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2    A gall-forming insect manipulates hostplant phytohormone synthesis, concentrations, and  
3    signaling

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21    *Running headline:* Phytohormones in an aphid gall

22

23    *Author Contributions:* MJAB collected the samples; MJAB, HMA, and JCS designed the overall  
24    study; MJAB setup the analytical method; MJAB extracted the phytohormones and performed  
25    statistical analyses; PPE performed the RNAseq data analysis; the interpretation and writing drafts  
26    were done by MJAB, HMA, and JCS, with help from PPE.

27

28    **Abstract**

29

30       Insect galls are highly specialized plant organs formed by an intimate biochemical  
31   interaction between the plant and a gall-inducing insect. Galls provide the insect enhanced  
32   nutrition and protection against natural enemies and environmental stresses. Because galls are  
33   plant organs, their development is likely to be governed by native phytohormones that function in  
34   organogenesis. We characterized concentrations of both growth and stress phytohormones in  
35   ungalled leaves and the leaf galls of *Daktulosphaira vitifoliae* on wild grape *Vitis riparia* at 4  
36   different developmental stages. We found clear evidence of hormone manipulation by phylloxera  
37   during gall initiation and development. Phylloxera suppressed accumulation of gibberellins,  
38   auxin, and jasmonates during the first two gall stages while abscisic acid concentrations were  
39   suppressed throughout development. Concentrations of 3 cytokinins and salicylate were greatly  
40   elevated during the earliest gall stage and declined sharply thereafter. We found no evidence of  
41   expression of cytokinin biosynthesis genes during the first gall stage, which strongly suggests that  
42   phylloxera supplied those phytohormones. High salicylate concentrations could have been caused  
43   by cytokinins, a response to microbes, or both. Our results suggest a central role for cytokinins in  
44   gall initiation and indicate the importance of the insect's ability to manipulate other hormones.

45

46

47    **Keywords**

48

49       Insect gall, phylloxera, grape, phytohormones, cytokinin, auxin, gibberellin, salicylate, jasmonate,  
50   brassinosteroid, ethylene, defense, development

51     **Introduction**

52         Galls are highly specialized structures arising from atypical development of plant tissue  
53         induced by another organism. Many different kinds of organisms can induce galls on plants,  
54         including viruses, fungi, bacteria, nematodes, mites, and insects (Redfern 2011). However, insects  
55         make galls that are more structurally complex and diverse than those made by other gall-inducing  
56         organisms (Imms 1947; Price et al. 1987). Galling has evolved repeatedly among and within insect  
57         orders: Hymenoptera, Diptera, Hemiptera, Lepidoptera, Coleoptera and Thysanoptera (e.g.,  
58         Roskam 1992, 2005; Stone and Schönrogge 2003; Gullan et al. 2005; La Salle 2005; Raman et al.  
59         2005). An estimated 15,000 insect species manipulate the development, morphology and  
60         physiology of their host-plants in a species-specific manner to generate galls within which the  
61         insect and/or its offspring feed. Insect galls are distinguished from other insect-generated shelters  
62         (such as rolled leaves or leaf mines) by the active differentiation and growth of plant tissues with  
63         features of a novel organ (Mani 1964; Stone and Schönrogge 2003; Shorthouse et al. 2005). Galls  
64         induced by insects range from simple stem swellings to complex structures that are ornamented  
65         with bright colors and spines. These structures are thought to provide adaptive advantages to gall  
66         feeders of enhanced nutrition and protection of the galling insect against natural enemies and  
67         environmental stresses (Hartley and Lawton 1992; Hartley 1998; Nyman and Julkunen-Tiitto  
68         2000; Nakamura et al. 2003; Stone and Schönrogge 2003; Allison and Schultz 2005; Motta et al.  
69         2005; Ikai and Hijii 2007; Diamond et al. 2008).

70         Gall formation is a complex and close interaction between the insect and the host-plant  
71         resulting from molecular hijacking; however, the mechanisms underlying gall induction and  
72         maintenance by gall-inducing insects remains poorly understood. Some work initially suggested  
73         that gall induction resulted only from mechanical stimulation (Madden and Stone 1984). However,

74 recent studies strongly suggest the involvement of chemical stimuli originating from salivary  
75 secretions and/or oviposition fluids to redirect normal plant development (Chen et al. 2010;  
76 Hogenhout and Bos 2011). These effectors (small molecules that alter host-cell structure and  
77 function, and modulate plant response) are injected into the wound by the galling insect to initiate  
78 the interaction with the host (Pascal-Avarado et al. 2008; Barnewall and De Clerck-Floate 2012;  
79 Yamaguchi et al. 2012). The chemical identity and mode of action of these effectors remain  
80 unclear. Unlike the host genetic transformation used by *Agrobacterium tumefaciens* to cause crown  
81 gall on plants, insect galls are not thought to involve host genetic transformation because insect  
82 gall development stops if the insect is removed. Diverse chemical signals have been proposed in  
83 insect gall systems, including phytohormones (especially plant growth factors, such as auxins  
84 and/or cytokinins) (Cornell 1983; Shorthouse and Rohfritsch 1992; Suzuki et al. 2014; Tooker and  
85 Helms 2014), amino acids (Stone and Schönrogge 2003), proteins (Higton and Mabberly 1994),  
86 mutualistic viruses (Cornell 1983) or bacterial symbionts (Yamaguchi et al. 2012). In some plant-  
87 gall inducer systems, the site of gall induction is different from the feeding sites of gall-inducing  
88 insects, indicating that a chemical signal is necessary to act over some distance (Sopow et al. 2003;  
89 Matsukura et al. 2009).

90 It is likely, of course, that mechanisms of induction vary among gall-inducing insect taxa,  
91 but because galls grow via cell hypertrophy and tissue hyperplasia (Mani 1964; Shorthouse et al.  
92 2005; Richardson et al. 2017), attention has focused on phytohormones that influence cell growth  
93 and division, such as auxins and cytokinins. Attempts to inject phytohormones into plants to  
94 produce artificial galls have met with limited success (Hough 1953; Mills 1969; Conner et al. 2012;  
95 Bartlett and Conner 2014). Moreover, auxins, cytokinins, or other plant hormones have been  
96 detected in insect saliva, and these insect-derived plant hormones may be responsible for

97 stimulating gall growth (Wood and Payne 1988; Hori 1992; Mapes and Davies 2001a,b; Tooker  
98 et al. 2008; Dorchin et al. 2009; Straka et al. 2010; Yamaguchi et al. 2012).

99 In this study, we focus on a gall-inducing insect, the grape phylloxera *Daktulosphaira*  
100 *vitifoliae*, an invasive pest species with worldwide economic importance (Boubals 1993; Granett  
101 et al. 2001; Yin et al. 2019). The phylloxera-grape system has been studied for several decades,  
102 mostly focusing on population dynamics (Powell et al. 2000, 2003; Herbert et al. 2006) and the  
103 genetic variability of the insect (Corrie et al. 2002, 2003; Vorwerk and Forneck 2006), but the  
104 underlying genetic mechanisms of gall formation remain poorly understood.

105 To gain insight into potential mechanisms of gall formation, we characterized how the  
106 galling insect *D. vitifoliae* alters the phytohormone concentrations, expression of host genes  
107 involved in phytohormone metabolism, and expression of genes known to respond to particular  
108 phytohormones at the site of gall induction on wild grape (*Vitis riparia*) during gall development.  
109 We attempted to identify changes in phytohormone concentration and activity characteristic of gall  
110 initiation and development.

111

## 112 **Material and methods**

113

### 114 **Study system**

115 This study was conducted using leaves of the wild grape *Vitis riparia* (Michx.) (Vitaceae)  
116 which are naturally infected by the phylloxera gall-inducing insect, *Daktulosphaira vitifoliae*  
117 (Fitch, 1855) (Hemiptera: Phylloxeridae: Aphidoidea) (Figure 1). *D. vitifoliae* is monophagous  
118 endophytophagous insect with a complex life cycle, including a number of life cycle variations

119 (see Forneck and Huber 2009). Grape Phylloxera are oviparous, and in the North America native  
120 habitat complete the full life cycle on American *Vitis* species.

121 Holocyclic reproduction (incorporating both asexual and sexual phases) begins in spring  
122 with the hatching of a sexual over-wintering egg deposited in the bark (Coombe 1963). The  
123 hatching fundatrix (or stem mother), which represents the first parthenogenetic generation  
124 following the sexual cycle, searches the grapevine for alternative feeding sites, preferably on the  
125 young leaves located at the tip of the stem, and once feeding begins typically remains at the same  
126 feeding location throughout the developmental life stages. The stem mother climbs onto a leaf and  
127 feeds on the adaxial (upper) surface of American *Vitis* leaves causing the formation of a pocket-  
128 like gall on the abaxial (underside) surface of the leaf. As the gall develops, the insect is effectively  
129 enclosed within and protected by the host-plant tissue. This multivoltine hemipteran insect lives  
130 concealed within the gall and feeds internally on nutritive tissues it induces on its host-plant. After  
131 developing into an apterous adult, the fundatrix parthenogenetically reproduces several hundred  
132 (up to 300) eggs, and dies. The eggs hatch, the nymphs (crawlers) develop through four molts into  
133 gall-forming apterous adults ("gallicolae") and will continue the parthenogenetic reproduction: up  
134 to 300 eggs for 3 to 5 (and up to 10) generations of eggs throughout spring and summer.

135

### 136 ***RNAseq experiment***

137 Methods for tissue sampling, RNA extraction, construction of Illumina library, processing of  
138 Illumina read, expression quantification, and validation of RNAseq results by digital droplet PCR  
139 are described in Schultz et al. (2019).

140 *Tissue sampling* – Galled and ungalled leaves were collected between 09:00 am and 10:00 am  
141 from, April to August 2014 and 2015 from wild *Vitis riparia* Michx. vines near Rocheport,

142 Missouri, USA ( $38^{\circ} 58' 16.424''$  N,  $92^{\circ} 32' 54.118''$  W). Galls from three different vines were  
143 separated by size into four developmental categories (Figure 1) and dissected on ice; midribs were  
144 removed from ungalled control leaves. Because the two earliest gall stages developed on the same  
145 leaves, there were only three control leaf size classes matched to the four gall stages. To obtain  
146 enough RNA, samples were pooled from three individual vines, producing three biological  
147 replicates ( $N = 3$ ) for each of the four gall developmental stages and three control leaf sizes (i.e.,  
148 originating from twelve independent grapevines for galled tissue, and nine for control leaves). All  
149 tissues were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ .

150 *RNA Extraction* – RNA was extracted and DNase1-treated, on column, using the Spectrum  
151 Plant Total RNA Kit (Sigma #STRN50-1KT; protocol A and Appendix). The resulting RNA was  
152 further purified and concentrated with the RNeasy MinElute Cleanup Kit (Qiagen #74204) and  
153 eluted with water. The quality of the resulting RNA was assessed using the Agilent 2100  
154 BioAnalyzer (Agilent, Santa Clara, CA, USA), and all RNA integrity number values were found  
155 to be above 8.

156 *Illumina Library and Construction* – The Illumina libraries (three biological replicates for  
157 each of the four gall developmental stages and three control leaf sizes, for a total of 21 libraries)  
158 were constructed using the RNA TruSeq Kit (Illumina, Inc., San Diego, CA, USA), barcoded  
159 (TACT ungalled; GTAT galled), and sequenced single-end with 100 bp reads on the  
160 Illumina HiSeq-2000 platform at the University of Missouri DNA Core  
161 (<http://dnacore.missouri.edu/>; University of Missouri, Columbia, MO, USA).

162 *Illumina read processing and expression quantification* – A custom Perl script was used to  
163 parse the libraries and remove barcode sequences resulting in approximately 40.9 million reads  
164 per biological replicate for the ungalled library and 40.3 million reads per biological replicate for

165 the galled library. NextGENe V2.3.3.1 (SoftGenetics, LLC., State College, PA, USA) was used to  
166 quality filter the fastq data, remove reads with a median quality score of less than 22, trim reads at  
167 positions that had three consecutive bases with a quality score of less than 20, and remove any  
168 trimmed reads with a total length less than 40 bp. The reads were aligned against *Vitis vinifera* V2  
169 genome (DOE-JGI; <ftp://ftp.jgi-psf.org/pub/compgen/phytozome/v9.0/Vvinifera/>), thus  
170 eliminating transcripts originating from phylloxera insects. Gene expression was quantified using  
171 TopHat/Cufflinks software (Ghost and Chan 2016).

172 Differential expression between galled and ungalled leaf tissue was analyzed for each  
173 mapping, using two discrete probability distribution based methods, DESeq and edgeR  
174 (<https://bioconductor.org>) and the annotated *Vitis vinifera* V2 genome (DOE-JGI; <ftp://ftp.jgi-psf.org/pub/compgen/phytozome/v9.0/Vvinifera/>). Read counts and RPKM values (reads per  
175 kilobase per million) were calculated for each library. An RPKM cutoff of 0.1 per gene model was  
176 applied for comparing expression values. Functional analyses were limited to genes with a  
177 differential expression significance < 0.05 and > 1.5-fold difference. Fold-change between galls  
178 and their respective ungalled control leaves was calculated for each gene by subtracting the base-  
179 2 logarithm of the RPKM value of galls to the base-2 logarithm of the RPKM value of ungalled  
180 control leaves.

182 Genome-wide syntenic analyses were performed to identify *Arabidopsis thaliana* – *Vitis*  
183 *vinifera* orthologs using CoGe (<http://genomevolution.org/CoGe/>). In addition, *Arabidopsis* –  
184 *Vitis* orthologs were identified using reciprocal BLASTp analyses (protein databases) with a  
185 0.00001 p-value cutoff resulting in the annotation of ~86.7 % of all coding sequences in the *Vitis*  
186 *vinifera* V2 genome. Because the *Vitis* genome is not yet fully annotated, we used orthology with  
187 *A. thaliana* genes to infer gene function to our *V. riparia* differentially expressed genes (DEG).

188 Gene Ontology (GO) enrichment analyses were performed for each of the gall and leaf gene  
189 expression sets using the PANTHER classification system (Mi et al. 2016). Statistical significance  
190 for enrichment scores was set at < 0.005.

191 RNAseq results were validated successfully using digital droplet PCR. The results are  
192 reported in Schultz et al. (2019).

193 *Data availability* – RNAseq data that were generated for this study are available at NCBI  
194 Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>) under study accession  
195 GSE118569.

196

197 ***Phytohormone quantification***

198 *Collect of biological material* – Galled (developmental stages 1-4) and ungalled (small: 0-  
199 2 cm; medium: 2-5 cm; large: 5-8 cm) leaves (Figure 1) were simultaneously collected from May  
200 to August 2014 and 2015 on wild grapes. All collections took place between 9:00 am and 10:00  
201 am in different locations in Columbia and Rocheport (MO, USA). The synchronization of  
202 sampling is crucial as levels of phytohormones, for example, vary during the day and between  
203 seasons. This required collecting galled and ungalled leaves at the same times and locations to  
204 make sure that any observed physiological differences were due to the impact of the gall-inducing  
205 insect on the plant, and not to phenological changes in the plants.

206 Collected galled and ungalled control leaves (N = 12 biological replicates per development  
207 stage) were kept on ice in the field because wounding can induce changes in plant hormones. Galls  
208 were individually dissected on ice with tools of different diameters: 0.60 mm for stage 1 galls, 1.19  
209 mm for stage 2, 1.70 mm for stage 3 and 3.57 mm for stage 4 (Figure 1). Edges of control leaves

210 were trimmed and the midrib was removed on ice as well. All samples were immediately frozen  
211 with liquid nitrogen and stored at -80 °C until analysis.

212       Each leaf sample consists in about 10 young/small leaves, 5 medium leaves, or 2 old/large  
213 leaves. Each gall sample consists in about 1,200 galls for stage 1 (on young/small leaves), 160  
214 galls for stage 2 (on young/small leaves), 60 galls for stage 3 (on medium leaves), or 10 galls for  
215 stage 4 (on old/large leaves).

216       *Phytohormones* – We chose to study phytohormones involved in growth and development  
217 (GAs, gibberellins; CKs, cytokinins; AUXs, auxins), source-sink relationships (CKs), response to  
218 stress (ABA, abscisic acid), and defenses (JA, jasmonates; SA, salicylate). To increase our ability  
219 to interpret results, we included several alternative forms of GAs, CKs, AUXs, and JAs.

220       *Chemicals* – Phytohormone standards – gibberellin A1 (GA1), gibberellin A4 (GA4),  
221 gibberellin A7 (GA7), 6-(Δ2-isopentenyl) adenine (iP), 6-(Δ2-isopentenyl) adenine riboside  
222 (iPR), *trans*-zeatin (*t*Z), *trans*-zeatin riboside (*t*ZR), indole-3-acetic acid (IAA), 2-*cis,4-trans*-  
223 abscisic acid (ABA), jasmonic acid (JA), 13-epi-12-oxo-phytodienoic acid (OPDA; JA precursor),  
224 jasmonoyl-isoleucine (JA-Ile; bioactive JA derivative), methyl jasmonate (MeJA) – and stable  
225 isotope-labeled compounds – [2H6]-*cis,trans*-abscisic acid (D6-ABA) and [2H6]-N6-  
226 isopentenyladenine (D6-iP) – were purchased from OlChemIm Ltd. (Olomouc, Czech Republic).  
227 Gibberellic acid (GA3), indole-3-butanoic acid (IBA), salicylic acid (SA), and methyl salicylate  
228 (MeSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Double distilled (MilliQ)  
229 water was used throughout the experiment.

230       *Sample preparation* – Phytohormone extraction was performed as described in Body et al.  
231 (2019). The large amount of tannins in grape samples required an additional purification step using  
232 a 96-well solid phase extraction (SPE) plate (Sep-Pak tC18 96-well plate, 25 mg sorbent per well,

233 37-55  $\mu\text{m}$  particle size; Waters, Milford, MA, USA). The SPE plate was conditioned using a  
234 vacuum manifold as follow: 250  $\mu\text{L}$  of 100 % methanol, 250  $\mu\text{L}$  of 50 % methanol with 0.1 %  
235 formic acid, 1 mL of 0.1 % formic acid. Each sample (diluted with 100 % methanol to reach 500  
236  $\mu\text{L}$ ) was ran through the SPE and phytohormones were eluted into a 96-well plate with 50  $\mu\text{L}$  of  
237 100 % methanol by centrifuging for 15 min at 3,500 x g.

238 *UPLC-ESI-MS/MS* – Ten  $\mu\text{L}$  of sample solution were injected into an ultrahigh-  
239 performance liquid chromatography – electrospray ionization-mass – spectrometry in tandem  
240 (UPLC-ESI-MS/MS) for quantification of all phytohormones. The phytohormones were separated  
241 by a reverse-phase C18 UPLC column (Supelco Ascentis Express 2.7  $\mu\text{m}$  C18, LC column 50 mm  
242 x 2.1 mm; Sigma-Aldrich, St. Louis, MO, USA) on a Waters Acquity H-class ultra-performance  
243 liquid chromatography (UPLC) system coupled with a Waters Acquity TQ triple quadrupole mass  
244 spectrometer (MS/MS) detector (Waters TQ Detector, Acquity Ultra Performance LC), controlled  
245 by the Waters Masslynx software (version 4.1). The binary solvent system used for phytohormone  
246 separation was 0.3 mM ammonium formate in water (negative-ion mode) or 0.1 % formic acid in  
247 water (positive-ion mode) (mobile phase A), 0.1 % formic acid in acetonitrile (mobile phase B),  
248 and set as follow for a 10-min run: initial conditions were 2 % B; gradient of 2-40 % B over 5 min,  
249 ramp 40-98 % B over 0.5 min, hold at 98 % B for 2 min, rapid ramp (0.25 min) to and hold at  
250 (2.25 min) initial conditions. The oven (holding UPLC column) temperature was set at 30 °C, the  
251 samples cooled to 10 °C in the autosampler, and the solvent flow rate at 0.4  $\text{mL}\cdot\text{min}^{-1}$ . For each  
252 phytohormone, Intellistart was used to generate a quantifying (best signal) transition (precursor >  
253 fragment pair) in which Q1 was set to 1 m/z filtering and Q3 set to 0.7. Optimized cone and  
254 collision energy voltages were then used to build a multiplexed MRM method. The ion source in  
255 the MS/MS system was electrospray ionization (ESI) operated in the positive or negative ion mode

256 (Supplement 1) with capillary voltages of 3.2 kV and 2.7 kV (positive and negative ion modes,  
257 respectively). The ionization sources were programmed at 150 °C and the desolvation temperature  
258 was programmed at 450 °C with a nitrogen gas flow rate of 800 L/hr. The MS/MS system was  
259 operated in the multiple reaction monitoring (MRM) mode with the optimized cone and collision  
260 energies. The optimized parameters for the method were reported in the Supplement 1.

261 *Statistical analysis* – Statistical analyses were performed using R version 3.2.1 and RStudio  
262 version 0.99.467 (The R Foundation for Statistical Computing, Vienna, Austria). Kruskal-Wallis  
263 tests were performed to compare phytohormone concentrations (*i*) between galls from different  
264 developmental stages, (*ii*) between ungalled control leaves from different developmental stages,  
265 as well as (*iii*) between galls and their respective ungalled control leaves for developmental stage.  
266 Where significant effects were observed, Mann-Whitney post-hoc tests were performed. The level  
267 of significance used in all tests was p-value ≤ 0.05. All phytohormone concentrations are presented  
268 as ng per mg of fresh weight (FW) (average ± S.E.M.).

269

270 **Results**

271

272 ***Broad patterns – Metabolic DEGs***

273 We examined changes gene expression for each phytohormone as gall development  
274 proceeded from initiation to organogenesis (Schultz et al. 2019). Patterns in the early galls –  
275 especially stage 1 – may provide clues about signaling involved in gall initiation, while patterns in  
276 later stages probably represent signaling in organogenesis. The number of up- and down-regulated  
277 differentially regulated genes (DEGs) involved in hormone metabolism was generally small in the  
278 early stages but then increased as the gall developed (Figures 2 and 3). The number of metabolic

279 DEGs that would increase the concentration of each hormone as expressed in the galls also  
280 increased as development proceeded (Figure 3).

281 The most abundant metabolic DEGs likely to increase hormone concentrations in earliest  
282 galls (gall stage 1; Figure 1) were for AUX (9) and SA (9) (Figure 2). The number of DEGs likely  
283 to enhance BR concentrations in the earliest galls was 4. This comprised 80% of the 5 early-  
284 expressed BR metabolism genes. There was little or no transcript evidence for known genes  
285 associated with the biosynthesis of GA, CK, ABA, or JA in these early stages (Figure 2). While  
286 the number of concentration-enhancing DEGs increased for all hormones as galls aged, this  
287 increase was proportionately greatest for GA (86 %), BR (80 %) and ABA (75 %) (Figure 2).

288

289 ***Broad patterns – Hormone concentrations***

290 We obtained accurate concentrations for all of the phytohormones we attempted to study.  
291 Our methods did not permit measuring BRs or ET. All targeted hormones were present in both  
292 leaves and galls except IBA, which was absent in both. Developmental patterns in concentrations  
293 were more or less phytohormone specific, with some tracking leaf concentrations, others following  
294 different trajectories. Several trajectories were relatively flat (GA3, GA7, IAA, OPDA), some  
295 increased with gall age (GA1, GA4) and some declined with gall age (iPR, iP, tZ, ABA, JA, JA-  
296 Ile, SA). The only phytohormones exhibiting significantly greater concentrations in galls than in  
297 leaves during at least one gall stage were iPR, iP, tZR, tZ, MeJA, SA and MeSA.

298

299 ***Broad patterns – Hormone responsive DEGs***

300 The number of DEGs responsive of several hormones also increased as gall development  
301 proceeded (Figure 3). The most abundant hormone-responsive DEGs in gall stage 1 were first for

302 ABA (62), then CK (41) and SA (30), all by a substantial margin over the other hormone-  
303 responsive DEGs (Figure 3). The number of responsive DEGs retained a similar pattern through  
304 gall stage 4 (ABA 211, then CK 124 and SA 106 plus AUX 103) (Figure 3). There were fewer  
305 than 11 responsive DEGs for any of the other hormones. The greatest proportionate increases in  
306 number of DEGs from stage 1 to stage 4 were for BR (86 %), JA (83 %), and AUX (82 %) (Figure  
307 3). Relative increases for all others were below 71 %.

308

309 ***Hormone-specific patterns***

310

311 ***Gibberellins (GAs)***

312 A list of DEGs involved in GA metabolism was constructed by querying Gene Ontology  
313 annotations with the term “*gibberellin metabolic process*” (GO:0009685). We found only 2 copies  
314 of one GA metabolism DEG in gall stages 1 and 2 (Figures 2A and 4A, Supplement 2). That DEG  
315 was *GERMINATION INSENSITIVE TO ABA MUTANT 2 (GIM2)*, and it was downregulated in  
316 galls (Supplement 2). GIM2 promotes GA synthesis while suppressing ABA synthesis, and  
317 functions in seed germination (Xiong et al. 2018). Hence, the only metabolic DEG found in gall  
318 stage 1 is likely to suppress GA accumulation in galls compared with leaves as expressed (Figure  
319 4A, Supplement 2). Downregulation of these 2 *GIM2* copies continued throughout gall  
320 development (Supplement 2). Three additional copies of *GIM2* were strongly upregulated in galls  
321 beginning in stages 3 and 4 (Supplement 2). This was accompanied by dramatic increases in  
322 expression of many other GA biosynthesis DEGs, including *ENT-KAURENOIC ACID*  
323 *HYDROXYLASE 2 (KAO2)*, *ENT-COPALYL DIPHOSPHATE SYNTHETASE 1*  
324 (*GA1*), *GIBBERELLIN 20-OXIDASE 1 (GA20OX1)*, *GIBBERELLIN 3-OXIDASE 1 (GA3OX1)*,

325 and *GIBBERELLIN 20 OXIDASE 2* (*GA20OX2*) (Figure 4A, Supplement 2). At the same time,  
326 catabolic DEGs *GIBBERELLIN 2-OXIDASE 1* (*GA2OX1*) and *GIBBERELLIN 20-OXIDASE 8*  
327 (*GA2OX8*) were downregulated in galls.

328 We measured concentrations of 4 forms of GAs, and all were present in both leaves and  
329 galls (Figure 4B). All GA levels decreased with leaf age (-50 % between small and large ungalled  
330 control leaves), while all GA concentrations increased with gall growth (+47 % for GA1, +31 %  
331 for GA4, and +21 % for GA7 between gall stage 1 and 4), except for GA3, which remained  
332 unchanged in galls (Figure 4B). GA levels were significantly lower in young galls (stages 1-2)  
333 than in their respective controls, similar in medium galls (stage 3) and higher (or similar for GA3  
334 and GA7) in older galls (stage 4), due to the opposite trends in galls and leaves during development  
335 (Figure 4B).

336 The number of GA-responsive DEGs drawn from GO:0009739 (“*response to gibberellin*”)  
337 in gall stages 1 and 2 was only 4, all downregulated (Figure 3A, Supplement 3). The number  
338 increased to 28 as gall and leaf development proceeded (Figure 3A, Supplement 3), with the  
339 number of downregulated DEGs in galls outnumbering the upregulated DEGs during stages 2 and  
340 3 (Figure 3A, Supplement 3). The majority (23 of 38 DEGs) are known to respond to other  
341 hormones in addition to GA (Supplement 3).

342

### 343 *Cytokinins (CKs)*

344 We examined the expression of DEGs in the GO category “*cytokinin metabolic process*”  
345 (GO:0009690). Altogether 18 DEGs involved in CK metabolism were found throughout gall  
346 development (Figures 2B and 5A, Supplement 4). We identified 4 DEGs during gall stage 1. Only  
347 1 of these, *CYTOCHROME P450 FAMILY 735 A1* (*CYP735A1*) which catalyzes the biosynthesis

348 of *trans*-zeatin (*tZ*), could increase concentration of a CK, likely at the expense of others (Figure  
349 5A, Supplement 4) (Takei et al. 2004). The other DEGs found in gall stage 1, *CYTOKININ*  
350 *OXIDASE 1* and *3* (*CKX1,3*) are catabolic and respond positively to CK concentrations (Figure  
351 5A, Supplement 4) (Armstrong 1994). *CYTOKININ OXIDASE 5* (*CKX5*) was activated in gall  
352 stage 2, while *LONELY GUY 3* (*LOG3*) was downregulated (Figure 5A, Supplement 4). Of the 18  
353 DEGs involved in CK metabolism, expression of only 3, *CYP735A1*, *RING DOMAIN LIGASE2*  
354 (*RGLG2*), and *ISOPENTENYLTRANSFERASE 5* (*IPT5*), would increase CK synthesis as  
355 expressed (Figure 5A, Supplement 4). All 3 were activated solely or primarily in gall stages 3 and  
356 4 (Figure 5A, Supplement 4). At the same time, expression of *IPT3* and *IPT9* was significantly  
357 less in galls than in leaves during stages 3 and 4 (Figure 5A, Supplement 4). Three CK  
358 activators, *LOG1,3,7*, were all downregulated in galls; one copy of *LOG8* was upregulated only in  
359 gall stage 3 while another *LOG8* was strongly downregulated at the same time (Figure 5A,  
360 Supplement 4). Expression of 4 of the 5 *LOGs* would suppress production of active CKs (Kuroha  
361 et al. 2009).

362 We measured concentrations of four CK forms, and all were found in both leaves and galls  
363 (Figure 5B). CK concentrations were higher in galls than in leaves only for stage 1 for iPR, iP and  
364 *tZ* (Figure 5B). Three of the four CKs (iPR, iP, and *tZ*) exhibited very high concentrations in gall  
365 stage 1 and a steep drop in gall stages 2-4 (-99 % between gall stage 1 and 4), while *tZR* in galls  
366 progressively increased during gall growth (+357 % between gall stage 1 and 3) (Figure 5B).

367 We found 152 CK-responsive DEGs (“*response to cytokinin*”, GO:0009735) (Figure 3B,  
368 Supplement 5). The 43 CK-responsive DEGs found in gall stage 1 included the catabolic cytokinin  
369 oxidases mentioned above, *TRYPTOPHAN AMINOTRANSFERASE 1* (*TAA1*), *TRYPOTPHAN*  
370 *SYNTHASE ALPHA CHAIN* (*TSA1*), *KNOTTED1-LIKE HOMEOBOX GENE* 3

371 (KNAT3), *WUSCHEL*                  *RELATED*                  *HOMEobox*                  9                  (*WOX9*)  
372 and *HPT PHOSPHOTRANSMITTER 4* (*AHP4*) (Supplement 5). TAA1 and TSA1 provide the  
373 substrates for auxin synthesis and are upregulated by CKs (Jones et al. 2011). *KNAT3* is repressed  
374 by CKs and is involved in root and root nodule development (Di Giacomo 2017). *WOX9* regulates  
375 activity of meristems (Wu et al. 2005). *AHP4* is a key element of the CK signaling network, which  
376 is upregulated by CKs and suppresses CK responses (Hutchison et al. 2006). The CK signaling  
377 repressor *HSP20-like chaperone* (*P23-2*) expression was more than 16-fold lower in galls than in  
378 leaves.

379                  Many of these genes continued to be actively expressed in gall stage 2, and the homolog  
380 of *ARABIDOPSIS RESPONSE REGULATOR 4* (*ARR4*) became active at stage 2 and continued  
381 throughout gall development. *ARR6*, *11*, and *18* became active in stages 3 and 4 while *ARR9*  
382 and *12* were repressed (Supplement 5). As the gall developed, we found CK-regulated DEGs that  
383 influence photosynthesis, glycolysis, respiration, cell division, and ribosome assembly among  
384 many functions. The CK-responsive DEGs we found are very narrowly regulated by CKs; of the  
385 152 DEGs, 136 are known to respond only to CKs (Supplement 5).

386

387 *Auxins (AUXs)*

388                  We established a list of DEGs by querying the GO category “auxin metabolic process”  
389 (GO:0009850). Altogether, we found 43 DEGs that participate in AUX metabolism among the 4  
390 gall stages (Figures 2C and 6A, Supplement 6). We identified 6 metabolic DEGs in stage 1  
391 increasing to 24 in stages 3 and 4 (Figure 2C). Three of the 6 gall stage 1 DEGs are directly  
392 involved in AUX synthesis: *YUCCA6*, *TAA1*, and *TRYPTOPHAN SYNTHASE BETA TYPE 2*  
393 (*TSBTYPE2*) (Figure 6A, Supplement 6). Expression of *GRETCHEN HAGEN 3.6*, (*GH3.6*), which

394 forms AUX conjugates, and *PHOSPHOLIPASE C 2* (*PLC2*), which suppresses AUX  
395 accumulation, was suppressed in galls (Supplement 6). These results suggest production of auxin  
396 in galls *via* the only complete DEG pathway going from tryptophan to indole-3-pyruvic acid  
397 (Figure 6A, Supplement 6).

398 As gall development continued, additional DEGs of similar types plus AUX transporters  
399 became activated or suppressed. These included *TRYPTOPHAN SYNTHASE ALPHA CHAIN 3*  
400 (*TRP3*), *TRYPTOPHAN AMINOTRANSFERASE-RELATED* 2 *(TAR2)*, *SHOOT*  
401 *GRAVITROPISM 5* (*SGR5*), *IAA-LEUCINE-RESISTANT (LR1)-LIKE 3* (*ILL3*), *ETTIN*  
402 (*ETT*), *RING DOMAIN LIGASE 2* (*RGLG2*), *ANTHRANILATE SYNTHASE ALPHA SUBUNIT 1*  
403 (*SA1*), *IAA-AMINO ACID HYDROLASE 1* (*ILR1*), (and *PIN-LIKES 2* (*PILS2*) (Supplement 6). At  
404 the same time, DEGs that would reduce AUX accumulation were activated,  
405 including *GRETCHEN HAGEN 3.17* (*GH3.17*) *GH3.3, GH3.9, SUPERROOT 2*  
406 (*SUR2*), *METHYL ESTERASE 17* (*MES17*), *INDOLE-3-ACETATE BETA-D-*  
407 *GLUCOSYLTRANSFERASE* (*IAGLU*), and *REVERSAL OF SAV3 PHENOTYPE 1* (*VAS1*) (Figure  
408 6A, Supplement 6). Other DEGS that normally promote AUX accumulation were downregulated  
409 in galls as they developed, such as *YUCCA8* and *10*, *SLOW MOTION* (*SLOMO*), *TRYPTOPHAN*  
410 *SYNTHASE BETA SUBUNIT HOMOLOG 2* (*TSB2*), *ALDEHYDE OXIDASE 1* and *2* (*AAO1,2*)  
411 and *LATERAL ROOT PRIMORDIUM* (*LRP1*) (Figure 6A, Supplement 6). Altogether, 22 of 43  
412 DEGs would increase AUX concentrations as expressed. However, on balance expression of  
413 metabolic DEGs was relatively unchanged through development while expression in leaves  
414 declined (Figure 6A).

415 We measured concentrations of two forms of AUXs, but only IAA was found in both leaves  
416 and galls (Figure 6B). IAA concentrations decreased in leaves as they grew (-51 % between small

417 and large ungalled control leaves), while they remained stable in galls during gall development  
418 (Figure 6B).

419 We found 154 AUX-responsive DEGs across all gall stages (“*response to auxin*”,  
420 GO:0009733). Of these, 10 occurred in stage 1 galls (Figure 3C, Supplement 7). The DEGs found  
421 upregulated in stage 1 galls are involved in diverse activities, including cell growth (*SMALL*  
422 *AUXIN UPREGULATED RNA 78, SAUR78*), cell cycle (*PATL4*), polar auxin transport (*POLAR*  
423 *AUXIN TRANSPORT INHIBITOR SENSITIVE 1, PISI*), defense (*PROHIBITIN 3, PHB3*), and  
424 cell wall development (*CHALCONE SYNTHASE, CHS*, and *CHALCONE*  
425 *FLAVANONE ISOMERASE, CFI*). All 3 downregulated AUX-responsive DEGs in gall stage 1 are  
426 involved in signaling: *MYB DOMAIN PROTEIN D (MYBD)*, *TRANSPORT INHIBITOR*  
427 *RESPONSE 1 (TIR1)*, and *INDOLE-3-ACETIC ACID INDUCIBLE 19 (IAA19)* (Supplement 7).  
428 Downregulating *MYBD* and *TIR1* would seem to suppress AUX signaling, while downregulating  
429 signaling repressor *IAA19* would enhance it (Tatematsu et al. 2004). Many DEGs regulating  
430 phenylpropanoid synthesis were activated (Supplement 7). For example, 7 copies of *CHS* were  
431 upregulated during gall stage 2, and an additional 4 were activated during gall stage 3 while *CFI*  
432 expression remained elevated in galls throughout gall development (Supplement 7). In addition, 3  
433 copies of *TRANSPARENT TESTA 7 (TT7)* and one copy of *TRANSPARENT TESTA GLABRA 1*  
434 (*TTG1*) became upregulated in galls during stages 3 and 4 (Supplement 7).

435 DEGs involved in AUX signaling also became very numerous during gall stages 3 and 4  
436 (Figure 3C, Supplement 7). Upregulated DEGs involved in auxin signaling included *AUXIN*  
437 *RESPONSE FACTOR 6 (ARG6)*, *AUXIN RESISTANT 1 (AUX1)*, *MYB DOMAIN PROTEINS 12,*  
438 *15, 61 and 93 (MYB12,15,61,93)*, *AUXIN RESPONSE FACTOR 11 (ARF11)*, *SUPPRESSOR OF*  
439 *AUXIN RESISTANCE 1 and 3 (SAR1,3)*, *ETTIN*, 9 different *AUXIN-INDUCED PROTEINS*

440 (*IAA4,11,13,16,26,27,29,31,33*), and 14 different *SMALL AUXIN UPREGULATED RNAs*  
441 (*SAUR1,4,5,6,14,42,53,59,64,65,67,68,76,78*) (Supplement 7).

442 At least 9 DEGs involved in auxin polar transport were found in gall stages 2-4  
443 (Supplement 7). These included *ATP-BINDING CASSETTE B1 AND B19 (ABCB1,19)*, *AUXIN*  
444 *RESISTANT 4 (AXR4)*, *LIKE AUX1 3 (LAX3)*, *PINOID-BINDING PROTEIN 1 (PBPI)*, *PINOID*  
445 (*PID*), *PILS 2,3,6*, *POLAR AUXIN TRANSPORT INHIBITOR SENSITIVE 1 (PISI)*, *SAUR 4,5,7,8*.

446 Gall stages 3 and 4 were also significantly enriched in auxin-responsive DEGs involved in  
447 flowering and fruiting (Supplement 7) (Schultz et al. 2019).

448 We identified DEGs that were enriched with functions specific to auxin response  
449 (Supplement 7). One hundred and ten (110) of the 154 putatively auxin-responsive DEGs are  
450 known to respond only to auxin (Supplement 7).

451

452 *Brassinosteroids (BR)*

453 We found 23 DEGs involved in brassinosteroid metabolism by querying the GO category  
454 “*brassinosteroid metabolic process*” (GO:0016131) in leaves and galls (Figures 2D and 7,  
455 Supplement 8). Two metabolic DEGs involved in BR synthesis were found in gall stage 1.  
456 *DWARF4 (DWF4)* was slightly downregulated while *CYTOCHROME P450, FAMILY 716,*  
457 *SUBFAMILY A, POLYPEPTIDE 1 (CYP716A1)* was strongly upregulated throughout gall  
458 development (Supplement 8). The number of BR metabolism DEGs increased rapidly, to 6 in gall  
459 stage 2 through gall stage 4 with 18 (Supplement 8). Throughout gall development 13 of the 23  
460 DEGs we found would increase BR concentrations if expressed as they were in galls.

461 We did not measure concentration of brassinosteroids in this study.

462 We found only 23 DEGs responsive to BR. As the number of metabolism DEGs in galls  
463 increased, so did the number BR-responsive DEGs drawn from GO:0009741 (“*response to*  
464 *brassinosteroid*”) (Figure 3D, Supplement 9). Only *DWF4* occurred in stage 1, and it was  
465 downregulated. By the 4th stage, 11 DEGs were upregulated in galls, while 5 were downregulated  
466 (Supplement 9). The functions of both up- and down-regulated DEGs were diverse, including cell  
467 wall development, cell division, and signaling. Thirteen of the 23 DEGs responsive to BRs are  
468 responsive only to brassinosteroids (Supplement 9).

469

470 *Abscisic acid (ABA)*

471 We found relatively few DEGs involved in ABA metabolism in gall tissues (“*abscisic acid*  
472 *metabolic process*”, GO:0009687). The numbers ranged from 3 DEGs in stage 1 to 21 in stage 4  
473 (Figures 2E and 8A, Supplement 10), and totaled 22 over the course of development. One copy of  
474 *CYTOCHROME P450, FAMILY 707, SUBFAMILY A, POLYPEPTIDE 4 (CYP707A4)* was  
475 strongly upregulated in all gall stages while another copy of the same gene was strongly  
476 downregulated in the same stages (Figure 8A, Supplement 10). This, together with downregulation  
477 of *ABA DEFICIENT 1 (ABA1)*, the only other DEG in stage 1, makes it appear unlikely that ABA  
478 would accumulate in gall stage 1 (Figure 8A, Supplement 10). Fifteen of the 21 DEGs involved in  
479 ABA metabolism at all stages were downregulated in galls; overall 15 DEGs would decrease ABA  
480 production if expressed as we found in this study, while 7 would increase it (Supplement 10).  
481 Among the strongly and significantly downregulated DEGs were several that encode enzymes that  
482 are key for ABA synthesis: *ABA1 (ZEAXANTHIN EPOXIDASE)*, *NCED3 (NINE-CIS-*  
483 *EPOXYCAROTENOID DIOXYGENASE 3)*, *AAO1 (ALDEHYDE OXIDASE 1)*, *AAO2*  
484 (*ALDEHYDE OXIDASE 2*), *AAO3 (ALDEHYDE OXIDASE 3)*, *NCED5 (9-CIS-*

485 *EPOXYCAROTENOID DIOXYGENASE 5*, and *ABA3 (MOLYBDIUMUM COFACTOR*  
486 *SULFURASE*) (Figure 8A, Supplement 10). The reduced expression of these key genes makes it  
487 unlikely that ABA concentrations in galls would exceed those in leaves, at least *via* biosynthesis.  
488 Differences between galls and leaves in the 3rd and 4th stages arose from a steeper decline in galls  
489 than leaves (Figure 8A).

490 ABA levels remained constant in leaves during development, while a decrease was  
491 observed in the galls as they developed (-31 % between gall stage 1 and 4) (Figure 8B).

492 Despite the equivocal picture of ABA metabolism from metabolic DEGs and concentration  
493 data, there were more ABA-responsive DEGs (“*response to abscisic acid*”, GO:0009737) in all  
494 gall stages (290) than DEGs responding to any other hormone (Figure 3E, Supplement 11). The  
495 putative biological functions of these DEGs were diverse. For example, a GO enrichment analysis  
496 indicated that 118 ABA-responsive DEGs can be described as responsive to stress, 69 responsive  
497 to osmotic stress, 67 involved in developmental processes including 25 in plant organ  
498 development, and 32 in reproduction. Interestingly, expression of several DEGs normally  
499 upregulated by ABA was strongly suppressed in galls (up to 16-fold). Examples include *MOTHER*  
500 *OF FT AND TFL1 (MFT)*, *GALACTINOL SYNTHASE 1 and 2 (GOLS1,2)*, *RAFFINOSE*  
501 *SYNTHASE 5 (RS5)*, *ABA INSENSITIVE 1 (ABI1)*, and *ATP-BINDING CASETTE G25 and G11*  
502 (*ABCG25,11*) (Supplement 11). At the same time, expression of additional copies of all of these  
503 DEGs except MFT was significantly increased in galls. Of 290 ABA-responsive DEGs, 58 are  
504 also responsive to one or more other hormones (Supplement 11).

505

506 *Jasmonic acid (JA)*

507 We found 37 DEGs involved in JA metabolism among all gall stages by querying the GO  
508 category “*jasmonic acid metabolic process*” (GO:0009694) (Figure 2F, Supplement 12). Fourteen  
509 DEGs related to JA metabolism were found in gall stages 1 and 2, of which 2 could contribute to  
510 JA accumulation (Figures 2F and 9A, Supplement 12). An *ALLENE OXIDE SYNTHASE (AOS)*  
511 DEG, which encodes a key step in JA synthesis (Dumin et al. 2018) was upregulated in gall stage  
512 1 only (Figure 9A, Supplement 12). Expression of several DEGs might increase JA concentration  
513 by liberating JA from other forms: *CYP94B3 (JASMONYL ISOLEUCENE-12-HYDROXYLASE)*  
514 expression was greater in galls, but 4 copies of *JMT (JASMONIC ACID CARBOXYL*  
515 *METHYLTRANSFERASE*) expression was reduced (Figure 9A, Supplement 12). Two copies of  
516 *LOX2* were significantly downregulated in gall stage 2 and thereafter (Figure 9A, Supplement 12).  
517 *OXOPHYTODIENOATE REDUCTASE 3 (OPR3)* is an essential step in JA synthesis (Stintzi et al.  
518 2001) and was unchanged or downregulated. Expression of *SULFOTRANSFERASE 2A (ST2A)*,  
519 which inactivates JA via conjugation, was strongly elevated in gall stages 1, 3, and 4 (Figure 9A,  
520 Supplement 12). Taken together, it appears unlikely that JA production would be greater in early  
521 stage galls than in leaves, and may be less. A similar pattern of downregulation of JA biosynthesis  
522 DEGs and expression of catabolic or conjugating DEGs continued through gall stage 4 (Figure  
523 9A, Supplement 12).

524 We measured concentrations of four components of the JA-pathway, and all were found in  
525 both leaves and galls (Figure 9B). OPDA levels remained constant during both leaf and gall  
526 development, and no difference was observed between galls and their respective controls (Figure  
527 9B). Both JA and JA-Ile showed the same patterns with a decrease of their concentrations during  
528 leaf (-73 % for JA and -69 % for JA-Ile between small and large control leaves) and gall growth  
529 (-55 % for JA and -37 % for JA-Ile between galls stage 1 and 4) (Figure 9B). JA and JA-Ile

530 concentrations were lower in galls than in ungalled control leaves during stages 1-2 and higher or  
531 similar in stages 3-4 (Figure 9B). MeJA showed a different pattern with no MeJA at all in small  
532 control leaves, and an increase in concentration as leaves aged (+157 % between galls stage 1 and  
533 2; -72 % between gall stage 3 and 4; Figure 9B). Difference in MeJA concentrations between galls  
534 and their respective controls were only statistically significant during stage 2-3 with higher  
535 concentrations in the galls (Figure 9B).

536 We found 119 JA-responsive DEGs during gall development (“*response to jasmonic acid*”,  
537 GO:0009753). Only 7 of these were seen in the first gall stage; that number increased to 29 in gall  
538 stage 2 (Figure 3F, Supplement 13). DEGs related to phenylpropanoid synthesis were prominent  
539 in these early stages, with 7 copies of *CHS* and one of *ANTHOCYANIN SYNTHASE* (*ANS*)  
540 upregulated in galls (Supplement 13). Otherwise, JA-responsive DEGs in all gall stages related  
541 primarily to oxylipin metabolism and signaling. After stage 1, more DEGs were downregulated  
542 than upregulated in galls, including many key genes involved in JA synthesis and signaling. The  
543 JA-responsive DEGs in our samples were not very specific to JA (Supplement 13). Two thirds  
544 (80) of the JA-responsive DEGs are also known to respond to other hormones, often to 4 or more  
545 (Supplement 13).

546

547 *Salicylic acid (SA)*

548 We examined the expression of DEGs in the GO category “*salicylic acid metabolic*  
549 *process*” (GO:0009696). We found 26 DEGs from this category. Fifteen of these would increase  
550 SA accumulation as expressed. Seven DEGs were found in gall stage 1, all but one of which could  
551 contribute to SA accumulation (Figures 2G and 10A, Supplement 14). The DEGs upregulated in  
552 galls included 4 copies of *ENHANCED DISEASE SUSCEPTIBILITY 1* (*EDS1*) plus one

of *PROHIBITIN 3 (PHB3)*. *BENZOIC ACID HYPERSENSITIVE 1 (BAH1)* expression was significantly lower in first stage galls (Supplement 14). *EDS1* promotes SA synthesis (Feys et al. 2001), and *PHB3* participates in it (Seguel et al. 2018), while *BAH1* mutants accumulate excess SA (Yaeno and Iba 2008). These results suggest that SA should accumulate in gall stage 1 (Supplement 14). However, expression of SA-catabolic *DMR6-LIKE OXYGENASE 1 (DLO1)* was elevated more than 64-fold in stage 1 galls (and even more thereafter) (Supplement 14). As the galls developed, expression of two more SA synthesis promoters, *CALMODULIN-BINDING PROTEIN 60B (CVP60B)* and *APETALA 2 FAMILY PROTEIN INVOLVED IN SA MEDIATED DISEASE DEFENSE 1 (APDI)* increased significantly, and *PHE AMMONIA LYASE 1 (PAL1)* expression increased, potentially providing more substrate for SA synthesis (Figure 10A, Supplement 14). The reduced expression of *NECROTIC SPOTTED LESIONS 1 (NSL1)*, *SIZ1 (SUMO)* E3 ligase and *CALMODULIN-BINDING PROTEIN 60A (CVP60A)* in later gall stages would presumably contribute to SA accumulation (Supplement 14). Three methyl esterases were downregulated in galls, which may also contribute to SA accumulation (Figure 10A, Supplement 14). However, the expression of catabolic *DLO1* had increased 156-fold by the 4<sup>th</sup> gall stage.

We measured concentrations of SA and MeSA, and both were found both in leaves and galls (Figure 10B). SA concentration in leaves decreased with growth (-20 % between small and large control leaves; Figure 10B). SA levels were much greater in stage 1 galls than leaves, then dropped during gall development (-89 % between gall stage 1 and 4) and MeSA (-99 % between gall stage 1 and 4) (Figure 10B). SA concentrations were statistically greater in galls than in ungalled control leaves during all developmental stages (Figure 10B). MeSA levels were higher in galls than in their respective controls at stage 1 and lower or similar in other stages (Figure 10B).

575 We found 160 SA-responsive DEGs across all gall stages, including 30 in gall stage 1  
576 (“*response to salicylic acid*”, GO:0009751) (Figure 3G, Supplement 15). Unsurprisingly, many of  
577 these are involved in defense against microbes of several types, e.g., *AGD2-LIKE DEFENSE*  
578 *RESPONSE PROTEIN 1 (ALD1)*, *RNA-DEPENDENT RNA POLYMERASE 1 (RDR1)*, *BASIC*  
579 *PATHOGENESIS-RELATED PROTEIN 1 (PRB1)*, *HYPER-SENSITIVITY-RELATED 4 (HSR4)*,  
580 *TRIPHOSPHATE TUNNEL METALLOENZYME 2 (TTM2)*, *DOWNY MILDEW RESISTANCE 6*  
581 (*DMR6*), *DLO1*, and *WALL-ASSOCIATED KINASE 2 (WAK2)* in the first stage (Supplement 15).  
582 An enrichment analysis of all SA-responsive DEGs found that 25 of the DEGs in our samples can  
583 be classified as “response to bacterium” and 13 as “response to fungus”. The specificity of these  
584 responsive DEGs was modest; 60 % of the SA-responsive DEGs are responsive only to SA  
585 (Supplement 15).

586

587 *Ethylene (ET)*

588 Twenty-one DEGs drawn from the GO category “*ethylene metabolic process*”  
589 (GO:0009692) were found in galls (Figure 2H and 11A, Supplement 16). Following the same  
590 pattern seen for the other hormones we studied, the number of DEGs increased from 4 in the 1<sup>st</sup>  
591 gall stage to 15 in the last stage (Figure 2H, Supplement 16). Two DEGs involved in ET  
592 biosynthesis (*ACC OXIDASE 1, ACO1*; *ACC SYNTHASE, ACS1*) were upregulated in gall stage  
593 1, while 2 others (*ETHYLENE FORMING ENZYME, EFE*; *ACC SYNTHASE 10, ACS10*) were  
594 downregulated in gall stage 1 and thereafter (Supplement 16).

595 We did not measure concentration of ethylene in this study.

596 We found 84 DEGs in galls and leaves drawn from GO:0009723 (“*response to ethylene*”)  
597 (Figure 3H, Supplement 17). Eight were upregulated in gall stage 1 while 3 were downregulated

598 (Figure 3H, Supplement 17). The putative functions of these early-expressed DEGs include AUX  
599 synthesis (*TAA1*) and defense (*PHB3*, *PRB1*, *YELLOW-LEAF-SPECIFIC GENE 2 – YSL2*). As  
600 the number of ET-responsive DEGs increased during gall and leaf development, their functions  
601 diversified, including signaling by other hormones (e.g., *AUXIN RESPONSE FACTOR 19*, *ARF19*;  
602 DELLA protein *RGL1*; *REPRESSOR OF GA*, *RGA1*), AUX synthesis (*TAA1*, *TAR2*, *TRP5*), and  
603 circadian rhythm (*MYB DOMAIN PROTEIN 32*, *MYB32*; *REVEILLE6*, *RVE6*; *CIRCADIAN*  
604 *CLOCK ASSOCIATED 1*, *CCA1*). Interestingly, 11 of 13 copies of *ATP-BINDING CASSETTE*  
605 *G40* (*ABCG40*), an ABA transporter, were strongly downregulated in 3<sup>rd</sup> and 4<sup>th</sup>-stage galls (Figure  
606 3H, Supplement 17).

607 The great majority – 64 of 84 – of the ET-responsive DEGs we found are also influenced  
608 by other hormones (Supplement 17).

609

## 610 Discussion

611

612 Researchers have tried to determine whether galling insects manipulate or inject plant  
613 hormones to elicit gall development for at least 70 years (Arrillaga 1949). Many studies have  
614 subsequently found phytohormonal changes in galled tissue (reviewed in Tooker and Helms, 2014  
615 and Giron et al. 2016). Most of these studies have focused on one or a few phytohormones, and  
616 even fewer have followed changes in phytohormone concentrations throughout gall development  
617 (cf. Wood and Payne 1988; Mapes and Davies 2001; Tooker et al. 2008; Tooker and De Moraes  
618 2011a). None of these studies examined the underlying transcriptional basis of phytohormone  
619 metabolism in developing galls, or presented evidence that the phytohormones present were  
620 actually functioning as signals. The present study is the first to examine transcription of

621 phytohormone metabolism genes, phytohormone concentrations, and transcriptional evidence of  
622 signaling by multiple hormones throughout the development of an insect gall.

623 Our study focused on galls developed on leaves of *V. riparia* by phylloxera *D. vitifoliae*  
624 (Schultz et al. 2019). We assessed the metabolic and response transcriptomes in leaves and galls  
625 of 4 classes of phytohormones involved in growth and development (GA, CK, AUX and BR), as  
626 well as 4 stress- and defense-related hormones (ABA, JA, SA and ET). We measured  
627 concentrations of each hormone in leaves and galls except BR and ET.

628 Numbers of DEGs involved in metabolism of each hormone ranged from 21 (cytokinin  
629 metabolism) to 44 (jasmonate metabolism). Numbers of metabolic genes differentially expressed  
630 in galls were lowest in the first gall stage and greatest in the 4th stage for all hormones studied.  
631 Numbers of DEGs known to respond to each hormone ranged from 23 (brassinosteroids) to 290  
632 (abscisic acid). As was the case for metabolic genes, responsive genes were fewest in the first gall  
633 stage and maximum in the 4<sup>th</sup> stage. Variation in actual concentrations was hormone-specific and  
634 is described for each hormone below.

635

636 ***Growth and development phytohormones***

637 Insect galls vary in complexity from simple swellings to highly complex organs, and the  
638 ontogeny of phylloxera leaf galls exhibits this range, beginning as simple swellings and  
639 culminating in a relatively complex organ (Figure 1; Schultz et al. 2019). It is not surprising, then,  
640 that concentrations of phytohormones involved in growth and organogenesis, their metabolism,  
641 and numbers of responsive DEGs might change dramatically as the galls develop. The increases  
642 in both metabolism-related and responsive DEGs with gall development for each

643 growth/development hormone, even when concentrations remained constant, reflects the increase  
644 in organ development as the galls aged.

645 **Gibberellins'** role in insect gall induction remains so far unclear, as their quantification  
646 has been neglected in most of the studies of gall induction and phytohormone profiles. Indeed,  
647 only two studies quantified GAs in insect-induced galls (Tokuda et al. 2013; Body et al. 2019),  
648 while their role has been studied more in depth at root knot nematode and cyst nematode's feeding  
649 sites (Klink et al. 2007; Kyndt et al. 2012, 2013). The GA biosynthesis pathway was strongly and  
650 constantly induced in giant cells and syncytia of nematode galls on both *Oryza sativa* and soybean  
651 *Glycine max* (Klink et al. 2007; Kyndt et al. 2012, 2013). In these galls, GAs stimulate both cell  
652 division and expansion (Richards et al. 2001).

653 Phylloxera suppressed GA accumulation in galls. We found no differential expression of  
654 genes that could contribute to GA synthesis or accumulation during the first two gall stages and  
655 GA concentrations in galls did not exceed those in leaves. The numbers of metabolic DEGs  
656 increased dramatically during gall stages 3 and 4, while GA concentrations in galls remained  
657 nearly constant throughout gall development as they declined in leaves. The later increase in GA-  
658 responsive DEGs suggests that GA signaling was active during stages 3 and 4 but not in stages 1  
659 or 2.

660 **Cytokinins** have been the focus of numerous studies of plant-manipulating insects. They  
661 have been found in high concentrations in many galls on many plant species (Mapes and Davies  
662 2001b; Dorchin et al 2009; Straka et al. 2010; Tokuda et al. 2013; Body et al. 2019).

663 Phylloxera appears to have provided CKs to the plant when initiating galls. Concentrations  
664 of 3 CKs (iP, iPR, tZ) were dramatically elevated in galls (up to 20-fold) during stage 1 and  
665 declined precipitously to leaf levels or lower immediately afterwards. These greatly elevated

666 concentrations in the earliest galls occurred despite no transcriptional evidence for enhanced  
667 biosynthesis in stage 1 or stage 2 galls (Figures 5A and 5B). We also observed increased expression  
668 of genes encoding CK-responsive catabolic enzymes involved in CK homeostasis in stage 1.

669 Evidence that galling insects produce and use CKs in gall elicitation has been accumulating  
670 for some time (McCalla 1962; Leitch 1994), and has grown stronger more recently (Straka et al.  
671 2010; Yamaguchi et al. 2012; Tanaka 2013; Tokuda 2013; Brutting et al. 2018). Our results suggest  
672 that phylloxera is another example of this phenomenon.

673 The elevated CK levels in early gall stages are consistent with the roles they are expected  
674 to play in gall development. CKs may maintain plant cells in an undifferentiated, meristematic  
675 stage (Weis et al. 1988), which is likely needed for gall development (Schultz et al. 2019). The  
676 early phylloxera gall stages are characterized primarily by cell division and tissue swelling, both  
677 of which are stimulated by CKs (Mok and Mok 1994). We found 78 CK-responsive DEGs in stage  
678 1 and stage 2 galls, indicating that CKs were present and signaling was occurring. While those  
679 numbers continued to increase dramatically as development went on, it seems clear that CK  
680 signaling was occurring at the earliest gall stages, as well as later. Finally, CKs have the ability to  
681 regulate AUX synthesis and transport (Jones et al. 2010). We found elevated expression of genes  
682 involved in AUX synthesis and known to be regulated by CKs in the earliest gall stages.

683 *tZR* was the only CK measured that accumulated above leaf levels in phylloxera galls of  
684 all stages (Figures 5A and 5B). *tZR* accumulated as *tZ* declined, suggesting that *tZR* accumulated  
685 at the expense of *tZ*. *tZR* is the major form of CKs transported in xylem (Osugi et al. 2017), and  
686 could have arrived from roots and accumulated later than the increases seen for the other CKs. It  
687 seems more likely that *tZR* was synthesized to reduce *tZ* activity *via* conjugation (Osugi et al.  
688 2017). The downregulation of 7 of the 8 LOG copies we found in galls is consistent with this

689 hypothesis, since LOG hydrolyzes *t*Z from *t*ZR (Osugi et al. 2017). Unfortunately, we did not find  
690 an ortholog in our grape vines of *Arabidopsis ADENINE PHOSPHORIBOSYL TRANSFERASE 1*,  
691 which ribosylates *t*Z (Allen et al. 2002).

692 **Auxin** has also received considerable scrutiny for its potential role in gall development due  
693 to its importance in organogenesis (Tooker and Herms 2014). IAA accumulation in galls has been  
694 found in several systems, sometimes to quite high levels (Mapes and Davies 2001b; Tooker and  
695 De Moraes 2011a,b; Tooker and Herms 2014). And recently evidence has appeared that insects  
696 may synthesize and inject it during gall elicitation (Suzuki et al. 2014; Yokoyama et al. 2017).

697 Phylloxera suppressed auxin accumulation during stages 1 and 2. Levels in galls were well  
698 below those in leaves during the early gall stages. While AUX concentrations in galls remained  
699 unchanged throughout development, concentrations in leaves declined to levels equal to galls in  
700 stages 3 and 4. Some copies of genes encoding early steps in AUX synthesis were upregulated in  
701 early stage galls, especially those involving modification of tryptophan (Figure 4A). These genes  
702 are regulated by CKs, which was elevated in the earliest galls. However, at the same time the gene  
703 encoding IAA AMIDO SYNTHETASE was upregulated, presumably conjugating IAA. These  
704 activities come together as a possible explanation for constant levels of IAA through phylloxera  
705 gall development. As was true for the other phytohormones we studied, the number of DEGs  
706 involved in IAA metabolism increased with gall development, including those that should increase  
707 IAA production and conjugation.

708 The number of auxin-responsive DEGs up- or downregulated in galls compared with leaves  
709 increased strongly through gall stage 4. While this is consistent with IAA's presumed role in  
710 organogenesis later in gall development (Schultz et al. 2019), we have no explanation for this  
711 increase while IAA concentration remained the same as it was in the earlier stages. Some of the

712 IAA-regulated DEGs in later gall stages are transporters; it is possible that IAA was transported  
713 away from the gall. It is also possible that these transporters changed distribution of IAA without  
714 altering total concentrations per gall. AUX action may be focused narrowly on small groups of  
715 cells (Zhao 2010). Measuring IAA in the entire gall is likely to have underestimated localized  
716 concentrations. Genes involved in defense against microbes and cell wall development were  
717 prominent among these AUX-responsive DEGs.

718 **Brassinosteroids** regulate a wide range of physiological processes including plant growth,  
719 development and immunity. While we did not measure BR concentrations in this study, we did  
720 examine genes involved in its biosynthesis and subsequent response in the cell. There was little  
721 evidence of either synthesis or response in the first two gall stages, with modest increases in both  
722 in stages 3 and 4. BR activity could be important in phylloxera gall development because BRs  
723 promote cell elongation, which becomes conspicuous as the phylloxera gall develops and grows  
724 (Witiak 2006). Although BRs are closely related to insect steroid hormones, there is as yet no  
725 evidence to suggest that insects provide them during gall initiation or growth.

726

727 **Stress and defense phytohormones**

728 **Abscisic acid** is an important regulator of plant responses to stresses, especially  
729 drought/osmotic stress (Tuteja 2007). ABA levels were shown to increase in galls of *Dryocosmus*  
730 *kuriphilus* on Chinese chestnut, *Lipara lucens* galls on common reed, *Cicadulina bipunctata* on  
731 corn, and *Pemphigus betae* galls on resistant poplar genotypes (Wood and Payne 1988; De Bruyn  
732 et al. 1998; Tokuda et al. 2013; Body et al. 2019).

733 Phylloxera suppressed ABA concentration in phylloxera galls during all 4 stages.  
734 Consistent with this, expression of very few genes (maximum of 7 per stage) promoting ABA

735 synthesis was found to be greater in galls at any point. This may be result from the very strong (up  
736 to 16-fold) upregulation of *CYP707A4* and *UGT71C5* in galls, proteins of which catabolize ABA  
737 in grape (Zheng et al. 2018). The latter results agree with several some other studies finding  
738 suppressed ABA concentrations in galls (Kai et al. 2017; Li et al. 2017).

739 Nabyt et al. (2013) showed that phylloxera galls require high rates of gas exchange. ABA  
740 treatments close stomata, which inhibit photosynthesis in grapevine leaves (Downton et al. 1988).  
741 A decrease of ABA concentration in galls could therefore lead to stomatal opening, which is  
742 consistent with the increased gas exchange reported by Nabyt et al. (2013) in phylloxera leaf galls.

743 Increased ABA responses are reported in plant defense against non-galling insect  
744 herbivores (Bodenhausen and Reymond 2007; Appel et al. 2014), but other studies have found an  
745 inhibition of ABA activity in *Pachypsylla celtidis* galls on hackberry trees, *Mayetiola destructor*  
746 galls on wheat and rice, and at the feeding site of the leaf-miner *Phyllonorycter blancardella* on  
747 apple trees (Straka et al. 2010; Zhu et al. 2011; Zhang et al. 2016). Bartlett and Connor (2014)  
748 found that injecting ABA in petioles of *Capsicum annuum* repressed formation of gall-like changes  
749 elicited by other hormones. Kai et al. (2017) and Li et al. (2017) found reduced ABA  
750 concentrations in other gall systems. Tooker and Helms (2014) have suggested that galling insects  
751 may suppress ABA production and signaling to avoid plant defenses.

752 Despite the apparent downregulation of ABA production in galls, we found more DEGs  
753 known to respond to ABA than to any other hormone. Only approximately 20 % of the ABA-  
754 responsive DEGs we found are also responsive to other hormones. In light of the concentration  
755 and metabolism transcription data, it is not clear how so many ABA-responsive genes were  
756 activated.

757           **Jasmonic acid** and its relatives comprise a family of phytohormones that coordinate  
758 defense responses against necrotrophic pathogens and chewing herbivores (Erb et al. 2012;  
759 Pieterse et al. 2012; Thaler et al. 2012). As a piercing-sucking herbivore that causes minimal  
760 wounding as it feeds, phylloxera might not be expected to elicit much JA production or signaling.

761           Phylloxera suppressed JA concentrations in the first 2 gall stages. Concentrations of JA  
762 and JA-Ile in galls began significantly elevated above those in leaves in early gall stages.  
763 Concentrations in both galls and leaves subsequently declined. The decline in JA and JA-Ile  
764 concentrations with age was slightly less steep in galls than in leaves, so that their concentrations  
765 in galls were slightly, but significantly, greater than leaves during the 4<sup>th</sup> stage. We found little  
766 transcriptional evidence for enhanced JA synthesis until gall stage 4. Phylloxera evidently  
767 suppressed the transcriptional basis of synthesis, and hence concentrations of JA and JA-Ile.

768           The number of DEGs responsive to JA signaling was substantial during gall development.  
769 Interestingly, there were up to twice as many down-regulations as up-regulations in galls, as well  
770 as responses to these signals during much of gall development. Here, too, transcriptional evidence  
771 of response was greatest in gall stage 4, when JA concentrations in galls exceeded those in leaves.  
772 This picture is clouded by the very large number of JA-related DEGs in our data that are  
773 coregulated by other hormones.

774           Concentrations of the JA precursor, OPDA, were unchanged in galls, and while not  
775 significant at p-value = 0.05, were generally lower in galls than in leaves throughout. We also  
776 observed downregulation of key biosynthetic steps *LOX2* and *OPR3*. Reduced JA/JA-Ile  
777 concentrations could also arise from methylating JA. MeJA concentrations were substantially  
778 greater in galls than in leaves during the first 3 stages and dropped below leaf levels in stage 4,  
779 when JA-Ile concentration in galls exceeded that in leaves. We found 5 copies of the gene encoding

780 jasmonic acid methyltransferase, necessary for MeJA production; all 5 were downregulated in  
781 galls. We thus have no convincing explanation for the very high concentrations of MeJA in galls.

782       **Salicylic acid** is a key signal coordinating plant responses to infection by biotrophic  
783 pathogens, particularly by bacteria, and to fluid-feeding herbivores like phylloxera (Erb et al. 2012;  
784 Pieterse et al. 2012; Thaler et al. 2012). SA and MeSA concentrations in galls exceeded those in  
785 leaves throughout development. This difference was related to strong upregulation of 4 copies of  
786 *EDS1* and 2 copies of *PAL1* in most gall stages. In keeping with strongly elevated SA  
787 concentrations, we found many (160) SA-responsive DEGs. While many of these are involved in  
788 defense, a substantial number are catabolic, representing homeostatic responses to elevated SA  
789 concentrations in galls. Overall, the JA/SA relationship in galls resembles what one often sees in  
790 responses to piercing-sucking herbivores (Lazebnik et al. 2014). High CK concentrations may  
791 stimulate SA synthesis in *Arabidopsis* (Argueso et al. 2014). The greatly elevated concentrations  
792 of SA and CK in the first gall stage could also represent a response to bacteria.

793       The elevated SA concentrations in galls might explain the low concentrations of jasmonate  
794 signals in the same galls. As an aphid-relative with similar feeding mechanisms, phylloxera would  
795 be expected to elicit SA-based defenses (Erb et al. 2012; Pieterse et al. 2012; Thaler et al. 2012),  
796 and block JA-based defenses, which have a negative impact on aphids (Kersch-Becker et al. 2019).  
797 There is evidence that SA blocks JA defense by suppressing JA synthesis (Pena-Cortes et al. 1993;  
798 Harms et al. 1998; Laudert and Weiler 1998; Sivasankar et al. 2000), although we did not find  
799 downregulation of *ALLENE OXIDE SYNTHASE*, a proposed SA target.

800       **Ethylene** formation in plants may be elicited by anoxia, drought, chilling, wounding, and  
801 pathogen attack. We found evidence of synthesis in the earliest galls in the form of 2 DEGs that  
802 could increase its concentrations. Expression of a few key biosynthesis genes continued to be

803 elevated throughout gall development. We did not measure ET concentrations, but found 83 ET-  
804 responsive DEGs in galls. However, the great majority of these DEGs are known to be regulated  
805 by other hormones as well. ET synthesis is suppressed by SA post-transcriptionally (Leslie and  
806 Romani 1988; Huang et al. 1993), so ET concentrations may not have been elevated in galls. Like  
807 ABA, ET interacts in complex fashion with many other hormone signaling networks.

808 It is clear that phytohormones must be involved in insect gall development. While galls are  
809 unique organs, they develop *via* processes common to normal plant organogenesis. Cell division,  
810 elongation, developmental trajectories, nutrient and hormonal transport and more are necessary  
811 elements of all organogenesis and are all regulated by phytohormones. So, it is no surprise that  
812 many phytohormones exhibit significant dynamics during gall development (Tooker and Helms  
813 2014). We have for the first time provided a detailed picture of such phytohormone concentration  
814 dynamics, as well as transcriptional dynamics, during the development of an insect gall.

815 The results clearly indicate elevated CK concentrations and signaling activity in the earliest  
816 galls without evidence of within-plant biosynthesis. The most logical explanation for this is that  
817 phylloxera is providing the CKs, something that has long been suspected. Eitle et al. (2017) also  
818 found elevated CK levels in phylloxera root galls. CK concentrations and dynamics have been  
819 related to the ability of bacteria, fungi, nematodes, and insects to form galls on resistant or  
820 susceptible plant genotypes (Akiyoshi 1984; Morrison 2015; Siddique et al. 2015; Li et al. 2017;  
821 Body et al. 2019). We concur with Giron et al. (2013) that cytokinins are central to many plant-  
822 insect relationships, especially galling.

823 Our results also reveal powerful evidence of synthesis and activity of salicylate. While this  
824 may be typical of plant responses to piercing-sucking, aphid-like insects, it may also reflect the  
825 presence and influence of microbes, especially bacteria, during gall formation. The high

826 concentrations of CKs in early galls may also be part of this response. Thus far, the only microbe  
827 shown to be associated with phylloxera is *Pantoea agglomerans*, which itself can elicit galls  
828 (Vorwerk et al. 2007).

829

830 **Conclusions**

831

832 The initiation and development of insect galls must be directed by phytohormones, just as  
833 they direct normal organ development. The ability to manipulate phytohormone concentrations is  
834 likely key for insects to induce gall formation. We examined the expression of genes involved in  
835 hormone metabolism and responses to hormones, as well as hormone concentrations. These data  
836 provide clear evidence of hormone manipulation during development of the phylloxera gall on  
837 grape leaves. Phylloxera suppressed accumulation of GAs, AUX, ABA, and JAs during the first  
838 two gall stages and ABA concentrations were suppressed throughout development. On the other  
839 hand, concentrations of CKs and SA were greatly elevated during the earliest gall stage. We found  
840 no evidence of expression of CK biosynthesis genes during the first gall stage, which strongly  
841 suggests that phylloxera supplied the CKs. High SA concentrations could have been due to high  
842 CK concentrations, or a response to microbes, or both. Our results suggest a key direct and indirect  
843 (*via* interactions with other hormones) role for CKs in gall initiation and indicate the importance  
844 of the insect's ability to manipulate other hormones.

845

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847

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855

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857

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- 1178

1179 **Table captions**

1180

1181 **Table 1. Phytohormone levels in phylloxera at three developmental stages (egg, crawler, and**  
1182 **stem mom).** For each phytohormone, statistical differences (p-value  $\leq 0.05$ ; Mann-Whitney test)  
1183 between different developmental stages are indicated by different letters (a, b, c). It is important  
1184 to note that no difference was found due to important variations between bioreplicates ( $n = 3$  for  
1185 each developmental stage). Each insect sample consists in about 6,000 eggs, 750 crawlers, or 250  
1186 stem moms. Data are presented in ng/mg FW, as average  $\pm$  S.E.M.

1187

1188 **Figure captions**

1189

1190 **Figure 1. Galls induced by phylloxera aphids, *Daktulosphaira vitifoliae*, on *Vitis riparia* grape**  
1191 **leaves.** Gall stages sampled and the stage-matched leaves on which they occurred. The fundatrix  
1192 female feeds on the adaxial surface of young leaf, initiating gall formation. The female is still  
1193 visible at stage 1, but disappears as adaxial leaf tissue grows over her, while the sack-like gall  
1194 expands beneath her. Very few galls are initiated on leaves wider than 2 cm. Photo credits: Melanie  
1195 J.A. Body.

1196

1197 **Figure 2. Number of sequences involved in phytohormone metabolism that were significantly**  
1198 **differentially expressed in galls compared with leaves, organized by GO category:** (A)  
1199 gibberellins, (B) cytokinins, (C) auxins, (D) brassinosteroids, (E) abscisic acid, (F) jasmonic acid,  
1200 (G) salicylic acid, (H) ethylene. N = 3 biological replicates per development stage for both galls  
1201 and ungalled control leaves. All the metabolism DEGs are available in Supplements 2, 4, 6, 8, 10,  
1202 12, 14, and 16.

1203

1204 **Figure 3. Number of sequences involved in phytohormone response that were significantly**  
1205 **differentially expressed in galls compared with leaves, organized by GO category:** (A)  
1206 gibberellins, (B) cytokinins, (C) auxins, (D) brassinosteroids, (E) abscisic acid, (F) jasmonic acid,  
1207 (G) salicylic acid, (H) ethylene. N = 3 biological replicates per development stage for both galls  
1208 and ungalled control leaves. All the response DEGs are available in Supplements 3, 5, 7, 9, 11, 13,  
1209 15, and 17.

1210

1211 **Figure 4. Changes in gibberellin metabolism and levels in grape ungalled control leaves vs.**  
1212 **galled tissues. (A)** Overview of the differentially expressed GA metabolism-related genes  
1213 (GO:0009685 “*gibberellin metabolic process*”) (adapted from Hedden and Kamiya 1997; Sun  
1214 2008; Hedden and Thomas 2012) in response to *Daktulosphaira vitifoliae*. Only genes with  
1215 significantly different expression levels ( $P \leq 0.05$ ) are shown. Ratios of expression levels of target  
1216 transcript in ungalled control and galled tissues are represented by a blue to orange palette, blue  
1217 colors reflecting higher expression in the ungalled controls and orange colors higher expression in  
1218 galls of different developmental stages (1-4). N = 3 biological replicates per development stage  
1219 for both galls and ungalled control leaves. See Supplement 2 for a complete list of GA-related  
1220 genes and ratio values. Numbers in circles indicate an enzyme while numbers in squares indicate  
1221 developmental stages. Enzymes: (1) *ent*-copalyl diphosphate synthase; (2) *ent*-kauren synthase;  
1222 (3) *ent*-kauren oxidase; (4) *ent*-kaurenoic acid oxidase; (5) gibberellin 13-oxidase (GA13ox); (6)  
1223 C20 gibberellin 2-oxidase (C20-GA2ox); (7) gibberellin 20-oxidase (GA20ox); (8) C19  
1224 gibberellin 2-oxidase (C19-GA2ox); (9) gibberellin 3-oxidase (GA3ox); (10) glucosyltransferase;  
1225 (11) salicylic acid methyltransferase (SAMT); (12) 16,17-epoxidase. Abbreviations: *ent*-CDP, *ent*-  
1226 copalyl diphosphate; GA, gibberellin; GA-GE, gibberellin glucosyl ester; GGDP, geranylgeranyl  
1227 diphosphate; MeGA, gibberellin methyl esters. Color code: black, compounds not measured; gray,  
1228 compounds measured but not detected; blue, compounds with lower level in galls or genes  
1229 downregulated in galls; orange, compounds with higher level in galls or genes upregulated in galls.  
1230 **(B)** Levels of gibberellin A1 (GA1), gibberellic acid (GA3), gibberellin A4 (GA4), and gibberellin  
1231 A7 (GA7) in ungalled control (white) and galled (black) tissues for different developmental stages  
1232 (1-4). N = 12 biological replicates per development stage for both galls and ungalled control leaves.  
1233 Statistical differences (p-value  $\leq 0.05$ ; Mann-Whitney test) between *different developmental*

1234 stages are indicated by different *lowercase* letters (a, b, c) for galls, and different *uppercase* letters  
1235 (A, B, C) for ungalled control leaves. Statistical differences (Mann-Whitney test) between *galls*  
1236 and *ungalled control leaves* for each developmental stage are shown as follow: \*\*\* when p-value  
1237  $\leq 0.001$ ; \*\* when p-value  $\leq 0.01$ ; \* when p-value  $\leq 0.05$ ; ns (non-significant) when p-value  $> 0.05$ .  
1238 Data are presented in ng/mg FW, as average  $\pm$  S.E.M.

1239

1240 **Figure 5. Changes in cytokinin (CK) metabolism and levels in grape ungalled control leaves**  
1241 **vs. galled tissues.** (A) Overview of the differentially expressed CK metabolism-related genes  
1242 (GO:0009690 “*cytokinin metabolic process*”) (adapted from Spíchal 2012; Zhang et al. 2016) in  
1243 response to *Daktulosphaira vitifoliae*. Only genes with significantly different expression levels ( $P$   
1244  $\leq 0.05$ ) are shown. Ratios of expression levels of target transcript in ungalled control and galled  
1245 tissues are represented by a blue to orange palette, blue colors reflecting higher expression in the  
1246 ungalled controls and orange colors higher expression in galls of different developmental stages  
1247 (1-4). N = 3 biological replicates per development stage for both galls and ungalled control leaves.  
1248 See Supplement 4 for a complete list of CK-related genes and ratio values. Numbers in circles  
1249 indicate an enzyme while numbers in squares indicate developmental stages. Enzymes: (1)  
1250 adenylate isopentenyl transferase; (2) phosphatase; (3) 50-ribonucleotide phosphohydrolase; (4)  
1251 adenosine nucleosidase; (5) cytokinin phosphoribohydrolase ‘lonely guy’; (6) purine nucleoside  
1252 phosphorylase; (7) adenosine kinase; (8) adenine phosphoribosyltransferase; (9) N-glucosyl  
1253 transferase; (10) cytokinin hydroxylase; (11) cytokinin dehydrogenase; (12) *trans*-zeatin-O-  
1254 glucosyltransferase; (13)  $\beta$ -glucosidase. Abbreviations: iP, N6-isopentenyladenosine; IPRDP, N6-  
1255 isopentenyladenosine-50-diphosphate; IPRTP, N6-isopentenyladenosine-50-triphosphate;  
1256 iPRMP, N6-isopentenyladenosine-50-monophosphate; iPR, N6-isopentenyladenosine riboside;

1257 iP7G, N6-isopentenyladenosine-7-glucoside; iP9G, N6-isopentenyladenosine-9-glucoside; tZ,  
1258 *trans*-zeatin; tZOG, *trans*-zeatin-O-glucoside; tZR, *trans*-zeatin riboside; tZROG, *trans*-zeatin-O-  
1259 glucoside riboside; tZOX, *trans*-zeatin-O-xyloside. Color code: black, compounds not measured;  
1260 gray, compounds measured but not detected; blue, compounds with lower level in galls or genes  
1261 downregulated in galls; orange, compounds with higher level in galls or genes upregulated in galls.  
1262 (B) Levels of 6-(Δ2-isopentenyl) adenine riboside (iPR), 6-(Δ2-isopentenyl) adenine (iP), *trans*-  
1263 zeatin riboside (tZR), *trans*-zeatin (tZ) in ungalled control (white) and galled (black) tissues for  
1264 different developmental stages (1-4). N = 12 biological replicates per development stage for both  
1265 galls and ungalled control leaves. Statistical differences (p-value ≤ 0.05; Mann-Whitney test)  
1266 between *different developmental stages* are indicated by different *lowercase* letters (a, b, c) for  
1267 galls, and different *uppercase* letters (A, B, C) for ungalled control leaves. Statistical differences  
1268 (Mann-Whitney test) between *galls and ungalled control leaves* for each developmental stage are  
1269 shown as follow: \*\*\* when p-value ≤ 0.001; \*\* when p-value ≤ 0.01; \* when p-value ≤ 0.05; ns  
1270 (non-significant) when p-value > 0.05. Data are presented in ng/mg FW, as average ± S.E.M.  
1271

1272 **Figure 6. Changes in auxin metabolism and levels in grape ungalled control leaves vs. galled**  
1273 **tissues.** (A) Overview of the differentially expressed AUX metabolism-related genes  
1274 (GO:0009850 “auxin metabolic process”) (adapted from Mano and Nemoto 2012; Korasick et al.  
1275 2013) in response to *Daktulosphaira vitifoliae*. Only genes with significantly different expression  
1276 levels ( $P \leq 0.05$ ) are shown. Ratios of expression levels of target transcript in ungalled control and  
1277 galled tissues are represented by a blue to orange palette, blue colors reflecting higher expression  
1278 in the ungalled controls and orange colors higher expression in galls of different developmental  
1279 stages (1-4). N = 3 biological replicates per development stage for both galls and ungalled control

leaves. See Supplement 6 for a complete list of AUX-related genes and ratio values. Numbers in circles indicate an enzyme while numbers in squares indicate developmental stages. Enzymes: (1) anthranilate synthase; (2) anthranilate phosphoribosyl transferase; (3) phosphoribosyl anthranilate isomerase; (4) indole-3-glycerol-phosphate synthase; (5) tryptophan synthase  $\alpha$ -subunit (TSA); (6) tryptophan synthase  $\beta$ -subunit (TSB); (7) monooxygenase/oxidoreductase (CYP79B2/CYP79B3); (8) indole-3-acetaldoxime dehydratase (CYP71A13); (9) tryptophan monooxygenase; (10) tryptophan aminotransferase (TAA); (11) indole-3-pyruvate decarboxylase (IPDC); (12) (27) tryptophan decarboxylase (TDC); (13) tryptophan side-chain oxidase (TSO); (14) nitrilase (NIT); (15) indole-3-acetamide hydrolase (AMI1); (16) indole-3-pyruvic acid decarboxylase (YUCCA); (17) aldehyde oxidase (AAO); (18) indole-3-butyric acid synthase; (19) short-chain dehydrogenase/reductase A (SDRA/IBR); (20) indole-3-acetic acid amido synthetase; (21) indole-3-acetic acid amino acid hydrolase; (22) UDP-glucosyltransferase (UGT74E2); (23) indole-3-acetate beta-glucosyltransferase (IAGlu); (24) indole-3-acetate beta-glucosyltransferase; (25) methyl esterase. Abbreviations: Ala, alanine; Asp, aspartic acid; Glc, glucose; Glu, glutamic acid; Gly, glycine; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; IPA, indole-3-pyruvic acid Leu, leucine; MeIAA, indole-3-acetic acid methyl ester; Phe, phenylalanine; Trp, tryptophan. Color code: black, compounds not measured; gray, compounds measured but not detected; blue, compounds with lower level in galls or genes downregulated in galls; orange, compounds with higher level in galls or genes upregulated in galls. **(B)** Levels of indole-3-acetic acid (IAA) in ungalled control (white) and galled (black) tissues for different developmental stages (1-4). Indole-3-butyric acid (IBA) was also quantified but not found. N = 12 biological replicates per development stage for both galls and ungalled control leaves. Statistical differences (p-value  $\leq$  0.05; Mann-Whitney test) between *different developmental stages* are indicated by different

1303 *lowercase* letters (a, b, c) for galls, and different *uppercase* letters (A, B, C) for ungalled control  
1304 leaves. Statistical differences (Mann-Whitney test) between *galls and ungalled control leaves* for  
1305 each developmental stage are shown as follow: \*\*\* when p-value  $\leq$  0.001; \*\* when p-value  $\leq$   
1306 0.01; \* when p-value  $\leq$  0.05; ns (non-significant) when p-value  $>$  0.05. Data are presented in ng/mg  
1307 FW, as average  $\pm$  S.E.M.

1308

1309 **Figure 7. Changes in brassinosteroid metabolism in grape ungalled control leaves vs. galled**  
1310 **tissues.** Overview of the differentially expressed BR metabolism-related genes (GO:0016131  
1311 “*brassinosteroid metabolic process*”) (adapted from Chung and Choe 2013) in response to  
1312 *Daktulosphaira vitifoliae*. Only genes with significantly different expression levels ( $P \leq 0.05$ ) are  
1313 shown. Ratios of expression levels of target transcript in ungalled control and galled tissues are  
1314 represented by a blue to orange palette, blue colors reflecting higher expression in the ungalled  
1315 controls and orange colors higher expression in galls of different developmental stages (1-4). N =  
1316 3 biological replicates per development stage for both galls and ungalled control leaves. See  
1317 Supplements 8 for a complete list of BR-related genes and ratio values. Numbers in circles indicate  
1318 an enzyme while numbers in squares indicate developmental stages. Enzymes: (1) steroid  
1319 oxidoreductase (CPD); (2) steroid 5-alpha reductase (DET2); (3-4) unknown; (5) steroid 22-alpha  
1320 hydroxylase (DWF4); (6) C-23 steroid hydroxylase (ROT3/CYP90D1); (7-13) unknown; (14) C-  
1321 6 steroid oxidation (CYP85A1); (15) steroid hydroxylase (CYP85A2). Abbreviations: 3DT, 6-  
1322 dehydroteasterone; BL, brassinolide; CS, castasterone; CT, cathasterone; TE, teasterone; TY,  
1323 typhasterol.

1324

1325 **Figure 8. Changes in abscisic acid metabolism and levels in grape ungalled control leaves vs.**  
1326 **galled tissues. (A)** Overview of the differentially expressed ABA metabolism-related genes  
1327 (GO:0009687 “*abscisic acid metabolic process*”) (adapted from Zhang et al. 2016) in response to  
1328 *Daktulosphaira vitifoliae*. Only genes with significantly different expression levels ( $P \leq 0.05$ ) are  
1329 shown. Ratios of expression levels of target transcript in ungalled control and galled tissues are  
1330 represented by a blue to orange palette, blue colors reflecting higher expression in the ungalled  
1331 controls and orange colors higher expression in galls of different developmental stages (1-4). N =  
1332 3 biological replicates per development stage for both galls and ungalled control leaves. See  
1333 Supplement 10 for a complete list of ABA-related genes and ratio values. Numbers in circles  
1334 indicate an enzyme while numbers in squares indicate developmental stages. Enzymes: (1)  $\beta$ -  
1335 carotene hydroxylase (BCH2); (2) zeaxanthin epoxidase; (3) neoxanthin synthase; (4) isomerase;  
1336 (5) 9-cis-epoxycarotenoid dioxygenase; (6) xanthoxin dehydrogenase; (7) abscisic aldehyde  
1337 oxidase (AAO); (8) abscisic acid 8-hydroxylase. Abbreviations: ABA-GE, abscisic acid glucosyl  
1338 ester. Color code: black, compounds not measured; gray, compounds measured but not detected;  
1339 blue, compounds with lower level in galls or genes downregulated in galls; orange, compounds  
1340 with higher level in galls or genes upregulated in galls. **(B)** Levels of abscisic acid (ABA) in  
1341 ungalled control (white) and galled (black) tissues for different developmental stages (1-4). N =  
1342 12 biological replicates per development stage for both galls and ungalled control leaves.  
1343 Statistical differences (p-value  $\leq 0.05$ ; Mann-Whitney test) between *different developmental*  
1344 *stages* are indicated by different *lowercase* letters (a, b, c) for galls, and different *uppercase* letters  
1345 (A, B, C) for ungalled control leaves. Statistical differences (Mann-Whitney test) between *galls*  
1346 and *ungalled control leaves* for each developmental stage are shown as follow: \*\*\* when p-value

1347  $\leq 0.001$ ; \*\* when p-value  $\leq 0.01$ ; \* when p-value  $\leq 0.05$ ; ns (non-significant) when p-value  $> 0.05$ .

1348 Data are presented in ng/mg FW, as average  $\pm$  S.E.M.

1349

1350 **Figure 9. Changes in jasmonate metabolism and levels in grape ungalled control leaves vs.**

1351 **galled tissues. (A)** Overview of the differentially expressed JA metabolism-related genes

1352 (GO:0009694 “jasmonic acid metabolic process”) (adapted from Miersch et al. 2007; Hu et al.

1353 2012; Zhang et al. 2016; Jimenez-Aleman et al. 2017) in response to *Daktulosphaira vitifoliae*.

1354 Only genes with significantly different expression levels ( $P \leq 0.05$ ) are shown. Ratios of expression

1355 levels of target transcript in ungalled control and galled tissues are represented by a blue to orange

1356 palette, blue colors reflecting higher expression in the ungalled controls and orange colors higher

1357 expression in galls of different developmental stages (1-4). N = 3 biological replicates per

1358 development stage for both galls and ungalled control leaves. See Supplement 12 for a complete

1359 list of JA-related genes and ratio values. Numbers in circles indicate an enzyme while numbers in

1360 squares indicate developmental stages. Enzymes: (1) phospholipase; (2) lipoxygenase (LOX); (3)

1361 lipoxygenase chloroplastic (13-LOX); (4) allene oxide synthase (AOS); (5) allene oxide cyclase

1362 (AOC); (6) 12-oxophytodienoic acid reductase (OPR3); (7) 3-oxo-2-(2-(Z)-pentenyl)-cyclo

1363 pentane-1-octanoic coA ligase (OPC-8:0-CoA ligase 1); (8)  $\beta$ -oxidation: acyl-CoA oxidase

1364 (ACX1, ACX5), 3-hydroxyacyl-CoA hydrolase (AIM1), and 3-ketoacyl-CoA thiolase

1365 (PED1/KAT2); (9) thioesterase (TE); (10) jasmonic acid methyltransferase (JMT); (11)

1366 jasmonoyl-isoleucine-12-hydroxylase (CYP94B1, CYP94B3); (12) jasmonic acid amido

1367 synthetase (JAR1); (13) sulfotransferase (ST2); (14) glucosylation; (15) amino acid hydrolase;

1368 (16) jasmonyl-isoleucine hydrolase (17) methyljasmonate esterase (MJE). Abbreviations: 12-

1369 OPDA, 12-oxo phytodienoic acid; OPC4:0, 3-oxo-2-(2-(Z)-pentenyl)-cyclopentane-1-butryric;

1370 OPC6:0, 3-oxo-2-(2-(Z)-pentenyl)-cyclopentane-1-hexanoic; OPC8:0, 3-oxo-2-(2-(Z)-pentenyl)-  
1371 cyclopentane-1-octanoic; c-J, *cis*-jasnone; JA, jasmonic acid; MeJA, methyl jasmonate; JA-Ile,  
1372 jasmonoyl-isoleucine; 12-OH-JA, 12-hydroxyjasmonic acid; 12-OH-JA-Ile, 12-hydroxy-  
1373 jasmonoyl-isoleucine; 12-COOH-JA-Ile, 12-dicarboxy-jasmonoyl-isoleucine; JA-Ile-Glc; O-  
1374 glucosyl-jasmonoyl-isoleucine; 12-HSO4-JA, 12-hydroxyjasmonic acid sulfate; 12-OH-JA-Glc,  
1375 12-hydroxy-O-glucosyl-jasmonic acid; 12-OH-JA-Ile-Glc, 12-hydroxy-O-glucosyl-jasmonoyl-  
1376 isoleucine. Color code: black, compounds not measured; gray, compounds measured but not  
1377 detected; blue, compounds with lower level in galls or genes downregulated in galls; orange,  
1378 compounds with higher level in galls or genes upregulated in galls. (B) Levels of 12-oxo-  
1379 phytodienoic acid (OPDA), jasmonic acid (JA), jasmonic acid methyl ester (MeJA), and  
1380 jasmonoyl-isoleucine (JA-Ile) in ungalled control (white) and galled (black) tissues for different  
1381 developmental stages (1-4). N = 12 biological replicates per development stage for both galls and  
1382 ungalled control leaves. Statistical differences (p-value  $\leq$  0.05; Mann-Whitney test) between  
1383 *different developmental stages* are indicated by different *lowercase* letters (a, b, c) for galls, and  
1384 different *uppercase* letters (A, B, C) for ungalled control leaves. Statistical differences (Mann-  
1385 Whitney test) between *galls and ungalled control leaves* for each developmental stage are shown  
1386 as follow: \*\*\* when p-value  $\leq$  0.001; \*\* when p-value  $\leq$  0.01; \* when p-value  $\leq$  0.05; ns (non-  
1387 significant) when p-value  $>$  0.05. Data are presented in ng/mg FW, as average  $\pm$  S.E.M.  
1388

1389 **Figure 10. Changes in salicylate metabolism and levels in grape ungalled control leaves vs.**  
1390 **galled tissues. (A)** Overview of the differentially expressed SA metabolism-related genes  
1391 (GO:00096896 “*salicylic acid metabolic process*”) (adapted from Dempsey et al. 2011; Hayat et  
1392 al. 2013; An and Mou 2014; Zhang et al. 2016; Kohli et al. 2017) in response to *Daktulosphaira*

1393 *vitifoliae*. Only genes with significantly different expression levels ( $P \leq 0.05$ ) are shown. Ratios  
1394 of expression levels of target transcript in ungalled control and galled tissues are represented by a  
1395 blue to orange palette, blue colors reflecting higher expression in the ungalled controls and orange  
1396 colors higher expression in galls of different developmental stages (1-4). N = 3 biological  
1397 replicates per development stage for both galls and ungalled control leaves. See Supplements 14  
1398 for a complete list of SA-related genes and ratio values. Numbers in circles indicate an enzyme  
1399 while numbers in squares indicate developmental stages. Enzymes: (1) 3-deoxy-D-arabino-  
1400 heptulosonate 7-phosphate synthase (DAHP synthase); (2) 3-dehydroquinate dehydratase; (3)  
1401 chorismate synthase; (4) isochorismate synthase (ICS); (5) isochorismate lyase (IPL); (6)  
1402 chorismate mutase (CM); (7) prephenate dehydratase; (8) prephenate aminotransferase (PPA-AT);  
1403 (9) phenylalanine dehydrogenase; (10) arogenate dehydratase (ADT); (11) phenylalanine  
1404 ammonia lyase (PAL); (12) 4-coumarate-CoA ligase (4CL); (13) cinnamate 4-hydroxylase (C4H);  
1405 (14) aldehyde oxidase (AAO); (15) benzoyl-CoA ligase (BZL); (16) benzoic acid-2-hydroxylase  
1406 (BA2H); (17) salicylic acid methyltransferase (SAMT); (18) salicylic acid esterase. Abbreviations:  
1407 PEP, phosphoenolpyruvate; DAHP, 3-deoxy-arabinohexitulosonate 7-phosphate. Color code:  
1408 black, compounds not measured; gray, compounds measured but not detected; blue, compounds  
1409 with lower level in galls or genes downregulated in galls; orange, compounds with higher level in  
1410 galls or genes upregulated in galls. **(B)** Levels of salicylic acid (SA) and methyl salicylate (MeSA)  
1411 in ungalled control (white) and galled (black) tissues for different developmental stages (1-4). N  
1412 = 12 biological replicates per development stage for both galls and ungalled control leaves.  
1413 Statistical differences (p-value  $\leq 0.05$ ; Mann-Whitney test) between *different developmental*  
1414 *stages* are indicated by different *lowercase* letters (a, b, c) for galls, and different *uppercase* letters  
1415 (A, B, C) for ungalled control leaves. Statistical differences (Mann-Whitney test) between *galls*

1416 and ungalled control leaves for each developmental stage are shown as follow: \*\*\* when p-value  
1417  $\leq 0.001$ ; \*\* when p-value  $\leq 0.01$ ; \* when p-value  $\leq 0.05$ ; ns (non-significant) when p-value  $> 0.05$ .  
1418 Data are presented in ng/mg FW, as average  $\pm$  S.E.M.

1419

1420 **Figure 11. Changes in ethylene metabolism in grape ungalled control leaves vs. galled tissues.**

1421 Overview of the differentially expressed ET metabolism-related genes (GO:0009692 “ethylene  
1422 metabolic process”) (adapted from Booker and DeLong 2015) in response to *Daktulosphaira*  
1423 *vitifoliae*. Only genes with significantly different expression levels ( $P \leq 0.05$ ) are shown. Ratios  
1424 of expression levels of target transcript in ungalled control and galled tissues are represented by a  
1425 blue to orange palette, blue colors reflecting higher expression in the ungalled controls and orange  
1426 colors higher expression in galls of different developmental stages (1-4). N = 3 biological  
1427 replicates per development stage for both galls and ungalled control leaves. See Supplements 16  
1428 for a complete list of ET-related genes and ratio values. Numbers in circles indicate an enzyme  
1429 while numbers in squares indicate developmental stages. Enzymes: (1) S-adenosyl methionine  
1430 (SAM) synthase; (2) 1-aminocyclopropane-1-carboxylate synthase (ACS); (3) 1-  
1431 aminocyclopropane-1-carboxylate oxidase (ACO); (4) ACC-malonyl transferase; (5)  $\gamma$ -glutamyl-  
1432 transpeptidase; (6) jasmonate-amino synthetase (JAR1). Abbreviations: ACC, 1-  
1433 aminocyclopropane-1-carboxylate; JA, jasmonic acid; ET, ethylene.

1434

1435 **Supplement captions**

1436

1437 **Supplement 1. UPLC-ESI-MS/MS features.** Reaction monitoring conditions for protonated or  
1438 deprotonated plant hormones ( $[M + H]^+$  or  $[M - H]^-$ ). Abbreviations: GA1, gibberellin A1; GA3,  
1439 gibberellic acid; GA4, gibberellin A4; GA7, gibberellin A7; iPR, 6-( $\Delta$ 2-isopentenyl) adenine  
1440 riboside; iP, 6-( $\Delta$ 2-isopentenyl) adenine; tZR, *trans*-zeatin riboside; tZ, *trans*-zeatin; IAA, indole-  
1441 3-acetic acid; IBA, indole-3-butanoic acid; ABA, 2-*cis,4-trans*-abscisic acid; OPDA, 13-epi-12-  
1442 oxo-phytodienoic acid; JA, jasmonic acid; JA-Ile, jasmonoyl-isoleucine; MeJA, methyl jasmonate;  
1443 SA, salicylic acid; MeSA, methyl salicylate. Internal standards (IS): D6-ABA, [2H6]- *cis,trans*-  
1444 abscisic acid; D6-iP, [2H6]-N6-isopentenyladenine.

1445 • Internal standards.

1446 \* Retention times listed in this table are obtained under UPLC and column conditions mentioned  
1447 in the protocol.

1448

1449 **Supplement 2. Differentially regulated genes involved in gibberellin metabolism.** Only genes  
1450 from GO:0009685 “*gibberellin metabolic process*” with significantly different expression levels  
1451 ( $P \leq 0.05$ ) are shown. Ratios of expression levels of target transcript in ungalled control and galled  
1452 tissues are represented by a blue to orange palette, blue colors reflecting higher expression in the  
1453 ungalled controls and orange colors higher expression in galls of different developmental stages  
1454 (1-4). N = 3 biological replicates per development stage for both galls and ungalled control leaves.

1455

1456 **Supplement 3. Differentially regulated gibberellin-responsive genes.** Only genes from  
1457 GO:0009739 “*response to gibberellin*” with significantly different expression levels ( $P \leq 0.05$ ) are

1458 shown. Ratios of expression levels of target transcript in ungalled control and galled tissues are  
1459 represented by a blue to orange palette, blue colors reflecting higher expression in the ungalled  
1460 controls and orange colors higher expression in galls of different developmental stages (1-4). N =  
1461 3 biological replicates per development stage for both galls and ungalled control leaves.

1462

1463 **Supplement 4. Differentially regulated genes involved in cytokinin metabolism.** Only genes  
1464 from GO:0009690 “*cytokinin metabolic process*” with significantly different expression levels (P  
1465  $\leq 0.05$ ) are shown. Ratios of expression levels of target transcript in ungalled control and galled  
1466 tissues are represented by a blue to orange palette, blue colors reflecting higher expression in the  
1467 ungalled controls and orange colors higher expression in galls of different developmental stages  
1468 (1-4). N = 3 biological replicates per development stage for both galls and ungalled control leaves.

1469

1470 **Supplement 5. Differentially regulated cytokinin-responsive genes.** Only genes from  
1471 GO:0009735 “*response to cytokinin*” with significantly different expression levels (P  $\leq 0.05$ ) are  
1472 shown. Ratios of expression levels of target transcript in ungalled control and galled tissues are  
1473 represented by a blue to orange palette, blue colors reflecting higher expression in the ungalled  
1474 controls and orange colors higher expression in galls of different developmental stages (1-4). N =  
1475 3 biological replicates per development stage for both galls and ungalled control leaves.

1476

1477 **Supplement 6. Differentially regulated genes involved in auxin metabolism.** Only genes from  
1478 GO:0009850 “*auxin metabolic process*” with significantly different expression levels (P  $\leq 0.05$ )  
1479 are shown. Ratios of expression levels of target transcript in ungalled control and galled tissues  
1480 are represented by a blue to orange palette, blue colors reflecting higher expression in the ungalled

1481 controls and orange colors higher expression in galls of different developmental stages (1-4). N =  
1482 3 biological replicates per development stage for both galls and ungalled control leaves.

1483

1484 **Supplement 7. Differentially regulated auxin-responsive genes.** Only genes from GO:0009733  
1485 “*response to auxin*” with significantly different expression levels ( $P \leq 0.05$ ) are shown. Ratios of  
1486 expression levels of target transcript in ungalled control and galled tissues are represented by a  
1487 blue to orange palette, blue colors reflecting higher expression in the ungalled controls and orange  
1488 colors higher expression in galls of different developmental stages (1-4). N = 3 biological  
1489 replicates per development stage for both galls and ungalled control leaves.

1490

1491 **Supplement 8. Differentially regulated genes involved in brassinosteroid metabolism.** Only  
1492 genes from GO:0016131 “*brassinosteroid metabolic process*” with significantly different  
1493 expression levels ( $P \leq 0.05$ ) are shown. Ratios of expression levels of target transcript in ungalled  
1494 control and galled tissues are represented by a blue to orange palette, blue colors reflecting higher  
1495 expression in the ungalled controls and orange colors higher expression in galls of different  
1496 developmental stages (1-4). N = 3 biological replicates per development stage for both galls and  
1497 ungalled control leaves.

1498

1499 **Supplement 9. Differentially regulated brassinosteroid-responsive genes.** Only genes from  
1500 GO:0009741 “*response to brassinosteroid*” with significantly different expression levels ( $P \leq$   
1501 0.05) are shown. Ratios of expression levels of target transcript in ungalled control and galled  
1502 tissues are represented by a blue to orange palette, blue colors reflecting higher expression in the

1503 ungalled controls and orange colors higher expression in galls of different developmental stages  
1504 (1-4). N = 3 biological replicates per development stage for both galls and ungalled control leaves.

1505

1506 **Supplement 10. Differentially regulated genes involved in abscisic acid metabolism.** Only  
1507 genes from GO:0009687 “*abscisic acid metabolic process*” with significantly different expression  
1508 levels ( $P \leq 0.05$ ) are shown. Ratios of expression levels of target transcript in ungalled control and  
1509 galled tissues are represented by a blue to orange palette, blue colors reflecting higher expression  
1510 in the ungalled controls and orange colors higher expression in galls of different developmental  
1511 stages (1-4). N = 3 biological replicates per development stage for both galls and ungalled control  
1512 leaves.

1513

1514 **Supplement 11. Differentially regulated abscisic acid-responsive genes.** Only genes from  
1515 GO:0009737 “*response to abscisic acid*” with significantly different expression levels ( $P \leq 0.05$ )  
1516 are shown. Ratios of expression levels of target transcript in ungalled control and galled tissues  
1517 are represented by a blue to orange palette, blue colors reflecting higher expression in the ungalled  
1518 controls and orange colors higher expression in galls of different developmental stages (1-4). N =  
1519 3 biological replicates per development stage for both galls and ungalled control leaves.

1520

1521 **Supplement 12. Differentially regulated genes involved in jasmonate metabolism.** Only genes  
1522 from GO:0009694 “*jasmonic acid metabolic process*” with significantly different expression  
1523 levels ( $P \leq 0.05$ ) are shown. Ratios of expression levels of target transcript in ungalled control and  
1524 galled tissues are represented by a blue to orange palette, blue colors reflecting higher expression  
1525 in the ungalled controls and orange colors higher expression in galls of different developmental

1526 stages (1-4). N = 3 biological replicates per development stage for both galls and ungalled control  
1527 leaves.

1528

1529 **Supplement 13. Differentially regulated jasmonate-responsive genes.** Only genes from  
1530 GO:0009753 “*response to jasmonic acid*” with significantly different expression levels (P ≤ 0.05)  
1531 are shown. Ratios of expression levels of target transcript in ungalled control and galled tissues  
1532 are represented by a blue to orange palette, blue colors reflecting higher expression in the ungalled  
1533 controls and orange colors higher expression in galls of different developmental stages (1-4). N =  
1534 3 biological replicates per development stage for both galls and ungalled control leaves.

1535

1536 **Supplement 14. Differentially regulated genes involved in salicylate metabolism.** Only genes  
1537 from GO:0009696 “*salicylic acid metabolic process*” with significantly different expression levels  
1538 (P ≤ 0.05) are shown. Ratios of expression levels of target transcript in ungalled control and galled  
1539 tissues are represented by a blue to orange palette, blue colors reflecting higher expression in the  
1540 ungalled controls and orange colors higher expression in galls of different developmental stages  
1541 (1-4). N = 3 biological replicates per development stage for both galls and ungalled control leaves.

1542

1543 **Supplement 15. Differentially regulated salicylate-responsive genes.** Only genes from  
1544 GO:0009751 “*response to salicylic acid*” with significantly different expression levels (P ≤ 0.05)  
1545 are shown. Ratios of expression levels of target transcript in ungalled control and galled tissues  
1546 are represented by a blue to orange palette, blue colors reflecting higher expression in the ungalled  
1547 controls and orange colors higher expression in galls of different developmental stages (1-4). N =  
1548 3 biological replicates per development stage for both galls and ungalled control leaves.

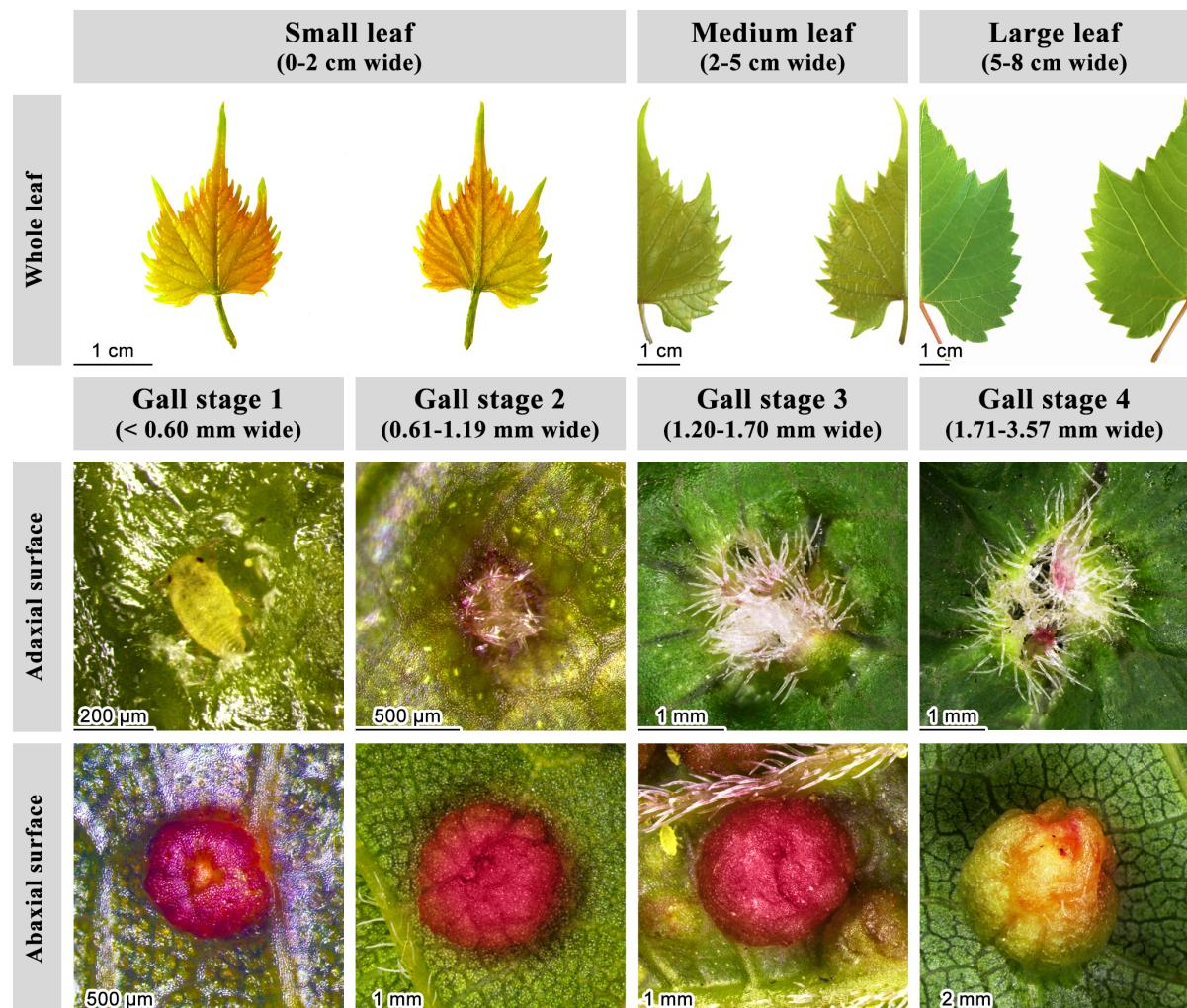
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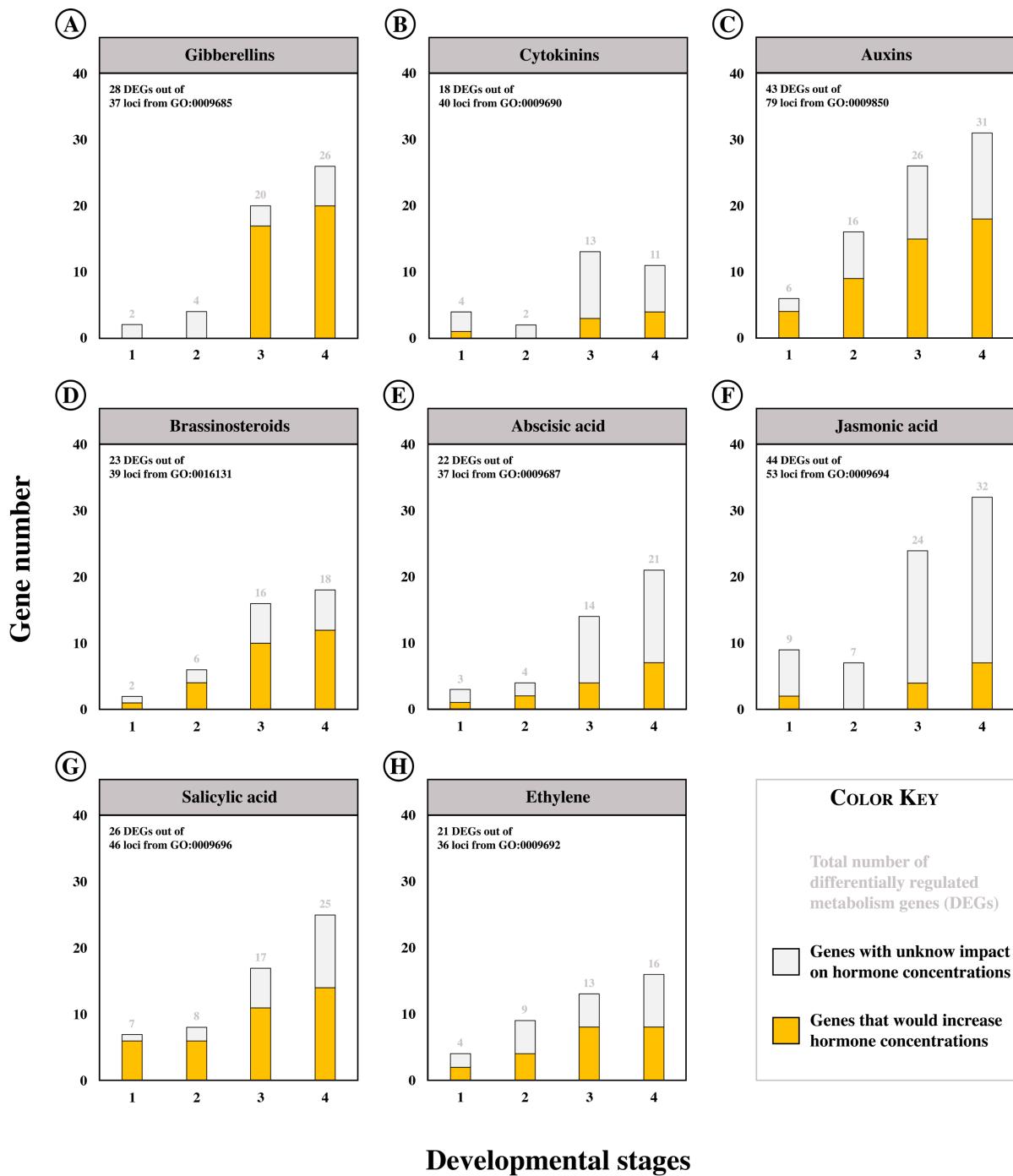
1550 **Supplement 16. Differentially regulated genes involved in ethylene metabolism.** Only genes  
1551 from GO:0009692 “*ethylene metabolic process*” with significantly different expression levels ( $P$   
1552  $\leq 0.05$ ) are shown. Ratios of expression levels of target transcript in ungalled control and galled  
1553 tissues are represented by a blue to orange palette, blue colors reflecting higher expression in the  
1554 ungalled controls and orange colors higher expression in galls of different developmental stages  
1555 (1-4).  $N = 3$  biological replicates per development stage for both galls and ungalled control leaves.

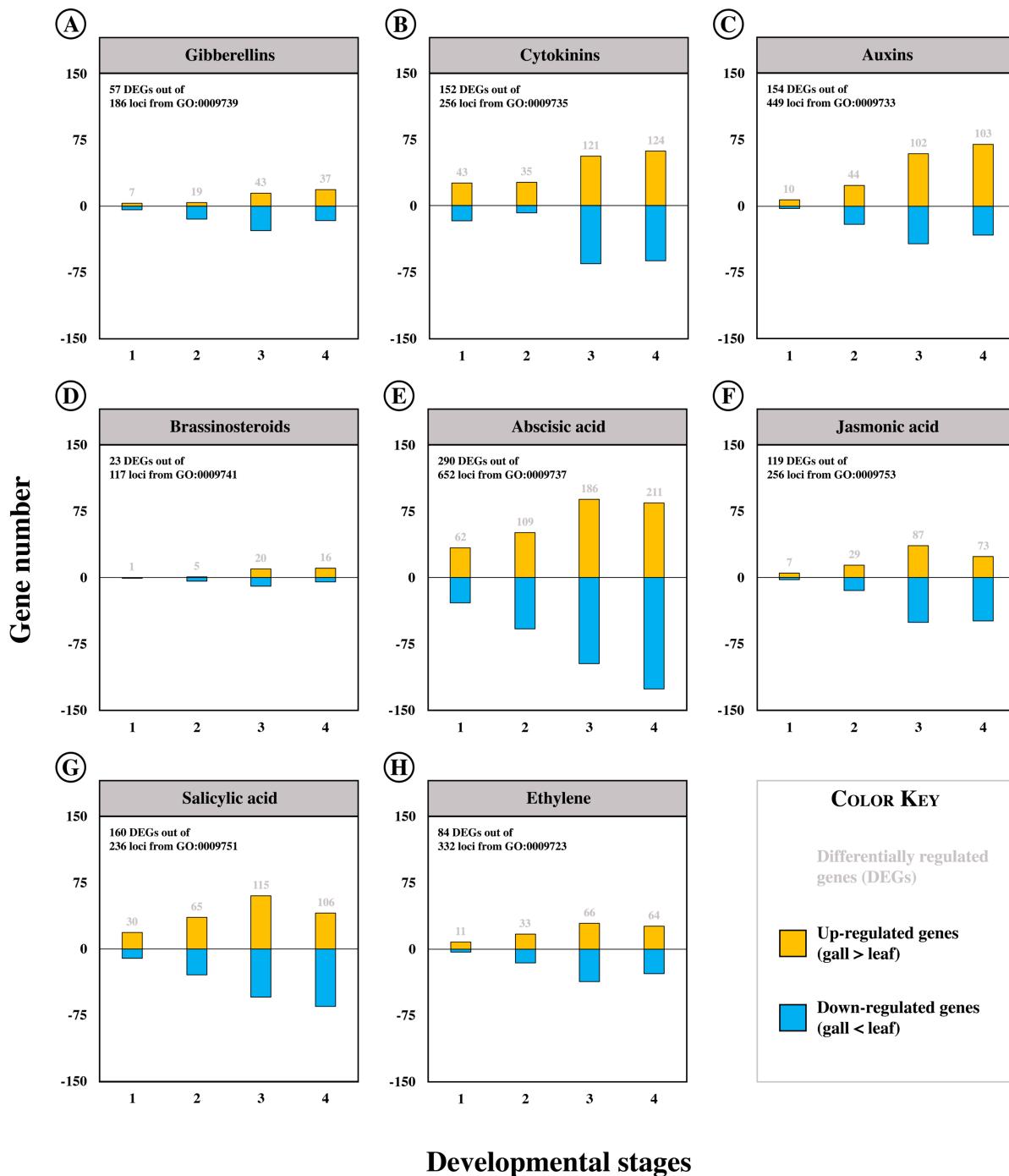
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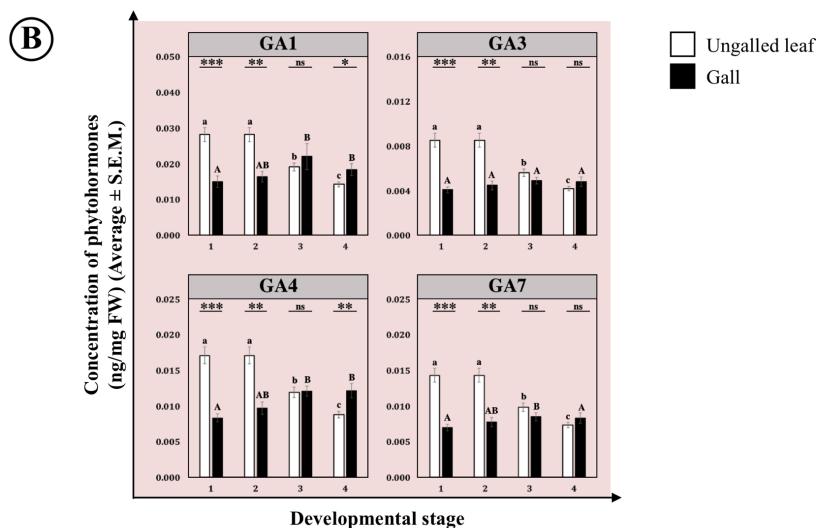
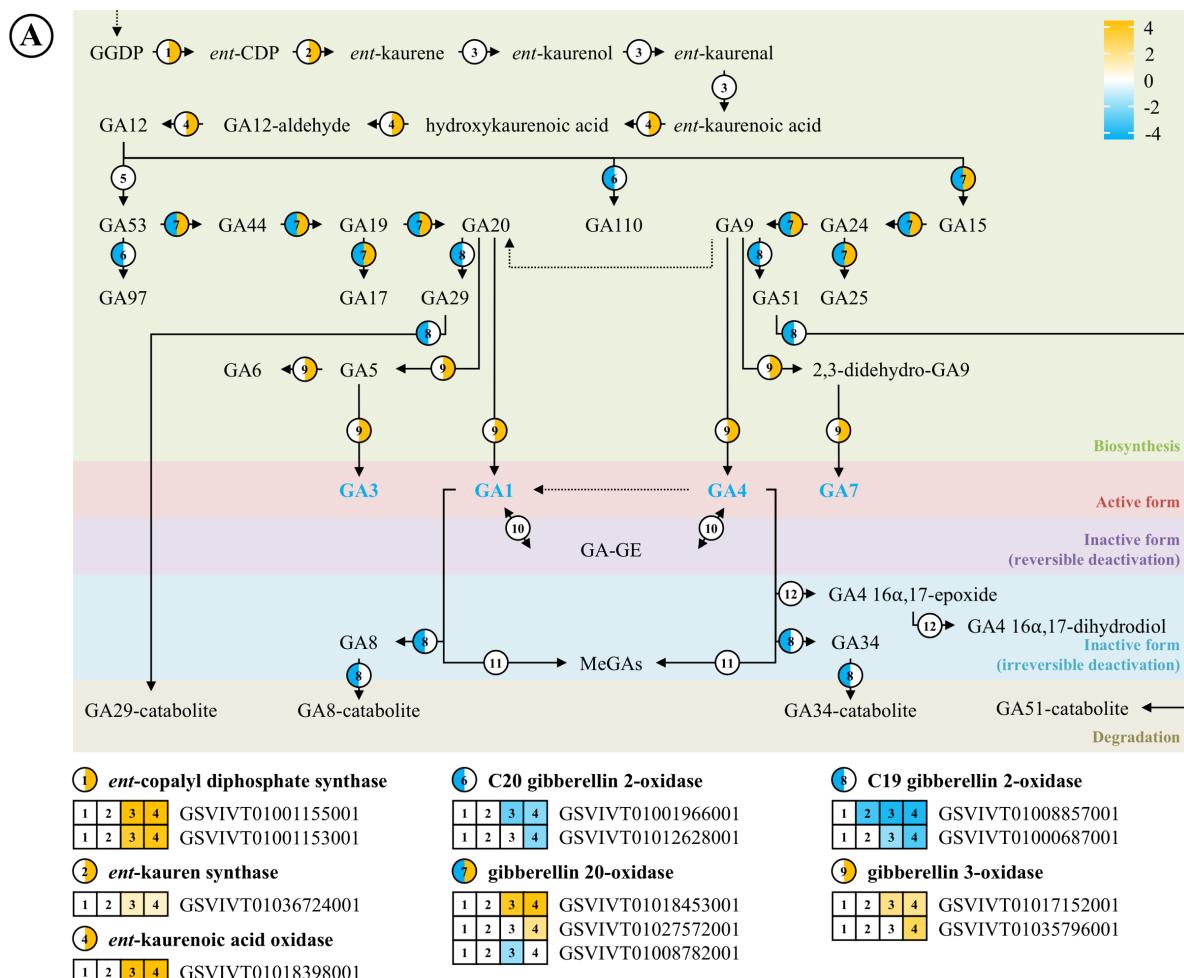
1557 **Supplement 17. Differentially regulated ethylene-responsive genes.** Only genes from  
1558 GO:0009723 “*response to ethylene*” with significantly different expression levels ( $P \leq 0.05$ ) are  
1559 shown. Ratios of expression levels of target transcript in ungalled control and galled tissues are  
1560 represented by a blue to orange palette, blue colors reflecting higher expression in the ungalled  
1561 controls and orange colors higher expression in galls of different developmental stages (1-4).  $N =$   
1562 3 biological replicates per development stage for both galls and ungalled control leaves.

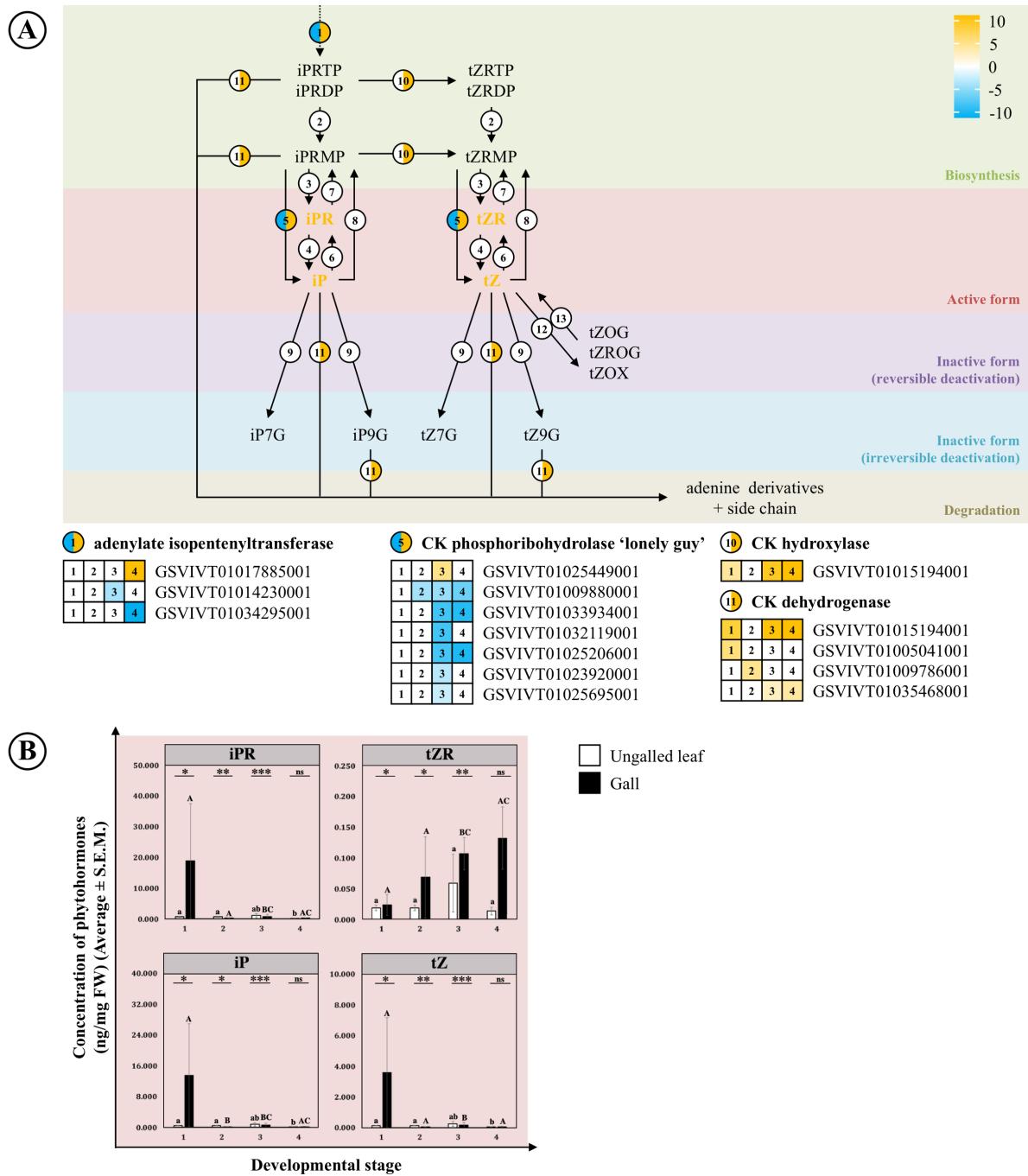
Figure 1.

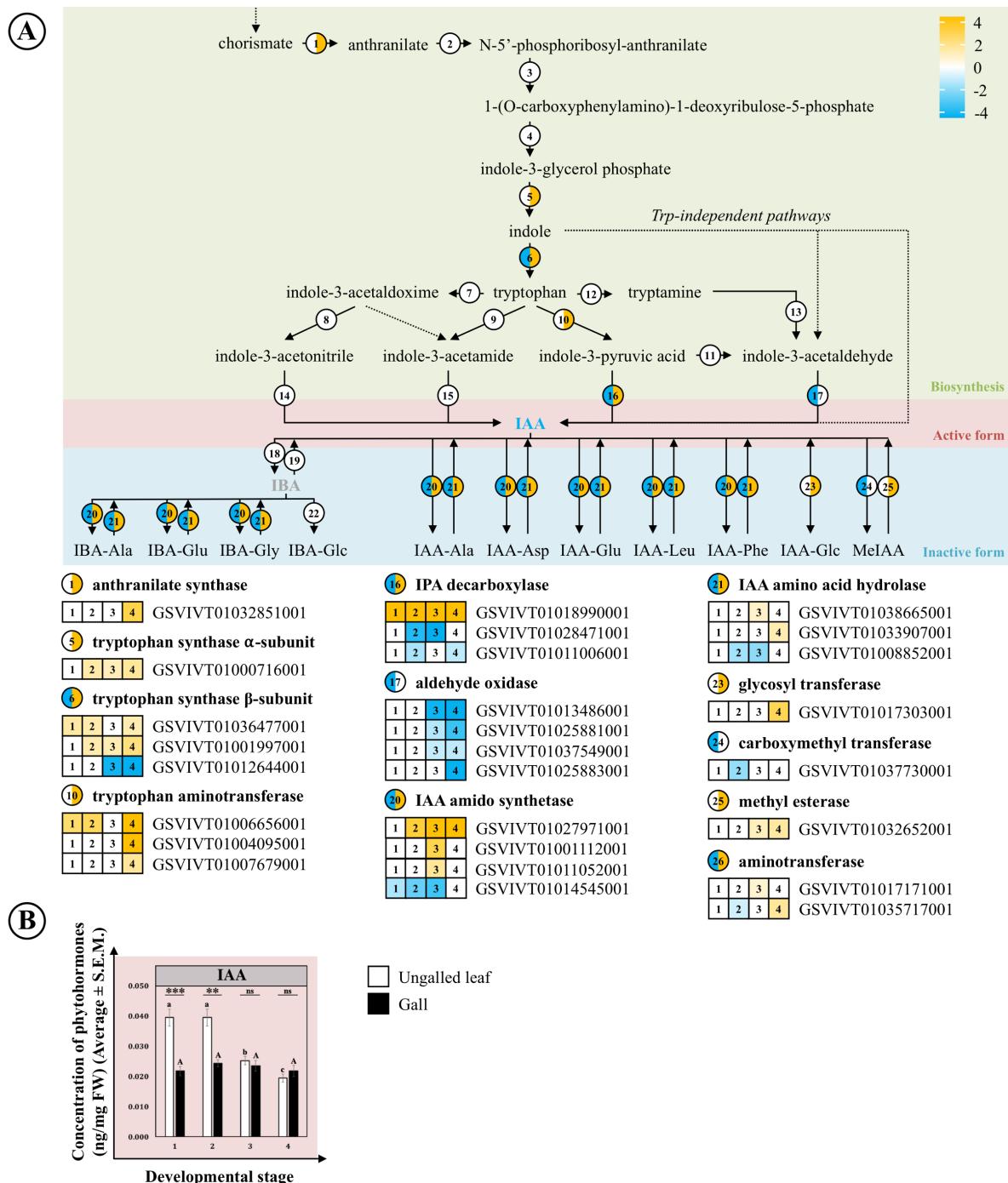


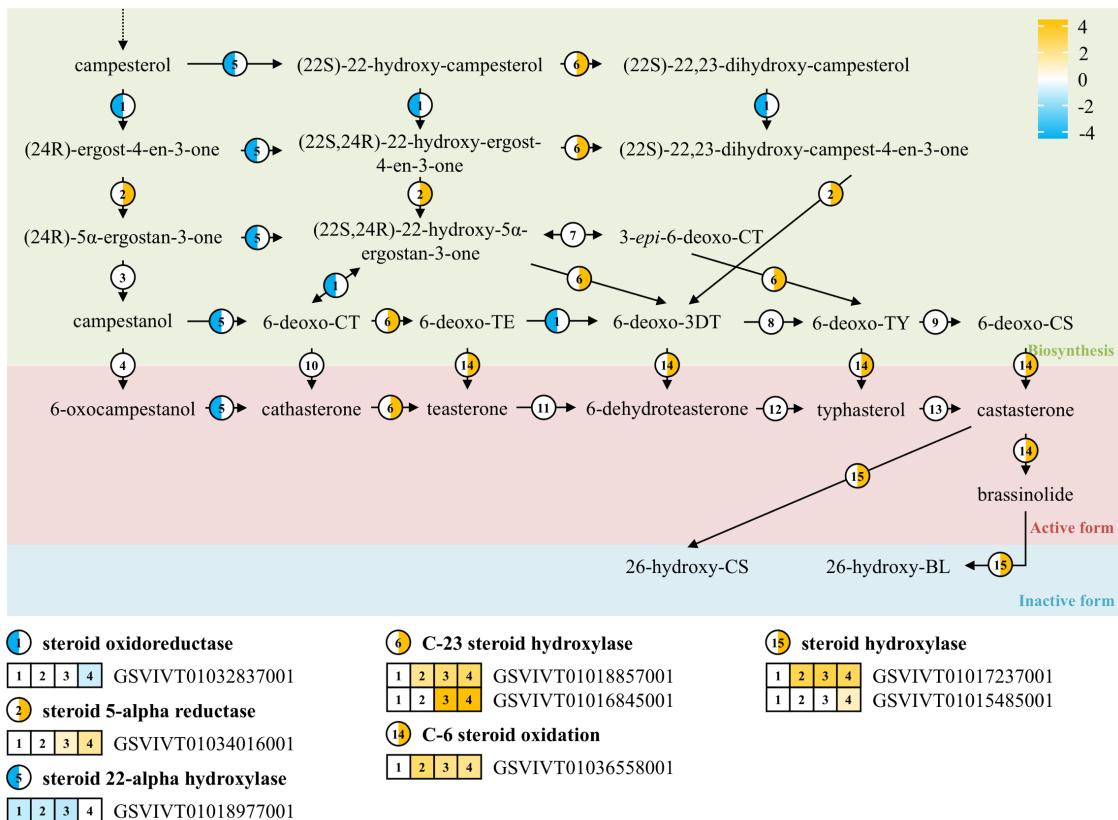
**Figure 2.**

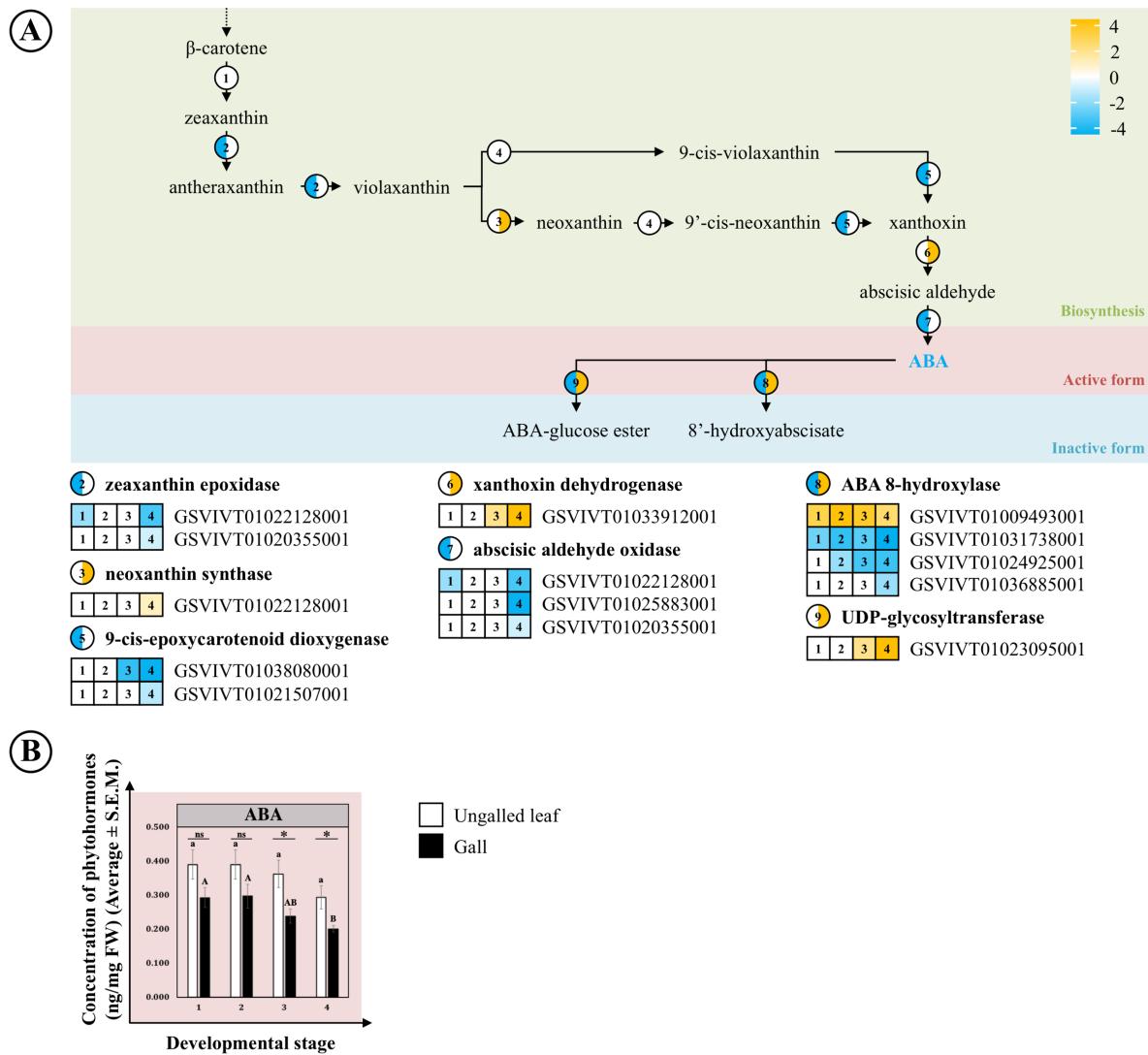
**Figure 3.**

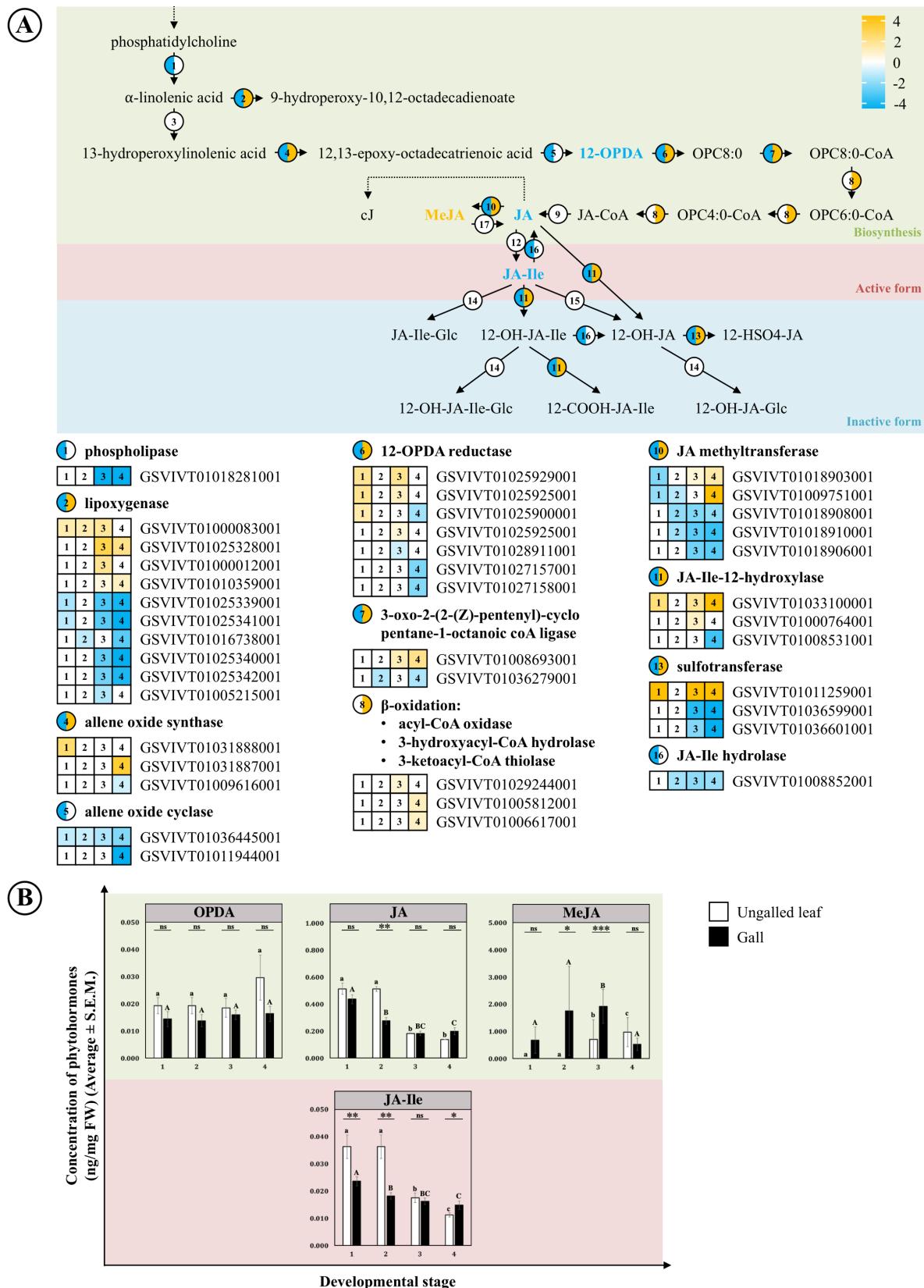
**Figure 4.****Figure 5.**

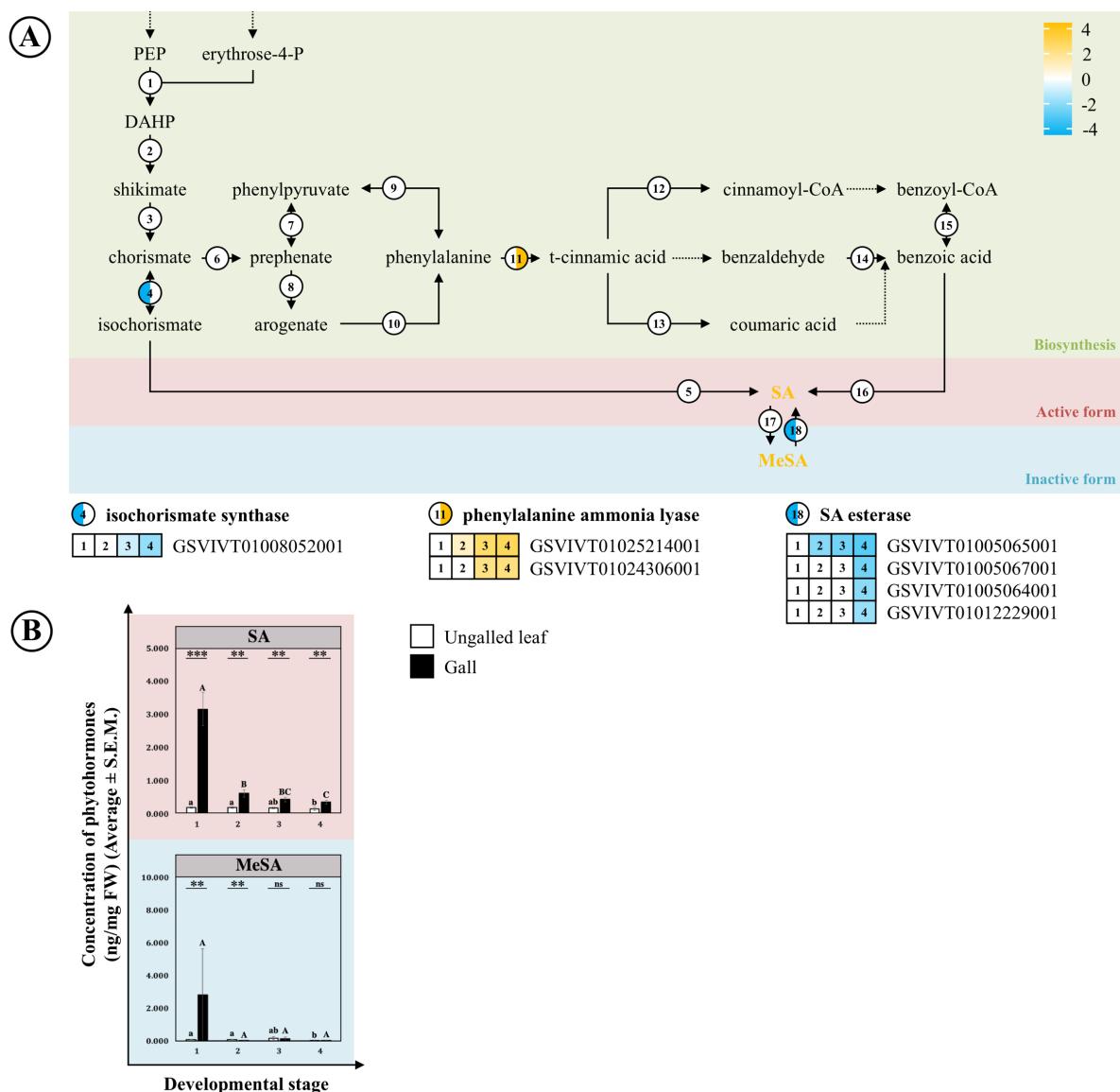


**Figure 6.**

**Figure 7.**

**Figure 8.**

**Figure 9.**

**Figure 10.**

**Figure 11.**