



**HAL**  
open science

## The penetration of sunflower root tissues by the parasitic plant *Orobanche cumana* is intracellular

Marie-Christine Auriac, Caitlin Griffiths, Alexandre Robin-Soriano, Alexandra Legendre, Marie-Claude Boniface, Stéphane Muños, Joëlle Fournier, Mireille Chabaud

### ► To cite this version:

Marie-Christine Auriac, Caitlin Griffiths, Alexandre Robin-Soriano, Alexandra Legendre, Marie-Claude Boniface, et al.. The penetration of sunflower root tissues by the parasitic plant *Orobanche cumana* is intracellular. *New Phytologist*, 2024, 241 (6), pp.2326-2332. 10.1111/nph.19495. hal-04377553

HAL Id: hal-04377553

<https://hal.inrae.fr/hal-04377553v1>

Submitted on 13 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.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 ***Title***

2 **The penetration of sunflower root tissues by the parasitic plant *Orobanche cumana* Wallr.**  
3 **is intracellular.**

4

5 Auriac MC, Griffiths C, Robin-Soriano A, Legendre A, Boniface MC, Muños S, Fournier J,  
6 and Chabaud M.

7

8

9 ***Key message***

10 Combination of *in vivo* confocal, large field and transmission electron microscopy approaches  
11 revealed how intimate the relationship between the parasitic plant broomrape (*Orobanche*  
12 *cumana* Wallr.) and its sunflower host (*Helianthus annuus* L.) is at very early stages of their  
13 interaction.

14

15

16 ***Key words***

17 Live-cell imaging, parasitic plant, *Orobanche cumana*, *Helianthus annuus* L., plant-plant  
18 interaction.

19

20

21

## 22 **Introduction**

23 Sunflower broomrape (*Orobanche cumana*) is one of the main pests for sunflower crops. This  
24 holo-parasitic plant, is specific to sunflower crops. Broomrape seeds perceive their host thanks  
25 to germination stimulants present in sunflower root exudates (Bouwmeester et al., 2021). Once  
26 germinated, the broomrape radicle grows toward the host root (Krupp et al. 2021) and develops  
27 papillae, which adhere to the host root and secrete mucilaginous compounds (Joel and Losner-  
28 Goshen, 1994). Subsequently, epidermal cells at the tip of the haustorium, a specific parasitic  
29 organ, differentiate into intrusive cells that penetrate the host root (Masumoto et al., 2021). This  
30 penetration combines physical pressure and degradation of sunflower root cell walls thanks to  
31 pectolytic activity enzymes released by the parasitic plant (Shomer-Ilan, 1993; Losner-Goshen  
32 et al., 1998). Intrusive cells make their way toward the host root vessels, crossing the successive  
33 host root tissues. Transcriptomic analyses showed that, in the case of a susceptible interaction,  
34 defense genes were activated only transiently and at a low level (Letousey et al., 2007; Dos  
35 Santos et al., 2003a, b). In addition, the expression of the putative defense suppressor gene *Par1*  
36 of various parasitic plants at the early stages of interaction (Yang et al., 2020; Qiu et al., 2022),  
37 suggests manipulation of their host by parasitic plants. Once in contact with the host xylem  
38 vessels, intrusive cells differentiate into vessel elements and vascular connections are  
39 established (xylem as well as phloem), to insure the nutrient supply of the parasite (Krupp et  
40 al., 2019). Although numerous studies have been performed on parasitic seed germination and  
41 haustorium development (Yoshida et al. 2016), most of them were focused on the parasitic  
42 plants, and the host cellular mechanisms involved during the intrusive cell development were  
43 poorly described (Mutuku et al., 2021). How the host cells behave during the massive expansion  
44 of the haustorium tissues across the outer root cell layers remained quite unknown. A few  
45 studies published in the 70-90s explored the host cellular reorganization during the early stages  
46 of the haustorium penetration of various *Orobanchaceae* parasitic plant species. It was shown  
47 that haustorium development is accompanied by unusual host cell proliferation (Kuijt 1977;  
48 Dörr and Kollmann, 1974). Whether the penetration is intra or intercellular in the root host was  
49 rarely stated. Dörr and Kollman (1974) and Kuijt (1977) mentioned intercellular growth only  
50 of the haustorial cells, with no observations of plasmodesmata interconnecting host and parasite  
51 cells for the interactions *O. crenata*/ *Vicia faba* and *O. ramosa*/ *Cannabis sativa*. Intercellular  
52 penetration between two cortical cells was shown during the interaction between *Striga*  
53 *gesnerioides* (another *Orobanchaceae* species) and cowpea (*Vigna unguiculata*; Reiss and  
54 Bailey, 1998). By contrast, the work by Dörr (1969) on the stem parasitic plant species *Cuscuta*

55 (*Convolvulaceae* family) on the host *Pelargonium zonale* revealed intracellular as well as  
56 intercellular penetrations preceding the vascular connection between the host and the parasite  
57 (Press et al., 1990). In addition, Musselmann and Dickinson (1975) showed an example of an  
58 intrusive cell of the parasitic plant *Agalinis aphylla* (*Orobanchaceae* family) penetrating  
59 intracellularly a cortical cell through a small opening in the cell wall. Thus, whether sunflower  
60 root penetration by the broomrape haustorium is intra and/or inter cellular remained an open  
61 question. This knowledge is required in the perspective of subsequently investigate and  
62 understand the sunflower cellular mechanisms associated with resistances to *O. cumana*. In this  
63 work, using an efficient selection of the early stages, and combining various microscopy  
64 approaches including live-cell imaging of transgenic fluorescent host tissues, we re-investigated  
65 the relationships between host and parasitic tissues at the cell level during the early stages of  
66 haustorium penetration. The questions we addressed were: (i) do intrusive cells penetrate the  
67 host root inter or intra-cellularly? (ii) do the sunflower root cells in the vicinity of the intrusive  
68 cells die or stay alive? (iii) are sunflower cell divisions induced at the very early stages of the  
69 penetration, and which are the root tissues involved?

#### 70 ***Early stage kinetics of the sunflower/ broomrape interaction in rhizotrons***

71 To answer these questions, we needed to observe attachments at very early stages, *i.e.*  
72 haustorium penetration sites sampled before the establishment of vessel connections. To this  
73 end, we used a dedicated growth and inoculation device called rhizotron, a plexiglass home-  
74 made box, which facilitates the observation of inoculated sunflower roots and selection of  
75 attachment sites (Le Ru et al., 2021, **Notes S1**). For large field and transmission electron  
76 microscopy (TEM) observation of stained longitudinal sections of attachments, we used root  
77 fragments from young inoculated wild-type sunflower plantlets (*i.e.* non-transformed). In  
78 addition, to get more information on the living status and the sub-cellular organization of the  
79 penetrated cells, we observed attachments using *in vivo* confocal imaging of living inoculated  
80 transgenic composite sunflower plants, *i.e.* obtained by *Agrobacterium rhizogenes*-mediated  
81 transformation. This method generated plants with fluorescent roots, expressing the Green  
82 Fluorescent Protein (GFP) targeted to the endoplasmic reticulum (ER) (**Fig. S1-S2; Table S1**).  
83 Observation of attachments was performed from 4 to 8 days after inoculation (dai) (**Table S2**).  
84 Broomrape rarely penetrated the host root before 6 dai, while most of the haustoria had reached  
85 the inner root tissues (inner cortex to the vessels) at 8 dai. The kinetics were very similar  
86 whatever the type of plants and microscopy approach. Interestingly, similarly to our

87 observations, Joel and Losner-Goshen (1994) observed the first stages of attachments at 5-7  
88 dai.

89 ***Broomrape enters into living sunflower root tissues intracellularly***

90 Germinated broomrape seeds developed papillae at the tip of the radicle when contacting the  
91 host root (**Fig. S2i**, Joel and Losner-Goshen, 1994). Mechanical pressure of the broomrape in  
92 contact with sunflower root epidermal cells led to cell wall deformation (**Fig. 1a-c**).  
93 Differentiated intrusive cells at the broomrape radicle tip were strongly stained by toluidine  
94 blue O. They displayed a very dense cytoplasm, a reduced vacuole and a large nucleus  
95 containing a darkly stained nucleolus, suggesting a high metabolic activity (**Fig. 1a, d, g, j**).  
96 Imaging early stages of broomrape penetration revealed that intrusive cells penetrated the  
97 epidermal layer as well as the successive outer cortical layers intracellularly (**Fig. 1d-i**). In our  
98 culture system, sunflower roots had 4 to 5 cortical cell layers between the epidermis and the  
99 endodermis (**Fig. S3**). Intracellular penetration of sunflower root cells was observed in all the  
100 analyzed penetration sites (21 sites for large field microscopy and 21 sites for confocal  
101 microscopy, **Table S2**). The use of the GFP-ER construct provided information about both the  
102 cytoplasmic organization and the nucleus position, thanks to the ER outline labelling the nuclear  
103 envelope (Genre et al. 2005). In many cases, the nucleus of the penetrated cell was strikingly  
104 positioned close to the intrusive cells (**Fig. 1b, c, h, i**). The nucleus re-positioning close to the  
105 intruder, is reminiscent of the cellular reorganization of plant cells during bacterial and fungal  
106 symbiotic or pathogenic interactions (Fournier et al., 2008; Genre et al., 2005, 2008; Genre et  
107 al., 2009). It suggests that the host nucleus perceives the intrusive cell, either through the  
108 exerted mechanical pressure (Genre et al. 2009) and/or through unknown chemical signals.  
109 However, in contrast to root penetration by symbiotic (Genre et al., 2005; 2008) or pathogenic  
110 biotrophic fungi (Koh et al., 2005; Kankanala et al., 2007; Genre et al., 2009), no cytoplasmic  
111 aggregation, nor specific ER re-organization were observed ahead of the penetration process.  
112 Interestingly, ER was surrounding the broomrape intrusive cells (**Fig. 1e, f, h, i, k, l**), showing  
113 active, though not massive, host intracellular re-organization along with the penetration  
114 process. These results suggested active membrane synthesis around intrusive cells requiring  
115 nucleus and ER activity in the host cell, and showed that the sunflower penetrated cells  
116 remained alive. Deeper root tissues (endodermis and pericycle) were also penetrated  
117 intracellularly by intrusive cells (**Fig. 1j**). In most cases, haustoria penetrated the host root with  
118 minimal host cell damage. However, the live-cell imaging approach revealed a few cases of cell  
119 death as shown by the absence of fluorescence (2 sites, **Fig. S4a, b**), or a severe ER disruption

120 (1 site, **Fig. S4c, d**). Similarly, change of the vacuole structure was observed using large field  
121 microscopy for a few sites (7 sites among 21 penetration sites): appearing as a blue smear (**Fig.**  
122 **1d; Fig. S4e**) or light blue material filling the cell (**Fig. S4e**). One or a few penetrated cells only  
123 were affected, adjacent to the intrusive cells in outer root tissues. These results suggested that  
124 in some cases, penetration of the intrusive cells got out of control and synchronization of the  
125 penetration process and the sunflower cellular re-organization failed, leading to sunflower cell  
126 death. This phenomenon remained cell-autonomous, without other defense reactions in the  
127 surrounding or the deepest root tissues. Furthermore, penetration could result in the separation  
128 of the host nucleus from the distal part of the penetrated cell, probably leading to cell death as  
129 well. Strikingly, broomrape intrusion was thicker in outer root tissues (**Fig. 1**) than in inner root  
130 tissues, in which only single elongated and separated intrusive cells were detected (**Fig. 1e, f,**  
131 **h, i, k, l**). Similarly, Dörr et al. (1969) reported intracellular “searching hyphae” for the *Cuscuta*  
132 stem parasite.

### 133 *Divisions are induced at very early stages*

134 Sunflower roots were known to swell locally at the site of broomrape attachment by means of  
135 cell division (Kuijt, 1977) and as early as 7 dai (Dörr and Kollamn, 1994). In the present study,  
136 sunflower root cell divisions were observed as early as 6 dai, close to attachments (respectively  
137 9 and 7 sites for sections and live-cell imaging). Divisions were mostly anticlinal in the cortex,  
138 and periclinal in the pericycle (**Fig. S5; Fig. 1d, e, h, i**). The number of dividing root cell layers  
139 and the length of the dividing zone were highly variable (for example from 1 cortical cell to  
140 more than 30 cells in a row). These divisions may account for root hypertrophy that was  
141 previously observed at the site of 14-dai attachments in rhizotrons (Chabaud et al., 2022). These  
142 divisions could be induced indirectly (host hormonal regulation) or directly by the parasitic  
143 plant (hormonal release: such as auxin [Ishida et al., 2016] or cytokinin [Spallek et al., 2017]).  
144 Whether germinated broomrape seed exudates would be sufficient for the induction of host cell  
145 divisions remains an open question.

### 146 *Intrusive cells penetrate a new apoplastic compartment*

147 The interface between intrusive cells and the sunflower penetrated root cells at early stages of  
148 the interaction was further characterized by TEM (**Fig. 2**). A 7 dai attachment with the  
149 haustorium reaching the 3<sup>rd</sup> cortical cell layer is illustrated **Fig. 2a, b**. Starch grains, a sign of  
150 the transition from the autonomous (germination stage) to the parasitical stage (Joel and Losner-  
151 Goshen, 1993), were present in the central part of the attachment (**Fig. 2a, c**). In the outer root  
152 cell layers, the interface appears as a thick layer surrounding broomrape (**Fig. 2b**), as already

153 described for *Striga* (Reiss and Bailey, 1998; Neuman et al., 1999). The intrusive cells were  
154 easily distinguished from sunflower root cortical cells thanks to their dense cytoplasm,  
155 containing Golgi stacks, large mitochondria as well as a reduced vacuole (**Fig. 2b, e, f**) as  
156 already reported by Kuijt and Toth (1976) and Kuijt (1977). By contrast, the host cortical cells  
157 whether penetrated or not, contained a large vacuole with a thin layer of surrounding cytoplasm  
158 (**Fig. 2b, d**). Mitochondria in the penetrated host cells were present all along the host  
159 plasmalemma, suggesting intense activity at the periphery of the host cell such as membrane  
160 biosynthesis (**Fig. 2d**). This dense cytoplasm confirmed that the penetrated host cells were alive  
161 at this stage. The parasitic cell wall was present all around the intrusive cells. By contrast, the  
162 presence of a host matrix along the anticlinal interface of the host penetrated cell was not always  
163 detectable and its appearance varied along the length of the haustorium. On the outermost  
164 anticlinal side of the host penetrated cell, the host interface with the haustorium appeared as a  
165 dark thick layer, in continuity with the existing periclinal host cell wall (**Fig. 2b, d**). It could  
166 partly result from the invagination of the existing periclinal host cell wall pushed in by the  
167 penetrating intrusive cells. The discontinuity of the staining suggests disorganization of this  
168 host cell wall/ matrix. On the innermost side of the cell, the host matrix was either too thin to  
169 be visible (**Fig. 2e, f**) or appeared as a low-density material (stars in **Fig 2. g, h**) separating the  
170 host cell plasmalemma from the parasitic cell wall, and differing from the existing darker  
171 periclinal host cell wall. The use of various fluorescent dyes to distinguish host cell wall from  
172 newly-made matrix would be interesting, as done for the symbiotic nitrogen-fixing bacterial  
173 infection thread (Rae et al., 2021). At the frontline of the haustorium penetration the existing  
174 periclinal host cell wall seemed also disorganized (**Fig. 2i**), suggesting progressive local  
175 enzymatic degradation of the host cell wall. This apparent dissolution of the nearby host cell  
176 walls (Kuijt, 1977) or a partial digestion of the cell wall at the interface (Kurotani et al., 2020,  
177 in the case of the interaction *Ptheirospermum japonicum/ Arabidopsis thaliana*) had been  
178 reported previously. While cell-wall degrading enzymes which might contribute to this process  
179 have been identified from the parasite (Shomer-Ilan et al., 1993; Loshen-Gosner et al., 1998),  
180 there is no evidence at the moment of the direct involvement of host enzyme activities involved  
181 in host cell wall degradation in this context (Mitsumatsu et al., 2015; Yang et al., 2020).  
182 Nevertheless, the host cell plasmalemma seemed to remain undisturbed and continuous (**Fig.**  
183 **2g; Fig. S6b**). Both host and parasitic plasma membranes were highly convoluted at the front  
184 line of the haustorium (**Fig. S6b**), suggesting membrane synthesis for the haustorium  
185 accommodation (host) and haustorium expansion (parasite). No plasmodesmata were observed  
186 on the interface at these early stages, indicating that molecular exchanges between the parasite

187 and the host happened at later stages, or through vessel connections, as interspecific  
188 plasmodesmata have been shown in the phloem (Krupp et al., 2019). In some cases, the  
189 penetration led to disaggregation of the vacuole of the host cell (**Fig. S6c-d**), with disruption  
190 of the host cell plasmalemma, leading to cell necrosis, as for *Striga* (Neuman et al., 1999).  
191 However as mentioned above this was not very common and remained cell autonomous. In  
192 addition, no evidence of cell death was observed at the later stages (Chabaud et al., 2022).  
193 Altogether, these results showed that the parasitic intrusive cells penetrate the host root cells  
194 intracellularly, as a result of degradation of the host cell wall and formation of a new host trans-  
195 cellular apoplastic compartment for haustorium accommodation.

### 196 ***Concluding remarks***

197 Most striking among our findings has been the observation of intracellular haustorium  
198 penetration of host root tissues, in contrast to most studies on *Orobanchaceae*. These studies  
199 relied mainly on the observation of transverse sections, by contrast to the longitudinal sections  
200 used in this work, which made it easier to distinguish between intra and intercellular processes.  
201 Our work showed the intimate broomrape penetration into its host, through the formation of a  
202 new apoplastic compartment. It suggested that although host cell wall integrity has been  
203 damaged by parasitic cell wall degrading enzymes (Shomer-Ilan et al., 1993; Losner-Goshen et  
204 al., 1998), only minor defense reactions were induced as previously reported for biotrophic  
205 pathogenic fungi (Mendgen and Hahn, 2002; Bellincampi et al., 2014). In that respect, genes  
206 encoding inhibitors of cell wall degrading enzymes could be good candidates for increasing  
207 resistance to broomrape. In addition, as *HaOr7* (Duriez et al. 2019) and *HaOr<sub>Deb2</sub>* (Fernandez-  
208 Aparicio et al., 2022) encode Leucine-Rich-Repeat Receptors Like Proteins, providing  
209 resistance to various *O. cumana* races, it would be of outstanding interest to characterize the  
210 cellular processes involved in these incompatible interactions. Comparing the cellular processes  
211 for various *O. cumana* races could highlight common or different mechanisms. Finally, using  
212 these approaches on other major parasitic plant species such as *Striga* will be of great interest  
213 for future resistance development in a larger host range.

214

215

### 216 ***Acknowledgments***

217 We thank P. Gresshoff (University of Queensland, Australia) for the *A. rhizogenes* strain K599.  
218 This study was supported by the “Laboratoires d’Excellences (LABEX)” “Towards a Unified  
219 theory of biotic interactions: roLe of environmental Pertubations” (TULIP; ANR-10-LABX-



220 41)” and/or by the “École Universitaire de Recherche (EUR)” TULIP-GS (ANR-18-EURE-  
221 0019). Slide scanning was performed using the Nanozoomer from the Imagery Platform of the  
222 Federated Research Institute AgroBiosciences-Interactions-Biodiversité (FRAIB; Castanet-  
223 Tolosan, France). This study was performed in the frame of a 2-year project (SunOrCell),  
224 funded by “Promosol/ Seleopro” (the association of French Sunflower and Rapeseed Breeders  
225 for promoting these crops). The International Consortium of Sunflower Genomics (ICSG)  
226 supported the grants for the training students CG and ARS.

227

### 228 *Competing interests*

229 None.

230

### 231 *Authors and contribution*

232 MCA performed cytological experiments (large field microscopy and TEM). CG, ARS and AL  
233 established sunflower transformation experiments. MCB produced sunflower and broomrape  
234 resources. SM: coordinated the ICSG, assisted with the construction of the project and the  
235 writing of the manuscript. JF gave technical and scientific advice, assisted with the construction  
236 and the writing of the manuscript. MC designed the experiments, carried out confocal  
237 microscopy and cytology experiments, wrote the manuscript.

238

### 239 *ORCID*

240 Mireille Chabaud: <https://orcid.org/0000-0002-7786-6895>

241 Joëlle Fournier : <https://orcid.org/0000-0003-3746-8080>

242 Stéphane Muños: <https://orcid.org/0000-0003-0261-2759>

243

### 244 *Data availability statement*

245 The original contributions presented in the study are included in the article/Supplementary  
246 Material. Further inquiries can be directed to the corresponding author.

247

248 **References**

- 249 **Bellincampi D, Cervone F, Lionetti V. 2014.** Plant cell wall dynamics and wall-related susceptibility in plant-  
250 pathogen interactions. *Frontiers in Plant Science* **5**: 228.
- 251 **Bouwmeester H, Li C, Thiombiano B, Rahmini M, Dong L. 2021.** Adaptation of the parasitic plant lifecycle:  
252 germination is controlled by essential host signaling molecules. *Plant Physiology* **185**: 1292-1308.
- 253 **Chabaud M, Auriac M-C, Boniface M-C, Delgrange S, Folletti T, Jardinaud M-F, Legendre A, Perez-Vich  
254 B, Pouvreau J-B, Velasco L, Delavault P, Muños S. 2022.** Wild *Helianthus* species: A reservoir of  
255 resistance genes for sustainable pyramidal resistance to broomrape in sunflower. *Frontiers in Plant  
256 Science* **13**.
- 257 **Dörr I. 1969.** Feinstruktur intrazellulär wachsender *Cuscuta*-Hyphen? *Protoplasma* **67**: 123-137.
- 258 **Dörr I, Kollmann R. 1974.** Strukturelle Grundlage des Parasitismus bei *Orobanch* I. Wachstum der  
259 Haustorialzellen im Wirtsgewebe. *Protoplasma* **80**: 245-279.
- 260 **Dos Santos CV, Delavault P, Letousey P, Thalouarn P. 2003a.** Identification by suppression subtractive  
261 hybridization and expression analysis of *Arabidopsis thaliana* putative defence genes during *Orobanch*  
262 *ramosa* infection *Physiology Molecular Plant Pathology* **62**: 297-303.
- 263 **Dos Santos CV, Letousey P, Delavault P, Thalouarn P. 2003b.** Defense Gene Expression Analysis of  
264 *Arabidopsis thaliana* Parasitized by *Orobanch* *ramosa*. *Phytopathology* **93**: 451-457.
- 265 **Duriez P, Vautrin S, Auriac MC, Bazerque J, Boniface MC, Callot C, Cauet S, Chabaud M, Gentoux F,  
266 Lopez-Sendon M et al. 2019.** A receptor-like kinase enhances sunflower resistance to *Orobanch*  
267 *cumana*. *Nature Plants* **5**: 1211-1215.
- 268 **Fernandez-Aparicio M, del Moral L, Muños S, Velasco L, Pérez-Vich B. 2022.** Genetic and physiological  
269 characterization of sunflower resistance provided by the wild-derived *OrDeb2* gene against highly virulent  
270 races of *Orobanch* *cumana* Wallr. *Theoretical Applied Genetics* **135** : 501-525
- 271 **Genre A, Chabaud M, Timmers ACJ, Bonfante P, Barker DG. 2005.** Arbuscular mycorrhizal fungi elicit a  
272 novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell* **17**:  
273 3489-3499.
- 274 **Genre A, Chabaud M, Faccio A, Barker DG, Bonfante P. 2008.** Prepenetration apparatus assembly precedes  
275 and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of  
276 both *Medicago truncatula* and *Daucus carota*. *Plant Cell* **20**: 1407-1420.
- 277 **Genre A, Ortu G, Bertoldo C, Martino E, Bonfante P. 2009.** Biotic and abiotic stimulation of root epidermal  
278 cells reveals common and specific responses to arbuscular mycorrhizal fungi. *Plant Physiology* **149**:  
279 1424-1434.
- 280 **Ishida JK, Wakatake T, Yoshida S, Takebayashi Y, Kashara H, Wafula E, dePamphilis CW, Namba S,  
281 Shirasu K. 2016.** Local auxin biosynthesis mediated by a YUCCA flavin monooxygenase regulates  
282 haustorium development in the parasitic plant *Phtheirospermum japonicum*. *Plant Cell* **28**: 1795-1814.
- 283 **Joel DM, Losner-Goshen D. 1994.** The attachment organ of the parasitic angiosperms *Orobanch* *cumana* and  
284 *O. aegyptiaca* and its development. *Canadian Journal Botany* **72**: 564-574.
- 285 **Kankanala P, Czymmek K, Valent B. 2007.** Roles for rice membrane dynamics and plasmodesmata during  
286 biotrophic invasion by the blast fungus. *Plant Cell*. **19**: 706-724.
- 287 **Koh S, Andrea A, Edwards H, Ehrhardt D, Somerville S. 2005.** *Arabidopsis thaliana* subcellular responses to  
288 compatible *Erysiphe cichoracearum* infections. *Plant Journal* **44**:516-529.
- 289 **Krupp A, Heller A, Spring O. 2019.** Development of phloem connexion between the parasitic plant *Orobanch*  
290 *cumana* and its host sunflower. *Protoplasma* **256**: 1385-1397.
- 291 **Krupp A, Bertsch B, Spring O. 2021.** Costunolide Influences Germ Tube Orientation in Sunflower Broomrape  
292 - A First Step Toward Understanding Chemotropism. *Frontiers in Plant Science* **12**.
- 293 **Kuijt J. 1977.** Haustoria of phanerogamic parasites. *Annual Review Phytopathology* **17**: 91-118.
- 294 **Kuijt J, Toth R. 1976.** Ultrastructure of angiosperm haustoria-A review. *Annals Botany* **40**: 1121-30.

- 295 **Kurotani KI, Wakatake T, Ichihashi Y, Okayasu K, Sawai Y, Ogawa S, Cui S, Suzuki T, Shirasu K,**  
 296 **notaguchi M. 2020.** Host-parasite tissue adhesion by a secreted type of beta-1,4-glucanase in  
 297 the parasitic plant *Phtheirospermum japonicum*. *Communications Biology* **3**.
- 298 **Le Ru A, Ibarcq G, Boniface MC, Baussart A, Munos S, Chabaud M. 2021.** Image analysis for the automatic  
 299 phenotyping of *Orobanche cumana* tubercles on sunflower roots. *Plant Methods* **17**.
- 300 **Letousey P, de Zelicourt A, Dos Santos CV, Thoiron S, Monteau F, Simier P, Thalouarn P, Delavault P.**  
 301 **2007.** Molecular analysis of resistance mechanisms to *Orobanche cumana* in sunflower. *Plant*  
 302 *Pathology* **56**: 536-546.
- 303 **Losner-Goshen D, Protnoy VH, Mayer AM, Joel DM. 1998.** Pectolytic Activity by the Haustorium of the  
 304 Parasitic Plant *Orobanche L.*(Orobanchaceae) in Host Roots. *Annals Botany* **81**: 319-326.
- 305 **Masumoto N, Suzuki Y, Cui S, Wakazaki M, Sato M, Kumaishi K, Shibata A, Furuta K, Ichihashi Y,**  
 306 **Shirasu K et al. 2021.** Three-dimensional reconstructions of haustoria in two parasitic plant species in  
 307 the Orobanchaceae. *Plant Physiology* **185**: 1429–1442.
- 308 **Mendgen K, Hahn M. 2002.** Plant infection and the establishment of fungal biotrophy. *Trends in Plant Science*  
 309 **7**: 352-356.
- 310 **Mitsumasu K, Seto Y, Yoshida S. 2015.** Apoplastic interactions between plants and plant-root intruders. *Frontiers*  
 311 *in Plant Science* **6**: 617.
- 312 **Mutuku JM, Cui S, Yoshida S, Shirasu K. 2021.** Orobanchaceae parasite–host interactions. *New Phytologist*  
 313 **230**: 46–59.
- 314 **Musselman LJ, Dickison WC. 1975.** The structure and development of the haustorium in parasitic  
 315 Scrophulariaceae. *Botanical Journal Linn. Society* **70**: 183-212.
- 316 **Neumann U, vian B, weber HC, Sallé G. 1999.** Interface between haustoria of parasitic members of the  
 317 Scrophulariaceae and their hosts: a histochemical and immunocytochemical approach. *Protoplasma* **207**:  
 318 84-97.
- 319 **Press MC, Gravest JD, Stewart GR. 1990.** Physiology of the interaction of angiosperm parasites and their higher  
 320 plant hosts. *Plant Cell and Environment* **13**: 91-104.
- 321 **Qiu S, Bradley JM, Zhang P, Chaudhuri R, Blaxter M, Butlin RK, Scholes JD. 2022.** Genome-enabled  
 322 discovery of candidate virulence loci in *Striga hermonthica*, a devastating parasite of African cereal crops.  
 323 *New Phytologist* **236**: 622–638.
- 324 **Rae AE, Rolland V, White RG, Mathesius U. 2021.** New methods for confocal imaging of infection threads in  
 325 crop and model legumes. *Plant Methods* **17**: 24.
- 326 **Reiss GC, Bailey JA. 1998.** *Striga genesrioides* parasiting cowpea: development of infection structures and  
 327 mechanisms of penetration. *Annals Botant* **81**: 431-440.
- 328 **Shomer-Ilan A. 1993.** Germinating seeds of the root parasite *Orobanche aegyptiaca* Pers. excrete enzymes with  
 329 carbohydrase activity. *Symbiosis* **15**: 61-70.
- 330 **Spallek T, Melnyk CW, Wakatake T, Zhang J, Sakamoto Y, Kiba T, Yoshida S, Matsunaga S, Sakakibara**  
 331 **H, Shirasu K. 2017.** Interspecies hormonal control of host root morphology by parasitic plants.  
 332 *Proceedings National Academy Science USA* **114**: 5283–5288.
- 333 **Yang C, Fu F, Zhang N, Wang J, Hu L, Islam F, Bai Q, Yun X, Zhou W. 2020.** Transcriptional profiling of  
 334 underground interaction of two contrasting sunflower cultivars with the root parasitic weed *Orobanche*  
 335 *cumana*. *Plant Soil* **450**: 303–321.
- 336 **Yoshida S, Cui S, Ichihashi Y, Shirasu K. 2016.** The Haustorium, a Specialized Invasive Organ in Parasitic  
 337 Plants. *Annual Review Plant Biology* **67**:643–67.
- 338

339 *Legends*

340 **Fig.1. Broomrape haustorium penetrates the sunflower root tissues intracellularly.**

341 Broomrape attachments (6-7 dai) were observed at the cellular level, using 2 approaches: (i)  
342 large field microscopy of thin sections of resin-embedded and toluidine blue O-stained  
343 attachments (right column, **a, d, g, j**); (ii) confocal microscopy of broomrape-inoculated  
344 transgenic fluorescent sunflower roots expressing the GFP targeted to the endoplasmic  
345 reticulum (ER) (middle and left columns, **b, c, e, f, h, i, k, l**). Confocal microscopy images show  
346 fluorescence alone (combined GFP fluorescence and auto-fluorescence channels, middle  
347 column, **b-e-h-k**) or fluorescence with the corresponding bright field image (left column, **c, f,**  
348 **i, l**).

349 **a-c.** Deformation of the epidermal cell wall (arrow) due to the mechanical pressure exerted by  
350 the haustorium. Re-positioning of the nuclei of the deformed epidermal cells in the vicinity of  
351 the haustorium tip (**b.** white arrowheads). **d-i** and **k-l.** Intrusive cells penetrated the root  
352 epidermal and cortical layers intracellularly. The penetrated cortical cells had wavy (**d.** black  
353 double arrowheads) or deformed (**g.** black arrow) cell wall in contact with the haustorium. ER  
354 surrounded the intrusive haustorium tip (**h, k.** white double arrowheads). Nuclei were  
355 positioned close to the intrusive cells (**h.** white arrowheads). **j.** Intrusive cells reached the host  
356 xylem vessels (xylem: black cross), and crossed intracellularly the deepest cortical cell layer as  
357 well as the endodermis and the pericycle. Divisions in the cortex (mostly anticlinal, squares)  
358 and the pericycle (mostly periclinal, double squares) in the vicinity of the haustorium (**d, e, j**).  
359 **c, f, i, l.** White dots outline the broomrape haustorium visible on the bright field image. **b-c, e-f**  
360 **, k-l** are z axis projections of serial optical sections.

361 Scale bar = 50  $\mu\text{m}$  (**a, b, c, d, e, f, g, j**); 20  $\mu\text{m}$  (**h, i, k, l**).

362

363 **Fig.2. Creation of a new apoplastic compartment for haustorium accommodation.**

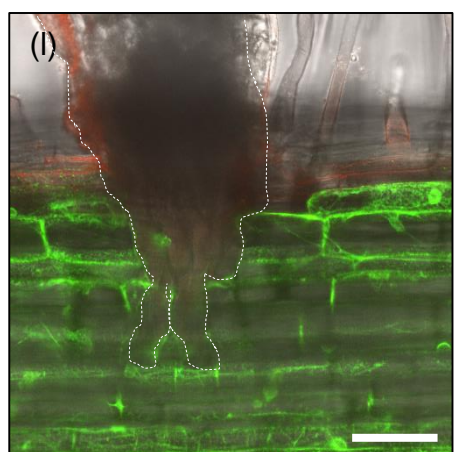
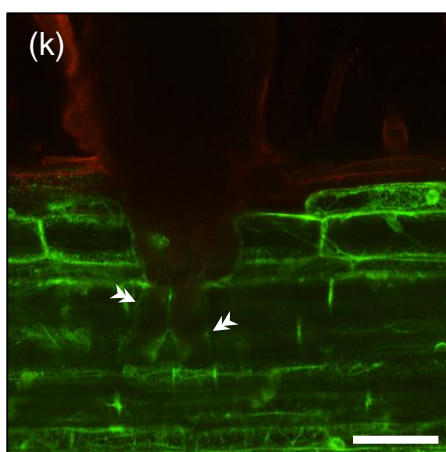
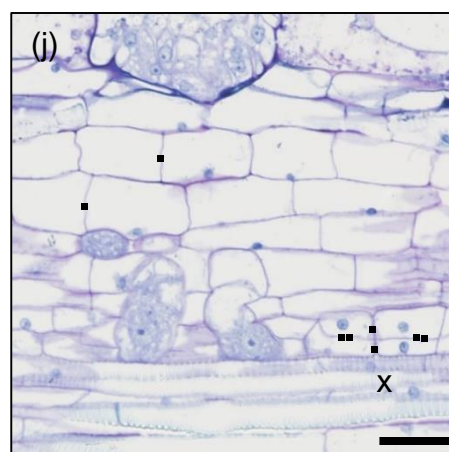
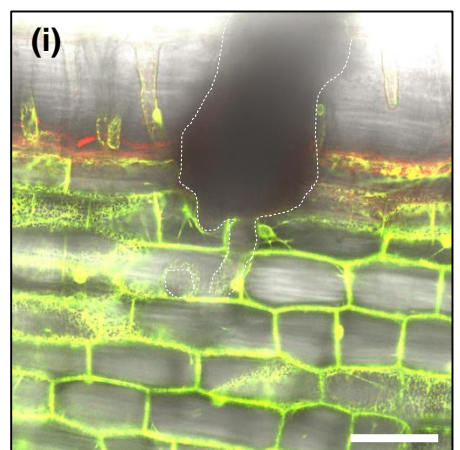
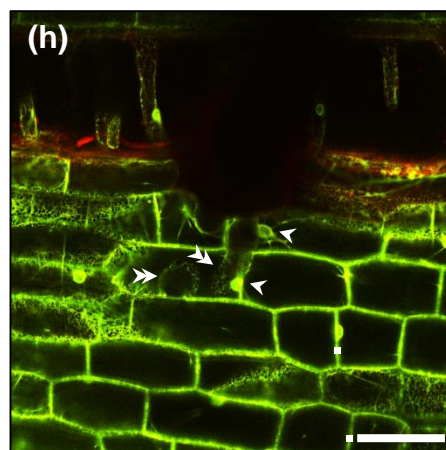
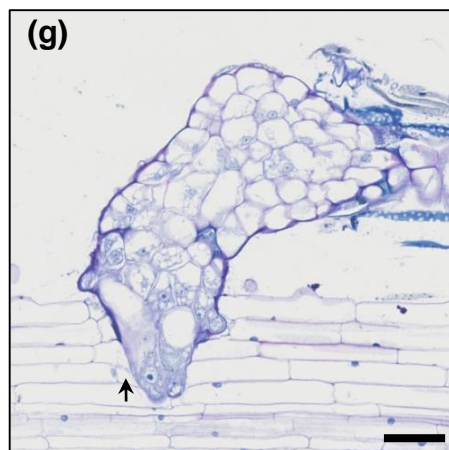
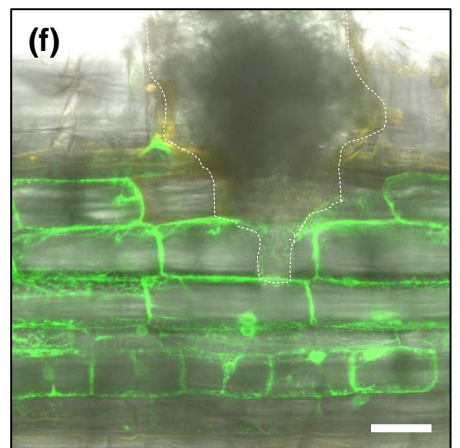
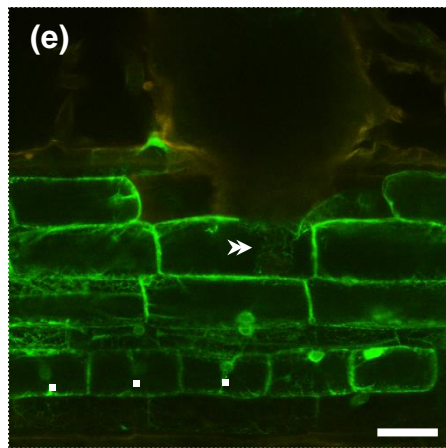
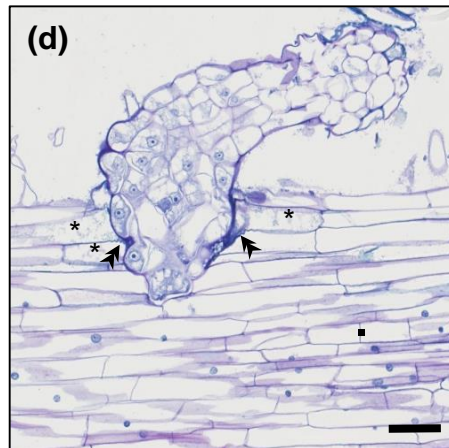
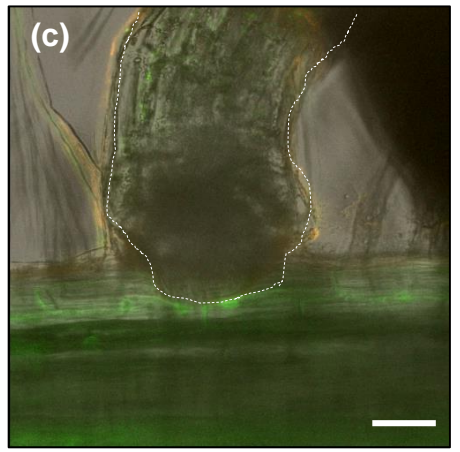
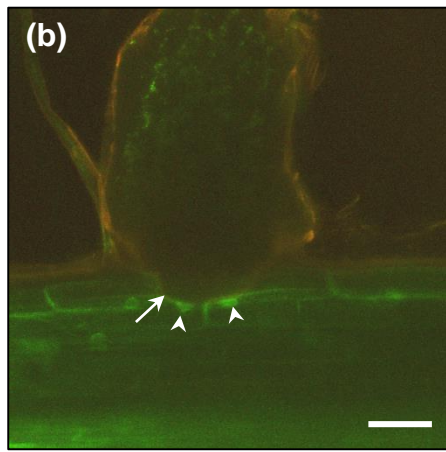
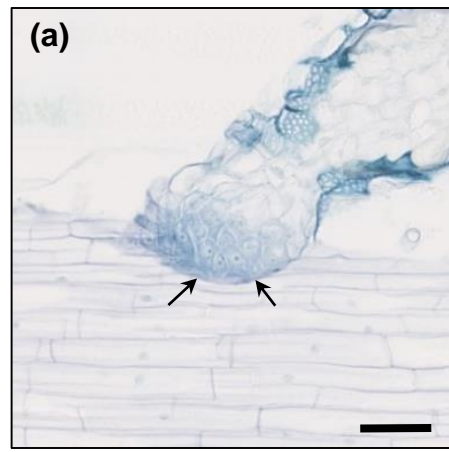
364 A 7 dai attachment, with intrusive cells reaching the 3<sup>rd</sup> cortical cell layer was imaged. **a.**  
365 General view of the attachment using large field microscopy. **b-i.** Transmission electron  
366 microscopy. IC intrusive cell, HC host cell.

367 **a, b.** Intrusive cells intracellular penetration in the host outer cortex cell. A thick stained layer  
368 surrounded the penetrating intrusive cells in outer host tissues (epidermis and outer cortex;  
369 black arrows in **a**, red arrows in **b**). **c.** Magnification of the central part of the broomrape, with  
370 starch grain-containing cells. **d.** In the outermost side of the anticlinal interface between the  
371 intrusive cell and the penetrated host outer cortical cell, disorganization of the host cell

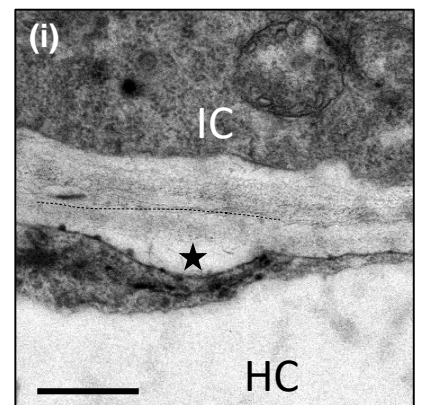
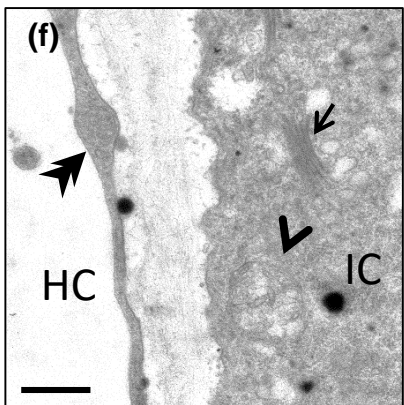
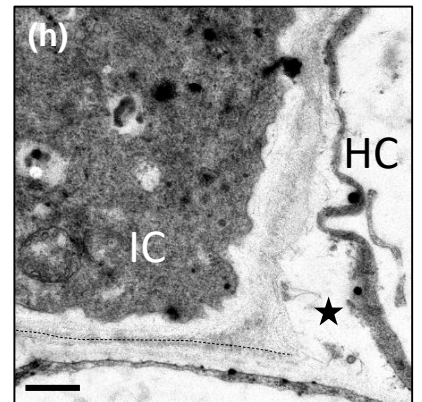
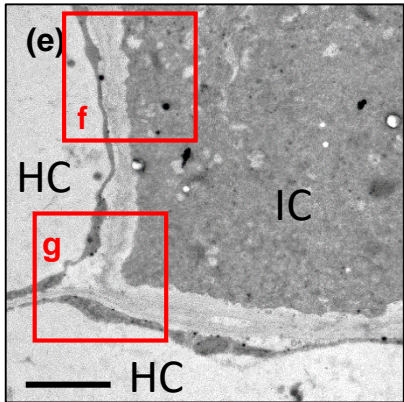
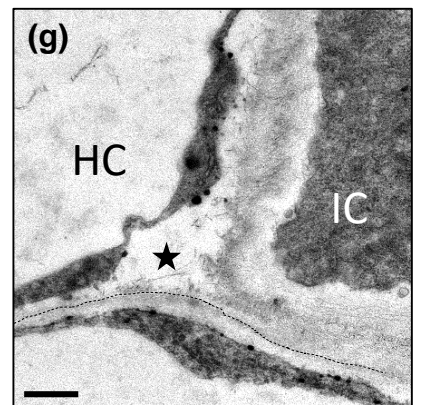
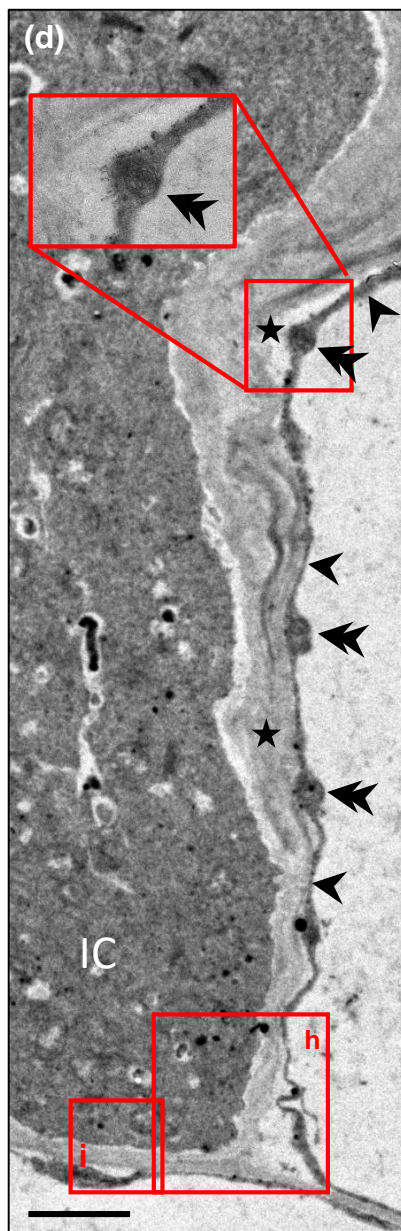
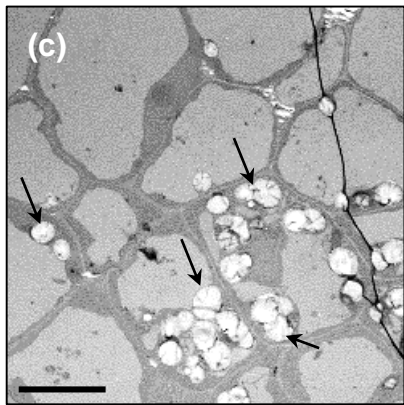
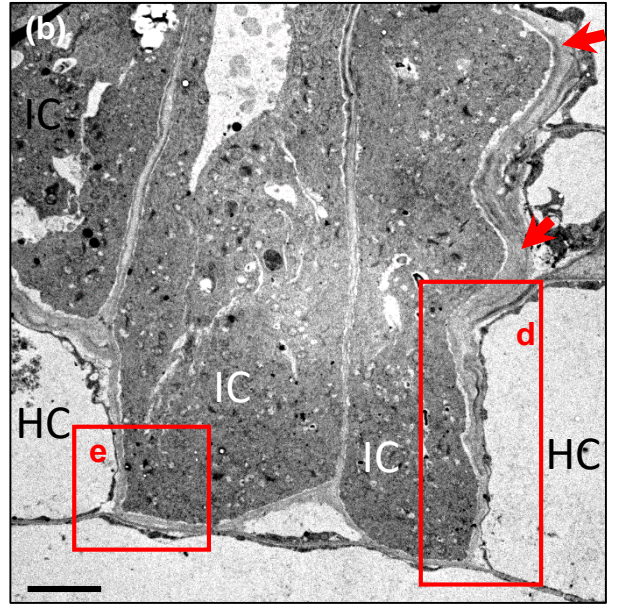
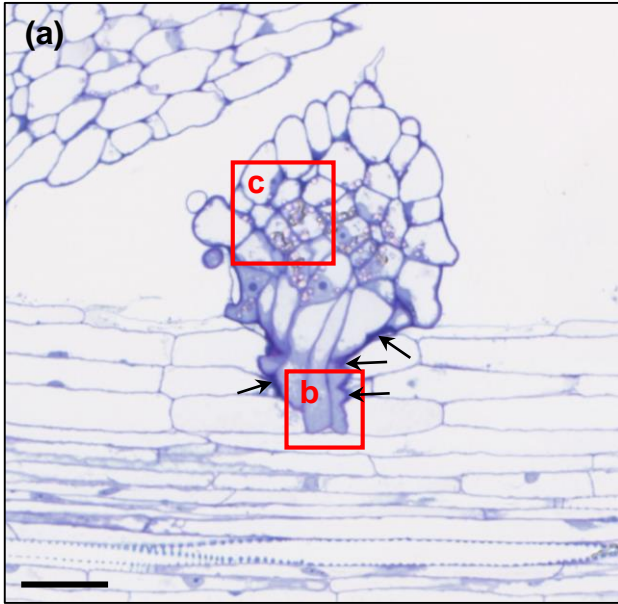
372 wall/matrix (star). Continuous thin layer of cytoplasm in the host cortical cell (black  
373 arrowheads), containing numerous mitochondria (double arrowheads). **e, f.** Presence of the  
374 parasitic cell wall but no visible host cell matrix in the outermost anticlinal side of the penetrated  
375 cell. **e.** Magnification of **b.** **f.** Magnification of **e.** **f.** The intrusive cell cytoplasm contains Golgi  
376 (thin arrow) and mitochondria (arrowhead). Mitochondria are also present in the thin host  
377 cytoplasm layer (double arrowhead). **g-h.** At the tip of the haustorium, presence of a host matrix  
378 (star) with fibrillae fragments. Convolution of the plasmalemma of the host cortical cells  
379 adjacent to the intrusive cells. **i.** At the front line of the intrusive cell tips (periclinal interface),  
380 local disorganization of the host cell wall (star), the plasmalemma of the host cell remaining  
381 intact and continuous. The dotted line shows the separation between the parasitic and host cell  
382 walls (**g-i**).

383 Scale bar = 50  $\mu\text{m}$  (**a**); 5  $\mu\text{m}$  (**b**); 10  $\mu\text{m}$  (**c**); 2  $\mu\text{m}$  (**d-e**); 0.5  $\mu\text{m}$  (**f, g, h, i**).









1 **Notes S1. Details of the materials and methods.**

2

3 ***Plant genotypes, bacterial strains, and constructs***

4 We used the susceptible cultivated *H. annuus* L., XRQ (Chabaud et al., 2022; Badouin et al.,  
5 2017) as the sunflower host for experiments in rhizotrons and for root transformation  
6 experiments. The *O. cumana* Wallr. population used in this study was the French race E-BOU  
7 of *O. cumana*, with a virulence level classified between E and F, and harvested in 2017 in  
8 Bourret (Tarn et Garonne, France; reference: LIPM-20734).

9 The *Agrobacterium rhizogenes* strain K599 (Savka et al. 1990) was used for sunflower root  
10 transformation. The binary vector used was derived from pBIN19 (Bevan, 1984) and carried  
11 the construct p35S-GFP-ER coding for the Cauliflower Mosaic Virus 35S promoter (p35S)  
12 driving the constitutive expression of the Green Fluorescent Protein targeted to the endoplasmic  
13 reticulum due to the presence of ER-targeting sequences (N-terminal signal peptide) and ER-  
14 retention (C-terminal tetra-peptide HDEL; Haseloff et al. 1997). Bacteria were grown on LB  
15 medium supplemented with Streptomycin 100 mg/l (bacterial selection) and kanamycin 50 mg/l  
16 (plasmid selection)

17

18 ***Cultivation of sunflower plants in rhizotrons***

19 Two types of sunflower plants were used: wild-type (non-transformed, non-fluorescent)  
20 sunflower plantlets for large field and TEM microscopy, and transgenic composite plants  
21 (expressing the fluorescent marker in their roots only) for confocal microscopy.

22 **Wild-type (*i.e.* non-transformed) sunflower plants**

23 Sunflower seeds of wild-type plants were surface sterilized for 10 min in a 4.8 % sodium  
24 hypochlorite solution, rinsed 3 times and sown in a 1/1 v/v mixture of sand/ vermiculite in  
25 alveoli. Seedlings were grown at 22 °C, 60% humidity, 16 h light 118  $\mu\text{E}/\text{m}^2/\text{s}$  for 7 days.  
26 Rhizotrons, 12x12 cm home-made plexiglass boxes were assembled as described in Le Ru et  
27 al. (2021), with water-soaked sterile rock-wool, a sterile glass fiber paper and a 7-day old  
28 sunflower plantlet. Rhizotrons were then watered with a ½ Long Ashton solution (Hewitt, 1966)  
29 containing 370  $\mu\text{M}$  phosphate.

30 **Transgenic composite sunflower plants**

31 To transform sunflower roots and obtain composite plants (with a non-transformed aerial part  
32 and transgenic roots), we used a modified version of the protocol from Parks and Yordanov  
33 (2020, **Fig. S1-S2**). Sunflower seeds were decontaminated and grown in alveolus-trays as above  
34 for 10 days. Transformation of plantlets was performed in soaked pre-cut rock-wool cubes



35 (reference ALR02G from GRODAN) with bacterial solution [final OD= 0.25 in ¼ (MS +  
36 Gamborg vitamins B5)], in Magenta boxes. After 3 days, Magenta boxes were slightly opened.  
37 Six days after transformation, plantlets were transferred to hydroponics, using sterile 1000µl  
38 cone boxes, as described in Morel et al. (2018) for another 6 days of culture in ¼ MS liquid  
39 medium (without vitamins; Sigma reference MS 5524). Culture in hydroponics, a new step  
40 compared to Parks and Yordanov's protocol (2020) was very beneficial to newly developed  
41 root growth. Finally, 14 to 18 days following transformation (6-7 days in rock-wool cubes and  
42 7-12 days in hydroponics; **Fig. S1**), transformation efficiency was recorded by measuring the  
43 percentage of plants with fluorescent roots and the number of fluorescent roots/ transformed  
44 plant (**Table S1**). Fluorescence expression was observed by epifluorescence microscopy using  
45 a stereomicroscope (Axiozoom V16; Zeiss), equipped with a GFP Long Pass filter (excitation  
46 485/12 nm and emission from 515 LP). Composite plants were transferred to rhizotrons as for  
47 non-transformed plantlets (see above).

48 Four experiments were performed. In the first experiment, 17-day-old plantlets were used for  
49 transformation, and the transfer to rhizotrons was done 18 days later, resulting in large 35-day-  
50 old plants that were difficult to handle under the confocal microscope. Hence the length of  
51 culture was progressively reduced in the following experiments to 10-day-old plantlets for  
52 transformation, culminating with transfer to rhizotrons as soon as 14 days later in experiment  
53 No. 4 (**Fig. S1**). The reduction of the age of the plants used for transformation facilitated the  
54 manipulation of the composite plants when they were removed from the rock-wool cubes and  
55 limited wounding of the transgenic roots. It also facilitated their transfer into rhizotrons and use  
56 in confocal microscopy.

57

### 58 ***Inoculation of sunflower plants in rhizotrons***

59 Broomrape seeds were surface sterilized for 5 min in a 3.2 % sodium hypochlorite solution,  
60 rinsed 3 times using a 40 µm cell strainer, and water-conditioned for 7 days in a 50 ml sterile  
61 tube at 23 °C in the dark, at a final concentration of 10 mg/ 3 ml of water. Each rhizotron was  
62 inoculated with 10 mg (3 ml) of conditioned broomrape seeds.

63 Seven day-old wild-type plants were inoculated the day of transfer in rhizotron. For transgenic  
64 composite plants the day of inoculation varied among the transformation experiments from 1  
65 to 8 days after the transfer in rhizotrons (*i.e.* 27 to 36 days after sunflower sowing; **Figure S1**).

66

### 67 ***Microscopic observation of young *O. cumana* attachments***

68 Young wild-type sunflower plantlets were inoculated and used for large field and transmission  
69 electron microscopy (TEM) observation of stained longitudinal sections of attachments.  
70 Selected composite sunflower plants with fluorescent roots were inoculated and used for *in vivo*  
71 confocal microscopy images of attachments.

## 72 **Large field microscopy observations**

73 This approach was inspired from the work of Xiao et al. (2014) on early symbiotic nodule  
74 development. Four to 8 days after inoculation (dai), root samples were prepared for cytological  
75 studies as described in Chabaud et al. (2022). Fixed samples were embedded in Technovit 7100  
76 resin (Heraeus Kulzer, Wehrheim, Germany), according to the manufacturer's  
77 recommendations. Thin (4-5  $\mu\text{m}$ ) sections, longitudinal to both haustorium and host root, were  
78 made using a microtome (2040 Reichert Jung), stained in 0.2% toluidine blue for 3 min,  
79 mounted in DePeX mounting medium (BDH Laboratories, Poole, England) and scanned using  
80 a Nanozoomer (NDP, Hamamatsu).

81

## 82 **Transmission electron microscopy (TEM) observations**

83 Samples were prepared as in Cerutti et al. (2017) with some modifications. Five to 7 dai,  
84 broomrape attachments on sunflower root fragments were fixed under vacuum for 30 min with  
85 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH = 7.2) containing 0.1 % Triton  
86 X100, and then at the atmospheric pressure in the same solution without Triton X100, washed  
87 in the same cacodylate buffer and post-fixed for one hour at room temperature with 1 % osmium  
88 tetroxide ( $\text{OsO}_4$ ) in the same buffer. They were then dehydrated in ethanol series, and embedded  
89 in Epon. Thin (1  $\mu\text{m}$ ) or ultra-thin (80-90 nm) sections were prepared on UltraCut E  
90 ultramicrotome (Reichert-Jung) equipped with a diamond knife (Reichert-Leica, Germany).  
91 The histological organization of tissues was observed on thin sections stained in a 1 % borax  
92 solution containing 0.2 % methylene blue and 0.1 % toluidine blue, rinsed in water and then in  
93 an aqueous solution of 0.07 % basic fuchsin. Ultrastructural observation was done on ultrathin  
94 sections stained with uranylless (Delta microscopy, Maressac France), and lead citrate (delta  
95 microscopies, Maressac France) using the electron microscope Hitachi-7700 (Japan) operating  
96 at 80 kV.

97

## 98 **Confocal microscopy**

99 This approach was inspired from the cytology work of Genre et al. (2005) for arbuscular  
100 mycorrhizal symbiosis studies on *Medicago* and carrot. Six days after broomrape inoculation

101 (dai) in rhizotrons, inoculated sunflower composite plants were transferred to a 12x12 cm  
102 square Petri dish containing 80 ml of solid medium ½ Long Ashton 370 µM phosphate, 3 g/l  
103 Phytigel, with the aerial part outside of the dish (**Fig. S2g**). The root system was covered with  
104 a gas-permeable plastic film (Lumox Film, Starsted) as described in Fournier et al. (2015).  
105 Attachments were imaged with a Leica TCS SP8 AOBS confocal laser scanning microscope  
106 equipped with a long-distance 25X HC FLUOTAR (numerical aperture, 0.95) water immersion  
107 objective. The 488 nm argon laser line was used to excite GFP and auto-fluorescence. Specific  
108 emission windows used for GFP and auto-fluorescence signals were 500 to 550 nm, and 580 to  
109 650 nm, respectively, and emitted fluorescence was false-coloured in green (GFP), and red  
110 (auto-fluorescence). The images shown are single confocal sections or maximal projections of  
111 selected planes of a z-stack. Images were acquired and projected using Leica confocal software  
112 and processed using Leica confocal software.

113

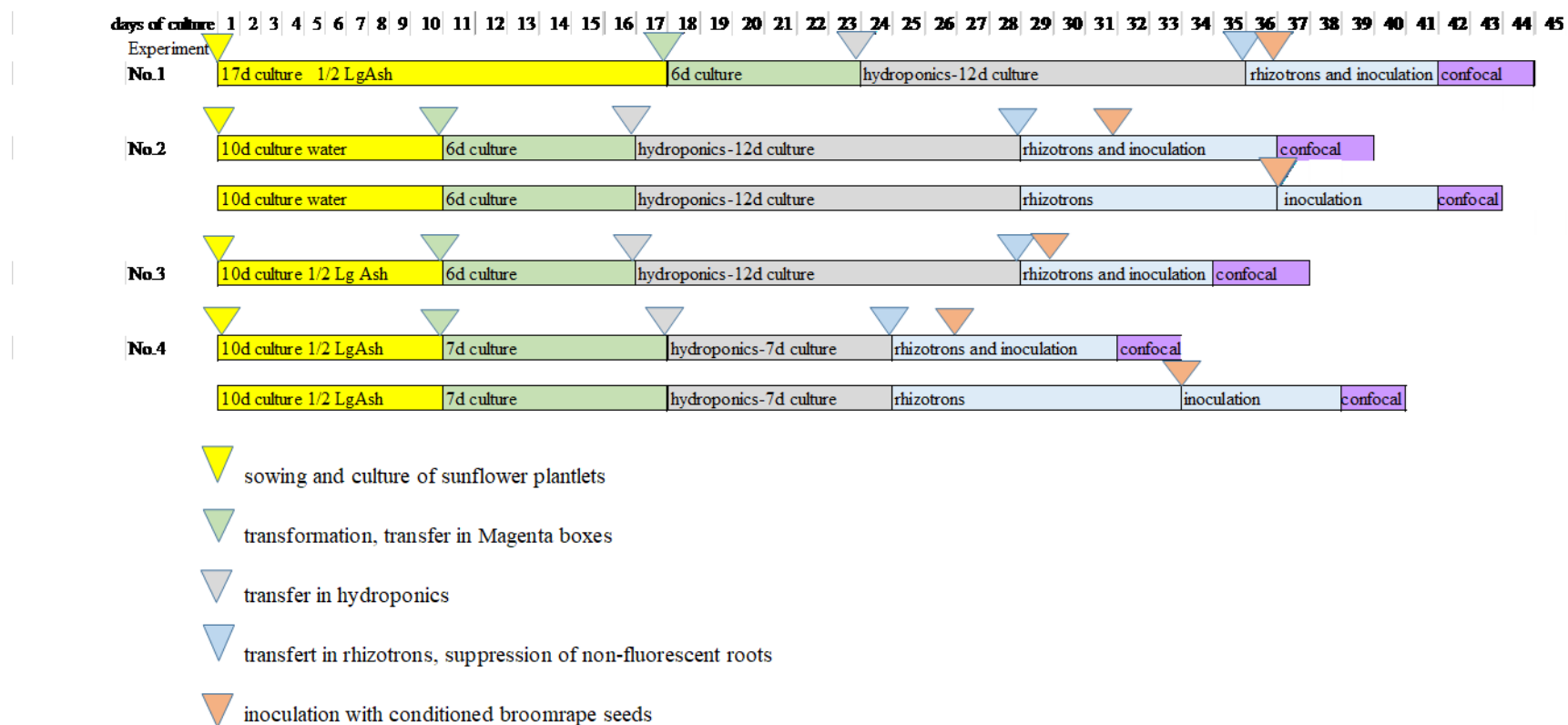
#### 114 References

- 115 **Badouin H, Gouzy J, Grassa, CJ, Murat F, Staton SE, Cottret L, Lelandais-Briere C, Owens GL, Carrere**  
116 **S, Mayjonade B et al. 2017.** The sunflower genome provides insights into oil metabolism, flowering  
117 and Asterid evolution. *Nature* **546**: 148
- 118 **Bevan M. 1984.** Binary Agrobacterium vectors for plant transformation. *Nuc. Ac. Res* 12:8711–8721
- 119 **Cerutti A, Jauneau A, Auriac MC, Lauber E, Martinez Y, Chiarenza S, Leonhardt N, Berthomé R, Noël**  
120 **LD. 2017.** Immunity at cauliflower hydathodes controls systemic infection by *Xanthomonas campestris*  
121 *pv campestris*. *Plant Physiology* **174**: 700–716.
- 122 **Chabaud M, Auriac MC , Boniface Mc , Delgrange S , Folletti T , Jardinaud MF , Legendre A, Perez-Vich**  
123 **B, Pouvreau JB , Velasco L , Delavault P, Muños S. 2022.** Wild *Helianthus* species: A reservoir of  
124 resistance genes for sustainable pyramidal resistance to broomrape in sunflower. *Frontiers in Plant*  
125 *Science* **13**.
- 126 **Fournier J, Teillet A, Chabaud M, Ivanov S, Genre A, Limpens E, de Carvalho-Niebel F, Barker B. 2015.**  
127 Remodeling of the Infection Chamber before Infection Thread Formation Reveals a Two-Step  
128 Mechanism for Rhizobial Entry into the Host Legume Root Hair. *Plant Physiology* **167** : 1233–1242.
- 129 **Genre A, Chabaud M, Timmers ACJ, Bonfante P, Barker DG. 2005.** Arbuscular mycorrhizal fungi elicit a  
130 novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell* **17**:  
131 3489-3499.
- 132 **Haseloff J, Siemering KR, Prasher DC, Hodge S. 1997.** Removal of a cryptic intron and subcellular localization  
133 of green fluorescent protein are required to mark transgenic Arabidopsis plants brightly. *Proceedings*  
134 *National Academy Science USA* **94**:2122–2127.
- 135 **Hewitt EJ. 1966.** Sand and water culture methods in the study of plant nutrition. In: Technical communication  
136 no.22. 2<sup>nd</sup> ed. Rev. Commonwealth Agr. Bur., London.
- 137 **Le Ru A, Ibarcq G, Boniface MC, Baussart A, Munos S, Chabaud M. 2021.** Image analysis for the automatic  
138 phenotyping of *Orobanche cumana* tubercles on sunflower roots. *Plant Methods* **17**.
- 139 **Morel A, Peeters N, Vailleau, F, Barberis P, Jiang G, Berthomé R, Guidot A . 2018.** Plant pathogenicity  
140 phenotyping of *Ralstonia solanacearum* strains. In: Medina C Lopez-Baena FJ, eds. *Host-Pathogen*  
141 *interactions: Methods and Protocols*. **1734**: 223-239.
- 142 **Parks T, Yordanov YS. 2020.** Composite plants for a composite plant: An efficient protocol for root studies in  
143 the sunflower using composite plants approach. *Plant Cell Tissue and Organ Culture* **140**: 647-659.

- 144 **Savka M., Ravillion B., Noe, G., Farrand S.1990.** Induction of hairy roots on cultivated soybean genotypes and  
145 their use to prop-agate the soybean cyst nematode. *Phytopathology*.**80**: 503–508.
- 146 **Xiao TT, Schilderink S, Moling S, Deinum EE, Kondorosi E, Franssen H, Kulikova O, Niebel A, Bisseling**  
147 **T. 2014.** Fate map of *Medicago truncatula* root nodules. *Development* **141**: 3517-3528.

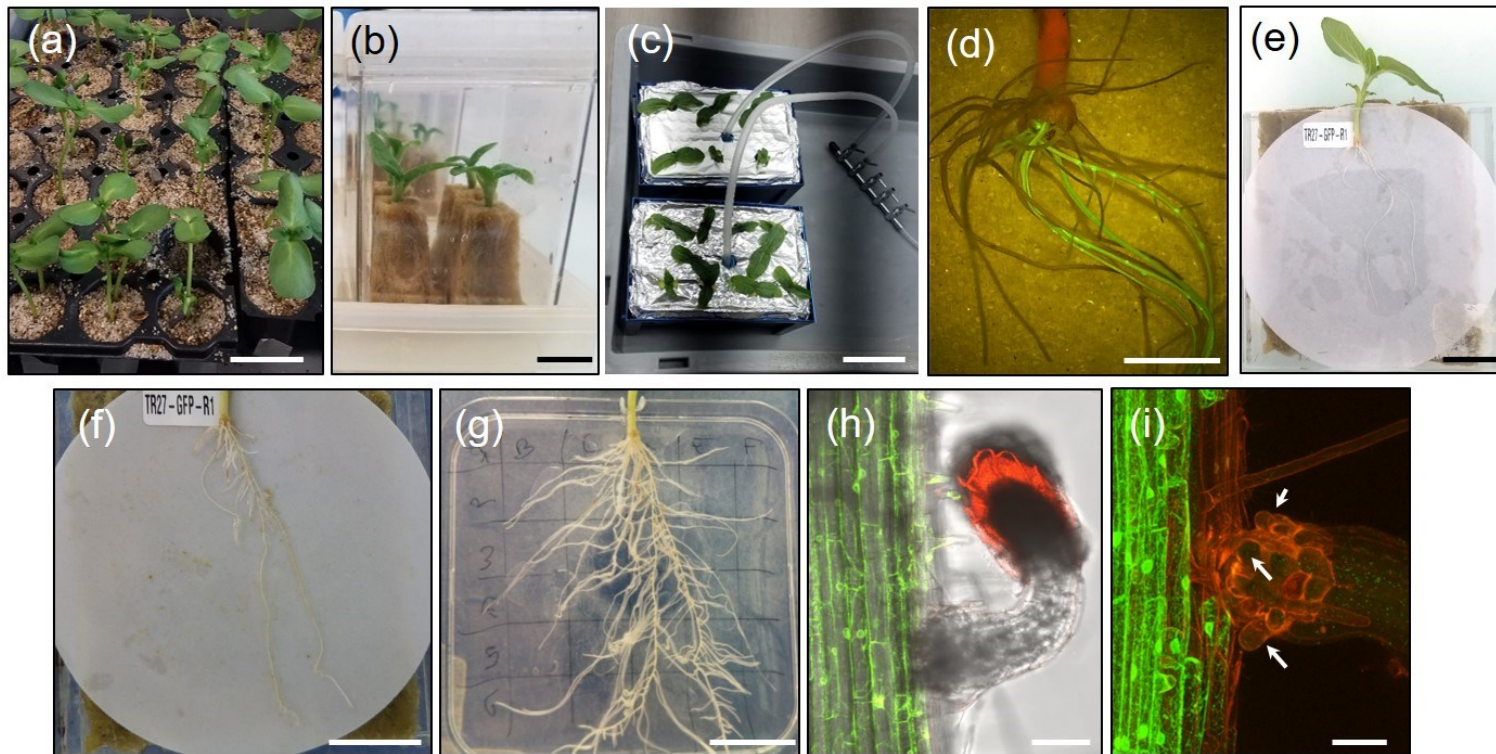
**Fig. S1. Sunflower transformation experiments and broomrape inoculation of composite plants for confocal microscopy observations.**

The initial experiment (No.1) led to broomrape inoculation of 36-day-old sunflower plants, which were very developed and not easy to handle under confocal microscopy. Hence, the length of culture was progressively reduced in the following experiments to finally reach broomrape inoculation of smaller, 26-day-old plantlets in experiment No.4. This also allowed to generate two series of inoculated plants according to their development in experiments No. 2 and 4, using the most developed composite plants for the 1<sup>st</sup> inoculation and the less developed composite plants for a 2<sup>nd</sup>, delayed inoculation 6 days later. Confocal microscopy was performed 5 to 8 dai.



**Fig. S2. Transformation of sunflower plants via *A. rhizogenes* and transfer of composite plants in rhizotrons for broomrape inoculation and observation using confocal microscopy.**

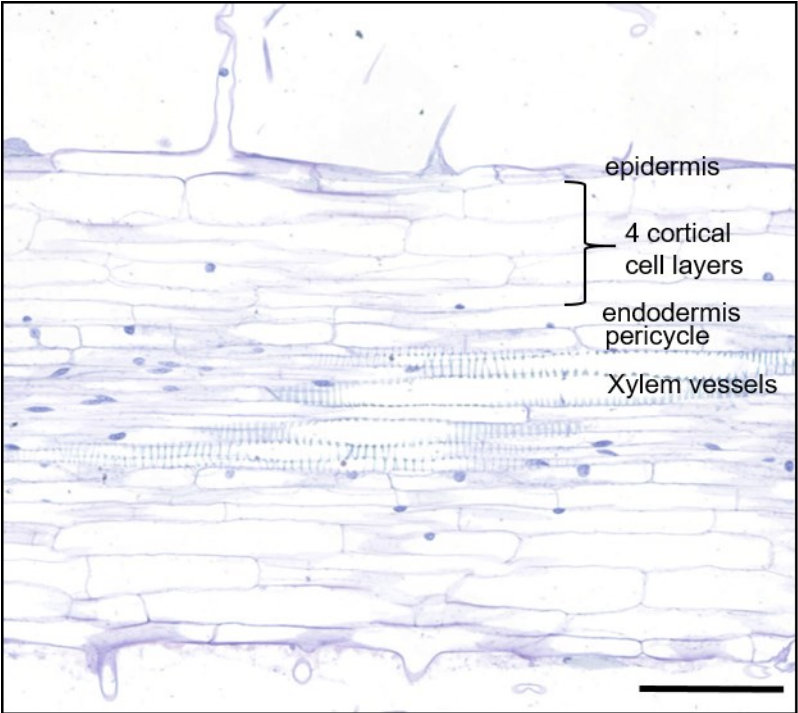
Cuttings without cotyledons were excised from 10-day-old plantlets (a), and transformed in pre-soaked rock-wool cubes with *A. rhizogenes* solution (b). Six days later, plantlets were transferred in hydroponics (c) for another 8 days of culture. Fourteen days after transformation, green fluorescent transgenic roots were counted under a binocular microscope (d, Table S1). Non-fluorescent roots were removed and composite plants were transferred to rhizotrons (e) and inoculated with pre-conditioned broomrape seeds 1 to 2 days later (f). Five to 8 days after inoculation, inoculated composite plants were transferred to a Petri dish (g- 5 dai) and attachments were selected and imaged by confocal microscopy (h-i). i. Papillae development (arrows). Scale bar = 4 cm (a); 2 cm (b); 5 cm (c); 0.5 cm (d); 3 cm (e-g); 100  $\mu$ m (h); 10  $\mu$ m (i).



**Fig. S3. Longitudinal section of a sunflower root.**

A thin section of a sunflower root fragment, without broomrape attachment, resin-embedded and stained with toluidine blue O, was observed using large field microscopy. In our growth conditions, roots displayed 4 to 5 cortical layers.

Scale bar = 100  $\mu\text{m}$ .

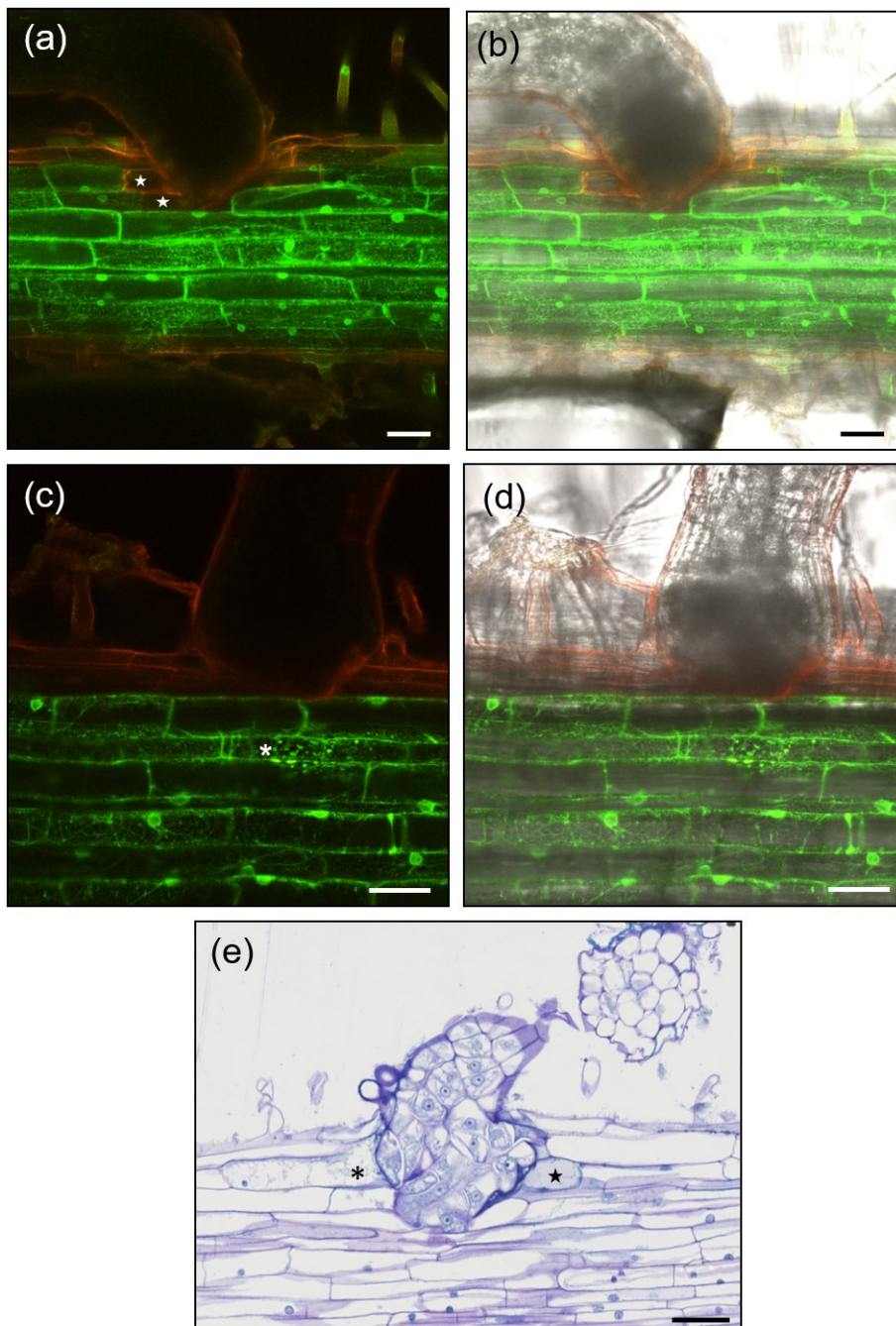




**Fig. S4. Cell death or ER de-structuring associated with haustorium penetration.**

Confocal microscopy imaging revealed that occasionally, sunflower cells in the vicinity of haustoria cells were either devoid of fluorescence (**a, b, star**) or showed a destructured ER (**c, d, asterisk**), suggesting cell death. **e.** Using bright field microscopy, similar events were observed as destructuring of the vacuole (blue smear, asterisk) or light blue material filling the cell (star). These observations suggested rare occurrence of dying or dead cells in contact or in the vicinity of the haustorium.

Scale bar = 10  $\mu\text{m}$  (**a, b**); 50  $\mu\text{m}$  (**c-e**).

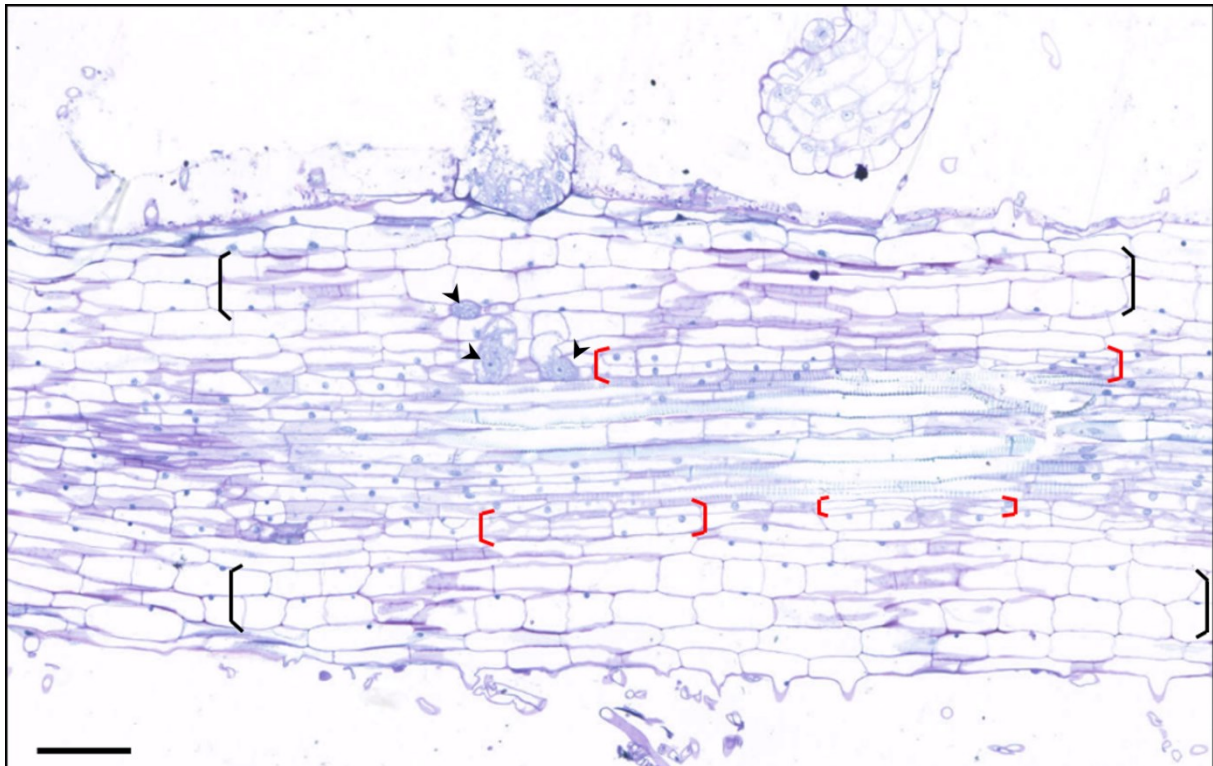




**Fig. S5. Multiple divisions in the host root at the site of broomrape intrusion.**

Lower magnification image of the section in **Fig. 1j**, showing a wider zone of the root. Cell division was observed around the intrusive cells (arrowheads). Anticlinal divisions affected the host cortex on both sides of the root (black square brackets). Periclinal divisions were visible in the pericycle (red square brackets).

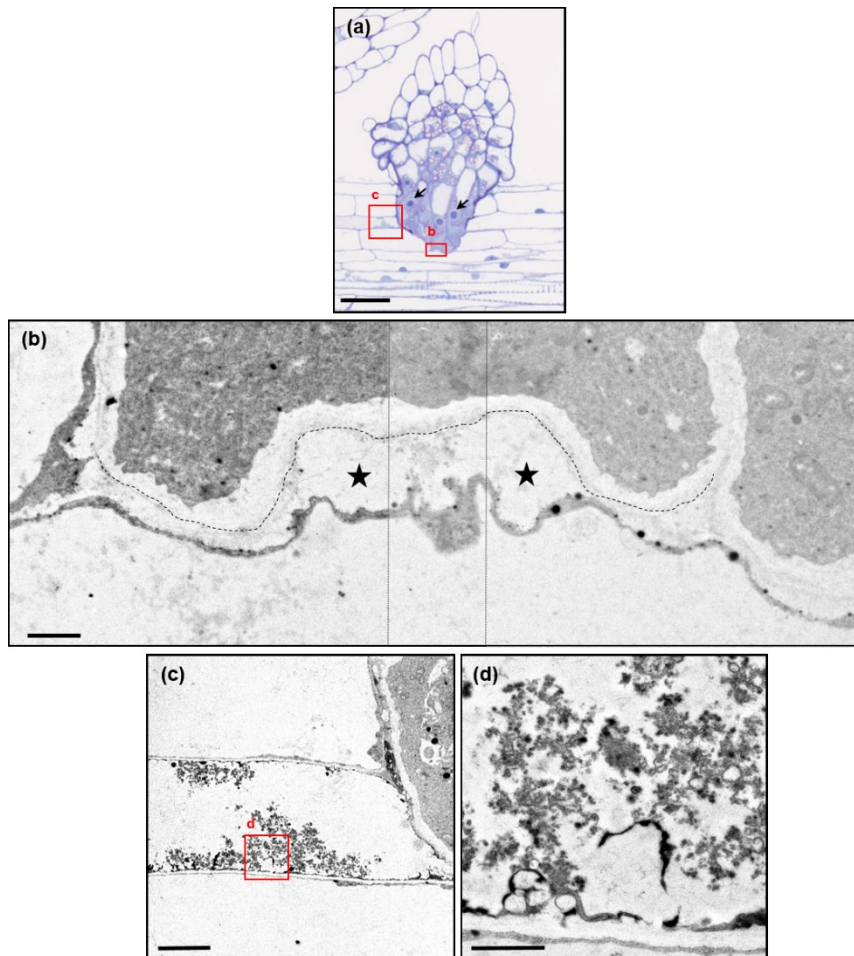
Scale bar = 100  $\mu\text{m}$ .



**Fig. S6. Transmission electron microscopy of a 7 dai attachment.**

The attachment shown in Fig. 2 was sectioned *c.* 9  $\mu\text{m}$  further. **a.** General view of the attachment using large field microscopy. **b-d.** TEM.

**a.** Intrusive cells showed a dense cytoplasmic content and a big nucleus (arrow), containing a large nucleolus (dark blue). **b.** Assembly of 3 TEM pictures at the interface of the penetrating intrusive cells. The periclinal cell wall of the host cortical cell contacted by the tip of the haustorium was deformed (stars), potentially digested by parasitic pectolytic enzymes. By contrast the parasitic cell wall surrounding the intrusive cells (dotted line) appeared intact and continuous. The plasmalemma of the host cell showed convolution, suggesting active membrane synthesis, preparing the apoplastic compartment for subsequent accommodation of the haustorium. Electron dense granules were present along the host plasmalemma. **c, d.** One of the penetrated cortical cells, adjacent to the attachment, showed abnormal accumulation of granular material inside the vacuole. Electron microscopy of this cell indicated disruption of the tonoplast and plasmalemma as well as cytoplasm degradation, likely causes of cell death. Scale bar = 50  $\mu\text{m}$  (**a**); 5  $\mu\text{m}$  (**b**); 1  $\mu\text{m}$  (**c-d**).



**Table S1. Efficiency of sunflower transformation via *A. rhizogenes*.**

Four experiments of sunflower transformation were performed, using the p35S-GFP-ER construct (**Fig. S1**). Transgenic fluorescent roots were observed using a stereomicroscope equipped with a Long Pass GFP filter, which allowed to discriminate transgenic green roots from non-transformed orange auto-fluorescent roots. Plants were counted as transformed when at least one green fluorescent root had developed.

NA: data not available. In experiment No.1, composite plants were too developed and consequently transgenic roots were intermingled and difficult to distinguish from one another.

experiment	number of plants	% of transformed plants	number of fluorescent root/ transformed plant	% of fluorescent root/ transformed plant
No.1	20	95	NA	NA
No.2	7	86	4,6	63
No.3	9	100	5,4	51
No.4	10	100	4,9	49

**Table S2. Overview of observed sites.**

Twenty five attachments (4 to 8 dai) from inoculated wild-type (non transformed) sunflower plants were scored using large field microscopy: 21 sites of penetration and 4 sites without penetration yet. Thirty eight attachments (5 to 7 dai) were scored under confocal microscopy on inoculated transgenic composite plants: 21 sites of penetration and 17 sites without penetration yet. Sites are organized according to time of observation (dai) and stage of colonization (*i.e.* deepest sunflower root cell layer reached by the intrusive cells).

Time	Stage of colonization	microscopy	
		large field	confocal
4 dai	no contact	1	
	epidermis	1	
5 dai	contact but no penetration		3
6 dai	contact but no penetration	3	10
	epidermis	1	1
	1st outer cortex	1	3
	2nd or 3rd outer cortex	1	8
	inner cortex	1	2
	vessels	1	
7 dai	contact but no penetration		4
	epidermis		1
	1st outer cortex	2	2
	2nd/ 3rd outer cortex	4	3
	inner cortex	3	1*
	vessels	1	
8 dai	1st layer outer cortex	2	
	2nd/ 3rd outer cortex		
	inner cortex	1	
	endodermis	1	
	vessels	1	
total number of sites		25	38

\*: inner cortex or deeper