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CUTICULAR HYDROCARBONS OF EIGHT SPECIES OF NORTH AMERICAN CONE BEETLES, *Conophthorus* HOPKINS[1]

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Abstract—A study to determine the degree of similarity and/or diversity among eight of the 15 described species of *Conophthorus* is reported. Cuticular hydrocarbons were evaluated for *C. conicolens*, *C. ponderosae*, *C. cembroides*, *C. edulis*, *C. radiatae*, *C. coniperda*, *C. resinosae*, and *C. banksianae*. Seventy-eight individual and isomeric mixtures of hydrocarbons were identified by gas chromatography–mass spectrometry, including *n*-alkanes, alkenes, alkadienes, 2- or 4-methylalkanes, 3-methylalkanes, and single-component and isomeric mixtures of internally branched mono-, di-, and trimethylalkanes. Differences in alkenes and mono-, di-, and trimethylalkanes can be used easily to separate the eight species. *Conophthorus conicolens* and *C. ponderosae* contain the most complex blends. Hydrocarbon patterns in three geographically separated populations of *C. ponderosae*, each from a different host, are qualitatively identical with the exception of a homologous series of 3,7-dimethylalkanes from adults collected from *Pinus lambertiana* cones. The latter could comprise a sibling species. Hydrocarbon mixtures of two eastern species, *C. resinosae* and *C. banksianae*, are qualitatively identical, supporting the suspicion that *C. banksianae* may not be a valid species. Closely related *C. cembroides* and *C. edulis* have similar combinations of hydrocarbons except for a unique and abundant alkene ($C_{27:1}$) in *C. edulis* and two dimethyhexacosanes in *C. cembriodes*.

[1] Coleoptera: Scolytidae.

Key Words—Cuticular lipids, chemotaxonomy, methyl-branched hydrocarbons, mass spectra, *Pinus* species, Coleoptera, *Conophthorus*, Scolytidae, insect integument.

INTRODUCTION

Beetles of the genus *Conophthorus* Hopkins (family Scolytidae) attack and kill female cones of *Pinus* species during the second year of cone development. In severe infestations up to three-fourths of the cones can be destroyed (Furniss and Carolin, 1977). Such sizable reductions in seed crops adversely affect natural and managed reforestation. Most species of the genus are monophagous; the most dramatic exception is *Conophthorus ponderosae* Hopkins. This polyphagous species reportedly breeds in cones of 8–13 species of *Pinus* and ranges from northern British Columbia to southern Mexico (Hedlin et al., 1980; Wood, 1982).

Wood (1982) synonomized *C. ponderosae* with five other species of *Conophthorus*: *C. scopulorum* Hopkins, *C. contortae* Hopkins, *C. monticolae* Hopkins, *C. flexilis* Hopkins, and *C. lambertianae* Hopkins. Previous species diagnoses were generally made on the basis of host, geography, and morphological features. "Taxonomic characters in *Conophthorus* species are so few and poorly developed that a search for non-conventional approaches is needed in order to find a means for the accurate identification of species" (S. L. Wood, personal communication). Wood (1982) found the collected material difficult to interpret. Perhaps *C. ponderosae* is in the process of speciation or it comprises two or more sibling species awaiting discovery. Wood (1982) candidly admits ". . . material from five-needle pines could be different."

Future control methods for *Conophthorus* will likely rely on mate-finding and host-acceptance behaviors, which are presumably under genetic control and are assumed to be species specific. Thus proper identification and understanding of the biology of *Conophthorus* species are fundamental to development of sound pest management programs. Since many of the *Conophthorus* species are difficult to separate morphologically, we decided to evaluate cuticular hydrocarbons as another set of taxonomic characters.

In this paper we report the identification of the hydrocarbon components in the cuticular wax of eight species of *Conophthorus*. This is the first step in determining the degree of similarity and/or diversity of cuticular hydrocarbon patterns among the species of this genus.

METHODS AND MATERIALS

Adult cone beetles characterized in this paper were collected as overwintering adults from infested cones or twig terminals. *C. ponderosae* was collected from three different species of pine: sugar pine, *Pinus lambertiana*

Dougl.; ponderosa pine, *P. ponderosae* Dougl. ex Laws.; and western white pine, *P. monticola* Dougl. All other *Conophthorus* were collected from a single host: *C. conicolens* Wood from Chihuahua pine, *P. leoiphylla* var. *chihuahua* (Engelm.) Shaw; *C. cembroides* Wood from Mexican pinyon pine, *P. cembroides* Zucc.; *C. edulis* Hopkins from pinyon pine, *P. edulis* Engelm.; *C. coniperda* (Schwartz) from eastern white pine, *P. strobus* L.; *C. radiatae* Hopkins from Monterey pine, *P. radiata* D. Don; *C. banksianae* McPherson from Jack pine, *P. banksiana* Lamb.; and *C. resinosae* Hopkins from red pine, *P. resinosa* Ait.

Pine cones infested with *Conophthorus* are not always easy to collect. Cones of sugar pine, western white pine, and eastern white pine fall to the ground at the end of the summer. Overwintering, adult *Conophthorus* remain in the cones on the ground and emerge as parent adults in the spring. Infested cones of these species are relatively easy to collect; cones are simply picked up off the ground and placed in a collection bag. Cones of Monterey pine, ponderosa pine, and Chihuahua pine are difficult to collect. Even after they are infested, they are retained in the crown of the tree for one or more seasons. By the time trees present their infested cones to the biologist (i.e., drop them on the ground), the beetles have already emerged. Cones are usually borne in the upper portions of the crown of trees ≥ 20 m in height. Biologists must resort to the use of a pole pruner to cut the cones down, a rifle to shoot the cones down, or we must collect from sexually mature, small trees or downed, mature trees in logging operations. Cones of the pinyon pines are relatively easy to collect; the trees are short (≤ 6 m in height) and bushy, and trees with cones usually have them distributed throughout the crown.

Hydrocarbon mixtures of *Conophthorus* reported in this paper represent data obtained from adult beetles that emerged from cones collected from the following locations: sugar pine from the Eldorado National Forest near Placerville, California; ponderosa pine from the Sierra National Forest ca. 40 km south of Yosemite National Park, California; western white pine from Sandpoint, Idaho; Chihuahua pine and Mexican pinyon pine cones from the Chiricahua Mountains in southern Arizona; pinyon pine from Prescott, Arizona and the Chiricahua Mountains; eastern white pine from Murphy, North Carolina and Petawawa, Ontario, Canada; Monterey pine from Albany and Aptos, California; jack pine from Aubrey Falls, Ontario, Canada, and red pine from Little Rapids, Ontario, Canada.

Infested cones from the western pine species were collected by three of the authors; cones from the eastern species were supplied by colleagues. In addition, we have examined cuticular hydrocarbons from museum specimens. Some of these beetles were collected before 1920; hydrocarbon patterns of these specimens are, remarkably, qualitatively identical to those collected and extracted by the authors. Hydrocarbon patterns of many of the *Conophthorus* species presented in this paper are representative of additional beetle collections made by the authors and of museum specimens.

Beetles were allowed to emerge from the cones or twigs. One to three days after emergence beetles were frozen and held at −20°C until their hydrocarbons were extracted. Beetles were removed from the freezer and allowed to warm to ambient temperature. Cuticular lipids were then extracted by immersing 15–50 beetles as a group once in 10 ml of hexane for 10 min. Sexes were not separated; males and females were extracted together. Extracted beetles were prepared as voucher specimens and submitted to the U.S. National Museum, Smithsonian Institution, Washington, D.C. After beetles were extracted, hydrocarbons were separated from other components by pipetting the 10-ml extract and an additional 8 ml of hexane through 3 cm of activated BioSil-A in Pasteur pipet mini-columns (Blailock et al., 1976). All hydrocarbon extracts were evaporated to dryness under a stream of nitrogen and redissolved in 30 μl of hexane for analysis.

Gas chromatography–mass spectrometry (GC-MS) analyses were performed on Hewlett Packard 5890 gas chromatograph equipped with a Hewlett Packard 5970B Mass Selective Detector, which was interfaced with a Hewlett Packard Chemstation computer. The GC-MS system was equipped with a fused silica capillary column (30 m × 0.2 mm ID, HP-1) and operated in split mode (with a split ratio of 20:1). Each mixture was analyzed by a temperature program from 200°C to 320° at 3°C/min with a final hold of 20 min. Electron impact (EI) mass spectra were obtained at 70 eV. Retention times and mass spectra of extracted *n*-alkanes were compared with external standards. Alkenes and methyl-branched alkanes were tentatively identified by calculating their equivalent chain lengths (ECL) (Nelson and Sukkestad, 1970; Jackson and Blomquist, 1976). Mass spectra of alkenes and methlyalkanes were interpreted as described by Nelson et al. (1972), Nelson (1978), Pomonis et al. (1978), and Blomquist et al. (1987).

RESULTS

Data presented in this report are the results of pooled samples (mixed sexes), from numerous cones, from several trees of the same species, from any location. In preliminary studies with other scolytid beetles, *Dendroctonus brevicomis* LeConte and *D. frontalis* Zimmerman, we determined that the hydrocarbon blends of pupae and callow adults are incomplete when compared to adults. However, hydrocarbon mixtures of fully tanned, emerged adults are fixed and do not change over the short lifetime of the beetles. In early, unpublished studies of *C. ponderosae*, we determined that there were no qualitative differences within beetles collected from the same host from several disparate geographical locations. Furthermore, we examined groups of males and females of *C. banksianae* and *C. resinosae* and found no qualitative differences in their hydrocarbon profiles. Therefore, we feel justified in presenting results of pooled samples of each species of *Conophthorus*.

All of the major hydrocarbon components (mean percent $\geq 0.5\%$ of the total hydrocarbon mixture) in the cuticular wax of eight species of *Conophthorus* were characterized (Table 1). Hydrocarbon components consist of homologous series of *n*-alkanes, alkenes, alkadienes, 2- or 4-methylalkanes, 3-methylalkanes, and single-component and isomeric mixtures of internally branched monomethylalkanes, dimethylalkanes, and trimethylalkanes. Unique and abundant species-specific hydrocarbon components were identified. Monomethyl-, dimethyl-, trimethylalkanes and unsaturated components can easily be used to separate the eight species of *Conophthorus* (Table 2). GC-MS total ion chromatograms for each species are displayed; closely related species, as inferred from Wood's (1982) key, are paired on the same figure (Figures 1–4).

The *n*-alkane composition in all species, except *C. conicolens*, is a continuous series from C_{21} (*n*-heneicosane) to C_{31} with C_{23}, C_{25}, and C_{27} predominating. In *C. conicolens* the *n*-alkane series includes small quantities of C_{32}, C_{33}, C_{35}, and C_{37}. All species have measurable quantities of *n*-alkanes of even-numbered chain length from C_{22} to C_{28}; C_{24} and C_{26} are the most abundant.

Alkenes were identified by their retention times and diagnostic mass ions. The molecular ion of an alkene is 2 mass units less than the molecular ion of the corresponding *n*-alkane. Alkadienes produce a molecular ion which is 4 mass units less. No structural information, position of double bond(s), or stereochemistry was obtained on alkenes. Each *Conophthorus* species contains $C_{25:1}$ (pentacosene) and $C_{27:1}$. *C. ponderosae* populations possess alkenes from $C_{23:1}$ to $C_{35:1}$. The major alkene in *C. conicolens*, *C. ponderosae*, *C. cembroides*, C. radiatae, *C. resinosae*, and *C. banksianae* is $C_{25:1}$, whereas $C_{27:1}$ is most abundant in *C. edulis* and *C. coniperda*. *C. resinosae* and *C. banksianae* both contain significant amounts of $C_{35:1}$ and $C_{37:1}$. Other alkenes are present in very small or trace amounts and occur only as a shoulder of the peak of a 3-(Me) C_n (3-methylalkane). The hydrocarbons from *C. ponderosae*, *C. coniperda*, *C. resinosae*, and *C. banksianae* contain the only alkadienes, $C_{25:2}$ (pentacosadiene, peak 12) and $C_{27:2}$ (peak 23). The mass spectra of peaks 12 and 23 showed the characteristic molecular ions, *m/z* 348 and 376, respectively, which are 4 mass units less than the molecular ions of C_{25} and C_{27}, respectively.

All *Conophthorus* species, except *C. conicolens*, *C. cembroides*, and *C. edulis*, contain mixtures of hydrocarbons with both 2-(Me) C_n and 3-(Me) C_n. It is difficult to distinguish 2-(Me) from 4-(Me) C_n (Blomquist et al., 1987). Peak 23 (Table 1) from *C. coniperda* illustrates this situation and is interpreted as 2- or 4-(Me) C_{26}. The high-abundance M-43 ion at *m/z* 337 is consistent with 2-(Me) C_{26}. The M-71 : M-72 ion pair from this spectrum, and others, varied in intensity, indicating the possible presence of 4-(Me) C_{26}. Therefore, all are designated as either 2- or 4-(Me) C_n (Tables 1 and 2). The 3-(Me) C_n were identified by their retention times and spectra, which gave a strong $(M-29)^+$ ion and a weaker $(M-57)^+$ ion.

TABLE 1. HYDROCARBONS IDENTIFIED FOR EIGHT *Conophthorus* SPECIES

Peak[a]	Hydrocarbon	ECL[b]	CN[b]	Diagnostic MS ions
1	2-Methyleicosane	20.70	21	252/253, 280/281, 296
2	*n*-Heneicosane	21.00	21	296
3	*n*-Docosane	22.00	22	310
4	2- or 4-; 3-Methyldocosane[c]	22.76	23	252/253, 280/281, 308/309, 324; 295/296, 266/267, 324
5	Tricosene	22.76	23	322
6	*n*-Tricosane	23.00	23	324
7	9-; 11-; 13-Methyltricosane[c]	23.39	24	140/141, 224/225; 168/169, 196/197; 196/197, 168/169
8	Tetracosene	23.70	24:1	336
9	3-Methyltricosane	23.73	24	280/281, 308/309, 338
10	*n*-Tetracosane	24.00	24	338
11	2- or 4-Methyltetracosane	24.63	25	308/309, 336/337, 352
12	Pentacosadiene	24.69	25:2	348
13	Pentacosene	24.70	25:1	350
14	*n*-Pentacosane	25.00	25	352
15	9-; 11-; 13-Methylpentacosane[c]	25.38	26	140/141, 252/253, 366; 168/169, 224/225, 366; 196/197, 366
16	5-Methylpentacosane	25.50	26	84/85, 308/309
17	11,15-Dimethylpentacosane	25.60	27	168/169, 239
18	2- or 4-Methylpentacosane	25.70	26	323, 351, 366
19	Hexacosene + 3-methylpentacosane[c]	25.73	26:1	364
		25.73	26	308/309, 336/337, 366
20	*n*-Hexacosane	26.00	26	366
21	12-; 13-Methylhexacosane[c]	26.34	27	182/183, 224/225; 196/197, 210/211
22	11,15-; 13,17-Dimethylhexacosane[c]	26.60	28	168/169, 182/183, 239, 253; 196/197, 154/155, 225, 267

23	2- or 4-Methylhexacosane + heptacosadiene[c]	26.63	27 27:2	336/337, 364/365, 308/309, 380 376
24	Heptacosene	26.70	27:1	378
25	3-Methylhexacosane	26.79	27	322/323, 350/351, 380
26	*n*-Heptacosane	27.00	27	380
27	9-; 11-; 13-Methylheptacosane[c]	27.32	28	140/141, 280/281; 168/169, 252/253; 196/197, 224/225, 394
28	9-Methylheptacosane	27.40	28	140/141, 280/281, 394
29	7-Methylheptacosane	27.42	28	112/113, 308/309
30	5-Methylheptacosane	27.50	28	84/85, 336/337, 394
31	9,13-; 9,15-; 11,15-Dimethylheptacosane[c]	27.63	29	140/141, 224/225, 211, 295; 140/141, 196/197, 239, 295; 168/169, 196/197, 239, 267
32	3-Methylheptacosane	27.74	28	336/337, 364/365, 394
33	*n*-Octocosane	28.00	28	394
34	9-; 11-; 13-; 15-Methyloctacosane[c]	28.32	29	140/141, 294/295 168/169, 266/267 196/197, 238/239 210/211, 224/225
35	11,15-; 13,17-Dimethyloctacosane[c]	28.62	29	168/169, 210/211, 239, 281; 196/197, 182/183, 253, 267
36	3-Methyloctacosane	28.70	29	350/351, 378/379, 408
37	*n*-Nonacosane	29.00	29	408
38	9-; 11-; 13-; 15-Methylnonacosane[c]	29.32	30	140/141, 308/309; 168/169, 280/281; 196/197, 252/253; 224/225
39	5-Methylnonacosane	29.50	30	84/85, 364/365
40	11,15-; 13,17-Dimethylnonacosane[c]	29.61	31	168/169, 224/225, 239, 295; 196/197, 267
41	3-Methylnonacosane	29.73	30	364/365, 392/393, 422
42	13,17,21-Trimethylnonacosane	29.90	32	196/197, 140/141, 211, 267, 281, 337
43	*n*-Triacontane	30.00	30	422
44	3,7-Dimethylnonacosane	30.10	31	126/127, 336/337, 407

TABLE 1. Continued

Peak[a]	Hydrocarbon	ECL[b]	CN[b]	Diagnostic MS ions
45	11,15-; 13,17-Dimethyltriacontane[c]	30.60	32	168/169, 238/239, 239, 309; 196/197, 210/211, 267, 281
46	*n*-Hentriacontane	31.00	31	436
47	7-; 9-; 11-Methylhentriacontane[c]	31.40	32	112/113, 364/365; 140/141, 336/337; 168/169, 308/309
48	11,15-; 13,17-Dimethylhentriacontane[c]	31.64	33	168/169, 252/253, 239, 323; 196/197, 224/225, 267, 295
49	Docotriacontene + 3-methyltriacontane[c]	31.71	32:1 32	448 421/422, 392/393, 450
50	11,15,19-Trimethylhentriacontane	31.90	34	168/169, 196/197, 267, 239, 309, 337
51	Docotriacontane	32.00	32	450
52	3,7-Dimethylhentriacontane	32.14	33	126/127, 364/365, 435
53	Tritriacontene	32.75	33:1	462
54	*n*-Tritriacontane	33.00	33	464
55	9-; 11-; 13-; 15-Methyltritriacontane[c]	33.34	34	140/141, 364/365; 168/169, 336/337; 196/197, 308/309; 224/225, 252/253
56	11,19-; 11,15-; 11,21-; 13,17-; 13,19-Dimethyltritriacontane[c]	33.57	35	168/169, 224/225, 295, 351; 168/169, 280/281, 239, 351; 168/169, 196/197, 323, 351; 196/197, 252/253, 267, 323; 196/197, 224/225, 295, 323
57	11,15,21-; 13,17,21-; 13,17,23-Trimethyltritriacontane[c]	33.86	36	168/169, 196/197, 239, 295, 337, 365; 196/197, 267, 337; 196/197, 168/169, 267, 337, 365

58	3,7-Dimethyltritriacontane	34.08	35	126/127, 392/393, 463
59	11-; 12-Methyltetracontane[c]	34.30	35	168/169, 350/351; 182/183, 336/337
60	Pentatriacontene	34.70	35:1	490
61	Pentatriacontane	35.00	35	492
62	9-; 11-; 13-Methylpentatriacontane[c]	35.34	36	140/141, 392/393; 168/169, 364/365; 196/197, 336/337
63	11,23-; 13,19-; 13,21-; 15,19-Dimethylpentatriacontane[c]	35.60	37	168/169, 224/225, 351, 379; 196/197, 252/253, 295, 351; 196/197, 323, 351; 224/225, 252/253, 295, 323
64	Heptatriacontene	36.70	37:1	518
65	11,15,23-; 13,17,23-Trimethylpentatriacontane[c]	36.78	38	168/169, 196/197, 239, 323, 365, 393; 196/197, 267, 295, 365
66	Heptatriacontane	37.00	37	520
67	9-; 11-; 13-Methylheptatriacontane[c]	37.30	38	140/141, 406/407; 168/169, 392/393; 196/197, 364/365
68	13,21-; 15,21-Dimethylheptatriacontane[c]	37.51	39	196/197, 252/253, 323, 379; 224/225, 252/253, 323, 351
69	13,17,21-; 13,17,23-Trimethylheptatriacontane	37.90	40	196/197, 252/253, 267, 323, 337, 393 196/197, 224/225, 267, 323, 365, 393
70	11-; 12-; 13-Methyloctatriacontane[c]	38.30	39	168/169, 406/407; 182/183, 392/393; 196/197, 378/379
71	9-; 11-; 13-; 15-Methylnonatriacontane[c]	39.30	40	140/141, 448/449; 168/169, 420/421; 196/197, 392/393; 224/225, 364/365

TABLE 1. Continued

Peak[a]	Hydrocarbon	ECL[b]	CN[b]	Diagnostic MS ions
72	13,17-; 13,21-; 15,19-Dimethylnonatriacontane[c]	39.60	41	196/197, 336/337, 267, 407; 196/197, 280/281, 323, 407; 224/225, 308/309, 295, 379
73	13,17,23-; 13,17,25-Trimethylnonatriacontane[c]	39.90	42	196/197, 252/253, 267, 365, 351, 421; 196/197, 224/225, 267, 351, 393, 421
74	11-; 13-; 15-Methylhentetracontane[c]	41.30	42	168/169, 448/449; 196/197, 420/421; 224/225, 392/393
75	13,17-; 13,21-Dimethylhentetracontane	41.60	43	196/197, 364/365, 267, 435 196/197, 308/309, 323, 435
76	13,17,21-Trimethylhentetracontane	41.90	44	196/197, 308/309, 267, 337, 379, 449
77	11-; 13-; 15-Methyltritetracontane[c]	43.30	44	168/169, 476/477; 196/197, 448/449; 224/225, 420/421
78	13,17-Dimethyltritetracontane	43.60	45	196/197, 392/393, 267, 463

[a] Peak numbers refer to peaks identified in Figures 1–4.
[b] ECL = equivalent chain length, CN = carbon number.
[c] An isomeric mixture or two or more components coelute in this peak.

TABLE 2. HYDROCARBONS IDENTIFIED FROM EIGHT SPECIES OF EIGHT SPECIES OF *Conophthorus*[A]

Peak No.	Hydrocarbon	ECL	*Conophthorus* species[b]							
			Coni	*Cpon*	*Ccem*	*Cedu*	*Crad*	*Ccon*	*Cres*	*Cban*
1	2-Methyleicosane	20.70	o	o	o	o	o	+	o	o
2	*n*-Heneicosane	21.00	o	o	o	o	o	tr	o	o
3	*n*-Docosane	22.00	tr	+	tr	tr	tr	+	tr	tr
4	2- or 4-; 3-Methyldocosane[c]	22.76	o	tr	o	o	o	o	o	o
5	Tricosene	22.76	o	tr	o	o	o	o	o	o
6	*n*-Tricosane	23.00	+++	+++	++	++	+++	+++	+++	+++
7	9-; 11-; 13-Methyltricosane[c]	23.39	tr	o	tr	o	o	o	o	o
8	Tetracosene	23.70	o	tr	o	o	o	o	tr	tr
9	3-Methyltricosane	23.73	tr	tr	tr	o	tr	o	o	o
10	*n*-Tetracosane	24.00	+	++	+	tr	++	+	+	+
11	2- or 4-Methyltetracosane	24.63	o	tr	o	o	o	o	o	o
12	Pentacosadiene	24.69	o	tr	o	o	++	tr	++	++
13	Pentacosene	24.70	+++	+++	+	+	+++	+++	+++	+++
14	*n*-Pentacosane	25.00	+++	+++	+++	+++	+++	+++	+++	+++
15	9-; 11-; 13-Methylpentacosane[c]	25.38	+	tr	+	+	tr	++	+	+
16	5-Methylpentacosane	25.50	tr	tr	tr	o	o	o	o	o
17	11,15-Dimethylpentacosane	25.60	o	o	tr	tr	o	o	o	o
18	2- or 4-Methylpentacosane	25.70	o	o	o	o	o	tr	o	o
19	Hexacosene + 3-methylpentacosane[c]	25.73	tr	tr	o	o	o	o	o	o
			tr	tr	tr	tr	tr	+	+	+
20	*n*-Hexacosane	26.00	++	++	++	++	++	++	++	++
21	12-; 13-Methylhexacosane	26.34	tr	tr	tr	tr	o	++	o	o
22	11,15-; 13,17-Dimethylhexacosane[c]	26.60	o	o	+	o	o	o	o	o
23	2- or 4-Methylhexacosane +	26.63	o	o	o	o	o	++	o	o
	heptacosadiene[c]		o	tr	o	o	o	++	tr	tr
24	Heptacosene	26.70	+	+	tr	++	+	+++	++	++

TABLE 2. Continued

Peak No.	Hydrocarbon	ECL	*Conophthorus species*[b]							
			Coni	*Cpon*	*Ccem*	*Cedu*	*Crad*	*Ccon*	*Cres*	*Cban*
25	3-Methylhexacosane	26.79	o	tr	o	o	o	o	o	o
26	*n*-Heptacosane	27.00	+++	+++	+++	+++	+++	+++	+++	+++
27	7-; 9-; 11-; 13-Methylheptacosane[c]	27.32	+	++	++	++	tr	+++	+	+
28	9-Methylheptacosane	27.40	o	o	o	o	o	+	o	o
29	7-Methylheptacosane	27.42	o	o	o	o	o	+	o	o
30	5-Methylheptacosane	27.50	tr	tr	o	o	o	+	o	o
31	9,13-Dimethylheptacosane + 9,15-dimethylheptacosane + 11,15-dimethylheptacosane[c]	27.63	tr	o	o	o	o	o	o	o
			o	o	+++	+++	o	o	o	o
			tr	o	+++	+++	o	o	o	o
32	3-Methylheptacosane	27.74	tr	tr	o	o	tr	x	+	+
33	*n*-Octacosane	28.00	+	+	++	+	tr	x	tr	tr
34	9-; 11-; 13-; 15-Methyloctacosane	28.32	o	tr	tr	tr	o	+	o	o
35	11,15-; 13,17-Dimethyloctacosane[c]	28.62	o	o	+	tr	o	o	o	o
36	3-Methyloctacosane	28.70	o	tr	o	o	o	o	o	o
37	*n*-Nonacosane	29.00	++	++	+++	++	+	x	+	+
38	9-; 11-; 13-; 15-Methylnonacosane[c]	29.32	tr[d]	tr	++	++	o	++[e]	o	o
39	5-Methylnonacosane	29.50	o	tr	o	o	o	o	o	o
40	11,15-; 13,17-Dimethylnonacosane[c]	29.61	tr	o	+++	+++	o	o	o	o
41	3-Methylnonacosane	29.70	o	tr	o	o	o	o	o	o
42	13,17,21-Trimethylnonacosane	29.90	o	o	+	tr	o	o	o	o
43	*n*-Triacontane	30.00	+	tr	tr	tr	o	o	o	o
44	3,7-Dimethylnonacosane	30.10	o	tr[g]	x	x	o	o	o	o
45	11,15-; 13,17-Dimethyltriacontane[c]	30.60	o	o	tr	x	o	o	o	o
46	*n*-Hentriacontane	31.00	++	+	++	++	o	o	++	++
47	7-, 9-, 11-, 13-, 15-Methylhentriacontane	31.40	tr	o	+	+	o	o	o	o

48	11,15-; 13,17-Dimethylhentriacontane	31.64	+	o	++	++	tr[f]	o	o	o
49	Docotriacontene + 3-methyltriacontane[c]	31.71	o	tr	o	o	o	o	o	o
			o	tr	o	o	o	o	o	o
50	11,15,19-Trimethylhentriacontane	31.90	+	o	o	o	o	o	o	o
51	*n*-Docotriacontane	32.00	tr	o	o	o	o	o	o	o
52	3,7-Dimethylhentriacontane	32.10	tr	+[g]	o	o	o	o	o	o
53	Tritriacontene	32.70	o	tr	o	tr	o	o	o	o
54	*n*-Tritriacontane	33.00	+	tr	tr	tr	o	o	o	o
55	9-; 11-; 13-; 15-Methyltritriacontane[c]	33.30	+	+	++	+	tr	x	o	o
56	11,15-; 11,19-; 11,21-; 13,19-Dimethyltritriacontane[c]	33.57	o	tr	o	o	o	x	o	o
	11,19-; 13,19-dimethyltritriacontane[c]		++	o	o	o	o	o	o	o
	13,17-Dimethyltritriacontane		o	o	tr	+	o	++	o	o
57	11,15,21-; 13,17,23-Trimethyltritriacontane[c]	33.86	++	+	o	o	o	o	o	o
	13,17,21-Trimethyltritriacontane		o	o	tr	++	o	++	o	o
58	3,7-Dimethyltritriacontane	34.08	tr	tr[g]	o	o	o	o	o	o
59	11-; 12-; 13-Methyltetratriacontane[c]	34.30	o	tr	tr	x	o	o	o	o
60	Pentatriacontene	34.70	o	tr	o	o	o	o	++	++
61	*n*-Pentatriacontane	35.00	++	o	x	x	o	o	o	o
62	9-; 11-; 13-Methylpentatriacontane[c]	35.31	++	++	++	++	++	o	+	tr
63	11,23-; 13,21-; 15,19-Dimethylpentatriacontane[c]	35.60	++	o	o	o	o	o	o	o
	11,23-; 15,19-Dimethylpentatriacontane[c]		o	+	o	o	o	o	o	o
	13,19-; 13,21-Dimethylpentatriacontane[c]		o	o	o	o	++	o	o	o
	13,21-; 15,19-Dimethylpentatriacontane[c]		o	o	o	o	o	+++	o	o
	13,19-Dimethylpentatriacontane		o	o	tr	+	o	o	o	o
64	11,15,23-Trimethylpentatriacontane	35.78	++	++	o	o	o	o	o	o
	13,17,23-Trimethylpentatriacontane		++	++	tr	tr	+	++	++	++
65	Heptatriacontene	36.70	o	o	o	o	o	o	++	++
66	Heptatriacontane	37.00	+	o	o	o	o	o	o	o
67	9-; 11-; 13-Methylheptatriacontane[c]	37.30	++	++	++	++	++	x	++	++

TABLE 2. Continued

Peak No.	Hydrocarbon	ECL	*Conophthorus species*[b]							
			Coni	*Cpon*	*Ccem*	*Cedu*	*Crad*	*Ccon*	*Cres*	*Cban*
68	13,21-; 15,21-Dimethylheptatriacontane[c]	37.59	++	+	o	tr[h]	+++	+++	+	+
69	13,17,21-Trimethylheptatriacontane[c]	37.90	+	++	o	o	++	o	++	++
	13,17,23-Trimethylheptatriacontane		o	++	o	o	++	x	o	o
70	11-; 12-; 13-Methyloctatriacontane[c]	38.30	tr	tr	tr	o	o	o	o	o
71	9-; 11-; 13-; 15-Methylnonatriacontane[c]	39.30	++	++	++	+++	++	o	++	++
72	13,17-; 13,21-; 15,19-Dimethylnonatriacontane[c]	39.60	++	+	++[h]	++[h]	+++[h]	o	x	x
73	13,17,23-; 13,17,25-Trimethylnonatriacontane[c]	39.90	++	+	o	o	++	o	++	++
74	11-; 13-; 15-Methylhentetracontane[c]	41.30	++	+	++	++	x	o	+	+
75	13,17-Dimethylhentetracontane	41.60	++	+	o	o	x	o	x	x
	13,21-Dimethylhentetracontane		+	o	+	+	o	o	x	x
76	13,17,21-Trimethylhentetracontane	41.90	++	+	o	o	x	o	x	x
77	11-; 13-; 15-Methyltritetracontane[c]	43.30	+	o	++	++	o	o	o	o
78	13,17-Dimethyltritetracontane	43.60	++	o	+	+	o	o	o	o

[a] Hydrocarbon identification by GC-MS (see Table 1 and Figures 1–4). A triple + indicates ≥5.0%, ++ from 1.0 to 5.0%, and + from 0.5 to 1.0% of the total hydrocarbon component. Some trace (tr) components appear infrequently or consistently in very small quantities (<0.5% of the total). A zero indicates the hydrocarbon was never identified for the species. Compounds with an x were not identified; mass spectra were insuffient to make positive identification.

[b] Coni = *Conophthorus conicolens*, Cpon = *C. ponderosae*, Ccem = *C. cembroides*, Cedu = *C. edulis*, Ccon = *C. coniperda*, Crad = *C. radiatae*, Cres = *C. resinosae*, Cban = *C. banksianae*.

[c] An isomeric mixture. These components coelute in this peak.

[d] The 9-methyl isomer is the only component present.

[e] The 13- and 15-monomethylalkanes coelute in this peak.

[f] Only the 13,17-dimethyl isomer was identified.

[g] The 3,7-dimethyl isomers were found only in adults from sugar pine cones.

[h] The 13,21-dimethyl isomer was the only component identified.

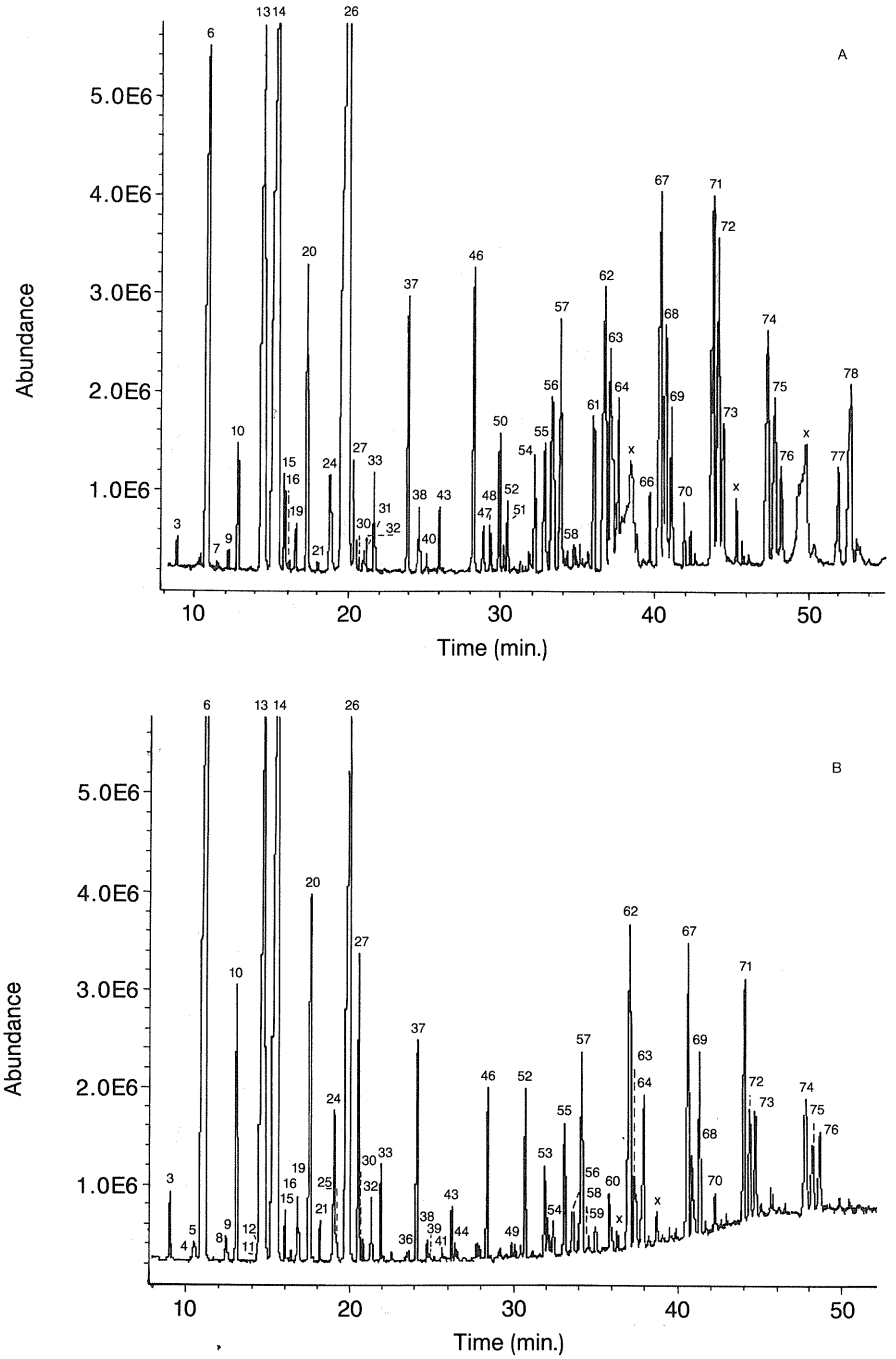

FIG. 1. Total ion chromatograms of cuticular hydrocarbons from: (A) *Conophthorus conicolens* collected from Chihuahua pine from the Chiricahua Mountains in southeastern Arizona, and (B) *C. ponderosae* collected from sugar pine from the Eldorado National Forest, California. Numbers identify peaks whose compositions are listed in Table 1. Compounds marked with an x were not identified.

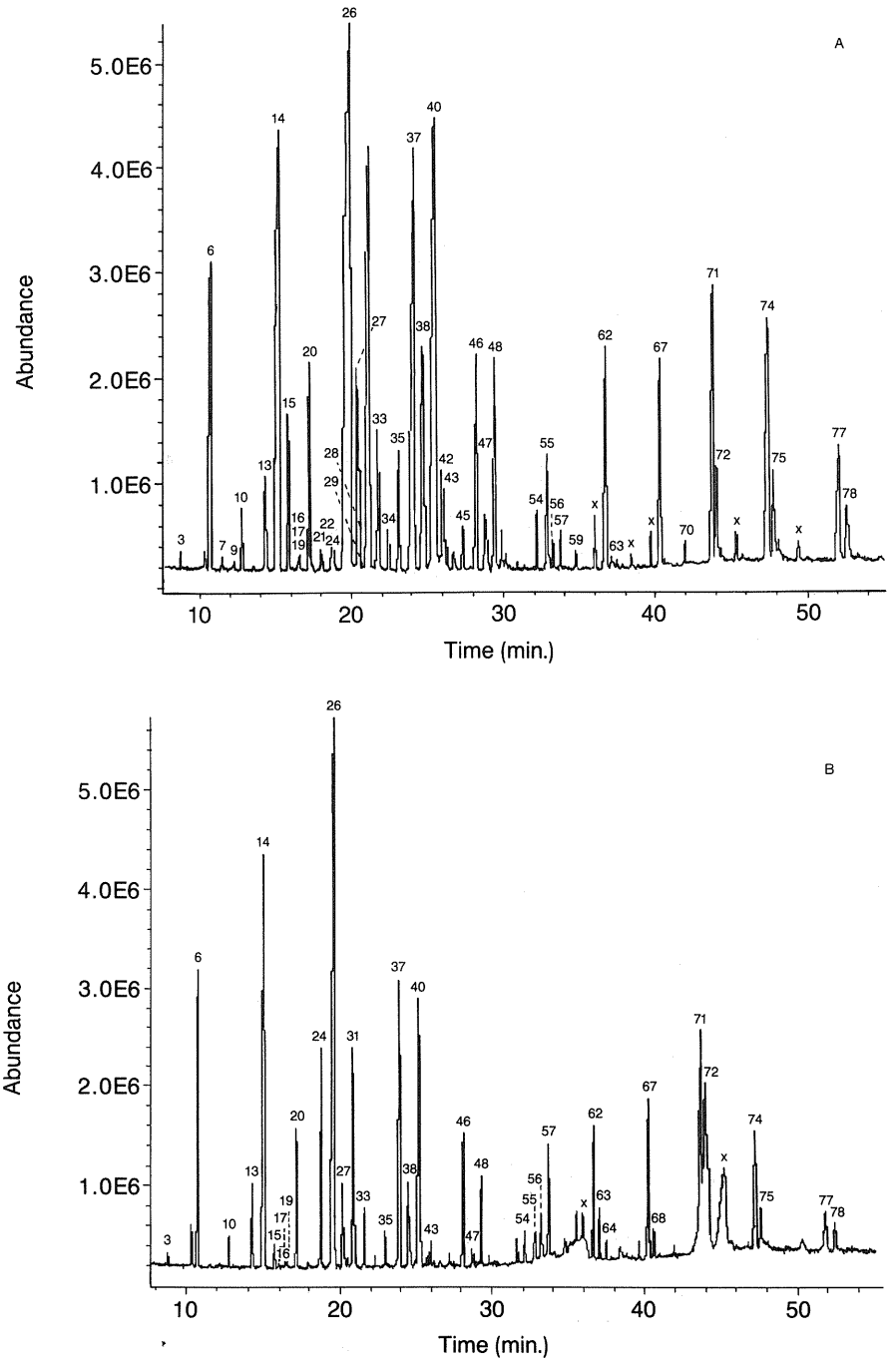

FIG. 2. Total ion chromatograms of cuticular hydrocarbons from: (A) *Conophthorus cembroides* collected from Mexican pinyon from the Chiricahua Mountains in southeastern Arizona, and (B) *C. edulis* collected from pinyon pine from Prescott, Arizona. Numbers identify peaks whose compositions are listed in Table 1. Compounds marked with an x were not identified.

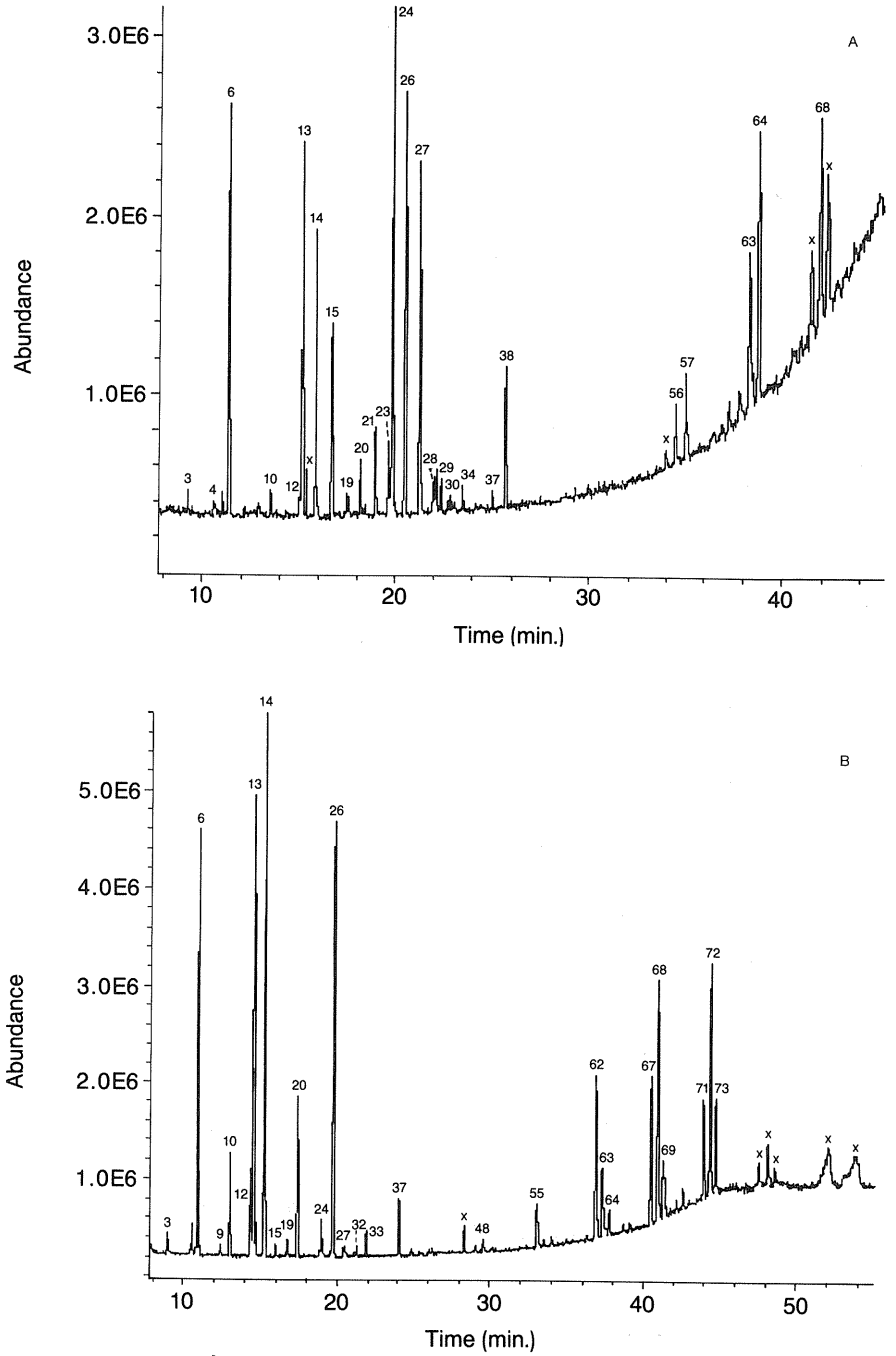

FIG. 3. Total ion chromatograms of cuticular hydrocarbons from: (A) *Conophthorus coniperda* collected from eastern white pine from Murphy, North Carolina, and (B) *C. radiatae* collected from Monterey pine from Albany, California. Numbers identify peaks whose compositions are listed in Table 1. Compounds marked with an x were not identified.

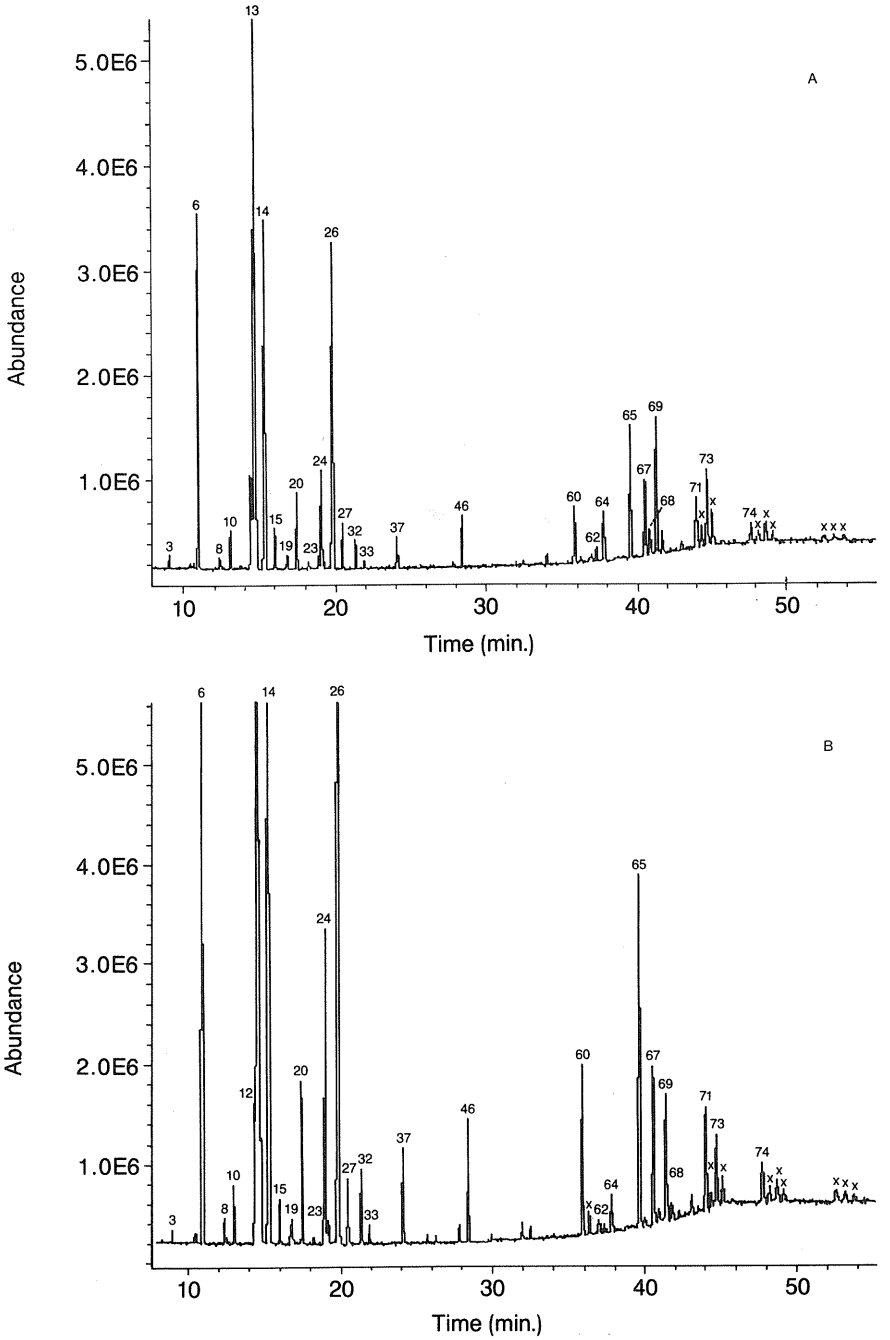

FIG. 4. Total ion chromatograms of cuticular hydrocarbons from: (A) *Conophthorus resinosae* collected from red pine from Little Rapids, Ontario, Canada, and (B) *C. banksianae* collected from jack pine twigs from Aubrey Falls, Ontario, Canada. Numbers identify peaks whose compositions are listed in Table 1. Compounds marked with an x were not identified.

Significant (>0.5% total hydrocarbon) internally branched monomethylalkanes occur in these *Conophthorus* species as complex isomeric mixtures. The mass spectra of peaks with ECL of approximately 0.7 before the corresponding C_n have been identified as having methyl branches on odd-numbered carbons (9 through 15). They have strong ions at *m/z* 140:141, 168:169, 196:197 and 224:225, indicating alpha cleavage internal to the branches at 9, 11, 13, and 15 carbons. *C. conicolens*, *C. cembroides*, and *C. edulis* produce the only homologous series of isomeric mixtures that includes all 11- and 13-(Me) C_n compounds having odd-numbered parent chains from 11-; 13-(Me) C_{25} to 11-; 13(Me) C_{34}. The 9-(Me) C_n isomers are present from 9-(Me) C_{25} to 9-(Me) C_{35}. The only exception is *C. conicolens*, which produces only 9-(Me) C_{29} and adds the 15-(Me) C_n isomers at 15-(Me) C_{33}, 15-(Me) C_{39}, 15-(Me) C_{41}, and 15-(Me) C_{43}.

There were only five individual or isomeric mixtures of internally branched monomethylalkanes identified with even-numbered parent chains (Tables 1 and 2). *C. ponderosae* and *C. coniperda* both produce 12-; 13-(Me) C_{26}. 15-(Me) C_{28} is found in *C. coniperda* and 11-; 12-; 13-(Me) C_{38} in *C. conicolens*. Traces of 11-; 12-(Me) and 13-(Me) C_{34} are found in *C. ponderosae* and *C. cembroides*. Small amounts of single-component 5- and 7-monomethylalkanes are present in *C. ponderosae* and *C. coniperda*. Trace amounts of 5-(Me) C_{25}, 5-(Me) C_{27}, and 5-(Me) C_{29} were identified in all of the *C. ponderosae* populations. *C. coniperda* is the only species with a single-component 7-(Me) C_n, identified as 7-(Me) C_{27}.

Dimethylalkanes in insects generally have methyl branches on odd-numbered carbons separated by an odd number of carbons (Blomquist et al., 1987). All of the dimethylalkanes produced by these species of *Conophthorus* fit this pattern. The majority of the methyl branches are separated by three carbons. They include the 3,7-$(Me)_2$ C_n (3,7-dimethylalkanes) (*C. ponderosae*, sugar pine, and *C. conicolens*), the 11,15- and 13,17-$(Me)_2$ C_n (*C. conicolens*, *C. ponderosae*, *C. cembroides*, *C. edulis*, *C. radiatae*), 15,19-$(Me)_2$ C_{35} (*C. conicolens*, *C. ponderosae*, *C. coniperda*) and 9,13-$(Me)_2$ C_{27} in *C. conicolens*. The remainder of the dimethylalkanes have methyl branches separated by 5, 7, 9, and 11 carbons. Dimethylalkanes with methyl branches separated by five carbons include 9,15-$(Me)_2$ C_{27} (*C. cembroides*, *C. edulis*), 13,19-$(Me)_2$ C_n (*C. conicolens*, *C. ponderosae*, *C. cembroides*, *C. edulis*, and *C. radiatae*), and 15,21-$(Me)_2$ C_{37} in all species except *C. cembroides* and *C. edulis*. All species include representatives of dimethylalkanes separated by 7 carbons: 11,19-$(Me)_2$ C_{33}; 13,21-$(Me)_2$ C_{35}; 13,21-$(Me)_2$ C_{37}, and 13,21-$(Me)_2$ C_{41}. The only component with methyl branches separated by nine carbons was 11,21-$(Me)_2$ C_{33} in an isomeric mixture in *C. ponderosae*. The one dimethylalkane with 11 carbons separating methyl branches was 11,23-$(Me)_2$ C_{35} in *C. conicolens* and *C. ponderosae*.

We identified isomeric mixtures of species specific dimethylalkanes in *C. ponderosae*, *C. conicolens*, *C. cembroides*, and *C. edulis* (Table 2). Dimethylalkanes that eluted about 0.9 carbon units in front of the corresponding *n*-

alkane were identified as 3,7-$(Me)_2$ C_n isomers. An example of the mass spectrum of this type of component is interpreted as 3,7-$(Me)_2$ C_{31} (Figure 5A). The $(M-29)^+$ and $(M-57)^+$ ions at *m/z* 435 and 407 localize one methyl branch on the 3-position. The ion fragments at *m/z* 364/365 and 127 show that the other methyl branch is on carbon 7. The absence of other diagnostic ions indicates that this isomer is the only prominent one in this peak. In *C. ponderosae* three 3,7-$(Me)_2$ C_n (peaks 44, 52, and 58) were unique to beetles infesting sugar pine cones. Thus far we have not found these components in adults infesting ponderosa pine or western white pine cones.

Dimethylalkanes with both methyl branches positioned internally elute ca. 1.4 ECL in front of the corresponding *n*-alkane. Unique species-specific dimethylalkanes with such ECLs are present in *C. conicolens*, *C. cembroides*, and *C. edulis* (Table 2). The hydrocarbon mixtures of *C. cembroides* and *C. edulis* contain significant amounts of 9,15- and 11,15-$(Me)_2$ C_{27} (peak 31); 11,15- and 13,17-$(Me)_2$ C_{29} (peak 40), and 11,15- and 13,17-$(Me)_2$ C_{31} (peak 48). An example of the mass spectrum of this type of dimethylalkane was interpreted as 11,15-$(Me)_2$ C_{27} (Figure 5B). The fragments at *m/z* 168/169 and 196/197, where the even-to-odd ratio is greater than one, arise from cleavage internal to each of the carbons bearing methyl branches. The ion fragment at *m/z* 239 and 267, in which the odd-numbered ions predominate, arise from cleavage external to the two branching methyl groups. The mass spectra of the other internally branched dimethylalkanes were interpreted in a similar manner (Tables 1 and 2).

Homologous series of high-molecular-weight trimethylalkanes are found in all eight species of *Conophthorus*. Each component has an ECL of approximately 2.1 less than the corresponding *n*-alkane. *C. cembroides* and *C. edulis* contain fewer trimethylalkanes than the other six species. *C. radiatae*, *C. coniperda*, *C. resinosae*, and *C. banksianae* appear to only produce trimethylalkanes with parent carbon chains of 35, 37, and 39. *C. conicolens* and *C. ponderosae* produce isomeric mixtures of trimethylalkanes from parent carbon chains 33–41. We identified a homologous series of 13,17,23-$(Me)_3$ C_n (13,17,23-trimethylalkanes) in *C. conicolens* and *C. ponderosae* starting with 13,17,23-$(Me)_3$ C_{33} (Figure 6A). The component 13,17,23-$(Me)_3$ C_{35} (peak 64) is found in all *Conophthorus* species characterized in this paper. The significant even-mass ions *m/z* 196 : 197, with an even-to-odd number ratio of about 1, are interpreted as arising from a cleavage internal to the methyl branches at either end of the molecule. The ions at *m/z* 267, 295, and 365 arise from cleavage external to the methyl branch on positions 13 and 23 and on both sides of the methyl branch on position 17. The odd-to-even ratio for these ions is greater than 1, as secondary carbonium ions containing two of three methyl branches supress formation of even-mass ions (Blomquist et al. 1987). *C. conicolens* and *C. ponderosae* clearly have the most complex series of trimethylalkanes. In these species, we have identified a unique trimethylalkane (peak 73) that occurs

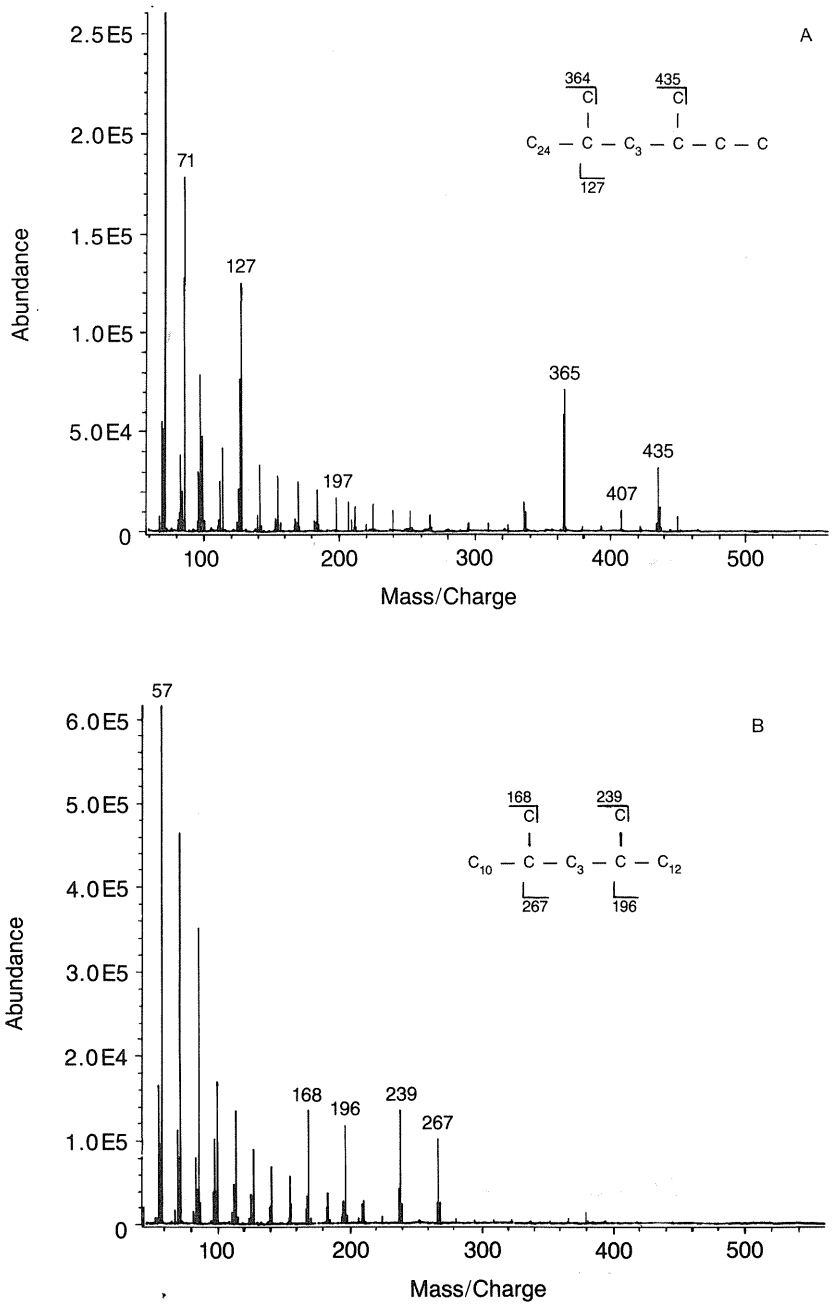

FIG. 5. EI mass spectra of (A) peak 52, Figure 1B, identified as 3,7-dimethylhentriacontane, and (B) peak 31, Figure 2A, identified as 11,15-dimethylheptacosane.

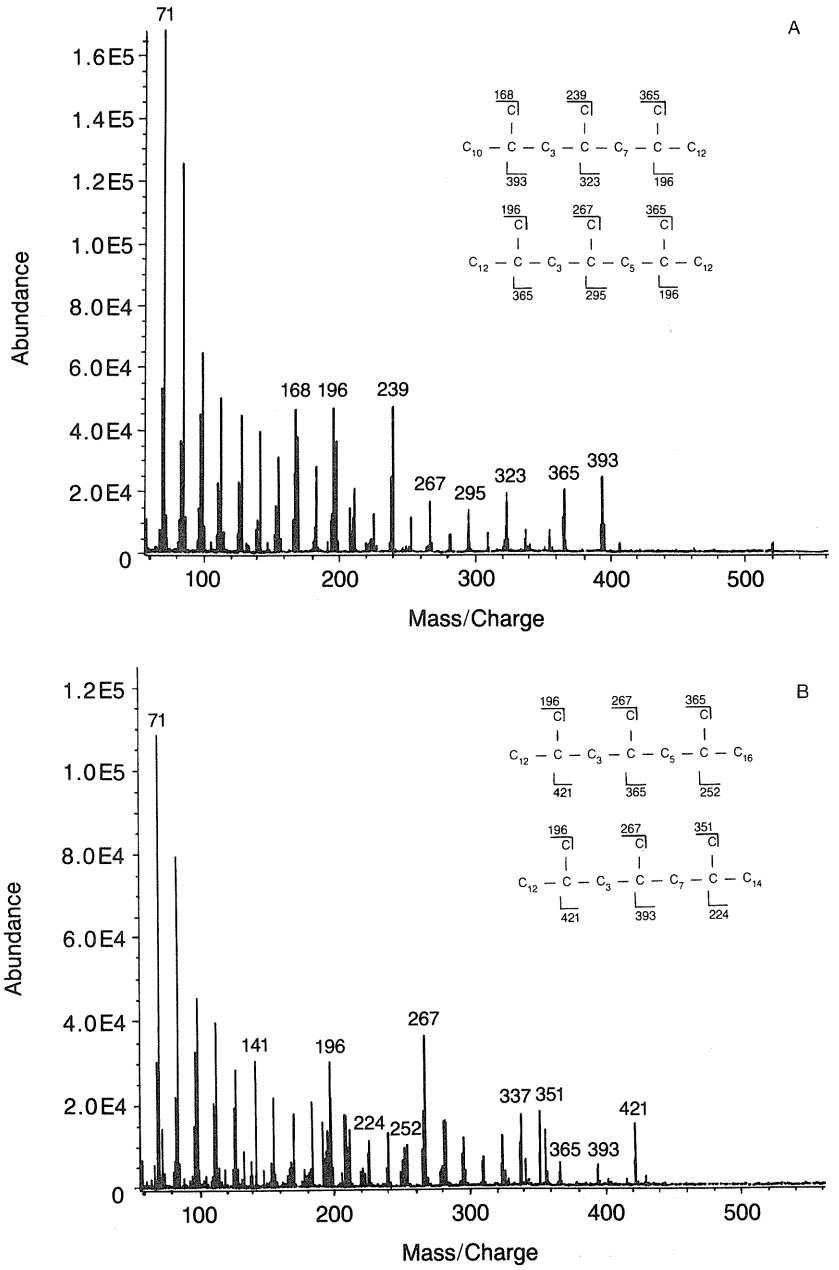

FIG. 6. EI mass spectrum of peak 65, Figure 1A and B, identified as an isomeric mixture of 11,15,23- and 13,17,23-trimethylpentatriacontane, and (B) peak 73, Figure 1B, identified as 13,17,25-trimethylnonatriacontane.

in an isomeric mixture whose mass spectrum is interpreted as 13,17,25-$(Me)_3$ C_{39} (Figure 6B). This component has even-mass ions at *m/z* 196/197 and 224/225 and significant odd ions at *m/z* 267, 351, 393 and 421.

DISCUSSION

The cuticular hydrocarbons of *Conophthorus*, as is the case for many insect species (Blomquist et al., 1987), consist of a complex mixture of unsaturated and straight-chain and methyl-branched, saturated components. The relatively large number of components, ease of analysis, and species-specific compositions for many insects make them attractive characters for use in chemotaxonomy (Carlson and Bolten, 1984; Gastner and Nation, 1986; Haverty et al., 1988; Howard et al., 1988; Lockey, 1982; Vander Meer, 1986).

The cuticular hydrocarbons, particularly the unsaturated and methyl-branched components, are synthesized by the insect (Blomquist et al., 1987). A small portion of the *n*-alkanes in several species can arise from the diet (Blomquist and Jackson, 1973; Nelson et al., 1971), but unsaturated and methyl-branched hydrocarbons are rare in plants. Thus, the prevalence of unsaturated and methyl-branched components in *Conophthorus*, while expected, is particularly useful.

Conophthorus is a genus in which many of the species are difficult to separate on the basis of morphological characters alone. In this report we have identified all the major cuticular hydrocarbons of eight of the 15 described species of *Conophthorus* (Flores and Bright, 1987; Wood, 1982). We hope to develop a new suite of characters for the entire genus, similar to our studies with the dampwood termites, *Zootermopsis* (Haverty et al., 1988; Haverty and Thorne, 1989; Thorne and Haverty, 1989). The cuticular waxes of these species of *Conophthorus* contain many diagnostic hydrocarbons, which are useful for separation of these species.

Reports of trimethylalkanes in cuticular lipids of Coleoptera are rare, although they have been reported from other groups of insects (Blomquist et al., 1987). So far, trimethylalkanes have been positively identified only in *Cylindrinotus laevioctostriatus* (Goeze) and *Phylan gibbus* (Fabricius) in the Tenebrionidae (Lockey, 1981). Trimethyalkanes may be ubiquitous in all species of *Conophthorus*. Each of the eight species we examined in this study produces significant amounts of trimethylalkanes after C_{30}. The spacing between methyl groups in other Coleoptera has been a [3,3] sequence. We have identified trimethylalkanes with [3,5] and [3,7] spacing. The [3,5] spacing of 11,15,21- and 13,17,23-$(Me)_3$ C_n isomers is the most common in all of these species of *Conqphthorus*. This spacing has been identified so far only in the components 11,15,21- and 13,17,23-$(Me)_3$ C_{35} and 11,15,21- and 13,17,23-$(Me)_3$ C_{37} in the female tsetse fly, *Glossina pallidipes* (Austen) (Nelson and

Carlson, 1986). We have identified the components 11,15,21- and 13,17,23-$(Me)_3$ C_{33} and 13,17,25-$(Me)_3$ C_{39} in several species of *Conophthorus*, in addition to those trimethylalkanes reported in *G. pallidipes*. This appears to be the first report of a trimethylalkane with a [3,7] spacing sequence between methyl branches.

The composition of the cuticular wax components in three populations of the polyphagous species *Conophthorus ponderosae* from three different hosts is one of the most complex. With one possible exception, the hydrocarbon blends are qualitatively the same in all three of the populations we examined; the exceptions are the 3,7-$(Me)_2$ C_n in the populations from sugar pine. It may be possible that *C. ponderosae* in ponderosae pine and western white pine produce these dimethylalkanes in significantly lower quantities. Future analyses with more adults from additional populatations may resolve their presence or absence. If one agrees that hydrocarbon profiles are species specific (Haverty et al., 1988; Howard and Blomquist, 1982; Howard et al., 1982; Vander Meer, 1986), we would infer that two populations evaluated here (from ponderosa pine and western white pine) are of the same species or at least very closely related.

The status of *C. cembroides* and *C. edulis* as distinct, separate species is in question (S.L. Wood, personal communication). Our analyses of their hydrocarbon components suggest that they are closely related. The only difference in hydrocarbon patterns is the replacement of a dimethylalkane at ECL = 26.6 with an alkene (ECL = 26.7) in *C. cemroides*. Extensive sampling of *Conophthorus* from the nut pines in the southwestern United States and northern Mexico should clarify the relationship of these two similar species.

The hydrocarbon patterns of *C. resinosae* and *C. banksianae* are qualitatively identical; no unique hydrocarbon components were found between these two species from many populations in Canada and the United States. They do possess several components that easily separate them from the other six species discussed in this paper. This evidence supports the suspicion that *C. banksianae* is not a valid species (S.L. Wood and P. de Groot, personal communications). Further elucidation with biological and genetic studies will be necessary to resolve this problem.

Future studies will involve further characterization of the cuticular hydrocarbons of additional species of *Conophthorus* and quantitative evaluation of intra- and interspecific variation. It is hoped these studies will clarify and/or validate the taxonomy of this genus, especially the polyphagous species, *C. ponderosae* and the *C. edulis* complex. Once we have characterized the cuticular hydrocarbons of all of the extant species of *Conophthorus*, we will develop a dichotomous key to all species. To be useful for dichotomous keys, hydrocarbons should be abundant, not minor, components (at least 1%, but preferably >5% of the total hydrocarbon mixture). They should be unique or present in only a few of the species, or conversely, they should be common in most of

the species yet completely absent, rare, or present in insignificant quantities in one or a few. Furthermore, they should have a unique elution time so that they do not coelute with another hydrocarbon in the same species, nor should they elute at a time similar to a different hydrocarbon in a different species.

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