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

Pauline Blin[1], Kévin Robic[2], Slimane Khayi[1], Jérémy Cigna[2], Euphrasie Munier[2], Pauline Dewaegeneire[2], Angélique Laurent[2], Yan Jaszczyszyn[1], Kar-Wai Hong[3], Kok-Gan Chan[3], Amélie Beury[4], sylvie Reverchon-Pescheux[5], Tatiana Giraud[6], Valérie Hélias[4], and Denis Faure[1]

[1]CNRS
[2]French Federation of Seed Potato Growers
[3]Jiangsu University
[4]French Federation of Seed Potato Growers
[5]INSA Lyon
[6]Université Paris-Sud

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Abstract

Invasive pathogens can be a threat when they affect human health, food production or ecosystem services, by displacing resident species, and we need to understand the cause of their establishment. We studied the patterns and causes of the establishment of the pathogen Dickeya solani that recently invaded potato agrosystems in Europe by assessing its invasion dynamics and its competitive advantages or disadvantages against the closely-related resident D.dianthicola species. Epidemiological records over one decade in France revealed the establishment of D.solani and the maintenance of the resident D.dianthicola in potato fields exhibiting blackleg symptoms. Using experimentations, we showed that D.dianthicola was more aggressive than D.solani on aerial parts, while D.solani was more aggressive on tubers. In co-infection assays, D.dianthicola outcompeted D.solani in aerial parts, while D.solani and D.dianthicola co-existed in tubers. A comparison of 76 D.solani genomes (56 of which having been sequenced here) revealed balanced frequencies of two uncharacterized alleles, VfmBPro and VfmBSer, at the vfmB virulence gene. Experimental inoculations showed that the VfmBSer population was more aggressive on tubers, while VfmBPro and VfmBSer populations exhibited a similar aggressiveness on stems. In stem co-infections, the VfmBPro population outcompeted the VfmBSer population, while they co-existed in tubers. This study thus brings novel insights allowing a better understanding of the pattern and causes of the D.solani invasion into potato production agrosystems, and the reasons why D.dianthicola nevertheless persisted. More broadly, this study contributes to our understanding the ecological determinants of pathogen invasion and of the conditions for the maintenance of endemic competitors.

KEYWORDS

competition; *Dickeya;* invasion; plant pathogens; population genomics; quorum-sensing; *Solanum tuberosum* ; transcriptomics; bacteria; competitive exclusion

1 Introduction

Biological invasions represent major threats to biodiversity as invasive species can replace native species. Evolutionary and ecological studies are of major importance for understanding the patterns and causes of

biological invasions and plant and animal invasions have been extensively studied since the 19th century (Burns, Murphy, & Zheng, 2018; Cadotte, Campbell, Li, Sodhi, & Mandrak, 2018; MacDougall, Gilbert, & Levine, 2009). Evolutionary and ecological approaches aim at elucidating the dynamics of invasions as well as the advantages of invaders that allow them to outcompete resident species (Facon et al., 2006; Gladieux et al., 2015). Although microbial invasions can represent important threats to ecosystems and human societies, they have been much less studied than invasive plants and animals (Gladieux et al., 2015; Kinnunen *et al* ., 2016; Litchman, 2010; Mallon, Elsas, & Salles, 2015; Perkins, Leger, & Nowak, 2011).

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

Dickeya solani has emerged in potato production systems in Europe much more recently, in the early 2000s (Toth et al., 2011; van der Wolf et al., 2014). Aside from potatoes, *D. solani* has been isolated from ornamental plants such as *Hyacinthus* , *Iris* and*Muscari* , but the primary host(s) of that species remain(s) uncertain (Chen, Zhang, & Chen, 2015; Slawiak et al., 2009; van der Wolf et al., 2014). Population genomics revealed little genetic variation in *D. solani* , in agreement with a recent spread of this species following a bottleneck during invasion (Golanowska et al., 2018; Khayi et al., 2015). An analysis of 20 *D. solani* genomes identified a non-synonymous single nucleotide polymorphism (SNP) in each of the two *fliC* and *fliN* genes that are involved in bacterial motility (Khayi et al., 2015). Horizontal gene transfer events from *D. dianthicola* to *D. solani* have been documented, adding or replacing genomic fragments (Khayi et al., 2015).

The *D. solani* invader, as well as the *D. dianthicola* and*Pectobacterium* spp. residents, are necrotrophic pathogens causing similar symptoms, resulting in blackleg disease on stems and soft-rot disease of tubers (Charkowski 2018; Toth et al., 2011). All these bacteria secrete plant cell-wall macerating enzymes (including the pectate lyases) and proliferate by assimilating the plant cell remains, including iron by producing the chrysobactin and achromobactin siderophores. Pectate lyases, i.e., PelA, PelD and especially PelE, play a key role in the initiation of the plant cell wall maceration. Using pectin as a substrate, these enzymes release oligosaccharides that elicit expression of other plant-cell wall macerating enzymes in a positive feedback loop (Duprey, Nasser, Léonard, Brochier-Armanet, & Reverchon, 2016). Noticeably, the *pelA* gene is truncated, hence non-functional, in *D. dianthicola* (Duprey et al., 2016; Raoul des Essarts et al., 2019). The secretion of these virulence factors is a costly process that is tightly regulated, being induced by the perception of chemicals that inform the pathogen on plant host physiology (mainly plant sugars) and on the size of its own population (quorum-sensing signals) (Leonard, Hommais, Nasser, & Reverchon, 2017).*Dickeya* pathogens synthetize and sense two types of quorum-sensing signals, the N-acylhomoserine lactones and Vfm compounds, which activate virulence gene expression when a threshold concentration of signals (reflecting the number of cells in the environment) is reached (Crépin et al., 2012; Nasser et al., 2013; Potrykus, Golanowska, Hugouvieux-Cotte-Pattat, & Lojkowska, 2014; Potrykus, , Hugouvieux-Cotte-Pattat, & Lojkowska, 2018). By modulating the synthesis of virulence factors, the quorum-sensing signals participate in the transition from an oligotroph lifestyle in soils and surface waters, where *Dickeya* and *Pectobacterium* bacteria are rare, to a copiotroph lifestyle on a plant host, where they increase in number by several orders of magnitude (Laurila et al., 2008; Potrykus et al., 2016). Functional differences such as metabolic capacities have been identified between *D. solani* and *D. dianthicola* by comparative genomics, transcriptomics and biochemical approaches (Bellieny-Rabelo et al., 2019; Raoul des Essarts et al., 2019), but fitness differences within host plants remain

only partially investigated (Czajkowski et al., 2013; Czajkowski, de Boer, Velvis, & van der Wolf, 2010; Raoul des Essarts et al., 2019; Shyntum et al., 2019). Whether the potato host could be differentially exploited by *D. dianthicola* and *D. solani* and whether the two pathogens stably coexist by using different ecological niches or compete directly when exploiting this host remain unsolved questions, despite their importance for understanding the *D. solani* invasion.

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

2 Material and Methods

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

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

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

Five *D. dianthicola* strains (RNS11-47-1-1A, CFBP1888, CFBP2982, CFBP2015, MIE34) and five *D. solani* strains (3337 = RNS08-23-3-1A, IPO2222, RNS05-1-2A, Ds0432.1, PPO9019) were used to produce plant inoculation assays. The strains were chosen to be different regarding phylogeny placement with neutral markers (**Figure S1**) and geographical locations, isolation years or host of collection (potato plants vs ornamentals, **Table S2**). Phylogeny analyses were conducted using MEGA7 (Kumar, Stecher, & Tamura, 2016). Bacterial strains were stored at -80°C in glycerol (25% vol/vol). They were cultivated in TY medium (tryptone 5 g.L^{-1}; yeast extract 3 g.L^{-1}) with or without agar (15 g.L^{-1}) at 28°C.

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

Each bacterial strain was cultivated individually before being used for plant and tuber inoculations, either separately or assembled in populations. To constitute experimental populations of *D. solani,* *D. dianthicola,* or mixtures of the two species, cell suspensions of different strains were assembled just before plant inoculation. Potato plants (*S. tuberosum* var. Bintje) and hyacinths (*Hyacinthus orientalis* var. Delft blue) were cultivated in horticultural compost in individual pots (2 L) in a greenhouse at around 23°C (minimum 20°C maximum 28°C) with a 12-hour photoperiod. Potato plants (three weeks post-tuber plantation) and hyacinths (four weeks post-bulb plantation) were inoculated by watering the substrate with a pathogen cell suspension at 10^9 colony-forming units (CFU) per pot. In a wounded condition, the potato roots were wounded with a sterile knife before infection. The symptom monitoring was adapted from Raoul des Essarts et al. (2015). The number of symptomatic potato plants (blackleg disease) and hyacinths (soft-rot disease) was recorded

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biweekly (see examples of symptoms in **Figure 1a-b**). For each strain and experimental population and unwounded and wounded conditions, the number of symptomatic and asymptomatic plants (hence two symptom classes) was counted at 60 days post infection (dpi) using 15 potato plants (up to 25 potato plants when indicated) and 8 hyacinths. A set of non-inoculated plants was used as an asymptomatic control. In order to facilitate visualization of the aggressiveness data in the figures, they are presented as a percentage of symptomatic plants.

Potato tubers (*S. tuberosum* var Bintje) were inoculated using a tip to inject 10 µL of pathogen strains and experimental populations at 10^7 (or 10^5 when indicated) CFU per tuber. After 5 days of incubation at 24°C, the tubers were cut in half and scored in five aggressiveness classes according to the symptom gravity in each tuber (from no symptom to the most severe symptoms;**Figure 1c**). For each strain and experimental population, at least 10 tubers were inoculated. A set of non-inoculated tubers was used as an asymptomatic control. In order to facilitate the presentation of the aggressiveness data in the figures, results are presented as normalized disease severity index (DSI) of which values, ranging from 0 to 100 (arbitrary unit), increase with symptom severity. DSI was calculated using the five aggressiveness classes assigned to 10 tubers, see formula in **Supplementary Methods 1 (SM1).**

Symptomatic tissues from potato stems and tubers, as well as from hyacinths, were also used to quantify *D. solani* and *D. dianthicola* pathogens by Taq Man qPCR as described in**Supplementary Methods 2 (SM2)** . Assessing *D. dianthicola*and *D. solani* in inoculum and plant tissues (potato stems and tubers, and hyacinths) permitted the calculation of competitive index (CI) values following the formula presented in **SM1** . A CI value equal to one indicated an equal fitness between *D. solani* and*D. dianthicola* , CI value greater than one indicated a fitness advantage to *D. solani* , and CI value below one indicated a fitness advantage to *D. dianthicola* .

2.4 *Dickeya solani* population genomics

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

2.5 Characterization of the fitness differences between*Dickeya solani* strains carrying the VfmB$_{Ser}$ and VfmB$_{Pro}$ alleles

Structure of the VfmB proteins was modeled and represented using the Phyr2 and EzMol web portals (Kelley, Mezulis, Yates, Wass, & Sternberg, 2015; Reynolds, Islam, Sternberg, 2018). Given the known role in virulence of the *vfm* gene cluster (Nasser et al., 2013), we assessed virulence differences between *D. solani* strains exhibiting either a serine (allele VfmB$_{Ser}$) or a proline (allele VfmB$_{Pro}$) at the position 55 of the VfmB protein. Using genomic data, we chose four isolates carrying VfmB$_{Pro}$ (IPO2222, MIE35, AM3a and 3337) and four isolates carrying VfmB$_{Ser}$ (Ds0432.1, RNS10-27-2A, Sp1a and M21a), the four isolates in each group differing at other positions in genomes (their characteristics in **Tables S3** and**S4**). Plant inoculation assays (on potato tubers and stems) were performed following the same protocols as described above using pure strains and mixtures as inocula. In the figures, aggressiveness data were presented as percentage of symptomatic plants (in potato stem infections assay) and DSI values (in potato tuber infection assays).

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

2.6 Statistical analyses

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

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

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

3 Results

3.1 A scarce co-occurrence of *D. dianthicola* and *D. solani* in potato fields

A monitoring of the *Pectobacterium* and *Dickeya* populations was performed over one decade (2004-2015) in France. Annual surveys were conducted in 541 different potato fields, all exhibiting symptomatic plants. The samplings resulted in the collection of over 1,600 isolates that were characterized taxonomically. Each year, we recorded the number of fields from which we isolated each taxon. These data informed on the dynamics of the different pathogen populations. Over the period,*Dickeya* was detected in 10% to 35% of the sampled fields (**Figure 2a**). A moderate increase of the percentage of*Dickeya* -positive fields was observed over time (F= 5.05; DFn= 1; DFd= 9; p= 0.05; $R^2 = 0.36$). Although decreasing in incidence, the resident *Pectobacterium* species remained the most widespread. As expected, the slopes of the dynamics of the percentage of*Dickeya*- and *Pectobacterium*- positive fields diverged (F= 10.11; DFn= 1; DFd= 18; p= 5 x 10^{-3}). Among the*Dickeya* isolates, only two species were identified, *D. dianthicola* and *D. solani* (**Figure 2b**). The slopes of*D. dianthicola* -positive (F= 1.35; DFn= 1; DFd= 9; p= 0.28) and*D. solani* -positive (F= 3.10; DFn=1; DFd= 9; p= 0.11) field percentages were not different from base line and were not different from each other (F= 0.26; DFn = 1; DFd = 18; p= 0.62). This suggests that the *D. solani* invasion did not occur at the expense of*D. dianthicola* in French potato agrosystems.

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

3.2 Compared to *D. solani*, *D. dianthicola* exhibited a fitness advantage in potato plants

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

In a first assay, each strain was inoculated individually on 15 plants, and the number of asymptomatic and symptomatic plants (the two symptom classes) were counted. A Kruskal-Wallis test revealed differences between *D. solani* and *D. dianthicola* in terms of symptom severity (k=7; DF=1; p=8 x 10^{-3}): *D. dianthicola* was found more aggressive than *D. solani* (percentages of symptomatic plants of 61% $\pm$ 10 versus 15% $\pm$ 11;

Figure 3a).

In a second plant assay, the five bacterial cultures (i.e., the five strains) of each species were assembled to constitute two experimental populations and each was inoculated on 15 plants. A mix of the two species (10 strains) was also inoculated on 15 plants. The assay was duplicated (2 x 15 plants per treatment). Kruskal-Wallis test revealed differences between the three treatments, i.e., *D. solani* strain mixture, *D. dianthicola* strain mixture and species mixture (k=6.1; DF=2; p=0.04). Pairwise comparison of the symptom class counts (Post-hoc Tukey test) showed that the aggressiveness of *D. solani* was higher than that of *D. dianthicola* (F=-2.5; p= 0.03) and that of the species mixture (F=1.9; p= 0.10). However, symptom incidence caused by the species mixture did not differ from those of the *D. dianthicola* mixture (F=-0.5; p= 0.86). Post-hoc Tukey p-values and mean value ($\pm$ SE) of the percentage of symptomatic plants inoculated by the *D. dianthicola* (53% $\pm$ 7), *D. solani* (23% $\pm$ 3) and mixed (47% $\pm$ 0) populations are presented in the **figure 3b** .

In a subsequent experiment, qPCR allowed to quantify pathogen loads. When the two species mixtures were inoculated separately, *D. dianthicola* and *D. solani* reached the mean values $\pm$ SE of 2 x 10^{11} $\pm$ 1 x 10^4 cells and 3 x 10^9 $\pm$ 1 x 10^2 cells per gram of symptomatic stem tissues, respectively. In eight co-infected symptomatic plants, quantifications of the pathogen abundance revealed an excess of *D. dianthicola* with a CI median value of 10^{-5} (**Figure 3c**). The eight CI values differed from one (Kruskal-Wallis test, k=9.5; DF=1; p= 2 x 10^{-3}), meaning that *D. dianthicola* outcompeted *D. solani* in blackleg tissues. Altogether, these data revealed a fitness advantage of *D. dianthicola* in terms of multiplication within lesions in the aerial parts of the host.

Pathogen mixtures were also inoculated in pots containing plants with wounded roots. Symptomatic and asymptomatic plants were counted among 15 plants per treatment. The assay was duplicated (2 x 15 plants per treatment). Kruskal-Wallis test revealed differences between the three treatments, i.e., *D. solani* strain mixture, *D. dianthicola* strain mixture and species mixture (k=29.2; DF=2; p=4.6x10^{-7}). Pairwise comparisons (post-hoc Tukey tests) showed that the number of plants with symptoms differed following inoculation

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by *D. solani* from inoculation by *D. dianthicola* (F=-4.5; p=1.9x10^{-5}) and by a mixture of *D. dianthicola* and *D. solani* (F=6.1; p=2.7x10^{-9}). Mean $\pm$ SE of the percentage of symptomatic plants reached 33% $\pm$ 0 for *D. solani* , 83% $\pm$ 10 for *D. dianthicola* and 93% $\pm$ 7 for a mix of *D. dianthicola* and *D. solani* , indicating a stronger aggressiveness of *D. dianthicola* as compared to *D. solani* . In 27 co-infected plant tissues, qPCR quantification of the pathogens revealed an excess of *D. dianthicola* with a CI median value of 10^{-5}. These CI values were different from one (Kruskal-Wallis test: k=19.5; DF=1; p=10^{-5}; **Figure S3)** .

Altogether these experimental data revealed fitness differences between *D. solani* and *D. dianthicola* for infecting and exploiting potato plants, the resident being fitter than the invader in all conditions and measured traits.

3.3. Compared to *D. dianthicola*, *D. solani* exhibited a fitness advantage in potato tubers

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

In a first tuber rotting assay, each strain was inoculated separately at 10^7 or at 10^5 CFU on each set of 10 tubers. The assay was performed five times, so we collected symptom class information from 50 tubers for each bacterial strain and for each inoculation load. Kruskal-Wallis tests revealed differences between the two treatments, *D. solani* and *D. dianthicola,* both a high (k=3.2; DF=1; p=0.07) or a low (k=43.6; DF=1; p= 4 x 10^{-11}) pathogen load. The DSI values were calculated for each set of 10 tubers and mean values $\pm$ SE across the five replicates reached 63 $\pm$ 13 for *D. solani* and 47 $\pm$ 4 for *D. dianthicola* with an inoculum of 10^7 CFU per tuber (**Figure 4a**) and 49 $\pm$ 8 for *D. solani* and 7 $\pm$ 4 for *D. dianthicola* with an inoculum of 10^5 CFU per tuber (**Figure 4d**).

In a second tuber assay, we used strain mixtures for each of the two species and an assembly of the two species to inoculate 10 tubers for each of 10^7 and 10^5 CFU per tuber. This assay was performed twice. Kruskal-Wallis tests revealed differences between the three treatments, i.e., *D. solani* ,*D. dianthicola* and the species mixture both at a high (k=51.3; DF=2; p=4.2x10^{-11}) and a low (k=22.4; DF=2; p=1.3x10^{-5}) pathogen load. At both bacterial loads, pairwise comparisons (Post-hoc Tukey tests) showed that symptom incidence was different between *D. solani* and *D. dianthicola* (at a high load: F=2.7; p=0.04; at a low load: F=8.6; p< 10^{-11}). Symptom incidence also differed between *D. solani* and the species mixture (at 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 each set of 10 tubers. Mean value $\pm$ SE of the DSI values reached 83 $\pm$ 0 (*D. solani*), 63 $\pm$ 3 (*D. dianthicola*) and 63 $\pm$ 8 (*D. solani* and *D. dianthicola*) with an inoculum of 10^7 CFU per tuber (**Figure 4b**) and 69 $\pm$ 14 (*D. solani*), 16 $\pm$ 6 (*D. dianthicola*) and 48 $\pm$ 10 (*D. solani* and *D. dianthicola*) with an inoculum of 10^5 CFU per tuber (**Figure 4e**). Altogether, these tuber assays revealed a higher aggressiveness of *D. solani* as compared to *D. dianthicola* and to the species mixture.

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

Overall, tubers constituted potato plant tissues in which *D. solani* initiated symptoms more efficiently, caused more damage and was more competitive than *D. dianthicola* . Remarkably, symptom severity of *D. solani* was decreased in the presence of *D. dianthicola* .

3.4. Compared to *D. dianthicola*, *D. solani* exhibited a fitness advantage in hyacinths

The aggressiveness and fitness of the two species was further compared in hyacinths, that constitute a reservoir or/and primary or intermediate host of *D. solani.* The five *D. dianthicola* and five *D. solani* bacterial strains

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were inoculated separately on eight plants per strain and the number of symptomatic and asymptomatic plants were counted. A Kruskal-Wallis test revealed differences between *D. solani* and *D. dianthicola* in terms of symptom incidence (k=3.03; DF=1; p=0.08). *D. solani* was found more aggressive than *D. dianthicola* ; means ± SE of the percentage of symptomatic plants reached 43% ± 17 and 23% ± 9, respectively (**Figure S4a**) .

In co-inoculation assays with species mixtures (**Figure S4b**), the qPCR quantification of the pathogens in five symptomatic tissues showed that the calculated CI were different from one (Kruskal-Wallis test: k=7.8; DF=1; p= 5 x 10^{-3}). The CI median value of 4 x 10^9 indicated a high competitive advantage of *D. solani* over *D. dianthicola* in rotted tissues of hyacinths. Bulb plants thus appeared promoting competitive exclusion of *D. dianthicola* by *D. solani* , which would lead to an enrichment in *D. solani* in the bulb plant agrosystems.

3.5 The expression of pectate lyase genes was higher in *D. solani* than in *D. dianthicola*

The capacity of *D. solani* to initiate rotting with a low bacterial load suggested a differential expression of pectate lyase genes between the two species. Expression levels of the *pelA* ,*pelD* and *pelE* genes were quantified by RT-qPCR in the five *D. solani* and five *D. dianthicola* strains grown separately in a rich medium (in the absence of pectin) and in rotted potato stems and tubers. The expression of two other virulence genes, *cbsE* and *acsD,* which are not involved in the plant cell wall degradation, but in iron-uptake, were also compared. Four RT-qPCR measurements were performed per condition and gene. For each gene, Kruskal-Wallis tests revealed differences between the six treatments (rich medium, stems and tubers for the two species, considering each strain as a replicate) for the different genes: *pelA* (k=17.1; DF=5 ; p=4x10^{-3}), *pelD* (k=25.1; DF=5 ; p=1x10^{-4})*, pelE* (k=24.9; DF=5; p=1x10^{-4})*, cbsE* (k=20.3; DF=5 ; p=1x10^{-3}) and *acsD* (k=21.3; DF=5; p=7x10^{-4}).

Pairwise post-hoc Tukey tests were used to compare gene expression between *D. solani* and *D. dianthicola* . In the absence of pectin (**Figure 5**), the *pelA* (F=864.3; p< 10^{-11}), *pelD* (F=864.3; p< 10^{-11}) and *pelE* (F=2.9; p= 4 x 10^{-3}) genes were expressed at a higher level in *D. solani* than in *D. dianthicola* . The expression levels of the *cbsE* (F=0.377; p=0.99) and *acsD* (F=-1.0; p=0.99) genes were not significantly different between species (**Figure S5**). In the absence of pectin, a higher expression of pectate lyase genes, especially *pelE* , could be considered as an advantage for initiating degradation of the plant cell wall polymers.

In symptomatic potato tubers and stems, gene expression was more variable among strains (**Figures 5 and S5**). The expression levels of *pelD* and *pelE* were higher in *D. solani* than in *D. dianthicola* (*pelD* : F=864.3; p< 10^{-11} in tubers and F=4.5; p= 10^{-4} in stems; *pelE* : F=4.5; p= 1.1 x 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. dianthicola* in stem tissues, but not in tubers (*pelA* : F=-0.09; p=1 and *cbsE* : F=0.67; p=0.99). The *acsD* expression level did not differed between the two species in diseased tubers (F=-1.5; p=0.9) and stems (F=0.5 p=0.9). Overall, in plant symptoms, *D. solani* maintained a higher expression of the virulence genes *pelD* and *pelE* compared to *D. dianthicola* .

3.6 The population genomic analysis of *D. solani* identified two alleles at the *vfmB* gene, at balanced frequencies

We studied SNPs and InDels in 76 *D. solani* genomes (**Figure S2**), 56 of which were assembled after Illumina sequencing in this study, while the 20 others had been previously acquired using the same sequencing approach (Khayi et al., 2015). Out of the 76 genomes, 66 exhibited from 2 to 11 SNPs and InDels when compared to the *D. solani* 3337 reference genome (**Figure S2**). In the nine others, more than 100 SNPs were observed: some of these SNPs were clustered in regions of the chromosome (RNS13-30-1A, PPO9134, PPO9019, RNS13-31-1A, RNS13-48-1A, RNS15-102-1A, RNS07-7-3B) while others were distributed along the genome (RNS05-1-2A and RNS10-105-1A).

Focusing on the 66 genomes exhibiting less than 12 SNPs compared to the strain 3337, 45 non-synonymous variations were identified in whole genomes (**Table S5)** . Two-third of them (30 out of 45) were private to a single genome, while the 15 others were found in at least two genomes. Among these 15 later, three variations

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were identical in four strains, two others in five strains and one was present in 19 genomes (**Table S5 and Figure 6a**) . The most balanced frequencies alternated a thymine (T) in 19 genomes and a cytosine (C) residue in the other 48 genomes at the position 2,930,940 in the reference *D. solani* 3337 genome (**Figure 6a**). The C to T modification caused a proline (VfmB$_{Pro}$ allele) to serine (VfmB$_{Ser}$ allele) change at the position 55 in the VfmB protein. A SNP-based tree of these 67 strains (**Figure 6b**) revealed that the *D. solani* isolates carrying VfmB$_{Ser}$ were placed in two different sub-clusters, one with only two VfmB$_{Ser}$ *D. solani* strains, EU3296 and Ds0432.1, and the other mainly VfmB$_{Ser}$ strains.

The VfmB$_{Ser}$ allele was present in 25% (19 out of 76) of all analyzed *D. solani* genomes. A similar percentage (27%, 17 out of 63) was observed when only the French isolates were considered. Using the isolation year of these 63 *D. solani* isolates, we drew the dynamics of the percentage of the VfmB$_{Ser}$ alleles, which was found to fluctuate over the past decade (2005-2015) with a peak in 2013-2014 (**Figure 6c**).

3.7 Characteristics of VfmB$_{Pro}$ and VfmB$_{Ser}$ strains

Alignments of VfmB proteins retrieved from genomic databases revealed the occurrence of the VfmB$_{Pro}$ allele in *D. dadantii* , *D. chrysanthemi* and *D. dianthicola* genomes, while the VfmB$_{Ser}$ allele was found in *D. zeae* genomes (**Figure S6**). However, the low number of genomes available let open the possibility of the existence of additional alleles in each of these species.

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

We used transcriptomics in *D. solani* IPO2222 (VfmB$_{Pro}$) and Ds0432.1 (VfmB$_{Ser}$) to test the existence of differential expression of virulence genes in tuber tissues (**Figure 7c** ; **Table S6**). The expression of four genes found differentially expressed based on transcriptomics (*pelE* , *impC* , *cytA* and *budA*) was further investigated by RT-qPCR in the same samples used for transcriptomics: they found to be also upregulated (**Figure S7**). Overall, 170 differentially expressed genes (adjusted p-value < 0.05 and absolute log2 fold change > 2) were identified by comparative transcriptomics. Most of them (150 genes) were upregulated in Ds0432.1 carrying the VfmB$_{Ser}$ allele (**Table S7**). We studied more specifically 35 well-characterized *D. solani* virulence genes (Raoul des Essarts et al., 2019). We observed an enrichment of these virulence genes among the upregulated genes in *D. solani* Ds0432.1, while none of them was found among the down-regulated genes (**Table S8**). These upregulated virulence genes in *D. solani* Ds0432.1 included the pectate lyase genes *pelB, pelC, pelD, pelE* and *pelL,* the protease genes *ptrA* , *prtB* and *prtC,* and the T6SS-related toxin/antitoxin genes *hcp* (**Table S8**). These upregulated genes encompassed virulence determinants that are already known to be regulated by the Vfm quorum-sensing in *D. dadantii* (Nasser et al., 2013).

3.8 Fitness costs and gains associated with VfmB alleles

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

In soft-rot assays (**Figure 8a-b**), each of the two experimental populations and their mix was inoculated on 10 tubers and a symptom class was assigned to each tuber. Two inoculation loads were used (10^7 CFU or 10^5 CFU per tuber). This assay was performed in triplicate (3 x 10 tubers per treatment). Kruskal-Wallis tests revealed differences between the three treatments, i.e., inoculation by *D. solani* VfmB$_{Pro}$, *D. solani* VfmB$_{Ser}$

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and their mixture, and the differences were significant at both high (k=17.6; DF=2; p=1.5x10^{-4}) and low (k=7.2; DF=2; p=0.03) pathogen loads. Whatever the bacterial load, pairwise comparisons (Post-hoc Tukey tests) showed that symptom severity was different between *D. solani* VfmB$_{Pro}$ and *D. solani*VfmB$_{Ser}$ (at a high load: F=4.6; p=9.5 x 10^{-6}; at a low load: F=0.8; p= 0.01). The *D. solani* VfmB$_{Ser}$ population was more aggressive than the*D. solani* VfmB$_{Pro}$ population (DSI mean value $\pm$ SE of 92 $\pm$ 2 for VfmB$_{Ser}$ vs 73 $\pm$ 3 for VfmB$_{Pro}$ at a high load and 45 $\pm$ 2 for VfmB$_{Ser}$ vs 26 $\pm$ 9 for VfmB$_{Pro}$ at a low load; **Figure 8a-b)** . VfmB$_{Ser}$ *D. solani*strains thus more efficiently damaged tubers than VfmB$_{Pro}$ *D. solani* strains.

When the VfmB$_{Ser}$ and VfmB$_{Pro}$*D. solani* experimental populations were co-infected in tubers, shotgun sequencing of the pathogens recovered from symptoms allowed to calculate CI values (**Figure 8a-b**). With a load of 10^7 CFU per tuber, the CI values (8 values; median = 1.3) were not different from one (Kruskal Wallis test; k=0.8; DF=1; p= 0.4). With a load of 10^5 CFU per tuber, the CI values (3 values; median = 2) differed from one (Kruskal Wallis test; k=4.4; DF=1 ; p= 0.04). In tubers, the *vfmB* allele thus appeared neutral or to confer a moderate advantage as compared to the VfmB$_{Pro}$ allele.

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

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

4 Discussion

Two successive invasions of *Dickeya* potato pathogens occurred in Europe: first *D. dianthicola* in the middle of the 20th century, this pathogen being now considered as endemic, and second, *D. solani* at the beginning of the 21th. *Dickeya solani* and *D. dianthicola*cause similar symptoms on potato plants, so that they are expected to compete for the same resources. Over ten years, our epidemiologic surveys revealed a successful establishment of *D. solani* together with a maintenance of *D. dianthicola* . Our experiments contributed to explain such puzzling co-existence by contrasted advantages in different parts of the plants and therefore partially different ecological niches.

By comparing ecological traits of *D. solani* and *D. dianthicola* , we indeed found contrasted behaviors in these species with respect to aerial parts versus tubers of *S. tuberosum* . The resident *D. dianthicola* more efficiently exploited host stems after an inoculation of the wounded and unwounded roots than did the invader *D. solani* in terms of symptom severity, proliferation and relative fitness in competition assays. In contrast, *D. solani*more efficiently initiated the rotting process (especially at a low bacterial inoculum) and competed in tubers, with potential consequences on tubers in the fields and during storage. The capacity of *D. solani* to cause rooting at a lower bacterial load than *D. dianthicola* is likely at least partly due to a higher expression of the*pel* enzymes, especially PelE that acts as an initiator of the plant cell wall degradation (Duprey et al., 2016). By comparing*pel* genes in several *Dickeya* species, Duprey et al. (2016) showed that *D. solani* evolved specific regulatory sequences that contributes to a different expression levels of the *pelD* and*pelE* genes compared to *D. dianthicola* . The *pelA*gene is truncated in *D. dianthicola* (Duprey et al., 2016; Raoul des Essarts et al., 2019), but full-length and expressed in *D. solani* , reinforcing their virulence arsenal. Other metabolic traits could also contribute to the fitness advantage of *D. solani* for exploiting

tubers: comparative transcriptomics revealed a higher expression of the glyoxylate shunt in *D. solani* than in *D. dianthicola,* a pathway that contributes to exploit alternative carbon sources when sugar availability is low (Raoul des Essarts et al., 2019).

Beside the tripartite interaction between potato plant host, *D. solani* and *D. dianthicola* , additional environmental (soil and climate) and biological factors (microbiota including *Pectobacterium* species) may facilitate or limit *D. solani* establishment (Charkowsky 2018; Shyntum et al., 2019; Toth et al., 2011). Competition and facilitation processes have been well studied in pathogenic fungi (Al-Naimi, Garrett, & Bockus, 2005; Abdullah et al., 2017; Gladieux et al., 2015; Zhan & McDonald, 2013). Another factor that could reduce competition between the two *Dickeya* species is their natural low abundance in soils and surface waters, as well as in tuber seeds that are subjected to prophylactic diagnosis. A small population size belonging to a single species could proliferate in a plant individual without any interactions with another *Dickeya* or *Pectobacterium* pathogen species. These effects linked to size population and dispersal are expected to delay invasion. In line with this hypothesis, the observed slow increase of the percentage of *Dickeya* -positive fields over one decade suggested that the *Dickeya* invasion was still ongoing (2004-2015). Among the emerging literature on microbial invasion, the *D. solani* pathogen appears as a good example illustrating how different ecological components (here, the plant host and a resident pathogen) should be considered to understand the biological invasion by a bacterial pathogen (Cadotte et al., 2018; Germain, Mayfield, & Gilbert, 2018).

The genome sequencing data indicating low diversity in *D. solani* suggest a bottleneck during introduction in Europe. The dispersal modes (either horizontally via soil, surface water, insects and some agricultural practices or vertically by asymptomatically contaminated tuber seeds) 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 has been 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 this work, we showed that the VfmB$_{Ser}$ strains were more aggressive than the VfmB$_{Pro}$ strains in potato tubers, but not in potato stem assays, and that the VfmB$_{Ser}$ strains were less competitive than the VfmB$_{Pro}$ strains in tuber and stem symptoms. This 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 past decade, in agreement with an increase of competition between *D. solani* VfmB alleles in the more recent sampling year.

In conclusion, this study brings novel insights allowing a better understanding of the pattern and causes of the *D. solani* invasion into potato production agrosystems, and the reasons why *D. dianthicola* nevertheless persisted. More broadly, this study contributes to our understanding the ecological determinants of pathogen invasion and of the conditions for the maintenance of endemic competitors.

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DATA ACCESSIBILITY

DNA sequences: Genbank accessions VYSC00000000; VZQE00000000; VZQF00000000; VZQG00000000; VZ-QH00000000; VZQI00000000; VZQK00000000; VZQJ00000000; VZQL00000000; VZQM00000000

AUTHOR CONTRIBUTION

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

CONFLICT OF INTEREST

No potential conflict of interest was disclosed.

ORCID

Denis Faure orcid.org/0000-0002-5379-8867

Figure legends

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

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

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

Figure 4 Aggressiveness and fitness of *Dickeya dianthicola* and *D. solani* in potato tubers. Rotting assays were conducted by inoculating each tuber by either 10^7 colony-forming units (CFU) (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 index (DSI) value was calculated using symptom classes observed on 10 tubers. In **a** and **d**, mean values and standard errors (SE) of DSI values were measured for each of the five *D. solani* strains (3337, IPO2222, RNS05-1-2A, Ds0432.1, PPO9019) and five *D. dianthicola* strains (RNS11-47-1-1A, CFBP1888, CFBP2982, CFBP2015, MIE34) using five (**a**) and two (**d**) independent experiments. The p-values of the Kruskal-Wallis tests comparing symptom classes are indicated below the graphs. In **b** and **e**, mean values and standard errors of DSI values were measured twice for each of the experimental populations of *D. dianthicola, D. solani* and the mixture

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of the two. The p-values of the pairwise Tukey tests comparing the symptomatic classes are indicated, when p [?] 0.1. In **c** and **f**, the competitive index (CI) between *D. solani* and *D. dianthicola* populations was calculated in 10 co-infected symptomatic tubers: the median values (indicated by a thick bar) reached 5.7 (**c**) and 5.8 (**f**) and the CI values were statistically different from one (Kruskal-Wallis test; $p = 5 \times 10^{-5}$ and $p = 0.05$ respectively), revealing a competitive advantage of *D. solani*. Legend: * for $0.05 < p[?]0.1$; ** for $0.01 < p[?]0.05$ and *** for p [?]0.01.

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

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

Figure 7 Characterization of the VfmB alleles. In **a**, using the *Escherichia coli* Aidb protein (the PDB accession is c3djlA) as a model, the Phyre2-predicted structure of the VfmB protein showed conformational differences in the beta-sheet associated with the $VfmB_{Pro}$ and $VfmB_{Ser}$ alleles; the Pro55 and Ser55 positions are in red color in the VfmB representations obtained using the EzMol web server. In **b**, mean values and standard error (SE) of disease severity index (DSI) values, which were calculated by recording symptom classes on 10 tubers infected by 10^7 CFU of each of the *D. solani* strains carrying either $VfmB_{Pro}$ (IPO2222, MIE35, AM3a and 3337) or $VfmB_{Ser}$ (Ds0432.1, RNS10-27-2A, Sp1a and M21a); the p-value of the Kruskal-Wallis test comparing the four $VfmB_{Pro}$ and four $VfmB_{Ser}$ strains using symptom classes is indicated below the graph. In **c**, comparative transcriptome of *D. solani* 3337 ($VfmB_{Pro}$) and Ds0432.1 ($VfmB_{Ser}$) recovered from soft-rot symptoms in potato tubers; upregulated virulence genes were enriched in Ds0432.1 ($VfmB_{Ser}$) as illustrated by the *pelB, pelC, pelC, hcpA* and *prtA* genes (closed circles).

Figure 8 Aggressiveness and fitness assays of two *Dickeya solani* experimental populations expressing the VfmB alleles. Rotting assays were conducted by inoculating each tuber by either 10^7 colony-forming units (CFU) (**a**) or 10^5 CFU (**b**) of the $VfmB_{Pro}$ and $VfmB_{Ser}$ *D. solani* populations and a mixture of the two. In **a** and **b**, each disease severity index (DSI) value was calculated using symptomatic classes observed on 10 tubers; the assays were performed in triplicate. The p-values of the pairwise Tukey tests comparing the symptom classes are indicated, when p [?] 0.1. In **c**, percentage (%) of plants exhibiting blackleg symptoms was measured on plants inoculated by the $VfmB_{Pro}$ and $VfmB_{Ser}$ *D. solani* populations and a mixture of the two. the assays were performed in triplicate. Kruskal-Wallis test revealed no difference between the three treatments using symptom classes ($p = 0.86$). In **a, b** and **c**, competitive index (CI) values of $VfmB_{Pro}$ and $VfmB_{Ser}$ populations were calculated using allele counts based on shotgun sequencing of bacterial populations recovered from co-infected tissues. Median values of CI are represented as thick lines and reached 1.7 (8 repeats in **a**), 2.0 (3 repeats in **b**) and 14.0 (16 repeats in **c**) in the three treatments. Statistical differences between CI values and 1 (Kruskal-Wallis test) revealed that neither *D. solani* $VfmB_{Pro}$ or *D. solani* $VfmB_{Ser}$ had significant advantage in tubers inoculated with a high load ($p = 0.3$ in **a**), but *D. solani* $VfmB_{Pro}$ was more competitive in tubers inoculated with a low load ($p = 0.04$ in **b**) and in stems ($p = 9 \times 10^{-3}$ in **c**). Legend: * for $0.05 < p[?]0.1$; ** for $0.01 < p[?]0.05$ and *** for p [?]0.01.

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