Pergamon

PII: S0031–9422(98)00105–8

Phytochemistry, Vol. 49, No. 5, pp. 1269–1277, 1998

Printed in Great Britain
0031–9422/98/$—see front matter

CONE AND FOLIAGE VOLATILES EMITTED BY *PINUS CEMBRA* AND SOME RELATED CONIFER SPECIES

LAURENT DORMONT,†‡* ALAIN ROQUES† and CHRISTIAN MALOSSE

†Station de Zoologie Forestière, CRF-Orléans, INRA, Ardon, 45160 Olivet, France; ‡Laboratoire Dynamique des Ecosystèmes d'Altitude, Université de Savoie, CISM, 73376 Le Bourget-du-Lac cedex, France; ¶Unité de Phytopharmacie et Médiateurs Chimiques, INRA, 78026 Versailles cedex, France

(*Received* 30 *July* 1997)

Key Word Index—*Pinus cembra*; *Picea abies*; *Abies alba*; pinaceae; cones; headspace; volatile monoterpene.

Abstract—Volatiles emitted by cones and foliage of the Swiss stone pine (*Pinus cembra*), as well as cone volatiles emitted by some other conifers (*Picea abies*, *Abies alba*, *Pinus uncinata*, *P. sylvestris*, *P. koraiensis*, *P. pumila*, *P. peuce*, *P. strobus*, *P. parviflora*, *P. wallichiana* and *P. armandi*) were collected using headspace techniques and analyzed by GC-mass spectrometry. The same major monoterpenes, i.e. α-pinene, β-pinene, and limonene $+$ β-phellandrene were found in both the cone and foliage volatiles of Swiss stone pine. The monoterpene profile did not vary among cones from the same tree, or among different geographic origins. A few differences were noted between trees from the same stand, and over cone development. In other conifers, cone emissions consisted of the same monoterpenes, plus 3-carene, with specific proportions related to tree species. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Volatile compounds emitted by plants have been shown to act as a key factor in recognition and selection of hosts by many pest insects [1, 2], especially by those exploiting flowers and fruits [3, 4]. The involvement of cone volatiles in long- and short-range host-selection process of insects specialized in seed cone exploitation has been suspected for some time [5]. However, the olfactory cues provided by conifer reproductive structures have received little attention, although the conifer terpenoids have been largely studied [6]. There have been only a few works intended to demonstrate that differences in volatile emissions could allow cone insects to distinguish cones of different species; cones from foliage, twigs, or shoots; and cones at various stages of development. Moreover, most studies focused on the composition of cone oleoresin, using solvent extraction or steam distillation methods, rather than analyzing volatiles. Thus, the terpene composition of seed cone oleoresin has been reported in *Pinus resinosa* Ait. [7], *Larix decidua* Mill. and *Pseudotsuga menziesii* [Mirb.] Franco [8], *Abies nordmanniana* Spach. and *Picea orientalis* L. [9], and *Pinus sylvestris* L. [10]. More recently, headspace techniques were developed to isolate volatile compounds from plant scents in order to determine the composition of the host odour which is attractive to insects [11]. Such headspace techniques were used in the study of seed cone volatiles of *Pinus taeda* L. [12], *Pinus sylvestris*, *Picea abies* Karst., and *Larix sibirica* Ledeb. [13], *Larix decidua* and *Pseudotsuga menziesii* [14].

Among conifers, some stone pines (*Pinus* spp., subgenus *Strobus*, section *cembra*, subsection *cembrae*) show specific relationships between cones and related insects. The alpine species, Swiss stone pine (*Pinus cembra* L.), hosts a cone entomofauna limited to three species [15] whereas the cones of other conifers native to Europe are usually attacked by from six (Juniperaceae) to more than 10 insect pests [16]. Two related Asian pines of the same *cembrae* subsection, *P. pumila* (Regel) and *P. sibirica* (Rupr.) Mayr, also present only 2–3 insect species in cones [17, 18]. Because host selection by cone insects is probably mediated by olfactory cues, the limited insect colonization of these stone pine cones has been suspected to result from a chemical barrier associated with volatile emissions of seed cones [15]. The composition of cone oleoresin and cone volatiles of *P. cembra* has been preliminarily analyzed [19], but remains to be compared to volatiles emitted by other parts of Swiss stone pines as well as to volatiles emitted by cone species showing a larger entomofauna.

The objectives of the present study were therefore

---

*Author to whom correspondence should be addressed.

as follows: i) to compare cone volatiles with volatiles emitted by foliage surrounding the Swiss stone pine; ii) to examine possible variations of cone volatiles of Swiss stone pines within a tree, among trees, among different geographical sites, and over cone development; iii) to compare cone volatiles of Swiss stone pines with cone volatiles of other conifers, either ecologically close (high altitude tree species in the Alps, e.g. spruce, mountain pine, fir), or taxonomically close (e.g. *Pinus* species of the *cembrae* and *strobi* subsections).

RESULTS

Comparison of cone volatiles with foliage volatiles in Swiss stone pine (*Table* 1)

In volatiles of both cones and surrounding foliage, we identified the same seven major monoterpenes plus traces of terpinolene and bornyl acetate. Cone volatiles additionally showed traces of tricyclene and of an unidentified sesquiterpene. α-Pinene was prevalent in the two profiles but the foliage volatiles differed from the cone volatiles by a significantly lower level of α-pinene and a much higher level of limonene + β-phellandrene (the two compounds were not separated in the quantitative analysis because of the GC temperature program we used). Cone and foliage volatiles thus appeared to contain much fewer compounds than the oleoresin extracts from fresh cones, which showed a total of 12 monoterpenes plus bornyl acetate and several sesquiterpenes (e.g., caryophyllene, humulene, but most remained unidentified). The seven major components of oleoresin were similar to the monoterpenes observed in the volatiles but the oleoresin profile significantly differed from both cone and foliage volatiles by a medium level in α-pinene and limonene + β-phellandrene and a higher level in β-pinene.

Variation in cone volatiles of Swiss stone pine with space and time (*Tables* 1 *and* 2)

Volatile profiles did not vary significantly, either between cones of the same tree collected at the same stage of cone development, or between trees of a Swiss stone pine stand. There was no evidence for a geographic variation in volatile emission by cones of Swiss stone pines in the French Alps, except a significantly lower level of myrcene observed in the Les Gaboureaux forest. Foliage volatiles did not show any variation between the three natural stands of Swiss stone pine. However, significant quantitative differences were observed in volatile emissions between foliage samples from alpine natural stands and those collected on planted trees at the "Chèvreloup" arboretum. Foliage volatiles of planted trees showed significant lower levels of α-pinene and β-pinene, and higher levels of myrcene and limonene + β-phellandrene (Table 1). The cone volatile profiles showed limited variation during cone development from June

Table 1. Mean composition of cone oleoresin, cone volatiles, and foliage volatiles of *Pinus cembra* sampled in the forest of Tueda, the northern French Alps, and of foliage volatiles from planted trees of *P. cembra* sampled at the "Chèvreloup" arboretum, north central France. Values are expressed as a percentage relative to total components of the solution (mean percent ± s.e.)

Compounds	Tueda			Chèvreloup
	cone oleoresin*[†] (*n* = 16)	cone volatile*[‡] (*n* = 32)	foliage volatile (*n* = 32)	foliage volatile (*n* = 8)
Tricyclene	tr[§]	tr	—	—
α-Pinene	52.2 ± 3.2 a[¶]	67.1 ± 3.4 b	38.9 ± 2.9 c	26.4 ± 2.1 d
Camphene	0.9 ± 0.2 a	0.7 ± 0.2 a	0.5 ± 0.1 a	0.6 ± 0.2 a
Sabinene	0.2 ± 0.1 a	0.3 ± 0.1 a	0.3 ± 0.1 a	0.5 ± 0.2 a
β-Pinene	24.7 ± 3.3 a	18.2 ± 1.7 b	20.7 ± 3.4 b	10.1 ± 2.4 c
Myrcene	1.8 ± 0.4 a	1.4 ± 0.3 a	1.4 ± 0.5 a	3.7 ± 1.6 b
α-Phellandrene	tr	—	—	—
Limonene/β-Phellandrene[‖]	17.0 ± 1.4 a	11.1 ± 3.0 b	34.0 ± 3.1 c	57.9 ± 4.1 d
γ-Terpinene	tr	—	—	—
Terpinolene	tr	tr	tr	tr
Azulène	tr	—	—	—
Bornyl acetate	tr	tr	tr	tr

* Data from Dormont *et al.* [19].

[†] Plus traces of sesquiterpenes.

[‡] Plus traces of an unidentified sesquiterpene.

[§] Traces.

[¶] For a compound, means in the same line followed by the same letter are not significantly different ($p > 0.05$; Kruskal–Wallis test).

[‖] Limonene and β-Phellandrene were not separated in the GC analysis.

Table 2. Comparison of the mean composition of cone volatiles of *Pinus cembra* sampled at three different locations in the northern French Alps (Tuéda forest, Les Gaboureaux forest) and the southern French Alps (Les Ayes forest).* Values are expressed as a percentage relative to total components of the solution

	Tuéda $n = 32$ cones (8 trees)				Les Ayes $n = 32$ cones (8 trees)				Les Gaboureaux $n = 24$ cones (4 trees)			
	Mean†	s.e.†	mini	maxi	Mean	s.e.	mini	maxi	Mean	s.e.	mini	maxi
α-Pinene	67.1a‡	3.4	65.4	73.3	66.9 a	5.3	62.3	77.3	70.1 a	2.6	67.8	72.0
Camphene	0.7 a	0.2	0.3	1.0	0.9 b	0.1	0.7	1.0	0.8 ab	0.1	0.7	1.0
Sabinene	0.3 a	0.1	0.2	0.4	0.4 a	0.2	0.1	0.9	0.3 a	0.1	0.2	0.4
β-Pinene	18.2 a	1.7	16.7	26.6	20.3 a	4.0	16.5	24.6	19.8 a	2.2	17.5	22.3
Myrcene	1.4 a	0.3	1.0	1.8	1.4 a	0.7	0.9	1.9	0.6 b	0.3	0.3	0.8
Limonene/β-Phell.§	11.1 a	3.0	4.7	12.0	10.0 a	4.7	7.8	15.6	8.3 a	2.9	7.2	11.1

* Plus traces of tricyclene, bornyl acetate, and an unidentified sesquiterpene.

† Data from Dormont *et al.* [19].

‡ For a compound, and for the three origins, means in the same line followed by the same letter are not significantly different ($p > 0.05$; Kruskal–Wallis test).

§ Limonene and β-Phellandrene were not separated in the GC analysis.

to late August in both the forests of Tuéda and Les Ayes. Only two compounds presented significant changes. The proportion of limonene + β-phellandrene declined from 15–18% in June to 7–8% in August, at the end of cone growth period (Fig. 1). By contrast, the proportion of β-pinene increased from 16% in June to 24% in August at the Les Ayes forest, but not in Tuéda.

Volatile emission of cones and foliage of other alpine conifers (*Table* 3)

Cone and foliage volatiles of *Picea abies*, *Abies alba*, *Pinus uncinata*, and *Pinus sylvestris* respectively showed eight, five, six, and five major monoterpenes. Cone volatile profiles largely varied with tree species, the dominant monoterpene being myrcene in *Picea abies*, and α-pinene in *Abies alba* and *Pinus sylvestris*. The presence of Δ-3-carene also distinguished volatiles of *Picea abies* and *Pinus sylvestris* from these of the two other alpine conifers. The cone volatiles of *Pinus uncinata* did not show any dominant monoterpene, the specific levels of myrcene, α-pinene, limonene + β-phellandrene, and β-pinene accounting for about 20% of the total volatiles. There were only slight quantitative differences between the respective emissions of cones and foliage in a same tree species, apart for *Picea abies* where a much fewer proportion of myrcene as well as higher levels of camphene, β-pinene, and 3-carene were found in foliage emissions.

Volatile emissions of cones and foliage of pine species taxonomically or ecologically close to Swiss stone pine (*Table* 4)

Volatiles largely differed between tree species for *Pinus pumila*, *P. koraiensis*, *P. peuce*, *P. strobus*, *P. parviflora*, *P. wallichiana*, and *P. armandi*. Δ-3-carene was observed in volatiles of all but the two *Pinus* species of the *cembrae* subsection, *Pinus pumila* and *P. koraiensis*. By contrast, an unidentified sesquiterpene was found in the cone volatiles of these two species. Volatiles of *Pinus koraiensis* also differed from these of all other pines by the presence of terpinolene in cones and the absence of β-phellandrene (confirmed by MS analysis) in cones and foliage. Unlike other pine species, *P. wallichiana* did not show camphene and sabinene in foliage emissions. Limonene (in *P. koraiensis*) and Limonene + β-phellandrene largely dominated the volatile profile in all species except *P. strobus*. Cone and foliage volatiles did not differ significantly in *Pinus pumila*, *P. parviflora*, *P. wallichiana*, and *P. armandi*. Traces of terpinolene and sabinene were detected in cone but not in foliage emissions of *P. koraiensis* and *P. peuce*, respectively. By contrast, the volatile profile of cones largely differed from that of foliage in *P. strobus*.

DISCUSSION

In the conifer species we studied, cone and foliage volatiles mostly consisted of monoterpenes. Such compounds are widely distributed in conifers, and the relative proportion of monoterpenes in oleoresin has been shown to provide a good diagnostic value for chemosystematic studies because it varies specifically with genera and species, often under genetic control [20, 21]. We also observed specific proportions of monoterpenes in cone volatiles of the conifer species, even when the species were taxonomically close (e.g., *P. cembra*, *P. pumila*, and *P. koraiensis*). The absence of geographic variation in volatile emissions of Swiss stone pines suggested that cone volatiles can similarly characterize a conifer species. However, no obvious trend was detected at higher taxonomic levels, e.g. for

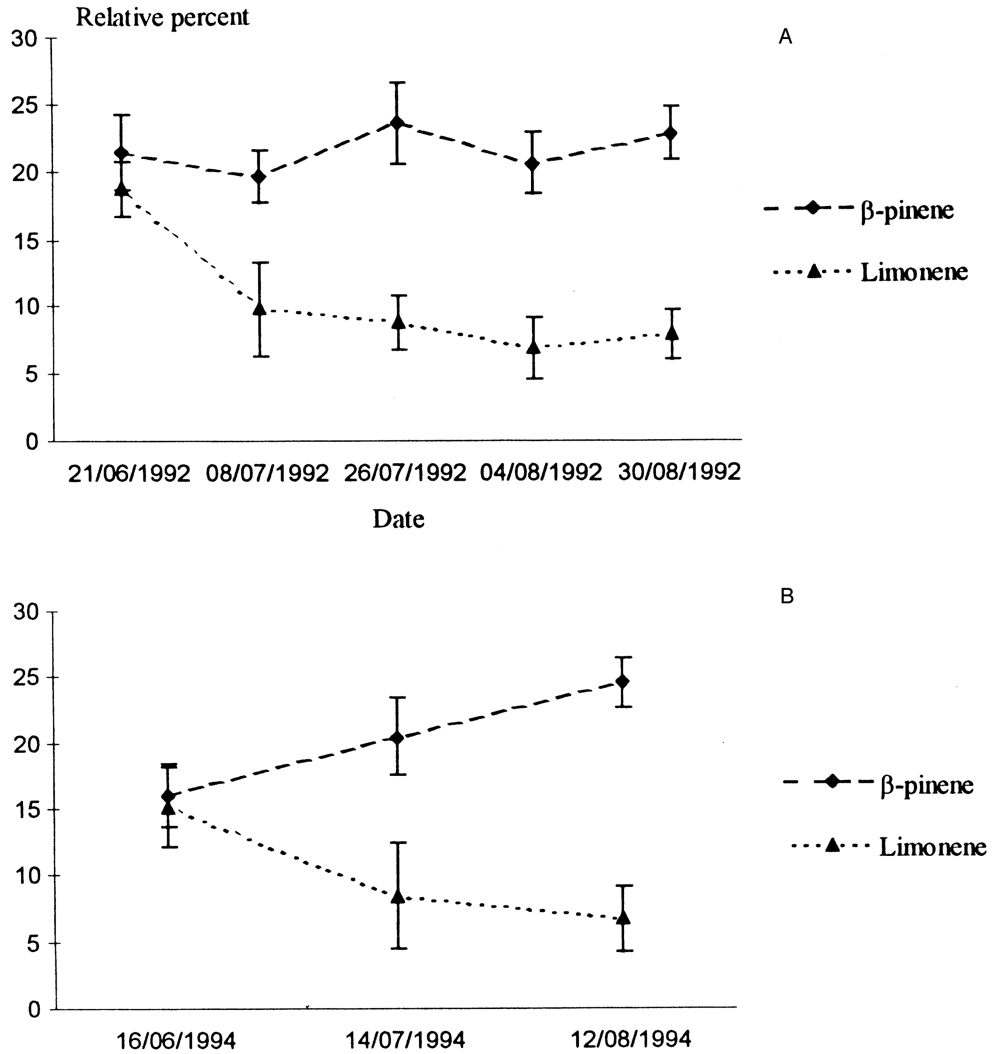

Fig. 1. Volatile emission of *β*-pinene (-•-) and limonene (-▲-) over the development of cones of *Pinus cembra* sampled at Tueda forest, the northern French Alps (A), and at Les Ayes forest, the southern French Alps (B) (8 cones collected on two trees at each date). Values are expressed as a percentage relative to total components of the solution (mean percent ± s.e.).

separating pines of the subsections *cembrae*, *strobi* and *flexilis*.

Comparing our results with the literature data required a number of precautions because of the large differences in both the sampling methods and the part of the tree that has been sampled. The volatile composition of cones and foliage largely differed from the oleoresin composition reported for the same conifer species following analyses by solvent extraction and steam distillation methods, even when the same organ was sampled (cf. cone oleoresin and cone volatiles of *Pinus cembra*). In fact, solvent extraction and steam distillation methods usually result in isolating non-volatile material from tissues and producing heat-produced rearrangements, respectively [11]. Moreover, comparisons of results regarding different parts of a tree are biased because monoterpene contents are known to vary among distinct anatomical organs [9, 21]. Thus, it is not surprising that the volatiles we analyzed did not include some of the monoterpenes reported from the previous oleoresin studies. The presence of Δ-3-carene was observed in foliage oil of *P. cembra* [22], *P. pumila* [23] and *P. koraiensis* [24], whilst we did not detect this compound in the foliage and cone volatiles of the three species, nor in the cone oleoresin of *P. cembra*. In some other species, the monoterpenes observed in volatiles were similar to these reported from the oleoresin but the profiles were entirely different. In *P. silvestris*, α-pinene was reported to be dominant in oleoresin extracts of needles [10, 25, 26], phloem [27] and sometimes even cones [10]. We observed similar levels of α-pinene in both cone and foliage volatiles. However, we detected only traces of 3-carene in volatile emissions of this conifer, whereas these authors found a high proportion of this monoterpene in the profile. In *Picea abies*, the main constituents of needle oleoresin were found to be camphene, then limonene and α-pinene [28, 29], but we observed only small amounts of camphene in the volatiles from cones (1%) and foliage (6.6%). In *Abies alba*, Von Rudloff [21] reported 3-carene from leaf oil analyses, but we did not find this terpene in our study. In *P. strobus*, this last monoterpene was observed to be dominant in foliage samples (41%), whilst Hanover [30] and Bridgen *et al.* [31] both reported only 10% of 3-carene in the oleoresin profile of shoots. In *P. wallichiana* and *P. armandi*, large variation also occured in the proportion of α-pinene, β-pinene and limonene + β-phellandrene between our results and those reported by Song *et al.* [24].

These differences may also result from geographic variation in the monoterpene composition although contrasting results exist. Schönwitz *et al.* [28] noted large quantitative differences in foliage monoterpenes between different stands of *Picea abies* in southern Germany, as did Nerg *et al.* [26] for seedlings of *Pinus sylvestris*. By contrast, only minor variation were reported for cortical monoterpenes of *Pseudotsuga macrocarpa* (Vasey) Mayr [32], and we observed no significant variation in the volatile emissions of *Pinus cembra* cones sampled at three different natural stands in the French Alps.

Results from headspace analyses carried out on cone and foliage provided more valuable comparisons than oleoresin studies. Qualitative and quantitative differences were also observed. Using adsorption on a different porous polymer, Tenax, Borg-Karlson *et al.* [10] did not detect tricyclene, camphene, and sabinene in the cone emissions of *Picea abies* and *Pinus sylvestris* in Sweden, but they additionally found traces of aliphatic alcohols and esters. Relative proportions of monoterpenes also differed. We similarly observed great amounts of myrcene and limonene in *Picea abies* volatiles. In *Pinus sylvestris*, 3-carene was reported to be dominant in cone and foliage volatiles, and α-pinene was found only as traces. By contrast, we observed only traces of 3-carene, and more than 40% of α-pinene in both cone and foliage emissions (Table 3). Using the solid phase microextraction method (SPME), Schäfer *et al* [33]. analyzed monoterpene composition from foliage emissions of *Pinus peuce* and *P. strobus* and found both qualitative and quantitative differences from our results. These authors reported additional monoterpenes, α-phellandrene, α-terpinene, and terpinolene, but not 3-carene. We observed this last monoterpene in foliage volatiles of both *P. peuce* (3%) and *P. strobus* (41%) (Table 4).

Differences may first result from the headspace method. For instance, SPME probably allowed the trapping of highly volatile compounds, such as alcohols, which were not trapped with Amberlite or evaporated during the elution process [34]. It is also possible that different stages of cone and foliage development were considered according to the various studies. Large variations in cone emissions have been shown over cone development in *Pinus cembra* (cf. Fig. 1), *Larix decidua* and *Pseudotsuga menziesii* [14]. As discussed above, the geographic origin of the sample may also be involved in the difference in volatile emissions. This could be especially verified when different growing conditions are compared; e.g., exotic pines planted in arboreta vs. pines in native areas. Differences in the volatile profile of *Pinus cembra* foliage between samples collected in natural areas and in arboreta confirmed these observations. However, it was possible to carry out a limited additional survey of cone volatiles of *Pinus koraiensis* using the same headspace technique in a natural Chinese stand (Moershan, Heilongjiang, 45°23′ N, 127°32′ E, 370 m elevation) during 1993. No significant difference in volatile profile was observed between the Chinese cones and those sampled at the "Les Barres" aboretum (Dormont, Sun and Roques, unpublished).

Table 3. Mean composition of volatiles emitted by cone and foliage of alpine conifer species ecologically close to *Pinus cembra*. Values are expressed as a percentage relative to total components of the solution (mean percent)

	Picea abies		*Abies alba*		*Pinus uncinata*		*Pinus sylvestris*	
	cone ($n=16$)	foliage ($n=16$)	cone ($n=16$)	foliage ($n=8$)	cone* ($n=16$)	foliage ($n=16$)	cone ($n=16$)	foliage ($n=8$)
Tricyclene	tr†	tr	tr	tr	—	—	tr	tr
α-Pinene	14.3	17.7	57.2	52.0	20.5	25.2	43.5	45.7
Camphene	1.0	6.6	1.3	0.9	3.5	2.3	tr	tr
Sabinene	1.6	1.9	tr	tr	6.1	5.0	0.9	1.4
β-Pinene	9.8	27.8	8.5	12.2	17.8	14.3	12.4	25.1
Myrcene	50.3	19.4	3.3	1.9	22.5	24.9	15.8	6.6
3-carene	3.6	10.5	—	—	—	—	tr	tr
Limonene/β-Phell.‡	18.6	13.6	27.7	32.1	22.2	19.9	24.7	18.4
Bornyl acetate	tr	tr	—	—	tr	tr	—	—

* Data from Dormont *et al.* [19].

† Traces.

‡ Limonene and β-Phellandrene were not separated in the GC analysis.

Table 4. Mean composition of volatiles emitted by cone and/or foliage of some exotic *Pinus* species taxonomically close to *Pinus cembra* (*cembra* section of the genus *Pinus*). Cone and foliage samples were collected in two arboreta of northern France: ‘‘Les Barres’’ arboretum (*P. koraiensis*) and ‘‘Chèvreloup’’ arboretum (other species). Values are expressed as a percentage relative to total components of the solution (mean percent)

Subsection of the genus *Pinus*:	*cembrae*			*strobi*							*flexilis*	
	*P. pumila**	*P. koraiensis**		*P. peuce*		*P. strobus*		*P. parviflora*		*P. wallichiana*	*P. armandi*	
	fol.[†] $n=8$	cone $n=5$	fol. $n=8$	cone $n=8$	fol. $n=8$	cone $n=8$	fol. $n=8$	cone $n=8$	fol. $n=8$	fol. $n=8$	cone $n=8$	fol. $n=8$
Tricyclene	—	tr‡	tr	—	—	—	—	tr	tr	—	—	—
α-Pinene	12.3	15.3	9.4	28.6	22.5	49.2	17.5	11.2	8.5	15.2	14.0	13.4
Camphene	tr	0.5	tr	tr	3.5	0.7	0.6	0.5	1.2	—	0.8	tr
Sabinene	0.5	tr	tr	tr	—	0.4	0.3	1.2	2.8	—	4.0	0.8
β-Pinene	7.8	2.9	12.3	19.3	9.6	33.2	14.4	8.7	12.1	28.1	22.2	26.0
Myrcene	3.6	4.8	2.1	4.1	2.2	7.7	17.1	15.6	14.2	12.2	2.3	1.9
3-carene	—	—	—	1.1	3.2	2.4	40.8	6.6	3.7	3.4	9.8	5.4
Limo/β-Phel.[§]	74.6	71.0[¶]	72.2[¶]	46.0	58.1	5.1	9.2	55.9	51.4	38.1	48.5	51.3
Terpinolene	—	tr	—	—	—	—	—	—	—	—	—	—
Bornyl acetate	tr	tr	tr	—	—	tr	—	tr	tr	tr	—	—

* Plus traces of an unidentified sesquiterpene for *P. pumila* and *P. koraiensis*.

[†] Foliage

[‡] Traces.

[§] Limonene and β-Phellandrene were not separated in the GC analysis.

[¶] Limonene only; β-Phellandrene was absent in the volatile emissions of *P. koraiensis* (confirmed by MS analysis).

The volatile profiles of the three species studied of stone pines did not include Δ-3-carene, unlike the five species of pines belonging to the subsections *strobi* and *flexilis* of the same subgenus, *strobi*. Is it possible that the *cembrae* subsection presents a specific volatile pattern that can be involved in the limitation of insect attack? It would be interesting to survey volatiles in the two pine species of the *cembrae* subsection that were not considered in our study, *Pinus sibirica* and *P. albicaulis* Engel., but Δ-3-carene was also absent in cone volatiles of *Pinus uncinata*, which belong to a different subgenus (subgenus *Pinus*), and of *Abies alba*. Moreover, the volatile profiles of both cones and foliage largely differed among species of stone pines, the relative proportion of α-pinene and limonene, the respective dominant monoterpene, being opposite in *P. cembra* and *P. koraiensis* (and *P. pumila* for foliage, at least).

Cone volatiles of *Pinus cembra* showed only few differences with those of other conifers growing in the Alps, except *Picea abies* which differed strongly by the dominance of myrcene and the presence of 3-carene, and *Pinus uncinata* which did not show any dominant compound. Apart from the apparent absence of bornyl acetate that is present as traces in *Pinus cembra*, and the presence of traces of Δ-3-carene in *Pinus sylvestris*, cone volatile profiles of *Abies alba* and *Pinus sylvestris* were roughly similar to that of Swiss stone pines in June, with a large dominance of α-pinene, followed by limonene + β-phellandrene. European larch, *Larix decidua*, which grows in the same altitudinal range as the Swiss stone pine, also presented a very close profile [14]. Minor differences concerned only the level of myrcene, that was lower in Swiss stone pines and the level of sabinene, that was higher in larch. We also showed that the volatile profile of Swiss stone pine cones is not stable with time, and significantly varied with cone development, but we did not survey variations in cone emissions with cone development in other conifer species. It was, thus, difficult to affirm whether the limited colonization of stone pine cones by insects results from a specific terpene profile at the time of insect attack, since the response of cone insects to varying levels of these compounds has been poorly investigated [5]. However, our studies were only aimed at precise volatile profiles. The total amount of volatiles emitted by cones was not measured in the different tree species, and we cannot exclude the possibility that similar volatile profiles result in a different quantity of monoterpenes present in the cone environment. This remains to be tested because chemicals normally attractive to an insect may, instead, be repellent to the same insect at higher concentrations [1, 4, 25].

Further investigations of cone volatiles with regard to insect attraction to the host must also consider the enantiomeric composition of the monoterpenes in the profile. Although the chiral composition has no influence on insect attraction to the host in *Hylobius abietis* L. [35] and *Trioza apicalis* Förster [36], the response of *Ips typographus* L. differs with enantiomers [37]. The importance of eniantomeric composition was suggested to mainly concern insects using certain constituents of the host-tree as precursors for their own pheromone production [29].

EXPERIMENTAL

Study sites

The study was carried out during 1993 and 1994. Cone and foliage samples of the Swiss stone pine were collected in three different natural stands. The two first stands were located in the northern French Alps (Tuéda forest, near Méribel-Les-Allues [45°21′ N, 6°38′ E], and Les Gaboureaux forest, near Chamrousse [45°07′ N, 5°53′ E]), whilst the third one was located in the southern French Alps, near Briançon (Les Ayes forest, 44°51′ N, 6°36′ E). The sample collection was realized at 1850, 1900, and 1900 m altitude, respectively. Cone samples of other alpine conifers were also collected in the French Alps. Norway spruce, *Picea abies*, was sampled in the Tuéda forest, at 1700 m elevation. White fir, *Abies alba* Mill., was sampled in the Collet d'Allevard forest (45°15′ N, 6°08′ E), at 1600 m elevation. Mountain pine, *Pinus uncinata* Ram., was sampled in the Montgenèvre forest (44°55′ N, 6°44′ E), at 1950 m elevation. Scots pine, *Pinus sylvestris*, was sampled in the Col de l'Echelle forest (45°20′ N, 6°39′ E), at 1450 m elevation. European larch, *Larix decidua*, was not considered in this study because Rappaport *et al.* [14] already analyzed volatiles from larch cones sampled in the same area, using similar headspace techniques. Cone (when available) and foliage collections of related, but exotic, *Pinus* species were carried out in two arboreta of north central France. Among the pines of the subgenus *Strobus* section *cembra*, two species of the *cembrae* subsection could have been sampled, *Pinus koraiensis* (Sieb. and Zucc.) at the "Les Barres" arboretum near Nogent-sur-Vernisson (47°50′ N, 2°45′ E), and *P. pumila* at the "Chèvreloup" arboretum near Versailles (48°49′ N, 2°04′ E). We also collected foliage samples from planted trees of *P. cembra* at Chèvreloup. Four species of the *strobi* subsection (*P. peuce* Griseb., *P. strobus* L., *P. parviflora* Sieb. et Zucc., and *P. wallichiana* A. B. Jacks) and one species of the *flexilis* subsection (*P. armandi* Franck.) were also sampled at the "Chèvreloup" arboretum.

Sample collection

For each tree species, four cone-bearing trees were randomly selected per site. Only two trees were chosen for exotic pine species growing in arboreta. The collection was designed to occur during the attack period of the major cone insects which differ with tree species. In Swiss stone pines and other *Pinus* species, insect pests mostly attack second-year cones during the spring–summer growth phase [16]. In all pine species, two branches bearing second-year cones (four branches in the Swiss stone pine) were sampled per tree on 15–20 May (arboretum), or between 1 June and 30 June (The French Alps), depending on both tree species and altitude. In the Swiss stone pine, additional samples were collected at different stages of development of the second-year cones: in both Tuéda forest and Les Ayes forest, two trees were selected and two cone-bearing branches per tree were regularly collected. In the Tuéda forest, collections were carried out every two weeks from mid-June to late August whilst they occurred in mid-June, mid-July, and late August in the Les Ayes forest. In spruce and fir, insects attack first-year cones [16]. In these two species, two branches bearing first-year cones were sampled in mid-June in the French Alps. In all species, the branches were brought to a "field laboratory" immediately after cutting. Two cones were then removed from each branch, and were processed immediately on the day they were harvested from the trees. In Swiss stone pines, a total of 32 cones were sampled per site (24 cones in Les Gaboureaux forest). Collections at different stages of cone development included 40 additional cones from Tuéda forest and 24 cones from Les Ayes forest. A total of 16 cones per tree species were sampled in *Picea abies*, *Abies alba*, *Pinus uncinata* and *P. sylvestris*, whilst 8 cones per species were collected in exotic pine species growing in arboreta (only 5 cones in *P. koraiensis*). No cone was available from the *P. pumila* and *P. wallichiana* trees. Foliage samples consisted of a 10 cm ramet from the apical part of a branch, involving 10–15 needle fascicles for pine species. Only branches without young shoots were selected, so that one-year-old needles were analyzed.

Headspace sampling of tree volatiles

Volatiles were analyzed using a manifold effluvial headspace sampler, designed by Rappaport *et al.* [14], that enabled us to process eight samples simultaneously. The sampling procedure was identical to that described by Dormont *et al.* [19]. Cones and foliage were individually sealed inside bags made of Tedlar® connected by Teflon® tubes to glass cartridges filled with Amberlite XAD2® (Supelco®). The glass cartridges were then connected by Teflon® tubes to a vacuum pump. Seven different vegetal samples and a control consisting of an empty Tedlar bag, were simultaneously connected to the pump during each experiment. The effluvial sampling ran for 20 minutes. The experiments were carried out during daytime, between 11:00 a.m. and 4:00 p.m., in order to match the flight period of cone insect pests. Once the effluvial sampling was completed, each glass cartridge was eluted with 3 ml (CH_2Cl_2) for a two-hour period.

Gas chromatography and gas chromatography–mass spectrometry of cone volatiles

Gas chromatography of the crude mixture was done using a Varian Star 3400 CX chromatograph equipped with a SPI injector, a flame-ionization detector, and a fused silica capillary column WCOT CPSil-8CB 0.32 mm × 25 m (Chrompack®, The Netherlands). The GC–MS analyses were performed using electronic impact ionization mode on a Nermag R10-10C quadrupole interfaced with a Varian 3300 apparatus. The Varian 3300 was equipped with a Ross injector (230°C) and a 25 m × 0.32 mm i.d. WCOT DB-5MS fused silica capillary column (J&W Scientifics, USA). In both apparati, the carrier gas was helium with a flow rate of 3 ml/min. The temperature programmes for injector, detector and oven followed those used by Dormont *et al.* [19]. Mass spectra were recorded in electronic impact at 70 eV and identified by comparison with the EPA/NIH software library. Peaks were quantified using Star Chromatography Software®. The proportions of each compound were expressed with respect to total volatiles in the analyzed sample in order to compare the volatile profile of the samples.

Extraction of oleoresin from cones of Swiss stone pine

The cone oleoresin was surveyed on the same trees as those assigned for the sampling of cone volatiles in the Tuéda forest. Four second-year cones were collected per tree in mid-June. Concurrent with the volatile sampling, each sampled cone was cut and 50 g of fresh cone was immediately extracted with 50 ml *n*-pentane for 24 hr. Extracts were then filtered and analyzed using GC and GC–MS as described above.

Data analysis

The volatile profiles were compared using the relative proportions of the compounds identified in the solutions. The transformation of the percentage data using the angular transformation ($\arcsin\sqrt{x}$) did not allow us to normalize and homogenize the variances. Therefore, the nonparametric Kruskal–Wallis test was applied on untransformed data in order to test for differences between the profiles ($p = 0.05$) (Statistica 5.0 Statsoft®).

Acknowledgements—We thank D. Piou, manager of "Les Barres" arboretum, M. Callen and M. Hachette, who were in charge of the "Chèvreloup" arboretum, B. Roman-Amat, A. Collas and P. Clauss, French National Forestry Office, and E. De Guillebon, National Park of Vanoise for having permitted this study. We also thank A. S. Mercier-Guyon, University of Savoie, Chambéry, J. P. Raimbault, I.N.R.A., Orléans, and J. H. Sun, Northeast Forestry University, Harbin, China, for technical assistance. We are much indebted to F. Pellissier, University of Savoie, for reviewing the manuscript.

REFERENCES

1. Visser, J. H., *Ann. Rev. Entomol.*, 1986, **21**, 121.

2. Bernays, E. A. and Chapman, R. F., *Host-Plant Selection by Phytophagous Insects*. Chapman & Hall, London, 1994.
3. Gabel, B., Thiery, D., Suchy, V., Marion-Poll, F., Hrafsky, P. and Farkas, P., *J. Chem. Ecol.*, 1992, **18**, 693.
4. Dobson, H. E. M. In *Insect–plant interactions*, ed. E. A. Bernays. CRC Press, Boca Raton, 1994, p. 47.
5. Turgeon, J. J., Roques, A. and De Groot, P., *Ann. Rev. Entomol.*, 1994, **39**, 179.
6. Harborne, J. B. and Tomas-Barberan, F. A. (eds.), Ecological chemistry and biochemistry of plant terpenoids. In *Proc. Phytochem. Soc. Europe*. Clarendon Press, Oxford, 1991.
7. Mattson, W. J. and Strauss, S. H., In *Proc.* 2*nd Int. Conf. Cone and Seed Insects Working Party* (*IUFRO S*2.07-01), ed. A. Roques. Briançon, France. INRA, Olivet, September 3–5, 1986, p. 185.
8. Roques, A., PhD Thesis. University Pau et des Pays de L'Adour, France, 1988.
9. Hafizoglu, H. and Reunanen, M., *Holzforschung*, 1994, **48**, 7.
10. Sjödin, K., Persson, M., Borg-Karlson, A.-K. and Norin, T., *Phytochemistry*, 1996, **41**, 439–445.
11. Knudsen, J. T., Tollsten, L. and Bergström, L. G., *Phytochemistry*, 1993, **33**, 253.
12. Hanula, J. L., Berisford, C. W. and DeBarr, G. L., *J. Chem. Ecol.*, 1985, **11**, 943.
13. Borg-Karlson, A.-K., Eidmann, H. H., Lindstrom, M., Norin, T. and Wiersma, N., *Phytochemistry*, 1985, **24**, 455.
14. Rappaport, N., Jenkins, M. J. and Roques, A., In *Proc.* 4*th Int. Conf. Cone and Seed Insects Working Party* (*IUFRO S*2.07-01), eds. G. L. DeBarr, A. Roques, J. H. Sun and J. J. Turgeon. Beijing and Harbin, USDA For. Serv. Southeast. For. Exp. Stn. Athens, GA, (in press), 1996.
15. Dormont, L., Roques, A. and Trosset, L., *Ann. Sci. For.*, 1996, **53**, 153.
16. Roques, A., *Les insectes ravageurs de cônes et graines de conifères en France*. INRA, Versailles, 1983.
17. Florov, D. N., Pests of *Pinus sibirica* Mayr. Obl. gosudarstv. Izdatel'stvo, Irkoutsk. 1951, p. 8.[In Russian].
18. Khomentovsky, A. and Efremova, L. S., In *Forest insect guilds*: *pattern of interaction with host-trees*, eds. Y. N. Baranchikov, W. J. Mattson, F. P. Hain and T. L. Pain. US For. Serv. Tech. Rep. NE-153., 1991, p. 316.
19. Dormont, L., Roques, A. and Malosse, C., *J. Chem. Ecol.*, 1997, (in press).
20. Zavarin, E. and Snajberk, K., *Forest. Sci.*, 1965, **9**, 141.
21. Von Rudloff, E., *Biochem. Syst. Ecol.*, 1975, **2**, 131.
22. Contini, L. and Lavarelo, Y., *Le Pin Cembro. Répartition*, *Écologie*, *Sylviculture et Production.* INRA, Versailles, 1982.
23. Yatagai, M. and Sato, T., *Biochem. Syst. Ecol.*, 1986, **14**, 469.
24. Song, Z. and Liang, Z., Liu, X., *Biochem. Syst. Ecol.*, 1995, **23**, 517.
25. Annila, E. and Hiltunen, R., *Ann. Entomol. Fenn.*, 1977, **43**, 87.
26. Nerg, A., Kainulainen, P., Vuorinen, M., Hanso, M., Holopainen, J. K. and Kurkela, T., *New Phytologist*, 1994, **128**, 703.
27. Delorme, L. and Lieutier, F., *Eur. J. For. Path.*, 1990, **20**, 304.
28. Schonwitz, R., Kloos, M., Merk, L. and Ziegler, H., *Trees*, 1990, **4**, 27–33.
29. Persson, M., Sjödin, K., Borg-Karlson, A.-K., Norin, T. and Ekberg, I., *Phytochemistry*, 1996, **42**, 1289.
30. Hanover, J. W., *For. Sci.*, 1975, **21**, 214.
31. Bridgen, M. R., Hanover, J. W. and Wilkinson, R. C., *For. Sci.*, 1979, **25**, 175.
32. Zavarin, E. and Snajberk, K., *Biochem. Syst. Ecol.*, 1976, **4**, 93.
33. Schäfer, B., Hennig, P. and Engewald, W., *J. High Res. Chromatogr.*, 1995, **18**, 587.
34. Mathieu, F., Ph.D. Thesis. University Paris VII, France, 1995.
35. Nordlander, G., *Entomol. Exp. Appl.*, 1991, **59**, 229.
36. Nehlin, G., Valterova, I. and Borg-Karlson, A.-K., *J. Chem. Ecol.*, 1994, **20**, 771.
37. Dickens, J. C., *Entomol. Exp. Appl.*, 1978, **24**, 136.