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Supporting Information

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for

Release of Volatile Aldehydes by the Brown Algal Kelp *Laminaria digitata* in Response to Both Biotic and Abiotic Stresses

Sophie Goulitquer, Andrés Ritter, François Thomas, Cyrille Ferec,
Jean-Pierre Salaün, and Philippe Potin*

Additionnal methods

Chemicals: 4-hydroxy-(*E*)-2-hexenal (4-HHE); 4-hydroxy-(*E*)-2-nonenal-d3 (4-HNE-d3) and 4-oxo-(*E*)-2-nonenal-d3 were purchased from Cayman Chemical (Spi-Bio, Montigny Le Bretonneux, France); 9-oxo-nonanoic acid was from Larodan Fine Chemicals (Malmö, Sweden). Silylating reagent Sylon BFT (bis(trimethylsilyl)trifluoroacetamide / trimethyl-chlorosilane, 99:1) was from Supelco (Bellefonte, PA, USA). All other chemicals and solvents were from Merck and Sigma. MDA was synthesized as described in Vollenweider and co-workers [38].

Elicitors : Copper treatment medium consisted in filtered seawater with addition of the nominal concentration of 100 $\mu g L^{-1}$ copper as $CuCl_2$ from Merck. Each treatment included three replicates.

Alginate oligosaccharides with a polymerization degree ranging from 15 to 25[40] were prepared in the laboratory according to Haug and co-workers[41] by using sodium alginate from *Laminaria hyperborea* stipes (provided by B. Larsen, Trondheim University, Norway) and yielding three categories of alginate oligosaccharides (GG, MM, and MG blocks). In elicitation experiments, GG were added to the seawater at final concentrations of 150 $\mu g\, mL^{-1}$ (3 mg). Incubation period was 1 h. At the end of the experiment thalli were frozen in liquid nitrogen and kept at -80 °C until further analyses.

Inhibitors : Several compounds were screened for their potential to inhibit enzyme activity possibly involved in the synthesis of aldehydes: quinacrine (20 μM; target,

flavin-dependent redox enzymes, in particular oxidases), and chlorpromazine-HCl (20 µM; target, phospholipase A) from stock solutions dissolved in ethanol, salicylhydroxamic acid (SHAM) at 1 mM final concentration (LOX, COX, peroxidase, CYP P450 activities). Plantlets were preincubated in seawater added with inhibitors dissolved in ethanol or ethanol alone during 30 min. Incubation medium was completely removed and algae rinsed with fresh filtered sea water. 20 mL of newly fresh filtered seawater was added in the flask prior to oliguluronates addition.

Algae treatment by aldehydes: Hexanal, nonanal, (*E*)-2-nonenal, 4-HHE, 4-HNE, (*E*,*E*)-2,4-nonadienal, (*E*,*E*)-2,4-decadienal were individually tested at both 1 µg.mL^{-1} and 100 ng mL^{-1} by addition into the surrounding seawater. After 1 h, the seawater was extracted as described in “seawater analyses” for aldehydes analyses. 20 mL of newly fresh filtered seawater was added in the flask. After 23 h at room temperature (24 h incubation), thalli were frozen in liquid nitrogen and kept at -80 ℃ until oxylipin extraction.

Oxylipin extraction and GC-MS-EI analysis : Oxylipins were extracted in *L. digitata* tissue according to ref. [43]. In each sample, 250 ng of 12-OH-lauric acid was added as an internal standard. The residue was dissolved in 100 µL of hexane.

Methyl esters were prepared by treatment with an excess of ethereal diazomethane. Silylation was achieved by treatment with a mixture of BSTFA (*N,N*-bistrimethylsilyltrifluoroacetamide) / TMCS (1% trimethyl-chlorosilane) for 1 h at 60 ℃, in order to obtain trimethylsilyl derivatives for compounds containing hydroxyl group.

GC-MS analyses were carried out on a HP 5890 Series II gas chromatograph equipped with a fused silica capillary column (HP-5MS 5% phenyl methyl siloxane; 30 m × 0.32 mm I.P, film thickness 0.25 µm) and combined to a quadrupole mass-selective detector (HP 5971A, Agilent Technology). Mass spectra (EI mode) were recorded at 70 eV. 2 µL were injected in the splitless mode at 60 ℃. After 5 min at 60 ℃, the oven temperature was increased to 200 ℃ at 50 ℃ min^{-1} and then linearly ramped to 280 ℃ at 2 ℃ min^{-1} that became stable for 10 min before returning to initial conditions. 13-HOTrE was identified using authentic standard and was quantified from standard curves.

Statistical analyses : Data analysis was performed using the Mann & Whitney non-parametric test with Prism 4.0 software (GraphPad, Inc., San Diego, CA, USA). Data are presented as the mean ± SD.

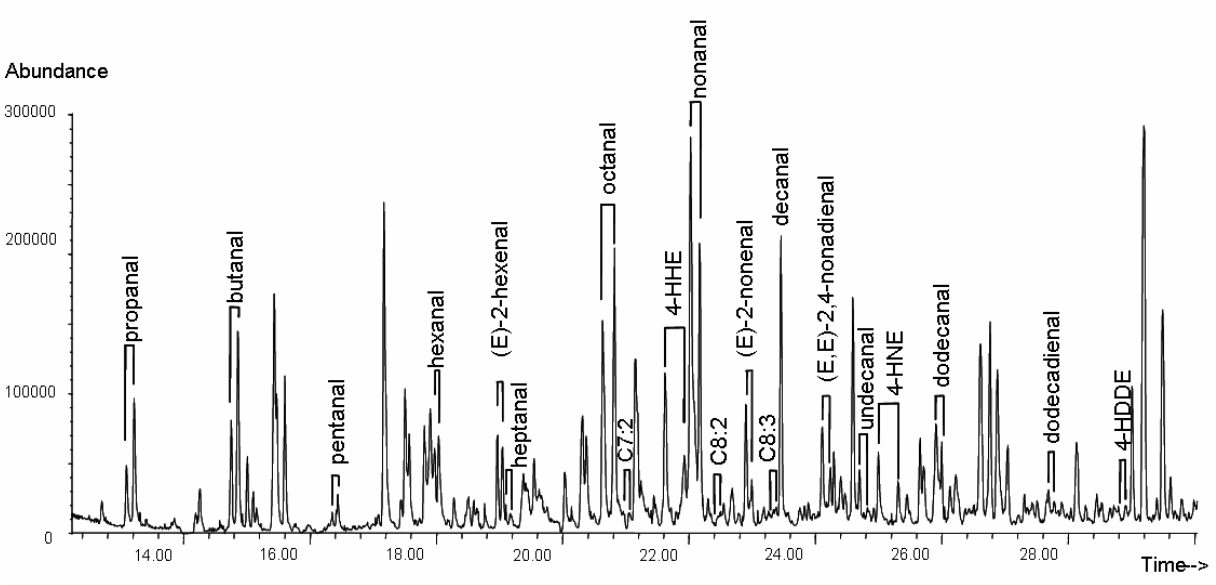


Figure S1. *m/z* 181 chromatogram obtained by GC-MS in the NICI mode for aldehydes extracted from 20 mL filtered seawater containing *L. digitata* elicited by 150 µg.mL^{-1} oligoguluronates. Aldehydes were monitored as PFB-oximes/TMS-ethers. *m/z* 181 is the corresponding ion for the fragment [CH_2-C_6F_5] loss by all aldehydes-*O*-PFB.

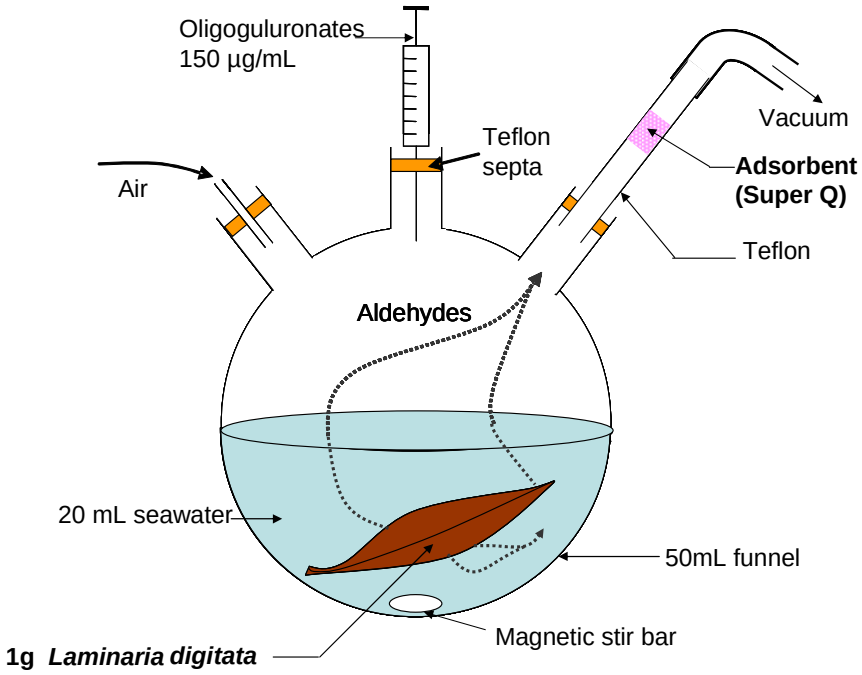
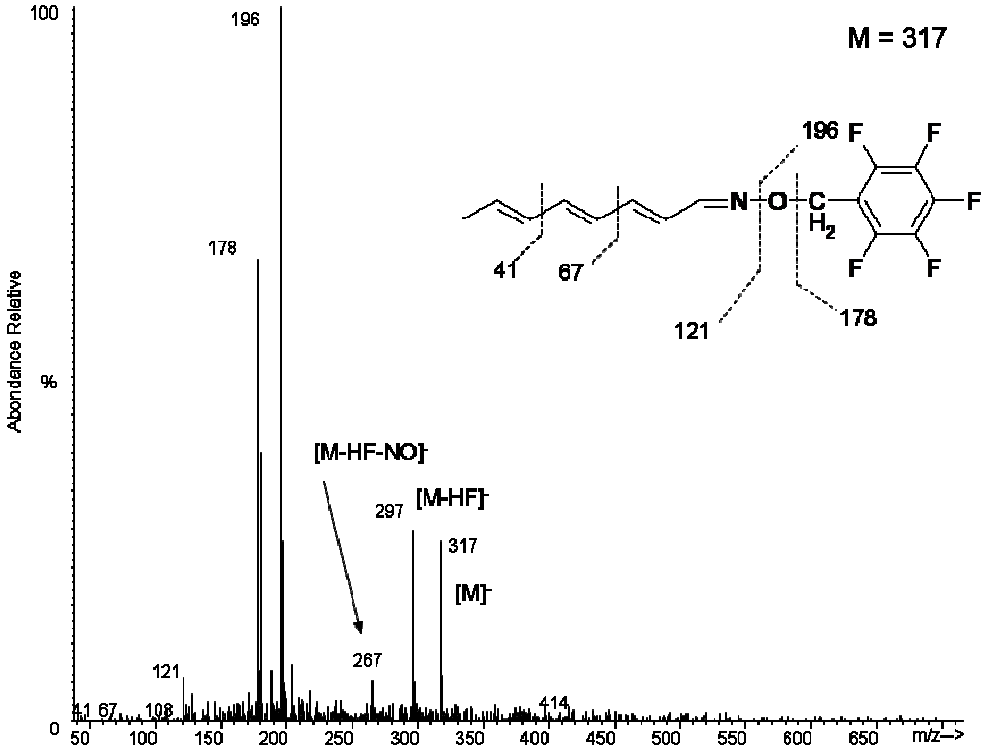


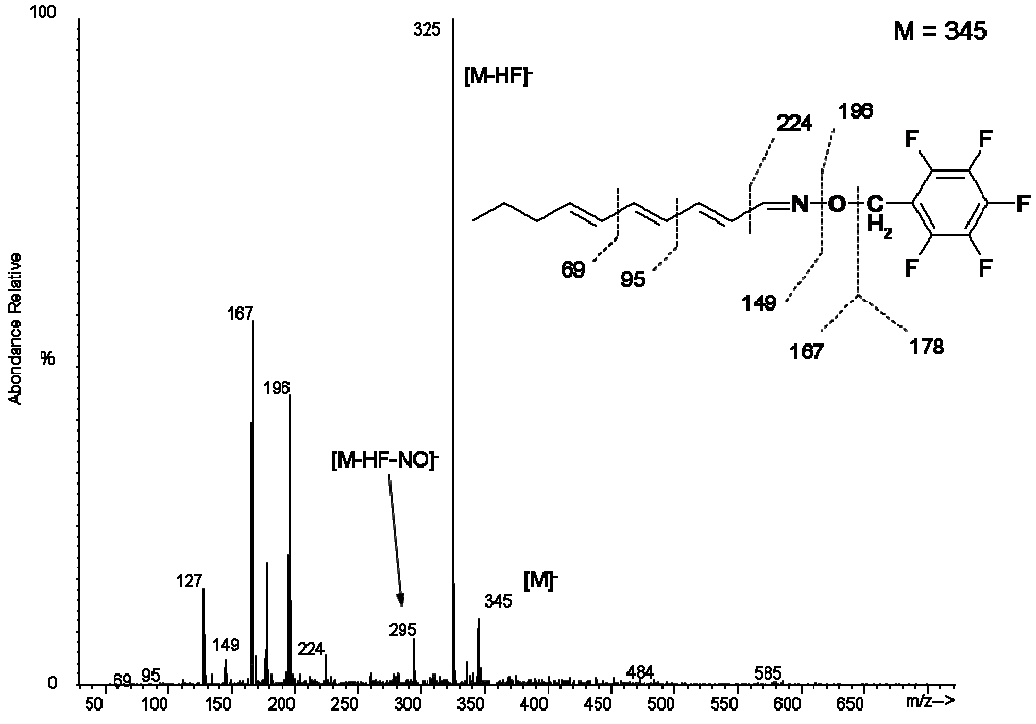
Figure S2. Trapping of volatile compounds emitted by *Laminaria digitata* on Super Q adsorbent in response to oligoguluronates (150 µg.mL^{-1}) elicitation. Both the head space and the surrounding sea water medium were submitted to PFB-oximation and TMS-etherification previous GC/MS analysis in the NCI mode.

Mass spectra of some identified compounds.

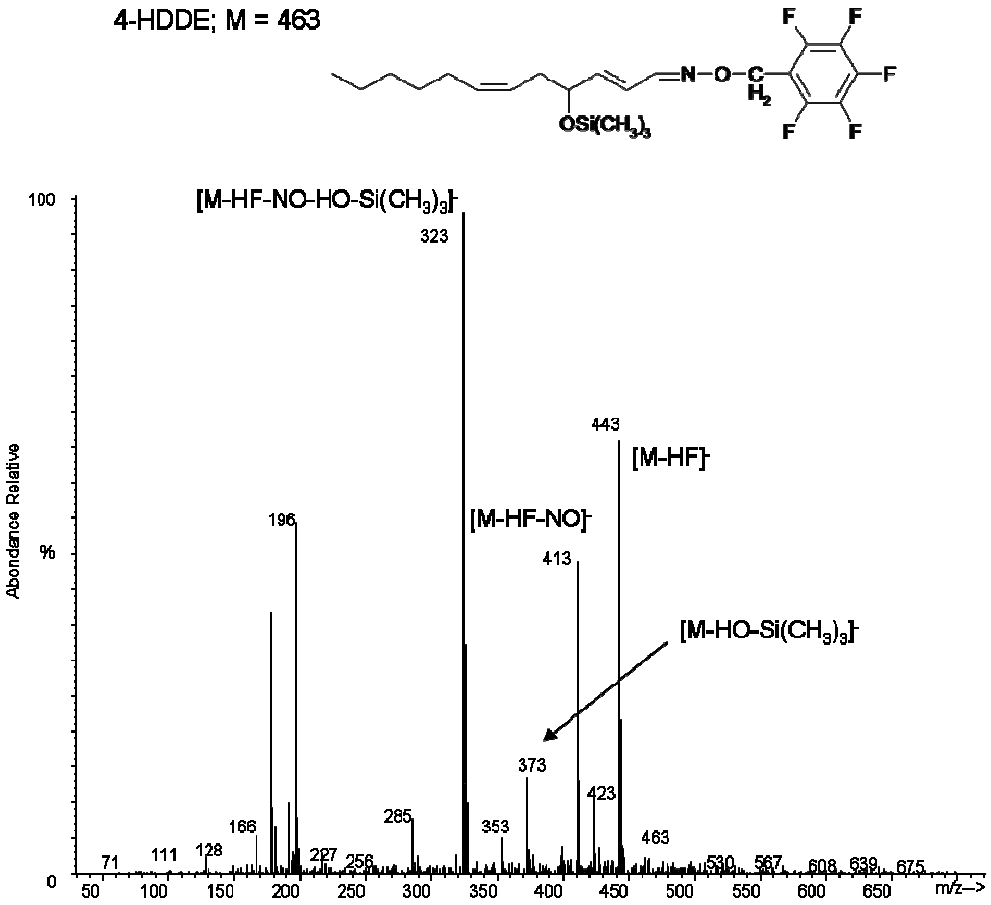
(*E,E*)-2,4,7-octatrienal-*O*-PFB (NCI mode)



(*E,E*)-2,4,7-decatrienal-*O*-PFB (NCI mode)



4-HDDE-*O*-PFB (NCI mode)



4-HDDE-*O*-PFB (EI mode)