Analytical Chemistry

Direct Plant Tissue Analysis and Imprint Imaging by Desorption Electrospray Ionization Mass Spectrometry

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Supporting Information

Materials. HPLC grade methanol and acetonitrile were from Acros Organics (Geel, Belgium). Formic acid puriss.p.a. was from Fluka (Buchs, Switzerland).

Methods. *HPLC:* Dionex Ultimate 3000 HPLC system equipped with an autosampler and a diode array detector. Injection loop 20 µl. Data were collected and processed with Chromeleon V6.80. *Analytical:* Phenomenex HyperClone ODS 5µm 250 x 4.6mm i.d. column at 20°C protected with a Phenomenex ODS 4 mm x 3 mm i.d. precolumn was used with a flow rate 0.5 $mlmin^{-1}$. Solvent A: water with 0,2% formic acid (v/v), solvent B: acetonitrile; standard solvent compositions: (A/B) as function of time (0 - 50 min): 0 - 5: 90/10; 5 - 43: 90/10 to 60/40; 43 - 47: 60/40 to 40/60; 47 - 50: 40/60 to 0/100.

Spectroanalytical Data *Ov*-NCC-1. HPLC: Retention time in analytical HPLC (Rt) = 32.4 min (see main text and Figure S1). UV/Vis (from diode array detector): λmax (rel ε) = 217 (ca. 1.81), 245sh (0.84), 314 (1.00).

Spectroanalytical Data *Ov*-NCC-2. HPLC: Retention time in analytical HPLC (Rt) = 34.4 min (see main text and Figure S1). UV/Vis (from diode array detector): λmax (rel ε) = 217 (ca. 1.35), 245sh (0.85), 314 (1.00).

Supporting Figures

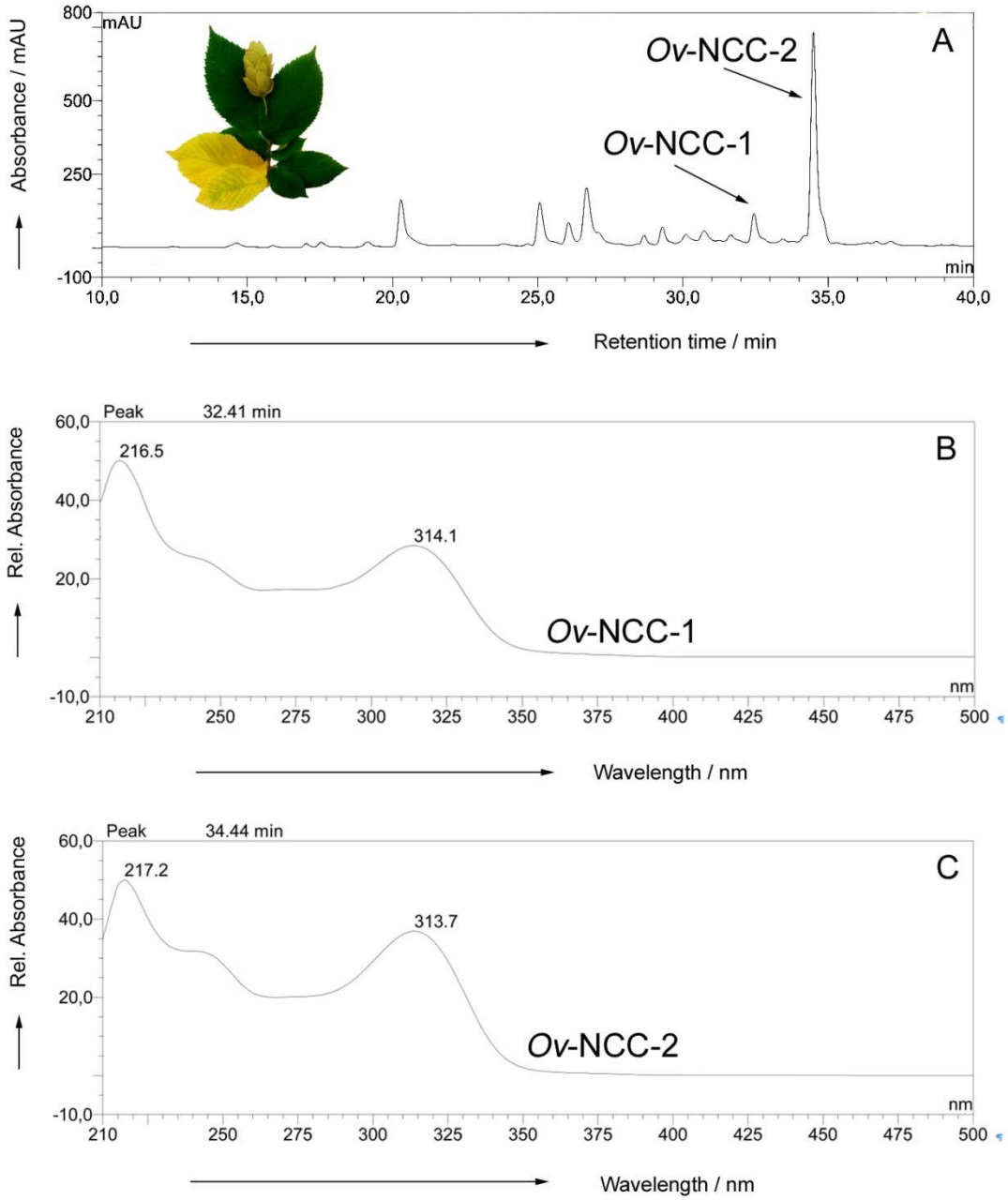

Figure S1. (A) HPLC analysis (320 nm trace) of NCCs in senescent Hophornbeam leaves (*Ostrya virginiana*) from the Purdue campus. The two NCC fractions at Rt = 32.4 min and Rt = 34.4 min are marked. Insert: Green and yellow (senescent) leaves as well as fruit from Hophornbeam tree. (B) UV spectrum of *Ov*-NCC-1 fraction at Rt = 32.4 min from diode array detector. (C) UV spectrum of *Ov*-NCC-2 at Rt = 34.4 min from diode array detector.

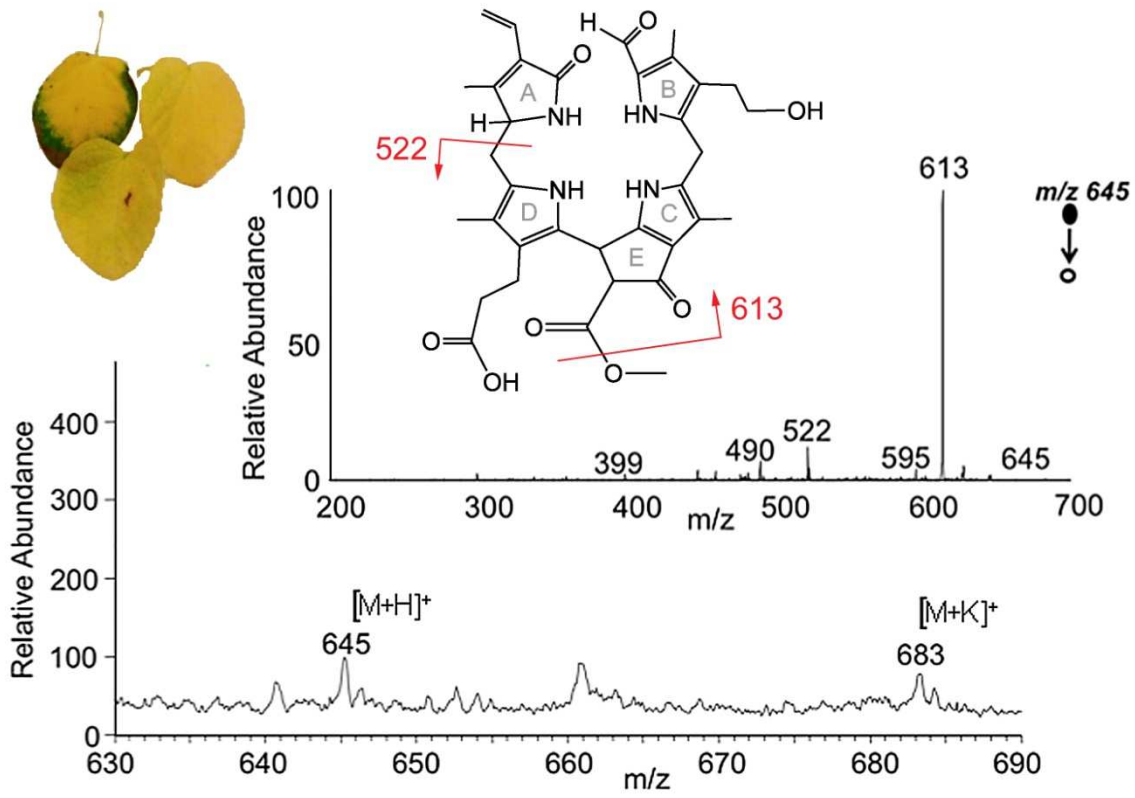

Figure S2. (A) Positive-ion mode DESI-MS spectra of a Katsura tree (*Cercidiphyllum japonicum*) leaf. Spray solvent was methanol/water (80:20) at a flow rate of 13µl/min. Protonated and potassiated molecules were detected at m/z 645 and *m/z* 683. (B) DESI-MS/MS spectrum of the isolated protonated molecule at *m/z* 645. (C) Structure of the chlorophyll catabolite Cj-NCC-1 [1] corresponding to the ion at m/z 645. Characteristic framentations due to the losses of methanol (fragment at *m/z* 613) and ring A (fragment at *m/z* 522) are marked.

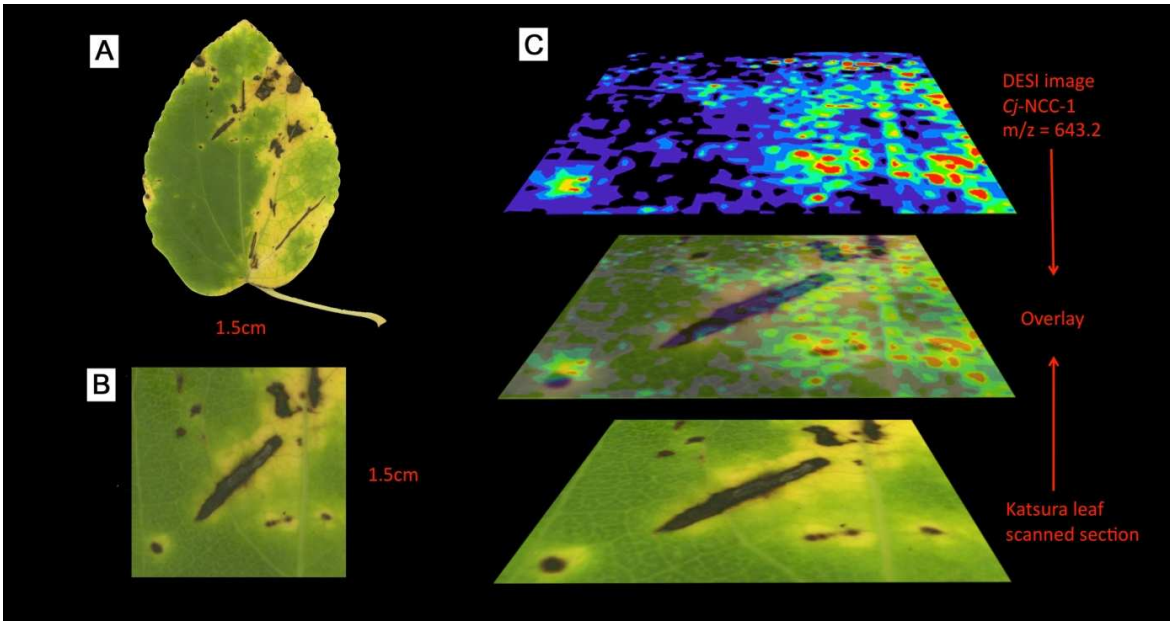

Figure S3. Negative-ion mode DESI imaging of a senescent Katsura tree leaf imprint on porous PTFE substrate. Spray solvent was 1% conc. Aqueous ammonia in methanol at a flow rate of 1.5 µl/min. Imaging parameters: 1.36 sec per scan; 61 scans per horizontal row; 60 rows; pixel size was 250 x 250 µm; total acquisition time was 83 min. (A) Senescent Katsura tree (*Cercidiphyllum japonicum*) leaf. (B) 15 x 15 mm section of the photographic image depicted in panel A. (C) Ion image of the chlorophyll catabolite *Cj*-NCC-1 [1] at *m*/*z* 643.2 (top), photographic image of the analyzed leaf section (bottom) and their overlay (center).

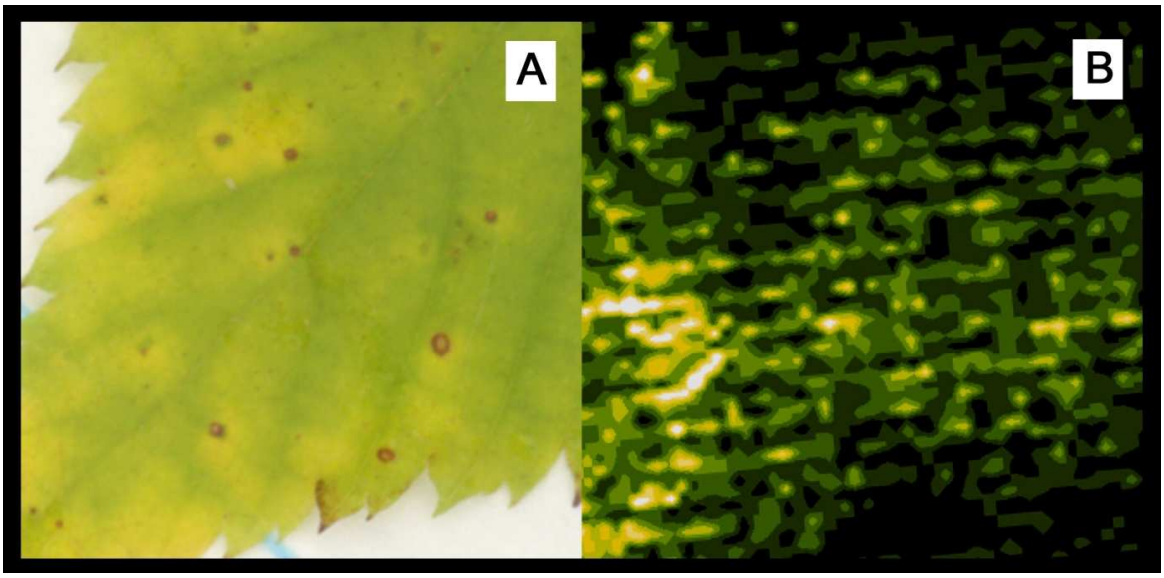

Figure S4. Negative-ion mode DESI imaging of a senescent Hophornbeam leaf imprint on porous PTFE substrate. Spray solvent was 1% conc. aqueous ammonia in methanol at a flow rate of 1.5

µl/min. Imaging parameters: 1.36 sec per scan; 61 scans per horizontal row; 60 rows; pixel size was 250 x 250 µm; total acquisition time was 83 min. (A) 15 x 15 mm section of a photographic image taken from a senescent Hophornbeam tree (*Ostrya virginiana*) leaf. (B) Ion image of the most abundant chlorophyll catabolites in Hophornbeam leaves at *m/z* 677.2. The image is plotted on a color scale, which visualizes relative ion intensities from 0 (black) to 50 (green) to 100 (white).

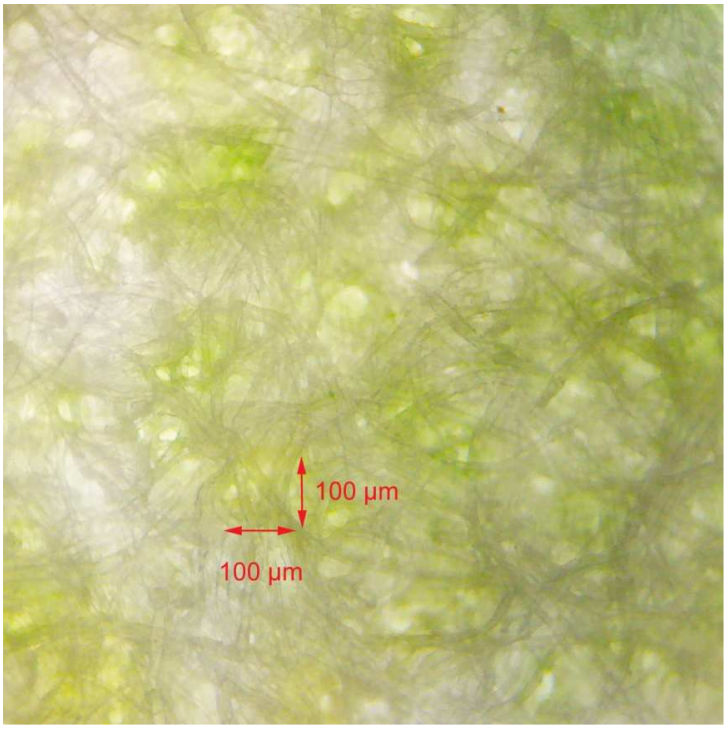

Figure S5. 1 mm^2 section of a light microscopic image taken from a *Cercidiphyllum japonicum* leaf imprint on filter paper. Leaf imprinting produces well-defined hotspots at diameters of around 100-150 µm on the printed material (e.g. filter paper, PTFE). 100 µm bars are highlighted in red.

Supporting References

[1] Curty, C.; Engel, N. *Phytochem.* 1996, *42*, 1531-1536.