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Nonacosan-10-ol and *n*-Alkanes in Needles of *Pinus halepensis*

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Abstract

In needle cuticular wax of *Pinus halepensis*, nonacosan-10-ol is high (77.08% on average). *n*-Alkanes ranged from C_{18} to C_{35} with the most dominant C_{27} and C_{29} (32.4% and 25.8%, respectively). The carbon preference index ranged from 3.2 to 5.4 (3.4 on average), while the average chain length ranged from 14.0 to 18.0 (17.2 on average). Long-chain *n*-alkanes strongly dominated (95.1%).

Keywords

Pinus halepensis, nonacosan-10-ol, *n*-alkanes, needles, waxes

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Introduction

Pinus halepensis Miller, also known as Aleppo pine or Jerusalem's oren, is a 2-needle pine which belongs to family Pinaceae, genus *Pinus*, subgenus *Pinus*, section *Pinus*, subsection *Pinaster* (classification of Gernandt et al).[1] It is distinctly Mediterranean species which spreads from Morocco to Tunisia and Libia as well as from Spain to France, Italy, former Yugoslavia, Greece, Israel, Jordan, and Corsica. It succeeds from sea level to altitude of 1500 m (in Morocco and Algeria).[2]

Cuticular waxes and *n*-alkanes have often been studied in trees[3] and herbaceous plants.[4-10] They are also used in chemosystematic and phylogenetic studies, hybrid detection, etc.[3-10] Cuticular waxes and *n*-alkanes of various *Pinus* species have already been investigated,[11-14] sometimes on population level (in case of *Pinus heldreichii*, *Pinus peuce*, *Pinus nigra*, etc.).[15-18] Sometimes, *n*-alkanes could detect varieties within conifer species.[19]. Other authors have already reported that pine epicuticular waxes have tube crystalloids[20] and that nonacosan-10 is the main component of epicuticular wax components.[21-25]

The aim of this study is to examine for the first time amount of nonacosan-10-ol and *n*-alkane profile of *P. halepensis* in needle cuticular waxes. Besides that, the chemotaxonomy of section *Pinus* was done by comparing our results of *P. halepensis* with other pines of section *Pinus*.

Results and Discussion

Nonacosan-10-ol content of *P. halepensis* is a little bit smaller in spring needles (76.7%) than in autumn needles (77.4%). In average, nonacosan-10-ol is higher (77.1%) than in wax of other species of subsection *Pinaster* (*P. heldreichii,* Bosnian pine, up to 73.2%, 55.5% on average).[15]

n-Alkane profile of spring needles of *P. halepensis* is C_{27}, C_{29}, and C_{25} while autumn needles are abundant in C_{29}, C_{27}, and C_{31} (Figure 1). On the species level, *n*-alkanes ranged from C_{18} to C_{35} with the most dominant C_{27} and C_{29} (32.4% and 25.8%, respectively) (Figure 1, Table 1). In *P. heldreichii*, this range is C_{18} to C_{33}, with the most dominant C_{23}.[15]

The carbon preference index (CPI_{total}) was much higher in spring than in autumn (Table 1). Mean values of CPI_{total} ranged from 3.2 to 5.4 (3.4 on average) (Table 1), while in *P. heldreichii*, it ranged from 0.8 to 3.1 (1.6 on average). Almost all CPIs (from Table 1), exhibited odd/even predominance (OEP) (because CPI >1 indicates OEP, CPI <1 denotes OEP).[26]

The average chain length (ACL_{total}) was much higher in autumn than in spring needles (Table 1). Mean ACL_{total} ranged from 14.0 to 18.0 (17.2 on average). Long-chain *n*-alkanes strongly dominated (95.1%). In *P. heldreichii*, it ranged

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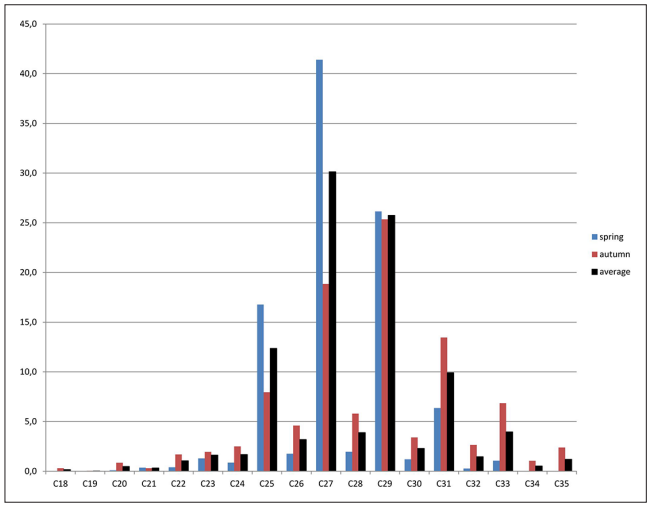

Figure 1. Profile of *n*-alkanes (in percentage) of *Pinus halepensis* needle waxes.

from 20.9 to 26.5 (24.4 on average) and long-chain *n*-alkanes did not strongly dominate (mid-chain: 37.9%, long-chain: 49.6%).[15]

Experimental

Plant Material

Twigs with needles from the lowest third of the full tree crown were collected in spring and autumn 2015 from Croatia, Island Korčula. The collected twigs were stored at –20°C prior to further needle analyses.

Extraction of Needle Wax for the Investigation of the Nonacosan-10-ol Content

A concentrated sample of epicuticular wax was collected from each tree by immersing 3 g of needles in 10 mL of *n*-hexane (high-performance liquid chromatography grade; Merck, Darmstadt) for 45 seconds. The samples were then dried under vacuum at 60°C, and aliquots of 1 mL of these samples were used to determine the nonacosan-10-ol content by gas chromatography (GC)–mass spectrometry (MS) analysis (Figure 2).

Extraction of Needle Wax for the Investigation of the n-*Alkanes*

The concentrated extracts, obtained as described above, were chromatographed on small-scale columns using a Pasteur pipette filled with silica gel 60 (SiO_2, 0.2-0.5 mm; Merck) previously activated at –20°C.[23] The wax samples were obtained by elution with 5 mL of hexane and stored at –20°C until further analysis.

Table 1. Variability of the Most Abundant *n*-Alkanes, CPIs, ACLs, and Relative Proportions of Short, Mid, and Long-Chain *n*-Alkanes in the Needle Wax of *Pinus halepensis*

	C_{range}	C_{max} [a]	CPI_{total} [b]	CPI_{25-33} [c]	CPI_{20-36} [d]	CPI_{15-21} [e]	CPI_{25-31} [f]	ACL_{total} [g]	ACL_{23-35} [h]	***n*-C_{18-20}** [i] Short-chain	***n*-C_{21-24}** [i] Mid-chain	***n*-C_{25-35}** [i] Long-chain
										In % of total *n*-alkanes (C_{18-35})		
Range	**18-35**	27, 29	3.2–5.4	1.1–1.2	1.0	0.0-2.2	1.1-1.2	14.0-18.0	28.4-36.2	0.0-1.9	1.5-8.5	89.6-98.5
Mean		**29**	**3.4**	1.1	1.0	1.3	1.1	**17.2**	32.6	**0.6**	**4.3**	**95.1**

CPI, carbon preference index; ACL, average chain length.
[a] C_{max}: the 3-4 most abundant *n*-alkanes are given in the row "Range" and the most abundant among them in the row "Mean".
[b] CPI_{total} = Σodd C_n/Σeven C_n; C_n is the concentration of alkane containing *n* C-atoms.[19]
[c] CPI_{25-33} = [Σ(C_{25}–C_{33})odd/Σ(C_{24}–C_{32})even + Σ(C_{25} –C_{33})odd/Σ(C_{26}–C_{34})even]/2.[20]
[d] CPI_{20-36} =[Σ(C_{20}–C_{36})odd/ Σ(C_{19}–C_{35})even+ Σ(C_{20}–C_{36})odd/ Σ(C_{21}–C_{37})even]/2.[20]
[e] CPI_{15-21} = [Σ(C_{15}–C_{21})odd/ Σ(C_{14} –C_{20})even+ Σ(C_{15}–C_{21})odd/ Σ(C_{16}–C_{22})even]/2.[20]
[f] CPI_{25-31} =[Σ(C_{25}–C_{31})odd/ Σ(C_{24}–C_{30})even+ Σ(C_{25}–C_{31})odd/ Σ(C_{26}–C_{32})even]/2.[20]
[g] ACL_{total}=(ΣC_n × *n*)/ΣC_n.[21]
[h] ACL_{23-35}= (23×C_{23} + 25×C_{25} + 27×C_{27} + 29×C_{29} + 31×C_{31} + 33×C_{33} + 35×C_{35})/(C_{23} + C_{25} + C_{27} + C_{29} + C_{31} + C_{33} + C_{35}).
[i] *n*-C_{18-20}, *n*-C_{21-24}, and *n*-C_{25-35}: relative proportions (%) of short, middle, and long-chain *n*-alkanes, respectively, calculated according to Mazurek and Simoneit[22] and expressed as percentage of the total *n*-alkanes (C_{18-35}).

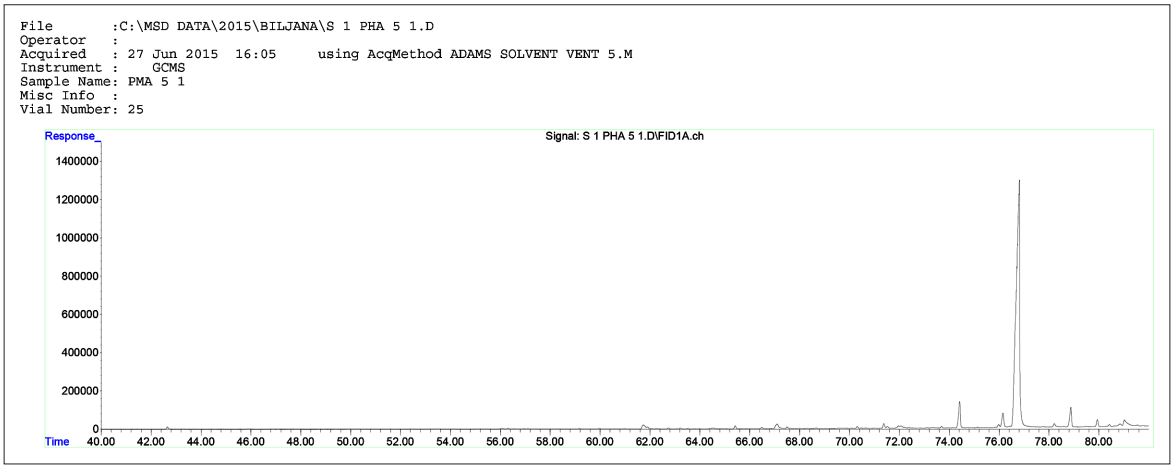

Figure 2. Nonacosan-10-ol of needles of *Pinus halepensis*.

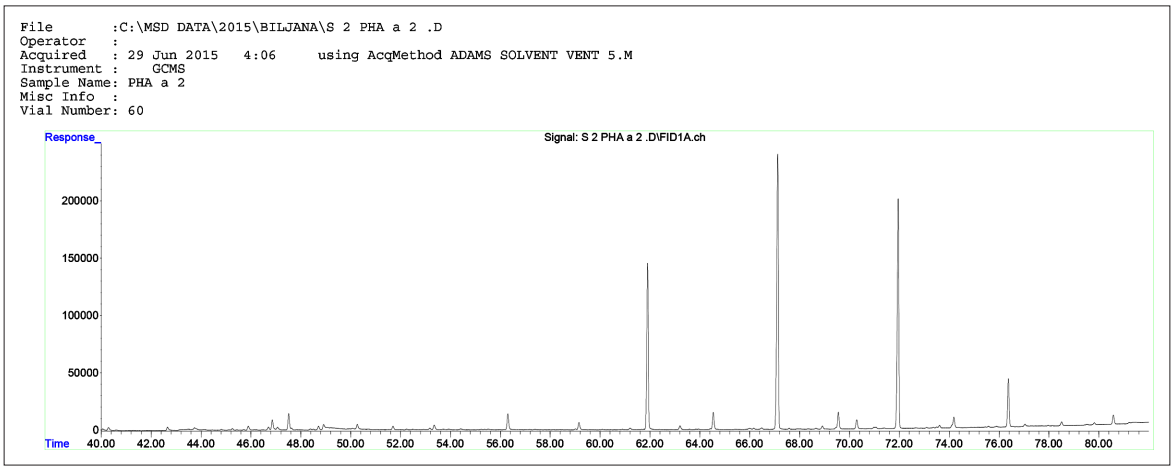

Figure 3. *n*-Alkanes of needles of *Pinus halepensis*.

GC and GC-MS Analyses of Needle Wax

GC and GC-MS analyses were performed using an Agilent 7890A GC equipped with an inert 5975C XL EI/CI mass selective detector and flame ionization detector (FID) connected by capillary flow technology 2-way splitter with make-up. An HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The GC oven temperature was programmed from 60°C to 315°C at a rate of 3°C/min and held for 15 minutes. Helium was used as the carrier gas at 16.255 psi (constant pressure mode). An auto-injection system (Agilent 7683B Series Injector) was employed to inject 1 μL of the sample. The sample was analyzed in the splitless mode. The injector and the detector temperature was 300°C. MS data were acquired in the EI mode with scan range 30-550 m/z, source temperature 230°C, and quadrupole temperature 150°C; the solvent delay was 3 minutes (Figure 3).

Identification of Needle Wax Components

The components were identified based on their retention indices and comparison with reference spectra (Wiley and NIST databases) as well as by the retention time locking (RTL) method and the RTL Adams database. The retention indices were experimentally determined using the standard method of Van Den Dool and Kratz[27] involving retention times of *n*-alkanes, injected after the sample under the same chromatographic conditions. The relative abundance of the *n*-alkanes was calculated from the signal intensities of the homologs in the GC-FID traces.

Declaration of Conflicting Interests

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