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

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Reminiscent of the important role that volatile C6 and C9 aldehydes play in wound healing and pest resistance, and of their so-called fresh green odour in higher plants,[1] the occurrence of volatile polyunsaturated aldehydes (PUAs) has also been reported in several marine diatoms[2–5] and other planktonic algae.[5,6] These organisms seem to use PUAs such as (2*E*,4*E*)-octa-2,4,7-trienal and (2*E*,4*E*,7*Z*)-deca-2,4,7-trienal as signal molecules related to defence mechanisms[7] and as a stress alarm systems.[8] Moreover, in the brown marine alga *Laminaria angustata* the capacity to produce long- and short-chain aldehydes enzymatically has also been demonstrated.[9,10] In *L. angustata*, the precursors of these aldehydes are $n-6$ fatty acids such as linoleic and arachidonic acid, whereas in diatoms they are derived mostly from $n-3$ and $n-4$ fatty acids.[11,12] Although numerous studies had demonstrated that PUAs could be produced enzymatically by algae, these experiments were carried out by cellular sonication, which would partially wound the alga, or by incubation of algal extracts with polyunsaturated fatty acids (PUFAs). Only in a recent report was it indicated that the diatom *Skeletonema marinoi* releases PUAs at some growth phases under culture conditions.[13] Therefore, to better establish that PUAs act as signalling chemicals in the marine environment, there is a need for evidence of their release from intact algae in their natural environment.

In this context, the brown algal kelp *Laminaria digitata* provides a model with which to investigate aldehyde release in a physiological context. The objective of this work was therefore to investigate aldehyde release by *L. digitata* under stress conditions, including: 1) subjection of algae to oligoguluronate (GG) treatment, which mimics a biotic stress by inducing an oxidative burst,[14,15] 2) exposure to excess copper, which acts as an abiotic stressor[16,17] and 3) natural subjection to the effects of low tide, which increases UV exposure, salinity and temperature.[18]

We first address the hypothesis that oxidative-burst-mediated peroxidation of lipids should involve the release of PUAs and other aldehydes into the surrounding seawater medium of young *L. digitata* sporophytes upon challenge with GG. As shown by GC-MS analyses in the negative ion chemical ionization (NICI) mode with *o*-pentafluorobenzyl (*o*-PFB) oxime derivatives, the production of aldehydes extracted from both seawater and air was increased after GG treatment (Figure 1).

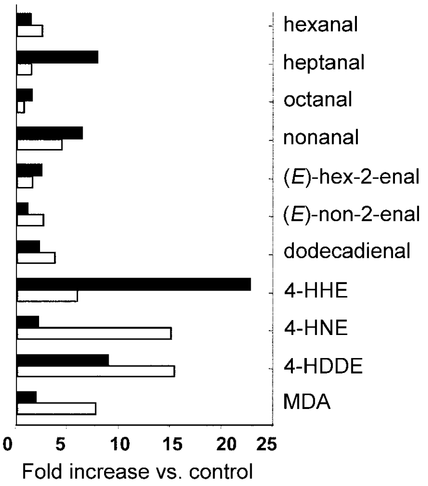

Figure 1. Comparative production of aldehydes by *L. digitata*, both in the surrounding seawater and in the headspace, after elicitation by oligoguluronates (150 μg mL^{-1}). Aldehyde levels in seawater (■) and in air (□) were determined after 1 h incubation. Results are expressed as fold increase versus control samples.

Challenging plantlets with GG elicitors indeed dramatically changed the emission (relative to control *L. digitata* sporophytes) of a bouquet of more than eleven compounds that ranged in chain length from hexanal to 4-hydroxydodeca-2,6-dienal (4-HDDE; Figure 1). The concentrations of (*E*)-hex-2-enal and (*E*)-4-hydroxyhex-2-enal (4-HHE) released by elicited *L. digitata* sporophytes were two and 24 times higher, respectively, in the water phase than in the controls. A few compounds—such as hexanal, (*E*)-4-hydroxynon-2-enal (4-HNE) and 4-HDDE—were much more abundant in the air phase, whereas (*E*)-hex-2-enal and 4-HHE were more abundant in the water phase. Particular differences were observed in the content, 4-HHE, which was 2.5 times more abundant in the aqueous

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phase than in the volatile fraction. In contrast, 4-HNE was six times more abundant in the volatile fraction.

Further quantification of the aldehyde releases in response to GG in seawater was then carried out (Figure 2). The retention times of the two peaks detected for each aldehyde-*o*-PFB in the chromatograms (Figure S1 in the Supporting Information) corresponding to the presence of *syn* and *anti* stereoisomers[19] are given in Table 1, which also provides the calibration equations for all the identified compounds. Malonaldehyde (MDA) aside, (*E*)-non-2-enal is the most abundant aldehyde (9.85 ± 2.46 ng ml^{-1} seawater). Therefore, in *L. digitata*, GG elicitation induces rapid regulation of the synthesis of aldehydes, as has also been observed for the release of iodide stores[20] and the concomitant emission of molecular iodine and volatile halocarbons that occurs during oxidative bursts.[21] This is reminiscent of the oligosaccharide-elicitor-induced modification in the emissions of aldehydes and of other volatile organic compounds (VOCs) recently reported in the plant model *Medicago trunculata*.[22] In addition, the natures of these aldehydes are likely to reflect their origin from $n-6$ fatty acids such as linoleic and arachidonic acid, which are the most abundant PUFAs in the dominant galactolipids and phospholipids of *L. digitata*.[23]

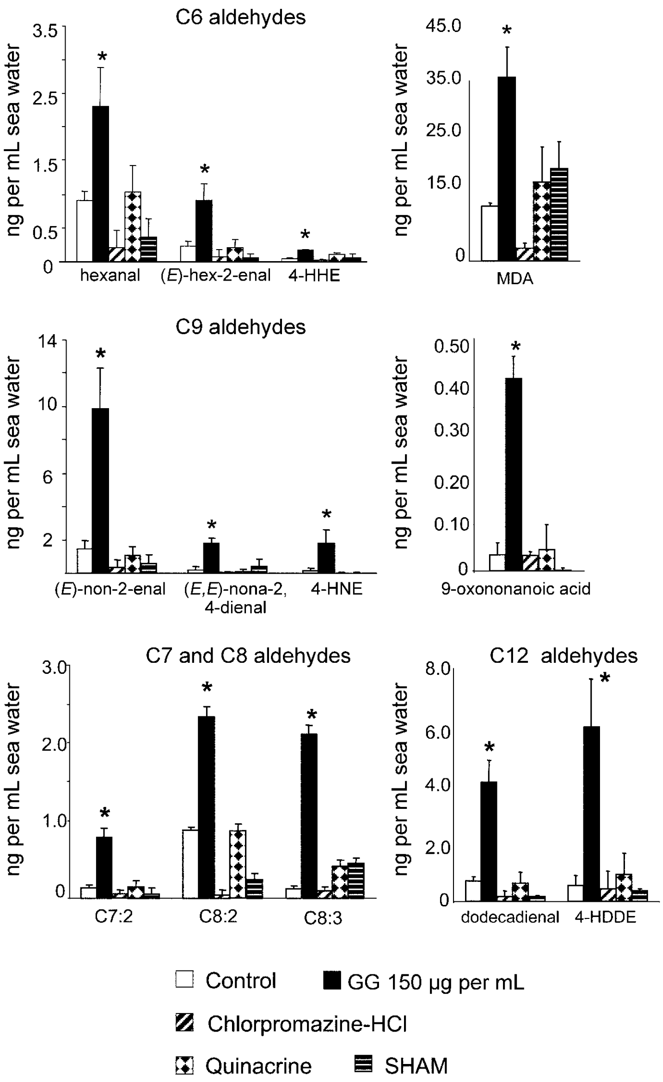

Figure 2. Aldehyde concentration and effect of inhibitors after GG treatment of *L. digitata* (1 h). The algae were either kept 1 h in seawater as control or were treated over 1 h with GG (150 µg mL^{-1}). Individual samples were preincubated with chlorpromazine•HCl (20 µM), quinacrine (20 µM) or SHAM (1 mM) and subjected to GG treatment. Values represent means of three independent replicates and bars represent SDs. * Significantly different from control ($p<0.05$).

Generation of aldehydes is known to be mediated both by enzymatic reactions and chemically through oxidative mechanisms.[24–26] We detected 9-oxononanoic acid, which is known as a byproduct of hydroperoxide lyase (HPL) action on the 9-hydroperoxides of linolenic and linoleic acids.[24] Quinacrine and chlorpromazine•HCl, which had previously been shown to interfere with the GG-induced oxidative burst in *L. digitata*,[14] also affected aldehyde production, suggesting the involvement of the oxidative burst in the lipoperoxidation process (Figure 2). However, chlorpromazine•HCl is also known to interfere with the oxidative burst through the inhibition of lipases, such as the phospholipase A2 in mammalian phagocytes, which release the arachidonic acid essential for the activation of the NAPDH oxidase.[27] Therefore, its strong inhibitory effect on the release of aldehydes is also likely to reflect the limitation of PUFA release to fuel a putative lipoxygenase (LOX) pathway. Salicylhydroxamic acid (SHAM), which does not prevent the oxidative burst in *L. digitata*,[14] repressed aldehyde formation. It is known as a non-specific inhibitor of LOXs in mammals and higher plants, but also inhibits cyclooxygenases, peroxidases and cytochrome P450 enzymes.

These preliminary inhibitor studies suggest that aldehydes are not only formed chemically but also through enzymatic pathways. Some of the observed compounds, such as (*E*)-non-2-enal, (*E*)-hex-2-enal and (*E,E*)-hepta-2,4-dienal, have already been described in *L. angustata*, in which synthesis of PUAs is believed to originate from HPL metabolism.[23] Production of (*E*)-4-hydroxyalk-2-enals—4-HHE, 4-HNE and 4-HDDE—was also strongly induced during GG elicitation. Because 4-HDDE is a biomarker of the 12-lipoxygenation of arachidonic acid in human plasma,[25] our data suggest that *L. digitata* generates aldehydes not only by enzymatic routes, but also by non-enzymatic pathways leading to MDA. Although biosynthetic pathways of brown algal aldehydes have hardly been investigated, it has been proposed that the synthesis of pheromones in brown algae involves the same steps as aldehyde synthesis.[28] Previous studies on diatoms have suggested that the biosynthesis of aldehydes[11,12] is the result of the breakdown of cellular compartments and the subsequent mixture of pre-existing enzymes with their corresponding substrates—a process that occurs after wounding of diatoms cells by sonication[2,6] or copepod grazing.[7] Similarly, this biosynthesis of aldehydes was only observed in crude algal extracts of the brown macroalga *L. angustata* or during incubation with exogenous PUFAs.[10] Our results might provide a link between a signal transduction pathway triggered by GG and the activation of lipase- and LOX-like enzymes in a physiological response.

Table 1. Characteristics and calibration parameters of the identified volatile compounds.

	t_R [min]	m/z M-*o*-PFB(-TMS)	 [M-HF]$^-$	Equation	Correlation coefficient
hexanal	17.90–17.98	295	275	$y = 5.736x + 5.5781$	0.9887
(*E*)-hex-2-enal	18.96–19.10	293	273	$y = 21.026x + 38.564$	0.9803
4-HHE	22.17–22.28	381	361	$y = 0.0878x + 0.4807$	0.9821
nonanal	21.68–22.17	337	317	$y = 6.7771x + 6.9614$	0.9710
(*E*)-non-2-enal	22.99–23.19	335	315	$y = 85.39x + 87.69$	0.9606
(*E,E*)-nona-2,4-dienal	24.08–24.21	333	313	$y = 0.8533x + 8.009$	0.9742
4-HNE	25.03–25.40	423	403	$y = 0.4299x + 0.1267$	0.9849
(*E,E*)-hepta-2,4-dienal	21.57–21.75	305	285	$y = 12.785x + 5.65$	0.9931
(*E,E*)-octa-2,4-dienal	22.46–22.51	319	299	$y = 9.298x + 4.5183$	0.9979
(*E,E*)-octa-2,4,6-trienal	23.19–23.32	317	297	cf. (*E,E*)-octa-2,4-dienal[a]	–
(*E,E*)-deca-2,4-dienal	25.02–25.23	347	327	$y = 1.3905x + 5.3616$	0.9770
(*E,E*)-deca-2,4,6-trienal	25.58–25.95	345	325	cf. (*E,E*)-deca-2,4-dienal[a]	–
(*E,E*)-dodeca-2,4-dienal	27.63–27.91	375	325	$y = 1.3353x + 12.984$	0.9629
4-HDDE	28.57–28.86	463	443	cf. 4-HNE[a]	–
MDA	25.28–25.64	462	442	$y = 43.042x + 155.68$	0.9524
9-oxononanoic acid	27.89–28.03	439	419	$y = 2.7736x + 2.1468$	0.9779

[a] Commercial standards are not available for these compounds and integration was based on the referred compound.

In marine environments, brown algae not only cope with biotic stresses, but also deal with abiotic stresses, such as heavy metal exposure and modification of physical factors of the environment (UV, salinity, temperature) associated with low and high tides. Copper stress was recently shown to induce the enzyme-mediated synthesis of fatty acid hydroperoxides and prostaglandins in *L. digitata*, as well as the chemically mediated formation of various oxylipins.[17] This study further shows that treatment of this kelp species with nominal copper (100 $\mu g\,L^{-1}$) enhanced the early release of a bouquet of more than 16 compounds including hexanal, (*E*)-non-2-enal, and 4-hydroxyalkenals such as 4-HHE and 4-HNE (Figure 3), whereas low amounts of these metabolites were observed in control sporophytes. In the water phase, 4-HHE and dodecadienal concentrations were 37 and ten times higher, respectively, than in the control. Within 60 min after addition of copper, the (*E*)-non-2-enal, MDA and hexanal concentrations produced by *L. digitata* sporophytes in the air phase were ten, four and three times higher, respectively, than in the control. It has been described that hexanal is derived from C18:2 $n-6$ and C20:4 $n-6$ fatty acids in the brown alga *L. angustata*.[23] Interestingly, no (*E*)-hex-2-enal could be detected after copper stress either in air or in water phase.

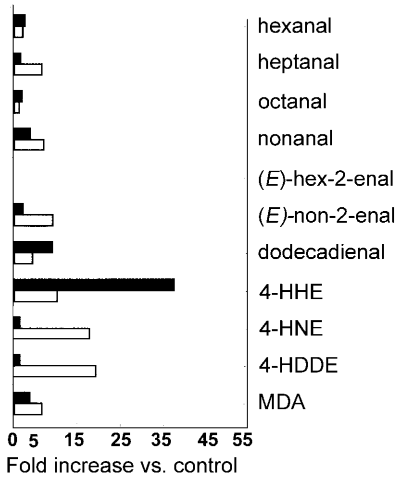

Figure 3. Comparative production of aldehydes by *L. digitata*, both in the headspace and in the surrounding seawater, after exposure to nominal copper (100 $\mu g\,L^{-1}$). Aldehyde levels in seawater (■) and in air (□) were determined after 1 h incubation. Results are expressed as fold increase versus control samples.

In addition, the occurrence of volatile emissions in the natural environment was tested under field conditions by monitoring aldehyde fingerprints in rock pools colonized by *L. digitata* subjected to environmental stress. The partial emersion of algae during spring tides involves desiccation, exposure to UV and ozone and rapid variations of temperature and salinity, leading to lipid peroxidation and oxidative stress.[18,29,30]

Results of some aldehyde measurements over a 2.5 h field experiment are summarized in Figure 4. Concentrations of C6 aldehydes, particularly (*E*)-hex-2-enal, increased dramatically 1 h after low tide. Levels of the C9 aldehydes (*E*)-non-2-enal and (*E,E*)-nona-2,4-dienal were also significantly increased. As would be expected, the levels of the corresponding (*E*)-4-hydroxyalk-2-enals were significantly increased with emersion time. MDA concentrations behaved similarly, with a maximum of 3.25 ± 0.50 ng mL^{-1} 1 h after low tide. Concentrations of all compounds decreased dramatically when the sea rose. This decrease could be attributed to a dilution effect when the seawater flooded the pool.

In contrast with their roles in mammals,[31] higher plants[1] and even diatoms,[4] the function of aldehydes in macroalgae have been poorly investigated. Their potential role as feeding attractants was suggested from bioassays with an essential oil of the green alga *Ulva pertusa*, which is known to contain some of these aldehydes.[32] The biological significance of aldehyde emission in *L. digitata* was therefore investigated by examining whether these compounds induce metabolic responses in the alga. Thalli of *L. digitata* were therefore exposed to

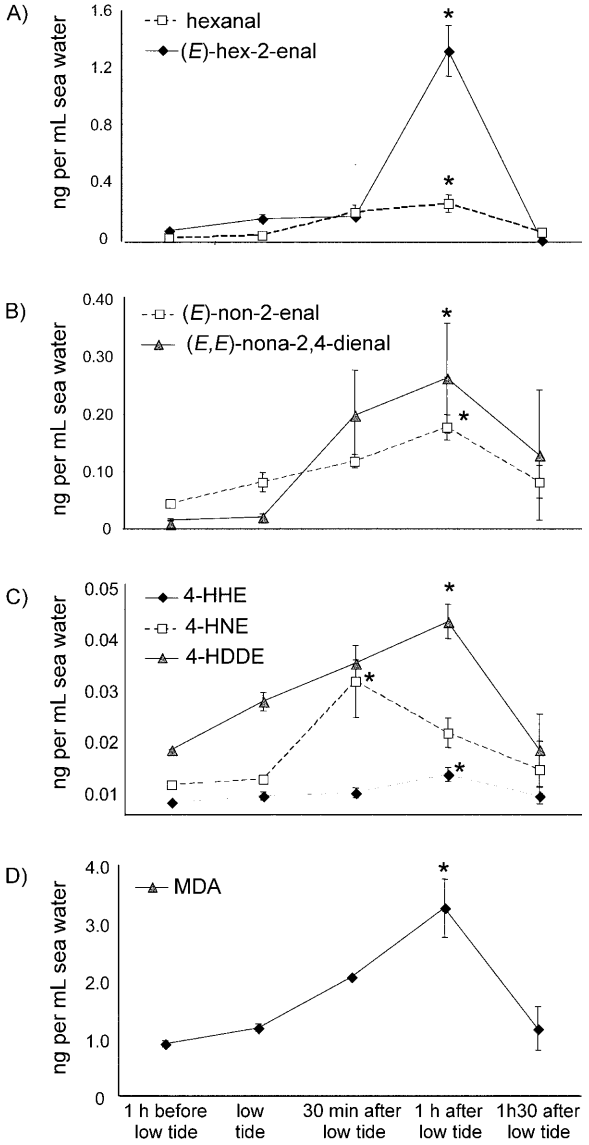

Figure 4. Variations in the emissions of various aldehydes during emersion of *L. digitata* at low tide. Seawater (20 mL) was sampled at 1 h before low tide, at low tide and at 30 min, 1 h and 1 h 30 min after low tide. A) C6 aldehydes, B) C9 aldehydes, C) 4-hydroxyalkenals, and D) malonaldehyde (MDA). Aldehydes were extracted in the field. * Significantly different from values at 1 h before low tide ($p < 0.05$).

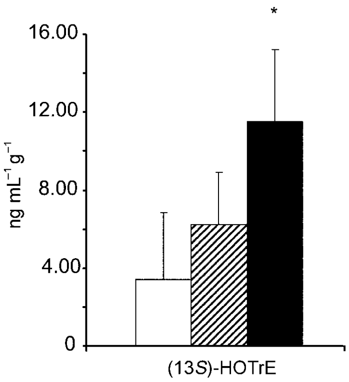

Figure 5. (13*S*)-HOTrE release after treatment of *L. digitata* with 4-HHE. Both aldehyde and oxylipin signature modification were monitored by GC/MS. Treatments with $100\,ng\,ml^{-1}$ (hatched) and $1\,\mu g\,mL^{-1}$ (■) of 4-HHE were compared to controls (□). $n = 3$. * Significantly different from control ($p < 0.05$).

stress-induced aldehydes by addition of the pure compounds to the surrounding seawater. Hexanal, nonanal, (*E*)-non-2-enal, 4-HHE, 4-HNE, (*E*,*E*)-nona-2,4-dienal and (*E*,*E*)-deca-2,4-dienal were individually tested at both $1\,\mu g\,mL^{-1}$ and $100\,ng\,mL^{-1}$. Both endogenous oxylipin signatures and aldehyde profiles in seawater were monitored by GC-MS in the NICI mode. Only 4-HHE, a C6-hydroxylated aldehyde, induced modifications of oxylipin profile (Figure 5). A release of (13*S*)-HOTrE was induced after 24 h incubation. Interestingly, this oxylipin is derived from the pathway leading to the production of 12-oxo-phytodienoic acid, which was recently characterized in copper-stressed *L. digitata*,[17] and from jasmonate in higher plants.[33] Therefore, its early synthesis may similarly involve the activation of a signalling cascade leading to late-defence mechanisms in *L. digitata*.

To the best of our knowledge, this is the first report showing that some aldehydes may induce the synthesis of oxylipins in algae and therefore act as inducers of metabolic responses. This is reminiscent of the early regulation of cell protective mechanisms suggested for reactive electrophile species in higher plants.[34] By analogy with higher plants[35–38] and diatoms,[8] these results strongly suggest that the release of aldehydes may play the role of an external and/or internal emergency signal in *L. digitata* confronted with physical modifications of their environment and exposed to pollutants.

In conclusion, we have shown that the brown algal kelp *L. digitata* naturally emits volatile aldehydes in response to both biotic and abiotic stress under laboratory conditions and in their natural environment. Together with previous reports showing temperature-dependent, species-dependent and light-dependent isoprene emissions in macro-algae, including *L. digitata*,[39] and the increased emission of iodinated and brominated VOCs in response to oligoguluronates, ozone and H_2O_2 in *L. digitata*,[21] our results extend the ensemble of VOCs naturally emitted by kelp during both biotic and abiotic stress to saturated, mono- and polyunsaturated and hydroxylated aldehydes. In addition, this is the first study that quantifies the production of PUAs by algae in their natural environment (Figure 4). Interestingly, previous knowledge about the release into seawater of oxidized fatty acid-derived compounds from brown algae was limited to sexual pheromones.[29]

These findings also raise the questions of which enzymes are involved with the generation of fatty acid aldehydes in brown algae: either hydroperoxide lyase CYP74 enzymes as in plants[1] and/or LOXs as in the moss *Physcomitrella patens*,[40] or otherwise, an enzyme machinery specific to the phylum of Heterokonta as suggested by recent results obtained with diatoms.[41] The recently provided access to whole genomes of brown algae[42] and diatoms[43,44] should afford new opportunities to express the cDNA of the conserved sequences and to characterize the products of the associated recombinant enzymes as conducted in the green lineage.[1,40]

Experimental Section

Material and treatment conditions: The marine brown alga *Laminaria digitata* V. Lamouroux (Laminariales, Laminariaceae) was collected in the intertidal zone close to the Station Biologique de Roscoff in Brittany, France, during low tide. Young fronds (2 to 15 cm in length) were chosen and transported to the laboratory. They were kept in cultivation tanks with seawater as previously described.[17]

Seawater analyses: Seawater medium was transferred into a funnel containing ethyl acetate (20 mL) and 4-HNE-d3 (50 ng) as internal standard. After vigorous shaking of the mixture, the water phase was discarded and the organic phase was recovered and gently dried under vacuum (rotary evaporator). The residues were dissolved in MeOH (2 mL) containing *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine•HCl (PFBHA•HCl, 0.05 м), transferred into a sylylated glass tube and incubated for 2 h at room temperature. Solvent was evaporated under a gentle stream of nitrogen (free of oxygen) and residues were dissolved in CH_3CN (100 μL) and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane (99:1, Sylon BFT, 200 μL). The mixture was further incubated for 1 h at 60 °C to form trimethylsilyl (TMS) derivatives and dried under a stream of nitrogen gas, and the residues were dissolved in hexane (100 μL).

Trapping of volatile compounds: The aldehyde trapping protocol was similar to that described by Engelberth and co-workers to trap methyl jasmonate.[45] *L. digitata* plantlets (1 g blotted fresh weight) were incubated in a flask (50 mL) containing fresh filtered seawater (20 mL; see Figure S2 in the Supporting Information). A Super Q filter trap, containing adsorbent (about 30 mg), and Teflon vent tubing were connected to a vacuum source at a flow rate of 500 mL min^{-1}. Constant agitation was maintained with a magnetic bar. Oligoguluronates were injected through a septum by syringe (200 μL). The compounds were eluted from the Super Q with MeOH (600 μL) containing PFBHA•HCl (0.05 м) and incubated for 2 h at room temperature. TMS-derivatization was then carried out as described above.

Tide pool measurements: Experiment took place at the Pointe Sainte-Barbe close to the CNRS Institute of Roscoff. A tide pool containing 102 thalli of *L. digitata* and only exposed at a very high tide coefficient was chosen for the experiment. The pH, temperature, salinity, brightness and O_2 levels were monitored during the whole experiment. A five-point study was carried out: at 1 h before low tide, at low tide and at 30 min, 1 h and 1 h 30 min after low tide. At this last point, the sea had risen enough to flood the pool. Seawater samples (20 mL), collected in triplicate with a glass pipette, were transferred into a Pyrex bottle containing ethyl acetate (20 mL) and 4-HNE-d3 (50 ng) and the mixture was vigorously shaken. Samples were cooled on ice and rapidly analysed in the laboratory as described above (see "seawater analyses").

GC-MS-NCI analyses: Aldehyde samples were analysed with a HP 5873 MSD interfaced to a HP 6890 Series+ gas chromatograph (Agilent, Les Ullis, France). A sample (volume of 2 μL) was injected into a capillary column (HP-5MS, J & W Scientific, 0.25 mm i.d., 30 m length, 0.25 mm film thickness). The temperatures of injection port and interface were 250 °C and 280 °C, respectively. The oven temperature was set at 60 °C for 5 min, increased at the rate of 8 °C min^{-1} to 300 °C and held for 5 min. The compounds were ionized by NICI with methane as reagent gas at 40 mL min^{-1}. For aldehyde identification, sample GC-MS analysis was carried out in both NICI and EI mode (70 eV) in the total ion current (TIC) mode. Calibration curves were prepared with increasing amounts of standards in distilled water analysed by GC-MS in the NICI mode. The samples were extracted and derivatized as described in the "seawater analyses" section. The calibration curves were constructed by plotting the area ratio of each analyte relative to the internal standard against the concentration of the analyte. For quantification of octa-2,4,7-trienal and deca-2,4,7-dienal, the calibration curves of the commercially available 2,4-dienals were used—they were assumed to behave similarly during derivatization[46] whereas for 4-HDDE quantification the calibration curve of 4-HNE was similarly used. Standard curves were linear ($r^2 > 0.95$) from 0.5 to 50 ng injected.

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Keywords: abiotic stress · aldehydes · biotic stress · environmental chemistry · marine alga

[1] K. Matsui, A. Minami, E. Hornung, H. Shibata, K. Kishimoto, V. Ahnert, H. Kindl, T. Kajiwara, I. Feussner, *Phytochemistry* **2006**, *67*, 649–657.

[2] G. d'Ippolito, O. Iadicicco, G. Romano, A. Fontana, *Tetrahedron Lett.* **2002**, *43*, 6137–6140.

[3] A. Miralto, G. Barone, G. Romano, S. A. Poulet, A. Ianora, G. L. Russo, I. Buttino, G. Mazzarella, M. Laabir, M. Cabrini, *Nature* **1999**, *402*,.

[4] G. Pohnert, *ChemBioChem* **2005**, *6*, 946–959.

[5] T. Wichard, S. A. Poulet, C. Halsband-Lenk, A. Albaina, R. Harris, D. Liu, G. Pohnert, *J. Chem. Ecol.* **2005**, *31*, 949–958.

[6] G. Pohnert, *Angew. Chem.* **2000**, *112*, 4506–4508; *Angew. Chem. Int. Ed.* **2000**, *39*, 4352–4354.

[7] A. Ianora, A. Miralto, S. A. Poulet, Y. Carotenuto, I. Buttino, G. Romano, R. Casotti, G. Pohnert, T. Wichard, L. Colucci-D'Amato, *Nature* **2004**, *429*, 403–407.

[8] A. Vardi, F. Formiggini, R. Casotti, A. De Martino, F. Ribalet, A. Miralto, C. Bowler, *PLoS Biol.* **2006**, *4*, e60.

[9] Y. Akakabe, K. Matsui, T. Kajiwara, *Bioorg. Med. Chem.* **2003**, *11*, 3607–3609.

[10] K. Boonprab, K. Matsui, Y. Akakabe, N. Yotsukura, T. Kajiwara, *Phytochemistry* **2003**, *63*, 669–678.

[11] A. Fontana, G. d'Ippolito, A. Cutignano, A. Miralto, A. Ianora, G. Romano, G. Cimino, *Pure Appl. Chem.* **2007**, *79*, 481–490.

[12] G. d'Ippolito, A. Cutignano, S. Tucci, G. Romano, G. Cimino, A. Fontana, *Phytochemistry* **2006**, *67*, 314–322.

[13] C. Vidoudez, G. Pohnert, *J. Plankton Res.* **2008**, DOI:10.1093/plankt/fbn085.

[14] F. C. Küpper, B. Kloareg, J. Guern, P. Potin, *Plant Physiol.* **2001**, *125*, 278–291.

[15] F. C. Küpper, D. G. Muller, A. F. Peters, B. Kloareg, P. Potin, *J. Chem. Ecol.* **2002**, *28*, 2057–2081.

[16] L. Contreras, M. H. Medina, S. Andrade, V. Oppliger, J. A. Correa, *Environ. Pollut.* **2007**, *145*, 75–83.

[17] A. Ritter, S. Goulitquer, J. P. Salaun, T. Tonon, J. A. Correa, P. Potin, *New Phytol.* **2008**, *180*, 809–821.

[18] M. J. Dring, *Adv. Bot. Res.* **2005**, *43*, 175–207.

[19] M. Kohlmann, A. Bachmann, H. Weichert, A. Kolbe, T. Balkenhohl, C. Wasternack, I. Feussner, *Eur. J. Biochem.* **1999**, *260*, 885–895.

[20] F. C. Küpper, L. J. Carpenter, G. B. McFiggans, C. J. Palmer, T. J. Waite, E.-M. Boneberg, S. Woitsch, M. Weiller, R. Abela, D. Grolimund, P. Potin, A. Butler, G. W. Luther, III, P. M. H. Kroneck, W. Meyer-Klaucke, M. C. Feiters, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6954–6958.

[21] C. J. Palmer, T. L. Anders, L. J. Carpenter, F. Kupper, G. B. McFiggans, *Environ. Chem.* **2005**, *2*, 282–290.

[22] M. Leitner, R. Kaiser, M. O. Rasmussen, H. Driguez, W. Boland, A. Mithofer, *Phytochemistry* **2008**, *69*, 2029–2040.

[23] S. V. Khotimchenko, I. V. Kulikova, *Chem. Nat. Compd.* **1999**, *35*, 17–20.

[24] K. Boonprab, K. Matsui, Y. Akakabe, M. Yoshida, N. Yotsukura, A. Chirapart, T. Kajiwara, *J. Appl. Phycol.* **2006**, *18*, 409–412.

[25] M. Guichardant, N. Bernoud-Hubac, B. Chantegrel, C. Deshayes, M. Lagarde, *Prostaglandins Leukotrienes Essent. Fatty Acids* **2002**, *67*, 147–149.

[26] C. Schneider, N. A. Porter, A. R. Brash, *J. Biol. Chem.* **2008**, *283*, 15539–15543.

[27] L. M. Henderson, J. B. Chappell, O. T. Jones, *Biochem. J.* **1989**, *264*, 249–255.

[28] G. Pohnert, W. Boland, *Nat. Prod. Rep.* **2002**, *19*, 108–122.

[29] J. Collen, I. A. Davison, *J. Phycol.* **1999**, *35*, 54–61.

[30] G. A. Pearson, I. A. Davison, *J. Phycol.* **1994**, *30*, 257–267.

[31] P. J. O'Brien, A. G. Siraki, N. Shangari, *Crit. Rev. Toxicol.* **2005**, *35*, 609–662.

[32] Y. Akakabe, T. Kajiwara, *J. App. Phycol.* **2007**, DOI: 10.1007s10811–007–9309–x.

[33] E. Blée, *Prog Lipid Res.* **1998**, *37*, 33–72.

[34] E. E. Farmer, C. Davoine, *Curr. Opin. Plant Biol.* **2007**, *10*, 380–386.

[35] I. T. Baldwin, A. Kessler, R. Halitschke, *Curr. Opin. Plant Biol.* **2002**, *5*, 351–354.

[36] Z. Chen, H. Silva, D. F. Klessig, *Science* **1993**, *262*, 1883–1886.

[37] A. Levine, R. Tenhaken, R. Dixon, C. Lamb, *Cell* **1994**, *79*, 583–593.

[38] M. L. Orozco-Cardenas, J. Narvaez-Vasquez, C. A. Ryan, *Plant Cell* **2001**, *13*, 179–191.

[39] W. J. Broadgate, G. Malin, F. Kupper, A. Thompson, P. S. Liss, *Marine Chemistry* **2004**, *88*, 61–73.

[40] M. Stumpe, J. Bode, C. Gobel, T. Wichard, A. Schaaf, W. Frank, M. Frank, R. Reski, G. Pohnert, I. Feussner, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2006**, *1761*, 301–312.

[41] A. Barofsky, G. Pohnert, *Org. Lett.* **2007**, *9*, 1017–1020.

[42] B. Charrier, S. M. Coelho, A. Le Bail, T. Tonon, G. Michel, P. Potin, B. Kloareg, C. Boyen, A. F. Peters, J. M. Cock, *New Phytol.* **2008**, *177*, 319–332.

[43] E. V. Armbrust, J. A. Berges, C. Bowler, B. R. Green, D. Martinez, N. H. Putnam, S. Zhou, A. E. Allen, K. E. Apt, M. Bechner, *Science* **2004**, *306*, 79–86.

[44] C. Bowler, A. E. Allen, J. H. Badger, J. Grimwood, K. Jabbari, A. Kuo, U. Maheswari, C. Martens, F. Maumus, R. P. Otillar, *Nature* **2008**, *456*, 239–244.

[45] J. Engelberth, E. A. Schmelz, H. T. Alborn, Y. J. Cardoza, J. Huang, J. H. Tumlinson, *Anal. Biochem.* **2003**, *312*, 242–250.

[46] T. Wichard, S. A. Poulet, G. Pohnert, *J. Chromatogr. B* **2005**, *814*, 155–161.

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