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Inferring the origin of primary inoculum of *Zymoseptoria tritici* from differential adaptation of resident and immigrant populations to wheat cultivars

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Abstract We investigated whether the origin of primary inoculum of the fungal wheat pathogen *Zymoseptoria tritici* can be inferred from evidence of adaptation to host cultivars. We compared the aggressiveness of two pathogen populations collected locally, the first considered as resident (collected from debris in a wheat cv. Soissons monoculture plot) and the second considered as immigrant (collected from leaf lesions in a 300 m apart wheat cv. Soissons plot containing no debris, exposed to an inoculum pool of distant origin), with the aggressiveness of a third population (collected from early leaf lesions in the same monoculture plot) the origin of which we wanted to determine (local vs. distant). The three populations were sampled twice, in 2009 and 2012, from a 6-year field trial. Latent period and sporulating area of 6 × 12 isolates were assessed in greenhouse on adult plants of cv. Soissons and of Apache, another cultivar commonly grown around the field for several years. Firstly, we detected a differential host adaptation: after several years of monoculture, the resident pathogen populations became less adapted to the other cultivar. Secondly, we showed that when the inoculum pressure was high (2009), the aggressiveness profile of the third pathogen population was more similar to that of the resident populations than of the immigrant. This indicates that early lesions in the monoculture plot were mostly caused by within-field (local) primary inoculum. The comparison of aggressiveness profiles when the inoculum pressure was lower (2012) was less conclusive, suggesting that the tested population could have a mixed origin.

Keywords Aggressiveness trait · Host adaptation · Inoculum source · Mycosphaerella graminicola · Wheat

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Introduction

Primary inoculum can be defined as the propagule (most often, a spore) at the origin of a disease outbreak observed in a given host area (for instance, a field plot). In annual crops the pathogen is faced at the end of a cropping season with unfavorable environmental conditions and the lack of a host. The pathogen can survive through different mechanisms, such as infection of an alternate (Roelfs 1985; Anikster et al. 1997; Jin et al. 2010) or accessory host (Holmes and Dennis 1985; Suffert et al. 2011), or on different substrates, such as crop debris (Landschoot et al. 2011; Suffert and Sache 2011). When local conditions do not permit survival, recolonization of the crop must be achieved every year by pathogen propagules spread over long distance from remote sources of inoculum, located in areas conducive to survival (Strange 2003; Wang et al. 2010). The origin of primary inoculum remains a matter of speculation in many plant diseases, because of the lack of biological

knowledge on non-epidemic (survival) stages of epidemics, of the potentially very high number of sources scattered over huge areas, and of the difficulty to detect the small amounts of inoculum theoretically able to initiate disease. Accordingly, accurately tracking back the origin of a disease outbreak has been for decades a challenge to epidemiologists, who have to find a needle in a haystack.

Direct evidence of the presence of inoculum can be obtained by trapping and quantifying airborne spores (Calderon et al. 2002; Fraaije et al. 2005; Kaczmarek et al. 2012; Karolewski et al. 2012). Such methods still have technical limitations, which often prevents the detection of the very low amounts of spores thought to initiate the first infections, and are, therefore, more suitable to identify inoculum peaks over the epidemic season (Carisse et al. 2009; Duvivier et al. 2013; Morais et al. 2015a). Indirect evidence of the origin of inoculum can be gained by comparing the genetic structure or the fitness traits of pathogen populations confronted to a local host.

Pathogen populations are usually considered to be locally adapted if they perform better on local (sympatric) hosts than on foreign (allopatric) hosts (Gandon and Van Zandt 1998; Kaltz and Shykoff 1998; Kawecki and Ebert 2004; Laine 2007). In contrast, pathogen populations are considered to be locally maladapted if they perform worse on sympatric than on allopatric hosts (Kaltz et al. 1999). On average, immigrants are expected to be at a competitive disadvantage compared with residents because they have not been selected for adaptation to the local environment (Giraud 2006; Gladieux et al. 2011). Accordingly, the local adaptation of a plant pathogen population can be thought of as the consequence of the specialization of the population on the most common local host genotypes (e.g., Ahmed et al. 1996; Montarry et al. 2006; 2008; Pariaud et al. 2009b). The demonstration of population adaptation to a host cultivar grown locally does not necessarily require testing "local vs. foreign" or "home vs. away" criteria; a reciprocal cross-inoculation experiment is sometimes impossible for practical reasons. For instance, samples corresponding to sympatric vs. allopatric deme × habitat combination (Kawecki and Ebert 2004) are not accessible at smaller spatial scales.

For pathogens with long distance dispersal, strains co-infecting host plants in a field are likely of different origin and possess different degrees of genetic relatedness. Some proportion of strains sampled from leaf lesions in a perennial or monoculture crop field at the beginning of a polycyclic epidemic should be considered as resident; they have been locally selected prolongedly through several reproduction cycles and are potentially adapted to the local host cultivar present in the field. Any remaining of the strains, landing in the field at the beginning of a epidemic, should be considered as immigrant, although collected locally; it is likely that a single infection cycle is not sufficient for the strains to be adapted to the host cultivar despite compatibility filter effect (Combes 2001). These strains, however, cannot be considered as representative of the total pool of immigrant because selection by the host has already removed several maladapted immigrants, which could not be sampled. This can explain the lack of signs of local adaptation at fine spatiotemporal scales (e.g., Montarry et al. 2006; 2008) despite theoretical expectations (Kaltz and Shykoff 1998; Kawecki and Ebert 2004). Adaptation of plant pathogens to different host cultivars can be assessed using fitness estimates (Laine 2008) and aggressiveness components (Lannou 2012) Aggressiveness is defined as a quantitative variation in the ability of pathogens to infect and grow on susceptible hosts (pathogenicity - Lannou 2012; Zhan and McDonald 2013b); its components (latent period, spore production rate, lesion size, infection efficiency) were experimentally assessed in several wheat pathogens (Pariaud et al. 2009a; Suffert et al. 2013; Azzimonti et al. 2013).

Epidemics of Septoria tritici blotch of wheat, caused by the fungus *Zymoseptoria tritici*, are mainly initiated by ascospores wind-dispersed from infected wheat debris (Shaw and Royle 1989), although other sources of inoculum such as pycnidiospores cannot be excluded in a wheat monoculture (Suffert et al. 2011). Whether primary inoculum originates mostly from local or remote sources is unknown yet. In this study, we investigated whether the main origin of the inoculum responsible of early infection in a monoculture wheat field (cultivated for several years with the same cultivar) can be inferred from evidence of adaptation to host cultivar of two pathogen populations collected locally, the one having a local origin (the "resident population") and the other having a distant origin (the "immigrant population"). A cross-inoculation experiment was performed first to investigate differences in aggressiveness between these two populations. Aggressiveness traits were assessed on the cultivar cultivated in the field where a part of the resident population has likely multiplied over several years and on another cultivar

commonly grown around the field for several years, which likely hosted the immigrant population. The sporulating area and the latent period, two main components of aggressiveness in Z. *tritici* (Suffert et al. 2013; Morais et al. 2015b), were measured in greenhouse conditions. In a first step, we addressed two questions: i) Can we detect differences in aggressiveness between the resident and immigrant populations; ii) Is there a differential competitive advantage between them on the two cultivars, which can be interpreted as sign of host adaptation? In a second step, we compared the aggressiveness of the resident and immigrant populations with the aggressiveness of a third local population (responsible of early infections in the same monoculture wheat field) whose origin has to be elucidated. In the third step, the final question was: iii) Can the origin of the third population be inferred from the comparison of its aggressiveness profile with those of the resident and immigrant populations?

Materials and methods

Sampling of pathogen populations

A field experiment (Suffert and Sache 2011; Morais et al. 2015a) was carried out from 2007–2008 to 2012–2013 at the Grignon experimental station (France; 48°51'N, 1°58'E). Two plots were sown every year in early autumn (e.g., on 19 October 2009 and on 16 October 2012) with wheat cv. Soissons (moderately susceptible to Septoria tritici blotch; rated 5 on a 1–10 scale of decreasing susceptibility, Arvalis Institut du Végétal-CTPS). The first plot (D+, 20 m × 100 m) had been grown with wheat cv. Soissons by direct drilling, without fungicide application, since 2007 (Figure S1a, b). Wheat debris were not removed after harvest; naturally infected with *Z. tritici*, the debris were expected to act in autumn as a local source of primary inoculum. The second plot (D_0, 40 m × 100 m), located 300 to 400 m away from plot D+, did not contain any wheat debris since it had been cropped during the previous season with another crop (e.g., oilseed rape in 2008–2009 and faba bean in 2011–2012); its location changed every year. The severity of Septoria tritici blotch epidemics, as quantified in the two wheat plots, was high during springs 2009, 2010 and 2013, moderate during springs 2008 and 2012, and low during spring 2011 (Suffert and Sache 2011; Morais et al. 2015a).

Two sets of three populations, each made of 12 isolates, were sampled from the wheat plots (Fig. 1; Table S1).

Two populations hereafter named "resident populations" were obtained from debris collected in plot D+ on 23 October 2009 and on 10 November 2012, respectively. They were considered as samples of the local inoculum pool, as their selective trajectory was likely impacted by several reproductive cycles on cv. Soissons cultivated for several years in the same field. Debris was cut into 1-cm long pieces kept at 18 °C for 24 h. A subset of these pieces (20 g) was evenly spread on wet filter paper enclosed in a moist box. Petri dishes (90 mm in diameter) filled with PDA medium (potato dextrose agar, 39 g L^{-1}) were placed upside down 1 cm above the debris pieces (Figure S1c). The boxes were placed for 6 h at 18 °C in the dark to trigger ascospore discharge. Thereafter the Petri dishes were closed and kept in the same conditions to promote yeast-like growth of the fungus (Suffert and Sache 2011; Morais et al. 2015a). Five days after ascospore discharge, *Z. tritici* colonies were isolated from the growing mycelium and purified by monospore culture to obtain 12 isolates.

Two populations hereafter named "immigrant populations" were obtained from infected leaves sampled in plot D_0 from 24 November to 8 December 2009 and from 11 December 2012 to 17 December 2012. They were considered as samples of an inoculum pool mainly of distant origin, that is, built-up by ascospores discharged from distant debris. Since seed transmission of *Z. tritici* is considered unlikely (Suffert and Sache 2011) and no infected wheat debris was present in the neighbourhood of plot D_0, infections were caused by wind-dispersed, distant ascospores. They were probably mainly discharged from wheat debris present in fields located a few hundred meters or a few kilometres away. As the dispersal gradient of ascospores and the spatial arrangement of the different commercial cultivars cannot be assessed precisely, we considered that the most probable origin of these ascospores was cv. Apache (the most commonly grown cultivar in the Paris Basin) and Soissons (cultivated in the nearest wheat field D+). Some ascospores released from debris of plot D+ could have infected plot D_0, but their contribution is negligible, taking into account the proximity between D_0 and other commercial wheat plots and the wind direction (see Fig. 1 in Morais et al. 2015a).

Two populations hereafter named "mixed-origin populations" were obtained from infected leaves

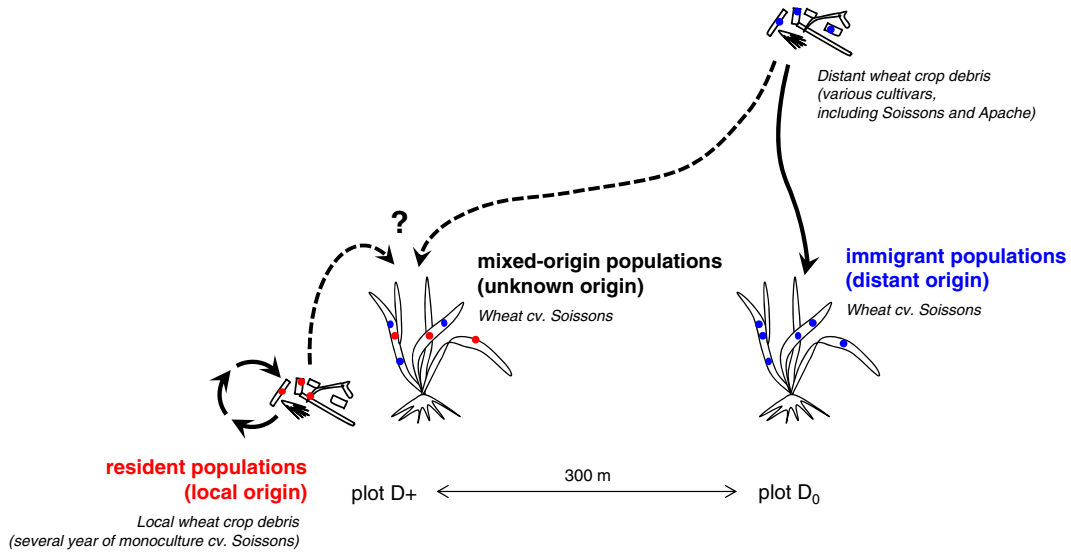

Fig. 1 Sketch of the origin of local *Zymoseptoria tritici* populations collected from wheat debris (resident population) and from leaf lesions (immigrant and mixed-origin populations) during the early stages of a Septoria tritici blotch epidemic surveyed in autumn 2009 and 2012. The resident populations, considered as samples of the local inoculum pool, were collected from wheat crop debris in 2009 and 2012 (D+; 3-year and 6-year cv. Soissons monoculture plot, respectively). The immigrant populations, considered as samples of an inoculum pool of distant origin, were collected from a cv. Soissons wheat plot without contaminated debris (D_0; previous crop, oilseed rape in 2008–2009 and faba bean in 2011–2012) from lesions caused by inoculum coming from distant debris. The mixed-origin populations were assumed to have been built-up by ascospores discharged from debris of either local (resident population) or/and distant origin (immigrant population), in an unknown proportion

sampled in plot D+ on 24 November 2009 and on 11 December 2012, during the early epidemic stages. Of unknown origin, the two populations were assumed to have a mixed origin, that is, built-up by ascospores discharged from debris of either local (resident population, present on the soil of plot D+) or/and distant origin (immigrant population) in an unknown proportion.

To obtain the isolates composing the immigrant and mixed-origin populations, leaves collected from plots D+ and D_0, respectively, were placed on wet filter paper enclosed in a moist box kept for 24 h at 18 °C in the dark to promote cirrus exudation (Suffert and Sache 2011). A single cirrus from a single pycnidium per lesion (Figure S1d) was transferred to a Petri dish filled with PDA and then streaked across the agar surface with a sterile glass rod to separate individual spores. Petri dishes were incubated at 18 °C in the dark to promote yeast-like growth of the fungus. *Z. tritici* colonies were isolated from the growing mycelium and purified by monospore culture to obtain 4 × 12 isolates.

The 72 isolates used in this study have distinct genotypes, as evidenced by distinct SSR neutral markers (unpublished data; Gautier et al. 2014). They were stored in a sterile glycerol-water (1:1) solution kept at −80 °C.

Plant material

Wheat adult plants of cv. Apache and Soissons were grown in a greenhouse for subsequent inoculation with the aforementioned fungal isolates. Like Soissons, Apache is moderately susceptible to Septoria tritici blotch (rated 5 on a 1–10 scale of decreasing susceptibility, Arvalis Institut du Végétal-CTPS). Wheat seeds were sown on 7 December 2011 and 18 December 2014 in Jiffy peat pots (Jiffypot®, Jiffy France SARL). Seedlings were vernalized for seven weeks at 8 °C with a 10 h light period and a 14 h dark period. Afterwards, seedlings were brought back to the greenhouse and left to acclimatize for one week. Each plant was then transplanted into 1.1 L pots filled with 1 L of commercial compost (Klasmann® Substrate 4; Klasmann-Deilmann France SARL) and fertilized with 5 g Osmocote® Exact (16 N-11P-11 K + 3MgO + TE). Plants were sprayed with Spiroxamine (Aquarelle SF at 2 mL L^{-1}; Bayer CropScience, France) against powdery mildew (*Blumeria graminis* f.sp. *tritici*). In addition, plants were fertilized with a commercial nutrient solution (Hydrokani C2®, Hydro Agri Spécialités) at a 1:100 dilution rate poured in the pot saucers five and seven weeks after seedling transplantation. During plant

growth, natural daylight was supplemented with 400 W sodium lamps to obtain 9 h of dark and 15 h of light, the greenhouse temperature being kept under 20 °C during the light period and above 12 °C during the dark period. Three stems per plant were kept. The air temperature in the greenhouse compartment was recorded every 15 min using a ventilated sensor (Greenhouse Humidity and Temperature Sensor type 224.401; RAM, Germany). The thermal time t, expressed in degree-days, was calculated, starting from the inoculation date (12 April 2012 and 24 April 2015, respectively), by summing the daily mean air temperature using a 0 °C base temperature.

Inoculation procedure

Populations collected in 2009 and in 2012 were tested in greenhouse experiments performed in 2012 and 2015, respectively. For each fungal isolate, subcultures were prepared on Petri dishes filled with PDA from the aforementioned stock cultures kept at −80 °C. Conidial suspensions were obtained on the day before inoculation by flooding the surface of 5 day-old subcultures with distilled water and scraping the agar surface with a sterilized glass rod to release conidia (Suffert et al. 2013). The suspensions were adjusted to a final concentration of 2.10^5 conidia mL^{-1} using a Malassez counting chamber (Figure S1e). Two drops of surfactant (Tween20®, Sigma, France) were added to the suspensions to ensure adequate coverage of the inoculated leaf surface.

Conidial suspensions were applied along a 25 mm long section of the adaxial face of the two upper leaves (F1 and F2) of the main tiller of each plant between growth stages Z50 (first head spikelet visible) and Z59 (head fully emerged; Zadoks et al. 1974). A paintbrush was used to apply each suspension, using a square plastic frame (25 × 25 mm) to delimit the surface to be inoculated. Then, the inoculated side of the leaf was turned over to avoid inoculum suspension trickling, and enclosed for 72 h in a transparent bag slightly wetted to maintain a high humidity and promote infection (Suffert et al. 2013). Each trial was set up as a randomized block design (216 plants) in a single greenhouse compartment. Each isolate × cultivar × leaf layer combination was replicated three times in 2012 and 2015.

Assessment of maximum sporulating area and latent period

Disease progress, as well as maximum sporulating area and latent period were assessed visually as described elsewhere (Suffert et al. 2013). Disease assessments were performed by two different assessors during the two experiments, preventing the direct comparison of the mean values of the aggressiveness components between 2009 and 2012. Lesion size, estimated by the sporulating area (SPO), was visually assessed on each leaf (Figure S1f) with a hand lens (magnification ×10) as the percentage of the inoculated leaf surface (1, 2, 3 and 5 %, then 5 % increments up to 100 %) bearing pycnidia (Suffert et al. 2013). The assessments were performed twice a week from the time of inoculation until leaf senescence (14 assessments). A Gompertz growth curve (Berger 1981) was fitted to the values of SPO(t) recorded on each leaf (Suffert et al. 2013; Fig. 2):

$$SPO(\mathrm{t}) = SPO_{\max}\exp\left(-\mathrm{B}_{spo}.\exp\left(-\mathrm{r}_{spo}\mathrm{t}\right)\right) \quad (1)$$

where SPO_{max} is the maximum sporulating area, r_{spo} is a rate parameter, and B_{spo} is a position parameter. $LatSPO_5$, the time elapsed from inoculation to 5 % of the maximum percentage of area bearing pycnidia (SPO_{max}) was calculated using eq. 1 as described in Suffert et al. (2013) and expressed in degree-days (dd). Hereafter, for the sake of simplicity, SPO_{max} and $LatSPO_5$, will be termed "sporulating area" and "latent period", respectively. Out of the 864 tested leaves, 29 in 2009 and 9 in 2012 were excluded from the statistical analysis due to either inoculation failure or lack of fit of the Gompertz model.

Data analysis

Firstly, the effects of year (2009, 2012), cultivar (Soissons, Apache), leaf layer (F1, F2), pathogen population (resident, immigrant and mixed-origin) and isolate (Table S1) were investigated overall in a factorial analysis of variance. Isolate identity was nested as a random factor within pathogen population. The variance of the sporulating area and of the latent period, assessed on three replicates per isolate and 12 isolates per population, was partitioned into sources attributable to year Y, isolate I(P), population P and cultivar C, taking into

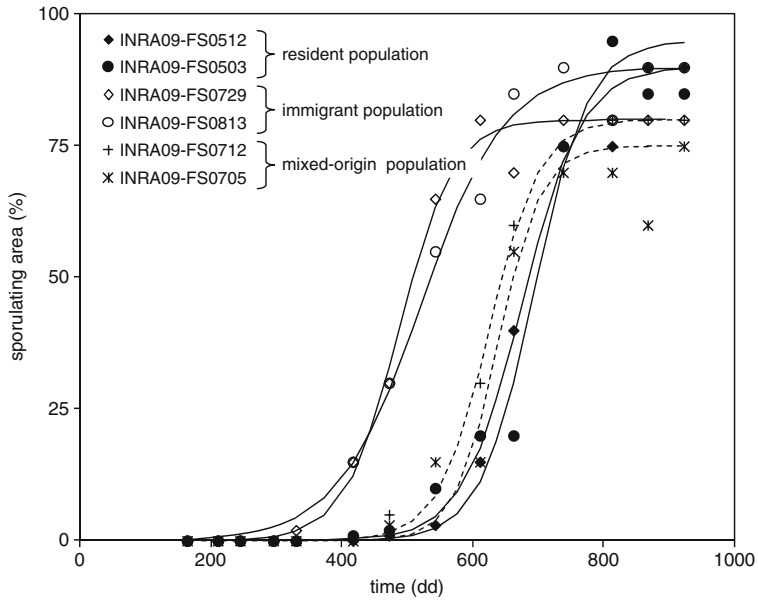

Fig. 2 Growth of the sporulating area of disease lesions caused by six *Zymoseptoria tritici* isolates belonging to the resident population (isolates INRA09-FS050 and INRA09-FS0503), the immigrant population (INRA09-FS0729 and INRA09-FS0806) and the mixed-origin population (INRA09-FS0705 and INRA09-FS0712). Data from the 2012 experiment, cv. Apache, leaf F1, block a. Curves were fitted using eq. 1 (see main text). Sporulating area is expressed in % of the inoculated area; time is expressed in degree-days (dd), starting from the sowing date (base temperature 0 °C)

account the effect of the leaf layer L, according to the model:

$$T_{yipcl} = M + Y + I(P) + P + C + L + Y \times C + Y \times L + I(P) \times C + I(P) \times L + P \times C + P \times L + C \times L + \varepsilon_{yipcl} \quad (2)$$

where T_{yipcl} is the value of fitness trait T (sporulating area or latent period) of isolate i, in population p, for cultivar c, on leaf layer l, for the year y. M indicates the overall mean and ε_{yipcl} the variance between replicates.

Disease symptoms were not assessed by the same person during the 2012 and 2015 experiments; accordingly, effect of year and assessor could not be differentiated. Traits derived directly from visual assessment such as the sporulating area may be assessor dependant. For these reasons, the effects of cultivar, leaf layer, pathogen population and isolate were investigated separately for each year by two additionnal factorial analyses of variance derived from eq. 2.

Secondly, the mean of the sporulating area and of the latent period were compared between populations, and then between cultivars, with isolate I(P) and leaf layer L as factors, separately for each year (Student-Newman-Keuls test).

Thirdly, the mean of the sporulating area and of the latent period were compared between populations for each wheat cultivar separately (Student-Newman-Keuls test); similarly, the mean of the sporulating area and of the latent period were compared between cultivars for each population, separately for each year.

Curve fitting and data analysis were performed with the S-PLUS 6.3 software (Lucent Technologies, Inc., USA).

Results

The two ANOVAs performed separately for each year show that the largest sources of variation for both sporulating area and latent period were leaf layer and isolate ($P < 0.01$; Table 1); this illustrates that the phenotypic diversity is high in the *Z. tritici* populations. The aggressiveness of the isolates also depended on the cultivar on which they were inoculated and on the population from which they originated (Table 1). The population effect was only significant for the sporulating area in 2009 ($P = 0.03$). The cultivar effect was significant for the latent period ($P < 0.01$ in 2009 and $P = 0.02$ in 2012) but not for the sporulating area ($P = 0.33$ in 2009 and $P = 0.91$ in 2012). Interactions between factors were not significant together for the sporulating area and latent period except for cultivar × isolate ($P < 0.01$). The cultivar × population interaction was not significant. These results are consistent with the overall ANOVA including a year effect. The year effect confounded with the assessor effect was significant ($P < 0.01$; Table S2). Mean comparison tests (data not

Table 1 Effect of population origin (resident, immigrant, mixed-origin), cultivar (Apache, Soissons), leaf layer (F1, F2), and isolate (2 × 36 *Zymoseptoria tritici* isolates collected in autumn 2009 and 2012) on sporulating area and latent period

2009						
Sporulating area						
Factor [a]		df	Sum of squares	Mean square	F	P
Population [c]	P	2	3744	1872	4.04	0.03
Cultivar	C	1	180	180	0.93	0.33
Leaf layer	L	1	11,229	11,229	58.44	< 0.01
Isolate(Population)	I(P)	33	15,283	463	2.41	< 0.01
Residual	R	294[d]	56,493	192		
Latent period						
Factor [a]		df	Sum of squares	Mean square	F	P
Population [c]	P	2	47,868	23,934	1.77	0.19
Cultivar	C	1	33,728	33,728	16.62	< 0.01
Leaf layer	L	1	49,680	49,680	24.47	< 0.01
Isolate(Population)	I(P)	33	447,598	13,564	6.68	< 0.01
Residual	R	294 [d]	592,722	2030		
2012						
Sporulating area						
Factor [b]		df	Sum of squares	Mean square	F	P
Population [c]	P	2	1203	602	1.35	0.27
Cultivar	C	1	2	2	0.01	0.91
Leaf layer	L	1	15,311	15,311	93.24	< 0.01
Isolate(Population)	I(P)	33	14,625	443	2.70	< 0.01
Residual	R	314 [d]	51,564	164		
Latent period						
Factor [b]		df	Sum of squares	Mean square	F	P
Population [c]	P	2	35,450	17,725	0.68	0.51
Cultivar	C	1	25,782	25,782	10.00	0.02
Leaf layer	L	1	142,820	142,820	55.42	< 0.01
Isolate(Population)	I(P)	33	861,100	26,094	10.13	< 0.01
Residual	R	314 [d]	801,513	2577		

[a] two-factor interactions were non-significant, expect C × L and P × L for SPOmax and C × I(P) for $LatSPO_5$

[b] two-factor interactions were non-significant, expect C × I(P) and L × I(P) for SPOmax and C × I(P) for $LatSPO_5$

[c] the "Population" effect was tested against "Isolate(Population)", with the F-ratio $_P$ = Mean Square $_P$/Mean Square $_{I(P)}$, such as the interactions P × C and P × L

[d] out of the 2 × 432 tested leaves, 29 in 2009 (294 df instead of 323 df) and 9 in 2012 (314 df instead of 323 df) were excluded from the statistical analysis due to either inoculation failure or lack of fit of the Gompertz model

shown) show that the difference between years was higher for the sporulating area (9.7 %; 82.9 % for isolates collected in 2009 vs. 91.8 % for those collected in 2012), a trait whose value is more assessor-dependent, than for the latent period (2.7 %, 465.8 dd for isolates collected in 2009 vs. 478.7 for those collected in 2012), a trait which is less assessor-dependent. The assessor effect was thus not negligible, ranging from 3 to 10 % so the effects of cultivar, leaf layer, pathogen population and isolate were assessed separately for each of the two years of study.

The latent period was significantly shorter on Soissons than on Apache (Table 2); the difference was 18.5 dd for isolates collected in 2009 (456.3 dd in Soissons vs. 474.8 dd in Apache) and 15.7 dd for isolates collected in 2012 (470.9 dd in Soissons vs. 486.6 dd in

Table 2 Mean sporulating area and latent period of two sets of three *Zymoseptoria tritici* populations (resident, immigrant, mixed-origin), collected in autumn 2009 and 2012 and assessed on two wheat cultivars (Apache and Soissons)

Variable	Population				Cultivar		
	Resident	Immigrant	Mixed-origin		Apache	Soissons	
2009							
Mean sporulating area	82.8 (ab)	86.6 (a)	79.1 (b)	**	83.6	82.3	ns
Latent period	465.3	453.1	480.0	ns	474.8 (a)	456.3 (b)	***
2012							
Mean sporulating area	93.7	89.6	92.1	ns	91.9	91.7	ns
Latent period	467.4	490.0	478.8	ns	486.6 (a)	470.9 (b)	***

[a] expressed in % of the inoculated leaf area

[b] expressed in degree-days post inoculation (base temperature 0 °C)

*Student-Newman-Keuls test: **: $P < 0.05$; ***: $P < 0.01$; ns: not significant

Apache). In 2009 the sporulating area was lower in the mixed-origin population (79.1 %) than in the immigrant population (86.6 %); the difference between the mixed-origin and resident populations was not significant whatever the aggressiveness trait (Table 2). When considering the two cultivars separately (Fig. 3a), the differences between the resident and the immigrant populations were significant for latent period both on Soissons and Apache, but not for sporulating area. The sporulating area of isolates collected in 2009 was lower in the mixed-origin population (79.1 %) than in the immigrant population (86.6 %); the latent period was longer in the mixed-origin population (480.0 dd) than in the immigrant population (453.1 dd). In 2012 there was no significant difference in either the sporulating area of the latent period between the three pathogen populations (Table 2). However, when considering the two cultivars separately, the latent period measured on Apache was significantly different between the mixed-origin and the immigrant populations, while it was similar on the mixed-origin and resident populations (Fig. 3a). No such differences were established on Soissons.

The mean latent period of isolates from the 2009 resident and mixed-origin populations was significantly longer when measured on Apache (476.7 dd and 492.1 dd, respectively) than on Soissons (453.5 dd and 466.6 dd, respectively); such a difference was not significant for the immigrant population (Fig. 3a). The mean sporulating area did not significantly differ when measured on Apache or Soissons whatever the population considered. The mean latent period of isolates from the 2012 mixed-origin population was also significantly longer when measured on Apache (491.3 dd) than on Soissons (466.5 dd); such a difference was not significant either for the immigrant or resident population (Fig. 3b). As in 2009, the mean sporulation area did not significantly differ when measured on Apache or Soissons whatever the population considered.

Discussion

In this study, evidence for adaptation of *Z. tritici* populations to wheat cultivars was revealed by significant differences detected between pathogen populations: the immigrant populations appeared to be similarly adapted to Soissons and to Apache, whereas the resident populations appeared to be better adapted to Soissons (shorter latent period). The lack of significant cultivar × isolate interaction should not be interpreted as a lack of local adaptation because the experiment is not a strict reciprocal transplant (Kawecki and Ebert 2004). In the immigrant populations sampled in 2009 and 2012 no difference was detected between the two cultivars for both aggressiveness traits (latent period and sporulating area; Fig. 3): these populations were not particularly maladapted to Apache, although they had been collected on Soissons, a likely biological filter. This result firstly reflects the similar disease resistance rating of those two cultivars. Secondly it suggests that the compatibility filter effect (Combes 2001), which determines infection success of the pathogen once it makes contact with the host, had limited consequences in the experimental conditions of our study: a single fungal infection cycle did

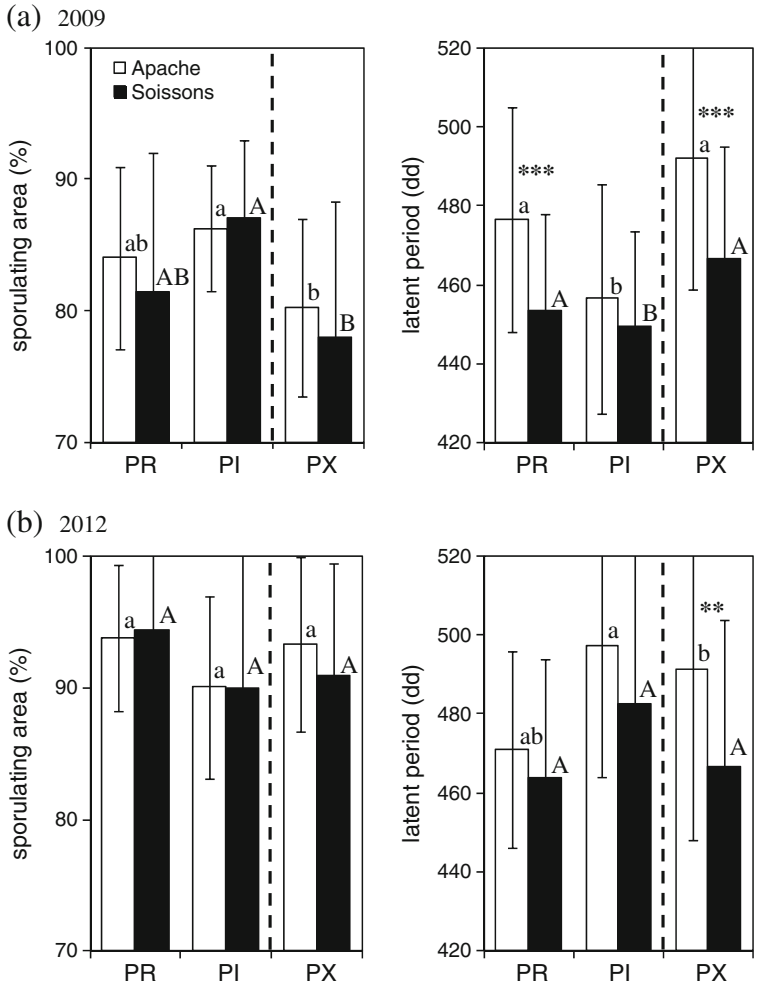

Fig. 3 Mean sporulating area (% of the inoculated leaf area) and latent period (degree-days post inoculation; dd) of two sets of three *Zymoseptoria tritici* populations (PR, resident population; PI, immigrant population; PX, mixed-origin population) collected in autumn **a** 2009 and **b** 2012, assessed on two wheat cultivars (Apache and Soissons). Significant differences (Student-Newman-Keuls test) between the two cultivars, considering separately each pathogen population, are indicated by *** ($P < 0.01$) and ** ($P < 0.05$). Significant differences ($P < 0.05$) between the pathogen populations, considering separately each cultivar, are indicated by different letters (small for Apache, capital for Soissons)

not result in differential selection for aggressiveness traits, although it is supposed to have effects through molecules produced by either the pathogen or the host, linked either to virulence or resistance (Combes 2001). The lack of significant difference in the susceptibility of Apache and Soissons to the immigrant population also confirms that the immigrant population likely had a selective trajectory in which Apache acted; it is likely that immigrant isolates collected on D_0 have at least one ancestor that infected at a certain time Apache, besides Soissons. This is consistent with the local use of the two cultivars on wide areas for several years.

A resident pathogen population is usually adapted, at least to some degree, to its local host (Burdon 1993). When parasites migrate more than hosts, local adaptation of parasites is expected, with a higher parasite relative fitness in sympatry than in allopatry (Kaltz and Shykoff 1998; Laine 2007). Several field experiments have established that populations of fungal plant pathogens are more aggressive on the cultivar from which they were isolated than on others (Clifford and Clothier 1974; Kolmer and Leonard 1986; Andrivon et al. 2007; Pariaud et al. 2009b). Our results are consistent with these findings, and, more specifically, with result previously obtained with *Z. tritici*. For example, Californian isolates of *Z. tritici* were found to induce more disease on Californian than on Oregonian wheat cultivars; similarly, Oregonian isolates induced more disease on Oregonian wheat cultivars (Ahmed et al. 1995). *Z. tritici* isolates were further showed to be better adapted to the host cultivar from which they originated than to other cultivars (Ahmed et al. 1996).

It cannot be concluded from the comparison of the latent period of the immigrant and resident populations

on the two cultivars that the resident population is better adapted to Soissons; rather, the comparison suggests that the resident populations is less maladapted to Apache. Finally, we showed that a resident pathogen population locally evolving on a given wheat cultivar experienced, within a few years under repeated epidemic pressure, a fitness penalty on another wheat cultivar grown in the same agricultural basin. This result is consistent with a field experiment that showed that fitness was nearly 10 % lower in immigrant than in resident isolates (Zhan and McDonald 2004). Lower competitive ability of immigrants may arise from a trade-off in performance of isolates carrying alleles that are selectively advantageous in a population but deleterious in another because of environmental heterogeneity (Zhan and McDonald 2013b). The resident isolates collected during autumn 2009 and 2012 in the wheat monoculture plot resulted from a sexual cycle on the local host. Their parents have been present in that plot for one year at least and have performed one asexual cycle at least during season 2008–2009 and 2011–2012, respectively. Therefore, the resident populations have probably been selected by the local host before they were sampled. Inversely, most of isolates of the immigrant populations, resulting from infection by ascospores of a distant origin, did not have enough time to be strongly selected by the local host before they were collected.

Consistent with the hypothesis of a more rapid pathogen evolution under monoculture (Zhan and McDonald 2013a), our findings illustrate the competitive disadvantage experienced by immigrant isolates compared with resident ones, the immigrant isolates having not been selected for adaptation to the local environment (Giraud 2006; Gladieux et al. 2011). Selection for quantitative traits in fungal pathogen populations has previously been shown to operate at the scale of a single-season epidemic and to result in a higher aggressiveness at the end of the crop season on the host genotype on which the pathogen population multiplied (Cowger and Mundt 2002; Suffert et al. 2015). Our findings suggest that selection for quantitative traits may be accentuated, at the scale of several crop seasons, by growing locally the same host cultivar.

The comparison of the aggressiveness profile of the resident and the immigrant populations to those of a third local population responsible of early infections in the monoculture wheat field can potentially be used to elucidate the main origin of the third population. Indeed, our results show that the aggressiveness of the mixed-origin population collected in 2009 was more similar to that of the resident than that of the immigrant population. Accordingly, the origin of inoculum for the mixed-origin population was probably more local than distant in autumn 2009: the main proportion of isolates belonging to the mixed-origin population would have been resident, resulting from the local inoculum pool, while a minor proportion would have been immigrant. The early lesions that appeared in autumn 2009 in plot D+ can be suspected to be caused mostly by local primary inoculum, here ascospores produced on debris within this plot. This interpretation is consistent with the estimation that 90 % of the isolates collected at the end of a field epidemic have a local origin (66 % as asexual progeny and 24 % sexual recombinants) and the remaining 10 % are immigrant isolates of distant origin (Zhan et al. 1998). Results obtained with populations sampled in 2012 were less conclusive: the aggressiveness profile of the mixed-origin population was not different from the aggressiveness profile either of the resident or the immigrant population, despite a significant difference in the latent period measured on Apache. The question arises whether the third population was actually of mixed origin, that is, composed of isolates having local and distant origins in indistinguishable proportions. No difference was observed in 2012 in the aggressiveness profile between the immigrant and the resident population on Soissons and Apache, contrary to 2009. The year effect may be explained by a nearly 20-fold difference in the pressure of primary inoculum in fall. The amount of ascospores produced on wheat debris in the D+ plot was high in 2009 (> 500 ascospores ejected from 1 g of debris during a single ejection event; Suffert and Sache 2011) but much lower in 2012 (< 30 ascospores; Morais et al. 2015a. The impact of the local inoculum in the beginning of the epidemic appears to be quite limited when the local inoculum pressure is low, as observed in the same experimental design (Suffert and Sache 2011): the local presence of inoculum had a strong quantitative effect, although transient, on the early epidemic dynamic. The more infected debris present in a field, the more severe initial disease was, emphasizing the preponderant role of the local primary inoculum compared to the distant inoculum.

Quantification of inoculum sources of *Z. tritici* has been mainly investigated by assessing ascospore production directly from wheat debris in field (Suffert and Sache 2011), that is, the amount

of locally produced inoculum able to infect more or less distant wheat fields. The assessment of the amount of airborne inoculum actually available for a target wheat field, for example by airborne trapping of ascospores (Bathgate and Loughman 2001; Fraaije et al. 2005; Duvivier et al. 2013), cannot track the origin of inoculum. Mark-release-recapture, consisting in co-inoculating a wheat plot with selected *Z. tritici* genotypes, then separated from non-inoculant genotypes by using molecular genetic markers (Zhan and McDonald 2013a), is a promising experimental approach. Population genetic analyses based on neutral SSR markers usually did not reveal a strong differentiation among populations collected at large or small spatial scales, whatever their evolutionary history (Zhan et al. 2001; Linde et al. 2002; Morais 2015c). To test the inference presented in this study, wheat debris, produced by crossing under semi-controlled conditions different couples of parent isolates bearing rare alleles, could be placed at different distances from a target wheat crop. Debris would act as different, identified sources of inoculum. Such an approach would maximize the probability to identify thereafter progeny isolates and so to quantify more accurately the origin of inoculum.

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