PNAS
www.pnas.org

Supplementary Information for

Oligogalacturonides production *upon Arabidopsis thaliana-Botrytis cinerea* interaction.

Aline Voxeur, Olivier Habrylo, Stéphanie Guénin, Fabien Miart, Marie-Christine Soulié, Christophe Rihouey, Corinne Pau-Roblot, Jean-Marc Domon, Laurent Gutierrez, Jérôme Pelloux, Grégory Mouille, Mathilde Fagard, Herman Höfte & Samantha Vernhettes

Samantha Vernhettes
Email: samantha.verhnettes@inra.fr

This PDF file includes:

Supplementary text
Figs. S1 to S19
Tables S1 to S3
References for SI reference citations

Other supplementary materials for this manuscript include the following:

Datasets S1

www.pnas.org/cgi/doi/10.1073/pnas.1900317116

Supplementary Information Text

Material and Methods

Plant Growth

A. thaliana wild-type Wassilewskija (Ws-0) plants were grown in soil in a growth chamber at 22 °C, 70% humidity, under irradiance of 100 µmol·m^{-2}·s^{-1} with a photoperiod of 8 h light/16 h dark.

Fungal strains and growth

The wild-type *B. cinerea* B05.10 strain and *Bcpg1-6 and Bcpme1/2* (**1,2**) mutants were grown on potato dextrose agar at 23 °C under continuous light. After 10 days, each strain produced a dense carpet of conidia.

The *BcPNL1* cloning

The coding sequence of BcPNL1(BofuT4_P032630) (**3**) including the native peptide signal part, was amplified by PCR using Phusion®Taq polymerase (Thermofisher scientific) from *B. cinerea* gDNA with two specific primers (**Table S3**). The expression vector pPICZαB (Invitrogen, Cat. No. V19520) was digested by *Bst*BI and *Not*I, and the insert was ligated into the vector. After transformation in *E. coli* TOP10 (Invitrogen, Cat. No. C404003), the insert was verified by sequencing, the linearized construct was used to transform *Pichia pastoris* X-33 strain as described in the instruction manual *P. pastoris* expression kit (Invitrogen, Cat. No. K1710-01). Transformants were selected on Zeocin.

Virulence assays

The spores were washed from the surface of the plate using potato dextrose broth medium, the concentration of spores was determined using a Malassez cell and adjusted to a final concentration of 3.10^5 conidia/mL. Twenty microliter drops of spore suspension were placed on *A. thaliana* leaves of 5-week-old or 6-week-old plants. Control leaves were spotted with droplets of potato dextrose broth medium. Fungal DNA was extracted and quantified according to Gachon and Saindrenan **(4)**.

OG production

The spores were washed from the surface of the plate using Gamborg's B5 basal medium, 2% (w/v) fructose and 10 mM phosphate buffer pH 6.4. Fungal hyphae were removed from the suspension by filtering. The concentration of spores was determined using a Malassez cell and adjusted. To analyze OGs released from citrus pectins (Sigma, P9135), a 0,8% pectic solution in Gamborg medium was mixed v/v with spore suspensions at 3 x 10^5 spores/ml and incubated on a rotary shaker at 100 rpm at 23 °C during 6, 16 or 24 h. To analyze OGs released during infection, isolated *A. thaliana* leaves of 5-week-old plants were either spotted with spore suspension drops or directly immersed in a *B. cinerea* suspension (6 leaves for 10 ml of suspension at 3 x 10^5 spores/ml) and incubated on a rotary shaker at 100 rpm at 23 °C during 12, 15, 18 or 20 h. To analyze OGs released from plant cell wall, alcohol insoluble residue from *A. thaliana* leaves were incubated with *B. cinerea* spore suspension. At last, DP3-DP15 were obtained from polygalacturonic acid and incubated with germinated spores. For all the experiments, the liquid media was then collected and an equal volume of 96% ethanol was added to precipitate the largest molecules. After centrifugation (5000 g during 10 min), the supernatant was collected and dried in a speed vacuum concentrator at room temperature. The obtained pellet was then diluted. For OGs produced from pectins, 2 ml were dried and diluted in 200 µl. For OGs released during infection, the equivalent of the digestate

of 3 leaves of 5-week-old *A. thaliana* plants was dried and diluted in 200 µl. 10 µl were injected for MS analysis.

Protein extraction and purification, enzymatic activities

Endopolygalacturonase M2 from *Aspergillus aculeatus* (Megazyme) was used as reference. *Pichia pastoris* lines expressing *B. cinerea* BcPG2, BcPG3 and BcPME1, were obtained from Jan A. L. Van Kan and grown for 3 days at 30 °C in Yeast Extract Peptone Dextrose (YEPD) solid medium (**5, 6**). The methods to purify BcPME1, BcPG2 and BcPG3 have been described previously (**5, 6**). Enzymatic activities were tested using commercial pectic substrates (Citrus peel pectins with a with a degree of methylesterification (DM) of 70% (Sigma), sugar beet pectins).

The *P. pastoris line expressing* BcPNL1 were grown in baffled flasks in 10 mL of buffered glycerol-complex medium, overnight at 30°C using the appropriate antibiotic. Cells were then collected by centrifugation and resuspended to an OD600 of 1.0 in 100 mL of buffered methanol complex medium. A final concentration of 0.5 % (v/v) methanol was added every 24 h to maintain induction. After 72h of induction, the culture was centrifuged at 1 500 g for 10 min. The supernatant was loaded onto a 1ml HisTrap excel column (GE Healthcare) to affinity purification. The eluate fractions were concentrated using centrifugal filter units (Amicon® Ultra-4, Millipore). 6 µg of eluate were loaded into a 10% SDS-PAGE with Coomassie blue staining. The protein concentrations were determined using the Bradford assay with bovine serum albumin as a standard. To identify the recombinant protein by Western blot, SDS-PAGE was transferred from resolving gel to PVDF blotting membrane using the appropriate cathode and anode buffers and a Trans-Blot TURBO Transfer System (Bio-Rad, Cat. No. 170-4155) at 0.1A for 30 min. TBS-T (0.5% Tween 20 in TBS) was used as washing buffer and 4% non-fat dried milk in TBS-T was used as blocking reagent.

Transferred proteins were incubated for 1 h at room temperature under shaking with 1:4000 dilution of anti-his antibody coupled with peroxidase (Sigma, Cat. No. A7058). After washes, the reagent DAB substrate (ThermoFisher Scientific, Cat. No. 34002) was used to detect the protein of interest according to the supplier's instructions. Substrate specificities of pectin lyase were tested on following substrates: polygalacturonic acid (Sigma, Cat. No. 81325); *Citrus* pectin, degree of methylesterification (DM) 20-34% (Sigma, Cat. No. P9311); *Citrus* pectin, DM 55-70% (Sigma, Cat. No. P9436); *Citrus* pectin, DM >85% (Sigma, Cat. No. P9561); apple pectin DM 70-75% (Sigma, Cat. No. 76282); sugar beet pectin, DM 42%, degree of acetylation 31% (CPKelco). Pectin lyase activity from purified BcPNL1 was determined adapted from Albersheim (1966). 25 µl of purified BcPNL1 (17.2 ng) was added into 100 µl of pre-heated 0.5 % (w/v) substrate in 50 mM Tris-HCl buffer (pH 7.8) and incubated at 40 °C. Pectin lyase activity of forming unsaturated products was determined by measuring a linear increase in absorbance at 235 nm for 20 min.

The optimal pH of BcPNL1 was assayed in 50 mM with glycine-NaOH buffer (pH 7.7-10.0) and Tris-HCl buffer (pH 6.8-8.2) at 40°C, according to the same conditions as previously described and using high DM *Citrus* pectin as substrate.

One enzyme unit is defined as the formation of 1 µmol unsaturated pectin per min, with a molar extinction coefficient of 5500 M^{-1} cm^{-1}. Each enzymatic measurement was performed in triplicate.

OG characterization and quantification

Hydrophilic interaction liquid chromatography (HILIC). Pectin digests, diluted to 1 mg/ml in 50% (v/v) acetonitrile, were analyzed using an UltiMate™ 3000 RSLCnano System system (Thermo Scientific, Waltham, MA, USA) coupled to an Impact II UHR-QqTOF (Bruker). Chromatographic separation was performed on an Acquity UPLC BEH HILIC column (1.7 µm, 2.1 mm × 150 mm, Waters Corporation, Milford, MA, USA). Elution was

performed at a flow rate of 500 µl/min and a column oven temperature of 40 °C. The injection volume was set to 1 µl.

The composition of the two mobile phase lines was (A) 99:1(v/v) water/acetonitrile 15 mM with (water/ACN) 0.1% formic acid, (B) 90% (v/v) ACN ammonium formate 15 mM/ formic acid 0.1%. The following elution profile was used: 0–1 min, isocratic 100% B; 1–30 min, linear from 100% to 60% B; followed by column re-equilibration; 35–45 isocratic 100% B. MS-detection was performed in negative mode with the end plate offset set voltage to 500 V, capillary voltage to 2500 V, Nebulizer 50 psi, dry gas 10 l/min and dry temperature 200 °C. Mass spectra were acquired over the scan range m/z 150–2000. Compass 1.8 software, (Bruker Daltonics) was used to acquire and process the data.

High-performance size-exclusion chromatography (HP-SEC). Samples were diluted at 1 mg/ml in ammonium formate 50 mM, formic acid 0.1%. Chromatographic separation was performed on an ACQUITY UPLC Protein BEH SEC Column (125Å, 1.7 µm, 4.6 mm X 300 mm, Waters Corporation, Milford, MA, USA). Elution was performed in 50 mM ammonium formate, formic acid 0.1% at a flow rate of 400 µl/min and a column oven temperature of 40 °C. The injection volume was set to 10 µl. MS-detection was performed in negative mode with the end plate offset set voltage to 500 V, capillary voltage to 4000 V, Nebulizer 40 psi, dry gas 8 l/min and dry temperature 180 °C.

Data analysis

Major peaks were annotated following accurate mass annotation, isotopic pattern and MS/MS analysis. The MS fragmentation pattern is indicated according to the nomenclature of Domon and Costello (**7**). The fragments are designated as X for cross-ring cleavages and Y, Z for glycosidic bond cleavages when charge is retained at the reducing end and A (cross ring cleavages) and B, C (glycosidic bond cleavages) when charge is retained at the non-reducing

end. Sugars, indicated as subscript number, are numbered from the reducing end for X, Y and Z ions and from the non-reducing end for the others. For cross-ring cleavages, the cleaved bonds are indicated by superscript numbers. At last, ions produced as a result of more than one cleavage are designated with a slash between cleavage sites (e.g. $^{0,2}A_4/^{1,5}X_2$). We took in account that Z- and C-type were found to be dominant over Y- and B-type ions in negative ion mode (**8-10**).

For the targeted analysis of 72 specific oligosaccharides (see **Table S2**), the theoretical exact masses were used with 4 significant figures with a scan width of 5 ppm. The resulting extracted ion chromatograms were integrated and the area under the curve was used for relative quantitation. Data were analyzed using principle component analysis (PCA). The heatmap presented in **Fig. 7a** was generated using ClustVis (http://biit.cs.ut.ee/clustvis).

Semi-purification of OGs and GUS quantification

OG-containing samples corresponding to 16 leaves infected either by WT strain or *Bcpme1/2* were desalted using Pierce Graphite spin column (Thermo Scientific, Waltham, MA, USA). After desalting, both samples were dried and then resuspended at a final concentration of 50 µg/ml; A final concentration of OGs were next infiltrated in leaves of 5-week-old seedlings expressing the defense reporter construct *pAtPGIP1::GUS*. GUS activity analyses were performed on the aerial part of 5-week-old seedlings as described (**11)** with some modifications: the GUS buffer does not contain any β-mercaptoethanol and the measures were performed with a fluoroscan ascent (Thermo Scientific, Waltham, MA, USA). Three pools of two leaves of two different replicates were analyzed. For GUS staining of infiltrated leaves, hypocotyls and leaves infected with fungal strains, leaves expressing the promoter *pGIP1-GUS* fusion were submerged in GUS buffer, infiltrated 3 times (2 min) under vacuum and incubated at 37°C for 5h (**12-14**).

Size-exclusion chromatography and multi-angle laser-scattering

Pectins (1 mg/ml in 0.1 M $LiNO_3$) were injected on an on-line size-exclusion chromatography (SEC) column coupled with multi-angle laser-light scattering (MALLS), a differential refractive index (dRI) detector and a viscometer (Viscostar, Wyatt Technology Inc. (Santa Barbara, USA). Experiments were performed as described (**15**).

Quantitative RT-PCR

After incubation for 6 and 16 h of WT strain on *A. thaliana* leaves, total fungal RNA was extracted from harvested biomass using Trizol reagent (Invitrogen, Carsbad, CA, U.S.A.). Reverse transcription was performed using an oligo-dT20 for a primer and Superscript II RnaseH-reverse transcriptase (Invitrogen). Real-time quantitative PCR analysis was performed using Bio-rad Cfx Connect. A 1:5 dilution of cDNA (2.5 µl) was amplified in a 7.5 µl reaction mix containing Power Sybr green PCR master mix (Applied Biosystems) and 10 µM of each primer (**Table S3**). Gene expression values were normalized to expression of *B. cinerea* actin gene.

After incubation for 3 h with semi-purified OGs (50 µg/mL), leaves were quick-frozen in liquid nitrogen and stored at -80°C. RNA was extracted using Qiagen RNAeasy kit (Qiagen, Paris, France). 1µg of RNA was treated with RNA-free DNAse and used for RT-PCR using the RevertAid H using ingredients from ThermoFisher (Villebon-sur-Yvette, France). 10 ng of RNA was used for each q-PCR. 3 technical repeats were performed for each primer pair (**Table S3)** and 3 biological repeats for each sample. Transcript levels were normalized using the reference gene *UBI4* according to the formula E*2^(Cq target – Cq ref). Gene transcript levels were expressed relative to the mock (treatment without OG), i.e. the calibrator for which expression level was set to 1.

Affymetrix Microarray analysis

24 h after infection with Bcpme or WT strains,leaves were collected and RNA was extracted. Total RNA extraction was carried out from three biological replicates of infected leaves using the Nucleospin RNA XS purification kit according to the manufacturer's instructions (Macherey Nagel, Germany), including the removal of genomic DNA. RNA samples were quantified using a Nanodrop spectrophotometer (Thermo Scientific, USA) and quality control (RIN>8) was assessed by a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA).

cRNAs, prepared from 100 ng of total RNA according to the GeneChip Whole Transcript (WT) PLUS protocol (Affymetrix, Thermo Fisher Scientific, USA), were used to generate single-stranded DNAs, which were fragmented and biotinylated according to the manufacturer's instructions. The labelled single-stranded DNAs were hybridized for 18 hours at 48 °C on Affymetrix four-arrays strips (Arabidopsis Gene 1.1 ST Array strip) in Affymetrix GeneAtlas hybridization station. After hybridization, strips were washed (Affymetrix GeneAtlas Fluidics Station) and imaged (GeneAtlas imaging station).

Data were normalized using the Expression Console software (Affymetrix) using default RMA-sketch normalization. Normalized files were then analyzed by Transcriptome Analysis Console (TAC) 4.0 software (Affymetrix) including Limma differential expression analysis with eBayes correction of ANOVA variance using default settings. Normalized expression values were filtered for statistical relevance of differential expression using FDR F-Test p-value<0,01. Affymetrix Microarray data will be available in the Gene expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE120933).

Statistical analyses

For testing the normality of distribution, the Shapiro-Wilk test was performed. For normally

distributed data, a Bartlett's test was performed to compare the variance of samples. Unpaired t-tests were used. For smaller set of data, we performed a Mann-Whitney test.

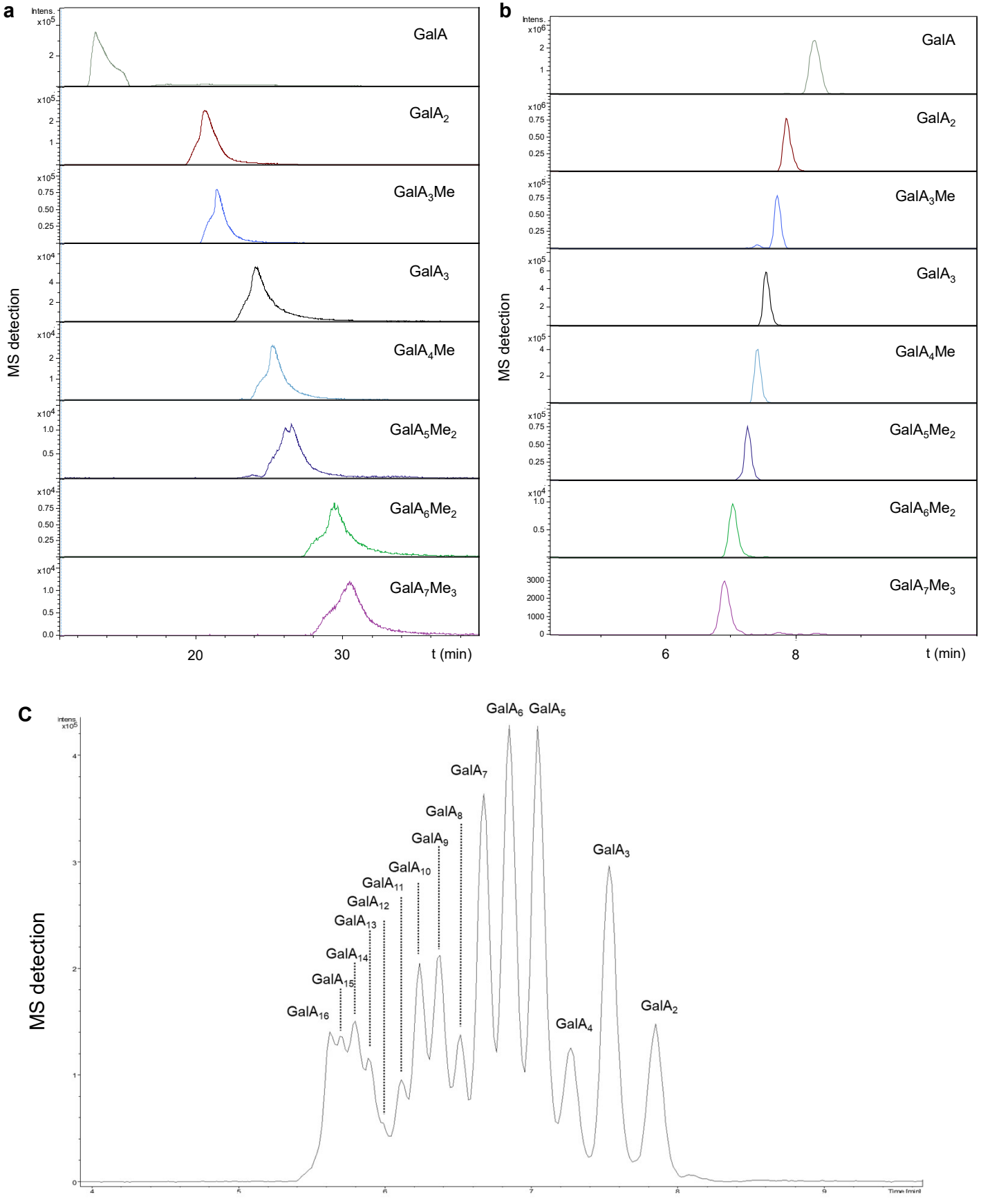

Fig. S1. Elution profile of various oligogalacturonides using MS-detection. (**a**) Separation of Oligogalacturonides (OG) **produced by *Aspergillus aculeatus* polygalacturonase from citrus pectins** on a BEH-HILIC column. (**b**) Separation of OGs **produced by *Aspergillus aculeatus* polygalacturonase from citrus pectins** on a HP-SEC column. (**c**) Separation of OGs from autoclaved polygalacturonic acid on a HP-SEC column. OGs are named $GalA_xMe_y$. Subscript numbers indicate the degree of polymerization and the number of methylester groups respectively. GalA: galacturonic acid; Me: methylester group; Intens.: signal intensity.

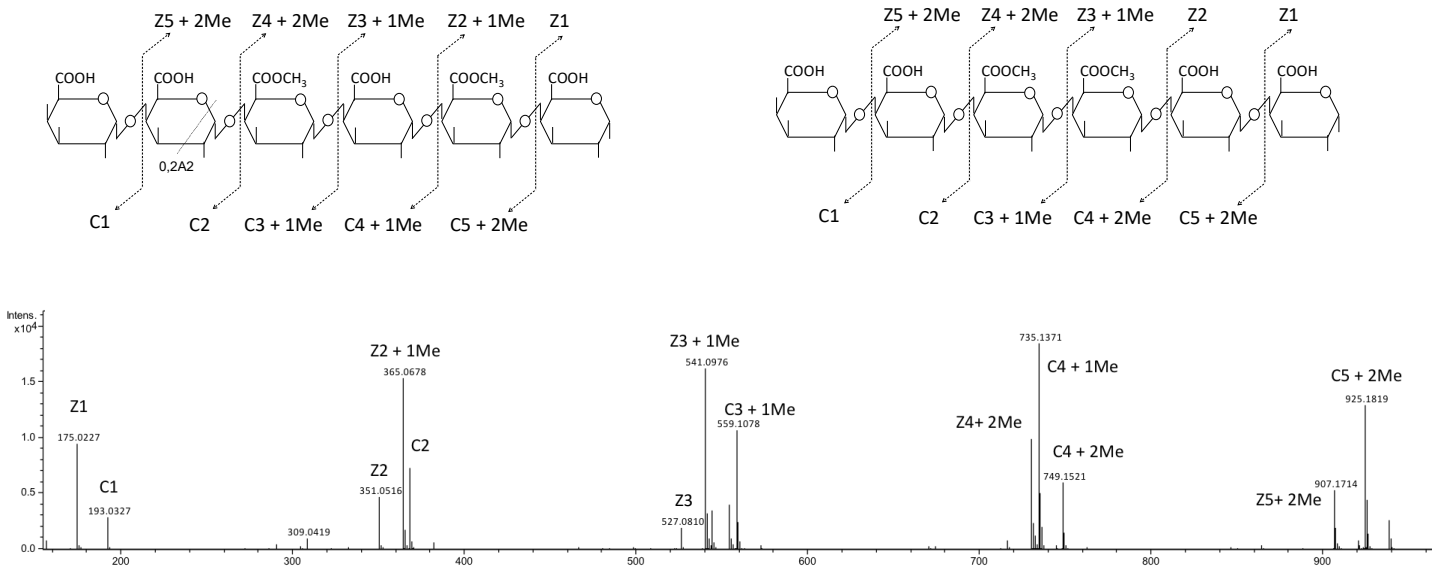

Fig. S2. Example of oligogalacturonides MS² fragmentation pattern. MS^2 fragmentation pattern of $GalA_6Me_2$ oligomer (m/z 550.118) produced by *Aspergillus aculeatus* polygalacturonase from sugar beet pectins. Subscript numbers indicate the degree of polymerization and the number of methylester groups respectively. GalA: galacturonic acid; Me: methylester group; Intens.: signal intensity.

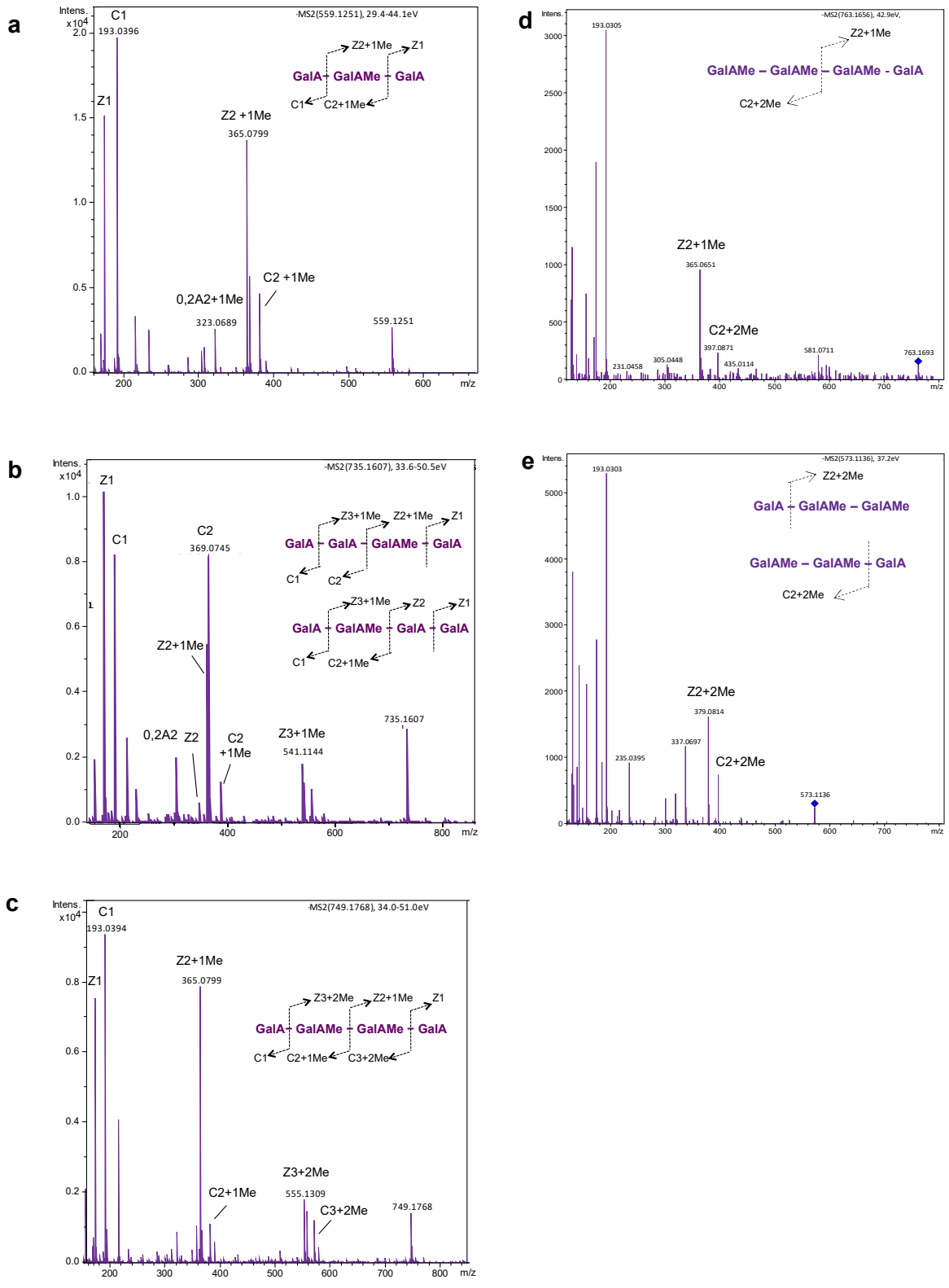

Figure S3. MS² fragmentation patterns of various short oligogalacturonides produced by *Aspergillus aculeatus* polygalacturonase from sugar beet pectins. (a) $GalA_3Me$, (**b**) $GalA_4Me$, (**c**) $GalA_4Me_2$, (**d**) $GalA_4Me_3$, (**e**) $GalA_3Me_2$. OGs are named $GalA_xMe_y$ Subscript numbers indicate the degree of polymerization and the number of methyl groups respectively. GalA: galacturonic acid; Me: methylester group; Intens.: signal intensity.

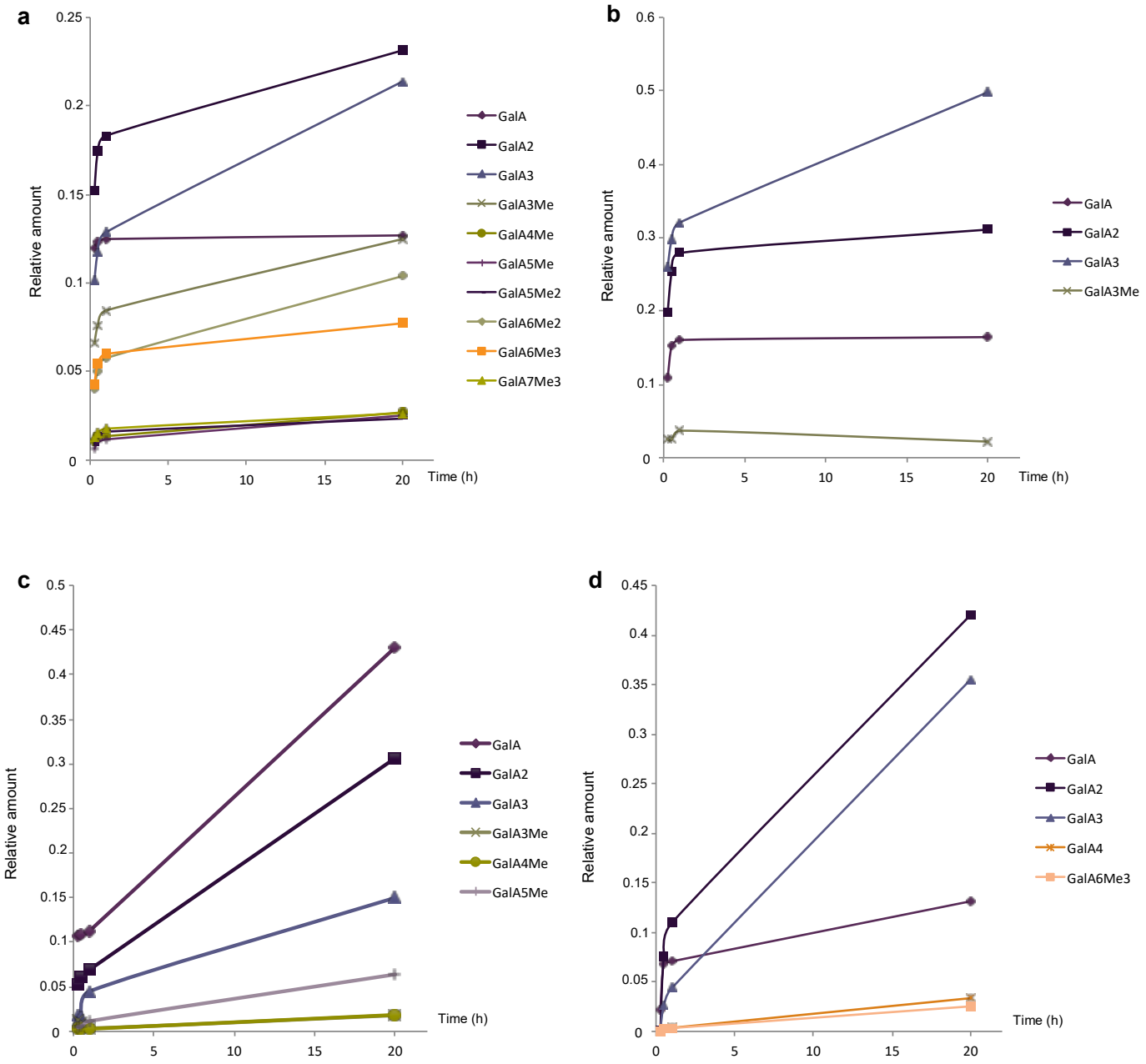

Figure S4. **Kinetics of oligogalacturonides produced over time by the pectinolytic activities of purified *Botrytis cinerea* enzymes from commercial citrus peel pectins.** (**a**) Oligogalacturonides (OG) released by BcPG2 over time. (**b**) Impact of BcPME1 on OGs released by BcPG2 over time. (**c**) OGs released by BcPG3 over time. (**d**) Impact of BcPME1 on OGs released by BcPG3 over time. OGs are named $GalA_xMe_yAc_z$. Subscript numbers indicate the degree of polymerization and the number of methyland acetyl-ester groups. GalA: galacturonic acid; Ac: acetylester group; Me: methylester group.

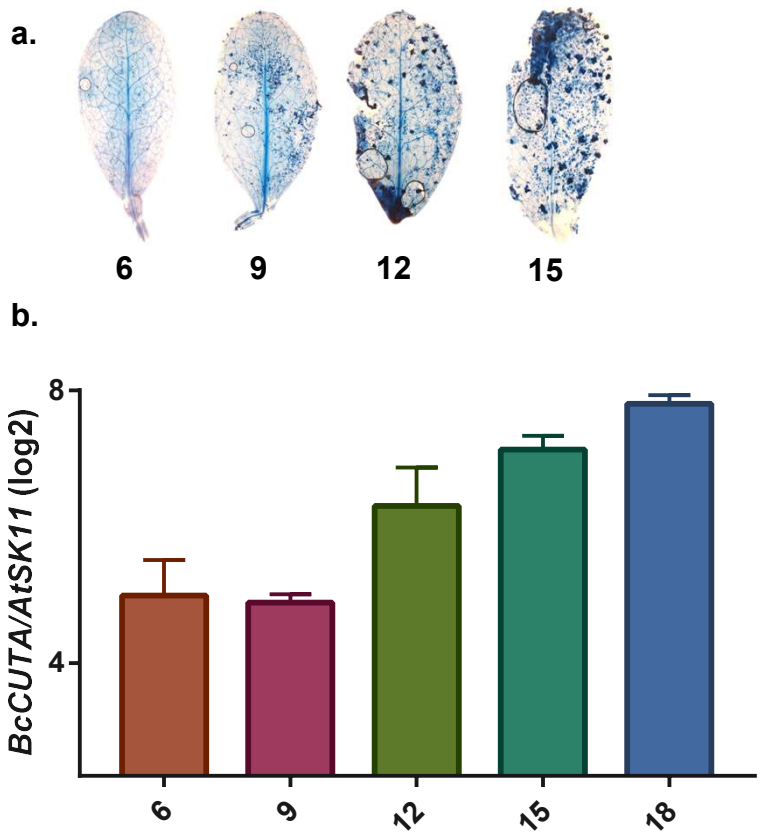

Figure S5 : *Botrytis cinerea* growth over *Arabidopsis thaliana* leaf infection in liquid medium. (a). *A. thaliana* leaves stained with trypan blue after 6, 9, 12 and 15 hours of incubation in solution containing 3.10^5 spore/mL . **(b).** Abundance of *B. cinerea CUTINASE* A (*BcCUTA*) quantified in 6, 9, 12, 15 and 18 hours -infected leaves and normalised with the plant *SHAGGY-RELATED KINASE 11* (*AtSK11*) gene.

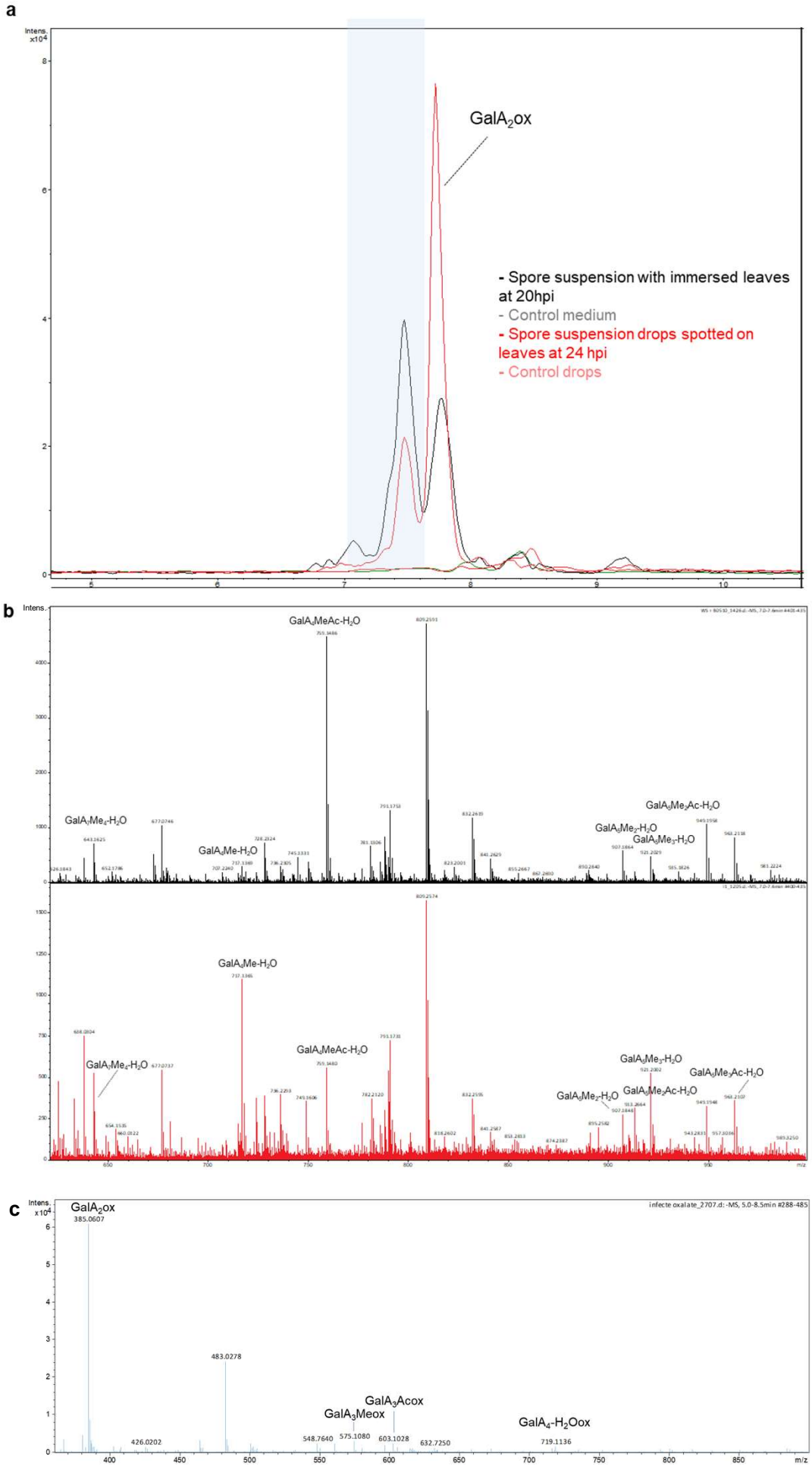

Figure S6. Comparison of OGs produced upon *Arabidopsis thaliana* - *Botrytis cinerea* interaction.
(**a**) Comparison of extracted ion chromatograms obtained by HP-SEC–MS analysis in negative ionization mode of oligogalacturonides (OG) endogenously produced from spore drops spotted on leaves 24 hpi (red) or from immersed leaves in spore medium 20 hpi (black) during infection of *A. thaliana* leaves infected by WT *B. cinerea* strain. Extracted ion chromatograms obtained for control drops (light red) and control medium (light dark) are also presented. (**b**) MS identification of OGs of DP4 and DP5 from spore drops spotted on leaves (red) or from immersed leaves in spore medium (black) during infection of *A. thaliana* leaves infected by WT *B. cinerea* strain. (**c**) MS identification of OGs detected from cell wall of leaves spotted with spore drops 24 hpi incubated in a strong chelating agent. OGs are named $GalA_xMe_yAc_z$. Subscript numbers indicate the degree of polymerization and the number of methyl and acetyl- ester groups respectively. GalA: galacturonic acid; Ac: acetylester group; Me: methylester group; Intens.: signal intensity.

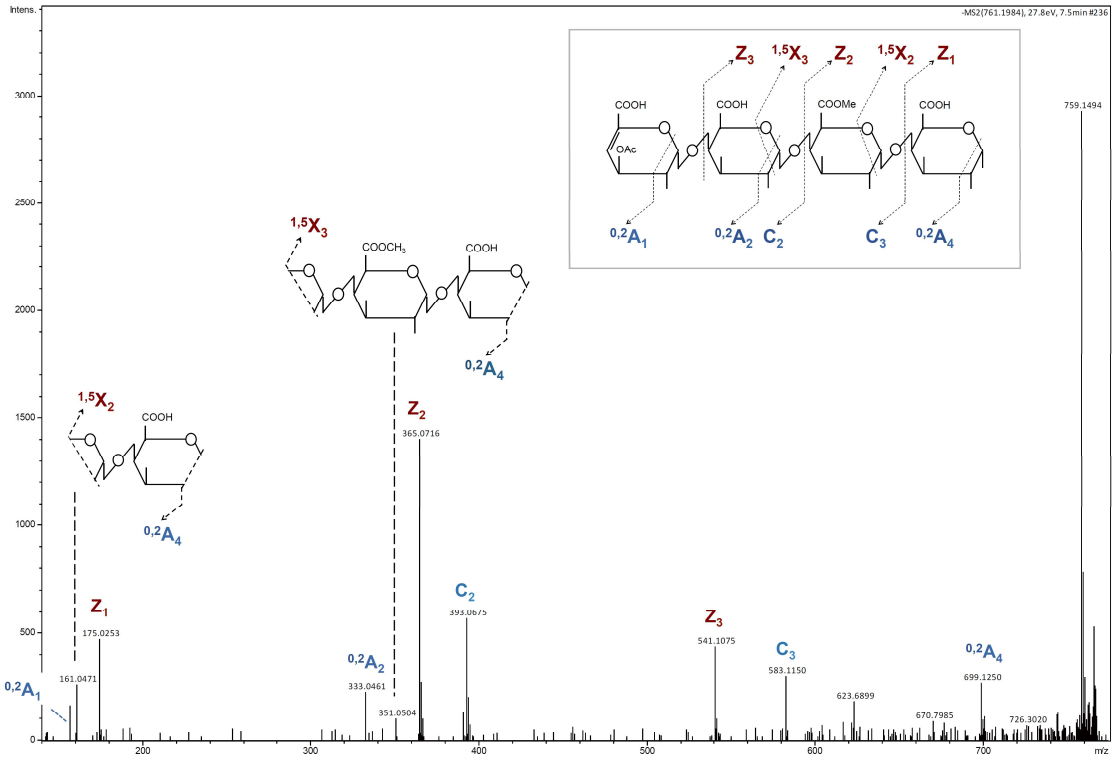

Figure S7. Fragmentation pattern of GalA4MeAc-H_2O.

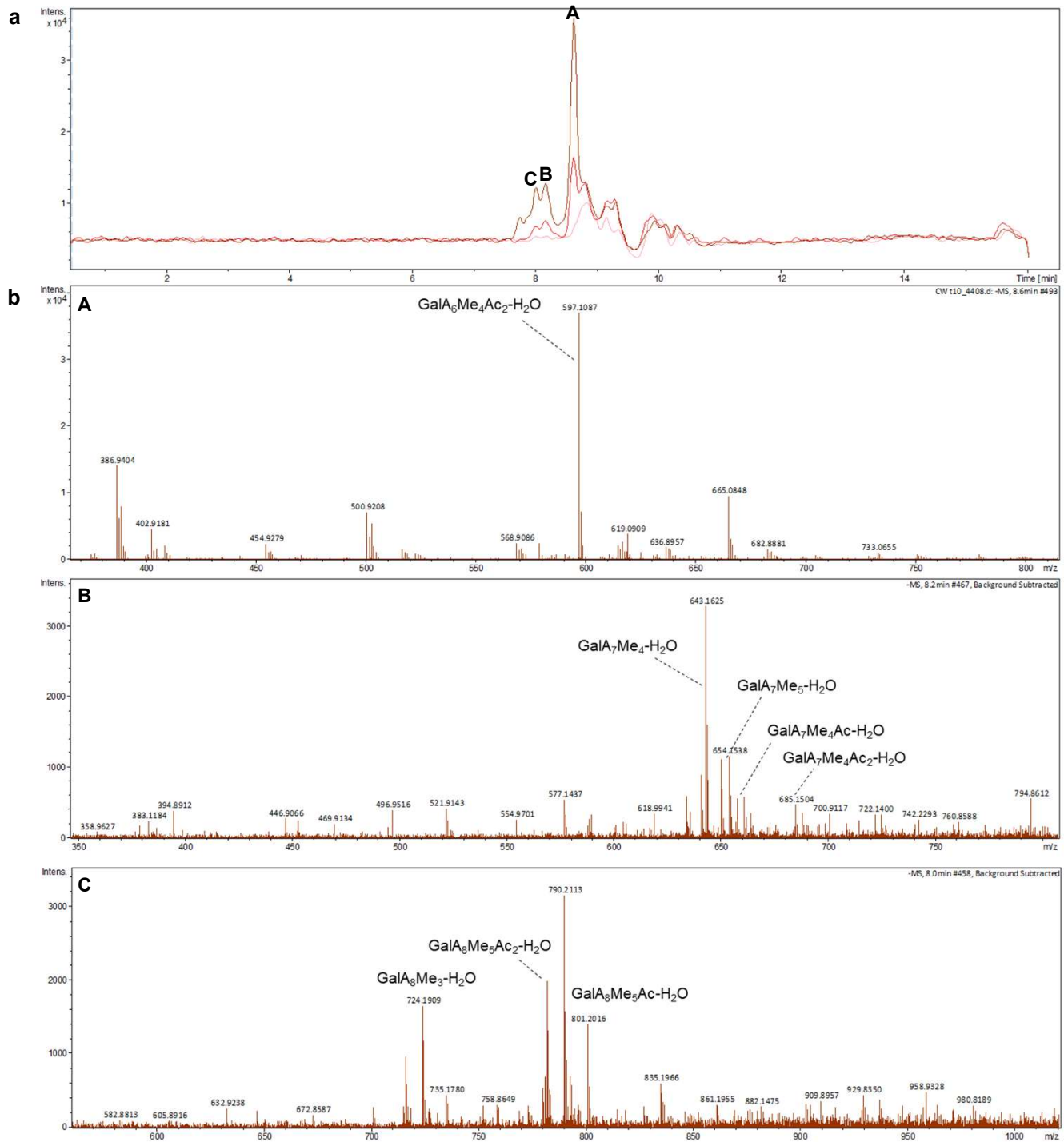

Figure S8. Analysis of OGs released by *Botrytis cinerea* from *Arabidopsis thaliana l*eaf cell wall.
(**a**) Comparison of extracted ion chromatograms obtained by HP-SEC–MS analysis in negative ionization mode of oligogalacturonides (OG) produced from immersed cell wall in *B. cinerea* spore medium after 0 (pink), 5 (red) and 10 hours (brown) of incubation (**b**) MS identification of OGs of peaks A,B and C. OGs are named $GalA_xMe_yAc_z$. Subscript numbers indicate the degree of polymerization and the number of methyl and acetyl-ester groups respectively. GalA: galacturonic acid; Ac: acetylester group; Me: methylester group; Intens.: signal intensity.

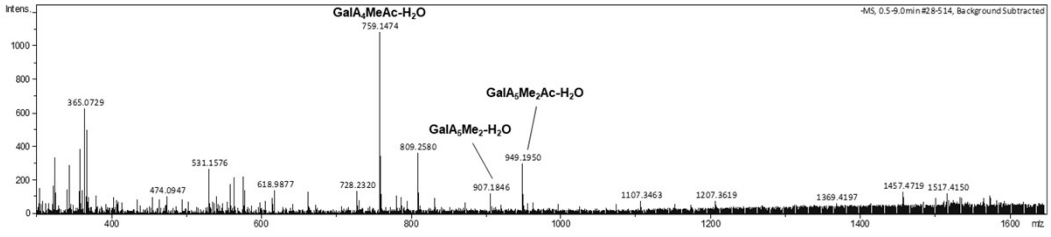

Figure S9. Spectrum of semi-purified OG preparations from infected leaves by *Botrytis cinerea*.

a

```
PelA - A. niger          MKYSTIFSAAAAVFA-GSAAAVGVSGSAEGFAEGVTGGGDATPVYPDTIDELVSYLGDDE
BcPNL1 - B. cinerea      --MVSIKTLAILLFSVIAVDAAAVIGKAEGFAASATGGGSAAGASPKDLTELVNWLSDGV
                             :* : *  :*:  :. *..* *.***** ..****.*: . *. : ***.:*.*.

PelA - A. niger          ARVIVLTKTFDFTDSEGTTTGTGCAPWGTAS---ACQVAIDQDDWCENYEPDA---PSVS
BcPNL1 - B. cinerea      ARTIVLDKTWDFTGSMGSKTEKGCTPLSNTCTNGAGQDSVDINGWCEQAANANQNLPKPT
                         **.*** **:***.* *:.* .**:* ..:.   * * ::* :.***:        *. :

PelA - A. niger          VEYYNAG--VLGITVTSNKSLIGEGSSGAIKGKGLRIVSGAENIIIQNIAVTDINPKYVW
BcPNL1 - B. cinerea      ITYDVAGIPPSAIKLGSNKSIIGVGSAGKIKGRGFYI-AGAKNIIIQNVEFIEMNPKYIW
                         : *  **    .*.: ****:** **:* ***:*: * :**:******: . ::****:*

PelA - A. niger          GGDAITLDDCDLVWIDHVTTARIGRQHYVLGTSADNRVSLTNNYIDGVSDYSATCDGYHY
BcPNL1 - B. cinerea      GGDAISVDGTDLLWIDHVKISRIGRQFITMGPGASNRVTISNSEFDGTTLWSAKCNNHHY
                         *****::*. **:*****. :*****. .:* .*.***:::*. :**.: :**.*:.:**

PelA - A. niger          WGIYLDGDADLVTMKGNYIYHTSGRSPKVQ-------DNTLLHCVNNYFYDISGHAFEIG
BcPNL1 - B. cinerea      WTLYFTGSQDTVTFKGNYIHNTSGRGPKVGGLHSSVTPNVFLQASNNYWSNVAGNAFQIG
                         * :*: *. * **:*****::****.***         *.:*:. ***: :::*:**:**

PelA - A. niger          EGGYVLAEGNVFQNVDTVLETYEGAAFTVPSTTAGEVCSTYLGRDCVINGFGCSGTFSED
BcPNL1 - B. cinerea      EGAKVLAEGNIFESVTTPVIFDITSNPLWSSSSGVAGCASILGRNCQANSLVSSGSFAGT
                         **. ******:*:.* * :     :     *::.   *:: ***:*  *.: .**:*:

PelA - A. niger          STSFLSDFEGKNIASASAYTSVASRVVANAGQGNL
BcPNL1 - B. cinerea      NTNVLTAAKGLSLASVADVGTTKASVLKNAGIGKI
                         .*..*:  :* .:**.:   :. : *: *** *::
```

b

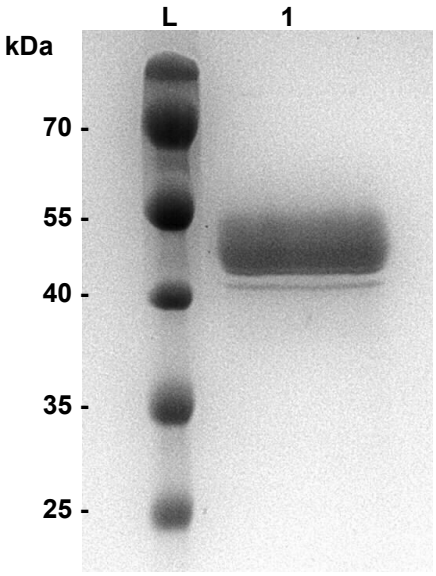

Figure S10. Sequences alignment and expression of BcPNL1.
(**a**) Sequence alignment of BcPNL1 and pectin lyase, family 1 (PelA) of *Aspergillus niger*. The residues underlined are involved in the enzyme-substrate interaction. The arginine residues, boxed in grey, are predicted to be N-glycosylated. (**b**) Western blot verification of the expression of BcPNL1 by using anti-his antibodies and chromogenic detection. L: molecular weight markers.

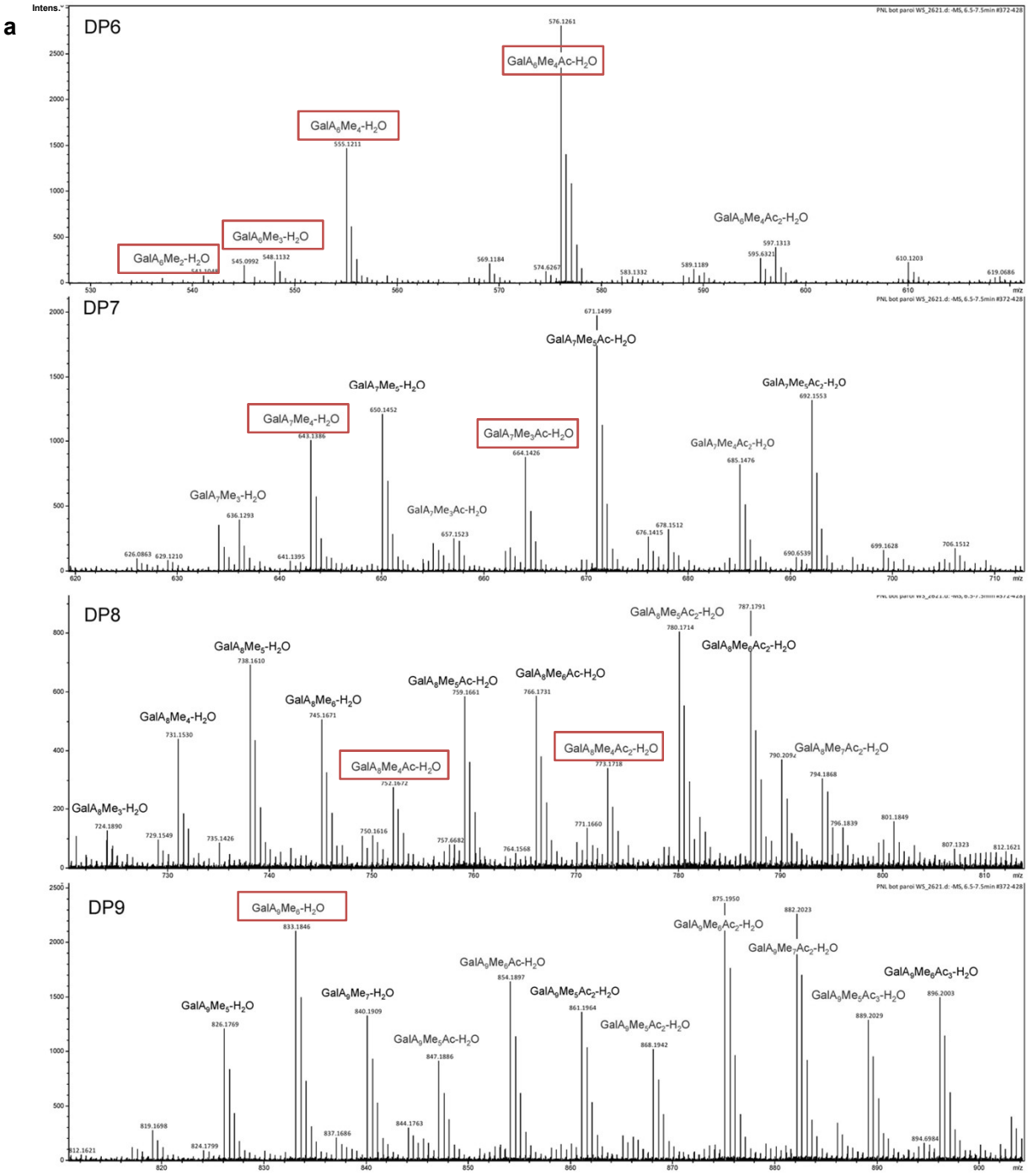

Figure S11. MS identification of OGs from DP6 to DP9 released by BcPNL1 from *Arabidopsis thaliana* leaf cell wall. Boxed oligogalacturonides (OG) are also detected in OGs produced during the *Arabidopsis thaliana* - *Botrytis cinerea* interaction. OGs are named $GalA_xMe_yAc_z$. Subscript numbers indicate the degree of polymerization and the number of methyl and acetyl- ester groups respectively. GalA: galacturonic acid; Ac: acetylester group; Me: methylester group; Intens.: signal intensity.

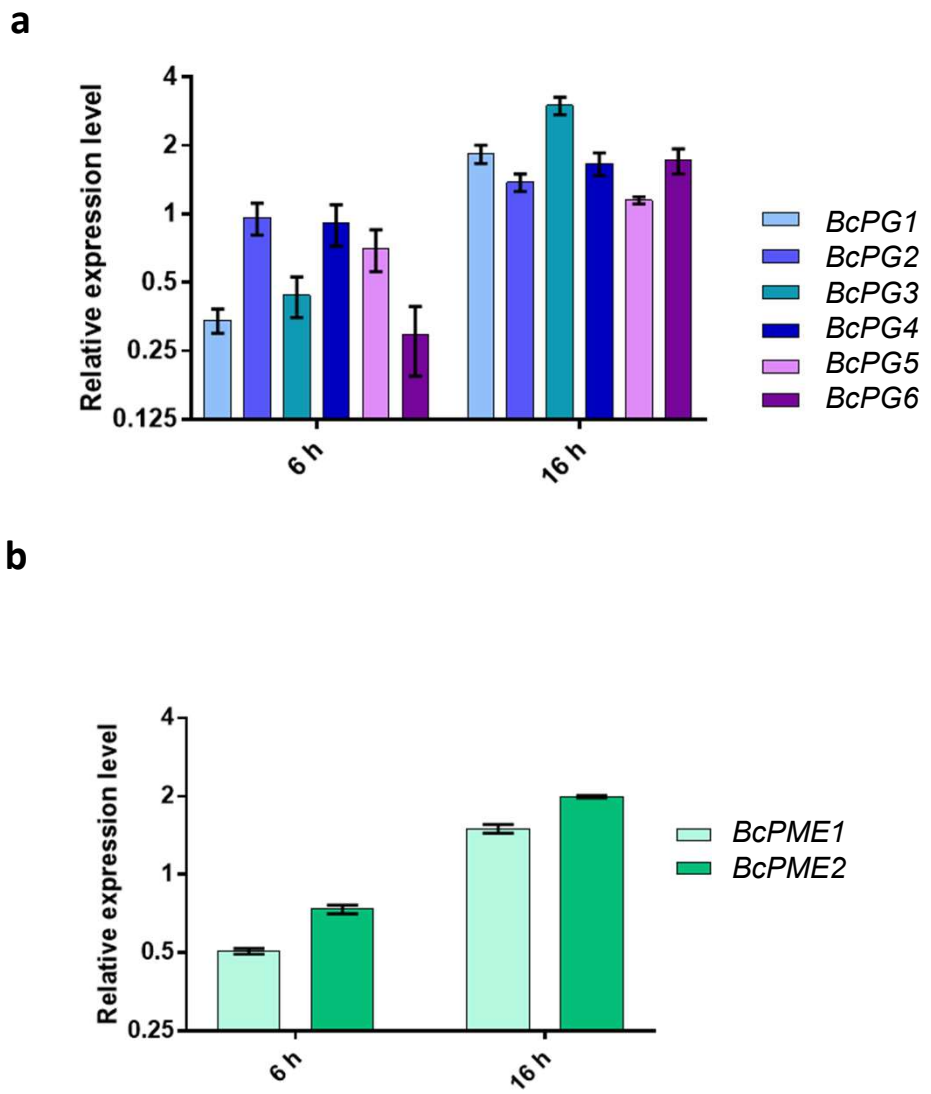

Figure S12. ***Botrytis cinerea BcPG1* to *BcPG6* and *BcPME1* and *BcPME2* gene expression** measured by RT-qPCR with the reference *ACTIN* gene as an internal control after 6 and 16 hours of incubation with citrus pectins n = 3.

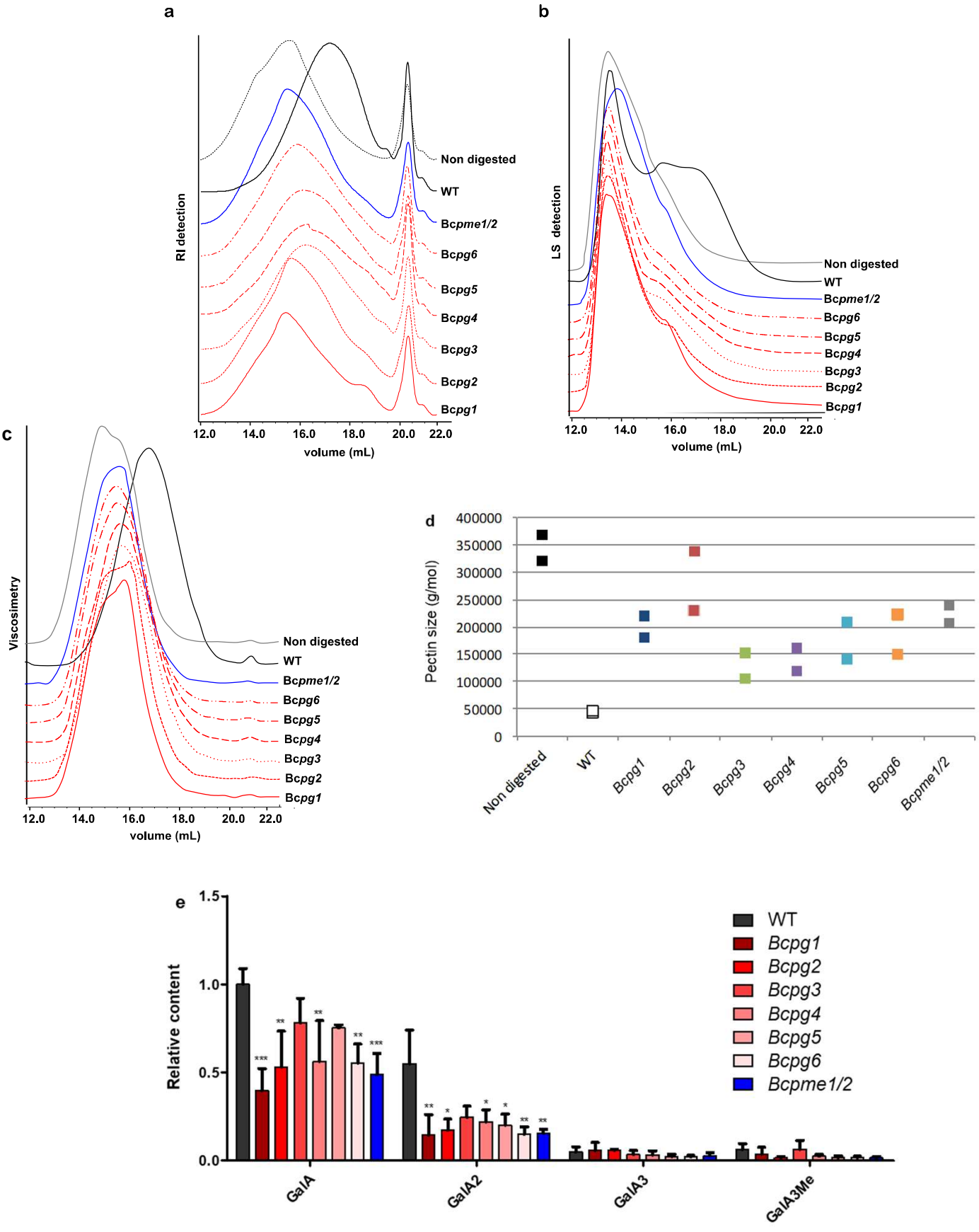

Figure S13. **Size-exclusion chromatographic (SEC) profiles of non-digested and digested citrus pectins by WT *Botrytis cinerea* strain, *Bcpme1/2* and *Bcpg1* to *Bcpg6* mutants after 6 h of incubation.** (**a**) Light scatter detection of size-exclusion chromatographic profile of non-digested and digested citrus pectins by WT *B. cinerea* and mutant strains after 6 h of incubation coupled to light scatter detection. (**b**) Refractive index detection of SEC profiles. (**c**) Viscosity detection of SEC profiles. (**d**) Pectin size after 6 h of incubation assessed by SEC-MALLS analysis. n= 2. (**e**) Characterization of oligogalacturonides released after 6 h of incubation. Data are means ± SD; n = 3. OGs are named $GalA_xMe_y$. Subscript numbers indicate the degree of polymerization and the number of methyl respectively. GalA: galacturonic acid; Me: methylester group.

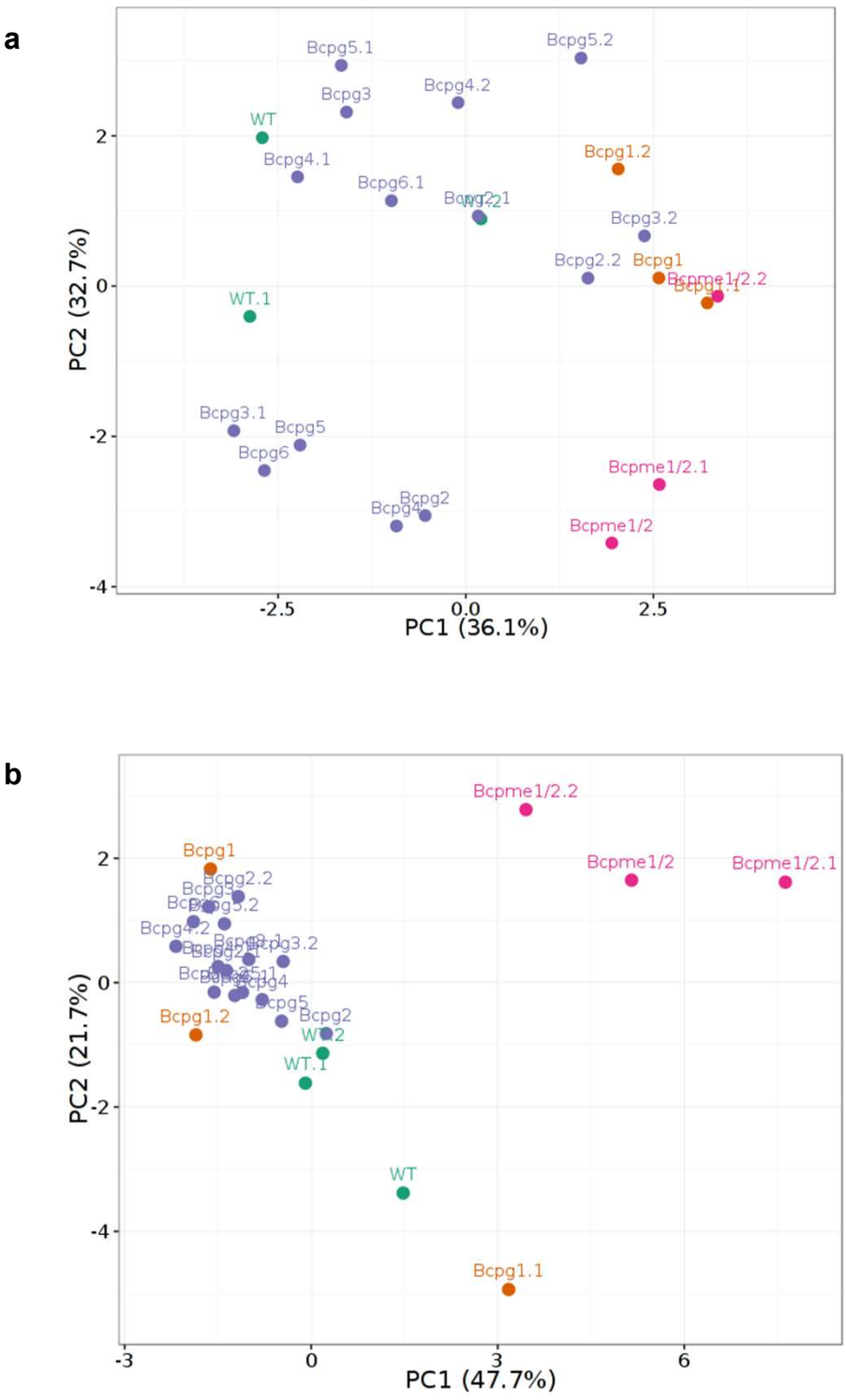

Figure S14. Principal component analysis (PCA) of oligogalacturonides production by WT *Botrytis cinerea* and mutant strains from citrus pectins. Unit variance scaling is applied to rows; SVD with imputation is used to calculate principal components. X and Y axis show principal component 1 (PC1) and principal component 2 (PC2) respectively. **(a**) PCA assuming genotypes as variables after 16 h of incubation. PC1 and PC2 explain 36.1 % and 32.7 % of the total variance respectively. (**b**) PCA assuming genotypes as variables after 24 h of incubation. PC1 and PC2 explain 47.7 % and 21.7 % of the total variance respectively.

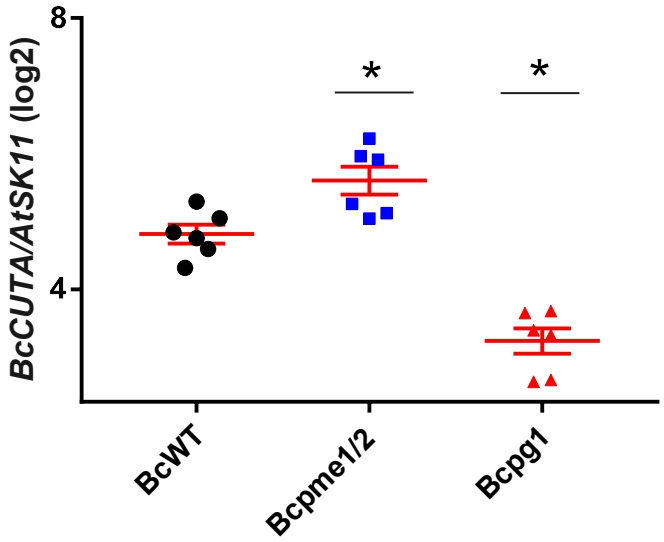

Figure S15 : Abundance of *B. cinerea CUTINASE A* (*BcCUTA*) quantified in *Arabidopsis thaliana* 5-week-old rosette leaves infected by BcWT, *Bcpme1/2* and *Bcpg1* 72 hours post infection and normalised with the plant *SHAGGY-RELATED KINASE 11* (*AtSK11*) gene.

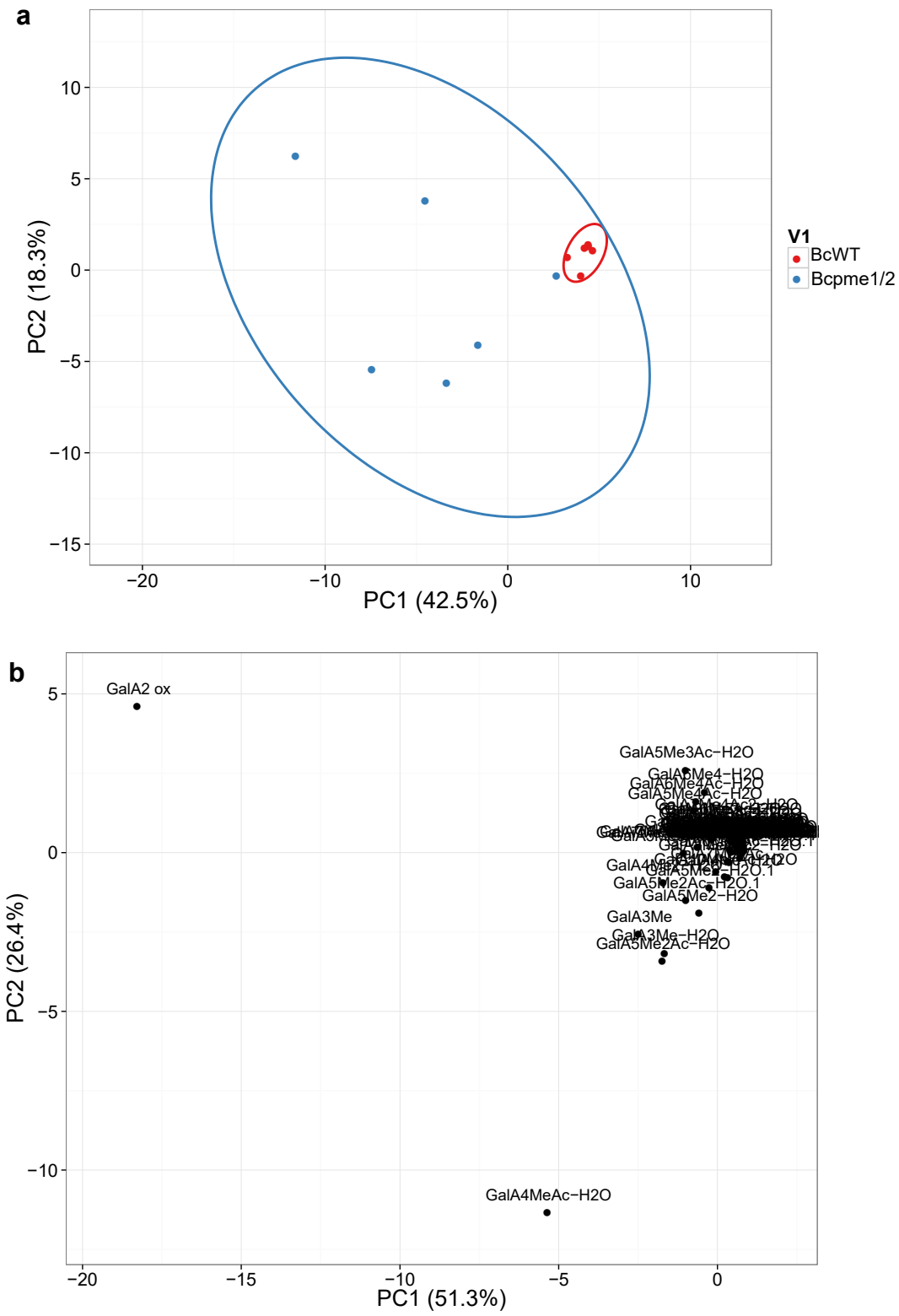

Figure S16. **Principal component analysis (PCA) of oligogalacturonides production by WT *Botrytis cinerea* strain and *Bcpme1/2* 20 hours post infection of *Arabidopsis thaliana* leaves.** Unit variance scaling is applied to rows; SVD with imputation is used to calculate principal components. X and Y axis show principal component 1 (PC1) and principal component 2 (PC2). (**a**) PCA assuming genotypes as variables. PC1 and PC2 explain 42.5 % and 18.3 % of the total variance, respectively. (**b**) PCA assuming OGs as variables. PC1 and PC2 explain 51.3 % and 26.4 % of the total variance, respectively. Oligogalacturonides (OGs) are named $GalA_xMe_yAc_z$. Subscript numbers indicate the degree of polymerization and the number of methyl- and acetyl-ester groups. GalA: galacturonic acid; Ac: acetylester group; Me: methylester group.

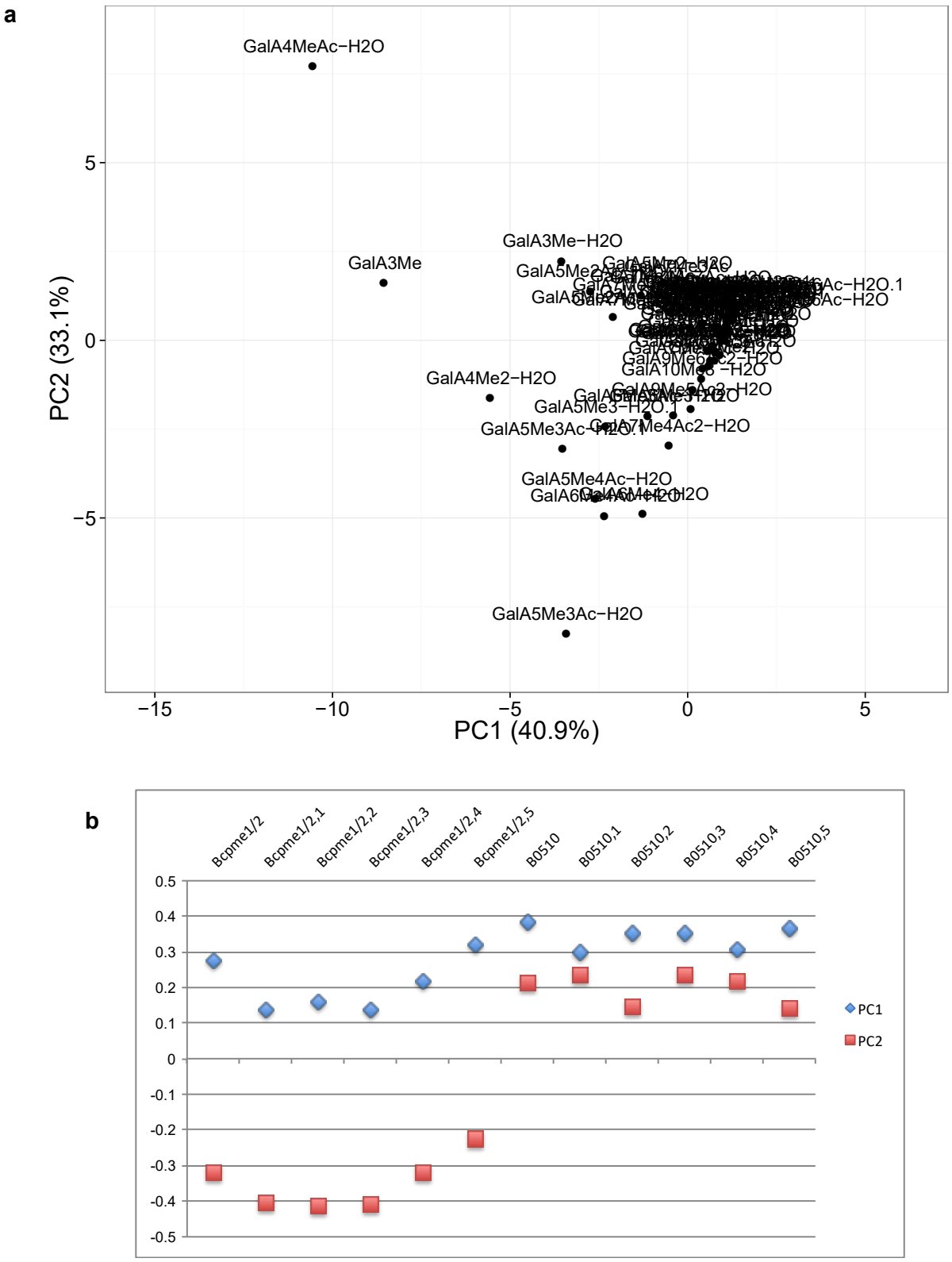

Figure S17. **Principal component analysis (PCA) of oligogalacturonides production by WT *Botrytis cinerea* strain and *Bcpme1/2* 20 hours post infection of *Arabidopsis thaliana* leaves after the exclusion of $GalA_2ox$.** Unit variance scaling is applied to rows; SVD with imputation is used to calculate principal components. X and Y axis show principal component 1 and principal component 2 respectively (**a**) PCA assuming Oligogalacturonides (OG) as variables. PC1 and PC2 explain 40.9 and 33.1 % repectively. (**b**) PCA loadings show that *Bcpme1/2* and the WT strains are separated according to PC2. OGs are named $GalA_xMe_yAc_z$. Subscript numbers indicate the degree of polymerization and the number of methyl- acetyl- ester groups. GalA: galacturonic acid; Ac: acetylester group; Me: methylester group.

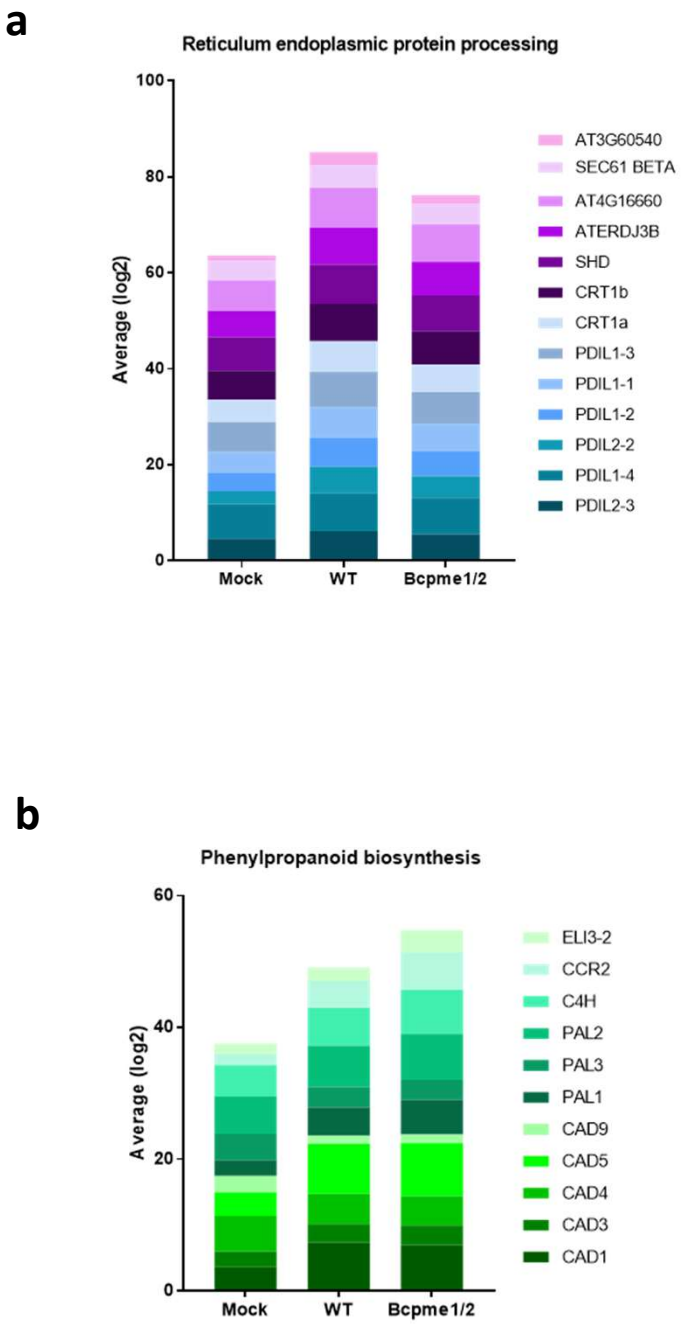

Figure S18. Different patterns of gene expression were detected in planta during *Bcpme1/2* or WT *Botrytis cinerea* infection. Quantification of transcripts involved in the processing of proteins in the endoplasmic reticulum (**a**) or in phenylpropanoid biosynthesis (**b**) in non-infected leaves (mock) and leaves infected by WT *Botrytis cinerea* and *Bcpme1/2* strains. Values of normalized transcript quantities from genes differentially expressed by WT *Botrytis cinerea* or *Bcpme1/2* strains compared to non-infected leaves (Log2, FDR F-Test p-value<0,01) are shown in supplementary dataset 1.

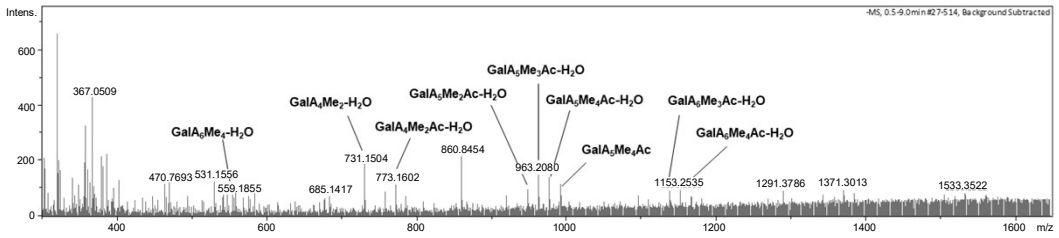

Figure S19. Spectrum of semi-purified OG preparations from *Bcpme1/2* infected leaves.

Supplementary Table 1. List of oligogalacturonides produced by the commercial *Aspergillus aculeatus* polygalacturonase from citrus and sugar beet pectins.

m/z	Ion formula	RT (sec)	Proposed structure	Citrus pectins	Sugar beet pectins
DP2					
369.0665	$C_{12}H_{17}O_{13}$	468.6	$GalA_2$	x	x
DP3					
545.0999	$C_{18}H_{25}O_{19}$	453.2	$GalA_3$	x	x
559.1154	$C_{19}H_{27}O_{19}$	463.3	$GalA_3Me$	x	x
573.1309	$C_{20}H_{29}O_{19}$	464.8	$GalA_3Me_2$		x
587.1102	$C_{20}H_{27}O_{20}$	452.5	$GalA_3Ac$		x
601.1255	$C_{21}H_{29}O_{20}$	463.9	$GalA_3MeAc$		x
615.1410	$C_{22}H_{31}O_{20}$	464.1	$GalA_3Me_2Ac$		x
643.1361	$C_{23}H_{31}O_{21}$	463.7	$GalA_3MeAc_2$		x
DP4					
735.1460	$C_{25}H_{35}O_{25}$	445.2	$GalA_4Me$	x	x
749.1611	$C_{26}H_{37}O_{25}$	453.9	$GalA_4Me_2$		x
763.1781	$C_{27}H_{39}O_{25}$	454.8	$GalA_4Me_3$		x
777.1575	$C_{27}H_{37}O_{26}$	444.5	$GalA_4MeAc$		x
791.1733	$C_{28}H_{39}O_{26}$	454.3	$GalA_4Me_2Ac$		x
819.1680	$C_{29}H_{39}O_{27}$	443.3	$GalA_4MeAc_2$		x
833.1837	$C_{30}H_{41}O_{27}$	454.2	$GalA_4Me_2Ac_2$		x
DP5					
911.1799	$C_{31}H_{43}O_{31}$	430.2	$GalA_5Me$	x	
462.0960	$C_{32}H_{44}O_{31}$	436.8	$GalA_5Me_2$	x	
476.0920	$C_{33}H_{44}O_{32}$	430.4	$GalA_5MeAc$		x
483.1001	$C_{34}H_{46}O_{32}$	436.9	$GalA_5Me_2Ac$		x
490.1077	$C_{35}H_{48}O_{32}$	445.2	$GalA_5Me_3Ac$		x
497.0983	$C_{35}H_{46}O_{33}$	428.6	$GalA_5MeAc_2$		x
504.1050	$C_{36}H_{48}O_{33}$	436.5	$GalA_5Me_2Ac_2$		x
511.1131	$C_{37}H_{50}O_{33}$	445.2	$GalA_5Me_3Ac_2$		x
518.1029	$C_{37}H_{48}O_{34}$	427.2	$GalA_5MeAc_3$		x
DP6					
550.1180	$C_{38}H_{52}O_{37}$	422.6	$GalA_6Me_2$	x	
557.1199	$C_{39}H_{54}O_{37}$	423.0	$GalA_6Me_3$		x
585.1139	$C_{41}H_{54}O_{39}$	416.2	$GalA_6MeAc_2$		x
592.1216	$C_{42}H_{56}O_{39}$	422.7	$GalA_6Me_2Ac_2$		x
599.1289	$C_{43}H_{58}O_{39}$	429.6	$GalA_6Me_3Ac_2$		x
606.1191	$C_{43}H_{56}O_{40}$	414.1	$GalA_6MeAc_3$		x
620.1345	$C_{45}H_{58}O_{40}$	428.7	$GalA_6Me_3Ac_3$		x

Supplementary Table 2. List of the oligogalactuturonides produced upon infection of *Arabidopsis thaliana* by *Botrytis cinerea.*

m/z	Ion formula	Proposed structure
DP2		
369.0665	$C_{12}H_{17}O_{13}$	$GalA_2$
385.0635	$C_{12}H_{17}O_{14}$	$GalA_2ox$
DP3		
545.0997	$C_{18}H_{25}O_{19}$	$GalA_3$
559.1876	$C_{19}H_{27}O_{19}$	$GalA_3Me$
575.1079	$C_{19}H_{27}O_{20}$	$GalA_3Meox$
603.1028	$C_{20}H_{27}O_{21}$	$GalA_3Acox$
DP4		
717.1356	$C_{25}H_{33}O_{24}$	$GalA_4Me\text{-}H_2O$
719.1136	$C_{24}H_{31}O_{25}$	$GalA_4\text{-}H_2Oox$
731.1516	$C_{26}H_{35}O_{24}$	$GalA_4Me_2\text{-}H_2O$
735.1484	$C_{25}H_{35}O_{25}$	$GalA_4Me$
749.162	$C_{26}H_{37}O_{25}$	$GalA_4Me_2$
759.1485	$C_{27}H_{35}O_{25}$	$GalA_4MeAc\text{-}H_2O$
379.0701	$C_{27}H_{34}O_{25}$	$GalA_4MeAc\text{-}H_2O$
773.1612	$C_{28}H_{37}O_{25}$	$GalA_4Me_2Ac\text{-}H_2O$
DP5		
453.0885	$C_{32}H_{42}O_{30}$	$GalA_5Me_2\text{-}H_2O$
907.1772	$C_{32}H_{43}O_{30}$	$GalA_5Me_2\text{-}H_2O$
460.0966	$C_{33}H_{45}O_{30}$	$GalA_5Me_3\text{-}H_2O$
921.1997	$C_{33}H_{45}O_{30}$	$GalA_5Me_3\text{-}H_2O$
925.1953	$C_{32}H_{45}O_{31}$	$GalA_5Me_2$
935.2148	$C_{34}H_{47}O_{30}$	$GalA_5Me_4\text{-}H_2O$
939.2096	$C_{33}H_{47}O_{31}$	$GalA_5Me_3$
474.0937	$C_{34}H_{44}O_{31}$	$GalA_5Me_2Ac\text{-}H_2O$
949.1931	$C_{34}H_{45}O_{31}$	$GalA_5Me_2Ac\text{-}H_2O$
481.102	$C_{35}H_{46}O_{31}$	$GalA_5Me_3Ac\text{-}H_2O$
963.2105	$C_{35}H_{47}O_{31}$	$GalA_5Me_3Ac\text{-}H_2O$
977.2264	$C_{36}H_{49}O_{31}$	$GalA_5Me_4Ac\text{-}H_2O$
981.2223	$C_{35}H_{49}O_{32}$	$GalA_5Me_3Ac$
995.2352	$C_{36}H_{51}O_{32}$	$GalA_5Me_4Ac$
DP6		
541.1041	$C_{38}H_{50}O_{36}$	$GalA_6Me_2\text{-}H_2O$
548.1125	$C_{39}H_{52}O_{36}$	$GalA_6Me_3\text{-}H_2O$
555.1203	$C_{40}H_{54}O_{36}$	$GalA_6Me_4\text{-}H_2O$
1111.2448	$C_{40}H_{55}O_{36}$	$GalA_6Me_4\text{-}H_2O$
569.1179	$C_{41}H_{54}O_{37}$	$GalA_6Me_3Ac\text{-}H_2O$
1139.2382	$C_{41}H_{55}O_{37}$	$GalA_6Me_3Ac\text{-}H_2O$
576.1243	$C_{42}H_{56}O_{37}$	$GalA_6Me_4Ac\text{-}H_2O$

1153.2527	$C_{42}H_{57}O_{37}$	$GalA_6Me_4Ac\text{-}H_2O$
1167.2759	$C_{43}H_{59}O_{37}$	$GalA_6Me_5Ac\text{-}H_2O$
590.123	$C_{43}H_{56}O_{38}$	$GalA_6Me_3Ac_2\text{-}H_2O$
597.1311	$C_{44}H_{58}O_{38}$	$GalA_6Me_4Ac_2\text{-}H_2O$
DP7		
636.1284	$C_{45}H_{60}O_{42}$	$GalA_7Me_3\text{-}H_2O$
643.137	$C_{46}H_{62}O_{42}$	$GalA_7Me_4\text{-}H_2O$
652.1416	$C_{46}H_{64}O_{43}$	$GalA_7Me_4$
657.134	$C_{47}H_{62}O_{43}$	$GalA_7Me_3Ac\text{-}H_2O$
664.1413	$C_{48}H_{64}O_{43}$	$GalA_7Me_4Ac\text{-}H_2O$
666.1371	$C_{47}H_{64}O_{44}$	$GalA_7Me_3Ac$
673.1468	$C_{48}H_{66}O_{44}$	$GalA_7Me_4Ac$
678.139	$C_{50}H_{68}O_{43}$	$GalA_7Me_6Ac\text{-}H_2O$
685.1473	$C_{50}H_{66}O_{44}$	$GalA_7Me_4Ac_2\text{-}H_2O$
DP8		
752.156	$C_{54}H_{72}O_{49}$	$GalA_8Me_4Ac\text{-}H_2O$
768.1696	$C_{55}H_{76}O_{50}$	$GalA_8Me_5Ac$
773.1612	$C_{56}H_{74}O_{50}$	$GalA_8Me_4Ac_2\text{-}H_2O$
780.1703	$C_{57}H_{76}O_{50}$	$GalA_8Me_5Ac_2\text{-}H_2O$
794.1666	$C_{58}H_{76}O_{51}$	$GalA_8Me_4Ac_3\text{-}H_2O$
826.1753	$C_{58}H_{78}O_{52}$	$GalA_8Me_4Ac_3\text{-}H_2O$
DP9		
833.1836	$C_{60}H_{82}O_{54}$	$GalA_9Me_6\text{-}H_2O$
847.1799	$C_{61}H_{82}O_{55}$	$GalA_9Me_5Ac\text{-}H_2O$
854.1887	$C_{62}H_{84}o_{55}$	$GalA_9Me_6Ac\text{-}H_2O$
856.1856	$C_{61}H_{84}O_{56}$	$GalA_9Me_5Ac$
868.1868	$C_{63}H_{84}O_{56}$	$GalA_9Me_5Ac_2\text{-}H_2O$
875.1938	$C_{64}H_{86}O_{56}$	$GalA_9Me_6Ac_2\text{-}H_2O$
882.2012	$C_{65}H_{88}O_{56}$	$GalA_9Me_7Ac_2\text{-}H_2O$
889.1927	$C_{65}H_{86}O_{57}$	$GalA_9Me_5Ac_3\text{-}H_2O$
DP10		
928.2088	$C_{67}H_{92}O_{60}$	$GalA_{10}Me_7\text{-}H_2O$
935.2148	$C_{68}H_{94}O_{60}$	$GalA_{10}Me_8\ \text{-}H_2O$
942.2052	$C_{68}H_{92}O_{61}$	$GalA_{10}Me_6Ac\text{-}H_2O$
949.2146	$C_{69}H_{94}O_{61}$	$GalA_{10}Me_7Ac\text{-}H_2O$
956.2204	$C_{70}H_{96}O_{61}$	$GalA_{10}Me_8Ac\text{-}H_2O$
963.2105	$C_{70}H_{94}O_{62}$	$GalA_{10}Me_6Ac_2.H_2O$
965.2236	$C_{70}H_{98}O_{62}$	$GalA_{10}Me_8Ac$
970.2173	$C_{71}H_{96}O_{62}$	$GalA_{10}Me_7Ac_2\text{-}H_2O$
977.2264	$C_{72}H_{98}O_{62}$	$GalA_{10}Me_8Ac_2\text{-}H_2O$
979.2227	$C_{71}H_{98}O_{63}$	$GalA_{10}Me_7Ac_2\text{-}H_2O$
984.2159	$C_{72}H_{96}O_{63}$	$GalA_{10}Me_6Ac_3\text{-}H_2O$
986.2301	$C_{72}H_{100}O_{63}$	$GalA_{10}M_{e8}Ac_2$

Supplementary Table 3. List of primers used for RT q-PCR experiments.

Act Fwd	5’-CGCCATTGCTCGTGTTGAC-3’
Act rev	5’-TCGGCAGTGGTGGAGAAAGT-3’
PME1fwd	5’-TATCTCTGCCCACACCAC-3’
PME1rev	5’-CGACGACGAGACATTTA-3’
PME2 fwd	5’-AAAACAACGAAGCCACCCTC-3’
PME2 rev	5’-CGTAGGAGGAGAGAGCAAGG-3’
PME3fwd	5’-TACCAGCGATGCAAGAACTG-3’
PME3 rev	5’-CTTCCCAAAACACCAGCAAT-3’
PG1 fwd	5’-CCCTCTCCGGCATTACATCC-3’
PG1 rev	5’-CGTTGGTAGCACTGGAGGAG-3’
PG2 fwd	5’-TCAAAACCGGCACCAAAGTT-3 ‘
PG2 rev	5’-CCAAGTCCATCCCACCATCT-3’
PG3 fwd	5’-CTCAGCTTCCACTGGTCTCC-3’
PG3 rev	5’-GCAACAGTAGCCAAGGTGGT-3’
PG4 fwd	5’-GCCCACGACTTGACTGATTC
PG4 rev	5’-TTGCCCAAAGAATCACCAGC-3’
PG5 fwd	5’-CGACCTTGGATTTGACAGGT-3’
PG5 rev	5’-GAGACCAAAGGACCAGACCA-3’
PG6 fwd	5’-GCCCGTCCATCTTTTCACTA-3’
PG6 rev	5’-CTGGACGAGCTGACATCAAA-3’
PL(12017) fwd	5’-AGCTTGTCAACTGGCTCTCC-3’
PL(12017) rev	5’-GTTGGAGATGGTGACACGGT-3’
PL(00912) fwd	5’-TGGTTTCGGATTGCTCGTCA-3’
PL(00912) rev	5’-ACCCTTCTCAGCTTCGTTGG-3’
JOX3 fwd	5’- GAACCAGCTCCTCATGCTTT-3’
JOX3 rev	5’- GGGTTCACATCACTCTGTG-3’
UBI4F fwd	5’-TGACACCATCGACAACGTGA-3’
UBI4R rev	5’-GAGGGTGGACTCCTTCTGGA-3’

References for SI reference citations

1. Ten Have A, Breuil WO, Wubben JP, Visser J, van Kan JAL (2001) *Botrytis cinerea* endopolygalacturonase genes are differentially expressed in various plant tissues. *Fungal Genet. Biol.* **33**:97–105.
2. Kars I, McCalman M, Wagemakers L, Van Kan JAL (2005) Functional analysis of *Botrytis cinerea* pectin methylesterase genes by PCR-based targeted mutagenesis: BcPME1 and BcPME2 are dispensable for virulence of strain B05.10. *Mol. Plant Pathol.* **6**:641–652.
3. Leroch M, Kleber A, Silva E, Coenen T, Koppenhöfer D, Shmaryahu A, Valenzuela PD, Hahn M (2013) Transcriptome profiling of Botrytis cinerea conidial germination reveals upregulation of infection-related genes during the prepenetration stage. *Eukaryot Cell*. **12**:614-626.
4. Gachon C, Saindrenan P. Real-time PCR monitoring of fungal development in Arabidopsis thaliana infected by Alternaria brassicicola and Botrytis cinerea. Plant Physiol Biochem. 2004 May;42(5):367-71
5. L'Enfant M et al. (2015) Substrate specificity of plant and fungi pectin methylesterases: Identification of novel inhibitors of PMEs. *Int. J. Biol. Macromol.* **81**:681–691.
6. Kars I et al. (2005) Necrotizing activity of five *Botrytis cinerea* endopolygalacturonases produced in *Pichia pastoris*: Necrotizing activity of BcPGs. *Plant J.* **43**:213–225.
7. Domon B, Costello CE (1988) A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. *Glycoconj. J.* **5**:397–409.
8. Körner R, Limberg G, Christensen TM, Mikkelsen JD, Roepstorff P (1999) Sequencing of partially methylesterified oligogalacturonates by tandem mass

spectrometry and its use to determine pectinase specificities. *Anal. Chem.* **71**: 421–1427.

9. Quéméner B, Cabrera Pino JC, Ralet MC, Bonnin E, Thibault JF (2003) Assignment of acetyl groups to O-2 and/or O-3 of pectic oligogalacturonides using negative electrospray ionization ion trap mass spectrometry: Assignment of oligogalacturonide acetyl groups by ESI-ITMS. *J. Mass Spectrom.* **38**:641–648.
10. Quéméner B, Désiré C, Lahaye M, Debrauwer L, Negroni L (2003) Structural characterisation by both positive- and negative-ion electrospray mass spectrometry of partially methylesterified oligogalacturonides purified by semi-preparative high-performance anion-exchange chromatography. *Eur. J Mass Spectr*om. **9**:45.
11. Elmayan, T. & Vaucheret, H. Expression of single copies of a strongly expressed 35S transgene can be silenced post-transcriptionally. *Plant J.* **9,** 787–797 (1996).
12. Davidsson P et al. (2017) Short oligogalacturonides induce pathogen resistance-associated gene expression in Arabidopsis thaliana. *BMC Plant Biol.* **17**:19.
13. Ferrari S, Vairo D, Ausubel FM, Cervone F, De Lorenzo G (2003) Tandemly duplicated Arabidopsis genes that encode polygalacturonase-inhibiting proteins are regulated coordinately by different signal transduction pathways in response to fungal infection. *Plant Cell* **15**:93-106.
14. Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J* **6 :**3901–3907.
15. Rihouey C, Paynel F, Gorshkova T, Morvan C (2017) Flax fibers: assessing the non-cellulosic polysaccharides and an approach to supramolecular design of the cell wall. *Cellulose* **24**:1985–2001.