Fatty Acids of the Seeds from Pine Species of the *Ponderosa-Banksiana* and *Halepensis* Sections. The Peculiar Taxonomic Position of *Pinus pinaster*

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ABSTRACT: The fatty acid compositions of pine seed oils were determined from 11 species of the *Banksiana* subsection and three species of the *Ponderosa* subsection. All were collected in North America (United States, Mexico, and Cuba). These analyses also included the seed oils from the unique European species of the *Ponderosa-Banksiana* section (*Banksiana* subsection), *Pinus pinaster*, and from three pine species of the *Halepensis* section, which are related to the *Banksiana* subsection. Emphasis was placed on their Δ5-olefinic acid content and profile. Principal-component analysis of fatty acid compositions showed that all North American species constituted a fairly homogeneous group. However, *P. jeffreyi* was slightly eccentric, and *P. pinaster*, a west-Mediterranean species, was completely isolated from the North American group. Other species from the *Banksiana* and *Ponderosa* subsections could not be distinguished on the basis of their seed oil fatty acid compositions. With respect to Δ5-olefinic acids, the North American species (except for *P. jeffreyi*) had 5,9-18:2, 5,9,12-18:3, 5,11-20:2, and 5,11,14-20:3 acid concentrations in the ranges 1.9 to 3.2, 17.7 to 22.9, 0.2 to 0.4, and 2.0 to 3.5%, respectively (sum, 22.7–28.5%). Levels of corresponding acids in *P. pinaster* were 0.9, 7.9, 0.9, and 7.0%, respectively (sum, 16.7%). Other differences were observed for linoleic acid (42.6 to 48.6% vs. 52.2%) and α-linolenic acid (0.3 to 0.6% vs. 1.4%). *Pinus pinaster* was close to species of the *Halepensis* section (5,9-18:2, 0.5 to 1.0%; 5,9,12-18:3, 3.1 to 4.4%; 5,11-20:2, 0.4 to 0.5%; 5,11,14-20:3, 3.6 to 5.4%; sum, 8.6–11.1%), which were clearly separated from the *Ponderosa-Banksiana* section. Among all pines analyzed, *P. pinaster* presented the highest level of sciadonic (5,11,14-20:3) acid, a component that has three ethylenic bonds in common with arachidonic and eicosapentaenoic acids.

JAOCS 75, 45–50 (1998).

KEY WORDS: *Banksiana, Halepensis*, multivariate analysis, Δ5-olefinic acids, pine seed oil, *Pinus pinaster, Ponderosa*, sciadonic acid, taxonomy.

Δ5-Olefinic acids (or Δ5-unsaturated polymethylene-interrupted fatty acids) are characteristic and systematic components of Gymnosperm seed oils (1–5) and also may occur in a few rare Angiosperm species. In conifer seeds, some of the following acids may be present, depending on the botanical family considered: 5,9-18:2, 5,9,12-18:3, 5,9,12,15-18:4, 5,11-20:2, 5,11,14-20:3, and 5,11,14,17-20:4 acids (1,3). For example, Pinaceae seed oils contain 5,9-18:2, 5,9,12-18:3, 5,9,12,15-18:4, 5,11-20:2, and 5,11,14-20:3 acids, whereas 5,11,14,17-20:4 acid is found almost exclusively in Cupressaceae and Taxodiaceae seed lipids (1,3,5). More than 100 conifer species analyzed to date contain Δ5-olefinic acids (Refs. 1–6, this study; and Wolff, R.L., and A.M. Marpeau, unpublished data). The total content of Δ5-olefinic acids varies from less than 1% [*Pinus cembroides edulis* (6)] to 33.9% [*Larix sibirica* (5)]. The upper value is apparently imposed by the fact that Δ5-olefinic acids are almost exclusively esterified to the *sn*-3 position of conifer seed triacylglycerols (TAG) (7).

Some of these acids may favorably alter lipid metabolism in rats (8,9). These effects, observed with *P. koraiensis* (Korean pine) and *Biota orientalis* (arborvitae, a Chinese Cupressaceae) seed oils, have been reviewed recently (10,11). Δ5-Olefinic acids from conifer seeds all share Δ5 unsaturation that also is found in arachidonic and eicosapentaenoic acids. It is hypothesized that this ethylenic bond might exhibit important functional properties. Recently, it was observed that the oil from *P. pinaster* (maritime pine), initially described by Wolff and Bayard (2), which contains both pinolenic (5,9,12-18:3) and sciadonic (5,11,14:20:3) acids (*ca.* 7% each), could profoundly alter the lipid and lipoprotein parameters in rat serum (Asset, G., B. Staëls, R.L. Wolff, E. Bauge, J.C. Fruchart, and J. Dallongeville, submitted for publication). When fed to rats as a dietary supplement (5% by weight in the diet) for 4 wk, this oil decreased TAG by 30%, very-low density lipoproteins (VLDL) TAG by 40%, VLDL phospholipids by 21%, and apo A-II by 18%, when compared to rats fed a diet in which pinolenic and sciadonic acids were quantitatively replaced by oleic acid. In the same study, *P. pinaster* seed oil was compared to *P. koraiensis* seed oil (15% of pinolenic acid), and it was noted that the former was more potent than the latter. The authors thus suggested that sciadonic acid might have a greater effect on serum lipids than pinolenic acid.

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The oil from *P. pinaster* seeds is available in France, at least upon request, on a multiliter-scale. However, seed stocks are limited. Assuming that the present French seed stock (which is principally reserved for reforestation) was exclusively used for oil production, only 3000 L could be recovered. This is an evident overestimate, because only a minor part of the seed stocks can be used for oil production. Consequently, new sources of sciadonic acid are needed.

As a first approach, we analyzed seeds of pine species that belong to the same section, *Ponderosa-Banksiana*, as *P. pinaster*, which is divided into two subsections, *Ponderosa* and *Banksiana* (12). We hoped that seed oils from these species would resemble that of *P. pinaster*. With the exception of *P. pinaster*, which is a west-Mediterranean species (principally European: France, Portugal, Spain, Italy), all other species of this section grow in North America. They represent the majority of pine species in North America, where they are of major economical importance in the wood industry. In addition, we have analyzed a few species from the *Halepensis* section (essentially circum-Mediterranean), because species from this section have some biological affinities with species of the *Banksiana* subsection (12). Both the *Ponderosa-Banksiana* and *Halepensis* sections belong to the subgenus *Pinus* (12).

The seeds from *P. pinaster* and from 14 pine species of the *Ponderosa-Banksiana* section collected in North America (United States, Mexico, and Cuba) were thus analyzed for Δ5-olefinic acid content and profile, along with three species of the *Halepensis* section. The fatty acid compositions were subjected to multivariate analysis (principal-component analysis). Our results indicate that *P. pinaster* is quite distinct from the North American species and that it is closer to the *Halepensis* section. It still remains the best present source of sciadonic acid.

TABLE 1
Oil Content (wt%) of the Seeds from *Pinus* Species Belonging to the *Banksiana* and *Ponderosa* Subsections (*Ponderosa-Banksiana* section), and to the *Halepensis* Section

Number[a]	Species	Trivial name	Origin	Oil Content
1	*P. banksiana*	Jack pine	USA	27
2	*P. contorta*	Shore pine	USA	29
3	*P. palustris*	Longleaf pine	USA	22
4	*P. elliotii*	Slash pine	USA	19
5	*P. caribaea*	—	Cuba	24
6	*P. echinata*	Shortleaf pine	USA	18
7	*P. occidentalis*	Cuban pine	Cuba	25
8	*P. attenuata*	Knobcone pine	USA	32
9	*P. muricata*	Bishop pine	USA	32
10	*P. radiata*	Monterey pine	USA	31
11	*P. taeda*	Loblolly pine	USA	13
12	*P. pinaster*	Maritime pine	France	16
13	*P. ponderosa*	Western yellow pine	USA	28
14	*P. michoacana*	Michoacán pine	Mexico	16
15	*P. jeffreyi*	Jeffrey pine	USA	29
16	*P. halepensis*	Aleppo pine	France	35
17	*P. brutia*	Calabrian pine	Greece	14
18	*P. eldarica*	Afgan pine	Caucasius	18

[a]Species numbers 1 to 12, and 13 to 15, belong to the *Banksiana* and *Ponderosa* subsections, respectively. Species 16 to 18 belong to the *Halepensis* section. The fatty acid compositions of species 10, 12, 13, 16, and 17 have already been described elsewhere (Refs. 2,5).

EXPERIMENTAL PROCEDURES

Seed samples and standards. Pine seeds were purchased from the Versepuy Society (Le Puy-en-Velay, France) or were kindly donated by the Vilmorin Society (La Ménitré, France) and AFOCEL (Moulis-en-Médoc, France). *Pinus pinaster* seeds were harvested in the Landes region of France. 14-Methylhexadecanoic acid methyl ester was purchased from the Sigma Chemical Company (St. Louis, MO).

Oil extraction. Seed oil was extracted according to Folch *et al*. (13). The seeds (undehulled) were finely ground in a household electric grinder. An aliquot (10 g) of the resulting powder was dispersed in 50 mL methanol with an Ultra-Turrax T-25 (Janke & Kunkel GmbH and Co. KG, Staufen, Germany), equipped with an S-25N shaft. Chloroform (100 mL) was added, and the suspension was dispersed a second time with the Ultra-Turrax. The suspension was then filtered into a separatory funnel. The vessels and the residue on the filter were rinsed with several portions (total: 25 mL) of chloroform/methanol (2:1, vol/vol). The clear filtrate was thoroughly mixed with 35 mL of a 1% (wt/vol) aqueous solution of KCl and allowed to stand for about 2 h. The lower phase was drained, and the solvents were removed in a rotary evaporator at 50°C. The oil was dried in an oven at 105°C for 15 min, cooled, and then weighed.

Fatty acid methyl ester (FAME) preparation. FAME were prepared according to Morrison and Smith (14). Two drops of oil, introduced in a Teflon-lined screw-capping tube, were dissolved in 1.5 mL of a methanolic solution of BF_3 (12%, wt/vol), and the mixture was homogenized with 1.5 mL benzene. The tubes were tightly capped, and the reaction was allowed to proceed for 1 h in a boiling water bath. FAME were extracted twice with 2 mL hexane, with water (2 mL) being added to the mixture.

Gas–liquid chromatography (GLC). FAME were analyzed in a Carlo Erba 4130 chromatograph (Carlo Erba, Milano, Italy), equipped with a DB Wax column (30 m × 0.32 mm i.d., 0.5 μm film; J&W Scientific, Folsom, CA). The oven temperature was 190°C, and the inlet pressure of the carrier gas (helium) was 140 kPa. The injector (split mode) and the flame-ionization detector were maintained at 250°C. Quantitative data were calculated by an SP 4290 integrator (Spectra Physics, San Jose, CA).

Peak identification. Δ5-Unsaturated polymethylene-interrupted fatty acids were identified by their equivalent chain-lengths (ECL) according to Wolff *et al*. (3). The use of ECL was supported by GLC–mass spectrometry of appropriate derivatives (15).

Data processing. Multivariate statistical treatment (principal-component analysis) of normalized peak areas, practically identical to fatty acid weight percentages, was performed by using the program STAT-ITCF (ITCF, Paris, France). Only

TABLE 2
Fatty Acid Compositions of the Oil (as wt% of total fatty acids[a]) from the Seeds of *Pinus* Species of the *Banksiana* Subsection

Species[b]	16:0	16:1[c]	*br*-17:0[d]	18:0	9-18:1	11-18:1	9,12-18:2	9,12,15-18:3	20:0	11-20:1
1	3.65	0.13	0.17	1.55	16.73	0.94	44.73	0.46	0.39	0.95
2	3.21	0.16	0.23	1.62	15.35	1.69	44.75	0.58	0.59	1.22
3	5.09	0.10	0.16	1.66	19.66	0.46	43.43	0.57	0.42	0.81
4	5.14	0.08	0.17	2.07	18.95	0.50	44.96	0.43	0.52	1.11
5	5.82	0.10	0.18	2.65	19.11	0.51	45.02	0.30	0.55	1.19
6	5.40	0.07	0.16	2.25	19.12	0.50	45.52	0.40	0.51	1.01
7	5.28	0.07	0.12	2.41	19.54	0.60	45.32	0.32	0.47	0.92
8	3.65	0.13	0.11	1.86	20.29	0.63	45.51	0.36	0.39	0.84
9	3.39	0.07	0.16	1.55	18.00	0.33	44.63	0.45	0.31	1.08
10	3.62	0.15	0.11	1.48	19.40	0.97	43.60	0.37	0.24	0.69
11	4.02	0.06	0.15	1.84	17.62	0.48	48.25	0.40	0.44	1.06
12	4.61	0.17	0.20	2.70	18.92	0.38	52.23	1.37	0.31	1.15

Species	11,14-20:2	22:0	7,11,14-20:3	5,9-18:2	5,9,12-18:3	5,9,12,15-18:4	5,11-20:2	5,11,14-20:3	Others	Σ Δ5[e]
1	0.84	0.14	0.56	2.37	22.90	0.07	0.29	2.87	0.26	28.50
2	0.82	0.15	0.68	1.87	22.75	0.11	0.35	3.46	0.44	28.54
3	0.56	trace[f]	0.34	3.20	19.93	0.06	0.35	2.42	0.78	25.96
4	0.90	0.22	0.37	2.50	18.47	0.06	0.34	2.89	0.32	24.26
5	0.88	trace	0.33	2.25	17.71	0.08	0.23	2.42	0.67	22.69
6	0.76	0.15	0.28	2.69	18.18	0.05	0.25	2.31	0.39	23.48
7	0.71	trace	0.22	2.57	18.50	trace	0.31	2.35	0.29	23.73
8	0.57	trace	0.32	2.56	20.37	0.06	0.28	2.01	0.18	25.28
9	0.96	trace	0.46	2.02	22.80	0.07	0.36	3.00	0.36	28.25
10	0.56	0.08	0.27	2.98	22.34	0.04	0.31	1.82	0.93	27.49
11	0.76	trace	0.32	2.19	18.34	trace	0.42	3.37	0.28	24.32
12	0.87	0.08	0.18	0.90	7.90	0.07	0.85	6.97	0.14	16.69

[a]Values are the means of analyses of two fatty acid methyl ester preparations, except for species number 12 where $n = 10$.
[b]The numbers refer to the species listed in Table 1.
[c]Sum of two isomers.
[d]Tentatively identified as 14-methylhexadecanoic acid by cochromatography with an authentic standard.
[e]Sum of all Δ5-olefinic acids.
[f]Trace amounts. Peaks visible on the chromatogram but not taken into account by the integrator.

those acids that were generally higher than 0.2% of total fatty acids (14 variables) were used for calculations.

RESULTS AND DISCUSSION

Fatty acid compositions. The oil content of the seeds analyzed in the present study is given in Table 1, and their fatty acid compositions in Tables 2–4. Total saturated acids represented between 5.4 and 9.3% of total fatty acids, with 16:0 and 18:0 being the major components. Other saturated acids that could be identified were 20:0 and 22:0, along with minute amounts (less than 0.05%) of 14:0 and 17:0 acids (not reported in Tables 2–4).

A branched-17:0 acid also was present, which was tentatively identified as 14-methylhexadecanoic acid by cochromatography with an authentic standard on two capillary columns with different polarities [DB-Wax and CP-Sil 88 (Chrompack, Middelburg, The Netherlands); results not shown]. It also could be isolated by argentation thin-layer chromatography in the saturated acid fraction (results not shown). This acid, uncommon in Angiosperm seed oils, was formally identified by mass spectrometry in *Ginkgo biloba* seed oil (16) and in the sapwood of several North-American pine species [*P. elliotii*, *P. palustris*, *P. echinata*, also analyzed here, and *P. virginiana* (17)]. It is also apparently a habitual component of conifer needle lipids (18), although *Pinu*s species contain the lowest levels of this acid among Pinaceae. In a previous study on pine seed oils (2), where a CP-Sil 88 capillary column was used, we erroneously reported this acid as a 16:1 acid because 14-methylhexadecanoic acid coeluted with one of the two 16:1 isomers generally present in pine seed oils (results not shown). On the DB-Wax capillary column, 14-methylhexadecanoic acid (ECL = 16.68) was quite distinct from the two 16:1 isomers (ECL = 16.26 and 16.32).

The major unsaturated acid was 9,12-18:2 acid in all species. For species of the *Banksiana* subsection, the concentration of this acid was almost constant, 43.4 to 48.3% (Table 2). Species of the *Ponderosa* section also exhibited approximately the same range, 45.4 to 48.5% (Table 3). *Pinus pinaster* had a slightly higher level of 18:2n-6 acid, 52.2%, but still less than species from the *Halepensis* section (55.5–60.5%) (Table 4). The second major unsaturated acid was 9-18:1 acid, which varied from a low of 15.4% to a high of 21.8% in the *Ponderosa-Banksiana* section (Tables 1 and 2), with *P. jeffreyi* being a major exception (30.9%). For species of the *Halepensis* section, the range was 18.8–23.7% (Table 4). Oleic acid was always accompanied by the 11-18:1 (*cis*-vaccenic) isomer. With the exception of *P. contorta*

TABLE 3
Fatty Acid Compositions of the Oil (as wt% of total fatty acids[a]) from the Seeds of *Pinus* Species of the *Ponderosa* Subsection

Species[b]	16:0	16:1[c]	*br*-17:0[d]	18:0	9-18:1	11-18:1	9,12-18:2	9,12,15-18:3	20:0	11-20:1
13	3.97	0.09	0.16	2.18	21.81	0.38	45.44	0.58	0.42	0.89
14	3.48	0.11	0.15	1.60	16.74	0.52	48.55	0.64	0.41	1.23
15	5.04	0.11	0.16	1.49	30.88	0.54	42.55	0.52	0.45	0.88

Species	11,14-20:2	22:0	7,11,14-20:3	5,9-18:2	5,9,12-18:3	5,9,12,15-18:4	5,11-20:2	5,11,14-20:3	Others	ΣΔ5[e]
13	0.45	trace[f]	0.20	2.92	18.36	0.07	0.26	1.62	0.20	23.23
14	0.80	trace	0.24	2.32	18.76	0.10	0.43	3.83	0.09	25.44
15	0.29	trace	trace	3.88	11.28	0.07	0.34	1.41	0.11	16.98

[a]Values are the means of analyses of two fatty acid methyl ester preparations.
[b]The numbers refer to the species listed in Table 1.
[c]Sum of two isomers.
[d]Tentatively identified as 14-methylhexadecanoic acid by cochromatography with an authentic standard.
[e]Sum of all Δ5-olefinic acids.
[f]Trace amounts. Peaks visible on the chromatogram but not taken into account by the integrator.

(1.7%), it generally accounted for less than 1% of the oil. Except for *P. pinaster*, where it reached 1.4%, the level of 18:3n-3 acid was less than 0.6% for all pine species of both sections.

On the basis of their ECL, Δ5-olefinic acids present in pine seed oils had the structures 5,9-18:2, 5,9,12-18:3, 5,9,12,15-18:4, 5,11-20:2, and 5,11,14-20:3, in agreement with studies of other pine species (1,2,4–6,16,19,20). Their total generally accounted for 22.7 to 28.5% in the *Banksiana-Ponderosa* section, with *P. jeffreyi* and *P. pinaster* being exceptions (17.0 and 16.7%, respectively), and was significantly less in the *Halepensis* section (8.6 to 11.1%).

The 5,9-18:2 acid, for which the trivial name taxoleic has been suggested (7), was in the range of 1.9 to 3.2% in the *Ponderosa-Banksiana* section, except for *P. pinaster*, where it was only 0.9%. This finding was similar to species of the *Halepensis* section (0.5–1.0%). The principal Δ5-olefinic acid was 5,9,12-18:3 acid, which varied from 18.5 to 22.9% in most species of the *Ponderosa-Banksiana* section. *Pinus jeffreyi* and *P. pinaster* were distinct from other species of the *Ponderosa-Banksiana* section, with only 11.3 and 7.9% substrates of the Δ5 desaturase, which catalyzes synthesis of 5,11-20:2 and 5,11,14-20:3 acids. However, there were no significant differences in the levels of 11-20:1 and 11,14-20:2 acids between *P. pinaster* and the North American pine species (1.2 vs. 0.8 to 1.2%, and 0.9% vs. 0.5 to 1.0%, respectively). In pines from the *Halepensis* section, the content of Δ5-olefinic acids with 20 carbon atoms was approximately the same as the content of species with 18 carbon atoms, a situation also observed in *P. pinaster*.

In addition to Δ5-olefinic acids, we noted the presence of a previously unidentified polymethylene-interrupted unsaturated fatty acid (ECL = 20.93 on the DB-Wax column) (2). It has been tentatively identified as 20:3n-6 (8,11,14-20:3) acid (4,18,19). However, except for the action of a Δ6 desaturase (which is apparently absent from pine seed oils because they do not contain 6,9,12-18:3 acid) on linoleic acid followed by a two-carbon atom elongation on the carboxylic end, there is no logical basis for such a structure in conifer seed oil fatty acids. On the other hand, bishomopinolenic (7,11,14-20:3) acid (the elongation product of pinolenic acid) has been identified in the wood of *Picea abies* (20), along with several other Δ5-olefinic acids. In unpublished experiments, we

TABLE 4
Fatty Acid Compositions of the Oil (as wt% of total fatty acids[a]) from the Seeds of *Pinus* Species of the *Halepensis* Section

Species[b]	16:0	16:1[c]	*br*-17:0[d]	18:0	9-18:1	11-18:1	9,12-18:2	9,12,15-18:3	20:0	11-20:1
16	4.51	0.09	0.11	3.67	23.68	0.36	55.49	0.69	0.50	0.53
17	4.73	0.09	0.09	3.54	18.81	0.32	60.45	0.66	0.50	0.87
18	3.97	0.05	0.07	3.32	19.94	0.28	57.61	0.70	0.49	0.87

Species	11,14-20:2	22:0	7,11,14-20:3	5,9-18:2	5,9,12-18:3	5,9,12,15-18:4	5,11-20:2	5,11,14-20:3	Others	ΣΔ5[e]
16	0.53	0.12	0.04	0.95	4.02	0.02	0.51	3.60	0.58	9.10
17	0.87	0.12	0.04	0.50	3.14	—[f]	0.38	4.60	0.29	8.62
18	1.09	trace[g]	trace	0.74	4.42	—	0.53	5.38	0.04	11.08

[a]Values are the means of analyses of two fatty acid methyl ester preparations.
[b]The numbers refer to the species listed in Table 1.
[c]Sum of two isomers.
[d]Tentatively identified as 14-methylhexadecanoic acid by cochromatography with an authentic standard.
[e]Sum of all Δ5-olefinic acids.
[f]Not detected.
[g]Trace amounts. Peaks visible on the chromatogram but not taken into account by the integrator.

demonstrated that the unknown fatty acid contained a Δ7-ethylenic bond by mass spectrometry of the 4,4-dimethyloxazoline derivative. Thus, this structure is most probably 7,11,14-20:3 acid and occurred in all pine seed oils studied here in the range of 0.2 to 0.7%.

On the basis of oil and Δ5-olefinic acid contents, several North American pine species of the *Ponderosa-Banksiana* section appeared to be potential sources of pinolenic acid. Generally, this acid is higher in these species than in the edible seeds of *P. koraiensis* (*ca.* 14.5%), which is the sole commercial source. Moreover, these species also contained sciadonic acid, though less than in seed oil of *P. pinaster* or of pines of the *Halepensis* section.

Multivariate analysis. In an earlier investigation (3), we noted that two groups of conifer families could be distinguished on the basis of their seed oil fatty acid compositions. Pinaceae and Taxaceae were characterized by low levels of 9,12,15-18:3 acid (generally less than 1.6%), whereas Cupressaceae and Taxodiaceae, with only one exception, contained more than 12% of this acid. Imbs and Pham (4) noted that Pinaceae constituted a distinct and rather homogeneous group among Gymnosperms, but no details were given for other families. Later, the model was improved through multivariate analyses of the seed oil fatty acid compositions of 82 conifer species (5), and it was possible to clearly individualize the four families Pinaceae, Taxaceae, Cupressaceae, and Taxodiaceae. It also was possible to divide the main Pinaceae genera (28 species analyzed) into *Pinus* (including *Tsuga* and *Pseudotsuga*), *Abies*, *Larix* plus *Picea*, and *Cedrus* (5). To our knowledge, this was the first time that fatty acid compositions were used as a chemometric means for the taxonomic systematization of a whole botanical class, the conifers, at the levels of families and genera.

However, the use of fatty acid compositions of seed oils from conifers for their taxonomy fundamentally relied on the invariancy of these compositions for each species. Alternately stated, it was hypothesized in earlier studies that the fatty acid compositions were genetically determined and stable. To support this hypothesis, we have analyzed the oil of *P. sylvestris* seeds collected in 10 different locations in France (Deluc, L.G., A.M. Marpeau, and R.L. Wolff, unpublished data). These fatty acid compositions were compared with those established for the same species from 10 different collecting areas in Finland (19). No major statistical differences were noted, and it was concluded that the fatty acid composition of *P. sylvestris* seed oil was invariable, at least between *ca.* 47°N and 69°N. Similar conclusions were reached with the study of *Picea abies* from 15 and 10 collecting areas from France and Finland, respectively. Consequently, on the basis of these two examples, it may be concluded that conifer seed oil fatty acid compositions are fixed and valid chemometric data for the taxonomic differentiation of conifers.

A principal-component analysis was achieved with the seed oil fatty acid compositions of the 18 species analyzed in the present study (Fig. 1). The three first axes explained 41.8, 24.1, and 13.2%, respectively, of the total variation. Axis 2 allowed separation of two principal groups: species of the *Ponderosa-Banksiana* (negative coordinates) and species of the *Halepensis* section, plus *P. pinaster* (positive coordinates). Axis 1 indicated a moderate heterogeneity in the *Ponderosa-Banksiana* group, but it was not possible to individualize the two subsections. *Pinus jeffreyi* and *P. ponderosa* appeared slightly eccentric in this group. Axis 1 allowed full discrimination between *P. pinaster* and species of the *Halepensis* section. Axis 3 did not add to these discriminations, except that it isolated slightly more *P. pinaster* (not shown in Fig. 1).

The most discriminant variables along axis 2 were: (i) 9,12-18:2 and 18:0 acids, which were higher in the *Halepensis* section and in *P. pinaster*; (ii) 5,11,14-20:3, 5,11-20:2, and 9,12,15-18:3 acids, which allowed strong discrimination of *P. pinaster*; (iii) 5,9,12-18:3 and 5,9-18:2 acids, which were higher in the *Ponderosa-Banksiana* section, and to a lesser degree, 7,11,14-20:3 and 11-18:1 acids. The only strong discriminant variable along axis 1 was the 9-18:1 acid, which slightly isolated *P. jeffreyi* from other species of the *Ponderosa-Banksiana* section. The moderate eccentricity of *P. ponderosa* was mostly explained by two minor variables, 11-18:1 and 7,11,14-20:3 acids.

In conclusion, two remarks can be made. On the basis of its seed oil fatty acid composition, *P. pinaster* is completely isolated from the North American species of the *Ponderosa-Banksiana* section, despite its membership of the *Banksiana* subsection founded on morphological, anatomical, and physiological criteria (12). On the other hand, it is close to the circum-Mediterranean section *Halepensis*, which appears homogeneous, and quite distinct from the *Ponderosa-Banksiana* section. To which extent this situation might correspond with an evolutive adaptation of *P. pinaster* to the Mediterranean climate remains to be established. Some other pine species

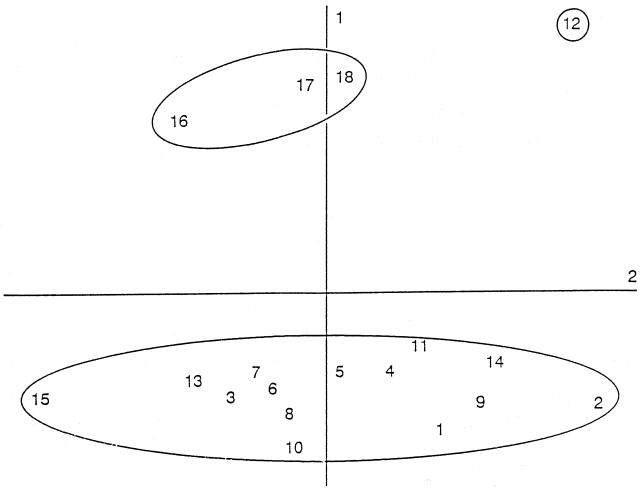

FIG. 1. Plot of chemometric data from the seeds of 18 pine species from the *Ponderosa-Banksiana* (numbers 1–15) and *Halepensis* (numbers 16–18) sections against the first two axes in principal-component analysis. The numbers refer to the species listed in Table 1. Note the remoteness of species number 12 (*Pinus pinaster*).

that are limited to warm regions, such as *P. merkusii*, *P. kremfii*, *P. dalatenis* [southeast Asia (4)], *P. edulis* ([southwest of the United States and Mexico (6)], *P. pinea* [a circum-Mediterranean pine (2)], and probably others, have significantly less Δ5-olefinic acids than most other pine species that grow in altitude or in colder regions. Perhaps this is indicative of some relationship between the Δ5 unsaturation and resistance to cold.

Finally, our study showed that the use of the fatty acid compositions of seed oils for the chemometric distinction of conifer families and genera can be extended to sections, at least to some of them. With few exceptions, such a chemosystematization generally supports the taxonomy based on other biological parameters.

ACKNOWLEDGMENT

The student engineers from ISTAB provided helpful assistance in extracting the oils and preparing fatty acid methyl esters.

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[Received February 18, 1997; accepted July 21, 1997]