



HAL
open science

Invasion of the stigma by oomycete pathogenic hyphae or pollen tubes: striking similarities and differences

Lucie Riglet, Sophie Hok, Naïma Kebdani-Minet, Joëlle Le Berre, Mathieu Gourgues, Frédérique Rozier, Vincent Bayle, Lesli Bancel-Vallée, Valérie Allasia, Harald Keller, et al.

► To cite this version:

Lucie Riglet, Sophie Hok, Naïma Kebdani-Minet, Joëlle Le Berre, Mathieu Gourgues, et al.. Invasion of the stigma by oomycete pathogenic hyphae or pollen tubes: striking similarities and differences. *Journal of Experimental Botany*, 2024, 75 (20), pp.6258-6274. 10.1093/jxb/erae308 . hal-04779975

HAL Id: hal-04779975

<https://hal.science/hal-04779975v1>

Submitted on 19 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

1 6695 words included figure legends. Material and methods excluded from word count

2 **Research article**

3

4 **Invasion of the stigma by oomycete pathogenic hyphae or pollen** 5 **tubes : striking similarities and differences**

6 **Lucie Riglet^{1, 2}, Sophie Hok³, Naïma Kebdani-Minet³, Joëlle Le Berre³, Mathieu Gourgues^{3, 4},**
7 **Frédérique Rozier¹, Vincent Bayle¹, Lesli Bancel-Vallée^{5, 6}, Valérie Allasia³, Harald Keller³,**
8 **Martine Da Rocha³, Agnès Attard^{3#*} and Isabelle Fobis-Loisy^{1#*}**

9 1 Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon, UCB Lyon1, CNRS, INRA, F-69342 Lyon,
10 France

11 2 Present Address: The Sainsbury Laboratory, Bateman Street, CB2 1LR, University of Cambridge, Cambridge, UK

12 3 INRAE, CNRS, Université Côte d'Azur, Institut Sophia Agrobiotech, 06903 Sophia Antipolis, France

13 4 Present Address: Bayer Crop Science France, 14, impasse Pierre Baizet CS 99163, F-69263 Lyon, France

14 5 Unité de Bordeaux, Bordeaux Imaging Center, 146 rue Léo Saignat CS 61292, F-33076 Bordeaux.

15 6 Present Address: Carl Zeiss SAS, 15 avenue Edouard Belin, F-92500 Rueil-Malmaison, France

16 # The authors contributed equally to this work and share the last authorship

17 * Correspondence: IFL : isabelle.fobis-loisy@ens-lyon.fr; AA: agnes.attard@inrae.fr.

18

19 **Running Title:** Stigmatic cells response to invader invasion

20

21 **Highlight**

22 Epidermal cells face constant environmental challenges. Comparing stigmatic cell responses
23 to pathogen hypha or pollen tube invasion, sheds light on how cells adjust the most relevant
24 responses to encountered invaders.

25

26 **Abstract**

27 Both filamentous pathogens' hyphae and pollen tube penetrate the host's outer layer and involve
28 growth within the host tissues. Early epidermal responses are decisive for the outcome of these two-
29 cell interaction processes. We identified a single cell type, the papilla of *Arabidopsis thaliana*'s stigma,
30 as a tool to conduct a comprehensive comparative analysis on how an epidermal cell responds to the
31 invasion of an unwanted pathogen or a welcomed pollen tube. We showed that *Phytophthora*
32 *parasitica*, a root oomycete, effectively breaches the stigmatic cell wall and develops as a biotroph
33 within the papilla cytoplasm. These invasive features resemble the behaviour exhibited by the
34 pathogen within its natural host cells, but diverge from the manner in which the pollen tube
35 progresses, being engulfed within the papilla cell wall. Quantitative analysis revealed that both
36 invaders trigger reorganisation of the stigmatic endomembrane system and the actin cytoskeleton.
37 While some remodelling processes are shared between the two interactions, others appear more
38 specific towards the respective invader. These findings underscore the remarkable ability of an
39 epidermal cell to differentiate between two types of invaders, thereby enabling it to trigger the most
40 suitable response during the onset of invasion.

41 **Keywords**

42 cell-cell interaction, invasive growth, oomycete, pollen, cell wall, EIHM, vesicular trafficking, actin,
43 Arabidopsis

44

45 **Abbreviations**

46 CW : cell wall

47 EIHM : extra-invasive hyphal membrane

48 HAI : hours after infection

49 MAP: minutes after pollination
50 LE : late endosome
51 MVB : multivesicular bodies
52 PM : plasma membrane
53 TGN : trans Golgi network
54

55 Introduction

56 The epidermis, as the outermost layer of plant cells, is in direct contact with the environment.
57 Epidermal cells must promptly react to potential external stresses to effectively mediate the most
58 relevant responses. Invaders can be infection structures such as hyphae of fungi or oomycetes but
59 also reproductive structures such as pollen tubes. The first contact between infection hyphae and
60 epidermal cells determine the outcome of the interaction, whether it leads to disease or resistance.
61 Similarly, the initial interaction between invading pollen tubes and the epidermal cells of the stigma
62 (papillae) is crucial for successful reproduction. In both scenarios, the early stages of the interaction
63 between the host and the invader are crucial for the outcome of these two cell-cell interaction systems.
64 Many points of convergence between plant defence and pollen recognition have already led some
65 authors to suggest that the two processes share common origins (Nasrallah, 2005; Kodera *et al.*,
66 2021). First, both fungal/oomycete hyphae and pollen tubes are tip-growing cells, secreting cell wall-
67 degrading enzymes to weaken and penetrate the host's surface layer (Chapman and Goring, 2010;
68 Kebdani *et al.*, 2010; Blackman *et al.*, 2014). Secondly subcellular reorganisation of intracellular
69 compartments and cytoskeleton occurs within epidermal cells at penetration sites (Takemoto *et al.*,
70 2003; Hardham, 2007; Iwano *et al.*, 2007; Samuel *et al.*, 2009, 2011). Thirdly, both hyphae and pollen
71 tubes uptake resources from invaded cells to support their growth (Oliveira-Garcia and Valent, 2015;
72 Rottmann *et al.*, 2018; Charrier *et al.*, 2019) (Oliveira-Garcia and Valent, 2015; Rottmann *et al.*, 2018;
73 Charrier *et al.*, 2019). Moreover, plant receptor-like kinases, like Feronia, are involved in both
74 processes, serving as a scaffold for assembling immune-receptor complex and regulating pathogen-
75 elicited burst of Reactive Oxygen Species (ROS) (Stegmann *et al.*, 2017). Feronia, along with other
76 members of the *Catharanthus roseus* receptor-like kinase 1-like (CrRLK1L) receptors, also controls
77 changes in ROS status in stigmatic cells, facilitating pollen grain hydration (Liu *et al.*, 2021; Zhang *et al.*,
78 2021; Huang *et al.*, 2023) and participates in the regulation of pollen tube penetration in the
79 stigmatic CW (Lan *et al.*, 2023). A transcriptomic analysis has predicted the activation of components
80 of the pattern-triggered immunity in the stigma upon pollination (Kodera *et al.*, 2021). Similarly, a study
81 comparing the transcriptomes of pollinated pistils with those infected by *Fusarium graminearum*
82 revealed that similar groups of genes were overexpressed in pistil responding to hyphae or pollen
83 tubes growth (Mondragón-Palomino *et al.*, 2017). This study, conducted at late stages of interaction, is
84 the only one involving a common host tissue, the pistil, to compare reproductive and immune
85 responses. Surprisingly, a comprehensive comparison of the cellular responses to intrusion and early
86 growth of these two types of invasive organisms has never been carried out.

87 Here, we aimed at identifying a cell-cell interaction system that allows to compare the behaviour of
88 a single responding cell facing two types of invasive growth. The pollen tube exhibits a host cell
89 specificity, penetrating only the papilla, while filamentous pathogens, such as oomycetes, can infect a
90 broader range of tissues. Therefore, we chose the papilla as the reference cell and conducted a
91 search for an oomycete capable of infecting this specific cell. We showed that *Phytophthora parasitica*,
92 a hemibiotrophic root pathogen breaches the stigmatic epidermis and develops as a biotroph within
93 the papilla cytoplasm, eliciting cellular responses similar to its natural host. When compared with the
94 pollen tube, some stigmatic responses are shared between both invaders (late endosome trafficking,
95 actin reorganisation), while others appear to be specific (EIHM formation, trans Golgi network
96 mobilisation).
97

98 Materials and methods

100 All *Arabidopsis thaliana* lines were in the Col-0 background and grown in growth chambers under long-
101 day conditions (16h light/8h dark at 21°C/19°C with a relative humidity around 60%). Three sets of
102 *Arabidopsis* marker lines were used; (i) For expression in stigmatic cells, we used the Brassica pSLR1
103 promoter (Rozier *et al.*, 2020). The pSLR1-LTI6b:GFP and the pSLR1-Lifeact:Venus lines were
104 previously described (Rozier *et al.*, 2020). We used the Gateway® technology (Life Technologies,
105 USA; <http://www.thermofisher.com>, (Karimi *et al.*, 2002) to generate the pSLR1-GFP:2xFYVE
106 construction. (ii) to control expression in root cells, two ubiquitous promoters, p35S and pUbiquite10,
107 were used; these promoters are poorly active in papillae. We generated the p35S-AtPIP2A:RFP
108 construction in the binary vector, pm-rk (Nelson *et al.*, 2007), using the Gateway® technology. The
109 pUbiquitine10-Citrine:2xFyve and the pUbiquitine10-Lifeact:YFP lines were previously described
110 (Simon *et al.*, 2014; Doumane *et al.*, 2021). The pVHAa1-AtVHAa1:GFP line was described previously
111 (Dettmer, 2006); the *VHAa1* promoter is active in both stigmatic and root cells. (iii) The pACT11-RFP
112 and the pLAT52-GFP lines, expressing a cytoplasmic fluorescent marker in pollen grain and tube,
113 were previously described (Rotman *et al.*, 2003; Rozier *et al.*, 2020).

114 *P. parasitica* Dastur isolate INRA-310 was maintained in the Phytophthora collection at INRAE, Sophia
115 Antipolis, France. The growth conditions and zoospores production were previously described
116 (Galiana *et al.*, 2005; Attard *et al.*, 2014). The *P. parasitica* transformant (pCL380-GFP:GUS)
117 expressing a GFP:GUS fusion protein was previously described (Attard *et al.*, 2014). The
118 *Hyaloperonospora arabidopsidis* isolate Noco was transferred weekly onto the susceptible accession
119 Col-0 as described (Hok *et al.*, 2014). Inoculated plants were kept in a growth cabinet at 16°C for 6
120 days with a 12 h photoperiod.

121

122 *Oomycetes pathogen assays and histochemical analysis*

123 Pathogen assays with the *P. parasitica* and *H. arabidopsidis* isolates on roots and leaves, respectively,
124 were performed as previously described (Hok *et al.*, 2014; Le Berre *et al.*, 2017). To infect pistil tissues
125 with *H. arabidopsidis*, *Arabidopsis* leaves with the sporulation oomycete on their surface were gently
126 rubbing over the pistil surface of manually opened flower buds (late stage 12; (Smyth *et al.*, 1990).
127 Alternatively, spores were applied in solution (5×10^5 zoospores/ml) directly on the stigma surface.
128 Inoculated pistils were observed by confocal microscopy in a period of 4h to 24h after inoculation.

129 Manually opened floral buds or naked pistils (late stage 12; Smyth *et al.*, 1990), were dipped in an
130 aqueous suspension of *P. parasitica* zoospores (5×10^5 zoospores/ml) obtained from the strain
131 pCL380-GFP:GUS (Attard *et al.*, 2014). In a period of 3h to 24h after infection, the GUS reporter
132 activity staining in plant tissues was performed as previously described (Hok *et al.*, 2014).

133

134 *Pollination assay and aniline blue staining*

135 Pistils (late stage 12; Smyth *et al.*, 1990) were emasculated and pollinated with mature pollen. Six
136 hours after pollination, stigmas were fixed in acetic acid 10%, ethanol 50% and stained with Aniline
137 Blue for epifluorescence microscopy observation as previously described (Rozier *et al.*, 2020).

138

139 *Transmission Electron microscopy*

140 Pistils (late stage 12; Smyth *et al.*, 1990) were emasculated and inoculated with *P. parasitica* for three
141 hours or pollinated with mature pollen for 60 minutes. Roots were inoculated with *P. parasitica* for
142 three hours. Pollinated or inoculated tissues were fixed in a solution containing 2.5% glutaraldehyde
143 and 2.5% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) and after four rounds of 30 min
144 vacuum, they were incubated in fixative for 12 hours at room temperature. Pistils or roots were then
145 washed in a phosphate buffer and further fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH
146 7.2) for 1.5 hours at room temperature. After rinsing in phosphate buffer and distilled water, samples
147 were dehydrated through an ethanol series, impregnated in increasing concentrations of SPURR resin

148 over a period of three days before being polymerised at 70°C for 18 h, sectioned (65 nm sections) and
149 imaged at 80 kV using an FEI TEM tecnaiSpirit with 4 k x 4 k eagle CCD.

150

151 *Confocal Laser Scanning Microscopy (CLSM)*

152 Pistils (late stage 12; Smyth et al., 1990) were emasculated and inoculated with *P. parasitica* for one
153 hour or pollinated with mature pollen for 30 minutes. Roots were inoculated with *P. parasitica* for one
154 hour. Pollinated pistils were observed with a Zeiss microscope (Zeiss 800 or AxioObserver Z1
155 equipped with a spinning disk module) with a 40x objective. Oomycetes infected tissues (stigma or
156 root) were observed with a Zeiss 880 confocal microscope with a 63x objective. Venus, Citrine, YFP
157 and GFP were excited at 488 nm and fluorescence detected between 500 and 550 nm. RFP was
158 excited at 561 nm and fluorescence detected between 550 and 600 nm. Stigmas or roots were imaged
159 every 0.4 µm, encompassing the entire volume of the stigma or half of the root thickness, using z-
160 stack confocal protocol. Pictures were taken with detector settings optimised for no pixel saturation.

161

162 *Fluorescence quantification*

163 All image processing, image analysis and fluorescence measurements were done using the
164 ImageJ/Fiji software (Schindelin et al., 2012).

165 To quantify fluorescence intensity at the contact site with the invader, we used a homemade Fiji
166 macro. From the serial confocal images, we generated an average intensity projection (Z project). We
167 manually choose one slide from the stack which corresponds to the focus plan of the contact site with
168 the invader. On this selected slide, we manually drew the stigmatic cell periphery and designated the
169 invader entry point. Then, we indicated the contact area length (ROI zone Length). From numerous
170 image observations, we defined this contact area as 16 µm. Next, the macro automatically depicted
171 two zones, the contact and the surrounding zones, with a series of circles of fixed diameter (ROI zone
172 thickness set at 2.6 µm). We estimated that 2.6 µm was an appropriate dimension compared to the
173 papilla sizes and the contact area length. The contact zone included five to seven circles depending
174 on its shape (straight or curved). The surrounding zone contained twice as many circles as the contact
175 zone equally distributed from each side of the contact area. Fluorescence intensity was measured in
176 each circle by the Fiji script, given as gray values and reported in an Excel file. The mean fluorescence
177 in contact and surrounding zones was calculated, then, a fluorescence difference [contact-
178 surrounding] was applied. For control stigmatic cells (non infected or non pollinated), as there was no
179 invader entry point, we introduced zero for the ROI zone Length, 2.6 µm for ROI zone thickness and
180 defined an arbitrary contact zone of six circles always positioned at the same distance from one
181 extremity of the drawn papilla periphery. This Fiji macro is available on demand.

182 For fluorescence quantification in root cells and vesicular marker lines, we followed the same
183 procedure except that we manually outlined one root edge. For actin fluorescence, quantification using
184 the Fiji macro was not possible. We then counted the number of images displaying a large actin
185 focalisation when a fluorescence patch was clearly visible at the contact site with the penetrating
186 hyphae. Statistical analyses of fluorescence intensity at the contact site with the invaders were based
187 on the paired sample *t*-test. The statistical analysis was carried out on control and inoculated or
188 pollinated cells (n =15).

189

190 *Deformation and diameter measurements*

191 Pistils (late stage 12; Smyth et al., 1990) expressing a GFP-tagged PM marker (LTI6b) were
192 emasculated and inoculated with *P. parasitica* or pollinated with mature pollen. Stigma were observed
193 under CLSM one hai or 30 map respectively. Internal (IntD) and external (ExtD) papilla deformation
194 were measured at the penetration site with the invaders as described (Riglet et al., 2020). The
195 statistical analysis compared IntD and ExtD (n= 21 hyphae, 20 pollen tubes) and were based on
196 unpaired *t*-test. On the same LTI6bGFP images, we measured two perpendicular diameters of the

197 hypha or pollen tube and calculated a mean diameter. The statistical analysis compared both
198 diameters (n= 21 hyphae, 20 pollen tubes) and were based on unpaired *t*-test.
199

200 **Results**

201 *P. parasitica* but not *H. arabidopsidis* successfully colonises the pistil

202 To investigate the ability of oomycete pathogens to breach the stigmatic barrier and infect *Arabidopsis*
203 *thaliana* pistils, we selected two oomycete species from different genera, each with distinct lifestyles
204 and host ranges. *P. parasitica* is a hemibiotrophic root pathogen with a broad host range, including *A.*
205 *thaliana* (Attard *et al.*, 2010), while *Hyaloperonospora arabidopsidis* (*H. arabidopsidis*) is an obligate
206 biotrophic foliar pathogen exclusively infecting *A. thaliana*. On their respective natural host organs,
207 both oomycetes penetrate within the first four hours after infection (hai). Mobile zoospores and
208 immobile conidiospores from *P. parasitica* (Fig. 1A) and *H. arabidopsidis* (Fig. 1B), respectively, emit
209 germ tubes on the plant surface, forming a specialised swelling structures, called appressoria, to
210 breach the epidermis through a penetrating hyphae (Attard *et al.*, 2010; Kebdani *et al.*, 2010; Boevink
211 *et al.*, 2020).

212 When we applied *H. arabidopsidis* conidiospores to the stigma, by gently rubbing *A. thaliana* leaves
213 with sporulating conidiophores over the surface of a mature pistil (from late stage 12 floral buds, just
214 prior to anthesis, Smyth *et al.*, 1990), spores started to germinate four hours after inoculation,
215 producing germ tubes encircling papillae (Fig. 2A). However, no appressoria were observed, indicating
216 that *H. arabidopsidis* failed to penetrate the stigma epidermis. *P. parasitica* produces motile zoospores
217 swimming towards roots under natural conditions. Thus, to infect pistil tissues, we dipped either entire
218 stage 12 flower buds or bare mature pistils in a suspension containing motile zoospores from a *P.*
219 *parasitica* strain expressing a Green Fluorescent Protein and β -glucuronidase (GFP:GUS) fusion
220 protein, which expression level significantly increases during penetration of plant tissue (Attard *et al.*,
221 2014). Remarkably, in both cases, the zoospores displayed a clear preference, accumulating at the
222 stigma surface and successfully penetrating the papillae, as indicated by the high expression of the
223 GUS reporter (Fig. 2B, C). This selective preference for specific host tissues is also observed on roots,
224 where zoospores expressing the GUS reporter aggregated around the elongation zone (Fig. 2D). We
225 then examined later stages of infection and noticed GFP-labelled *P. parasitica* hyphae penetrating the
226 pistil twenty-four hours after infection (Fig. 2E). In contrast to pollen tubes, whose elongation was
227 restricted to the central transmitting tract (Fig. 2F), *P. parasitica* hyphae invaded the entire pistil body.
228 Thus, the root pathogen *P. parasitica*, unlike the leaf pathogen *H. arabidopsidis*, is able to overcome
229 the stigmatic barrier and invade the pistil, despite the stigmatic epidermis not being its natural host
230 tissue.

231

232 *P. parasitica* forms appressoria for penetration and induces a PM-derived membrane around 233 the invading hyphae

234 To gain deeper insights into *P. parasitica* infection, we conducted a comparative analysis of the early
235 stages of infection in both the root and stigma epidermis. As previously published (Attard *et al.*, 2010)
236 we confirmed that one hour after root inoculation, zoospores initiated a germ tube that formed an
237 appressorium (white arrow head) on the root surface to penetrate the host epidermis and enter the
238 cells (Fig. 3A-C). On the stigma epidermis, we observed that zoospores initiated a germ tube that grew
239 at the papilla surface (Fig. 3G; Supplementary Fig. S1) and formed a swelling appressorium-like
240 structure, likely serving as an entry point for the penetrating hyphae to grow into the papilla cells (Fig.
241 3H). Given the limited knowledge regarding PM behaviour in epidermal cells invaded by *P. parasitica*,
242 we examined its fate in cells undergoing hyphal penetration. Using an *A. thaliana* line expressing
243 fluorescent-tagged plasma membrane (PM) markers (GFP-LTI6b in stigma, RFP-AtPIP2A in root), we
244 observed that in both root and papilla cells a structure labelled with the PM markers encased the
245 penetrating hypha within the cytoplasm (Fig. 3D-F, 3I-K).

246 Taken together, our findings indicate that *P. parasitica* uses comparable infection mechanisms to
247 invade both root and papillae cells.

248

249 *Penetration of stigmatic cells by pollen tube or infectious hyphae involves distinct processes*

250 To gain a deeper understanding of the invader-host cell interface, we conducted a comparative TEM
251 analysis. After three hours of inoculation with *P. parasitica*, we observed the digestion of the root cell
252 wall (CW) beneath the appressorium at the penetration sites (Fig. 4F, G) and the hyphae to be
253 embedded within the root cytoplasm (Fig. 4H). In the case of *P. parasitica* infection on stigma, three
254 hours after inoculation, the stigma cuticle and CW were no longer visible beneath the appressorium-
255 like structure on the papilla surface (Fig. 4A-B). This strongly suggests that both layers had been
256 digested at the penetration site, mirroring what was observed for the root CW. Within the papilla, we
257 found the hypha either between the cuticle and the CW, with the stigmatic CW partially digested (Fig.
258 4C-D) or entirely embedded within the stigmatic cytoplasm (Fig. 4E). Since cell penetration occurs
259 asynchronously, we assume that the gradual digestion of the CW, progressing from partial (Fig. 4C) to
260 complete (Fig. 4E), likely corresponds to different stages of infection.

261 Then, we aim at comparing infection with pollination. The early events occurring after contact between
262 a compatible pollen grain and a stigmatic cell are well documented and presented in Figure 1. Shortly
263 after contact, proteins and lipids from both cell surfaces fused, forming a hydrophilic environment
264 (called the foot) essential for pollen acceptance (Fig. 1C; (Chapman and Goring, 2010). This initial
265 contact is followed by pollen hydration and emergence of a pollen tube (germination) that penetrates
266 the stigmatic cell, growing towards the stigma base (Fig. 1C). When analysing pollinated papilla cells
267 by TEM, we found that the pollen tube breached the cuticle layer 30 minutes after pollination (map;
268 Fig. 5A, B). In contrast to hyphal penetration, we did not observe complete digestion of the stigmatic
269 CW. Instead, the pollen tube grew between the inner and outer CW layers of the papillae (Figs 1C,
270 5C-E), a mode of invasive growth characteristic of *Brassicaceae* species (Riglet *et al.*, 2020).

271 Our TEM analysis further supports that *P. parasitica* uses comparable infection processes to invade
272 both root and papillae cells, but also reveal fundamental differences when comparing infection with the
273 pollination process. While oomycete hyphae and pollen tubes penetrate the papilla cuticle, the pollen
274 tube remains engulfed within the CW, whereas oomycete hyphae digest the two CW layers to grow in
275 contact with the papilla PM.

276

277 *Penetration causes different constraints to the papilla surface*

278 Penetration imposes constraints on the epidermis surfaces. In order to breach a plant tissue, both
279 hyphae and pollen tubes have to soften and deform the invaded tissue, causing substrate radial
280 expansion (Sanati Nezhad and Geitmann, 2013). To evaluate the constraints exerted by both invaders
281 as they progressed through the stigmatic cells, we quantified papilla deformations shortly after hyphae
282 or pollen tubes penetration. Following the approach from Riglet *et al.*, (2020), we assessed two
283 measures: (i) the deformation towards the interior of the papilla (intD, inward), which we estimated by
284 quantifying the inward invagination of the stigmatic PM, labelled with the membrane marker LTI6B-
285 GFP and (ii) the deformation towards the exterior of the papilla (extD, outward), assessed through
286 analysis of bright field images (Fig. 6A, B). We found that the *P. parasitica* hypha induces a significant
287 external deformation during penetration (2.8 μm ; Fig. 6C; Supplementary Table S1) while causing only
288 slight deformation within the papilla interior (0.6 μm ; Fig. 6C; Supplementary Table S1). In contrast,
289 pollen tube growth resulted in nearly equal extD and intD values (2.1 μm and 1.8 μm respectively; Fig.
290 6C; Supplementary Table S1). Thus, although both the pollen tubes and the hyphae are capable of
291 piercing and penetrating the stigmatic surface, they exert different levels of constraints on the papilla
292 cells.

293

294 *Penetration triggers subcellular rearrangements in epidermal cells*

295 Vesicle trafficking within infected host cells and cytoskeletal remodelling are crucial for plant defence
296 responses (Ruano and Scheuring, 2020; Lu *et al.*, 2023). Likewise, early events linked to pollen
297 acceptance involve polarised secretion (Samuel *et al.*, 2009; Safavian and Goring, 2013) and actin
298 reorganisation (Iwano *et al.*, 2007; Rozier *et al.*, 2020) in stigmatic cells, targeted towards the pollen
299 grain. Therefore, we conducted a comparative analysis of the subcellular remodelling processes
300 triggered in stigmatic cells upon intrusion by either a hypha or a pollen tube. To address this objective,
301 we specifically focused on two functionally distinct populations of endomembrane compartments, (i)
302 the trans-Golgi network (TGN), a critical compartment at the crossroads of secretion and endocytosis
303 (Zhuang *et al.*, 2024), (ii) the late endosome (LE), also known as multivesicular bodies (MVB), serving
304 dual roles in cargo transport to the lytic vacuole and polarised secretion through the release of internal
305 vesicles into the extracellular space (Zhuang *et al.*, 2024) and (iii) the actin network, which provides
306 tracks to drive vesicular transport (Geitmann and Nebenführ, 2015). To monitor the behaviour of these
307 cellular components, we used specific marker lines expressing distinct fluorescent tags: a GFP-tagged
308 vacuolar ATPase $\alpha 1$ subunit (VHAa1-GFP, TGN-VHAa1), a GFP-tagged tandem FYVE domain (GFP-
309 FYVE, LE-FYVE), and a fusion protein combining the Lifeact peptide and the Venus fluorochrome
310 (Lifeact-Venus, actin cytoskeleton). We quantified the fluorescence intensity within the papilla
311 surrounding the site of invader penetration using a customised Fiji macro. By calculating the difference
312 in fluorescence intensity between the invader penetration site (contact zone) and the surrounding
313 region where no penetration occurred (surrounding area). A positive difference [contact-surrounding]
314 indicates a focalisation of the component of interest towards the penetration site. Pistils were
315 inoculated with *P. parasitica* for one hour or pollinating for 30 minutes, and cellular component
316 dynamics were tracked by confocal microscopy during early stages of interactions. Our analysis
317 revealed a significant increase in both TGN-VHAa1 and LE-FYVE fluorescence intensity at the contact
318 zone with the growing hyphae (Fig 7; Supplementary Figs S3, S4; Supplementary Table S2). LE-
319 FYVE fluorescence intensity was enhanced in response to the pollen tube intrusion around the
320 penetration site (Fig. 7; Supplementary Fig. S5; Supplementary Table S2), while the intensity of TGN-
321 VHAa1 labelling at the contact zone did not significantly differ from the area without contact (Fig. 7;
322 Supplementary Fig. S6; Supplementary Table S2). As control, we detected no significant fluorescence
323 intensity changes in non-infected and non-pollinated papillae (Supplementary Fig. S7; Supplementary
324 Table S2). In stigmatic cells, the actin cytoskeleton formed a network of fine cables uniformly
325 distributed along the papillae (Supplementary Fig. S7A; Rozier *et al.*, 2020). However, upon infection,
326 Lifeact-Venus fluorescence intensity significantly increased at the contact zone, resulting in a dense
327 and brightly fluorescent patch beneath the growing hypha (Fig. 7; Supplementary Fig. S8;
328 Supplementary Table S2). Similarly, significant focal accumulation of actin was observed in pollinated
329 papillae at the contact zone with the growing pollen tube (Fig. 7; Supplementary Fig. S9;
330 Supplementary Table S2).

331 Dynamic changes in the endomembrane system and the cytoskeleton during the invasion of the
332 natural target for *P. parasitica* infection remain poorly documented. Consequently, we expanded our
333 comparison to include the root epidermis. Similarly to the infected stigmatic cells, we detected a
334 significant increase in TGN-VHAa1 and LE-FYVE fluorescence intensity at the contact zone with the
335 growing hyphae in infected root cells (Fig. 8A, B; Supplementary Figs S10, S11; Supplementary Table
336 S2). Such fluorescence focalisation was not observed in control root cells (Supplementary Fig. S12A,
337 B). Our customised Fiji macro was not suitable for actin-Lifeact quantification due to the high
338 concentration of actin filaments at the cortical region of the entire root (asterisk in Fig. 8C), possibly
339 masking any focal accumulation at the contact zone. To address this issue, we counted the number of
340 images displaying a large actin-Lifeact focalisation patch at the tip of the penetrating hypha (red arrow
341 in Fig. 8C). Our observations revealed that the oomycete triggered actin focalisation at the contact
342 zone in 11 out of 15 infected root cells out (Supplementary Fig. S13). In contrast, no such fluorescent
343 patches were detected in control cells (Supplementary Fig. S12C).

344 Collectively, our results demonstrate that although *P. parasitica* is not a natural pathogen of the
345 stigmatic epidermis, it triggers cellular responses in papillae that closely resemble those induced in its
346 natural host, the root cell. Interestingly, when compared with the pollen tube, some of these responses
347 are shared between both invaders such as late endosome trafficking and actin reorganisation.

348 However, others, like trans Golgi network mobilisation, are more specific to likely orchestrate an
349 appropriate response.

350

351 **Discussion**

352 We aimed at deciphering the response of a single epidermal cell, the papilla, when facing the intrusion
353 of either a pollen tube or the hypha of pathogenic oomycetes from two distinct genera, *P. parasitica*
354 and *H. arabidopsidis*.

355 Upon *P. parasitica* inoculation of papilla or roots, we observed a preferential accumulation of
356 swimming zoospores at the elongation zone of the root and also at the stigma epidermis of the pistil.
357 This suggests that both zones share common features that specifically facilitate zoospore aggregation.
358 While the precise mechanisms behind the attraction of zoospores of several *Phytophthora* species by
359 root exudates is not fully elucidated, a various components, such as carbohydrates, amino-acids and
360 hormones, have been identified as potential attractant (Bassani *et al.*, 2020; Kasteel *et al.*, 2023). *A.*
361 *thaliana* belongs to a plant family characterised by a dry stigma lacking surface exudates (Hiscock and
362 Allen, 2008) suggesting that secreted chemical cues are unlikely to play a role in spore aggregation on
363 the stigma surface. However, we can not rule out that components from the outermost layer of the
364 papilla surface (the proteinaceous pellicle) may serve as attractants (Hiscock and Allen, 2008).
365 Electrostatic forces have been suggested to be involved in both root interactions with *Phytophthora*
366 *palmivora* and pollination (Van West *et al.*, 2002; Clarke *et al.*, 2017). While the mechanisms through
367 which these electrical signals are perceived by zoospores or pollen grains remain unknown, we can
368 speculate that electrostatic forces may be involved in the preferential accumulation of *P. parasitica*
369 spores at the stigmatic surface.

370 Upon reaching the epidermal surface, filamentous pathogens form appressoria to penetrate the tissue.
371 Thereby, the appressorium-like structure differentiated by *P. parasitica* at the outer surface of the
372 stigmatic epidermis (Figs 3, 4) is likely employed to breach both the cuticle and the CW, granting
373 access to penetration. Physical and chemical properties of the epidermis, such as surface hardness,
374 hydrophobicity, wax composition and cutin, are strong triggers for appressorium formation (Bircher and
375 Hohl, 1997; Ryder *et al.*, 2022). It is worth noting that stigmas as well as leaves, are coated with a
376 hydrophobic cuticle whereas the roots are not (Heizmann *et al.*, 2000; Schreiber, 2010). Despite this,
377 we have never observed appressorium formation on the stigma surface by *H. arabidopsidis*, a leaf
378 pathogen (Fig. 2). This suggests that the papillae may either lack the requisite triggering signals or
379 inhibit the differentiation of *H. arabidopsidis* appressorium. Furthermore, *H. arabidopsidis* is an
380 obligate pathogen exclusively infecting *A. thaliana* leaves, whereas *P. parasitica* has a broader host
381 range, infecting over 72 plant species and forming appressoria on both root and leaf tissues in natural
382 conditions (Meng *et al.*, 2014). It is therefore likely that *H. arabidopsidis* relies on specific host stimuli
383 to initiate appressorium differentiation, while *P. parasitica* is less specific, forming appressoria on
384 various tissues, including stigma.

385 To overcome the plant barrier formed by the cuticle and the CW, filamentous pathogens and pollen
386 grains secrete degrading enzymes capable of digesting their main polymers (Kubicek *et al.*, 2014;
387 Robinson *et al.*, 2021). Our TEM analysis of the penetration process (Figs 4, 5) reveals a major
388 difference between both invaders regarding their ability to digest the stigmatic CW. Whereas the
389 hypha passes through the bilayer papilla wall to grow in between the inner face of the CW and the PM,
390 pollen tube penetration is restricted to the outer wall layer, confining the tube growth within the CW.
391 This distinction suggests that the two papilla wall layers may possess distinct chemical properties,
392 thereby requiring diverse cocktails of secreted enzymes for their digestion. Notably, a mechanical
393 heterogeneity within the stigmatic cell wall, which can influence the pollen tube behaviour, has
394 previously been suggested (Riglet *et al.*, 2020). In this context, the interaction between the papillae
395 and the invader entails a dialogue that leads to digestion of either both stigmatic CW layers (in the
396 case of interaction with *P. parasitica*) or only the outer layer (in the case of interaction with the pollen
397 tube). One particularly intriguing aspect that remains enigmatic is the process by which a stigmatic cell
398 envelops the pollen tube within its CW. The available knowledge of the papilla CW is very limited,
399 except for its bilayered structure that distinguishes it from other plant cells. Based on our findings, it is

400 tempting to speculate that the selective digestion of the CW, limited to the outer layer, and the
401 controlled confinement of pollen tube growth within the two wall layers, could represent a specialised
402 adaptation of plants to discriminate a pollen tube from an unwanted invasive agent, such as a
403 pathogen.

404 As the hyphae and pollen tubes penetrate, they exert pressure on the interior of the stigmatic cell. We
405 estimated the compression intensity by measuring the deformation of the papilla shortly after
406 penetration (Fig. 6). We found that the pollen tube deforms the interior of the cell to a greater extent
407 compared to the hypha. This quantitative difference could stem from several factors. Firstly, the
408 invaders have different diameters, with the pollen tube being significantly larger than the hypha (*i.e.*
409 4.8 μm and 3.8 μm respectively) potentially resulting in greater compressive forces exerted by the
410 tube. Secondly, the way the invader softens the invaded tissue, either enzymatically or mechanically,
411 may differ, leading to differences in the magnitude of penetrating forces. Lastly, the resistance exerted
412 by the papilla cell against penetration may differ depending on the location or the nature of the
413 invader.

414 Gentle pressure applied with a microneedle on the surface of an epidermal cell induces actin
415 microfilaments and cables focusing beneath the needle tip (Hardham *et al.*, 2008). This observation
416 has led the authors to propose that the actin response might be triggered by physical detection of
417 pressure exerted by a penetrating invader rather than molecules associated with the invader. In our
418 study, we detected a similar actin reorganisation around the penetration site in stigmatic cells
419 challenged by either a hypha or a pollen tube (Fig. 7). This indicates comparable actin remodelling,
420 despite differences in the localisation of the growing hypha and the pollen tube, as well as variations in
421 the compression intensity they impose to the papilla surface. We could postulate that a threshold value
422 for the physical forces required to initiate actin reorganisation may have been reached in both
423 interaction systems. Such a mechanical threshold has been proposed to elicit subcellular
424 reorganisation in epidermal cells infected with *H. arabidopsidis* (Branco *et al.*, 2017). Alternatively, we
425 can not rule out that the chemical signatures of oomycete hyphae and pollen tubes, while distinct,
426 could have a similar impact on the actin network in invaded stigmatic cells.

427 In the cytoplasm of infected roots or stigmatic cells, the growing hypha is surrounded by a membrane
428 envelope labelled with fluorescent markers localised to the PM in non-infected cells (Fig. 3). Such
429 membrane envelope has been previously described in *A. thaliana* and rice leaves to surround the
430 hyphae of the hemibiotrophic fungi *Magnaporthe oryzae* or *Colletotrichum higginsianum* upon infection
431 (Yi and Valent, 2013; Qin *et al.*, 2020). This specialised membrane, called the extra-invasive hyphal
432 membrane (EIHM), is considered as a typical hallmark of the biotrophic phase, required to escape the
433 plant recognition system while uptaking nutrients from the host (Oliveira-Garcia and Valent, 2015;
434 Jones *et al.*, 2021). The EIHM forms a continuum with the plant PM but its composition differs as its
435 content is modified during infection (Qin *et al.*, 2020). To our knowledge, such specialised membranes
436 have never been reported in pathosystems involving flower pathogens (Brewer and Hammond-
437 Kosack, 2015; Andargie and Li, 2016; Mondragón-Palomino *et al.*, 2017). The presence of an EIHM-
438 like membrane within papilla cells infected by *P. parasitica* suggests that the oomycete adopts a
439 biotrophic lifestyle during the first hours of infection, similarly to its behaviour in roots. Further
440 investigation is needed to determine whether this envelope serves as a functional interface and
441 whether its composition differs from the rest of the PM.

442 The mobilisation of TGN and LE/MVB vesicles contributes significantly to plant defence mechanisms
443 by delivering defence proteins and antimicrobial compounds to the extracellular space, reinforcing the
444 CW to prevent pathogen entry and participating in the expansion of the host-derived membrane during
445 infection (Bozkurt *et al.*, 2015; Gu *et al.*, 2017). Thereby, the presence of VHA-TGN and LE-FYVE
446 concentrated at the vicinity of *P. parasitica* penetration site in root epidermal cells was not surprising
447 (Fig. 8). Interestingly, the focalisation of TGN-VHAa1 and LE-FYVE was also induced by *P. parasitica*
448 in infected stigmatic cells (fig. 7). This indicates the pathogen's capability to manipulate a non-natural
449 target, the papilla, to hijack the host cellular machinery similar to its strategy during root infection.
450 Upon pollination, intense vesicular trafficking in stigmatic cells is essential to sustain pollen
451 germination and subsequent pollen tube growth. Ultrastructural studies focused on pollen–stigma
452 interactions in *Brassica napus* identified LE/MVB fusion with the stigmatic PM and the release of

453 internal vesicles into the extracellular space adjacent to the pollen grain (Safavian and Goring, 2013;
454 Indriolo *et al.*, 2014). In pollinated *A. thaliana* stigma, instead of LE/MVB, secretory vesicles likely
455 originating from the TGN were detected attached to the stigmatic PM beneath the pollen grain
456 (Safavian and Goring, 2013; Indriolo *et al.*, 2014). In our experiments conducted on *A. thaliana*, we did
457 not detect polarised movement of the TGN-VHAA1 compartments during pollen tube penetration. This
458 discrepancy could be attributed to the methods used between our study, using fluorescent probes and
459 confocal imaging, and the techniques used in Safavian and Goring (2013) and Indriolo *et al.* (2014),
460 which relies on chemically fixed material and TEM. Alternatively, the observed differences may also
461 stem from the timing after pollination. Indeed, while we focused on pollen tube penetration at 30
462 minutes after pollination, Goring's group concentrated on earlier stages of interaction 10 minutes after
463 pollen deposition (Safavian and Goring, 2013; Indriolo *et al.*, 2014). Besides, the TGN constitutes a
464 complex cellular compartment at the interface of the secretory and endocytic pathways, divided into
465 subdomains or sub-populations (Aniento *et al.*, 2022). Thus, deciphering the precise mechanisms
466 behind its involvement in the pollination process would require further live-cell imaging with an
467 extensive collection of fluorescent-tagged markers for endomembrane compartments. Nevertheless,
468 our study highlights the intriguing possibility that trafficking of FYVE-labelled LE/MVB compartments
469 may play a role in pollen acceptance in *A.thaliana*, supplementing the TGN-associated secretory
470 pathway previously suggested by Goring and colleagues (Safavian and Goring, 2013; Indriolo *et al.*,
471 2014).

472 Recent studies proposed an attractive lock-and-key model in which a stigmatic barrier is established
473 through receptors from the CrRLK1L family and their corresponding stigmatic ligands to exclude
474 unwanted pollen. This barrier is disrupted when a compatible pollen grain carrying competing ligands
475 for the same receptor complexes lands on the papillae, thus gaining access to papilla entrance (Liu *et*
476 *al.*, 2021; Zhang *et al.*, 2021; Huang *et al.*, 2023; Lan *et al.*, 2023). The current challenge lies in
477 understanding how two distinct sets of ligands can trigger opposite downstream signalling responses,
478 leading to rejection or acceptance. An attractive hypothesis is that the LE/MVB-FYVE trafficking we
479 observed in this study may be involved in the acceptance pathway, delivering necessary factors
480 required for pollen germination and/or pollen tube penetration. In this context, it is plausible to
481 speculate that *P. parasitica*, which induces LE/MVB-FYVE trafficking and penetrates the papilla, might
482 possess the key ligands necessary to disrupt the stigmatic lock. MVBs are fascinating compartments
483 that not only serve as a sorting station directing proteins towards vacuolar for degradation but also
484 play a role in secretion, by releasing their intraluminal vesicles into the extracellular space upon fusion
485 with the plasma membrane. While the exact nature of the cargoes transported by these vesicles
486 remains poorly understood, exosomes are believed to play a crucial role in delivering signalling
487 molecules for intercellular communication (Zhuang *et al.*, 2024).

488
489 To conclude, we identified the papilla as a valuable model for comprehensively comparing on how
490 a single epidermal cell responds to two opposite scenarios: the invasion of a pathogenic hyphae and
491 the beneficial process of pollination. We have identified shared subcellular changes triggered in
492 stigmatic cells upon *P. parasitica* infection and in responses to pollination (LE trafficking and actin
493 remodelling). These findings support the longstanding assumption that common or evolutionary
494 related host-encoded functions exist between plant defence and pollen recognition. Besides, we
495 identified distinct cellular events specific to each type of interaction highlighting a finely tuned dialog
496 established during early stages of invasions. These include the mobilisation of the TGN-VHAA1
497 compartments and the formation of an EIHM-like membrane. Such interaction-specific features are
498 likely to be pivotal triggering the appropriate response, depending on the nature of the invader and/or
499 the established entry strategy (*i.e.* appressorium formation and complete CW digestion vs foot
500 formation and partial CW digestion). Our findings suggest that numerous crosstalks exist between the
501 processes of infection and pollination. The interaction system we have developed holds promise as a
502 framework for further exploration, offering insights on how an epidermal cell perceives and responds to
503 an invader in order to fine-tune the most relevant responses.

504
505

506

507 **Supplementary data**

508 The following supplementary data are available at JXB online

509 *Table S1.* Papilla deformation and hypha/pollen tube diameters

510 *Table S2.* Fluorescence quantification

511 *Fig. S1.* *P. parasitica* zoospores infecting stigmatic cells

512 *Fig. S2.* Invader features.

513 *Fig. S3.* Pattern of TGN-VHAA1 compartments in stigmatic cells in response to *P. parasitica*.

514 *Fig. S4.* Pattern of LE-FYVE compartments in stigmatic cells in response to *P. parasitica*.

515 *Fig. S5.* Pattern of LE-FYVE compartments in stigmatic cells in response to pollen tubes.

516 *Fig. S6.* Pattern of TGN-VHAA1 compartments in stigmatic cells in response to pollen tubes.

517 *Fig. S7.* Subcellular arrangement of the TGN, the LE, and the actin network in control stigmatic cells
518 as observed by CLSM.

519 *Fig. S8.* Pattern of the actin cytoskeleton (Actin-Lifeact) in stigmatic cells upon infection with *P.*
520 *parasitica*.

521 *Fig. S9.* Pattern of the actin cytoskeleton (Actin-Lifeact) in stigmatic cells in response to pollen
522 tubes.

523 *Fig. S10.* Pattern of TGN-VHAA1 compartments in root cells in response to *P. parasitica*.

524 *Fig. S11.* Pattern of LE-FYVE compartments in root cells in response to *P. parasitica*.

525 *Fig. S12.* Subcellular arrangement of the TGN, the LE, and the actin network in control root cells, as
526 observed by CLSM.

527 *Fig. S13.* Dynamics of the actin cytoskeleton (Actin-Lifeact) in root cells in response to infection with
528 *P. parasitica*, as analysed by CLSM at one hai.

529

530

531 **Acknowledgments**

532 We thank all the members of the Cell Signaling and Endocytosis (SiCE) group (Laboratoire de
533 Reproduction et Développement des Plantes, ENS Lyon, France), especially Thierry Gaude at the
534 origin of this story, and the Interactions Plantes-Oomycètes (IPO) team (Institut Sophia Agrobiotech,
535 Sophia Antipolis, France) for fruitful discussions. We thank Y. Jaillais for his comments on the
536 manuscript. We thank P. Bolland, A Lacroix, J. Berger (RDP) for plant care. We thank for the
537 microscopy facility, the PLATIM of the SFR Biosciences Gerland-Lyon Sud, the “Institut Sophia
538 AgroBiotech” (PlantBIOs) and the “Institut de Pharmacologie Moléculaire et Cellulaire”, both part of the
539 “Microscopie Imagerie Cytométrie Côte d’Azur” GIS IBiSA labelled platform, and We thank C. Lionnet
540 at the PLATIM and F. Brau at the “Institut de Pharmacologie Moléculaire et Cellulaire”. We thank the
541 Bordeaux imaging center for TEM imaging and analysis.

542

543 **Author contribution**

544 LR was responsible for all experiments and analysis performed for pollination. SH developed the
545 pathosystem for papilla infection and performed the confocal imaging. LBV performed the image
546 acquisition by SEM. NM performed and analysed *P. parasitica* root and pistil infections. JL performed
547 and analysed *P. parasitica* root infections. VA and HK performed *H. arabidopsidis* leaf and pistil
548 infections. VB designed the Fiji macro. LR, SH, HK, MG, IFL and AA designed the study; LR, IFL and
549 AA wrote the manuscript. All the authors contributed to the discussion, reviewed and edited the
550 manuscript. The authors read and approved the final manuscript.

551

552 **Conflict of interest**

553 No conflict of interest declared

554

555 **Funding**

556 This work was supported by the French Government (National Research Agency, ANR) through Grant
557 ANR-14-CE11-0021, the "Investments for the Future" LABEX SIGNALIFE program reference ANR-11-
558 LABX-0028-01.

559

560 **Data availability**

561 All data supporting the findings of this study are available within the paper or within its supplementary
562 data published online. The custom Fiji macro is available upon request to the corresponding authors
563 Isabelle Fobis-Loisy and Agnès Attard.

564

References

Andargie M, Li J. 2016. *Arabidopsis thaliana*: A Model Host Plant to Study Plant–Pathogen Interaction Using Rice False Smut Isolates of *Ustilaginoidea virens*. *Frontiers in Plant Science* **7**.

Attard A, Evangelisti E, Kebdani-Minet N, Panabières F, Deleury E, Maggio C, Ponchet M, Gourgues M. 2014. Transcriptome dynamics of *Arabidopsis thaliana* root penetration by the oomycete pathogen *Phytophthora parasitica*. *BMC Genomics* **15**, 538.

Attard A, Gourgues M, Callemeyn-Torre N, Keller H. 2010. The immediate activation of defense responses in *Arabidopsis* roots is not sufficient to prevent *Phytophthora parasitica* infection. *New Phytologist* **187**, 449–460.

Bassani I, Larousse M, Tran QD, Attard A, Galiana E. 2020. *Phytophthora* zoospores: From perception of environmental signals to inoculum formation on the host-root surface. *Computational and Structural Biotechnology Journal* **18**, 3766–3773.

Bircher U, Hohl HR. 1997. Environmental signalling during induction of appressorium formation in *Phytophthora*. *Mycological Research* **101**, 395–402.

Blackman LM, Cullerne DP, Hardham AR. 2014. Bioinformatic characterisation of genes encoding cell wall degrading enzymes in the *Phytophthora parasitica* genome. *BMC Genomics* **15**, 785.

Boevink PC, Birch PRJ, Turnbull D, Whisson SC. 2020. Devastating intimacy: the cell biology of plant–*Phytophthora* interactions. *New Phytologist* **228**, 445–458.

Bozkurt TO, Belhaj K, Dagdas YF, Chaparro-Garcia A, Wu C-H, Cano LM, Kamoun S. 2015. Rerouting of Plant Late Endocytic Trafficking Toward a Pathogen Interface: Rerouting of Endocytic Pathway to Pathogen Interface. *Traffic* **16**, 204–226.

Branco R, Pearsall E-J, Rundle CA, White RG, Bradby JE, Hardham AR. 2017. Quantifying the plant actin cytoskeleton response to applied pressure using nanoindentation. *Protoplasma* **254**, 1127–1137.

Brewer HC, Hammond-Kosack KE. 2015. Host to a Stranger: *Arabidopsis* and *Fusarium* Ear Blight. *Trends in Plant Science* **20**, 651–663.

Chapman LA, Goring DR. 2010. Pollen-pistil interactions regulating successful fertilization in the Brassicaceae. *Journal of Experimental Botany* **61**, 1987–1999.

Charrier A, Vergne E, Dousset N, Richer A, Petiteau A, Chevreau E. 2019. Efficient Targeted Mutagenesis in Apple and First Time Edition of Pear Using the CRISPR-Cas9 System. *Frontiers in Plant Science* **10**, 40.

- Clarke D, Morley E, Robert D.** 2017. The bee, the flower, and the electric field: electric ecology and aerial electroreception. *Journal of Comparative Physiology A* **203**, 737–748.
- Dettmer J.** 2006. Vacuolar H⁺-ATPase Activity Is Required for Endocytic and Secretory Trafficking in Arabidopsis. *THE PLANT CELL ONLINE* **18**, 715–730.
- Doumane M, Lebecq A, Colin L, et al.** 2021. Inducible depletion of PI(4,5)P₂ by the synthetic iDePP system in Arabidopsis. *Nature Plants* **7**, 587–597.
- Galiana E, Rivière M-P, Pagnotta S, Baudouin E, Panabières F, Gounon P, Boudier L.** 2005. Plant-induced cell death in the oomycete pathogen *Phytophthora parasitica*. *Cellular Microbiology* **7**, 1365–1378.
- Geitmann A, Nebenführ A.** 2015. Navigating the plant cell: intracellular transport logistics in the green kingdom. *Molecular Biology of the Cell* **26**, 3373–3378.
- Gu Y, Zavaliev R, Dong X.** 2017. Membrane Trafficking in Plant Immunity. *Molecular plant* **10**, 1026–1034.
- Hardham AR.** 2007. Cell biology of plant-oomycete interactions. *Cellular Microbiology* **9**, 31–39.
- Hardham AR, Takemoto D, White RG.** 2008. Rapid and dynamic subcellular reorganization following mechanical stimulation of Arabidopsis epidermal cells mimics responses to fungal and oomycete attack. *BMC Plant Biology* **8**, 63.
- Heizmann P, Luu DT, Dumas C.** 2000. Pollen-stigma adhesion in the Brassicaceae. *Annals of Botany* **85**, 23–27.
- Hiscock SJ, Allen AM.** 2008. Diverse cell signalling pathways regulate pollen-stigma interactions: the search for consensus. *New Phytologist* **179**, 286–317.
- Hok S, Allasia V, Andrio E, et al.** 2014. The Receptor Kinase IMPAIRED OOMYCETE SUSCEPTIBILITY1 Attenuates Abscisic Acid Responses in Arabidopsis1[C][W]. *Plant Physiology* **166**, 1506–1518.
- Huang J, Yang L, Yang L, et al.** 2023. Stigma receptors control intraspecies and interspecies barriers in Brassicaceae. *Nature*, 1–6.
- Indriolo E, Safavian D, Goring DR.** 2014. The ARC1 E3 Ligase Promotes Two Different Self-Pollen Avoidance Traits in Arabidopsis. *The Plant Cell* **26**, 1525–1543.
- Iwano M, Shiba H, Matoba K, et al.** 2007. Actin Dynamics in Papilla Cells of Brassica rapa during Self- and Cross-Pollination. *PLANT PHYSIOLOGY* **144**, 72–81.
- Jones K, Zhu J, Jenkinson CB, Kim DW, Pfeifer MA, Khang CH.** 2021. Disruption of the Interfacial Membrane Leads to Magnaporthe oryzae Effector Re-location and Lifestyle Switch During Rice Blast Disease. *Frontiers in Cell and Developmental Biology* **9**, 681734.
- Karimi M, Inzé D, Depicker A.** 2002. GATEWAY vectors for Agrobacterium-mediated plant transformation. *Trends in Plant Science* **7**, 193–195.
- Kasteel M, Ketelaar T, Govers F.** 2023. Fatal attraction: How Phytophthora zoospores find their host. *Seminars in Cell & Developmental Biology* doi: 10.1016/j.semcdb.2023.01.014.

- Kebedani N, Pieuchot L, Deleury E, Panabières F, Le Berre J-Y, Gourgues M.** 2010. Cellular and molecular characterization of *Phytophthora parasitica* appressorium-mediated penetration. *New Phytologist* **185**, 248–257.
- Kodera C, Just J, Da Rocha M, Larrieu A, Riglet L, Legrand J, Rozier F, Gaude T, Fobis-Loisy I.** 2021. The molecular signatures of compatible and incompatible pollination in *Arabidopsis*. *BMC Genomics* **22**, 268.
- Kubicek CP, Starr TL, Glass NL.** 2014. Plant Cell Wall-Degrading Enzymes and Their Secretion in Plant-Pathogenic Fungi. *Annual Review of Phytopathology* **52**, 427–451.
- Lan Z, Song Z, Wang Z, et al.** 2023. Antagonistic RALF peptides control an intergeneric hybridization barrier on Brassicaceae stigmas. *Cell* **186**, 4773-4787.e12.
- Le Berre J-Y, Gourgues M, Samans B, Keller H, Panabières F, Attard A.** 2017. Transcriptome dynamic of *Arabidopsis* roots infected with *Phytophthora parasitica* identifies VQ29, a gene induced during the penetration and involved in the restriction of infection. *PloS one* **12**, e0190341.
- Liu C, Shen L, Xiao Y, et al.** 2021. Pollen PCP-B peptides unlock a stigma peptide–receptor kinase gating mechanism for pollination. *Science* **372**, 171–175.
- Lu Y, Zhang Y, Lian N, Li X.** 2023. Membrane Dynamics Regulated by Cytoskeleton in Plant Immunity. *International Journal of Molecular Sciences* **24**, 6059.
- Meng Y, Zhang Q, Ding W, Shan W.** 2014. *Phytophthora parasitica*: a model oomycete plant pathogen. *Mycology* **5**, 43–51.
- Mondragón-Palomino M, John-Arputharaj A, Pallmann M, Dresselhaus T.** 2017. Similarities between Reproductive and Immune Pistil Transcriptomes of *Arabidopsis* Species. *Plant Physiology* **174**, 1559–1575.
- Nasrallah J.** 2005. Recognition and rejection of self in plant self-incompatibility: comparisons to animal histocompatibility. *Trends in Immunology* **26**, 412–418.
- Nelson BK, Cai X, Nebenführ A.** 2007. A multicolored set of in vivo organelle markers for co-localization studies in *Arabidopsis* and other plants: Fluorescent organelle markers. *The Plant Journal* **51**, 1126–1136.
- Oliveira-Garcia E, Valent B.** 2015. How eukaryotic filamentous pathogens evade plant recognition. *Current Opinion in Microbiology* **26**, 92–101.
- Qin L, Zhou Z, Li Q, et al.** 2020. Specific Recruitment of Phosphoinositide Species to the Plant-Pathogen Interfacial Membrane Underlies *Arabidopsis* Susceptibility to Fungal Infection. *The Plant Cell* doi: 10.1105/tpc.19.00970.
- Riglet L, Rozier F, Kodera C, Bovio S, Sechet J, Fobis-Loisy I, Gaude T.** 2020. KATANIN-dependent mechanical properties of the stigmatic cell wall mediate the pollen tube path in *Arabidopsis*. *eLife* **9**, e57282.
- Robinson R, Sollapura V, Couroux P, Sprott D, Ravensdale M, Routly E, Xing T, Robert LS.** 2021. The Brassica mature pollen and stigma proteomes: preparing to meet. *The Plant Journal* **107**, 1546–1568.

- Rotman N, Rozier F, Boavida L, Dumas C, Berger F, Faure J-E.** 2003. Female control of male gamete delivery during fertilization in *Arabidopsis thaliana*. *Current Biology* **13**, 432–436.
- Rottmann T, Fritz C, Sauer N, Stadler R.** 2018. Glucose Uptake via STP Transporters Inhibits in Vitro Pollen Tube Growth in a HEXOKINASE1-Dependent Manner in *Arabidopsis thaliana*. *The Plant Cell* **30**, 2057–2081.
- Rozier F, Riglet L, Kodera C, Bayle V, Durand E, Schnabel J, Gaude T, Fobis-Loisy I.** 2020. Live-cell imaging of early events following pollen perception in self-incompatible *Arabidopsis thaliana*. *Journal of Experimental Botany* **71**, 2513–2526.
- Ruano G, Scheuring D.** 2020. Plant Cells under Attack: Unconventional Endomembrane Trafficking during Plant Defense. *Plants* **9**, 389.
- Ryder LS, Cruz-Mireles N, Molinari C, Eisermann I, Eseola AB, Talbot NJ.** 2022. The appressorium at a glance. *Journal of Cell Science* **135**, jcs259857.
- Safavian D, Goring DR.** 2013. Secretory Activity Is Rapidly Induced in Stigmatic Papillae by Compatible Pollen, but Inhibited for Self-Incompatible Pollen in the Brassicaceae. (D Bassham, Ed.). *PLoS ONE* **8**, e84286.
- Samuel MA, Chong YT, Haasen KE, Aldea-Brydges MG, Stone SL, Goring DR.** 2009. Cellular Pathways Regulating Responses to Compatible and Self-Incompatible Pollen in Brassica and *Arabidopsis* Stigmas Intersect at Exo70A1, a Putative Component of the Exocyst Complex. *THE PLANT CELL ONLINE* **21**, 2655–2671.
- Samuel MA, Tang W, Jamshed M, Northey J, Patel D, Smith D, Siu KWM, Muench DG, Wang Z-Y, Goring DR.** 2011. Proteomic Analysis of Brassica Stigmatic Proteins Following the Self-incompatibility Reaction Reveals a Role for Microtubule Dynamics During Pollen Responses. *Molecular & Cellular Proteomics* **10**, M111.011338-M111.011338.
- Sanati Nezhad A, Geitmann A.** 2013. The cellular mechanics of an invasive lifestyle. *Journal of Experimental Botany* **64**, 4709–4728.
- Schindelin J, Arganda-Carreras I, Frise E, et al.** 2012. Fiji: an open-source platform for biological-image analysis. *Nature Methods* **9**, 676–682.
- Schreiber L.** 2010. Transport barriers made of cutin, suberin and associated waxes. *Trends in Plant Science* **15**, 546–553.
- Simon MLA, Platre MP, Assil S, van Wijk R, Chen WY, Chory J, Dreux M, Munnik T, Jaillais Y.** 2014. A multi-colour/multi-affinity marker set to visualize phosphoinositide dynamics in *Arabidopsis*. *The Plant Journal* **77**, 322–337.
- Smyth DR, Bowman JL, Meyerowitz EM.** 1990. Early flower development in *Arabidopsis*. *The Plant Cell* **2**, 755–767.
- Stegmann M, Monaghan J, Smakowska-Luzan E, Rovenich H, Lehner A, Holton N, Belkhadir Y, Zipfel C.** 2017. The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. *Science* **355**, 287–289.

Takemoto D, Jones DA, Hardham AR. 2003. GFP-tagging of cell components reveals the dynamics of subcellular re-organization in response to infection of Arabidopsis by oomycete pathogens. *The Plant Journal* **33**, 775–792.

Van West P van, Morris BM, Reid B, Appiah AA, Osborne MC, Campbell TA, Shepherd SJ, Gow NAR. 2002. Oomycete plant pathogens use electric fields to target roots. *Molecular plant-microbe interactions* **15**, 790–798.

Yi M, Valent B. 2013. Communication Between Filamentous Pathogens and Plants at the Biotrophic Interface. *Annual Review of Phytopathology* **51**, 587–611.

Zhang L, Huang J, Su S, et al. 2021. FERONIA receptor kinase-regulated reactive oxygen species mediate self-incompatibility in Brassica rapa. *Current Biology* **0**.

Zhuang X, Li R, Jiang L. 2024. A century journey of organelles research in the plant endomembrane system. *The Plant Cell*, koae004.

565 **Figure legends**

566 **Fig. 1.** Invasion features of *P. parasitica*, *H. arabidopsidis*, and *A. thaliana* pollen tube in root, leaf,
567 and stigma, respectively. (A) Arabidopsis root infected with the oomycete *P. parasitica*. Schematic
568 representations (upper row) and merged CLSM images between the green channel and bright-field of
569 *P. parasitica* strain pCL380-GFP:GUS expressing a cytoplasmic GFP marker (lower row). The zoospore
570 (sp) germinates a germ tube (gt) that grows along the plant surface and forms an appressorium
571 (white arrow head) to penetrate the epidermis. Penetration starts 30 min after infection (left panel).
572 The penetrating hypha (ph) grows intercellularly and develops haustoria (ha) (6 hai, right panel). (B)
573 Arabidopsis leaf infected with the oomycete *H. arabidopsidis*. Schematic representations (upper row)
574 and bright-field CLSM images (lower row). The conidiospore (sp) germinates a germ tube (gt) that
575 grows along the plant surface and forms an appressorium (white arrow head) to penetrate the
576 epidermis (8 hai, left panel). The penetrating hypha (ph) grows intercellularly and develops haustoria
577 (ha) (18 hai, right panel). (C) Arabidopsis stigma with germinating pollen. Schematic representations
578 (upper row) and merged CLSM images between green fluorescence and bright-field of a pollen grain
579 and a pollen tube expressing the GFP marker (pLAT52-GFP Line; lower row). Rapidly after pollen
580 capture, a specific structure, called the foot (depicted in orange), is formed at the pollen-papilla
581 interface. A pollen tube (pt) emerges from the grain, passes through the foot and penetrates the
582 papilla CW (dark grey layer) 12 map (left panel). The pollen tube grows inside the papilla CW towards
583 the basis of the stigma (right panel). Scale bar=10 µm.

584
585 **Fig. 2.** *P. parasitica* but not *H. arabidopsidis* invades *A. thaliana* pistil. (A) *H. arabidopsidis* spores (sp)
586 were deposited on the stigma surface of mature pistils and observed by CLSM. The upper and lower
587 images are both extracted from the same Z-stack showing a spore and a germ tube (gt) growing
588 around the papilla cells (s) without penetration, 18 hai. Scale bar=10 µm. (B, C) The entire flower bud
589 (B) or the naked pistil (C) were dipped in a suspension of GUS-expressing zoospores of *P. parasitica*
590 (strain pCL380-GFP:GUS) and observed by transmission light microscopy. Zoospores preferentially
591 attached to the stigma epidermis, three hai. (D) *A. thaliana* roots dipped in a suspension of *P.*
592 *parasitica* strain pCL380-GFP:GUS and observed by TEM. Zoospores preferentially attached to the
593 elongation zone of the root, three hai. (E) Entire flower buds were dipped in a suspension of GFP-

594 expressing zoospores of *P. parasitica* and observed by CLSM. Median longitudinal optical section of
595 the entire pistil. Growing hyphae were detected inside the pistil tissues 24 hai. (F) Pollinated pistils
596 stained with aniline blue and observed under epifluorescence microscopy. Six hours after pollen
597 grain (pg) deposition at the stigma surface, pollen tubes (pt) were present within the central
598 transmitting track. Scale bar=100 μ m. Each experiment was repeated at least three times.

599
600 **Fig. 3.** Appressorium-mediated penetration of the pistil and the root epidermis by *P. parasitica*. (A)
601 Transversal optical sections of a zoospore (sp, delimited by a white dashed line) of *P. parasitica*
602 germinating a germ tube (gt) on the stigmatic cell surface, one hai. The germ tube forms an
603 appressorium-like structure at its extremity (white arrow head). (B) Longitudinal optical sections of *P.*
604 *parasitica* infecting a papilla cell. The penetrating hypha (ph) emerges from an appressorium (white
605 arrow head). (C-E). An inoculated papilla expressing a GFP-tagged PM marker (LTI6b) four hai. A
606 Lti6b-labelled membrane encircles the penetrating hypha, as observed in bright field (C), green
607 fluorescence (D) and the merged image (E). (F-K), Arabidopsis root expressing a RFP-tagged PM
608 aquaporin AtPIP2A upon invasion by *P. parasitica*. The roots were dipped in a zoospore suspension
609 for one hour and the epidermis was analysed by CLSM. The images show optical sections of the same
610 infection site, with F, G and H focused on the root (r) surface, and I, J and K on the cell interior. The
611 zoospore (sp) germinates a germ tube that differentiates an appressorium (white arrow head) to
612 penetrate between two adjacent cells. Inside the epidermis, the AtPIP2A-labelled membrane
613 surrounds the penetrating hypha. Left column, bright field, middle column, RFP channel, and right
614 column, merged channel. Scale bar=10 μ m. Each experiment was repeated at least three times.

615
616 **Fig. 4.** Invasion by *P. parasitica* hypha depends on the host epidermis. (A-E) Stigmatic cell infected
617 with *P. parasitica* observed by TEM, three hai. (A) The germ tube (gt) emitted from the zoospore is
618 located outside the papilla (s). The extremity of the germ tube forms an appressorium-like structure
619 (white arrow head). A penetrating hypha (ph) enters the host cell. (B) Magnification detail depicted
620 by the white square in A showing the stigmatic cuticle (SC; electron dense black layer) and the
621 stigmatic CW (SCW) digested at the penetration site. The oomycete CW (OCW) appears as an
622 electron transparent white layer. (C) The hypha locates between the stigmatic cuticle and the stigma
623 CW (SCW). (D) Magnification detail depicted by the white square in C showing the stigmatic CW
624 degradation (degSCW) occurring at the contact area with the hypha. (E) *P. parasitica* penetration
625 hypha (ph) embedded in the stigmatic cytoplasm (s). (F-H) Root cells (R) infected with *P. parasitica*,
626 as observed by TEM, three hai. (F) An appressorium (white arrowhead) is visible at the root surface
627 and a penetration hypha (ph) inside the host cell. (G) Detailed view depicted by the white square in F
628 showing degradation of the root CW(deg RCW). (H) A penetration hypha embedded in the root
629 cytoplasm. Scale Bar=1 μ m. Each experiment was repeated at least three times.

630
631 **Fig. 5.** The pollen tube grows within the papilla CW. The images show the pollinated stigma as
632 observed by TEM, 60 map, with general and detailed views in the left and right columns, respectively.
633 (A) Transversal section showing the pollen tube (pt) emerging from a pollen grain (pg) and
634 penetrating a stigmatic cell (s). (B) close-up view depicted by the white square in A shows stigmatic
635 cuticle (SC) digestion underneath the pollen tube. Scale bar=2 μ m. (C) The progressing pollen tube
636 grows between two CW layers of the stigmatic cell (s), the inner layer (SCW.in) and the outer layer
637 (SCW.out), as indicated in the close-up view (D) depicted by the white square in C. Cell walls appear
638 as electron transparent white/light grey layers. (E) Transversal section of the pollen tube within the

639 stigmatic CW. (F) close-up view depicted by the white square in (E) shows the inner stigmatic I
640 surrounds the pollen tube. PCW, pollen CW. Scale bar=1 μ m. Each experiment was repeated at least
641 three times.

642

643 **Fig. 6.** Pathogen and pollen tubes apply different mechanical stresses onto the papilla. Arabidopsis
644 stigma expressing a GFP-tagged PM marker (Lti6b) were infected with *P. parasitica* (A) or pollinated
645 (B), and observed by CLSM at one hai or 30 map, respectively. To quantify the papilla deformation, a
646 red line was drawn on merged images (inset) between the two external points of the deformation.
647 Distances from the red line towards the cuticle (blue line, external deformation, extD) and towards
648 the cytoplasm (yellow line, internal deformation, intD) were determined. Scale bar=10 μ m on the full
649 images and 5 μ m on the insets. Sp, spore; pg, pollen grain. (C) Quantitative analysis of external (extD)
650 and internal (intD) papilla deformations upon infection or pollination, for 21 hyphae or 20 pollen
651 tubes. In the plots, the cross corresponds to the mean value. t-test; *** pVal<0,0005; n.s., not
652 significant.

653

654 **Fig. 7.** The pathogen and pollen tube trigger both similar and different subcellular rearrangements
655 upon penetration of stigmatic cells. (A) Stigmatic cells from fluorescent marker lines for the trans
656 Golgi network (TGN; TGN-VHAa1), the late endosome (LE/MVB; LE-FYVE), and the actin network
657 (Actin-Lifeact) were infected with *P. parasitica* or pollinated. CLSM at one hai or 30 map allowed to
658 visualize the papillae (green or yellow fluorescence) and the invader (bright field for *P. parasitica*, red
659 fluorescence for pollen). The papilla periphery was manually outlined on the obtained images (white
660 lines), and the Fiji macro automatically depicted two zones, (i) a contact zone (yellow circles)
661 including the invader entry point (red arrow/red circle), and (ii) a surrounding zone (green circles).
662 Fluorescence intensities (gray values) were automatically measured in each circle. Scale bar=10 μ m.
663 (B) Quantification of fluorescence intensity differences between zones (i) and (ii). For each
664 interaction, 15 stigmatic cells on at least four independent stigma were analysed. In the plots, the
665 cross corresponds to the mean value. Statistical analysis of fluorescence intensity was based on a
666 paired T-test. * pVal<0,05; ** pVal<0,005; *** pVal<0,000.5; n.s., not significant. Detailed
667 measurements are shown on supplementary Figures S3 to S6, S8 and S9.

668

669 **Fig. 8.** *P. parasitica* triggers reorganisation of subcellular components in root cells. Roots from
670 fluorescent marker lines for the trans Golgi network (TGN; VHAa1), the late endosome (LE/MVB;
671 2xFYVE), and the actin network (Actin; LifeAct) were infected with *P. parasitica* and analysed by
672 CLSM at one hai. (A) For the TGN and LE marker lines, fluorescence was quantified using the Fiji
673 macro as described in Figure 7 to determine fluorescence differences between contact zones and
674 surrounding zones (B) Quantification of fluorescence differences. For each interaction, 15 root cells
675 on at least 10 independent roots were analysed. In the plots, the cross corresponds to the mean
676 value. Statistical analysis of fluorescence intensity was based on a paired T-test; * pVal<0,05; ***
677 pVal<0,0005. Detailed measurements are shown on supplementary Figures S10 and S12. (C) The Fiji
678 macro was not applicable to quantify actin fluorescence, because the high concentration of actin
679 filaments at the cortical region (asterisk) distorted the quantification of fluorescence. We thus
680 visually determined actin focalisation when a fluorescence patch (red arrow, delimited by a red
681 dashed line) was clearly visible at the contact site with the penetrating hyphae (black arrow). Among
682 15 root cells on 12 independent roots we determined a frequency of 11/15 events of actin
683 focalisation at penetration sites (see Supplementary Figure S13). BF, bright field. Scale bar=10 μ m.

684

685