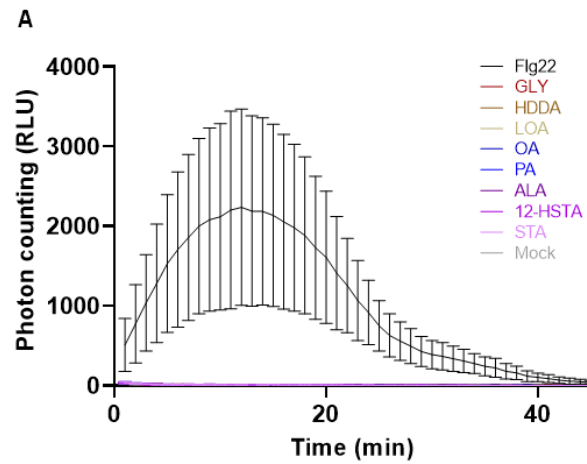
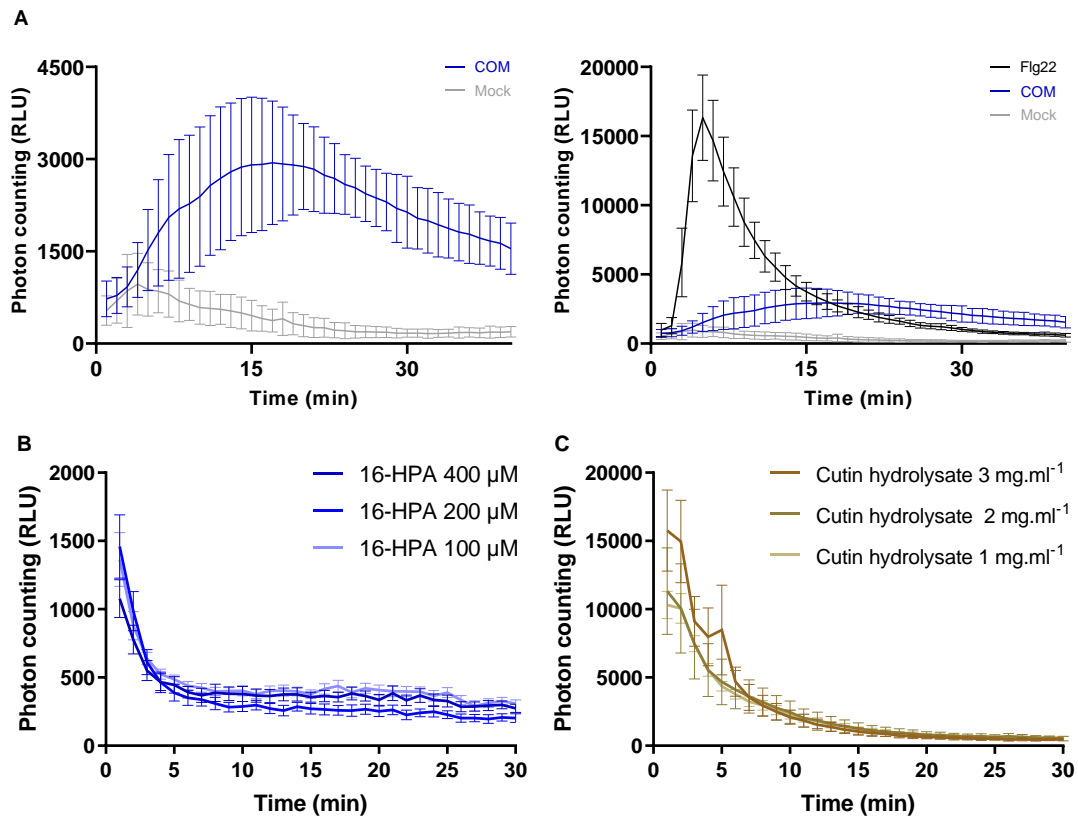


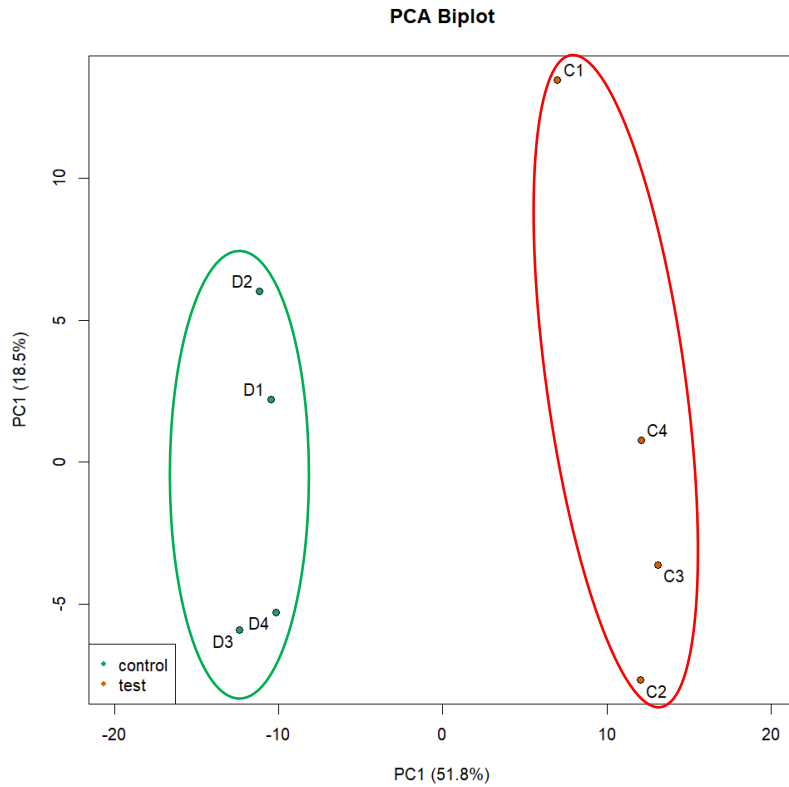
Supplementary Fig. S1 - Luminescence-based detection of apoplastic ROS in *Arabidopsis thaliana* Col-0 seedlings upon treatment with $2 \text{ mg} \cdot \text{mL}^{-1}$ of cutin in MiliQ water for 30 min (**A**) and 45 min (**B**). The Mock consists of the solvent. The positive control was Flg22 (100 nM).



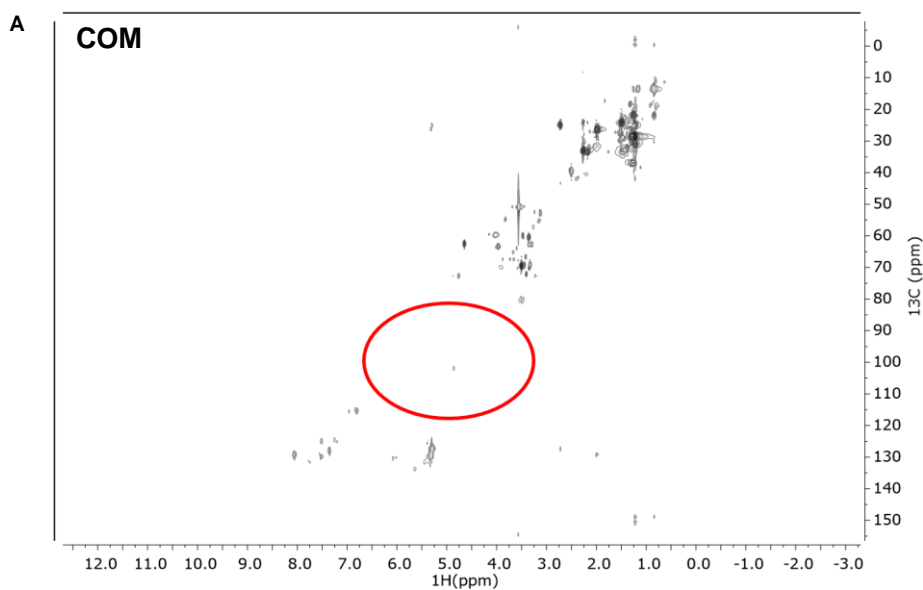
Supplementary Fig. S2 – Luminescence-based detection of apoplastic ROS in *Arabidopsis thaliana* Col-0 seedlings upon treatment with 1 mM of commercially available pure compounds (glycerol (GLY), hexadecanedioic acid (HDDA), linoleic acid (LOA), oleic acid (OA), palmitic acid (PA), aleuritic acid (ALA), 12-hydroxystearic acid (12-HSTA) and stearic acid (STA)), in 10 % ethanol in MiliQ water for 45 min. The Mock consists of the solvent. The positive controls was Fig22 (100 nM).



Supplementary Fig. S3 – (A) Luminescence-based detection of calcium influx in *Nicotiana benthamiana* leafdiscs expressing the calcium reporter aequorin, upon treatment with 2 $\text{mg}\cdot\text{mL}^{-1}$ of COM in 2 % DMSO in MiliQ water **(B)** Luminescence-based detection of calcium influx in *Arabidopsis thaliana* seedlings expressing the calcium reporter aequorin, upon treatment with increasing concentrations of 16-hydroxypalmitic acid (100 μ M, 200 μ M and 400 μ M) in 0.5 % DMSO in MiliQ; and **(C)** increasing concentrations of the hydrolysate obtained from the total alkaline hydrolysis of cutin (1 $\text{mg}\cdot\text{mL}^{-1}$, 2 $\text{mg}\cdot\text{mL}^{-1}$ and 3 $\text{mg}\cdot\text{mL}^{-1}$) in 0.5 % DMSO in MiliQ. The Mock consists of the solvent. The positive control was Flg22 (100 nM).



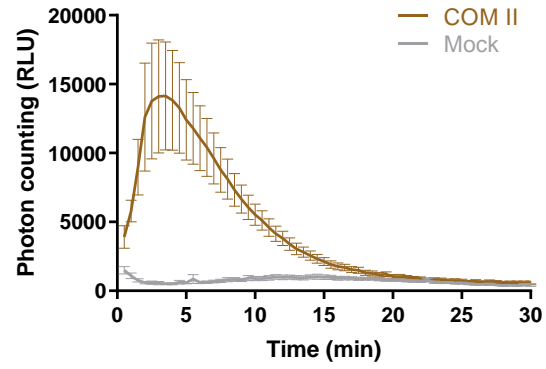
Supplementary Fig. S4 – Principal components analysis performed with all eight samples used for the RNA-seq experiment. The figure depicts the first two main components. Ellipses enclose the regions of the space comprising the four biological replicates of each treatment: **COM (C1-C4)** and 0.5 % **DMSO** in water – mock control (**D1-D4**).



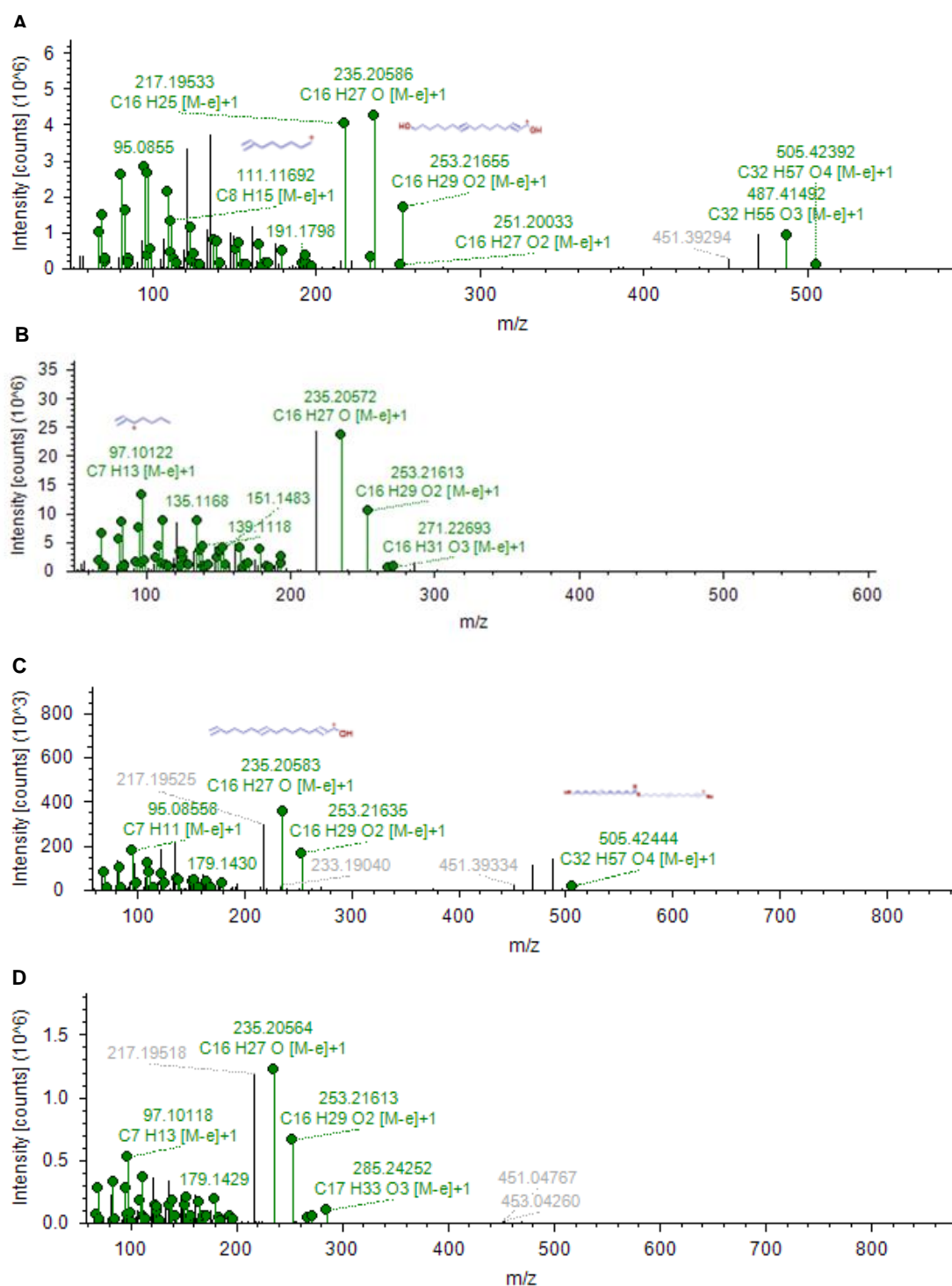
B

	pg (carbohydrate) / mg (COM)	Carbohydrate content (%)
COM	41.05 ± 7.25	4.11x10 ⁻⁷ ± 7.25x10 ⁻⁸
COM II	43.83 ± 25.05	4.38x10 ⁻⁷ ± 2.51x10 ⁻⁷

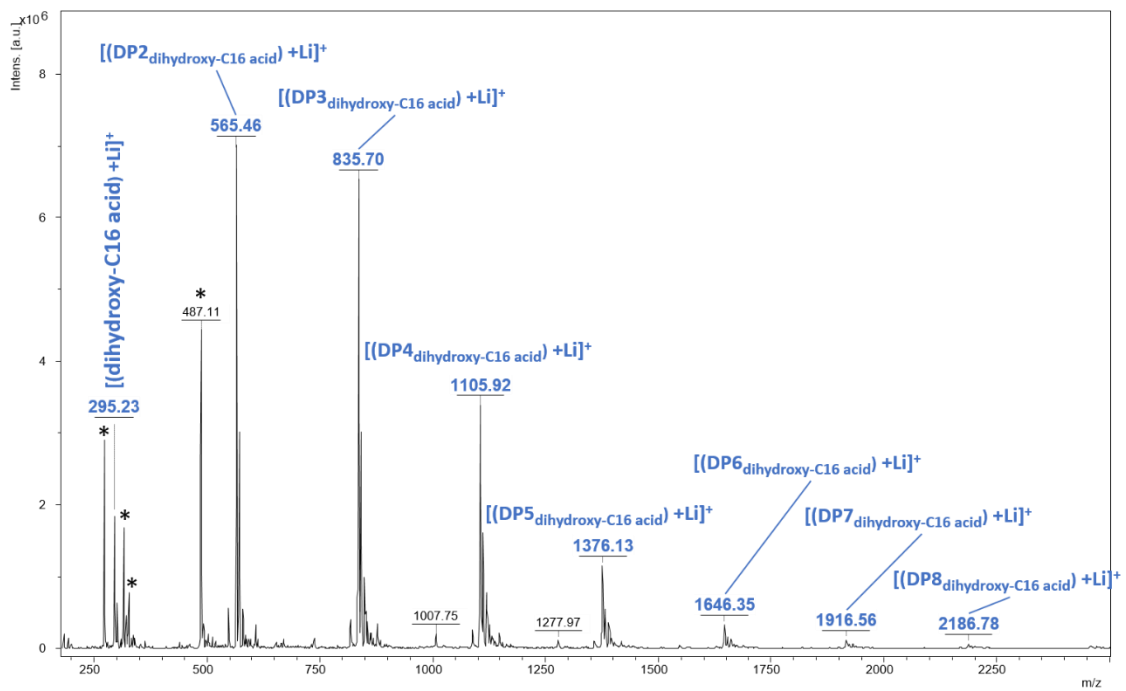
Supplementary Fig. S5 – (A) NMR characterization of 40 mg of COM. Representative magnification of the HSQC spectral region corresponding to glycosidic linkages for the COM. **(B)** Quantification of the total hydrolysable carbohydrates content present in cutin oligomeric mixtures (COMs).



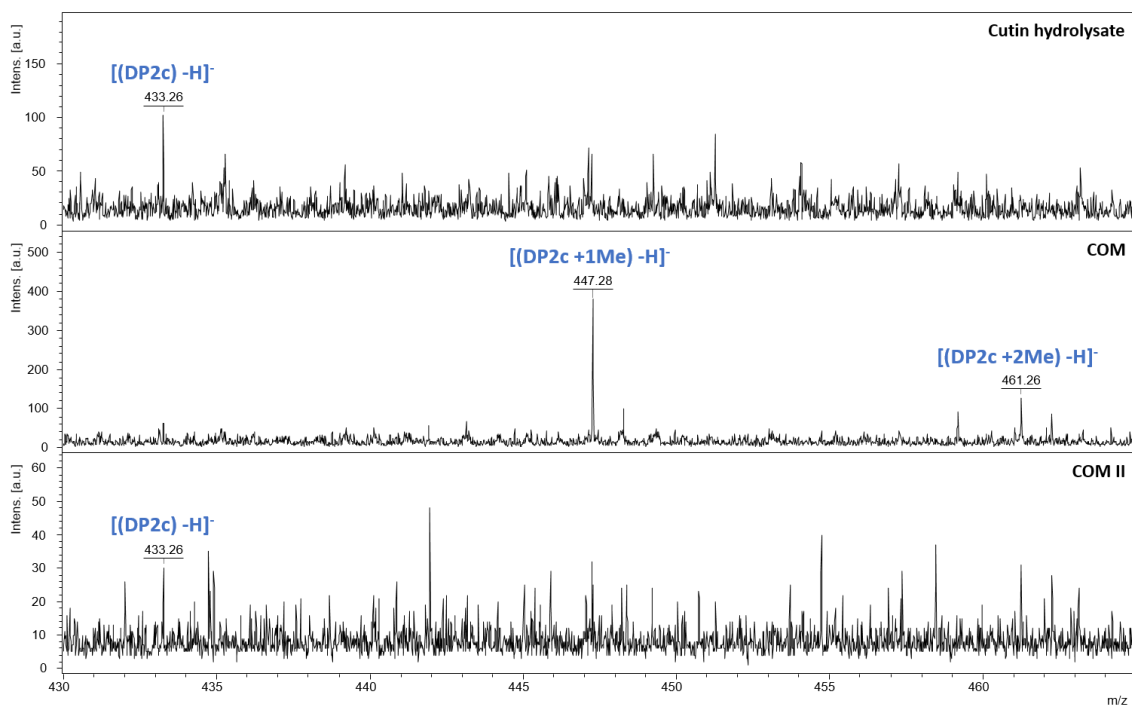
Supplementary Fig. S6 – Luminescence-based detection of calcium influx in *Arabidopsis thaliana* seedlings expressing the calcium reporter aequorin, upon treatment with 2 mg·mL⁻¹ of COM II in 0.5 % DMSO in MiliQ water. In this assay the Mock consists of the solvent.



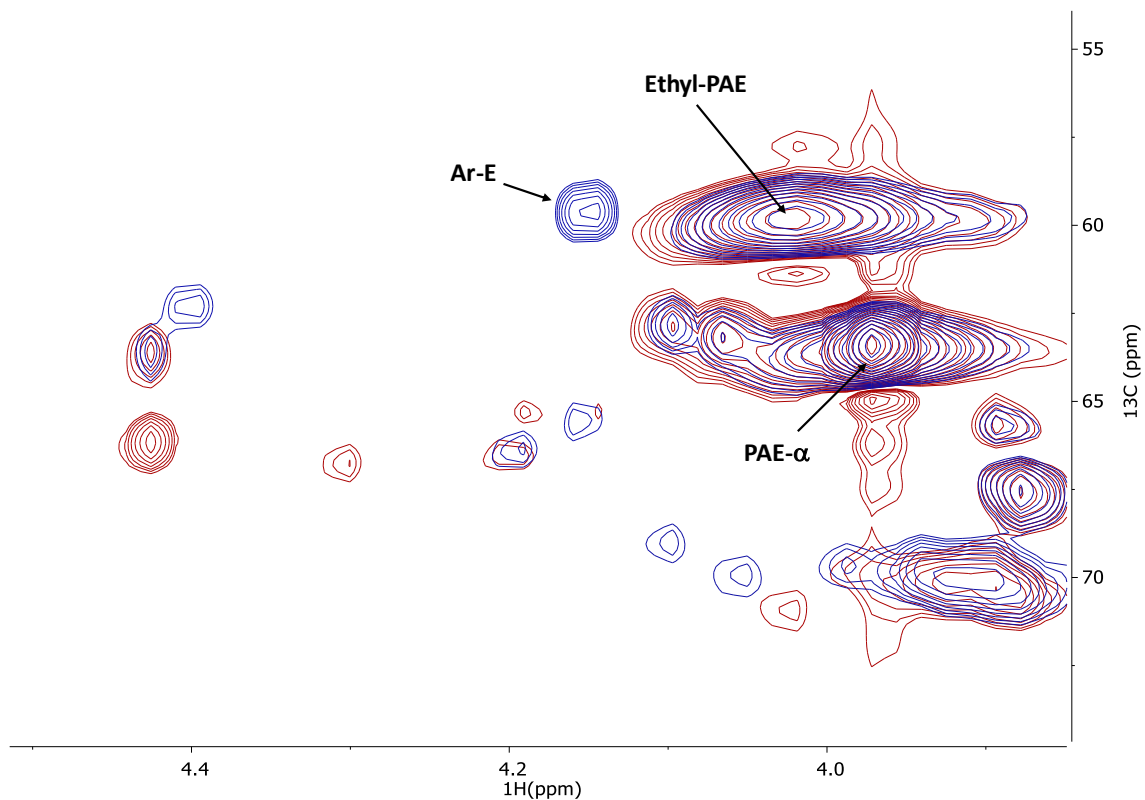
Supplementary Fig. S7 – LC-MS/MS characterization of COM II (representative spectra for both COMs) in positive mode. MS2 spectra of the detected dimer composed by ester linked molecules of 10,16-dihydroxyhexadecanoic acid (DP2) in the non-methylated (**A**) and methylated (**B**) forms. MS2 spectra of the detected trimer composed by ester linked molecules of 10,16-dihydroxyhexadecanoic acid (DP3) in the non-methylated (**C**) and methylated (**D**) forms.



Supplementary Fig. S8 – MALDI-TOF (+) spectrum of a reference sample containing DP's of 10,16-dihydroxyhexadecanoic acid. Annotations were deduced from exact mass measurements. Black star indicates an ion from the MALDI matrix.



Supplementary Fig. S9 – LDI-TOF (-) spectra of cutin hydrolysate and COMs. The range 430-465 Da corresponds to expected masses for DP2s made of one 10,16-dihydroxyhexadecanoic acid and one p-coumaric acid. Annotations were deduced from exact mass measurements.



Supplementary Fig. S10 – NMR characterization of 40 mg of COM (Blue) and COM II (Red) showing a magnification of the HSQC spectral regions comprised between 3.82 and 4.48 ppm (^1H). Samples were 2.6-fold concentrated compared to samples used in all standard NMR analyses (15 mg).

Supplementary Table S1 – GC-MS characterization of COM before (NH) and after alkaline hydrolysis (AH). List of the monomeric hydrolysable constituents of each sample after derivatization. Monomers that were not detected in a specific sample are labelled as *n.d.*

Compound (X)	COM NH	COM AH
	m (X) / m COM (g)	m (X) / m COM (g)
Alkanoic acids	244.36 ± 7.17	203.23 ± 11.79
hexadecanoic acid methyl ester	26.85 ± 0.71	n.d.
9,12-octadecadienoic acid methyl ester	41.08 ± 0.92	n.d.
9-octadecenoic acid methyl ester	33.92 ± 0.67	n.d.
octadecanoic acid methyl ester	25.35 ± 0.74	n.d.
hexadecanoic acid tms	26.48 ± 0.91	37.73 ± 1.80
9,12-octadecadienoic acid tms	40.02 ± 1.60	86.01 ± 4.96
9-octadecenoic acid tms	27.77 ± 1.09	53.09 ± 3.60
octadecanoic acid tms	22.88 ± 0.84	26.40 ± 2.04
ω-Hydroxyalkanoic acids	111.15 ± 3.99	182.86 ± 10.33
16-hydroxyhexadecanoic acid methyl ester		
10,16-Dihydroxyhexadecanoic acid methyl ester	37.42 ± 1.39	16.43 ± 2.78
9,10-epoxy-18-hydroxyoctadecanoic acid methyl ester		
16-hydroxyhexadecanoic acid tms	27.71 ± 0.72	18.16 ± 1.39
10,16-Dihydroxyhexadecanoic acid tms	52.02 ± 2.14	140.32 ± 8.53
9,10-epoxy-18-hydroxyoctadecanoic acid tms		23.86 ± 0.52
α, ω-Alkanedioic acids	10.66 ± 0.63	51.85 ± 1.97
Nonanedioic acid methyl ester		
Hexadecanedioic acid methyl ester		
Nonanedioic acid tms	10.66 ± 0.63	39.49 ± 0.90
Hexadecanedioic acid tms	n.d.	12.36 ± 1.33
Aromatics	15.02 ± 2.87	13.00 ± 0.59
4-hydroxybenzaldehyde methyl ester		
4-coumaric acid methyl ester	5.19 ± 0.13	n.d.
4-hydroxybenzaldehyde tms	4.31 ± 0.20	3.77 ± 0.44
4-coumaric acid tms	5.87 ± 0.17	9.23 ± 0.19

Supplementary Table S2 – List of genes uniquely induced by the COM treatment

after comparison with seven different elicitors of PTI and seven different types of abiotic stress conditions. Genes for which there was no attributed name on the TAIR database were labelled as NA. ASI stand for the number of abiotic stresses

Gene	Log2 FC	padj	ASI	Name	Short Description
AT3G19700	4.752	9.67E-03	0	IKU2	Leucine rich repeat (LRR) kinase
AT1G27080	3.582	2.03E-02	0	ATNPF2.12	Protein with low-affinity nitrate transporter activity
AT5G07880	3.419	6.19E-10	0	ATSNAP29	Member of mammalian SNAP25 Gene Family
AT3G46070	3.407	1.37E-04	0	NA	C2H2-type zinc finger family protein
AT3G56980	2.815	6.64E-05	0	BHLH039	Basic helix-loop-helix transcription factor protein
AT1G64940	2.637	2.91E-03	0	CYP89A6	Member of the CYP89A family
AT4G36040	2.290	1.14E-37	0	DJC23	Chaperone DnaJ-domain superfamily protein
AT5G22890	1.967	7.40E-19	0	STOP2	Sensitive to proton rhizotoxicity 2.
AT5G42600	1.940	3.45E-02	0	MRN1	Oxidosqualene synthase that produces monocyclic triterpenes
AT3G04530	1.651	5.10E-04	0	ATPPCK2	Arabidopsis phosphoenolpyruvate carboxylase kinase
AT3G21260	1.646	2.60E-06	0	GLTP3	Glycolipid transfer protein (GLTP) family protein
AT3G21270	1.560	5.18E-15	0	ADOF2	Dof zinc finger protein
AT5G54630	1.534	3.48E-09	0	NA	Zinc finger protein-like protein
AT4G19865	1.474	1.34E-04	0	NA	Galactose oxidase/kelch repeat superfamily protein
AT5G66690	1.434	2.82E-09	0	UGT72E2	UDPG:coniferyl alcohol glucosyltransferase
AT5G51500	1.397	3.88E-02	0	NA	Plant invertase/pectin methylesterase inhibitor superfamily
AT2G36090	1.394	6.92E-04	0	NA	F-box family protein
AT1G66800	1.385	2.90E-02	0	NA	NAD(P)-binding Rossmann-fold superfamily protein
AT1G28470	1.382	5.47E-04	0	ANAC010	NAC domain containing protein 10
AT1G35260	1.355	3.01E-11	0	MLP165	MLP-like protein 165
AT2G28690	1.257	6.11E-06	0	NA	TOX high mobility group box protein
AT1G64780	1.225	1.78E-02	0	AMT1	Ammonium transporter protein
AT3G25290	1.194	1.29E-05	0	NA	Auxin-responsive family protein
AT3G09270	1.090	1.38E-04	0	ATGSTU8	Glutathione transferase belonging to the tau class of GSTs
AT5G03670	1.082	5.70E-04	0	TRM28	Histone-lysine N-methyltransferase SETD1B-like protein
AT3G18400	1.071	3.32E-03	0	ANAC058	NAC domain containing protein 58
AT5G03240	1.063	4.55E-04	0	UBQ3	Ubiquitin that is attached to proteins destined for degradation
AT1G05530	1.055	3.40E-02	0	UGT2	Protein with glucosyltransferase activity
AT3G52360	1.027	1.80E-04	0	NA	Transmembrane protein
AT3G14680	1.008	7.13E-03	0	CYP72A14	Putative cytochrome P450
AT3G61410	1.008	3.84E-02	0	NA	U-box kinase family protein
AT1G68795	1.005	1.07E-02	0	CLE12	Putative ligand homologous to the Clavata3 gene

inducing the same effect.

Supplementary Table S3 – List of genes uniquely induced by the COM treatment after comparison with seven other different elicitors of PTI, but also induced by one or more different of abiotic stress conditions. Genes for which there was no attributed name or description on the TAIR database were labelled as NA, and genes which were either not represented on the ATH1 microarray or were represented by a probe with multiple gene matches are labelled ND.

Gene	Log2 FC	padj	ASI	Name	Short Description
AT3G27880	3.3280	5.38E-66	1	NA	Hypothetical protein
AT3G04200	3.0262	3.89E-03	1	NA	C2H2-type zinc finger family protein
AT2G20670	2.3084	1.01E-05	1	NA	Sugar phosphate exchange
AT5G65790	2.1720	1.12E-09	1	ATMYB68	Putative MYB transcription factor
AT2G27010	2.0167	8.85E-11	1	CYP705A9	Member of CYP705A
AT1G67030	1.8268	3.82E-06	1	ZFP6	Zinc finger protein containing only a single zinc finger
AT1G31050	1.7533	2.28E-05	1	PFA1	Pericycle factor type-a 1
AT4G21510	1.7418	4.24E-14	1	ATFBS2	F-box family protein
AT1G58170	1.6905	1.11E-02	1	NA	Disease resistance-responsive family protein
AT5G42580	1.5640	3.99E-02	1	CYP705A12	Member of the cytochrome P450 family
AT1G22110	1.5603	2.04E-05	1	NA	Structural constituent of ribosome
AT2G36050	1.4361	2.24E-09	1	ATOFP15	Ovate family protein 15
AT1G67340	1.4226	1.19E-07	1	NA	HCP-like superfamily protein with MYND-type zinc finger
AT1G35515	1.3600	9.30E-03	1	HOS10	Nuclear localized R2R3-type MYB transcription factor
AT2G17080	1.2715	1.55E-04	1	NA	Hypothetical protein
AT3G14660	1.1079	8.28E-05	1	CYP72A13	Putative cytochrome P450
AT1G17360	1.0928	9.71E-05	1	NA	Protein phosphatase 1 regulatory subunit-like protein
AT5G07870	3.5385	7.84E-83	2	NA	HXXXD-type acyl-transferase family protein
AT5G05220	3.2064	4.63E-02	2	NA	Hypothetical protein
AT3G28740	2.6127	1.80E-04	2	CYP81D11	Member of the cytochrome p450 family
AT5G07860	2.6096	3.66E-59	2	NA	HXXXD-type acyl-transferase family protein
AT4G34138	2.3470	8.31E-11	2	UGT73B1	UDP-glucosyl transferase 73B1
AT3G49690	1.9282	2.73E-07	2	ATMYB84	Putative homolog of the Blind gene in tomato
AT1G54120	1.7080	7.66E-04	2	NA	Hypothetical protein
AT2G39330	1.5299	2.13E-02	2	JAL23	Jacalin-related lectin 23
AT1G02205	1.4895	3.06E-02	2	CER1	Gene associated with production of stem epicuticular wax
AT5G48010	1.4732	5.37E-05	2	ATTHAS1	Oxidosqualene cyclase involved in the biosynthesis of thalianol
AT3G13310	1.4728	3.88E-13	2	DJC66	Chaperone DnaJ-domain superfamily protein
AT3G03840	1.3663	4.61E-03	2	SAUR27	SAUR-like auxin-responsive protein family
AT5G67420	1.2773	8.30E-03	2	ASL39	LOB-domain protein involved in N metabolism and leaf morphogen
AT5G53500	1.2322	1.54E-16	2	NA	Transducin/WD40 repeat-like superfamily protein
AT1G18330	1.2301	8.86E-03	2	EPR1	Early phytochrome responsive 1
AT1G68440	1.2035	6.49E-19	2	NA	Transmembrane protein
AT3G16800	1.1923	9.57E-03	2	EGR3	Regulates microtubule organization
AT1G15550	1.1772	1.52E-02	2	ATGA3OX1	Involved in the gibberellic acid biosynthetic pathway
AT4G05070	1.0033	1.73E-03	2	WIP2	Member of the wound-induced polypeptide (WIP) family
AT3G59940	2.7494	8.68E-21	3	KFB50	Member of the F-box KISS ME DEADLY (KMD) protein family
AT1G68360	1.7719	2.77E-02	3	GIS3	Nuclear localized member of the C2H2 family of transcription factors
AT2G30130	1.6642	1.55E-05	3	ASL5	Gene similar to asymmetric leaves (AS)/lateral organ boundary (LOB) genes
AT5G16600	1.5649	3.06E-03	3	ATMYB43	Putative transcription factor (MYB43)
AT3G47600	1.5635	1.87E-09	3	ATMYB94	Putative transcription factor (MYB94)
AT1G78600	1.5492	3.70E-05	3	BBX22	Light-regulated zinc finger protein
AT1G21680	1.4751	1.29E-03	3	NA	DPP6 N-terminal domain-like protein
AT5G01820	1.3611	1.60E-03	3	ATCIPK14	CBL-interacting serine/threonine protein kinase
AT5G63970	1.2545	7.16E-04	3	RGLG3	Calcium-dependent phospholipid-binding protein
AT3G24310	2.5638	2.05E-08	4	ATMYB71	Snapdragon myb protein 305 homolog (myb)
AT5G54230	2.3699	3.64E-10	4	ATMYB49	Putative transcription factor (MYB49)
AT3G48510	1.9524	9.78E-04	5	AITR2	ABA - induced transcription repressor acting as feedback regulator in ABA
AT2G47890	1.5210	1.02E-07	5	NA	B-box type zinc finger protein with CCT domain
AT4G25480	3.4252	4.48E-04	6	ATCBF3	Member of the DREB subfamily A-1 of ERF/AP2 transcription factor family
AT1G05100	2.6703	2.87E-17	6	MAPKKK18	Member of the MEKK subfamily
AT5G35688	5.2093	3.42E-03	ND	NA	Small protein with evidence of transcription or purifying selection
AT4G22210	4.6102	1.81E-02	ND	LCR85	Member of a family of small, secreted, cysteine rich proteins
AT2G30615	4.2291	2.97E-03	ND	NA	F-box/LRR protein
AT1G10220	3.2335	2.69E-06	ND	ZCF37	NA
AT4G15248	3.0468	7.29E-03	ND	BBX30	B-box type zinc finger family protein
AT1G72490	2.3606	2.59E-09	ND	DRO1	Member of the IGT gene family with unknown function
AT2G26370	2.2571	2.03E-02	ND	NA	MD-2-related lipid recognition domain-containing protein
AT5G63595	2.1890	2.26E-10	ND	ATFLS4	Flavonol synthase 4
AT4G37700	1.9244	1.77E-02	ND	NA	Hypothetical protein
AT1G64950	1.7784	5.54E-18	ND	CYP89A5	Member of CYP89A
AT5G65030	1.7624	3.92E-02	ND	NA	Nitric oxide synthase-interacting protein
AT2G28160	1.6114	3.29E-03	ND	ATBHLH029	Putative transcription factor regulating iron uptake responses
AT2G30230	1.3264	1.96E-03	ND	NA	6,7-dimethyl-8-ribityllumazine synthase
AT2G23690	1.3238	1.10E-03	ND	NA	HTH-type transcriptional regulator
AT4G06534	1.0011	3.85E-02	ND	NA	Transmembrane protein
AT4G02950	5.4744	1.15E-04	ND	NA	Ubiquitin family protein
AT5G23360	2.4024	6.64E-05	ND	NA	GRAM domain-containing protein / ABA-responsive protein-related
AT5G23350	1.9571	4.41E-04	ND	NA	GRAM domain-containing protein / ABA-responsive protein-related
AT1G05660	1.3890	1.14E-02	ND	NA	Pectin lyase-like superfamily protein

Supplementary Table S4A – List of putative oligomeric targets for LC-MS/MS analysis, their chemical formulas and the corresponding exact mass (neutral species only).

Oligomer	Chemical Formula	Mass (Da)
DP2: 10,16-dihydroxyhexadecanoic acid (2x)	C ₃₂ H ₆₂ O ₇	558.444839
DP2-me: 10,16-dihydroxyhexadecanoic acid (2x) + Me	C ₃₃ H ₆₄ O ₇	572.4604891
DP3: 10,16-dihydroxyhexadecanoic acid (3x)	C ₄₈ H ₉₂ O ₁₀	828.6596185
DP3-me: 10,16-dihydroxyhexadecanoic acid (3x) + Me	C ₄₉ H ₉₄ O ₁₀	842.6752686

Supplementary Table S4B – List of all putatively identified oligomers (DP2 and DP3) by LC-MS/MS (+) in COMs and the corresponding detected masses.

Species	Detected <i>m/z</i>	COM	COM II
<i>Observed in LC-MS/MS (+)</i>			
[DP2(dihydroxy-C ₁₆ acid) + H] ⁺	559.46	+	+
[DP2(dihydroxy-C ₁₆ acid) + NH ₄] ⁺	576.49	-	+
[DP2(dihydroxy-C ₁₆ acid + Na) ⁺	581.44	+	+
[DP2(hydroxy-C ₁₆ -dioic acid) + Me + H – H ₂ O] ⁺	555.46	-	+
[DP2(hydroxy-C ₁₆ -dioic acid) + Me + H] ⁺	573.47	+	+
[DP2(hydroxy-C ₁₆ -dioic acid) + Me + NH ₄] ⁺	590.50	+	+
[DP2(hydroxy-C ₁₆ -dioic acid) + Me + Na] ⁺	595.45	+	+
[DP2(hydroxy-C ₁₆ -dioic acid) + Me + K] ⁺	611.43	-	+
[DP3(dihydroxy-C ₁₆ acid) + H] ⁺	829.68	-	+
[DP3(dihydroxy-C ₁₆ acid) + Na] ⁺	851.66	-	+
[DP3(dihydroxy-C ₁₆ acid) + Me + H] ⁺	843.69	-	+
[DP3(dihydroxy-C ₁₆ acid) + Me + NH ₄] ⁺	860.72	-	+
[DP3(dihydroxy-C ₁₆ acid) + Me + Na] ⁺	865.67	-	+
[DP3(dihydroxy-C ₁₆ acid) + Me + K] ⁺	881.65	-	+

Supplementary Table S5 – List of all putatively identified monomers and oligomers by MALDI-TOF (+) and LDI-TOF (-) in cutin hydrolysates, COMs hydrolysates and COMs.

Species	Theoretical <i>m/z</i>	Cutin hydrolysate	COM hydrolysate	COM II hydrolysate	COM	COM II
Observed in MALDI-TOF(+)						
[hydroxy-C ₁₆ acid +Li] ⁺	279.25	-	+	+	-	+
[C ₁₆ -dioic acid +Li] ⁺	293.23	+	+	+	-	+
[dihydroxy-C ₁₆ acid +Li] ⁺	295.25	+	+	+	-	+
[dihydroxy-C ₁₆ acid -H+2Li] ⁺	301.25	+	+	+	-	+
[hydroxy-C ₁₆ -dioic acid +Li] ⁺	309.22	+	+	+	-	-
[dihydroxy-C ₁₆ acid +1.Me +Li] ⁺	309.26	-	-	-	+	+
[C ₁₈ -dioic acid +Li] ⁺	321.26	-	+	+	-	-
[hydroxy-C ₁₈ -dioic acid +Li] ⁺	337.26	+	+	+	+	+
[DP2(dihydroxy-C ₁₆ acid) +Li] ⁺	565.47	+	-	-	-	+
[DP2(dihydroxy-C ₁₆ acid) -H+2Li] ⁺	571.47	+	-	-	-	+
[DP2(dihydroxy-C ₁₆ acid +1.Me) +Li] ⁺	579.48	-	-	-	+	+
[DP2(hydroxy-C ₁₆ -dioic acid) +Li] ⁺	593.42	+	-	-	-	-
[DP3(dihydroxy-C ₁₆ acid) +Li] ⁺	835.68	-	-	-	-	+
[DP3(dihydroxy-C ₁₆ acid +1.Me) +Li] ⁺	849.70	-	-	-	-	+
Observed in LDI-TOF(-)						
[narengenin -H] ⁻	271.06	+	-	-	-	+
[DP2(dihydroxy-C ₁₆ acid - p-coum) -H] ⁻	433.26	+	-	-	-	+
[DP2(dihydroxy-C ₁₆ acid - p-coum +1.Me) -H] ⁻	447.28	-	-	-	+	-
[DP2(dihydroxy-C ₁₆ acid - p-coum +2.Me) -H] ⁻	461.29	-	-	-	+	-