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**Pacific Southwest
Research Station**

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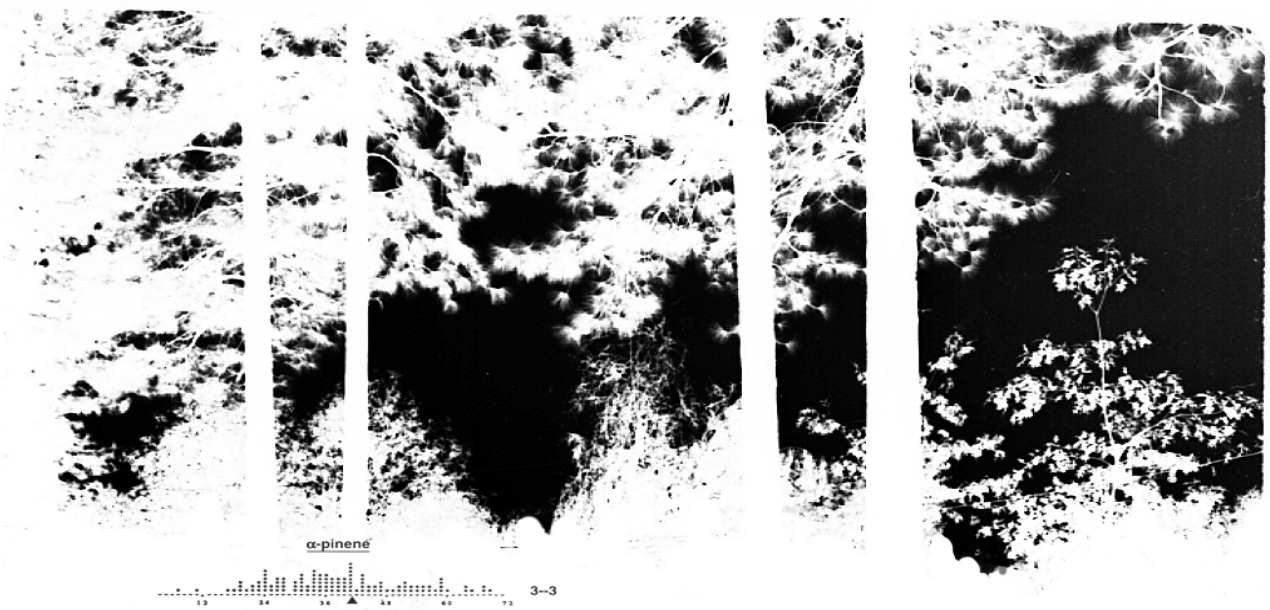
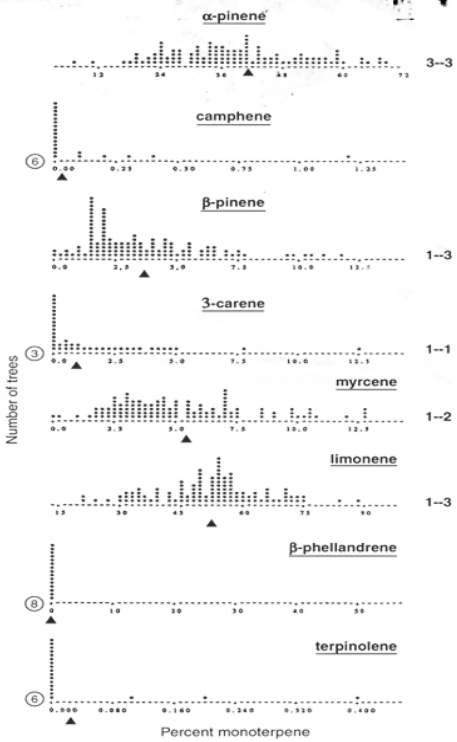
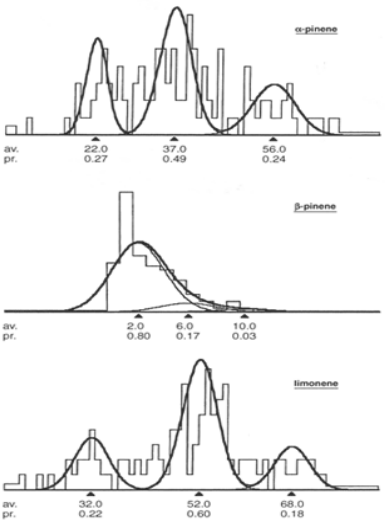
PSW-GTR-177

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

Richard H. Smith







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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 Insitute 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; an (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–terpenene, ocimene, α–phellandrene, and terpinolene.

The Author:

Richard H. Smith, a research emtomologist, was Project Leader—Forest Insect Research, headquartered in Berkeley, California, until his retirement in 1989. Much of his 33-year career at the Pacific Southwest Research Station was spent on studying the resistance of pines to bark beetles, including action of pine resins in resistance, and on the biology, ecology, and control of forest insect pests. Before joining the Pacific Southwest Station staff in 1955, he served as a research entomologist for 8 years at three locations in the eastern and southern United States.

Cover Image: Chapter 6, Figure 6-2.

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In Brief

Smith, Richard H. 2000. **Xylem monoterpenes of pines: distribution, variation, genetics, function**. Gen. Tech. Rep. PSW-GTR-177. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture; 454 p.

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 classes 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 may be 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.

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