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Phenotypic and genotypic diversity of *Fusarium proliferatum* in France; an emerging cause of dry rot of garlic

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Summary. Garlic production is affected by dry rot, which is an emerging disease caused by several *Fusarium* spp., including *Fusarium proliferatum* (the main species). There is currently no effective method to prevent garlic dry rot during storage of garlic bulbs. Increased knowledge of the characteristics of *F. proliferatum* linked to dry rot development and pathogen population genetics would increase understanding of Fusarium dry rot epidemics in garlic. The present study aimed to assess genotypic and phenotypic diversity of *F. proliferatum* isolates collected from garlic in southern France and elsewhere from other plant species. Among the 185 *Fusarium* isolates genotyped using eight microsatellite markers, 54 complete multilocus haplotypes were obtained, of which five were identified as distinct. All of 55 isolates assessed for their mycelium growth and sporulation capacities were able to grow and to produce conidia at 15, 21, 30 and 35 °C, while none grew at 4 or 40 °C. All of 20 assessed isolates were similarly pathogenic to garlic when assessed at 23 or 30 °C. All assessed isolates grew well at basic pH. No differences were detected among *F. proliferatum* isolates collected from French garlic or from other countries or other host plant species. The *F. proliferatum* isolates collected from garlic in France had low genotypic diversity and little phenotypic difference, compared with isolates obtained from garlic in other countries or from other host plants.

Keywords. *Allium sativum*, mycelium growth, conidiation, temperature, pH.

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INTRODUCTION

Garlic (*Allium sativum* L.) is a valued vegetable because of its organoleptic and antimicrobial properties. Production of garlic has increased over the last 20 years, reaching 29 million tons in 2022 (Food & Agriculture Organization of the United Nations (FAO), 2025). In Europe, 800,000 tons are produced from 97,000 ha. In EU countries, garlic is usually grown to high quality standards (Galvez and Palmero, 2022).

Garlic produced in France (19,430 tons from 4,360 ha; FAO, 2025), mainly in the southern half of the country, benefits from high quality standards and certification, which ensure control of production including restric-

tion of production to particular regions (Chrétien *et al*., 2020). Despite intensive control and certification of seed and plants, French garlic is affected by Fusarium dry rot (FDR). Symptoms of this disease mainly manifest during garlic storage: brown discolourations and white/pink mycelia appear on cloves, and can extend until the affected plant heads collapse. Garlic heads with these symptoms can no longer be sold, and can cause severe income losses for producers (Chrétien *et al*., 2021). These causes and effects have been observed in neighbouring European countries (Galvez and Palmero, 2022).

In the past 20 years, FDR has emerged internationally. The disease has been reported in many countries in Europe (Germany, Spain, Italy, France, Serbia, Slovakia, Bulgaria), the Americas (United States of America, Argentina, Mexico), Asia (Russia, India, China, Turkey), Africa (Egypt), and more recently in Australia (Harper *et al*. 2025). Several *Fusarium* species have been isolated from garlic with FDR symptoms, but *F. proliferatum* is the most frequently identified causal agent (Dugan *et al*., 2003; Stankovic *et al*., 2007; Palmero *et al*., 2010; Sankar and Babu, 2012; Tonti *et al*., 2012; Moharam *et al*., 2013; Ochoa Fuentes *et al*., 2013; Salvalaggio and Ridao, 2013; Ignjatov *et al*., 2018; Leyronas *et al*., 2018; Mondani *et al*., 2021; Chrétien *et al*., 2021; Gálvez and Palmero, 2021; Horáková *et al*., 2021; Dedecan *et al*., 2022).

At present, there is no efficient method to prevent outbreaks of garlic dry rot. This is due to lack of knowledge about factors affecting dry rot development (e.g. nature of inoculum reservoirs, time and place of infections, conditions for symptom expression). Increased knowledge is required on epidemiology of FDR, including enhanced understanding of the biology and ecology of *F. proliferatum* as the main pathogen, and the conditions that promote its development. Since FDR is emerging internationally, comparison of the phenotypic characters that affect pathogen fitness and the genotypic profile of *F. proliferatum* isolates obtained in different countries could indicate reasons for the increasing importance of this pathogen.

Fusarium proliferatum is a polyphagous fungus, with a host range including more than 25 dicot and monocot plant species, that are herbaceous plants and trees. This fungus can cause different host symptoms including wilt, blight, rot, and dieback. The pathogen can also be endophytic in some without inducing symptoms or only causing diseases under particular conditions (Kwon *et al*., 2001; Proctor *et al*., 2010). In garlic, *F. proliferatum* can be isolated from symptomatic and asymptomatic cloves (Chrétien *et al*., 2021; Mondani *et al*., 2021), making it difficult to establish how disease develops. The conditions underlying the transition of *F. proliferatum* from the endophytic to symptomatic phases in garlic are not known.

In contrast to its diversity of hosts and the symptoms it causes, *F. proliferatum* has low genetic diversity as shown from sequencing of several genes (*tef-1α, rpb1, rpb2*, ITS, and IGS). Chretien *et al*. (2021) reported low diversity of isolates from French garlic collected from geographically separated growing regions over several years. The same low genetic diversity was observed for *F. proliferatum* isolates collected from Spanish garlic (Gálvez *et al*., 2017). Limited intraspecies divergence of the *tef-1α* sequences was also observed in isolates collected from different host species (Stępień *et al*., 2011).

The present study aimed to genotypically and phenotypically characterize *F. proliferatum* isolates to increase understanding of development of dry rot in garlic. A collection of *F. proliferatum* isolates was assessed for genetic characteristics with microsatellites markers. These neutral markers enable comparison between groups of isolates, and assessment of their possible differentiation. The study also assessed isolate phenotypic traits possibly linked to epidemic development (mycelium growth, conidiation, aggressiveness) at different temperatures and pH values.

MATERIALS AND METHODS

Fungal material

Several sets of isolates were used to assess indices of genetic diversity and effects of temperature and pH on mycelium growth, conidium production, and pathogenic aggressiveness. All the isolate sets were selected to represent the diversity present in the overall collection at the time the experiments were carried out.

One hundred and eighty-five isolates of *F. proliferatum* were genotyped (Supplementary material SI 1). Of these isolates, 178 were from the collection of the Pathologie Végétale Research Unit (Chrétien *et al*., 2021). These isolates originated from France, and their sources were distributed among garlic types (pink, white or purple), from southwestern and southeastern production areas, and among years of production (2017, 2018, or 2019). Two isolates, also collected from garlic and originating from Spain, and two from the United States of America were also provided, respectively, by the Universidad Politécnica de Madrid and the Agricultural Research Service (United States Department of Agriculture). Three *F. proliferatum* isolates were also provided by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) had been isolated from, respectively, *Triticum* sp., *Zea mays*, or *Hordeum vul-*

gare. One isolate of *F. solani* (FA574-E04) and one of *F. oxysporum* (FA112-E04), both obtained from French garlic, were also included in the isolate set that was genotyped in an assessment of the specificity of microsatellites markers.

To characterize phenotypic traits linked to epidemic development, 55 *F. proliferatum* isolates were studied for their mycelium growth on Potato Dextrose Agar (PDA; Difco). Among these, 25 isolates had been collected from French garlic. To compare life history traits of French *F. proliferatum* isolates with those of isolates of different origins, seven isolates collected in Spain and 11 isolates collected in the United States of America (USA) were included in this experiment (Supplementary material SI 1). In addition, 12 *F. proliferatum* isolates originating from plants other than garlic were also assessed; from onion (*Allium cepa*), soybean (*Glycine max*), barley (*Hordeum vulgare*), wheat (*Triticum* sp.), maize (*Zea mays*), *Populus* sp., palm tree (*Bactris gasipaes*), or banana (*Musa* sp.).

A sub sample of 25 *F. proliferatum* isolates representing the diversity of the main sample (15 from garlic, including six from France, six from the USA, three from Spain; and ten from other plants), were characterized for their conidiation capacities. Twenty *F. proliferatum* isolates collected from French garlic (13 from south eastern France and seven from the southwest) were assessed for their pathogenicity at 23 °C and at 30 °C.

Isolate genotyping

Genomic DNA was extracted in 96-well plates from 100 mg fresh weight amounts of frozen mycelium (collected from 14-d-old colonies), following the Dneasy Plant Extraction Kit protocol (Qiagen). Eight microsatellite markers designed for *F. proliferatum* by Moncrief *et al*. (2016) were amplified with forward primers conjugated with the following fluorescent dyes: 6FAM for loci SSR37 and SSR45, NED for loci SSR18 and SSR 68, VIC for loci SSR32 and SSR109, and PET for loci SSR38 and SSR92. Reverse primers did not carry any fluorescent dye. The SSR32 locus could not be amplified with the initial protocol tested on 15 isolates representing the diversity of the main sample (geographical origin, garlic type, year of isolation). Despite modifications to the protocol, no amplification product could be obtained for SSR32, so this locus was excluded from further analyses.

The seven remaining loci were amplified using 55 °C as the hybridization temperature. To determine the size of the microsatellites, the PCR products were diluted and multiplexed prior to scanning using an ABI 3730XL sequencer (Applied Biosystems). The multiplexing consisted of mixing the PCR products of several markers in each microplate well. The PCR products were separated in two mixes: one for markers SSR18, SSR37, SSR38 and SSR108, and the second for markers SSR45, SSR68 and SSR92. In each well, 500LIZ was used as a standard size marker. PCR and product scanning were carried out using Genoscreen. GeneMapper software version 4.1 (Applied Biosystems) was then used for microsatellite size analyses. Complete microsatellite size profiles (referred hereafter as "haplotypes") were obtained for 54 *F. proliferatum* isolates.

Genetic characterization and comparison of Fusarium proliferatum *isolates*

Unbiased gene diversity (Hnb) was computed separately for the isolates collected from the three garlic types (pink, purple, or white) and areas of production (southeast of southwest France), the Genetix software (Belkhir *et al*., 1996–2004).

The index of clonal richness (R) was calculated, using the following formula:

$$R = (G-1)/(N-1),$$

where G is the number of distinct haplotypes and N is the number of individuals with complete haplotypes. This index estimates the proportion of clonal haplotypes present in a population, and is equal to 1 when a population is composed exclusively of unique haplotypes (Arnaud-Haond *et al*., 2007).

The level of genetic differentiation between the *F. proliferatum* isolates from different types of garlic and different production areas was assessed by computing Weir & Cockerham's fixation indices (FST), using the software Arlequin version 3.5 (Excoffier *et al*., 2005). The monomorphic locus (SS92) was removed for these analyses.

Evaluation of mycelium growth and conidiation of Fusarium proliferatum *isolates*

To evaluate temperature effects on mycelium growth and conidiation, six temperatures were used (4, 15, 21, 30, 35 or 40 °C). In this experiment, PDA of pH 5.5 was used. Three replicate PDA plates were used for each isolate and for each temperature. To evaluate effects of pH, six values (4.5, 5.0, 6.0, 7.0, 8.0 or 9.0) were assessed. Medium pHs were adjusted with HCl and NaOH before sterilization, and were verified after sterilization. The Petri inoculated plates were stored in a climate chamber at 21 °C in the dark. Three replicate plates were used for each isolate and each pH value.

Agar plugs (4 mm diam.) were taken from the growing margins of 7-d-old colonies, and placed in the centres of PDA Petri plates (90 mm diam.). All Petri plates for every modality were then stored in the dark. Seven days after inoculation of Petri plates, the diameter of each resulting colony was measured in two directions at right angles to each other. Mycelium growth after 7 d was calculated as the average of the two diameters divided by seven, and expressed in mm d^{-1}. After 14 d, mycelium and conidia were collected from Petri plates by gently rubbing each agar surface with a cotton bud. All the collected material was then placed in 30 mL of water and homogenized. The concentration of conidia in each suspension was assessed using hemocytometer, and the total number of conidia in 30 mL was calculated. Conidium production was expressed as number of conidia produced per Petri plate.

Evaluation of Fusarium proliferatum *aggressiveness at 23 and 30 °C*

This assessment aimed to evaluate the aggressiveness of *F. proliferatum* at a temperature near 20 °C and at a higher temperature similar to those that occur in southern France in summer, when garlic is harvested, dried, and then stored. This experiment was carried out using plants growing in climate chambers set at 23 and 30 °C. Twenty isolates of *F. proliferatum* were inoculated onto garlic cloves (variety Edenrose, pink type), using the protocol of Chrétien *et al*. (2021). In brief, asymptomatic garlic cloves were surface disinfected and were then soaked in conidium spore suspensions (10^5 conidia mL^{-1}) for 24 h. Twenty-four cloves were inoculated with each isolate, and 12 cloves were then incubated in the dark at 23 °C and 12 cloves were incubated at 30 °C. The areas of resulting lesions were monitored and scored at 4, 6, 8, 11, 13, 15, and 18 d after inoculation, using the notation scale of Chrétien *et al*. (2021). These data were used to compute areas under disease progress curves (AUDPC). Isolate FA3-E01 was used as a reference for pathogen aggressiveness. For each isolate, an index of aggressiveness (IA) was calculated relative to that from isolate FA3-E01, as follows: $IA_{isolate} = 100 \times (AUDPC_{isolate}/AUDPC_{FA3\text{-}E01})$.

Statistical analyses

Statistical analyses were carried out using R software (R Core team, 2013) and Statistica (version 12, Statsoft). Mycelium growth and conidiation data were compared with analyses of variance (ANOVAs) and Tukey's HSD *post hoc* test. Dunn's tests were carried out to determine which groups were different. Statistical inferences were made at $P > 0.05$. Indices of aggressiveness were compared using Mann-Whitney tests. Kruskall-Wallis tests were used to compare mycelium growth and conidium production of the different groups of isolates for each assessed temperature or pH.

RESULTS

Genetic diversity of Fusarium proliferatum *isolates*

Locus SSR32 was removed from the genotyping since no amplifications were obtained from the 15 isolates used in the experiment, even using up to 40 amplification cycles. Locus SSR92 was monomorphic giving a single 359 bp allele, while the other loci were polymorphic with the number of alleles being 2 from isolates SSR18, SSR38, SSR68 and from SSR37 (Table 1). Some of the microsatellite markers lacked specificity: all except SSR109, were amplified for *F. oxysporum*, while none amplified for the *F. solani* isolate.

Despite successive trials to amplify the seven microsatellites loci, complete haplotypes were obtained for only 54 *F. proliferatum* isolates out of 185. Only five different multilocus haplotypes (MLH) were identified among the 54 haplotypes, leading to a low value index of clonal richness (0.07). The most frequent MLH was represented by 46 isolates, among which 24 isolates were from the southeastern region of France, 21 were from the southwestern basin, and one was from Spain (Table 2). The number of alleles per locus did not exceed 1.71 for any group of isolates.

There was no evidence of genetic differentiation between *F. proliferatum* isolates from southeast and southwest France, and the FST index statistic was not significant ($P > 0.05$). Also, there was no genetic differentiation between *F. proliferatum* isolates from pink,

Table 1. Polymorphism of the seven microsatellite markers (Moncrief *et al*., 2016) used in the present study to characterize the genetic diversity of the 185 *Fusarium proliferatum* isolates.

Microsatellite locus	Number of alleles	Size range (bp)
SSR18	2	380–382
SSR37	4	271–273–275–279
SSR38	2	380–382
SSR45	3	148–150–152
SSR68	2	116–121
SSR92	1	359
SSR109	2	401 - 404

Table 2. Number, origin and genetic diversity of *Fusarium proliferatum* isolates collected from garlic and other plant species in France, Spain or the United States of America (USA).

Origin	Number of isolates	Number of complete haplotypes	Hnb [a]	Number of alleles per locus	Number of distinct MLHs [b]	R[c]
France	178	53	0.0606 (0.07)	1.71	5	0.07
Southeast of France	91	28	0.0393 (0.05)	1.43	4	0.11
Southwest of France	87	25	0.0821 (0.11)	1.43	3	0.08
Pink garlic type	56	21	0.0252 (0.06)	1.14	2	0.05
White garlic type	61	16	0.1014 (0.08)	1.71	5	0.26
Purple garlic type	57	16	0.0668 (0.09)	1.43	3	0.13
USA	2	0	nd	nd	nd	nd
Spain	2	1	nd	nd	1	nd
Plant species other than garlic	3	0	nd	nd	nd	nd
Total	185	54	0.0595 (0.07)	1.71	5	0.07

[a] Unbiased gene diversity (standard deviation in parentheses).
[b] Multilocus haplotype.
[c] Clonal richness: (number of distinct MLH-1)/(total number of complete haplotypes-1).
nd: no data.

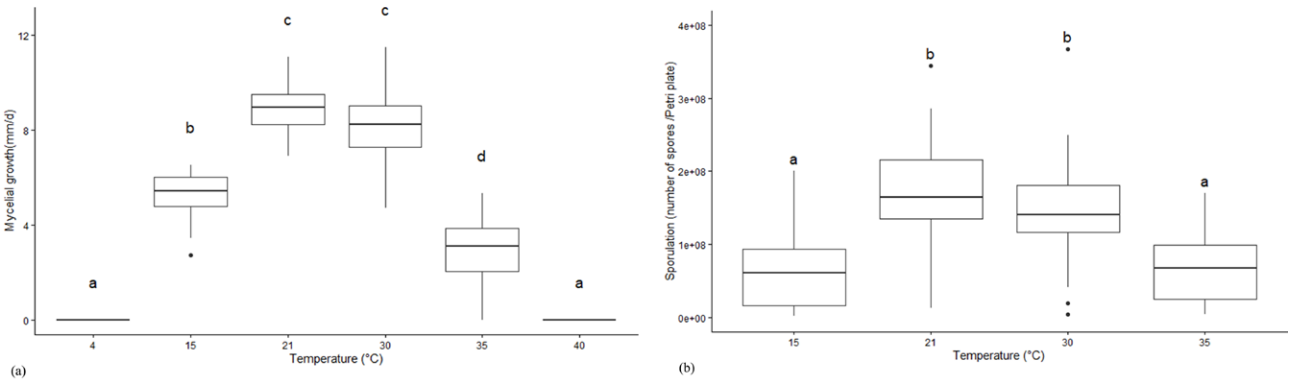

Figure 1. Mean mycelium growth rates (mm d-1; a) and numbers of conidia per Petri plate (b) for *Fusarium proliferatum* isolates collected from garlic in France, Spain or the United States of America (USA), or and from other plant hosts, grown at different temperatures. Fifty-five isolates were used in the mycelium growth assessment (a), and 25 isolates were used in the conidium production assessment. No mycelium growth or conidium production occurred at 4 or 40°C. For each temperature, the bold horizontal line inside the rectangle is the median, the horizontal borders of the rectangle are the 25 and 75% confidence intervals and the vertical lines indicate the maximum and minimum values. Outlier data are indicated by black dots. Means accompanied by the same letters are not different ($P \leq 0.05$).

purple or white garlic host types ($P > 0.05$) for pairwise combinations.

Effects of temperature on mycelium growth, conidium production and aggressiveness on Fusarium proliferatum *isolates*

None of the 55 *F. proliferatum* isolates collected from garlic in France, or from the USA or Spain, or from other plant hosts, were able to grow or produce conidia at 4 or 40 °C (Figure 1a). Mean mycelium growth rates were 5.2 mm d^{-1} at 15 °C, 8.7 mm d^{-1} at 21 °C, 7.6 mm d^{-1} at 30 °C, and 3.74 mm d^{-1} 35 °C, with significant differences ($P < 0.003$) between temperatures.

The 25 *F. proliferatum* isolates assessed for conidium production at different temperatures showed greater production at 21 °C (1.32×10^7 to 3.44×10^8 conidia per Petri plate after in 14 d), and at 30 °C (from $3.9\ 10^6$ to 3.7×10^8) than at 15 °C (1.8×10^6 to 2×10^8) or 35 °C (from 3.7×10^6 to 1.7×10^8) ($P < 0.001$) (Figure 1b).

When grouped according to their origins, the isolate mycelium growth rates for isolate from garlic in France were not different ($P > 0.05$) from isolates from other origins. No differences ($P = 0.238$) were found between

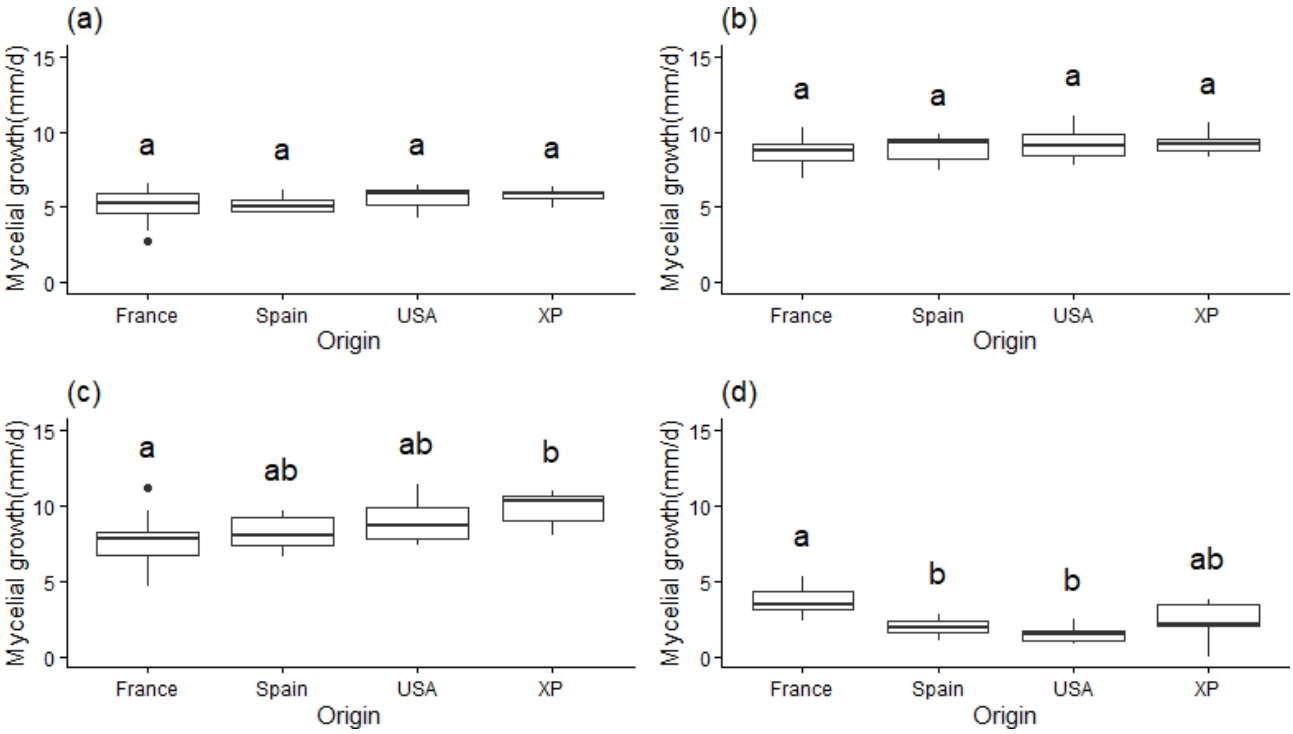

Figure 2. Mean mycelium growth rates (mm d-1) of *Fusarium proliferatum* isolates collected from garlic in France (25 isolates), Spain (seven isolates) and in the USA (11 isolates), or from other plant species (XP; 12 isolates), at 15°C (a), 21°C (b), 30°C (c) or 35°C (d). For each plot, the bold horizontal line inside the rectangle is the median, the horizontal borders of the rectangle are the 25 and 75% confidence intervals, and the vertical lines indicate the maximum and minimum values. Outlier data are indicated by black dots. Means accompanied by the same letters are not different ($P \leq 0.05$).

the isolate groups. All the isolates grew more at 21 or 30 °C than at 15 or 35 °C ($P < 0.001$ for Tukey test comparisons of means).

When comparing data from each temperature, there were significant differences between the mycelium growth of the four groups of isolates at 30 °C ($P_{Kruskall-Wallis} = 0.03$) and 35 °C ($P_{Kruskall-Wallis} < 0.001$). At 30 °C, the French isolates were the slowest-growing (Figure 2c), whereas at 35 °C they were the fastest-growing. (Figure 2d). However, there were no significant differences of conidium production between the different groups of isolates at each temperature ($0.056 < P_{Kruskall-Wallis} < 0.219$), except at 35 °C between the isolates from France and from USA (Figure 3d).

Aggressiveness at 23 and 30 °C

When inoculated onto plants of the Edenrose variety, all 20 *F. proliferatum* isolates collected from French garlic produced disease symptoms. The index of aggressiveness (IA) ranged from 67 to 142% of the reference isolate at 23 °C, and from 66 to 125% at 30 °C (Figure 4). Isolates IAs did not differ ($P_{Mann-Whitney} = 0.96$) when the garlic cloves were incubated at 23 °C, from when they were incubated at 30 °C. The IA values at 23 °C were positively correlated with the equivalent values at 30 °C ($R_{Pearson} = 0.89$, $P < 0.05$). This showed that the more aggressive a *F. proliferatum* isolate was at 23 °C, the more aggressive it was at 30 °C on garlic.

Six isolates (with IAs ranging from 72 to 142% at 23 °C) were characterized for aggressiveness and mycelium growth. There were no correlations between their mycelium growth rates and their aggressiveness at 30 °C ($R^2 = 0.01$). However, there was significant correlation between mycelium growth at 21 °C and aggressiveness at 23 °C ($R^2 = 0.7$).

Effects of pH on mycelium growth and conidium production by Fusarium proliferatum

All 55 *F. proliferatum* isolates were able to grow at pHs between 4.5 and 9.0, with mean mycelium growth rates ranging from 5.2 to 11.9 mm d^{-1}. Growth rate differences between pHs were significant ($P < 2 \times 10^{-16}$)

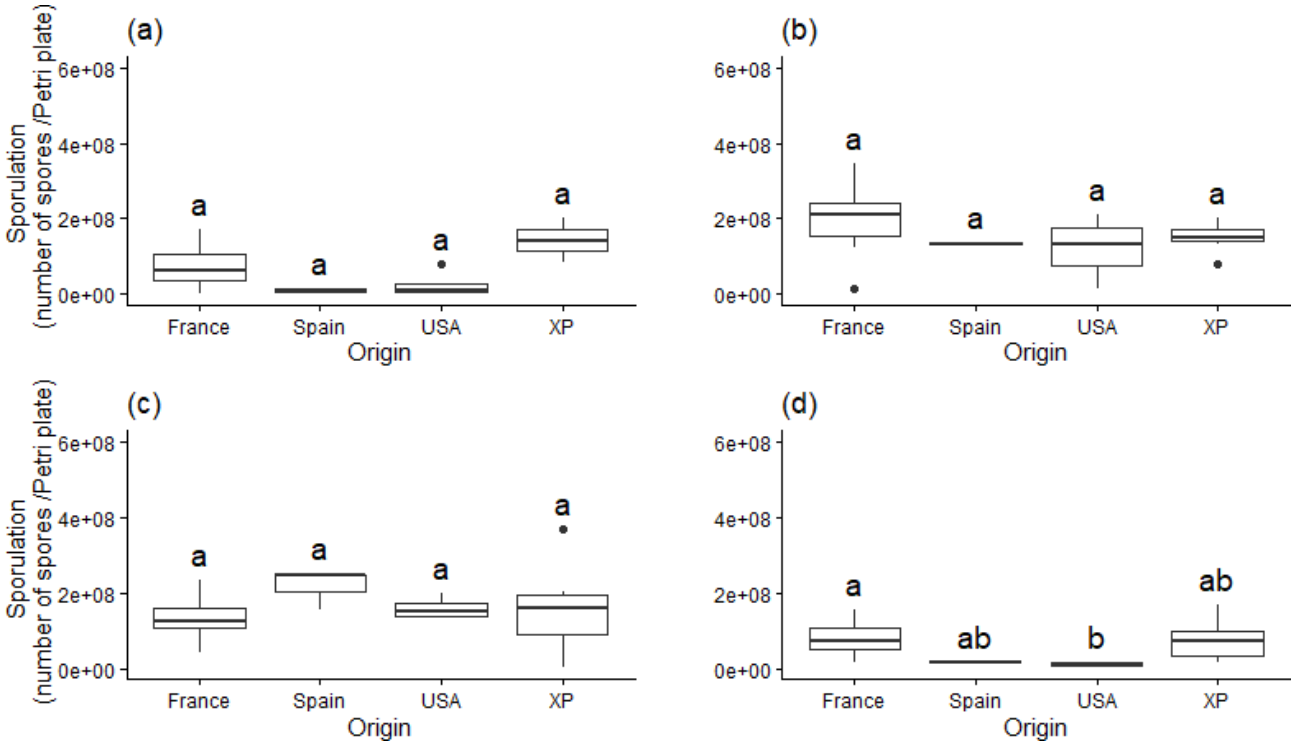

Figure 3. Mean numbers of conidia per Petri plate for cultures of *Fusarium proliferatum* isolates from garlic from France (six isolates), in Spain (three) or the USA (six), or from other plant species (XP; ten), at 15°C (a), 21°C (b), 30°C (c) or 35°C (d). For each plot, the bold horizontal line inside the rectangle is the median, the horizontal borders of the rectangle are the 25% and 75% confidence intervals, and the vertical lines indicate the maximum and minimum values. Outlier data are indicated by black dots. Means accompanied by the same letters are not different ($P \leq 0.05$).

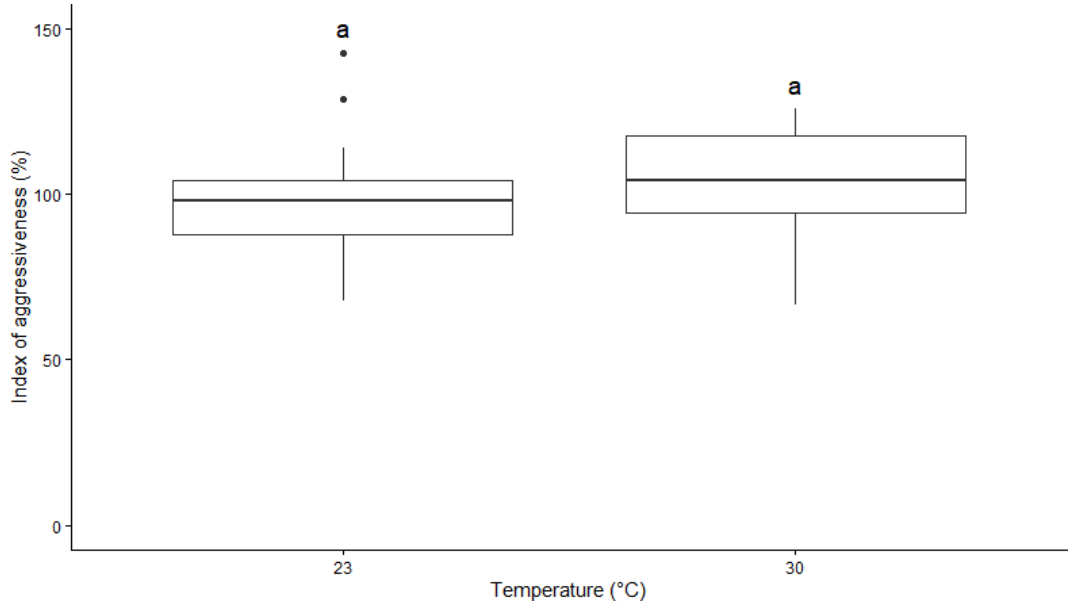

Figure 4. Mean indices of aggressiveness of 20 isolates of *Fusarium proliferatum* obtained from garlic in France, as determined at 23 or 30°C when inoculated onto bulbs of the Edenrose variety. Isolate FA3-E01 was a reference. For each plot, the bold horizontal line inside the rectangle is the median, the horizontal borders of the rectangle are the 25 and 75% confidence intervals, and the vertical lines indicate the maximum and minimum values. Outlier data are indicated by black dots. Means accompanied by the same letters are not different ($P \leq 0.05$).

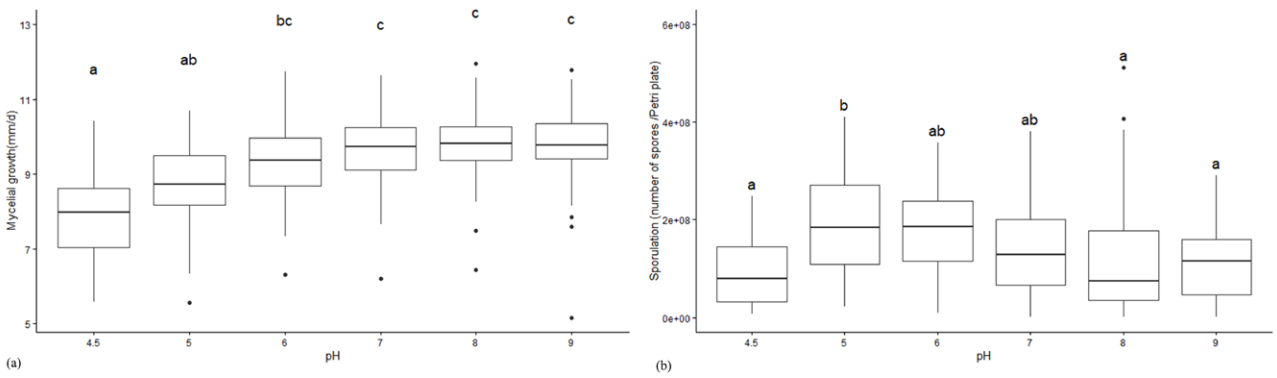

Figure 5. (a) Mean mycelium growth rates (mm d-1), and (b) mean numbers of conidia, after 7 d in Petri plates containing PDA of different pHs, for *Fusarium proliferatum* isolates obtained from garlic in France, Spain the USA, or from other plant species. Fifty-five isolates were used in the mycelium growth assessments, and 25 were used in the conidium production experiment. For each plot, the bold horizontal line inside the rectangle is the median, the horizontal borders of the rectangle are the 25 and 75% confidence intervals, and the vertical lines indicate the maximum and minimum values. Outlier data are indicated by black dots. Means accompanied by the same letters are not different ($P \leq 0.05$).

(Figure 5a). The isolates grew more slowly at pH 4.5 than at all the other pHs (all $P_{Tukey} < 0.001$). They also grew more slowly at pH 5.0 than at pH 6.0 ($P_{Tukey} = 0.049$), pH 7.0, pH 8.0, and pH 9.0 (all $P_{Tukey} < 0.001$). There were no differences ($P_{Tukey} > 0.4$) for mycelium growth at pH 9.0, pH 8.0, pH 7.0 or pH 6.0.

All the isolates of the subsample assessed for conidium production produced conidia at pHs between 4.5 and 9.0, with numbers of conidia produced per Petri plate after 14 d ranging from 4.5×10^5 at (pH 7.0, 8.0 or 9.0) to 5.11×10^8 (at pH 8.0) (Figure 5b) Conidium production was different at pH 5 compared to pH 4.5 ($P = 0.003$), pH 8.0 ($P = 0.019$) or pH 9.0 ($P = 0.002$). There were no other statistically significant differences in conidium production at the other pH values assessed.

When comparing data at a particular pH, there were no significant differences in mycelium growth between the four groups of isolates at pH 4.5 ($P_{Kruskall\text{-}Wallis} = 0.50$), pH 5.0 ($P_{Kruskall\text{-}Wallis} = 0.63$), pH 7.0 ($P_{Kruskall\text{-}Wallis} = 0.63$), pH 8.0 ($P_{Kruskall\text{-}Wallis} = 0.46$), or pH 9.0 ($P_{Kruskall\text{-}Wallis} = 0.44$) (Figure 6). There was a small difference ($P = 0.0505$) at pH 6.0. The isolates from Spain grew more slowly than the isolates from the other three regions.

When each pH value was considered separately, there were statistically significant differences among the four geographical groups of isolates at pH 5.0 ($P = 0.0019$) and pH 7.0 ($P = 0.017$) (Figure 7).

DISCUSSION

The present study aimed to characterize genetic and phenotypic characteristics of *F. proliferatum* isolates collected from different origins, to provide increased knowledge of development of dry rots in garlic bulbs.

Isolates of *F. proliferatum* were well-adapted to the cultural conditions for garlic crops, including soil pH and ambient temperatures during plant growth, harvest and storage. Garlic is cultivated in soil with basic pH (Villeneuve and Erard, 2012), and the present study results showed that isolates of *F. proliferatum* grew more rapidly at basic than at acid pHs. In Europe, garlic is harvested in early summer, and is then dried and stored. In the south of France, temperatures usually reach 30 °C and above during summer. The present study showed that *F. proliferatum* can grow and can produce conidia at these temperatures, that are generally not optimal for the development of fungi growing in temperate regions. These results are similar to those of Stępień *et al.* (2011), and Galvez *et al.* (2017). In the present study, the *F. proliferatum* isolates did not grow at low temperature but remained viable, confirming that low temperatures are suitable for garlic storage (Mondani *et al.*, 2021), to prevent or to slow appearance of FDR symptoms. As soon as garlic bulbs are back at ambient temperature, disease symptoms may develop or increase.

Internationally, there are few or no differences among *F. proliferatum* isolates collected from French garlic and those from garlic Spain or the USA, or from isolates other plants (except for mycelium growth, where the French isolates grew more slowly at 30 °C, and more rapidly at 35 °C). Phenotypic traits were investigated in the present study to reveal potential diversity among *F. proliferatum* from different regions. Although limited genetic diversity was shown, phenotypic diversity would indicate adaptation of *F. proliferatum* to different regions, and determining if there were biogeographi-

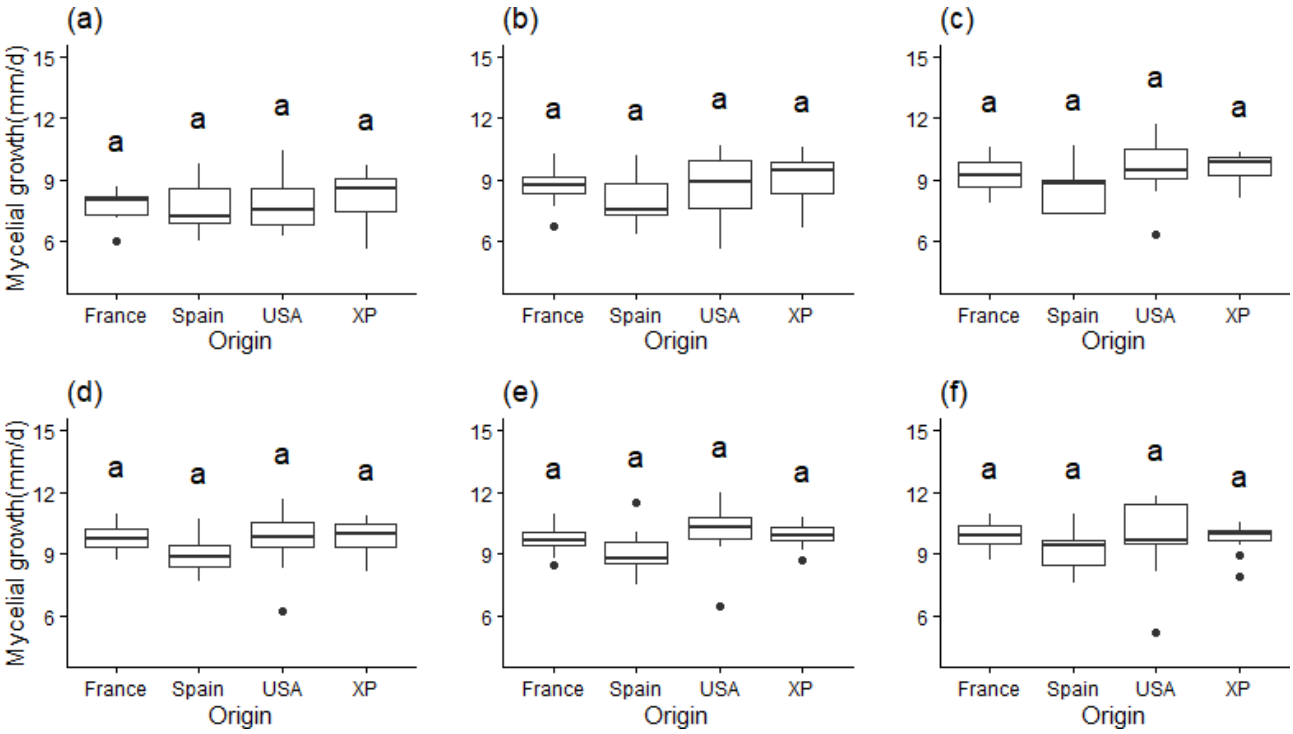

Figure 6. Mean mycelium growth rates for *Fusarium proliferatum* isolates collected from garlic in France (25 isolates), Spain (seven) or the USA (11), or from other plant species (XP; 12 isolates), at different pHs of 4.5 (a), 5.0 (b), 6.0 (c), 7.0 (d), 8.0 (e), or 9.0 (f). For each plot, the bold horizontal line inside the rectangle is the median, the horizontal borders of the rectangle are the 25 and 75% confidence intervals, and the vertical lines indicate the maximum and minimum values. Outlier data are indicated by black dots. Means accompanied by the same letters are not different ($P \leq 0.05$).

cal specializations. The regions of isolate origin in the present study were probably not sufficiently contrasting. Further research should assess isolates originating from regions where FDR is present, but with contrasting soils and climates (e.g. cold winters as in Canada or Russia, or very hot summers as in Egypt).

In the present study, microsatellite markers were used to possibly differentiate pathogen isolates. When these markers are well designed, they are suitable for genotypic characterization of fungi, enabling different groups to be compared on the basis of indices of genetic diversity (Dutech *et al.*, 2007). Despite the attempts used here, difficulty was experienced for amplifying the microsatellites designed for *F. proliferatum* by Moncrief *et al.* (2016) to the 188 *F. proliferatum* isolates. The isolates that were amplified showed low numbers of alleles. This low number, and the consequent low numbers of haplotypes, could be due to low haplotypic diversity of *F. proliferatum*, or this could be an artefact of the low polymorphism of the microsatellite markers.

Fusarium proliferatum is a polyphagous species, and the host symptoms it generates are occurring on *Allium* host species (Stankovic *et al.*, 2007; Alberti *et al.*, 2018; Haapalainen *et al.*, 2023). Therefore, study of genetic similarities or differences among groups of isolates from diverse hosts and origins would be useful. This could increase understanding of population dynamics and provide insights for possible methods for protecting garlic and other *Allium* species. If host specificities were detected, new plant rotations could be defined and utilized. In the South of France, garlic is often cultivated in rotation with wheat or maize, but these crops can harbour *F. proliferatum* (Proctor *et al.*, 2010). It would be worthwhile to determine if there is one population on the different hosts, and if *F. proliferatum* inhabiting a particular host species can produce inoculum for neighbouring or following crops, crops through crop residues. Therefore, future research should: 1) develop methods to detect endophytic forms of *F. proliferatum* in diverse hosts (we are currently developing molecular methods for detection); 2) define more microsatellites markers with high polymorphism and specificity for *F. proliferatum*; and 3) inoculate garlic with *F. proliferatum* isolates from different hosts to assess their pathogenicity.

Factors causing emergence of FDR on garlic and other alliaceous plants remain to be clarified. Emer-

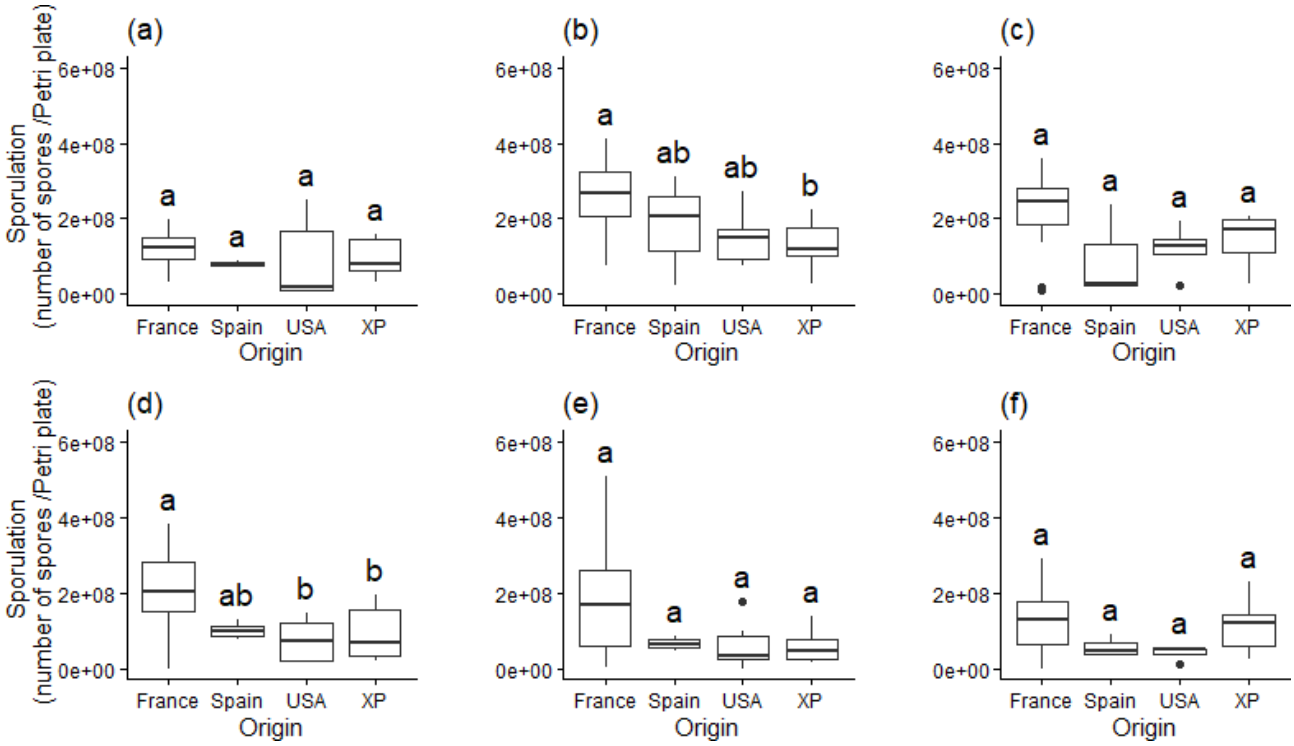

Figure 7. Mean numbers of *Fusarium proliferatum* conidia per Petri plate produced by isolates collected from garlic in France (six isolates), in Spain (three) or the USA (six), or from other plant species (XP; ten isolates), at different pHs of 4.5 (a), 5.0 (b), 6.0 (c), 7.0 (d), 8.0 (e), or 9.0 (f). For each plot, the bold horizontal line inside the rectangle is the median, the horizontal borders of the rectangle are the 25% and 75% confidence intervals, and the vertical lines indicate the maximum and minimum values. Outlier data are indicated by black dots. Means accompanied by the same letters are not different ($P \leq 0.05$).

gence of plant diseases can be driven by different factors, including introduction of new pathogens (trade in plant products, dissemination by extreme climate events), agricultural changes (intensification, globalization), evolution of host and pathogen relationships, and/or climate change (Anderson *et al*., 2004). However, most of these processes would be associated with geographic patterns, and would unlikely lead to international disease emergence. Since *F. proliferatum* is often endophytic in garlic cloves (Chrétien *et al*., 2021; Mondani *et al*., 2021), and because isolates have low genetic diversity, it is possible that this fungus has always lived in symbiosis with garlic (and other *Allium* hosts). However, the *Fusarium*/garlic association has recently been of increasing importance, probably due to climate change that is common to all areas where FDR is emerging. This situation is unlikely to improve. A recent modelling study based on different climate change scenarios has shown that increased temperatures will expand the geographic range of *F. proliferatum*, particularly in regions that are not presently suitable for this pathogen (Tagyan *et al*., 2025). Research is therefore required to determine how to maintain the *Fusarium*/garlic association as asymptomatic and without mycotoxin production.

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https://eng-pathologie-vegetale.paca.hub.inrae.fr/infrastructures/prophyle/experimental-facilities

AUTHOR CONTRIBUTIONS

The projects in which the data presented in this paper were collected were managed by Christel Leyronas. All authors contributed to conception and design of the study. Material preparation, laboratory experiments and data collection were carried out by Christel Leyronas, Paul Chrétien, Magali Duffaud, Jean-François Bourgeay and Claire Troulet. Data analyses was carried out by Christel Leyronas. The first draft of the manuscript was written by Christel Leyronas, and all authors commented on versions of the manuscript. All the authors read and approved the final manuscript.

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