Can higher aggressiveness effectively compensate for a virulence deficiency in plant pathogen? A case study of *Puccinia triticina*'s fitness evolution in a diversified varietal landscape

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Abstract

Plant resistances impose strong selective pressure on plant pathogen populations through the deployment of major resistance genes, which leads to the emergence of new virulences. The pathogen adaptation also involves other life-history or parasitic fitness traits, especially aggressiveness components. A previous study on *Puccinia triticina*, the causal agent of wheat leaf rust, revealed that the distribution frequency of virulences in the French pathogen population cannot be fully explained by the major resistance genes deployed in the landscape. From 2012 to 2015, two major pathotypes (groups of isolates with the same combinations of virulences) — 166 317 0 and 106 314 0 — were equally represented in the landscape, despite the theoretical advantage conferred to 166 317 0 by its virulence to *Lr3* frequent in the cultivated landscape, whereas 106 314 0 is avirulent to this gene. To explain this apparent contradiction, we assessed three components of aggressiveness — infection efficiency, latency period and sporulation capacity — for 23 isolates representative of the most frequent pathotype-genotype combination (named 'pathogenotype') within each pathotype. We tested these isolates on seedlings of Michigan Amber, a 'naive' wheat cultivar that has never been grown in the landscape, Apache, a 'neutral' cultivar with no selection effect on the landscape-pathotype pattern, and several cultivars that were frequently grown. We found that pathogenotype 106 314 0-G2 was more aggressive than 166 317 0-G1, with a consistency for the three components of aggressiveness. Our results show that aggressiveness plays a significant role in driving evolution in pathogen populations by acting as a selective advantage, even offsetting the disadvantage of

lacking virulence towards a major *Lr* gene. Higher aggressiveness represents a competitive advantage that is likely even more pronounced when exhibited at the landscape scale as the expression of its multiple components is amplified by the polycyclic nature of epidemics.

Keywords

Aggressiveness, fitness, fungus, host adaptation, leaf rust, plant disease epidemiology, *Puccinia triticina*, quantitative phenotyping, virulence, wheat disease

Introduction

Pathogenicity or the ability of plant pathogens to cause a disease is generally broken down into a qualitative term, 'virulence', and a quantitative term, 'aggressiveness' (Lannou, 2012). Virulence is the capacity of the pathogen to infect its host (compatibility) as opposed to avirulence, which expresses a resistance (incompatibility). This follows the gene-for-gene model involving a specific interaction between an avirulence gene (*Avr*) on the pathogen side and a corresponding qualitative resistance gene (*R*) in the plant (Flor, 1971; Dangl & Jones, 2001). A pathotype, or race, is defined by a virulence profile, which consists in a unique combination of virulences, i.e. two pathogenic isolates are considered to belong to the same pathotype if they have the same combination of virulences. The term aggressiveness is used to describe both the parasitic fitness (Shaner et al., 1992) and the amount of damage caused to the host plant. It is formally defined as the quantitative variation of pathogenicity of a pathogen on its host plant (Pariaud et al., 2009a; Lannou, 2012) and is related to several life-history traits of the pathogen, specific to its biology and the nature of the symptoms it induces.

In field conditions, *R* genes exert a high selection pressure, which contributes to shape pathogen populations. This pressure is all the higher as the *R* genes are deployed at high frequency in the landscape (Rouxel et al., 2003; Goyeau et al. 2006; Fontyn et al., 2022; Mundt et al., 2014; Rimbaud et al., 2018), favoring the selection of pathotypes carrying the corresponding virulences, hence resulting in a rapid decline of the immunity of the cultivated varieties. Quantitative resistance, based in most cases on several quantitative trait loci or QTL (Niks et al., 2015), characterizes an incomplete

immunity expressed by a reduction in symptoms intensity. This type of resistance has gained a great interest because of the lower selection pressure it exerts on pathogen populations, thus considered to be more durable as compared to qualitative resistance genes (Mundt et al., 2014; Cowger & Brown, 2019). In the literature, analysis of the determinants and consequences of differences in aggressiveness levels is often linked to certain sources of quantitative resistance, but these traits are not necessarily interrelated and can evolve independently. Studying aggressiveness as a general aspect of the pathogen's fitness is both possible and useful, even if knowledge of its interaction with QTL of the host plant is not (yet) available.

Among all life-history traits, the most widely assessed aggressiveness components for rust pathogens are infection efficiency, latency period and sporulation capacity (Pariaud *et al.*, 2009a; Lannou, 2012; Azzimonti *et al.*, 2013). Infection efficiency is the proportion of spores able to cause a new infection when deposited on the host plant tissues (Sache, 1997). Latency period, expressed in thermal time as it is highly temperature-dependent (Lovell et al., 2004), is the length of time between the deposition of a spore and the appearance of most (usually 50%) of the sporulating structures (Parlevliet, 1975; Johnson, 1980; Pfender, 2001). Sporulation capacity is the number of spores produced per individual sporulation structure and per unit of time (Sache, 1997; Pariaud *et al.*, 2009a).

Pathogen aggressiveness acts on the pathogen population dynamics, as it determines the rate at which a given intensity — incidence and severity — can be reached by a polycyclic disease (Azzimonti et al., 2022). Field experiments have shown that the most aggressive individuals tended to be selected over the course of an epidemic (e.g. Laloi et al., 2016; Suffert et al., 2018), highlighting that aggressiveness can be a significant component of the short-term evolution of pathogen populations. Several studies conducted under controlled conditions have suggested that the aggressiveness of fungal wheat pathogens also increase after repeated cycles on the same host leading to host-specific adaptation. A heterogeneous population of *Puccinia graminis* f. sp. *avenae* was inoculated on two different oat genotypes for seven asexual generations, and the infection efficiency of each population was assessed on the host on which it had been maintained and on another host genotype (Leonard, 1969). By the end of the experiment, the infection efficiency of the population increased on the host on which it had been maintained. Another experimental evolution with Fusarium head blight has compared the evolution of the aggressiveness of *Fusarium graminearum* after serial infections on susceptible and partially resistant wheat cultivars, and after their growth

in axenic conditions (Sakr, 2022). Increase of aggressiveness was detected after serial infections on the plant, but not *in vitro*, highlighting that the change in the latency period of the pathogen was the result of an interaction with the host plant.

The postulate that aggressiveness plays a role in the evolution of fungal plant pathogen populations is based on observations made under controlled conditions, in the field at the annual scale, but also at larger spatiotemporal scales with theoretical modelling approaches (Van den Berg et al., 2014; Rimbaud et al., 2018). However, this statement has not been formally demonstrated experimentally at the pluriannual scale for a varietal landscape. Indeed, the pressure exerted by host resistance is distributed over several pathogen fitness traits, making it more difficult to quantify and to estimate its consequences at large spatiotemporal scales. Quantification is a crucial issue because the long-term effectiveness of resistance might be compromised by the possible increase in pathogen aggressiveness, regardless of the virulence combinations in the pathogen population.

Leaf rust caused by *Puccinia triticina* is one of the most damaging wheat diseases, causing high yield losses worldwide (Huerta-Espino et al., 2011; Savary et al., 2019). Both qualitative and quantitative resistances play a role in the adaptive dynamics of *P. triticina* populations, which is broadly determined by the evolution of clonal lineages (Goyeau et al., 2007; Pariaud et al., 2009a; Kolmer, 2019; Zhang et al., 2020). The deployment of a new qualitative resistance gene (*Lr*) — among the 82 that have been permanently designated in wheat so far (Bariana et al., 2022) — in the cultivar landscape is most of the time followed by the rapid emergence of pathotypes carrying the corresponding virulence, such as *Lr*28 in France, which has been overcome within two years after its introduction in the varietal landscape (Fontyn et al., 2022). This adaptive dynamic results in 'boom-and-bust' cycles of resistance deployment (McDonald & Linde, 2002). Recently, Fontyn et al. (2022) showed that the domination of two *P. triticina* pathotypes 106 314 0 and 166 317 0 in the French landscape during the decade 2006-2016 could not be fully explained by the deployment of *Lr* genes. Several compatible pathotypes, other than the two aforementioned, virulent against the *Lr* genes carried by the most widely grown cultivars, were present in the landscape but never reached substantial frequencies. Focusing on the comparative dynamics of these two dominant pathotypes (Figure 3 in Fontyn et al., 2023) highlighted three periods: (i) until 2011, the frequency of the pathotype 106 314 0 remained higher than the frequency of 166 317 0; (ii) then, from 2012 to 2015, the two pathotypes were at similar frequencies; and (iii) from 2016, 106 314 0 strongly decreased and finally disappeared,

while 166 317 0 became dominant. Considering the *Lr* genes present in the French varietal landscape from 2011 to 2015 (Fontyn et al., 2022), the four most frequent *Lr13*, *Lr37*, *Lr14a* and *Lr10* were overcome by both pathotypes 106 314 0 and 166 317 0. However, *Lr3,* the fifth most frequent *Lr* gene, should have limited the prevalence of the pathotype 106 314 0, avirulent to *Lr3*, as compared to 166 317 0, virulent to *Lr3*. Yet 106 314 0 remained at a frequency superior or equal to that of 166 317 0 until 2015. In their study, Fontyn et al. (2022) proposed that the longer than expected persistence of 106 314 0 in the landscape could be due to the fact that the aggressiveness, as defined above, is a significant evolutionary driver of *P. triticina* populations, leading to changes in pathotype frequencies. Under controlled conditions, Lehman and Shaner (1997) have previously demonstrated that the composition of a *P. triticina* population can be changed by selecting isolates with a shorter latent period after several cycles of asexual reproduction on a partially resistant cultivar, indicating that the pathogen has the potential to adapt to wheat resistances by increasing its aggressiveness. Additionally, Azzimonti et al. (2013) found that *P. triticina* isolates can adapt to quantitative resistance, as evidenced by their specificity towards QTLs. Despite these evidences, the impact of aggressiveness components in agronomic context at a large spatiotemporal scale has not been previously established for leaf rust, until Fontyn et al. (2023) compared the aggressiveness of 'old' and 'new' *P. triticina* pathogenotypes. A pathogenotype is defined as a unique pathotype × genotype combination. They found that the ‘new’ pathogenotypes, within each pathotype 106 314 0 and 166 317 0, exhibited higher levels of aggressiveness compared to the ‘old’ ones, and concluded that this fitness advantage can explain the replacement of isolates by others with the same virulence combinations.

Building upon the aforementioned, the aim of the present study was to investigate the impact of aggressiveness on the comparative dynamics of the two predominant *P. triticina* pathotypes within the cultivated French landscape. To this end, we assessed three aggressiveness components of the two pathotypes (166 317 0 and 106 314 0), from representative isolates of their respective dominant pathogenotypes. These assessments were conducted on two cultivars considered 'neutral' and 'naïve', as defined by Fontyn et al. (2023), meaning that they have no selection effect on the cultivated landscape-pathotype pattern, and on five of the most commonly grown cultivars from 2006 to 2016, which includes cultivars grown before and during the studied period.

Material and methods

Selection and purification of isolates

The 23 isolates used in this study were collected in 2012-2013 during annual surveys of *P. triticina* populations carried out throughout the wheat-growing areas in France over the last two decades (Goyeau et al., 2006; Fontyn et al., 2022). Eight isolates were selected from pathogenotype 106 314 0-G2 and 15 from pathogenotype 166 317 0-G1 (Table S1), which were identified as the most prevalent within each pathotype from 2012 to 2015 (Figures 4 and 5 in Fontyn et al., 2023), justifying their choice as case studies.

The recovery of these isolates was achieved as follows. Urediniospores were bulk-harvested from numerous infected leaves collected in a single field plot, purified from a single pustule and pathotyped on wheat differential lines at the seedling stage (i.e. phenotyped to determine their combination of virulences) according to the method described in Goyeau et al. (2006). Among the bulks of urediniospores initially sampled in 2012 and 2013, 58 corresponded to the pathotype 106 314 0 (carrying virulences 1, 10, 13, 14a, 15, 17, 37) and 56 to the pathotype 166 317 0 (carrying virulences 1, 3, 3bg, 10, 13, 14a, 15, 17, 26, 17b, 37). One isolate from each of these 114 bulks stored at -80°C has been purified once again in 2020 and genotyped with 19 microsatellite markers (Duan et al., 2003; Szabo & Kolmer, 2007). Different genotypes were identified within each pathotype from these markers leading to the distinction of several pathogenotypes, i.e. different pathotype × genotype combinations (Fontyn et al., 2023). Indeed, identical genotypes may differ in one or several virulences, and, conversely, that different genotypes can have the same virulence profile while potentially expressing differences in their aggressiveness.

Experimental design

Three aggressiveness components, infection efficiency (IE), latency period (LP) and sporulation capacity (SP), were assessed for the 23 selected isolates (Table S1). The 23 isolates were characterized in three successive Series, according to the same protocol and under the same experimental conditions (Table 1). In Series 1, we compared the aggressiveness of pathogenotypes 106 314 0-G2 and 166 317 0-G1 (collected on cultivars Apache, Arezzo, Aubusson and Premio) on two 'neutral' wheat varieties: (i) Apache, a commercial French cultivar shown in a previous study (Fontyn et al., 2022) to

have no selection effect on the cultivated landscape-pathotype pattern, and (ii) Michigan Amber, considered intrinsically 'naive' both because it carries no known leaf rust resistance gene and because it has never been cultivated in France. In Series 2 and 3, we compared the aggressiveness components of both pathogenotypes on five of the most frequently grown cultivars in the French landscape from 2006 to 2016: Aubusson, Premio, Sankara, Expert and Bermude. The isolates used in Series 2 and 3 were collected on cultivars Apache and were tested on Aubusson and Premio (for Series 2) and Bermude, Expert and Sankara (for Series 3). To test the isolate × cultivar interaction in all series, eight wheat seedlings were used and the experiment was replicated three times.

Evaluation of aggressiveness components

The pathotyping tests were performed in a greenhouse on eight-day-old wheat seedlings grown in a plastic box containing potting soil placed under standardized conditions as described in Fontyn et al. (2023). The inoculation was performed with 10 fresh (two-week-old) spores, picked one by one with a human eyelash under a dissecting microscope, then deposited on a 3 cm-long segment of the second leaf of each seedling, maintained with double-sided tape on a rigid plate coated with aluminum foil. The plants were then placed in a dew chamber. Uredinia were counted twice a day at 10- to 14-hour intervals, after the first uredinia break through the leaf epidermis and until no new uredinia appeared. Once the final number of uredinia had been reached, i.e. 9 days after inoculation, the spores that had already been produced were removed from the leaf with a small brush. Slightly incurved aluminum gutters (2 × 7cm) made from blind slats were positioned under each inoculated leaf (Figure 1) and all the newly produced spores after four days were removed by suction with a cyclone collector into a portion of plastic straw. Each portion of straw was weighed before and after spore harvesting. Infection efficiency (IE) was estimated for each leaf as the ratio between the final number of uredinia and the number of spores deposited (10 spores). Latency period (LP), expressed in degree-days based on the air temperature measured in the greenhouse every ten minutes, was estimated as the time between inoculation and the appearance of 50% of the total number of uredinia. Sporulation capacity (SP) was calculated by dividing the total weight of the spores collected from a single leaf by the number of uredinia on that leaf.

Statistical analyses

An ANOVA model (1) was used to examine each of the three aggressiveness components (the response variable Y), evaluated separately for each cultivar (Apache and Michigan Amber) in Series 1. Our objective here was not to assess the effect of the cultivar since Michigan Amber was never grown in fields contrary to Apache. Thus, the ANOVA model was used to investigate each component of aggressiveness for each cultivar independently.

$$(1)\ Y_{ijklm} = \mu + R_i + P_j + P_j/Cs_k + P_j/I_l + \varepsilon_{ijklm}$$

where Y_{ijklm} are the value of the aggressiveness component in replicate (R) *i*, of pathogenotype (P) *j,* sampled cultivar (Cs) *k* nested within pathogenotype, and isolate (I) *l* nested within pathogenotype. μ is the overall mean value for this trait and ε is the residual, representing the measurement error, with $\varepsilon \sim N(o, \sigma 2)$.

A linear mixed model (2) was used to analyze the data of Series 2 and 3 separately using the R package *nlme* (Pinheiro et al., 2017). The mixed model enables simultaneous multiple comparisons of two fixed effects, allowing for the analysis of the effect of each sampled cultivar on the two pathogenotypes.

$$(2)\ Y_{ijklm} = \mu + R_i * P_j * Ct_k + 1/P_j/I_l + \varepsilon_{ijklm}$$

where Y_{ijklm} are the value of the aggressiveness component in replicate (R) *i*, of pathogenotype (P) *j,* tested cultivar (Ct) *k*, and isolate (I) *l* nested within pathogenotype.

R, P, Cs and Ct were fixed effects as well as the interaction between them, while I was a random effect. Significance for the fixed effect was calculated using the Satterthwaite method to estimate degrees of freedom and generate *p*-values for mixed models. Significance for the random effect was calculated based on the likelihood ratio chi-squared test. *Post hoc* test was performed using the R package *emmeans* (Lenth *et al*., 2018) for single effect, and the R package *multcomp* (Hothorn *et al*., 2016) for multiple comparisons of two fixed effects.

Log, $\sqrt{}$ or 1/x transformation was applied to IE, LP and SP when necessary, to obtain a normalized distribution of residuals. When the distribution of residuals could not be normalized by any transformation, a non-parametric Kruskal-Wallis test was performed

to analyze the effect of genotype on the aggressiveness components. All the analyses were performed with R software version 4.1.0.

Results

Differences in aggressiveness between the two most frequent pathogenotypes, on neutral or naïve cultivars

When tested in Series 1, pathogenotype 106 314 0-G2 appeared overall more aggressive than 166 317 0-G1. On the 'neutral' cultivar Apache, this result was obtained for latency period (LP) and sporulation capacity (SP) (Figure 2B and 2C; Table S3; *p*-values provided in Table S2). On the 'naïve' cultivar Michigan Amber, this result was obtained for infection efficiency (IE) and SP (Figure 2A and 2C). IE was higher for pathogenotype 106 314 0-G2 (69%) than for 166 317 0-G1 (64%) on Michigan Amber only. SP was higher for pathogenotype 106 314 0-G2 (0.092 mg.uredinium^{-1} on both Apache and Michigan Amber) than for 166 317 0-G1 (0.084 mg.uredinium^{-1} on Apache and 0.085 mg.uredinium^{-1} on Michigan Amber). LP was shorter for pathogenotype 106 314 0-G2 (135.5 degree-days) than for 166 317 0-G1 (141.5 degree-days) on cultivar Apache only.

Within each pathogenotype, we also evidenced a significant isolate effect on Apache and Michigan Amber (Table S2). Within pathogenotype 106 314 0-G2, the isolate effect was significant for LP and IE on both cultivars. Within pathogenotype 166 317 0-G1, the isolate effect was significant for the three aggressiveness components on Apache, and for IE and SP only on Michigan Amber.

Differences in aggressiveness between the two most frequent pathogenotypes, on five of the mostly grown cultivars

When tested on five of the mostly grown French cultivars in Series 2 (Aubusson and Premio) and Series 3 (Bermude, Expert and Sankara), pathogenotype 106 314 0-G2 appeared more aggressive than 166 317 0-G1 (Table 3), but the difference was statistically significant only for LP on Aubusson (135.5 vs 138.8 degree-days) and Sankara (140.7 vs. 143.9 degree-days). In addition, significant differences in varietal

susceptibility were identified, with the effects of aggressiveness components showing consistent directionality. This consistency enhances the robustness of the conclusions drawn. In Series 2, both pathogenotypes appeared more aggressive on Premio than on Aubusson (Table 3): 106 314 0-G2 had a significantly shorter LP and higher SP on Premio, and 166 317 0-G1 was significantly more aggressive on Premio than on Aubusson for all three components LP, IE and SP. In Series 3, both pathogenotypes appeared more aggressive on Expert with higher IE and shorter LP as compared to Bermude and Sankara (Table 3). For both pathogenotypes, SP was not significantly different between Expert and Sankara, but was significantly lower on Bermude. Bermude was slightly less susceptible than Expert and Sankara to both pathogenotypes.

Discussion

Despite a theoretical benefit related to virulence on *Lr3*, two *P. triticina* pathogenotypes dominated similarly the cultivated landscape from 2012 to 2015

In the 2012-2015 period, the two *P. triticina* pathotypes that extensively dominated the French landscape differed in their virulence profile with the presence of four more virulences in 166 317 0 than in 106 314 0, preventing the latter pathotype to infect varieties carrying the genes *Lr3*, *Lr3bg*, *Lr17b* and *Lr26* (Fontyn et al., 2022). While *Lr3bg*, *Lr17b* and *Lr26* are not present in French wheat cultivars, the frequency of *Lr3* has continuously increased in the landscape since 2006, reaching 14% in 2014 and thus becoming the fifth most common *Lr* gene in France after *Lr13*, *Lr37*, *Lr14a* and *Lr10* on which both pathotypes are virulent (Fontyn et al., 2022). Thus, the presence of *Lr3* in the cultivars confers a theoretical selective advantage to 166 317 0, virulent to *Lr3*, as compared to 106 314 0, avirulent to *Lr3*. However, during the 2012-2014 period, 166 317 0 was not more frequent than 106 314 0, as might have been expected based on both this theoretical statement and the large-scale field results from previous studies on *P. triticina* populations (Papaïx et al., 2011; Kolmer, 2019; Zhang et al., 2020). The difference in aggressiveness between representative isolates of 166 317 0-G1 and 106 314 0-G2 might explain this apparent contradiction. While it is unlikely that a fitness trade-off between aggressiveness and virulence will persist permanently, our research has demonstrated that there are specific points in the evolutionary trajectory of pathogen populations where this trade-off becomes apparent. The period from 2012 to 2014 was one such moment, and our findings indicate that this dynamic can endure

for some years, particularly in the case of leaf rust.

The pathogenotype avirulent on *Lr3* exhibited higher aggressiveness, which should confer a significant selective advantage

The aggressiveness of the pathogenotypes 106 314 0-G2 and 166 317 0-G1 was assessed accurately on cultivar Apache (the mostly grown cultivar in France from 2009 to 2013), Michigan Amber (not cultivated), and five of the mostly grown cultivars in France from 2012 to 2015. Significant differences in latency period (LP), infection efficiency (IE) and sporulation capacity (SP) were found, not only on the 'neutral' cultivar Apache and on the 'naive' cultivar Michigan, but also on Aubusson, Sankara, Premio, Bermude and Expert, for certain components at least. Although the difference between both pathogenotypes was not significant for all the aggressiveness components, the ones that were significant all indicated that 106 314 0-G2, which does not carry the *Lr3* virulence, is more aggressive than 166 317 0-G1. LP, IE and SP, by acting as life-history traits at complementary steps of the pathogen cycle, impact the development of certain isolates and thus modify their involvement in leaf rust epidemics (Pringle & Taylor, 2002; Lannou, 2012; Azzimonti et al., 2022). All other immunity-related traits remaining equal, especially virulence profile, each of the three aggressiveness components confers a significant advantage to certain *P. triticina* isolates. LP, which presents the most pronounced difference between 106 314 0-G2 and 166 317 0-G1, is likely the aggressiveness component having the strongest impact on epidemic dynamics since it drives the number of disease cycles during one epidemic season (Pringle & Taylor, 2002; Milus et al., 2006; Lannou, 2012). On cultivar Apache, the difference in LP was approximately half a day (11 h) at 15°C. As rust spores are known to be mainly dispersed towards the middle of the day (Pady et al., 1965), this difference may almost give a one-day advance in their dispersal if the environmental conditions (humidity, wind) are favorable. The more components are concerned by a higher aggressiveness, the more it will be advantageous for a pathogenotype. Indeed, severe leaf rust epidemics are mostly associated with a high sporulation capacity, high infection efficiency and a short latency period (Azzimonti et al., 2022).

The higher expression of aggressiveness in pathogenotype 106 314 0-G2 did not depend on the cultivar

Pathogenotype 166 317 0-G1 was never found more aggressive than 106 314 0-G2 whatever the cultivar on which it was tested (Apache, Michigan Amber, Aubusson or Sankara) and whatever the aggressiveness component considered. However, the significant differences in the aggressiveness of both pathogenotypes between the five cultivars tested in Series 2 and 3 suggest that these cultivars differed in their susceptibility to *P. triticina*, consistently with the findings of Azzimonti *et al.* (2013) who highlighted differences in quantitative resistance between cultivars, both in field and in greenhouse conditions.

We found here an isolate effect within a given pathogenotype, using 6 isolates for pathogenotype 106 314 0-G2 (with a significant effect on IE and LP on Apache and Michigan Amber) and 12 isolates for pathogenotype 166 317 0-G1 (with a significant effect on the three aggressiveness components, except for LP on Michigan Amber). Pariaud *et al.* (2012) also evidenced such an aggressiveness variability between *P. triticina* isolates within a given pathotype, despite the clonal reproduction of *P. triticina* in Europe. Study on *Phytophthora infestans* revealed similar differences in aggressiveness traits (latency period, infection efficiency, sporulation capacity and lesion expansion rate) between isolates sharing an identical genotype (Carlisle et al., 2002). Specificity of quantitative resistance with regard to pathogen isolates, and thus the cultivar dependence of the aggressiveness expression without considering the effect of *Lr* genes, remains a matter of debate in the case of wheat leaf rust. Interactions between *P. triticina* isolates and wheat lines were found for latent period (Broers, 1989; Lehman & Shaner, 1996) and for sporulation capacity (Milus & Line, 1980), but Denissen (1991) did not find any specificity for these components. Later, Singh et al. (2011) stated that there was no isolate-specificity for quantitative resistance to the three wheat rust diseases in a large collection of CIMMYT breeding material.

It is worth noting that Fontyn et al. (2023) already found differences in aggressiveness within a given pathotype, i.e. between pathogenotypes sharing the same virulence profiles. For instance, within the pathotype 106 314 0, the pathogenotype 106 314 0-G2 replaced 106 314 0-G1, which dominated the landscape between 2006 and 2012 but completely disappeared by 2014. Such a replacement of 'old' pathogenotypes by 'young' ones was explained by differences in aggressiveness. It is important to keep in mind that the hypothesis tested here was based on data presented in the aforementioned study, which already discussed the methodological aspects of assessing aggressiveness components under similar experimental conditions.

Higher aggressiveness under controlled conditions represents a competitive advantage that may be even more pronounced when exhibited at the landscape scale

Although Apache was considered as a 'neutral' cultivar and has a relatively high quantitative resistance level, its high proportion in the French landscape (> 8% over the decade 2003-2013) might have contributed to the selection of the most aggressive pathotype 106 314 0. Aubusson, Sankara, Premio, Bermude and Expert were the most frequently grown cultivars before 2012, and together they accounted for 11.1% of the cultivated landscape in 2012 (20.3% including Apache), 6.6% in 2013 (14.6% including Apache) and 3.0% in 2014 (9.0% including Apache). Therefore, the earliest pathotype 106 314 0 appears to be significantly advantaged over 166 317 0 on a significant part of the varietal landscape, and it is likely that the differences highlighted under controlled conditions have had a real impact in field conditions as the difference in aggressiveness might be expressed more intensely on adult plants than on seedlings (Milus and Line 1980). Knott (1991) found a higher (+25%) infection efficiency and a shorter (-4%) latent period of *P. triticina* on the upper wheat leaves than on the lower leaves, likely due to greater susceptibility of the upper leaves or to physiological effects.

Our experimental findings suggest that a cultivar with a good level of quantitative resistance could select for more aggressive isolates, consistently with results obtained on other plant pathogens (e.g. Delmas et al., 2016; Sakr, 2022). It has been shown that aggressiveness confers a selective advantage resulting in pathotype or race replacement in stripe rust populations (Milus et al., 2009). In leaf rust, it has already been shown that the *P. triticina* pathotype 073 100 0, which dominated the landscape between the late 1990s and early 2000s, had a higher aggressiveness on the cultivar Soissons as compared to other virulent pathotypes present in minor frequencies (Pariaud et al., 2009b). Soissons was the mostly grown cultivar from 1991 to 1999, with a frequency in the French landscape going from 40.5% in 1993 to 15.3% in 1999. This result was interpreted as an adaptation of pathotype 073 100 0 to Soissons, which explained its domination within the pathogen population during this period. Similarly, the advantage provided by the higher aggressiveness of 106 314 0-G2 on the mostly grown cultivars may explain the high frequency of pathotype 106 314 0 in the landscape in 2013-2014, despite the disadvantage conferred by its avirulence to *Lr3*.

The aforementioned selective advantages, considered trait by trait (LP, IE, SP), may seem weak compared to those conferred theoretically by a single *Lr* gene. Indeed, the pathotype 106 314 0-G2 cannot infect at all wheat varieties carrying *Lr3*, which

occupied up to 14% of the wheat surfaces in 2014. However, the smaller advantage of a few hours of shortened infection cycle (LP) combined with a few percent more released spores (SP) from each uredinia can be expressed across all other varieties, that is, 86% of the surface area. If we attempt to generalise, the frequency equilibrium between 106 314 0-G2 and 166 317 0-G1 observed in 2012, 2013 and 2014 exemplified that a 'moderate advantage' expressed in a large majority of situations and a 'strong advantage' expressed in a small number of situations may have equivalent consequence for short-term evolutionary dynamics. Furthermore, higher aggressiveness observed under controlled conditions represents a competitive advantage that is likely even more pronounced when exhibited at the landscape scale as the expression of its multiple components is amplified by the polycyclic nature of epidemics.

While it is important to approach this conclusion with caution as it was established on a single case study, it signifies a noteworthy progression in plant disease epidemiology as our findings corroborate assumptions and parameterizations utilized in theoretical models that underscore the significance of aggressiveness (e.g. Van den Berg et al., 2014; Rimbaud et al., 2018, 2021). These models suggest that the most promising trait for quantitative resistance is the latency period, as it directly affects the number of epidemic cycles that the pathogen can complete during a given season. Our experimental results support this assumption, and the significant differences observed between major pathogenotypes for other aggressiveness components will help to parameterize more biologically realistic predictive models, for leaf rust at least.

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Data Availability Statement

Data, scripts, and code are available in the INRAE Dataverse online data repository at https://doi.org/10.57745/QDF06W.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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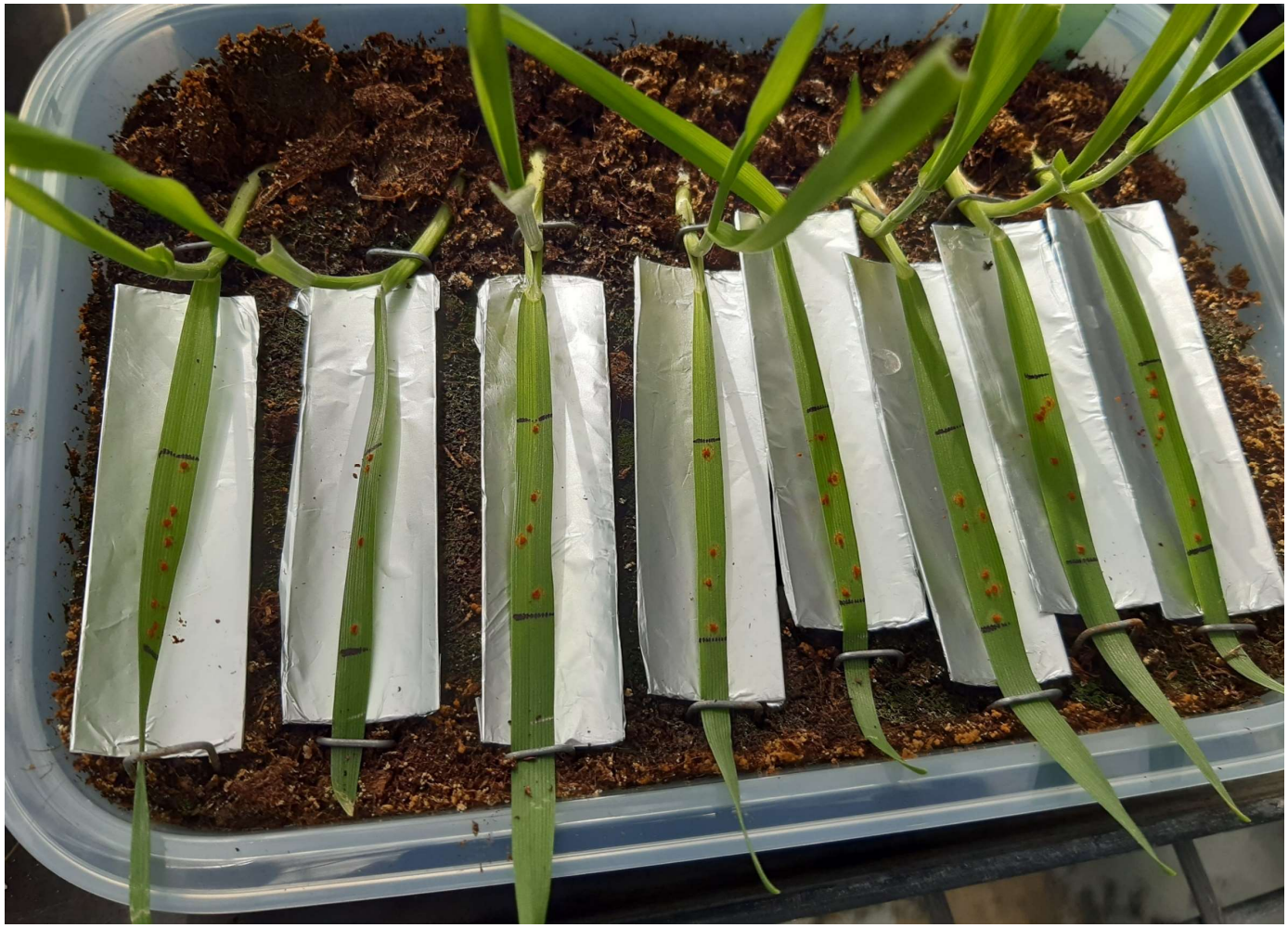

Figure 1. Experimental device consisting of incurved aluminium gutters positioned under wheat leaves presenting uredina, used to measure the sporulation capacity of each *Puccinia triticina* isolate.

Table 1. Experimental design for comparison of the aggressiveness of pathogenotypes 106 314 0-G2 and 166 317 0-G1.

Series	Pathogenotype	Number of isolates	Sampled cultivar (Cs)	Tested cultivar (Ct)
1	106 314 0-G2	1	Apache	Apache, Michigan Amber
		2	Arezzo	
		2	Aubusson	
		1	Premio	
	166 317 0-G1	3	Apache	
		3	Arezzo	
		3	Aubusson	
		3	Premio	
2	106 314 0-G2	3	Apache	Aubusson, Premio
	166 317 0-G1	6		
3	106 314 0-G2	2	Apache	Bermude, Expert, Sankara
	166 317 0-G1	4		

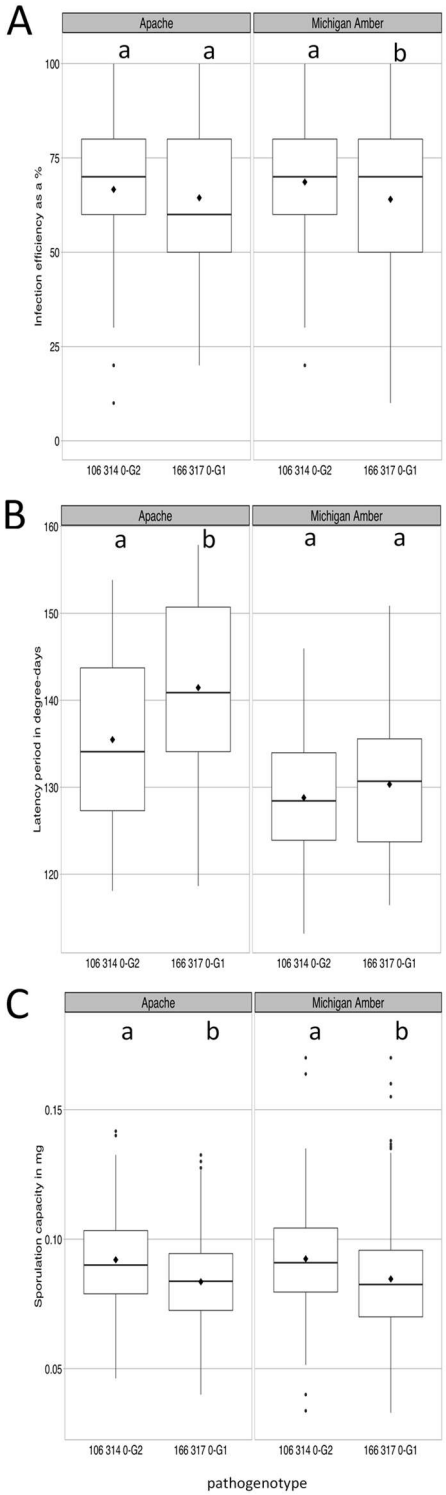

Figure 2. Comparison of three aggressiveness components of *Puccinia triticina* pathogenotypes 106 314-G2 and 166 317-G1 — infection efficiency in % (A), latency period in degree-days (B) and sporulation capacity in mg of spores.uredinium^{-1} (C) — measured on cultivars Apache and Michigan Amber. Within a box plot, black diamonds represent the mean value and horizontal bars the median value. Letters indicate statistical difference between pathogenotypes, resulting from Tukey test.

Table 3. Comparison of the aggressiveness components of the pathogenotypes 106 314 0-G2 and 166 317 0-G1 assessed on five of the most widely grown French cultivars during the 2006-2016 period. Infection efficiency (IE) was measured as a percentage, latency period (LP) in degree-days, and sporulation capacity (SP) in mg of spores.uredinium^{-1}.

Pathogenotype	Aggressiveness component	Series 2		Series 3		
		Premio	Aubusson	Bermude	Expert	Sankara
106 314 0-G2	LP	132.22 a	135.35 b*	142.09 b	138.17 a	140.74 b*
	IE	51.83	51.12	46.96	48.72	46.89
	SP	0.134 a	0.120 b	0.121 a	0.132 ab	0.134 b
166 317 0-G1	LP	131.23 a	138.80 b*	142.89 b	138.44 a	143.88 b*
	IE	54.04 a	47.23 b	41.94 a	52.21 b	45.53 a
	SP	0.127 a	0.113 b	0.119 a	0.129 b	0.132 b

* indicates a significant difference between the two pathogenotypes on the tested cultivar after a Tukey multiple-comparison test or a Wilcoxon-Mann-Whitney test.

Letters indicate a significant difference between cultivars within the same pathogenotype and series based on a multiple-comparison test.

SUPPLEMENTARY MATERIAL

Table S1. List of the 23 *Puccinia triticina* isolates of the two pathogenotypes (106 314 0-G2 and 166 317 0-G1) collected in 2012-2013 and characterized for their agressiveness.

Isolate	Pathotype	Pathogenotype	Series	Cultivar sampled (Cs)
BT13M108	106 314 0	106 314 0-G2	1	Aubusson
BT13V026	106 314 0	106 314 0-G2	1	Aubusson
BT13V143	106 314 0	106 314 0-G2	1	Arezzo
BT12M024	106 314 0	106 314 0-G2	1	Arezzo
BT12M302	106 314 0	106 314 0-G2	1	Premio
BT12M293	166 317 0	166 317 0-G1	1	Aubusson
BT12V078	166 317 0	166 317 0-G1	1	Aubusson
BT13V066	166 317 0	166 317 0-G1	1	Aubusson
BT13M239	166 317 0	166 317 0-G1	1	Arezzo
BT13M259	166 317 0	166 317 0-G1	1	Arezzo
BT12M042	166 317 0	166 317 0-G1	1	Arezzo
BT12M371	166 317 0	166 317 0-G1	1	Premio
BT12M264	166 317 0	166 317 0-G1	1	Premio
BT12M079	166 317 0	166 317 0-G1	1	Premio
BT12M033	106 314 0	106 314 0-G2	2	Apache
BT12M391	166 317 0	166 317 0-G1	2	Apache
BT12M192	166 317 0	166 317 0-G1	2	Apache
BT12M326	106 314 0	106 314 0-G2	2+3	Apache
BT12M321	166 317 0	166 317 0-G1	2+3	Apache
BT13V189	106 314 0	106 314 0-G2	1+2+3	Apache
BT12M202	166 317 0	166 317 0-G1	1+2+3	Apache
BT12M378	166 317 0	166 317 0-G1	1+2+3	Apache
BT12M109	166 317 0	166 317 0-G1	1+2+3	Apache

Table S2. P-values of the ANOVA model used to analyze the source of variations (R for replicate, P for pathogenotype, P/I for isolate and P/Cs for sampled cultivar) on the latency period (LP), infection efficiency (IE) and sporulation capacity (SP) in Series 1.

Tested cultivar	Aggressiveness component	R	P	P/I	P/Cs
Apache	LP	<0.0001	<0.0001	<0.0001	0.4
	IE	<0.0001	0.27	<0.0001	0.6
	SP	<0.0001	<0.0001	0.002	0.001
Michigan	LP	<0.0001	0.01	<0.0001	0.001
	IE	<0.0001	0.01 *	<0.0001	<0.0001
	SP	<0.0001	0.0002	<0.0001	0.001

* significant interaction with replicate

Table S3. Comparison of the aggressiveness component of the pathogenotypes 106 314 0-G2 and 166 317 0-G1 assessed on cultivars Apache and Michigan collected in 2012-2013 (Series 1). Infection efficiency (IE) was measured as a %, latency period (LP) in degree-days, and sporulation capacity (SP) in mg of spores.uredinium^{-1}.

Pathogenotype	Isolate	Apache			Michigan Amber		
		LP	IE	SP	LP	IE	SP
106 314 0-G2	BT12M024	141.3 a	69.6 ab	0.087 a	133.5 a	76.4 a	0.091 a
	BT12M302	135.8 ab	55.7 ab	0.096 a	127.2 bc	63.7 ab	0.082 a
	BT13M108	133.7 ab	72.5 ab	0.090 a	128.5 bc	69.2 ab	0.092 a
	BT13V026	137.1 ab	74.8 a	0.092 a	130.4 ab	76.7 a	0.100 a
	BT13V143	133.7 ab	57.4 b	0.095 a	128.5 bc	60.0 b	0.099 a
	BT13V189	131.4 b	70.4 ab	0.091 a	124.8 c	64.8 ab	0.092 a
166 317 0-G1	BT12M042	138.8 ab	48.2 c	0.090 a	127.8 a	56.5 cdef	0.096 a
	BT12M079	139.3 ab	74.8 a	0.079 ab	131.2 a	80.9 a	0.086 a
	BT12M109	140.9 ab	67.3 ab	0.087 a	132.6 a	70.0 abcd	0.087 a
	BT12M202	146.3 a	72.7 a	0.083 a	132.1 a	67.9 abcde	0.089 a
	BT12M264	141.7 ab	63.8 abc	0.081 ab	130.1 a	60.9 bcdef	0.092 a
	BT12M293	141.7 ab	62.2 abc	0.086 a	132.4 a	57.6 bcdef	0.086 a
	BT12M371	142.2 ab	71.8 a	0.080 ab	128.1 a	75.5 ab	0.076 a
	BT12M378	143.3 ab	68.7 ab	0.067 b	129.7 a	72.2 abc	0.063 b
	BT12V078	136.8 b	66.7 ab	0.091 a	128.7 a	50.0 ef	0.087 a
	BT13M239	143.4 ab	59.1 abc	0.082 a	132.7 a	53.68 def	0.075 ab
	BT13M259	142.6 ab	52.9 bc	0.088 a	126.6 a	48.18 f	0.096 a
	BT13V066	141.6 ab	66.36 ab	0.089 a	132.6 a	73.0 abc	0.087 a

Letters indicate a significant difference between isolates within the same pathogenotype based on a multiple-comparison test.