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► To cite this version:

Pauline Blin, Kévin Robic, Slimane Khayi, Jérémy Cigna, Euphrasie Munier, et al.. Pattern and causes of the establishment of the invasive bacterial potato pathogen *Dickeya solani* and of the maintenance of the resident pathogen *D. dianthicola*. *Molecular Ecology*, 2021, 30 (2), pp.608-624. 10.1111/mec.15751 . hal-03376174

HAL Id: hal-03376174

<https://hal.science/hal-03376174>

Submitted on 1 Dec 2021

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1 **Pattern and causes of the establishment of the invasive bacterial potato pathogen**
2 ***Dickeya solani* and of the maintenance of the resident pathogen *D. dianthicola***

3

4 Short title: coexistence of invasive and resident pathogens on potatoes

5

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25

26 **Abstract**

27 Invasive pathogens can be a threat when they affect human health, food production or
28 ecosystem services, by displacing resident species, and we need to understand the cause of
29 their establishment. We studied the patterns and causes of the establishment of the
30 pathogen *Dickeya solani* that recently invaded potato agrosystems in Europe by assessing
31 its invasion dynamics and its competitive ability against the closely-related resident *D.*
32 *dianthicola* species. Epidemiological records over one decade in France revealed the
33 establishment of *D. solani* and the maintenance of the resident *D. dianthicola* in potato fields
34 exhibiting blackleg symptoms. Using experimentations, we showed that *D. dianthicola*
35 caused a higher symptom incidence on aerial parts of potato plants than *D. solani*, while *D.*
36 *solani* was more aggressive on tubers (i.e. with more severe symptoms). In co-infection
37 assays, *D. dianthicola* outcompeted *D. solani* in aerial parts, while the two species co-existed
38 in tubers. A comparison of 76 *D. solani* genomes (56 of which having been sequenced here)
39 revealed balanced frequencies of two previously uncharacterized alleles, VfmB_{Pro} and
40 VfmB_{Ser}, at the *vmB* virulence gene. Experimental inoculations showed that the VfmB_{Ser}
41 population was more aggressive on tubers while the VfmB_{Pro} population outcompeted the
42 VfmB_{Ser} population in stem lesions, suggesting an important role of the *vmB* virulence gene
43 in the ecology of the pathogens. This study thus brings novel insights allowing a better
44 understanding of the pattern and causes of the *D.solani* invasion into potato production
45 agrosystems, and the reasons why the endemic *D.dianthicola* nevertheless persisted.

46

47 **KEYWORDS**

48 competitive exclusion; *Dickeya*; invasion; plant pathogens; population genomics; quorum-
49 sensing.

50

51

52

53 **1 Introduction**

54 Biological invasions represent major threats to biodiversity as invasive species can replace
55 native species. Evolutionary and ecological studies are of major importance for
56 understanding the patterns and causes of biological invasions and plant and animal
57 invasions have been extensively studied since the 19th century (Burns, Murphy, & Zheng,
58 2018; Cadotte, Campbell, Li, Sodhi, & Mandrak, 2018; MacDougall, Gilbert, & Levine, 2009).
59 Evolutionary and ecological approaches aim at elucidating the dynamics of invasions as well
60 as the advantages of invaders that allow them to outcompete resident species (Facon et al.,
61 2006; Gladieux et al., 2015). Although microbial invasions can represent important threats to
62 ecosystems and human societies, they have been much less studied than invasive plants
63 and animals (Gladieux et al., 2015; Kinnunen *et al.*, 2016; Litchman, 2010; Mallon, Elsas, &
64 Salles, 2015; Perkins, Leger, & Nowak, 2011).

65 In this work, we used evolutionary and ecological approaches for understanding the patterns
66 and causes of the establishment of the bacterial pathogen *Dickeya solani* in potato
67 agrosystems and the maintenance of the resident, related competitor *D. dianthicola*. There
68 are two major clades of potato bacterial pathogens in Europe, *Pectobacterium* and *Dickeya*,
69 two sister genera among the order Enterobacterales (Gamma-proteobacteria). While some
70 *Pectobacterium* species had settled since before the 1970s in potato agrosystems in Europe
71 (Pérombelon, 2002), *Dickeya* species invaded later, in two successive waves. Mostly
72 isolated from ornamental plants such as *Dianthus*, *Dahlia* and *Begonia*, *D. dianthicola* has
73 been reported in Europe as early as the 1950s. Isolation of *D. dianthicola* from *Solanum*
74 *tuberosum* dates back to the 70's (Toth et al., 2011; Parkinson et al., 2009). Nowadays,
75 *D. dianthicola* is considered an endemic pathogen of potato plants in Europe. It is present in
76 all continents, including North-America, where it was associated with disease outbreaks in
77 potato farms in 2015 (Oulghazi et al., 2017; Patel, Baldwin, Patel, Kobayashi, & Wyenandt,
78 2019; Sarfraz et al., 2018; Toth et al., 2011; Wright et al., 2018).

79 *Dickeya solani* has emerged in potato production systems in Europe much more recently, in
80 the early 2000s (Toth et al., 2011; van der Wolf et al., 2014). Aside from potatoes, *D. solani*

81 has been isolated from ornamental plants such as *Hyacinthus*, *Iris* and *Muscari*, but the
82 primary host(s) of that species remain(s) uncertain (Chen, Zhang, & Chen, 2015; Slawiak et
83 al., 2009; van der Wolf et al., 2014). Population genomics revealed little genetic variation in
84 *D. solani*, in agreement with a recent spread of this species with a bottleneck associated to
85 the introduction and/or invasion (Golanowska et al., 2018; Khayi et al., 2015). An analysis of
86 20 *D. solani* genomes identified a non-synonymous single nucleotide polymorphism (SNP) in
87 each of the two *fliC* and *fliN* genes that are involved in bacterial motility (Khayi et al., 2015).
88 Horizontal gene transfer events from *D. dianthicola* to *D. solani* have been documented,
89 adding or replacing genomic fragments (Khayi et al., 2015).

90 The *D. solani* invader, as well as the *D. dianthicola* and *Pectobacterium* spp. residents, are
91 necrotrophic pathogens causing similar symptoms, resulting in blackleg disease on stems
92 and soft-rot disease of tubers (Charkowski 2018; Toth et al., 2011). All these bacteria secrete
93 plant cell-wall macerating enzymes (including the pectate lyases) and proliferate by
94 assimilating the plant cell remains, including iron by producing the chrysobactin and
95 achromobactin siderophores. Pectate lyases, i.e., *PeIA*, *PeID* and especially *PeIE*, play a key
96 role in the initiation of the plant cell wall maceration. Using pectin as a substrate, these
97 enzymes release oligosaccharides that elicit expression of other plant-cell wall macerating
98 enzymes in a positive feedback loop (Duprey, Nasser, Léonard, Brochier-Armanet, &
99 Reverchon, 2016). Noticeably, the *peIA* gene is truncated, hence non-functional, in
100 *D. dianthicola* (Duprey et al., 2016; Raoul des Essarts et al., 2019). The secretion of these
101 virulence factors is a costly process that is tightly regulated, being induced by the perception
102 of chemicals that inform the pathogen on plant host physiology (mainly plant sugars) and on
103 the size of its own population (quorum-sensing signals) (Leonard, Hommais, Nasser, &
104 Reverchon, 2017). *Dickeya* pathogens synthesize and sense two types of quorum-sensing
105 signals, the N-acylhomoserine lactones and Vfm compounds, which activate virulence gene
106 expression when a threshold concentration of signals (reflecting the number of cells in the
107 environment) is reached (Crépin et al., 2012; Nasser et al., 2013; Potrykus, Golanowska,
108 Hugouvieux-Cotte-Pattat, & Lojkowska, 2014; Potrykus, , Hugouvieux-Cotte-Pattat, &

109 Lojkowska, 2018). By modulating the synthesis of virulence factors, the quorum-sensing
110 signals participate in the transition from an oligotroph lifestyle in soils and surface waters,
111 where *Dickeya* and *Pectobacterium* bacteria are rare, to a copiotroph lifestyle on a plant
112 host, where they increase in number by several orders of magnitude (Laurila et al., 2008;
113 Potrykus et al., 2016). Functional differences such as metabolic capacities have been
114 identified between *D. solani* and *D. dianthicola* by comparative genomics, transcriptomics
115 and biochemical approaches (Bellieny-Rabelo et al., 2019; Raoul des Essarts et al., 2019),
116 but fitness differences within host plants remain only partially investigated (Czajkowski et al.,
117 2013; Czajkowski, de Boer, Velvis, & van der Wolf, 2010; Raoul des Essarts et al., 2019;
118 Shyntum et al., 2019). Whether the potato host could be differentially exploited by *D.*
119 *dianthicola* and *D. solani* and whether the two pathogens stably coexist by using different
120 ecological niches or compete directly when exploiting this host remain unsolved questions,
121 despite their importance for understanding the *D. solani* invasion.

122 Here, we combined epidemiological records in potato fields, experimental inoculations and
123 population genomics to contribute to our understanding of the patterns and ecological
124 determinants of *D. solani* invasion. Epidemiological records over one decade in France
125 allowed to compare the relative abundances of the *D. solani* invader, the *D. dianthicola*
126 resident and *Pectobacterium* spp. Using experimental populations and plant assays in
127 greenhouse, with a diversity of strains and of ecological conditions, we then focused on *D.*
128 *solani* and *D. dianthicola* to determine the ecological traits that could facilitate or constrain *D.*
129 *solani* establishment, including competition or ecological niche differences between the two
130 *Dickeya* species. Finally, we obtained the genomes of 56 *D. solani* isolates that we analyzed
131 together with 20 genomes previously published (Khayati et al., 2015), revealing novel
132 variations in *D. solani* population. We focused on the quorum-sensing gene *vfmB*, because it
133 is known to be involved in virulence, to investigate whether its variation, which has not been
134 studied so far, could be associated to invasion traits in *D. solani*.

135

136 **2 Material and Methods**

137 **2.1 Pathogen sampling and identification in potato fields with blackleg disease**
138 **symptoms**

139 Pectinolytic bacteria were isolated from symptomatic plant tissues on crystal violet pectate
140 medium (Hélias, Hamon, Huchet, Wolf, & Andrivon, 2012). Isolates were purified on agar
141 plates and characterized at the genus (*Dickeya* and *Pectobacterium*) and species (*D. solani*
142 and *D. dianthicola*) levels using the PCR primers listed in **Table S1**. Two sampling strategies
143 were used. From 2004 to 2015, a national inventory was conducted in French potato
144 producing areas. Each year (with the exception of the year 2006), fields exhibiting blackleg
145 symptoms were randomly sampled by collecting diseased plants from which isolates were
146 recovered and characterized. From 541 fields, around 1600 isolates were collected and
147 taxonomically characterized. In parallel, from 2013 to 2016, 19 of the potato fields exhibiting
148 more than 1% of blackleg incidence were deeply sampled. Around 30 plants with blackleg
149 symptoms were collected along a transect in each field, and a single isolate was retained
150 from each of the 548 plants. The resulting 548 isolates were characterized taxonomically.

151

152 **2.2 *Dickeya solani* and *D. dianthicola* strains used in plant assays**

153 Five *D. dianthicola* strains (RNS11-47-1-1A, CFBP1888, CFBP2982, CFBP2015, MIE34)
154 and five *D. solani* strains (3337 = RNS08-23-3-1A, IPO2222, RNS05-1-2A, Ds0432.1,
155 PPO9019) were used to produce plant inoculation assays. The strains were chosen to be
156 representative of the diversity of the populations, i.e. from different geographical locations,
157 isolation years or host of collection (potato plants vs ornamentals), and regarding phylogeny
158 placement with neutral markers (**Figure S1** and see characteristics and genome accession
159 numbers in **Table S2**). Phylogeny analyses were conducted using MEGA7 (Kumar, Stecher,
160 & Tamura, 2016). Bacterial strains were stored at -80°C in glycerol (25% vol/vol). They were
161 cultivated in TY medium (tryptone 5 g.L⁻¹; yeast extract 3 g.L⁻¹) with or without agar (15 g.L⁻¹)
162 at 28°C.

163

164 **2.3 Plant and tuber inoculations by *D. solani* and *D. dianthicola* strains and** 165 **experimental populations**

166 In our plant and tuber assays, we use the term virulence as the capacity of the pathogen to
167 parasitize a host (symptom incidence) and the term aggressiveness as the degree of
168 damage caused by a pathogen on a host (symptom or disease severity). The terms used to
169 describe pathogens behaviors (virulence, symptom incidence, aggressiveness, disease
170 severity index, fitness and competitive index) are defined more extensively in
171 **Supplementary Methods 1 (SM1)**. We designed the whole plant experiments based on our
172 knowledge of the pathosystem, to investigate contrasting conditions relevant for the disease
173 expression in fields. Each bacterial strain was cultivated individually before being used for
174 plant and tuber inoculations, either separately or assembled in populations. To constitute
175 experimental populations of *D. solani*, *D. dianthicola*, or mixtures of the two species, cell
176 suspensions of different strains were assembled just before plant inoculation. Potato plants
177 (*S. tuberosum* var. Bintje) and hyacinths (*Hyacinthus orientalis* var. Delft blue) were
178 cultivated in horticultural compost in individual pots (2 L) in a greenhouse at around 23°C
179 (minimum 20°C maximum 28°C) with a 12-hour photoperiod. For mimicking plant infection by
180 soil pathogens, potato plants (three weeks post-tuber plantation) and hyacinths (four weeks
181 post-bulb plantation) were inoculated by watering the substrate with a pathogen cell
182 suspension at 10⁹ colony-forming units (CFU) per pot. This inoculation load was calibrated to
183 obtain a large range (20 to 60%) of symptom incidence in the unwounded potato plant
184 condition (Raoul des Essarts et al., 2015). In a wounded condition, the potato roots were
185 wounded with a sterile knife before infection. The symptom monitoring was adapted from
186 Raoul des Essarts et al. (2015). The number of symptomatic potato plants (blackleg disease)
187 and hyacinths (soft-rot disease) was recorded twice a week (see examples of symptoms in
188 **Figure 1a-b**). For each strain and experimental population and unwounded and wounded
189 conditions, the number of symptomatic and asymptomatic plants (hence two symptom
190 classes that were used in statistical comparisons) was counted at 70 days post infection (dpi)
191 using 15 potato plants (up to 25 potato plants when indicated) and 8 hyacinths. A set of non-

192 inoculated plants was used as an asymptomatic control. In order to facilitate visualization of
193 the virulence data in the figures, they are presented as a percentage of symptomatic plants
194 (symptom incidence).

195 In tuber assays, potato tubers (*S. tuberosum* var Bintje) were inoculated using a tip to inject
196 10 µL of calibrated pathogen suspensions. Based on previous results (Raoul des Essarts et
197 al., 2019), we chose two different inoculum loads of strains and experimental populations
198 (10^7 CFU and 10^5 CFU per tuber) to obtain a large range of symptom severity, to compare
199 the capacity to initiate maceration at different population sizes, to investigate fitness of the
200 two pathogens in two contrasting ecological conditions. After 5 days of incubation at 24°C,
201 the tubers were cut in half and scored based on five aggressiveness classes according to the
202 symptom severity in each tuber (from no symptom to the most severe symptoms; **Figure 1c**).
203 For each strain and experimental population, at least 10 tubers were inoculated. A set of
204 non-inoculated tubers was used as an asymptomatic control. In order to facilitate the
205 presentation of the aggressiveness data in the figures, results are presented as normalized
206 disease severity index (DSI) of which values, ranging from 0 to 100 (arbitrary unit), increase
207 with symptom severity. DSI was calculated using the five aggressiveness classes assigned
208 to 10 tubers, see formula in **Supplementary Methods 1 (SM1)**.

209 Emerging lesions from potato stems and hyacinths and rotting tissue recovered from tubers
210 at 5 dpi were collected and frozen at -80°C, and then used to quantify the abundance *D.*
211 *solani* and *D. dianthicola* pathogens within hosts by Taq Man qPCR as described in
212 **Supplementary Methods 2 (SM2)**. Assessing *D. dianthicola* and *D. solani* in inoculum and
213 plant tissues (potato stems and tubers, and hyacinths) permitted the calculation of
214 competitive index (CI) values following the formula presented in **SM1**. A CI value equal to
215 one indicated an equal fitness between *D. solani* and *D. dianthicola*, CI value greater than
216 one indicated a fitness advantage to *D. solani*, and CI value below one indicated a fitness
217 advantage to *D. dianthicola*.

218

219 **2.4 *Dickeya solani* population genomics**

220 Total DNA was purified from each of 56 *D. solani* isolates (their characteristics in **Figure S2**)
221 using MasterPure complete DNA and RNA purification kit (Epicentre, Illumina). After quantity
222 and quality controls of extracted DNA using a NanoDrop (ND 1000) device and agarose gel
223 electrophoresis at 1.0 % (w/v), paired-end libraries were constructed for each strain and then
224 sequenced using High Output Sequencing kit with 75 × 2 cycles on and Illumina NextSeq500
225 sequencer (CNRS, Gif-sur-Yvette, France). For each strain, 2.4 to 18 million reads were
226 obtained, corresponding to an average coverage ranging from 37× to 270×. Paired-end
227 reads were trimmed (quality score threshold 0.05) and mapped against the *D. solani*
228 reference genome, from the 3337 strain, at mild stringency threshold (80% identity on 50%
229 read length) using CLC Genomics Workbench version 10.1.3 (Khayri *et al.* 2015). The
230 mapping was used for the detection of SNPs and InDels using the variant calling tool from
231 CLC genomic workbench version 10.1.3. Only SNPs and InDels (1 to 9 nucleotides) with a
232 high occurrence (≥ 99 % of the reads) in the mapping step were retained. The representation
233 of allelic variants in *D. solani* population was performed using PHYLOViZ (Francisco *et al.*,
234 2012).

235

236 **2.5 Characterization of the fitness differences between *Dickeya solani* strains carrying** 237 **the VfmB_{Ser} and VfmB_{Pro} alleles**

238 The structure of the VfmB proteins was modeled and represented using the Phyr2 and EzMol
239 web portals (Kelley, Mezulis, Yates, Wass, & Sternberg, 2015; Reynolds, Islam, Sternberg,
240 2018). Given the known role in virulence of the *vfm* gene cluster (Nasser *et al.*, 2013), we
241 investigated the impact on virulence of a SNP detected in the *vfm* gene cluster : we
242 assessed virulence differences between *D. solani* strains exhibiting either a serine (allele
243 VfmB_{Ser}) or a proline (allele VfmB_{Pro}) at the position 55 of the VfmB protein. Using genomic
244 data, we chose four isolates carrying VfmB_{Pro} (IPO2222, MIE35, AM3a and 3337) and four
245 isolates carrying VfmB_{Ser} (Ds0432.1, RNS10-27-2A, Sp1a and M21a), the four isolates in
246 each group differing at other positions in genomes (genome accession numbers and

247 characteristics in **Tables S3** and **S4**). Plant inoculation assays (on potato tubers and stems)
248 were performed following the same protocols as described above using pure strains and
249 mixtures as inocula. In the figures, virulence was presented as the percentage of
250 symptomatic plants (in potato stem infections assay) and aggressiveness by DSI values (in
251 potato tuber infection assays).

252 To measure their relative fitness, co-inoculation assays were also performed with VfmB_{ser}
253 and VfmB_{pro} experimental populations, and their relative abundance was quantified by shot
254 gun sequencing of DNA extracted from inoculum and from lesions. From 12 to 28 million
255 reads were obtained for each sample, corresponding to an average coverage of *D. solani*
256 genomes ranging from 180x to 420x. Sequencing (75 × 2 cycles) was performed using an
257 Illumina NextSeq500 at the I2BC platform (CNRS, Gif-sur-Yvette, France) and Illumina
258 MiSeq platform (University of Malaya, Kuala Lumpur, Malaysia). The trimmed reads were
259 mapped on *D. solani* *vfmB* gene for quantifying the relative abundance of the two VfmB_{ser}
260 and VfmB_{pro} alleles in each sample using the CLC genomic workbench version 10.1.3. The
261 relative abundances of the alleles permitted calculation of CI values of the VfmB_{ser} and
262 VfmB_{pro} populations (see **SM1**). A CI value equal to one indicated an equal fitness between
263 *D. solani* VfmB_{pro} and VfmB_{ser} populations, CI value greater than one indicated a fitness
264 advantage to VfmB_{pro}, and CI value less than one indicated a fitness advantage to VfmB_{ser}.
265 Finally, we used transcriptomics to identify the genes that were differentially expressed
266 between IPO2222 (VfmB_{pro}) and Ds0432.1 (VfmB_{ser}) *D. solani* strains when they grew inside
267 potato tubers (**SM2**). Transcriptomes were compared as described by Raoul des Essarts et
268 al. (2019).

269

270 **2.6 Statistical analyses**

271 The slopes of the dynamics of the percentages of *Dickeya*-, *Pectobacterium*-, *D. solani*- and
272 *D. dianthicola*-positive fields were analyzed using F-tests and ANCOVAs using GraphPad
273 Prism version 8.4.2 for MacOS, GraphPad Software (www.graphpad.com). A Chi-squared
274 method was used to test the non-random distribution of taxa in each field.

275 In all the plant assays, the non-inoculated plants and tubers remained asymptomatic, and
276 this control condition was not considered in statistical analyses. The two symptom levels
277 (with or without symptoms) of potato plants and hyacinths virulence assays, the five
278 symptom classes in tuber aggressiveness assays, and gene expression levels were
279 compared among treatments (the different pathogens) by Kruskal-Wallis tests ($\alpha = 0.05$)
280 because the data significantly deviated from normality. Then, post-hoc Tukey tests were run
281 to assess what pairwise differences were significant. When the bacterial strains were
282 inoculated separately on plants and tubers, we compared the *D. solani* and *D. dianthicola*
283 species traits using each bacterial strain as a replicate of the considered species. When the
284 bacterial strains were inoculated altogether to constitute an experimental population, we
285 compared the *D. solani* and *D. dianthicola* species traits using at least two independent
286 replicates of the experiments.

287 In competition assays, the hypothesis of balanced abundances of the two competitors was
288 tested by comparing the calculated CI values to one using a Kruskal-Wallis test. Analyses
289 were carried out with the software R (<http://www.R-project.org>) using the packages Rcmdr
290 and nparcomp.

291

292

293 **3 Results**

294 **3.1 Little co-occurrence of *D. dianthicola* and *D. solani* in potato fields**

295 A monitoring of the *Pectobacterium* and *Dickeya* populations was performed over one
296 decade (2004-2015) in France. Each year, we recorded the number of fields from which we
297 isolated each taxon (**Table S5**). These data informed on the dynamics of the different
298 pathogen populations. Over the period, *Dickeya* was detected in 10% to 35% of the sampled
299 fields (**Figure 2a**). A moderate increase of the percentage of *Dickeya*-positive fields was
300 observed over time ($F= 5.05$; $DFn= 1$; $DFd= 9$; $p= 0.05$; $R^2 = 0.36$). Although decreasing in
301 incidence, the resident *Pectobacterium* species remained the most widespread (67% in the
302 most recent sampling year 2015). As expected, the slopes of the dynamics of the percentage

303 of *Dickeya*- and *Pectobacterium*-positive fields diverged ($F= 10.11$; $DFn= 1$; $DFd= 18$; $p= 5 \times$
304 10^{-3}). Among the *Dickeya* isolates, only two species were identified, *D. dianthicola* and
305 *D. solani* (**Figure 2b**). The slopes of *D. dianthicola*-positive ($F= 1.35$; $DFn= 1$; $DFd= 9$; $p=$
306 0.28) and *D. solani*-positive ($F= 3.10$; $DFn=1$; $DFd= 9$; $p= 0.11$) field percentages were not
307 different from base line and were not different from each other ($F= 0.26$; $DFn = 1$; $DFd = 18$;
308 $p= 0.62$). This suggests that the *D. solani* invasion did not occur at the expense of *D.*
309 *dianthicola* in French potato agrosystems.

310 Aside from this national survey, we zoomed at the field level using a more extensive
311 sampling strategy over a four-year period (2013-2016). Along a transect in each of 19
312 sampled fields, we collected ca. 30 plants with blackleg symptoms and a single isolate was
313 characterized from aerial symptoms of each plant, resulting in the sampling of 548 isolates
314 (**Figure 2c**). The *Pectobacterium* populations were present in all the 19 sampled fields. In
315 each of the 19 fields, the null hypothesis that *Pectobacterium* and *Dickeya* were randomly
316 distributed was rejected (Chi-squared test: $DF= 1$; $p< 0.05$), meaning that *Pectobacterium*
317 and *Dickeya* co-occurred less often than expected under random distribution. The hypothesis
318 that *D. solani* and *D. dianthicola* were randomly distributed along the transect was also
319 rejected in each of the 16 *Dickeya*-positive fields (Chi-squared tests: $DF= 1$; $p< 0.05$) but one
320 (field #13; $DF= 1$; $p= 0.24$). These field data showed little co-occurrence of taxa at the field
321 levels, both for genera (*Dickeya* and *Pectobacterium*) and species (*D. solani* and *D.*
322 *dianthicola*). This bias in the symptomatic plants can result from a non-random distribution of
323 the taxa among populations in seed tubers or/and soils and surface waters before plant
324 infection, or from competitive exclusion during the infection process of potato plants and
325 tubers. In the following, we focused on the two species *D. solani* and *D. dianthicola* to
326 compare experimentally their fitness in the course of plant and tuber infection, and in
327 particular to test the hypothesis of competitive exclusion.

328

329 **3.2 *Dickeya dianthicola* exhibited a fitness advantage over *D. solani* in potato plants.**

330 Plant assays were performed in the greenhouse to compare the incidence of blackleg
331 symptoms after inoculation by *D. dianthicola* or *D. solani* on non-wounded potato plants. Five
332 *D. dianthicola* strains (RNS11-47-1-1A, CFBP1888, CFBP2982, CFBP2015, MIE34) and five
333 *D. solani* strains (3337 = RNS08-23-3-1A, IPO2222, RNS05-1-2A, Ds0432.1, PPO9019)
334 were used in these assays.

335 In a first assay, each strain was inoculated individually on 15 plants and the number of
336 asymptomatic and symptomatic plants were counted at 70 days post inoculation (dpi). A
337 Kruskal-Wallis test revealed differences between *D. solani* and *D. dianthicola* in terms of
338 symptom incidence ($k=7$; $DF=1$; $p=8 \times 10^{-3}$): *D. dianthicola* was found more virulent than *D.*
339 *solani* (percentages of symptomatic plants of $61\% \pm 10$ versus $15\% \pm 11$; **Figure 3a**).

340 In a second plant assay, the five bacterial cultures (i.e., the five strains) of each species were
341 assembled to constitute two experimental populations and each was inoculated on 15 plants.
342 A mixture of the two species (10 strains) was also inoculated on 15 plants. The assay was
343 duplicated (2 x 15 plants per treatment). We compared symptom incidence at 70 dpi.
344 Kruskal-Wallis test revealed differences between the three treatments, i.e., *D. solani* strain
345 mixture, *D. dianthicola* strain mixture and species mixture ($k=6.1$; $DF=2$; $p=0.04$). Pairwise
346 comparison (Post-hoc Tukey test) showed that *D. solani* was less virulent than *D. dianthicola*
347 ($F=-2.5$; $p= 0.03$) and the species mixture ($F=1.9$; $p= 0.10$). However, symptom incidence
348 caused by the species mixture did not differ from those of the *D. dianthicola* mixture ($F=-0.5$;
349 $p= 0.86$). Post-hoc Tukey p-values and mean value (\pm SE) of the percentage of symptomatic
350 plants inoculated by the *D. dianthicola* ($53\% \pm 7$), *D. solani* ($23\% \pm 3$) and mixed ($47\% \pm 0$)
351 populations are presented in the **figure 3b**. To assess the dynamics of the appearance of
352 symptomatic plants, we also drew a disease progress curve for each replicate (**Figure S3a**)
353 and compared the areas under disease progress curve (**Figure S3b**). Pairwise comparisons
354 (Post-hoc Tukey tests) confirmed that *D. solani* was less virulent than *D. dianthicola* ($F=-336$;
355 $p<10^{-11}$) and the species mixture ($F=386$; $p<10^{-11}$).

356 To evaluate which pathogen had emerged at the beginning of the infection process, hence to
357 identify the most probable causative agent of the observed symptoms, we used qPCR to

358 quantify pathogen loads. When the two species mixtures were inoculated separately, *D.*
359 *dianthicola* and *D. solani* reached the mean values \pm SE of $2 \times 10^{11} \pm 1 \times 10^4$ cells and $3 \times$
360 $10^9 \pm 1 \times 10^2$ cells per gram of symptomatic stem tissues, respectively. Among the 14 co-
361 infected plants exhibiting symptoms, eight lesions were randomly chosen for qPCR
362 quantification of the pathogen abundance: this analysis revealed an excess of *D. dianthicola*
363 with a CI median value of 10^{-5} (**Figure 3c**). The eight CI values differed from one (Kruskal-
364 Wallis test, $k=9.5$; $DF=1$; $p= 2 \times 10^{-3}$), meaning that *D. dianthicola* outcompeted *D. solani* in
365 blackleg tissues. Altogether, these data revealed a fitness advantage of *D. dianthicola* in
366 terms of multiplication within lesions in the aerial parts of the host in either the absence or
367 presence of the *D. solani* invader.

368

369 **3.3 *Dickeya dianthicola* exhibited a fitness advantage over *D. solani* in potato plants** 370 **with wounded roots.**

371 To evaluate whether plant wounding could benefit to the *D. dianthicola* resident or to the *D.*
372 *solani* invader, pathogen mixtures were inoculated in pots containing plants with wounded
373 roots. Kruskal-Wallis test revealed differences between the three treatments, i.e., *D. solani*
374 strain mixture, *D. dianthicola* strain mixture and species mixture ($k=29.2$; $DF=2$; $p=4.6 \times 10^{-7}$).
375 Pairwise comparisons (post-hoc Tukey tests) showed that the numbers of plants with
376 symptoms differed between inoculations with *D. solani* and *D. dianthicola* ($F=4.5$; $p=1.9 \times 10^{-5}$)
377 and with the mixture of *D. dianthicola* and *D. solani* ($F=6.1$; $p=2.7 \times 10^{-9}$). Mean \pm SE of the
378 percentage of symptomatic plants reached $33\% \pm 0$ for *D. solani*, $83\% \pm 10$ for *D. dianthicola*
379 and $93\% \pm 7$ for a mixture of *D. dianthicola* and *D. solani*, indicating a stronger virulence of
380 *D. dianthicola* as compared to *D. solani* (**Figure S4a**). Pairwise comparisons (post-hoc Tukey
381 tests) of the areas under the disease curves also confirmed that *D. solani* was less virulent
382 than *D. dianthicola* ($F=386$; $p<10^{-11}$) and than a mixture of *D. dianthicola* and *D. solani*
383 ($F=386$; $p<10^{-11}$) (**Figure S3c-d**). Because the virulence measured using symptom incidence
384 at the final time point (70 dpi) and area under disease progress curve (from 1 to 70 dpi) gave
385 similar results when comparing the two pathogens, only the final point of symptom incidence

386 was retained for the analysis of the following plant assays, in order to facilitate the
387 presentation of the data.

388 In the 27 emerging lesions collected from co-infected plants, qPCR quantification of the
389 pathogens revealed an excess of *D. dianthicola* with a CI median value of 10^{-5} . These CI
390 values were different from one (Kruskal-Wallis test: $k=19.5$; $DF=1$; $p=10^{-5}$; **Figure S3b**).

391 Altogether, these experimental data revealed that the resident *D. dianthicola* was more
392 effective at exploiting unwounded and wounded potato host plants than the *D. solani* invader,
393 *i.e.* *D. dianthicola* had a higher fitness regarding this component of its ecological niche.

394

395 **3.4 *Dickeya solani* exhibited a fitness advantage over *D. dianthicola* in potato tubers.**

396 We compared the *D. dianthicola* and *D. solani* ecological traits in potato tubers.
397 Aggressiveness of the pathogens (*i.e.*, disease severity) was compared using five symptom
398 classes and a disease severity index (DSI) was calculated summarizing the observations of
399 the symptom classes on 10 tubers.

400 In a first tuber rotting assay, each strain was inoculated separately at 10^7 or at 10^5 CFU on
401 each set of 10 tubers. The two different inoculum loads allowed comparing the capacity of
402 the pathogens to initiate maceration and exploit tubers in two contrasting ecological
403 conditions. The assay was performed five times, so we collected symptom class information
404 from 50 tubers for each bacterial strain and for each inoculation load. Kruskal-Wallis tests
405 revealed differences between the two treatments, *D. solani* and *D. dianthicola*, both at high
406 ($k=3.2$; $DF=1$; $p=0.07$) or at low ($k=43.6$; $DF=1$; $p=4 \times 10^{-11}$) inoculum dose. The DSI values
407 were calculated for each set of 10 tubers and mean values \pm SE across the five replicates
408 reached 63 ± 13 for *D. solani* and 47 ± 4 for *D. dianthicola* with an inoculum of 10^7 CFU per
409 tuber (**Figure 4a**) and 49 ± 8 for *D. solani* and 7 ± 4 for *D. dianthicola* with an inoculum of 10^5
410 CFU per tuber (**Figure 4d**).

411 In a second tuber assay, we used strain mixtures for each of the two species and an
412 assembly of the two species to inoculate 10 tubers for each of 10^7 and 10^5 CFU per tuber.
413 This assay was performed twice. Kruskal-Wallis tests revealed differences between the three

414 treatments, i.e., *D. solani*, *D. dianthicola* and the species mixture both at a high ($k=51.3$;
415 $DF=2$; $p=4.2 \times 10^{-11}$) and a low ($k=22.4$; $DF=2$; $p=1.3 \times 10^{-5}$) pathogen load. At both bacterial
416 loads, pairwise comparisons (Post-hoc Tukey tests) showed that aggressiveness was
417 different between *D. solani* and *D. dianthicola* (at a high load: $F=2.7$; $p=0.04$; at a low load:
418 $F=8.6$; $p < 10^{-11}$). Aggressiveness also differed between *D. solani* and the species mixture (at
419 a high load: $F=2.9$; $p=0.01$; at a low load: $F=2.2$; $p=0.07$). A DSI value was calculated for
420 each set of 10 tubers. Mean value \pm SE of the DSI values reached 83 ± 0 (*D. solani*), 63 ± 3
421 (*D. dianthicola*) and 63 ± 8 (*D. solani* and *D. dianthicola*) with an inoculum of 10^7 CFU per
422 tuber (**Figure 4b**) and 69 ± 14 (*D. solani*), 16 ± 6 (*D. dianthicola*) and 48 ± 10 (*D. solani* and
423 *D. dianthicola*) with an inoculum of 10^5 CFU per tuber (**Figure 4e**). Altogether, these tuber
424 assays revealed a higher aggressiveness of *D. solani* compared to *D. dianthicola* and to the
425 species mixture.

426 The qPCR analyses of 10 symptomatic tubers revealed that the abundance of the *D. solani*
427 and *D. dianthicola* strain mixtures ranged from $2 \times 10^9 \pm 3 \times 10^9$ to $9 \times 10^9 \pm 9 \times 10^9$ cells per
428 gram of rotted tissues (mean values \pm SE). These data indicated no substantial differences in
429 the capacity to exploit tubers in terms of population yield. In co-infection assays, qPCR
430 quantification of the pathogens in 10 symptomatic tubers revealed an advantage in favor of
431 *D. solani* regardless of the initial load (CI median value = 5.7; **Figure 4c and 4f**). The CI
432 values were different from one (Kruskal-Wallis tests: $k=16.3$; $DF=1$; p-value of 5×10^{-5} with a
433 high load; $k=7.8$; $DF=1$; $p=0.05$ with a low load).

434 Overall, tubers constituted potato plant tissues in which *D. solani* initiated symptoms more
435 efficiently (even with a low pathogen load), caused more damage (higher disease severity
436 index) and was more competitive than *D. dianthicola* (outcompeting *D. dianthicola*).
437 Remarkably, the disease severity caused by *D. solani* was decreased in the presence of *D.*
438 *dianthicola*.

439

440 **3.5 *Dickeya solani* exhibited a fitness advantage over *D. dianthicola* in hyacinths.**

441 The symptom incidence and competition were further evaluated in hyacinths, that represent
442 important hosts as they constitute a reservoir or/and primary or intermediate host of *D.*
443 *solani*. We expected a higher symptom incidence and growth advantage of *D. solani* on
444 hyacinth host as compared to *D. dianthicola*. The five *D. dianthicola* and five *D. solani*
445 bacterial strains were inoculated separately on eight plants per strain and the number of
446 symptomatic and asymptomatic plants were counted. At 70 dpi, a Kruskal-Wallis test
447 revealed differences between *D. solani* and *D. dianthicola* in terms of symptom incidence
448 ($k=3.03$; $DF=1$; $p=0.08$): *D. solani* was found more virulent than *D. dianthicola*; means \pm SE
449 of the percentage of symptomatic plants reached $43\% \pm 17$ and $23\% \pm 9$, respectively
450 **(Figure S5a)**.

451 In co-inoculation assays with species mixtures **(Figure S5b)**, the qPCR quantification of the
452 pathogens in five symptomatic tissues showed that the calculated CI were different from one
453 (Kruskal-Wallis test: $k=7.8$; $DF=1$; $p= 5 \times 10^{-3}$). The CI median value of 4×10^9 indicated a
454 high competitive advantage of *D. solani* over *D. dianthicola* in rotted tissues of hyacinths.
455 Bulb plants thus appeared promoting competitive exclusion of *D. dianthicola* by *D. solani*,
456 which would lead to an enrichment in *D. solani* in the bulb plant agrosystems.

457

458 **3.6 Pectate lyases gene expression is higher in *Dickeya solani* than in *D. dianthicola***

459 The capacity of *D. solani* to initiate rotting with a low bacterial load suggested a differential
460 expression of pectate lyase genes between the two species. To test this hypothesis, the
461 expression levels of the *peIA*, *peID* and *peIE* genes were quantified by RT-qPCR in the five
462 *D. solani* and five *D. dianthicola* strains grown separately in a rich medium (in the absence of
463 pectin) and in emerging lesions of potato stems and tubers (see a detailed protocol in SM2).
464 The expression of two other virulence genes, *cbsE* and *acsD*, which are not involved in the
465 plant cell wall degradation, but in iron-uptake, were also compared. Four RT-qPCR
466 measurements were performed per condition and gene. For each gene, Kruskal-Wallis tests
467 revealed differences between the six treatments (rich medium, stems and tubers for the two
468 species, considering each strain as a replicate) for the different genes: *peIA* ($k=17.1$; $DF=5$;

469 $p=4 \times 10^{-3}$), *pelD* ($k=25.1$; $DF=5$; $p=1 \times 10^{-4}$), *pelE* ($k=24.9$; $DF=5$; $p=1 \times 10^{-4}$), *cbsE* ($k=20.3$;
470 $DF=5$; $p=1 \times 10^{-3}$) and *acsD* ($k=21.3$; $DF=5$; $p=7 \times 10^{-4}$).

471 Pairwise post-hoc Tukey tests were used to compare gene expression between *D. solani*
472 and *D. dianthicola*. In the absence of pectin (**Figure 5**), the *pelA* ($F=864.3$; $p < 10^{-11}$), *pelD*
473 ($F=864.3$; $p < 10^{-11}$) and *pelE* ($F=2.9$; $p=4 \times 10^{-3}$) genes were expressed at a higher level in *D.*
474 *solani* than in *D. dianthicola*. The expression levels of the *cbsE* ($F=0.377$; $p=0.99$) and *acsD*
475 ($F=-1.0$; $p=0.99$) genes were not significantly different between species (**Figure S6**). In the
476 absence of pectin, a higher expression of pectate lyase genes, especially *pelE*, could be
477 considered as an advantage for initiating a rapid degradation of the plant cell wall polymers.
478 In symptomatic potato tubers and stems, some remarkable gene expression patterns could
479 be noticed (**Figures 5 and S6**). The expression levels of *pelD* and *pelE* were higher in *D.*
480 *solani* than in *D. dianthicola* (*pelD*: $F=864.3$; $p < 10^{-11}$ in tubers and $F=4.5$; $p=10^{-4}$ in stems;
481 *pelE*: $F=4.5$; $p=1.1 \times 10^{-4}$ in tubers and $F=8.1$; $p < 10^{-11}$ in stems). The *pelA* ($F=864.3$; $p < 10^{-11}$) and *cbsE* ($F=3.4$; $p=0.01$) genes were expressed at higher levels in *D. solani* than in *D.*
482 *dianthicola* stem tissues, but not in tubers (*pelA*: $F=-0.09$; $p=1$ and *cbsE*: $F=0.67$; $p=0.99$).
483 The *acsD* expression level did not differ between the two species in diseased tubers ($F=-1.5$;
484 $p=0.9$) and stems ($F=0.5$ $p=0.9$). Overall, in plant symptoms, *D. solani* maintained a higher
485 expression of the virulence genes *pelD* and *pelE* compared to *D. dianthicola*.
486

487

488 **3.7 *Dickeya solani* genomics revealed two *vfmB* alleles at balanced frequencies**

489 We used a population genomic approach to investigate additional ecological traits of the *D.*
490 *solani* invading population. We studied SNPs and InDels in 76 *D. solani* genomes (**Figure**
491 **S2**), 56 of which were assembled after Illumina sequencing in this study, while the 20 others
492 had been previously acquired using the same sequencing approach (Khayri et al., 2015). Out
493 of the 76 genomes, 66 exhibited from 2 to 11 SNPs and InDels when compared to the *D.*
494 *solani* 3337 reference genome (**Figure S2**). In the nine others, more than 100 SNPs were
495 observed: some of these SNPs were clustered in regions of the chromosome (RNS13-30-1A,

496 PPO9134, PPO9019, RNS13-31-1A, RNS13-48-1A, RNS15-102-1A, RNS07-7-3B) while
497 others were distributed along the genome (RNS05-1-2A and RNS10-105-1A).

498 Focusing on the 66 genomes exhibiting less than 12 SNPs compared to the strain 3337, 45
499 non-synonymous variations were identified in whole genomes (**Table S6**). Two-third of them
500 (30 out of 45) were present in one genome only, while the 15 others were found in at least
501 two genomes. Among these 15, three SNPs were identical in four strains, two others in five
502 strains and one was present in 19 genomes (**Table S6 and Figure 6a**). The most balanced
503 frequencies consisted in a thymine (T) in 19 genomes and a cytosine (C) residue in the other
504 48 genomes at the position 2,930,940 in the reference *D. solani* 3337 genome (**Figure 6a**).
505 The C to T modification caused a proline (VfmB_{Pro} allele) to serine (VfmB_{Ser} allele) change at
506 the position 55 in the VfmB protein. Such SNPs affecting an amino-acid in the *vfmB* gene
507 retained our attention because the Vfm quorum-sensing system is involved in the regulation
508 of some virulence traits in *Dickeya* (Nasser et al., 2013; Potrykus et al., 2018).

509 A SNP-based tree of these 67 strains (**Figure 6b**) revealed that the *D. solani* isolates
510 carrying VfmB_{Ser} were placed in two different sub-clusters, one with only two VfmB_{Ser} *D.*
511 *solani* strains, EU3296 and Ds0432.1, and the other mainly VfmB_{Ser} strains. This observation
512 suggests that the VfmB_{Ser} allele could have appeared independently in two different *D. solani*
513 lineages, thus representing convergence. The VfmB_{Ser} allele was present in 25% (19 out of
514 76) of all analyzed *D. solani* genomes. A similar percentage (27%, 17 out of 63) was
515 observed when only the French isolates were considered. Using the isolation year of these
516 63 *D. solani* isolates, we drew the dynamics of the percentage of the VfmB_{Ser} alleles, which
517 was found to fluctuate over the past decade (2005-2015) with a peak in 2013-2014 (**Figure**
518 **6c**).

519 We also investigated the presence of the VfmB_{Pro} and VfmB_{Ser} alleles in other *Dickeya*
520 species. The alignments of VfmB proteins retrieved from genomic databases revealed the
521 occurrence of the VfmB_{Pro} allele in *D. dadantii*, *D. chrysanthemi* and *D. dianthicola* genomes,
522 while the VfmB_{Ser} allele was found in *D. zea* genomes (**Figure S6**). However, the low

523 number of genomes available let open the possibility of the existence of additional alleles in
524 each of these *Dickeya* species.

525

526 **3.8 VfmB_{Ser} strains were more aggressive than VfmB_{Pro} strains in soft-rot assays**

527 The modeling of the *D. solani* VfmB_{Pro} and VfmB_{Ser} protein structures predicted a
528 conformational difference in the beta-sheet structure of the virulence regulator VfmB (**Figure**
529 **7a**). This change is explained by the exceptional conformational rigidity of proline that
530 strongly affects secondary structures such as alpha helices and beta sheets. The modeling
531 data prompted us to test whether VfmB_{Pro}-VfmB_{Ser} variation could be associated with a
532 change in aggressiveness. To compare the aggressiveness of *D. solani* VfmB_{Ser} and VfmB_{Pro}
533 isolates, we used genomic data to identify a set of 8 isolates carrying either VfmB_{Pro}
534 (IPO2222, MIE35, AM3a and 3337) or VfmB_{Ser} (Ds0432.1, RNS10-27-2A, Sp1a and M21a),
535 in which the other variations were at different positions (**Tables S3 and S4**). Soft-rot assays
536 using 10 inoculated potato tubers per strain revealed that the set of the VfmB_{Ser} strains was
537 more aggressive (Kruskal-Wallis test; $k=9.5$; $DF=1$; $p= 2 \times 10^{-3}$) than that of the VfmB_{Pro}
538 strains (**Figure 7b**).

539 To test the existence of differential expression of virulence genes in tuber tissues, we
540 compared transcriptomes of *D. solani* IPO2222 (VfmB_{Pro}) and Ds0432.1 (VfmB_{Ser}) (**Figure**
541 **7c**; **Table S7**). The expression of four genes found differentially expressed based on
542 transcriptomics (*pelE*, *impC*, *cytA* and *budA*) was further investigated by RT-qPCR in the
543 same samples used for transcriptomics: they found to be also upregulated (**Figure S8**).
544 Overall, 170 differentially expressed genes (adjusted p-value < 0.05 and absolute log2 fold
545 change > 2) were identified by comparative transcriptomics. Most of them (150 genes) were
546 upregulated in Ds0432.1 carrying the VfmB_{Ser} allele (**Table S8**). We studied more specifically
547 35 well-characterized *D. solani* virulence genes (Raoul des Essarts et al., 2019). We
548 observed an enrichment of these virulence genes among the upregulated genes in *D. solani*
549 Ds0432.1, while none of them was found among the downregulated genes (**Table S9**).
550 These upregulated virulence genes in *D. solani* Ds0432.1 included the pectate lyase genes

551 *pelB*, *pelC*, *pelD*, *pelE* and *pelL*, the protease genes *ptrA*, *prtB* and *prtC*, and the T6SS-
552 related toxin/antitoxin genes *hcp* (**Table S9**). These upregulated genes encompassed
553 virulence determinants that are already known to be regulated by the Vfm quorum-sensing in
554 *D. dadantii* (Nasser et al., 2013).

555

556 **3.9 Fitness costs and gains associated with VfmB alleles in potato stems and tubers**

557 We used the same eight isolates carrying either VfmB_{Pro} (IPO2222, MIE35, AM3a and 3337)
558 or VfmB_{Ser} (Ds0432.1, RNS10-27-2A, Sp1a and M21a) to constitute VfmB_{Pro} and VfmB_{Ser}
559 experimental populations for comparing their fitness in plant assays.

560 In soft-rot assays (**Figure 8a-b**), each of the two experimental populations and their mixture
561 was inoculated on 10 tubers and a symptom class was assigned to each tuber. Two
562 inoculation loads were used (10^7 CFU or 10^5 CFU per tuber). This assay was performed in
563 triplicate (3 x 10 tubers per treatment). Kruskal-Wallis tests revealed differences between the
564 three treatments, i.e., inoculation by *D. solani* VfmB_{Pro}, *D. solani* VfmB_{Ser} and their mixture,
565 and the differences were significant at both high ($k=17.6$; $DF=2$; $p=1.5 \times 10^{-4}$) and low ($k=7.2$;
566 $DF=2$; $p=0.03$) pathogen loads. Whatever the bacterial load, pairwise comparisons (Post-hoc
567 Tukey tests) showed that aggressiveness (i.e. symptom severity) was different between *D.*
568 *solani* VfmB_{Pro} and *D. solani* VfmB_{Ser} (at a high load: $F=4.6$; $p=9.5 \times 10^{-6}$; at a low load:
569 $F=0.8$; $p=0.01$). The *D. solani* VfmB_{Ser} population was more aggressive than the *D. solani*
570 VfmB_{Pro} population (DSI mean value \pm SE of 92 ± 2 for VfmB_{Ser} vs 73 ± 3 for VfmB_{Pro} at a
571 high load and 45 ± 2 for VfmB_{Ser} vs 26 ± 9 for VfmB_{Pro} at a low load; **Figure 8a-b**). VfmB_{Ser}
572 *D. solani* strains thus more efficiently damaged tubers than VfmB_{Pro} *D. solani* strains.

573 When the VfmB_{Ser} and VfmB_{Pro} *D. solani* experimental populations were co-infected in tubers,
574 shotgun sequencing of the pathogens recovered from symptoms allowed to calculate CI
575 values (**Figure 8a-b**). With a load of 10^7 CFU per tuber, the CI values (8 values; median =
576 1.3) were not different from one (Kruskal Wallis test; $k=0.8$; $DF=1$; $p=0.4$). With a load of 10^5
577 CFU per tuber, the CI values (3 values; median = 2) differed from one (Kruskal Wallis test;

578 $k=4.4$; $DF=1$; $p= 0.04$). In tubers, the *VfmB_{Pro}* allele thus appeared neutral or to confer a
579 moderate advantage as compared to the *VfmB_{Ser}* allele.

580 In blackleg symptom assays (**Figure 8c**), the *D. solani VfmB_{Pro}* and *D. solani VfmB_{Ser}*
581 experimental populations and their mixture were inoculated on non-wounded plants and the
582 number of symptomatic and asymptomatic plants were counted. The assay was performed in
583 triplicate: two assays with 25 plants and one assay with 15 plants per treatment. Kruskal-
584 Wallis tests revealed no difference between the three treatments, i.e., the *D. solani VfmB_{Pro}*
585 strain mixture, the *D. solani VfmB_{Ser}* strain mixture and their mixture ($k=0.29$; $DF=2$; $p=0.86$).
586 Shotgun sequencing of bacteria recovered from symptoms allowed calculation of CI values in
587 16 co-infected plants (**Figure 8c**). These CI values were different from one (Kruskal-Wallis
588 test; $k=6.6$; $DF=1$; $p= 9 \cdot 10^{-3}$) with a median CI of 14, showing a fitness advantage of the
589 *VfmB_{Pro}* allele in emerging lesions in the stems.

590 Altogether, the plant assays indicated that the *VfmB_{Ser}* allele was associated to a higher
591 aggressiveness in the rotted tubers compared to the *VfmB_{Pro}* allele. This suggested that the
592 *VfmB_{Ser}* allele could more efficiently initiate maceration of tubers in the field and storage
593 conditions. In contrast, the *VfmB_{Ser}* allele seemed to confer a lower competitive fitness than
594 the *VfmB_{Pro}* allele when *D. solani* proliferates in rotted tubers and, particularly, in stem
595 symptoms. Such opposite gains across different conditions could contribute to the
596 maintenance of balanced frequencies of the two alleles in *D. solani* populations.

597

598 **4 Discussion**

599 Two successive invasions of *Dickeya* potato pathogens occurred in Europe: first *D.*
600 *dianthicola* in the middle of the 20th century, this pathogen being now considered as endemic,
601 and second, *D. solani* at the beginning of the 21st century. *Dickeya solani* and *D. dianthicola*
602 cause similar symptoms on potato plants, so that they are expected to compete for the same
603 resources. Over ten years, our epidemiologic surveys revealed a successful establishment of
604 *D. solani* together with a maintenance of *D. dianthicola*. Our experiments contributed to

605 explain such puzzling co-existence by contrasted advantages in different parts of the plants
606 and therefore partially different ecological niches.

607 By comparing ecological traits of *D. solani* and *D. dianthicola*, using multiple strains
608 representative of the population diversity, we indeed found contrasted behaviors in these
609 species with respect to aerial parts versus tubers of *S. tuberosum*. The resident *D.*
610 *dianthicola* more efficiently exploited host stems after an inoculation of the wounded and
611 unwounded roots than did the invader *D. solani*, *i.e.*, causing higher symptom incidence, and
612 exhibiting greater proliferation and relative fitness in competition assays. In contrast, *D.*
613 *solani* more efficiently initiated the rotting process (especially at a low bacterial inoculum) and
614 outcompeted *D. dianthicola* in tubers, with potential consequences on tubers in the fields and
615 during storage. Hence, our assays suggest that infected tubers could facilitate the entry and
616 establishment of the *D. solani* invader into the potato agrosystems. A contrasting defense
617 response of potato foliage and tuber against *Phytophthora infestans* has been reported (Gao
618 & Bradeen, 2016). In addition to the intrinsic characteristics of *D. solani* and *D. dianthicola*,
619 an organ-specific response of the plant defense system could similarly also contribute to their
620 difference in behavior.

621 The capacity of *D. solani* to cause rotting at a lower bacterial load than *D. dianthicola* is likely
622 at least partly due to a higher expression of the *pel* enzymes, especially PelE that acts as an
623 initiator of the plant cell wall degradation (Duprey et al., 2016). By comparing the expression
624 of some *pel* genes in emerging lesions in potato stems and tubers, we observed a higher
625 expression of the virulence genes *pelD* and *pelE* in *D. solani* compared to *D. dianthicola*.
626 Remarkably, by comparing *pel* genes in several *Dickeya* species, Duprey et al. (2016)
627 showed that *D. solani* evolved specific regulatory sequences that contributes to a different
628 expression level of the *pelD* and *pelE* genes compared to *D. dianthicola*. The *pelA* gene is
629 truncated in *D. dianthicola* (Duprey et al., 2016; Raoul des Essarts et al., 2019), but full-
630 length and expressed in *D. solani* (as shown in this study), reinforcing their virulence arsenal.
631 Other metabolic traits could also contribute to the fitness advantage of *D. solani* for exploiting
632 tubers: comparative transcriptomics revealed a higher expression of the glyoxylate shunt in

633 *D. solani* than in *D. dianthicola*, a pathway that contributes to exploit alternative carbon
634 sources when sugar availability is low (Raoul des Essarts et al., 2019).

635 Beside the tripartite interaction between potato plant host, *D. solani* and *D. dianthicola*,
636 additional environmental (soil and climate) and biological factors (microbiota including
637 *Pectobacterium* species, other hosts and non-host plants) may facilitate or limit *D. solani*
638 establishment (Charkowsky 2018; Shyntum et al., 2019; Toth et al., 2011). We showed that
639 *D. solani* efficiently outcompeted *D. dianthicola* in hyacinths, in line with the epidemiologic
640 data supporting the bulb plants as potential intermediate hosts with an important role in the
641 recurrent invasion of potato agrosystems (Chen, Zhang, & Chen, 2015; Slawiak et al., 2009;
642 van der Wolf et al., 2014). Prophylactics may allow circumventing the propagation of *D.*
643 *solani* from an agrosystem to another. Competition and facilitation processes have been well
644 studied in pathogenic fungi (Al-Naimi, Garrett, & Bockus, 2005; Abdullah et al., 2017;
645 Gladieux et al., 2015; Zhan & McDonald, 2013). Another factor that could reduce competition
646 between the two *Dickeya* species is their natural low abundance in soils and surface waters,
647 as well as in seed tubers that are subjected to prophylactic diagnosis. A small population size
648 belonging to a single species could proliferate in a plant individual without any interactions
649 with another *Dickeya* or *Pectobacterium* pathogen species. These effects linked to
650 population size and dispersal are expected to delay invasion. In line with this hypothesis, the
651 observed slow increase of the percentage of *Dickeya*-positive fields over one decade (2004-
652 2015) suggested that the *Dickeya* invasion was still ongoing. Among the emerging literature
653 on microbial invasion, the *D. solani* pathogen appears as a good example illustrating how
654 different ecological components (here, the plant host and a resident pathogen) should be
655 considered to understand the biological invasion by a bacterial pathogen (Cadotte et al.,
656 2018; Germain, Mayfield, & Gilbert, 2018).

657 The genome sequencing data indicating low diversity in *D. solani* suggest a bottleneck during
658 introduction in Europe. The dispersal modes (either horizontally via soil, surface water,
659 insects and some agricultural practices or vertically by asymptotically contaminated seed
660 tubers) could contribute to further bottlenecks in *D. solani* (Charkowsky 2018; Toth et al.,

2011). Selection on some genes may also have further reduced genetic diversity through selective sweeps across the whole genomes as these bacteria are clonal. Allelic changes related to quorum-sensing systems were already observed in different plant and animal pathogens along the host-colonization process (Feltner et al., 2016; Guidot et al., 2014; Tannières, Lang, Barnier, Shykoff, Faure, 2017), indicating that balancing selection contributed to maintain their variability. Remarkably, we observed balanced frequencies of two alleles in the VfmB protein involved in the Vfm-type quorum-sensing in *Dickeya* bacteria. The chemical structure of the Vfm signal is not known, but it is known to be involved in tuber rotting and upregulation of some virulence genes including pectate lyases (*pel* genes), proteases (*prt* genes) and cellulases (*cel* genes) (Nasser et al., 2013; Potrykus et al., 2018). Using tuber assays, we observed a slightly higher aggressiveness in VfmB_{ser} strains compared to VfmB_{pro} strains. Using transcriptomics of *D. solani* pathogens recovered from tuber symptoms, we confirmed an enrichment of upregulated genes, including *pel* and *prt* genes, in the VfmB_{ser} strain Ds0432.1 compared to VfmB_{pro} strain IPO2222. Because of the role of the VfmB protein in Vfm signaling is not completely elucidated, it was premature to go deeper into the mechanistic characterization of the VfmB_{ser} and VfmB_{pro} alleles. In a near future, the reconstruction of allelic mutants and comparison with the wild strain with otherwise the same genetic background should help characterizing the role of VfmB in Vfm quorum-sensing and ecology of *D. solani*.

In this work, we strongly suggest that the VfmB_{ser} strains are more aggressive than the VfmB_{pro} strains in potato tubers, but not more virulent in potato stem assays, and that the VfmB_{ser} strains were less competitive than the VfmB_{pro} strains in tuber and stem symptoms. Such an environment-dependent advantage of VfmB_{ser} and VfmB_{pro} alleles in plant infection assays predicts balancing selection in natural populations. The VfmB_{ser} allele could thus provide an advantage when competition is reduced at the beginning of the establishment in potato agrosystems and under a high dispersal condition. In contrast, VfmB_{ser} could be outcompeted by VfmB_{pro} when *D. solani* is already established. Our epidemiologic data revealed up and down variation of VfmB_{ser} relative abundance in field populations over the

689 past decade, in agreement with an increase of competition between *D. solani* VfmB alleles in
690 the more recent sampling year. There is a very low number of SNPs/InDels (<12) in the
691 genomes beyond those in Vfm, however, functional genetics will be required to fully validate
692 the causal role of the Vfm alleles.

693 In conclusion, this study using complementary approaches, multiple strains representative of
694 the population diversity and multiple ecological conditions, brings novel insights allowing a
695 better understanding of the pattern and causes of the *D. solani* invasion into potato
696 production agrosystems, and the reasons why *D. dianthicola* nevertheless persisted. More
697 broadly, this study contributes to our understanding the ecological determinants of pathogen
698 invasion and of the conditions for the maintenance of endemic competitors.

699

700 **ACKNOWLEDGEMENTS**

701 PB was supported by a PhD grant from Université Paris-Sud (Université Paris-Saclay), KR
702 was supported by a PhD grant from FN3PT/innov3PT with the support of ANRT
703 (N°2016/0800). KGC thanks the financial support from the University of Malaya (FRGS grant
704 FP022-2018A and HIR grant H-50001-A000027). This work has benefited from the facilities
705 and expertise of the high throughput sequencing platform and the plant culture facilities at
706 I2BC (Gif-sur-Yvette). This work was supported by CNRS (SE2016-2017-2018), French
707 Federation of Seed Potato Growers (FN3PT/innov3PT), Agence Nationale de la Recherche
708 (ANR-15-CE21-0003) and LabEx Saclay Plant Sciences-SPS (ANR-10-LABX-0040-SPS).

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859 DATA ACCESSIBILITY

860 DNA sequences: Genbank accessions VYSC00000000; VZQE00000000; VZQF00000000;
861 VZQG00000000; VZQH00000000; VZQI00000000; VZQK00000000; VZQJ00000000;
862 VZQL00000000; VZQM00000000

863

864 **AUTHOR CONTRIBUTION**

865 DF, PB, VH conceived experiments; JC, EM, PD, AL, AB and VH performed field sampling
866 and molecular diagnosis; PB characterized fitness traits in *D. solani* and *D. dianthicola*; PB,
867 KR and EM characterized fitness traits in *D. solani vfmB* variants; PB performed RNA
868 analyses (RT-qPCR and transcriptomics); YJ, KWH, KGC, AL and PB prepared DNA
869 samples and performed sequencing; SK compared *D. solani* genomes; PB, KR and DF
870 performed statistical analyses; DF, TG, PB, VH, SR wrote the manuscript.

871

872 **CONFLICT OF INTEREST**

873 No potential conflict of interest was disclosed.

874

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879 **Figure legends**

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883 **Figure 1 Symptoms caused by *Dickeya* pathogens.** Symptoms (white arrows) caused by
884 *Dickeya solani* and *Dickeya dianthicola* on potato stems (a) and hyacinths (b). The five symptom
885 classes (0 to 4) used to compare the aggressiveness of *Dickeya solani* and *Dickeya dianthicola*
886 on potato tubers (c).

887

888 **Figure 2 *Dickeya* and *Pectobacterium* prevalence from potato fields exhibiting blackleg**
889 **symptoms.** Percentage of *Pectobacterium*-containing and *Dickeya*-containing fields (a) and that
890 of *D. solani*-containing fields and *D. dianthicola*-containing fields (b) were calculated each year
891 from 2004 to 2015 (with the exception of 2006). (c) Number and relative abundance of the
892 *Pectobacterium*, *D. dianthicola* and *D. solani* isolates collected in 19 symptomatic fields sampled
893 from 2013 to 2016. A hierarchical clustering paired group method delineated four pathogen
894 population groups, i.e. *Pectobacterium* only (I: 3 fields), *D. solani* and *Pectobacterium* (II: 9 fields),
895 *D. dianthicola* and *Pectobacterium* (III: 3 fields), and *D. dianthicola*, *D. solani* and *Pectobacterium*
896 (IV: 4 fields).

897

898 **Figure 3 Symptom incidence and fitness of *Dickeya dianthicola* and *D. solani* in potato**
899 **plants.** In a, mean value and standard error (SE) of the percentage (%) values of plants exhibiting
900 blackleg symptoms, which were measured after inoculation of each of the five *D. solani* strains
901 (3337, IPO2222, RNS05-1-2A, Ds0432.1, PPO9019) and five *D. dianthicola* strains (RNS11-47-1-
902 1A, CFBP1888, CFBP2982, CFBP2015, MIE34) on 15 plants. The p-value of the Kruskal-Wallis
903 test comparing of the symptomatic classes between the two species is indicated below the graph.
904 In b, mean value and SE between two replicates of the percentages (%) of plants exhibiting
905 blackleg symptoms which was measured on 15 plants inoculated by *D. dianthicola* and *D. solani*
906 populations and their mixture. The p-values of the pairwise comparisons (Post-hoc Tukey tests) of
907 the symptomatic classes are indicated below the graph, when $p \leq 0.1$. In c, competitive index (CI)
908 values between *D. solani* and *D. dianthicola* populations were calculated in eight emerging lesions
909 of co-infected plants and revealed a competitive advantage of *D. dianthicola*: the CI median (= 2.4
910 10^{-5}) is represented by a thick bar; p-values resulting from Kruskal-Wallis tests testing difference
911 from one are indicated. Legend: * for $0.05 < p \leq 0.1$; ** for $0.01 < p \leq 0.05$ and *** for $p \leq 0.01$.

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918 **Figure 4 Aggressiveness and fitness of *Dickeya dianthicola* and *D. solani* in potato tubers.**
919 Rotting assays were conducted by inoculating each tuber by either 10^7 colony-forming units (CFU)
920 (in **a**, **b** and **c**) or 10^5 CFU of pathogens (in **d**, **e** and **f**). In **a**, **b**, **d** and **e**, each disease severity
921 index (DSI) value was calculated using symptom classes observed on 10 tubers. In **a** and **d**, mean
922 values and standard errors (SE) of DSI values were measured for each of the five *D. solani* strains
923 (3337, IPO2222, RNS05-1-2A, Ds0432.1, PPO9019) and five *D. dianthicola* strains (RNS11-47-1-
924 1A, CFBP1888, CFBP2982, CFBP2015, MIE34) using five (**a**) and two (**d**) independent
925 experiments. The p-values of the Kruskal-Wallis tests comparing symptom classes are indicated
926 below the graphs. In **b** and **e**, mean values and standard errors of DSI values were measured
927 twice for each of the experimental populations of *D. dianthicola*, *D. solani* and the mixture of the
928 two. The p-values of the pairwise Tukey tests comparing the symptomatic classes are indicated,
929 when $p \leq 0.1$. In **c** and **f**, the competitive index (CI) between *D. solani* and *D. dianthicola*
930 populations was calculated in 10 co-infected symptomatic tubers: the median values (indicated by
931 a thick bar) reached 5.7 (**c**) and 5.8 (**f**) and the CI values were statistically different from one
932 (Kruskal-Wallis test; $p = 5 \cdot 10^{-5}$ and $p = 0.05$ respectively), revealing a competitive advantage of
933 *D. solani*. Legend: * for $0.05 < p \leq 0.1$; ** for $0.01 < p \leq 0.05$ and *** for $p \leq 0.01$.

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936 **Figure 5 Expression of the pectate lyase genes *pelA*, *pelD* and *pelE*.** The expression level of
937 the *pelA*, *pelD* and *pelE* genes was evaluated in each of the five *D. solani* strains (3337, IPO2222,
938 RNS05-1-2A, Ds0432.1, PPO9019) and five *D. dianthicola* strains (RNS11-47-1-1A, CFBP1888,
939 CFBP2982, CFBP2015, MIE34), grown in three conditions: a rich culture medium in the absence
940 of pectin (exponential growth phase) and symptomatic tubers (at 5 days post infection) and
941 emerging lesions in stems. Relative expression was measured four times and normalized using
942 the *rpoB* and *yafS* gene expression. In the graphs, the mean values and standard error (SE) of
943 gene expression from all strains of a given species are indicated, as well as p-values of pairwise
944 comparisons by Tukey tests. Legend: * for $0.05 < p \leq 0.1$; ** for $0.01 < p \leq 0.05$ and *** for $p \leq 0.01$.

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946

947 **Figure 6 Population genomics of *Dickeya solani*.** In **a**, scan of 67 *D. solani* genomes revealed
948 that the genome position 2,930,940, (according the *D. solani* 3337 genome), in the *vfmB* gene,
949 was the most balanced non-synonymous variation, with alternated $VfmB_{Pro}$ (71%) and $VfmB_{Ser}$
950 (29%) alleles. In **b**, single nucleotide polymorphism (SNP)-based tree of *D. solani* strains using
951 PHYLOViZ; the name of the French isolates is indicated in blue font; the $VfmB_{Ser}$ and $VfmB_{Pro}$
952 strains used in the plant assays are underlined. In **c**, dynamics of the $VfmB_{Ser}$ allele (%) among
953 the *D. solani* isolates along the sampling period 2005-2015 in France.

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958 **Figure 7 Characterization of the VfmB alleles.** In **a**, using the *Escherichia coli* AidB protein (the
959 PDB accession is 3DJL) as a model, the Phyre2-predicted structure of the VfmB protein showed
960 conformational differences in the beta-sheet associated with the VfmB_{Pro} and VfmB_{Ser} alleles; the
961 Pro55 and Ser55 positions are in red color in the VfmB representations obtained using the EzMol
962 web server. In **b**, mean values and standard error (SE) of disease severity index (DSI) values,
963 which were calculated by recording symptom classes on 10 tubers infected by 10⁷ CFU of each of
964 the *D. solani* strains carrying either VfmB_{Pro} (IPO2222, MIE35, AM3a and 3337) or VfmB_{Ser}
965 (Ds0432.1, RNS10-27-2A, Sp1a and M21a); the p-value of the Kruskal-Wallis test comparing the
966 four VfmB_{Pro} and four VfmB_{Ser} strains using symptom classes is indicated below the graph. In **c**,
967 comparative transcriptome of *D. solani* 3337 (VfmB_{Pro}) and Ds0432.1 (VfmB_{Ser}) recovered from
968 soft-rot lesions in potato tubers; upregulated virulence genes were enriched in Ds0432.1 (VfmB_{Ser})
969 as illustrated by the *pelB*, *pelC*, *pelC*, *hcpA* and *prtA* genes (closed circles).

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973 **Figure 8 Aggressiveness, symptom incidence and fitness assays of two *Dickeya solani***
974 **experimental populations expressing the VfmB alleles.** Soft-rot assays were conducted by
975 inoculating each tuber by either 10⁷ colony-forming units (CFU) (**a**) or 10⁵ CFU (**b**) of the VfmB_{Pro}
976 and VfmB_{Ser} *D. solani* populations and a mixture of the two. In **a** and **b**, each disease severity
977 index (DSI) value (measuring aggressiveness) was calculated using symptomatic classes
978 observed on 10 tubers; the assays were performed in triplicate. The p-values of the pairwise
979 Tukey tests comparing the symptom classes are indicated, when p ≤ 0.1. In **c**, percentage (%) of
980 plants exhibiting blackleg symptoms was measured on plants inoculated by the VfmB_{Pro} and
981 VfmB_{Ser} *D. solani* populations and a mixture of the two. These assays measuring symptom
982 incidence were performed in triplicate. Kruskal-Wallis test revealed no difference between the
983 three treatments using symptom classes (p = 0.86). In **a**, **b** and **c**, competitive index (CI) values of
984 VfmB_{Pro} and VfmB_{Ser} populations were calculated using allele counts based on shotgun
985 sequencing of bacterial populations recovered from co-infected tissues. Median values of CI are
986 represented as thick lines and reached 1.7 (8 repeats in **a**), 2.0 (3 repeats in **b**) and 14.0 (16
987 repeats in **c**) in the three treatments. Statistical differences between CI values and 1 (Kruskal-
988 Wallis test) revealed that neither *D. solani* VfmB_{Pro} or *D. solani* VfmB_{Ser} had significant advantage
989 in tubers inoculated with a high load (p = 0.3 in **a**), but *D. solani* VfmB_{Pro} was more competitive in
990 tubers inoculated with a low load (p = 0.04 in **b**) and in stems (p = 9 x 10⁻³ in **c**). Legend: * for
991 0.05 < p ≤ 0.1; ** for 0.01 < p ≤ 0.05 and *** for p ≤ 0.01.

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