Supplementary Material

Vicariance between *Cercis siliquastrum* L. and *Ceratonia siliqua* L. unveiled by the physical-chemical properties of the leaves’ epicuticular waxes

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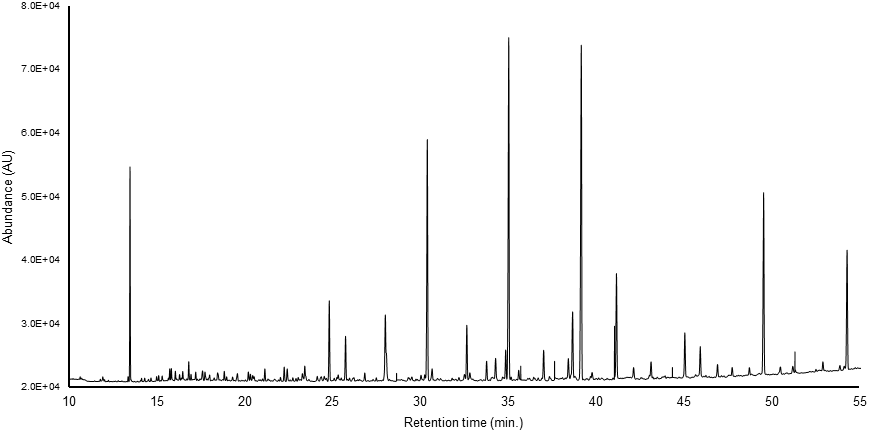
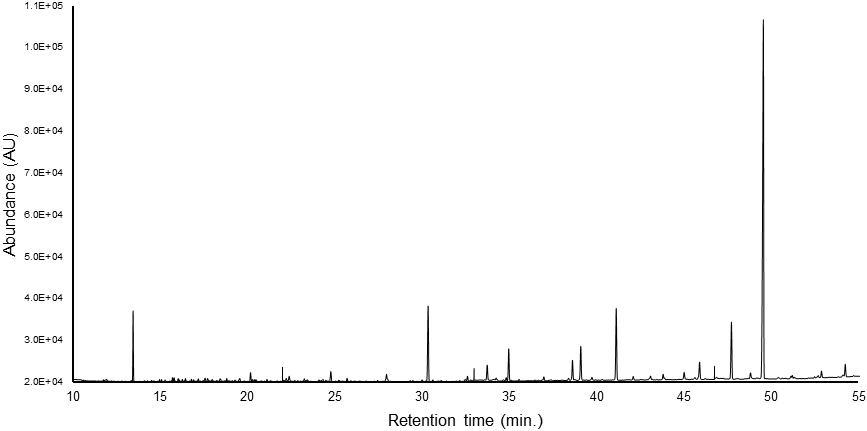
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**Supplementary Table 1.** Relative amounts (%, mean + standard deviation) of the most abundant compounds in the cuticular waxes of the *C. siliquastrum* leaf.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **Formula** | **chain length**  **Cn** | **Log Kowa** | **abaxial** | |  | **adaxial** | |
| **epicuticular** | **intracuticular** |  | **epicuticular** | **intracuticular** |
| 1-octadecanol | CH3(CH2)n-1OH | C18 | 7.7 | 1.9 ± 0.5 | 2.6 ± 0.8 |  | 4.9 ± 3.0 | 5.8 ± 5.0 |
| 1-eicosanol | C20 | 8.7 | 0.8 ± 0.7 | 3.4 ± 0.8 |  | 4.5 ± 2.3 | 6.8 ± 5.0 |
| 1-octacosanol | C28 | 12.6 | 3.8 ± 0.6 | 3.3 ± 0.6 |  | 4.6 ± 1.3 | 1.8 ± 1.8 |
| 1-triacontanol | C30 | 13.6 | 57.5 ± 6.4 | 54.2 ± 3.0 |  | 18.7 ± 5.4 | 7.4 ± 5.8 |
| **Total alcohols** | |  |  | **65.0 ± 6.0 A** | **65.64 ± 2.5 A** |  | **34.6 ± 8.1 B** | **26.1 ± 14.3B** |
| n-docosane | CH3(CH2)n-2CH3 | C22 | 11.1 | 1.5 ± 2.6 | 2.4 ± 1.5 |  | 5.9 ± 3.4 | 8.8 ± 4.2 |
| n-tricosane | C23 | 11.6 | *n.d.* | *n.d.* |  | 7.1 ± 5.9 | 11.0 ± 9.7 |
| n-pentacosane | C25 | 12.6 | 1.9 ± 2.5 | 1.8 ± 0.7 |  | 8.5 ± 3.3 | 9.8 ± 7.6 |
| n-nonacosane | C29 | 14.6 | 11.9 ± 2.4 | 6.8 ± 0.6 |  | 14.0 ± 5.7 | 5.0 ± 3.2 |
| **Total alkanes** | |  |  | **20.1 ± 6.4 A** | **13.64 ± 3.6 A** |  | **57.4 ± 10.2 B** | **58.7 ± 18.2 B** |
| 1-monopalmitin | CH3(CH2)n-5C(=O)OCH2CH(OH)CH2OH - | C19 | 5.6 | *n.d.* | *n.d.* |  | 1.9 ± 1.2 | 2.5 ± 2.1 |
| 1-monostearin | C21 | 6.6 | 4.1 ± 1.5 | 9.5 ± 5.1 |  | 4.9 ± 2.9 | 7.0 ± 3.4 |
| **Total esters** | |  |  | **4.1 ± 1.5 A** | **9.5 ± 5.1 BC** |  | **6.8 ± 3.0 B** | **10.8 ± 2.4 C** |
| triacontanal | CH3(CH2)n-2C(=O)H | C30 | 13.6 | 10.8 ± 1.6 | 9.9 ± 0.7 |  | *n.d.* | *n.d.* |
| squaleneb | (C5H8)6 | C30 | 14.1 | 0.08 ± 0.02 | 1.8 ± 0.3 |  | 1.2 ± 0.5 | 2.3 ± 0.5 |
| Different letters in a row (A, B or C) correspond to statistically significant (*p*<0.05) differences between means of major chemical classes. | | | | | | | | |
| n.d., not detected. | | | | | | | | |
| aPredicted data generated using the US Environmental Protection Agency’s EPISuite™.  b 2,6,10,15,19,23-hexametil-2,6,10,14,18,22-tetracosahexeno | | | | | | | | |

A

B



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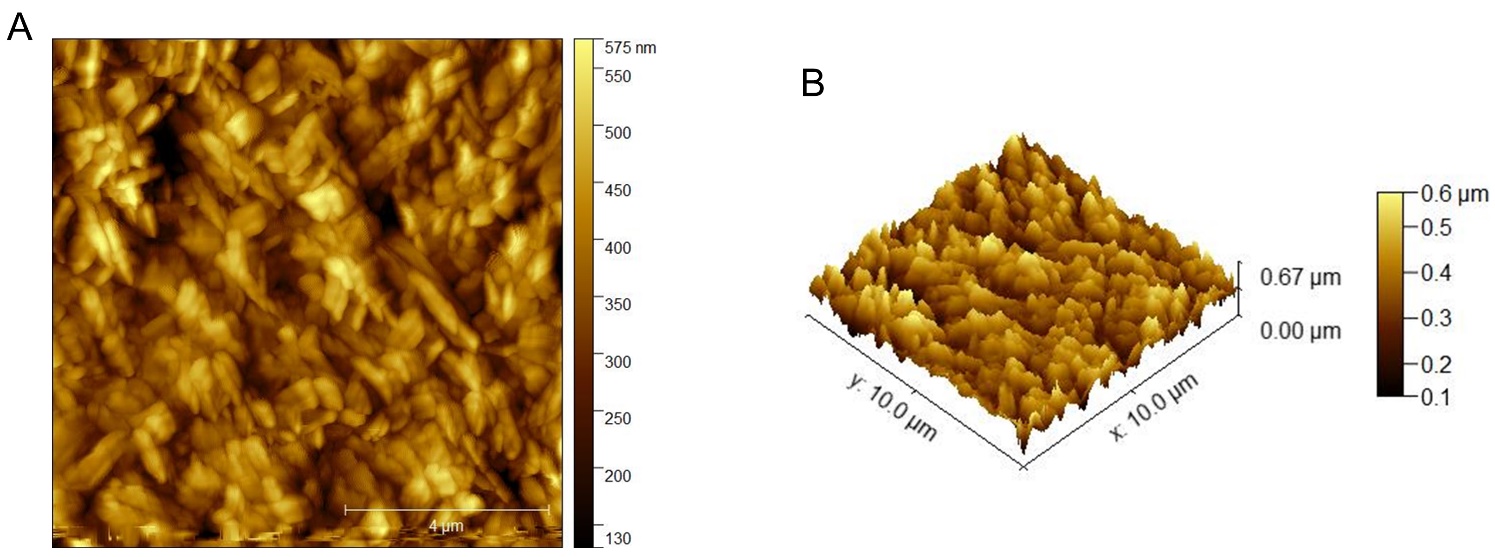
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**Supplementary Figure 1.** GC-FID Chromatograms of the epicuticular waxes of the adaxial (A) and abaxial (B) surfaces of the *C. siliquastrum* leaf: (1) hexadecane; (2) 1-octadecanol\*; (3) 1-eicosanol\*; (4) tetracosane (internal standard); (5) 1-monopalmitin\*; (6) 1-monostearin\*; (7) nonacosane; (8) 1-triacontanal; (9) 1-triacontanol\*; (10) tris(2,4-di-tert-butylphenyl) phosphate. Note: \* TMS derivative.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Theoretical**  **retention index** | **Match** | **Reversed match** |
|
| 1-Octadecanol, TMS derivative | 2152 | 941 | 941 |
| Docosane\* | 2200 | 900 | 902 |
| 1-Eicosanol\* | 2281 | 828 | 880 |
| Tricosane\* | 2300 | 870 | 871 |
| Pentacosane\* | 2500 | 900 | 900 |
| Hexacosane\* | 2600 | 935 | 940 |
| 2-Monostearin, 2TMS derivative | 2772 | 876 | 891 |
| Nonacosane\* | 2900 | 865 | 865 |
| Hentriacontane\* | 3100 | 900 | 905 |
| 1-Octacosanol, TMS derivative\* | 3138 | 934 | 918 |
| Triacontanal\* | 3251 | 824 | 864 |
| 1-Triacontanol, TMS derivative\* | 3334 | 923 | 925 |
| \*Compounds whose retention times and *m/z* spectra were compared to those of reference standards. | | | |

**Supplementary Table 2.** Chromatographic information regarding the most abundant compounds identified in *C. siliquastrum* leaf cuticular waxes.



**Supplementary Figure 2.** AFM topographical (A) 2D and (B) 3D images of a representative region of the adaxial surface of a *C. siliquastrum* leaf.

Diagram

Description automatically generated

**Supplementary Figure 3.** Schematic representation of the spectrometer setup to measure absorbance of light of the *C. siliquastrum* leaf. The sample is placed on a rotating central mount. The angle of incident light is varied by rotating the leaf sample with respect to the incoming light. The integrating sphere collects both reflected (R) and transmitted (T) light and the absorbance (A) is then calculated as 1−R−T.