

SUPPLEMENTARY MATERIAL

Chemical compositions of essential oils of five introduced conifers in Corsica

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Abstract: The aim of this survey was to determine the chemical composition of essential oils (EO) of five conifers acclimated in Corsica by GC(RI), GC-MS and ¹³C NMR. *L. decidua* needle and wood EOs contained as majors components: α - and β -pinenes, germacrene D (needles) and bornyl acetate (wood). The EOs of needles, wood and cones of *P. menziesii* were characterized by β - and α -pinenes, terpinen-4-ol, sabinene, terpinolene (needles and wood), Δ -3-carene (wood) and limonene (cones). Needles and wood EOs of *P. ponderosa* contained as major components: β - and α -pinenes, Δ -3-carene (wood) and estragole (needles). *S. giganteum* EOs of foliage and wood were rather similar and dominated by α -pinene, and safrole. The EOs of leaf, wood and cones from *C. japonica* were very similar, and exhibited α -pinene, sabinene, β -elemol and kaurene as major constituents. It appeared that EO compositions of some species were different from reported literature data.

Keywords: *Larix decidua*, *Pseudotsuga menziesii*, *Pinus ponderosa*, *Sequoiadendron giganteum*, *Cryptomeria japonica*, essential oils.

Experimental

Plant material, isolation of essential oil

The essential oil samples have been isolated by hydro distillation using a Clevenger-type apparatus from different parts of individual tree (500 g; yields given in table 1) harvested on the experimental forest plot, in which each planted tree is individually numbered, of l'Ospedale – southeast of the Corsica (*P. menziesii*, *P. ponderosa*, *S. giganteum*, *C. japonica*), and the managed natural forests of Vizzavona– center of Corsica (*P. menziesii*) and Verde - center of Corsica (*L. decidua*) (Figure S1). Plant material was identified by Ms J. Gross, G. Paolacci and J.M. Morin foresters from the ONF (National Forestry Office) in charge of the sampled forests. Voucher specimens have been deposited at the Conservatoire Botanique National de Corse, (Corte, France), under accession numbers: GG2770 (*P. menziesii*), GG2772 (*P. ponderosa*), GG2773 (*S. giganteum*), GG2774 (*C. japonica*) for experimental forest and GG2771 (*P. menziesii*, Vizzavona), GG2775 (*L. decidua*, Verde), for managed natural forests.

Gas Chromatography

Analyses were performed on a Clarus 500 PerkinElmer (PerkinElmer, Courtaboeuf, France) system equipped with a FID and two fused-silica capillary columns (50m x 0.22 mm, film thickness 0.25 mm), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min; injector temperature: 250 °C; temperature detector: 250 °C; carrier gas: helium (0.8 mL/min); split: 1/60; injected volume: 0.5 µL (50 µL in 350 µL of CHCl₃). The relative amounts of the oil constituents were expressed as percentages obtained by peak-area normalisation, without using correcting factors. Retention indices (RI) were determined relative to the retention times of a series of n-alkanes with linear interpolation (Target Compounds software from PerkinElmer).

Gas Chromatography-Mass Spectrometry in Electron Impact Mode

The essential oil sample was analyzed with a Perkin-Elmer TurboMass detector (quadrupole), directly coupled to a Perkin-Elmer Autosystem XL, equipped with a fused-silica capillary column (50 m x 0.22 mm i.d., film thickness 0.25 µm), BP-1 (polydimethylsiloxane). Carrier gas, helium at 0.8 mL/min; split: 1:74; injection volume: 0.5 µL; injector temperature: 250°C; oven temperature programmed from 60°C to 220°C at 2°C/min and then held isothermal at 220 °C for 20 min. Ion source temperature: 250°C; energy ionisation: 70 eV; electron ionisation mass spectra were acquired over the mass range 35-350 Da.

Nuclear Magnetic Resonance

All NMR spectra were recorded on a Bruker AVANCE 400 (100.623 MHz for ^{13}C) equipped with a 5-mm probe, in deuterated chloroform (CDCl_3), with all shifts referred to internal tetramethylsilane (TMS). The ^1H NMR spectra were recorded with the following parameters, pulse width (PW), 4.3 ms; acquisition time, 2.6 s for 32 K data table with a spectral width (SW) of 6000 Hz (15 ppm). ^{13}C NMR spectra were recorded with the following parameters: pulse width (PW), 4 μs (flip angle 45°); acquisition time, 2.7 s for 128 K data table with a spectral width of 24000 Hz (240 ppm); total repetition time, 2.8 s; CPD (composite pulse decoupling) mode decoupling; digital resolution, 0.183 Hz/point.

Identification and quantification of individual components

Identification of individual components was based: i) on comparison of their GC retention indices (RI) on polar and apolar columns, with those of authentic compounds (König et al. 2001) ii) on computer search using digital libraries of mass spectral data and comparison with published data (McLafferty & Stauffer 1994, König et al. 2001, Adams 2007) iii) on comparison of the signals in the ^{13}C NMR spectra of the mixtures with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (Tomi and Casanova 2006, Bighelli and Casanova 2009).

Statistical analysis

The principal component analysis (PCA) and hierarchical clustering analysis (HCA; *Ward's method*) (Figure S2) were performed with PAST (3.02a), (Hammer et al. 2001).

References

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Figure S1. Locations of harvest of coniferous.

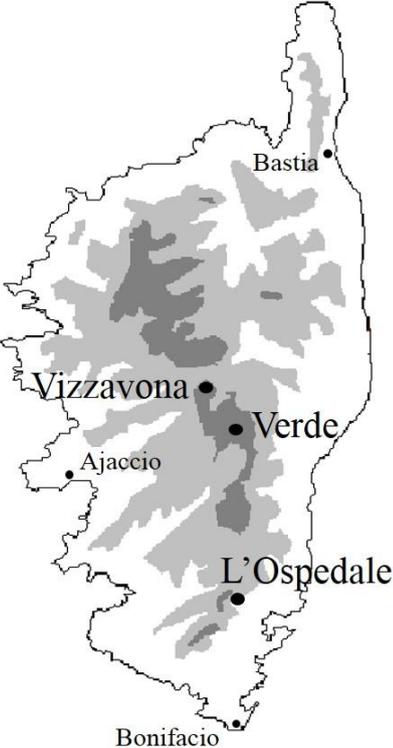
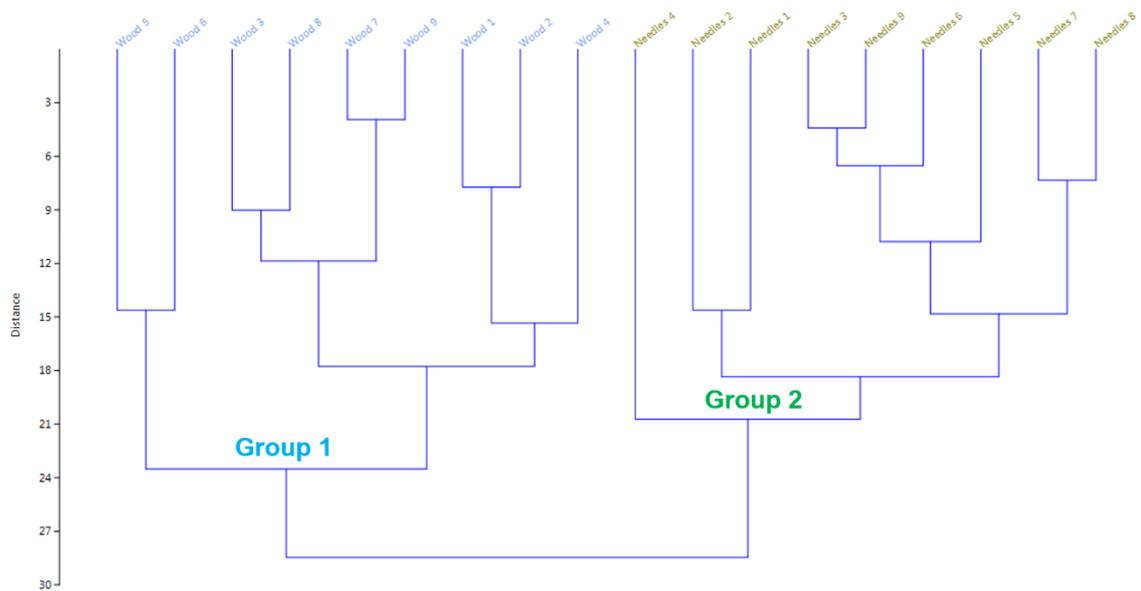
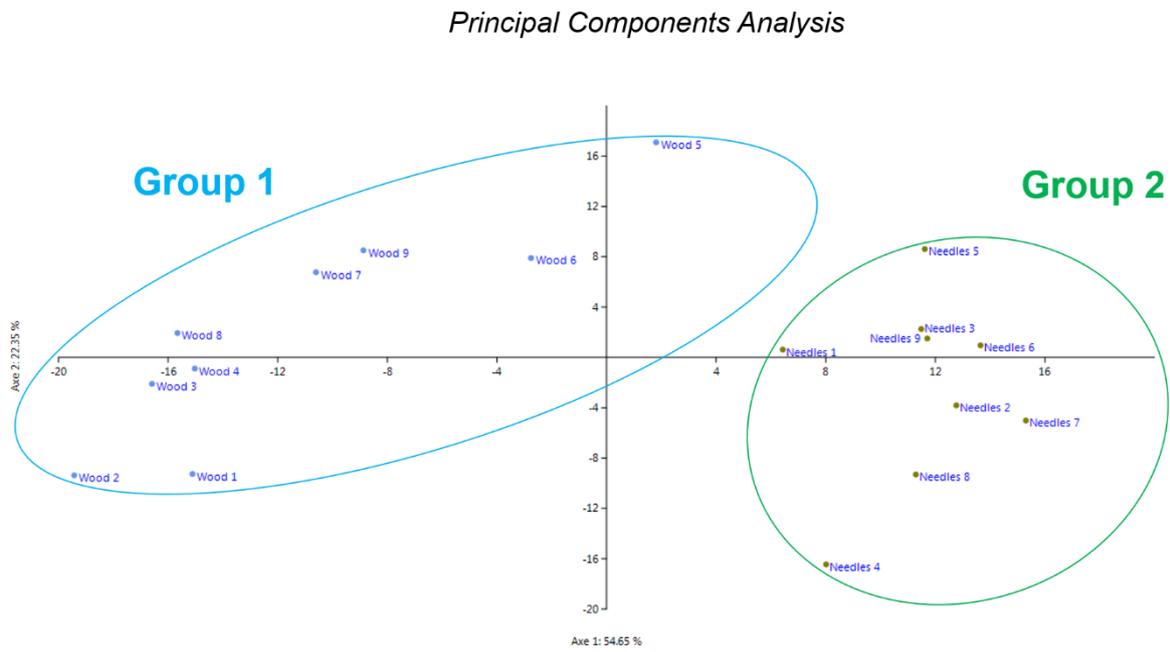


Figure S2. Statistical analysis of the nine samples (needles and wood) of *Larix decidua*.



Hierarchical Clustering Analysis