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Seasonal Changes Drive Short-Term Selection for Fitness Traits in the Wheat Pathogen *Zymoseptoria tritici*

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In a cross-infection experiment, we investigated how seasonal changes can affect adaptation patterns in a *Zymoseptoria tritici* population. The fitness of isolates sampled on wheat leaves at the beginning and at the end of a field epidemic was assessed under environmental conditions (temperature and host stage) to which the local pathogen population was successively exposed. Isolates of the final population were more aggressive, and showed greater sporulation intensity under winter conditions and a shorter latency period (earlier sporulation) under spring conditions, than isolates of the initial population. These differences, complemented by lower between-genotype variability in the final population, exhibited an adaptation pattern with three striking features: (i) the pathogen responded synchronously to temperature and host stage conditions; (ii) the adaptation concerned two key fitness traits; (iii) adaptation to one trait (greater sporulation intensity) was expressed under winter conditions while, subsequently, adaptation to the other trait (shorter latency period) was expressed under spring conditions. This can be interpreted as the result of short-term selection, driven by abiotic and biotic factors. This case study cannot yet be generalized but suggests that seasonality may play an important role in shaping the variability of fitness traits. These results further raise the question of possible counterselection during the interepidemic period. While we did not find any trade-off between clonal multiplication on leaves during the epidemic period and clonal spore production on debris, we suggest that final populations could be counterselected by an Allee effect, mitigating the potential impact of seasonal selection on long-term dynamics.

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Thermal variation, including seasonal fluctuations and also climate change, appears to be one of the main drivers of the predicted change in plant disease distributions (1–3). The adaptive response of plant pathogens to thermal variations may involve the movement of pathogen populations, as well as phenotypic plasticity and selection, so that full characterization requires extensive measurements of fitness traits under various environmental conditions. How the seasonality of disease development (e.g., the duration of the epidemic and interepidemic periods, through modifications of the biotic and abiotic environment) will be modified by climate change is a question increasingly addressed in the literature (4–6). In contrast, how seasonal climate variation within a year currently affects the dynamics of pathogen populations has received less attention. However, this knowledge would be very useful for characterizing the potential effects of warming on future population dynamics. Indeed, the effects of seasonal variation are measurable at this time and offer very interesting prospects for experimentation. Investigation of such current effects of seasonality is particularly interesting because they might induce short-term selection in the fitness traits of pathogen populations that affect the course of epidemics, which should therefore be taken into account in disease management. The connection between current seasonal patterns and long-term climate change has been highlighted recently for human pathogens (7), but not yet for plant pathogens. Several examples of relationships between disease and seasonal climate drivers have been demonstrated on relatively small scales in human and animal infectious diseases (8). Analyses of life history responses to seasonal changes are still lacking (9). New insights into seasonal selection pressures throughout the life cycle of plant pathogens and how they are influenced by environmental conditions need to be developed.

The goal of this case study was to explore how life history traits respond to seasonal variation in a local plant pathogen population over the course of an annual epidemic. We investigated the interaction between the haploid heterothallic pathogenic fungus (*Zymoseptoria tritici*) and its host plant (wheat) using a cross-infection experiment. The active part of the pathogen population (i.e., strains just below the emergent leaf layer and so primarily responsible for subsequent secondary infections) is potentially influenced by the environmental conditions to which it has been successively exposed. After spring, this active part of the population consisted of isolates belonging to the initial population that went through the epidemic by asexual multiplication. We postulate that the isolates that were best adapted to winter conditions, and then those that were best adapted to spring conditions, were selected. To test this hypothesis, the fitness traits of pathogen populations sampled before winter, at the beginning of an annual epidemic, and after spring, at the end of that epidemic (here called "initial" and "final" populations, respectively), were compared under two sets of environmental conditions mimicking, as much as experimentally possible, those to which the initial pathogen population was successively exposed (winter and spring).

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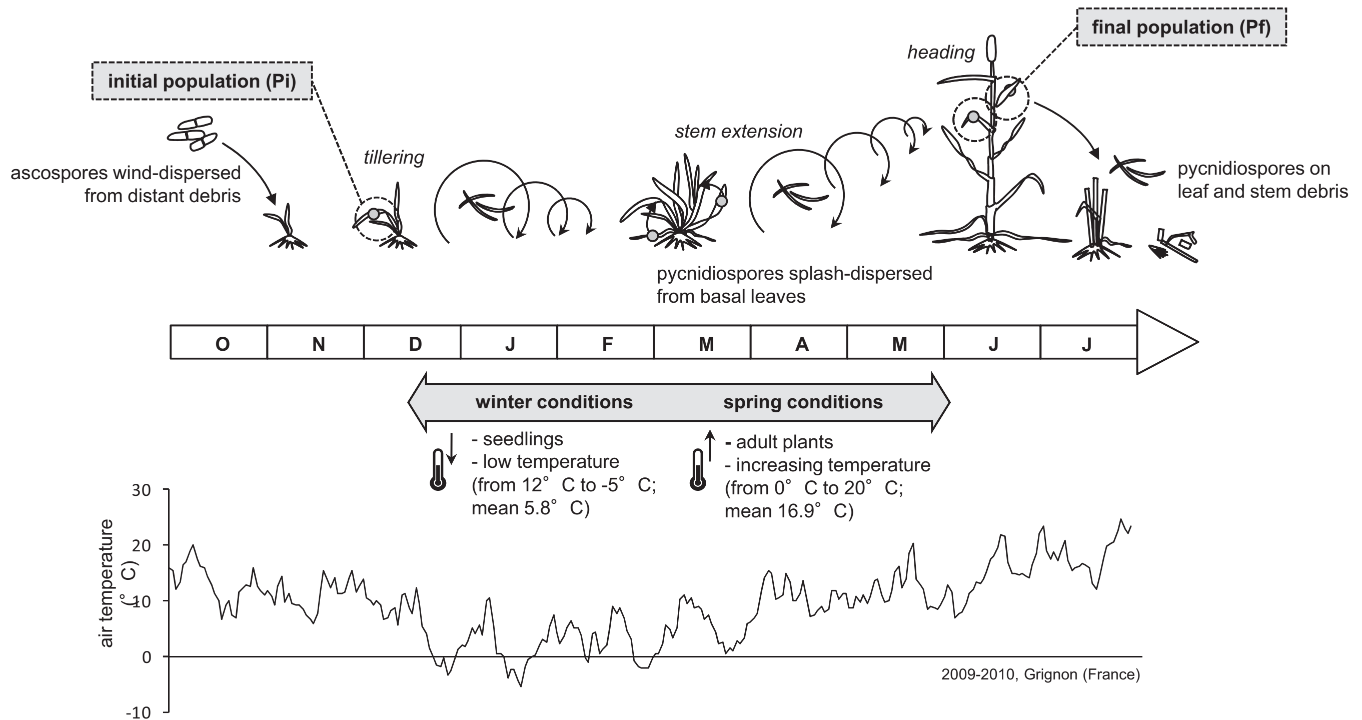

FIG 1 Overview of the development of a Septoria tritici blotch epidemic and environmental conditions (air temperature and wheat stage) during the 2009-2010 cropping season (Grignon, France). The first letter of each month, starting with October, is given in the open arrow.

The biological characteristics of the wheat–*Z. tritici* system are particularly suitable for investigating the effects of the most perceptible sources of seasonal variations expected to affect disease development (temperature and host stage). Pathogen populations are characterized by high phenotypic plasticity with signs of thermal adaptation (10). Variation in quantitative fitness traits (also considered to be quantitative traits of pathogenicity or components of aggressiveness by plant pathologists [11]) was shown to be accurate enough to establish differences in phenotypic expression *in planta* (12). During the epidemic period (from late autumn to early summer in the Northern Hemisphere), pathogen populations are exposed to large seasonal changes in thermal amplitude. Fungal development is temperature dependent (13, 14), so that seasonal fluctuations, which are suspected to constitute short-term selection pressure for fitness traits, might modify the dynamic of annual epidemics even within a year. Moreover, several infection cycles can amplify short-term selection pressure due to competition between pathogen strains (15–17).

To test whether seasonal changes could induce short-term selection, modify adaptation patterns in a local pathogen population, and affect disease development during the course of an epidemic, we compared the fitness of *Z. tritici* isolates sampled on wheat leaves at the beginning and at the end of a field epidemic under environmental conditions to which the active part of the population was successively exposed. Three specific questions were addressed in this case study, as follows.

(i) Is there potential for seasonal short-term selection in the populations examined? A prerequisite for the occurrence of such selection is between-genotype variability. We thus examined whether there was individual phenotypic variation in fitness traits in the initial and final pathogen populations. We also looked for plasticity of fitness traits in the face of environmental change and investigated whether the reaction norms were variable, too.

(ii) Can the final population be considered better adapted to winter and/or spring conditions? We investigated the phenotypic responses of populations to winter and spring conditions, and then to temperature and host stage separately in a complementary trial, to investigate the respective influence of each factor.

(iii) Is the observed seasonal change in the composition of the population reset at the end of the year, for example, by a trade-off between clonal multiplication on leaves during the epidemic period and clonal spore production on plant debris during the interepidemic period? We investigated differences in the dynamics of asexual sporulation on decaying leaves between the initial and final populations.

MATERIALS AND METHODS

Host-pathogen system. The ascomycete fungus *Zymoseptoria tritici* (Desm.) Quaedvl. & Crous (18) (formerly known as *Mycosphaerella graminicola*; anamorph *Septoria tritici*) causes Septoria tritici blotch, a foliar disease of wheat (*Triticum aestivum*) present in most wheat-growing areas worldwide. The disease is clonally propagated among wheat plants during the growing season by pycnidiospores, which are splash-dispersed over short distances upward in the canopy. The progress rate of the epidemic and the final disease intensity are determined by the number of asexual, embedded infection cycles completed by the pathogen (usually 4 to 6), depending mostly on temperature and rain events (Fig. 1). Wind-dispersed sexual ascospores, produced on wheat debris mainly during the intercrop period, initiate subsequent epidemics (19). Due to its very active sexual cycle, *Z. tritici* is considered a rapidly evolving pathogen with high genomic plasticity and genetic diversity, which is usually distributed on a fine spatial scale within a field (20–22).

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TABLE 1 Thirty *Zymoseptoria tritici* isolates sampled during the 2009-2010 season in a wheat field (Grignon, France), considered representative of initial and final populations

Population and isolate	Code	Date of collection
Pi		
INRA09-FS0729	I01	24 November 2009
INRA09-FS0731	I06	30 November 2009
INRA09-FS0732	I07	
INRA09-FS0798	I02	8 December 2009
INRA09-FS0799	I08	
INRA09-FS0800	I09	
INRA09-FS0802	I03	
INRA09-FS0803	I10	
INRA09-FS0805	I11	
INRA09-FS0806	I12	
INRA09-FS0808	I04	
INRA09-FS0809	I13	
INRA09-FS0811	I14	
INRA09-FS0813	I05	
INRA09-FS0814	I15	
Pf		
INRA09-FS01000	I16	12 July 2010
INRA09-FS01002	I22	
INRA09-FS01003	I23	
INRA09-FS01006	I24	
INRA09-FS01008	I25	
INRA09-FS01013	I17	
INRA09-FS01015	I26	
INRA09-FS01018	I18	
INRA09-FS01019	I27	
INRA09-FS01021	I19	
INRA09-FS01022	I28	
INRA09-FS01023	I29	
INRA09-FS01024	I20	
INRA09-FS01025	I21	
INRA09-FS01026	I30	

Pathogen populations. Two *Z. tritici* populations were taken into account during the 2009-2010 season in a wheat field (*Triticum aestivum* cv. Soissons), 200 m away from the nearest field where wheat had been grown in the previous year (Grignon, France, 48°51′N, 1°58′E). Fifteen isolates representative of the initial population (Pi) was sampled by destructive harvesting (five plants randomly collected in five 1-m^2 quadrats per plot, located 20 m away from each other) at the beginning of the epidemic, in late autumn (from 24 November to 8 December 2009), from the first 15 lesions detected on the first leaves of seedlings. The first contaminations probably occurred in late October as soon as the first wheat leaves emerged and originated exclusively from wind-dispersed ascospores of distant origin, because no wheat residues were present in the field (23, 24). Fifteen isolates representative of the final population (Pf) was sampled at the end of the epidemic, in early summer (12 July 2010), from the first 15 lesions found on either the antepenultimate (F3) or penultimate (F2) leaf of the main tiller. Since new immigrants (ascospores) from contaminated debris in distant fields may have continued to arrive throughout the course of the epidemic, it is possible that the final population contained some strains of distant origin, with a nonlocal evolutionary history. However, the presence of several lesions on F4 leaves and the absence of disease on ultimate leaves (F1) at the time of sampling (23) provided good circumstantial evidence that lesions on F3 and F2 leaves were caused by local, secondary reinfection of asexual progeny (or local sexual recombinants). This is consistent with the results obtained by Zhan et al. (25), who estimated that 90% of the isolates collected at the end of a field epidemic had a local origin (66% as asexual progeny and 24% as sexual recombinants) and the remaining 10% were immigrants of distant origin. Therefore, the final population could reasonably be considered to have resulted from the selective trajectory of the initial local population. This selection regime consisted of a winter period of decreasing temperatures on juvenile host plants followed by a spring period of increasing temperatures on erect plants (considered "adult") (Fig. 1). The 30 sampled isolates (Table 1), genotyped with 16 SSR (simple sequence repeat) neutral markers (multiplex 1 and multiplex 2 [26]), were genetically unique (see Table S1 in the supplemental material), as expected from the well-known very high diversity of *Z. tritici* populations on a local scale (20, 21). The phylogenetic tree presented in Fig. 2 illustrates the lack of correlation between the branching pattern of the clades and the origins of the isolates (initial and final populations).

Experimental design. We tested the adaptation of the initial and final populations to the seasonal conditions (temperature and host stage) to which the pathogen population was successively exposed in the field from the beginning to the end of the epidemic. A cross-infection experiment was conducted under semicontrolled conditions mimicking early-winter

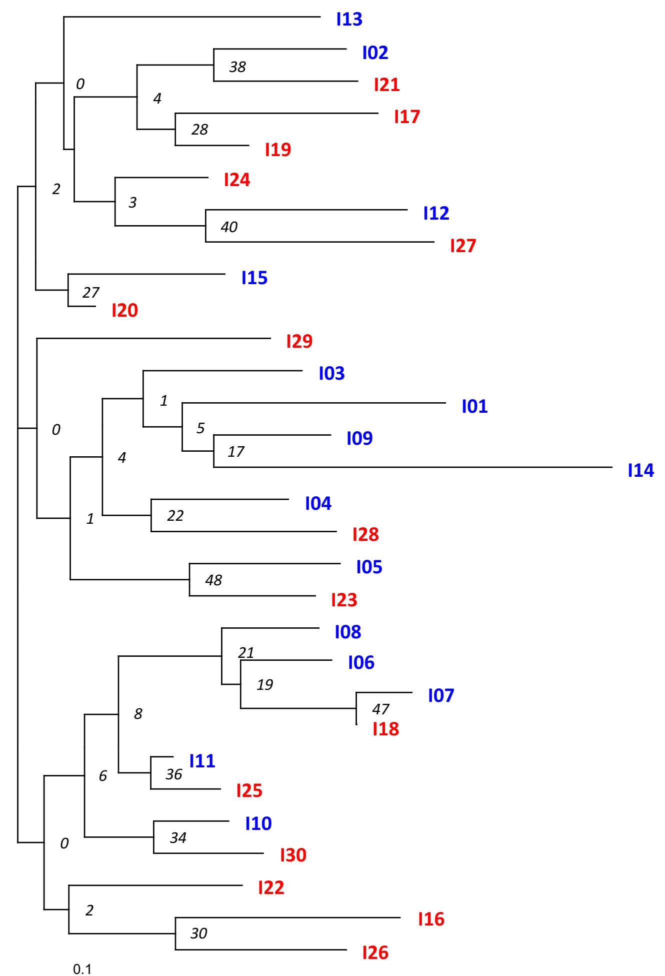

FIG 2 Phylogenetic tree for 30 *Zymoseptoria tritici* isolates (I01 to I15, initial population [n blue]; I16 to I30, final population [in red]) generated by neighbor-joining analysis based on the DA distance of Nei (62). The tree was computed using the Populations 1.2.31 program (http://bioinformatics.org/~tryphon/populations/) with SSR markers ST1E7, St2, St3B, St3C, St4, St5, St6, St7, St9, St10, St11, St12, and St13 and the mating-type locus (see Table S1 in the supplemental material) (25). Bootstrap values, calculated after 100 repetitions, are given in italics at nodes.

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TABLE 2 Experimental design of the trials conducted to investigate how seasonal changes in temperature and host stage can affect the adaptation patterns in a local *Zymoseptoria tritici* population

Trial	Condition[a]	Location	Factor				Population[b]	Isolates	Replicates[c]
			Host stage (S)	Temp (T) (°C)	Light	Leaf (L)[d]			
In planta, seasonal conditions	Ew	Cabinet	Seedling	8.9	Spotlight	L3	Pi	I01–I15	8
	Ew	Cabinet	Seedling	8.9	Spotlight	L3	Pf	I16–I30	8
	Es	Greenhouse	Adult plant	18.1	Daylight	F1	Pi	I01–I15	4
	Es	Greenhouse	Adult plant	18.1	Daylight	F1	Pf	I16–I30	4
	Es	Greenhouse	Adult plant	18.1	Daylight	F2	Pi	I01–I15	4
	Es	Greenhouse	Adult plant	18.1	Daylight	F2	Pf	I16–I30	4
In planta, fully crossed S × T conditions	Ew	Cabinet	Seedling	8.9	Spotlight	L3	Pi	I01–I05	8
	Ew	Cabinet	Seedling	8.9	Spotlight	L3	Pf	I16–I20	8
	Es	Greenhouse	Adult plant	18.1	Daylight	F1	Pi	I01–I05	4
	Es	Greenhouse	Adult plant	18.1	Daylight	F1	Pf	I16–I20	4
	Es	Greenhouse	Adult plant	18.1	Daylight	F2	Pi	I01–I05	4
	Es	Greenhouse	Adult plant	18.1	Daylight	F2	Pf	I16–I20	4
	Crossed conditions	Cabinet	Adult plant	8.9	Spotlight	F1	Pi	I01–I05	4
	Crossed conditions	Cabinet	Adult plant	8.9	Spotlight	F1	Pf	I16–I20	4
	Crossed conditions	Cabinet	Adult plant	8.9	Spotlight	F2	Pi	I01–I05	4
	Crossed conditions	Cabinet	Adult plant	8.9	Spotlight	F2	Pf	I16–I20	4
	Crossed conditions	Greenhouse	Seedling	18.1	Daylight	L3	Pi	I01–I05	8
	Crossed conditions	Greenhouse	Seedling	18.1	Daylight	L3	Pf	I16–I20	8
In vitro (petri dishes)		Cabinet		8.9	Darkness		Pi	I01–I15	3
		Cabinet		8.9	Darkness		Pf	I16–I30	3
		Greenhouse		18.1	Darkness		Pi	I01–I15	3
		Greenhouse		18.1	Darkness		Pf	I16–I30	3

[a] Ew, winter conditions; Es, spring conditions.
[b] Pi, initial population; Pf, final population.
[c] The number of replicates is the number of times a given isolate (from I01 to I30) was inoculated onto a given leaf layer (F1, F2, or L3) or was deposited on a petri dish, all other conditions being equal.
[d] Decaying leaves were collected to estimate the decrease in residual sporulation capacity.

and spring conditions (Table 2). The fitness traits of the 15 isolates from Pi and the 15 isolates from Pf were simultaneously assessed on seedlings subjected to a low temperature and on adult plants submitted to a higher temperature. For the sake of simplicity, such environmental conditions are referred to here as "winter conditions" (Ew) and "spring conditions" (Es), respectively. In this winter-versus-spring test, each isolate was inoculated onto the F1 and F2 leaves of four adult plants (2 × 4 replicates) (Table 2) kept in a greenhouse (spring thermal conditions) and onto the third leaves of eight seedlings (8 replicates) (Table 2) kept in a growth cabinet (winter thermal conditions).

To test whether better adaptation of one of the populations to one of the sets of environmental conditions was related to the temperature and/or host stage, the first trial was completed by assessing the fitness traits of some isolates under fully crossed host stage–temperature conditions (Table 2). Specifically, five isolates from Pi and five isolates from Pf were randomly selected and inoculated onto the F1 and F2 leaves of four adult plants (2 × 4 replicates) (Table 2) kept in the growth cabinet under winter thermal conditions and onto the third leaves of eight seedlings (8 replicates) (Table 2) kept in the greenhouse under spring thermal conditions.

A third *in vitro* trial compared the growth of the 30 isolates on an artificial medium under thermal conditions mimicking winter and spring conditions. The effect of temperature on the *in vitro* development of the fungus was then compared with that obtained by assessing *in planta* host-pathogen interactions. The objective of this comparison was to learn whether *in planta* assessment of different complementary fitness traits was a necessity or superfluous and whether a simplified method could be used for further studies.

Environmental conditions. Seedlings and adult plants of *T. aestivum* cv. Soissons were grown synchronously in a greenhouse and a cabinet so that the same spore suspensions could be used. The temperature and host stage were close to those observed under field conditions at the early- and late-epidemic stages.

To grow adult plants, wheat seeds were sown on 8 December 2010 and were vernalized for 8 weeks at 8°C with a 10-h light period and a 14-h dark period. Plants were then transplanted into 1.1-liter pots and were kept in a greenhouse for the whole experiment, as described by Suffert et al. (12): natural daylight was supplemented with 400-W sodium vapor lamps, and the temperature was kept below 20°C during the 15-h light period and above 12°C during the 9-h dark period (see Fig. S1 in the supplemental material). To grow seedlings, wheat seeds were sown, 2 per 0.4-liter pot, on 21 March 2011 and were kept in a cabinet at 11.5°C with a 10-h light period (photosynthetically active radiation [PAR], 75 μmol m^{-2} s^{-1}) and at 7.5°C with a 14-h dark period (see Fig. S1).

The air temperature was recorded every 15 min in the greenhouse and in the cabinet using a thermal sensor (digital probes with a SPY RF N recorder; JRI, Argenteuil, France) placed within the canopy. The thermal time (t), expressed in degree-days, was calculated in the two enclosures, starting from the inoculation date, by summing the daily mean air temperatures using a 0°C base temperature. The mean temperature recorded in the field in the "winter" period (basis, −2.4°C, i.e., excluding the temperatures on days when *Z. tritici* was suspected of not being able to grow [27]), from the sampling of the initial population (November) to the beginning of stem extension (April), was 5.8°C (Fig. 1). The daily mean PAR (2.7 mol m^{-2} day^{-1}) was equal to 33% of the mean PAR (8.3 mol m^{-2} day^{-1}) recorded in the field over the same period (see Fig. S1 in the

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supplemental material). In the cabinet, the daily mean temperature under the conditions mimicking "winter" was 8.9°C (Tw). Ideally, Tw should have been 5.8°C, but preliminary tests showed that at such a mean temperature, it was not possible to keep seedlings and adult plants alive concomitantly under good physiological conditions over the duration of the experiment. The mean temperature recorded in the field during the "spring" period, from stem extension to the sampling of the final population (early July), was 16.9°C (Fig. 1). The daily mean PAR (14.2 mol m^{-2} day^{-1}) was equal to 35% of the mean PAR (40.5 mol m^{-2} day^{-1}) recorded in the field over the same period. In the greenhouse, the daily mean temperature of the conditions mimicking "spring" was 18.3°C (Ts) instead of the target temperature of 16.9°C, because the climatic environment was regulated using a cooling system driven by threshold temperatures.

Inoculation. Wheat plants were inoculated in the greenhouse on 12 April 2011, after the head had fully emerged, when seedlings had the third leaf unfurled. An inoculum was obtained for each isolate (Table 1) from stock conidial suspensions stored at −80°C for 10 and 16 months for Pi and Pf, respectively. Such a difference probably had no effect, since *Z. tritici* can usually be stored for several years in this way without any change in its ability to grow (this was the case, e.g., for *Z. tritici* isolates IPO323 and IPO94269, isolated in 1984 and 1994 [28]). Aqueous suspensions (10^5 conidia ml^{-1}), prepared as described by Suffert et al. (12), were applied using a paintbrush along 25-mm-long sections of the upper sides of the F1 and F2 leaves of each main tiller of the adult plants and along 70-mm-long sections of the upper sides of the third leaves of the seedlings. To promote infection, the inoculated plant material was enclosed for 72 h in a sealed transparent polyethylene bag moistened with distilled water.

For each isolate, six petri dishes (diameter, 55 mm) containing potato dextrose agar medium (39 g $liter^{-1}$) were plated with a drop (300 μl) of the conidial suspension. To induce colony growth, the dishes were enclosed in an opaque plastic box for 5 days. Half of the dishes (3 × 30) were placed in the greenhouse (spring thermal conditions), while the other half were placed in the cabinet (winter thermal conditions) (Table 1).

Assessment of fitness traits. (i) *In planta* assessment. Four quantitative fitness traits of the plant-pathogen interaction were selected as the most relevant for our study, based on our previous knowledge of their impact on epidemic development (12). The maximum sporulating area (SPOmax) was estimated by the maximum percentage of the area covered by fruiting bodies (pycnidia). The latency period (LAT), the interval between inoculation and the appearance of the first sporulating fruiting body, was estimated for a given leaf as the time that elapsed from inoculation to the point at which coverage by pycnidia equaled 5% of SPOmax. The fruiting body density (PYCdens), was calculated as the surface density of pycnidia within the sporulating area. The sporulation capacity of a fruiting body (nbSPO) was assessed by the number of pycnidiospores produced by a pycnidium. Finally, SPOmax, PYCdens, and nbSPO could be considered as three components of sporulation intensity.

SPOmax, LAT, and PYCdens were estimated on each inoculated leaf of the adult plants. A Gompertz model was fitted to the temporal dynamics of lesion growth, assessed twice a week from mid-April to mid-June (14 assessments), as described by Suffert et al. (12). This assessment method was adapted to seedlings as follows. The rapidity of lesion development on seedlings precluded the assessment of LAT through curve fitting; instead, LAT was assessed using the date when the first pycnidia were observed. Moreover, SPOmax assessed on adult plants and SPOmax assessed on seedlings could not be analyzed jointly by a single variance analysis because of differences in the inoculation procedure and the appearance of symptoms; this could be a source of artifacts in testing of the effects of environmental conditions (Ew, Es).

On the day after the last disease assessment, the eight inoculated leaves that had been subjected to the same treatment (2 × 15 isolates × 2 environments) (Table 1) were pooled and were vortexed in water (10 ml). The total number of spores in the suspension, estimated using a Malassez counting chamber, was divided by the total number of pycnidia present on the eight source leaves to obtain the sporulation capacity of a pycnidium (nbSPO). Decaying leaves were then placed in open pillboxes that were positioned outdoors and exposed to sun and rainfall from 21 June to 27 September 2011. The residual sporulation capacity of the decaying leaves was assessed by following the same procedure 2, 4, 8, 13, and 16 weeks after the initial spore collection carried out to estimate nbSPO.

(ii) *In vitro* assessment. For each isolate, 6 plugs (diameter, 20 mm) colonized by the fungus were vortexed in water (10 ml). The concentration of conidia in the resulting suspension was assessed using a Malassez counting chamber. The mean daily conidial multiplication rate of isolates grown in petri dishes, adopted as the *in vitro* fitness trait, was then calculated by dividing the total number of conidia collected after 5 days by 1.5×10^5 (considering that the number of conidia deposited in each petri dish was 3×10^4; i.e., 300 μl of the suspension containing 10^5 conidia ml^{-1}).

Data analysis. (i) Between-genotype variability for fitness traits in the initial and final populations. For each trait in each environment, the between-genotype variance of fitness traits (within-population phenotypic variance) was estimated in Pi and in Pf using the mean square of the one-way analysis of variance (ANOVA) of the trait with the isolate [I(P)] as a factor. Whether the between-genotype variance decreased significantly between Pi and Pf was assessed by computing the null distribution of the ratio of variances using permutation tests (10,000 permutations).

(ii) Differences in fitness traits between populations. Because the fitness traits assessed on adult plants and seedlings could not be jointly analyzed, the overall effect of the environment was not tested by ANOVA on the whole data set. In the *in planta* trial of seasonal conditions, for each environment (Ew and Es) separately, the variance of fitness traits measured on eight replicates per isolate was divided into sources attributable to the isolate [I(P)] and population (P), taking into account the effect of the leaf layer [L(S)] (only in environment Es), according to the following model:

$$Y_{iplr} = M + I(P) + P + L(S) + [P \times L(S)] + [I(P) \times L(S)] + \varepsilon_{iplr} \quad (1)$$

where Y_{iplr} is the value of fitness trait Y of isolate i, in population p, on leaf layer l, for replicate r. S is the host stage, M indicates the overall mean, and ε_{iplr} is the variance between replicates.

Comparisons of trait means between Pi and Pf were performed with the population (P) and isolate [I(P)] as factors in Ew and then with P, I(P), and the leaf layer [L(S)] as factors in Es (with significance determined by Student tests) (Fig. 3).

(iii) Dissociated effects of temperature and host stage on fitness traits. In the *in planta* trial with fully crossed S × T conditions, the variance of fitness traits measured on eight replicates per isolate and five isolates per population under fully crossed conditions was divided into sources attributable to the isolate [I(P)], population (P), host stage (S), and temperature (T), taking into account the effect of the leaf layer [L(S)], according to the following model:

$$\begin{aligned} Y_{ipstlr} = {} & M + I(P) + P + S + T + L(S) + [P \times T] + [P \times S] \\ & + [T \times S] + [P \times L(S)] + [T \times L(S)] + [I(P) \times T] + [I(P) \times S] \\ & + [I(P) \times L(S)] + \varepsilon_{ipstlr} \quad (2) \end{aligned}$$

where Y_{ripst} is the value of fitness trait Y of isolate i, in population p, at host stage s, at temperature t, on leaf layer l, for replicate r. M indicates the overall mean, and ε_{ipstlr} is the variance between replicates.

In the *in vitro* trial, the effect of temperature (T) on the mean daily conidial multiplication rate was analyzed by applying a similar model [equation 2, factors S and L(S) excluded] to the 30 isolates. For each thermal condition (Tw and Ts), comparisons of the mean daily conidial multiplication rates between Pi and Pf (see Fig. 5a) were carried out with only the population (P) and isolate [I(P)] as factors (with significance determined by Student tests). In order to compare the thermal adaptation of the two populations estimated *in vitro* with that estimated *in planta*, similar comparisons of the means of LAT and SPOmax (equation 2) were carried out only for the 10 isolates tested under fully crossed temperature–host stage conditions.

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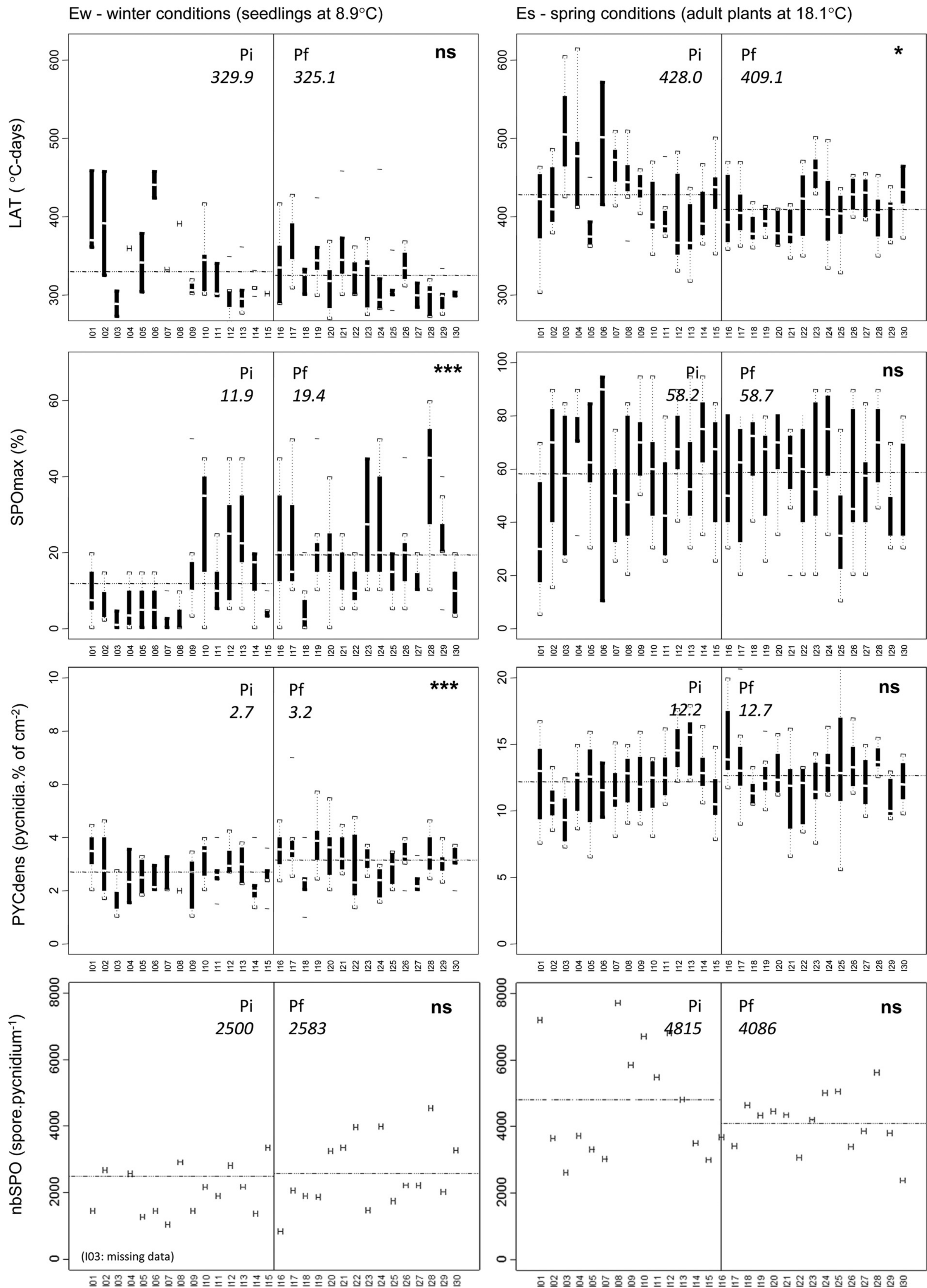

FIG 3 Fitness traits (LAT, the interval between inoculation and the appearance of the first pycnidia; SPOmax, the maximum sporulating area; PYCdens, the pycnidium density; nbSPO, the sporulation capacity of a pycnidium) of 30 *Zymoseptoria tritici* isolates (I01 to I15, initial population [Pi]; I16 to I30, final population [Pf]) assessed under winter (Ew) and spring (Es) conditions. Asterisks indicate that the means (indicated by horizontal dotted lines and given in italics above the data) differ significantly (*, $P < 0.1$; ***, $P < 0.01$) between Pi and Pf, as revealed by Student tests. ns, not significant.

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TABLE 3 Analysis of variance for four fitness traits of 30 *Zymoseptoria tritici* isolates assessed under winter and spring conditions in the *in planta* trial of seasonal conditions in order to characterize the effects of the population and isolate[a]

Condition and source of variance	df	LAT			SPOmax			PYCdens			nbSPO		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*[c]
Ew													
Population (P)	1	676	0.45	0.505	2,979	26.1	**<0.001**	10	13.49	**<0.001**	51,171	0.02	0.884
Isolate [I(P)]	28	3,736	2.48	**<0.001**	509	4.5	**<0.001**	1.4	2.0	**0.005**			
Residual		1,506			114			0.7			2,374,590		
Es													
Population (P)	1	22,036	2.9	**0.087**	10	0.02	0.881	13	2.2	0.141	3,843,966	2.0	0.165
Isolate [I(P)]	28	6,990	4.5	**<0.001**	693	1.5	**0.052**	14	2.3	**<0.001**			
Leaf layer [L(S)]	1	19,031	12.2	**0.002**	5,357	11.9	**<0.001**	55	9.2	**0.003**			
Residual		1,562			483			6			1,888,417		

[a] LAT, SPOmax, PYCdens, and nbSPO are defined in the text and in the legend to Fig. 3. Interactions between P and L(S) and between I(P) and L(S) were nonsignificant. MS, mean squares; Ew, winter conditions; Es, spring conditions.

[b] The effect of P was tested against I(P) in Ew, with the *F* ratio for MS_P calculated as $MS_P/MS_{I(P)}$, as with P × L(S).

[c] The significance level for nbSPO cannot be compared with the significance levels for other traits because spores were collected from a pool of eight leaves, precluding replicated measures of sporulation for each isolate.

(iv) Residual sporulation on decaying leaves. The decrease in residual sporulation capacity for isolates of Pi and Pf was characterized by fitting an exponential-decay model to data recorded from 4 to 13 weeks after the first spore collection.

RESULTS

Between-genotype variability for fitness traits in the initial and final populations. All the traits measured showed ample between-genotype variability: in each population; LAT ($P \leq 0.001$), SPOmax ($P \leq 0.052$), and PYCdens ($P \leq 0.005$) differed significantly between isolates when assessed in Ew and Es (Table 3). The variability of sporulation capacity (nbSPO) could not be similarly assessed because spores were collected from a pool of eight leaves, precluding replicated measurements of sporulation for each isolate. No difference in between-genotype variance (V_g) could be detected between Pi and Pf for traits SPOmax [V_g(Pi)/V_g(Pf), 1.346 ($P = 0.288$) for Ew and 0.830 ($P = 0.632$) for Es] and PYC_{dens} [V_g(Pi)/V_g(Pf), 1.514 ($P = 0.224$) for Ew and 0.671 ($P = 0.671$) for Es]. The between-genotype variance of LAT decreased between Pi and Pf [V_g(Pi)/V_g(Pf), 3.238 ($P = 0.022$) for Ew and 2.082 ($P = 0.112$) for Es], though without collapsing at the end of epidemic (Fig. 3 and 4).

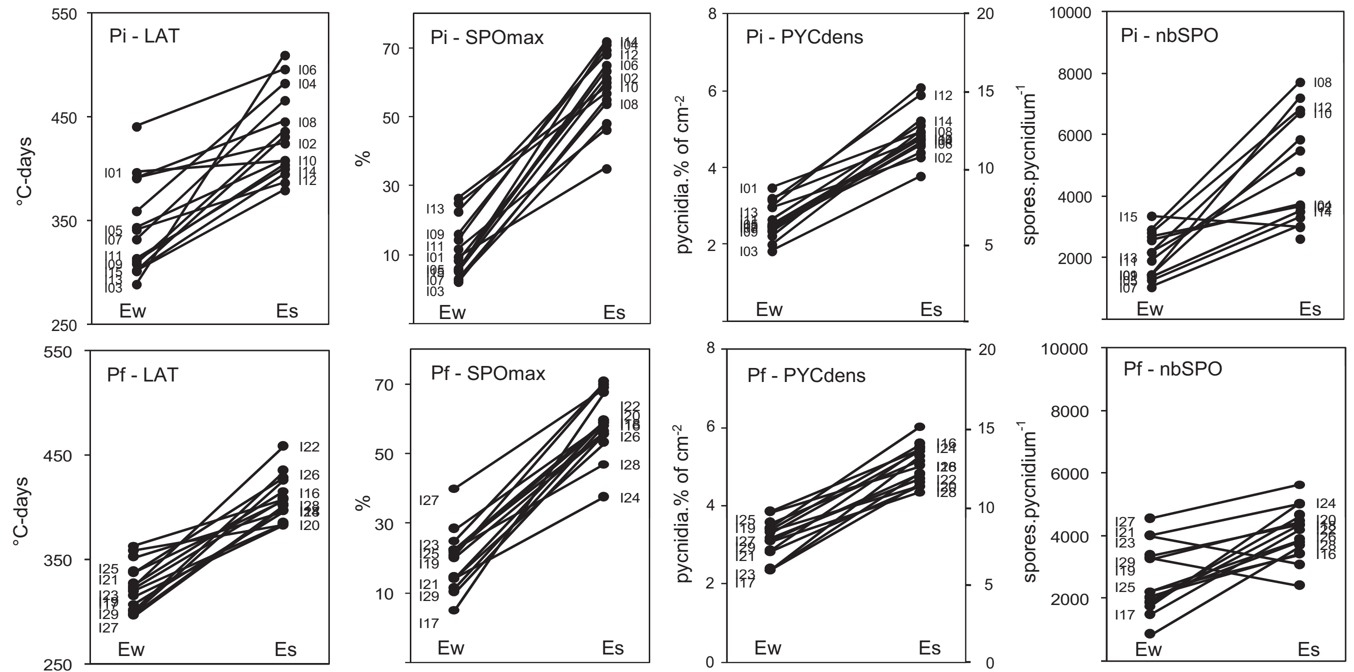

FIG 4 Reaction norms of 30 *Zymoseptoria tritici* isolates (I01 to I15, initial population [Pi]; I16 to I30, final population [Pf]) for four fitness traits (LAT, SPOmax, PYCdens, nbSPO; defined in the legend to Fig. 3) assessed under winter (Ew) and spring (Es) conditions. Each line (the reaction norm) joins the fitness trait values of a single genotype.

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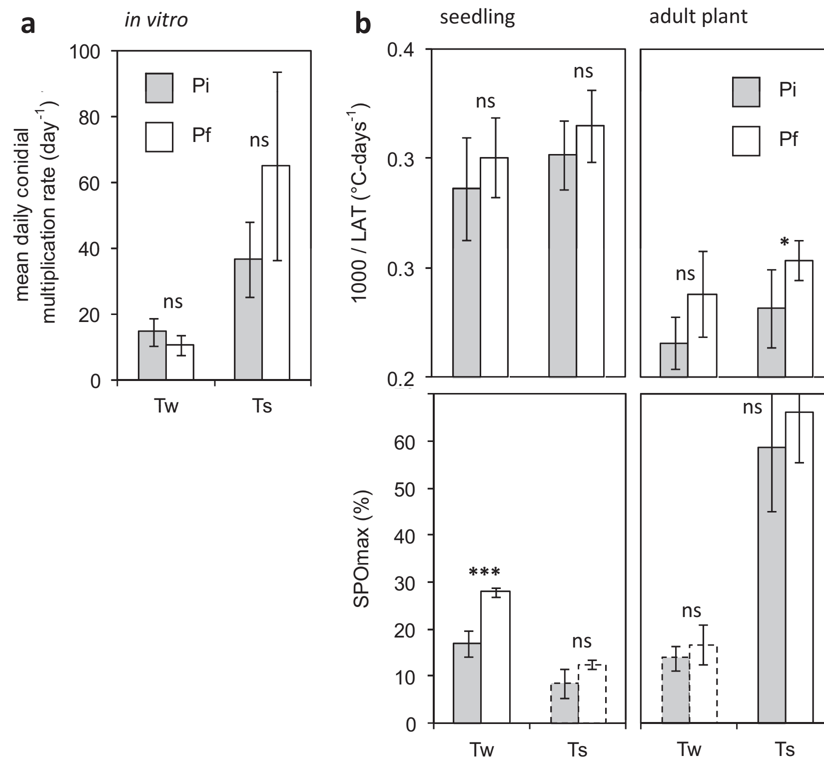

FIG 5 (a) Thermal adaptation of development (mean daily conidial multiplication rate) of 30 *Zymoseptoria tritici* isolates (I01 to I15, initial population [Pi]; I16 to I30, final population [Pf]) (Table 1) assessed *in vitro* (in petri dishes). (b) Dissociation between host stage (seedling versus adult plant) and temperature (Tw, 8.9°C; Ts, 18.1°C) adaptation of two fitness traits (LAT and SPOmax; defined in legend to Fig. 3) of 2 × 5 isolates assessed *in planta* (complete cross-infection trial). Asterisks indicate that the means differ significantly between Pi and Pf, as revealed by Student tests (*, $P < 0.1$; ***, $P < 0.01$); ns, not significant. Dashed-line bars indicate that the environmental cross-conditions (seedling × Ts and adult plant × Tw) under which the trait (SPOmax) was assessed were too artificial and that thus, the results were excluded from the analysis.

Effects of winter and spring conditions on fitness traits. Although the overall effect of the environment (E) could not be statistically tested on the whole data set, the effect of the environmental conditions (Ew versus Es) on the four fitness traits indicated a phenotypic plasticity in both populations (Fig. 4). Whatever their origin, isolates sporulated earlier and had a smaller sporulating area, a lower density of pycnidia, and lower sporulation capacity under winter conditions than under spring conditions. In the two populations, the between-genotype variability of the reaction norm of the latency period (LAT) was shown by the interaction between E and I(P); the between-genotype variability of the reaction norms of SPOmax and PYCdens should also be noted.

Change in fitness traits between initial and final populations. Differences in LAT, on the one hand, and SPOmax and PYCdens, on the other hand, showed that the fitness of Pi and Pf differed depending on their environmental conditions of expression. When exposed to winter conditions (on seedlings at 8.9°C), Pf isolates had a greater sporulating area (SPOmax, 11.9% for Pi versus 19.4% for Pf [$P < 0.001$] [Fig. 3; Table 3]) and a higher density of pycnidia (PYCdens, 2.7 for Pi versus 3.2 for Pf [$P < 0.001$]). No significant difference was detected in the latency period (LAT, 329.9°C-days for Pi versus 325.1°C-days for Pf) or sporulation capacity (nbSPO, 2,500 spores/pycnidium for Pi versus 2,583 spores/pycnidium for Pf). When exposed to spring conditions (on an adult plant at 18.1°C), Pf had a shorter latency period than Pi (LAT, 428.0°C-days for Pi versus 409.1°C-days for Pf [$P = 0.087$] [Table 3; Fig. 3]). No significant difference was detected in the sporulating area (SPOmax, 58.2% for Pi versus 58.7% for Pf), the density of pycnidia (PYCdens, 12.2 for Pi versus 12.7 for Pf), or the sporulation capacity (nbSPO, 4,815 spores/pycnidium for Pi versus 4,086 spores/pycnidium for Pf).

No significant correlation was established between the four fitness traits (SPOmax, LAT, PYCdens, and nbSPO) assessed with data obtained under Ew and Es separately (r, >-0.30 and <0.27, except for the SPOmax–nbSPO correlation under Ew [$r = 0.55$] [data not shown]). In a previous study especially dedicated to such analyses (12), correlations were higher (e.g., r was >-0.50 and <-0.32 for the LAT-SPOmax correlation and >-0.78 and <-0.48 for the LAT-PYCdens correlation). The choice of ANOVA rather than analysis of covariance (ANCOVA) (where a mean fitness trait was associated in all possible combinations with the other fitness traits) was thus guided by the principle of parsimony.

Dissociation between temperature and host stage effects on fitness traits. The mean latency period assessed *in planta* was lower on seedlings than on adult plants, and lower, too, at the highest temperature (Ts) than at the lowest temperature (Tw) (Fig. 5b; Table 4). Similarly, the mean sporulating area was greater on adult plants than on seedlings at the highest temperature, but not at the lowest temperature; the mean sporulating area was greater at the highest temperature than at the lowest temperature on adult plants, but not on seedlings. Under "seedling–Ts" and "adult plant–Tw" cross conditions, the sporulating area, unlike the latency period, had little biological meaning, since it was obtained under very artificial conditions. In any case, Pf was more aggressive than Pi (a shorter latency period and a larger sporulation area), whatever the temperature or the host stage.

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TABLE 4 Analysis of variance for three fitness traits of 10 *Zymoseptoria tritici* isolates[a] assessed in the trial of fully crossed S × T conditions in order to characterize the *in planta* effects of the population, isolate, host stage, and temperature

	LAT[b]				SPOmax[b]			PYCdens[b]		
Factor	df	MS	*F*	*P*	MS	F	*P*	MS	F	*P*
Population (P)[c]	1	66,705	8.4	**0.020**	3,831	35.9	**<0.001**	27	1.4	0.277
Temp (T)	1	32,402	18.3	**<0.001**	28,923	143.1	**<0.001**	1,354	433.4	**<0.001**
Host stage (S)[c]	1	449,248	253.8	**<0.001**	52,660	260.5	**<0.001**	1,609	515.0	**<0.001**
Isolate [I(P)]	8	7,919	4.5	**0.001**	107	0.5	0.835	20	6.3	**<0.001**
Leaf layer [L(S)]	1	13,622	7.7	**0.006**	1,024	5.1	**0.025**	15	4.9	**0.028**
Residual		1,770			202			3		

[a] LAT, SPOmax, and PYCdens are defined in the text and the legend to Fig. 3. Isolates I01 to I05 and I16 to I20 (Tables 1 and 2) were used.
[b] Interactions were nonsignificant, except for interactions between S and I(P) for SPOmax ($P = 0.009$) and PYCdens ($P = 0.023$) and interactions between T and S for SPOmax ($P < 0.001$) and PYCdens ($P < 0.001$).
[c] The effect of P was tested against I(P), with the *F* ratio for MS_P calculated as $MS_P/MS_{I(P)}$, as with P × T and P × L(S); similarly, the effect of S was tested against L(S), with the *F* ratio for MS_S calculated as $MS_S/MS_{L(S)}$, as with S × T and S × I(P).

The mean daily conidial multiplication rate assessed *in vitro* was about four times greater at the highest temperature (Fig. 5a; Table 5). This difference, which was expected because that temperature matched the thermal optimum of the pathogen (14), was consistent with assessments obtained *in planta*. However, these differences in the mean daily conidial multiplication rate between the initial and final populations were not statistically significant (the mean daily conidial multiplication rate was greater for Pf than for Pi when assessed at the highest temperature and quite similar when assessed at the lowest temperature [Fig. 5a]); the differences were consistent with results obtained *in planta* (LAT was shorter for Pf than Pi when assessed on adult plants at the highest temperature, and SPOmax was higher on seedlings at the lowest temperature). A similar consistency between *in planta* and *in vitro* results was also found for PYCdens and nbSPO (data not shown).

Residual sporulation on decaying leaves. For 4 weeks after nbSPO had been assessed, the residual sporulation capacity increased considerably on decaying leaves of seedlings (from 2,500 to 15,000 spores/pycnidium), while it decreased on decaying leaves of adult plants (from 4,500 to 2,500 spores/pycnidium) (Fig. 6). Seedling leaves were not entirely senescent when they were detached, and lesions still developed on them, while adult plant leaves were entirely senescent, and lesions had already stopped developing on them. The residual sporulation capacity subsequently decreased on all leaves. Whatever the time of assessment (2, 4, 8, 13, or 16 weeks), the residual sporulation capacity did not significantly differ between Pi and Pf, either on decaying leaves of seedlings or on those of adult plants (Fig. 6). After 16 weeks, the residual sporulation capacity was very low (<25 spores/pycnidium for Ew and <2 spores/pycnidium for Es) and did not differ between the conditions tested. No correlation was found between the clonal fitness traits (SPOmax, LAT, PYCdens, or nbSPO) and the residual sporulation capacity calculated for each set of isolates and each environmental condition at 13 and 16 weeks (*r*, >−0.21 and <0.29 [data not shown]).

TABLE 5 Analysis of variance for the mean daily conidial multiplication rate of 30 *Z. tritici* isolates[a] assessed on petri dishes in order to characterize the *in vitro* population and temperature effects

	Mean daily conidial multiplication rate			
Factor	df	MS	*F*	*P*
P[b]	1	89,259	0.7	0.398
T	1	1,491,030	339.5	**<0.001**
I(P)	28	121,187	27.6	**<0.001**
P × T[b]	1	229,738	1.9	0.184
I(P) × T	20	148,267	33.8	**<0.001**
Residual		4,392		

[a] Isolates I01 to I15 and I16 to I30 (see Tables 1 and 2) were assessed.
[b] The effect of P was tested against I(P), with the *F* ratio for MS_P calculated as $MS_P/MS_{I(P)}$, as with P × T.

DISCUSSION

An increase in *Z. tritici* aggressiveness was found over the course of the epidemic. This was consistent with the increase in disease intensity recorded in different fungal pathogens after only a few asexual cycles (29–32). Isolates of the final population (Pf) showed greater sporulation intensity under winter conditions, and a shorter latency period under spring conditions, than isolates of the initial population (Pi). This result suggests that such an increase in aggressiveness can be driven by seasonal fluctuations in environmental conditions. The difference in the latency period between the two populations, which was not significant under winter conditions but significant under spring conditions ($P =$

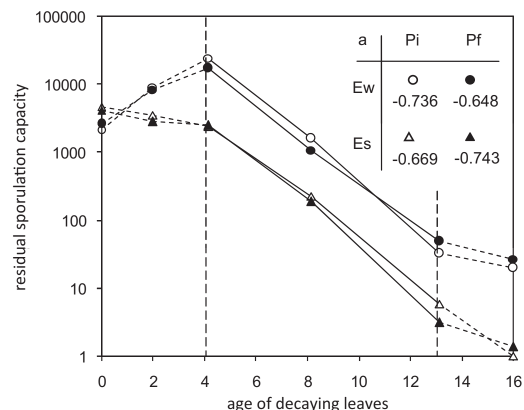

FIG 6 Decrease in residual sporulation capacity (in number of spores per pycnidium) on decaying wheat leaves inoculated with initial (Pi) and final (Pf) *Zymoseptoria tritici* populations. *a* is the slope parameter of the exponential-decay model of residual sporulation capacity [$\exp(-at + b)$] fitted to experimental data from 4 to 13 weeks after the first spore collection, where *t* is the age of decaying leaves, expressed in the number of weeks after leaf detaching. Ew, decaying L3 leaves (from seedlings placed at 8.9°C); Es, decaying F3 and F2 leaves (from adult plants at 18.1°C).

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0.1) (Table 3; Fig. 3), suggests that isolates with a shorter latency period might have been selected during the second part of the epidemic (spring), when the disease was propagated upward by splash-dispersed spores. During the period of stem extension, the longer the pathochron (a measurement of the number of leaves that emerge per latency period [27]), the more rapidly the disease spreads upward in the canopy (33). This may explain why the isolates with the shortest latency period were collected from upper lesions. At this epidemic stage, a short latency period is a key fitness trait that affords some isolates a definitive competitive advantage. Similarly, the difference in the maximum sporulating area and the density of pycnidia between the two populations, which was not significant under spring conditions but was significant under winter conditions ($P = 0.01$) (Table 3; Fig. 3), suggests that isolates with a greater sporulation intensity (a larger sporulating area and a higher density of pycnidia) might have been selected during the first part of the epidemic (winter), when disease progress is limited by the quantity of spores acting as a secondary inoculum (23). The difference between initial and final populations in the mean of fitness traits thus exhibits three striking features: (i) the pathogen responded synchronously to a biotic (host stage) and an abiotic (temperature) factor that characterized the two periods, winter and spring; (ii) the adaptation concerned two key fitness traits, sporulation intensity and latency period; (iii) adaptation to one trait (greater sporulation intensity) was expressed under winter conditions, while subsequently, adaptation to the other trait (shorter latency period) was expressed under spring conditions. Finally, these results, supplemented by the observed decrease in the between-genotype variance of the latency period under winter conditions, form a body of interrelated evidence for an adaptation pattern. This pattern suggests that short-term selection within the local population for key fitness traits is probably driven by seasonal environmental variations.

We established that changes in life history strategies can occur during a single epidemic, and we suggest that this is a result of selection. Only one population sampled on two dates (15 isolates per date) was included in this case study. However, this relatively small number of isolates was reasonable considering the complexity of the experimental design, which required all plant units (seedlings and adult plants) to be treated under the same conditions within the same period. The same spore suspensions had to be used, and all the isolates tested had to enter the experiment on the same day, placing constraints on the number of isolates that could be included in the experiment. An adaptation pattern was observed in 1 year only (2009 to 2010) and cannot be generalized. However, this year was characterized by particularly low inoculum pressure (23) and unfavorable climatic conditions. Greater epidemic intensity would probably facilitate stronger selection on a yearly scale. In particular, the difference in the latency period mean between populations observed under spring conditions might have been statistically significant at a higher significance level. Cowger and Mundt (15) showed that final populations exhibited greater fitness traits than initial populations in a particular year, while no difference could be detected in the next year, when the epidemic was considerably less intense. The high genetic diversity of *Z. tritici* populations and the geographic differentiation in important quantitative traits (e.g., thermal adaptation) suggest that the response to selection might be different for populations sampled in another area.

In line with some other studies (e.g., reference 34), we demonstrated that *in planta* investigation is required to identify fitness traits subjected to selection, epidemiological mechanisms involved in selective processes, and practical consequences of adaptive dynamics for disease development. Evidence of evolution within a local *Z. tritici* population was found under field conditions where there was competition between strains (22, 35). Strains of *Z. tritici* and *Rhynchosporium commune*, sampled either across different continents (10) or along a climatic gradient (36), respectively, showed thermal adaptation when grown in petri dishes. In the current study, the difference between results obtained *in planta* and *in vitro* demonstrates that the colony multiplication rate is probably not a relevant fitness trait. *In vitro* tests failed to account for the three specific features of thermal adaptation mentioned above: (i) the interaction of temperature and host stage is not accounted for; (ii) the sporulation capacity and latency period cannot be assessed separately; and (iii) the selection for different traits according to the season is not accounted for.

The development of complementary approaches would be more conclusive and informative than replication of the current experiment with a new set of *Z. tritici* field populations. *In vitro* miniaturized methods (e.g., in microtiter plates [37]) could be developed to increase the number of isolates or the number of populations tested. It should be taken into account that thermal adaptation can be investigated in two ways: first, by assessing differences in thermal performance estimated *in vitro* (using a more artificial fitness assessment, such as the growth rate) or *in planta* (using aggressiveness components such as lesion size, latency period, and sporulation capacity [see, e.g., reference 14]) and second, by assessing temperature sensitivity, derived by taking the ratio of performance trait values at high and low temperatures estimated *in vitro* (e.g., based on the growth rate [10]) or *in planta*. Sensitivity can be estimated by comparing key parameters of entire thermal performance curves (38) or comparing the temperature coefficient Q_{10} (39), for example, using just two temperatures. Here we compared different thermal performance trait values estimated *in planta* at high and low temperatures (the first way). The assessment of temperature sensitivity (the second way) will be carried out in further experiments by establishing thermal performance curves for the *in vitro* growth rate. This second experimental approach would be complementary to the current study.

Within-season effects on epidemiological dynamics. Our results challenge the implicit assumption that fitness traits of the active part of the pathogen population remain constant over the course of epidemics. Due to variations in key fitness traits, such as the latency period, sporulating area, and density of pycnidia (two components of sporulation intensity), the rate of disease development can be expected to increase slightly from the beginning to the end of an epidemic. Conversely, Shaw (13) observed an increase in the latency period at higher temperatures; he suggested that this may reflect adaptation of *Z. tritici* to cool summers and a physiological trade-off, in which the ability to grow fast at high temperatures would be traded for the ability to grow fast at low temperatures. We believe that this could be due to an artifactual field effect of the host stage (a shorter latency period on seedlings than on adult plants).

Our experiment set out to assess the fitness of isolates sampled from a local pathogen population causing a natural epidemic. "Mark-release-recapture" experiments based on artificial inoculations were performed with fungal pathogens of wheat, including

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Z. tritici (15, 35, 40, 41; see also the reviews by Zhan and McDonald [16, 17]). Under natural conditions, given the high diversity of *Z. tritici* populations (20), the earliest cohort of lesions is caused by different genotypes, and the probability of recapturing one of them on the upper leaves at the end of the epidemic is almost zero. With such a natural-conditions approach, it is even more difficult to detect competition in a single epidemic, since competition between strains is weak (13). One can be certain, however, that competition that is detected does exist under natural epidemic conditions and does not result from an artificial selection process. The mark-release-recapture approach could be useful, in a further step, for checking under different seasonal conditions whether the best competitors are the isolates with the shortest latency period or those with the highest sporulation capacity.

Between-season effects on epidemiological dynamics. We could hypothesize that selection occurs yearly, as shown by the potential increase in the rate of disease propagation through faster cycles and a higher sporulation capacity over the course of the season. The question is then, what happens during the interepidemic period? Are trait values and between-genotype variance reset, and if so, how? We raised the question of a trade-off between asexual reproduction during the epidemic period and subsequent survival. We showed that the residual clonal sporulation capacity on plant debris decreased similarly in the two populations studied, and we found no trade-off between the fitness of *Z. tritici* on green tissues and its ability to continue to reproduce clonally on decaying tissues and persist (lack of correlation between clonal fitness traits and residual sporulation). The pathogenicities of sexual and asexual spores of *Z. tritici* on wheat leaves were recently compared (42); significant differences were established. It can be further hypothesized that some trade-offs might exist between fitness traits on green tissues, relevant for the epidemic period (latency period and intensity of asexual sporulation), and the intensity of sexual reproduction on plant debris (quantity of ascospores) during the interepidemic period. Indeed, in a cyclical heterothallic pathogen with a dual sexual-asexual reproductive mode (43), such as *Z. tritici*, an Allee effect (disproportionate reduction in reproductive success at low population densities), interpreted as the difficulty in finding mates at low densities (44), can be suggested. At the end of epidemics of moderate intensity, a low pathogen density on upper leaves would decrease the likelihood that strains with compatible mating types will meet and mate, thus generating a low rate of sexual reproduction (22, 45) and low transmissibility of the strains selected during the season for their ability to propagate clonally. Moreover, crop debris left in the field comes from the very bottom parts of plants, while the upper parts are usually exported during harvesting (used as bedding for cattle). In some cases, the straw is left in the field and tilled into the soil, but this is not common practice in France. This could explain why the strains selected over the course of an epidemic and present on the upper leaf layers actually have a lower probability of persisting and of being transmitted, since the majority of the recombinants are present on the bottom parts of plants. The local, year-to-year perpetuation of these strains would be even more jeopardized by the fact that the next crop will not be wheat in most situations. Additionally, a possible genetic trade-off between clonal multiplication and an intrinsic ability sexually to reproduce cannot be ruled out.

Directional selection for increased aggressiveness during the epidemic period was established experimentally for *Phytophthora infestans*, but no evidence of any trade-off between aggressiveness and overwinter survival was found (31, 46). Further experimental investigations taking into account the pathogenic and saprophytic phases of the disease cycle (40, 41), guided by theory (43, 47), would be appropriate for characterizing the effect of seasonality on year-to-year disease transmission. Indeed, seasonality plays a role in short-term pathogen evolution by causing alternating periods of high transmission and population bottlenecks (8).

An alternative hypothesis to explain a kind of resetting of trait means and intergenotype variances during the interepidemic period relies on the immigration of novel genotypes into the local population from distant populations (22, 48, 49), strengthening the influence of the putative Allee effect.

Consequences of seasonal selection for long-term dynamics. The geographical variation in traits under thermal selection that many fungal pathogens show (50) has been widely explored in the context of climate change. Three types of responses to climate change have been described (51): dispersal to suitable habitats elsewhere (migration), change in phenotype distribution without a change in genotypes (phenotypic plasticity), and adaptation (change in population genetic composition), when some genotypes with greater fitness increase, while others decline, in frequency. There has been ample debate about which of these mechanisms contributes most to the observed shifts in phenotype distribution (see, e.g., references 52 and 53). In the case of *Z. tritici*, we showed that fitness traits can be both plastic and variable in local pathogen populations, so that their response to climate change can be hypothesized to imply both plasticity and adaptation by selection. We further detected high between-genotype variability in reaction norms, i.e., genotypes differ significantly in the way they respond to host stage–temperature combinations. Such a result is important, since it suggests that pathogen adaptation to a new set of environmental conditions (e.g., warmer winters) does not jeopardize adaptation to other conditions (e.g., summer conditions).

Experimental studies in "seasonality" would be useful for studying how plant pathogen populations may adapt to climate change. A consensual framework for studying how climate change will affect infectious disease recommends "incorporating intrinsic dynamics, spatiotemporal confounders, and small-scale temperature variability into models, and developing better concurrent data sets of disease, especially using combinations of laboratory, field, and mesocosm experiments to improve experimental realism" (54). Predictive models based on broad mean temperatures have been suspected of providing poor predictions of disease outcomes (55), because they fail to account for the effects of temperature fluctuations on pathogen development and the consequences of the nonlinearity of its response curves (14, 56). For Septoria tritici blotch, climate change was predicted to reduce the intensity of the disease in France by 2 to 6% (57); the significance of the prediction of such a small change is questionable, since this model does not take into account the adaptive potential of the pathogen population. Future plant disease response to climate change is still estimated by plant pathologists according to the assignment of the pathogen to different categories based on factors such as epidemic type, dissemination mode, and infection biology (58), a method that does not include the effect of biological features on adaptive potential (e.g., dual sexual-asexual reproductive mode). Such oversimplifying assumptions can be viewed as resulting from a lack of available experimental data. In this context, studies of seasonality enable the collection of relevant

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epidemiological data and the testing of hypotheses of short-term selection, because it is possible to experiment at the actual time on a small scale. Assessing adaptation by conducting cross-infection experiments, in which the mean fitness levels of sympatric and allopatric fungal populations are compared (59, 60), would be useful for predicting responses to thermal selection. This is crucial because recent models and experiments have suggested that the impact of temperature fluctuations on fitness may amplify the impact of climate warming on different species (61).

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