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# FURTHER STUDIES OF TURPENTINE COMPOSITION OF *PINUS MURICATA* IN RELATION TO ITS TAXONOMY

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**Abstract**—*Pinus muricata* D. Don is a species unique in the infraspecific variability of chemical composition of its turpentine. In this article previous findings on that subject are reviewed and additional analytical data are reported. The problem of this variability is discussed on the background of the Tertiary geomorphology and the climate of the Pacific Coast of California. These environmental factors alone, however, are not totally responsible for the chemical (and morphological) variability. Heredity apparently has had an equal importance.

## INTRODUCTION

### *Botanical Aspects*

*Pinus muricata* D. Don is an endemic California species, commonly known as Bishop pine because it was discovered and described from San Luis Obispo (i.e., Bishop) County. It belongs to the group Insignes Shaw of the subgenus Diploxylon.[1] With two other species *P. attenuata* Lemm. and *P. radiata* D. Don it forms the well defined subgroup of closed cone pines of California.

At present *P. muricata* extends about 800 miles along the California coast, from near the Oregon border to Baja California, Mexico. It clings to the coast, forming a series of discontinuous groves, large and small, and rarely penetrates inland for more than 1 or 2 miles. It is found also on the two islands, Santa Rosa and Santa Cruz, off the California coast approximately at the 34th parallel. A pine growing on Cedros Island (latitude 28°15′ North) was described by Howell[2] as a variety of *P. muricata*, but it was found later to be closer to *P. radiata*.[3] *P. attenuata* is distributed more widely than is *P. muricata*, and occurs not only in the coastal ranges of California, but also inland, from central Oregon to Baja California, where the summers are drier and the winters are colder than on the coast. On the mainland, *P. radiata* has a more restricted area than *P. muricata*, being found only in three places: one at Monterey and the other two not far from there to the north at Pt. Año Nuevo, Santa Cruz County, and to the south at Cambria, San Luis Obispo County. It is found also on Guadalupe Island off the coast of Baja California but does not occur on the Santa Rosa and Santa Cruz Islands.

In his monograph on *P. muricata*, Duffield[4] subdivided the species into four varieties (Table 1).

The variety *borealis* is characterized by bluish foliage (caused by accumulations of waxy substance filling the stomata), by large size of the trees, dense narrow crown, rough bark, and

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[1] G. R. SHAW, *The Genus* Pinus, Publ. 5. Arnold Arboretum (Cambridge Riverside Press), Cambridge, Mass. (1914).
[2] J. T. HOWELL, *Leaflet Western Botany* 3 (1), 1 (1941).
[3] J. M. FIELDING, *Australian Forestry* 25 (2), 62 (1961).
[4] J. W. DUFFIELD, Ph.D. thesis, University of California, Berkeley (1951).

TABLE 1. SUBDIVISION OF *Pinus muricata*[4]

| Variety | Occurrence |
|---|---|
| *P. borealis* Duffield | Del Norte to Sonoma Counties,* California |
| *P. muricata* D. Don | San Luis Obispo County to Baja California (on mainland) |
| *P. remorata* Mason (Duffield) | Santa Rosa and Santa Cruz Islands |
| *P. cedroensis* Howell | Cedros Island |

* More specifically, from Crescent City to Annapolis Junction.

incompletely serotinous cones. As a whole it is a better forest tree than the more southern varieties. The variety *muricata* possesses green foliage with no accumulation of wax in the stomata; the trees are smaller, with spreading crown, smooth bark, and serotinous cones more elongated than in variety *borealis*. The populations located along the coast between the varieties *borealis* and *muricata*, i.e. in Inverness (Tomales Bay) and Monterey localities (see Map, Fig. 1) were found by Duffield to be intermediate in some characteristics. Both populations belong to the 'green foliage' variety, and differed one from the other; the Inverness population possessed bark as rough as that of the variety *borealis*, but the cone shape index was similar to that of the var. *muricata*. On the other hand the Monterey population of

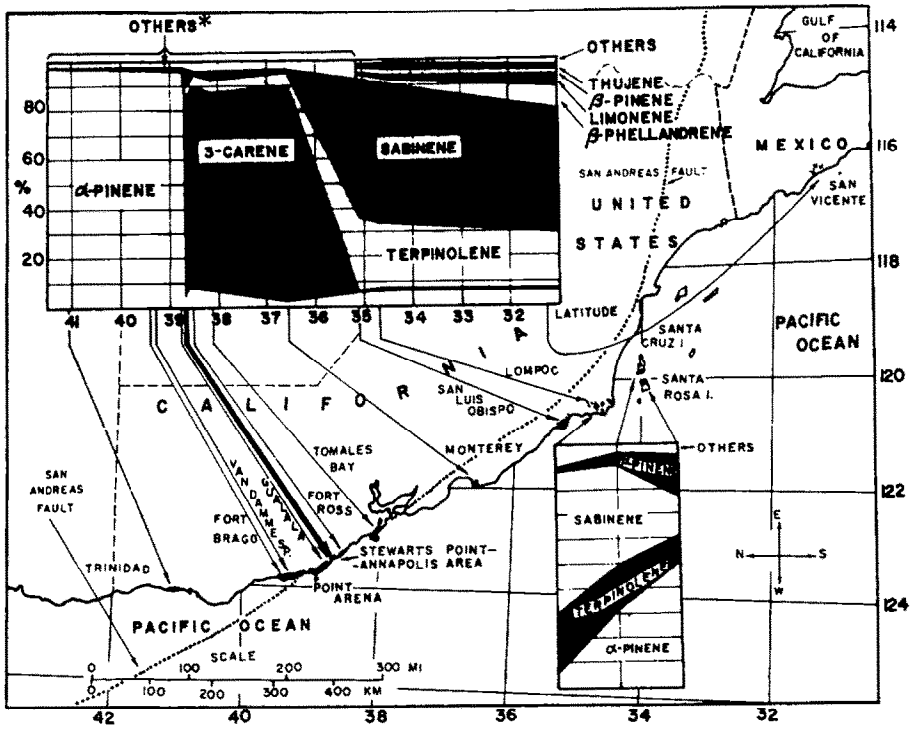


FIG. 1. LOCATION OF *P. muricata* STANDS AND TURPENTINE COMPOSITIONS OF THE POPULATIONS STUDIED.

In northern and central populations the percentage of other terpenes includes thujene, β-pinene, limonene and β-phellandrene.

*P. muricata* had the smooth bark typical of that of the (more southern) var. *muricata*. Generally, morphological characters of the Monterey population were found to be similar to those of the var. *muricata*.

The variety *remorata* was distinguished from the other varieties of *P. muricata* by its almost symmetrical cones and the less prominent apophyses. The variety *cedroensis* was described by Howell[2] as having smooth or even shallowly excavated apophyses.

*Chemical Aspects*

In 1947 Mirov[5] reported on the composition of *P. muricata* turpentine obtained from near Fort Bragg, California. The turpentine consisted almost entirely (98–99 per cent of the total volatile oil) of (+), (±)-α-pinene, $[\alpha]_4^{20} = +11{\cdot}6$, with only a small amount of camphene.

In 1949 Duffield, in connection with his studies of *P. muricata*[4] collected samples of its oleoresin from several localities. These collections were made in the Eddy Arboretum, Placerville, California. Because of the small size of the samples only the optical properties of their volatile oils were determined.

The results (Table 2) suggested that the chemical composition of *P. muricata* turpentine from these different localities might have been different.[4, 6]

TABLE 2. OPTICAL ROTATIONS OF SAMPLES OF OLEORESIN FROM *P. muricata* FROM DIFFERENT LOCALITIES

| Locality | Samples | Optical rotation $\alpha_D^{25}$ (degrees) |
|---|---|---|
| Mendocino Coast | 2 | +16·2, +35·0 |
| Monterey | 2 | + 5·6 + 9·6 |
| Santa Rosa I. | 2 | −43·5, −44·5 |
| Santa Cruz I. | 4 | −43·2 to −45·5 |

Thirteen years later Dr. Margot Forde of New Zealand collected a sample of *P. muricata* oleoresin in Monterey. The turpentine was analyzed in New Zealand[7] and was found to contain mainly 3-carene. This finding caused Dr. Forde[8] to investigate the turpentine composition of *P. muricata* over its whole range and led her to a most spectacular discovery of a chemical variability of this species rarely found in other pines. Fifty-eight samples were studied; twenty-one from the wild trees at three localities (Monterey, San Luis Obispo, Lompoc), one from San Vicente trees, planted in Placerville, California, and the rest from trees of known provenance planted in Australia.* On the basis of these analyses Forde and Blight[8] subdivided *P. muricata* into three distinct "chemical races"; (1) a northern race characterized by turpentine consisting almost entirely of (+), (±)-α-pinene; (2) a central race (Inverness and Monterey populations) with predominance of (+)-3-carene in the turpentine and (3) a southern race, with turpentine composed of (−)-sabinene and terpinolene

* To analyze turpentines for this purpose from trees grown away from their original habitat is perfectly justifiable, as chemical composition of pine turpentine does not change with the environment.[6] It has the disadvantage that in many cases it is not known whether or not the seeds came from one or from several trees; thus a statistical bias of close parentage is potentially present.

[5] N. T. MIROV, *J. Forestry* **45**, 659 (1947).
[6] N. T. MIROV, *U.S. Dep. Agr. Forest Serv., Tech. Bull.* No. 1239 (1961).
[7] A. L. WILLIAMS and M. H. BANNISTER, *J. Pharm. Sci.* **51**, 970 (1962).
[8] M. B. FORDE and M. M. BLIGHT, *New Zealand J. Botany* **2**, 44 (1964).

TABLE 3. COMPOSITION OF TURPENTINES IN DIFFERENT POPULATIONS OF *P. muricata*

| Population | Number of trees analyzed | Constituents (per cent) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | α-Pinene | Camphene | β-Pinene | 3-Carene | Sabinene | Myrcene | Limonene | β-Phellandrene | γ-Terpinene | Terpinolene | Thujene |
| Trinidad | 6 | 97·0–98·5 | 0·5–1·0 | 0·0–1·0 | — | — | 0·5 | tr–0·5 | — | — | — | — |
| Ft. Bragg | 11 | 92·5–97·5 | 1·0–1·5 | 1·0–3·0 | — | — | 0·5–1·0 | tr–0·5 | tr–2·0 | — | — | — |
| Van Damme S.P. | 8 | 94·5–97·5 | 1·0 | 1·0–2·0 | — | 0·0–2·0 | 0·5–1·0 | tr–0·5 | tr–0·5 | — | 0·0–0·5 | — |
| Gualala | 6 | 91·0–99·0 | 0·5–1·0 | 0·5–1·5 | — | tr–4·0 | tr–0·5 | tr–0·5 | tr·1·0 | — | tr–1·5 | — |
| 2 Mi. N. Ann. | 7 | 90·5–98·0 | 0·5–1·0 | 1·0–3·0 | — | 0·0–3·5 | 0·5–1·0 | tr–1·0 | tr–1·5 | — | tr–1·5 | — |
| Annapolis | 10 | 2·0–97·5 | tr–1·0 | tr–1·5 | 0·0–84·0 | 0·0–3·5 | 0·5–2·5 | tr–2·5 | tr–0·5 | 0·0–0·5 | tr–9·5 | 0·0–0·5 |
| 1 Mi. S. Ann. | 3 | 4·0–5·5 | tr | tr | 79·0–83·5 | 2·0–2·5 | 2·0–2·5 | tr | 0·5 | 0·5 | 7·0–7·5 | 0·0–tr |
| Stewarts Point | 5 | 4·5–13·5 | tr | tr–0·5 | 73·0–79·5 | 3·5–7·0 | 2·0–2·5 | tr–0·5 | 0·5 | 0·0–0·5 | 4·0–8·0 | — |
| Fort Ross | 8 | 3·0–18·5 | — | tr–1·5 | 71·0–90·5 | 0·0–4·5 | 0·5–2·5 | tr–0·5 | tr–1·0 | 0·0–0·5 | 4·5–6·5 | 0·0–0·5 |
| Inverness (Tomales Bay) | 9 | 2·5–20·0 | 0·0–tr | tr–2·0 | 69·0–86·0 | 0·1–6·0 | 2·0–2·5 | tr–0·5 | 0·5 | 0·5 | 5·0–7·5 | 0·0–0·5 |
| Monterey | 10 | 1·0–5·0 | tr–0·5 | tr–1·5 | 84·5–94·5 | tr–1·0 | 1·5–3·0 | tr–0·5 | tr–1·5 | 0·0–0·5 | tr–7·0 | 0·0–0·5 |
| San Luis Obispo | 10 | 4·5–9·0 | tr | 0·5–1·5 | tr–1·0 | 53·5–62·5 | 0·5–1·5 | tr–2·0 | 1·0–1·5 | 0·5–3·5 | 25·5–35·0 | 1·0–2·0 |
| Lompoc | | 4·0–12·5 | tr–1·0 | 1·5–4·0 | 0·5–2·0 | 44·0–73·5 | 0·0–1·5 | 0·5–1·5 | 0·5–1·5 | tr–2·5 | 8·0–31·0 | 1·0–11·0 |
| Santa Cruz I. | 10 | 11·5–54·5 | tr–2·0 | 1·5–13·5 | 0·0–1·0 | 25·0–62·0 | tr–1·0 | 0·0–1·0 | 0·5–2·0 | 0·0–2·5 | 3·5–32·5 | tr–3·0 |
| Santa Rosa I. | 5 | 36·5–59·5 | 0·5–1·5 | 14·5–20·0 | 0·0–1·0 | 8·5–30·0 | tr–1·0 | tr–1·5 | 0·0–1·5 | tr–2·0 | 8·5–12·0 | tr–1·0 |
| San Vicente | | 5·5–8·5 | 0·0–1·0 | 1·5–2·5 | 0·0–1·0 | 41·0–62·0 | 0·0–4·0 | tr–10·0 | 1·5–15·0 | tr–1·0 | 3·0–30·5 | 1·0–2·0 |

TABLE 4. ANALYSES OF TURPENTINES COLLECTED FROM TEN TREES NEAR ANNAPOLIS ROAD

| Tree no. | Composition in per cent | | | | | | | | | | | Morpho-logical* evalu-ation | Chemical evalu-ation† |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | α-Pinene | Camphene | β-Pinene | 3-Carene | Sabinene | Myrcene | Limonene | β-Phellan-drene | γ-Ter-pinene | Terpin-olene | Thujene | | |
| 87 | 7·5 | tr | tr | 82·0 | 1·5 | 2·5 | tr | 0·5 | 0·5 | 6·0 | tr | A | A |
| 88 | 3·0 | tr | tr | 84·0 | 2·0 | 2·5 | 0·5 | 0·5 | 0·5 | 6·5 | 0·5 | A | A |
| 89 | 4·0 | tr | tr | 84·0 | 1·5 | 2·0 | 0·5 | 0·5 | 0·5 | 6·5 | 0·5 | B | A |
| 90 | 38·0 | tr | 0·5 | 54·0 | 0·5 | 2·0 | 0·5 | 0·5 | tr | 4·0 | tr | B | H |
| 91 | 43·5 | tr | 1·0 | 47·5 | 1·0 | 2·0 | 0·5 | 0·5 | tr | 4·0 | tr | B | H |
| 92 | 97·5 | 1·0 | 1·0 | — | — | 0·5 | tr | tr | — | tr | — | B | B |
| 93 | 42·0 | 0·5 | 1·5 | 48·0 | 1·0 | 2·5 | 0·5 | 0·5 | tr | 3·5 | — | A | H |
| 94 | 2·5 | tr | 0·5 | 80·0 | 0·5 | 2·5 | 0·5 | 0·5 | 0·5 | 7·5 | 0·5 | A | A |
| 95 | 2·0 | tr | tr | 83·5 | 3·5 | 2·5 | 0·5 | 0·5 | 0·5 | 7·0 | tr | A | A |
| 96 | 29·5 | tr | 0·5 | 53·0 | 3·5 | 2·0 | 0·5 | 0·5 | 0·5 | 9·5 | — | H | H |

* Identification checked by Professor J. W. Duffield. A—Green-leaf variety; B—blue-leaf variety; H—hybrid.
† A—3-carene variety; B—α-pinene variety; H—hybrid.

(which is optically inactive). The honor of reporting sabinene (4[10]-thujene) in the turpentine from the southern race of *P. muricata*, the first time for the entire genus Pinus, belongs also to New Zealand research workers.[9] About the same time sabinene was independently detected by Dr. Richard H. Smith of the Pacific Southwest Forest and Range Experiment Station, Berkeley, California.

Despite the work already reported, several aspects of the *P. muricata* problem remained obscure. The populations between Fort Bragg and Inverness were not investigated nor were those of Santa Rosa Island. The turpentine constituents other than α-pinene, 3-carene, sabinene, and terpinolene were practically ignored. Most of the material was obtained from arboreta, and only slightly more than a third of the trees were samples in their original habitat. For these reasons we reinvestigated the turpentine composition of *Pinus muricata* from 127 trees in sixteen different localities, ten of which were in northern California to and including Inverness. With only one exception we used wild trees for this purpose.

*Results of the Present Chemical Investigations*

Supporting our previous finding[5] the results (Tables 3, 4, Figs. 1-4) indicate that north of the junction of State Highway 1 with the road at Annapolis *P. muricata* turpentine consists largely of α-pinene. The turpentine composition does not seem to vary much from population to

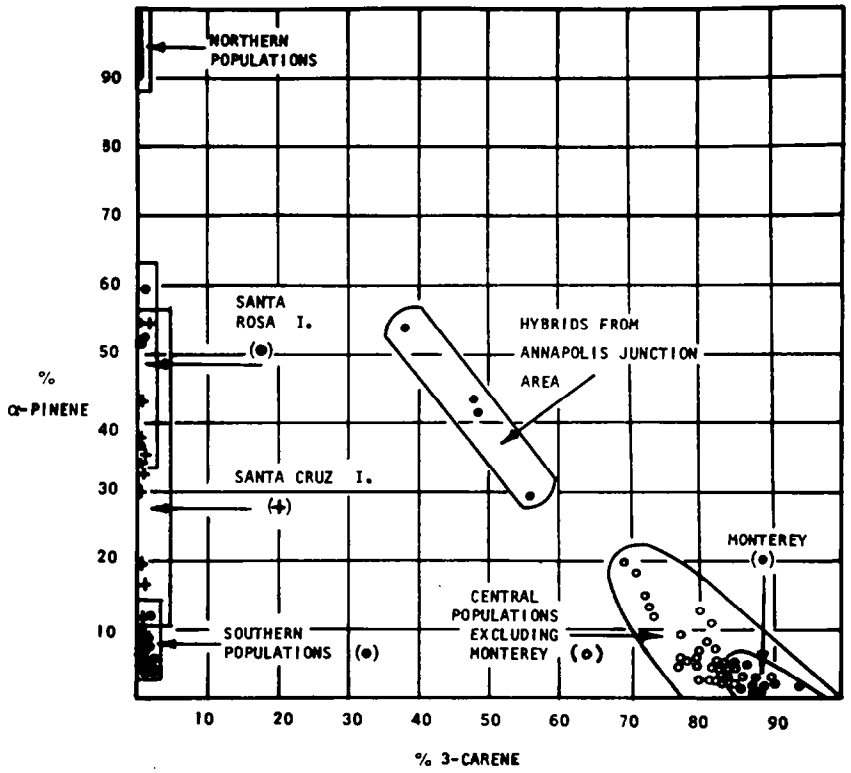


FIG. 2. PLOT OF α-PINENE VS. 3-CARENE PERCENTAGES IN THE TURPENTINES OF THE TREE INDIVIDUA STUDIED.

[9] M. M. BLIGHT and I. R. C. MCDONALD, *New Zealand J. Sci.* 6, 229 (1963).

population in that region, although a very slight, gradual decrease in α-pinene content from Trinidad (98 per cent) to just north of the Annapolis junction (95 per cent) is discernible. As anticipated by the work of Forde and Blight[8] to the south of this region the populations separate into two "chemical races"—a central race (Stewarts Point, Fort Ross, Inverness, Monterey), with turpentine composed largely of 3-carene (69–91·5 per cent) with moderate amounts of terpinolene (up to 8 per cent)—and a southern race (San Luis Obispo, Lompoc, San Vicente), with turpentine composed largely of sabinene (50–60 per cent on the average) and terpinolene (20–40 per cent on the average). As already reported by the New Zealand workers,[7] the insular populations (Santa Cruz and Santa Rosa Islands) form a separate group within

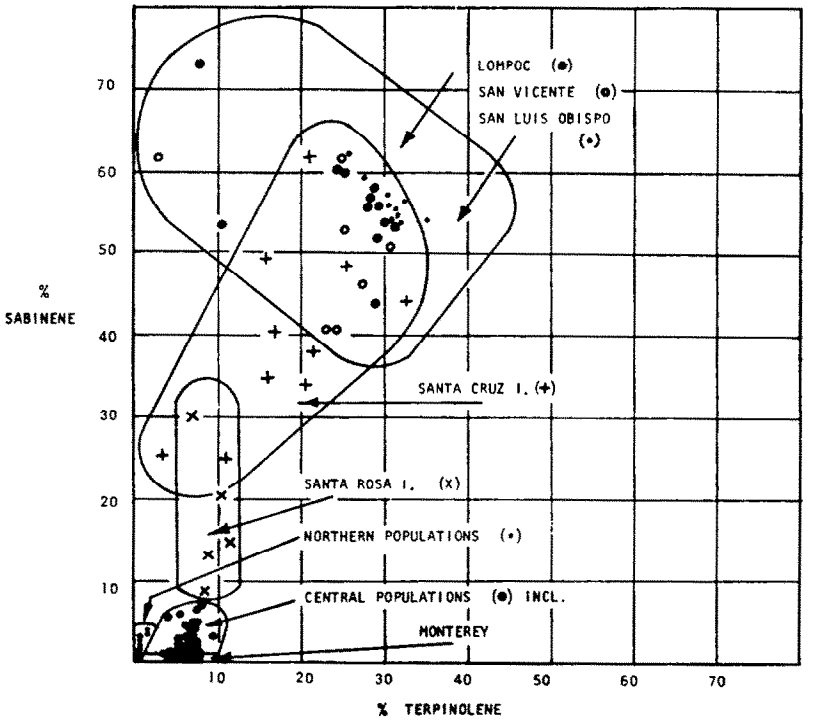


FIG. 3. PLOT OF SABINENE VS. TERPINOLENE PERCENTAGES IN THE TURPENTINES OF THE TREE INDIVIDUA STUDIED.

the southern race by virtue of their higher α-pinene and lower sabinene and terpinolene contents.

Since the central race and the southern races are geographically separated, there was no difficulty in determining ranges of their distribution. On the other hand it was not certain whether there is a geographical discontinuity between the northern race and the central race. We inquired into this problem more closely than previous investigators and found that the two races come in contact in an extremely limited area along the road from the coast to the town of Annapolis (Fig. 1). This is the area where Duffield's blue-leaf var. *borealis* meets the more southern green-leaf var. *muricata*. Here we found not only trees of both varieties but also hybrids between the two. However, the extent of hybridization seemed to be extremely limited. Ten trees were sampled in this area. Their morphological and chemical characters are shown in Table 4; the morphological assignments agree in some but not in all cases with

chemical assignments. Partial disagreement of the chemical and morphological identifications in case of hybridization was found also by Mirov in *Pinus contorta* × *Pinus banksiana* hybrids.[10]

Among the populations belonging to the central race, the Monterey population was found to occupy chemically a somewhat separate position in that its turpentine contains more 3-carene, less α-pinene,[7] and less sabinene. Contrary to the report of Forde and Blight,[8]

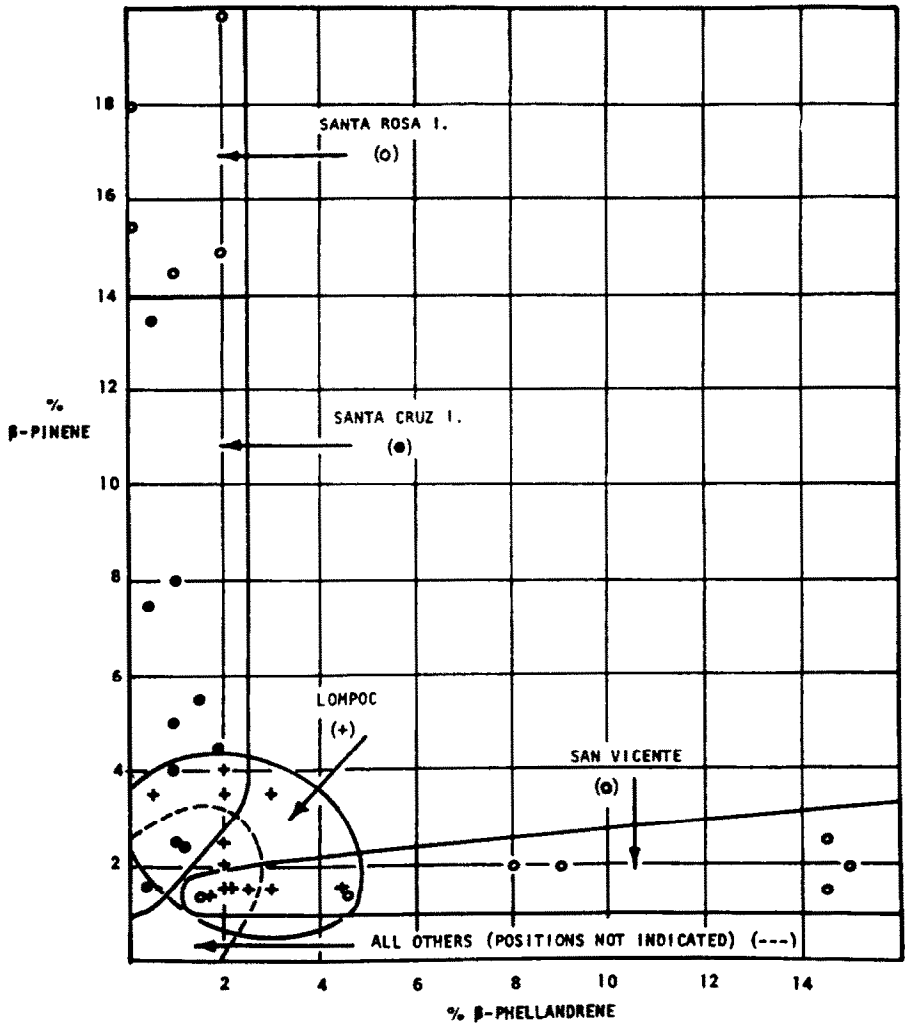


FIG. 4. PLOT OF β-PINENE VS. β-PHELLANDRENE PERCENTAGES IN THE TURPENTINES OF THE TREE INDIVIDUA STUDIED.

we found that sabinene was not restricted entirely to the populations of the southern race. Although in Trinidad samples it was detected only in trace amounts, in more southern populations of the var. *borealis* it was found in larger quantities, up to 4 per cent at Gualala. In the populations of the central race it was found in even higher amounts—as much as 7 per cent near Stewarts Point and 6 per cent at Inverness.

[10] N. T. MIROV, *Can. J. Botany* 34, 443 (1956).

According to Forde and Blight[8] "minor constituents including β-pinene and camphene" (reported together) were found in most of the samples, in some to an extent of 6·5, 8·8, or even 11·8 per cent. We found that these minor constituents were composed, in addition to β-pinene and camphene, of myrcene, limonene, β-phellandrene, γ-terpinene and α-thujene with the composition following definite patterns. Thus, β-pinene (Fig. 4) seems to be present in turpentines of the southern race in larger quantities than in those of the other two races. Among southern populations it occurs in still larger amounts in samples from the insular populations —5·5 per cent on Santa Cruz Island and 16·5 per cent on Santa Rosa Island, 2·5 per cent at Lompoc and 2·0 per cent at San Vicente (Fig. 4). α-Thujene was absent from the northern variety but was present in traces in central populations. However, in southern populations it appears in appreciable amounts, reaching 11·0 per cent at Lompoc. Thus its distribution seems to parallel that of sabinene. This is not surprising as it is chemically closely related to this terpene.

β-Phellandrene (Fig. 4), a monocyclic, very unstable terpene, presumably in its levo-form, was detected in particularly large quantities in San Vicente turpentine: 9·5 per cent on the average and reaching 15 per cent in the case of one tree. However at Lompoc it averaged only 2·0 per cent, and in other populations less than 1·0 per cent. Limonene, although present in somewhat smaller quantities, followed the β-phellandrene pattern closely (Figs. 1 and 4). These findings seem to contradict the results of Forde and Blight[8] who reported between 2·1 and 3·3 per cent for the *sum* of "minor constituents" in San Vicente samples. Of the other terpenes, γ-terpinene seems to be absent from the turpentines of the northern race and present only in rather small quantities in central and southern races. Myrcene occurs in all three races, but in somewhat increased amounts in the central race.

## DISCUSSION

*Pinus muricata* is a unique species among the pines. Although it grows under climatic conditions more or less unchanged since the Tertiary period, it shows a chemical diversification that no other pine species possesses. For example, throughout its extensive range, barring individual variations common to all plant species, *P. ponderosa* always yields turpentine with an abundance of 3-carene; *P. palustris*, no matter where it grows, is always a predominantly 'α-pinene/β-pinene' pine. *P. pinea* is a 'limonene pine' in all localities of its area, from Portugal to Turkey. *P. contorta* from different localities of its enormous area always contains in its turpentine appreciable quantities of (−)-β-phellandrene. On the other hand, the differences among the chemical races of *P. muricata* are greater than the differences between many species of *Pinus*, even those rather distantly placed in the present system of classification. In order to understand, at least partly, this unusual chemical behavior of *P. muricata* it seems expedient to inquire into its paleobotany as well as into the past and present geomorphology of the California coast, where this pine has developed.

Pines came to California from the North probably from the area around what is now Bering Sea,[11] as indicated by ample paleobotanical, geomorphological, and geographical evidence. Apparently the migration took place during the Mesozoic era. The scarcity of plant-bearing Mesozoic strata in western North America is well known, hence there is almost no fossil record of that era for the Pacific coast. The only Jurassic pine species on the Pacific coast was described from Oregon, and the only Low Cretaceous from Northern California.[12]

[11] N. T. MIROV, *Proc. Intern. Botan. Congr. Montreal*, I, II, IIA (1959).
[12] W. M. FONTAINE, In L. F. WARD, *Monogr. U.S. Geol. Survey* **48** (1905).

Jurassic and Cretaceous pines have been found also in the eastern United States[13] and in Eurasia.[14,15] This causes us to conclude that pines inhabited at least some parts of northern California already during the Cretaceous period of the Mesozoic era. The distribution and migration of pines in California during the Cenozoic era was determined chiefly by the changes in the geomorphology and climate during the Tertiary period.

In the Tertiary period land masses in California fluctuated considerably, both inland and along the coast, especially south of Point Arena (Fig. 1), as witnessed at present by numerous fault lines, both dead and alive, extending longitudinally either parallel to the coast or at an angle to it. The renowned San Andreas Fault, which caused the San Francisco earthquake of 1906 is the most extensive and is still active. There are also many shorter, latitudinal fault lines on the coast. As a result of these Tertiary land movements extending well into the Pleistocene, numerous blocks of land were severed from the mainland, and their submergence and subsequent uplifts caused repeated changes in the geomorphology of the California coast. There is ample evidence that an extensive Tertiary archipelago extended along what is now the coast,[16] and that moreover the coast was indented with numerous inlets, some penetrating deeply inland, similar to San Francisco Bay. The general uplift of the coast during the Pleistocene epoch occurred first in the north and later in the south.

Toward the end of the Tertiary period the climate of interior California became colder and drier. The climate along the coast has not changed much, as witnessed by many Tertiary relict species still growing there. The Tertiary predecessor of the closed-cone pines of California was *P. masoni* Dorf, which was found all along the coast of California but which is now extinct. *P. attenuata*, *P. radiata*, and *P. muricata*[17] developed from this species during the Pleistocene and Holocene eras.

*P. attenuata* extended inland adapting itself to the cold winters and dry summers. At present it grows not only in the coastal ranges (not too close to the ocean, however) but also in the inner mountain ranges of southern Oregon and California.

Variability of *P. attenuata* has not yet been studied thoroughly, but apparently in its morphology it is relatively uniform throughout its extensive range. The turpentine of *P. attenuata* from Sierra Nevada is extremely simple, consisting almost entirely of (+), (±)-α-pinene, being similar to that of *P. muricata*, var. *borealis*. The morphological uniformity and assumed general simplicity (subject to verification) of *P. attenuata* turpentine may be explained by the absence of the Cenozoic water barriers in inner California; there were no geomorphological factors inducive to the isolation of different populations of *P. attenuata* throughout its extensive range.

On the other hand the cold-tender species *P. radiata* and *P. muricata* clung to the coast where the climate was and still is relatively mild, but where the geomorphological catastrophes of the Tertiary period caused insular development of these two pines. *P. radiata* survived only on one piece of the continental land-mass, hence, there is a considerable morphological uniformity of the continental *P. radiata*, and relatively constant chemical composition of its turpentine, which consists predominantly of α-pinene and β-pinene, thus being almost as simple as the turpentine of *P. attenuata*. *P. radiata* of the Guadalupe and Cedros Islands is still little known either morphologically or chemically.

[13] J. S. PENNY, *Am. J. Botany* **34**, 281 (1947); E. G. BERRY, *Bull. Torrey Botan. Club* 37, 181 (1910); R. W CHANEY, *Ecology* **35**, 145 (1954).
[14] A. KRISHTOFOVICH, Prodromus florae fossilis. *Paleontologya SSSR* **12**, Suppl. *Akad. Nauk SSSR* (1941).
[15] W. STUDT, MITTELL. *Inst. Allgem. Bot. Hamburg* **6**, 167 (1926).
[16] H. L. MASON, *Carnegie Inst. Wash. Publ.* **415**, 81 (1934).
[17] S. A. CAIN, *Foundations of Plant Geography*. p. 113. Harper, New York (1944).

The history of *P. muricata* has been different. This species developed from *P. masoni* much earlier than the other two pines, probably in the north, where the late Tertiary land movements were less drastic than farther south. The similarity in turpentine composition of *P. muricata* var. *borealis* and *P. attenuata* suggests this possibility. In the north, *P. muricata* either formed pure groves[18] or occurred with other conifers, chiefly *Sequoia sempervirens*. Gradually *P. muricata* spread south along the coast but its expansion inland was prevented by its cold-tenderness. The Pleistocene geomorphological catastrophes resulted in formation of numerous islands on the coast and deep inlets which segregated *P. muricata* into separate populations; isolation was inducive to development of the morphological varieties and chemical races.

After the subsequent uplift of the coast, almost all islands again became parts of the mainland, and most of the inlets disappeared. *P. muricata* once more found itself on the continent and only on Santa Cruz and Santa Rosa Islands does it retain its insular habitat.

The Holocene history of distribution of *P. muricata* has been secondary. In some places there has been encroachment of other types of vegetation,[15] while *P. muricata*, as did many other pine species, readily invaded areas where competition from other types of vegetation had been destroyed by denudation (floods, fire, grazing, logging). Recent human activities, both protective (fire prevention) and destructive (real estate development) are contributing to the further redistribution.

In the north, where the continuity of the *P. muricata* area had not been disturbed as much as in the south, we found evidence that the var. *borealis* ($\alpha$-pinene race) had come in contact with the central (carene) race of *P. muricata* and the two had hybridized. This probably happened rather recently as indicated by the narrow area of the intercrossing and by the relatively few trees which exhibit intermediate chemical and morphological characters. That the area of intercrossing is located just south of the San Andreas Fault, while the main area of the var. *borealis* is north of it, also suggests the recent occurrence of its intercrossing with the central race.

From Annapolis south it is impossible to determine whether or not the now disjoined groves once presented a more continuous forest strip along the coast in some localities. Possibly some of these insular populations coalesced or even hybridized becoming separated later.

$\alpha$-Pinene may be considered as the generic, ancestral terpene of the pines belonging to the group *Insignes* Shaw. It is found in all three species of California closed-cone pines as well as in almost all species of *Pinus*,[6] in large quantities in most. The replacement of $\alpha$-pinene with other terpenes in a variety thus could indicate a more recent origin of this variety. The investigations show that the turpentine of *P. muricata* var. *borealis* is similar to that of *P. attenuata* and is composed almost entirely of $\alpha$-pinene. The turpentines of the central and southern varieties of this species contain this terpene only in small amounts, and the turpentine composition becomes more diversified farther south. Besides the spectacular appearance of 3-carene in the central race and the manifold increase of sabinene and terpinolene in the southern race the lesser components also show an increase. This is true for thujene, $\beta$-pinene, $\beta$-phellandrene, limonene, and even for the open chain terpene, $\beta$-myrcene (Fig. 1). All this diversity in turpentine composition seems to indicate that the southern races are more recent and, thus that *P. muricata* originated in the northern part of its present range.

Forde and Blight[10] suggested that the relatively high percentage of $\alpha$-pinene in the turpentine of the insular populations of *P. muricata* (Duffield's var. *remorata*) represents either a

[18] W. METCALF, *J. Forestry* **19**, 1 (1921).

local development of the southern race or, following the hypothesis of Mason,[19] is the result of hybridization between the southern race of *P. muricata* and *P. remorata*, originally an α-pinene pine. However there might be still another explanation. Our results indicate that the insular populations of *P. muricata* differ from the mainland populations by the increased percentage of both α- and β-pinene. If we assume that these increased amounts of α- and β-pinenes represent what is left of the characteristic *P. remorata* terpenes in the present day populations, it would follow that original *P. remorata* was an α-pinene/β-pinene pine. This immediately ties it with *P. radiata* which is also an α-pinene/β-pinene pine. *P. radiata* occurs at Año Nuevo, Monterey, and near Cambria (coastal, central California), and on Guadalupe and Cedros Islands. Santa Cruz and Santa Rosa Islands are exactly between these locations. From the chemical point of view it seems reasonable to assume that *P. radiata* also occurred on Santa Cruz and Santa Rosa Islands but was subjected to *P. muricata* gene flow to such an extent that it lost its species status. The fact that the Santa Rosa Island population seems to contain more of both α- and β-pinenes than does the Santa Cruz Island population supports this. Santa Rosa Island is farther from the mainland and presumably it was subjected to a less intense gene flow. It is planned to investigate this problem more thoroughly in the near future.

However unusually high chemical variability of *P. muricata* cannot be explained solely by the geographical isolation of its several populations during the Pliocene and Pleistocene. There are numerous examples of populations of a pine species isolated geographically one from another since the Tertiary, yet retaining essentially the same composition of their turpentines, e.g. *P. nigra*, *P. ponderosa*, or *P. sylvestris*.[6] *P. khasya* of Viet Nam and *P. insularis* of the Philippines considered by many as one species, have been separated since the Pleistocene by the wide distance of the South China Sea, yet composition of their turpentines is almost identical.[20]

It appears then, that factors other than geographical isolation, have been responsible for diversification of chemical composition of *P. muricata* turpentine. Apparently when this species emerged from its Pliocene ancestry it developed a capacity for chemical variability, such as is not found in most species of the genus *Pinus*.

Breeding experiments with *P. muricata* performed at the Institute of Forest Genetics, Placerville, California, showed that blue-foliage var. *borealis* could not be crossed, despite numerous attempts, with the green-foliage (southern) varieties of the species.[21] Thus *P. muricata* seems to possess strong infraspecific barriers to crossing, which apparently have been instrumental in maintaining the morphological and chemical diversity within the species.

It is combined effect of the environment (geomorphology and climate) and the genetic characteristics of *P. muricata* that have been responsible for its remarkable chemical diversity.

## EXPERIMENTAL

Samples were collected at the following locations (latitude indicated in parentheses): near Trinidad, Humboldt County (41° 3·5′); Jackson State Forest near Fort Bragg (39° 25′); Van Damme State Park (39° 18′), near Gualala (38° 46′), all in Mendocino County; about 0·5 miles inland from the junction of Highway 1 and the road to Annapolis (38° 41·5′), 2 miles north from Annapolis junction (38° 42·5′), 1 mile south from the Annapolis junction (38° 41′), 1 mile north from Stewarts Point (38° 39′), near Fort Ross (38° 31·5′), all in Sonoma County;

[19] H. L. MASON, In *Genetics, Paleontology and Evolution* (Edited by G. L. JEPSON, E. MAYR and G. G. SIMPSON), p. 356. Princeton University Press, Princetown (1949).
[20] E. ZAVARIN, N. T. MIROV and K. SNAJBERK, *Phytochem.* **5**, 91 (1966)
[21] W. B. CRITCHFIELD, Private communication.

at Tomales Bay near Inverness (38° 8′), Marin County; at Huckleberry Hill near Monterey (36° 33·5′), Monterey County; near San Luis Obispo (35° 10·5′), San Luis Obispo County; at the top of Harris grade near La Purisima Mission not far from Lompoc (34° 41′), at Santa Cruz Island (34° 00′), both in Santa Barbara County; and near San Vicente (31° 15′), Baja California. Oleoresins from the Santa Rosa Island (34° 00′) provenance were obtained from trees grown at the Institute for Forest Genetics, Placerville, Eldorado County, California.

In most cases the procedure for securing oleoresin was as follows: A hole was drilled in the sapwood of a tree 1–3 in. deep and of such diameter that a 1 dr or, at times a 4-dr screw-neck vial could be screwed in. The vial was left in the tree overnight, then removed; a few grains of pyrogallol were added to the oleoresin as a preservative. In the laboratory the air from the vials was displaced with nitrogen and the vials were stored in a refrigerator.

The oleoresins collected were analyzed quantitatively by gas–liquid chromatography using the β,β-oxydipropionitrile column. Details of the procedure were published in our study of Mediterranean pines.[22] The results are presented in Tables 3 and 4, in Fig. 1 and in α-pinene/3-carene, sabinene/terpinolene and β-pinene/β-phellandrene plots (Figs. 2–4).

*Note added in proof:* Recently a natural hybrid tree between central and southern *P. muricata* races was discovered at Lompoc. Anal. α-pinene—10·0; thujene—3·0; camphene—0·5; β-pinene—3·5; 3-carene 21·0; sabinene—48·0; myrcene—tr.; limonene1·0; β-phellandrene—0·5; γ-terpinene—1·5; and terpinolene—11·0%.

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[22] N. T. Mirov, E. Zavarin and K. Snajberk, *Phytochem.* 5, 97 (1966)