedure, the change in resin flow was fairly predictable; flow decreased as each successive section was cut from the tree. This did not prove to be so with the

Figure 9-2—Regression of gallery length of western pine beetle on resin flow of *P. ponderosa* with sets of high- and low-limonene trees in the 1974, 1975, 1976, 1979 nurseries; each plot point is the average gallery value for one replicate (i.e., one log); ▲ = high limonene, ○ = low limonene.





small, nursery-grown trees. Though flow did change from section to section of the same tree, it was not at all as regular and predictable as in larger trees. The nearness of the trees to each other and the possible intertwining of the root systems are the most logical explanations of this phenomenon.

It was observed in these series of experiments that nearly all galleries, short or long, were oriented in a vertical direction, rather than the sinuous, typically inclined, direction for WPB. Short vertical galleries were typical of forced attacks of WPB on non-hosts, hybrids of host x non-host, and on ponderosa pine with a large flow of resin. The large variation in gallery length also could have been caused by the irregular response of the resin system to the sequence of cutting. Variation in gallery length was not as great when using larger trees and larger sections (Smith 1975). The factor of phloem thickness may be important for typical gallery construction. Phloem thickness was 2 to 3 mm in larger trees and about 2 mm in smaller trees. Beetles will construct galleries in phloem less than 2 mm thick, but their boring may not be typical.

The range in gallery length was usually greater for low-limonene replicates than for high-limonene replicates. This might have been caused by greater variation in qualitative monoterpene composition of the low-limonene replicates. That is, low-limonene trees were selected only on that basis, with the remainder of the composition varying widely among the other four major components: α -pinene, β -pinene, 3-carene, and myrcene. Earlier work (Smith 1965) showed a difference in toxicity between the four monoterpenes and the adult WPB. On the other hand, in selecting for high limonene, the remaining composition was almost always α -pinene and myrcene, and very little β -pinene and 3-carene. Of course, the factor of beetle quality could be important in causing such great variation in gallery length. The factor of beetle quality has not yet been addressed in this work.

In early exploratory work on testing trees for resistance to WPB attack, attempts were made to cage individual females or pairs instead of groups of beetles so that the performance of individual beetles could be measured; i.e., each beetle was a replicate. The size of the cage area ranged from about 0.4 cm² to about 6.0 cm² and varied from a circular to a rectangular shape. No effort was made to encourage boring in the small cages by starting an entry into the bark, but pheromones were used in some tests to encourage boring. However, the results were not as satisfactory as group testing, and the use of small cages and individual females was not pursued.

One might also question the absence of male beetles in the tests. All reports agreed that the female initiated the attack and did all the boring and that the male may help a bit in moving frass and boring particles about the galleries. My observations of males with females in 1985 and 1986 suggested that the males were not important in attack initiation and gallery excavation for the 2-week testing period and that the addition of males could add another variable.

The results of the 1987 experiments, when compared to prior studies, were not as indicative as expected in proving that reliable bioassay could be made with small trees and projected to large trees. There may be limitations on this type of testing on small trees. However, the results should be compared with what has been obtained from tests on large trees and with what will be obtained for this same genetic stock when they are tested as larger trees. In time, both chemical assay and bioassay of young trees could be highly indicative of the results which might be expected from tests with large trees. When this is accomplished, then testing at a young age will be adequate.

Procedures other than no-choice testing on irreversibly stressed trees were tried early in the program, but none proved to be suitable. Four of these are reviewed very briefly as follows:

- (a) Pairs of beetles were caged on a small portion of the bark of a large *P.*

ponderosa, quantity and quality of resin were determined, and boring success was determined. The procedure failed because bark beetle attack functioned as a mass attack phenomenon, and individual attacks are impossible to evaluate under these conditions.

(b) Natural populations of beetles were attracted to groups of trees selected on the basis of resin properties. Progress of attack was monitored and associated with resin properties. Evaluation was poor because beetle quantity was not controlled, and the mass attack phenomenon masked the effects of the resin characteristics. Attempts were not successful in "turning off the beetle attack" when it seemed that some trees would live and others would die.

(c) Regulated numbers of beetles were caged on unaltered trees of the proper size. The beetles were added to successive caged units working from the top of the caged portion, i.e., much like the course of a natural attack. The plan was to halt the addition of beetles when some trees were judged as near to being successfully attacked based on the occurrence of beetle oviposition. This procedure also failed because the time to stop the attack could not be determined accurately or reliably. Oviposition was not a reliable sign that the attack was successful, and all trees survived when the attack sequence was stopped.

(d) Small trees about 6 inches in diameter were selected for resin properties and were cut off at about 5 feet above the ground. A rigid, portable cage was placed over the "high" stump and beetles introduced into the cage on the basis of bark area of the stump. The stump was cut off at the ground after 3 weeks, and all beetle activity was evaluated by shaving off the bark. Again evaluation was difficult. There were insufficient replicates, and there was usually a very strong and irregular flow of resin the first few weeks after the tree was cut. With this unpredictable flow of resin and the lack of replicates, it was difficult to evaluate beetle activity. However, these unsuccessful results led to the no-choice, irreversibly stressed, multiple-section procedure because the active tree physiology was largely nullified, beetle quantity was regulated, there were several replicates for a given tree, and resin quality was constant within a given tree but differed from tree to tree by choice. Resin quantity usually varied with each successive section so that it could be treated as a variable.

The nursery bed may not be the most appropriate place for this type of testing. However, it is very convenient and most economical with regard to time and money expended. Future tests in a nursery might consider a sun-screening structure over the bed or other means for controlling the micro environment. A plantation-type area should be tried. Though this would cost more, it could be more reliable. Pheromones might be used to attract beetles to uncaged and unmodified trees in plantations. Other procedures that should be pursued are: (a) further attempts to use individual females or pairs despite the poor results with preliminary testing; (b) other measurements of resin flow; (c) as mentioned earlier, use of larger trees; and (d) testing periods other than 2 weeks. With procedural improvements, it might be possible to use a larger array of compositions to associate boring success with various resin qualities.

Miller (1950) may have been correct that good and confident testing may require trees greater than 15 cm in diameter. However, one purpose of the experiments was to shorten the period between seed germination and testing from 15-20 years to between 8 and 10 years. The results show that the time interval for testing can be shortened but that improved culturing of trees might help.

These experiments culminated a long program of (a) identifying xylem resin limonene as a component of ponderosa pine that increases its resistance to western pine beetle attack, (b) similarly testing native trees, (c) searching for parent trees for breeding, and (d) culturing and testing the progeny of the

breeding program. The results of the testing, in general, supported the resistance model, demonstrating that other factors being equal, the success of attack of WPB on *P. ponderosa* is significantly less in trees with greater limonene content of xylem resin and in trees with greater flow of resin. It was also obvious that there is much room for improving the procedure. Resin flow seemed to be less of a factor in trees with a diameter well below that associated with normal beetle activity. The resistance model might be used in pest management as follows: (a) keep resin flow high by regulating stand density and composition; (b) keep beetle numbers low whenever economically and ecologically possible; (c) consider high-limonene sources of ponderosa pine for planting stressful sites. And it is quite likely that there are a number of other factors that could bear upon each of the four primary elements of the resistance model.

Chapter 10

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Xylem Monoterpenes of Pines: Distribution, Variation, Genetics, Function