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

Short title: coexistence of invasive and resident pathogens on potatoes

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Accepted Article

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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 ability 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* caused a higher symptom incidence on aerial parts of potato plants than *D. solani*, while *D. solani* was more aggressive on tubers (i.e. with more severe symptoms). In co-infection assays, *D. dianthicola* outcompeted *D. solani* in aerial parts, while the two species co-existed in tubers. A comparison of 76 *D. solani* genomes (56 of which having been sequenced here) revealed balanced frequencies of two previously uncharacterized alleles, VfmB_{Pro} and VfmB_{Ser}, at the *vfmB* virulence gene. Experimental inoculations showed that the VfmB_{Ser} population was more aggressive on tubers while the VfmB_{Pro} population outcompeted the VfmB_{Ser} population in stem lesions, suggesting an important role of the *vfmB* virulence gene in the ecology of the pathogens. 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 the endemic *D. dianthicola* nevertheless persisted.

KEYWORDS

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

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 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. Mostly 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 *Solanum tuberosum* dates back to the 70's (Toth et al., 2011; Parkinson et al., 2009). Nowadays, *D. dianthicola* is considered an endemic pathogen of potato plants 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 with a bottleneck associated to the introduction and/or 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 synthesize 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, with a

diversity of strains and of ecological conditions, we then focused on *D. solani* and *D. dianthicola* to determine the ecological traits that could facilitate or constrain *D. solani* establishment, including competition or ecological niche differences between the two *Dickeya* species. Finally, we obtained the genomes of 56 *D. solani* isolates that we analyzed together with 20 genomes previously published (Khayati et al., 2015), revealing novel variations in *D. solani* population. We focused on the quorum-sensing gene *vfmB*, because it is known to be involved in virulence, 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 of the potato fields exhibiting more than 1% of blackleg incidence were 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 representative of the diversity of the populations, i.e. from different geographical locations, isolation years or host of collection (potato plants vs ornamentals), and regarding phylogeny placement with neutral markers (**Figure S1** and see characteristics and genome accession numbers in **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 and tuber inoculations by *D. solani* and *D. dianthicola* strains and experimental populations

In our plant and tuber assays, we use the term virulence as the capacity of the pathogen to parasitize a host (symptom incidence) and the term aggressiveness as the degree of damage caused by a pathogen on a host (symptom or disease severity). The terms used to describe pathogens behaviors (virulence, symptom incidence, aggressiveness, disease severity index, fitness and competitive index) are defined more extensively in **Supplementary Methods 1 (SM1)**. We designed the whole plant experiments based on our knowledge of the pathosystem, to investigate contrasting conditions relevant for the disease expression in fields. 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. For mimicking plant infection by soil pathogens, 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 10⁹ colony-forming units (CFU) per pot. This inoculation load was calibrated to obtain a large range (20 to 60%) of symptom incidence in the unwounded potato plant condition (Raoul des Essarts et al., 2015). 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 twice a week (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 that were used in statistical comparisons) was counted at 70 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 virulence data in the figures, they are presented as a percentage of symptomatic plants (symptom incidence).

In tuber assays, potato tubers (*S. tuberosum* var. Bintje) were inoculated using a tip to inject 10 µL of calibrated pathogen suspensions. Based on previous results (Raoul des Essarts et al., 2019), we chose two different inoculum loads of strains and experimental populations (10⁷ CFU and 10⁵ CFU per tuber) to obtain a large range of symptom severity, to compare the capacity to initiate maceration at different population sizes, to investigate fitness of the two pathogens in two contrasting ecological conditions. After 5 days of incubation at 24°C, the tubers were cut in half

and scored based on five aggressiveness classes according to the symptom severity 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)**.

Emerging lesions from potato stems and hyacinths and rotting tissue recovered from tubers at 5 dpi were collected and frozen at -80°C , and then used to quantify the abundance *D. solani* and *D. dianthicola* pathogens within hosts 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 (Khayri *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 ($\geq 99\%$ 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

The 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 investigated the impact on virulence of a SNP detected in the *vfm* gene cluster : 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 (genome accession numbers and 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, virulence was presented as the percentage of symptomatic plants (in potato stem infections assay) and aggressiveness by DSI values (in potato tuber infection assays).

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 lesions. From 12 to 28 million reads were obtained for each sample, corresponding to an average coverage of *D. solani* genomes ranging from 180× to 420×. Sequencing (75 × 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 identify 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 levels (with or without symptoms) of potato plants and hyacinths virulence assays, the five symptom classes in tuber aggressiveness 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 Little 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. Each year, we recorded the number of fields from which we isolated each taxon (**Table S5**). 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 (67% in the most recent sampling year 2015). As expected, the slopes of the dynamics of the percentage of *Dickeya*- and *Pectobacterium*-positive fields diverged ($F= 10.11$; $DFn= 1$; $DFd= 18$; $p= 5 \times 10^{-3}$). 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.

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 little co-occurrence 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 seed tubers or/and soils and surface waters before plant infection, or from competitive exclusion during the infection process of potato plants and tubers. In the following, 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 *Dickeya dianthicola* exhibited a fitness advantage over *D. solani* in potato plants.

Plant assays were performed in the greenhouse to compare the incidence 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 were counted at 70 days post inoculation (dpi). A Kruskal-Wallis test revealed differences between *D. solani* and *D. dianthicola* in terms of symptom incidence ($k=7$; DF=1; $p=8 \times 10^{-3}$): *D. dianthicola* was found more virulent 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 mixture of the two species (10 strains) was also inoculated on 15 plants. The assay was duplicated (2 x 15 plants per treatment). We compared symptom incidence at 70 dpi. 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 (Post-

hoc Tukey test) showed that *D. solani* was less virulent than *D. dianthicola* ($F=-2.5$; $p= 0.03$) and 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**. To assess the dynamics of the appearance of symptomatic plants, we also drew a disease progress curve for each replicate (**Figure S3a**) and compared the areas under disease progress curve (**Figure S3b**). Pairwise comparisons (Post-hoc Tukey tests) confirmed that *D. solani* was less virulent than *D. dianthicola* ($F=-336$; $p<10^{-11}$) and the species mixture ($F=386$; $p<10^{-11}$).

To evaluate which pathogen had emerged at the beginning of the infection process, hence to identify the most probable causative agent of the observed symptoms, we used qPCR to quantify pathogen loads. When the two species mixtures were inoculated separately, *D. dianthicola* and *D. solani* reached the mean values \pm SE of $2 \times 10^{11} \pm 1 \times 10^4$ cells and $3 \times 10^9 \pm 1 \times 10^2$ cells per gram of symptomatic stem tissues, respectively. Among the 14 co-infected plants exhibiting symptoms, eight lesions were randomly chosen for qPCR quantification of the pathogen abundance: this analysis 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, $k=9.5$; $DF=1$; $p= 2 \times 10^{-3}$), 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 in either the absence or presence of the *D. solani* invader.

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

To evaluate whether plant wounding could benefit to the *D. dianthicola* resident or to the *D. solani* invader, pathogen mixtures were inoculated in pots containing plants with wounded roots. Kruskal-Wallis test revealed differences between the three treatments, i.e., *D. solani* strain mixture, *D. dianthicola* strain mixture and species mixture ($k=29.2$; $DF=2$; $p=4.6 \times 10^{-7}$). Pairwise comparisons (post-hoc Tukey tests) showed that the numbers of plants with symptoms differed between inoculations with *D. solani* and *D. dianthicola* ($F=-4.5$; $p=1.9 \times 10^{-5}$) and with the mixture of *D. dianthicola* and *D. solani* ($F=6.1$; $p=2.7 \times 10^{-9}$). 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 mixture of *D. dianthicola* and *D. solani*, indicating a stronger virulence of *D. dianthicola* as compared to *D. solani* (**Figure S4a**). Pairwise comparisons (post-hoc Tukey tests) of the areas under the disease curves also confirmed that *D. solani* was less virulent than *D. dianthicola* ($F=-386$; $p<10^{-11}$) and

than a mixture of *D. dianthicola* and *D. solani* ($F=386$; $p<10^{-11}$) (**Figure S3c-d**). Because the virulence measured using symptom incidence at the final time point (70 dpi) and area under disease progress curve (from 1 to 70 dpi) gave similar results when comparing the two pathogens, only the final point of symptom incidence was retained for the analysis of the following plant assays, in order to facilitate the presentation of the data.

In the 27 emerging lesions collected from co-infected plants, 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 S3b**).

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

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

We compared the *D. dianthicola* and *D. solani* ecological traits in potato tubers. Aggressiveness of the pathogens (i.e., disease severity) 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 two different inoculum loads allowed comparing the capacity of the pathogens to initiate maceration and exploit tubers in two contrasting ecological conditions. 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 at high ($k=3.2$; $DF=1$; $p=0.07$) or at low ($k=43.6$; $DF=1$; $p=4 \times 10^{-11}$) inoculum dose. The DSI values were calculated for each set of 10 tubers and mean values \pm SE across the five replicates reached 63 ± 13 for *D. solani* and 47 ± 4 for *D. dianthicola* with an inoculum of 10^7 CFU per tuber (**Figure 4a**) and 49 ± 8 for *D. solani* and 7 ± 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 ($k=51.3$; $DF=2$; $p=4.2 \times 10^{-11}$) and a low ($k=22.4$; $DF=2$; $p=1.3 \times 10^{-5}$) pathogen load. At both bacterial loads, pairwise comparisons (Post-hoc Tukey tests) showed that aggressiveness was different between *D. solani* and *D. dianthicola* (at a high load: $F=2.7$; $p=0.04$; at a low load: $F=8.6$; $p<10^{-11}$). Aggressiveness 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 ± 0 (*D. solani*), 63 ± 3 (*D. dianthicola*) and 63 ± 8 (*D. solani* and *D. dianthicola*) with an inoculum of 10^7 CFU per tuber (**Figure 4b**) and 69 ± 14 (*D. solani*), 16 ± 6 (*D. dianthicola*) and 48 ± 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* 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 $2 \times 10^9 \pm 3 \times 10^9$ to $9 \times 10^9 \pm 9 \times 10^9$ cells per gram of rotted tissues (mean values \pm SE). These data indicated no substantial 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 5×10^{-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 (even with a low pathogen load), caused more damage (higher disease severity index) and was more competitive than *D. dianthicola* (outcompeting *D. dianthicola*). Remarkably, the disease severity caused by *D. solani* was decreased in the presence of *D. dianthicola*.

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

The symptom incidence and competition were further evaluated in hyacinths, that represent important hosts as they constitute a reservoir or/and primary or intermediate host of *D. solani*. We expected a higher symptom incidence and growth advantage of *D. solani* on hyacinth host as compared to *D. dianthicola*. The five *D. dianthicola* and five *D. solani* bacterial strains were inoculated separately on eight plants per strain and the number of symptomatic and asymptomatic plants were counted. At 70 dpi, 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 virulent than *D. dianthicola*; means \pm SE of the percentage of symptomatic plants reached $43\% \pm 17$ and $23\% \pm 9$, respectively (**Figure S5a**).

In co-inoculation assays with species mixtures (**Figure S5b**), 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= 5×10^{-3}). The CI median value of 4×10^9 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.

3.6 Pectate lyases gene expression is higher in *Dickeya 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. To test this hypothesis, the 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 emerging lesions of potato stems and tubers (see a detailed protocol in SM2). 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* ($k=17.1$; $DF=5$; $p=4 \times 10^{-3}$), *pelD* ($k=25.1$; $DF=5$; $p=1 \times 10^{-4}$), *pelE* ($k=24.9$; $DF=5$; $p=1 \times 10^{-4}$), *cbsE* ($k=20.3$; $DF=5$; $p=1 \times 10^{-3}$) and *acsD* ($k=21.3$; $DF=5$; $p=7 \times 10^{-4}$).

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* ($F=2.9$; $p=4 \times 10^{-3}$) 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 S6**). In the absence of pectin, a higher expression of pectate lyase genes, especially *pelE*, could be considered as an advantage for initiating a rapid degradation of the plant cell wall polymers.

In symptomatic potato tubers and stems, some remarkable gene expression patterns could be noticed (**Figures 5 and S6**). 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*: $F=4.5$; $p=1.1 \times 10^{-4}$ 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. dianthicola* 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 differ 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*.

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

We used a population genomic approach to investigate additional ecological traits of the *D. solani* invading population. 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 (Khayati 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 S6**). Two-third of them (30 out of 45) were present in one genome only, while the 15 others were found in at least two genomes. Among these 15, three SNPs were identical in four strains, two others in five strains and one was present in 19 genomes (**Table S6 and Figure 6a**). The most balanced frequencies consisted in 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. Such SNPs affecting an amino-acid in the *vfmB* gene retained our attention because the Vfm quorum-sensing system is involved in the regulation of some virulence traits in *Dickeya* (Nasser et al., 2013; Potrykus et al., 2018).

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. This observation suggests that the VfmB_{Ser} allele could have appeared independently in two different *D. solani* lineages, thus representing convergence. 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**).

We also investigated the presence of the VfmB_{Pro} and VfmB_{Ser} alleles in other *Dickeya* species. The 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. zae* genomes (**Figure S7**). However, the low number of genomes available let open the possibility of the existence of additional alleles in each of these *Dickeya* species.

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

The 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**). Soft-rot assays using 10 inoculated potato tubers per strain revealed that the set of the VfmB_{Ser} strains was more aggressive (Kruskal-Wallis test; $k=9.5$; $DF=1$; $p=2 \times 10^{-3}$) than that of the VfmB_{Pro} strains (**Figure 7b**).

To test the existence of differential expression of virulence genes in tuber tissues, we compared transcriptomes of *D. solani* IPO2222 (VfmB_{Pro}) and Ds0432.1 (VfmB_{Ser}) (**Figure 7c**; **Table S7**). 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 S8**). Overall, 170 differentially expressed genes (adjusted p -value < 0.05 and absolute \log_2 fold change > 2) were identified by comparative transcriptomics. Most of them (150 genes) were upregulated in Ds0432.1 carrying the VfmB_{Ser} allele (**Table S8**). 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 downregulated genes (**Table S9**). 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 S9**). 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).

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

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 mixture 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} and their mixture, and the differences were significant at both high ($k=17.6$; $DF=2$; $p=1.5 \times 10^{-4}$) and low ($k=7.2$; $DF=2$; $p=0.03$) pathogen loads. Whatever the bacterial load, pairwise comparisons (Post-hoc Tukey tests) showed that aggressiveness (i.e. symptom severity) was different between *D. 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: $F=0.8$; $p=0.01$). The *D. solani* VfmB_{Ser} population was more aggressive than the *D. solani* VfmB_{Pro} population (DSI mean value \pm SE of 92 ± 2 for VfmB_{Ser} vs 73 ± 3 for VfmB_{Pro} at a high load and 45 ± 2 for VfmB_{Ser} vs 26 ± 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_{Pro} allele thus appeared neutral or to confer a moderate advantage as compared to the VfmB_{Ser} allele.

In blackleg symptom assays (**Figure 8c**), the *D. solani* VfmB_{Pro} and *D. solani* VfmB_{Ser} experimental populations and their mixture 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 mixture ($k=0.29$; $DF=2$; $p=0.86$). 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; $k=6.6$; $DF=1$; $p=9 \times 10^{-3}$) with a median CI of 14, showing a fitness advantage of the VfmB_{Pro} allele in emerging lesions in the stems.

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 rotted 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 21st century. *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*, using multiple strains representative of the population diversity, 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*, *i.e.*, causing higher symptom incidence, and exhibiting greater proliferation and relative fitness in competition assays. In contrast, *D. solani* more efficiently initiated the rotting process (especially at a low bacterial inoculum) and outcompeted *D. dianthicola* in tubers, with potential consequences on tubers in the fields and during storage. Hence, our assays suggest that infected tubers could facilitate the entry and establishment of the *D. solani* invader into the potato agrosystems. A contrasting defense response of potato foliage and tuber against *Phytophthora infestans* has been reported (Gao & Bradeen, 2016). In addition to the intrinsic characteristics of *D. solani* and *D. dianthicola*, an organ-specific response of the plant defense system could similarly also contribute to their difference in behavior.

The capacity of *D. solani* to cause rotting 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 the expression of some *pel* genes in emerging lesions in potato stems and tubers, we observed a higher expression of the virulence genes *pelD* and *pelE* in *D. solani* compared to *D. dianthicola*. Remarkably, 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 level 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* (as shown in this study), 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, other hosts and non-host plants) may facilitate or limit *D. solani* establishment (Charkowsky 2018; Shyntum et al., 2019; Toth et al., 2011). We showed that *D. solani* efficiently outcompeted *D. dianthicola* in hyacinths, in line with the epidemiologic data supporting the bulb plants as potential intermediate hosts with an important role in the recurrent invasion of potato agrosystems (Chen, Zhang, & Chen, 2015; Slawiak et al., 2009; van der Wolf et al., 2014). Prophylactics may allow circumventing the propagation of *D. solani* from an agrosystem to another. Competition and facilitation processes have been well studied in pathogenic fungi (Al-Naimi, Garrett, & Bockus, 2005; Abdullah et al., 2017; Gladioux 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 seed tubers 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 population size 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 (2004-2015) suggested that the *Dickeya* invasion was still ongoing. 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 asymptotically contaminated seed 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 *pri* 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 past decade, in agreement with an increase of competition between *D. solani* VfmB alleles in the more recent sampling year. There is a very low number of SNPs/InDels (<12) in the genomes beyond those in Vfm, however, functional genetics will be required to fully validate the causal role of the Vfm alleles.

In conclusion, this study using complementary approaches, multiple strains representative of the population diversity and multiple ecological conditions, 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 VYSC000000000; VZQE000000000; VZQF000000000; VZQG000000000; VZQH000000000; VZQI000000000; VZQK000000000; VZQJ000000000; VZQL000000000; VZQM000000000

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 performed 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.

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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 Symptom incidence 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 mixture. The p-values of the pairwise comparisons (Post-hoc Tukey tests) of the symptomatic classes are indicated below the graph, when $p \leq 0.1$. In c, competitive index (CI) values between *D. solani* and *D. dianthicola* populations were calculated in eight emerging lesions of co-infected plants and revealed a competitive advantage of *D. dianthicola*: the CI median ($= 2.4 \cdot 10^{-5}$) is represented by a thick bar; p-values resulting from Kruskal-Wallis tests testing difference from one are indicated. Legend: * for $0.05 < p \leq 0.1$; ** for $0.01 < p \leq 0.05$ and *** for $p \leq 0.01$.

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 of the two. The p-values of the pairwise Tukey tests comparing the symptomatic classes are indicated, when $p \leq 0.1$. 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; $p = 5 \cdot 10^{-5}$ and $p = 0.05$ respectively), revealing a competitive advantage of *D. solani*. Legend: * for $0.05 < p \leq 0.1$; ** for $0.01 < p \leq 0.05$ and *** for $p \leq 0.01$.

Figure 5 Expression of the pectate lyase genes *peIA*, *peID* and *peIE*. The expression level of the *peIA*, *peID* and *peIE* 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 (exponential growth phase) and symptomatic tubers (at 5 days post infection) and emerging lesions in 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 $0.05 < p \leq 0.1$; ** for $0.01 < p \leq 0.05$ and *** for $p \leq 0.01$.

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 3DJL) 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 10⁷ 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 lesions 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, symptom incidence and fitness assays of two *Dickeya solani* experimental populations expressing the VfmB alleles. Soft-rot assays were conducted by inoculating each tuber by either 10⁷ colony-forming units (CFU) (**a**) or 10⁵ 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 (measuring aggressiveness) 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 $p \leq 0.1$. 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. These assays measuring symptom incidence 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 $0.05 < p \leq 0.1$; ** for $0.01 < p \leq 0.05$ and *** for $p \leq 0.01$.

Figure 1

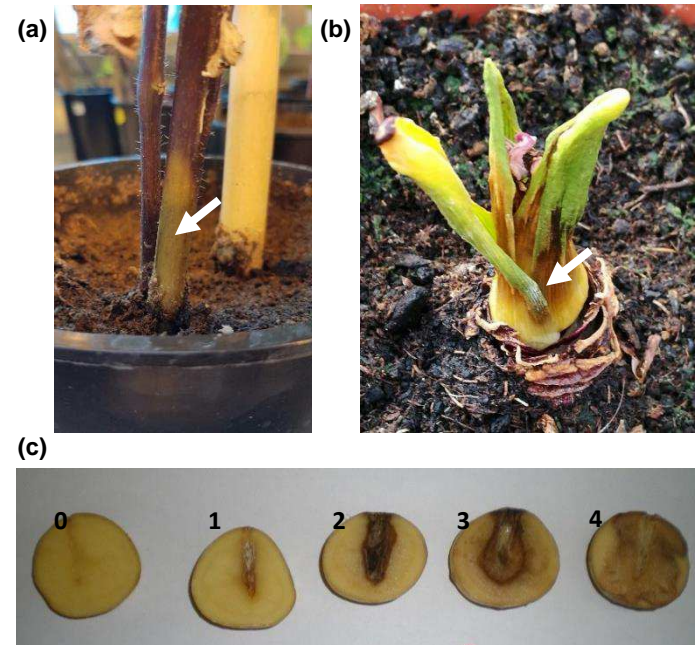


Figure 2

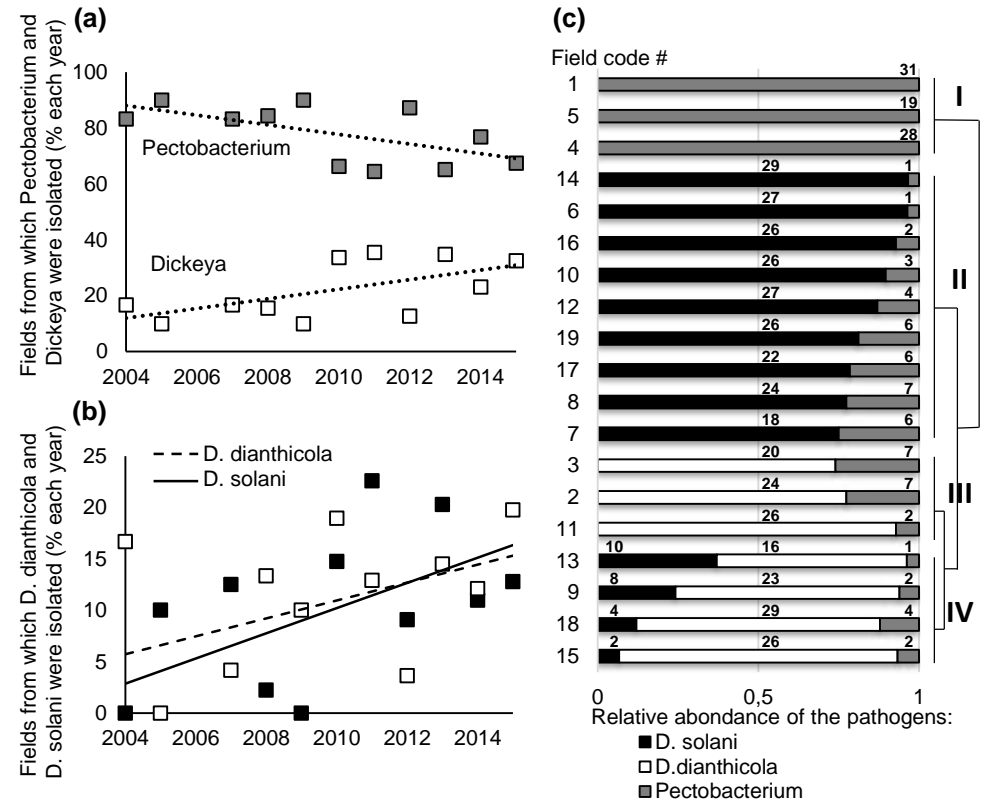


Figure 3

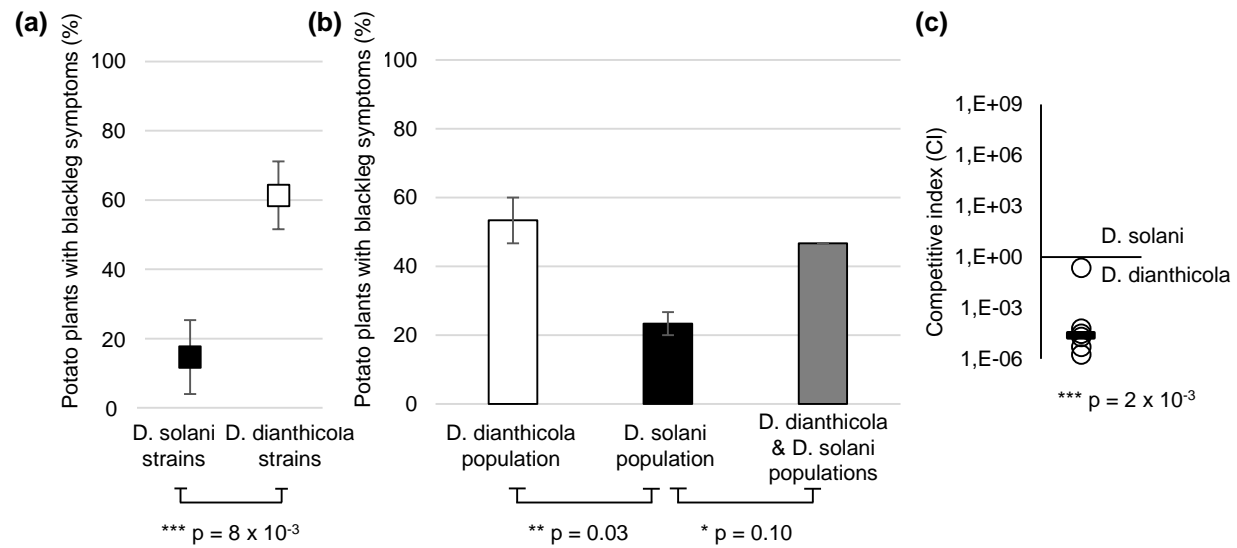


Figure 4

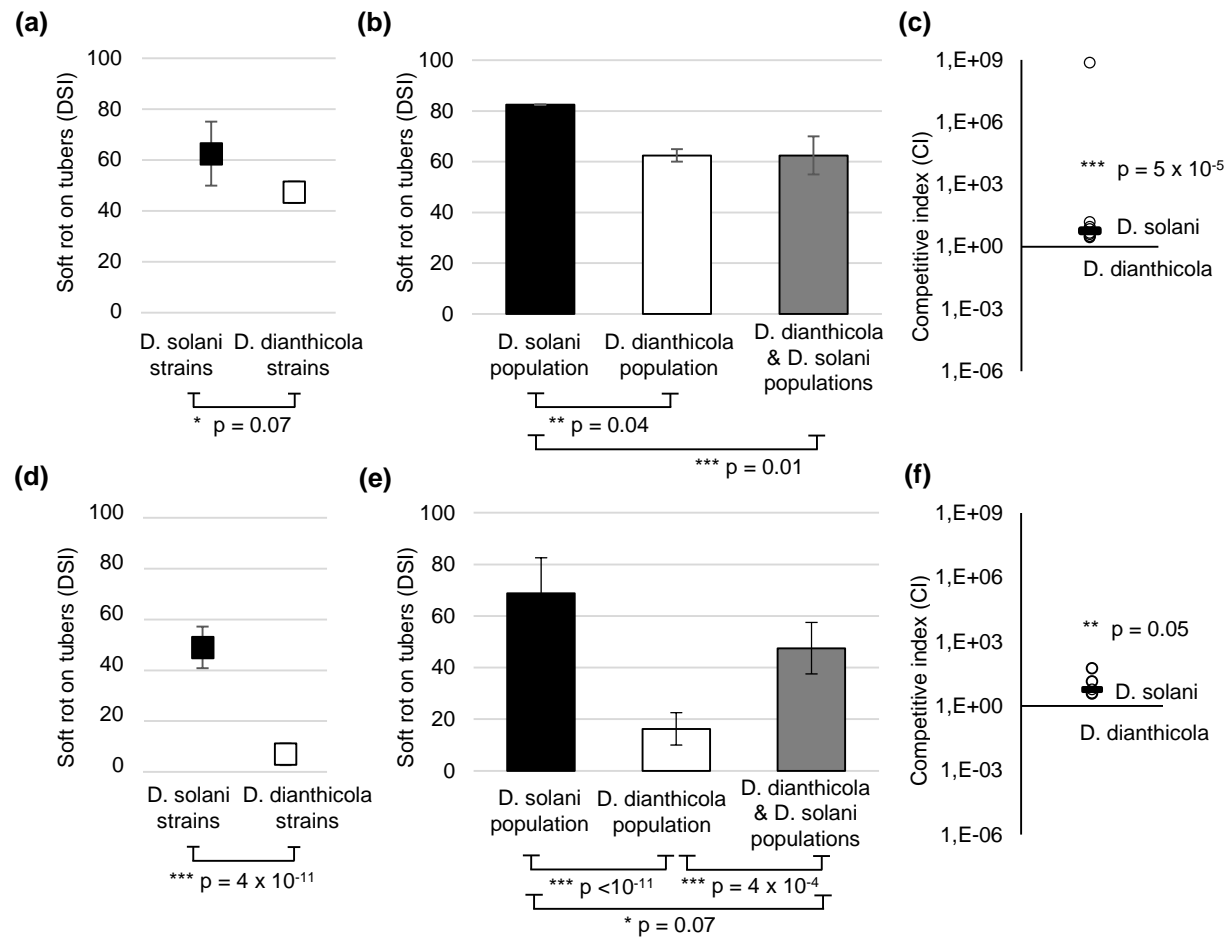


Figure 5

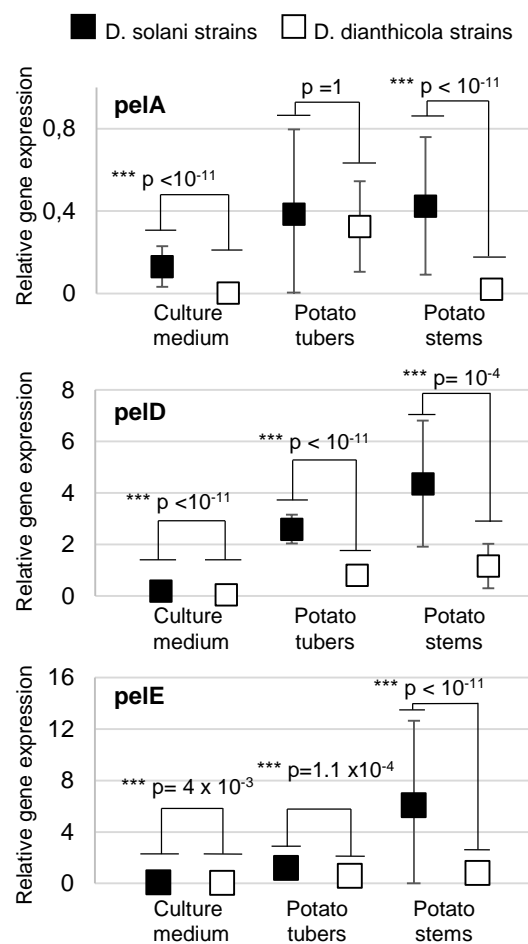


Figure 6

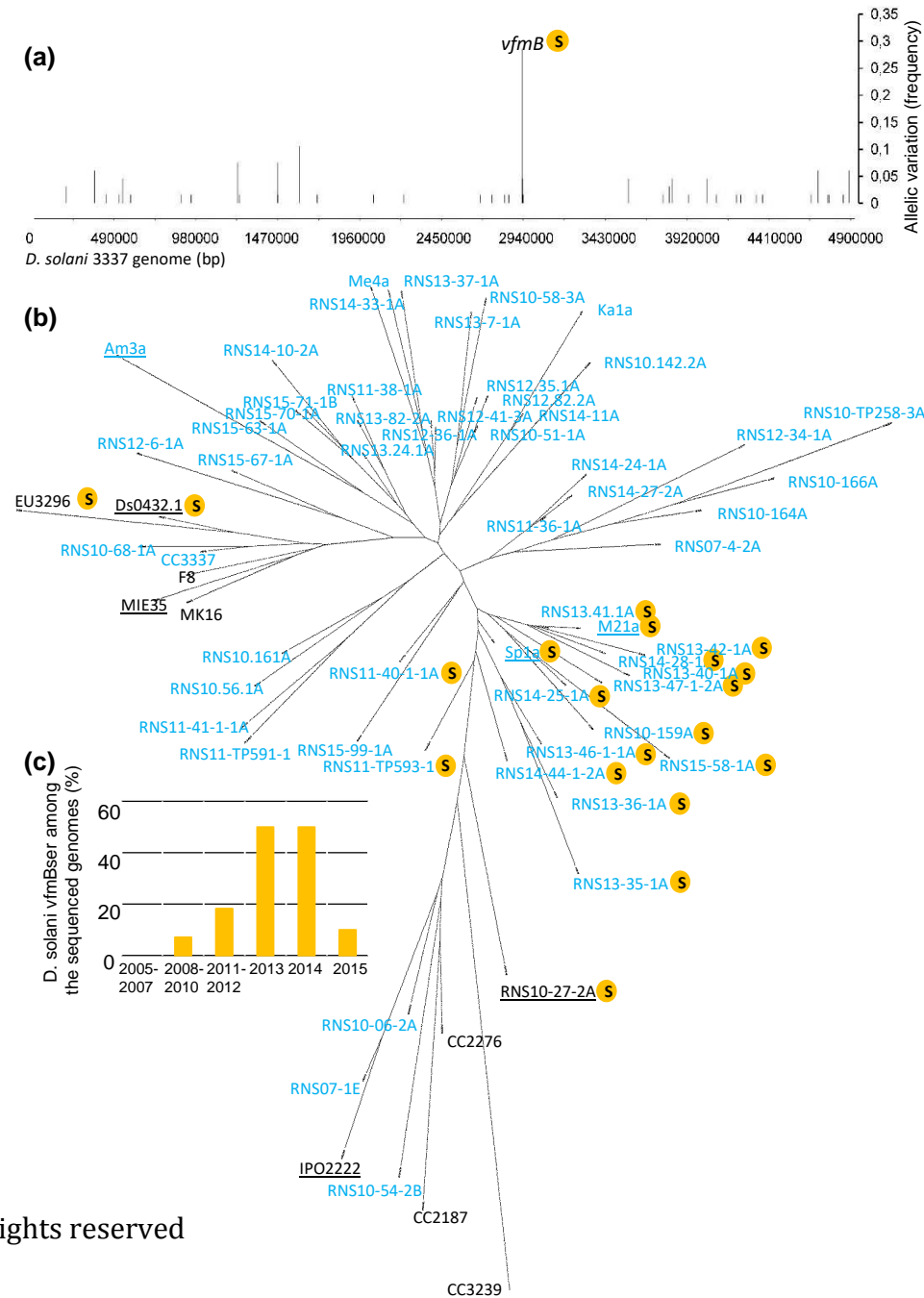


Figure 7

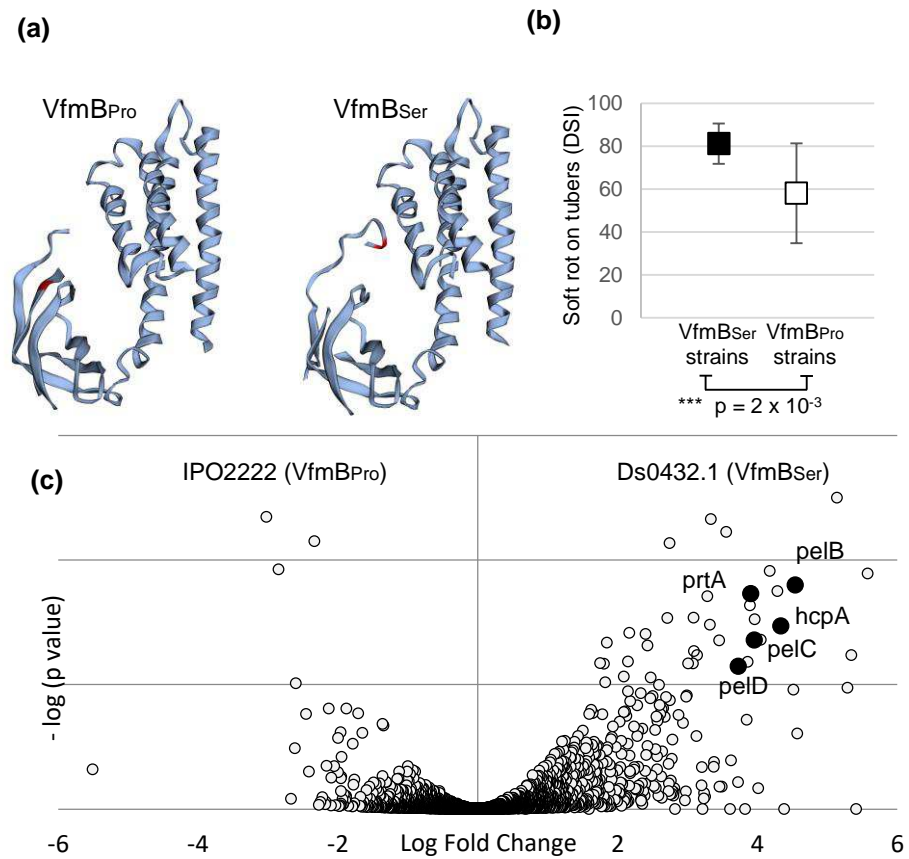


Figure 8

