

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

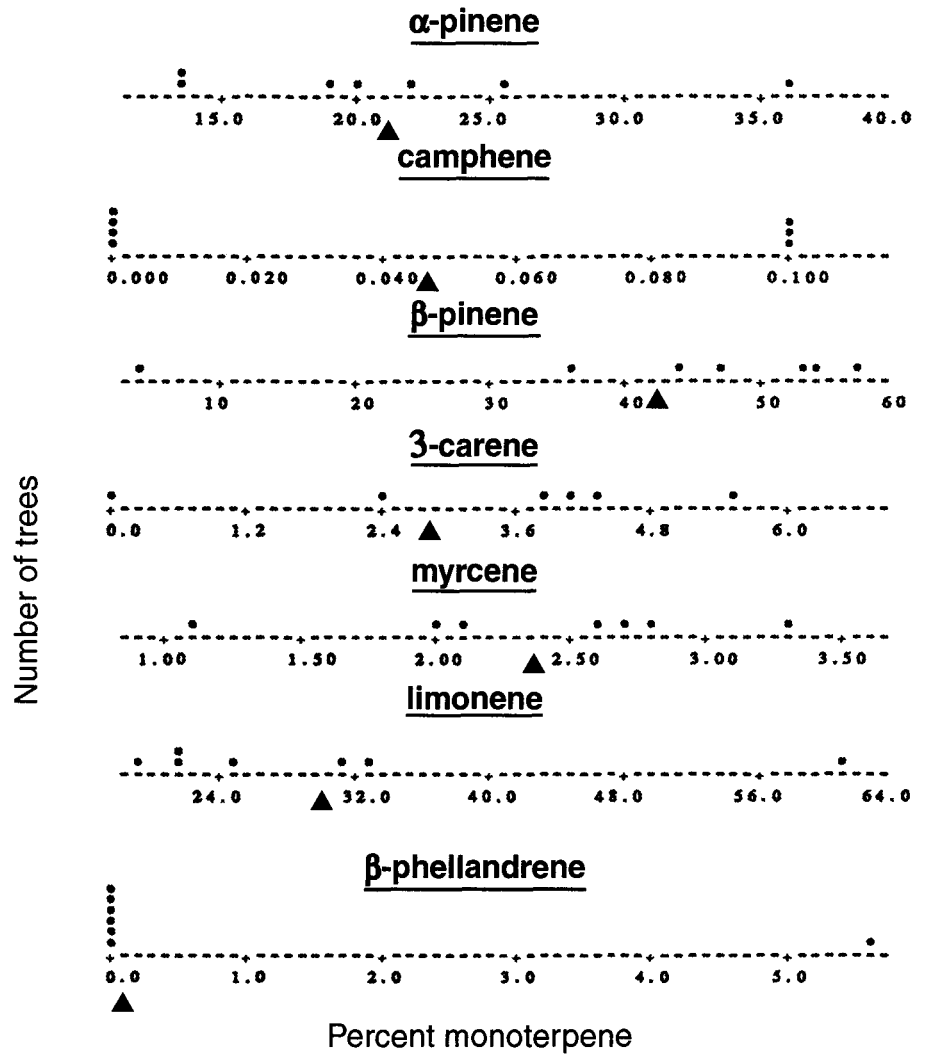
This was a cross of a near-high with a high limonene tree that were growing near each other.

Normalized percent compositions of parents and progeny were as follows:

<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-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.

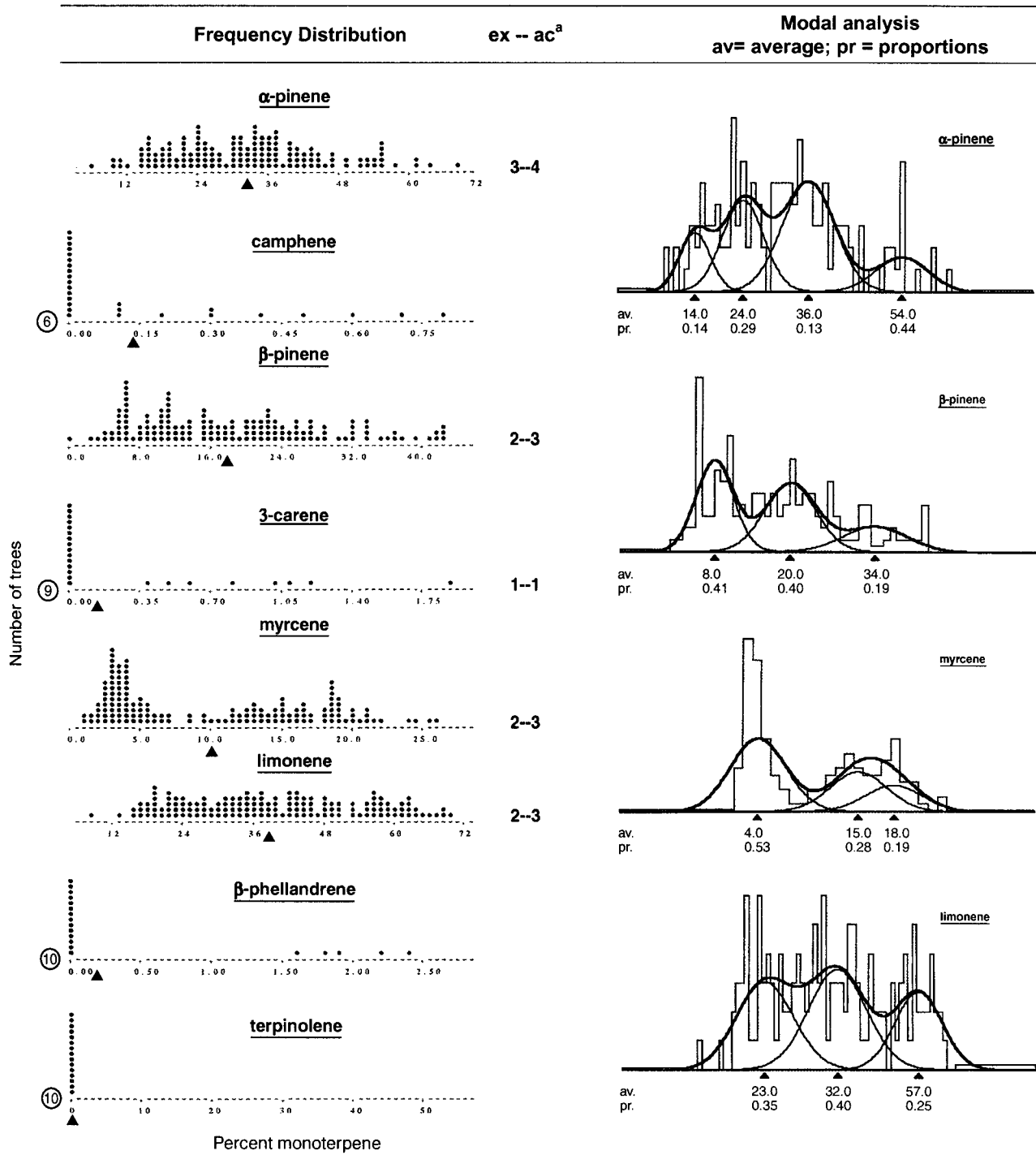
Male parent	Growth place ¹	N	Monoterpene composition of progeny ²							
			α -p	cam	β -p	car	myr	lim	β -ph	ter
			<i>percent</i> ³							
AS-3139	nur	145	32.2	0.1	18.2	0.1	10.0	39.3	0.1	0
AS-3139	con	16	25.2	0.1	34.7	9.5	5.6	23.9	0.3	0.5
AS-3139	copl	17	43.9	0.1	29.2	0	7.6	18.7	0.4	0
WP-3347	nur	71	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.

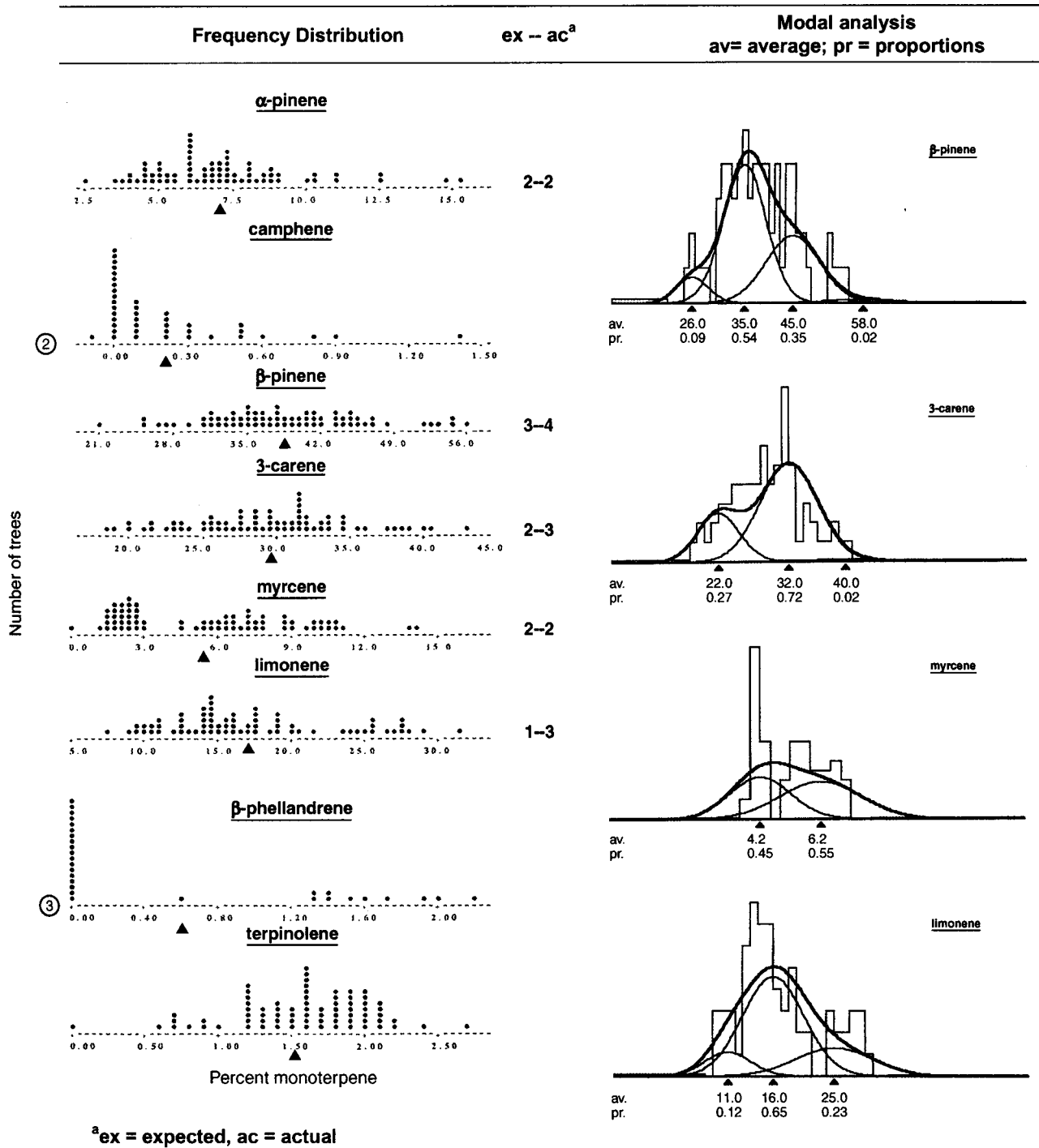
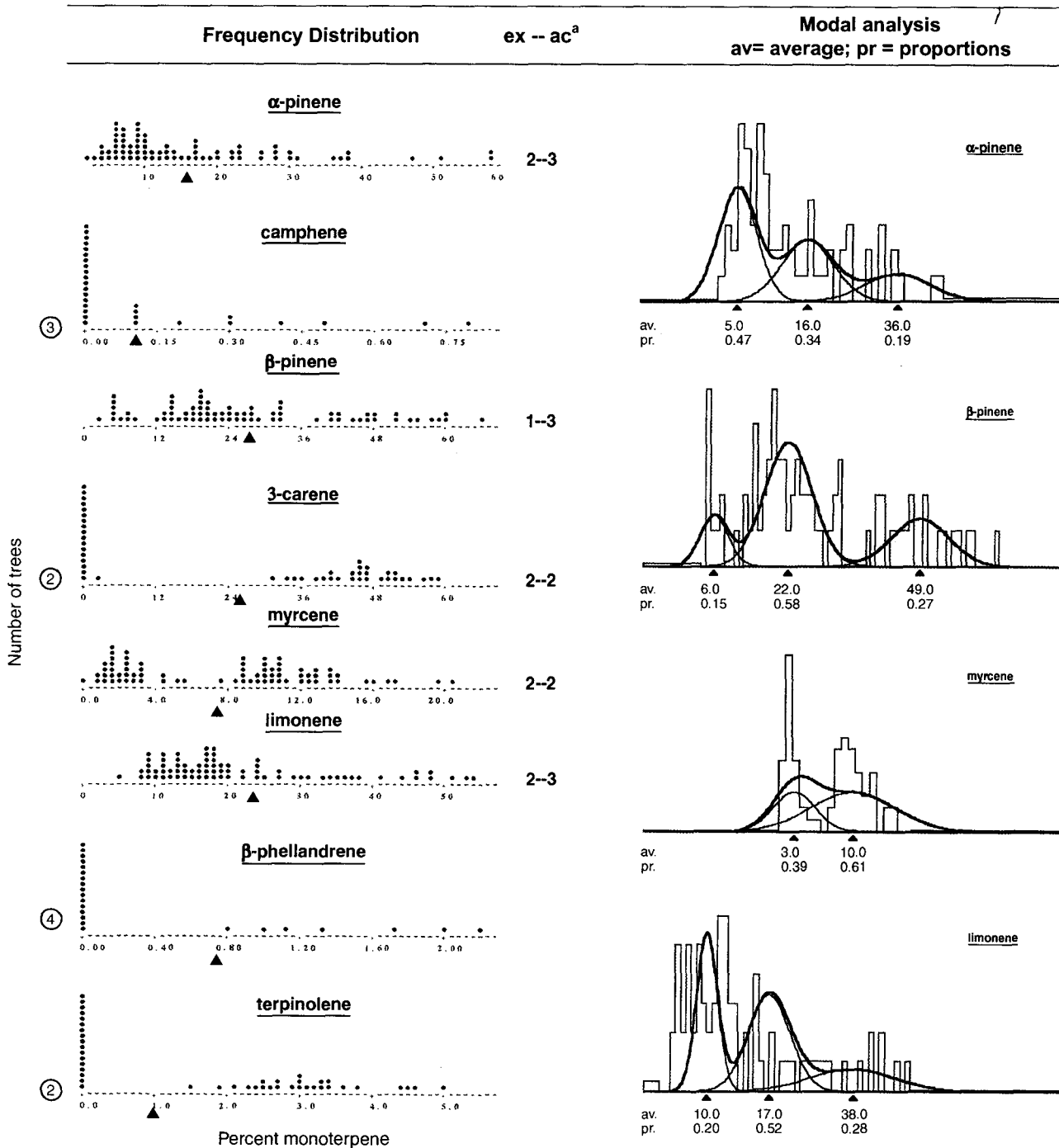


Figure 6-30—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-5789 x AS-3 194; see caption of figure 6-2 for explanation of the format below.



^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

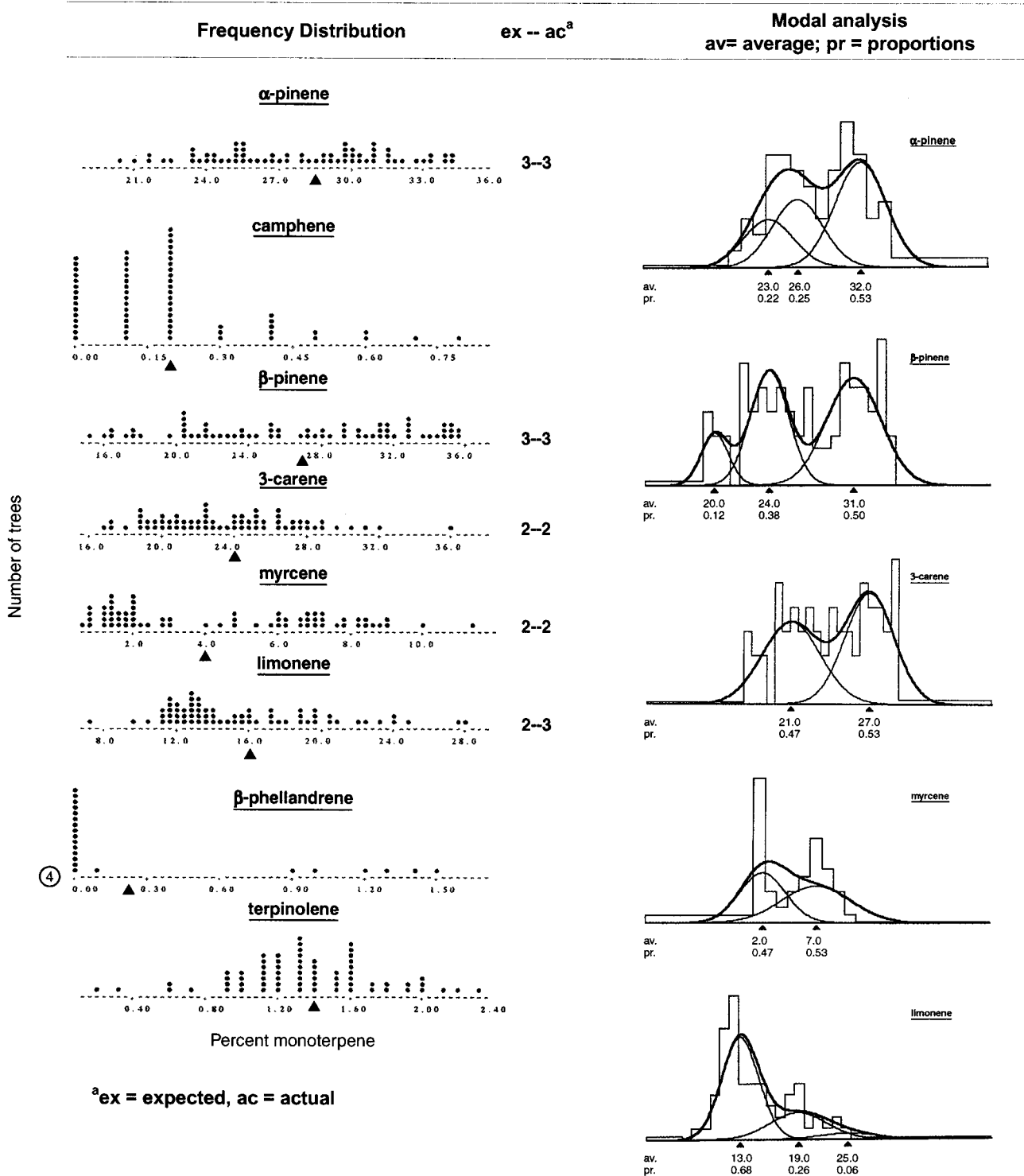
This was another cross of near-high limonene with high limonene, as in the previous cross; however the parents were about 400 miles apart.

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.

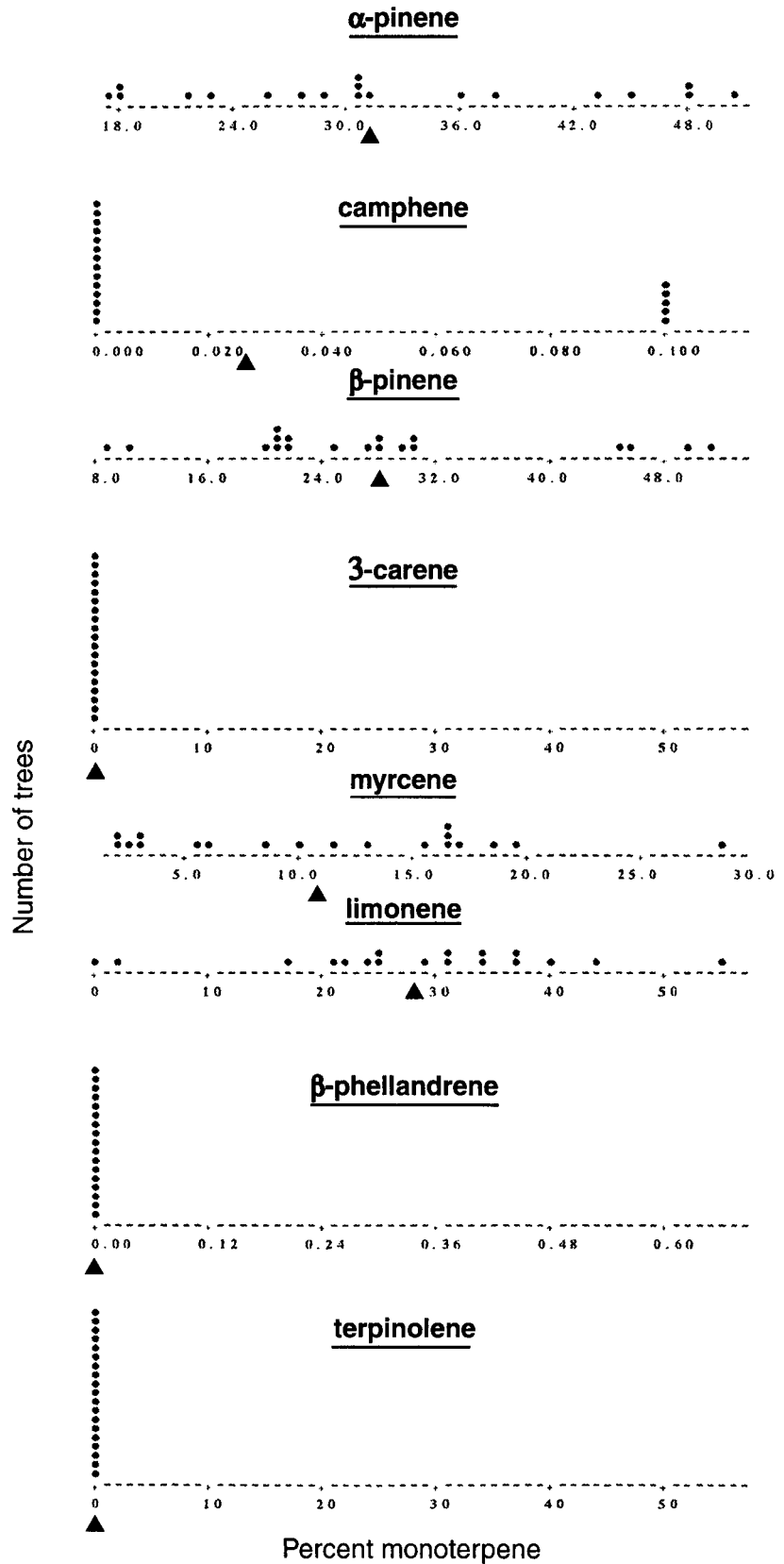
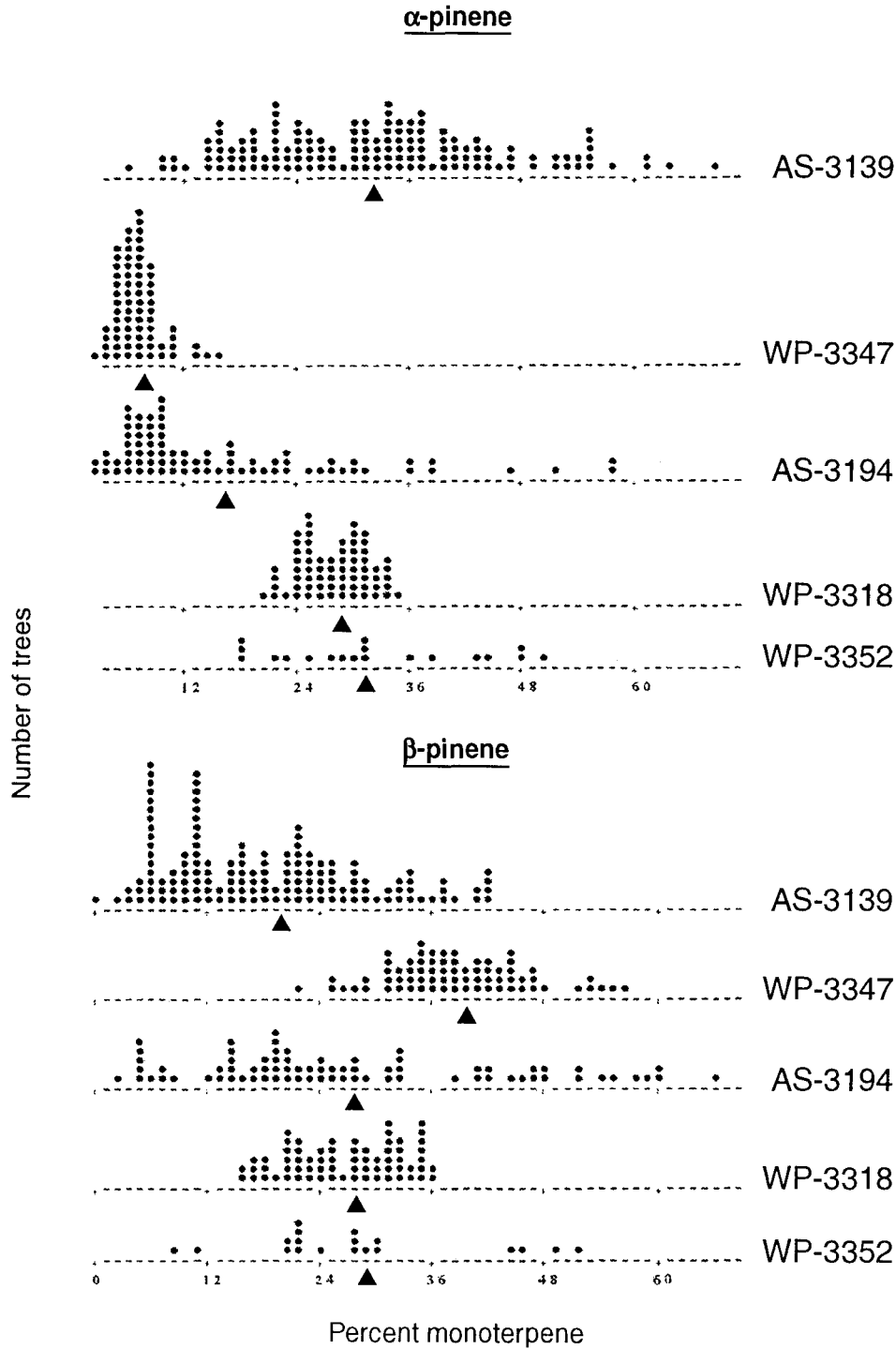
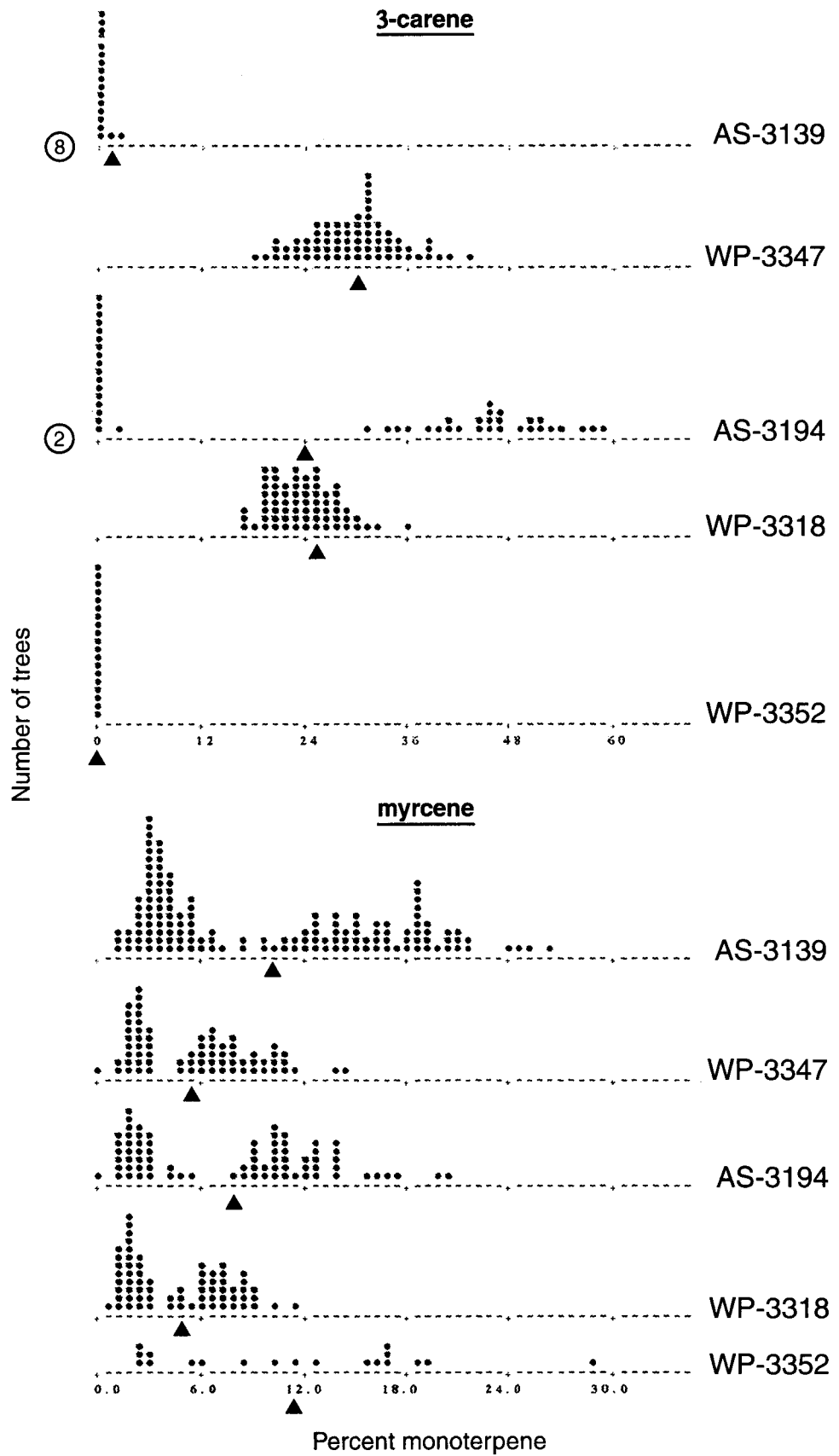


Figure 6-33—Comparative frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-5789 when crossed with the five males listed on the right; each dot is measurement of one tree except where \bar{n} indicates each is n trees; [▲] is average of set.

(fig 6-33 continues on next 3 pages)





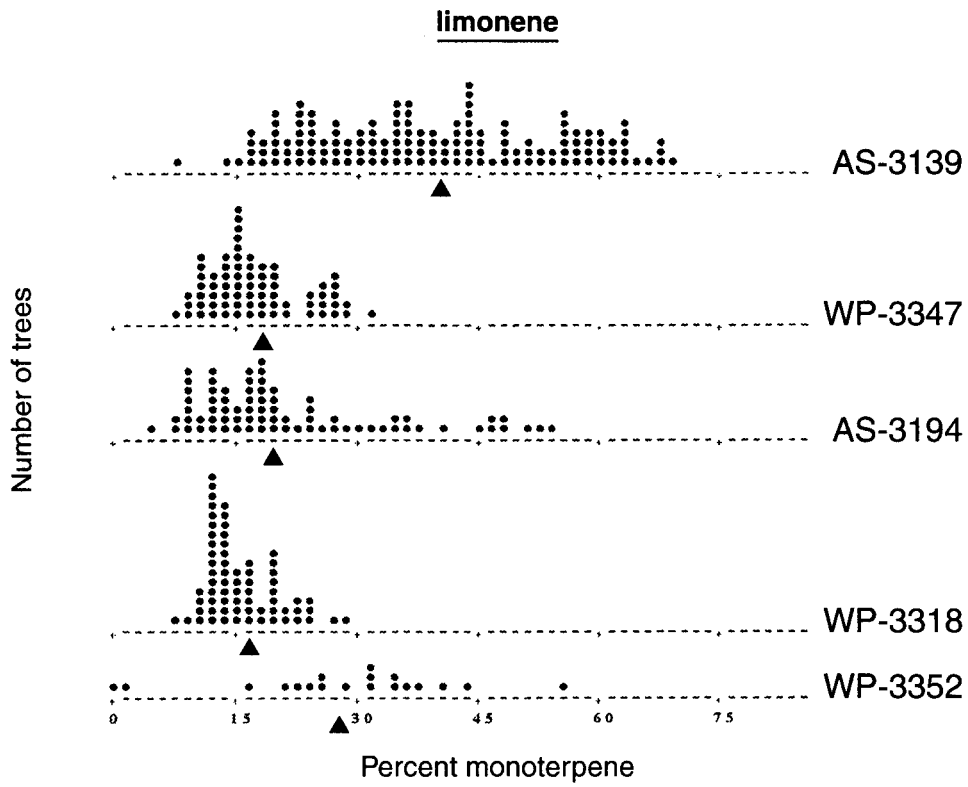


Table 6-9—Ratio of limonene in male parent tree to average limonene in four sets of progeny of AS-5789: two sets with nearby male (AS-3139, AS-3194), two sets with distant males (WP-3347, WP-3318)

Male tree	Distance: female-male <i>miles</i>	Limonene in:		Ratio for limonene: progeny to male
		male	progeny	
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:

<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
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 β -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:

<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
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.

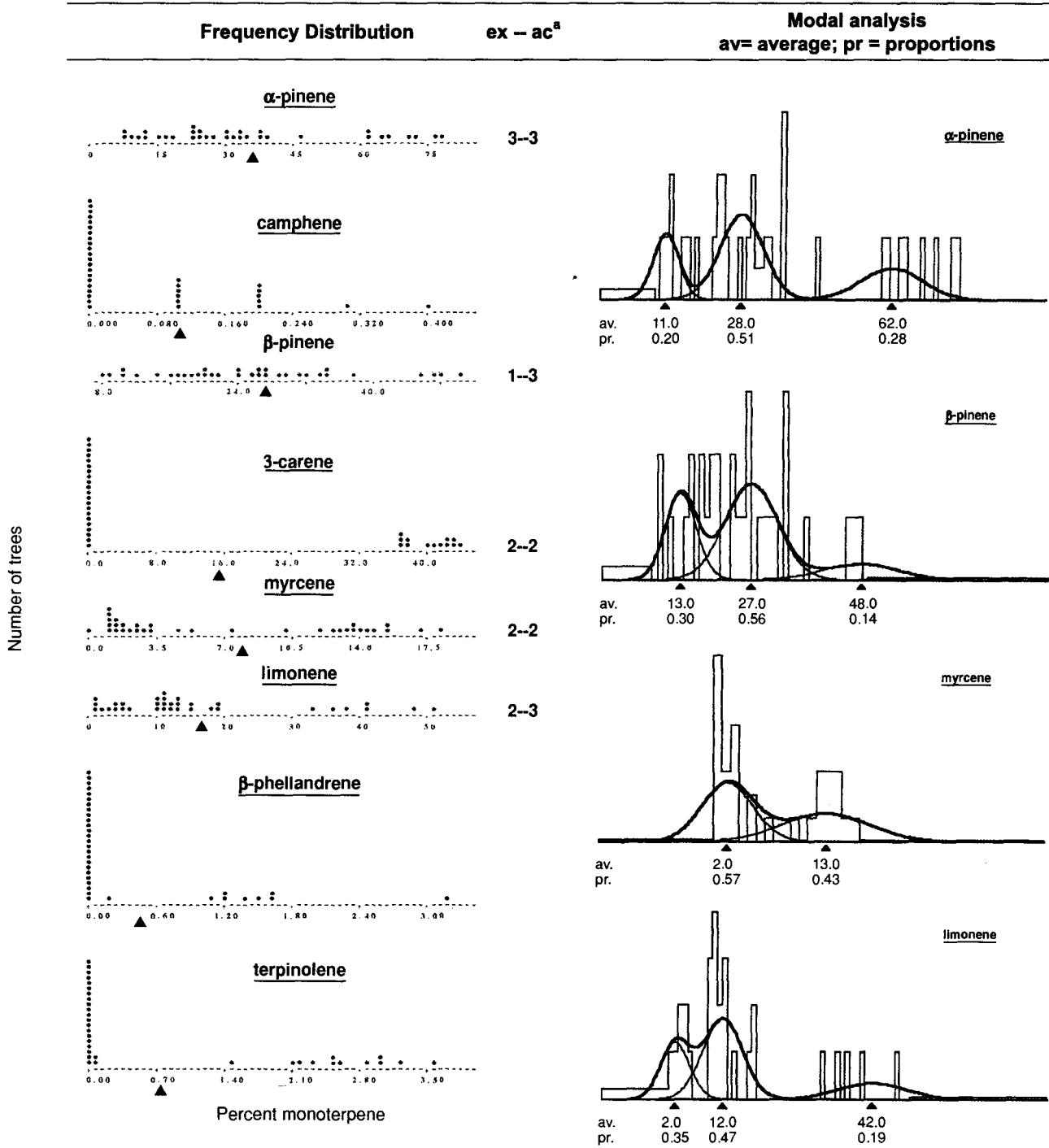
Male parent	N	Place ¹ of growth	Monoterpene composition of progeny ²							
			α -p	cam	β -p	car	myr	lim	β -ph	ter
			<i>percent</i> ³							
AS-5789	34	nur	34.8	0.1	25.4	15.3	7.2	15.9	0.4	0.9
AS-5789	6	con	24.4	0.1	39.6	24.4	4.8	3.9	0.8	1.9
AS-5789	4	copl	51.4	*	26.3	9.6	7.1	4.2	0.6	0.8
AS-A95	8	con-4	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

¹nur = nursery; con = container; copl = container plantation.

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

³Normalized; * = trace.

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.

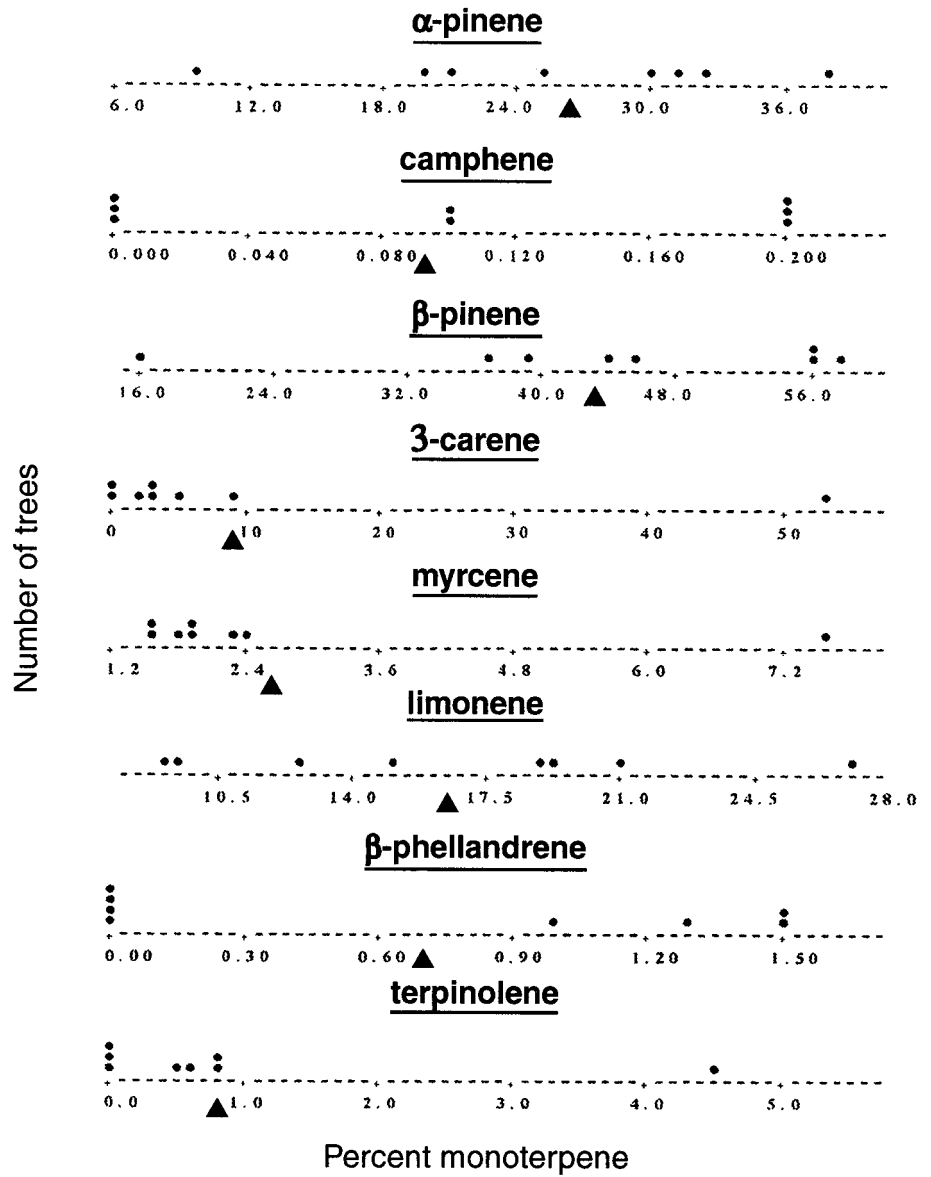
Discussion: Full Siblings of Tree AS-A295

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.



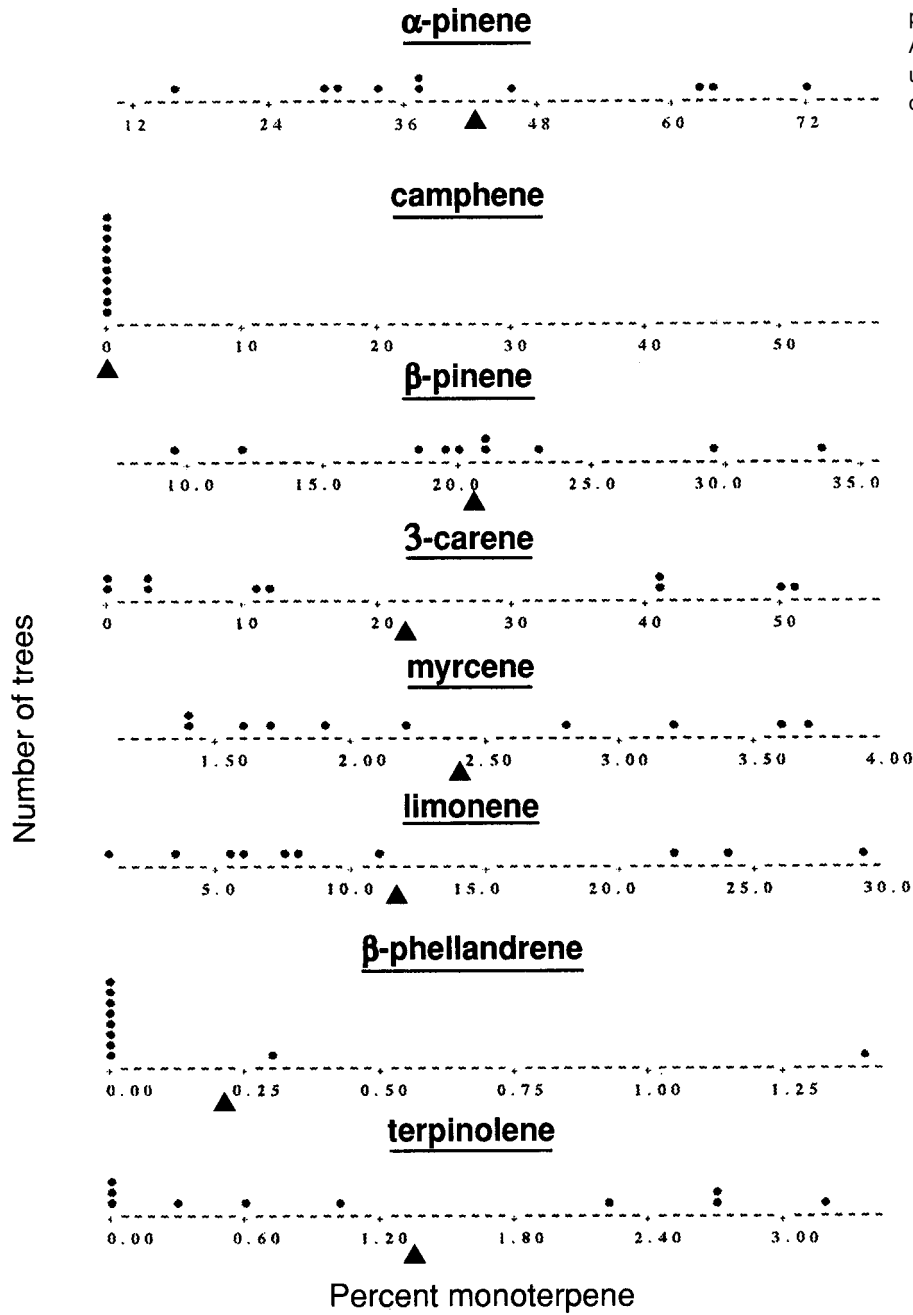


Figure 6-36—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-A295 x WP-3298; each dot is measurement of one tree; [▲] is average of set.

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	N	Monoterpene composition of progeny ¹							
			α -p	cam	β -p	car	myr	lim	β -ph	ter
			percent 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.²Normalized; * = 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.

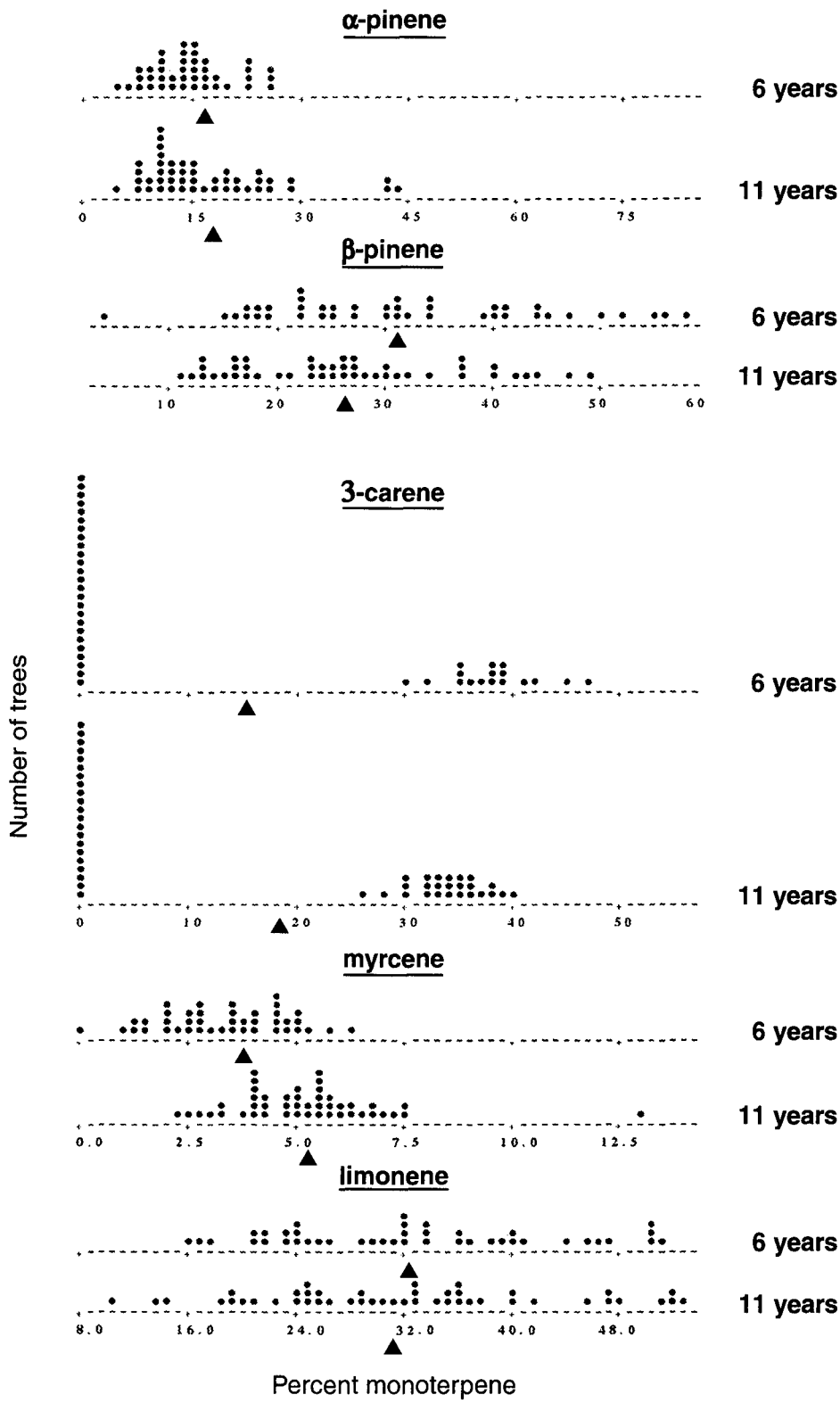
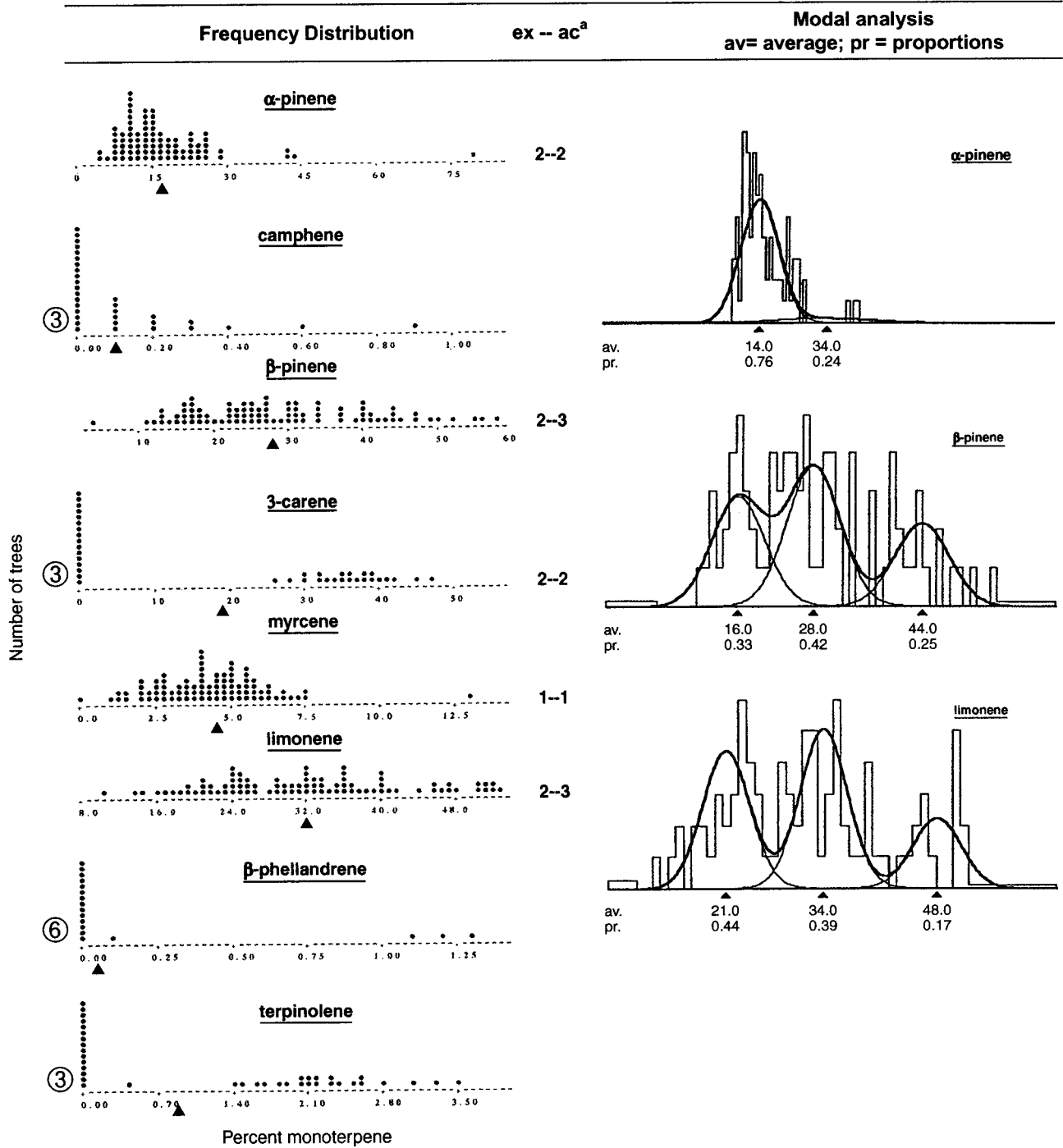


Figure 6-37—Comparative frequency distribution of the noted monoterpenes in the progeny of *P. ponderosa* AS-3187 x AS-3139 for two periods (years) of nursery growth listed on the right; each dot is measurement of one tree; [▲] is average of set.

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.

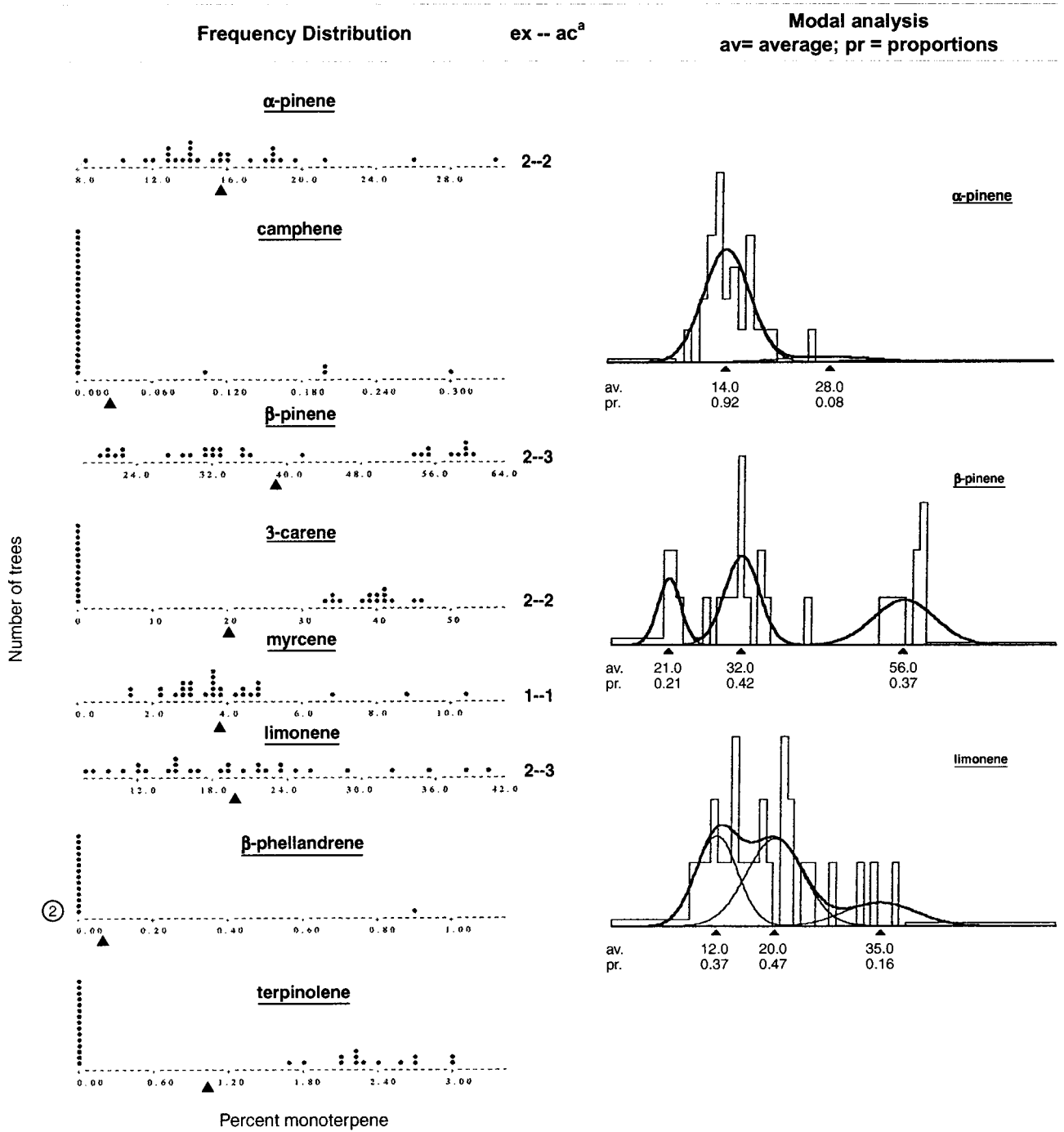


Figure 6-40—Comparative frequency distribution of the noted monoterpenes in the progeny of *P. ponderosa* AS-3187 x WP-3298 for two periods (years) of nursery growth listed on the right each dot is measurement of one tree; [▲] is average of set.

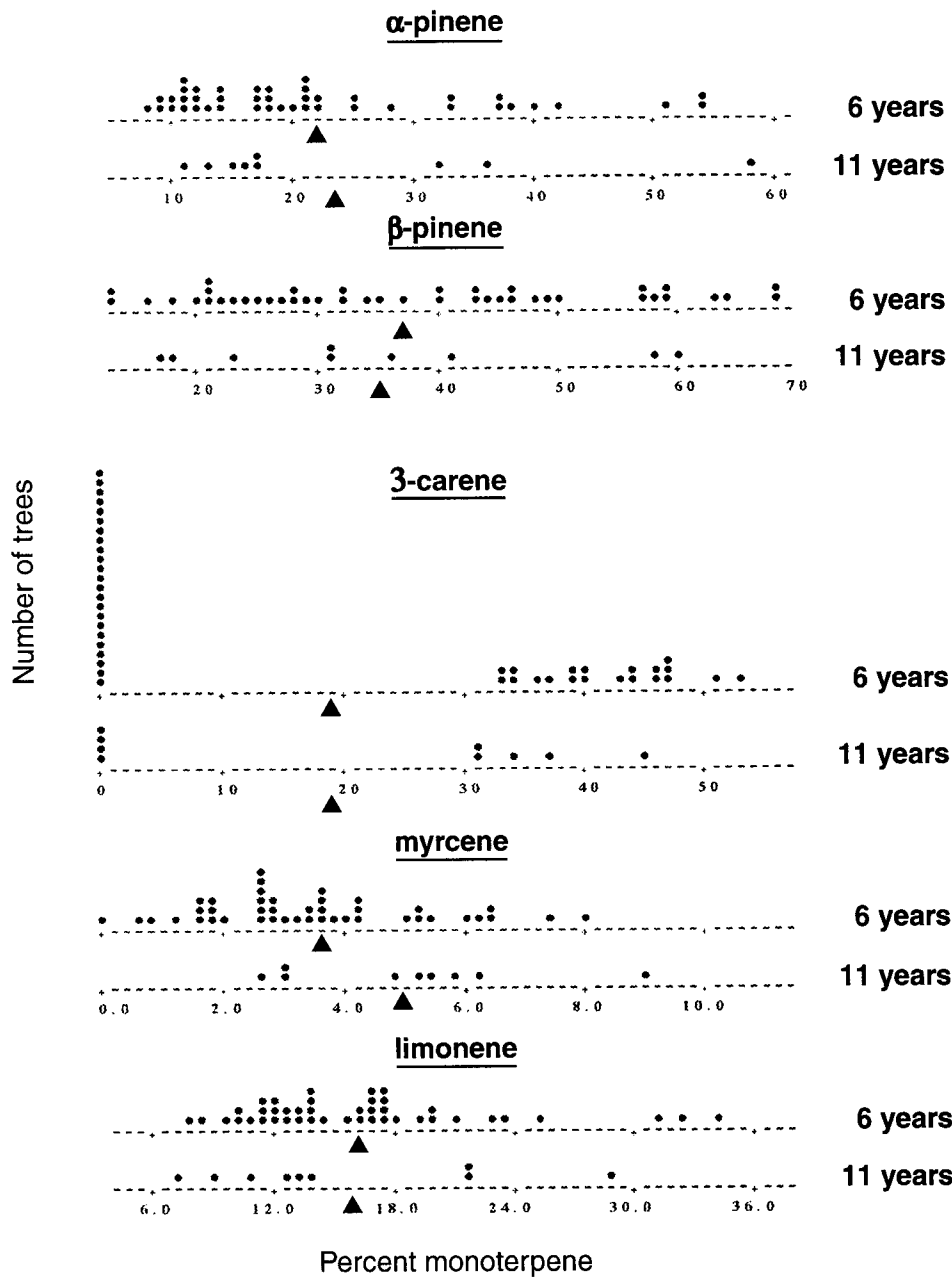
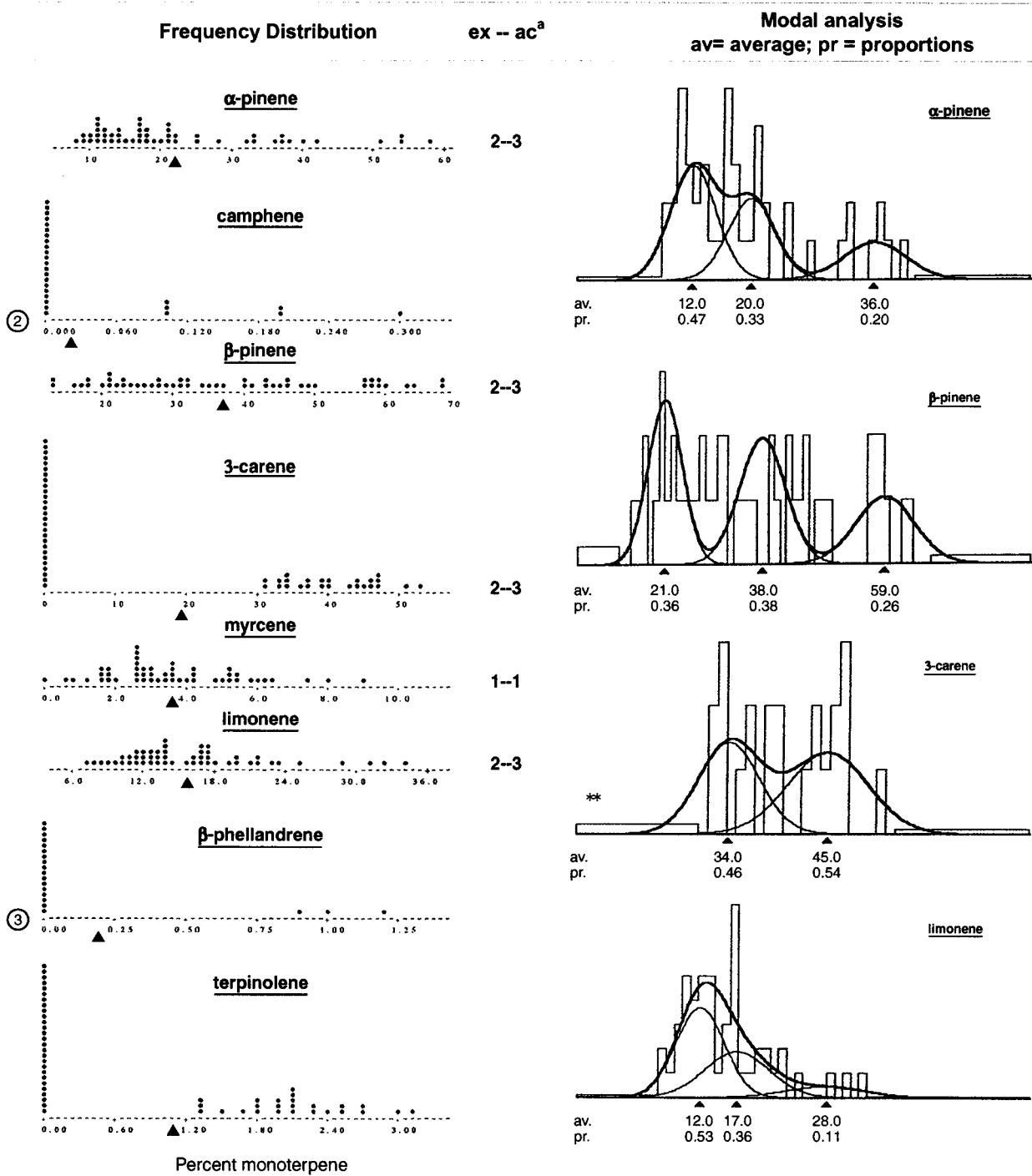


Figure 6-41—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x WP-3298; see caption of figure 6-2 for explanation of the format below; ** = mode at zero was not used in the modal analysis, but was included in the mode count.



^aex = expected, ac = actual

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

Figure 6-42—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x WP-3299 ; 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.

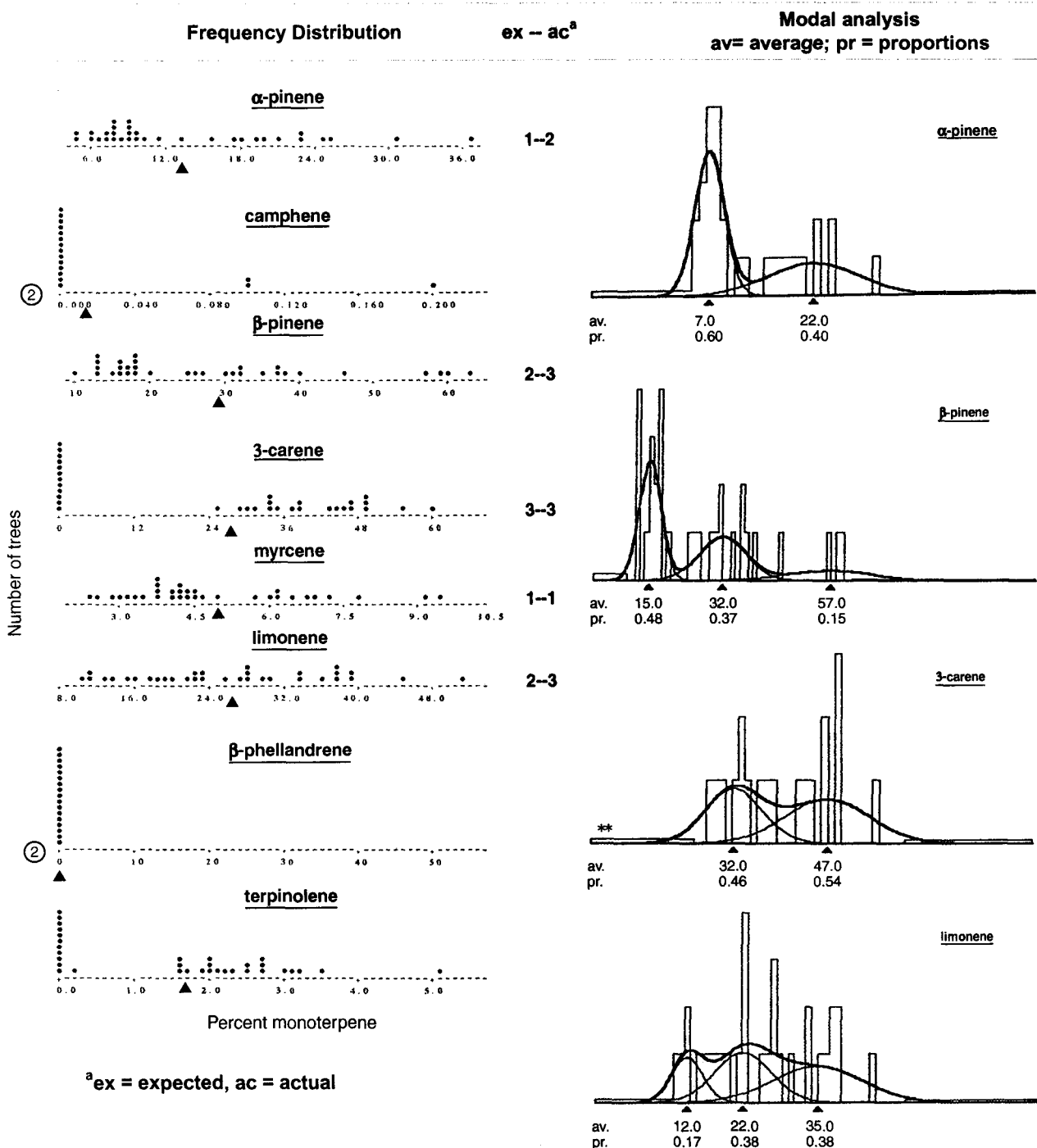


Figure 6-43—Comparative frequency distribution of the noted monoterpenes in the progeny of *P. ponderosa* AS-3187 x AS-3194 for two periods (years) of nursery growth, listed on right; each dot is measurement of one tree; [▲] is average of set.

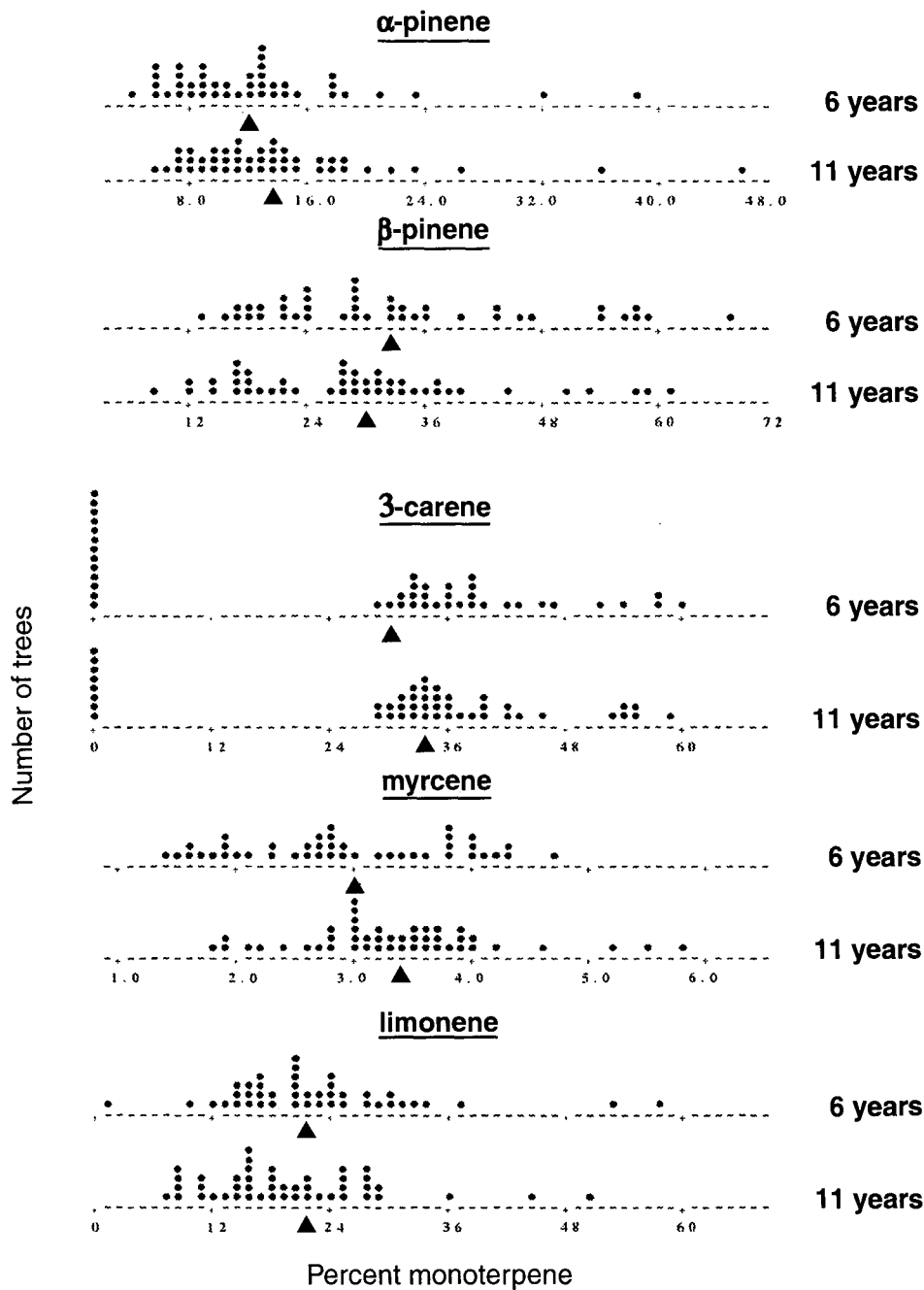
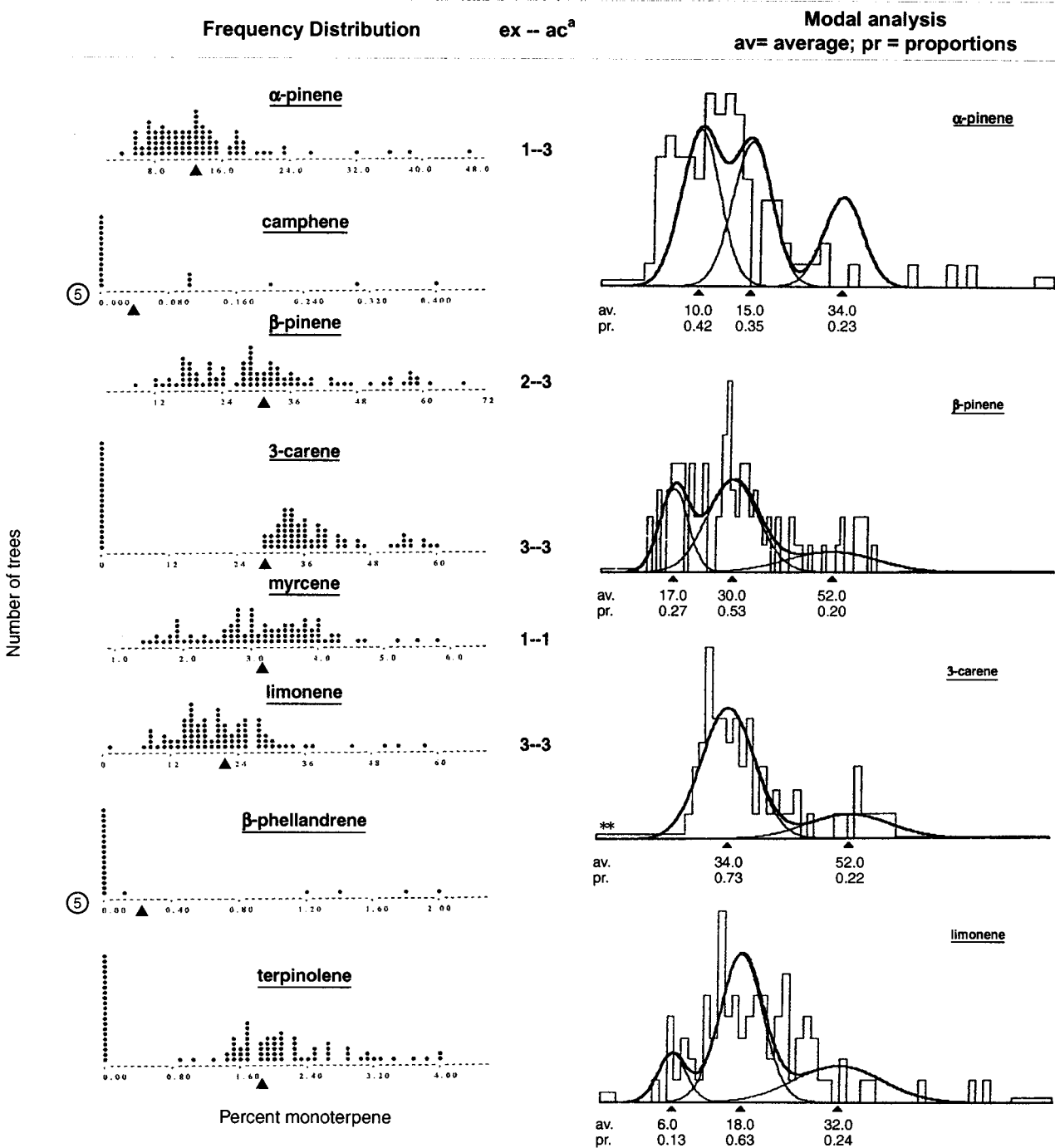


Figure 6-44—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* AS-3187 x AS-3194; see caption of figure 6-2 for explanation of the format below; ** = mode at zero was not used in the modal analysis, but was included in the mode count.



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.

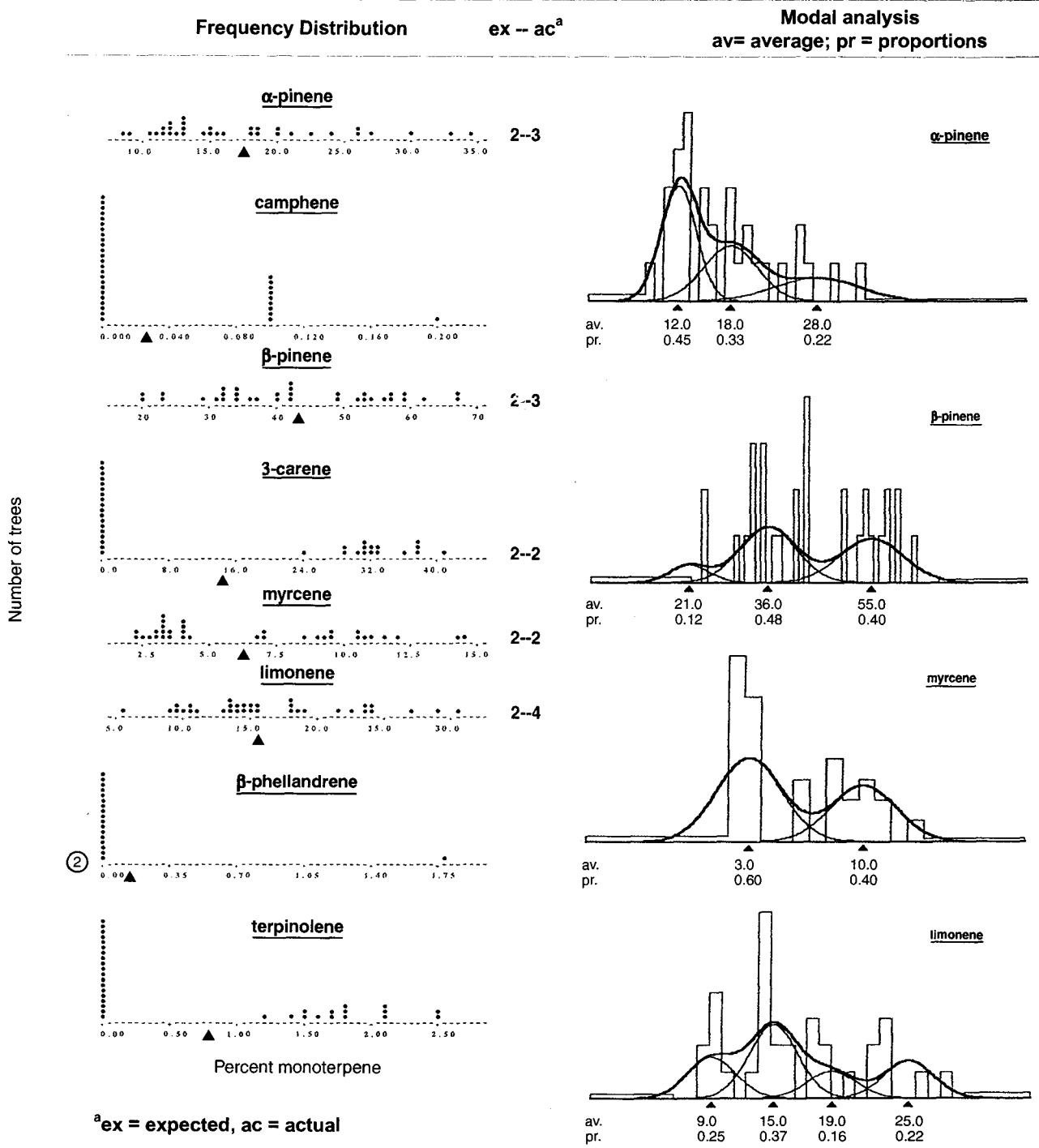
Normalized percent compositions of parents and progeny were as follows:

<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

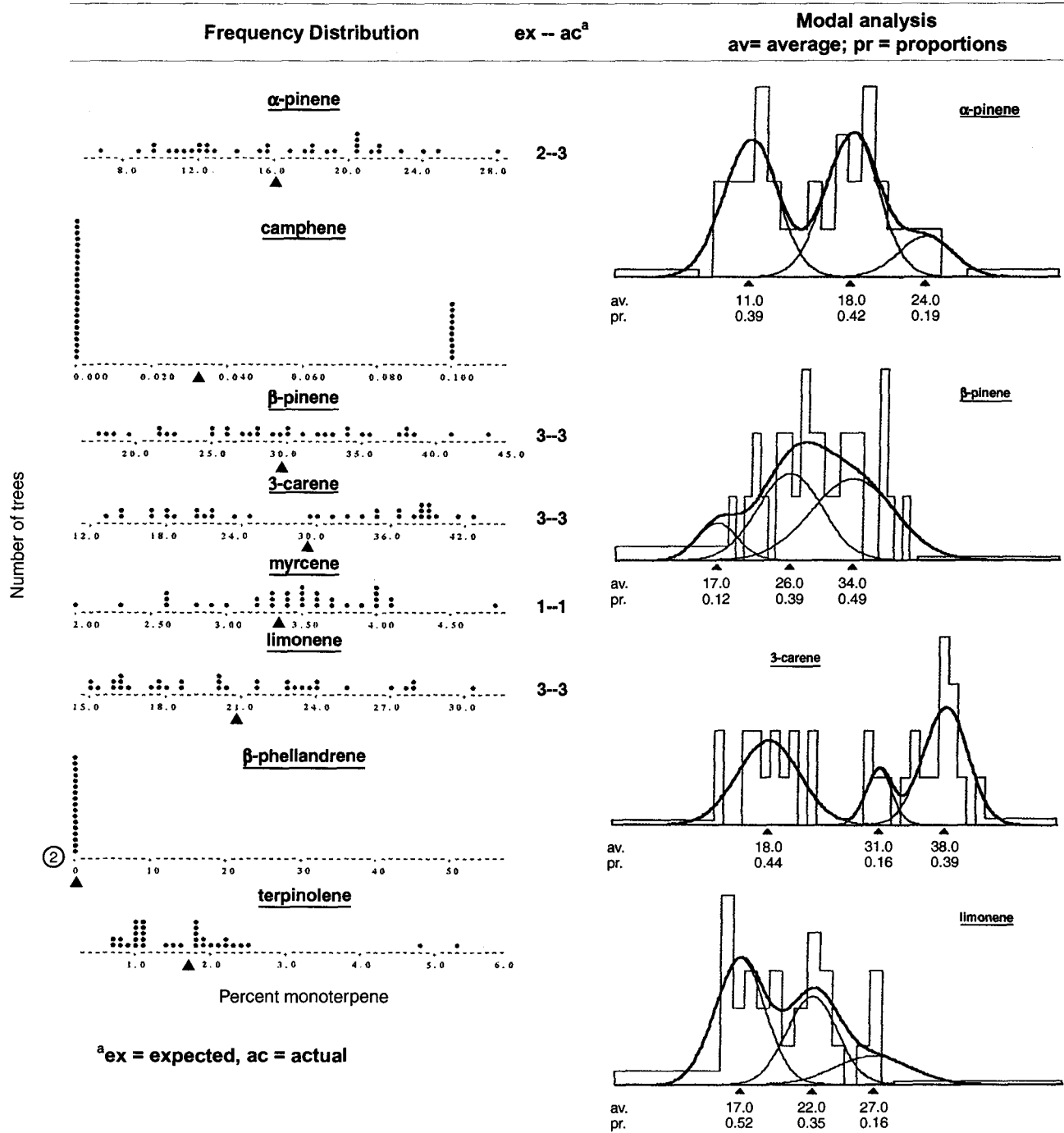
Tree WP-3312 was also classed as near-high limonene.

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

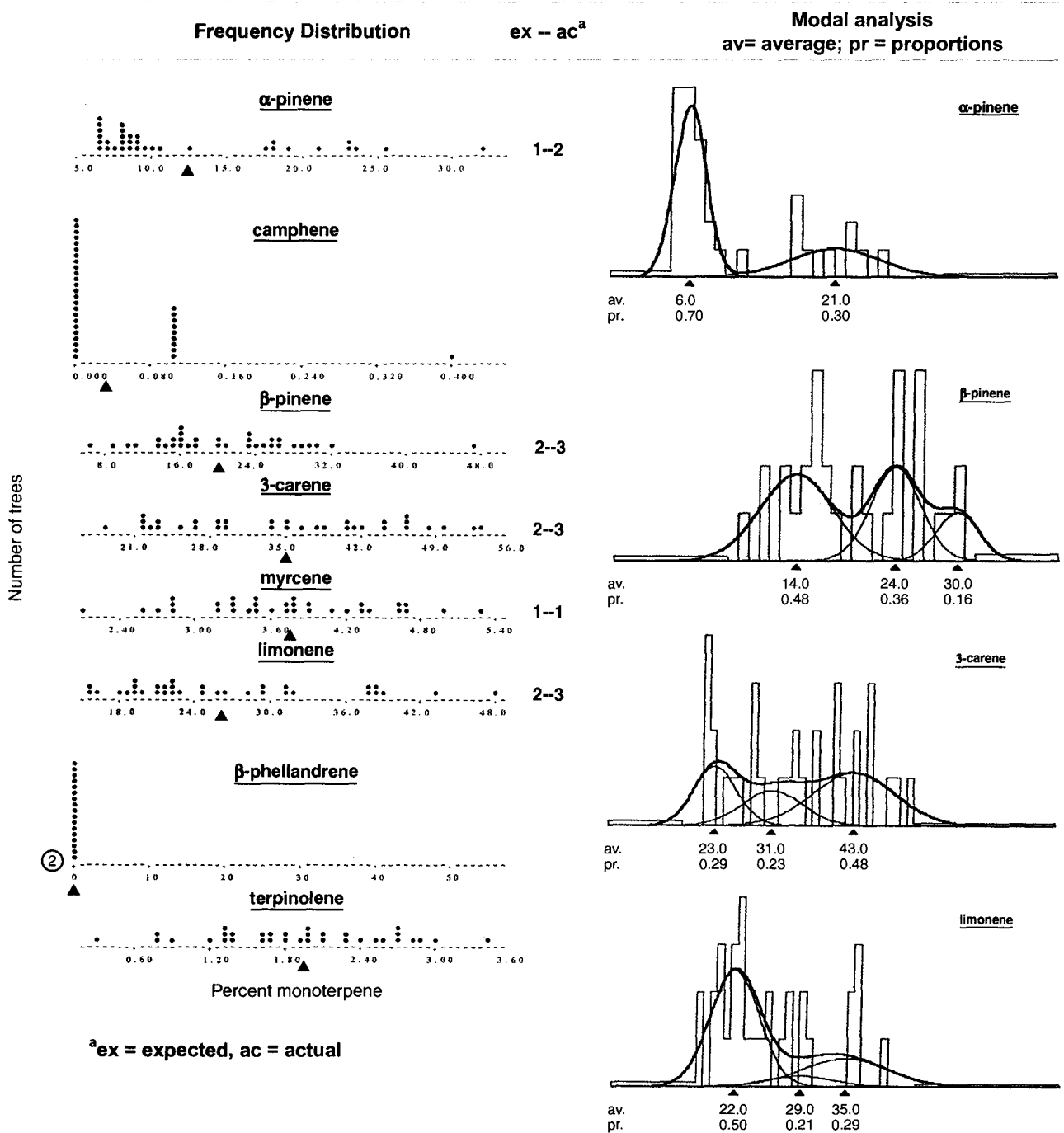
Tree WP-3347, growing at Wheeler Peak, was classed as near-high limonene.

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.



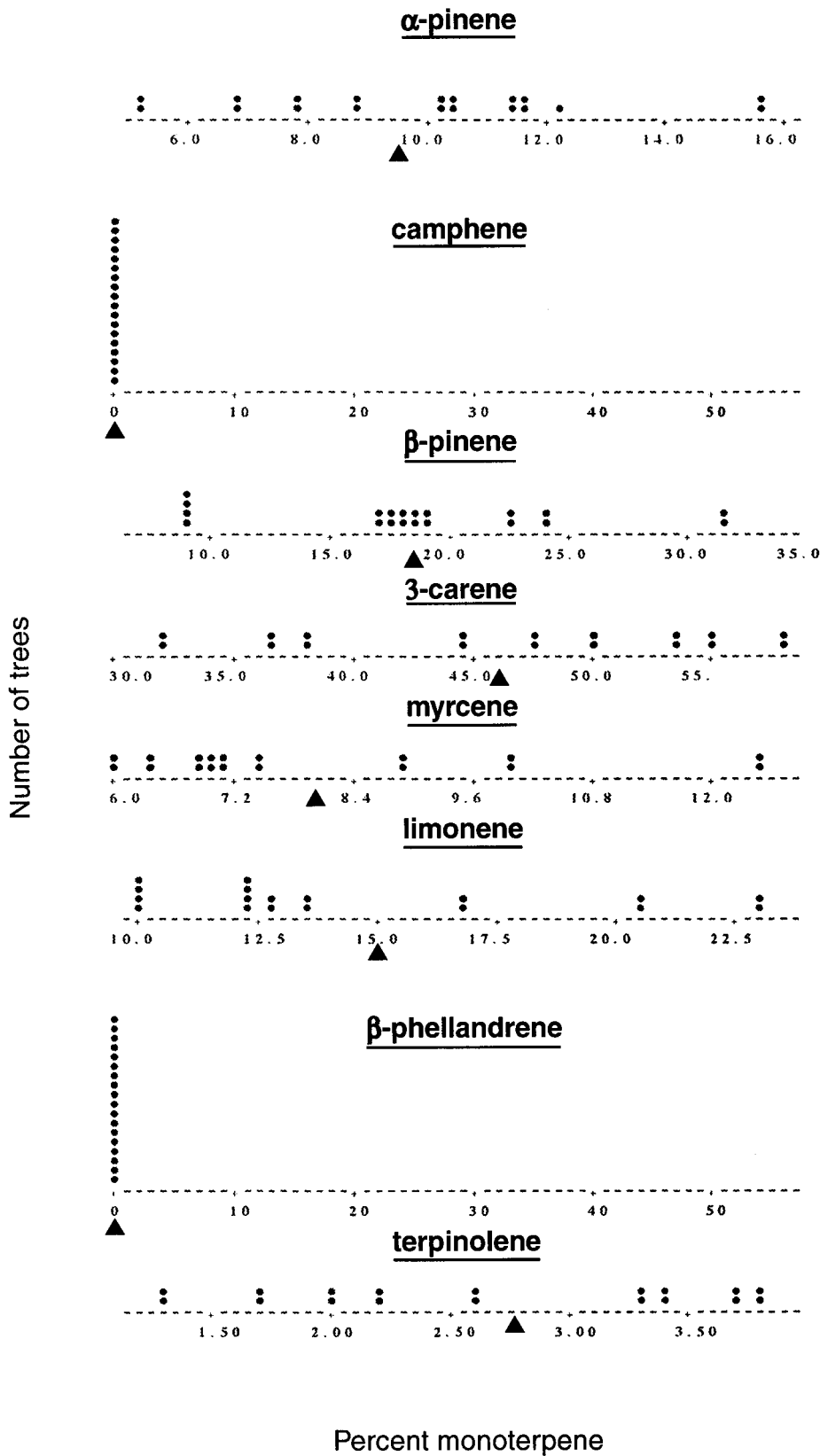
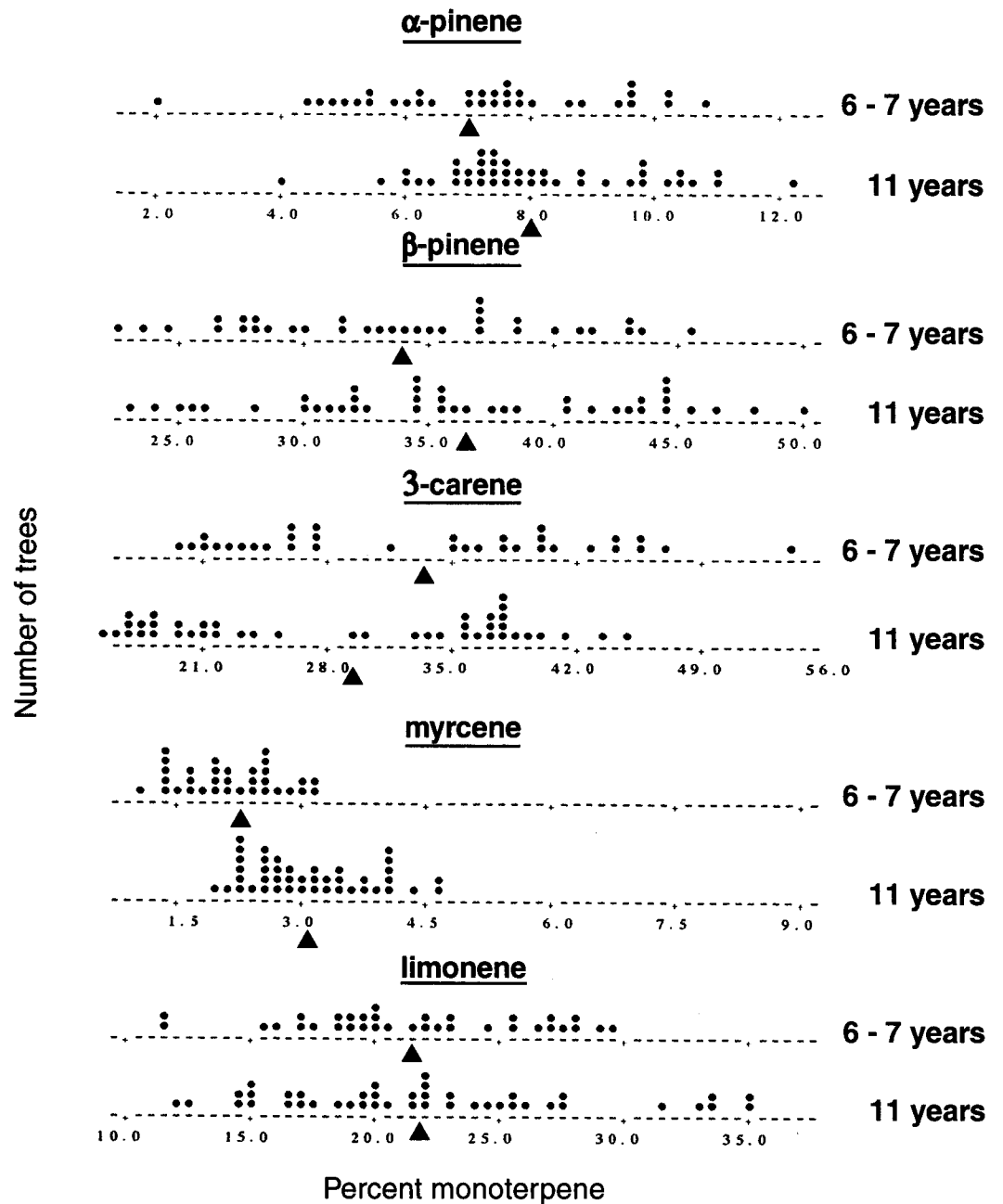


Figure 6-48—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* AS-3187 x AS-3178; each dot is measurement of one tree; [▲] is average of set.

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.

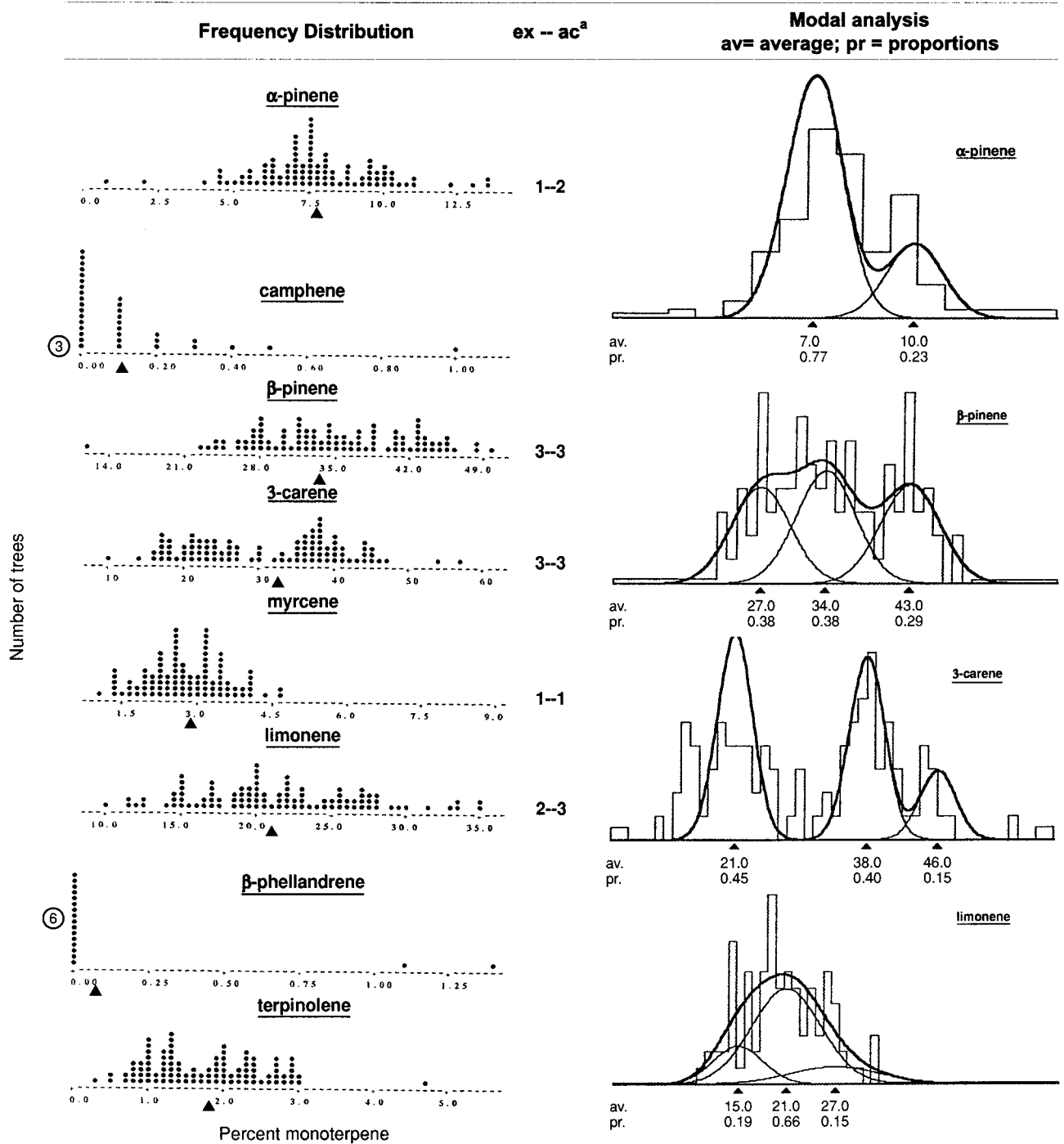
The average for limonene and the number of high limonene trees were much higher in sets of progeny with nearby males than with distant males. Again, a stress factor and the distance between parents were postulated as the causes.

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.

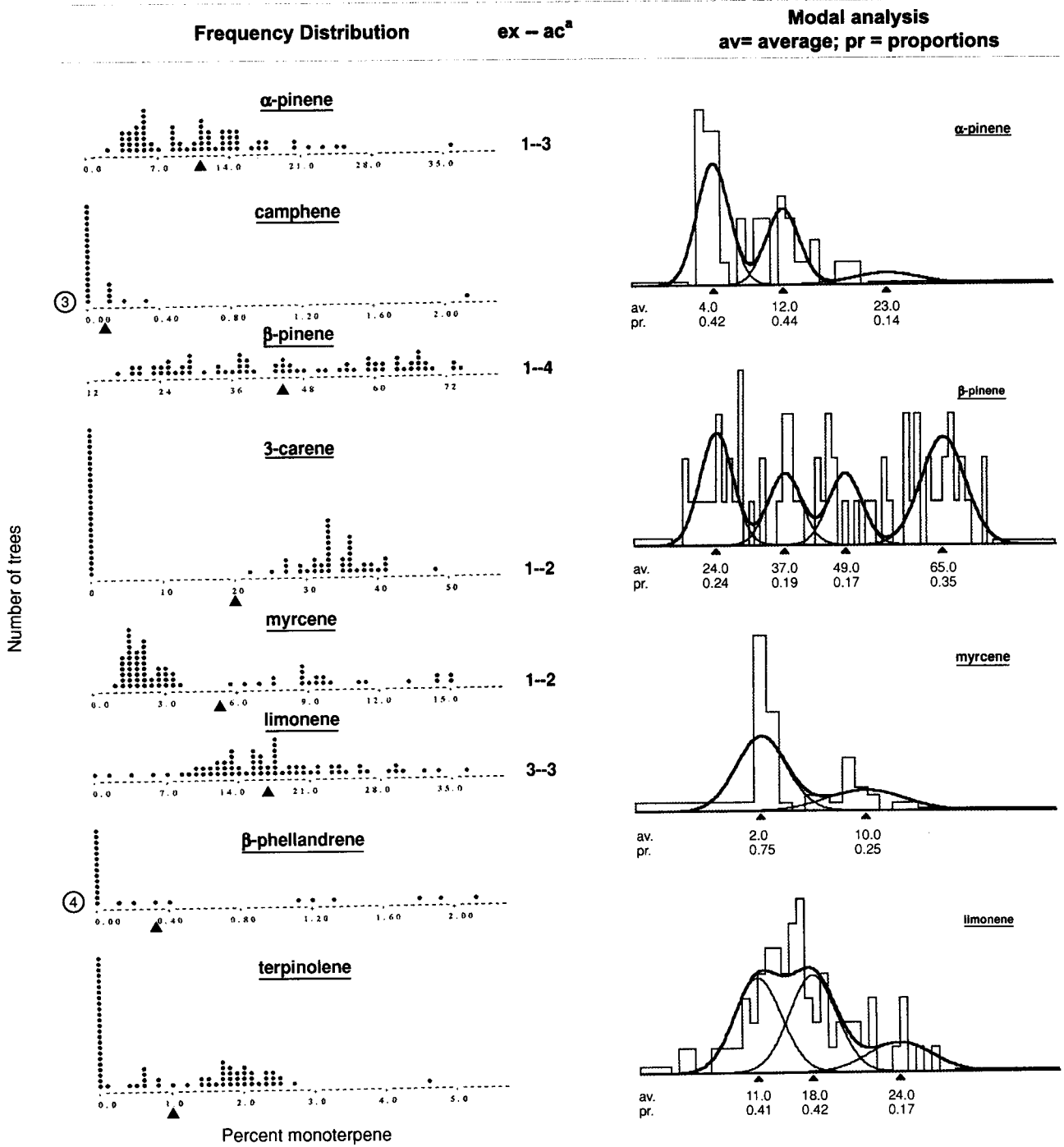
The frequency distribution of 3-carene had two modes, one at 0 and one at about 30 percent, where one mode was expected; i.e., with a (1-1) set of alleles for 3-carene in AS-3178, all progeny should have at least one allele for 3-carene. The one mode at 30 percent was expected, but not the mode at 0. 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
			percent 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.



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.

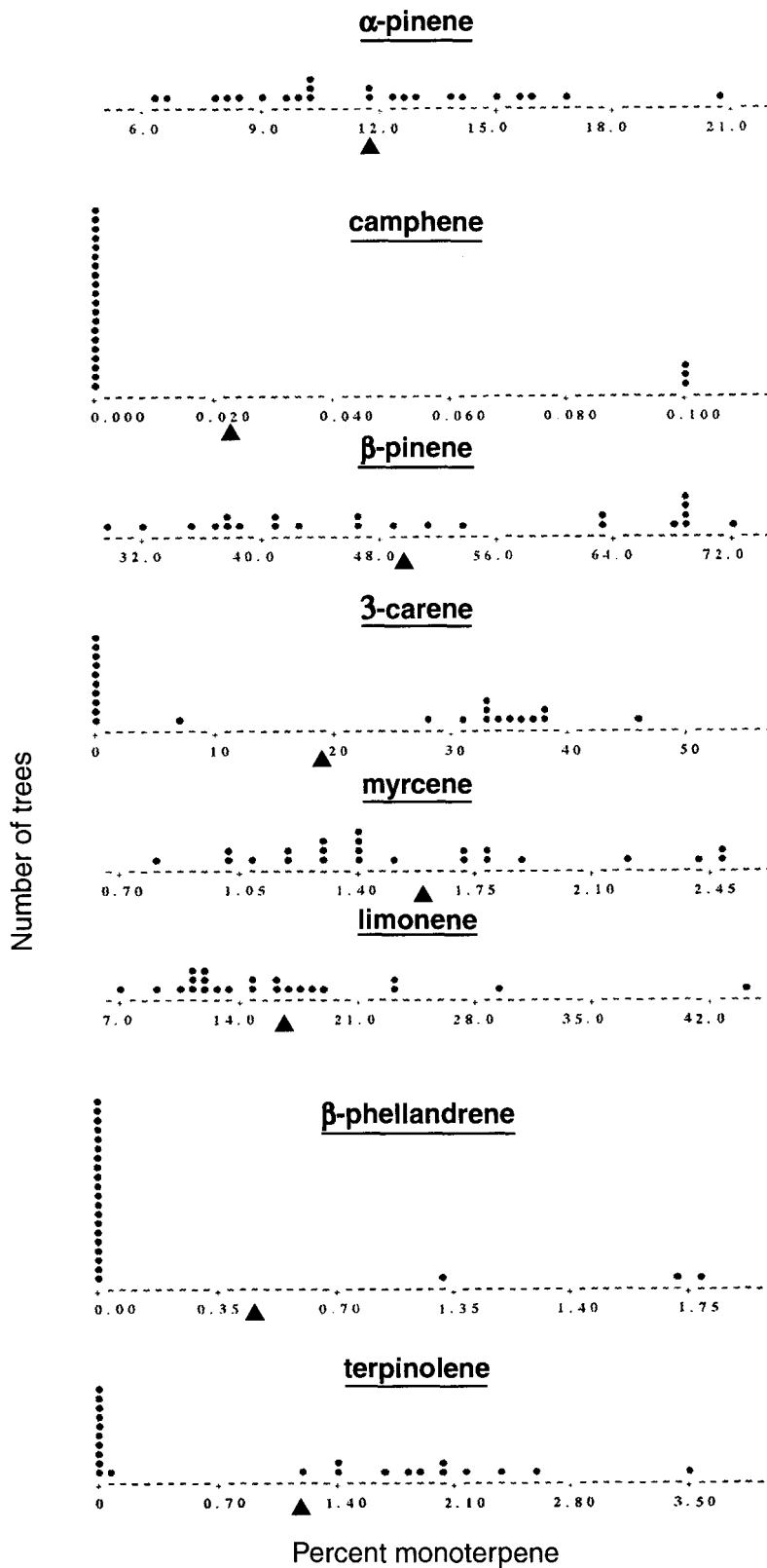
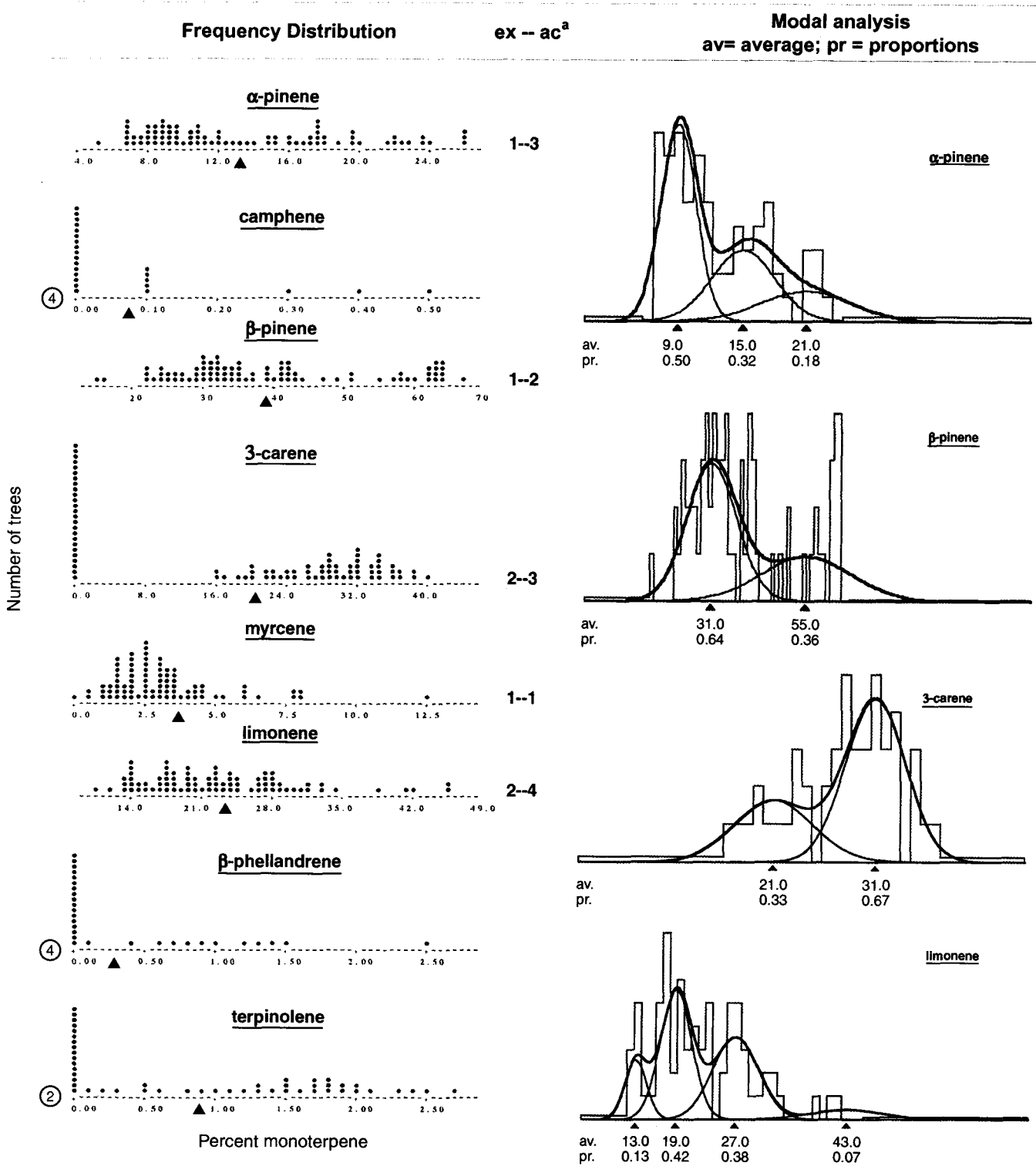


Figure 6-52—Frequency distribution of the amount of the noted monoterpenes in the progeny of *P. ponderosa* IFG-4B63 x AS-3194; each dot is measurement of one tree; [▲] is average of set.

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.



^aex = expected, ac = actual

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.

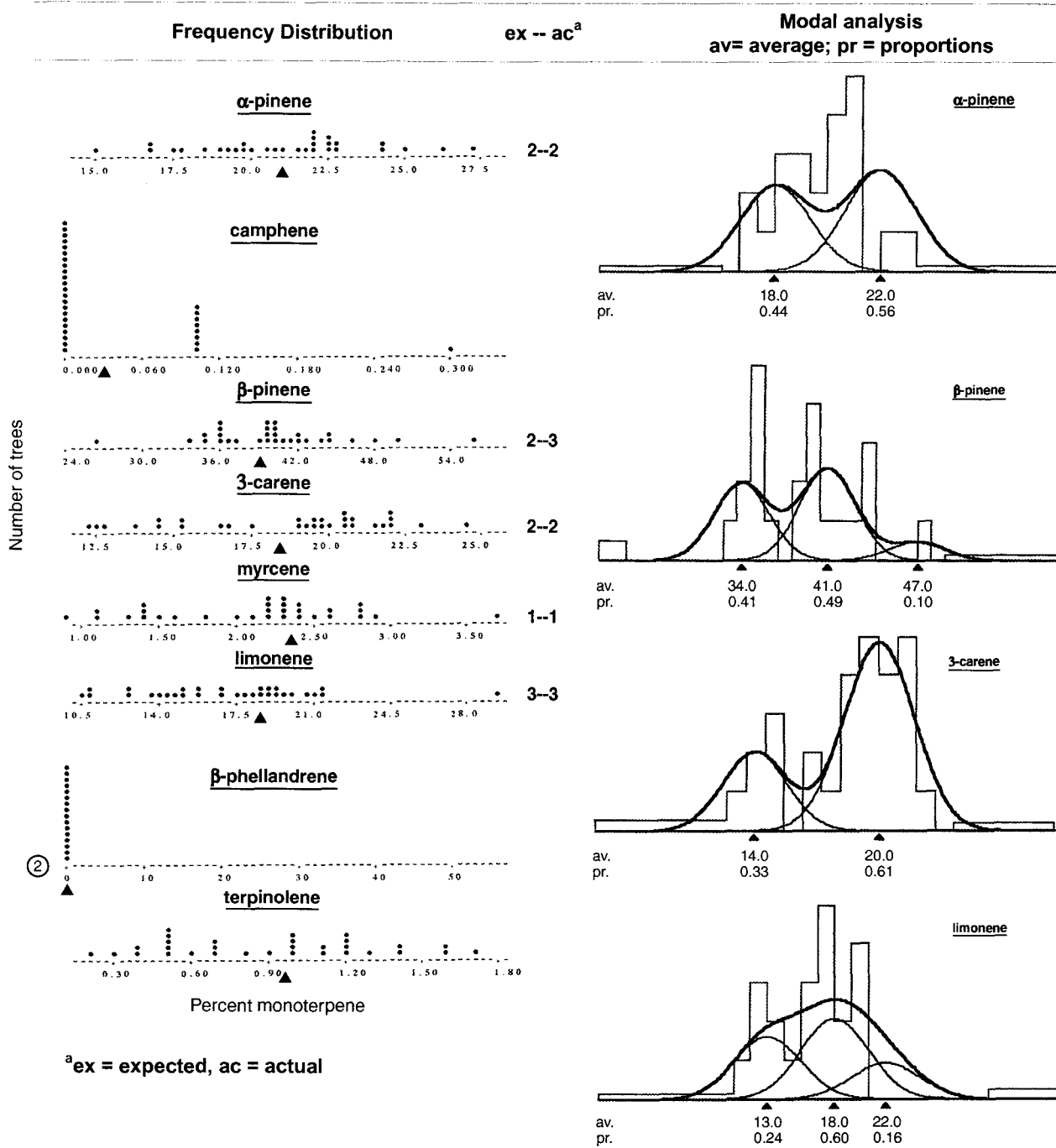
Normalized percent compositions of parents and progeny were as follows:

<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

Figure 6-54—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* IFG-4B63 x WP-3318; see caption of figure 6-2 for explanation of the format below.



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

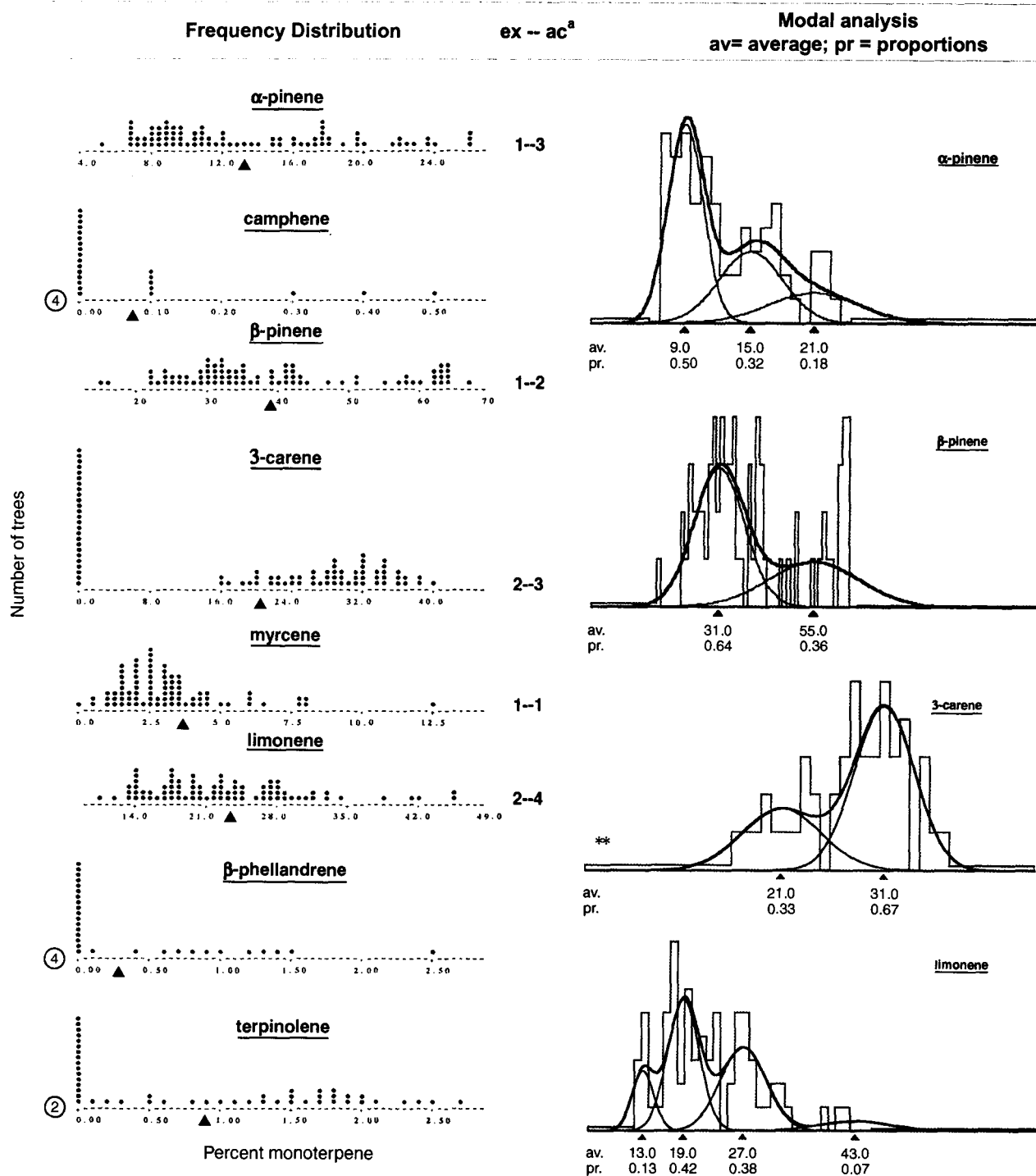
Tree AS-5789, like Tree IFG-4B63, had no 3-carene, but had near-high limonene.

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

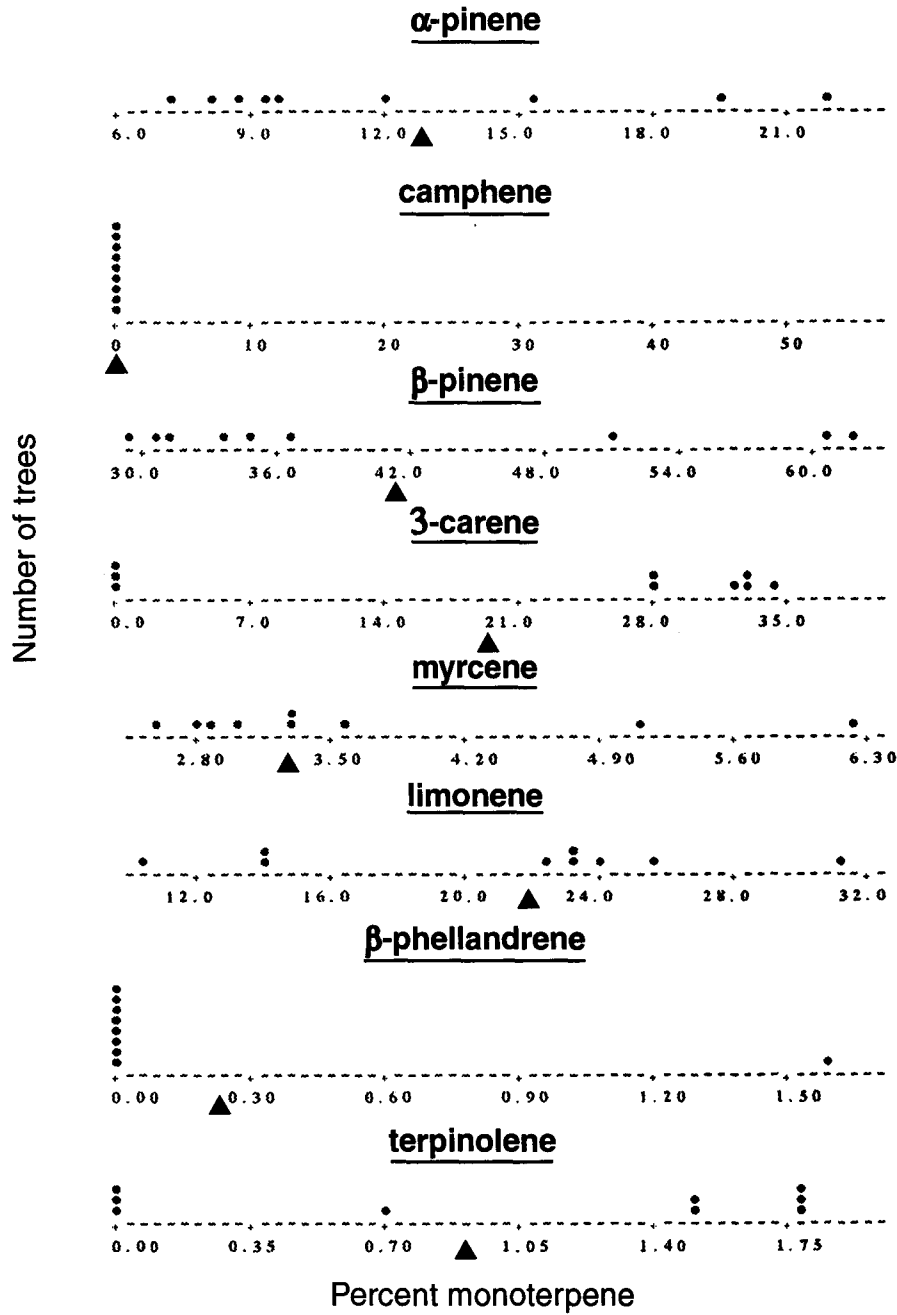
Data on nursery and 4-year containers data were aggregated. The average of the aggregated 30 nursery and container trees was not as expected (*table 6-13*) when compared with the previous sets of progeny. Limonene was only about half as much as expected, and myrcene was much lower than expected. The 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.



^aex = expected, ac = actual

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.

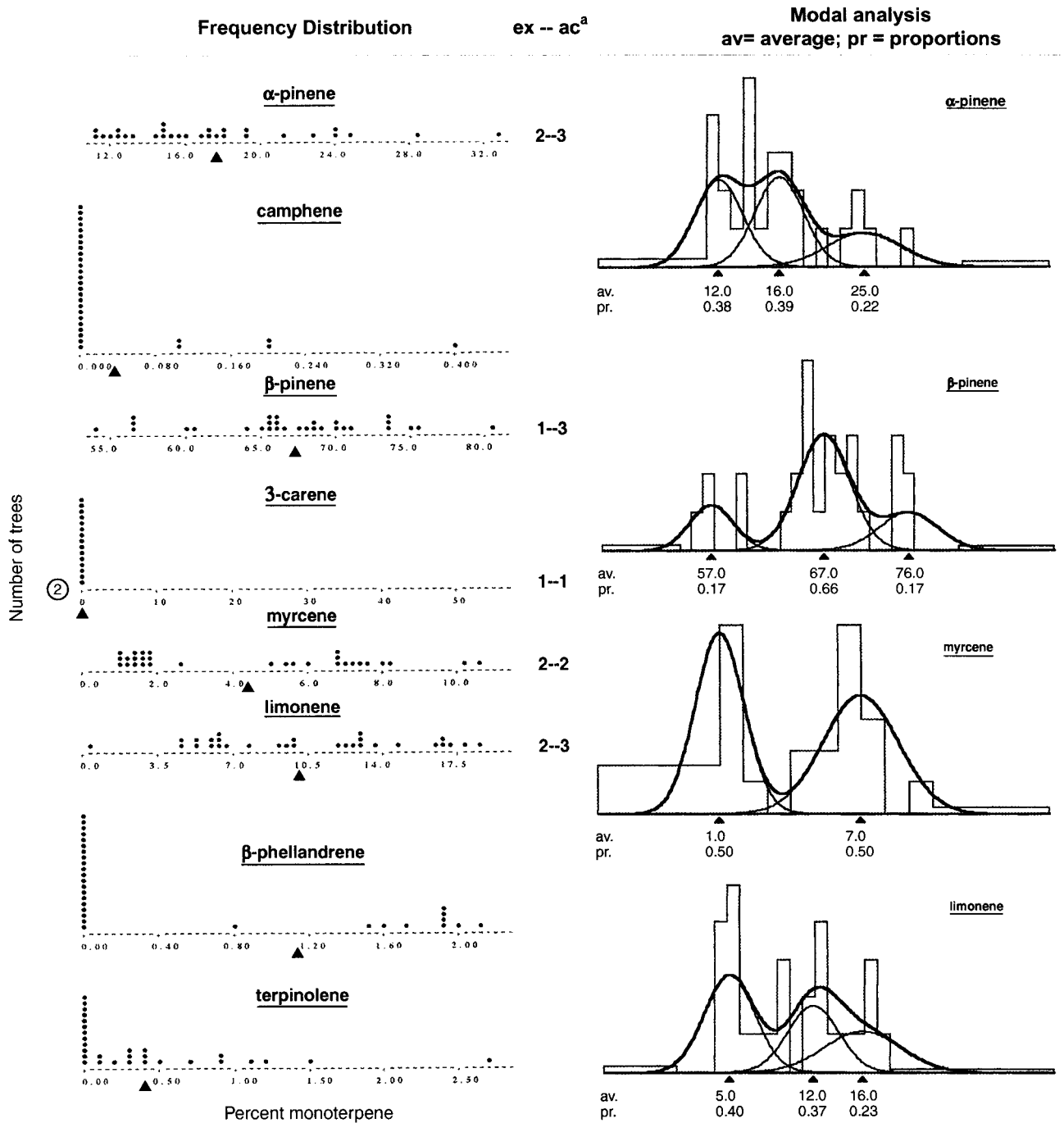
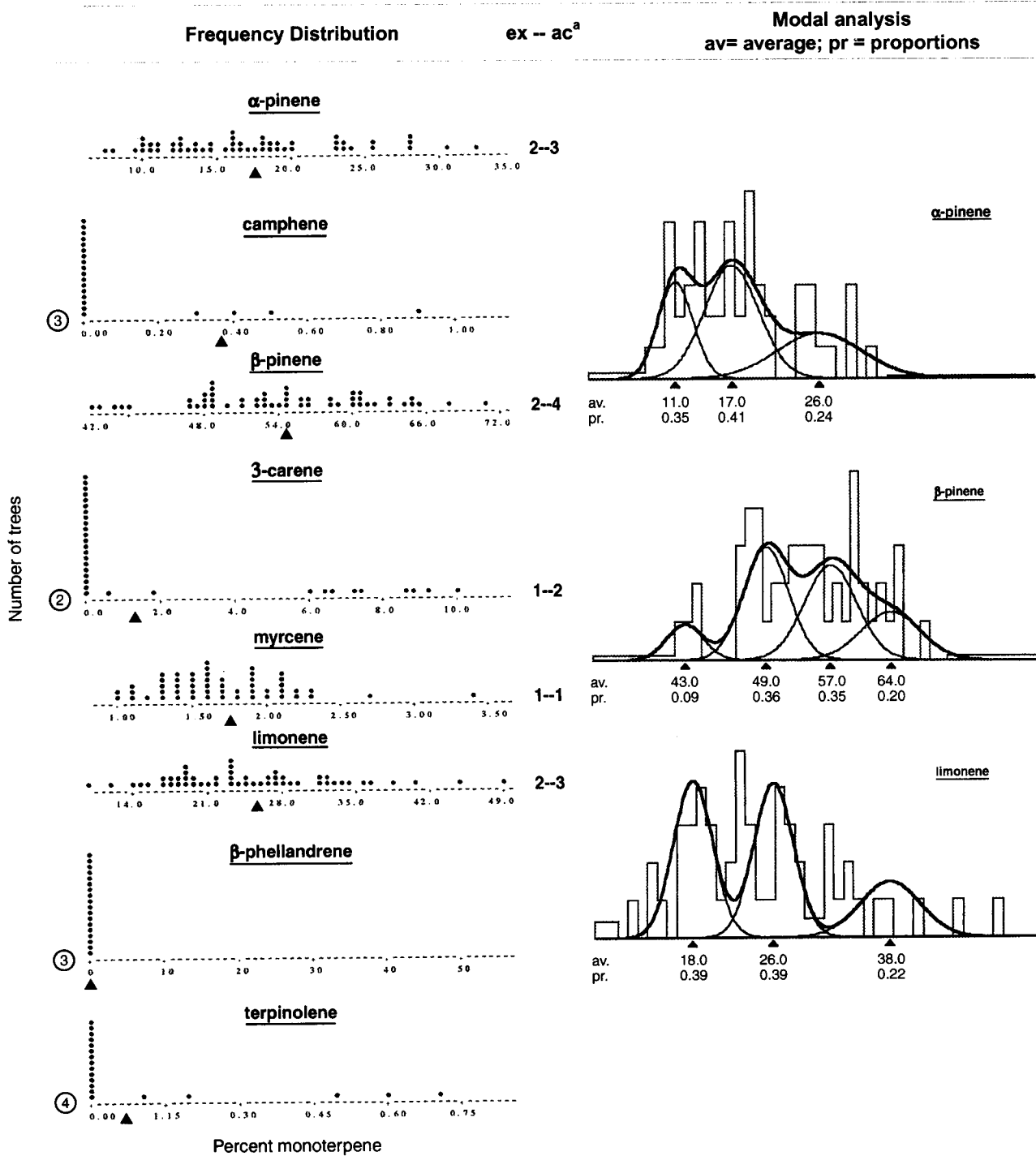


Figure 6-58—Distribution and analyses of the monoterpenes in the progeny of *P. ponderosa* IFG-4B63 x AS-A95; see caption of *figure 6-2* for explanation of the format below.



^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-4863 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.

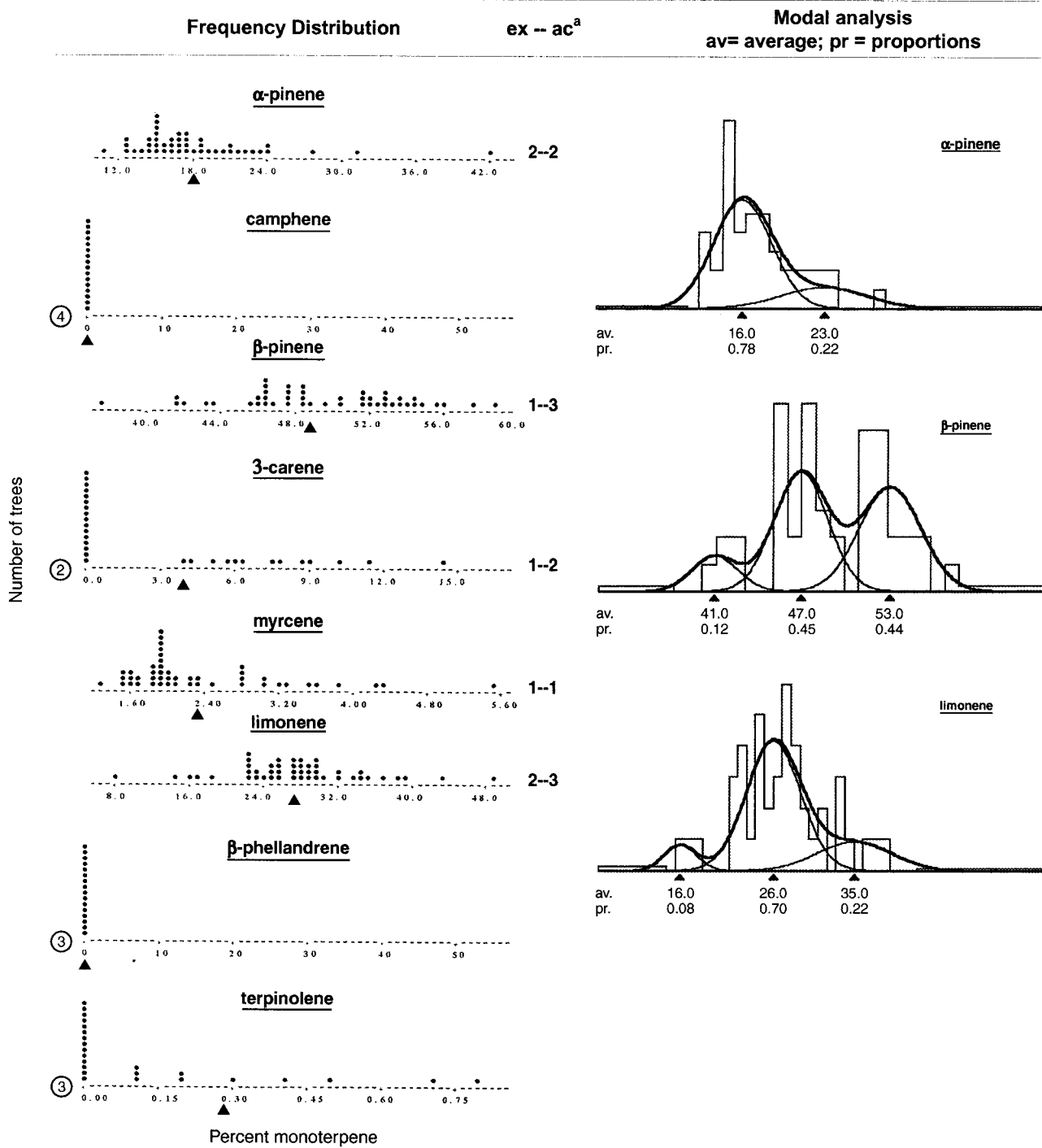
Normalized percent compositions of parents and progeny were as follows:

<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

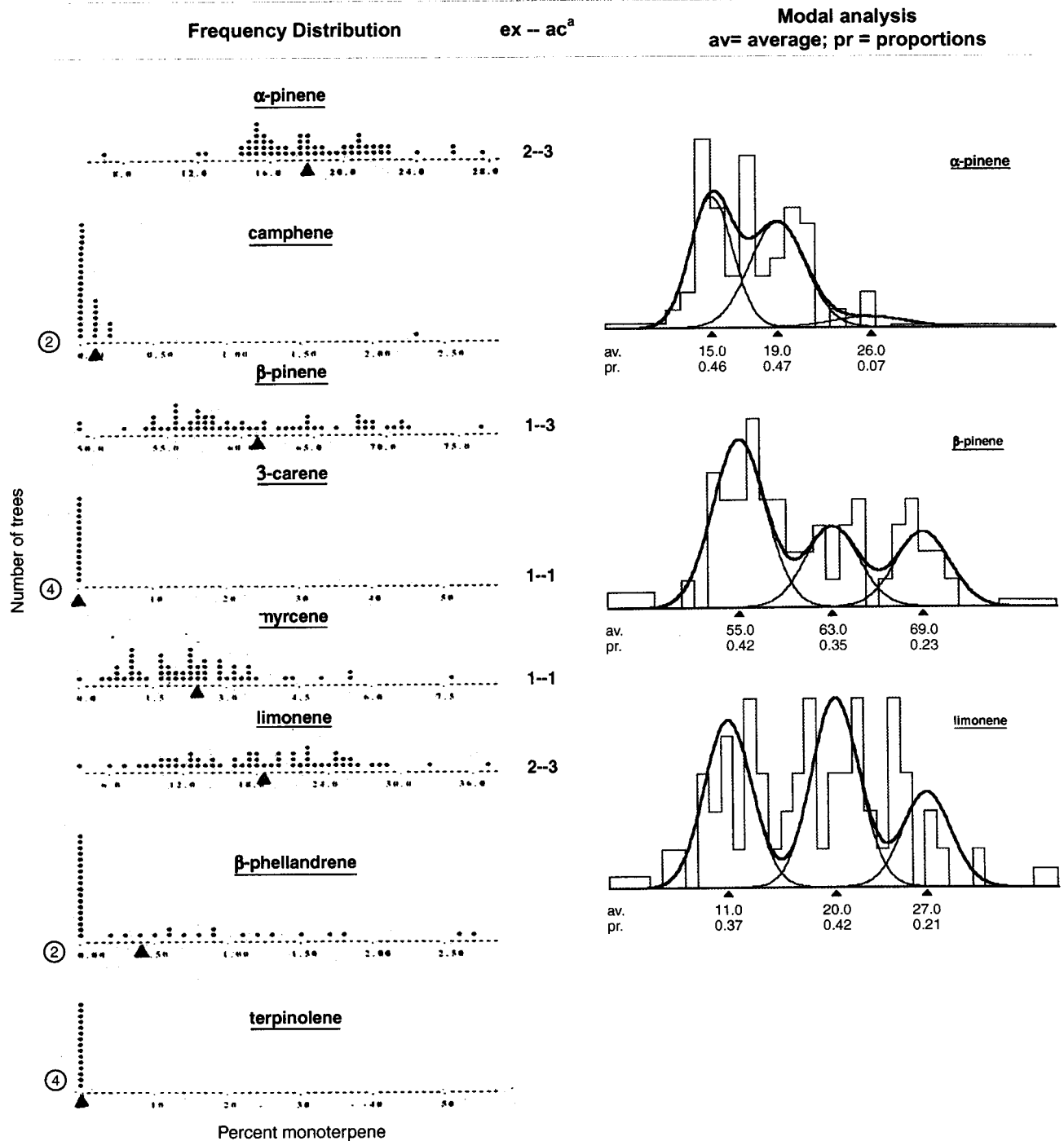
Tree BL-6074 was high limonene, 57 percent, with a small amount of α -pinene and β -pinene (*table 6-1*). It was reported earlier in this chapter as a pollen source with other females.

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 6-61—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 3-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

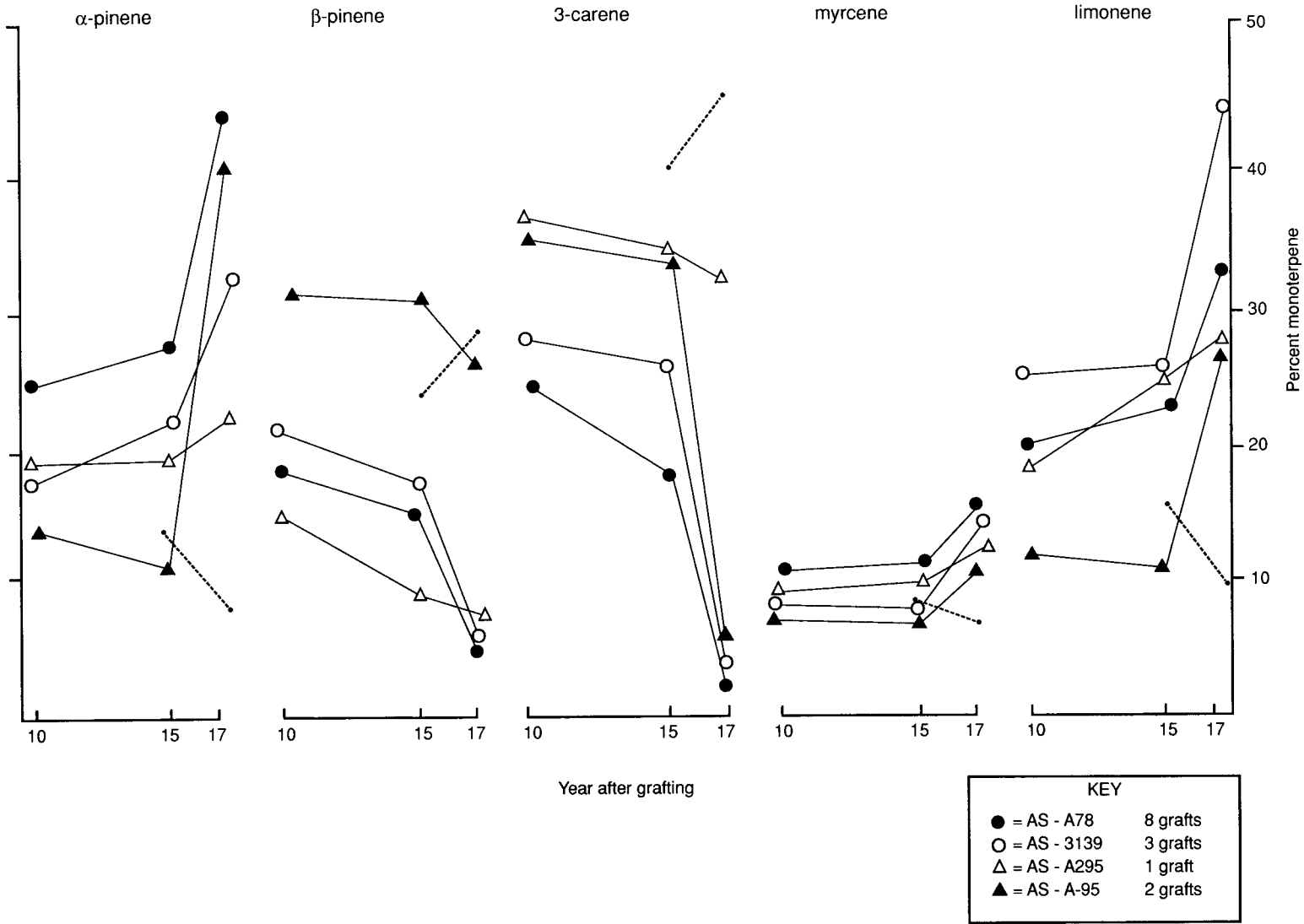


Figure 7-1—Trends in the average percentage of 5 monoterpenes in the basal internode of the scions of 4 grafted *P. ponderosa*, as noted in the key, at 10, 15, 17 years after grafting, and of the internode above the union of the 2 root stocks of the forked tree grafts, indicated by dashed line; see Table 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		
		percent ³						in	
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.

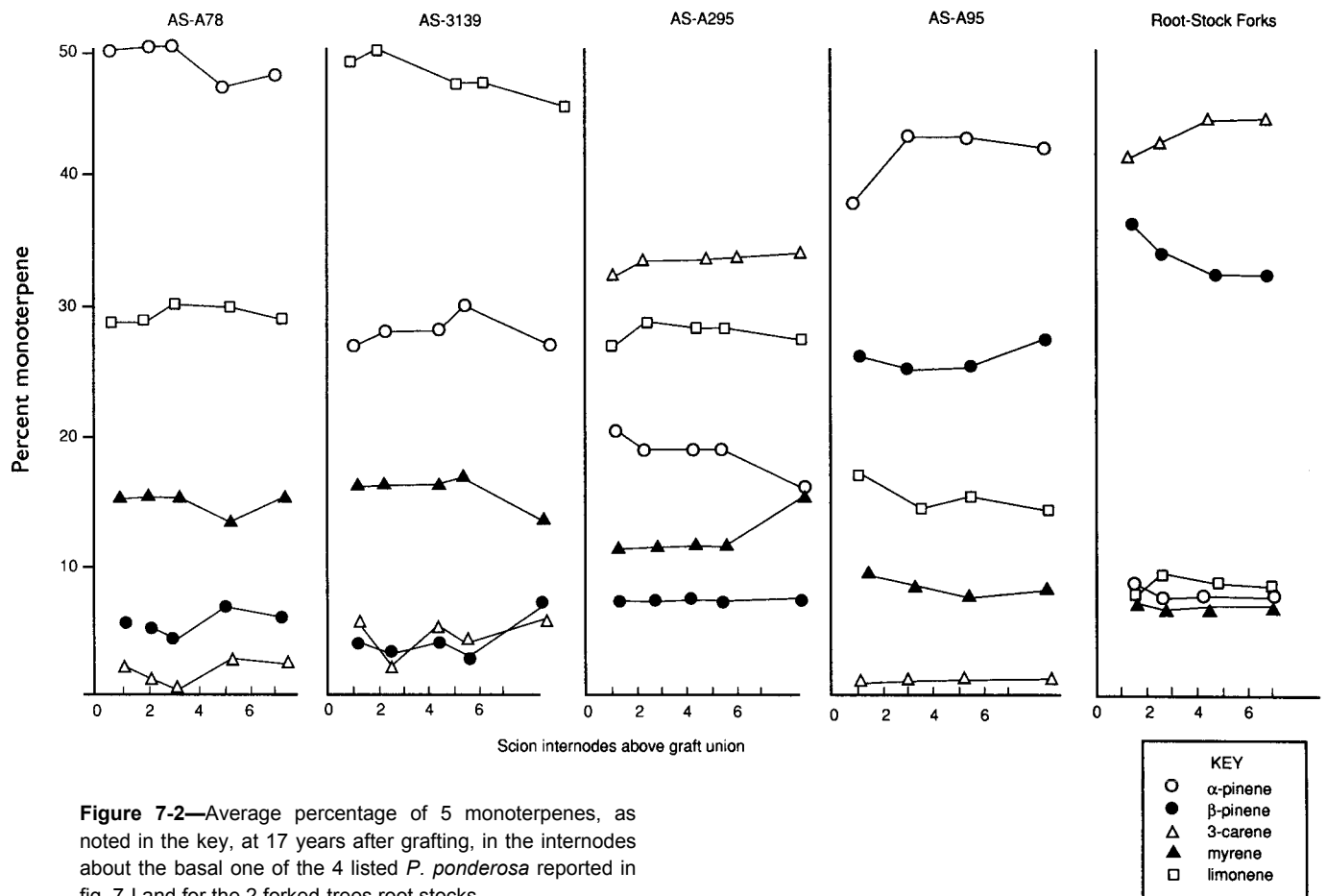


Figure 7-2—Average percentage of 5 monoterpenes, as noted in the key, at 17 years after grafting, in the internodes about the basal one of the 4 listed *P. ponderosa* reported in fig. 7-1 and for the 2 forked-trees root stocks.

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.

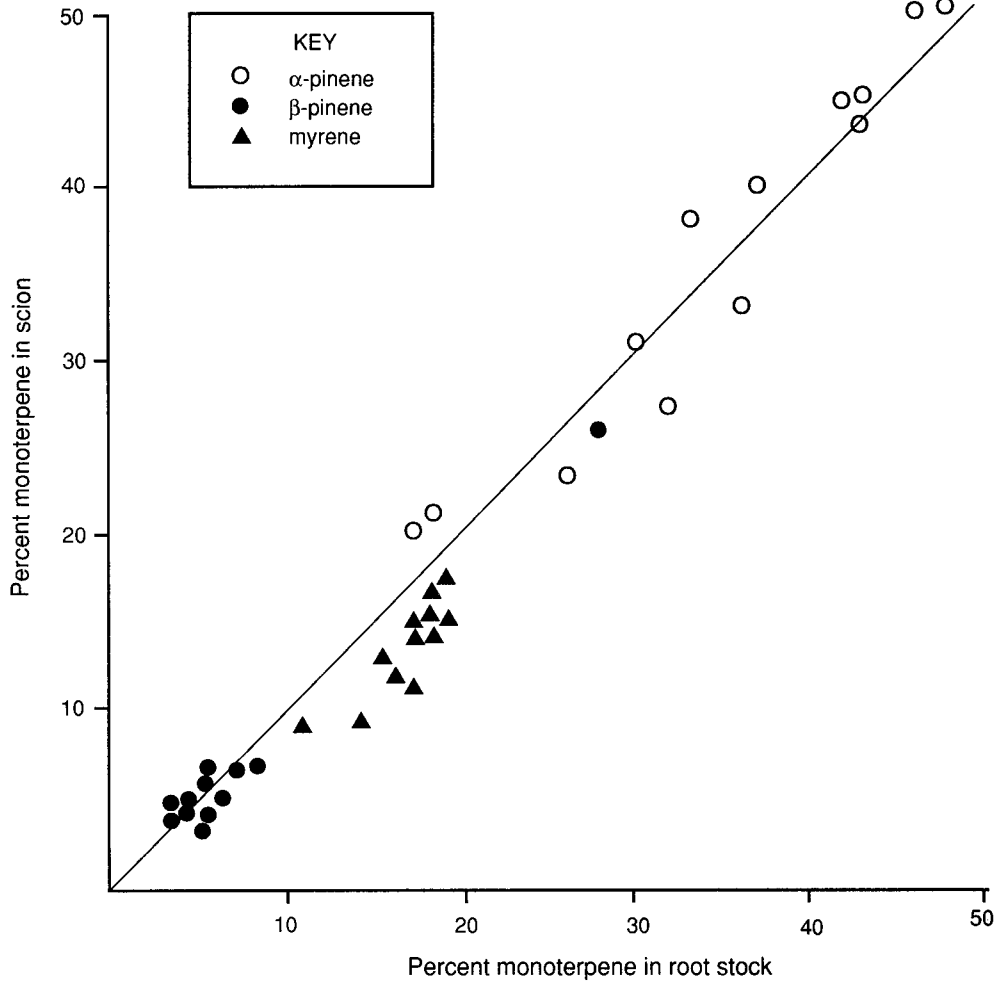


Figure 7-3—Regression of the amount of monoterpene, at 17 years after grafting, in the basal internode of the scion with the amount in the root stocks; limonene and 3-carene not plotted but fall within the ranges of the plotted components.

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.

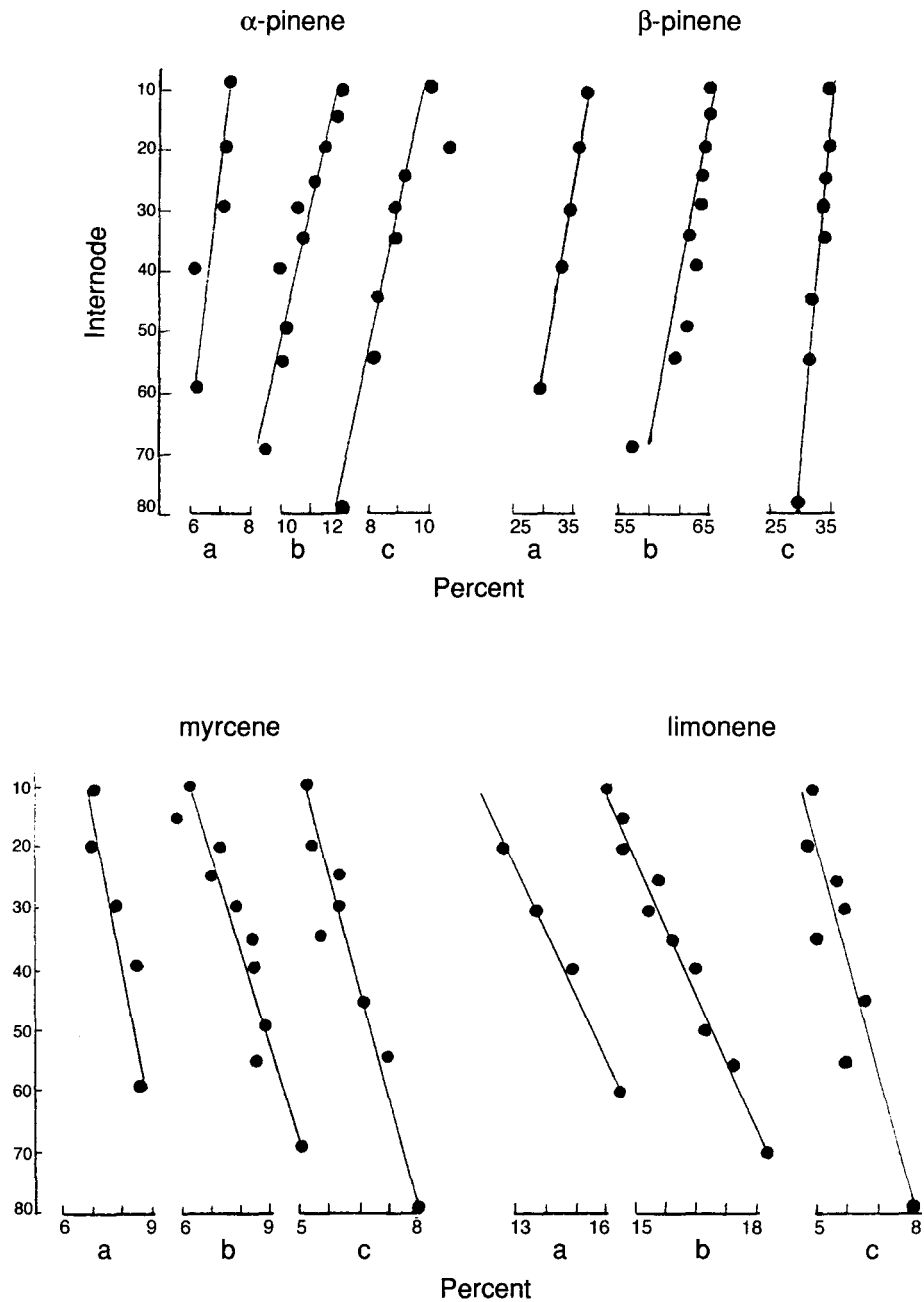
Tabulation was used to record data not applicable to either of the above procedures, or to summarize large amounts of data.

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.

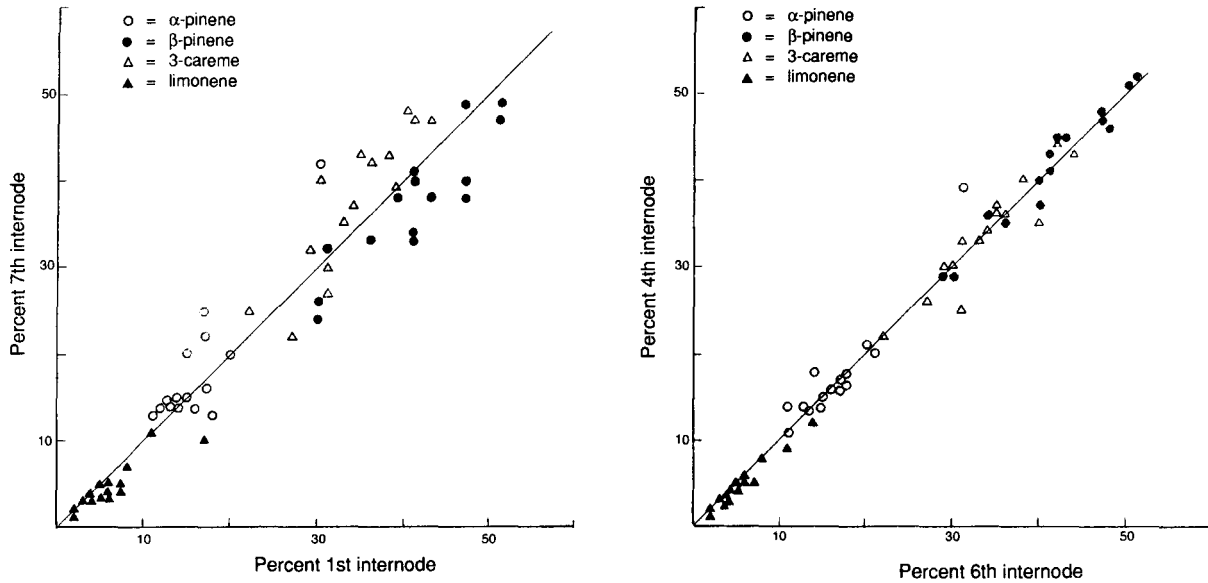
The report was divided into two sections: (a) sampling parameters and (b) operational parameters.

The basic GLC procedures are given in Chapter 2.

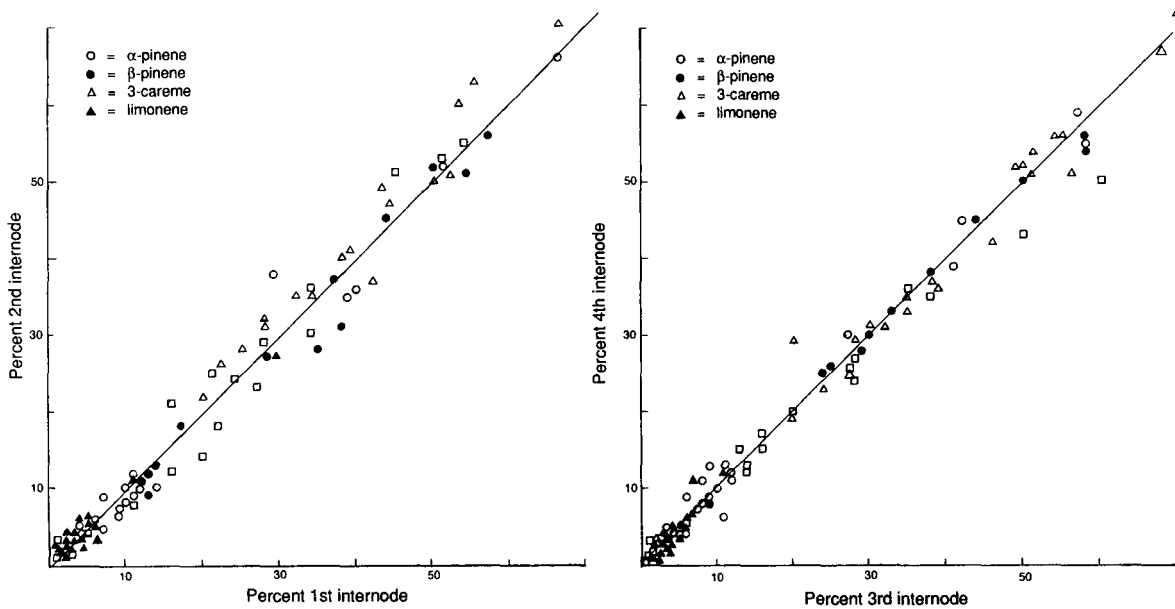
Study 8-2. Height of sample: Trend lines were drawn for the amount of the individual monoterpenes in the internodes of three *P. ponderosa* (a,b,c) 60 to 80 years old; internodes were counted from the top of the tree to the base, thus were essentially tree age at a given internode; each trend line has 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.



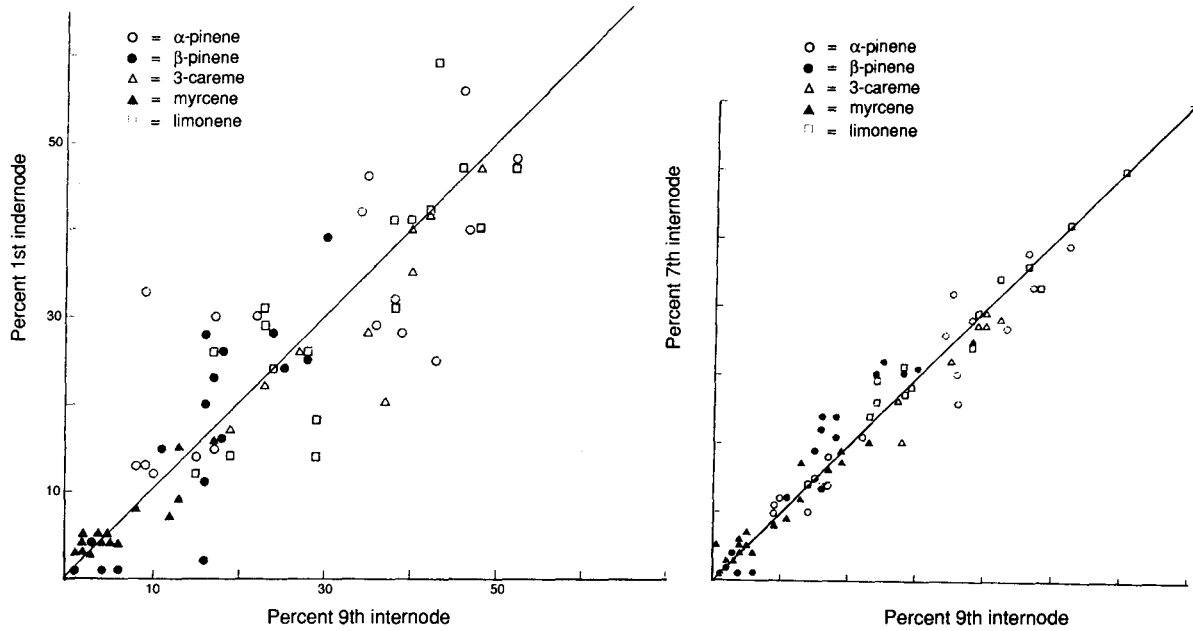
Study 8-3. Height of sample: Comparisons were made of the monoterpenes (in percent) between pairs of internodes in a population of 7-year old *P. ponderosa*. All samples were obtained by severing the tree at the internodes; internodes were counted from top of tree to base which was equivalent to tree age at a given internode. Diagonal line in regression of 1.0. Regression improved with age of internode.



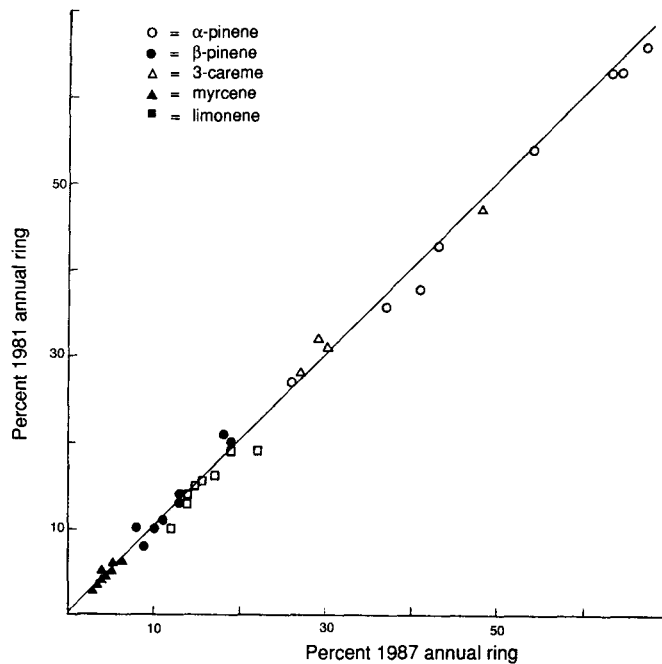
Study 8-4. Height of sample: Internode comparisons were made of the amount of the individual monoterpenes with different internode source of resin of 4-year old *P. ponderosa*; internodes were counted from the top to base of the tree, which is equivalent to tree age at an internode; diagonal line is regression of 1.0. Again, regression improved with age of internode.



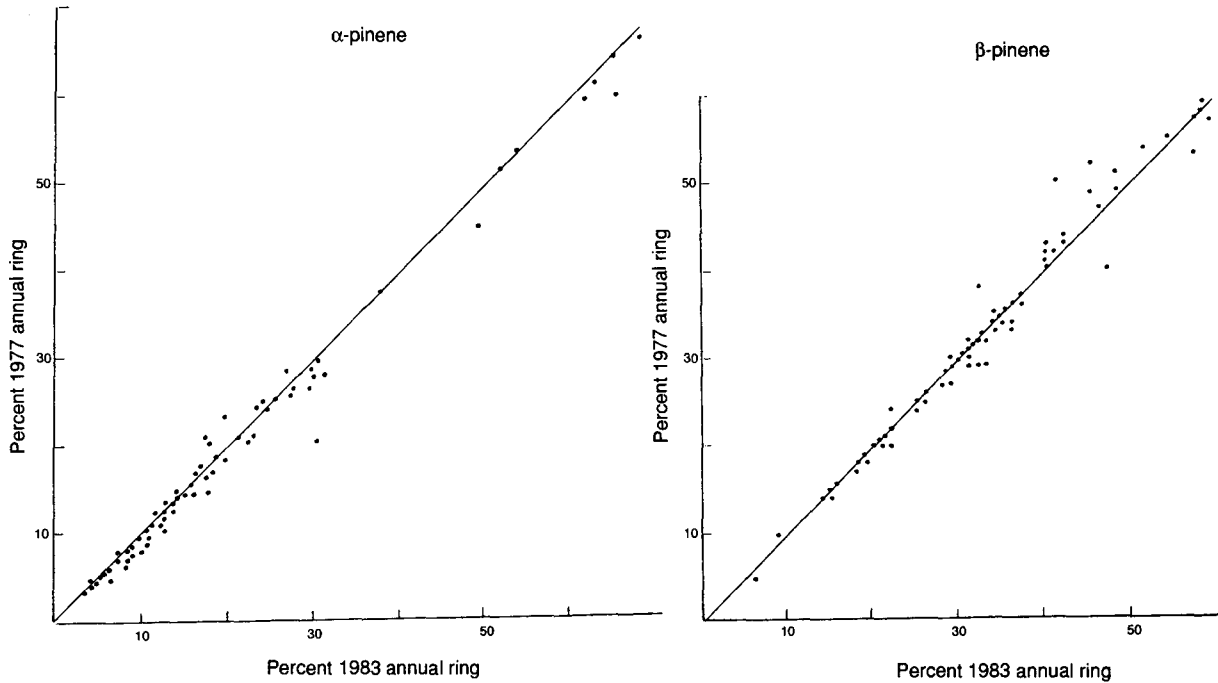
Study 8-5. Height of sample: Internodal comparison of the monoterpenes of pairs of internodes of a 9-year old full sibling population of *P. ponderosa*. Samples obtained by severing trees at internodes; internodes counted from top of tree to base. Again, regression improved with age of internode. Regression 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.



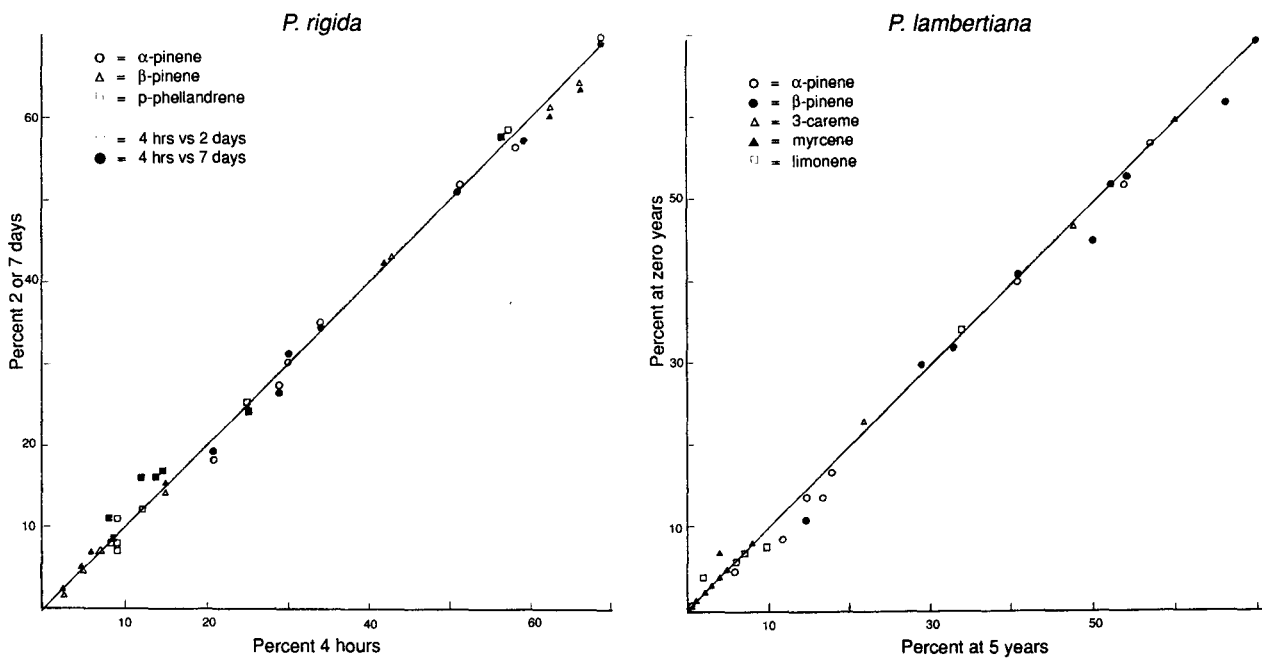
Study 8-6. Annual ring: Comparisons were made of the monoterpene composition of the 1981 and 1987 annual ring of the nursery-grown full siblings of 6-year old *P. ponderosa* IFG-4B63 x AS-5789; diagonal line is slope of 1.0. Clearly, all points were on the 1.0 diagonal or within 2 percent of it.



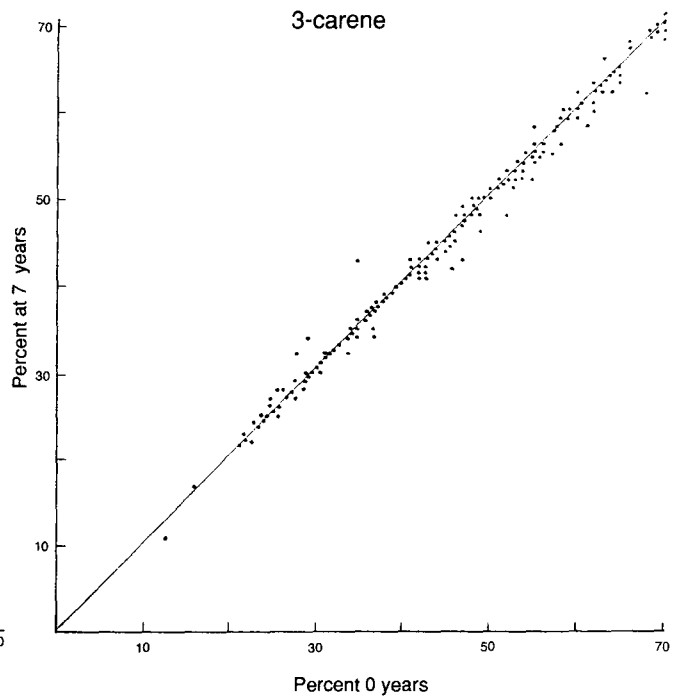
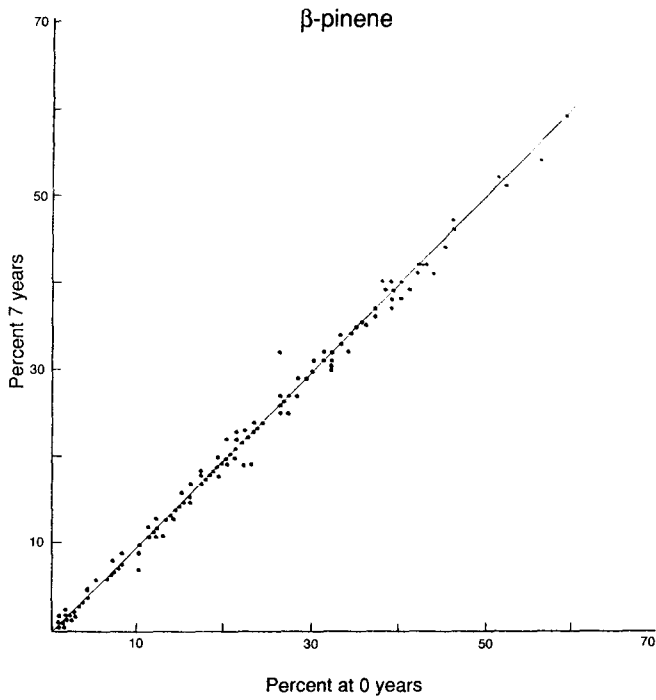
Study 8-7. Annual ring: Comparison were made of the monoterpene composition of the 1977 and 1983 annual ring at the basal internode of 72 6-year old *P. ponderosa*. Diagonal line is regression of 1.0. Over all there was very little difference, if any at all, between the composition of the 1977 and 1983 annual ring of the basal internode of these young trees; the results with myrcene and limonene were very similar.



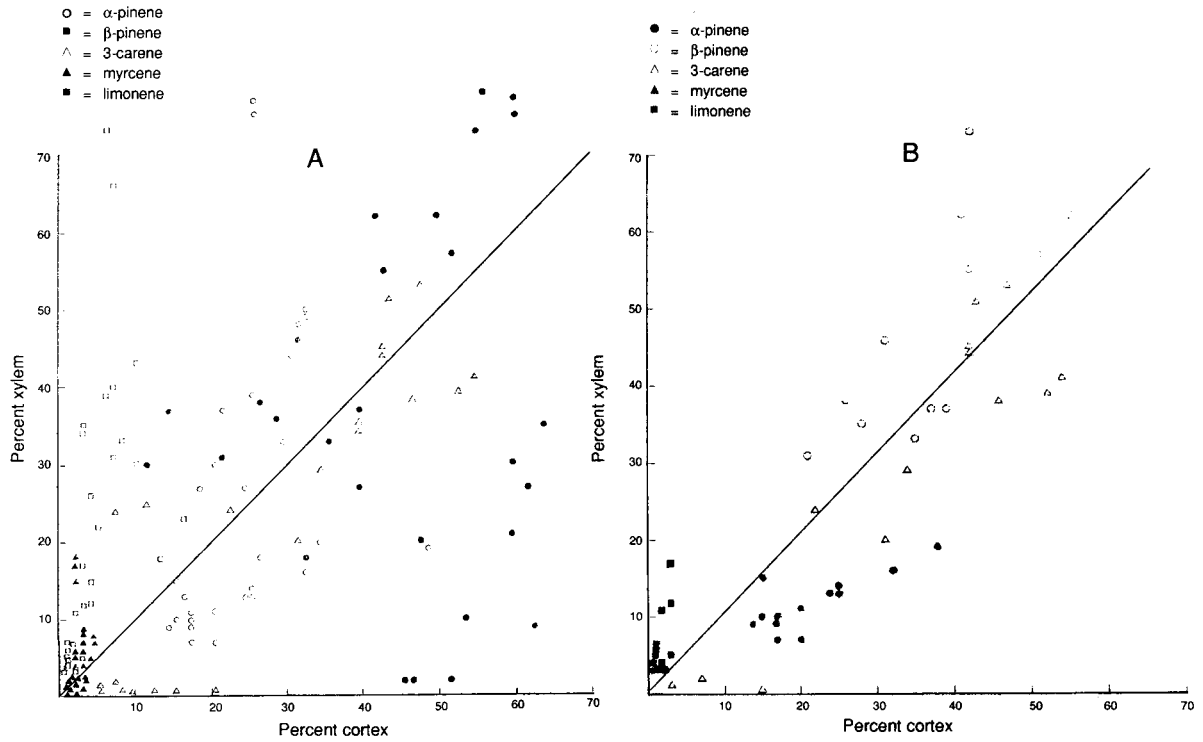
Study 8-8. Time of resin flow and sample storage: Comparisons were made of the monoterpene composition with differing time of resin flow and time of storage of prepared sample. Resin of seven *P. rigida* more than 40 years old was obtained at 4 hours, 2 days, and 7 days; and for 5 years of storage of 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.



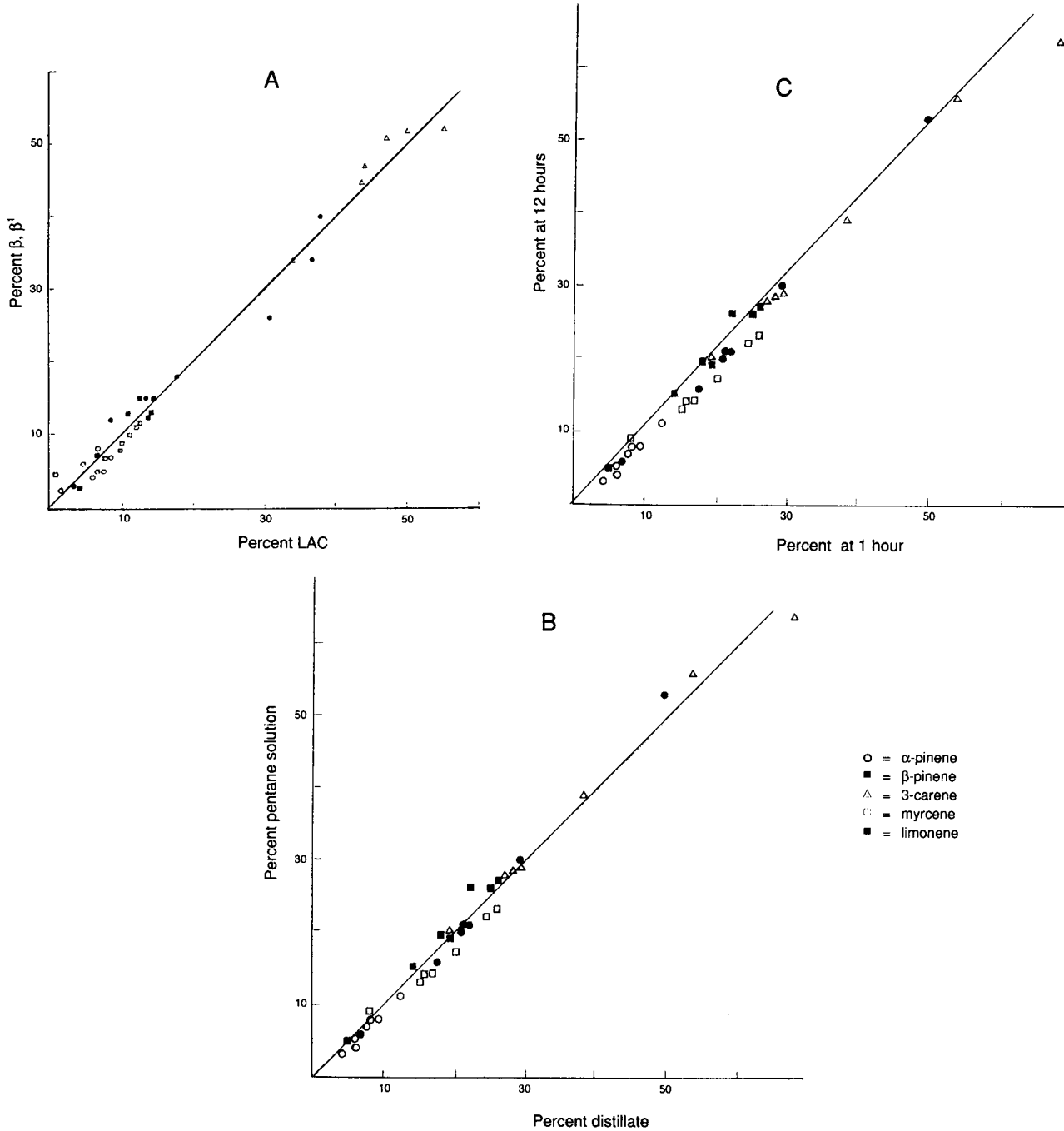
Study 8-9. Time of storage--Effect of storage time of pentane-prepared samples of 226 *P. ponderosa* was determined. Diagonal is regression of 1.0. A regression was made of the first analysis and the analysis after 7 years storage at 0 c. There was little, if any, change during storage. The data for 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>	Trees <i>number</i>	Replicate	Average Monoterpene Composition				
				α -pinene	β -pinene	3-carene	myrcene	limonene
				<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.

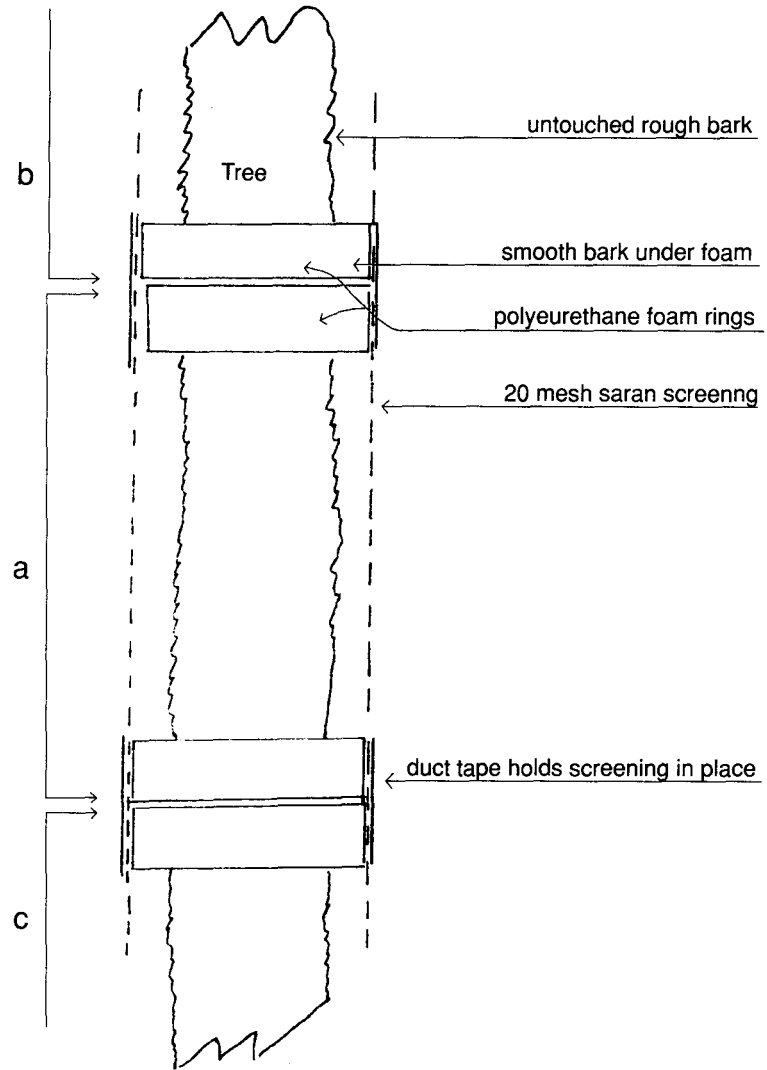
Beetles used in the tests were reared from infested bark taken from trees on the Sierra and Eldorado National Forests in California. The bark was gathered in early spring when the over-wintering brood was in the late larval stage. The brood bark was held in a cold room at 0 °C. As beetles were needed, 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 <i>number</i>	Gallery length		Resin flow cc/10 cm circumference	
			X	S.D. ²	y ²	S.D. ²
			<i>mm</i>		<i>cc</i>	
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.

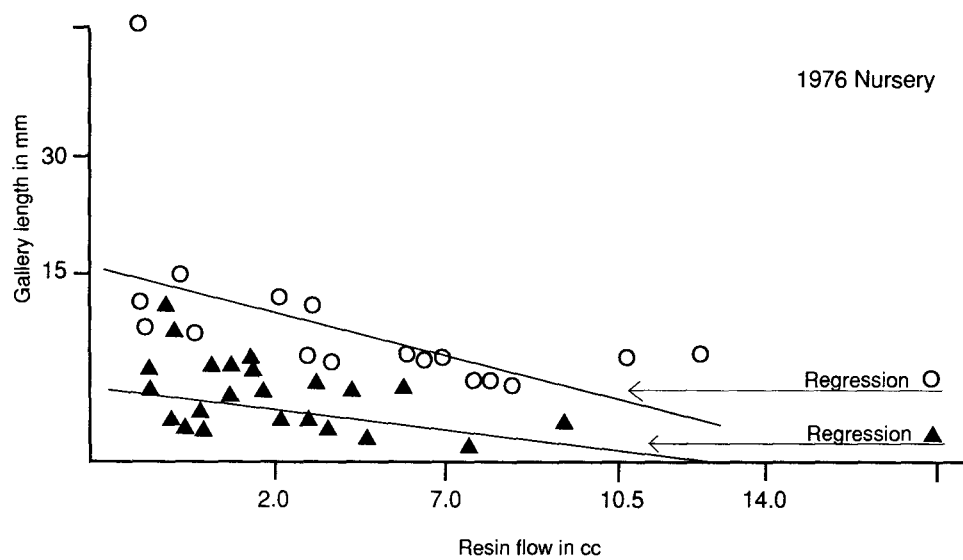
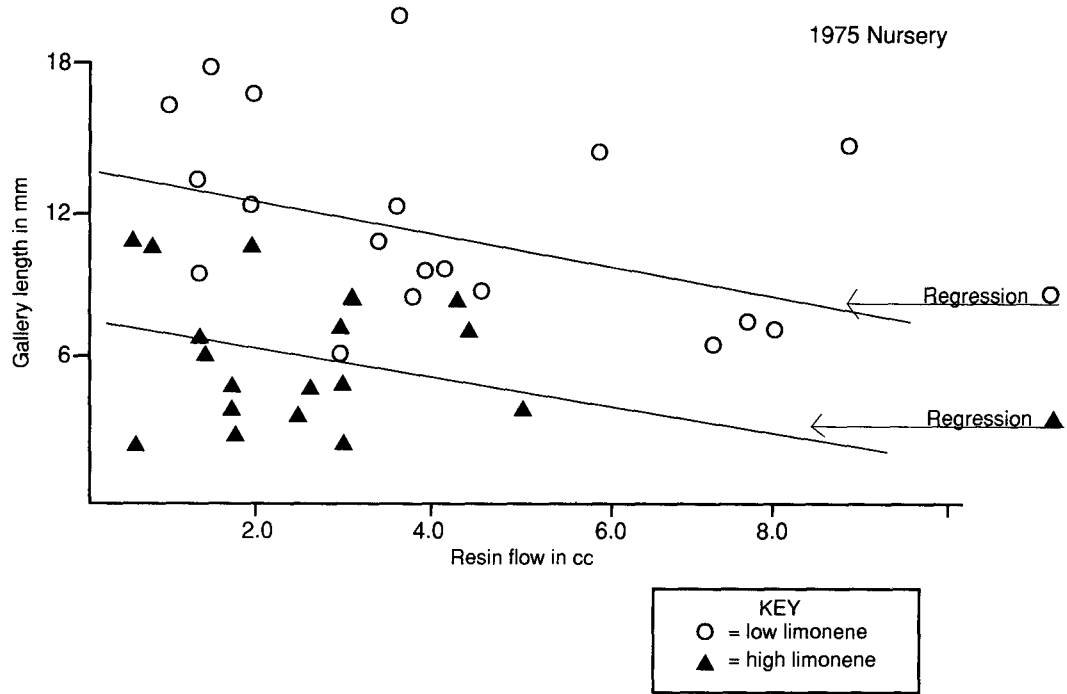
Conclusions

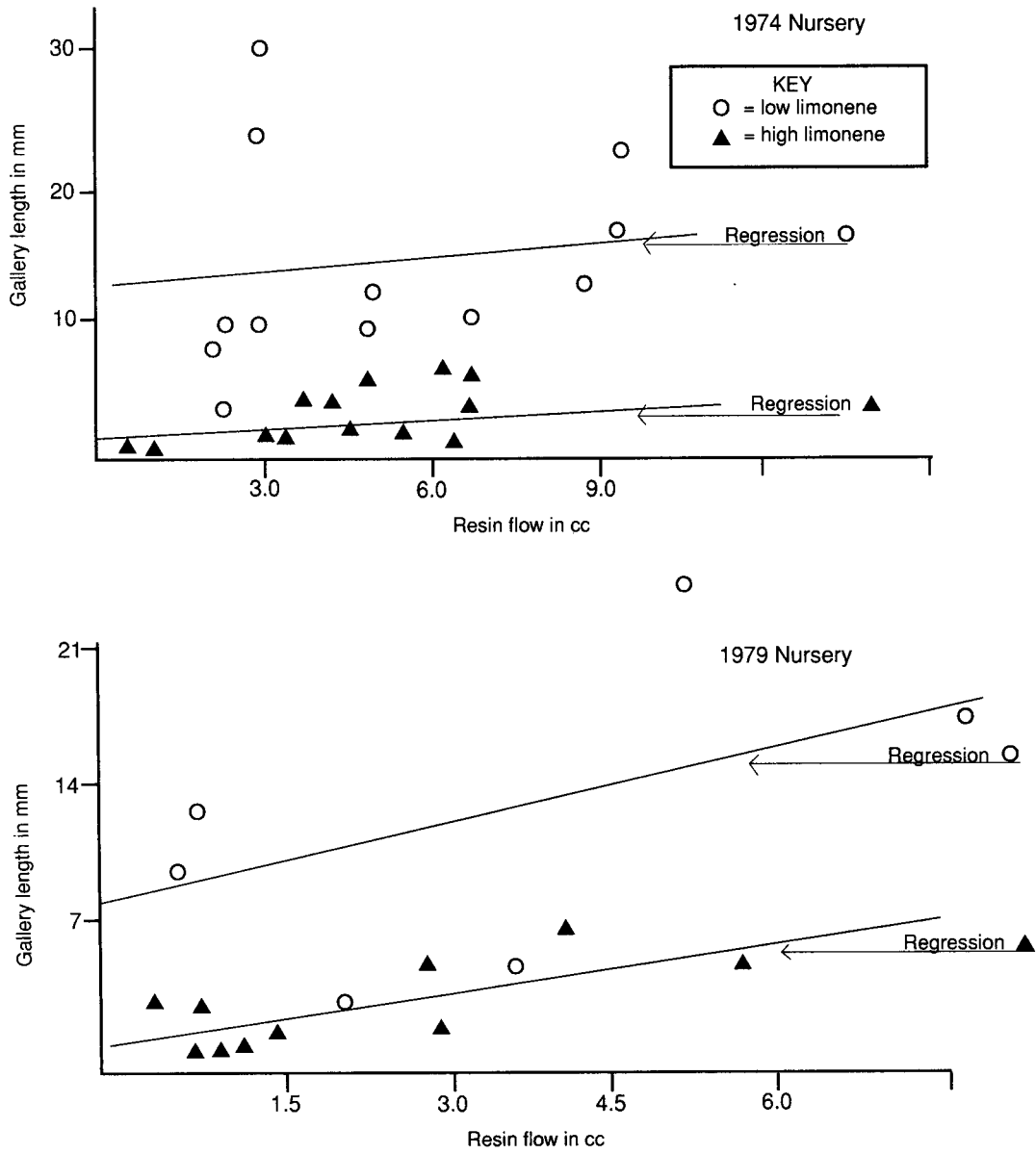
From the results and my observations of all previous work with trees greater than 15 cm in diameter and of the trees used for this report, it appeared that trees with a diameter less than 7.5 cm were not suitable, that trees with diameters greater than 7.5 cm but less than 12.5 cm are adequate for comparative 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