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Etiology of garlic rot, an emerging disease in France.

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Abstract

The incidence of garlic rot has constantly increased in France since the early 2000s. To set up an efficient method of garlic protection against this disease, we have clarified the etiology of this disease. This was achieved by surveying garlic from the two main French basins of garlic production during 3 years. Fungi were isolated from 5493 garlic cloves belonging to pink, purple and white garlic types. Sequencing of the translation elongation factor 1 alpha gene of 1171 strains revealed that 94 % of the strains belong to the species *Fusarium proliferatum* and 6 % belong to *F. oxysporum*. The pathogenicity of both species on garlic was confirmed by artificial inoculations and re-isolations. There was significantly more *F. oxysporum* in symptomatic garlic cloves coming from the southeast basin (9.44 %) than from the southwest basin (2.76 %). This study confirms that garlic rot is present in pink, purple and white types. However, pink type garlic harbors *F. oxysporum* significantly less frequently (1.59 %) than do white (9.39 %) and purple (7.34 %) types. Sequencing of *rpb1*, *rpb2*, *ITS* and *IGS* regions of a subsample of strains revealed that there is little genetic diversity of the French population of *F. proliferatum*.

Keywords

Allium sativum, molecular identification, *Fusarium proliferatum*, *Fusarium oxysporum*

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Introduction

The worldwide production of garlic (*Allium sativum* L.) has been regularly increasing over the last twenty years. In 2018, more than 28 million of tons garlic were produced on more than 1.5 million ha mostly located in tropical and temperate regions (Food and Agriculture Organization of the United Nations). In addition to its organoleptic contribution to various cuisines, garlic has been reported to have properties that promote human health, including about twenty organosulfur compounds with antimicrobial properties. Recently, garlic extract has been used as a biocontrol product to protect crops against fungal diseases (Curtis et al., 2004; Slusarenko et al., 2008). The antimicrobial properties of garlic constitute quite a paradox since garlic can be attacked by several fungal diseases (rust, white rot…). The most recently reported disease is garlic rot. This disease manifests itself during storage and inside collapsed garlic heads, where cloves show brown discoloration and the development of white/pink mycelia. Garlic heads with such symptoms can no longer be commercialized. Since 1986 when these symptoms were first described in Japan (Matuo et al., 1986), similar symptoms have been observed in every garlic production area. It has been reported in North America (Dugan et al., 2003; Ochoa-Fuentes et al., 2013), Europe (Koleva, 2004; Stankovic et al., 2007; Palmero et al., 2010; Tonti et al., 2012; Ignjatov et al., 2017), North Africa (Moharam et al., 2013) and Asia (Sankar & Babu, 2011).

In France, ranking sixth among European producers, garlic rot disease appeared in 2006. Thirty-three certified cultivars that belong to three types of garlic are cultivated in France. Cultivars of the white type (named white cultivars in the rest of the text) are planted in late autumn. They have large heads with about ten cloves per head and have the sweetest taste of all garlic cultivars. The cultivars of the purple type (names purple cultivars in the rest of the text) are planted in early autumn. They have a stronger taste and an intense purple color. The cultivars of the pink type (named pink cultivars in the rest of the text) are planted in spring. They have smaller, yet more numerous cloves per head. Some pink cultivars maintain a vestige of the floral stalk right in the middle of the head whereas floral stalks are completely absent from cultivars belonging to white and purple types. All these garlic cultivars are cultivated in two major basins of production (the southwest basin and the southeast basin), that are 200 km apart, separated by the Massif Central Mountain range. The southeast basin is subject to Mediterranean climate whereas the southwest basin is subject to a modified oceanic climate. Both basins soil is mostly acid and calcareous. Some garlic cultivars are cultivated in a single basin but the majority is cultivated in both.

In spite of intensive control and certification of seeds and plants, losses due to this disease are

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increasing. It can put French garlic producers in situations where they stop production of some cultivars and incur drastic losses of income (Chrétien et al., 2020). Producers of pink cultivars of garlic are particularly concerned since they have the impression that pink cultivars are more sensitive to the disease. In order to protect high quality standards and labels of French garlic there is a need to set up efficient, sustainable and environment-friendly methods of avoiding infection of garlic and the concomitant losses. To avoid infection it is necessary to determine when and how initial infection occurs. This will require early tracing of garlic health that is founded on comprehensive knowledge of the causal agent(s). To date, seven species of *Fusarium* have been isolated from garlic rot symptoms in different countries: *F. proliferatum* (Dugan et al., 2003; Stankovic et al., 2007; Palmero et al., 2010; Sankar & Babu, 2011; Tonti et al., 2012; Moharam et al., 2013; Ochoa-Fuentes, 2013; Ignjatov et al., 2018), *F. acuminatum* (Ignjatov et al., 2017), *F. culmorum* and *F. graminearum* (Koleva, 2004), *F. oxysporum* and *F. solani* (Moharam et al., 2013) and *F. tricinctum* (Ignjatov et al., 2016). In France, we have reported the presence of *F. proliferatum* on pink garlic (Leyronas et al., 2018).

The objective of the present study is to elucidate the etiology of garlic rot disease in France. The specific goals were to 1) identify *Fusarium* species isolated from different garlic types produced in the two main French basins of production, 2) verify the virulence and aggressiveness of these species on garlic, 3) assess their intra and inter specific genetic diversity and 4) determine if the causal agent(s) is(are) uniformly distributed between the two main French production regions and the different cultivars produced.

Materials and methods

1. Origin of garlic cloves

Sampling was carried out in the southwest basin and the southeast basin of garlic production in 2017, 2018 and 2019. Garlic heads were collected during the storage period (i.e. 3 weeks after harvest) and sent to the laboratory. For each basin, several cultivars of white (n=3), pink (n=4) and purple (n=1) garlic were studied. The cultivar names are not disclosed here to respect confidentiality agreements that are required when collaborating with the garlic production industry. For each cultivar, several batches (n=1 to 15) were used. A batch correspond to a field where the garlic cultivar is cultivated. Disease incidence on heads in a batch was determined by the number of garlic heads needed to be peeled in order to obtain three symptomatic heads. A symptomatic head was defined by the presence of at least three cloves showing discoloration from

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brown to orange with dry or water-soaked lesions to complete degradation. The clove incidence was determined based on the number of symptomatic cloves inside a garlic head out of the total number of cloves in that head. These two types of disease incidence were calculated for the 2018 and 2019 crop seasons.

2. Fungal isolation and purification

One isolate was collected from each symptomatic clove. All cloves were surface-disinfected in a 1 % chlorine bleach bath for 1 minute. They were then rinsed in 3 successive baths of sterile water for 1 minute each. The excess water was removed with sterile absorbent paper. The symptomatic region was cut out and placed on Potato Dextrose Agar medium (PDA; Difco Laboratories). Petri plates were stored at room temperature (20 °C) and under natural light until fungal colonies appeared (from 2 days to 2 weeks). The *Fusarium*-like colonies (white to slightly pink, orange or purple mycelium and cottony appearance) growing out from the samples were transferred to new PDA plates. Isolates were then purified on agar-water medium. A mycelial plug carrying a single piece of hyphal tip was excised from the growing margin of each *Fusarium*-like colony and transferred to PDA. After 14 days of incubation at 21 °C, mycelium and spores were collected from the surface of the agar and stored at -20 °C in 25 % glycerol buffer before further analyses.

3. Molecular identification of strains

Among the 2729 strains isolated from symptomatic garlic, 1366 were chosen for molecular identification to represent the two basins of production, the 8 garlic cultivars and the three sampling years (see Table 1 for distribution) in order to determine if the causal agents were uniformly distributed between the different basins, cultivars and years. The mycelium was collected from 2-week-old colonies growing on PDA by gently rubbing the agar surface with a sterile cotton swab. DNA was extracted in 96 well-plate according to the DNeasy® 96 Plant Kit Qiagen protocol. For each strain, a partial region of the translation elongation factor 1 alpha gene (*tef-1α*) was amplified with the primers EF1 (5'-ATG GGT AAG GAR GAC AAG AC-3') and EF2 (5'-GGA RGT ACC AGT SAT CAT GTT-3') (O'Donnell & Cigelnik, 1997). The PCR program was as follow: of 15 min at 95 °C, 35 cycles at 94 °C for 30 s, 57 °C for 90s and 72 °C for 60 s then 60 °C for 30 min. Reactions were performed in 30 µL of reaction volume with 1X Qiagen Multiplex PCR Master Mix (Qiagen, Venlo, The Netherlands), 0.4 µM of each primer and 2 µL of DNA. The amplified fragments were visualized on 1.5 % agarose electrophoresis gels

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after a 45 min run at 100 V. The amplified regions were sent to GenoScreen (Lille, France) for direct Sanger Sequencing with primer EF1.

The sequences were analyzed with Geneious prime v10.0.4 (Biomatters, Auckland, New Zealand). Raw sequences showing more than 15 % of Low-Quality bases were removed from further analyses. The remaining sequences were aligned with Geneious using default parameters and species were identified using both databases of Fusarium MLST (https://Fusarium.mycobank.org/) and Fusarium ID (http://isolate.Fusariumdb.org/blast.php). Neighbor-joining trees were built with the Geneious Tree Builder default parameters (1000 bootstrap replicates and 50% consensus support threshold) to visually confirm the molecular identification (not shown). The sequence of *tef-1α* of *F. dimerum* from the *MIAE strain collection* (Héraud et al., 2010) was used to root the tree where necessary.

4. *Fusarium* sp. genetic diversity

In addition to the *tef-1α* region (~600 bp), 4 other DNA regions were selected to assess the genetic diversity of the *Fusarium proliferatum* strains: *rpb1* (RNA polymerase subunit 1) (~1400 bp), *rpb2* (RNA polymerase subunit 2) (~1100 bp), *ITS* (internal transcribed spacer) (~500 bp) and *IGS* (intergenic spacer) (~550 bp). A sub-sample of 95 strains representing the different types of garlic, the two production basins and the 3 years of sampling was chosen for the *rpb1* and *rpb2* regions. They were amplified for all of these strains with the primers RPB1-Af (5'-GAR TGY CCD GGD CAY TTY GG-3') / RPB1-Cr (5'-CCN GCD ATN TCR TTR TCC ATR TA-3') (Matheny et al., 2002) and RPB2-5F2 (5'-GGG GWG AYC AGA AGA AGG C-3') / fRPB2-7cR (5'-CCC ATR GCT TGY TTR CCC AT-3') (Liu et al., 1999; Šišić et al., 2018). The *rpb1* PCR program consisted of 15 min at 95 °C, 40 cycles at 94 °C for 30 s, 55 °C for 90 s and 72 °C for 90 s then 1 final elongation of 10 min at 72 °C. The *rpb2* PCR program consisted of 15 min at 95 °C, 5 cycles at 94 °C for 30 s, 60 °C for 90 s and 72°C for 2 min, 5 cycles at 94 °C for 30 s, 58 °C for 90 s and 72 °C for 2 min, 30 cycles at 94 °C for 30 s, 54°C for 90 s and 72 °C for 2 min then final elongation of 10 min at 72 °C.

The *ITS* region of 19 strains, representing the 2018 sampling campaign of pink garlic, was amplified with the primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White *et al*., 1990). The following program was used: 1 cycle of 15 min at 95 °C, 30 cycles at 94 °C for 30 s, 55 °C for 90 s and 72 °C for 1 min then 1 cycle of 30 min at 60 °C.

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A total of 192 strains representing the different types of garlic, the 2 production basins and the first 2 years of sampling underwent amplification for the *IGS region*. Gib2-F (5'-GAG GCG CGG TGT CGG TGT GCT TG-3') and Fgc-R (5'-CTC TCA TAT ACC CTC CG-3') primers were used (Jurado et al., 2006). The following program was applied: 1 cycle of 15 min at 95 °C, 35 cycles at 94 °C for 30 s, 58 °C for 1 min and 72 °C for 2 min then 1 cycle of 10 min at 72 °C. The presence of all PCR products was confirmed by gel electrophoresis previously to sequencing by the Sanger One Shot Sequencing method (GenoScreen, Lille, France). We were able to correctly amplified 14 *IGS* sequences and compiled them with the 52 sequences used by Jurado et al. (2012).

Due to lack of polymorphisms for the *rpb1*, *rpb2* and *ITS* region sequences, three consensus trees were constructed with sequences of the *tef-1α* and *IGS* regions. All *tef-1α* sequences obtained during the molecular identification of strains (n=1171) were included in the analysis in addition to 139 sequences of *F. proliferatum* strains and 118 sequences of *F. oxysporum* strains isolated from garlic, maize (*Zea mays*), onion (*Allium cepa*), welsh onion (*Allium fistulosum*) and wheat (*Triticum* sp.) from the GenBank collection (https://www.ncbi.nlm.nih.gov/genbank/). These reference strains had been isolated from China, Finland, France, Iran, Japan, Malaysia, Mexico, Serbia, South Africa, Spain and in the USA (Table 2).

Sequences were analyzed with Geneious prime v10.0.4 (Biomatters, Auckland, New Zealand). They were aligned (Pairwise alignment) and ends were trimmed. Unique sequences were extracted and underwent iterative pairwise alignment and trimming until all sequence lengths were strictly identical. Trees of the *F. proliferatum* and the *F. oxysporum* sequences were constructed with MEGA-X v10.1.8 software using the maximum-likelihood method in order to get a more detailed picture of the strains distribution. For each tree, the best model was chosen using ModelTest include in the software. All trees were constructed using the bootstrap method with 1000 replicates. Gaps and missing data were treated as deletions. The neighbor joining method was used in cases where the objective was to distinguish groups of strains.

To the 1171 *tef-1α* sequences of strains isolated from French garlic, we added 12 *tef-1α* sequences from strains isolated from Spanish garlic, non-garlic sources or garlic leaf debris. Spanish strains were provided by D. Palmero from the Madrid Polytechnic University (Universidad Politécnica de Madrid). Non-garlic hosts or strains were provided by R. Ioos from the ANSES Plant Health Laboratory (ANSES Laboratoire de la Santé des Végétaux) in Malzéville. Haplotypes analyzes were conducted on these 1183 strains and compared with aggressiveness tests.

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5. Confirmation of pathogenicity and symptom assessment

A total of 122 strains of *F. proliferatum* were used to represent the two basins of production, the 8 garlic cultivars and the three sampling years (Table 1). Four strains of *F. oxysporum* were also tested. Explants (n=3) of 7-day-old colonies were transferred into 300 ml Erlenmeyer flasks containing 150 ml of PDB (Potato Dextrose Broth, Difco Laboratories). The strains grew under constant agitation (100 rpm) at 21 °C, with a 12 h-photoperiod (~ 25 $\mu mol.m^{-2}.s^{-1}$). After 8 days, the suspensions were filtered through cheesecloth filters (pores 25-35 µm in diameter). The spore concentrations were determined using a Malassez cell and then adjusted to 1.0×10^6 $spores.mL^{-1}$.

Garlic cloves were provided by Top'Alliance Alinéa. The same pink garlic cultivar (Agri-Obtentions INRA) was used throughout all the experiments. The healthy cloves (n=12 per *F. proliferatum* strain) were peeled and disinfected (3 min in 1% bleach + 1 min in sterile water thrice). They were then placed in a beaker containing the calibrated suspension of spores with constant stirring (100 rpm) at 21 °C and with a photoperiod of 12 h (~ 25 $\mu mol.m^{-2}.s^{-1}$). The cloves serving as negative control were soaked in sterile water. After 24 h each garlic clove was placed in a cell culture plate well, basal plate downward, in a moisture-saturated plastic box and then stored at 23 °C in total darkness for 18 days.

The area of resulting garlic lesions was monitored from the 4 to 18 days after inoculation and the disease scores were noted on days 4, 6, 8, 11, 13, 15 and 18. We developed a notation scale, from 0 to 5, based on the extent of brown lesions on the cloves (Figure 1). These notes were used to compute the area under the disease progression curves (AUDPC). The strain FA3-E01 was used as a reference for aggressiveness. For each strain, an index of aggressiveness (IA) was calculated relative to that of strain FA3-E01 as follows: $IA_{strain} = 100 * (AUDPC_{strain} / AUDPC_{FA3\text{-}E01})$.

6. Statistical analyses

All statistical analyzes were conducted with RStudio software (v 1.2.1335) and Statistica software (v. 10). Incidences of garlic rot on all garlic types were compared with ANOVAs and Tukey's HSD *post hoc* test. Relative aggressiveness of haplotypes were compared with ANOVAs. A generalized linear model (GLM) was used to explore the proportional differences of *Fusarium* species. A linear model (LM) was used to compare the link between aggressiveness and several factors.

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Results

1. Garlic rot incidence

A total of 132 batches (from 132 fields) were analyzed in 2018 and 2019 representing 394 garlic heads and 5493 cloves. Symptoms were observed on cultivars belonging to all garlic types (white, pink and purple) and on each batch. Globally, 55 % of the peeled cloves presented typical *Fusarium* symptoms. There were no significant differences among garlic types in the head incidence (number of heads needed to be peeled in order to obtain three symptomatic heads): 3.46 heads for pink cultivars (n=48 batches), 3.94 for purple cultivars (n=31 batches) and 3.70 for white cultivars (n=53 batches) (p=0.34). No significant differences were observed among garlic types (p>0.31). However, contrary to expectations, the clove incidence in heads was significantly lower on pink cultivars than on the cultivars of the two other types: 44 % of symptomatic garlic cloves per infected head for pink cultivars (n=144 heads), 59 % for purple cultivars (n=92) and 60 % for white cultivars (n=158) ($p<10^{-12}$).

2. *Fusarium* species isolated from garlic symptoms

From the 3853 symptomatic cloves selected for isolation, 2729 strains were obtained. Of these strains, 1366 underwent molecular characterization that led to identification of 1171 of them (Table 1). The majority of strains isolated from garlic belonged to the species *F. proliferatum* (94.03 %): at least one strain of *F. proliferatum* was isolated from each garlic batch. The rest of the strains were identified as *F. oxysporum* (5.97 %). A single strain identified as *F. solani* was isolated from a purple cultivar harvested in the southwest basin in 2018.

The proportions of *F. proliferatum* and *F. oxysporum* isolated from the three garlic types (pink, white and purple) differed between the two production areas (Figure 2). Significantly more *F. oxysporum* strains were isolated from garlic cloves harvested in the southeast basin (9.44 %) than in the southwest basin (2.76 %) ($p<10^{-8}$). Significantly fewer *F. oxysporum* strains were isolated from pink type garlic (1.59 %) than from white type (9.39 %) or purple type garlic (7.34 %) ($p<10^{-6}$). No significant differences of proportion of *F. oxysporum* were observed between strains isolated from white and purple garlic cloves (p=0.31).

The 1171 strains identified originated from 289 garlic heads. We observed *F. proliferatum* strains as the only species present in 234 garlic heads (81.0 %), *F. oxysporum* strains as the only species

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present in 18 garlic heads (6.2 %) and the co-presence of both species in 37 garlic heads (12.8 %).

3. Intra and inter specific diversity of *Fusarium* strains isolated from garlic.

The 19 *ITS* sequences (492 bases) did not show any polymorphism and were not included in further analyzes. Both *rpb1* (592 bases) and *rpb2* (904 bases) region alignments were made with 86 and 93 *F. proliferatum* DNA sequences respectively. We observed only two haplotypes for both regions. One major haplotype of *rpb1* and *rpb2* was present in 81 and 88 strains respectively and one minor present in 5 strains (FA84-E01, FA85-E08, FA92-E03, FA110-E05 and FA122-E11). These 5 strains carried one identical mutation in both loci.

We added our *F. proliferatum* partial *IGS* sequences (n=14) to all the *F. proliferatum* sequences obtained by Jurado et al. (2012) leading to two clusters (Figure 3) that each represented distinct monophyletic groups. One contained strain FA122-E11 and the other contained the 13 others strains.

The strains collected in this study were dominated by a *tef-1α* gene haplotype of *F. proliferatum* that accounted for more than 75 % of the strains collected from garlic (Haplotype 1, Table 3). Strains from garlic (from cloves or leaf debris from France and elsewhere) constituted 25 additional haplotypes of which 17 haplotypes represented less than 1 % of the strains collected from garlic. The strains tested for aggressiveness on garlic represented 8 of the 9 haplotypes with more than 10 strains in the collection and 3 of the haplotypes with fewer than 10 strains.

To those sequences, we added 80 *tef-1α* GenBank database sequences corresponding to strains isolated from different hosts and geographical areas. Of these 119 *tef-1α* haplotypes, 66 corresponded to strains of *F. proliferatum*. The 505 bp sequences of these 66 haplotypes showed high nucleotide identity with each other, ranging from 99.8 to 98.41 % except for the sequence of KU847789 sequence whose similarity to other sequences ranged from 98.03 to 96.45 %. Sequences of *F. proliferatum* strains from French garlic cloves were partially clustered but no correlation with geographic origin of host could be determined (Figure 4a). The 5 strains representing the minor *rpb1*/*rpb2* haplotype were also clustered in a unique branch on the tree obtained with the partial *tef-1α* sequences. *F. oxysporum* sequences (574 bp) showed 99.87 % to 96.03 % nucleotide identity. Sequences of *F. oxysporum* strains isolated on French garlic cloves showed affiliation across the whole range of available sequences of *F. oxysporum* from the GenBank database (Figure 4b).

Out of the 289 garlic heads used to obtain the 1171 *Fusarium* strains, 193 (67 %) hosted a single

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haplotype among the strains isolated from that head. More specifically, 154 garlic heads hosted strains from the *F. proliferatum* haplotype 1, 22 garlic heads hosted strains from a single other *F. proliferatum* haplotype and 17 garlic heads hosted strains from a single *F. oxysporum* haplotype. The rest of the garlic heads (n=96, 33 %) were infected by multiple haplotypes at the same time. Up to 58 garlic heads were infected by several *F. proliferatum* haplotypes, 37 garlic heads were infected by *F. proliferatum* and *F. oxysporum* haplotypes, and 1 head was infected by multiple *F. oxysporum* haplotypes.

4. Pathogenicity of *F. proliferatum* and *F. oxysporum* strains isolated from garlic

After 4 days of inoculation, thick white aerial mycelium appeared on the basal plate of the inoculated cloves. After a maximum of 6 days, all tested strains caused dry brown-orange lesions on soft tissues cloves, starting just above the basal plate. Depending on the strain inoculated, the lesions progressed upwards until 25 to 100 % of the surface was covered. Globally, the cloves of negative controls did not show any lesions. However, after 15 days, some of them started to develop small brown lesions and some mycelium. This mycelium was identified as *F. proliferatum*.

All tested strains of *F. proliferatum* were able to cause symptoms on cloves of pink type garlic, regardless of their basin of origin and their garlic type of origin (pink, white, violet). However, they displayed a wide range of aggressiveness from 51.8 % to 154.8 % compared to a reference strain (Figure 5). Differences among strains were significant ($p<10^{-16}$).

In order to determine which factors were responsible for the variability of aggressiveness of strains, linear models were applied. In the first model (GLM) we evaluated the influence of three factors concerning the origin of the strains: the type of garlic (pink, purple or white) from which the strain was isolated, the basin of production and the cropping season. All factors and interactions were significant except for the type factor but they explained only 15% of the variability of aggressiveness observed ($R^2 = 0.15$) (Table 4). In the second model (LM) we evaluated the influence of strains alone and obtained a significant impact of this factor on the variability of aggressiveness explaining 59 % of the variability observed ($R^2 = 0.59$) (Table 4).

We then assessed the variability in aggressiveness of strains in terms of the *tef-1α* haplotypes we identified. Although most of the haplotypes inoculated on garlic were as aggressive as the reference strain (Figure 6), there was a significant effect of haplotype on aggressiveness (ANOVA, $p<0.00$). Most of the haplotypes that corresponded to *F. oxysporum* were only about 60

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% as aggressive as their *F. proliferatum* counterparts in artificial inoculation (Figure 6). Nevertheless, some of the rarer haplotypes of *F. proliferatum* were markedly less aggressive than the dominant haplotypes. These results confirmed what we observed with the linear models: the genetic diversity of strains seems to be the key to the variability in aggressiveness observed among strains.

Discussion

Our results confirm that garlic rot disease is well established in France and it concerns all types of garlic cultivars produced in France: pink, purple and white. Based on the frequency of symptomatic cloves in symptomatic heads, pink cultivars show a lower incidence of damage for individual cloves compared to heads of purple and white types. This is surprising since growers in the French garlic industry have the impression that pink cultivars are the most sensitive. This impression might be due to the discrepancy between what is observed on farms and what we noted in the laboratory when we systematically peeled and dissected garlic heads. We peeled the cloves and noted symptoms as small as brown spots, undetectable by the methods used by producers when evaluating their stocks, thereby likely explaining this discrepancy. We can also assume that lesions do not evolve in the same way in the different types of garlic. For example, in the case of garlic seed, since pink cultivars are sown later in the season, lesions have more time to evolve between harvest and sowing, sometimes toward complete clove destruction.

The molecular identification of 1171 strains, completed with inoculation tests of a representative subsample of strains, showed that *F. proliferatum* and *F. oxysporum* are responsible for garlic rot in France. A population of *F. proliferatum* with little genetic diversity represents the dominant species. This result is consistent with the French first report (Leyronas et al. 2018) and with the report of *F. proliferatum* as the responsible agent for garlic rot in Serbia, Spain, Mexico and Egypt (Stankovic et al., 2007; Palmero et al., 2012; Ochoa-Fuentes et al., 2013; Elshahawy et al., 2017). Considering *F. oxysporum*, it has also been reported as responsible for garlic symptoms in the USA and Egypt (Dugan et al., 2007; Moharam et al., 2013). Even though *F. oxysporum* represents a small percentage of the strains isolated from French garlic, garlic producers need to be wary of a possible emergence of this species. The proportions of *F. oxysporum* in the South East basin were 4%, 16% and 5% in 2017, 2018 and 2019 respectively Furthermore, in Italy, *F. oxysporum* is isolated more frequently from garlic symptoms compared to what we now observe in France (Mondani et al., 2020).

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In this work, we highlighted the low diversity of *tef-1α*, *rpb1*, *rpb2*, *ITS* and *IGS* regions of *F. proliferatum* strains collected from French garlic. The same low genetic diversity of the *tef-1α* region has been described among different *Fusarium* species (Stepien et al., 2011) and different garlic strains in Spain (Gálvez et al., 2017). Two different monophyletic groups were defined with the *IGS* region for the 14 strains sequenced in the present study. These groups matched to the two types observed by Jurado et al. (2012) with strains collected in Europe, America and Saudi Arabia without correlation with geographical origin or host origin (11 different hosts from 5 botanical families). The first group contained 13 of the 14 strains sequenced for the *IGS* region. We were not able to amplify more sequences because of the variability of the IGS region (Gálvez *et al*., 2020) that prevented us to have a more detailed understanding of the phylogeny of *F. proliferatum* strains. The second group contained only one strain (FA122-E11) that also belonged to the minor haplotype in the *rpb1* and *rpb2* regions. Despite the low diversity in both *rpb1* and *rpb2* regions, this seems to confirm the presence of at least two groups inside *F. proliferatum* isolated on French garlic. Nevertheless, this slight genetic diversity should not pose a problem for *F. proliferatum* detection of the pathogen nor be an impediment to understanding the epidemiology of the disease. Identification of *F. proliferatum* based on the *tef-1α* gene is carried out routinely (O'Donnell, 2000). All strains of *F. proliferatum* are potential pathogens according to our results. The differences we observed in aggressiveness are likely to vary in the face of other environmental factors that will come into play during real-life epidemics. Therefore, for the time being we feel it is important to be inclusive during detection of the fungus.

We described 31 haplotypes among the strains isolated in the collection from different garlic sources and other hosts in France and other countries. For a third of studied garlic heads, we found multiple haplotypes on the same head and sometimes multiple species at the same time. This observation implies that the presence of one haplotype does not prevent other pathogens from infecting the same head. The inoculum, therefore, is not necessarily limited to one strain at one time and multiple independent infections of a same head are possible. This raises questions about the window of opportunity for infection and if it is prolonged throughout the cropping season.

The present study is the first step of a more ambitious research project aiming to set up a method to protect garlic against this emerging disease. Here we have clarified the disease etiology in France. The next step is to gain a better understanding of the disease epidemiology in order to determine how disease development can be reduced or even stopped. *F. proliferatum* is a species known to have a broad host range (including dicots, monocots and conifers) with a marked genetic

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homogeneity among strains from these different hosts and different geographic locations (Proctor et al., 2010). Broad host range is a feature shared by many emerging pathogens (Woolhouse & Gowtage-Sequeria, 2005). In this light, it is very plausible that *F. proliferatum* will eventually emerge on other crops including other *Allium* species. This suggest that our observation of *F. proliferatum* on onion is not just anecdotal but rather portends future emergences. The broad host range of *F. proliferatum* complicates the search for reservoirs of inoculum and points to the need for a comprehensive approach to find them.

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Conflict of interest

The authors have no conflict of interest to disclose.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding

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author. The data are not publicly available due to privacy or ethical restrictions.

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Figure 1. Symptoms notation scale for garlic cloves, pictured (a) and schematized (b). 0: Absence of symptoms. 1: Mycelium on basal plate. 2: Browning on 25% of the clove. 3: Browning of 50% of the clove. 4: Browning of 75% of the clove. 5: Browning of the entire surface of the clove. A bonus of 0,5 was added if the browning also started at the top of the clove or if more than 3 brown spots were found on the clove.

Figure 2. Proportions of *Fusarium proliferatum* and *F. oxysporum* isolated from symptoms of pink, white and purple garlic cloves harvested in the two major French basins of production. Proportions represented by bars associated with the same letter are not significantly different ($p>0.05$) based on a Generalized Linear Model.

Figure 3. Maximum-Likelihood tree obtained from the *F. proliferatum* partial IGS sequences with the Kimura-2 parameter model. Strains isolated from garlic cloves in France are highlighted. Bootstrap values (n=1000) are indicated for each node. The scale bar represents a genetic distance of 0.02. *Fusarium verticillioides* FvMM2-4 sequence (Jurado *et al.,* 2012) was used as an outgroup. Type I and II are monophyletic but non-orthologous groups previously described by O'Donnell et al. (1997).

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Figure 4. Maximum-Likelihood trees obtained from the *Fusarium proliferatum* (a) and *F. oxysporum* (b) partial *tef-1α* region sequences with the Kimura-2 model and discrete gamma distribution. Strains isolated from garlic cloves in France are highlighted. Asterisks indicate strains that carried a SNP in both the *rpb1* and *rpb2* regions. Bootstrap values (n=1000) are indicated for each node. The scale bar represents a genetic distance of 0.01. *F. verticillioides* strain NRRL 25117 sequence from the ARS culture collection (https://nrrl.ncaur.usda.gov/) was used as an outgroup in both trees.

Figure 5. Distribution of the aggressiveness in % relative to the reference strain FA3-01 (in black) of 122 *Fusarium proliferatum* strains and 4 *F. oxysporum* strains (in blue) on pink garlic. Each histogram represents the mean of twelve values. Verticals bars indicate the standard deviation of the mean.

Figure 6. Neighbor-joining tree obtained with the *tef-1α* sequences of 117 out of 126 *Fusarium* spp. strains tested for aggressiveness on garlic. The amount of disease induced, expressed as the percent of the area under the disease progress curve (% AUDPC) relative to reference strain is indicated for the 11 haplotypes described in the tree. The mean and 95 % confidence intervals of the % AUDPC for all strains tested (accounting of the 12 replicate inoculations per strain for all strains per haplotype) are indicated for each haplotype (ANOVA, $p < 10^{-2}$).

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Table 1. Distribution of fungal strains among production basin, garlic type and year for which molecular identification was completed. Numbers in brackets refers to the number of strains for which aggressiveness tests were carried out.

Garlic type	South-East basin			South-West basin			Type total
	2017	*2018*	*2019*	*2017*	*2018*	*2019*	
White	13 (3)	155 (10)	39 (6)	12 (6)	28 (6)	71 (4)	318 (35)
Pink	13 (6)	150 (4)	34 (2)	170 (31)	72 (2)	71 (4)	510 (49)
Violet	65 (13)	103 (3)	51 (6)	6 (6)	91 (6)	27 (4)	343 (38)
Yearly total	91 (22)	408 (17)	124 (14)	188 (43)	191 (14)	169 (12)	
Totals	623 (53)			548 (69)			**1171 (122)**

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Table 2. Characteristics of species and strains of *Fusarium* including GenBank accession numbers, used for construction of phylogenetic trees. The strains and sequences obtained in this research are in bold face. *Ac*: *Allium cepa*, *Ad*: *Arundo donax*, *Af*: *Allium fistulosum*, *Ao*: *Asparagus officinalis*, *As*: *Allium sativum*, *Ch*: *Chamaerops humilis*, *Hv*: *Hordeum vulgare*, *Ms*: *Musa sapientum*, *Ta*: *Tricitum aestivum*, *P*: *Pinus*, *Pc*: *Phoenix canariensis*, *Pd*: *Phoenix dactylifera*, *Pr*: *Phoenix reclinata*, *W*: *Washingtonia*, *Zm*: *Zea mays*. NA: Not Available.

Loci	Species	Strain	Host	Origin	GenBank acc.
tef-1α	*F. proliferatum*	**FA3-E01**	*As*	France	MW051796
tef-1α	*F. proliferatum*	**FA44-E14**	*As*	France	MW051797
tef-1α	*F. proliferatum*	**FA64-E07**	*As*	France	MW051798
tef-1α	*F. proliferatum*	**FA66-E08**	*As*	France	MW051799
tef-1α	*F. proliferatum*	**FA76-E05**	*As*	France	MW051800
tef-1α	*F. proliferatum*	**FA84-E01**	*As*	France	MW051801
tef-1α	*F. proliferatum*	**FA85-E08**	*As*	France	MW051802
tef-1α	*F. proliferatum*	**FA92-E03**	*As*	France	MW051803
tef-1α	*F. proliferatum*	**FA96-E04**	*As*	France	MW051804
tef-1α	*F. proliferatum*	**FA110-E05**	*As*	France	MW051805
tef-1α	*F. proliferatum*	**FA111-E04**	*As*	France	MW051806
tef-1α	*F. proliferatum*	**FA122-E11**	*As*	France	MW051807
tef-1α	*F. proliferatum*	**FA122-E12**	*As*	France	MW051808
tef-1α	*F. proliferatum*	**FA122-E13**	*As*	France	MW051809
tef-1α	*F. proliferatum*	**FA160-E03**	*As*	France	MW051810
tef-1α	*F. proliferatum*	**FA161-E04**	*As*	France	MW051811
tef-1α	*F. proliferatum*	**FA165-E06**	*As*	France	MW051812
tef-1α	*F. proliferatum*	**FA239-E09**	*As*	France	MW051813
tef-1α	*F. proliferatum*	**FA253-E07**	*As*	France	MW051814
tef-1α	*F. proliferatum*	**FA442-E01**	*As*	France	MW051815
tef-1α	*F. proliferatum*	**FA537-E09**	*As*	France	MW051816
tef-1α	*F. proliferatum*	**FA575-E02**	*As*	France	MW051817
tef-1α	*F. proliferatum*	**SPR 11 A Sci2**	*As*	USA	NA
tef-1α	*F. proliferatum*	**OB3**	*Ac*	France	MW051818
tef-1α	*F. proliferatum*	**LSVM674**	*Ta*	NA	NA
tef-1α	*F. proliferatum*	1	*As*	USA	KJ128964
tef-1α	*F. proliferatum*	B1	*As*	Serbia	KX092460

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tef-1α	*F. proliferatum*	BL11	*As*	Serbia	KX611147
tef-1α	*F. proliferatum*	BL8	*As*	Serbia	KX611149
tef-1α	*F. proliferatum*	JBL6	*As*	Serbia	MH161452
tef-1α	*F. proliferatum*	PBL70/1-1	*As*	Serbia	MH496028
tef-1α	*F. proliferatum*	UR01	*Ac*	NA	EU220408
tef-1α	*F. proliferatum*	P57G	*Ac*	Malaysia	KU847789
tef-1α	*F. proliferatum*	WF22	*Ta*	China	KP054293
tef-1α	*F. proliferatum*	WF29	*Ta*	China	KP054292
tef-1α	*F. proliferatum*	WF49	*Ta*	China	KP054291
tef-1α	*F. proliferatum*	T1.12	*Ta*	Mexico	KU508347
tef-1α	*F. proliferatum*	G16FX3-16	*Ta*	China	KY081485
tef-1α	*F. proliferatum*	129b	*Ta*	China	KY466788
tef-1α	*F. proliferatum*	wxwh63	*Ta*	USA	MG826912
tef-1α	*F. proliferatum*	R11	*Zm*	NA	KF562151
tef-1α	*F. proliferatum*	R24	*Zm*	NA	KF575334
tef-1α	*F. proliferatum*	R31	*Zm*	NA	KF575336
tef-1α	*F. proliferatum*	R37	*Zm*	NA	KF575339
tef-1α	*F. proliferatum*	R44	*Zm*	NA	KF575340
tef-1α	*F. proliferatum*	15	*Zm*	NA	KM583805
tef-1α	*F. proliferatum*	B52c	*Zm*	Malaysia	KP340030
tef-1α	*F. proliferatum*	Fp_hap1	*Zm*	China	KT716224
tef-1α	*F. proliferatum*	Fp_hap4	*Zm*	China	KT716227
tef-1α	*F. proliferatum*	Fp_hap6	*Zm*	China	KT716229
tef-1α	*F. proliferatum*	magh26	*Zm*	Iran	MG734635
tef-1α	*F. proliferatum*	prp2-1	*Zm*	China	MH448807
tef-1α	*F. proliferatum*	wx8-4	*Zm*	China	MH448808
tef-1α	*F. proliferatum*	ynx10-2	*Zm*	China	MH448809
tef-1α	*F. proliferatum*	ynx8-4	*Zm*	China	MH448810
tef-1α	*F. proliferatum*	dz1-1	*Zm*	China	MH448814
tef-1α	*F. proliferatum*	w2-2-3	*Zm*	China	MH448816
tef-1α	*F. proliferatum*	C12171	*Zm*	China	MN696137
tef-1α	*F. proliferatum*	C17009	*Zm*	China	MN696128
tef-1α	*F. proliferatum*	C17059	*Zm*	China	MN696108
tef-1α	*F. proliferatum*	C17068	*Zm*	China	MN696103
tef-1α	*F. proliferatum*	C17076	*Zm*	China	MN696098
tef-1α	*F. proliferatum*	C17091	*Zm*	China	MN696088

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tef-1α	*F. proliferatum*	C17136	*Zm*	China	MN696069
tef-1α	*F. proliferatum*	EF21	*Zm*	Spain	MN861758
tef-1α	*F. proliferatum*	F71	*Zm*	Spain	MN861790
tef-1α	*F. oxysporum*	**FA23-E08**	*As*	France	MW051819
tef-1α	*F. oxysporum*	**FA105-E02**	*As*	France	MW051820
tef-1α	*F. oxysporum*	**FA154-E05**	*As*	France	MW051821
tef-1α	*F. oxysporum*	**FA155-E02**	*As*	France	MW051822
tef-1α	*F. oxysporum*	**FA261-E01**	*As*	France	MW051823
tef-1α	*F. oxysporum*	**FA318-E14**	*As*	France	MW051824
tef-1α	*F. oxysporum*	**FA340-E03**	*As*	France	MW051825
tef-1α	*F. oxysporum*	**FA531-E04**	*As*	France	MW051826
tef-1α	*F. oxysporum*	**FA537-E04**	*As*	France	MW051827
tef-1α	*F. oxysporum*	**A-P4-T-Pl2**	*As* (leaves)	France	MW051828
tef-1α	*F. oxysporum*	**A-P5-T-Pl4**	*As* (leaves)	France	MW051829
tef-1α	*F. oxysporum*	**O2A**	*Ac*	France	MW051830
tef-1α	*F. oxysporum*	**O4A**	*Ac*	France	MW051831
tef-1α	*F. oxysporum*	**MR 20 B Sci1**	*As*	USA	NA
tef-1α	*F. oxysporum*	JBL1	*As*	Serbia	MH161445
tef-1α	*F. oxysporum*	Fs-N1	*Af*	Japan	AB898831
tef-1α	*F. oxysporum*	Fs-N4	*Af*	Japan	AB898832
tef-1α	*F. oxysporum*	Fs-N8	*Af*	Japan	AB898833
tef-1α	*F. oxysporum*	AF31	*Af*	Japan	AB938026
tef-1α	*F. oxysporum*	AF74	*Af*	Japan	AB938032
tef-1α	*F. oxysporum*	AF96	*Af*	Japan	AB938042
tef-1α	*F. oxysporum*	EZA	*Ac*	NA	EU220394
tef-1α	*F. oxysporum*	NL104-2	*Ac*	NA	EU220398
tef-1α	*F. oxysporum*	UR17-8	*Ac*	NA	EU220399
tef-1α	*F. oxysporum*	Fot-Yoko3	*Ac*	NA	EU220400
tef-1α	*F. oxysporum*	UR17-5	*Ac*	NA	EU220401
tef-1α	*F. oxysporum*	Foc-06	*Ac*	NA	EU220402
tef-1α	*F. oxysporum*	NL106-2	*Ac*	NA	EU220404
tef-1α	*F. oxysporum*	STEU6639	*Ac*	South Africa	GU165891
tef-1α	*F. oxysporum*	STEU6643	*Ac*	South Africa	GU165895
tef-1α	*F. oxysporum*	STEU6663	*Ac*	South Africa	GU165934
tef-1α	*F. oxysporum*	STEU6665	*Ac*	South Africa	GU165936
tef-1α	*F. oxysporum*	STEU6669	*Ac*	South Africa	GU165940

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tef-1α	*F. oxysporum*	STEU6671	*Ac*	South Africa	GU165941
tef-1α	*F. oxysporum*	STEU6690	*Ac*	South Africa	GU165960
tef-1α	*F. oxysporum*	Fox006	*Ac*	Finland	KT239475
tef-1α	*F. oxysporum*	Fox072a	*Ac*	Finland	KT239476
tef-1α	*F. oxysporum*	Fox125a	*Ac*	Finland	KT239473
tef-1α	*F. oxysporum*	Fox194	*Ac*	Finland	KT239474
tef-1α	*F. oxysporum*	Fox212	*Ac*	Finland	KT239479
tef-1α	*F. oxysporum*	Fox215f	*Ac*	Finland	KT239478
tef-1α	*F. oxysporum*	LMSA 1.09.131	*Ta*	NA	JF278593
tef-1α	*F. oxysporum*	WF42	*Ta*	China	KP054290
tef-1α	*F. oxysporum*	WF50	*Ta*	China	KP054288
tef-1α	*F. oxysporum*	G13AY2-31	*Ta*	China	KX663603
tef-1α	*F. oxysporum*	G14WX2-5	*Ta*	China	KX702531
tef-1α	*F. oxysporum*	R6.7	*Ta*	Mexico	KU508359
tef-1α	*F. oxysporum*	N369	*Ta*	South Africa	MG588085
tef-1α	*F. oxysporum*	TK22	*Zm*	Malaysia	KF575348
tef-1α	*F. oxysporum*	798CS	*Zm*	Mexico	KR905564
tef-1α	*F. oxysporum*	mdae57	*Zm*	Iran	MG734611
tef-1α	*F. oxysporum*	c7-3	*Zm*	China	MH448804
tef-1α	*F. oxysporum*	x1-4	*Zm*	China	MH448805
tef-1α	*F. proliferatum*	NRRL 52743	NA	NA	JF740819
tef-1α	*F. oxysporum*	NRRL 46589	NA	NA	FJ985438
tef-1α	*F. verticillioides*	NRRL 25117	NA	NA	JF740743
IGS	*F. proliferatum*	**FA20-E01**	*As*	France	MW051832
IGS	*F. proliferatum*	**FA37-E09**	*As*	France	MW051833
IGS	*F. proliferatum*	**FA74-E05**	*As*	France	MW051834
IGS	*F. proliferatum*	**FA76-E08**	*As*	France	MW051835
IGS	*F. proliferatum*	**FA79-E05**	*As*	France	MW051836
IGS	*F. proliferatum*	**FA89-E01**	*As*	France	MW051837
IGS	*F. proliferatum*	**FA89-E02**	*As*	France	MW051838
IGS	*F. proliferatum*	**FA90-E01**	*As*	France	MW051839
IGS	*F. proliferatum*	**FA122-E11**	*As*	France	MW051840
IGS	*F. proliferatum*	**FA205-E01**	*As*	France	MW051841
IGS	*F. proliferatum*	**FA207-E08**	*As*	France	MW051842
IGS	*F. proliferatum*	**FA216-E08**	*As*	France	MW051843
IGS	*F. proliferatum*	**FA256-E05**	*As*	France	MW051844

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IGS	*F. proliferatum*	**FA315-E08**	*As*	France	MW051845
IGS	*F. proliferatum*	Fp101	*Ta*	France	GQ495206
IGS	*F. proliferatum*	Fp102	*Ta*	France	GQ495207
IGS	*F. proliferatum*	Fp103	*Ta*	France	GQ495208
IGS	*F. proliferatum*	Fp175	*Ta*	France	GQ495209
IGS	*F. proliferatum*	FpB12	*Hv*	Spain	GQ495212
IGS	*F. proliferatum*	FpB15	*Hv*	Spain	GQ495213
IGS	*F. proliferatum*	FpB20	*Hv*	Spain	GQ495214
IGS	*F. proliferatum*	FpB21	*Hv*	Spain	GQ495215
IGS	*F. proliferatum*	FpB22	*Hv*	Spain	GQ495216
IGS	*F. proliferatum*	FpB23	*Hv*	Spain	GQ495217
IGS	*F. proliferatum*	FpC3	*Zm*	Spain	GQ495218
IGS	*F. proliferatum*	FpC24	*Zm*	Spain	GQ495219
IGS	*F. proliferatum*	FpO24	*Zm*	Spain	GQ495220
IGS	*F. proliferatum*	FpMM1-1	*Zm*	Spain	GQ495193
IGS	*F. proliferatum*	FpMM1-2	*Zm*	Spain	GQ495211
IGS	*F. proliferatum*	FpMM1-3	*Zm*	Spain	GQ495194
IGS	*F. proliferatum*	FpMM3-1	*Zm*	Spain	GQ495195
IGS	*F. proliferatum*	FpMM4-1	*Zm*	Spain	GQ495196
IGS	*F. proliferatum*	FpMM4-2	*Zm*	Spain	GQ495197
IGS	*F. proliferatum*	FpMM6-1	*Zm*	Spain	GQ495198
IGS	*F. proliferatum*	FpMM6-2	*Zm*	Spain	GQ495199
IGS	*F. proliferatum*	ITEM 1506	*Zm*	Italy	GQ495181
IGS	*F. proliferatum*	ITEM 2191	*Zm*	Italy	GQ495183
IGS	*F. proliferatum*	ITEM 2298	*Zm*	Italy	GQ495184
IGS	*F. proliferatum*	ITEM 1682	*Zm*	Canada	GQ495182
IGS	*F. proliferatum*	ITEM 2620	*Zm*	Slovakia	GQ495187
IGS	*F. proliferatum*	ITEM 2644	*Zm*	Slovakia	GQ495188
IGS	*F. proliferatum*	Gf26	*P*	Spain	GQ495200
IGS	*F. proliferatum*	Gf29	*P*	Spain	GQ495201
IGS	*F. proliferatum*	Gf31	*P*	Spain	GQ495202
IGS	*F. proliferatum*	Gf33	*P*	Spain	GQ495203
IGS	*F. proliferatum*	Gf34	*P*	Spain	GQ495204
IGS	*F. proliferatum*	Gf37	*Ms*	Ecuador	GQ495205
IGS	*F. proliferatum*	ITEM 1451	*Ao*	Italy	GQ495178
IGS	*F. proliferatum*	ITEM 1456	*Ao*	Italy	GQ495179

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IGS	*F. proliferatum*	ITEM 1486	*Ao*	Italy	GQ495180
IGS	*F. proliferatum*	ITEM 2341	*Pd*	Saudi Arabia	GQ495185
IGS	*F. proliferatum*	ITEM 2343	*Pd*	Saudi Arabia	GQ495186
IGS	*F. proliferatum*	ITEM 4285	*Pc*	Spain	GQ495189
IGS	*F. proliferatum*	ITEM 4291	*Pr*	Spain	GQ495190
IGS	*F. proliferatum*	ITEM 4293	*Ch*	Spain	GQ495191
IGS	*F. proliferatum*	ITEM 4306	*W*	Spain	GQ495192
IGS	*F. proliferatum*	Fp2287	NA	NA	GQ495210
IGS	*F. proliferatum*	MPD 4853	NA	NA	GQ495221
IGS	*F. verticillioides*	FvMM2-4	*Zm*	Spain	GQ495173

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Table 3. Distribution of the strains used in this study in the different haplotypes according to the nucleotide sequence of a 574-bp region of the translation elongation factor 1 alpha gene (tef-1 α). Nearly all strains were isolated from garlic cloves from French production regions unless indicated otherwise in the table.

Haplotype	Number of strains in this study					Species[b]
	Total	Tested for pathogenicity[a]	From non-garlic source	From garlic leaf debris	From garlic outside of France	
1	892	96	0	0	6	*F. proliferatum*
2	74	4	0	0	1	*F. proliferatum*
3	38	6	4	0	0	*F. proliferatum*
4	31	5	1	2	3	*F. oxysporum*
5	28	1	2	2	1	*F. oxysporum*
6	24	1	0	0	1	*F. proliferatum*
7	24	4	0	0	5	*F. proliferatum*
8	15	0	1	0	0	*F. oxysporum*
9	14	1	1	0	0	*F. proliferatum*
10	7	1	0	0	1	*F. proliferatum*
11	5	0	4	0	1	*F. proliferatum*
12	5	1	2	0	0	*F. proliferatum*
13	3	0	0	0	0	*F. oxysporum*
14	2	0	0	0	0	*F. oxysporum*
15	2	0	0	0	0	*F. oxysporum*
16	2	0	0	1	0	*F. oxysporum*
17	2	0	0	0	1	*F. oxysporum*
18	2	0	0	0	0	*F. proliferatum*
19	1	0	0	0	0	*F. proliferatum*
20	1	0	0	0	0	*F. proliferatum*
21	1	0	0	0	1	*F. proliferatum*
22	1	0	1	0	0	*F. proliferatum*
23	1	0	1	0	0	*F. proliferatum*
24	1	0	0	0	0	*F. proliferatum*
25	1	0	0	0	0	*F. proliferatum*
26	1	0	0	0	0	*F. proliferatum*
27	1	0	1	0	0	*F. proliferatum*

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28	1	1	0	0	0	*F. proliferatum*
29	1	0	0	1	0	*F. tricinctum / F accuminatum*
30	1	0	1	0	0	*F. solani*
31	1	0	1	0	0	*F. solani*

[a]Strains were randomly selected for tests of aggressiveness on garlic without prior knowledge of their haplotype. Aggressiveness tests were conducted in 18 blocks over 1 year under comparable conditions (on cultivar Edenrose, inoculum of 10^6 spores.ml^{-1}, incubation at 23 °C) and the behavior of all strains was compared to the same reference strain FA3-E01 used in all blocks.

[b]Species identification is based on similarity with reference strains in phylogenetic analyses and according to comparison with databases of *Fusarium* MLST (https://*Fusarium*.mycobank.org/) and *Fusarium* ID (http://isolate.*Fusarium*db.org/blast.php).

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Table 4. Variability in the aggressiveness index of *F. proliferatum* strains in relation with different factors: the type of garlic, the basin of production and the cropping season or with the strain factor alone. df: degree of freedom. MS: Mean Square.

Factor	**df**	**MS**	**p-value**
Generalized linear model ($p<10^{-16}$, $R^2 = 0.15$)			
Year	2	13560.3	10^{-11}
Basin	1	27216.1	10^{-12}
Type	2	2822.6	0,07
Year x Basin	2	15391.1	10^{-12}
Year x Type	4	10552.7	10^{-15}
Basin x Type	2	11379.5	10^{-9}
Linear model ($p<10^{-16}$, $R^2 = 0.59$)			
Strain	121	5185	$<10^{-16}$

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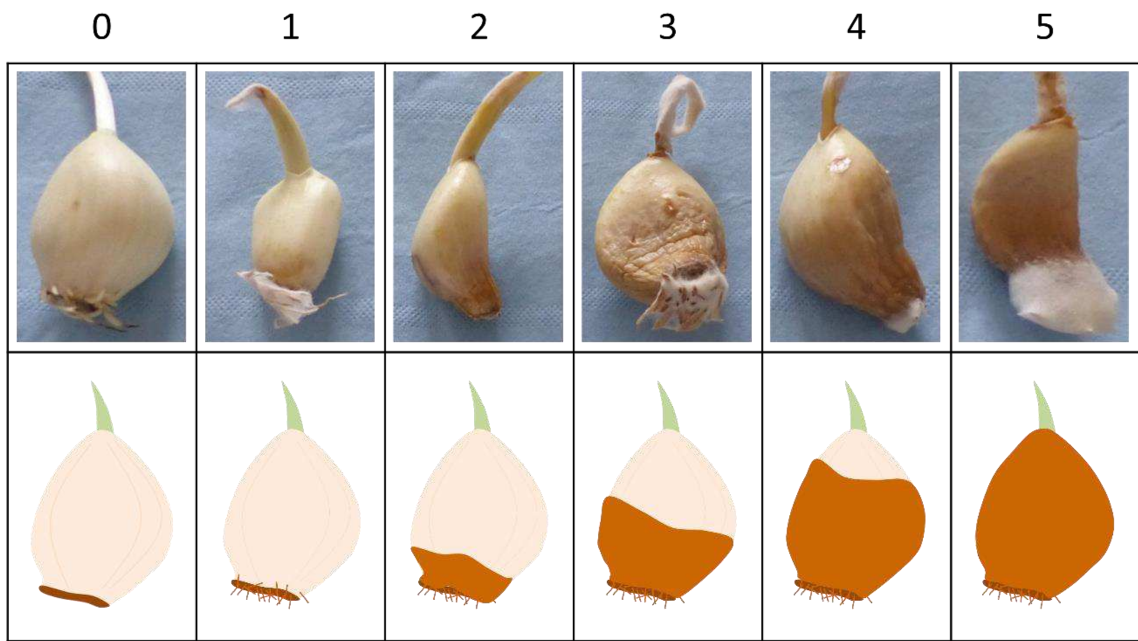

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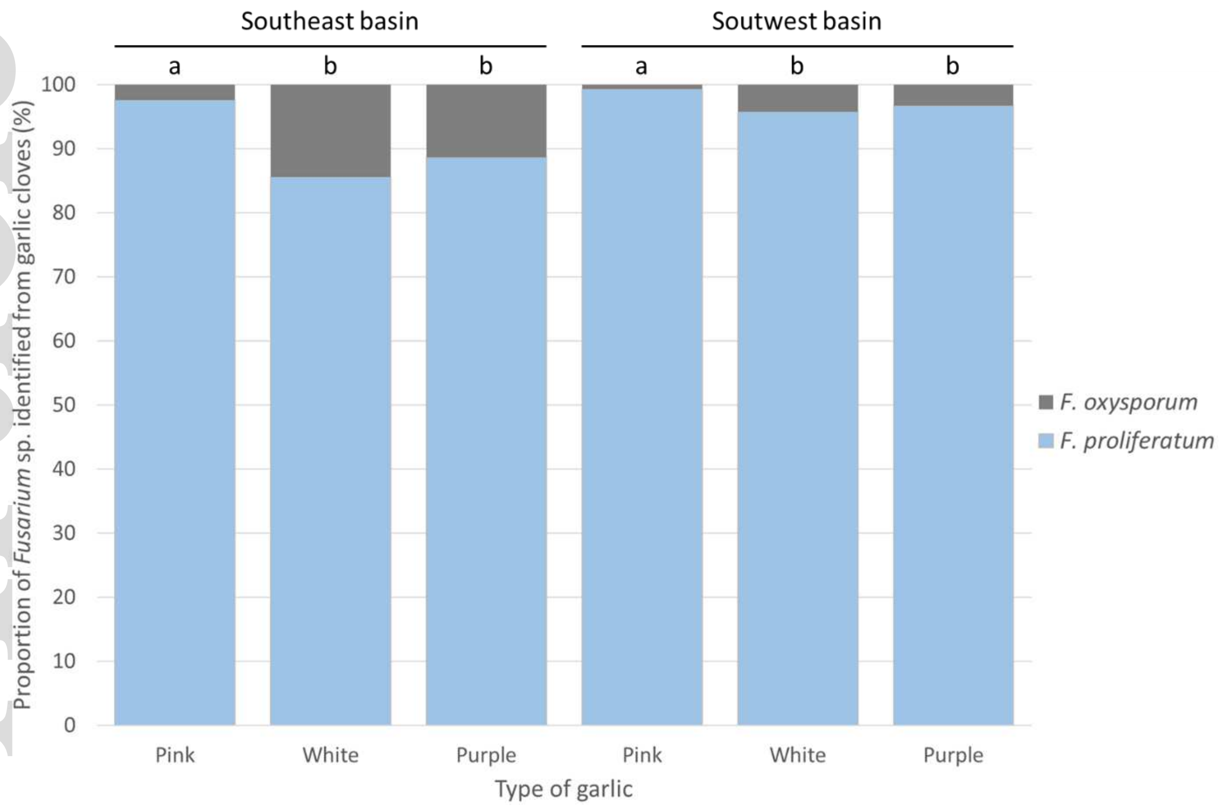

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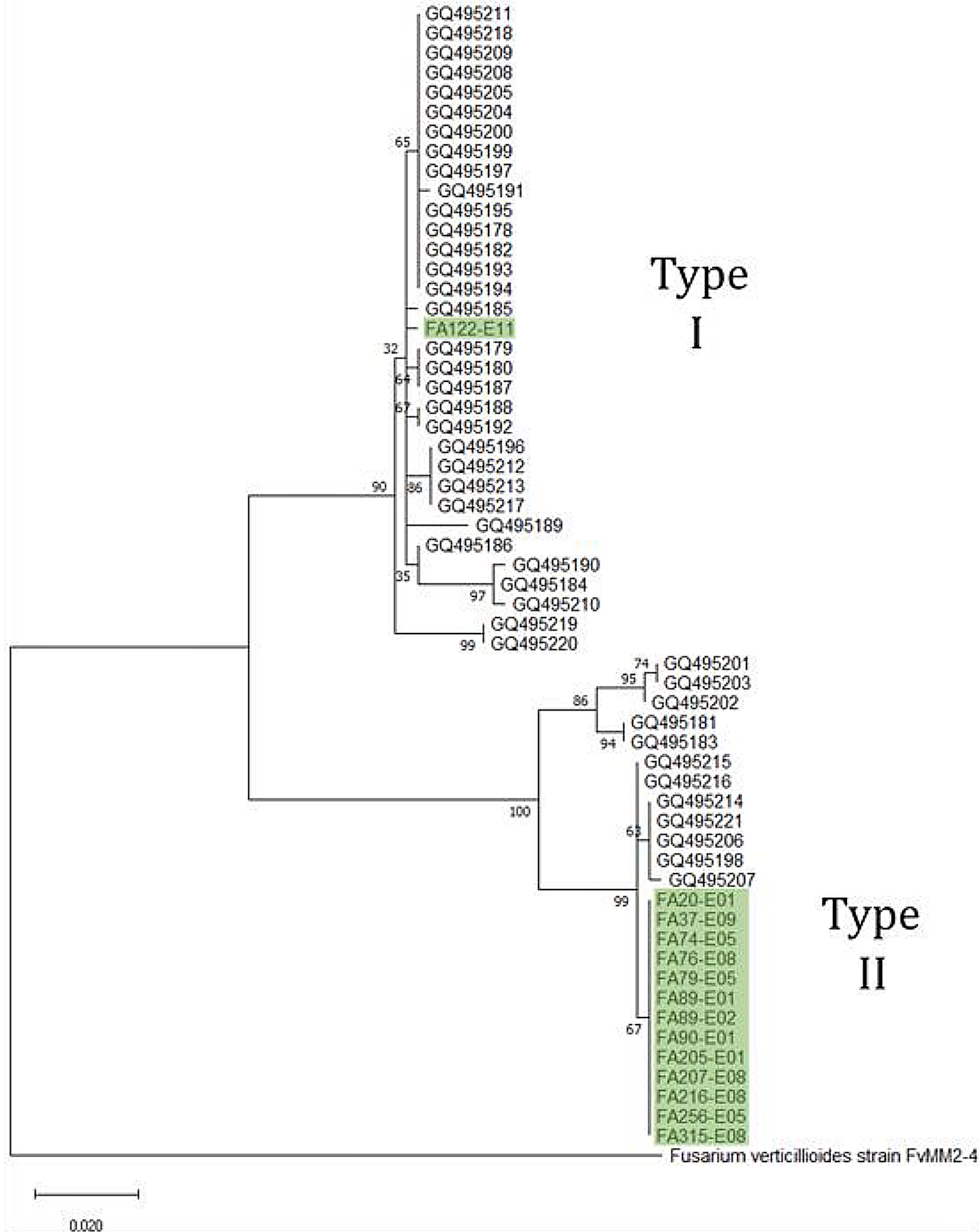

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GQ495214
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GQ495221
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GQ495207
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FA37-E09
FA74-E05
FA76-E08
FA79-E05
FA89-E01
FA89-E02
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FA90-E01
FA205-E01
FA207-E08
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FA256-E05
FA315-E08
Type
II
Fusarium verticillioides strain FvMM2-4
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FA442-E01
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MH496028
FA537-E09
FA3-E01
FA96-E04
FA64-E07
FA44-E14
FA66-E08
LSVM674
FA239-E09
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FA76-E05
FA253-E07
FA122-E13
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FA122-E11 *
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Fusarium oxysporum strain NRRL 46589
Fusarium verticillioides strain NRRL 25117
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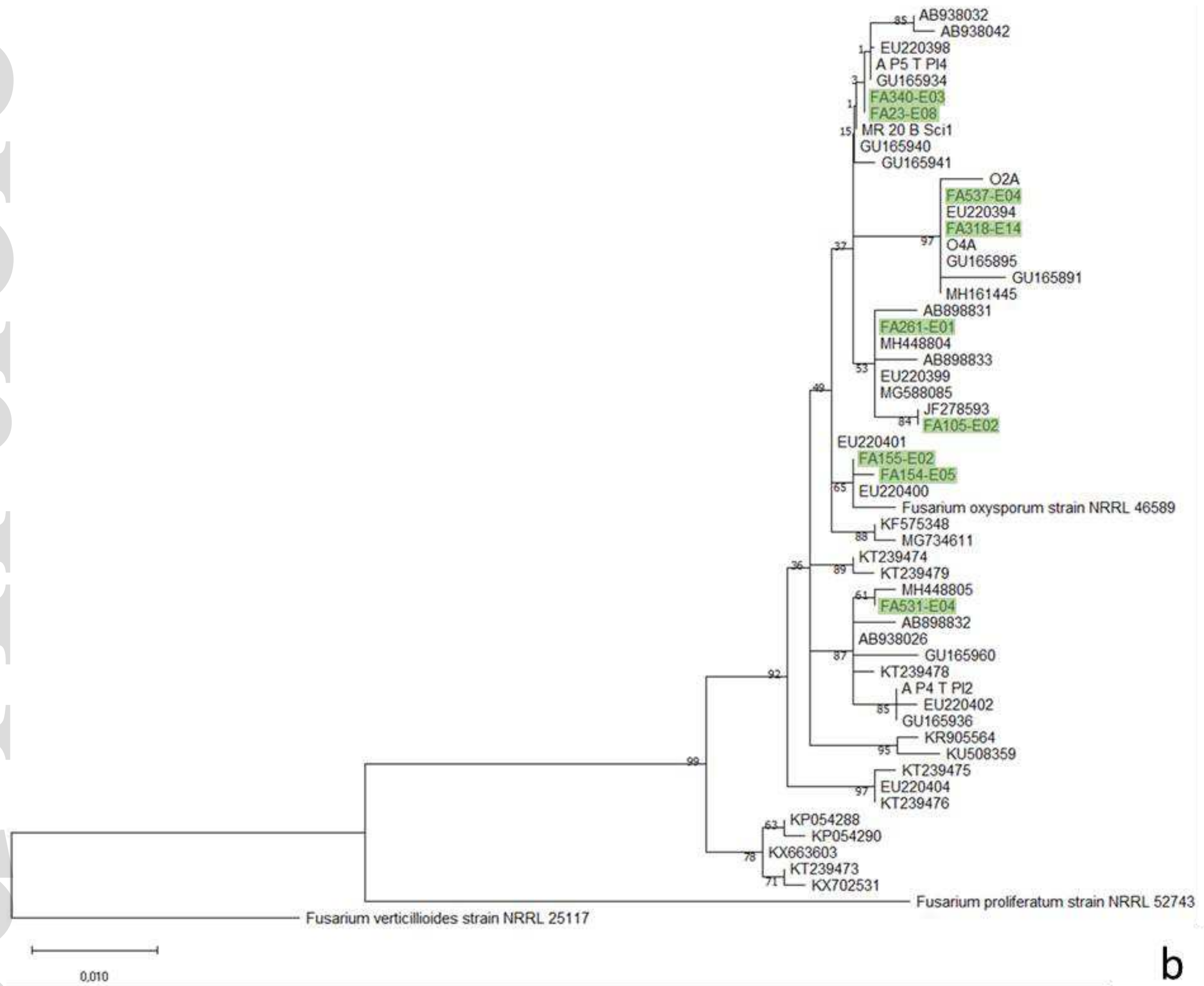

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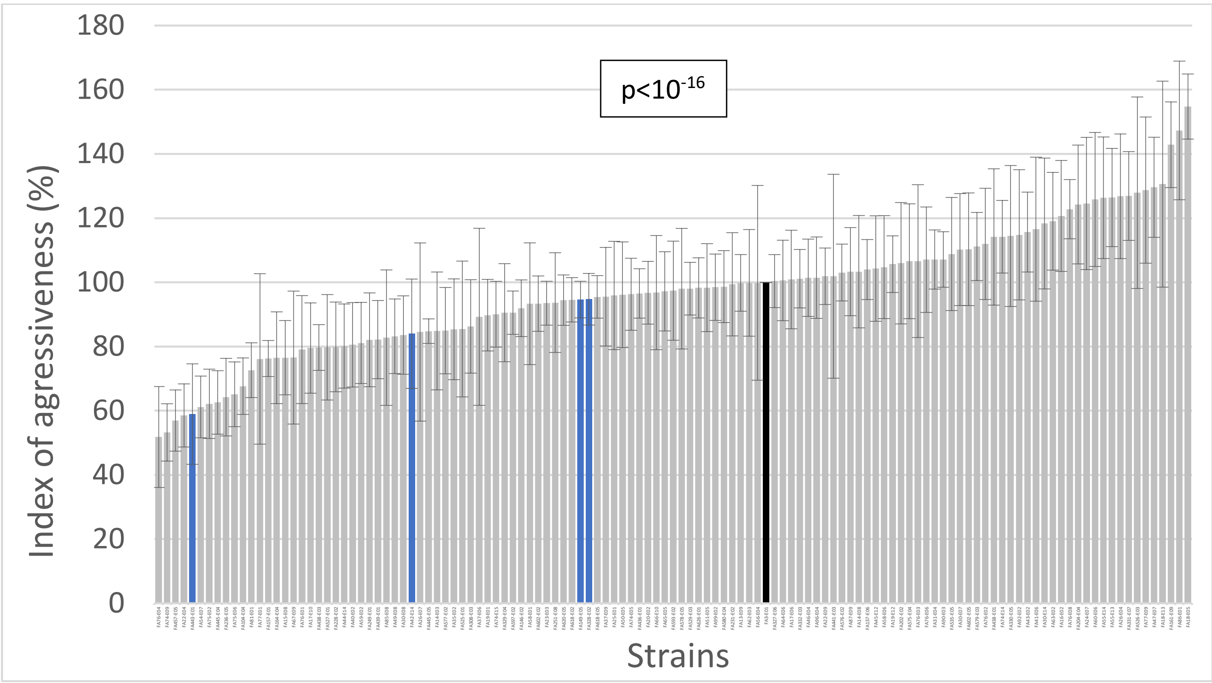
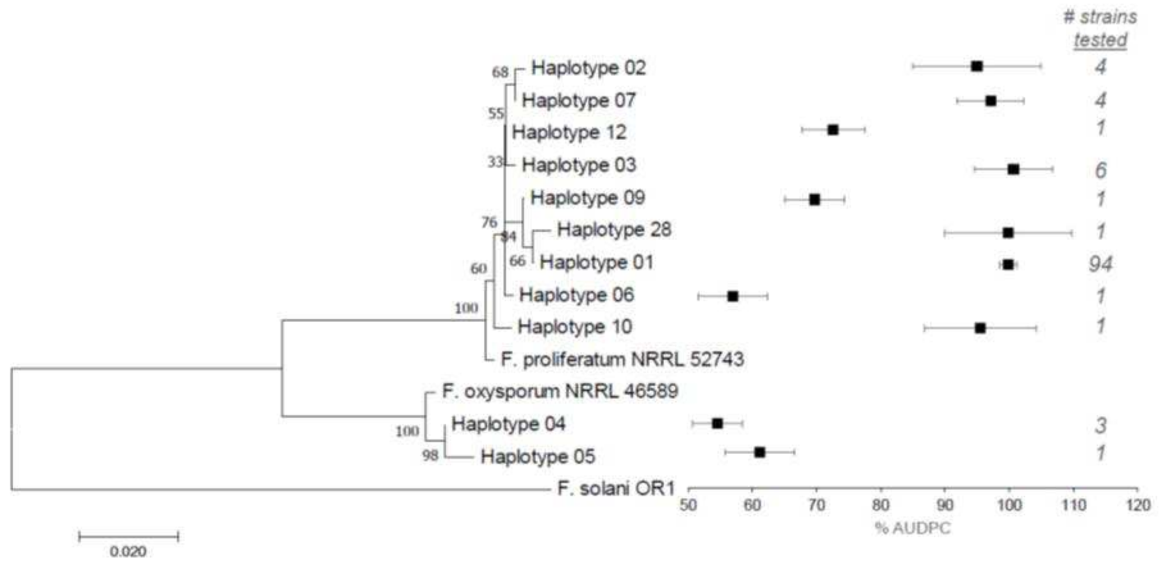


180
160
140
120
100
80
60
40
20
0
Index of agressiveness (%)
p<10-16
Strains



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