

# Variation in aggressiveness is detected among *Puccinia triticina* isolates of the same pathotype and clonal lineage in the adult plant stage

Bénédicte Pariaud · Henriette Goyeau · Fabien Halkett ·  
Corinne Robert · Christian Lannou

Accepted: 26 July 2012 / Published online: 1 September 2012  
© The Author(s) 2012. This article is published with open access at Springerlink.com

**Abstract** *Puccinia triticina* reproduces asexually in France and thus individual genotype is the unit of selection. A strong link has been observed between genotype identities (as assessed by microsatellite markers) and pathotypes (pools of individuals with the same combination of qualitative virulence factors). Here, we tested whether differences in quantitative traits of aggressiveness could be detected within those clonal lineages by comparing isolates of identical pathotype and microsatellite profile. Pairs of isolates belonging to different pathotypes were compared for

their latent period, lesion size and spore production capacity on adult plants under greenhouse conditions, with a high number of replicates. Isolates of the same pathotype showed remarkably similar values for the measured traits, except in three situations: differences were obtained within two pathotypes for latent period and within one pathotype for sporulation capacity. One of these differences was tested again and confirmed. This indicates that the average aggressiveness level of a leaf rust pathotype may increase without any change in its virulence factors or microsatellite profile.

---

B. Pariaud  
Vilmorin Costière,  
30210 Ledenon, France

H. Goyeau  
INRA,  
UMR 1290,  
78850 Thiverval Grignon, France

F. Halkett  
INRA,  
UMR 1136,  
54280 Champenoux, France

C. Robert  
INRA,  
UMR 1091,  
78850 Thiverval Grignon, France

C. Lannou (✉)  
INRA Bioger,  
BP 01, 78850 Thiverval Grignon, France  
e-mail: lannou@grignon.inra.fr

**Keywords** Genetic diversity · Quantitative trait · Leaf rust

## Introduction

Many plant pathogens reproduce asexually in natural populations. This can be due to the fact that sexual reproduction does not occur in nature (e.g. *Magnaporthe grisea*) or only in a restricted part of the pathogen distribution area (e.g. *Puccinia triticina*). Because in the absence of sexual reproduction the whole genome is the unit of selection, such pathogens often form clonal lineages in which a tight relationship can be found between measured phenotype and genotype identity (Goyeau et al. 2007). Considering avirulence to virulence changes according to a gene-for-gene pattern (Flor 1971), this leads to pathotype structures, pathotypes being groups of individuals with the

same combination of avirulence genes (Roumen et al. 1997; Pilet et al. 2005; Goyeau et al. 2006). For such clonal species, the pathotype is sometimes considered as a basic genetic unit in population studies, given the large selection pressure exerted by the qualitative resistance genes, and the diversity for pathogenicity-related traits at a lower scale is often ignored or neglected. Pathotypes were sometimes compared for quantitative traits on the basis of a single isolate per pathotype (Katsuya and Green 1967). Quantitative variation in pathogenicity among individuals can be the consequence of a change from avirulence to virulence (Vera Cruz et al. 2000), or related to the number of qualitative virulence factors (Thrall and Burdon 2003) but it is likely that aggressiveness variations also occur independently of qualitative virulence (Villaréal and Lannou 2000; Pilet et al. 2005; Lannou 2012). From the available literature, this is however not easy to establish for pathogens with clonal population structures.

The significance of measured aggressiveness differences among isolates of the same clonal lineage or pathotype is often difficult to evaluate. Several authors who have detected such differences also found that they were not always consistent from one trial to another. In order to take into account the experiment effect on *P. infestans* aggressiveness, Miller et al. (1998) included a reference isolate in a series of tests and they found that the aggressiveness level of the tested isolates relative to the reference isolate was not consistent across experiments. In a study of genetic variation in latent period of *P. triticulturae*, Lehman and Shaner (1996) compared seven isolates on five varieties in two different experiments. They found no difference between mean latent periods for isolates across experiments, varieties and replications but the interaction experiment  $\times$  isolate was significant because certain isolates reversed rank between the two experiments. Similarly, in a study on *Cochliobolus heterostrophus* adaptation to corn hosts with partial resistance, Kolmer and Leonard (1986) found a significant isolate-by-trial interaction. In a previous study on *P. triticulturae* (Pariaud et al. 2009a), in which significant differences in several aggressiveness traits were found among pathotypes, an “isolate” effect was detected within the pathotypes as a global effect but the number of replicates did not allow production of reliable differences between

two specific isolates and the relevance of this isolate effect remained questionable.

Differences in aggressiveness among isolates that are genetically close are likely to be small and therefore difficult to measure. In particular, genotype-by-environment interactions may strongly influence the expression of quantitative traits. In an obligate fungal pathogen (*Podosphaera plantaginis*) of *Plantago lanceola*, genotype-by-temperature interactions were measured for spore infectivity and spore production (Laine 2008). Quantitative measurements of aggressiveness may be influenced by the environment sensu lato (Lannou 2012), including climatic parameters (Eyal and Peterson 1967; Tomerlin et al. 1983), host physiological state (Milus and Line 1980; Tomerlin et al. 1983) and pathogen maintenance (Jeffrey et al. 1962; Mundt et al. 2002).

*P. triticulturae* reproduces asexually in France and its populations can be described as a set of pathotypes, each pathotype corresponding to a single multilocus genotype as assessed with microsatellite markers (Goyeau et al. 2007). The existence of variability for quantitative traits of pathogenicity within a *P. triticulturae* pathotype would mean that individuals which are genetically very close can yet differ in their aggressiveness level, and that a pathotype can increase in aggressiveness on a host through selection of the most aggressive individuals without any change in its qualitative virulence factors (i.e. without occurrence of a new pathotype).

Our objective here was to establish whether or not differences in aggressiveness between two isolates of *P. triticulturae* of same pathotype and same clonal lineage can be detected. For this, we measured several aggressiveness components with a high number of replicates on a restricted set of isolates and we confirmed the relatedness level of the isolates with 20 microsatellite markers. Since the probability of finding by chance isolates with different phenotypes within pathotypes presenting such a strong clonal structure was small, and since there is a trade-off between the number of individuals tested and the number of replicates in such experiments, the isolates were chosen based on the information provided by Pariaud et al. (2009a). Our approach was not that of a population study: the objective was not to establish differences among a large number of isolates but rather to show that at least a significant difference can be found among two isolates of same pathotype and clonal lineage in *P. triticulturae*. In

this paper, the term “aggressiveness” simply refers to the quantitative variation of pathogenicity on susceptible hosts (Pariaud et al. 2009b).

## Materials and methods

Greenhouse experiments were conducted to compare the aggressiveness levels of three pairs of *P. triticina* isolates, each pair belonging to a different pathotype (073100, 014103 and 166336, referred to as P1, P2 and P3, respectively, in the paper). The choice of the isolates was based on a previous population study (Pariaud et al. 2009a). Working with a small number of isolates allowed doing a high number of replicates and thus maximizing the precision of the measurements at the individual level. These experiments did not allow comparison of the pathotypes (since the isolates were not chosen to be representative of each pathotype’s sub-population) but were rather designed to identify differences among isolates. The isolates, within each pair, were compared in experiment 1 for their infection efficiency, latent period, lesion size and spore production capacity. For technical reasons, we were not able to replicate all these measurements and, in experiment 2a, we chose to focus on one of the significant differences that were detected. Additional data of a preliminary experiment are also presented as experiment 2b.

### Multilocus genotypes of the *P. triticina* isolates

DNA extraction was performed following the procedure described in Goyeau et al. (2007). The isolates were profiled with 20 SSR primer pairs developed for genetic analysis of *P. triticina*: RB8, RB11, RB12, RB17, RB25, RB26, RB29 (Duan et al. 2003), PtSSR13, PtSSR50, PtSSR55, PtSSR61, PtSSR68, PtSSR91, PtSSR92, PtSSR152, PtSSR154, PtSSR158, PtSSR164, PtSSR173, PtSSR186 (Szabo and Kolmer 2007). Two experiments were performed to check for the consistency of allele scoring. In the first experiment, for each of the 20 loci, one member of the primer pair had a 5′-end M13 extension. In the second experiment, the seven least polymorphic loci (RB12, RB25, RB26, PtSSR13, PtSSR55, PtSSR91, PtSSR152) were again tested with M13-labelled primers, while fluorescent dye-labeled primers were designed for the remaining 13 loci. These 13 loci were

amplified in a single multiplex PCR, whereas the loci tested with M13-labelled primers were pooled by two to three. Since two techniques have been used for loci amplification, we harmonized allele size among loci by deducing 19 bp (the length of the M13 tail) for loci which primers were not directly dye-labelled. PCR amplifications were performed using a PTC-100 thermocycler (MJ Research) under the following conditions: 15 min at 95 °C; 45 cycles of 30 s at 94 °C, 90 s at 57 °C, 60 s at 72 °C; and a final extension of 10 min at 72 °C. Each reaction (10 µl) contained 3 µl of DNA solution (approximately 5 ng/µl), 5 µl of Qiagen Multiplex PCR Master Mix plus, 1 µl of Q-solution (Qiagen Multiplex PCR Kit, ref 206143), and 1 µl of 10X primer mix: when using an M13-labelled extension, the primer mix contained 1.6 µM of forward primer complemented with M13 tail, 2 µM of the reverse primer, 1.2 µM fluorescent M13 primer labelled with one of the fluorescent dyes NED or FAM (Applied Biosystems); otherwise it contained 0.2 mM of each primer pairs, with one primer of the pair labeled with a fluorescent dye NED, HEX or FAM. Dyes were assigned to loci in such a way that loci with the same dye had non-overlapping ranges of allele sizes. Two micro litres of diluted amplified products (1/40) were mixed with 8 µl of MegaBACE ET 400-R size standard (diluted 1/40). PCR products were separated on a MegaBACE 1000 DNA sequencing apparatus (Amersham).

### Plant material

Plants of the wheat cultivar Soissons (carrying the resistance gene Lr14a) were sown separately in Jiffy pots on 18 November and 13 January, and vernalized in a growth chamber for 7.9 and 7 weeks at 8 °C for experiments 1 and 2a, respectively. After vernalization, seedlings were individually transplanted into pots made from sanitary PCV tubes (height: 25 cm; diameter: 6.5 cm). The pots were filled with a commercial substrate (Klasmann Substrat 4, Klasmann France SARL) mixed with 3 g/l of slow-release fertilizer (Osmocote 10-11-18 N-P-K). During plant growth, natural light was supplemented with 400-W sodium vapour lamps between 06:00 and 21:00 h, and temperature was maintained between 12 and 18 °C until inoculation. During this period, plants were treated for powdery mildew (Ethyrimol 2 ml/l, Syngenta) or insects (Methomyl, Lannate 24 ml/l, Dupont solution)

as needed. Ethirimol controls specifically powdery mildew and is commonly used in experiments on rusts. In addition to the slow-release fertilizer, nutritive solution was added once or twice a week from the third week after transplanting. Tillers were progressively eliminated to keep only the main stem, one replication consisting in a single flag leaf. Homogeneous plant material was obtained from a strong selection process, which led to discard about 50 % of the plants. Despite this, slight differences in growth stages occurred but a preliminary Anova analysis showed that they did not affect the aggressiveness components measured.

### Fungal material

The fungal material is described in Table 1. The choice of the isolates was made such as to maximize the expected difference within each pair for the spore production capacity ( $\mu\text{g}$  of spores produced per  $\text{mm}^2$  of sporulating surface), based on previous results (Pariaud et al. 2009a). For each pathotype, the isolates chosen had been collected in different years (for P1 and P2) and from locations at least 600 km apart. All isolates resulted from monospore isolations. Prior to adult plant inoculation, spores of each isolate were increased on seedlings in order to produce fresh spores.

### Adult plant inoculation

Inoculations were performed on flag leaves by applying mixtures of leaf rust uredospores and *Lycopodium*

spores to a leaf section with a soft brush. A leaf section of 9 cm was inoculated using a stencil to protect the remaining leaf surface. Immediately after inoculation, plants were placed in a dew chamber (15 °C) for 24 h, then put back in the greenhouse until the end of the experiment. In experiment 1, 23 plants were inoculated with each isolate on 03 March (18.6 weeks after sowing). Three spore concentrations were used in order to obtain a range of lesion densities: 1/20, 1/60 and 1/160. We were not able to entirely repeat the experiment because of space limitations (adult plant experiments are highly space-consuming). We therefore focused on one of the significant differences that was detected in experiment 1 (namely the difference between the P3 isolates) and attempted to confirm this difference in an independent experiment (experiment 2a). In experiment 2a, 15 plants were inoculated with each P3 isolate on 23 May, with a single leaf rust spore concentration of 1/10. A preliminary experiment designed to set up the experimental procedure for the latent period measurement was performed with the same protocol as in experiment 2a but with three isolates of pathotype P1. For the result presentation, this experiment will be referred to as experiment 2b. Inoculation took place on 30 September. At that time, the information for choosing the isolates was not fully available and we simply used three isolates of pathotype P1 : isolate 1–257, which was already identified as a good candidate, and two other P1 isolates. Therefore, only 1–257 is common to experiment 2b and experiment 1.

**Table 1** Fungal material description

Pathotype <sup>a</sup>	Virulence factors <sup>b</sup>	Isolate	genotype <sup>c</sup>	Year of sampling	Sampling location <sup>d</sup>
P1 (073100)	<i>Lr2c, Lr3, Lr3bg, Lr3ka, Lr10, Lr14a</i>	1–257	1	2001	80 (Somme)
		9–191	1	1999	26 (Drôme)
P2 (014103)	<i>Lr2c, Lr13, Lr14a, Lr26, Lr17b</i>	0–010	5	2000	44 (Loire-Atlantique)
		7–17	5	1997	32 (Gers)
P3 (166336)	<i>Lr1, Lr3, Lr3bg, Lr10, Lr13, Lr14a, Lr15, Lr17, Lr17b, Lr20, Lr37, Lr27+Lr31</i>	1–303	17	2001	22 (Côtes d'Armor)
		1–228	17	2001	03 (Allier)

<sup>a</sup> Six-digit code based on a 18-*Lr* genes differential set (see Goyeau et al. (2006) for details)

<sup>b</sup> Virulence factors at seedling stage (see Goyeau et al. (2006) for details)

<sup>c</sup> genotype according to 20 microsatellite markers (see Goyeau et al. (2007) for profile numbers)

<sup>d</sup> Administrative district

## Measurement of aggressiveness components

The greenhouse temperature was maintained between 18 and 22 °C. Four aggressiveness components were measured: infection efficiency, latent period, lesion size, and sporulation capacity ( $\mu\text{g}$  of spores produced per  $\text{mm}^2$  of sporulating surface). Infection efficiency was estimated from the lesion density on the inoculated surface, with 23 replicates per isolate. The latent period was measured as the time between infection and production of secondary inoculum and was expressed in degree-days (Lovell et al. 2004). Lesions were counted once or twice a day until their number stabilized on a leaf section of  $1\text{ cm}^2$ . The time by which half of the lesions were sporulating (T50) was estimated with a linear interpolation around the 50 % count (Knott and Mundt 1991). Latent period was estimated with 18 replicates in experiment 1 and 15 in experiments 2a and 2b. For the sporulation and lesion size measurements (experiment 1) we selected the leaves with the most homogeneous lesion distribution and, taking into account space constraints, the number of replicates was reduced to 15 per isolate. These variables were measured as follows: just before the sporulation onset, the selected leaves were placed into bent transparent plastic sheets forming open tubes ( $\sim 10\text{-cm}$  diameter) and maintained horizontal with a wooden frame. At each collection date, leaves were brushed and the spores allowed to fall into the tubes. Then the spores were collected, transferred into hermetically-closed Eppendorf tubes and weighed. Digital pictures of the leaves were taken with a scanner (Hewlett Packard 4670; 400 ppi) before putting them back in the tubes. These pictures were used to determine the number of lesions and the sporulating surface area by image analysis (Optimas 5, Media Cybernetics). Spores were collected at 15 and 23 days after inoculation (dai). Lesion size (in  $\text{mm}^2$ ) was estimated by the average sporulating area between those dates, divided by the number of lesions. The sporulation capacity (in  $\mu\text{g}$  of spores per  $\text{mm}^2$  of sporulating surface) was estimated by dividing the amount of spores produced between 15 and 23 dai by the area of the sporulating surface.

## Statistical analyses

All analysis were done with Splus (Lucent Technologies, Inc.). Lesion density was first analyzed as a

response variable to estimate potential differences in infection efficiency and then was introduced as a quantitative co-variable in the analysis of latent period, spore production and lesion size, which are potentially density-dependent variables (Mehta and Zadoks 1970; Shaner 1983; Robert et al. 2004). In the lesion size analysis, log-transformation of lesion density was needed to linearize the data. For the latent period analysis, the density used as a co-variable was that on the restricted leaf section used for latent period measurements. The effect of the greenhouse compartment was tested and found to be not significant. As well, second degree interactions were never found to be significant and were withdrawn from the models. Since the isolates had to be compared within each pair, a pathotype effect was included in the Anova models and the isolate effect was tested within the pathotypes. Specific comparisons between two isolates were subsequently done with Student's tests.

For each aggressiveness component (Y), the Anova models thus included the pathotype (P), the isolate (I), nested within pathotype, and the lesion density (D):

$$Y_{ijk} = \mu + \alpha D + P_j + P_j/I_i + \varepsilon_{ijk}$$

An anova analysis was done on the latent period of the P3 isolates, combining data from experiments 1 and 2a. The isolate (I), density (D) and experiment (E) effects were included in the model:

$$Y_{ijk} = \mu + \alpha D + I_i + E_j + I^*E_{ij} + \varepsilon_{ijk},$$

The latency of the three P1 isolates used in experiment 2b was compared the same way but without an experiment effect. Since the comparison was done on specific isolates, both isolate and pathotype were fixed effects. In all tests, the significance threshold was set at 5 %. The range of lesion density was not always the same for all isolates because of differences in infection efficiency. The anova models took into account this density-dependence effect and allowed to estimate average values of the other aggressiveness components at a fixed lesion density (predicted means) (Lannou and Soubeyrand 2012). The figures presented in the paper show the predicted means for a fixed lesion density (the average lesion density of the experiment).

## Results

The three pathotypes presented distinct genetic profiles but, within each pathotype, both isolates were of identical multilocus profile for the 20 microsatellite loci. The analysis confirmed, with a greater number of markers, the genetic profile of these pathotypes as established in a population study of *P. tritici* (Goyeau et al. 2007).

The mean values for each aggressiveness component are presented in Table 2, along with the anova outputs. In experiment 1, the anova analysis revealed differences among isolates within pathotypes, for latent period and spore production capacity, but not for lesion size. The isolate comparison showed three significant differences: within pathotypes P1 for the latent period and for the spore production capacity, and within pathotype P3 for the latent period (Fig. 1). For all other aggressiveness components  $\times$  isolate pair combinations, nearly identical values were obtained for both isolates. No difference between isolates of the same pathotype was found to be significant for infection efficiency (not shown).

Experiment 2a confirmed the significant difference in latency between isolates 1–303 and 1–228 of

pathotype P3. The experiment and the lesion density had no significant effect on the latent period (Table 3). The measured latent periods were on average 159.7 degree-days for isolate 1–303 and 149.8 degree-days for isolate 1–228. Very consistent values of latent period were obtained in experiments 1 and 2a. The difference (10 degree-days) is equivalent to 16 h at 15 °C. In experiment 2b, the anova analysis detected an isolate effect within pathotype P1: the latent period of isolate 1–257 (152.2 degree-days) was found approximately 14 degree-days greater than that of both 0–048 (138.4 degree-days) and 2–127 (137.7 degree-days), which were found not different (Fig. 2).

## Discussion

In most papers dealing with quantitative traits of aggressiveness, the focus is on the comparison of different groups or sub-populations (different pathotypes, groups that are resistant or susceptible to a fungicide, etc.) and not on individual isolates. In such a context, it is logical to consider a number of individuals large enough to be representative of a group and a reasonably small number of replicates for each individual.

**Table 2** Aggressiveness components<sup>a</sup>: mean values and sources of variation

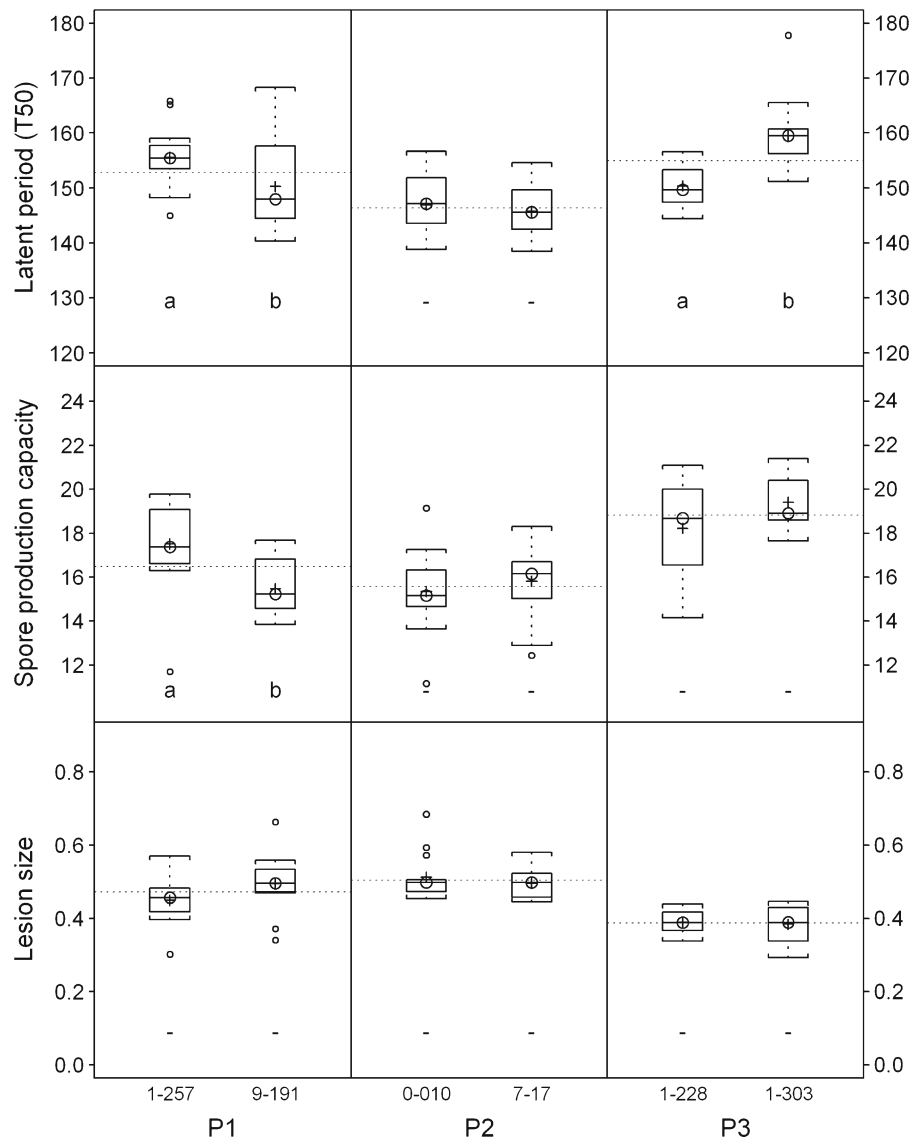
Pathotype	Isolate	Latent period (exp. 1)	Latent period (exp. 2a)	Spore production capacity	Lesion size
P1	1–257	<b>155.6</b>	–	<b>17.5</b>	0.45
	9–191	<b>150.3</b>	–	<b>15.4</b>	0.49
P2	0–010	146.9	–	15.3	0.51
	7–17	145.8	–	15.8	0.49
P3	1–228	<b>150.4</b>	<b>149.1</b>	18.2	0.39
	1–303	<b>159.5</b>	<b>159.7</b>	19.4	0.38
Source of variation <sup>b</sup>		Latent period (exp. 1)		Spore production capacity	Lesion size
P	F	21.57	– <sup>c</sup>	27.55	116.04
	<i>p</i>	0.0000	–	0.0000	0.0000
I/P	F	9.70	–	5.55	2.15
	<i>p</i>	0.0000	–	0.0016	0.1007
d	F	0.84	–	44.80	501.30
	<i>p</i>	0.3622	–	0.0000	0.0000

<sup>a</sup> Latent period is in degree-days, spore production capacity is in  $\mu\text{g}$  of spores/ $\text{mm}^2$  of sporulating tissue and lesion size is in  $\text{mm}^2$ . Latent period was measured twice for P3 isolates, in experiments 1 and 2a. Bold numbers indicate significant differences between two isolates of identical pathotype

<sup>b</sup> P: pathotype effect; I/P: isolate effect, nested in pathotype; d: lesion density effect; F: F-value; *p*: p-value ( $\alpha=0.05$ )

<sup>c</sup> See Table 3 for comparison of P3 isolates in experiments 1 and 2a

**Fig. 1** Latent period (T50, in degree-days), spore production capacity ( $\mu\text{g}$  spores/ $\text{mm}^2$  of sporulating tissue) and lesion size ( $\text{mm}^2$ ) of pairs of *P. triticina* isolates of pathotypes P1, P2 and P3, measured in experiment 1. The *boxplots* indicate the 25 % and 75 % quantils, the median (*circle*) and the mean (*cross*) for each isolate. Outliers, above or below 1.5 IQR (interquartile range) are indicated by *dots*. The *dotted horizontal line* represents the average value for the pathotype. The *letters* indicate significant differences between isolates ( $\alpha=5\%$ )



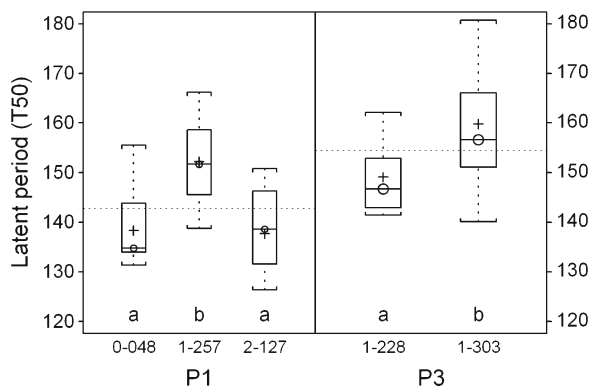
This is an optimal design to provide information at the group level, but does not allow to clearly establish

**Table 3** Comparison of P3 isolates for their latent period

Effects <sup>a</sup>	Df	Sum of Sq	F value	Pr(F)
Exp	1	4.705	0.094	0.7601
Iso	1	1588.721	31.751	<0.0001
d	1	21.371	0.427	0.5158
Iso*Exp	1	4.742	0.094	0.7592
Residuals	61	3052.203		

<sup>a</sup> Exp: experiment (1 or 2a); Iso: isolate (1–303 or 1–228); d: lesion density. There were 18 replicates per isolate in experiment 1 and 15 in experiment 2a

whether specific isolates are different. The level of variability in quantitative trait measurements is generally high and comparisons based on a small number of replicates may lead to type 1 errors. Another difficulty is that environmental effects interact with quantitative traits, which may cause inconsistencies between trials. In the case of pathogens such as *P. triticina* that present a highly clonal structure, it is nevertheless of importance to establish whether or not aggressiveness traits are able to evolve within a pathotype. For instance, in a modelling approach of the evolution of pathogen populations, one may have to decide whether pathotypes are a basic genetic unit or include a certain level of diversity for quantitative traits (Papaix



**Fig. 2** Latent period (T50, in degree-days) of two *P. triticina* isolates of pathotype P3 (experiment 2a) and three isolates of pathotypes P1 (experiment 2b). The boxplots indicate the 25 % and 75 % quantiles, the median (circle) and the mean (cross) for each isolate. Outliers, above or below 1.5 IQR (interquartile range) are indicated by dots. The dotted horizontal line represents the average value for the pathotype. The letters indicate significant differences between isolates ( $\alpha=5\%$ )

et al. 2011). The fact that these populations have a clonal structure is a priori in support of a genetic uniformity of the pathotypes but the data presented here, although based on a limited number of comparisons, strongly suggest that variability for aggressiveness within pathotypes should be considered.

Each pair of isolates that were compared in this study shared both the same virulence factors (since they belong to the same pathotype) and the same genetic profile based on 20 microsatellites. Nevertheless, significant differences for quantitative traits were detected among these isolates. A significant difference in the latent period was detected for isolates 1–228 and 1–303 of pathotype P3 in experiment 1 and was confirmed in experiment 2a. The difference in latency between isolates 1–303 and 1–228 was of approximately 16 h (at 15 °C). Since rust spores are mainly dispersed towards the middle of the day (Pady et al. 1965), this can result in differences of 1 day in the timing of parasite transmission in the field.

In experiment 1, isolates of pathotype P1 were found to be different for both the latent period and the spore production capacity. Note that the isolate with a longer latency was more efficient for spore production, which is consistent with the idea of a trade-off between latency and spore production capacity (Heraudet et al. 2008; Andrivon et al. 2009; Pariaud et al. 2012). The measured differences between the P1 isolates could not be tested again, mainly because these experiments are highly time

and resource consuming, but the additional data provided by experiment 2b also suggest differences in latency between isolate 1–257 and other isolates of pathotype P1.

By using an unusually large number (15 to 18) of replicates for each isolate, we obtained a good level of precision in the isolate comparisons: (i) the latency values for the P3 pathotypes were remarkably close in experiments 1 and 2a; (ii) except in the three cases where a clear difference was found, the measured values were practically identical for the isolates of same clonal lineage (Fig. 1); (iii) the level of variation was rather small in these experiments: for comparison, the coefficient of variation for the isolate (defined as the standard deviation divided by the mean) was between 0.03 and 0.05 for the latent period measurements presented here whereas it varied between 0.05 and 0.10 in another experiment with a large number of isolates but only six replicates per isolate (Pariaud et al. 2009a). Note that using thermal time for the latent period measurements probably made easier the comparison of experiments 1 and 2a by reducing the environmental variability (Lovell et al. 2004).

At the pathotype level, P3 produces smaller lesions and has a longer latency than P1 and P2, and P2 has a lower spore production capacity than P1 and P3 (Pariaud et al. 2009a). In the present study, the pathotype effect in the Anova analysis indicated that P3 had a greater sporulation capacity and produced smaller lesions than P1 and P2. This is consistent with the population study except that, at the population scale, P3 had the same spore production capacity than P1. With the same analysis, pathotype P2 had a shorter latent period than P1 and P3, whereas at the population scale, P1 and P2 had the same latent period, shorter than that of P3. There is no major contradiction here since in the present study the isolates were a priori not representative of the mean aggressiveness level of their respective pathotypes. This underlines the need to consider a large enough number of isolates to compare pathotypes or lineages in quantitative measurements, even for pathogens with a strong clonal structure.

This work shows that significant differences in quantitative traits may be found among *P. triticina* isolates belonging to the same pathotype and sharing the same microsatellite profile. The measured differences were not linked to virulence factors, as often suggested, since they occurred among isolates of identical pathotypes.



Differences in aggressiveness among genetically close individuals have been obtained in other species with a relatively good level of confidence. In a very detailed study, Carlisle et al. (2002) found differences in lesion expansion rate, latent period, spore production rate and infection efficiency among 17 *P. infestans* isolates, with nine replicates per isolate. These isolates shared an identical multilocus genotype (allozyme profiles), the same mating type, the same capacity to overcome the R1 specific resistance gene and the same sensitivity to a fungicide. Mundt et al. (2002), in a nested factor analysis, detected significant differences in aggressiveness between isolates of *Xanthomonas oryzae* pv. *oryzae* of the same clonal lineage, and even between isolates of the same RFLP haplotype within a clonal lineage, suggesting that mutations leading to increased aggressiveness had rapidly accumulated within the phylogenetic lineages.

In leaf rust, the existence of variability for quantitative traits of pathogenicity among individuals belonging to the same pathotype and clonal lineage indicates that a selection for higher levels of aggressiveness may occur independently of qualitative virulence factors and under strict clonal replication. The genetic support of quantitative traits of aggressiveness in plant pathogens is not known but QTL analyses of host resistance (e.g., Ballini et al. 2008) and pathogen aggressiveness (Cumagun et al. 2004; Lind et al. 2007) corroborate the idea that quantitative traits of pathogenicity are under the shared control of the pathogen, the host and their interaction (see Lannou 2012 for a review). The genetic determinism of the quantitative traits is complex and still barely understood from the plant side and even less information is available from the pathogen side. Isolate-specific QTLs are often found in quantitative resistance studies (e.g. Calenge et al. 2004; Marcel et al. 2008). Such QTLs in the host could result in a differential expression of quantitative traits among different isolates but our approach does not allow determining whether the differences in aggressiveness resulted from the pathogen itself or from a host-pathogen interaction.

Although the measured differences in aggressiveness were small, they may have significant evolutionary consequences. The difference in latency between isolates 1–303 and 1–228 was of approximately 16 h (at 15 °C) and, given the diurnal periodicity of rust spore release, this may even result in differences of 1 day in the parasite transmission. In a field epidemic,

this allows 1–228 to disperse earlier and occupy the available host surface first. Recently, van den Berg et al. (2012) have shown that a high level of autoinfection at the leaf level may facilitate the selection of mutants with a shorter latency. In leaf rust, autoinfection allows a fast saturation of infected leaves with new lesions (Lannou et al. 2008) and this may favour mutants with a shorter latent period, even if the difference with the resident population is small. These theoretical results, along with the results of the present study, suggest that quantitative traits such as latent period can be selected for within a leaf rust pathotype, even though these pathotypes present a strong genetic homogeneity when regarded through neutral markers and qualitative virulence.

**Acknowledgments** We are grateful to the GNIS (Groupement National Interprofessionnel des Semences et des plantes) for funding this work through the FSOV (Fonds de Soutien à l'Obtention Végétale) project, "Durabilité de la résistance partielle à la rouille brune du blé". We thank P. Belluomo for image analysis programming.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

## References

- Andrivon, D., Montarry, J., Corbiere, R., Glais, I., Magalon, H., Pasco, C. (2009). *Phytophthora infestans*: a life of evolutionary compromises. *10th International Epidemiology Workshop*. New York State Agricultural Experiment Station
- Ballini, E., Morel, J. B., Droc, G., Price, A., Courtois, B., Notteghem, J. L. & Tharreau, D. (2008). A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Molecular Plant-Microbe Interactions*, *21*, 859–868.
- Calenge, F., Faure, A., Goerre, M., Gebhardt, C., Van de Weg, W. E., Parisi, L. & Durel, C-E. (2004). Quantitative trait loci (QTL) analysis reveals both broad-spectrum and isolate-specific QTL for scab resistance in an apple progeny challenged with eight isolates of *Venturia inaequalis*. *Phytopathology*, *94*, 370–379.
- Carlisle, D. J., Cooke, L. R., Watson, S., & Brown, A. E. (2002). Foliar aggressiveness of Northern Ireland isolates of *Phytophthora infestans* on detached leaflets of three potato cultivars. *Plant Pathology*, *51*, 424–434.
- Cumagun, C. J. R., Bowden, R. L., Jurgenson, J. E., Leslie, J. F. & Miedaner, T. (2004). Genetic mapping of pathogenicity and aggressiveness of *Gibberella zeae* (*Fusarium graminearum*) toward wheat. *Phytopathology*, *94*, 520–526.

- Duan, X., Enjalbert, J., Vautrin, D., Solignac, M., & Giraud, T. (2003). Isolation of 12 microsatellite loci, using an enrichment protocol, in the phytopathogenic fungus *Puccinia triticina*. *Molecular Ecology Notes*, *3*, 65–67.
- Eyal, Z., & Peterson, J. L. (1967). Uredospore production of five races of *Puccinia recondita* as affected by light and temperature. *Canadian Journal of Botany*, *45*, 537–540.
- Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology*, *9*, 275–296.
- Goyeau, H., Park, R., Schaeffer, B., & Lannou, C. (2006). Distribution of pathotypes with regard to host cultivars in French wheat leaf rust populations. *Phytopathology*, *96*, 264–273.
- Goyeau, H., Halkett, F., Zapater, M.-F., Carlier, J., & Lannou, C. (2007). Clonality and host selection in the wheat pathogenic fungus *Puccinia triticina*. *Fungal Genetics and Biology*, *44*, 474–483.
- Heraudet, V., Salvaudon, L., & Shykoff, J. A. (2008). Trade-off between latent period and transmission success of a plant pathogen revealed by phenotypic correlations. *Evolutionary Ecology Research*, *10*, 913–924.
- Jeffrey, S. I. B., Jinks, J. L., & Grindley, M. (1962). Intra-racial variation in *Phytophthora infestans* and field resistance to potato blight. *Genetica*, *32*, 323–338.
- Katsuya, K., & Green, G. J. (1967). Reproductive potentials of races 15B and 56 of wheat stem rust. *Canadian Journal of Botany*, *45*, 1077–1091.
- Knott, E. A., & Mundt, C. C. (1991). Latent period and infection efficiency of *Puccinia recondita* f. sp. *tritici* populations isolated from different wheat cultivars. *Phytopathology*, *81*, 435–439.
- Kolmer, J. A., & Leonard, K. J. (1986). Genetic selection and adaptation of *Cochliobolus heterostrophus* to corn hosts with partial resistance. *Phytopathology*, *76*, 774–777.
- Laine, A. L. (2008). Temperature-mediated patterns of local adaptation in a natural plant–pathogen metapopulation. *Ecology Letters*, *11*, 327–337.
- Lehman, J. S. & Shaner, G. (1996). Genetic variation in latent period among isolates of *Puccinia recondita* f. sp. *tritici* on partially resistant wheat cultivars. *Phytopathology*, *86*, 633–641.
- Lannou, C. (2012). Variation and selection of quantitative traits in plant pathogens. *Annual Review of Phytopathology*, *50*, 319–338.
- Lannou, C., & Soubeyrand, S. (2012). Measure of life-cycle traits of a biotrophic pathogen. In K. L. Stevenson & M. J. Jeger (Eds.), *Exercises in plant disease epidemiology*. St. Paul: APS Press. in press.
- Lannou, C., Soubeyrand, S., Frezal, L., & Chadoeuf, J. (2008). Autoinfection in leaf rust dispersal. *New Phytologist*, *177*, 1001–1011.
- Lind, M., Dalman, K., Stenlid, J., Karlsson, B. & Olson, A., (2007). Identification of quantitative trait loci affecting virulence in the Basidiomycete *Heterobasidion annosum* s.l. *Current Genetics*, *52*, 35–44.
- Lovell, D. J., Hunter, T., Powers, S. J., Parker, S. R., & Van den Bosch, F. (2004). Effect of temperature on latent period of *Septoria* leaf blotch on winter wheat under outdoor conditions. *Plant Pathology*, *53*, 170–181.
- Marcel, T. C., Gorguet, B., Ta, M. T., Kohutova, Z., Vels, A. & Niks R. E. (2008). Isolate specificity of quantitative trait loci for partial resistance of barley to *Puccinia hordei* confirmed in mapping populations and near-isogenic lines. *New Phytologist*, *177*, 743–55.
- Mehta, Y. R., & Zadoks, J. C. (1970). Uredospore production and sporulation period of *Puccinia recondita* f. sp. *tritricina* on primary leaves of wheat. *Netherlands Journal of Plant Pathology*, *76*, 267–276.
- Miller, J. S., Johnson, D. A., & Hamm, P. B. (1998). Aggressiveness of isolates of *Phytophthora infestans* from the Columbia basin of Washington and Oregon. *Phytopathology*, *88*, 190–197.
- Milus, E. A., & Line, R. F. (1980). Characterization of resistance to leaf rust in Pacific Northwest wheat lines. *Phytopathology*, *70*, 167–172.
- Mundt, C. C., Nieva, L. P., & Vera Cruz, C. M. (2002). Variation for aggressiveness within and between lineages of *Xanthomonas oryzae* pv. *oryzae*. *Plant Pathology*, *51*, 163–168.
- Pady, S. M., Kramer, C. L., Pathak, V. K., Morgan, F. L., & Bhatti, M. A. (1965). Periodicity in airborne cereal rust urediospores. *Phytopathology*, *55*, 132–134.
- Papaix, J., Goyeau, H., du Cheyron, P., Monod, H., & Lannou, C. (2011). Influence of cultivated landscape composition on variety resistance: an assessment based on the wheat leaf rust epidemics. *New Phytologist*. doi:10.1111/j.1469-8137.2011.03764.x.
- Pariaud, B., Robert, C., Goyeau, H., & Lannou, C. (2009). Aggressiveness components and adaptation to a host cultivar in wheat leaf rust. *Phytopathology*, *99*, 869–878.
- Pariaud, B., Ravigne, V., Halkett, F., Goyeau, H., Carlier, J., & Lannou, C. (2009). Aggressiveness and its role in the adaptation of plant pathogens. *Plant Pathology*, *58*, 409–424.
- Pariaud, B., van den Berg, F., van den Bosch, F., Powers, S. J., Kaltz, O., & Lannou, C. (2012). Shared influence of pathogen and host genetics on a trade-off between latent period and spore production capacity in the wheat pathogen, *Puccinia triticina*. *Evolutionary Applications* (accepted)
- Pilet, F., Pellé, R., Ellissèche, D., & Andrivon, D. (2005). Efficacy of the R2 resistance gene as a component for the durable management of potato late blight in France. *Plant Pathology*, *54*, 723–732.
- Robert, C., Bancal, M.-O., & Lannou, C. (2004). Wheat leaf rust uredospore production on adult plants: influence of leaf nitrogen content and *Septoria tritici* blotch. *Phytopathology*, *94*, 712–721.
- Roumen, E., Levy, M., & Notteghem, J. L. (1997). Characterisation of the European pathogen population of *Magnaporthe grisea* by DNA fingerprinting and pathotype analysis. *European Journal of Plant Pathology*, *103*, 363–371.
- Shaner, G. (1983). Growth of uredinia of *Puccinia recondita* in leaves of slow- and fast-rusting wheat cultivars. *Phytopathology*, *73*, 931–935.
- Szabo, L. J., & Kolmer, J. A. (2007). Development of simple sequence repeat markers for the plant pathogenic rust fungus *Puccinia triticina*. *Molecular Ecology Notes*, *7*, 708–710.
- Thrall, P. H., & Burdon, J. J. (2003). Evolution of virulence in a plant host-pathogen metapopulation. *Science*, *299*, 1735–1737.

- Tomerlin, J. R., Eversmeyer, M. G., Kramer, C. L., & Browder, L. E. (1983). Temperature and host effects on latent and infectious periods and on urediniospore production of *Puccinia recondita* f. sp. tritici. *Phytopathology*, *73*, 414–419.
- van den Berg, F., Lannou, C., Gaucel, S., Gilligan, C. A., & van den Bosch, F. (2012). High levels of auto-infection in plant pathogens favour short latent periods: a theoretical approach. *Evolutionary Ecology* (accepted).
- Vera Cruz, C. M., Bai, J., Oña, I., Leung, H., Nelson, R. J., Mew, T. W., & Leach, J. E. (2000). Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. *Proceedings of the National Academy of Science, USA*, *97*, 13500–13505.
- Villaréal, L. M. M. A., & Lannou, C. (2000). Selection for increased spore efficacy by host genetic background in a wheat powdery mildew population. *Phytopathology*, *90*, 1300–1306.