Title

Phenotypic and transcriptomic analyses reveal major differences between apple and pear scab nonhost resistance

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Abstract

Background. Nonhost resistance is the outcome of most plant/pathogen interactions, but it has rarely been described in Rosaceous fruit species. Apple (*Malus x domestica* Borkh.) ~~is~~ have a nonhost ~~for~~ resistance to *Venturia pyrina*, the scab species attacking European pear (*Pyrus communis* L.). Reciprocally, *P. communis* ~~is~~ have a nonhost ~~for~~ resistance to *Venturia inaequalis*, the scab species attacking apple. The major objective of our study was to compare the scab nonhost resistance in apple and in European pear, at the phenotypic and transcriptomic levels.

Results. Macro- and microscopic observations after reciprocal scab inoculations indicated that, after a similar germination step, nonhost apple/*V. pyrina* interaction remained nearly symptomless,

whereas more hypersensitive reactions were observed during nonhost pear/*V. inaequalis* interaction. Comparative transcriptomic analyses of apple and pear nonhost interactions with *V. pyrina* and *V. inaequalis*, respectively, revealed ~~considerable~~ differences. Very few differentially expressed genes were detected during apple/*V. pyrina* interaction, preventing the inferring of underlying molecular mechanisms~~which is consistent with a symptomless type I nonhost resistance~~. On the contrary, numerous genes were differentially expressed during pear/*V. inaequalis* interaction, ~~as expected in a type II nonhost resistance involving visible hypersensitive reaction~~allowing a deep deciphering. Pre-invasive defense, such as stomatal closure, ~~was detected~~could be inferred, as well as several post-invasive defense mechanisms (apoplastic reactive oxygen species accumulation, phytoalexin production and alterations of the epidermis composition). In addition, a comparative analysis between pear scab host and nonhost interactions indicated that, although specificities were observed, two major defense lines ~~were~~ seems to be shared in these resistances: cell wall and cuticle potential modifications and phenylpropanoid pathway induction.

Conclusion. This first deciphering of the molecular mechanisms underlying a nonhost scab resistance in pear offers new possibilities for the genetic engineering of sustainable scab resistance in this species. Concerning nonhost scab resistance in apple, further analyses must be considered with the aid of tools adapted to this resistance with very few cells engaged.

Keywords: apple, pear, nonhost resistance, transcriptomics

Background

Apple (*Malus x domestica* Borkh.) and European pear (*Pyrus communis* L.) are two closely related species belonging to the *Rosaceae* family. Reclassification of the *Rosaceae* placed both *Pyrus* and *Malus* genera in the subfamily *Spiraeoideae*, tribe *Pyreae* and subtribe *Pyrinae,* this subtribe corresponding to the long-recognized subfamily *Maloideae* [1]. Efforts to resolve relationships within this subtribe have frequently failed, and Campbell et al. [2] concluded that the genera of this subtribe

52 *Pyreae* have not diverged greatly genetically. The recent sequencing of the pear genome [3] allowed
53 a precise comparison with the apple genome [4] and led to the estimation of a divergence time
54 between the two genera of ≈ 5.4 – 21.5 million years ago. Furthermore, apple and pear genomes
55 share similar chromosome number (n=17), structure and organization.

56 Scab disease, caused by *Venturia* spp., affects several rosaceous fruit tree species. These
57 hemibiotrophic pathogens can infect only a limited host-range during their parasitic stage, but they
58 can overwinter as saprophytes in the leaf litter of a larger range of plant species [5]. Scab disease is
59 caused by *V. inaequalis* on apple, by *V. pyrina* (formerly named *V. pirina* [6]) on European pear, and
60 by *V. nashicola* on Japanese (*P. pyrifolia* Nakai) and Chinese (*P. ussuriensis* Maxim) pears. Cross
61 inoculations of *Venturia* spp. on different rosaceous fruit trees indicates that these pathogens are
62 highly host specific, probably indicating a close co-evolution of these pathogens with their hosts [7].
63 A plant species unable to be successfully infected by all isolates of a pathogen species is considered
64 as a nonhost for this pathogen. Nonhost interactions of *Venturia* spp. on apple and pear have rarely
65 been described. Microscopic observations have been made on *P. communis / V. nashicola* [8] as well
66 as *M. domestica* / *V. pyrina* and *P. communis* / *V. inaequalis* [5, 9]. In all cases, conidia germinated
67 and produced appressoria and runner hypheae, but failed to establish a network of stroma. No
68 macroscopic symptoms were visible.

69 Because of its durability, nonhost resistance has attracted numerous studies over the last
70 decade, which have uncovered its multiple and complex defense components. The underlying
71 mechanisms of nonhost resistance comprise pre-invasion resistance with preformed or induced cell-
72 wall defenses, metabolic defense with phytoanticipin or phytoalexin accumulation, pattern-triggered
73 immunity (PTI) as well as elicitor-triggered immunity (ETI) and various signaling pathways [10].

74 To our knowledge, the molecular bases of scab nonhost resistance of apple and pear have
75 never been investigated. We were not able to find reports on large-scale fungi nonhost resistance
76 analyses in pear and the few available in apple are about *Penicillium digitatum* and are conducted on
77 fruit [11, 12].

78 However, it is possible to find genome-wide molecular analyses of scab host resistance in
79 apple and pear. Thus, Perchepied et al. [13] performed a detailed transcriptomic analysis of the host
80 resistance of pear against *V. pyrina* strain VP102, deployed in a transgenic pear bearing the well-
81 known apple *Rvi6* resistance gene against *V. inaequalis*. They reported the modulation of expression
82 of 4170 genes and revealed that downstream of the pathogen recognition, the signal transduction
83 was triggered with calcium, G-proteins and hormonal signaling (jasmonic acid (JA) and
84 brassinosteroids), without involvement of salicylic acid (SA), and that this led to the induction of
85 defense responses such as a remodeling of primary and secondary cell wall, cutin and cuticular waxes
86 biosynthesis, systemic acquired resistance (SAR) signal perception in distal tissues, and the
87 biosynthesis of phenylpropanoids (flavonoids and lignin). Only four other transcriptomic studies
88 involving pear/pathogen host interactions have been published so far but are not concerning scab.
89 Yan et al. [14] reported the modulation of expression of 144 pear genes after fruit treatment by
90 *Meyerozyma guilliermondii*, an antagonistic yeast used for biocontrol of natural pear fruit decay.
91 Zhang et al. [15] similarly reported the modulation of expression of 1076 pear genes after treatment
92 with *Wickerhamomyces anomalus*, another biocontrol agent. Using RNA-seq, Wang et al. [16]
93 reported a major role of ethylene signalization during the compatible interaction between *P. pyrifolia*
94 and *Alternaria alternata*, a necrotrophic pathogen. Finally, Xu et al. [17] applied RNA-seq to
95 characterize the genes of *Penicillium expansum* activated after infection of pear fruits.
96 Concerning host resistance of apple against *V. inaequalis*, subtractive hybridization [18, 19]
97 and cDNA-AFLP [20] led to the identification of a limited set of differentially expressed genes in *Rvi6*
98 natural resistant 'Florina' variety (scab inoculated 'Florina' versus mock, [19]), or in *Rvi6* resistant
99 transgenic 'Gala' lines (*Rvi6* transgenic 'Gala' versus non-transformed 'Gala', after scab inoculation,
100 [18]; *Rvi6* transgenic 'Gala' before versus post scab inoculation, [20]). Recently, Perchepied et al. [13]
101 also performed a transcriptomic analysis of the *Rvi6* resistance in a transgenic 'Gala' line (transgenic
102 versus non-transformed, before and after scab inoculation). They reported the modulation of
103 expression of 2977 genes and revealed that downstream of the pathogen recognition, signal

transduction was triggered with calcium and interconnected hormonal signaling (auxins and brassinosteroids), without involvement of SA, and that this led to the induction of defense responses such as a remodeling of primary and secondary cell wall, galactolipids biosynthesis, SAR signal generation and the biosynthesis of flavonoids. Genome-wide molecular analyses of apple scab host resistance have also been achieved in other context than the *Rvi6* resistance. A RNA-seq analyze identified five candidate genes putatively involved in the ontogenic scab resistance of apple [21]. In addition, nuclear proteome analysis identified 13 proteins with differential expression patterns among varying scab resistance 'Antonovka' accessions [22]. Recently, Masoodi et al. [23] performed a RNA-seq analyze comparing three scab-resistant ('Florina', 'Prima', and 'White Dotted Red') and three susceptible ('Ambri', 'Vista Bella', and 'Red Delicious') apple genotypes out to mine new scab resistance genes. They reported the modulation of expression of 822 genes related to various pathways, i.e., metabolic, protein processing, biosynthesis of secondary metabolites, plant hormone signal transduction, autophagy, ubiquitin-mediated proteolysis, plant-pathogen interaction, lipid metabolism, and protein modification pathways.

Thus, if large-scale analyses of pear and apple scab host resistance can be found, in-depth knowledge of transcriptional patterns and gene functions involved in apple and pear scab nonhost resistance is still needed. The objectives of our study were 1) to precisely describe nonhost resistance symptoms in *M. domestica* / *V.pyrina* and *P. communis* / V. *inaequalis* interactions 2) to analyze the underlying molecular mechanisms of both nonhost interactions through a transcriptomic study 3) to compare the mechanism of host [13~~1~~, 23] and nonhost scab resistance in apple and European pear.

Results

Macroscopic and microscopic symptoms analyze

Nonhost interactions were observed in a test performed on leaves of 'Gala' apple and 'Conference' pear cultivars, inoculated by a *V. pyrina* strain (VP102) and a *V. inaequalis* strain (VI EUB05) respectively. At the macroscopic level, a total absence of sporulation was observed on all

nonhost interactions (Table 1), on the contrary to host interactions (Fig. 1 A and B). Very few pear plants inoculated with *V. inaequalis* presented resistance symptoms such as pin points (Fig. 1C) and chlorotic lesions (Fig. 1D), whereas the apple 'Gala' remained completely symptomless after *V. pyrina* inoculations (Fig. 1E).

Table 1: Scab qualitative note of pear and apple lines inoculated with *V. pyrina* and *V. inaequalis.*

Percentage (number) of plants in the different classes of symptoms, 42 days after inoculation				
Class of symptoms	*V. pyrina* strain VP102		*V. inaequalis* strain EUB05	
	'Conference'	'Gala'	'Conference'	'Gala'
0: absence of symptoms	0	100 (28)	90 (17)	0
1: hypersensitivity (pin points)	0	0	5 (1)	0
2: resistance (chlorotic lesions, slight necrosis, crinkled aspect)	0	0	5 (1)	0
3a: weak resistance (necrotic or chlorotic lesions with occasional very light sporulation)	0	0	0	0
3b: weak susceptibility (clearly sporulating chlorotic or necrotic lesions)	0	0	0	0
4: susceptibility (sporulation only)	100 (19)	0	0	100 (28)

At the microscopic level, three days after inoculation, there was no clear difference between host and nonhost interactions: the conidia of *V. inaequalis* and *V. pyrina* germinated equally on both hosts forming one or two appressoria (Fig. 1 F and ~~H~~G). However, 14 days after inoculation, there was a clear reaction of the plant cells in contact with the appressoria (accumulation of red autofluorescent compounds and enlargement of these cells), which could indicate very small scale hypersensitive reactions (HR) (Fig. 1 ~~G~~ H and I) in both plant species, more frequently in pear than in apple (Fig1. J and K). No formation of subcuticular stroma and no conidiogenesis were observed in the nonhost interactions.

147 Global gene expression analyze

148 Differentially expressed genes (DEGs) were ~~analyzed~~ obtained by comparing transcript
149 abundance in leaves between T0 and 24 hours post inoculation (hpi) and between T0 and 72 hpi, in
150 the nonhost interactions 'Gala' / *V. pyrina* VP102 and 'Conference' / *V. inaequalis* EUB05. These time
151 points were chosen in order to cover the period of establishment of the first intimate contacts
152 between fungal and plant cells: conidia germination and appressoria formation. For each
153 comparison, the experimental design is a dye switch approach [24] between the two biological
154 repeats made by condition (genotype x treatment x time). Each biological repeat is a pool of three
155 leaves from three different plants.

156 In total, 60 DEGs in apple and 1857 DEGs in pear were identified, which amounts to 0.19 % of
157 all apple genes on the apple AryANE v2.0 microarray, and 4.23 % of all pear genes on the Pyrus v1.0
158 microarray (Table 2). Among the 1857 pear DEGs, 80.2 % were only detected at 24 hpi and 15.4 %
159 only at 72 hpi, whereas 4.2 % were up-regulated or down- regulated similarly at both time points of
160 the ~~kinetics~~experiment. Among all the pear DEGs observed at 24 and 72 hpi, the proportion of up-
161 regulated DEGs was higher (68.8 %) than the proportion of down-regulated DEGs (31.2 %).

162

163 **Table 2. Number of DEGs identified during apple and pear nonhost response to *V. pyrina* and *V.***
164 ***inaequalis***

	'Gala' / VP102		'Conference' / EUB05	
	24 hpi	72 hpi	24 hpi	72 hpi
Total # of DEGs*	49	11	1570	364
DEGs in % of all genes on the microarray**	0.16	0.03	3.58	0.83
% of up-regulated DEGs	67.3	36.4	74.5	25.5
% of down-regulated DEGs	32.7	63.6	25.5	74.5
% of DEGs without *Arabidopsis* homolog	27.1	30.4	0.70	1.09

165 *: DEGs numbers were calculated using the p-adj values ≤ 0.01 as selection threshold
166 **: 31311 genes on the apple Ariane V2 microarray, 43906 genes on the pear V1 microarray
167

168 To ~~basically~~ validate the transcriptomic data, 12 ~~DEGS~~ DEGs with varied ratios (between -1.9
169 and 2.9) ~~have been~~were tested by quantitative RT-PCR (qPCR; Table S1), on the two biological
170 repeats used for transcriptomic analyses. Considering the low~~weak~~ number of DEGs found for apple
171 in this study, we only tested two of them in qPCR. As seen in Table 2 for pear, at 24 hpi, a majority of
172 DEGs are up-regulated and at 72 hpi, a majority of DEG are down-regulated. qPCR was then
173 performed essentially on DEGs with positive ratios at 24 hpi~~phi~~ and negative ratios at 72 hpi (Table
174 S1). The qPCR results confirmed the induced or repressed status of all tested DEGs.

175 Among the 1857 pear DEGs, the 1845 DEGs with *Arabidopsis* homologs (TAIR identifier) have
176 been classified according to MapMan functional categories (Fig. 2). In order to highlight the enriched
177 classes, the numbers of DEGs per category have been normalized to the numbers of *Arabidopsis*
178 genes in each MapMan category and bootstraps have been done to provide a confidence estimate
179 for the accuracy of the output (Fig. 2). We then more particularly explored the DEGs present in the
180 following enriched classes: Hormone metabolism, Stress, Lipid metabolism, Signaling, Secondary
181 metabolism, Cell wall, and depending on the defense pathways and responses identified, search for
182 others related DEGs in enriched wider classes: Protein, RNA and Miscellaneous. The Table 3 gives the
183 defense pathways and responses identified and the functional categories in which DEGs related to
184 these pathways/responses have been found. The analyze of the 184 DEGs found (corresponding to
185 158 different functions) is expanded in the Discussion section (Metadata of the 184 DEGs in Table
186 S2).

187

188 **Table 3. Numbers of DEGs in the defense pathways and responses identified among the enriched**
189 **functional categories analyzed in pear nonhost response to *V. inaequalis***

MapMan functional categories	Defense pathways and reponses identified												
	JA	SA/SAR	Calcium	ROS	Stomatal closure	HR	Cell Wall	Cuticle and waxes	Terpens and isoprenoids	Phenylpropanoids	Lignin	Coumarin and hydroxycinnamic acid	TOTAL
Protein	1					6						2	9
RNA	3					1		1		2			7
Miscellaneous		5				1		6			3	6	21
Hormone metabolism	10	2					1	1	3				17
Stress	4	11	2	1		3							21
Lipid Metabolism	5	1				8		7					21
Signalling		4	5	4	9	3							25
Secondary metabolism								4	9	5	11	2	31
Cell Wall							32						32
TOTAL	23	23	7	5	9	22	33	19	12	7	14	10	184

190

191 Among the 60 apple DEGs found, 17 have no *Arabidopsis* homolog, 12 more have
192 unpredicted function and for 11 more we could not find information (Metadata of the 60 DEGs in
193 Table S3). In view of our findings in pear / *V. inaequalis* nonhost interaction, 9 of the 20-remaining
194 apple DEGS could be relevant in apple / *V. pyrina* nonhost interaction and are further analyzed in the
195 Discussion section (Metadata of the 9 DEGs in Table S4).

196

197 Discussion

198 ~~Variable~~ More frequent symptoms of nonhost resistance ~~between apple and pear~~in pear versus
199 apple at microscopic level

200 ~~Nonhost interactions were observed in a test performed on leaves of 'Gala' apple and~~
201 ~~'Conference' pear cultivars, inoculated by a *V. pyrina* strain (VP102) and a *V. inaequalis* strain (VI~~
202 ~~EUB05) respectively. At the macroscopic level, a total absence of sporulation was observed on all~~
203 ~~nonhost interactions (Table 1).~~ The apple 'Gala' remained completely symptomless after *V. pyrina*
204 inoculations (Fig. ~~1C~~1E). This is similar to the observation of Chevalier et al. [9] after inoculation of
205 'Gala' with another *V. pyrina* strain. On the contrary, pear plants inoculated with *V. inaequalis*
206 presented occasional pin points symptoms (Fig. ~~1A~~1D) and chlorotic lesions (Fig. ~~1B~~1E). Chlorotic
207 lesions had already been observed by Chevalier et al. [9] after inoculation of the pear 'Pierre
208 Corneille' with the *V. inaequalis* strain EUB04, but pin points had never been reported in this nonhost

209 interaction. ~~According to our observations, apple nonhost resistance could be classified as type I and~~
210 ~~pear as type II according to Mysore and Ryu [12] definition based on the absence/presence of visible~~
211 ~~HR reaction.~~
212
213 At the microscopic level, ~~three days after inoculation, there was no clear difference between~~
214 ~~host and nonhost interactions: the conidia of *V. inaequalis* and *V. pyrina* germinated equally on both~~
215 ~~hosts forming one or two appressoria (Fig. 1 D and F). However,~~ 14 days after inoculation, there was
216 a clear reaction of the plant cells in contact with the appressoria (accumulation of red
217 autofluorescent compounds and enlargement of these cells), which could indicate very small scale
218 hypersensitive reactions (HR) (Fig. 1 ~~E~~ H and ~~G~~I, I and K) in both plant species. No formation of
219 subcuticular stroma and no conidiogenesis were observed in the nonhost interactions, contrary to
220 the host-resistance reactions [~~11~~13]. These observations are similar to the collapsed cells described
221 by Chevalier et al. [9] in apple and pear nonhost reactions, and to the rare HR-like reactions observed
222 by Stehmann et al. [5] on apple inoculated by *V. pyrina*.
223 Our results seems to indicate that the leaf surface morphology of apple and pear is equally
224 compatible with *V. pyrina* and *V. inaequalis* conidia germination, without specific inhibition at this
225 stage. Recognition probably occurs only at the appressorium site, leading to the cellular reactions
226 observed. These reactions were limited to a few cells without visible symptoms in apple / *V. pyrina*
227 interaction, but more extended in pear / *V. inaequalis* interaction and could occasionally
228 ~~produced~~produce macroscopic symptoms ~~in pear / *V. inaequalis* interaction~~.
229
230 Different patterns of global gene expression in nonhost resistance in pear versus apple
231 ~~Differentially expressed genes (~~DEGs~~)~~ were analyzed by comparing transcript abundance in
232 leaves between T0 and 24 ~~hours post inoculation (~~hpi~~)~~ and between T0 and 72 hpi, in the nonhost
233 interactions 'Gala' / *V. pyrina* VP102 and 'Conference' / *V. inaequalis* EUB05. This ~~kinetic~~
234 experimental design is open to criticism because it ~~does not exclude~~could include in results ~~data~~

235 ~~corresponding on one hand to genes~~DEGs responding ~~not to the infection but~~ to the inoculation
236 method (spray of water), and ~~on the other hand to genes~~DEGs ~~whose~~ which expression varies due to
237 leaves ageing. Water spray is effectively perceived as a mechanical stimulus by plants, the
238 *Arabidopsis* response being largely regulated by the JA pathway induction under the control of
239 MYC2/MYC3/MYC4 transcription factors [~~13~~25]. But this response is transient and really fast as most
240 of genes differentially~~differently~~ regulated peak within 30 minutes regain untreated transcriptional
241 levels within 3 hours [~~13~~25]. It seems therefore very unlikely that our analysis at 24 and 72 hpi
242 includes genes responding to water spray. Resistance due to leaves ageing i.e. ontogenic resistance
243 has been investigated in *Malus*-*Venturia* pathosystem at 72 and 96 hpi, and 5 genes have been
244 identified whose modulation could be linked to this resistance [~~14~~21]. None of these apple genes, or
245 homologs in pear, were found differentially expressed at 72 hpi in our interactions (Table S5), which
246 argue against the presence of ageing responding genes in our results. In total, 60 DEGs in apple and
247 1857 DEGs in pear were identified, which amounts to 0.19 % of all apple genes on the apple AryANE
248 v2.0 microarray, and 4.23 % of all pear genes on the Pyrus v1.0 microarray (Table 2).
249
250 The very small number of DEGs (60) detected in the apple/ *V. pyrina* nonhost interaction at
251 24 or 72 hpi ~~is~~ seems in agreement with the total absence of macroscopic symptoms observed during
252 this interaction~~.~~, and the few small HR-like reactions detected at the microscopic level 14 days post
253 inoculation. ~~However, at the microscopic level, small HR-like reactions were detected in the apple /~~
254 ~~*V. pyrina* interaction at 14 days post inoculation.~~ Because these reactions involve only a few cells in
255 the leaves, the changes in gene expression are probably below the threshold of DEG detection
256 applied in this experiment. It is also possible that the mild response of apple to *V. pyrina* occurs later
257 than 72 hpi, between 72 hpi and 6 days after inoculation. Indeed, Chevalier et al. [9] observed these
258 rare HR from 6 days post inoculation, with no more evolution until 14 days after inoculation. A ~~longer~~
259 later time (6 days post inoculation) would have been necessary to conclude on this last hypothesis.

260 On the contrary to apple/ *V. pyrina* nonhost interaction, the number of DEGs (1857) detected
261 during the pear / *V. inaequalis* interaction is in the same order of magnitude as the number of DEGs
262 detected during pear host resistance to *V. pyrina* (see [1113]). This could be consistentThis is in
263 agreement with the more frequent observation in this interaction of macroscopic microscopic HR-
264 like reactions detected 14 days post inoculation, and of occasional macroscopic symptoms of
265 resistance (chlorotic lesions or pin points) in this interaction. Among the 1857 pear DEGs, 80.2 %
266 were only detected at 24 hpi and 15.4 % only at 72 hpi, whereas 4.2 % were up-regulated or down-
267 regulated similarly at both time points of the experiment. Among all the pear DEGs observed at 24
268 and 72 hpi, the proportion of up-regulated DEGs was higher (68.8 %) than the proportion of down-
269 regulated DEGs (31.2 %). The 1845 DEGs with *Arabidopsis* homologs (TAIR identifier) have been
270 classified according to MapMan functional categories (Fig. 2). In order to highlight the enriched
271 classes, the numbers of DEGs per category have been normalized to the numbers of *Arabidopsis*
272 genes in each MapMan category and bootstraps have been done to provide a confidence estimate
273 for the accuracy of the output (Fig. 2). We then more particularly explored the DEGs present in the
274 following enriched classes: Hormone metabolism, Stress, Lipid metabolism, Signaling, Secondary
275 metabolism, Cell wall, and depending on the defense pathways and responses identified, search for
276 others related DEGs in enriched wider classes: Protein, RNA and Miscellaneous. The Table 3 gives the
277 defense pathways and responses identified and the functional categories in which DEGs related to
278 these pathways/responses have been found. The analyze of the 184 DEGs found (corresponding to
279 158 different functions) is expanded in the Discussion section.
280
281 Weak involvement of hormone Hormone signaling pathways classically associated to resistance
282 seems weakly involved in pear / *V. inaequalis* interaction
283 Pear We found pear DEGs were found that seems to indicate that the jasmonic acid (JA)
284 pathway was repressed. The JA biosynthesis and metabolic conversions were reviewed by
285 Wasternack et al. [1526]. In our data, at 24 hpi, the first step of JA biosynthesis, that iscorresponding

286 to the conversion of linoleic acid in 12-oxo-phytodienoic acid (OPDA), ~~is~~ seems to be compromised:
287 six out of seven lipoxygenases (LOX) (three LOX1, two LOX2 and two LOX5) are repressed, the last
288 one being induced (Fig. 3). OPDA produced in the chloroplast is then transported to the peroxisome
289 for subsequent conversion to JA via the action of OPR3 (12-oxo-phytodienoic acid reductase) and β-
290 oxidation enzymes (reviewed in [~~16~~27] and in [~~15~~26]). In pear, three β-oxidation enzymes were found
291 activated more or less rapidly: ACX4 (24 hpi), MFP2 (72 hpi) and the thioesterase homolog to
292 *At2g29590* (72 hpi), which could suggest~~s~~ that constitutive OPDA stocks were turned into JA. But the
293 early and long-lasting induction of *JMT* and *ST2A* genes ~~is~~ could be in favor of a rapid conversion of JA
294 in inactive compounds, *JMT* induction potentially being reinforced by *BBD1* repression (24 hpi). *BBD1*
295 is actually known as a negative regulator of *JMT* [~~17~~28].
296 ~~The~~ The behavior of some DEGs seemed indicate that the defense response depending on JA
297 was also ~~clearly~~ repressed in pear (Fig. 3). The transcription activator MYC2 of JA-induced genes is
298 known to be repressed by its interaction with JAZ proteins (reviewed in [~~15~~26]), and two *JAZ1* and
299 one *JAZ3* coding genes were found activated at 24 hpi in pear. UBP12 is known as a stabilizer of
300 MYC2 [~~18~~29]. In our data, *UBP12* was found repressed at 72 hpi, which could reinforce~~s~~ the
301 inactivation of MYC2. WRKY33 is known as an activator of the JA defense pathway [~~19~~30] and WRK70
302 [~~20~~31] or AS1 (or MYB91; [~~21~~32]) as inhibitors, and among JA-responsive proteins, the pathogenesis-
303 related PR3, PR4 and PR12 act downstream MYC2 activation [~~22~~33]. In our data, accordingly with the
304 repression of the activator WRKY33 and the activation of the inhibitors WRK70 and AS1, some JA-
305 responsive genes were also found repressed, such as the chitinase coding genes *PR4* (also called *HEL*)
306 and *ATEP3*. Furthermore, no DEGs were found for PR3 and PR12 functions. To conclude, in the
307 nonhost interaction between pear and *V. inaequalis*, some JA ~~seems to~~could be produced, but is
308 rapidly converted into inactive compounds and the subsequent defense response ~~is clearly~~seems to
309 be repressed.
310 Pear DEGs were found that ~~seems to~~could indicate that the ~~salicylic acid (~~SA~~)~~ pathway was
311 slightly engaged and rapidly repressed (Fig. 3). *WRKY70* was induced at 72 hpi in our data. This

312 transcription factor is known as a negative regulator of SA biosynthesis but a positive regulator of SA-
313 mediated defense genes in *Arabidopsis* ~~([23]; [24]; [25])~~[34, 35, 36], among them *PR2*, *PR5* but not
314 *PR1* [~~26~~37]. *WRKY33* which is known as a negative regulator of SA-responsive genes [~~27~~38], was also
315 repressed at 72 hpi in our data. PR2 and 5 are well-known anti-fungal proteins ~~([28]; [29]; [30])~~[39,
316 40, 41]. At 24 hpi a *PR2*, two *PR2-like*, a *PR5* and a *PR5-like* coding genes were found induced in our
317 work, another *PR2-like* and two others *PR5-like* being repressed. The differential expression was
318 maintained at 72 hpi for only two of the previously activated ones. Furthermore no DEG was found
319 for the PR1 function but three *PR1-like* genes were found repressed: *ATPRB1* and genes homolog to
320 *At5g57625* and *At4g33720*. *ATPRB1* was already reported as repressed by SA treatment [~~31~~42]. In
321 our data, the WRKY70 transcription factor was induced later than the induced PR genes so we could
322 imagine that induced PR genes were activated by another precocious regulation, such as an oxidative
323 burst (see below), rather than by WRKY70. Furthermore, WRKY70 induction seems not sufficient to
324 enable a long lasting induction of these defense genes.

325 Other pear DEGs seems to indicate that SA accumulation ~~was also rather mixed~~was transient.
326 *CBP60a* [~~32~~43], *ACA11* [~~33~~44], *EICBP.B* (or *CATMA1*; [~~34~~45]), all three coding calcium-sensor proteins,
327 are known as negative regulators of SA accumulation and biosynthesis, as well as the light signaling
328 factor *FAR1* [~~35~~46] or the SA glucosyltransferase *UGT74F1* which convert SA in inactive SA 2-O-beta-
329 D-glucoside or the glucose ester of SA [~~36~~47]. On the contrary *EDS1*, *PAD4* (reviewed in [~~37~~48]) and
330 *MKS1* [~~38~~49] are known as positive regulators of SA accumulation. In our data, the repression of
331 *CBP60a*, *ACA11* and *EICBP.B* genes could sustain~~ed~~ a SA biosynthesis and accumulation. In addition,
332 *EDS1* activation could allow~~ed~~ to consider a positive feedback loop likely to potentiate SA action via
333 EDS1 cytosolic homodimers, even though *PAD4* was repressed. But, as well as *WRKY70* induction, the
334 repression of the MAPK *MKS1* and the activation of the light signaling factor *FAR1* (2 times) or the SA
335 glucosyltransferase *UGT74F1* ~~were~~ could be in favor of less free SA. Concerning SAR, *MES1* is known
336 as required in healthy systemic tissues of infected plants to release the active SA from methyl-SA,
337 which serves as a long-distance signal for ~~systemic acquired resistance (~~SAR~~)~~ [~~37~~48] and *ACBP6* may

338 be involved in the generation of SAR inducing signal(s) [~~39~~50]. In our data, SAR seemed compromised
339 given the repression of *ACBP6* at 24 hpi and *MES1* at 72 hpi. To conclude, in the nonhost interaction
340 between pear and *V. inaequalis*, the behavior of some DEGs led us to the hypothesis that the SA
341 pathway could be engaged but transiently and presumably reduced to the few infection sites and not
342 spread by SAR in healthy systemic tissues.
343
344 Calcium influx and reactive oxygen species (ROS) production seems to act as secondary messengers
345 and could lead to stomatal closure in pear / *V. inaequalis* interaction
346 Early responses of plants upon pathogen perception include calcium influx and ROS
347 production, which both act as secondary messengers ([~~40~~51], reviewed in [10]). Three pear DEGs
348 were found that seems to indicate early increased cytosolic calcium level. The CSC (Calcium
349 permeable Stress-gated cation Channel) ERD4 (found two times) and the two glutamate receptors
350 GLR3.4 and GLR2.7, are known as calcium permeable channels ~~([41]; [42])~~[52, 53]. They were
351 induced at 24 hpi in our data. An increased cytosolic calcium level can lead to a pre-invasive defense
352 response by stomatal closure and promote the post-invasive defense response ROS accumulation
353 [~~43~~54].
354 Calcium influx has been reported to promote stomatal closure through the regulation of
355 potassium flux and the activation of anion channels in guard cells (reviewed in [10]). The stomata
356 closure is known to be induced via the inhibition of inward potassium currents which is achieved via
357 activation of calcium dependent protein kinases (CDPK) such as CPK13 and CPK8/CDPK19 ~~([44];~~
358 ~~[45])~~[55, 56]; but also via activation of CBL1 of the CBL1-CIPK5 complex, which activates the GORK
359 potassium outward channel [~~46~~57]. CPK13, CPK8/CDPK19 and CBL1 were all activated at 24 hpi in our
360 data.
361 A NADPH oxidase *RBOHB* (respiratory burst oxidase homologs, RBOH) is early (i. e. 24 hpi)
362 and sustainably (through to 72 hpi) induced in the pear/*V. inaequalis* nonhost interaction, which
363 could suggest~~ing~~ a rapid and maintained apoplastic ROS production. Indeed, the apoplastic ROS are

364 mainly produced by plasma membrane localized NADPH oxidases, cell wall peroxidases and amine
365 oxidases [~~47~~58]. In addition, posttranslational regulation of RBOH is required for its activation and
366 ROS production. Calcium, phosphatidic acid, and direct interactors such as Rac1 GTPase and RACK1
367 (Receptor for Activated C-Kinase 1) have been reported to be positive regulators of RBOHs (reviewed
368 in [~~48~~59]). For example, the Rac-like/ROP GTPase ARAC3 is known to interact with a RBOH to
369 promote ROS production [~~49~~60]. In our data, RBOHB activity ~~was~~ could also be supported by the
370 presence of positive regulators such as Rac-like/ROP GTPase. The three Rac-like/ROP GTPase *ARAC1*,
371 *ARAC3* and the homolog of *At4g03100* were induced at 24 hpi. CDPKs such as CPK1 are also known to
372 activate RBOHs in response to increased cytosolic calcium level [~~50~~61]. But repression of *CPK1* in our
373 data ~~seems to~~could indicate that this way of activation did not function.

374 In response to abscisic acid (ABA) or microbe-associated molecular pattern (MAMP)
375 immunity, stomatal closure is known to be regulated by apoplastic ROS production (reviewed in
376 [~~51~~62]) and cysteine-rich receptor-like kinases (CRK) are also known to be elements between ROS
377 production and downstream signaling leading to stomatal closure, sometimes activated (CRK10),
378 sometimes inhibited (CRK2 and CRK29; [~~52~~63]). Three DEGs coding for CRK were found in our data
379 and the repression of *CRK2* and *CRK29* (found two times) ~~was~~ could be consistent with the stomata
380 closure previously ~~found~~hypothesized, but the repression of *CRK10* (found two times) was not.
381 Beyond closure, inhibition of stomatal development could be seen as an extreme defense. YODA
382 (found two times) and MPK6 (found two times) MAPKs belong to a pathway involved in the negative
383 regulation of stomata development [~~53~~64]. These two genes were induced early in our data.

384 To conclude, in pear/*V. inaequalis* nonhost interaction, a calcium influx could lead~~s~~ to the
385 development of the stomatal closure pre-invasive defense, but could also promote~~s~~ a post-invasive
386 defense: apoplastic ROS accumulation. Apoplastic ROS, acting themselves as messengers, could come
387 to strengthen the stomatal closure (Fig. 4).
388

389 Transcription factors and sphingolipids could be implicated in maintaining HR under control in pear /
390 *V. inaequalis* interaction

391 ROS are known to mediate cellular signaling associated with defense-related gene
392 expression, ~~hypersensitive response (~~HR~~)~~ i.~~-~~e. the programmed cell death (PCD) at the site of
393 infection during a pathogen attack, and phytoalexin production [~~54~~65]. *Arabidopsis thaliana* RCD1
394 regulator has been proposed to positively regulate cell death in response to apoplastic ROS by
395 protein-protein interactions with transcription factors (reviewed in [~~55~~66]) and WRKY70 and SGT1b
396 were identified as cell death positive regulators functioning downstream of RCD1 [~~55~~66]. *RCD1* and
397 *WRKY70* genes were found induced in our data, at 24 hpi and 72 hpi respectively.

398 In *Arabidopsis*, the F-box protein CPR1, in association with the Skp1-Cullin-F-box (SCF)
399 ubiquitin ligase complex, targets for degradation NLR (nucleotide-binding domain and leucine-rich
400 repeats containing proteins) resistance protein such as SNC1, RPM1 or RPS2, to prevent
401 overaccumulation and autoimmunity (reviewed in [~~56~~67]). A *Skp1-like* (*ASK19*; 72 hpi) gene and *CPR1*
402 (24 hpi) gene were found induced in our data. A gene coding for RPM1 function was also found
403 repressed at 24 hpi. These results ~~are~~ could be in favor of the hypothesis that NLR receptors do not
404 take part in the HR development observed in the pear/*V. inaequalis* nonhost interaction (Fig. ~~1A~~1C,
405 H, J). In addition, the induction of an *AtSerpin1* gene homolog at 24 hpi (found two times) in our data
406 ~~is~~ could be consistent with that hypothesis. Indeed, AtMC1 is a pro-death caspase-like protein
407 required for full HR mediated by intracellular NB-LRR immune receptor proteins such as RPP4 and
408 RPM1 [~~57~~68] and AtSerpin1 is a protease inhibitor which block AtMC1 self-processing and inhibit
409 AtMC1-mediated cell death [~~58~~69].

410 The differential expression of two others components of the proteasome pathway ~~is~~ could be
411 in favor of ~~an~~ the HR development: the induction of the *RIN3* ubiquitin E3 ligase (24 hpi) and the
412 repression of the *BRG3* ubiquitin E3 ligase (24 hpi). Indeed, RIN3 is known as positive regulator of
413 RPM1 dependent HR [~~59~~70]. And BRG3 is known as a negative regulator of HR in plant/necrotrophic
414 pathogen interactions [~~60~~71].

Sphingolipids are involved in the control of PCD, either as structural components of membranes but also as initiators in the cell death regulatory pathway. According to Huby et al. [~~61~~72], free ceramides and long chain/sphingoid base components (LCBs) are able to trigger cell death, via ROS production, whereas their phosphorylated counterparts, ceramide phosphates and long chain base phosphate components (LCB-Ps) promote cell survival. The induction of PCD by LCB is based on the activation of protein kinases, among them MPK6 [~~62~~73]. As already mentioned, *MPK6* was found early induced in our data and we found numerous DEGs in the nonhost interaction between pear and *V. inaequalis* that seems to indicate the presence of free ceramides and LCB, which could possibly participate to the HR development. Free LCB presence is ~~demonstrated~~ suggested by the activations of *SBH1* (24 hpi), *SLD1* (24 hpi) and another sphingolipid Δ8 long-chain base desaturase homolog to *At2g46210* (24 hpi; found two times), and their relative conversion in ceramides is suggested ~~demonstrated~~ by the differential expressions of the ceramide synthases *LOH2* (repressed at 24 hpi) and *LOH3/LAG13* (induced at 24 and 72 hpi). LCB non-conversion in phosphorylated counterparts ~~is~~ could be shown by the *AtLCBK1* repression (72 hpi) and free ceramides maintenance ~~is attested~~could be suggested by their non-conversion in glycosyled ones given the repression of a glucosyl ceramide synthase homolog to *At2g19880* (24 hpi).

The differential expression of numerous known regulators of HR in our data ~~is~~ seems again consistent with the HR phenotype observed. The mechanosensor MSL10 and the calmodulin-activated Ca^{2+} pump (autoinhibited Ca^{2+}-ATPase [ACA]) ACA11 were found ~~engaged~~differentially expressed: at 24 hpi *MSL10* was induced and *ACA11* was repressed. MSL10 is known as a positive regulator of cell death [~~63~~74] and ACA11 is known as a negative regulator of SA-dependent ~~programmed cell death~~PCD [~~33~~44]. Their modulation ~~is~~ could be linked with the hypothesized~~noticed~~ calcium influx discussed above ~~([33]; [64])~~[44, 75]. The participation of the SA pathway in the development of the ~~hypersensitive response~~HR could also be supported by the repression of *EDR1* (at 72 hpi). Indeed, the MAPKKK EDR1 is known as a negative regulator of the SA-dependent HR (reviewed in [~~65~~76]).

441 Three other regulators of HR were found modulated in our data. The transcription factor *AS1*
442 (*MYB91*) was found induced at 24 hpi. It is known as a positive regulator of HR and implicated in the
443 JA pathway (reviewed in [~~20~~31]). The transcription factor *WRKY40* was found repressed at 72 hpi. It is
444 known as a negative regulator of HR [~~66~~77] and implicated in PTI [~~67~~78]. Another negative regulator
445 of HR is the lipid-binding domains containing protein VAD1 [~~68~~79]. It was found repressed at 72 hpi.
446 The behavior of another gene in our data, *UGT73B3,* ~~seems to~~could indicate that the
447 developed HR was contained due to intracellular ROS production and damages. The function
448 *UGT73B3* was thus activated (24 hpi). UGT73B3 is known as a restrictor of HR expansion via its action
449 in detoxification of ROS-reactive secondary metabolites (UGT73B3; [~~70~~80]).
450 To conclude, in pear/*V. inaequalis* nonhost interaction, HR was spread out, ~~in~~ potentially in
451 link with ~~the~~ a calcium influx~~, but especially following apoplastic ROS production~~ and ROS production
452 via free sphingolipids accumulation and not via NLR receptors. Furthermore, the behavior of ~~not less~~
453 ~~than~~ eight regulators seems to indicate that the developed HR is under control (Fig. 4).
454
455 Cell wall carbohydrates content and cuticle composition ~~are~~ could be altered in pear / *V. inaequalis*
456 interaction
457 The first obstacle encountered by host as well as nonhost pathogens attempting to colonize
458 plant tissues is the plant cell wall, which is often covered with a cuticle. ~~Preinvasive penetration~~
459 ~~barrier, as a preformed physical barrier, or as the onset place of defensive signaling pathways, is~~
460 ~~considered an important factor, especially i~~In nonhost resistance, in which non adapted pathogens
461 normally fail to penetrate nonhost plant cells, ~~when blocked by~~ the cell wall is considered an
462 important factor and seen as a preinvasive penetration barrier, or as the onset place of defensive
463 signaling pathways ~~([10]; [43])~~[10, 54]. Plant cell wall alterations ~~, of the carbohydrates or the~~
464 ~~phenolic components, either by impairing or overexpressing cell wall-related genes,~~ have been
465 demonstrated to have a significant impact on disease resistance and/or on abiotic stresses ~~(reviewed~~

466 ~~in [71] and [72])~~. These alterations can concern the carbohydrates or the phenolic components, and
467 results in impairing or overexpressing cell wall-related genes (reviewed in [81] and [82]).
468 We found numerous genes related to the cell wall with a modified expression during nonhost
469 interaction between pear and *V. inaequalis*, among them about thirty related to the biosynthesis or
470 the modification of carbohydrates. These ~~genes~~ DEGs are presented in ~~table~~ Table ~~3~~4~~,~~ except those
471 related to the lignin and other phenolic compounds, which will be discussed later. We saw in
472 particular several ~~genes~~ DEGs related to cellulose (8) and even more ~~genes~~ DEGs related to pectin
473 (14) but no ~~genes~~ DEGs related to callose.
474 Concerning these particular carbohydrate components, the model proposed by Bacete et al.
475 [8~~7~~1] is as follows. Firstly, alterations in cellulose biosynthesis from primary or secondary cell wall
476 trigger specific defensive responses, such as those mediated by the hormones JA, ET or abscisic acid
477 (ABA), activate biosynthesis of antimicrobial compounds, but also might attenuate pattern triggered
478 immunity (PTI) responses. Secondly, alterations of cell wall pectins, either in their overall content,
479 their degree of acetylation or methylation, activate specific defensive responses, such as those
480 regulated by JA or SA, and trigger PTI responses, probably mediated by damage-associated molecular
481 patterns like oligogalacturonides. Thus, even though our results do not completely support a role of
482 these ~~genes~~DEGs, we ~~think~~ suppose that the modified expression of cell wall related genes during
483 nonhost interaction between pear and *V. inaequalis* ~~is~~ could be meaningful.
484
485
486 **Table 4~~3~~: Main DEGs related to cell wall carbohydrates synthesis/modification detected during**
487 **non~~-~~host interaction pear/*V. inaequalis*.**
488

	Gene	Action	Expression*
	Primary cell wall		
Cellulose	*CSLA2*	synthesis	I
	PNT1	synthesis	I
	COBL2	deposition (GPI-anchored protein)	R
	AtGH9A4	catabolism	I

	XTR7	loosening	I
Hemi-cellulose (xyloglucan)	*At5g15490*	synthesis	I
Pectin	*At3g42180*	synthesis	I
	At4g01220	synthesis	I
	GHMP kinase	synthesis	I
	RHM1	synthesis	R
	PME At2g45220	methylesterification	I
	PME At2g46930	methylesterification	I
	PME At3g05910	methylesterification	R
	PME At1g02810	methylesterification	R
	PME44	methylesterification	R
	PG At3g16850	depolymerisation	I
	PG At3g59850	depolymerisation	I
	PG At4g13710	depolymerisation	I
	PG At3g62110	depolymerisation	R
	IDA	degradation	R
Arabinogalactan protein	*AGP11*	_	I
	AGP1	_	R
Secondary cell wall			
Cellulose	*CESA09*	synthesis	I
	CESA10	synthesis	I
	CSLG1	synthesis	I
Hemi-cellulose (xylan)	*FRA8*	synthesis	I
Undetermined			
Expansin	*EXP15*	loosening	I
	EXPB3	loosening	I
Hemi-cellulose	*ATFUC1*	modification	I
	XTH33	growth and assembling	R

*I: induced, R: repressed

Concerning the cuticle layer, most cuticles are composed largely of cutin, an insoluble polyester of primarily long-chain hydroxy fatty acids. This lipophilic cutin framework is associated with hydrophobic compounds collectively referred to as waxes. The cuticle is also thought to contain varying amounts of intermingled cell wall polysaccharides and sometimes also a fraction termed cutan (reviewed in [~~73~~83]). Cutin monomers are synthesized by the modification of plastid-derived 16C and 18C fatty acids in the endoplasmic reticulum (ER), yielding variously oxygenated fatty acid–

497 glycerol esters referred to as monoacylglycerols, which polymerize upon arrival at the growing cuticle
498 (Fig. 5, reviewed in [~~73~~83]).
499 C16 and C18 fatty acids are also important precursors of cuticular wax synthesis (Fig. 5).
500 Upon transport to the ER, the C16 and C18 fatty acids are extended to form very-long-chain fatty
501 acids (VCLFAs; C>20), and this extension is carried out by the fatty acid elongase (FAE) complex
502 located on the ER membrane. The very-long-chain FAs are then converted into the varied cuticular
503 waxes (primary alcohols, aldehydes, alkanes, secondary alcohols, ketones) by many ways (reviewed
504 in [~~74~~84]).
505 Interestingly, we found three genes up-regulated 24 hpi belonging to the FAS (fatty acid
506 synthase) chloroplastic complex implicated in the production of the C16 precursor (Fig. 5): *ACCD*,
507 *FabG* and *MOD1* (found two times). ACCD encodes the carboxytransferase beta subunit of the
508 Acetyl-CoA carboxylase complex which catalyzes the first committed step in fatty acid synthesis: the
509 carboxylation of acetyl-CoA to produce malonyl-CoA. FabG and MOD1 are respectively a β-ketoacyl
510 ACP-reductase and an enoyl-ACP-reductase which catalyze respectively the conversion of
511 acetoacetyl-ACP into β-hydroxyacyl-ACP and the second reductive step from enoyl-ACP to butyryl-
512 ACP (reviewed in [~~74~~84]).
513 In the ER, the four functions we found related to waxes biosynthesis in our data were
514 repressed at 24 hpi: *KCS4* (found two times), *CER1* and *CER3*, or 72 hpi: *ECR/CER10*. KCS4 and
515 ECR/CER10 belong to the FAE complex ([~~75~~85]; [~~76~~86]). The last two genes are implicated in
516 aldehydes (*CER1*) and alkanes (*CER1 and 3*) generation (reviewed in [~~74~~84]). On the contrary, the
517 eight genes we found ~~connected~~ related to cutin biosynthesis were induced at 24 hpi except a gene
518 homolog to *At5g14450*, which was induced at 72 hpi. One of them is a glycerol-3-phosphate
519 acyltransferase (GPAT) coding gene: GPAT8, which catalyzes the transfer of a fatty acid from
520 coenzyme A (CoA) to glycerol-3-phosphate (Fig. 4; reviewed in [~~73~~83]). GPAT8 function in cutin
521 formation has been functionally confirmed in association with GPAT4 [~~77~~87]. The seven others genes
522 code GDSL-lipases enzyme (*At1g28600*, *At1g28660*, *At1g54790*, *At3g16370*, *At3g48460*, *AtCUS4*:

523 *At4g28780*, *At5g14450*), some of which have been shown to function as cutin synthase (Fig. 4;

524 [~~78~~88]; reviewed in [~~73~~83]) and polymerize monoacylglycerols.

525 We also found induced respectively at 24 and 72 hpi two genes involved in waxes and cutin

526 biosynthesis positive regulation: *MYB16* and *SHN1*. The SHN genes (*SHN1–SHN3*), a set of three

527 largely redundant APETALA 2 family transcription factors from *A. thaliana*, are regulators of floral

528 cutin and epidermal cell morphology. SHN1 is regulated by the MYB family transcription factor

529 MYB106, which, along with its paralog MYB16, controls many aspects of cuticle and epidermis

530 formation in *A. thaliana* (reviewed in [~~79~~89] and [~~73~~83]).

531 Cutin and cuticular waxes play an important role in plant-insect and plant-microbe

532 interactions. Numerous *Arabidopsis* mutants in cutin and waxes biosynthetic or transport genes, such

533 as Acyl-CoA binding proteins (ACBP), show varying degrees of cuticle impairment, alterations in cutin

534 and/or wax composition, and defects in SAR (reviewed in [~~74~~84]). We found *ACBP6* repressed at 24

535 hpi. That repression is not inconsistent with the previously described possible amplification of cutin

536 biosynthesis and polymerization, given that *acbp6* KO mutation is not associated with a defect in that

537 pathway [~~39~~50]. That repression is also consistent with the SAR repression ~~observed~~ hypothesized

538 above as the *acbp6* KO mutant show compromised SAR [~~39~~50].

539 To conclude, our analysis of nonhost pear/V. *inaequalis* interaction ~~identified~~ could show an

540 alteration of the cuticle composition with more cutin and less waxes synthesis. The potential increase

541 in cutin polymerization could lead to a thickening of the cuticular layer to prevent fungus penetration

542 via its appressoria.

543

544 Secondary metabolism seems to lead~~s~~ to G unit lignin polymerization and simple coumarin or

545 hydroxycinnamic~~hydrocinnamic~~ acid amine phytoalexins synthesis in pear / *V. inaequalis* interaction

546 As distinguished from primary metabolism, plant secondary metabolism refers to pathways

547 and small molecule products of metabolism that are non-essential for the survival of the organism.

548 But they are key components for plants to interact with the environment in the adaptation to both

549 biotic and abiotic stress conditions. Plant secondary metabolites are usually classified according to
550 their chemical structure. Several groups of large molecules, including phenolic acids and flavonoids,
551 terpenoids and steroids, and alkaloids have been implicated in the activation and reinforcement of
552 defense mechanisms in plants (reviewed in [~~80~~90]).

553 Terpenoids and steroids, or isoprenoids, are components of both the primary and secondary
554 metabolisms in cells, and mono-, tri-, sesqui- and polyterpenes are considered as secondary
555 metabolites (reviewed in [~~81~~91]). Our results on pear identified seven DEGs and five DEGs which
556 could belong~~ing~~ to the chloroplastic methylerythritol posphate (MEP) and to the cytosolic mevalonic
557 acid (MVA) pathway of isoprenoids production respectively (Table ~~4~~5), which results, among others
558 compounds, in tri- and sesquiterpenes secondary metabolites. The majority of these genes
559 contribute to produce primary metabolites according to Tetali [~~81~~91]. Except *SMT2*, that we found
560 induced at 24 hpi, there is no report concerning a putative implication of others genes in plant biotic
561 resistance. SMT2 is involved in sterols production and *smt2* mutation was reported to compromise
562 bacterial resistance in *Nicotiana benthamiana* [~~82~~92]. The hypothesis is that sterols regulate plant
563 innate immunity against bacterial host and nonhost infections by regulating nutrient efflux into the
564 apoplast. *V. inaequalis* is a hemi~~-~~biotrophic pathogen which colonizes only the apoplast compartment
565 at the beginning of the interaction. ~~strong~~ Strong relative induction of *SMT2* in our data could
566 indicate that a similar mechanism of nutrient efflux regulation via sterols could take place to limit the
567 fungus growth in pear nonhost resistance against *V. inaequalis*.

568

569

570 **Table ~~5~~4: Main DEGs involved in biosynthetic pathways for terpenes and isoprenoids during**
571 **pear/*V. inaequalis* non~~-~~host interaction.**

	Gene	Function	Expression*
Cytosolic MVA (mevalonic acid) pathway enzymes	*HMGS*	catalyze the second step of the pathway	R
	HMGR1	catalyze the third step of the pathway	R
	SMT2	sterols production	I
	FLDH	sesquiterpenes production	R

	SQE2	triterpenes production	I
Chloroplastic MEP (methylerythritol posphate) pathway enzymes	*DXR*	catalyzes the second step of the pathway	I
	GG reductase	chlorophylls production	R
	VTE4	tochopherols production	I
	KAO1	gibberellins production	R
	PDS2	plastoquinones production	I
	LYC	carotenoids production	I
	PGGT1	covalent attachment of a prenyl group to a protein	I

572 *I: induced, R: repressed

573

574 In our data, the other DEGs that were linked to secondary metabolism belong to the
575 phenylpropanoid pathway production (Fig. 6). Among them we found four genes which could
576 belonging to the flavonoid production, all repressed, at 24 hpi (*DFR* and *DRM6*) or 72 hpi (*TT7* and
577 *UGT71D1*). DFR (dihydroflavonol reductase) is involved in flavan-3,4-ol production and TT7 (flavonoid
578 3' hydroxylase) in dihydroquercetin production from dihydro-kaempferol, and UGT71D1 (glucosyl
579 transferase) in quercetin-glycoside production from quercetin (TAIR database;
580 https://www.arabidopsis.org/index.jsp). DMR6 (flavone synthase) is involved in flavone production
581 from naringenin [8393]. Thus flavonoid production does not seem to be favored, which is not
582 consistent with the induction of *MYB12* at 24 hpi, but consistent with *MYB4* induction at 72 hpi.
583 MYB12 is actually known as a positive regulator of flavonol biosynthesis in pear and apple fruits ([84];
584 [85])[94, 95] whereas MYB4 is known as a negative regulator of this biosynthetic pathway [8696].
585 Concerning the production of monolignols, precursors of lignin synthesis, some genes
586 potentially related were found induced, others repressed. We found *CYP98A3* and *CAD9* (found two
587 times) induced at 24 hpi and *HCT*, *CCR1* and a gene homolog to *At2g23910* (found two times, one
588 time repressed at 24 hpi, one time repressed at 72 hpi) repressed at 24 hpi, Fig. 6). *CYP98A3* encodes
589 a C3H (coumarate 3-hydroxylase), *CAD9* encodes a CAD (cinnamyl alcohol dehydrogenase), HCT is an
590 hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase, *CCR1* encodes a CCR
591 (cinnamoyl-CoA reductase) and *At2g23910* encodes a CCR-related protein. (TAIR and KEGG
592 databases (https://www.genome.jp/kegg/)).

Lignification is obtained by cross-linking reactions of the lignin monomers or by polymer–polymer coupling via radicals produced by oxidases such as peroxidases [~~87~~97] and laccases [~~88~~98]. However, while peroxidases are able to oxidize monolignols to produce H, G and S units of lignin, laccases only generate G units [~~87~~97]. In our data, we found two laccases induced at 24 hpi: *LAC11* (found two times, one time induced at 24 and 72 hpi) and *LAC17* (found two times), and three peroxidases repressed at 24 hpi: *PRX17*, *PER47* and *PRX52* (also repressed at 72 hpi), which ~~can~~ could be linked to lignin biosynthetic process (Fig. 6). According to Zhao et al. [~~88~~98], LAC11 and LAC17, along with LAC4, play a critical role in lignification, and their results suggests that peroxidase and laccase do not serve redundant functions in lignification, at least in the vascular tissues of the stem and root of *Arabidopsis*. Participation in lignin formation has also been proved for PRX17 [~~89~~99], PER47 [~~90~~100] and PRX52 [~~91~~101]. But there are currently no reports about a possible involvement of all these genes in lignification linked to biotic or abiotic stresses. Concerning non~~-~~host resistance, two reports describe lignin accumulation/deposition involvement: one in apple fruit [~~92~~102] and the other one in cowpea [~~93~~103]. In the latest, authors showed that preferentially generated lignin units in this nonhost interaction are G units, just as it ~~seems to~~could be the case in our pear / *V. inaequalis* study. To summarize, it is tempting to think that modifications of expression observed for genes linked to lignin polymerization are relevant for the pear nonhost resistance against *V. inaequalis*, but further functional analysis should be conducted to conclude about the lignin status, such as histochemical staining [104], content measure by absorbance [104] or Fourier-transform infrared (FTIR) spectroscopy analysis [105].

The biosynthesis of two others types of phenylpropanoid compounds could appear~~s~~ to be favored during pear nonhost resistance against *V. inaequalis*: simple coumarin on one hand and hydroxycinnamic acid amides on the other hand. We found four *BGLU*-like genes induced: *BGLU47* and *BGLC3* (at 24 hpi), *BGLU16* (at 72 hpi); *BGLU42* (at 24 and 72 hpi) (Fig. 6). These β-glucosidases could be implied in simple coumarin path production from the cinnamic acid (KEGG database). Some natural simple coumarins are known as antifungal compounds *in vitro* and have been developed as

fungicides [~~94~~106]. Previous work on Hevea also reports the correlation between the resistance against pathogenic fungi and the production of some coumarins, with antifungal activity *in vitro* [~~95~~107]. We also found induced at 24 hpi the genes *AACT1/ACT1*, *ATPAO5* and genes homologs to *At4g17830* and *At4g38220* (Fig. 6). AACT1/ACT1 catalyze the first specific step in branch pathway synthesizing hydroxycinnamic acid amides from the p-Coumaroyl CoA or the feruloyl CoA and amines agmatine or putrescine [~~96~~108]. Hydroxycinnamic acid amides are produced in response to pathogenic infections [~~96~~108] and surface exported. Hydroxycinnamic acid amides are reported to participate in *Arabidopsis* nonhost resistance against *Phytophthora infestans* via their inhibitory activity on spore germination [~~97~~109]. The three others genes belong to the arginine biosynthesis path (homologs to *At4g1783* and *At4g38220*) and the arginine and proline metabolisms which produce the amines agmatine and putrescine (*ATPAO5*) (KEGG database). Agmatine is directly produced from arginine thanks to an ADC activity (arginine decarboxylase) and putrescine can be produced from spermidine thanks to a PAO activity (polyamine oxidase). ATPAO5 catalyzes the conversion of spermine in spermidine. The induction of these three last genes is therefore consistent with the hypothesis of amines production in order to enable hydroxycinnamic acid amides synthesis. The induction of *C4H* at 24 hpi could also favor hydroxycinnamic acid amides synthesis via p-Coumaroyl CoA biosynthesis promotion. C4H (cinnamate 4-hydroxylase) catalyzes the production of p-Coumaric acid from Cinnamic acid and p-Coumaric acid gives p-Coumaroyl CoA thanks to 4CL (4-coumarate-CoA ligase) (KEGG database).

Among the suite of defense components synthetized in nonhost as in host context, a chemical barrier can be established via accumulation of a diverse array of secondary metabolites rapidly produced upon pathogen infection, named phytoalexins, with toxic or inhibitory effects (reviewed in [10]). Phytoalexins can be flavonoids, such as the pisatin of pea (in [~~98~~110]) but also varied phenylpropanoid compounds. In the nonhost interaction pear / *V. inaequalis*, the production of flavonoid type phytoalexins does not seem to be favored, except possibly simple coumarin and hydroxicinnamic acid amines.

645

646 Very limited transcriptomic modulation during apple / *V. pyrina* nonhost interaction

647 Only 60 DEGs were detected in the apple / *V. pyrina* nonhost interaction at 24 or 72 hpi, in
648 agreement with the total absence of macroscopic symptoms and few cells engaged in an HR-like
649 reaction observed at the microscopic level. Among these 60 DEGs, 36 have ~~no known~~unpredicted
650 function. Among the 24 remaining DEGs, nine DEGS could be relevant in apple / *V. pyrina* nonhost
651 interaction in view of our findings in pear / *V. inaequalis* nonhost interaction. *ORG2* (*BHLH038*), a
652 putative integrator of various stress reactions [~~99~~111] was induced at 24 hpi. Three genes were
653 related to an oxidative stress: *GASA10* was repressed at 24 hpi and *NRAMP3* and *AOR* were induced
654 at 24 hpi. GASA proteins have been suggested to regulate redox homeostasis via restricting the levels
655 of $OH^{\cdot}$ in the cell wall [~~100~~112]. The repression of this gene ~~is~~ could be thus in favor of more $OH^{\cdot}$ in
656 the cell wall. The oxidoreductase coding gene *AOR* is known in the chloroplast to contribute to the
657 detoxification of reactive carbonyls produced under oxidative stress [~~101~~113]. *NRAMP* genes
658 function as positive regulators of ROS accumulation, especially during *Arabidopsis Erwinia*
659 *chrisanthemi* resistance [~~102~~114]. The induction (at 24 and 72 hpi) of another gene could suggest~~s~~
660 modifications at the cell wall level: *EXP8*, an expansin coding gene involved in cell wall loosening (Tair
661 database). We also found two genes related to hormone pathways, one induced at 24 hpi: *WIN1* and
662 the other one repressed at 72 hpi: *UBP12*. WIN1 is known as a negative regulator of SA pathway
663 [~~103~~115] and UBP12 as a positive regulator of JA pathway via the stabilization of MYC2 [~~18~~29]. In
664 possible link with the JA pathway, we also found *TPS21* induced at 24 hpi. TPS21 is involved in
665 sesquiterpenes production and is promoted by JA signal via MYC2 [~~104~~116]. TPS21 is especially
666 involved in the jasmonate-dependent defensive emission of herbivore-induced volatiles in
667 cranberries [~~105~~117]. Finaly the last DEG we found potentially relevant in apple / *V. pyrina* nonhost
668 interaction could promote HR via ceramides accumulation. *ACD11* is repressed at 24 hpi in our data.
669 In *acd11* mutants, the relatively abundant cell death inducer phytoceramide rises acutely [~~106~~118].

Because nonhost resistance of apple against *V. pyrina* ~~is of a type I, with~~ has resulted in a very limited number of cells engaged in an HR-like reaction, it has not been possible for us to exhaustively describe ~~how this interaction is expressed~~its outcome at the transcriptomic level in the first three days of the interaction. Further insight with later points of kinetic and more adapted techniques such as laser-assisted cell picking, prior to micro arrays or RNA sequencing analysis (review in [~~107~~119]) could provide more information in the future.

Comparison of pear resistances against the host pathogen *V. pyrina* and the nonhost pathogen *V. inaequalis*

Perchepied et al. [~~11~~13] performed a detailed transcriptomic analysis of the host resistance of pear against *V. pyrina* strain VP102, deployed in a transgenic pear bearing the well-known apple *Rvi6* resistance gene against *V. inaequalis*. Comparing this work to ours gives us the rare opportunity to analyze similarities and differences between a host and a nonhost resistance in the same plant. ~~Only four transcriptomic studies involving pear/pathogen interactions have been published so far. Yan et al [108] reported the modulation of expression of 144 pear genes after fruit treatment by *Meyerozyma guilliermondii*, an antagonistic yeast used for biocontrol of natural pear fruit decay. Zhang et al [109] similarly reported the modulation of expression of 1076 pear genes after treatment with *Wickerhamomyces anomalus*, another biocontrol agent. Using RNA-seq, Wang et al. [110] reported a major role of ethylene signalization during the compatible interaction between *P. pyrifolia* and *Alternaria alternata*, a necrotrophic pathogen. Finally, Xu et al. [111] applied RNA-seq to characterize the genes of *Penicillium expansum* activated after infection of pear fruits. None of these studies can be directly compared to our work on host and nonhost scab pear resistance.~~

Concerning the recognition and early signaling steps of the interactions, many receptors and co-receptors have been found induced in the host pear resistance, especially damage-associated molecular patterns receptors such as RLK7, revealing that PTI and ETI must be engaged. We did not find evidence of the mobilization of such receptors in the pear nonhost resistance. PTI and ETI

696 receptors are nonetheless reported as implicated in nonhost resistance (reviewed in [~~112~~120] and
697 [10]). As we only analyzed post infection transcriptional modulations in the nonhost pear/*V.*
698 *inaequalis* interaction (at 24 and 72 hpi), one hypothesis to explain the lack of PTI and ETI receptors
699 in our data could be that these receptors were already present as preformed defenses and not
700 particularly induced by the infection onset. In pear nonhost interaction, the earliest likely signaling
701 pathways we were able to highlight are calcium influx and apoplastic ROS production. Calcium
702 signaling seems to be also implicated in pear host resistance, but less obviously than it seems in
703 nonhost resistance.

704 Regarding the hormonal signaling pathways, the JA defense signaling pathway ~~was~~
705 ~~found~~seems to be repressed in pear nonhost resistance but was found quite activated in pear host
706 resistance. The JA/ethylene (ET) defense signaling pathway is known as an effective defense against
707 necrotrophic fungi in *Arabidopsis* [~~113~~121]. Thus, it is not ~~surprising~~ inconsistent to find the JA
708 pathway potentially repressed in the development of the pear nonhost resistance against the hemi-
709 biotrophic pathogen *V. inaequalis*. But it is very interesting to find this pathway rather induced in the
710 development of the pear host resistance against the other hemi-biotrophic pathogen *V. pyrina*. The
711 SA signaling pathway is commonly seen as the classical one triggered to resist biotrophic fungi in
712 *Arabidopsis* [~~113~~121], but it seems only a little ~~engagement~~ engaged in pear nonhost resistance ~~has~~
713 ~~been observed~~, ~~SA signaling being~~and is repressed in pear host resistance. If this absence of SA
714 implication is quite unexpected in pear host resistance against a hemi-biotrophic fungus, it ~~is~~ could
715 be consistent with the report that the exact role of these key defense phytohormones is unclear in
716 nonhost resistance and remains to be established [~~43~~54]. As shown by Tsuda et al. [~~114~~122], an
717 explanation for the hormone pathways behavior in pear host resistance could be that: as both the SA
718 and JA/ET pathways positively contribute to immunity, a loss of signaling flow through the SA
719 pathway can be compensated by a rerouting signal through the JA/ET pathways. In addition,
720 independently of SA signaling, but in positive connection with JA signaling, SAR seems to be engaged
721 in distal tissues during pear host resistance. To conclude, in pear host as well as nonhost resistances,

722 classical resistance hormones SA and JA/ET, and the correlative PR gene defenses, seems differently
723 involved than in *Arabidopsis*.

724 The carbohydrate content of the cell-wall ~~is~~ seems to be modified in response to the attacks
725 by the pathogens. Regarding cell-wall and cuticle, in pear host as well as nonhost resistances, the
726 numerous DEGs found could highlight important modifications ~~were highlighted~~. Similar
727 modifications affected the cellulose and mainly the pectin contents, but no callose production was
728 observed. Regarding cuticle, waxes production was induced in host resistance whereas it ~~was~~ seems
729 to be repressed in nonhost resistance, in favor of cutin production / polymerization, which was also
730 induced in host resistance. To conclude, as a first obstacle encountered by host, as well as nonhost~~,~~
731 pathogens attempting to colonize plant tissues, the plant cell wall and its cuticle seem to play a
732 leading role in both pear host and nonhost resistances.

733 Finally, the production of secondary metabolites and phenylpropanoids compounds in
734 particular, ~~seems to~~could be a major line of defense, in pear host as well as nonhost resistances, but
735 with divergences. If lignin and flavonoid productions are preponderant in pear host resistance against
736 *V. pyrina*, lignin implication in pear nonhost resistance ~~is~~ seems less clear and flavonoids production
737 ~~is obviously~~seems repressed. But the biosynthesis of two other types of phenylpropanoid-derived
738 phytoalexins ~~appears to~~could be favored during pear nonhost resistance: simple coumarin on one
739 hand and hydroxycinnamic acid amides on the other hand.

740 The comparative analysis between a host and a nonhost resistance in pear shows that, even
741 though specificities are observed, ~~the~~ two major defense lines engaged ~~are~~ seems shared: the cell
742 wall and its cuticle on one hand, the secondary metabolism with the phenylpropanoid pathway on
743 the other hand. Moreover, these defenses seem deployed ~~largely~~ independently of the SA signaling
744 pathway, yet widely recognized as the main defense hormone against biotrophic pathogens.

745

Conclusion

~~As far as we know~~To our knowledge, our work is the first one published regarding a transcriptomic analysis of post-infections events of a nonhost resistance to *Venturia sp.* in apple and pear. ~~Velho and Stadik [115] recently published a detailed description of the apple / *Colletotrichum higginsianum* nonhost resistance, highlighting the accumulation of callose at the sites of penetration of the fungus. But no data on gene expression was included.~~ Here, our molecular work on apple / *V. pyrina* nonhost resistance remains preliminary and in order to allow a deeper deciphering, further analyses must be considered with the aid of tools adapted to this ~~type I~~ nonhost resistance with very few cells engaged in an HR-like reaction, only visible at a microscopic level. In pear, this deciphering allowed us to show that nonhost resistance against *V. inaequalis* ~~is a type II one, which~~ involves enough pathogen penetration in plant tissue to occasionally trigger visible HR, and develops post-invasive defenses.

To summarize our findings on pear with a notion of cascading effect, we can propose the following scenario (Fig. 4): once *V. inaequalis* presence is recognized by pear, a calcium cellular influx ~~is~~ could be induced and could lead~~s~~ to the possible development of a pre-invasive defense, the stomatal closure, but could also promote~~s~~ ~~an~~ a possible early post-invasive defense, an apoplastic ROS accumulation. Apoplastic ROS, acting themselves as ubiquitous messengers, could come to reinforce the stomatal closure but could also mediate cellular signaling possibly resulting in two post-invasive defenses: HR development at infection sites, along with phytoalexin (simple coumarin and hydroxicinnamic acid amines) production. The ~~observed~~ inferred alterations of the epidermis composition (cellulose, pectin, lignin for the cell wall, and cutin for the cuticle), are presumed to strengthen this physical barrier and ~~can~~ could be seen as the development of another pre-invasive defense. ~~The calcium (action on pectin reviewed in [116]) and the ROS (action on lignin, [117]; [118]; action on cuticle, [119]) have been linked to some type of epidermis modifications and may participate in the proceeding of these defense in pear / *V. inaequalis* nonhost interaction.~~

Nonhost resistance is defined as the resistance of an entire plant species against a specific parasite or pathogen ~~[120]~~ and is seen as the most durable resistance of plant. Thus, understanding the molecular mechanisms underlying nonhost resistance can open up some interesting avenues to create sustainable host resistances in the same plant species. Considering pear, in order to stop the germination and entrance of hemibiotrophic host fungi such as *V. pyrina*, strengthening the cuticle initial barrier via more cutin production and cross-link, or promoting the biosynthesis of phytoalexins like hydroxycinnamic acid amines, appear as promising solutions~~, relatively easy to engineer regarding recent advances in biotechnology tools on this species ([121]; [122]; [123])~~.

Material and methods

Biological material

Apple plants from the cultivar 'Gala' and pear plants from the cultivar 'Conference' were chosen because of their susceptibility to *V. inaequalis* and *V. pyrina*, respectively. The apple and pear genotypes were multiplied *in vitro*, rooted and acclimatized in greenhouse as described previously ~~([124]; [125])~~[123, 124].

For apple scab inoculation, the *V. inaequalis* monoconidial isolate used was EU-B05 from the European collection of *V. inaequalis* of the European project Durable Apple Resistance in Europe [~~126~~125]. For pear scab inoculation, the monoconidial strain VP102 of *V. pyrina* was chosen for its aggressiveness on 'Conference' [~~127~~126].

Scab inoculation procedure

Greenhouse growth conditions and mode of inoculum preparation were as described in Parisi and Lespinasse [~~128~~127] for apple and Chevalier et al. [~~129~~128] for pear. Briefly, the youngest leaf of actively growing shoots was tagged and the plants inoculated with a conidial suspension (2×10^5 conidia ml^{-1}) of *Venturia pyrina* strain VP102 for apple and *Venturia inaequalis* strain EUB04 for pear. Symptoms were recorded at 14, 21, 28, 35 and 42 days after inoculation on 20 actively growing

797 shoots (one shoot by plant) for each interaction. The type of symptoms was scored using the 6 class-
798 scale of Chevalier et al. [130129].
799
800 Microscopic observations
801 Histological studies were made on samples stained with the fluorophore solophenylflavine
802 [131130]. In brief, leaf discs were rinsed in ethanol 50° before staining in a water solution of
803 solophenylflavine 7GFE 500 (SIGMA-Aldrich, St Louis USA) 0.1% (v/v) for 10 min. The samples were
804 first rinsed in deionized water, then in 25% glycerol for 10 min. Finally, the leaf samples were
805 mounted on glass-slides in a few drops of 50% glycerol. They were examined with a wide-field
806 epifluorescence microscope BH2-RFC Olympus (Hamburg, D) equipped with the following filter
807 combination: excitation filter 395 nm and emission filter 504 nm.
808
809 Transcriptomics experiment
810 Leaf samples were immediately frozen in liquid nitrogen and kept at -80°C until analysis.
811 Sampling concerned the youngest expanded leaf of each plant labeled the day of the inoculation.
812 Each biological repeat is a pool of three leaves from three different plants and two biological repeats
813 (n=2) have been made by condition (genotype x treatment x time). Leaf samples taken just before
814 inoculation (T0) and at 24 and 72 hpi, were then used to perform transcriptomics analyses.
815 For RNA extraction, frozen leaves were ground to a fine powder in a ball mill (MM301,
816 Retsch, Hann, Germany). RNA was extracted with the kit NucleoSpin RNA Plant (Macherey Nagel,
817 Düren, Germany) according to the manufacturer's instructions but with a modification: 4% of PVP40
818 (4 g for 100 ml) were mixed with the initial lysis buffer RAP before use. Purity and concentration of
819 the samples were assayed with a Nanodrop spectrophotometer ND-1000 (ThermoFisher Scientific,
820 Waltham, MA, USA) and by visualization on agarose gel (1% (weight/volume) agarose, TAE 0.5x, 3%
821 (volume/volume) Midori green). Intron-spanning primers (forward primer:
822 CTCTTGGTGTCAGGCAAATG, reverse primer: TCAAGGTTGGTGGACCTCTC) designed on the *EF-1α* gene
823 (accession AJ223969 for apple and PCP017051 for pear, available at https://www.rosaceae.org/, with

the datasets on "*Pyrus communis* v1.0 draft genome") were used to check the absence of genomic DNA contamination by PCR. The PCR reaction conditions were as follows: 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, 60°C for 45 s, 72°C for 1 min, with a final extension at 72°C for 5 min. The PCR products were separated on a 2% agarose gel.

Amplifications (aRNAs) were produced with MessageAmpII aRNA Kit (Ambion Invitrogen, Waltham, MA, USA), from 300 ng total RNA. Then 5 µg of each aRNA were retrotranscribed and labelled using a SuperScript II reverse transcriptase (Transcriptase inverse SuperScript™ II kit, Invitrogen, Carlsbad, CA, USA) and fluorescent dyes: either cyanine-3 (Cy3) or cyanine-5 (Cy5) (Interchim, Montluçon, France). Labeled samples (30 pmol each, one with Cy3, the other with Cy5) were combined two by two, depending on the experimental design (i. e. for example, the CF/EUB05/24 hpi sample and the CF/non inoculated sample are labeled with Cy3 and Cy5 respectively and pooled to co-hybridize the microarray) . For each comparison two biological replicates were analyzed in dye-switch as described in Depuydt et al. [~~132~~131]. Paired labeled samples were then co-hybridized to Agilent microarray AryANE v2.0 (Agilent-070158_IRHS_AryANE-Venise, GPL26767 at GEO: https://www.ncbi.nlm.nih.gov/geo/) for apple, or Pyrus v1.0 (Agilent-078635_IRHS_Pyrus, GPL26768 at GEO) for pear, containing respectively 133584 (66792 sense and 66792 anti-sense probes) and 87812 (43906 sense and 43906 anti-sense probes) 60-mer oligonucleotide probes. The hybridizations were performed as described in Celton, Gaillard et al. [~~133~~132] using a MS 200 microarray scanner (NimbleGen Roche, Madison, WI, USA).

For microarray analysis we designed two new chips. For apple we used a deduplicated probeset from the AryANE v1.0 ([~~133~~132]; 118740 probes with 59370 in sense and 59370 in anti-sense) augmented by 14844 probes (7422 in sense and 7422 in anti-sense) designed on new gene annotations from *Malus x domestica* GDDH13 v1.1 (https://iris.angers.inra.fr/gddh13 or https://www.rosaceae.org/species/malus/malus_x_domestica/genome_GDDH13_v1.1). These probes target new coding genes with UTRs when available, manually curated micro-RNA precursors and transposable elements. For transposable elements we used one consensus sequence for each

850 family and a randomly peaked number of elements proportional to their respective abundance in the
851 genome. The microarrays used in this study also have probes for coding genes of *V. inaequalis* but
852 they have not been taken into account.

853 For pear the design was done on the *Pyrus communis* Genome v1.0 Draft Assembly &
854 Annotation available on GDR
855 (https://www.rosaceae.org/species/pyrus/pyrus_communis/genome_v1.0) web site. We have
856 downloaded the reference genome and gene predictions fasta files and structural annotation gff file
857 the 21st of September 2015. Using home-made Biopython scripts we have extracted spliced CDS
858 sequences with 60 nucleotides before start and after stop codons to get UTR-like sequences likely to
859 be found on transcripts resulting in a fasta file containing 44491 sequences. These 60 nucleotides
860 increase the probability of finding specific probes on genes with high similarity. This file was sent to
861 the eArray Agilent probe design tool (https://earray.chem.agilent.com/earray/) to generate one
862 probe per gene prediction. Options used were: Probe Length: 60, Probe per Target: 1, Probe
863 Orientation: Sense, Design Options: Best Probe Methodology, Design with 3' Bias. The probeset was
864 then reverse-complemented to generate anti-sense probes and filtered to remove duplicated
865 probes. The final probeset contains 87812 unique probes targeting 1 (73612 probes) or more (14200
866 probes) potential transcript both in sense and anti-sense.

867 Normalization and statistical analyses performed to get normalized intensity values have
868 been done as in Celton, Gaillard et al. [~~133~~132]. Briefly, data were normalized with the lowess
869 method, and differential expression analyses were performed using the lmFit function and the Bayes
870 moderated t-test with the LIMMA package in R software [133, 134]. The pipeline AnaDiff used for
871 these differential analyses is available at https://zenodo.org/record/6477918#.Yn5O3XVBzIE [135].
872 For each comparison and each probe, we retrieved a ratio of the logarithms of the fluorescence
873 intensities (one per compared sample: T0 versus 24 hpi or T0 versus 72 hpi in our case) and an
874 associated p-value. The applied p-value threshold to determine DEGs (differentially expressed genes)
875 was 0.01. Through blast analysis, a TAIR accession number (The *Arabidopsis* Information Resource;

876 https://www.arabidopsis.org/; [~~134~~136]) has been linked to a majority of apple or pear
877 “probe/corresponding gene”. Thanks to the Functional Classification SuperViewer tool [137] ~~and~~ , the
878 ~~couple “~~TAIR accession~~s/ratio value”~~ ~~has~~ have then been used to ~~make a global analysis of~~class DEGs
879 in functional categories ~~observed in the~~according to ~~Mapman~~ MapMan software
880 (https://mapman.gabipd.org/homemapman.gabipd.org; file Ath_AGI_LOCUS_TAIR10_Aug2012.txt;
881 [~~135~~138]), and to highlight the enriched ones by calculating the frequency of DEGs per category,
882 normalized to the numbers of *Arabidopsis* genes in each MapMan category, and bootstrapping the
883 dataset to provide a confidence estimate for the accuracy of the result. The detailed analysis of DEGs
884 has been done through TAIR and KEGG (https://www.genome.jp/kegg/) databases, and bibliography.
885 Metadata for the ~~172~~ 193 (~~162~~ 184 for pear, corresponding to 158 different functions and ~~10~~ 9 for
886 apple) DEGs discussed in this work are available in Table ~~S3~~ S2 and S4 (Online only).
887
888 qPCR validation of transcriptomic data
889 In order to validate transcriptomic data, qPCR was performed on a selection of gene/sample
890 associations. First-strand cDNA was synthesized using total RNA (2.0 µg) in a volume of 30 µl of 5×
891 buffer, 0.5 µg of oligodT15 primer, 5 µl of dNTPs (2.5 mM each), and 150 units of MMLV RTase
892 (Promega, Madison, WI, USA). The mixture was incubated at 42°C for 75 min. qPCR was then
893 performed. Briefly, 2.5 µl of the appropriately diluted samples (1/16 dilution) were mixed with 5 µl of
894 PerfeCTa SYBR Green SuperMix for iQ kit (Quantabio, Beverly, MA, USA) and 0.2 or 0.6 µl of each
895 primer (10 µM) in a final volume of 10 µl. Primers were designed with Primer3Plus, their volumes
896 were according to their optimal concentration (determined for reaction efficiency near to 100%;
897 calculated as the slope of a standard dilution curve; [~~136~~139]). Accessions, primer sequences and
898 optimal concentrations are indicated in Table ~~S2~~S1. The reaction was performed on a CFX Connect
899 Real-Time System (BIO-RAD, Hercules, CA, USA) using the following program: 95°C, 5 min followed by
900 40 cycles comprising 95°C for 3 s, 60°C for 1 min. Melting curves were performed at the end of each
901 run to check the absence of primer-dimers and nonspecific amplification products. Expression levels

902 were calculated using the ΔΔCT method [~~137~~140] and were corrected as recommended in
903 Vandesompele et al. [~~138~~141], with three internal reference genes (GADPH, TUA and ACTIN 7 for
904 apple, GADPH, TUA and EF1α for pear) used for the calculation of a normalization factor. For each
905 couple DEG/sample (sample defining a plant, time, treatment and biological repeat combination), the
906 ratio was obtained by dividing the mean value of CT calculated from 3 technical repeats by the
907 normalization factor obtained for this sample.
908

909 Supplementary information

910 **Additional File 1: Table S1, S2, S3~~ and~~, S4 and S5.**
911

912 Abbreviations

913 ABA: abscisic acid; CDPK: calcium dependent protein kinase; CRK: cysteine-rich receptor-like kinase;
914 DEG: differentially expressed gene; DFR: dihydroflavonol 4-reductase; DGDG:
915 digalactosyldiacylglycerol; ET: ethylene; ER: endoplasmic reticulum; ETI: effector triggered immunity;
916 FAE: fatty acid elongase; GPAT: glycerol-3-phosphate acyltransferase; hpi: hours post inoculation; HR:
917 hypersensitive reaction; JA; jasmonic acid; LCB: long chain/sphingoid base component; LCB-Ps: long
918 chain base phosphate component; LOX: lipoxygenase; MAMP: microbe-associated molecular pattern;
919 OPDA: 12-oxo-phytodienoic acid; PCD: programed cell death; PTI: pattern triggered immunity; RBOH:
920 respiratory burst oxidase homolog; ROS: reactive oxygen species; SA: salicylic acid; SAR: systemic
921 acquired resistance
922

923 Declarations

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927 Authors contribution

928 EC, LP, and EV conceived the study. EC and EV supervised the study. ER and MB performed the
929 biological experiments. SG and SP performed the database work and assisted with the bioinformatics
930 analysis. EV wrote the original manuscript. EV and EC edited the manuscript. All authors have read
931 and agreed to the published version of the manuscript.

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935 Availability of data and materials

936 The datasets supporting the conclusion of this article are available in the Gene Expression Omnibus
937 (GEO) repository [https://www.ncbi.nlm.nih.gov/geo/] with GSE159179 and GSE159180 accession
938 numbers for apple and pear respectively [142, 143]. The pipeline AnaDiff used for differential
939 analyses is available at https://zenodo.org/record/6477918#.Yn5O3XVBzIE [135].

940 Ethics approval and consent to participate

941 Experimental research on plants in this work comply with relevant institutional, national, and
942 international guidelines and legislation.

943 Consent for publication

944 This section is not applicable.

945 Competing interests

946 The authors declare that they have no competing interests.

947

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1374

1375 **Figure legends**

1376

1377 **Fig. 1**: **Macro- and microscopic observations of nonhost interactions.**

1378 *V. pyrina* VP102 strain / pear 'Conference' (A) and *V. inaequalis* EUB05 strain / apple 'Gala' (B) 21
1379 days symptoms are shown as classical ones of susceptible host interactions, to compare to nonhost

1380 ones. Binocular observation 21 days after *V. inaequalis* EUB05 strain inoculation on 'Conference' (~~A~~C)

1381 and (~~B~~D) and *V. pyrina* VP102 strain inoculation on 'Gala' (~~C~~E). Wide field fluorescence observations

1382 of: 'Conference' 3 days (~~D~~F) and 14 days (~~E~~H and J) after *V. inaequalis* EUB05 strain inoculation, 'Gala'

1383 3 days (~~F~~G) and 14 days (~~G~~I and K) after *V. pyrina* VP102 strain inoculation. Ap: appressorium, C:

1384 conidia, Gf: germination filament, Pp: pin point

1385

1386 **Fig. 2**: **Functional categories of DEGs at 24 or 72 hpi during pear response to *V. inaequalis*.**

1387 ~~The number of up- or down-regulated DEGs is expressed as a percentage of the total number of~~

1388 ~~genes present in the Pyrus v1.0 (87812 probes) microarray.~~ The 1845 DEGs with TAIR names have

1389 been ~~are~~ classified ~~in functional categories~~ according to MapMan ~~3.5.1R2 bins~~functional categories.

1390 In order to highlight the enriched ones, the numbers of DEGs per category have been normalized to

1391 the numbers of *Arabidopsis* genes in each MapMan category and ~~. Only bins with ≥ 6 DEGs are~~

1392 ~~presented.~~bootstraps have been done to provide a confidence estimate for the accuracy of the

1393 output.

1394

1395 **Fig. 3: DEGs involved in hormonal pathways during pear/*V. inaequalis* non-host interaction**.

1396 A: DEGs involved in JA pathway; B: DEGs involved in SA pathway. Genes written in red are induced,

1397 genes written in blue are repressed. ACA11: autoinhibited $Ca2^{+}$-ATPase, calmodulin-activated $Ca2^{+}$

1398 pumps at the plasma membrane, endoplasmic reticulum, and vacuole. ACBP6: acyl-CoA-binding

1399 protein. ACX4: acyl-CoA-oxidase1. AS1/MYB91: Asymmetric leaves 1 transcription factor, CAMTA1:

1400 calmodulin-binding transcription activator, CBP60a: calmodulin-binding protein 60a, EDS1: enhanced

1401 disease suceptibility 1. FAR1: FAR-red impaired response 1. G-box: cis-element in the promoter. JAZ:

1402 jasmonate-zim domain protein, JMT: jasmonic acid carboxyl methyltransferase. LOX: lipoxygenase,

1403 MES1: methylesterase 1. MFP2: multifunctional protein 2. MKS1: MAP kinase substrate 1. MYC2:

1404 transcription factor. NINJA: novel interactor of JAZ. PAD4: phytoalexin deficient 4. UGT74F1:

1405 glucosyltransferase. PR1-like (with ATPRB1), PR2, PR3, PR4 (HEL and ATEP3), PR5, PR12:

1406 pathogenesis-related proteins. ST2A: sulfotransferase 2A. TPL: TOPLESS co-repressor. UBP12: ubiquitin-specific protease 12. WRKY: transcription factor.
1407

1408

1409 **Fig. 4**: **Scenario of major events observed at three first days of pear/*V. inaequalis* non-host**
1410 **interaction.**

1411 On the left side, events observed in a typical cell, on the right side, events observed in guard cells of a
1412 stomata. A: apoplasm, AP: appressorium, C: cuticle, CBL1: calcineurin B-like protein 1, CDPK: Ca_2^+-
1413 dependent protein kinases, CRK: cysteine-rich receptor-like kinase, CY: cytoplasm, CW: cell wall, HAA:
1414 hydroxycinnamic acid amines, HR: hypersensitive response, JA: jasmonic acid, MB: plasma
1415 membrane, LCB: Long Chain/sphingoid Base components, MPK6: Mitogen activated protein kinase 6,
1416 MSL10: mechano-sensitive like 10, N: nucleus, PH: penetration hypha, PR: pathogenesis related
1417 proteins, RBOHB: respiratory burst oxidase homolog B, ROS: reactive oxygen species, S: stomata, SA:
1418 salycilic acid, SC: simple coumarins, SP: spore.

1419

1420 **Fig. 5: Main DEGs involved in cutin and wax biosynthesis during pear/V. inaequalis non-host**
1421 **interaction**.

1422 In green the chloroplast, in brown the endoplastic reticulum (ER) and in yellow the nucleus. Genes
1423 written in red are induced, genes written in blue are repressed. FAS: Fatty Acid Synthase complex to
1424 which belong ACCD (carboxytransferase beta subunit of the Acetyl-CoA carboxylase complex), FabG
1425 (β-ketoacyl ACP-reductase) and MOD1 (enoyl-ACP-reductase) functions. FAE: fatty acid elongase
1426 complex. KCS4 (3-ketoacyl-CoA synthase 4) and ECR/CER10 (trans-2-enoyl-CoA reductase) belong to
1427 the FAE complex. CER1 (octadecanal decarbonylase) and CER3 are implicated in aldehydes (CER1)
1428 and alkanes (CER1 and 3) generation in waxes biosynthesis. In cutin monomers synthesis, the ω-
1429 hydroxylation of C16:0 and C18:1 is catalyzed by cytochrome P450 monooxygenase (CYP86A) and
1430 LACS-encoded acyl-CoA synthetase may be required either to synthesize 16-hydroxy 16:0-CoA, a
1431 substrate for ω-hydroxylase, or for membrane transfer of monomers. Finally, the mature

monoacylglycerol cutin monomers are generated by transfer of the acyl group from acyl-CoA to glycerol-3-phosphate by glycerol-3-phosphate acyltransferase (GPAT) enzymes such as GPAT8. Some GDSL-lipases enzyme (such as At1g28600, At1g28660, At1g54790, At3g16370, At3g48460, AtCUS4: At4g28780, At5g14450) are then functioning as cutin synthase and polymerize cutin monoacylglycerols. Transcription factors such as MYB16 and SHN1 are positive regulators of wax and cutin biosynthesis. Adapted from Xia et al., 2009, [~~73~~83] and [~~74~~84].

Fig. 6: Main DEGs involved in the phenylpropanoid pathway during Pear / *V. inaequalis* non-host interaction.

Genes framed in red are induced, genes frames in blue are repressed. Framed in black, the detail of genes involved in flavonoids production and found in this interaction. Abbreviations: 4CL, 4-coumarate-CoA ligase; AACT, anthocyanin 5-aromatic acyltransferase; ANR, anthocyanidin reductase; ANS, anthocyanin synthase; BGLC or BGLU, β-glucosidases; C3H, coumarate 3-hydroxylase; C4H, cinnamate 4-hydroxylase; CAD, cinnamyl alcohol dehydrogenase; CCoAOMT, caffeoyl-CoA O-methyltransferase; CCR, cinnamoyl-CoA reductase; CHI, chalcone isomerase; CHS, chalcone synthase; COMT, caffeic acid 3-O-methyltransferase; CPK, calcium-dependent protein kinase ; DFR, dihydroflavonol reductase; DMR6, downy mildiou resistant 6; F3H, flavanone 3-hydroxylase; F3’H flavonoid 3’-hydroxylase; FLS, flavonol synthase; FNS, flavone synthase; GGT1, gamma-glutamyl transpeptidase 1; GT, glucosyl transferase; HCT, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase; LAC, laccase; LAR, leucoanthocyanidin reductase; OMT1, O-methyltransferase 1; PAL, phenylalanine ammonia-lyase; PER or PRX, peroxidase; TT7, transparent testa 7; UGFT, UDP-glucose flavonoid-3-O-glucosyltransferase; UGT71D1, UDP-glycosyltransferase 71D1.