Environmental heterogeneity, a driver of adaptation to temperature in foliar plant pathogen populations?

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This dissertation is submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Paris-Saclay. The research therein describes a body of work performed at the French National Research Institute for Agriculture, Food, and Environment (INRAE) within the BIOGER (Biology and risk management in agriculture) and ECOSYS (Functional ecology and ecotoxicology of agroecosystems) research units from the Ile-de-France - Versailles-Grignon research centre.

CONTENTS

Chapter 1

Introduction

1

contents

preamble

Drawing up on phytopathology, micrometeorology, and ecology to investigate the responses of plant pathogen populations to their environment

Extensive prior research has shown that temperature is a climatic factor affecting all stages of infection of plants by pathogens. This environmental variable has therefore been taken into account in disease prediction and management models (Teng, 1985; Hau, 1990) in order to better understand, predict, and manage epidemics and thus reduce the crop losses they cause (Zadoks & Schein, 1979; Madden et al., 2017; Burdon & Laine, 2019). These models aim at describing the development of plant diseases in time and space but they tend to simplify the system, both on the side of the plant and on the side of the pathogen. Indeed, pathogen populations are often described using average responses, disregarding differences between individual phenotypes. Individuals composing a population are thus considered to be similar enough to be aggregated into a single-state variable glossing over the contemporary adaptive potential of pathogen populations in new environments (West et al., 2012). Moreover, host canopies are considered as a homogeneous abiotic environment, disconsidering for example the difference in temperature between canopy top and bottom. The impact of thermal heterogeneity on both demographic and adaptive dynamics is thus only limitedly considered.

These simplifications adopted in disease prediction models are challenged by a growing number of phytopathological, micrometeorological, and ecological studies. First, marked phenotypic plasticity and inter-individual variation in performance in response to temperature have significant fitness consequences (Wu & O'Malley, 1998; Bolnick et al., 2011). Second, spatial and temporal patterns of microclimatic variation in plant canopies are highly heterogeneous and deviate from standard mesoclimatic measurements commonly powering the models (Potter et al., 2013). Finally, microbial populations can adapt to their environments by natural selection (Houle, 1992; Stanton et al., 2000). This raises the question of the validity range to gloss over both heterogeneity of the environment and of the characteristics of individuals in aforementioned epidemiological models, especially in the context of climate change. This also argues in favour of an in-depth analysis of the consequences of ignoring vs. integrating more details of individual variation and environmental heterogeneity on population processes and adaptive dynamics. The aim of my thesis was to better understand the relative importance of these influencing factors, and investigate their consequences with a view to understand how they mediate the evolutionary trajectories of populations in variable environments.

1. Crop phyllosphere, a challenging environment for leaf microbiota?

1.1. The surface of leaves as a microbial habitat

Together with a diverse array of microorganisms, foliar plant pathogens inhabit the phyllosphere (Lindow & Brandl, 2003; Vorholt, 2012), i.e. the biome comprised of the aerial parts of plants (Ruinen, 1956). Once being considered as relatively inert, the leaf surface as a habitat for microbial growth went from being a 'neglected milieu' (Ruinen, 1961; Fokkema, 1991) to a model microbial habitat (Doan & Leveau, 2015). There are several explanations for this growing interest. First, the phyllosphere constitutes one of the largest habitats of microorganisms, with a global terrestrial surface estimated between 5.10^8 and 6.10^8 km² (Morris & Kinkel, 2002; Woodward & Lomas, 2004). Second, the phyllosphere microbiota has a critical role in plant function (e.g. nutrient acquisition, stress response, plant productivity; Stone et al., 2018) and in plant health (Box 1) going from suppression to enhancement of disease symptoms (Newton et al., 2010). Finally, the phyllosphere is particularly suitable for testing ecological concepts dealing with heterogeneity due the spatial organisation of the different microbial communities into discrete and dynamic patches (leaves). As such, plants can be seen as landscapes for leaf-associated microorganisms (Kolasa & Pickett, 1991; Billings et al., 1992; Meyer & Leveau, 2012).

Box 1. Plants get sick too!

Plant disease can be defined as any disruption of the normal development of a plant, preventing it from reaching its maximum potential, over a duration that is sufficiently long to cause disturbance or cessation of vital activity (Bos & Parlevliet, 1995; Döring et al., 2012). With effects ranging from mild symptoms of suboptimal plant growth to destruction of large crop areas accounting for major economic losses to agricultural production, this deviation from normal functioning of physiological processes result in significant crop losses (Cooke, 2006; Oerke, 2006; Savary et al., 2012) and pose a threat to global food security (Strange & Scott, 2005). This vision of 'plant health', the one of agronomists, may be seen as anthropomorphic for an ecologist for which both plant and pathogen have an interest for the functioning of the ecosystem. Although plant disease can also be caused by conditions external to the plant (e.g. nutritional deficiencies), my focus here is on the disruption which arises owing to microbial infection of a pathogen that establish a nutritional parasitic relationship with the plant (Van Der Plank, 1963).

1.2. In the face of multi-scale thermal heterogeneity

Microbial life in the phyllosphere is subjected to extreme and continuously changing spatio-temporal variation of environmental factors. This variation affects the experience of microorganisms and their interactions during their residence on the leaf (reviewed in Whipps et al., 2008; Meyer & Leveau, 2012; Vorholt, 2012). For instance, hot and dry weather conditions coupled with heterogeneities in solar UV-B radiation contribute to the change in the bacterial community composition in the peanut phyllosphere (Jacobs & Sundin, 2001).

Phylloclimate, the climate of leaves within a plant population (Chelle, 2005), includes temperature, UV radiation, relative humidity, and leaf wetness within the leaf boundary layer (Hirano & Upper, 2000; Lindow & Brandl, 2003; Whipps et al., 2008). Phylloclimate differs from mesoclimate at the local scale, but also from local microclimate at the scale of a plant canopy (Boulard & Wang, 2002; Fig. 1).

In particular, the deviation of leaf temperature from the characteristics of its surrounding physical environment (e.g. air temperature in the leaf boundary layers; Chelle, 2005) is due to the climate filtering by the plant (Nobel, 1999) through biophysical processes integrated into a leaf energy budget (Box 2; Gates, 1980; Nobel, 1999).

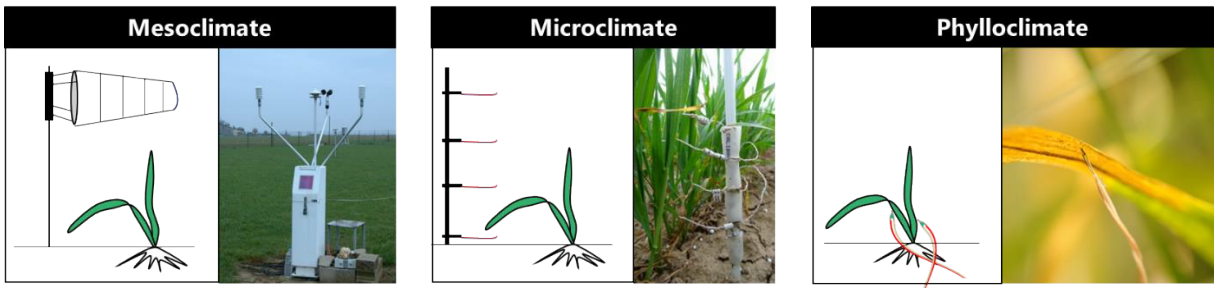
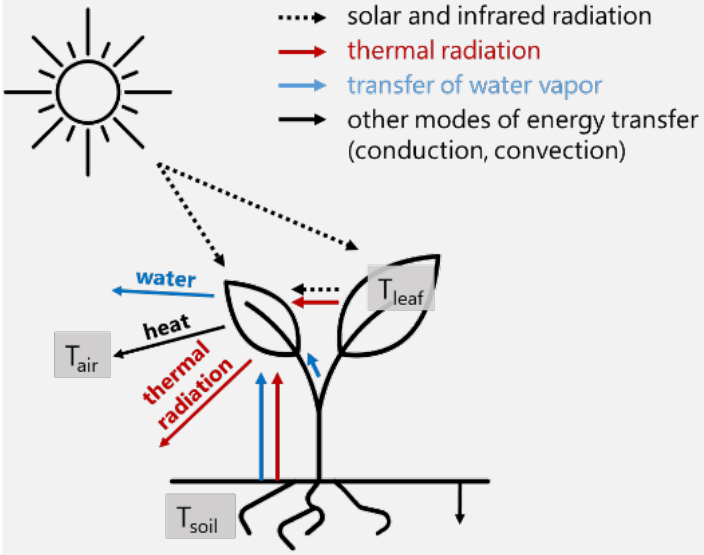


Fig. 1 Diving into the physical environment of crop canopies: meso-, micro-, and phylloclimate. Mesoclimate is defined by all the climatic factors at a height of 2 metres above a lawn. Microclimate corresponds to the same climatic factors measured at all levels of a given air layer inside or above a plant canopy. Phylloclimate deals with the climatic factors actually measured at the scale of aerial organs of a plant population (Chelle, 2005).

Phylloclimate is constantly changing, sometimes drastically, with strong and erratic patterns of variability both temporally (variation through time of environmental conditions) and spatially (variation in environmental patterns; Hirano & Upper, 2000; Whipps et al., 2008). Both aspects are included in this thesis under the umbrella term 'environmental heterogeneity' (Stein et al., 2014). This heterogeneity can be studied at multiple scales, from leaf to continent, and from a single point in time to seasonal or pluriannual changes. For example, variation ranges of temperature as high as 1 °C to 4°C have been reported within the same leaf of tomato and bean plants (Cook et al., 1964; Jones, 1999; Aldea et al., 2005) while leaf tissue temperature across apple tree canopies were measured to be up to 12°C warmer than the ambient air (Pincebourde & Woods, 2012)!

Box 2. How leaves produce phylloclimates

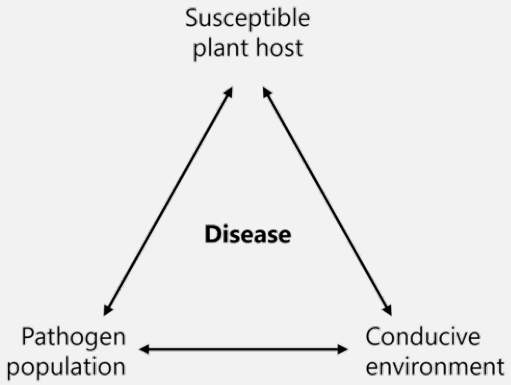


The temperature of individual leaves within crop canopies is the result of energy transfer (heat budget equation: absorbed radiation = reradiation + convection + transpiration; Gates, 1968, 1980). Many factors affect leaf temperature: local topography (Sears et al., 2011), canopy and plant structure (Sinoquet et al., 2001), surrounding environment (radiation level, wind speed, air temperature, and moisture; Chelle, 2005) and leaf-specific factors (size, orientation, shape, stomatal opening; Nobel, 1999; Michaletz et al., 2016).

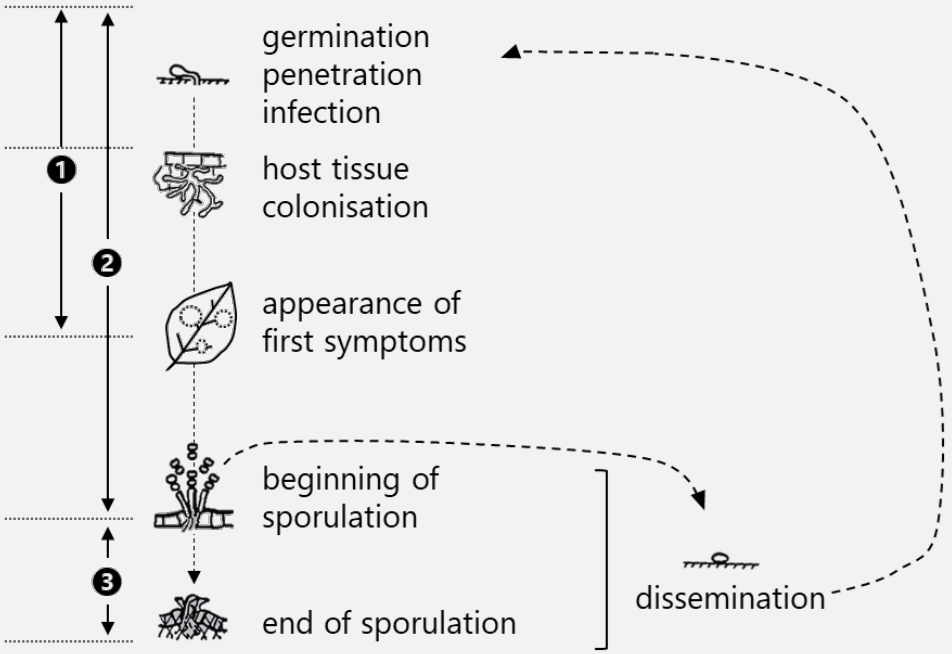
Box 3. Epidemiology, or disease development in plant populations

Disease development can be described as a spatio-temporal dynamic process whose epidemiological outcomes are conditioned by two conceptual frameworks (Zadoks & Schein, 1979):

- the disease triangle, which displays the three-way interaction between the factors that interact to cause a plant disease epidemic: a susceptible host plant, a virulent pathogen, and a conducive environment (see vertices);



- the disease cycle, which displays the continuous sequence or cycle of biological events leading to disease (repeated series of interconnected stages of pathogen life cycle).



❶ **Incubation period:** time between infection and symptom expression
❷ **Latent period:** time between infection and production of infectious units
❸ **Infectious period:** time between the start and end of production of infectious units

1.3. Impacts of temperature on foliar pathogens and diseases

Plant disease development is highly dependent upon weather factors (Friesland & Schrödter, 1988) that enable host infection (Colhoun, 1973; see the vertice 'conducive environment' in Box 3). For instance, plant diseases are expressed at lower levels when the environment is less favorable and even may not occur at all under non-conducive conditions (Dickson & Holbert, 1928; De Weille, 1965). Temperature (e.g. Shaw, 1990; Bernard et al., 2013), moisture (e.g. Hess & Shaner, 1987), free water on leaves (e.g. Mwakutuya & Banniza, 2010), wind (e.g. Pauvert, 1986), radiation (e.g. Austin & Wilcox, 2012), greenhouse gases such as CO_2 or ozone (e.g. Percy et al., 2002) are critical for a wide range of pathosystems. This sensitivity, which sometimes takes the form of threshold-level responses, can be linked to direct effects. These effects impact the host (e.g. rate of healthy leaf appearance; Parent et al., 2010), host plant resistance responses (e.g. expression of resistance genes; Huang et al., 2006; Melloy et al., 2014; Dossa et al., 2016), pathogenesis and survival of plant pathogens (e.g. lower infection success under extreme conditions; e.g. Karnosky et al., 2002), pathogen changes in parasitic behaviour (e.g. biotrophic or necrotrophic type of Septoria lactucae; Rapilly, 1991), and host-pathogen interaction (e.g. different response of host-pathogen gene pairs; e.g. Browder, 1986).

The climatic environment is characterised by a number of interdependent physical components. Among them, temperature is a major driving force affecting the growth and survival of microorganisms (Kinkel, 1997) through physiological processes due to the thermal sensitivity of most of biochemical reactions (Belehrádek, 1926; Campbell & Norman, 1998; Pörtner, 2002). Temperature has direct effects on all the developmental stages of foliar pathogens and on their interaction with plants (subsequent expression of disease). Temperature affects in particular the duration of incubation, latency, and infectiousness (Hallaire et al., 1969; Box 3). This dependence to temperature is such that the duration of certain stages is expressed in degree-days with a threshold that depends on each pathogen species. For instance, the range of latent periods reported in a recent meta-analysis of 18 pathogen species was found to be between 45 and 623 degree-days (Précigout et al., 2020).

Interestingly, while each pathogen species has its own ecological requirements (Andrews & Harris, 2000), there is a variability in temperature response within a species, and even between isolates of the same population (Milus et al., 2006). This affects competitive outcomes (Mizubuti & Fry, 1998) that may change over time and space due to the inherent environmental heterogeneity of leaf temperature. The problem at hand is thus more complex than at first glance: leaf microbiota must be viewed as communities of diverse populations inhabiting heterogeneous environments. The consequences of this perception have been mainly studied in theoretical population ecology. A growing number of studies have documented rapid species and community responses to environmental heterogeneity notably in terms of temperature conditions (Jacobs & Sundin, 2001). Interesting applications were also developed in predictive models of the evolution of antibiotic, fungicide, and host resistance. The challenge is now to better understand how both the diversity in populations and the heterogeneity of their environment may drive population adaptive dynamics.

2. Ecological consequences of living in a heterogeneous environment

2.1. Individual differences in response to temperature

Trait-based approaches (Violle et al., 2007; Zanne et al., 2019) tend to consider mean trait values to compare interspecific variability at the expense of intraspecific variability (within and between individual variability; Albert et al., 2011). This is problematic with regard to the question at hand given the evidence of phenotypic variation in a range of physiological traits across all organizational levels within a single species (populations → genotypes → individuals → instances; Fredrickson et al., 2011; Kreft et al., 2013).

This microbial diversity refers here to the degree of variation within a group and encompasses the plurality of individuals, distinguished among them by a differential characteristic that may be genotypic or phenotypic. At the population level, changes in the frequencies of the different variants reflect the evolutionary processes operating in a given environment. This is of particular ecological importance as this variation drives population performance particularly under stressful conditions and the extent of phenotypic adaptation to rapid environmental change (Forsman & Wennersten, 2016).

Phenotypic plasticity is the ability of genetically identical organisms to change their phenotype in response to environmental changes in space and time (Pigliucci, 2001). One interesting way to characterise it, together with inter-individual variation (Dall et al., 2012), is through comparative-based studies of reaction norms (Nussey, 2005; Dingemanse et al., 2010; Westneat et al., 2015). A thermal performance curve (TPC; Gibert et al., 1998; Kingsolver et al., 2001) is a reaction norm where the environmental variable related to an individual phenotype is temperature. Thus, TPCs highlight how individual performance varies as a function of the temperature to which individuals are exposed (Schlichting & Pigliucci, 1998). In the case of plant pathogens, this temperature-dependency of performance is typically well described by nonlinear and unimodal functions with the increase of the performance of the trait with temperature up to a peak value (single optimum) before decreasing with high temperature (e.g. Shearer & Zadoks, 1972; Pedersen & Morrall, 1994; Souza et al., 2012; Bernard et al., 2013 in the case of the latent period; Box 4).

Comparative studies of TPCs have provided evidence of individual variations in thermal responses for many organisms (Angilletta, 2009). In particular, previous studies have highlighted variations of three main modes of variations (Fig. 2): horizontal shifts, discriminating individuals adapted to cold and warm conditions; vertical shifts, discriminating high and low performers; simultaneous horizontal and vertical shifts, discriminating specialists and generalists (Kingsolver et al., 2001).

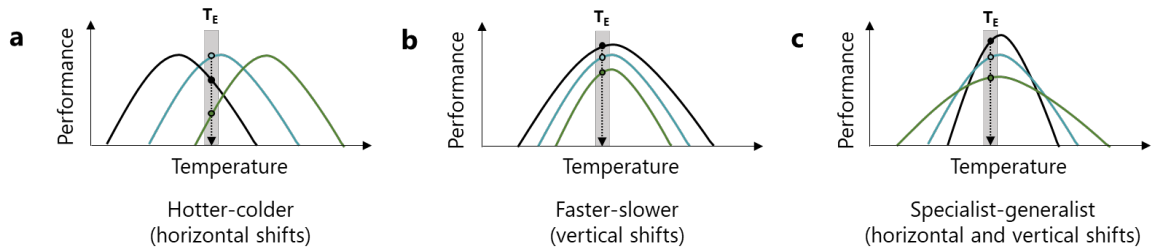
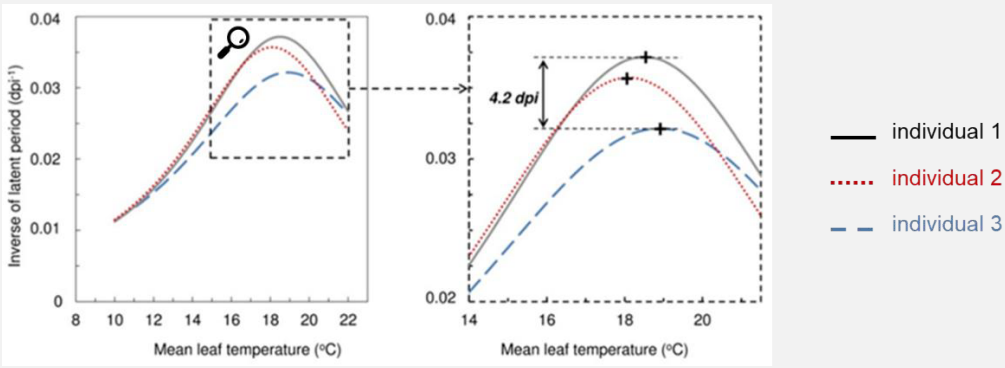


Fig. 2 Phenotypic variation is the norm. Documented patterns of individual variation in response to temperature: (a) hotter-colder; (b) faster-slower; (c) specialist-generalist shifts. These shifts in TPC highlight differences in thermal sensitivities that are expected to lead to differential selective pressures as illustrated by the differential performance of individual phenotypes along each shift at an arbitrary temperature of the environment T_E (arrow highlighted in grey).

Box 4. Thermal sensitivity (plasticity) vary between individuals within a same species: the case of the TPC for the latent period of Zymoseptoria tritici.

Such nonlinear TPCs have been highlighted in phyllosphere microorganisms notably in plant pathogens regarding their latent period, growth, and sporulation. For instance, the inverse of latent period of the ascomycetes Zymoseptoria tritici and Botrytis cinerea increases with increasing temperature, to a maximum value reached around 18-20°C, then it decreases rapidly as temperature increases further (Sosa-Alvarez et al., 1995; Bernard et al., 2013).

In Z. tritici, a differential relationship between latent period and mean leaf temperature for three individuals of a foliar Z. tritici pathogen population was found with an interindividual difference of up to 4.2 days with respect to their optimum latent period (figure from Bernard et al., 2013 where the latent period is expressed as the number of days post-inoculation to reach 37% of final sporulating area).



2.2. Differential selective pressures act on the thermal sensitivities of individuals

The differential plasticity of individual traits may lead to differential selective pressures under a given set of environmental conditions (as depicted in Fig. 2). Indeed, the environment determines the performance level that is expressed by an individual and affects the relationship between individual performance and fitness. As such, environment and its fine-grained variation may determine natural selection on performance curves operating on fitness landscapes of thermal sensitivities (Kingsolver & Gomulkiewicz, 2003).

Natural selection, as a driver of evolution, occurs when heritable phenotypic variation exists and has differential effects on fitness between individuals. It may favour one phenotype over another (Vellend, 2010). Selection thus concerns individuals but affects the phenotypic composition of a population. In nature, phenotypic selection is both common and strong (Kingsolver et al., 2001; Hoekstra et al., 2001), to the extent that it represents a significant proportion of the variation in fitness (Hereford et al., 2004). Selection on phenotypic plasticity occurs on the variation of reaction norms in a population and eliminates the ones that produce less efficient phenotypes for the range of occupied environments (Fig. 3). Three main types of selection pressure exist in the phyllosphere: those exerted by climate, by the plant and by the microbiota already present on the leaf (Vacher et al., 2016).

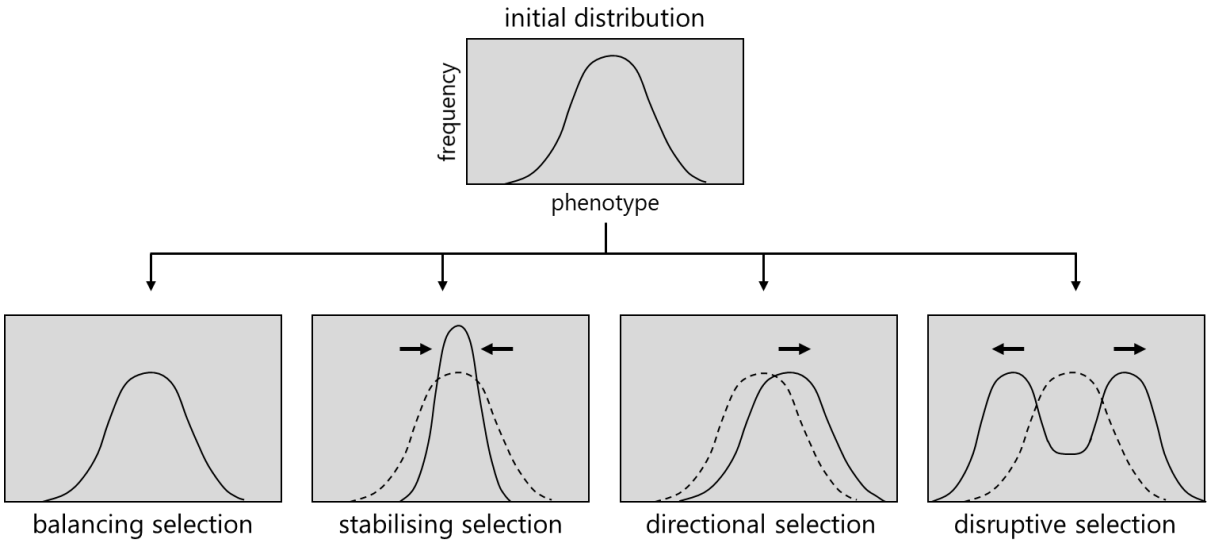


Fig. 3 Changes in the population-level distributions of quantitative phenotypes depending on the main types of selection acting on quantitative phenotypes. The different types of selection are categorised based on their impact on initial phenotypic distributions. The arrows illustrate the direction selection is shifting population distribution (i.e. the trait values that will be positively selected).

2.3. Current simplifications in disease prediction models

Heterogeneity in environmental factors may have substantial ecological implications with respect to diversity at different spatial and temporal scales. This heterogeneity has long been theorized to contribute to the maintenance of diversity with extensive work on plant communities (Ricklefs, 1977; Tilman, 1982; Palmer, 1994), notably by providing refuges from adverse conditions thus promoting persistence and coexistence (e.g. Kallimanis et al., 2010; Fjeldså et al., 2012).

Here, I consider 'population responses' to heterogeneous environments, with respect to their dynamics (changes in size) and their evolutionary trajectories (changes in composition; Hanski, 1998; Alexander et al., 2012). This topic has been a long standing focus and remains a central goal in the field of theoretical ecology (Fisher, 1937) whether based on: (i) quantitative genetics models aiming to study the maintenance of a polymorphism, under a balance between diffusion and selection (Endler, 1973; Slatkin, 1973; Nagylaki, 1975; Gavrilets, 1997); (ii) demographic model focusing on population dynamics and deviating from Fisher (1937) and Skellam (1951) models.

To date, the research on responses of plant pathogen populations to environmental conditions has focused on population changes in size rather than in composition despite evidence of local adaptation in nature (Kraemer & Boynton, 2017). Local adaptation results from divergent selection to local conditions (Kawecki & Ebert, 2004) with sometimes striking adaptation patterns to temperature (Boenigk et al., 2007; Zhan & McDonald, 2011; Mboup et al., 2012). This generally occurs when there is a genotype-by-environment interaction and when the ranking of phenotype-fitness change occurs in the right direction (home-field advantage of resident vs. immigrants; Fig. 4).

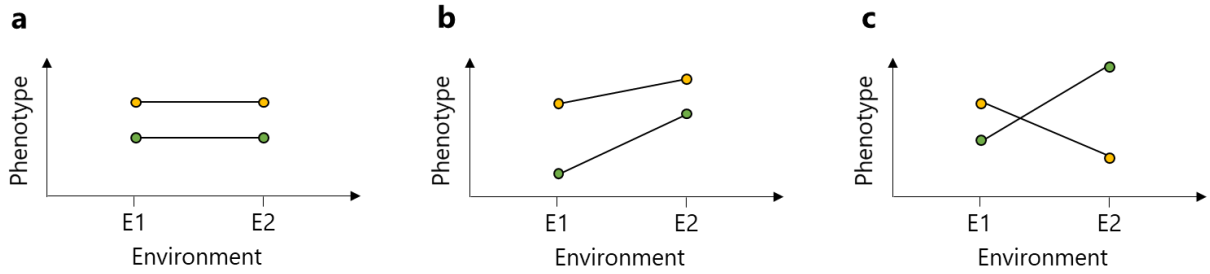


Fig. 4 Illustration of (mal)adaptation patterns to local conditions. (a) no local adaptation; (b) asymmetric local adaptation with only one of the populations experiencing higher fitness in its home environment; (c) local adaptation or maladaptation both in the sense of the home vs. away and local vs. foreign framework depending on if the 'yellow genotype or population' ('green genotype or population', respectively) was collected in 'environment E1 or E2'.

Plant disease prediction models are generally based on three simplifying assumptions which may cause limitations for prediction:

1 - 'One species, one thermal response': the thermal plasticity of all representatives of the pathogen species is summarized in a single thermal optimum value, threshold function or reaction norm, neglecting within- and inter-individual variability in most phenotypic trait databases (Albert et al., 2010; Sierra et al., 2019). All individuals are however not identical in their response to temperature (Logan et al., 2014).

2 - 'Invariance of responses to local environmental conditions or changes in climate': no consideration of adaptive potential over both geographical range and evolutionary time (West et al., 2012) despite experimental evidence of substantial variation in local conditions (Santini & Ghelardini, 2015). Environmental heterogeneity is however considered as one of the main drivers of the maintenance of genetic diversity in natural populations and their evolution (Hedrick, 1986; Ravigné et al., 2009; Mboup et al., 2012). It can thus lead to local adaptation patterns in host-parasite interactions (Nuismer & Gandon, 2008).

3 - 'No consideration of variation in temperature': mean temperatures acquired at large scale (e.g. weather station data) are not very revealing of those that prevail in the various strata of crop canopies. They also fail to account for the impact of temperature fluctuations that have been shown to affect pathogen development (Scherm & Van Bruggen, 1994; Truscott & Gilligan, 2003) and may amplify the impact of climate warming on different species (Vasseur et al., 2014).

As possible sources of error, the three aforementioned simplifications may affect predictions of population dynamics at range edges, especially in the context of climate change (Pearson et al., 2009). Overcoming the current lack of experimental data (Chakraborty, 2013; Fig. 5) is needed to better understand inconsistent findings (Krenek et al., 2012; Sternberg & Thomas, 2014) and finally to estimate how sensitive the models are to those simplifications. This gap can be narrowed based on a combination of "laboratory, field, and mesocosm experiments to improve experimental realism" (Rohr et al., 2011).

This would help to inform the effect of climate change on plant diseases (Boonekamp, 2012) whether regarding their development, their occurrence, and their geographical distribution (Chakraborty & Newton, 2011).

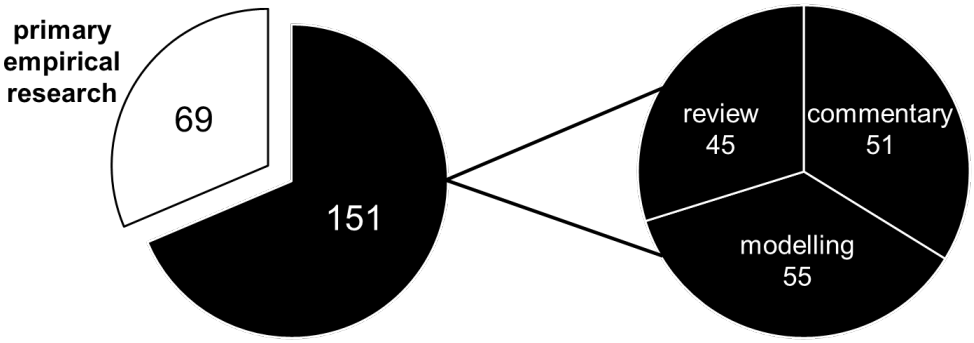


Fig. 5 State-of-the-art highlighting the lack of experimental data to tackle thermal adaptation of plant pathogen populations in the climate change context (adapted from Chakraborty, 2013)

3. From individual-level processes to population responses

3.1. Adopting an individual-based, bottom-up approach

Inter-individual variation has often been neglected in favour of the search for general patterns at the interspecific level (Wright et al., 2004). This went hand in hand with the establishment of protocols tailored to collect measurements minimising variations contained within species in favour of variations between species (Cornelissen et al., 2003). One can however observe phenotypic variation across all organization levels such as among individuals within populations, among populations within species and among species themselves. This is of particular ecological interest and importance as this variation may drive population performance and adaptation to rapid environmental change (Fox et al., 2019), particularly under stressful conditions (Forsman & Wennersten, 2016).

Given the important role individual characteristics (e.g. spatial location and fitness; Kinkel et al., 2002; Monier & Lindow, 2003; Grimm & Railsback, 2005) play in microbial interactions and in ecological processes in the phyllosphere (Durrett & Levin, 1994), individual variation must be accounted for.

3.2. The value of a phenotypic approach

As pointed above, selection acts on individual phenotypes and occurs in population when heritable phenotypic variation exists and has differential effects on the fitness between individuals. Comparing phenotypes based on TPCs is thus a particularly powerful approach as the concept of thermal performance curve:
- forms an unifying, conceptual framework for characterising differences in thermal plasticity at different organizational levels;
- allows to quantify functional diversity (Walker et al., 1999), that is to say functional richness, evenness, and divergence (Mason et al., 2005) based on the computation of similarity and

differentiation measures (Chao & Chiu, 2016), and to establish functional groups highlighting specialization levels (Devictor et al., 2010) on which selection can exert (Farine et al., 2015);
- facilitates the detection of selective dynamics (genotype frequency changes; Lande & Arnold, 1983) based on strength, mode, and occurrence of phenotypic selection (Brodie et al., 1995), using quantitative measures of selection (Table 1) exerted on a trait, acting both on phenotypic polymorphism or molecular diversity (Futuyma, 1998).

Table 1 Quantitative indicators of selection

Quantitative measure	Definition
Opportunity for selection	Variance in relative fitness, i.e. survival and/or reproductive rate of a phenotype relative to the maximum rate of other phenotypes in the population
Selection differential	Total change in the mean phenotype within a generation, i.e. difference in mean trait value between those that reproduce and the original population
Selection gradient	Gradient of a regression of deviation of a trait against fitness (e.g. negative values indicate stabilizing selection, which reduces the variance of the trait)
Selection coefficient	Difference in relative fitness expressing the relative strength of selection acting against a genotype, i.e. a measure of the fitness of one phenotype relative to a reference phenotype

3.3. Current concepts and challenges to characterise population responses to heterogeneous thermal environments

To avoid error in measurement of phenotypic traits that could affect estimates of selection (Kingsolver & Diamond, 2011; Morrissey & Hadfield, 2012), the current challenges to gain insight into microbial population responses to the thermal conditions occurring in crop phyllosphere are three-fold. They concern the accurate characterisation of the habitat (thermal environment), of its inhabitants (individual phenotypes), and of underlying adaptation/selective patterns and processes. They are summarised in Table 2.

Table 2 Current stumbling blocks to consider when investigating microbial population responses to leaf thermal environments

Challenge	Insight	Further reference
Characterisation of the thermal environment		
Nature of measurement	Incident radiation raises the surface temperature of the leaf above the air temperature (excess leaf temperature) → need for monitoring thermal conditions at the scale actually perceived by phyllosphere microorganisms	(Potter et al., 2013; e.g. for the phyllosphere: Cook et al., 1964; Pincebourde et al., 2007)
Resolution grain	Meteorological conditions vary greatly over very small scales between canopy microenvironments → spatio-temporal variation in environmental conditions, disturbance and connectivity (dispersal) must be accounted for	(Stein et al., 2014)
Methods	Quantifying local variation in temperature can be done through field instrumentation but the direct and indirect experimental characterisation of phylloclimate remains challenging (e.g. invasiveness, logistic constraints, modification of plant growth, estimation of many leaf parameters)	(Chelle, 2005)
Characterisation of individual phenotypes (TPC): see Box 5 for past and current overview of relevant thermal biology studies		
Choice of proxy for fitness	The fitness traits under study will not necessarily have a similar effect on the components of performance (growth, reproduction and survival) or be affected similarly by temperature	(Huey & Stevenson, 1979; Wiser & Lenski, 2015; Sinclair et al., 2016)
Lack of high-throughput phenotyping protocol	Particular attention should be given to measurement error, repeatability and standardized conditions to avoid potential biases in the measurement of performance likely to magnify or counteract differences between replicates and/or individuals	(Birgander et al., 2018)
Range and resolution of the thermal conditions to be studied	Particular attention should be given to the range and resolution of the thermal conditions under which data is acquired (notably with regard to the location of the thermal optimum) as well as to the choice of models to represent TPC so that they accurately describe experimental data (strong influence of TPC curve shape such as skewness on population responses)	(Low-Décarie et al., 2017)
Choice of model to establish TPC		(Quinn, 2017; Hurford et al., 2019)

How to group functional responses?	ecological specialization is highly context-dependent and inconsistently used in applied ecology → how to account for population-level outcomes? how to modify the covariance between groups of highly similar individuals?	(Devictor et al., 2010)
Characterisation of adaptation/selective patterns and processes		
Definition of the sampling scheme for estimation of baseline frequencies	Sampling snapshots should consider both temporal and spatial adaptation when warranting investigation and follow a rationale sampling to test specific hypotheses (instead of a culture collection approach randomly taking few isolates from each of many disparate locations)	(Gosden & Svensson, 2008; Siepielski et al., 2009, 2013)
Adequate replication or sampling size	Statistical inference quality (unperfect detection inherent to sampling) has consequences on estimates of selection (e.g. the tragedy of the uncommons), and as such, has to be tackle (conditional ability to detect a process)	(Conner, 2001; Bent & Forney, 2008; Waller & Svensson, 2016)
Choice of approach to detect adaptation	Different approaches of contrasting power to detect local adaptation can be distinguish: from low (population genetics and structure) to high (common-garden or reciprocal transplant experiments) → which one to choose? for which question?	(Kawecki & Ebert, 2004; Latimer et al., 2015)
Design of selection experiments	The experimental design should be able to attribute the selective response to the selective pressures or genetic drift under investigation to not miss the response to selection	see review for field studies in Pujol et al. (2018)

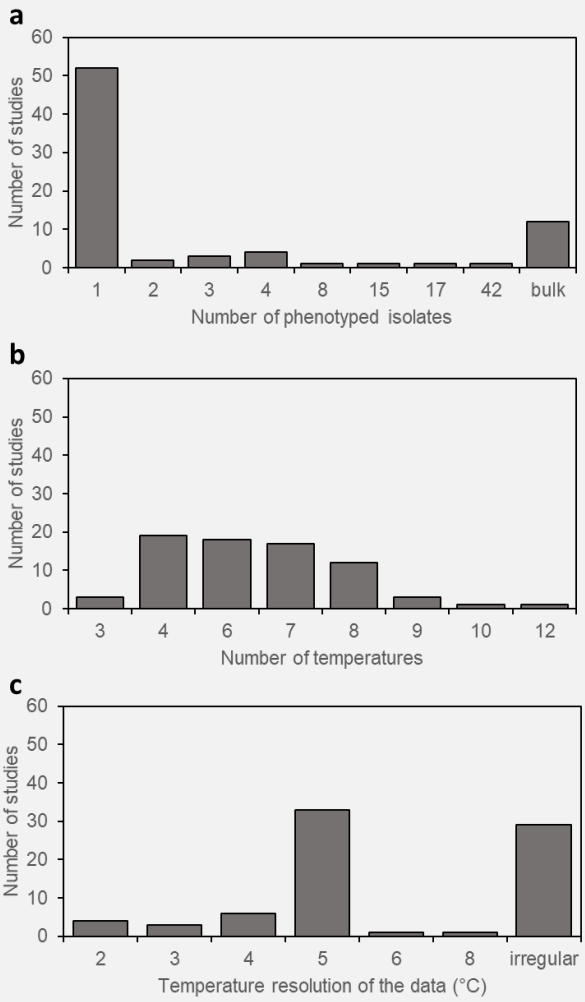
Box 5. Literature searching for experimental designs used to investigate in planta thermal responses of plant pathogens.

The aim of this review of literature was to comprehensively investigate how thermal responses of plant pathogens are determined in experimental studies. Relevant peer-reviewed articles were searched on the 'Web of Science' and 'Google Scholar' using the query "fung* AND plant disease AND (temperature effect OR thermal response) AND inoculation AND visual assessment AND development". Only studies that focused on standardised inoculation procedure on entire plants or detached plant organs under characterised environmental conditions were retained. The results presented here focused on a remaining of 77 articles (see supplementary references S1) after subsequent exclusion of studies screening less than three temperatures. This review of the literature gives an overview of the different experimental approaches adopted to determine thermal response curves of plant pathogens and their limitations:

- (i) they generally monitor a proxy of the temperature actually perceived by fungal pathogens (air instead of leaf temperature, but see Bernard et al., 2013);

- (ii) they are often conducted for a single isolate whose response is taken as representative of the species specificities (a; no consideration of individual variation, but see Roustaee et al., 2000; Mariette et al., 2016);

- (iii) the temperature range and resolution over which data are collected are often insufficient to avoid overfitting (number of temperatures measured vs. number of parameters in the TPC equation) and/or accurately quantify thermal responses (b-c; supra and suboptimal temperature ranges are often under-represented in existing data sets; Quinn, 2017; Low-Décarie et al., 2017).



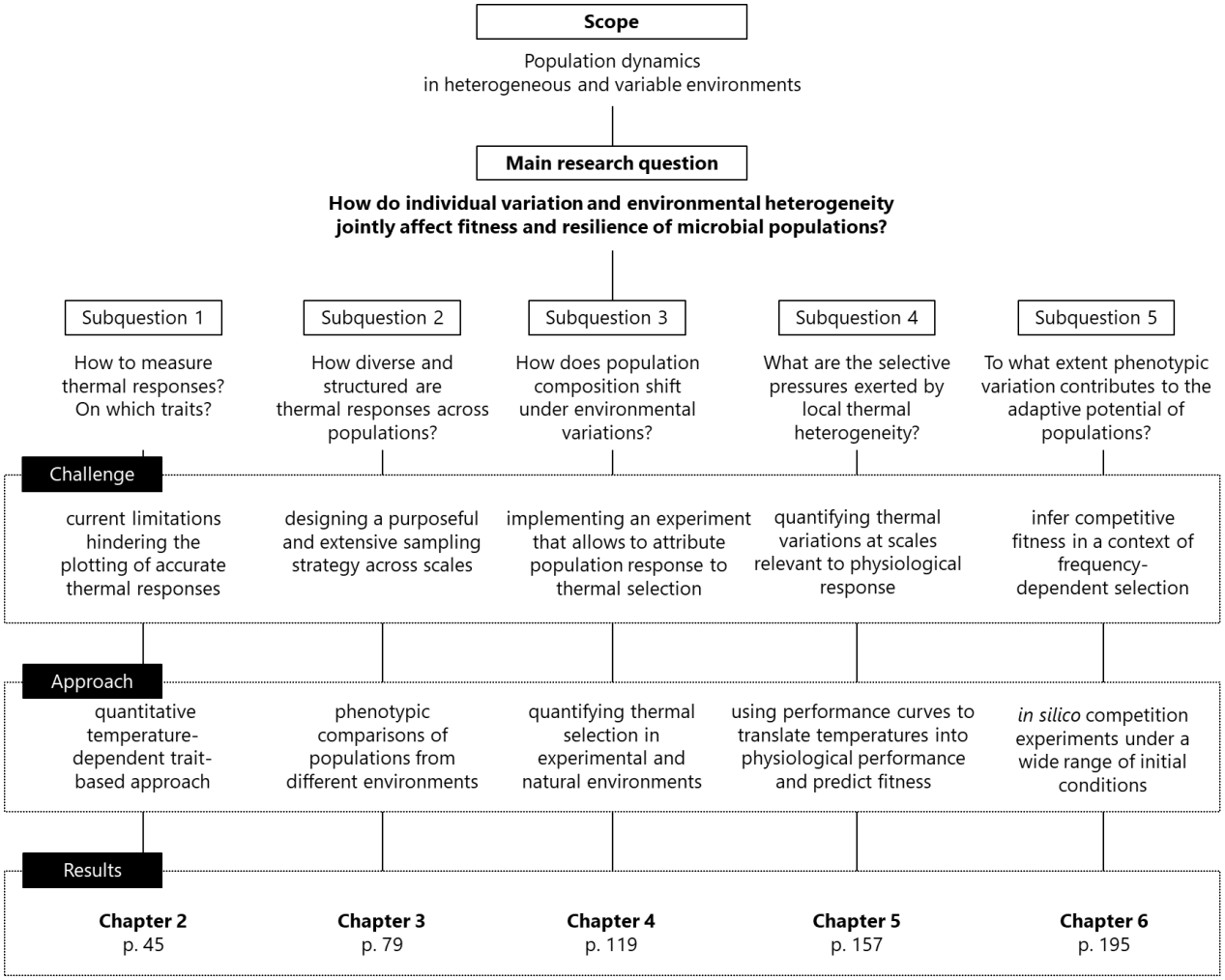


Fig. 6 Research question-driven structure of the thesis.

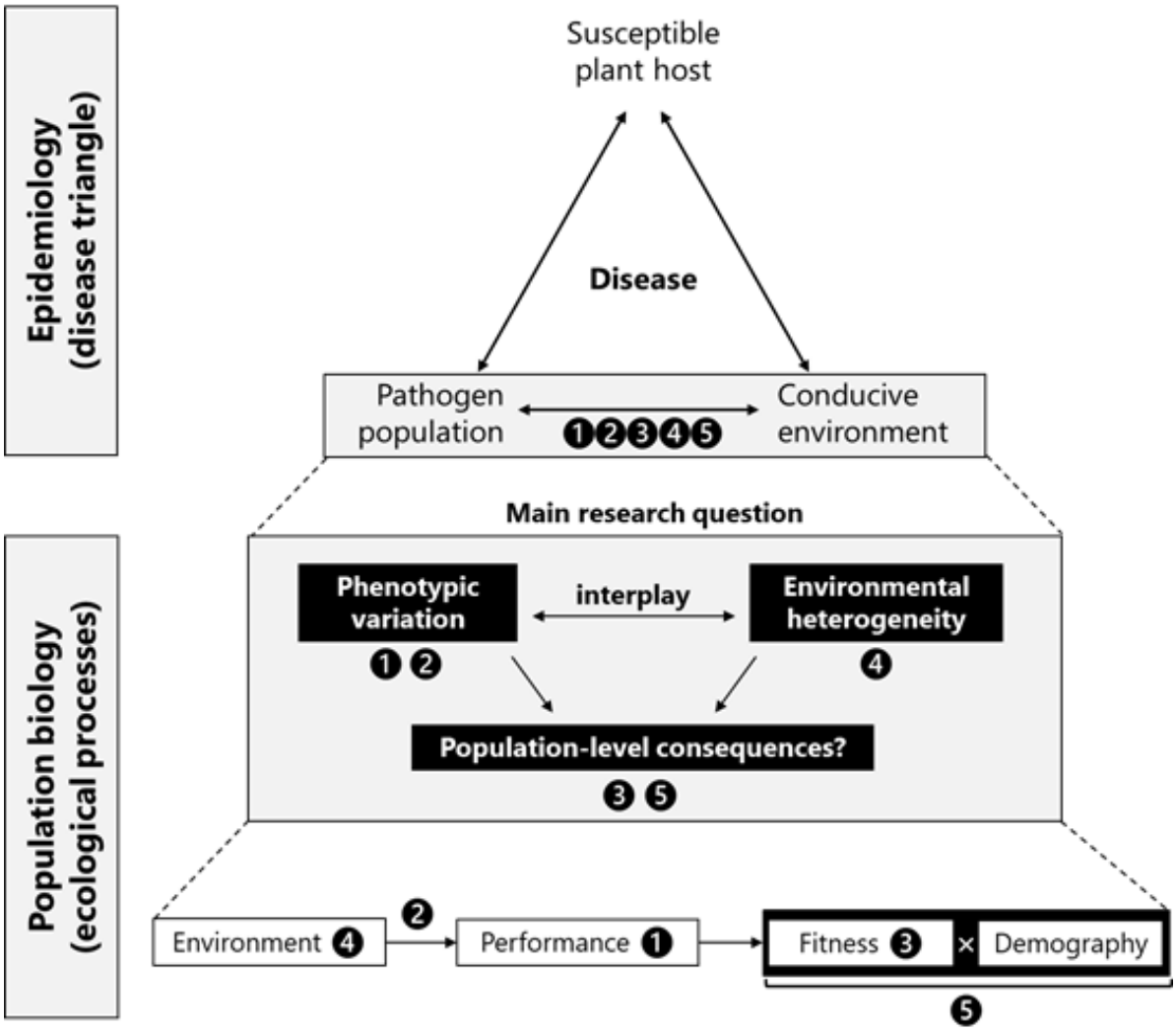


Fig. 7 Position of the research questions ❶ to ❺ with respect to the strategy of the thesis.

4. Research strategy

4.1. This thesis in context

The overall goal of this thesis was to investigate how populations respond to heterogeneous environments. In particular, my objective was to explore how within- and inter-individual phenotypic variation may drive the local adaptation of populations in response to environmental variations, focusing on natural selection and population-level fitness. This required to investigate patterns, processes, and functions, following five main roads that correspond to the following nested research questions:

❶ How to quantify, describe, and categorise phenotypic variation? (Chapter 2)

❷ What is the level of phenotypic variation in natural populations and how is it structured along spatio-temporal gradients? To what extent does it reflect adaptation to the environment? (Chapter 3)

❸ Are there changes in the phenotypic composition of local populations in response to environmental variations over short time-scales? If so, what are their magnitude and direction? (Chapter 4)

❹ What are the selective pressures exerted by a heterogeneous environment at a small spatial scale? (Chapter 5)

❺ How and to what extent does individual variation affect dynamics, fitness, and resilience of local populations exposed to a heterogeneous environment? (Chapter 6)

In the five main chapters of the thesis, in vitro, in planta, in natura, and in silico experiments were successively, sometimes concomitantly, conducted to address the aforementioned research questions by bringing each a slight change in focus from descriptive patterns to ecological processes. The multi-faceted approaches chosen for this exploration of population adaptation to their thermal environment are complementary in the way they (i) frame the topic in different ways (Fig. 6), (ii) address the different challenges inherent to tackle those questions across a range of spatio-temporal scales (Fig. 7), and (iii) provide different strengths of evidence under multiple and increasingly complex selective environments (Table 1).

Table 3 Overview of the experimental approaches used for investigating phenotypic variation in thermal sensitivity and its population-level consequences

	Chapter 2	Chapter 3	Chapter 4	Chapter 5	Chapter 6
General settings					
Approach	in vitro-in planta	in vitro	in vitro-in planta-in natura	in silico	in silico
Thermal regimes	constant	constant	constant - fluctuating	fluctuating	fluctuating
Environmental conditions[1]	laboratory	laboratory	laboratory, growth chamber, -field	field	field
Populations under investigation (individual-to-group-to-population covariance)					
Reseach sample	natural populations	natural populations	experimental populations	experimental populations	virtual populations
Sampling scales	French climate regions	European climatic zones	local site	local site	local site
Measures of adaptation of individuals and populations to their local environment					
Matter of investigation	patterns (comparisons)	patterns (comparisons)	processes (competition)	mechanisms (competition)	mechanisms (competition)
Time frame[2]	monocyclic	monocyclic	polycyclic	polycyclic	polycyclic
Fitness components[3]	major in vitro and in planta quantitative traits	in vitro growth rates	in planta development rate and offspring production	in planta development rate	in planta offspring production
Scope					
Main disciplines	Thermal biology	Functional ecology	Epidemiology Evolutionary ecology	Bioclimatology	Evolutionary ecology

[1] simplicity and control of experiments to reach complexity and ecological realism depending on if the investigations were conducted in laboratory (batch cultures), growth chamber (phytotrons) or field (wheat microplots) investigations

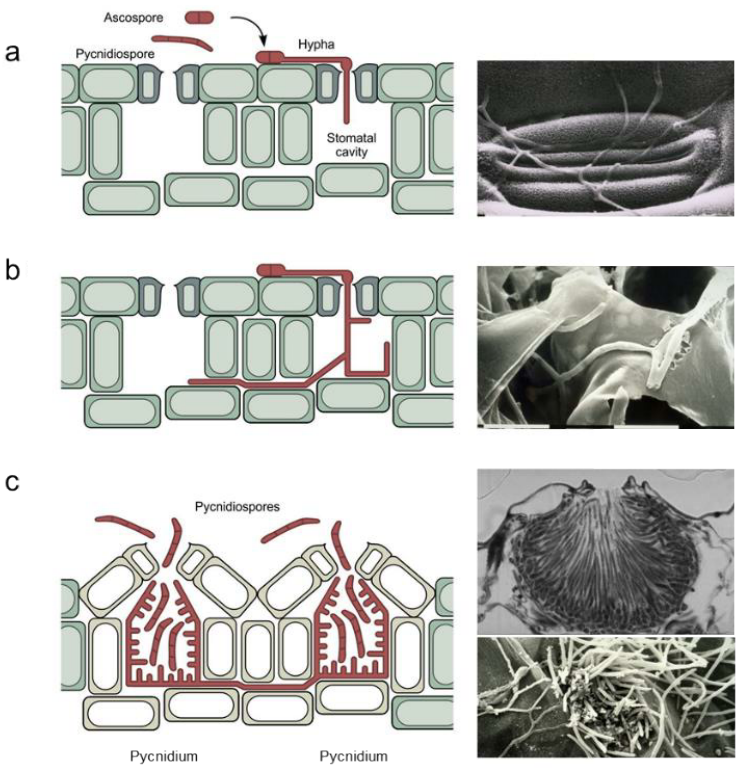
[2] duration of the experiment: monocyclic (intrinsic development or single infection cycle) or polycyclic (multiple infection cycles)

[3] traits on which competitive ability of strains is inferred

4.2. Relevance of the Z. tritici – wheat – temperature case study system

This research being conducted at the French National Research Institute for Agriculture, Food and Environment (INRAE), agronomic issues first motivated this work with a view to better understand the responses of plant pathogen populations to the environmental variations they experience, keeping in mind the context of global climate change. I addressed this topic on the specific case of the fungal foliar pathogen Zymoseptoria tritici (formerly Mycosphaerella graminicola; Quaedvlieg et al., 2011), the causal agent of septoria tritici blotch (STB; Box 6). This wheat disease, whose symptoms are necrotic lesions, filled with black fructifications (Box 7), that coalesce over time, causes recurrent yield losses on a pluriannual scale (Shaw & Royle, 1993; Dean et al., 2012; Suffert et al., 2018). Z. tritici is exposed to a wide range of temperature variations in space and time. In particular, its worldwide distribution in wheat growing areas make more relevant and effective the analyses of adaptive patterns of populations sampled in contrasted climatic zones. STB epidemic period spans from late fall to early summer (Box 7; Eyal, 1999; Fones & Gurr, 2015; Suffert et al., 2018). This allows to investigate the impact of fluctuating environmental conditions arising from seasonality. Furthermore, Z. tritici appears as a good candidate given the prior state-of-the-art of its responses to temperature. Indeed, previous studies have provided important information on several salient facts suggesting that thermal selection is likely to occur and results in signatures of local thermal adaptation: (i) existence of phenotypic variation with wide variability in thermal responses across individuals, populations, and scales (from local seasonal dynamics to wide geographic range; Zhan & McDonald, 2011; Suffert et al., 2015); (ii) heritability of phenotypes in terms of thermal responses (Lendenmann et al., 2016); (iii) temperature-dependent variation in competitive in planta fitness among strains notably in latent period (Bernard et al., 2013) which is particularly long in the case of Z. tritici (Précigout et al., 2020).

Box 6. The ascomycete fungus Zymoseptoria tritici, the causal agent of Septoria tritici blotch, a globally distributed foliar disease on wheat. Three phases form the infection cycle of Z. tritici: (a) Ascospores or pycnidiospores land on a wheat leaf, where they germinate and enter the leaf tissue via stomata (germination and penetration, 1 dpi), (b) colonize surrounding plant tissue (colonization, 2-4 dpi), (c) grow spikes and pycnidia which begin to develop and mature under stomatal apertures (pycnidium formation, 5-9 dpi, and maturation, 10-21 dpi). Adapted from Steinberg (2015) and G.H.J. Kema (photographs).



Box 7. Septoria tritici blotch symptoms and epidemics.

The Z. tritici – wheat – temperature system was chosen as a biological model to study the impact of multi-scale environmental variations on the annual dynamics of crop phyllosphere microbial populations. Z. tritici populations cause recurrent epidemics of Septoria tritici blotch (STB) on wheat which consist of repeated complete infection cycles of pathogen development (Box 6). STB epidemics are initiated by wind-dispersed Z. tritici ascospores (sexual spores), mostly discharged from fruiting bodies produced on wheat debris from the previous season during the period between successive crops (Suffert et al., 2011). During the plant-growing season, Z. tritici is clonally propagated by pycnidiospores (asexual spores), which are splash-dispersed to new susceptible sites upwards, initiating new infections from old, lower diseased leaves to younger, higher leaves (Shaw, 1987). The rate of spread of the epidemic is determined by the number of asexual, embedded secondary cycles of infection (usually estimated to range from 5 to 7, depending on climatic conditions). One to two sexual cycles and hence several ascospore generations can occur in a growing season (Kema et al., 1996). This polycyclic dynamics (see symptoms and disease cycle of Z. tritici on wheat below; figure from O'Driscoll et al., 2014) leads to the segregation of new from older generations facilitating successive samplings over separate generations in natural field settings.

4.3. A multi-faceted, interdisciplinary approach

In this work, I have chosen to dive into different factors driving population responses to temporally and spatially heterogeneous thermal environments. Going from tackling phenotypic variation between individuals to population patterns and processes has required to mobilize a population biology viewpoint that integrated plant disease epidemiology and ecological concepts, using approaches picked up from thermal biology, functional ecology, evolutionary ecology, and bioclimatology. This progression in the research strategy was made possible and even fostered by the choice of the wheat – Z. tritici – temperature pathosystem as a case study, which facilitated the characterisation of:

- the environmental variations experienced by an individual as Z. tritici is an ectotherm (its internal temperature directly reflects the surrounding environment; Angilletta, 2009) and sessile organism (this second characteristic makes it easier to quantify the actual environmental conditions to which they are subjected, e.g. in comparison to arthropods for which one has to consider also behavioural thermoregulation; Caillon et al., 2014). Furthermore, Z. tritici has a patchy distribution in the wheat canopy at the field scale (upward progression from lower diseased to upper healthy leaves; Shaw, 1987) for which there are available measurement systems of leaf temperature (the phylloclimatic conditions actually perceived by phyllosphere organisms; Chelle, 2005);

- the temperature dependence of multiple fitness traits for which the Z. tritici research community has available in vitro and in planta phenotyping protocols (Pijls et al., 1994; Zhan & McDonald, 2011; Bernard et al., 2013; Suffert et al., 2013, 2015);

- adaptation patterns as Z. tritici populations have large population sizes and exhibit substantial genetic diversity at all scales (Linde et al., 2002);

- the eco-evolutionary processes underlying the dynamics of Z. tritici natural populations as these latter are characterised by several generations (embedded infection cycles; Eyal, 1999) over a growing season and by successional dynamics (vertical progression of STB within wheat canopies, thus facilitating segregated samplings of new from older Z. tritici generations);

- population changes under different environmental regimes by experimental evolution as Z. tritici is characterised by large populations, an ease of culture both in vitro in the laboratory and in planta in growth chambers and in the greenhouse, short generation times, and genetic tractability; all contribute to making it a useful model organism for conducting in-depth investigations of population processes (Elena & Lenski, 2003; Fisher & Lang, 2016).

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Chapter

2

Addressing the challenges in phenotyping thermal responses at the individual and population level

Foreword. Focusing on life-history traits is crucial to determine how organisms respond to environmental factors. Yet, there are technical challenges regarding robustness, reproducibility, and quantification analysis of these phenotypic responses that need to be addressed before standardised methods can be used in large-scale phenotyping. I performed preliminary development steps to address this phenotyping bottleneck as a first step towards characterising the thermal responses of Zymoseptoria tritici at both the individual and population level. This chapter therefore intends to provide opportunities for exploration of the ways in which yeast-like micro-organisms interact with their environment: On which traits should one measure these responses? How to quantify the interindividual phenotypic diversity of these thermal responses? What insight does it give us?

This chapter was published in Microbial Ecology under the heading:
Phenotyping thermal responses of yeasts and yeast-like micro-organisms at the individual and population levels: proof-of-concept, development and application of an experimental framework to a plant pathogen

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contents

abstract

Background and Aims

Deciphering the responses of microbial populations to spatiotemporal changes in their thermal environment is instrumental in improving our understanding of their eco-evolutionary dynamics. Recent studies have shown that current phenotyping protocols do not adequately address all dimensions of phenotype expression. Therefore, these methods can give biased assessments of sensitivity to temperature, leading to misunderstandings concerning the ecological processes underlying thermal plasticity. We describe here a new robust and versatile experimental framework for the accurate investigation of thermal performance and phenotypic diversity in yeasts and yeast-like micro-organisms, at the individual and population levels.

Key Results

In addition to proof-of-concept, the application of this framework to the fungal wheat pathogen Zymoseptoria tritici resulted in detailed characterisations for this yeast-like micro-organism of: (i) the patterns of temperature-dependent changes in performance for four fitness traits; (ii) the consistency in thermal sensitivity rankings of strains between in planta and in vitro growth assessments; (iii) significant interindividual variation in thermal responses, with four principal thermotypes detected in a sample of 66 strains; (iv) the ecological consequences of this diversity for population-level processes through pairwise competition experiments highlighting temperature-dependent outcomes.

Conclusion

These findings extend our knowledge and ability to quantify and categorise the phenotypic heterogeneity of thermal responses. As such, they lay the foundations for further studies elucidating local adaptation patterns and the effects of temperature variations on eco-evolutionary and epidemiological processes.

Keywords: phenotyping, responses to temperature, thermal performance curve, diversity metrics, yeast-like micro-organisms, Zymoseptoria tritici

INTRODUCTION

Micro-organisms inhabit diverse environments differing considerably in terms of their temperature conditions (mean, range, variance, dynamics). For example, foliar pathogens live in patchy environments subject to thermal variations, the magnitude and rate of which vary over space (heterogeneity) and time (dynamics) (Lindow & Brandl, 2003; Chelle, 2005). Temperature has a large effect on the growth of micro-organisms, and is thus a crucial factor that must be taken into account when deciphering the ecological and epidemiological processes in which these micro-organisms are involved (Angilletta, 2009), e.g. disease development for foliar pathogens of plants (Bernard et al., 2013). The response to thermal conditions differs between individuals (Angilletta, 2009); therefore, it is vital to characterise the diversity of these responses in natural populations to predict changes, such as epidemic outbreaks, particularly in a context of climate change (Sutherst et al., 2011). To that end, a set of efficient, reproducible and standardised thermal phenotyping protocols is necessary to quantify these responses accurately and to ensure that they can be compared between individuals, populations and experiments.

In addition to the fact that protocols meeting these requirements are still lacking to date (Boyd et al., 2013), the phenotyping of thermal responses is currently subject to six types of limitation. These limitations may be inherent to the experimental design (i-ii), or may arise in either the measurement (iii-v) or data analysis (vi) phases namely:

- (i) suboptimal choice of the proxy for fitness when investigating phenotypic heterogeneity between individuals in the absence of prior multiple trait-based approaches. Indeed, the fitness traits under study will not necessarily have a similar effect on the components of performance (growth, reproduction and survival) or be affected similarly by temperature (Huey & Stevenson,1979; Sinclair et al., 2016);
- (ii) fragmented range and disparate resolution of the thermal conditions studied, leading to errors in the prediction of key thermal parameters (Low-Décarie et al., 2017);
- (iii) potential biases in the measurement of performance likely to magnify or counteract differences between replicates and/or individuals. This can be seen in the case of fungi, for which conventional protocols rely on radial growth rate measurements on solidified agar surfaces in Petri dishes. Indeed, colony diameter and area do not capture growth in three dimensions and are affected by both the heterogeneity of the moisture content of the solid medium and the differences in surface tension between plates (Zhan & McDonald, 2011);
- (iv) suboptimal consideration and monitoring of time-dependent effects inherent to labile fitness traits (Wiser & Lenski, 2015), with measurements conducted at a single time point;
- (v) use of an incorrect indicator of the temperature actually perceived by the micro-organism during the experiment (e.g. micro- vs. phyllo-climate; Chelle, 2005). For example, air temperatures are widely used in studies carried out under controlled (incubator or phytotron units), semi-controlled (greenhouse) or natural conditions (field) but do not correspond to the temperatures of the immediate environment of micro-organisms i.e. culture medium (in vitro assays) or leaf (in planta assays) temperatures (Bernard et al., 2013);
- (vi) incorrect description of the experimental data when establishing TPCs (Angilletta, 2006) (thermal performance curves, which depict the variation of performance over a range of temperatures; Huey & Stevenson, 1979) due to the inappropriate selection of candidate models (e.g. a linear model with only two temperatures) and/or an underappreciation of model constraints on the confidence estimates of key thermal parameters (Quinn, 2017).

All these factors result in a partial view of the relationship between trait, fitness and temperature, by hindering the plotting of accurate and robust TPCs.

Based upon these limitations, the design of phenotyping protocols is a potential stumbling block in the development of future high-throughput phenotyping assays assessing the phenotypic heterogeneity of thermal responses. This is particularly evident in the case of Zymoseptoria tritici (formerly Mycosphaerella graminicola), the causal agent of Septoria tritici blotch (STB), one of the most important foliar diseases of wheat worldwide (Eyal, 1999). For this biological model, research on thermal adaptation has been restricted to in vitro comparisons of two temperatures, with growth measured on solidified agar, at one to three time points (Zhan & McDonald, 2011; Suffert et al., 2015; Lendenmann et al., 2016; Chen et al., 2017), resulting in the inherent biases mentioned above. A phenotyping protocol has been proposed in planta for the latent period (Bernard et al., 2013), defined as the time interval between infection and sporulation, but it is suitable only for small numbers of samples (time-consuming and intensive procedures for disease assessment over a wide range of temperatures). Furthermore, in the case of in planta assessments, differential responses of performance (the expression of aggressiveness in this instance) between strains can take the form of a cross effect between host resistance and temperature adaptation (Ahmed et al., 1996).

This study aimed to bridge these gaps by providing a generic experimental framework for phenotyping the responses of a micro-organism to its temperature at the individual (responses of quantitative traits to temperature) and population (interindividual variability for a given trait) levels. This framework was designed to be suitable for use with micro-organisms whose growth predominantly results from budding or fission (e.g. yeasts and yeast-like micro-organisms). It was implemented here for Z. tritici, to provide both proof-of-concept and new research findings concerning the thermal responses of this pathogen.

We present here: (i) a laboratory-standardised and miniaturised method for accurately characterising the full functional thermal responses of Z. tritici over a wide range of temperatures, with practical recommendations for its use for yeasts and other yeast-like micro-organisms; (ii) an investigation of the interdependence of Z. tritici phenotypic traits regarding individual thermal sensitivity (phenotypic integration), to assess the biological relevance and quality of the information acquired in this framework; (iii) a characterisation of the diversity of thermal responses in Z. tritici between individuals and within and between populations; (iv) an assessment of the consequences of this diversity for population-level processes.

METHODS

The responses of Z. tritici to its thermal environment were analysed by establishing TPCs for sampled strains under laboratory-standardised and miniaturised conditions. This method involves measuring differences in growth kinetics (i.e. the increase in the number of yeast-like spores over time) within aliquots of different spore suspensions exposed to 12 temperatures. We assessed its veracity and biological relevance, and then applied it to investigate the diversity of thermal phenotypes in Z. tritici and its consequences for population-level processes.

A STEP-BY-STEP GUIDE TO THE THERMAL PHENOTYPING FRAMEWORK

An in vitro approach (to eliminate the interaction between Z. tritici and wheat plants) was developed to screen a large number of strains, using growth kinetics as a proxy for fitness. The steps in the thermal phenotyping process are summarised in Fig. 1.

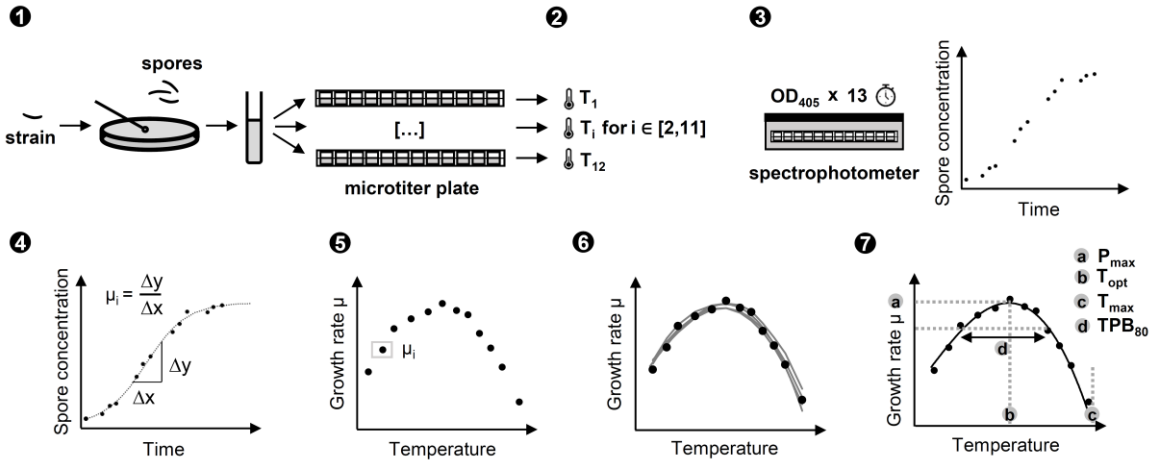

Fig. 1 Overview of the steps in the thermal phenotyping framework for a given strain. (1.1) Preparation of fungal cultures by adjusting spore suspensions and dispensing them in 12 microtiter plates; (1.2) Incubation of microtiter plates in the dark for four days at 12 temperatures; (1.3) Kinetic monitoring of fungal growth for each temperature through OD_{405} measurements with a spectrophotometer; (1.4) Calculation of the maximum growth rate μ (slope at the inflection point) for each temperature; (1.5) Expression of μ as a function of temperature; (1.6) Establishment of the strain's thermal performance curve (TPC) by the fitting of mathematical models to the growth rate data illustrated in step 5; (1.7) Capture of TPC features by retrieving key thermal parameters: maximum performance (P_{max}), thermal optimum (T_{opt}), maximum temperature (T_{max}) and 80% thermal performance breadth (TPB_{80}).

Preparation of fungal cultures (Fig. 1.1) – Inoculum was prepared from stock tubes of Z. tritici spore suspensions stored at -80 °C in a 1:1 glycerol–water mixture. Subcultures were grown on PDA (potato dextrose agar, 39 $g.L^{-1}$) in a Petri dish and kept at 18 °C in the dark for six days. Spores were scraped from the agar surface and resuspended in a glucose peptone liquid medium (14.3 $g.L^{-1}$ dextrose, 7.1 $g.L^{-1}$ bactopeptone and 1.4 $g.L^{-1}$ yeast extract). Concentrations of the resulting suspensions were adjusted to 2.5 x 10^5 $spores.mL^{-1}$ in a Malassez counting chamber. For each spore suspension, eight culture aliquots were dispensed in the eight wells of a column of a 96-well microtiter plate (Microtest Plate, Sarstedt), with an eight-channel pipette (150 µL per well). The first column was reserved for the blank (eight wells containing non-inoculated liquid medium), to take into account the contribution of the medium to optical density. With our experimental design, it was possible to study 11 strains simultaneously on each microtiter plate of a given experimental series. Microtiter plates were prepared in 12 replicates, each of which was assigned to one of the 12 temperatures studied. Plates were sealed with a sterile gas-permeable membrane (Breathe-Easy, Diversified Biotech) to prevent contamination and liquid medium evaporation. Preliminary tests were conducted to select the optimal culture conditions for studying Z. tritici growth at various temperatures (see SM1a).

Initiation of the growth experiment (Fig. 1.2) - Each plate was statically incubated in the dark for four days at one of the 12 study temperatures from 6.5 to 33.5 °C (6.5, 9.5, 11.5, 14.5, 17.5, 20.0, 22.5, 24.5, 26.5, 28.5, 30.5 and 33.5 °C). The number, resolution and range of these temperatures were chosen so as to ensure the accurate capture of responses to temperature (Low-Décarie et al., 2017). We chose to stop monitoring growth at four days for two reasons: to capture the growth kinetics as precisely as possible until saturation (stationary phase) whilst avoiding the phase during which experimental artefacts would be likely to occur. A biofilm-like structure (Donlan, 2002) formed at the surface of the liquid medium after five days, and this structure altered spore number estimates in the microplate wells (see SM1b). The 12 constant thermal environments were obtained by placing the plates in plastic boxes in an incubator (Heratherm Incubator IMC 18, Thermo Fisher Scientific) or in a water bath under thermostatic control (Polystat 24 Immersion Circulator, Thermo Fisher Scientific).

Kinetic monitoring of fungal growth (Fig. 1.3) - Plates were removed from their thermal environment to measure the optical density of each well at 405 nm (OD_{405}) three times per day, at five-hour intervals, with a microplate spectrophotometer (Multiskan FC and dedicated SkanIt Software, Thermo Fisher Scientific). Plates were shaken before each reading (horizontal shaking for 5 seconds at 5 Hz, with an amplitude of 15 mm) to aerate the medium and ensure a uniform distribution of spores within the suspension.

Establishment of TPCs - Optical density (OD_{405}) measurements were converted into spore numbers for each suspension, with a calibration curve (see Eq. 1). This calibration curve, which relates OD_{405} values to the actual spore concentration of 30 dilutions (1.25x dilution series, as recommended in Hall et al., 2014), was established for four reference strains and was shown to be suitable for broad-spectrum application when assessed with a set of 68 strains (no significant difference in curve coefficients).

$OD = 4.2 \times 10^{-7} S + 0.06$ (Eq.1) where OD is the OD_{405} value subtracted by the intrinsic absorbance of the medium (blank value) and S is the number of spores per millilitre estimated with an automated particle counter (Flow-Cell FC200S+, Occhio).

The TPC for a given strain was obtained by: (i) fitting logistic curves to its growth kinetics curve at each of the 12 temperatures studied (Fig. 1.4); (ii) extracting the mean maximum growth rate for each fitting (Fig. 1.5); (iii) fitting a non-linear model to biological growth rate data (Fig. 1.6). Since the goodness-of-fit of models to data varies with temperature (i.e. on the x-axis of TPCs), the systematic errors in predictions of performance may differ between models at a given temperature. Thus, as population-based studies are more focused on deciphering interindividual variations in TPCs than in obtaining absolute performance values for each strain, we decided to use a single model to describe TPCs for a given trait. After initial fitting tests on the experimental data which was obtained for Z. tritici, 15 mathematical models were chosen from a set of 50 models published in previous studies as descriptors of trait responses to temperature (see SM2). These 15 models were classified according to their goodness and statistical relevance of fit to experimental data. The criteria for discriminating between models were the residual sum of squares, Akaike and Schwarz weights (Burnham & Anderson, 2004).

We then selected the best of the top-ranking candidate models by focusing successively on: (i) the distribution of residuals across the temperature range, with greater weight given to the estimation accuracy of mid-range rather than extreme temperatures; (ii) model constraints, including in particular dependence of parameters (Jassby & Platt, 1976) and symmetry (Angilletta, 2006); (iii) practicality, i.e. whether the model has biologically meaningful parameters and is frequently used to describe TPCs in the literature (see SM2).

Capturing key thermal parameters from the TPC (Fig. 1.7) - The most appropriate model was adjusted separately for each strain studied. The characteristics of each TPC were captured with four key thermal parameters: maximum performance (P_{max}), thermal optimum (T_{opt}), maximum temperature (T_{max}) and thermal performance breadth (TPB_{80}: temperature range over which performance exceeded 80% of P_{max}) (Huey & Stevenson, 1979; Angilletta, 2009).

METHOD VALIDATION

The method was validated by assessing the accuracy, repeatability, reproducibility and suitability of the thermal phenotyping framework as defined by ISO international standards (International Organization for Standardization, 1994, 2017). The framework phenotyping steps (see Fig. 1) were applied to two Z. tritici reference strains (CBS-KNAW-IPO323 and INRAE-FS0932) in an experiment that was repeated three times consecutively (repetitions of experiment). For both strains, spore concentrations were measured for eight aliquots (technical replicates) at each of the 13 time steps for the analysis of growth kinetics with two methods: turbidity (OD) and automated (Flow-Cell FC200S+ particle counter and dedicated CALLISTO software, Occhio) measurements.

The veracity of growth estimates from OD_{405} measurements was evaluated by assessing agreement and precision between optical density (OD method) and particle counter (PC method) measurements. As extreme temperatures can modify spore melanisation (Butler & Day, 1998; Lendenmann et al., 2016) and as Z. tritici displays dimorphism (potential transition from yeast-like to hyphal growth forms, depending on growth conditions; Steinberg, 2015), we also checked that changes in OD were not due to shifts in the optical properties of spores during growth monitoring. Spore size (length and width), shape (straightness), melanisation (greyscale level) and growth form (visual discrimination procedure) were analysed from morphometric data and images of all spores passing through the particle counter (accuracy assessment; see SM3a). Differences in spore concentrations were investigated between the technical replicates for each set of growth conditions (strain x time x temperature) and between the three independent and consecutive repetitions of the experiment (repeatability and reproducibility assessments, respectively). We ensured that thermal growth conditions were homogeneous in the wells of each microtiter plate, by carrying out a specific experiment at 11.5, 18.0 and 24.5 °C, respectively: 20 T-type thermocouples (copper-constantan; diameter 0.2 mm) were immersed in the culture medium of 20 wells and connected to a datalogger (Campbell Scientific), which recorded temperature every 10 minutes (suitability assessment; see SM4).

CONSISTENCY IN THERMAL SENSITIVITY BETWEEN DIFFERENT FITNESS TRAITS

Is the thermal sensitivity inferred from in vitro growth rate representative of in planta trait-based thermal responses? 'Phenotypic integration' (Pigliucci, 2003) of thermal responses was studied for a set of 18 Z. tritici strains in liquid medium (in vitro) and on wheat plants (in planta). In vitro assessment involved monitoring the growth kinetics of each strain at 12 temperatures (see Fig. 1), whereas in planta assessment was based on three conventional aggressiveness traits (AUDPC, latent period and sporulation capacity; see Table 1). Each strain was inoculated by applying a spore suspension (calibrated at 2 x 10^5 spores.mL^{-1}) to the median part of the flag leaf (adaxial face) of six adult wheat plants (cv. Apache, head fully emerged stage) (Suffert et al., 2013). This experiment was conducted simultaneously under five thermal regimes at mean temperatures of 12.4 (± 0.4), 14.3 (± 0.5), 17.1 (± 0.4), 18.9 (± 0.4) and 21.5 (± 0.4) °C. Leaf temperature was recorded throughout the experiment with T-type thermocouples positioned under the leaf in contact with the inoculated area (Bernard et al., 2013). Disease symptoms were quantified twice weekly until total leaf senescence, through visual assessments of the sporulating area and the quantification of spore production (Suffert et al., 2013), to provide information about in planta traits (see Table 1). For each trait, TPCs were established with the best mathematical model from 15 candidates (see SM2). Based on these TPCs, we conducted (i) a multi-trait analysis to assess matches in thermal response patterns between the four traits; (ii) a consistency assessment in which we ranked strains based on the position of TPCs on the temperature axis for growth rate (in vitro) and AUDPC (in planta).

Table 1 Definition, measurement and calculation of the three aggressiveness traits for which response to temperature was studied in the case of 18 Z. tritici strains.

Trait	AUDPC	LAT	nbSPO
Definition	Area under the sporulating area progress curve	Latent period, i.e. estimation of the time elapsed from inoculation to appearance of the first pycnidia	Final number of pycnidiospores [b] produced per unit sporulating area [a]
Measurement	Sporulating area [a]	Sporulating area [a]	Total number of pycnidiospores [b] collected in lesion washing suspensions [c] and counted in a Malassez counting chamber
Calculation	Midpoint rule (trapezoidal integration method)	Number of days post-inoculation required to reach 5% of the maximum sporulating area [a]	Total number of pycnidiospores [b] divided by maximum sporulating area [a]

[a] leaf area covered by pycnidia (asexual fruiting bodies)

[b] asexual spores liberated from pycnidia

[c] suspensions obtained by sweeping leaf sporulating area with a paintbrush into a fixed volume of water

PRACTICAL APPLICATIONS

Application 1: Capturing thermal phenotypic diversity between individuals and within and between populations

How much do thermal responses vary between individuals and populations of Z. tritici? Six Z. tritici populations of 11 strains each, collected on wheat cv. Apache in fields located along two transects in France (a north-south increasing gradient of mean annual temperature and a west-east increasing gradient of annual temperature range; see SM5a), were phenotyped. The TPCs of all strains were established with a modified version of the thermal phenotyping framework (growth measured 72 h after deposition i.e. shortly after reaching the inflection point in the growth kinetics) and were summarised by the calculation of P_{max}, T_{opt}, T_{max} and TPB80. Overall thermal phenotypic diversity was assessed by establishing an average TPC template and determining the extent to which it varied between strains. We then conducted a hierarchical clustering on principal components (HCPC) analysis on P_{max}, T_{opt}, T_{max} and TPB80, to group together thermal responses with similar characteristics (entities hereafter referred to as 'thermotypes'). We then summarised this diversity, using the corresponding clusters silhouette plot (Rousseeuw, 1987) to visualise the number, proportion and features (compactness, separation, connectivity) of each thermotype. We assessed the variation of TPCs within and between populations, by analysing both divergence in the means and variances of thermal parameters and the composition patterns of thermotypes.

Application 2: Inferring relative fitness at the individual (performance) and population (competitive advantage) levels

TPCs show how temperature affects a given component of fitness (applied here to growth rate) at the individual level. Can we upscale this knowledge to group or population levels using additive rules? We conducted a pairwise competition experiment to determine whether the actual relative fitness of two strains grown together at a given temperature can be inferred from their respective individual TPCs. Five Z. tritici strain pairs, selected on the basis of their (dis)similar TPCs, were cocultured in 150 µL of GPL, with an initial total spore concentration of 2.5×10^5 spores.mL^{-1}, at eight temperatures (7.0, 11.5, 15.0, 18.5, 21.0, 25.0, 28.5 and 30.5 °C), in five replicates. This proof-of-concept experiment involved the monitoring of changes in strain frequencies 72 h after deposition, by measuring the final proportions of each strain by fluorescence microscopy and image analyses (Abràmoff et al., 2004) (see Fig. 2). Strain pairs were constituted by coculturing a GFP-expressing IPO323 transformant (IPO323* strain, T_{opt} = 25.8 °C) with one of the following non-GFP-expressing strains: (i) a strain with a similar TPC and genetic background (WT-IPO323 corresponding to IPO323* wild-type, T_{opt} = 25.8 °C) at initial proportions of 25, 50 and 75% in the coculture; (ii) a strain with a similar TPC but a contrasting genetic background (FS0917, T_{opt} = 25.7 °C) at an initial proportion of 50%; (iii) three strains (analyses conducted separately) with similar TPCs but differing from IPO323* (FS0678, FS0922, FS1014; T_{opt} = 19.1, 19.2, 19.3 °C, respectively), at an initial proportion of 50% (see Fig. 7a).

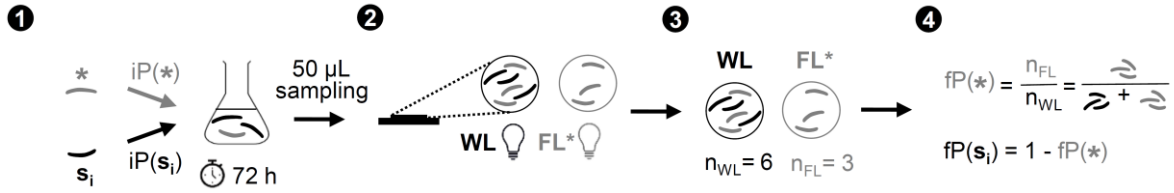

Fig. 2 Relative fitness measurements in pairwise competition experiments at eight constant temperatures. (1) We tested pairs of strains consisting of a GFP-expressing strain (IPO323* annotated as *), the spores of which were visible under both white (WL) and fluorescent (FL*) light and a non-GFP-expressing strain (s_i), the spores of which were visible only under WL. IPO323* spores were cocultured with s_i spores at initial proportions [iP(*)] of 25, 50 and 75%, in a 96-well microtiter plate (Microtest Plate, Sarstedt), with each well containing 150 µL of inoculated GPL liquid medium. After 72 hours of growth, 50 µL aliquots of each coculture replicate were sampled and deposited on a microscope slide; (2) Slides were photographed under both WL and FL*, at 10x magnification, with a fluorescence microscope equipped with a camera (Leica DM5500 B, Leica Microsystems) and LAS-AF software (Leica Microsystems). GFP fluorescence was captured with a filter, with excitation at 450-490 nm and emission in the 500-550 nm range; (3) All acquired images were analysed with ImageJ 1.48v software. After conversion into black and white images, spores were counted on WL- (n_{WL}) and FL*- (n_{FL}) photographs with the built-in Subtract Background, Threshold and Analyze Particles functions; (4) Calculation of the final proportions (fP) of each strain in the coculture.

STATISTICAL ANALYSES

Four types of statistical analyses were conducted with R software (R Core Team, 2018): (i) comparisons of means between groups based on analysis of variance (ANOVA) or its non-parametric alternative (Kruskal-Wallis test); followed, when necessary, by appropriate post hoc tests (P-value threshold: 0.05); (ii) fitting and analysis of a generalised linear model quantifying the extent to which OD values may be affected by experimental variables and their interactions with time and temperature (see method validation); (iii) nonlinear fitting and selection of mathematical models to establish TPCs (Pinheiro et al., 2018); (iv) exploratory multivariate analyses (PCA and HCPC; Lê et al., 2008) to define thermotypes (see application 1), the characteristics of which were explored further with clustering validation indices (Hennig, 2010).

RESULTS

METHOD VALIDATION

Veracity and precision of spore concentration measurements - The results obtained with the OD and PC methods displayed a high degree of agreement, as shown by the strong correlation between paired measurements (Pearson's correlation coefficient: 0.99; $P < 0.01$), although the OD method tended to give slightly higher estimates (by 0 to 7% of the total number of spores in the sample). The OD method displayed less variation in spore number estimates between successive replicate measurements (Student's t-test; $P < 0.01$) and, as such, appeared to be more reliable for the detection of small but significant differences between strains (see SM6).

The OD method is repeatable but requires normalisation in sequential test series - An ANCOVA was performed on the log-transformed growth kinetics of IPO323 and FS0932 (see Fig. 3). Spore concentration values were expressed as the combined effect of strain ($P < 0.01$), OD reading time ($P < 0.01$), technical replicates ($P = 0.99$), well position (core or edge wells; $P = 0.95$) and repetitions of experiment ($P < 0.01$). In other words, the OD method was highly repeatable, with no significant variation in spore concentration between aliquots or between positions on the microtiter plate. However, consecutive repetitions of the experiment were not strictly equivalent. Thus, when the set of strains cannot be phenotyped in a single batch, a control (reference strain) should be grown in each experimental run for subsequent normalisation.

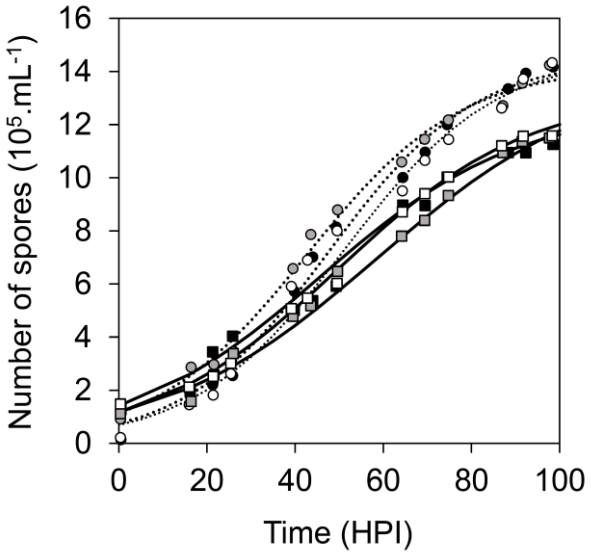

Fig. 3 Changes in spore concentration within the suspension over four days. Growth kinetics of two Z. tritici strains (here represented as a function of time in hours post-inoculation - HPI - and at their respective T_{opt}) were analysed by fitting a logistic regression model to the experimental data obtained for IPO323 (squares and solid lines) and FS0932 (circles and dotted lines). Each data point corresponds to the mean of eight technical replicates in an experiment repeated three times (each repetition being shown in a different colour).

The OD method is suitable for the thermal phenotyping of Z. tritici - We assessed the homogeneity of the system in terms of temperature and potential biological artefacts, to check that the phenotyping results obtained with the OD method were truly representative. We first checked the thermal homogeneity of in vitro growth conditions between wells of the same microtiter plate at three temperatures (deviation of the temperature of individual wells from the overall mean temperature of the microtiter plate < maximum residual error of measurements; see SM4b). Regarding spore optical properties during growth monitoring, spore length, width and shape had no significant effect on the change in OD values. Suspensions exposed to extreme temperatures (below 11.5 °C and above 30.5 °C) contained a slightly higher proportion of hyphal forms (not statistically significant). This change in the proportion of hyphal forms, which remained below 1% in all experimental conditions (see SM3b), had no effect on OD at any temperature except at 9.5 °C (interaction Hyphae:Temperature; $P = 0.02$), at which the hyphal forms presented more signs of branching filamentous structures. Melanisation affected OD, with a gradual increase in melanin levels detected after day 2 of the experiment (see SM3c), but this effect was not dependent on the temperature of the liquid medium (see Table 2 and SM3d).

Table 2 Impact of experimental variables on OD measurements across the experiment.
We investigated the experimental factors which can affect OD values, by fitting a generalized linear model to growth kinetics data over a four-day period, during which we monitored spore concentration and optical properties. The main effects and interactions with time and temperature are summarised by P-values (F-test).

Variables	Effect on OD	Interaction with time	Interaction with temperature
Number of spores	< 0.01	-	-
Time	< 0.01	-	-
Temperature	< 0.01	-	-
Spore length	0.88	0.93	0.17
Spore width	0.19	0.12	0.19
Spore shape	0.20	0.73	0.37
Spore melanisation	0.49	< 0.01	0.07
Presence of hyphal growth forms	0.26	0.42	0.02 ∗

∗ The corresponding post-hoc test indicates that the limited presence of hyphal growth forms affected OD values only at 9.5 °C (at which temperature there were more pseudohyphae i.e. growth forms presenting the first signs of branching of the filamentous structures). This interaction was not significant at the other 11 temperatures.

CONSISTENCY IN THERMAL SENSITIVITY BETWEEN DIFFERENT FITNESS TRAITS

Difference in TPCs between the four fitness traits - The mean TPCs for in vitro growth rate, AUDPC, LAT and nbSPO were described by different non-linear functions and key thermal parameter values (see Fig. 4). These differences reflect variations of the shape and position of performance distribution along the temperature axis (e.g. T_{opt} was 4.3 °C higher for in vitro growth rate than for AUDPC; 20.3 vs. 16.0 °C), with large differences in the rate and magnitude of changes in performance per degree change in temperature over the range. The overall correlation between in vitro growth rate and latent period or sporulation capacity was weak, but the one between in vitro growth rate and AUDPC was strong, for T_{opt} values (see Fig. 5b) and for full responses (Pearson's correlation coefficient: global 0.76, T_{opt} 0.92; $P < 0.05$).

Consistency of the thermal sensitivity rankings of strains for in vitro growth rate and AUDPC - In vitro growth rate was found to be a reliable proxy for the assessment of thermal sensitivity, with the results obtained highly consistent with aggressiveness in planta. As TPC shape did not differ significantly between the 18 strains (Kruskal-Wallis, $P < 0.05$), T_{opt} can be considered to reflect the position of the TPC along the temperature axis. This parameter was used to rank strains for both in vitro growth rate and AUDPC (see Fig. 5a). In total, 11 of 18 strains were assigned to the same thermal sensitivity rank, and the ranking errors between the two assessments ranged from -2 to +2. Consequently, strain categorisation (e.g. 'cold-adapted' vs. 'warm-adapted' strains) was conserved whether estimated in vitro or in planta. A clear benefit of the in vitro method is the magnification of the T_{opt} distribution relative to in planta disease assessment (4.8 vs. 2.2 °C; see Fig. 5c). This difference was particularly pronounced for cold-adapted strains, as there was a slight imbalance (distribution tail of the kernel density plot) in T_{opt} distribution for lower values.

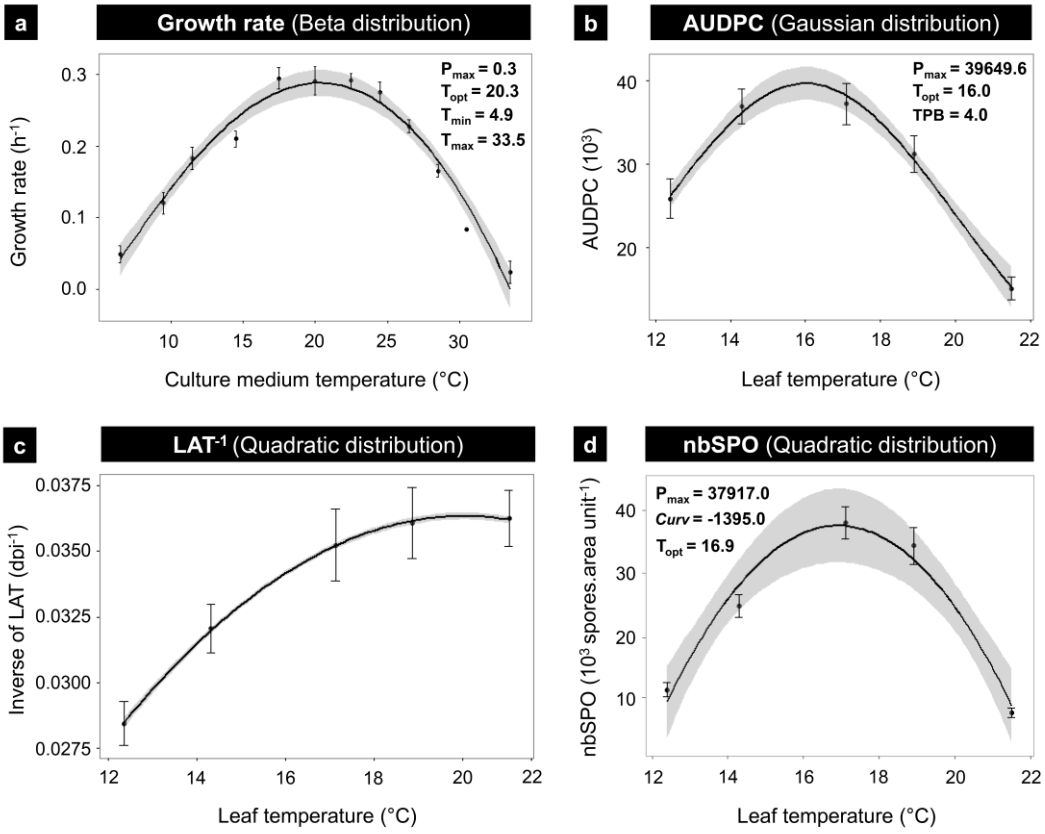

Fig. 4 TPCs of four Z. tritici phenotypic traits. (a) In vitro growth rate; (b) In planta AUDPC (area under the sporulating area progress curve); (c) Inverse of in planta latent period expressed in dpi^{-1} (number of days post-inoculation^{-1} required to reach 5% of the maximum sporulating area); (d) In planta sporulation capacity (final number of pycnidiospores produced per unit of maximum sporulating area). Data points correspond to the mean trait performance calculated for 18 Z. tritici isolates (mean ± SEM). TPCs were obtained by fitting the most appropriate mathematical model (solid line) to each data set and are displayed with their 95% confidence intervals (shaded area).

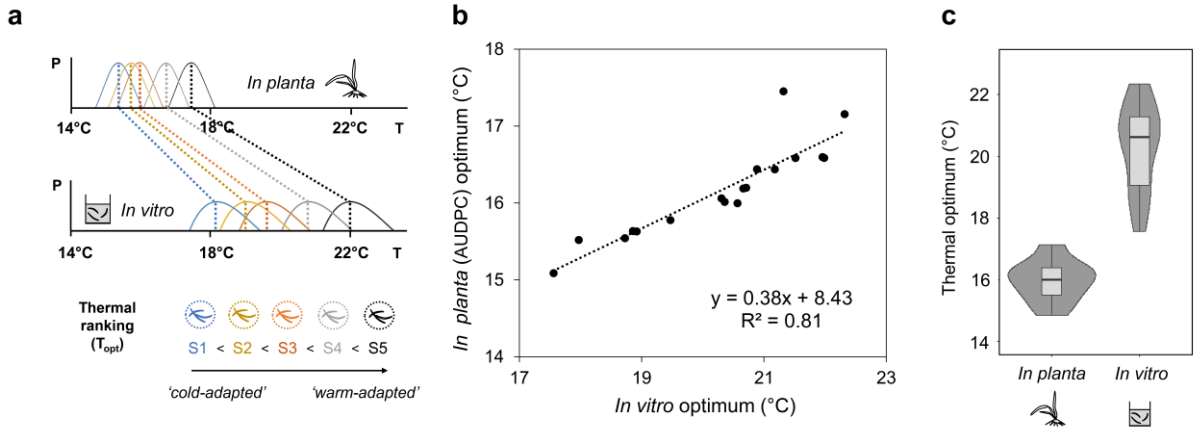

Fig. 5 Consistency in the ranking of thermal responses for 18 strains. (a) Theoretical representation of the information provided by the position of T_{opt} (dotted lines) along the temperature axis (horizontal shift of TPCs showing performance P as a function of temperature T) and consecutive thermal sensitivity rankings of five strains (S1-S5) for in vitro and in planta assessments; (b) Relationship between in vitro growth rate T_{opt} and in planta AUDPC T_{opt} of 18 Z. tritici strains illustrated by a linear model (dashed line) fitted to experimental data (points); (c) Full distribution (violin plot) of in planta AUDPC T_{opt} (mean ± SEM: 16.0 ± 0.1 °C) and in vitro growth rate T_{opt} (mean ± SEM: 20.3 ± 0.3 °C).

APPLICATION 1: CAPTURING THERMAL PHENOTYPIC DIVERSITY

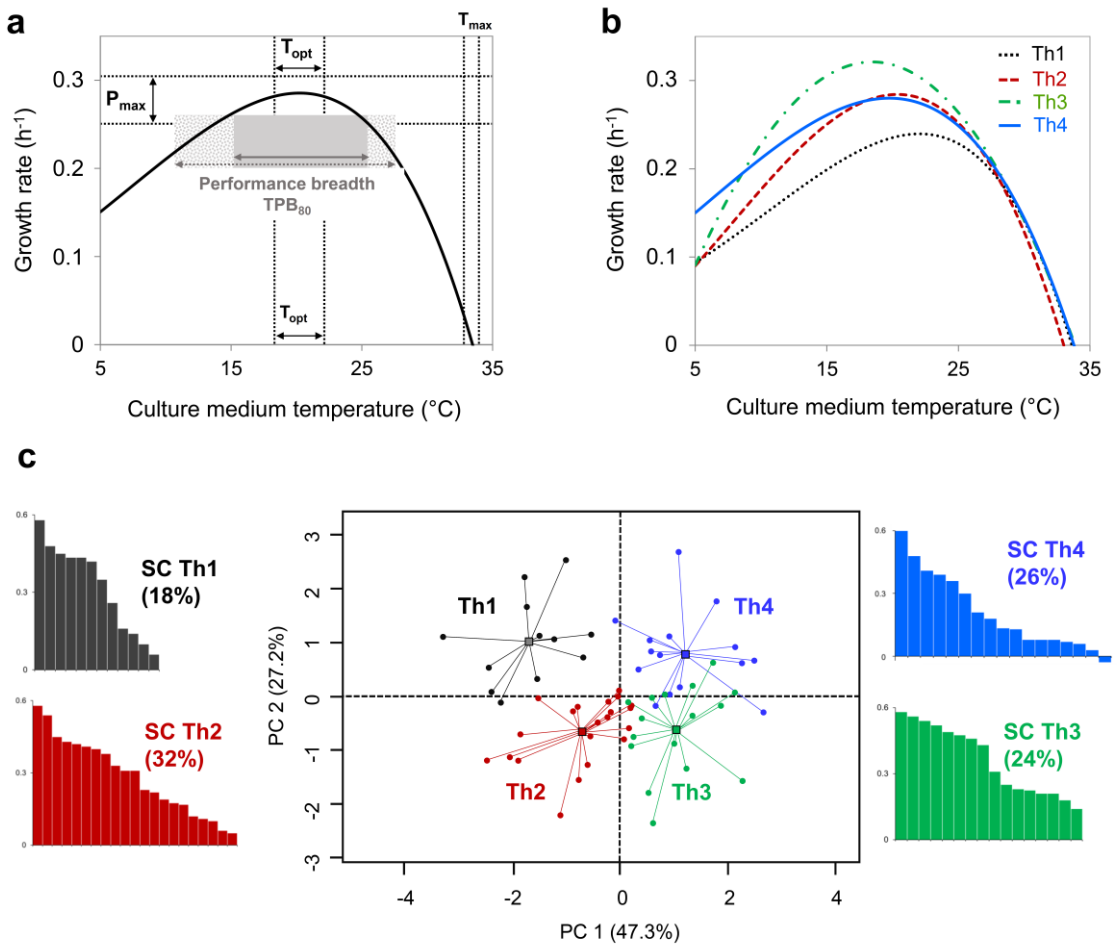

Fig. 6 Assessment of the diversity of Z. tritici thermal responses encountered within a set of six French populations (6 x 11 strains) collected along north-south and west-east transects. (a) Representative mean TPC obtained for in vitro growth rate and variation of its characteristics across strains (movement and shift in TPC position along the x- and y-axes). The curve was obtained by fitting a beta distribution to the whole data set (n = 66 strains). Interindividual variation around the average function is displayed for four thermal parameters capturing TPC features: maximum performance (P_{max}), thermal optimum (T_{opt}), maximum temperature (T_{max}) and 80% thermal performance breadth (TPB_{80}). Dotted lines and rectangles represent the lower and upper bounds of the standard deviation for each parameter. (b) Representative TPCs (paragons for each cluster) for each Z. tritici thermotype (Th1: 'adapted to warmer conditions', Th2: 'specialist', Th3 'high performer' and Th4 'generalist') obtained by hierarchical clustering on principal components (HCPC) analysis for the four thermal parameters indicated above (see Table 3). (c) Clustering of thermal responses based on Pmax, Topt, Tmax and TPB80. Factorial plan of the principal component analysis (PCA), with dots representing strains and coloured according to thermotype (HCPC cluster). The distance between each individual and the centre of the corresponding cluster is indicated by a line. Cluster silhouette plots (distribution of silhouette coefficient - SC - quantitative measurements of how well each strain lies within its cluster — for each individual of a given cluster) are also provided to illustrate and quantify disparities within (compactness) and between (connectivity) thermotypes. On these plots, the numbers in brackets refer to the proportion of the set of 66 strains corresponding to the thermotype concerned.

Classification and description of thermal responses: modes of variation and thermotypes The beta distribution was the preferred candidate model for describing TPCs for in vitro growth rate as it provided the most accurate and efficient description of the thermal response of this trait (see SM2). Therefore, this model was used to establish the mean TPC template and determine the degree to which it varied between strains (see Fig. 6a). Interindividual differences in TPCs were significant across all strains for the parameters P_{max}, T_{opt}, and TPB_{80} (ANOVA, $P < 0.05$). T_{max} was largely conserved across Z. tritici strains, with a confined value centered around 33.5 ± 0.7 °C. The minimum temperature values (T_{min}) given by the equation were not considered as they fell outside the range of experimental values (6.5 – 33.5 °C). Four thermotypes were identified across populations (see Fig. 6b and Table 3): Th1 ('adapted to warmer conditions'), Th2 ('specialist'), Th3 ('high performer') and Th4 ('generalist').

Table 3 Characteristics and proportions of the four thermotypes identified in HCPC analysis (French Z. tritici populations; n = 66 strains). Thermal responses were clustered on the basis of the following four TPC parameters: P_{max}, T_{opt}, T_{max} and TPB_{80}. T_{max} does not appear in the table as its value was largely conserved across Z. tritici strains (no variation between clusters). Numbers in brackets indicate the value for the barycentre of each thermotype (corresponding to an HCPC cluster; see Fig. 6b). Inter-cluster variations are summarised as follows, based on a statistical comparison with the overall mean of each parameter: '-' no deviation; '↗' higher value; '↘' lower value; '↘↘' even lower value. Bold text identifies the TPC parameter characterising each thermotype most appropriately. The frequency of each thermotype is provided across (overall proportion) and within (occurrence within populations) the six sampled populations.

Thermotype	TPC parameters			Overall proportion (%)	Occurrence within populations (ratio)
	P_{max}	T_{opt}	TPB_{80}		
Th1	0.24 (↘)	22.0 (↗)	13.7 (-)	18.2	5/6
Th2	0.28 (-)	20.4 (-)	13.1 (**↘**)	31.8	5/6
Th3	0.32 (↗)	18.5 (↘↘)	13.7 (-)	24.2	6/6
Th4	0.28 (-)	19.8 (↘)	15.5 (↗)	25.8	6/6

Compactness, separation and connectivity of thermotypes - All TPCs discriminated well between the four thermotypes (classification conducted without assumptions and with all retained PCA dimensions) except for two strains with a negative or almost null silhouette width coefficient. Thermotypes were well separated (distance between cluster centers and between strains in different clusters), with Th3 and Th4 less differentiated from each other. All thermotypes displayed substantial within-cluster variations, which were larger for Th1 and Th2 (higher within-cluster distance metrics) and particularly well structured for Th3 (bimodal distribution of silhouette coefficients: two distinct groups at about 0.55 and 0.25; see Fig. 6c).

Within and between population variation of TPCs - A similar diversity of thermal responses (similar means and variances) was observed for T_{opt}, T_{max} and TPB80 between the six populations (ANOVA and Bartlett's test: $P > 0.05$). The four thermotypes were represented in all populations except for Fp5, which was composed of Th1 and Th2 only. With a higher proportion of Th1 (poor-performance strains) and no Th3 (highly performant strains) in its population, Fp5 had a lower P_{max} (ANOVA and Tukey HSD, $P < 0.01$) due to the difference in thermotype composition compared with the other populations (see SM5b).

APPLICATION 2: INFERRING RELATIVE FITNESS

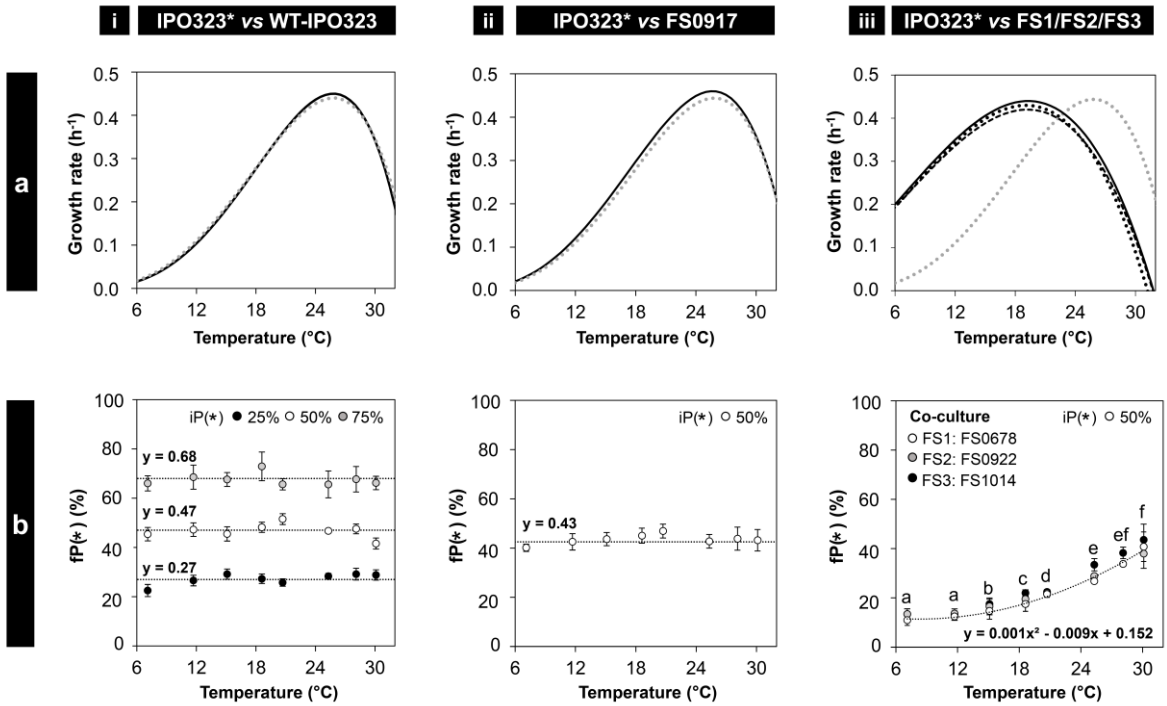

Fig. 7 Impact of individual TPCs on the outcome of pairwise competition between Z. tritici strains. (a) TPCs for in vitro growth rate for the five strains used in pairwise cocultures with IPO323* (GFP-expressing IPO323 transformant; T_{opt} = 25.8 °C; grey dotted curve). The various strains (black curves) paired with IPO323* have either (i) a matching TPC and identical genetic background (wild-type WT-IPO323; T_{opt} = 25.8 °C) or (ii) a matching TPC but different genetic background (FS0917; T_{opt} = 25.7 °C) or (iii) both a contrasting TPC and a different genetic background (FS1: FS0678, FS2: FS0922, FS3: FS1014; T_{opt} = 19.1, 19.2, 19.3 °C, respectively). (b) Competition outcomes expressed as the final proportion of fluorescent spores (IPO323*), fP(*), obtained after the separate exposure of each coculture to eight liquid culture medium temperatures. Experimental data are presented as means (data points) ± SEM (error bars). Dotted lines correspond to the trend line for change along the temperature axis for each initial proportion of IPO323*, iP(*), i.e. 25, 50, 75%, when applicable. Different letters above data points in Fig. 7biii indicate significant differences in final spore proportions as a function of temperature (Kruskal-Wallis test followed by post hoc pairwise comparisons, $P < 0.05$).

No competitive advantage for strains with similar TPCs - The final proportion of IPO323* spores was not affected by temperature in coculture with WT-IPO323 (ANOVA, $P > 0.05$), regardless of the initial ratio (see Fig. 7i). Furthermore, no significant difference was found between the initial and final proportions of each strain (ANOVA, $P > 0.05$). This suggests that neither strain had a competitive advantage over the other. Likewise, the final proportion of IPO323* spores did not vary significantly over the temperature range in coculture with FS0917 (ANOVA, $P > 0.05$). However, FS0917 slightly outcompeted IPO323*, with final spore proportions of 57% and 43% (ANOVA, $P = 0.02$), respectively (see Fig. 7ii).

Expression of a competitive advantage between strains with different TPCs - The final proportion of IPO323* spores increased significantly along the temperature axis (Kruskal-Wallis, $P < 0.01$) in coculture with FS0678, FS0922 or FS1014, whose TPCs are characterised by a lower T_{opt} (19.2 ± 0.1 vs. 25.8 °C; see Fig. 7aiii). The same trend (described by a second-order

polynomial equation) was observed for the three strains, indicating that the TPC dissimilarity of each strain with the TPC of IPO323* was responsible for the outcome of competition (see Fig. 7biii).

DISCUSSION

THE PHENOTYPING FRAMEWORK: PROPERTIES, APPLICATIONS & SCALING-UP

The thermal phenotyping framework developed here satisfactorily overcomes the six common limitations of such phenotyping methods (see introduction). It is miniaturised (microtiter plate method), standardised (balanced and homogeneous growth conditions), accurate (more reliable spore quantification, yielding a slight overestimate), robust (sound repeatability and potential of comparison, if a reference strain is included in the experimental design of sequential test series for subsequent normalisation) and suitable for thermal phenotyping (no skewed confounding effect related to shifts in the optical properties of spores and magnification of the T_{opt} distribution compared with in planta disease assessment). On this last point, dimorphism and melanisation, both of which have been reported to be temperature-dependent (Butler & Day, 1998; Gauthier, 2015), notably in Z. tritici (Lendenmann et al., 2016), could have affected OD measurements. Consequently, they were taken into account in the definition of the validity range of the method. Spore melanisation was not involved in any confounding effect or interaction with temperature on changes in OD. This seeming contradictory result (absence of interaction between melanisation and temperature) with those presented in Lendenmann et al. (2016) likely stems from the substantial differences in our methodological approaches since the use of liquid medium and the particle counter allowed us to measure melanisation directly at the spore level and not at the single-spore colony level (inherent to the use of solid medium, with possible differential effects of the air-colony-medium interface on melanisation). Regarding Z. tritici dimorphism, temperature did not affect the proportion of hyphal growth forms, which can be explained by the optimised culture conditions of our framework (in particular consideration of the characteristics of culture medium regarding nutrient deprivation rate and colony ageing; see SM1), but shifts from the yeast-like to the hyphal growth form might significantly affect OD at low temperatures (i.e. around 9.5 °C - as was the case in our experimental conditions - and probably below). This all the more supports our decision not to include T_{min} values in our analyses of thermal responses given their low confidence levels.

This framework is a significant improvement over those used in previous studies investigating thermal adaptation in Z. tritici (Zhan & McDonald, 2011; Suffert et al., 2015; Lendenmann et al., 2016; Chen et al., 2017). The use of a liquid medium makes higher-throughput screening assays possible (further design miniaturisation) and avoids the problems associated with physical differences between plates (moisture content and surface tension of solid medium), which can affect colony deposition and distort growth measurements (Zhan & McDonald, 2011).

It also covers a wide range of temperatures, with a finer-grained resolution, providing a more complete picture of thermal responses, which can be efficiently captured only by TPCs. In the case of Z. tritici, the use of 12 temperatures rather than two may overcome the problems

associated with simple tests of 'thermal sensitivity' based on two temperatures (Lendenmann et al., 2016), in which it can be questionable to draw firm conclusions given the non-linearity of thermal responses. Indeed, depending on the position relative to T_{opt} of the two temperatures considered, slope comparisons could lead to a misinterpretation of the results. Finally, growth rate measurements were not segregated at one to three single time points, but measured over four days to take time-dependent effects into account when reporting maximum growth rates inferred over the kinetics.

This framework can be applied to the study of other micro-organisms whose spores grow exclusively through cell division (e.g. yeasts and yeast-like micro-organisms in general), by following the step-by-step directions presented here: tailoring of the framework to the target micro-organism (preliminary growth monitoring experiments to define the optimal growth conditions, the experimental time frame and the range and resolution of thermal conditions; see SM1), method validation (verification of accuracy, repeatability, reproducibility and suitability) and a thorough assessment of the information obtained (cross-comparisons between the thermal responses of other fitness traits studied in the natural environment of the micro-organism).

CAPTURING PLASTICITY IN Z. TRITICI THERMAL RESPONSES

Phenotypic variation in thermal responses within and between French populations – In this study, we quantified the intraspecific phenotypic variability of thermal responses within populations of a fungal pathogen with a high level of genetic and phenotypic diversity (Linde et al., 2002; Zhan et al., 2005). Significant variation in TPCs between individuals was reported across strains from all six populations sampled. TPCs were classified into four thermotypes, each of which distinguished a particular category of thermal responses with its own intrinsic features (e.g. generalist or specialist strains) and differentiation (e.g. compactness and separation from other thermotypes on the basis of cluster distance metrics). This categorisation is a promising way to summarise diversity at different levels (individual, population, species). It should be noted that this constitutes not an absolute but a relative classification varying with the populations which are sampled. Given the differences in interindividual variation within thermotypes (e.g. scattered distribution for Th2 vs. bimodal distribution for Th3), thermal response diversity should be considered not only between, but also within thermotypes. This is of particular importance as the phenotypic composition of groups can drive the adaptation of a population to its environment through eco-evolutionary processes (Farine et al., 2015).

Spatial structure of thermal responses in French populations - No spatial phenotypic structure of thermal responses was detected between the six French Z. tritici populations, although one population displayed a considerably lower level of thermal response diversity (only two of the four identified thermotypes present). These populations were not sampled over the same time period during the wheat-growing season (at the end of winter for Fp5 and Fp6 and at the end of spring for Fp1, Fp2, Fp3 and Fp4).

Since we are analysing a potentially blurred picture with seasonal variations as a confounding factor, it is not possible to conclude that there is absolutely no pattern of thermal adaptation in France. Indeed, diversity in the thermal responses of Z. tritici at the population level may

emerge not only at a worldwide scale (significant differences in sensitivity to temperature linked to genetic differentiation; Zhan & McDonald, 2011), but also locally (selection pressure exerted by seasonal changes; Suffert et al., 2015). Consequently, future investigations of phenotypic diversity in Z. tritici populations and its potential structuration by thermal environments should involve careful consideration of the most appropriate sampling strategy (van de Pol, 2012) along spatial and temporal gradients because the traits studied here are labile. The populations sampled over a same period namely winter (extreme points of the west-east increasing gradient of annual temperature range) and spring (north-south increasing gradient of mean annual temperature) populations can be analysed separately. Interestingly, the Fp5 population, which displays the lowest richness level of diversity in thermal responses (only two thermotypes), was devoid of the generalist thermotype compared to the Fp6 population in which we detected both the specialist and the generalist thermotypes. Fp5 is characterised by a marked distinction in the annual range of its thermal environment as its range was the lowest among all sampling locations especially when compared with Fp6 (11.9 vs. 19.9 °C, respectively, whilst they share the same annual thermal mean). This finding is consistent with the assumption that more variable environments favour thermal generalists (Angilletta, 2009). Despite the difference in mean annual temperature along the north-south gradient, the same level of thermal response diversity and, in particular, a similar proportion of 'warm-adapted' strains were found in Fp1, Fp2, Fp3 and Fp4. This deviates from the assumption that there is a shift in the optimal range of thermal responses in relation to the mean temperature encountered within an environment. This high local diversity but non-geographically based population structure along the north-south gradient may be explained by the high gene flow among Z. tritici populations at large spatial scales due to the long-distance wind-dispersal of ascospores (Linde et al., 2002).

Differential effects of leaf temperature on aggressiveness traits - Leaf temperature effects on three fitness traits expressed in planta were studied through the establishment of TPCs, as described in a previous study focusing on latent period (Bernard et al., 2013). The knowledge of the range and distribution of TPC parameters acquired here for 18 Z. tritici strains could be incorporated into epidemiological models to investigate how and to what extent pathogen development and epidemic dynamics are affected by variations of temperature (potentially opposite effects of temperature on different aggressiveness traits; see Fig. 4) and the diversity of responses between individuals within a population (not accounted for in current models, despite the demonstration of a significant impact in epidemic forecasting; Ferrandino, 2012). A comprehensive multi-trait approach is particularly important because: (i) selection pressure does not necessarily operate on the same traits over the course of an epidemic (Suffert et al., 2015); (ii) strains can be regarded as patchworks of traits (Ghalambor et al., 2007) with different levels of phenotypic plasticity that can be reliably estimated from the differences in TPC shape.

Ranking consistency of thermal responses between in vitro and in planta assessments - Interestingly, the thermal optima for growth rate (in vitro) and AUDPC (in planta) were found to be strongly related. In particular, thermal sensitivity rankings were highly consistent whether estimated in vitro or in planta, but with an important shift in the thermal optimum and its distribution breadth. It is likely that this observed shift (4.3 °C higher for the in vitro growth rate optimum) results from the difference in the nutritional environment and in Z. tritici growth

forms between in vitro culture (predominantly yeast-like growth) and in planta leaf medium (hyphal growth, from epiphytic growth on the leaf surface to the intercellular growth within plant tissues; Steinberg, 2015) and/or from an effect of temperature on the host–pathogen interaction. This ranking consistency between in vitro and in planta assessments is in line with previous findings (Paisley et al., 2005; Zhan et al., 2016) and suggests some sort of interdependence between phenotypic traits in individual thermal responses, consistent with the concept of phenotypic integration. This also constitutes an ultimate step of methodological validation considerably expanding the scope of the experimental framework by confirming its biological relevance, even though the results are acquired in vitro. Additionally, this finding has important implications as it suggests that relative ranking of thermal responses between strains might be sufficient to draw conclusions on the thermal adaptation of groups and populations based on rank-based analyses of variance. However, it should be underlined that studies aiming to assess and to project performance in response to changes in temperature continue to require inference from the full TPC (position, curvature and skew of the curve along the temperature axis).

INFERRING RELATIVE PERFORMANCE FROM TPCs

How well can overall performance and competitive advantage be predicted from TPCs? An extension of the thermal phenotyping framework (see application 2) makes it possible to explore this question by inferring the performance of a given Z. tritici strain within a group or a population in a given thermal environment. We showed that the significance of these findings and the confidence that can be placed in them depend strongly on the properties of the experimental methods used (accuracy, repeatability, reproducibility, suitability of phenotyping assessment) and the correct integration of thermal biology and microbial ecology concepts. In our experiments, the relative proportions of the strains in pairwise cocultures of strains with similar TPCs were not affected by temperature regardless of genetic background. By contrast, pairwise cocultures of strains with dissimilar TPCs resulted in temperature-dependent outcomes with an increase in the proportion of the warm-adapted strains along the temperature axis. An expansion of this in vitro experiment with additional competition conditions (e.g. starting from different initial conditions and/or with more thermotypes in competition) would improve our understanding of the response of microbial populations to natural thermal variations. By extending the conditions (in planta) and the epidemiological framework (e.g. polycyclic dynamics) of the experiment, we could also try to improve characterisation of the thermal signal filter (time step and rate of the foliar fungal pathogen response to the environment; Sinclair et al., 2016) and the speed of natural selection in constant or fluctuating thermal environments (Niehaus et al., 2012).

FRAMEWORK VERSATILITY: APPLICATIONS FOR FUTURE RESEARCH

This integrated framework from experimental design to data interpretation allows detailed phenotyping of the thermal responses of micro-organisms. Through the quantification of interindividual phenotypic variation, it opens up new possibilities for exploring not only the diversity of thermal responses and its structuration (e.g. by highlighting patterns of local adaptation in natural populations; Giraud et al., 2017) but also its causes and consequences.

Indeed, this framework could help to provide deeper insight into the genetic basis of adaptation to temperature, by moving from phenotype to genotype (quantitative trait loci mapping or genome-wide association study of contrasting phenotypic profiles e.g. thermotypes) to identify genes involved in thermal adaptation (association of SNPs with given performance traits; Bazakos et al., 2017) or from genotype to phenotype (phenotyping of specific temperature-sensitive allelic variants or mutant lines) to facilitate the functional annotation of genomes (Brown et al., 2009).

This framework can also be used to explore the consequences of phenotypic variation between individuals, through the use of TPCs to infer individual performance in a given population and thermal environment (see application 2) and to predict changes in population composition in response to thermal changes (Reed et al., 2011), drawing on mark-release-recapture (Zhan & McDonald, 2013), selection (Fisher & Lang, 2016) or modelling (Chevin et al., 2010) experiments.

CONCLUDING REMARKS

By directly addressing the technical and analytical challenges in quantifying and describing thermal responses, this study provides a new methodology for inferring and categorising such responses in the case of yeasts and yeast-like micro-organisms. As such, the proposed framework can assist in shedding more light on the phenotypic heterogeneity of thermal responses between individuals, populations, species and communities of these micro-organisms, but also on the ways in which they interact with their natural or engineered environment. Among other applications, it can be used extensively to understand, predict and control the design, optimisation and conduct of food (e.g. strain selection), biomedical (e.g. product stabilisation), industrial (e.g. anaerobic digestion process for the production of biomethane), epidemiological (e.g. pathogen growth and epidemic development) and ecological processes (e.g. population dynamics in response to thermal variations).

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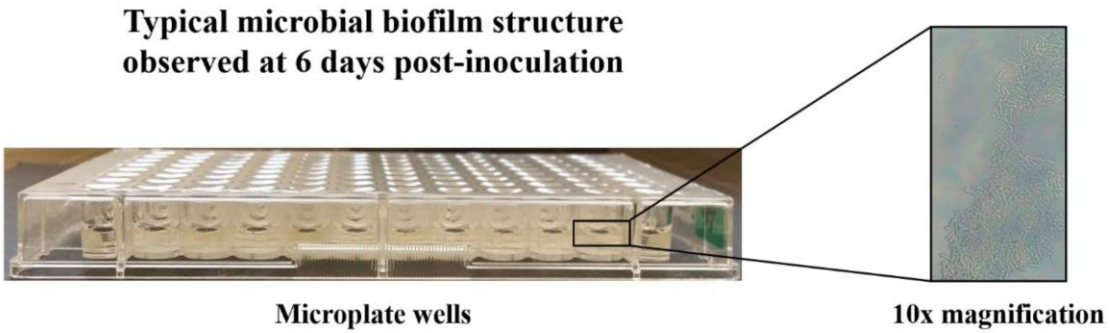
SUPPLEMENTARY MATERIAL

SM1a Tested culture conditions and parameter levels adopted for the thermal phenotyping of Z. tritici (the final chosen levels are highlighted in grey)

Parameter	Level	Experimental observation and decision-making
Liquid culture medium	• GPL (glucose peptone liquid) *14.3 g.L^{-1} dextrose, 7.1 g.L^{-1} bactopeptone and 1.4 g.L^{-1} yeast extract* • YM (yeast malt) *20 g.L^{-1} malt and 5 g.L^{-1} yeast extract* • MM-*Zt* (minimal) *20 g.L^{-1} dextrose, 2 g.L^{-1} $NaNO_3$, 1.5 g.L^{-1} K_2HPO_4, 1 g.L^{-1} KH_2PO_4, 0.5 g.L^{-1} $MgSO_4.7H_2O$, 0.5 g.L^{-1} KCl, 0.1 g.L^{-1} $CaCl_2$ $2H_2O$, 1 mL microelement and vitamin stocks* • PDB (potato dextrose broth) *24 g.L^{-1} potato dextrose broth* • YPD (yeast extract-peptone-dextrose) *10 g.L^{-1} yeast extract, 20 g.L^{-1} peptone and 20 g.L^{-1} dextrose*	Medium / Predominant *Z. tritici* growth form(s) / Biofilm formation **GPL** / Yeast-like cells / - (beyond 5 days) YM / Yeast-like and pseudo-hyphal forms / + (beyond 4 days) MM-*Zt* / Hyphal forms / + (beyond 3 days) PDB / Yeast-like and a small proportion of pseudo-hyphal forms / + (beyond 3 days) YPD / Yeast-like cells / - (beyond 5 days) • GPL was preferred over YPD as it permits a better monitoring of growth rate kinetics (growth ratio allowing a larger number of readings during the exponential phase)
Culture volume	100, 150, 200 µL per well	• Greater reproducibility was obtained with a **culture volume of 150 µL per well**
Initial spore concentration	5, 7.5, 10, 25, 50, 75, 100, 250, 500, 750 spores.µL^{-1}	• **Initial concentration of 250 spores.µL^{-1}**: lowest initial concentration presenting the lowest coefficient of variation between replicates (to provide reproducible conditions) and reaching saturation point as late as possible during the experiment (to ensure precision in the assessment of growth rates)
Luminosity during growth	Light *vs.* dark conditions	• Luminosity had no significant effect on growth kinetics. • **Dark conditions** were selected to facilitate standardisation.
OD wavelength	340, 405, 450, 560, 620, 820 nm	• **OD_{405}:** easy detection of light scattering (longer wavelengths generate less scattering)
Run time	1, 2, 3, 4, 5, 6, 7 days	• **Selected time window of four days:** sufficient to reach the kinetic saturation point and to prevent inherent biases due to spore melanisation and biofilm formation, which occurs at the surface of GPL liquid medium after more than five days (see ESM 1b)

SM1b Visual (culture medium aspect) and microscopic (at 10x magnification) observations of a biofilm-like structure (highly aggregated swollen yeast-like cells) formation at the surface of GPL liquid medium after more than five days of culture.



SM2. CHARACTERISTICS OF CANDIDATE MATHEMATICAL MODELS TO ESTABLISH TPCs

SET OF CANDIDATE MODELS

We conducted a literature review to identify models previously used to describe thermal response traits. This set of mathematical models was reduced to 15 candidates during a first fitting to Z. tritici growth kinetics data. These 15 models are listed in the table on the following page. Model equations relating performance (P) to temperature (T) by means of K parameters were harmonised using the following key thermal parameters, when relevant: P_{max} (maximum performance), T_{opt} (thermal optimum), T_{min} (minimum temperature), T_{max} (maximum temperature), Curv (shape parameter) and TPB (thermal performance breadth).

GRID OF MODEL SELECTION CRITERIA TO ESTABLISH TPCs FOR A GIVEN TRAIT

The most appropriate model (i.e. that most accurately and efficiently describing the data) was selected by detailed testing of all models with a predetermined set of criteria applied in the following order of importance: ❶ Goodness-of-fit, determined by an information-theoretical approach [1] based on the following statistical metrics: (i) residual sum of squares (RSS); (ii) Akaike weight (w_{AIC}) calculated by correcting the Akaike information criterion for finite sample size (AICc) [1,2] and (iii) Schwarz weight (w_{BIC}) calculated from the Bayesian-Schwarz information criterion (BIC) [1,3]. ❷ Dissimilarity between equation predictions of thermal responses over the temperature range: Are there significant differences in the quality of data adjustment between models relative to the apparently best-fitting model based on step 1 (see column 'Prediction dissimilarity'; NS: not significant, ✱: $P < 0.05$)? Where are residuals localised along the temperature axis? The aim is to maximise the accuracy of estimation for Topt- and its surrounding supra- and suboptimal estimates (i.e. mid-temperature range) over thermal extremes (T_{min} and T_{max}). We therefore favour models with residuals located at one or both ends of the temperature range over models with residuals in the mid-temperature range (see column 'Distribution of residuals'). ❸ Model constraints: is there any assumption forcing the fitting process that appears unjustified or problematic (see column 'Model features')? ❹ Consideration of parameter features: independence (necessary condition) and biological meaningfulness of parameters (not essential) (see column 'Parameters') ❺ Model popularity or frequency of use: when required to choose between two equivalent models, we favour the most widely used model (see column 'Use').

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ILLUSTRATIVE EXAMPLE: THE FRENCH Z. TRITICI POPULATIONS DATASET

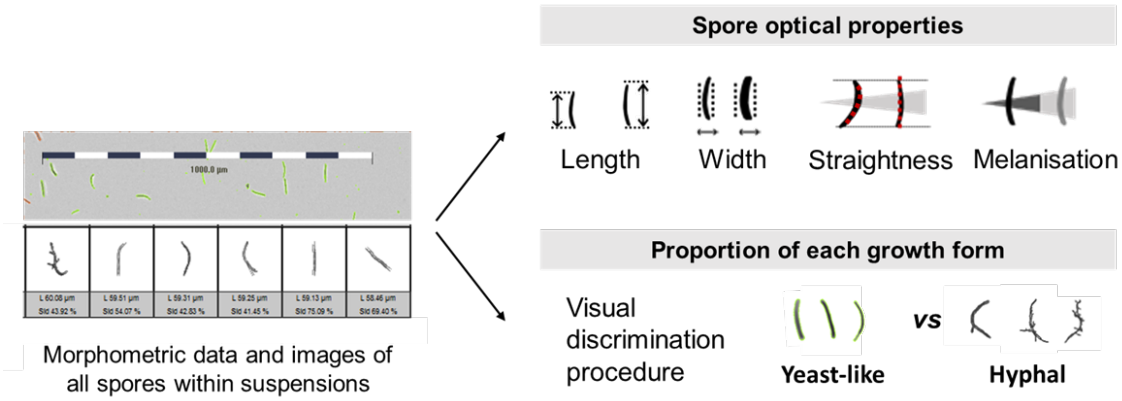
In the table below, the 15 candidate models were ranked and weighed for the French Z. tritici populations dataset (6 x 11 strains; see 'application 1' in the main text of the article or SM5). Five models (highlighted in grey) provided a similar goodness-of-fit over the temperature range (no significant difference in the shape and position of established TPCs and a similar distribution of model residuals). To illustrate the selection process, when a model is discarded at a given step, the fields corresponding to the subsequent steps (moving from the left to the right side of the table) are not filled in (shaded black).

Mathematical models	Relationship P(T) between temperature (T) and performance (P)	K	Goodness-of-fit over the temperature range					Model features	Parameters		Use	Final choice
			RSS	w_{AICc}	w_{BIC}	Prediction dissimilarity	Distribution of residuals		Mutually independent	Biologically meaningful		
Tauti [4]	$\frac{1}{a \times e^{bT}}$	2	9.24	0.00	0.00	*						
Exponential [4]	$a \times e^{bT}$	2	9.24	0.00	0.00	*						
Power [5]	$\frac{1}{a \times T^{b}}$	2	10.36	0.00	0.00	*						
Arrhenius [4]	$a \times e^{\frac{b}{T}}$	2	11.34	0.00	0.00	*						
Yan and Hunt [6] **(modified beta distribution)**	$P_{max}(\frac{T_{max}-T}{T_{max}-T_{opt}})(\frac{T}{T_{opt}})^{T_{opt}/(T_{max}-T_{opt})}$	3	3.96	0.21	0.28	NS	outer edge	T_{min}=0				
Kontodimas [7] **(modified Analytis equation)**	$a \times (T-T_{min})^2 \times (T_{max}-T)$	3	3.96	0.20	0.27	NS	outer edge	n/a	✓	2/3	-	
Quadratic [8]	$P_{max} + Curv(T-T_{opt})^2$	3	3.97	0.19	0.25	NS	mid-temperature ranges					
Gaussian [9]	$P_{max}e^{(-0.5(\frac{\lvert T-T_{opt}\rvert}{TPB})^2}$	3	4.53	0.00	0.00	*						
Lactin [10]	$e^{aT} - e^{aT_{max}-\frac{T_{max}-T}{b}}$	4	12.41	0.00	0.00	*						
Modified Gaussian [9]	$P_{max}e^{(-0.5(\frac{\lvert T-T_{opt}\rvert}{TPB})^d}$	4	3.88	0.13	0.07	NS	mid-temperature ranges					
Baker *et al.* [11]	$ae^{bT}(T_{max}-T)(T-T_{min})$	4	3.95	0.07	0.04	NS	outer edge	b: Eppley curve coefficient[1]				
Third-order polynomial [12]	$a + bT + cT^2 + dT^3$	4	3.96	0.07	0.04	NS	outer edge	symmetry				
Beta distribution [13]	$P_{max}((\frac{T_{max}-T}{T_{max}-T_{opt}})(\frac{T-T_{min}}{T_{opt}-T_{min}})^{\frac{(T_{opt}-T_{min})}{(T_{max}-T_{opt})}})$	4	3.96	0.07	0.03	NS	outer edge	highly flexible[2]	✓	4/4	+	✓
Square-Root model (Ratkowsky equation) [14]	$(a(T-T_{min})(1-e^{b(T-T_{max})}))^2$	4	4.06	0.03	0.01	NS	mid-temperature ranges					
Four-order polynomial [15]	$a + bT + cT^2 + dT^3 + eT^4$	5	3.94	0.02	0.00	*						

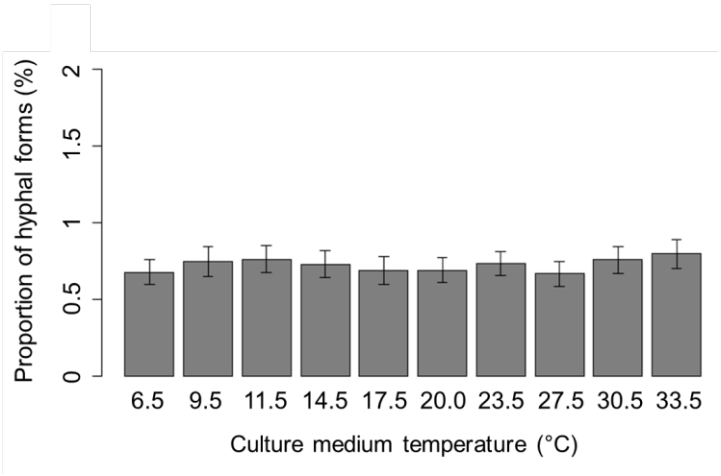
[1] Coefficient of an exponential function defining the maximum attainable daily phytoplankton growth rate as a function of temperature: 0.81 [16]

[2] Unimodal probability density function that is asymmetric, allowing the inflexion of responses on either side of the mode (sub- or supra-optimal range)

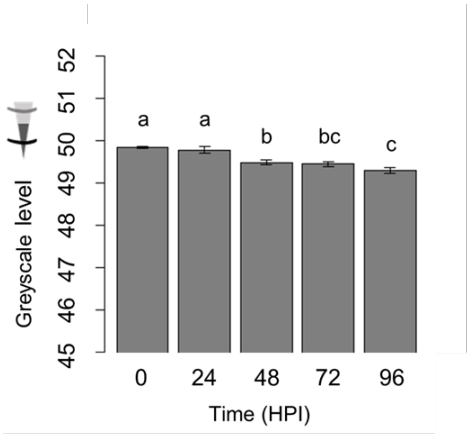
SM3. MONITORING OF SPORE CHARACTERISTICS OVER THE EXPERIMENT

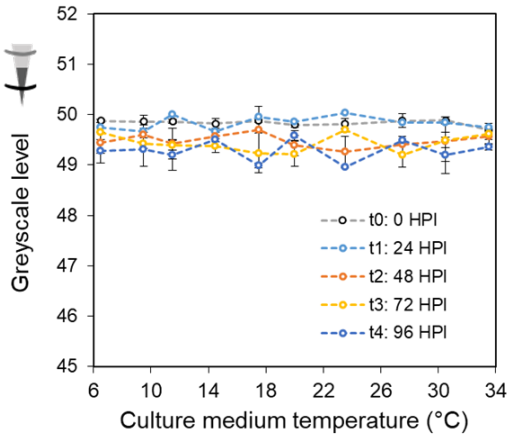


SM3a. Acquisition of spore morphometric data and images. At each time point, a volume of spore suspension is sampled and characterised with the particle counter (Flow-Cell FC200S+ morphogranulometer, Occhio). This machine provides morphometric data (spore length, width, straightness and greyscale level) and images for all spores passing through its flow cell.



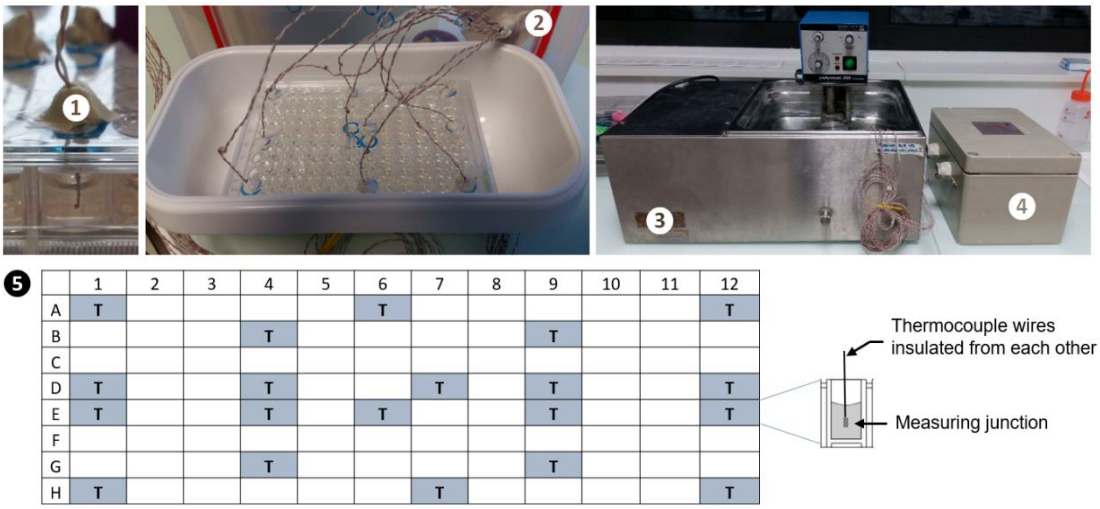
SM3b. Effect of temperature on hyphal growth forms. Temperature had no significant effect on the proportion of hyphal growth forms (mean ± SEM; Kruskal-Wallis, P = 0.79), which remained below 1% in all experimental conditions although spore suspensions subjected to extreme temperatures (< 11.5°C or > 30.5°C) were characterised by a higher number of hyphal growth forms (fewer spores at these temperatures).





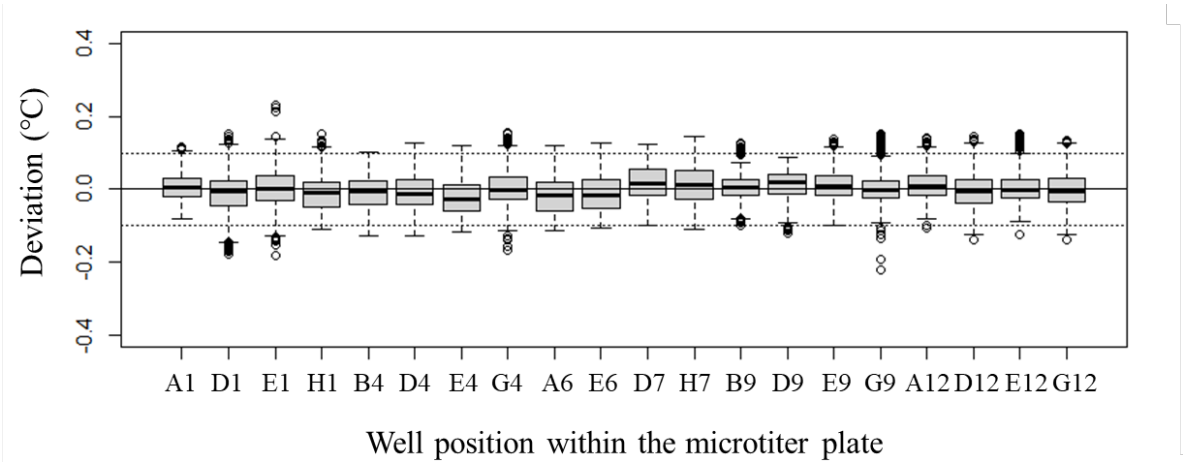
SM3c. Changes in spore melanisation (as measured by the greyscale level of spores, with values ranging from 0 for black particles to higher values for clearer particles) over time in Hours Post-Inoculation (melanisation levels gradually increased after day 2; Kruskal-Wallis, P < 0.01) and at the different studied temperatures (melanisation levels were not affected differently by the temperature of the culture liquid medium and the interaction of melanisation with temperature did not show any significant effect on changes in OD during growth monitoring; F-test, P = 0.07).

SM4. TEMPERATURE MONITORING SYSTEM IN MICROTITER PLATES



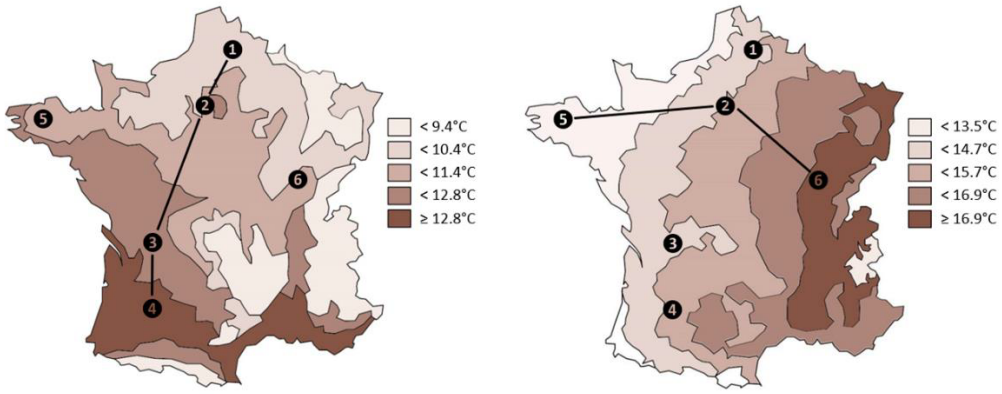
	1	2	3	4	5	6	7	8	9	10	11	12
A	T					T						T
B				T					T			
C												
D	T			T			T		T			T
E	T			T		T			T			T
F												
G				T					T			
H	T						T					T

SM4a. Temperature monitoring system for the liquid culture medium. The system shown here includes 10 T-type (copper-constantan) thermocouples used to monitor temperature in the wells of a microtiter plate positioned in a set-point thermal environment in a thermostatically controlled water bath (❸). Each thermocouple was immersed in a well to measure the temperature in the centre of the medium volume. It was secured in place with Blu Tack (❶). All thermocouples were centralised at one entry point to the box (❷), also sealed with Blu Tack to ensure that the system was hermetic. Thermocouples were linked to a data logger (❹) recording temperature values every 10 minutes. Plate layout (❺), showing the location of the 20 T-type thermocouples (used for the experiment presented in this paper) within the plate. Wells identified by the 'T' icon indicate wells in which a thermocouple was immersed and for which the temperature of the liquid culture medium was logged.



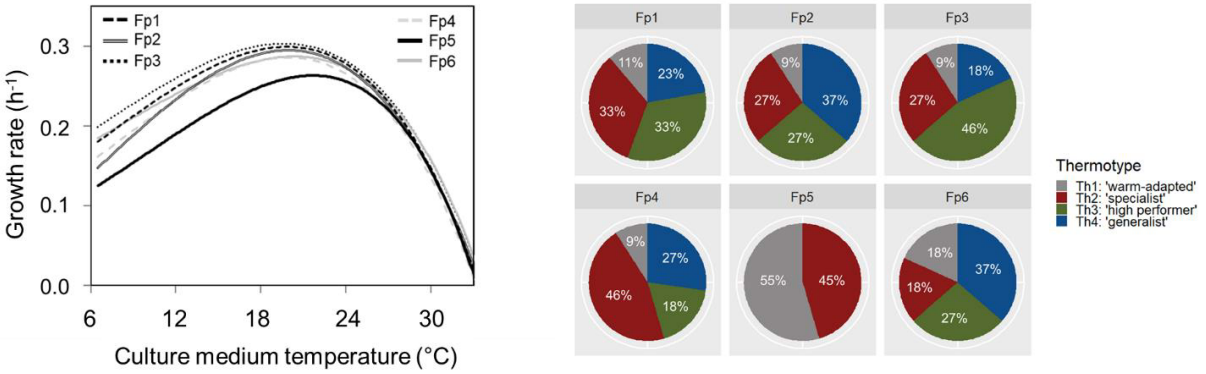
SM4b. Thermal homogeneity of in vitro growth conditions between the wells of a given microtiter plate. Boxplots displaying the range of temperature deviation from the overall mean temperature for the microtiter plate (averaged over the 20 monitored wells) are represented for each of the wells monitored (see plate layout in SM4a) over a four-day recording experiment. Temperature measurements were corrected based on calibrations of T-type thermocouples before and after the experiment, to take differences in accuracy between sensors and potential measurement drifts into account. This system made it possible to measure temperature deviations of more than 0.1°C (maximum error after calibration). Given that most of the temperature recordings fell in the range [-0.1; +0.1] (bounded by dotted lines), the temperature of the liquid medium was considered to be homogeneous between wells regardless of their position in the microtiter plate.

SM5. SAMPLING AND COMPOSITION OF THE SIX FRENCH Z. TRITICI POPULATION



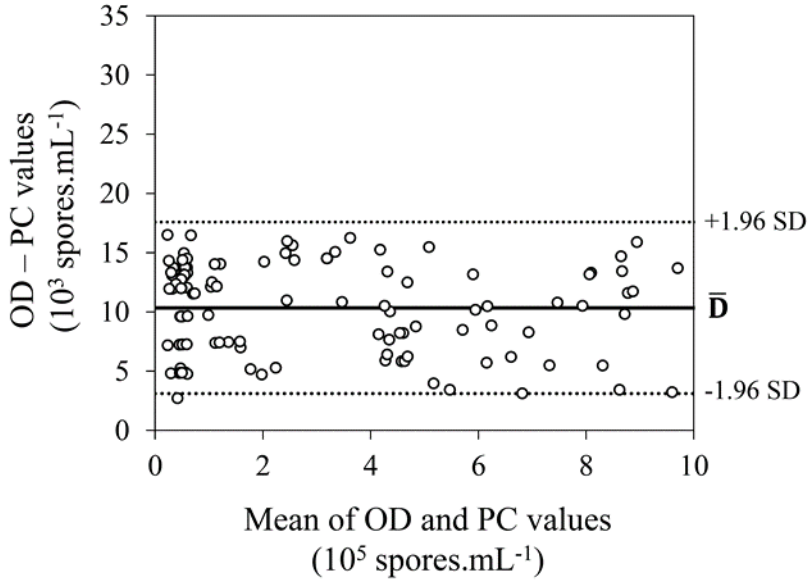
Population ID		Actual thermal environment over the sampling year		Sampling characteristics		
		Annual mean	Annual range	Location	Date	Period
1	Fp1	11.5 °C	13.9 °C	Villers-lès-Cagnicourt	25/06/2014	Spring
2	Fp2	11.9 °C	13.5 °C	Versailles	30/06/2014	
3	Fp3	13.6 °C	15.2 °C	Bergerac	10/06/2014	
4	Fp4	14.1 °C	15.4 °C	Lectoure	28/05/2014	
5	Fp5	10.8 °C	11.9 °C	Ploudaniel	27/03/2013	Winter
6	Fp6	10.8 °C	19.9 °C	Bretenière	22/02/2013	

SM5a. Sampling design and respective thermal conditions of the areas where the six French Z. tritici populations (11 strains each) were collected on cv. Apache. The sampling was performed along two thermal gradients (long-term average climatic parameters; maps were adapted from Joly et al., 2010): a north-south increasing gradient of mean annual temperature; a west-east increasing gradient of annual temperature range (difference between mean temperatures in July and January). Main thermal and sampling characteristics are summarised for each location in the opposite table. The thermal conditions over the sampling year (mean and range) were calculated using data from local weather stations. Additional reference: Joly D, Brossard T, Cardot H, Cavailhes J, Hilal M, & Wavresky P. 2010. Les types de climats en France, une construction spatiale. Cybergeo: European Journal of Geography.

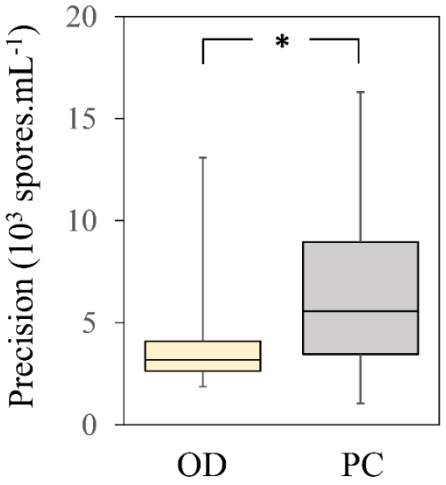


SM5b. Overview of the diversity of thermal responses across the six French Z. tritici populations. (a) Representative mean template thermal performance curve (TPC) for each of the six populations; (b) Pie charts displaying the composition in thermotypes (proportions expressed as percentages) of each population: Th1 ('adapted to warmer conditions'), Th2 ('specialist'), Th3 ('high performer') and Th4 ('generalist').

SM6. COMPARISONS BETWEEN MEASUREMENTS OF SPORE CONCENTRATION



SM6a. Bland-Altman plot showing the degree of agreement between optical density (OD) and particle counter (PC) results by plotting differences against means for each paired measurement. Solid and dotted lines represent the mean (D) and limits of agreement (± 1.96 SD) between the two methods, respectively (n = 124).



SM6b. Boxplots displaying the distribution (closeness of agreement) of three successive replicate measurements (n = 53) for each method. These findings provide an overview of the ability of these two methods to generate consistent results (∗: significant differences between OD and PC methods - $P < 0.05$ - highlighted by a Student's t-test).

Chapter 3

Quantifying the extent and patterns of phenotypic diversity in thermal responses across scales

Foreword. Using the thermal phenotyping framework for Z. tritici, I examine the diversity and shifts in the thermal responses of field populations collected across time (local seasonal dynamics) and space (wheat growing areas). Two questions were addressed: How diverse and structured are thermal responses across populations? Can we detect local adaptation to thermal conditions? This chapter therefore intends to provide new insight into the diversity of thermal responses encountered within pathogen populations living on plant leaves and investigate their potential to adapt to environmental variation and climate change.

Chapter 3 was deposited as preprint in bioRxiv under the heading: Patterns of thermal adaptation in a worldwide plant pathogen: local diversity and plasticity reveal two-tier dynamics

bioRxiv DOI: https://doi.org/10.1101/2019.12.16.877696

3

contents

abstract

Background and Aims

Plant pathogen populations inhabit patchy environments with contrasting, variable thermal conditions. We investigated the diversity of thermal responses in populations sampled over contrasting spatiotemporal scales, to improve our understanding of their dynamics of adaptation to local conditions. Samples of natural populations of the wheat pathogen Zymoseptoria tritici were collected from sites within the Euro-Mediterranean region subject to a broad range of environmental conditions. We tested for local adaptation, by accounting for the diversity of responses at the individual and population levels on the basis of key thermal performance curve parameters and 'thermotype' (groups of individuals with similar thermal responses) composition.

Key Results

The characterisation of phenotypic responses and genotypic structure revealed: (i) a high degree of individual plasticity and variation in sensitivity to temperature conditions across spatiotemporal scales and populations; (ii) geographic adaptation to local mean temperature conditions, with major alterations due to seasonal patterns over the wheat-growing season.

Conclusion

The seasonal shifts in functional composition suggest that populations are locally structured by selection, contributing to shape adaptation patterns. Further studies combining selection experiments and modelling are required to determine how functional group drives population dynamics and adaptive potential in response to thermal heterogeneity.

Keywords: ecological patterns, environmental heterogeneity, functional diversity, interindividual variation, plasticity, seasonal changes, reaction norm, Zymoseptoria tritici

INTRODUCTION

Environmental heterogeneity (Li & Reynolds, 1995) is regarded as one of the most important elements driving the emergence and maintenance of genetic variation within populations (Levins, 1974; Hedrick, 1986; Hughes et al., 2008; Ravigné et al., 2009) as it dictates physiological responses (Cavieres & Sabat, 2008) and can drive the emergence of local adaptation patterns (Thompson, 2005; Nuismer & Gandon, 2008). Gathering information about the way a given community, species or population copes with this environmental heterogeneity is crucial for the understanding and prediction of its distribution and responses to current and future environmental changes (Austin, 2007).

The adequate capture of eco-evolutionary responses requires an integration of physiological variation across biological (individual, group, population, species) and spatiotemporal (seasonal, geographic) scales, given the significant implications of this variation for dynamics (Saloniemi, 1993; Vindenes et al., 2008; Schreiber et al., 2011). It is therefore important to go beyond summarising diversity through average trait values (Bolnick et al., 2011; Violle et al., 2012), and to account for the individual specialisation of phenotypic responses by taking into account both phenotypic plasticity (within-individual differences, Pigliucci, 2001) and interindividual variation (between-individual differences; Dall et al., 2012). This paradigm shift has been made possible by progress in the measurement and analysis of this specialisation at the individual and population level.

The ecological concept of a 'reaction norm', describing phenotypic plasticity i.e. the set of phenotypes generated by a given genotype in response to environmental cues (Schlichting & Pigliucci, 1998) is particularly effective as a tool for accounting for individual specialisation (Bolnick et al., 2002; Araújo et al., 2008). Most of the comparisons of the thermal sensitivity of a given phenotypic trait across individuals under different environmental conditions have been conducted to date on reaction norm descriptors (e.g. comparisons of phenotypic mean and range values at the maximum; Gibert et al., 1998) or degree of plasticity (e.g. breakdown of the shifting and stretching of non-linear reaction norms into non-exhaustive biological modes, i.e. lower-higher, faster-slower, specialist-generalist directions; Izem & Kingsolver, 2005; Martin et al., 2011; van de Pol, 2012). Such approaches have proved highly valuable, but may not be suitable for decomposing the overall variation or distinguishing differential responses between populations (Bulté & Blouin-Demers, 2006) or including intra- and interindividual sources of error (Angilletta, 2006) in ANOVA and random regression approaches (Lynch & Gabriel, 1987; Gilchrist, 1995).

One possible complementary approach to the description of variation between reaction norms involves the use of functional ecology to describe significant variations in the intensity of individual specialisation within populations and species (Garnier & Navas, 2012). The idea is to translate reaction norms into functional traits (Violle et al., 2007) by grouping individual reaction norms into 'functional groups' (Gitay & Noble, 1997). Each of these functional groups responds to the environment in its own way (e.g. low- or high-performance specialists), according to a classification system that is not predetermined (no constrained modes of variation). This approach accounts more effectively for diversity and adaptation patterns,

through the characterisation of three functional components: richness, evenness, and divergence (Mason et al., 2005).

This approach is particularly useful for deciphering variation in continuous reaction norms describing performance as a function of temperature (thermal performance curves or TPC; Huey & Stevenson, 1979), and for documenting patterns of thermal adaptation to prevailing local conditions (Kawecki & Ebert, 2004) across a range of environments (e.g. Mitchell & Lampert, 2000). These patterns play an important role in the case of micro-organisms impacting ecosystems, human health, and food security (Fisher et al., 2012) as local adaptation to temperature conditions governs their geographic distribution, phenology, and abundance (Kraemer & Boynton, 2017). This results in impacting the expansion ranges of plant pathogens (e.g. Milus et al., 2009; Robin et al., 2017), as well as the onset and severity of disease epidemics (e.g. Ferrandino, 2012).

The studies of thermal responses in plant pathogenic micro-organisms performed to date have focused mostly on either summarising the individual variance of aggressiveness traits as population-scale averages (problematic use of single mean species values; Suffert & Thompson, 2018) or phenotyping individuals under a limited set of temperatures when considering variances (generally about three temperatures in thermal biology studies; Dell et al., 2013; Low-Décarie et al., 2017). These strategies have provided useful information about species distribution, making it possible to detect signatures of interindividual variation and adaptation within species and populations (Milus et al., 2006). However, they cannot be used to infer selection driving population dynamics (Lavergne et al., 2010) or to assess the relevant scales of functional diversity (Woodcock et al., 2006; Martiny et al., 2011). Such analyses go well beyond simple comparisons of mean trait values and would require the characterisation of entire TPCs and their variation across different scales.

This study explored the extent of variation in thermal responses of a globally-distributed wheat pathogen across space (geographic range) and time (local seasonal dynamics), and uncover the role that adaptation to local environmental conditions (dynamic evolutionary process) plays in generating this diversity. The analysis of the plasticity and variation of thermal sensitivity across individuals, populations and scales was conducted in the case of Zymoseptoria tritici (formerly Mycosphaerella graminicola; Steinberg, 2015), the causal agent of one of the most economically important wheat diseases (Septoria tritici blotch or STB; Dean et al., 2012; Fones & Gurr, 2015). Besides its agronomic relevance, we chose to study this fungal pathogen as its aggressiveness traits are empirically known to be temperature-sensitive (Shaw, 1990; Lovell et al., 2004) and to display interindividual variation (Bernard et al., 2013; Boixel et al., 2019). Furthermore, its populations present signatures of adaptation to a wide range of contrasted environments over space (globally-distributed pathogen across wheat-growing areas worldwide; Zhan & McDonald, 2011) and time (covering seasonal changes e.g. from late autumn to early summer in Europe; Suffert et al., 2015). Drawing on previous local adaptation studies conducted by Zhan & McDonald (2011) and Suffert et al. (2015), we designed a sampling scheme to grasp the levels of functional diversity shaping responses of Z. tritici populations to contrasted environments.

METHODS

Geographic and seasonal variations in the thermal sensitivity of Z. tritici were investigated by identifying two contrasting scales along which strains (i.e. monospore isolates corresponding to individuals), were collected from a particular site at a particular time. These strains were phenotyped for their thermal responses (asexual growth in vitro) and genotyped for 12 neutral microsatellite markers. The extent to which phenotypic and genotypic variation across individuals, populations and scales reflected thermal adaptation to local conditions was evaluated by cross comparisons of spatiotemporal patterns in thermal responses, allele frequency and temperature conditions (Fig. 1).

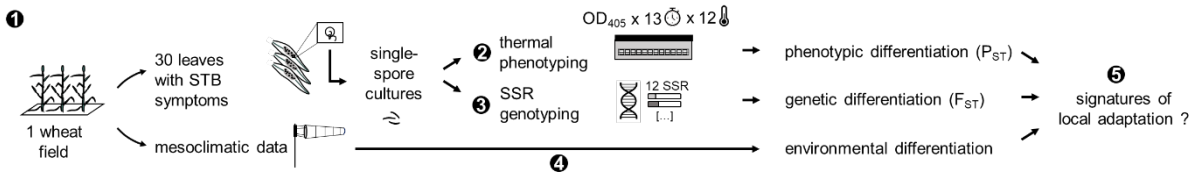


Fig. 1 Overview of the methodology for characterising diversity and adaptive patterns of thermal responses in the sampled Zymoseptoria tritici populations. (1.1) Twelve populations, each composed of either 25 or 30 strains, were collected from diseased leaves in different spatiotemporal locations (8 Euro-Mediterranean populations collected along geographic thermal gradients and 4 French seasonal subpopulations) with the corresponding mesoclimatic conditions (temperature data). All strains were: (1.2) phenotyped in an in vitro growth experiment conducted over a range of 12 temperatures to capture thermal performance curves from growth kinetics involving 13 measurement time points (experimental framework detailed in Boixel et al., 2019); (1.3) genotyped for 12 neutral microsatellite (SSR) markers to quantify phenotypic (P_{ST}) and genetic (F_{ST}) differentiation. (1.4) The thermal conditions experienced by individuals over the wheat growing season were characterised for each spatiotemporal site. (1.5) The local adaptation of individuals and populations to temperature was assessed by cross comparisons of the spatiotemporal patterns of thermal responses, allele frequency and thermal conditions.

Sampling strategy (Fig. 1.1) – Samples were collected from 12 Z. tritici populations for the exploration of spatial and temporal components of thermal adaptation. Spatial variation was investigated for 8 populations sampled within the Euro-Mediterranean region (see detailed sampling information of the geographical scale in Table 1) representative of the contrasting climatic conditions over this large geographic area (covering three – Cfb, Csa, Dfb - out of seven Köppen-Geiger climate zones in which Z. tritici is reported as a notable pathogen; Fig. S1). One of these sites (Grignon, France) was selected for a comparison of the thermal responses of two pairs of winter and spring subpopulations sampled from neighbouring fields, to capture seasonal dynamics over a wheat growing season (i.e. over the course of an annual epidemic; see the 4 populations of the seasonal scale in Table 1 and Fig. S2). These pairs of subpopulations were subject to seasonal variation from November to February and from March to June, respectively. For each of the 12 populations, we collected from 25 to 30 isolates at random from wheat leaves with STB symptoms, from which single-spore isolates were prepared (Methods S1) and which were later confirmed to be genetically unique strains with the microsatellite analysis. We chose to consider 25 or 30 strains (i.e. individuals) per population instead of the minimum level of 15 identified on the basis of a rarefaction analysis (Fig. S3) for estimating the diversity of thermal responses in Z. tritici with sufficient power, accuracy and precision (Dale & Fortin, 2014).

Table 1 Summary information for the 12 Z. tritici populations. These populations were specifically sampled along geographic (8 Euro-Mediterranean populations) and seasonal (4 local French winter (WIN) and spring (SPR) subpopulations) scales with contrasting temperature conditions. Strains were collected from naturally infected wheat fields characterised by a spatial location, a climate zone, a wheat cultivar and sampling conditions (time window and sample size).

Scale	ID	Sampling site	Coordinates[1]	Climate zone[2]	Cultivar	Time[3]	Sample[4]
Geographic	RU	Russia (Moscow)	55.649, 36.958	Dfb	Moskovskaya 56	Spring (booting-heading)	30
	KZ	Kazakhstan (Penkovo)	54.975, 69.226	Dfb	Lutescens		30
	LV	Latvia (Jelgava)	56.542, 23.726	Dfb	Zentos		30
	DK	Denmark (Flakkebjerg)	55.308, 11.388	Cfb	Hereford		30
	FR	France (Grignon)	48.843, 1.946	Cfb	Soissons		30
	IR	Ireland (Carlow)	52.860, -6.909	Cfb	JB Diego		30
	TN[5]	Tunisia (Manouba)	36.923, 9.839	Csa	Karim		30
	IS[6]	Israel (Kiryat-Tivon)	32.696, 35.125	Csa	Galil		30
Seasonal	WIN1	France (Grignon)	48.840, 1.945	Cfb	Soissons	Post-winter (tillering)	25
	WIN2		48.843, 1.946				30
	SPR1		48.840, 1.945			Post-spring (ripening)	25
	SPR2		48.843, 1.946				30

[1] latitude and longitude in decimal degrees format

[2] Köppen-Geiger classification: Cfb (temperate oceanic climate); Csa (hot-summer Mediterranean climate); Dfb (warm-summer humid continental climate)

[3] seasonal conditions and BBCH-scale coding system for cereal phenological growth stages (Zadoks et al., 1974)

[4] number of strains

[5] all individuals from a given location were collected from a single pure-stand field of a bread wheat cultivar susceptible to STB, except for the TN population, which was sampled from a durum wheat cultivar.

[6] Z. tritici populations were sampled during the 2015-2016 wheat growing season, except for the IS population, which was sampled during the 2016-2017 growing season.

Phenotypic variations in thermal responses (Fig. 1.2) – Thermal responses were phenotyped by determining the in vitro growth rates of the strains in liquid glucose peptone medium (14.3 $g.L^{-1}$ dextrose, 7.1 $g.L^{-1}$ bactopeptone and 1.4 $g.L^{-1}$ yeast extract) over a four-day period at 12 constant temperatures ranging from 6.5 to 33.5°C (6.5, 9.5, 11.5, 14.5, 17.5, 20.0, 22.5, 24.5, 26.5, 28.5, 30.5 and 33.5°C). The growth rate μ of each strain at each temperature was calculated according to the standardised specific experimental framework developed by Boixel et al. (2019) which have been validated to be representative of in planta responses with respect to discrimination between cold- and warm-adapted individuals. TPCs describing in vitro growth rate as a function of temperature were established by fitting a quadratic function to the temperature–growth rate (or performance P) estimates for each strain: $P(T)=P_{max}+Curv(T-T_{opt})^2$ where Curv is a shape parameter (Table S1 for more information on the model selection process). The key properties of TPCs were estimated through thermal traits commonly used to compare thermal sensitivities (Kingsolver, 2004; Angilletta, 2006). We have retained three parameters to describe the shape of these TPCs and quantify their characteristics: first, maximum performance (P_{max}) which informs on TPC height ('vertical shift' modes of variation); second, thermal optimum (T_{opt}) which informs on TPC position at the peak performance

('horizontal shift' modes of variation); third, thermal performance breadth (TPB_{80}) which informs on the sensitivity of the response to temperature change around T_{opt} ('width shift' modes of variation). The estimates of T_{min} and T_{max} were not retained for further analysis as they fell outside the range of temperatures tested. TPC variation was assessed in two ways: (i) differences in the range and mean values of P_{max}, T_{opt}, and TPB_{80}, assessed with parametric or non-parametric (depending on whether the assumptions of normality and homoscedasticity were verified) statistical tests for comparing variances and means; (ii) typological comparisons grouping together TPCs with similar thermal characteristics (functional thermal groups, referred to hereafter as 'thermotypes') based on a K-means clustering procedure applied to the covariation of P_{max}, T_{opt}, and TPB_{80} for all TPCs (Methods S2). The magnitude and distribution of diversity for thermal traits and thermotypes were analysed across individuals, populations and scales, to detect differentiation in phenotypic patterns, in Chi-squared tests on the observed frequency distribution of thermotypes.

Neutral genetic variation and population differentiation (Fig. 1.3) – To assess population genetic differentiation, the 350 individuals composing the 12 Z. tritici populations were genotyped for 12 neutral genetic markers on DNA extracted from 50 mg of fresh fungal material from five-day cultures, following SSR amplification and sequencing in one multiplex PCR sample, and allele size annotation (Gautier et al., 2014; Methods S3a). Population structure was inferred with a Bayesian clustering approach under an admixture and correlated allele frequencies model implemented in STRUCTURE (Pritchard et al., 2000). The degree and significance of genetic variability within a population (genetic diversity and allele richness) and differentiation between populations (pairwise estimates of Weir and Cockerham's F-statistic – F_{ST} – and hierarchical analyses of molecular variance – AMOVA) were evaluated with random allelic permutation procedures in GENETIX (Belkhir, 2004) and Arlequin (Excoffier & Lischer, 2010) software (Methods S3b-d).

Characterisation of local climates (Fig. 1.4) – Air temperature data for the closest weather stations within a mean 30-km radius of the eight sampling sites were retrieved from archives of global historical weather and climate data, to obtain: (i) monthly-averaged values of 1961-1990 climate normals (Norwegian Meteorological Institute, 2019); (ii) daily data over the sampling year (US National Climatic Data Center NCDC-CDO, 2019). Local variation in mesoclimatic temperatures were summarised for four climatic time windows (1961-1990 climate normals and thermal conditions for the year of sampling averaged over the calendar year, the wheat growing season, the winter/spring periods) and for five metrics (thermal mean, range, maximum, minimum and variance), giving a total of 20 thermal variables at each site. We then established a thermal niche (i.e. temperature conditions of a given sampling site) classification, by assessing the importance of each of these variables for discriminating between the three contrasting Köppen-Geiger climatic zones prospected, with a nonlinear and nonparametric random forest algorithm (RF; Breiman, 2001) in the 'randomForest' package of R (Liaw & Wiener, 2002). The importance of variables was compared on the basis of two metrics assessing the inaccuracy of RF zone classification if the variable concerned is not accounted for (RF mean decrease in prediction accuracy and node impurity).

Testing for signatures of local adaptation (Fig. 1.5) – Two steps were taken to detect genetic and phenotypic signatures of local adaptation underlying the observed differentiation between populations. First, the degree of genetic differentiation for the set of neutral markers (F_{ST} index; Weir & Cockerham, 1984) was compared with that for phenotypic traits (P_{ST} index; Leinonen et al., 2006). This made it possible to infer departures from neutral expectations (Merilä & Crnokrak, 2001), to determine whether thermal traits were under selection rather than subject to genetic drift (Brommer, 2011). F_{ST}-P_{ST} comparisons were conducted separately for seasonal (on T_{opt}) and geographic populations (on T_{opt} and TPB_{80}), on the basis of sensitivity analyses assessing the robustness of the conclusions to variations in the approximation of Q_{ST} by P_{ST} (Methods S3e). Second, correlations between local climate conditions and Z. tritici thermal sensitivity were evaluated, to detect signatures of adaptation. Pearson correlation coefficients and their statistical significance were established for all possible combinations of thermal traits or thermotypic compositions and for the 20 spatiotemporal thermal variables defining the thermal niche of a climatic site.

RESULTS

Marked individual variation in thermal traits at all scales - We observed a very high level of interindividual variation for the three thermal traits chosen to describe TPCs, within a range of 0.17 to 0.46 h^{-1} for P_{max} (in vitro growth rate), 9.6 to 25.1°C for T_{opt}, and 2.8 to 30.9°C for TPB_{80}, across all 350 strains (Fig. 2). The average metapopulation-level responses in the seasonal and geographic data sets were remarkably similar in terms of their quadratic parameters (Welch's two-sample t-test, $P > 0.05$): $P_{seasonal}(T) = 0.30 - 0.00077 \times (T - 18.3)^2$ vs. $P_{geographic}(T) = 0.30 - 0.00088 \times (T - 18.2)^2$. Interindividual variation around this average TPC was greater for the seasonal than for the geographic scale, as demonstrated by the standard shift in TPC position along the x- and y-axes (Fig. 2a) and the distinctly larger density distributions of the three thermal traits at the seasonal scale (Fig. 2b-d; Levene's test for homogeneity of variance: $P = 0.01$ for P_{max}; $P < 0.01$ for T_{opt}; $P = 0.02$ for TPB_{80}). Interindividual variation within populations was similar at both the geographic and seasonal scales, with equivalent variances for P_{max} ($\bar{x} \pm 0.06$ h^{-1} [SD] on average), T_{opt} ($\bar{x} \pm 2.59$ °C [SD] on average) and TPB_{80} ($\bar{x} \pm 5.72$ °C [SD] on average) within the 12 populations (Levene's test for homogeneity of variance: $P = 0.07$; 0.51; 0.13, respectively). The populations may therefore be considered similar in terms of their individual variances for thermal traits. By contrast, they were not similar in terms of the corresponding population means, as significant differences were detected for T_{opt} and TPB_{80} ($P < 0.05$) but not for P_{max} ($P_{geographic} = 0.09$; $P_{seasonal} = 0.75$; contrary to what would be expected under the 'warmer is better' hypothesis in thermal biology - this is not surprising given the fact that this is a fungus with limited growth at high temperatures; Bennett, 1987).

A reading grid for functional diversity in individual thermal responses - TPCs were classified into thermotypes with similar thermal responses (Hopkins' statistic of 0.71, indicating clustered data and justifying the establishment of such a typology; Methods S2a). The diversity of TPCs encountered in the data set was optimally partitioned into 13 thermotypes (Th1 to Th13; Fig. S4), for which relative degrees of temperature specialisation were described in terms of the T_{opt} (cold- vs. warm-adapted), TPB_{80} (specialist vs. generalist), and P_{max} (low vs. high

performer) dimensions (Fig. 3a). These thermotypes illustrated two commonly documented non-exclusive shifts in TPC along thermal gradients: a horizontal shift (low-temperature vs. high-temperature generalists or low-temperature vs. high-temperature specialists; e.g. Th1 vs. Th13 in Fig. 3b) and a generalist-specialist shift without (Th8 vs. Th9 in Fig. 3c) or with (Th1 vs. Th3 or Th11 vs. Th13 in Fig. 3d) trade-offs between P_{max} and TPB_{80} (i.e. when one cannot increase without a decrease in the other). Indeed, regression analysis revealed a significant negative correlation between P_{max} and TPB_{80} across all individuals (Pearson's correlation coefficient: $R = -0.44$; $P < 0.01$). About 10% of individuals did not follow this pattern, with high values of both P_{max} and TPB_{80}. These individuals (i.e. the strains of Th8), are both 'jack-of-all-temperatures' and 'masters of all', as they perform well at all temperatures (Huey & Hertz, 1984; Fig. 3e). Each cluster included strains from both geographic and seasonal populations (Fig. S4), but with an uneven distribution (difference in Jaccard distance, with a highest pairwise difference of 0.62 between WIN1 and SPR1) and an uneven relative abundance of the 13 thermotypes over the two scales. This relative abundance varied by a factor of up to two for the slightly adapted thermotypes Th5 and Th7. The various thermotypes were not equally distributed across the 12 populations either (Chi-squared test for given probabilities, $P < 0.01$). This heterogeneous distribution was particularly pronounced for high-temperature generalists (see the contributions of Th12 and Th13 to the total Chi-squared score for the comparison of distributions across seasonal and geographic populations in Fig. S5d and S6c). Four thermotypes together accounted for almost half the entire data set (Th5, Th6, Th7 and Th10). The distinguishing features of these four thermotypes were their average behaviour with respect to T_{opt} (Th5, Th6, Th7), TPB_{80} (Th5, Th10) and P_{max} (Th7, Th10).

Thermal phenotypic differentiation of Euro-Mediterranean populations - For population-level TPCs, significant variation was observed for thermal trait means for T_{opt} (Kruskal-Wallis, $P < 0.01$) and TPB_{80} (Kruskal-Wallis, $P < 0.01$), but not for P_{max} (Kruskal-Wallis, $P = 0.09$), for which no population differentiation was detected (Table 2). P_{max} values may have been constrained by the detection thresholds for optical density (smoothing of data for individuals with 'extreme performance phenotypes'). There was a two-degree difference in Topt between the IS population and the 7 other populations (Table 2). The IS population consisted of individuals performing best at higher temperatures (Fig. 4a) with a higher proportion of high-temperature generalists (Th12 and Th13; 1:3 vs. 1:15 on average for the other geographic populations), accounting for 20.7 % of the imbalance in the distribution of thermotypes between populations (see contributions to the total Chi-squared score in Fig. S5d). The thermotypes best adapted to colder conditions (CA+, Th1-Th2-Th3) were particularly abundant in the Dfb populations (RU-KZ-LV). This adaptation to colder conditions can be seen by their long-tailed distributions skewed towards lower temperatures (with 6 highlighted individuals in Fig. S5a presenting a T_{opt} of about 10.4 ± 0.7°C, i.e. about 7°C below the mean value). The IS population was characterised by a higher TPB_{80} for its average population response than the other populations, particularly DK (19.5 vs. 12.7°C; Table 2). These two populations had opposite patterns in terms of their respective proportions of thermal specialists and generalists (Fig. 4b and Fig. S5b). More broadly, the individuals with the greatest thermal breadth (G+, Th1-Th13) were less abundant in Cfb populations (DK-FR-IR), which were characterised by a higher proportion of more highly specialist individuals (S+, Th4 and Th9) than the average (accounting for 10% of the total Chi-squared score; Fig. S5d).

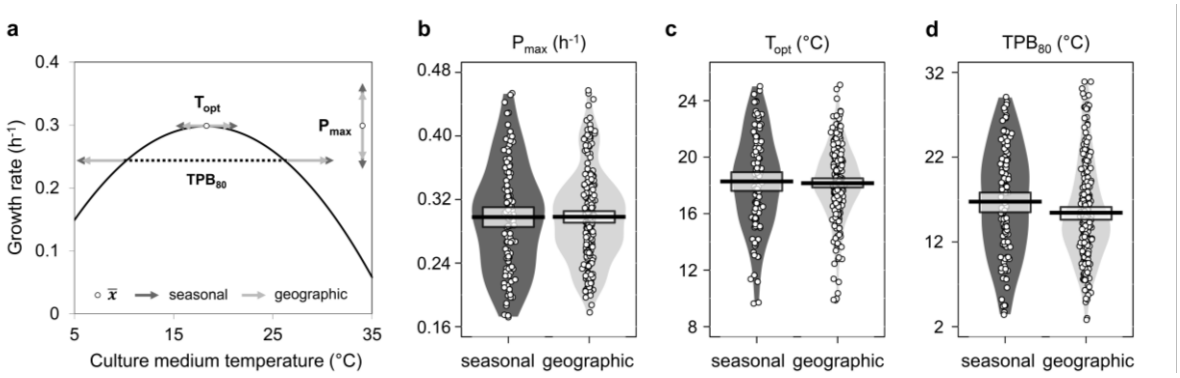


Fig. 2 Comparisons of individual variation in Zymoseptoria tritici thermal performance curves (TPCs) established for in vitro growth rate for the seasonal and geographic scales. (a) The proportion of individual variation around the average TPC for all strains (n = 350) is displayed for three key thermal parameters: maximum performance (P_{max}), thermal optimum (T_{opt}), thermal performance breadth (TPB_{80}: temperature range over which performance exceeds 80% of P_{max}). The plot displays the population-level response (black solid line), the mean value over the 350 individuals for each parameter (open circles and dashed horizontal line) and the spread of the parameter (movement and shift in TPC position along the x- and y-axes) within the seasonal (n = 110) and geographic (n = 240) data sets (colour-coded arrows indicating the standard deviation around the mean). The individual variation in TPCs across strains is further broken down into the distribution of (b) P_{max}, (c) T_{opt} and (d) TPB_{80}, visualised as their raw individual values (open circles), means (black thick lines), distributions (smoothed density curves) and 95% Bayesian highest density intervals (central rectangular boxes enclosing the means).

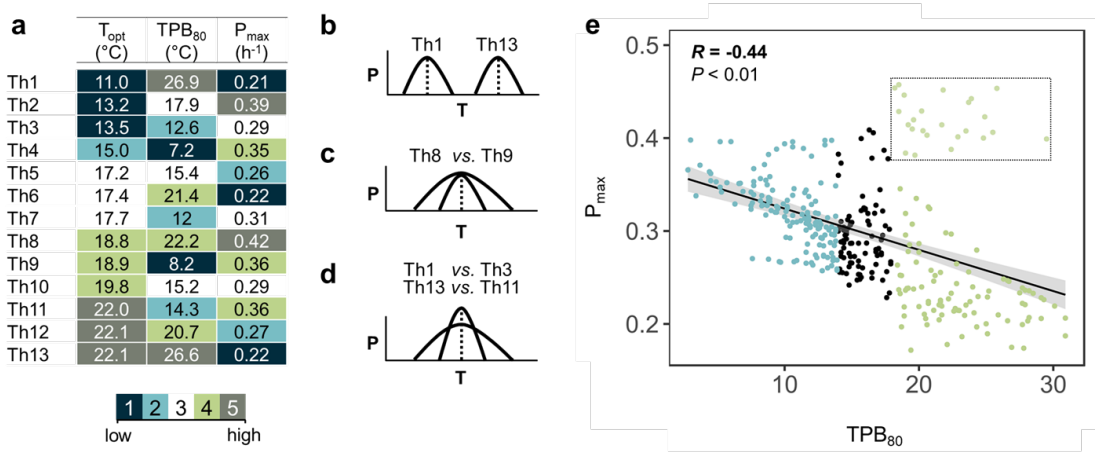


Fig. 3 Analysis of the functional differences in thermal performance curves (TPCs) across Z. tritici strains. (a) Heatmap highlighting the intrinsic features of the 13 Z. tritici thermotypes (Th) defined on the HCPC clustering of the 350 individual TPCs (Fig. S4). A five-level scale was defined to summarise the overall difference in low and high values of P_{max} (low vs. high performance strains); T_{opt} (cold- vs. warm-adapted strain); TPB_{80} (specialist vs. generalist strain): statistically significant (1) much lower, (2) lower, (3) no deviation, (4) higher, (5) much higher value, relative to the overall mean of each parameter over the whole data set. The indicated T_{opt}, TPB_{80} and P_{max} values correspond to the 'barycentre' of each thermotype. (b,c,d) Three common documented shifts in thermal biology studies were identified: (b) a horizontal shift with variations in the position of TPCs along the temperature-axis distinguishing 'cold-adapted' vs. 'warm-adapted' thermotypes; a horizontal stretch distinguishing 'generalist' vs. 'specialist' thermotypes (c) without or (d) with trade-offs between P_{max} and TPB_{80} (TPC axes: P: Performance; T: Temperature). (e) Scatter plot highlighting a trade-off between P_{max} and TPB_{80}. P_{max} is generally negatively related to TPB_{80} except for a group of TPCs with both high TPB_{80} and P_{max} (green points surrounded by a rectangle). The regression is displayed as a solid line, with its 95% confidence interval (shaded area) and its Pearson's correlation coefficient R.

Seasonal adaptive shifts within local populations - Spring subpopulations (SPR1 and SPR2) had a higher thermal optimum than winter subpopulations (ANOVA, $P < 0.01$), with a horizontal shift of T_{opt} towards higher temperature of the order of 5°C (SPR1) and 2.3°C (SPR2) on average (see Table 2, Fig. 5a). In terms of thermotype composition, these two pairs of subpopulations differed principally in their relative proportions in strains highly adapted to warm conditions (WA+). WA+ strains were significantly more abundant in SPR populations (Fig. 5b) than in WIN populations, accounting for 33.4% of the total Chi-squared score for difference in thermotype distributions between WIN and SPR populations. Conversely, WIN populations had a higher proportion of individuals highly adapted to cold conditions (CA+; Fig. S6).

Signatures of local adaptation to mean annual temperature conditions - Neutral molecular markers revealed that all strains were genetically different. We observed no difference in the genetic structure of the 12 populations, with similar allele frequencies at each locus (see Fig. S7 and Table S2), suggesting a constant mixing of populations through substantial continental gene flow, as underlined in previous studies for Z. tritici (Boeger et al., 1993). Strong evidence of local adaptation was detected, with the occurrence of strong phenotypic divergence (Fig. 6) and a robust P_{ST} - F_{ST} difference for the T_{opt} of both geographic and seasonal populations and for the TPB_{80} of geographic populations (Fig. S8). An analysis of possible correlations between these thermal traits and the temperature conditions of the eight sampling sites (monthly averaged values of 1961-1990 climate normals) indicated that the mean thermal optimum of geographic populations increased with mean annual temperature (Fig. 7a). The level of cold adaptation of these populations (measured as the ratio of highly cold-adapted to highly warm-adapted strains) was negatively and significantly correlated with the same environmental variable (Fig. 7b). The mean annual temperature over the 1961-1990 period to which populations seemed to be adapted was one of the three thermal variables most strongly differentiating between the three Köppen-Geiger climatic zones considered in this study (highest random forest mean decrease in accuracy; Fig. S9), together with mean annual temperature over the year of sampling and seasonal contrasts. This difference between mean spring and mean winter temperatures gave the highest mean decrease in Gini index (0.27 vs. 0.25 for mean annual temperature over the 1961-1990 period). This finding highlights the potential importance of seasonal conditions in structuring the thermal responses of these geographic populations.

Table 2 Differentiation in the averaged thermal performance curves (TPCs) of the 12 Z. tritici populations. Each population is characterised by the population mean values for maximum performance (P_{max}), thermal optimum (T_{opt}) and thermal performance breadth (TPB_{80}) of individual TPCs. Significant differences in the parameters of TPCs between populations were assessed separately for geographic and seasonal populations, through mean comparisons. The Latin (geographic analysis) and Greek (seasonal analysis) letters in brackets indicate significant differences in post-hoc tests.

Thermal trait		Geographic scale								Seasonal scale			
		RU	KZ	LV	DK	FR	IR	TN	IS	WIN1	SPR1	WIN2	SPR2
P_{max}	h^{-1}	0.31	0.29	0.29	0.32	0.31	0.28	0.29	0.29	0.31	0.3	0.3	0.29
T_{opt}	°C	17.1 (a)	17.8 (a)	17.8 (a)	17.7 (a)	18.1 (a)	18.2 (a)	18.5 (a)	20.0 (b)	15.9 (α)	20.9 (β)	17.0 (α)	19.3 (β)
TPB_{80}	°C	15.4 (b)	14.9 (bc)	16.4 (b)	12.7 (c)	14.1 (bc)	14.9 (bc)	15.7 (b)	19.5 (a)	14.8	18.9	17	16.4

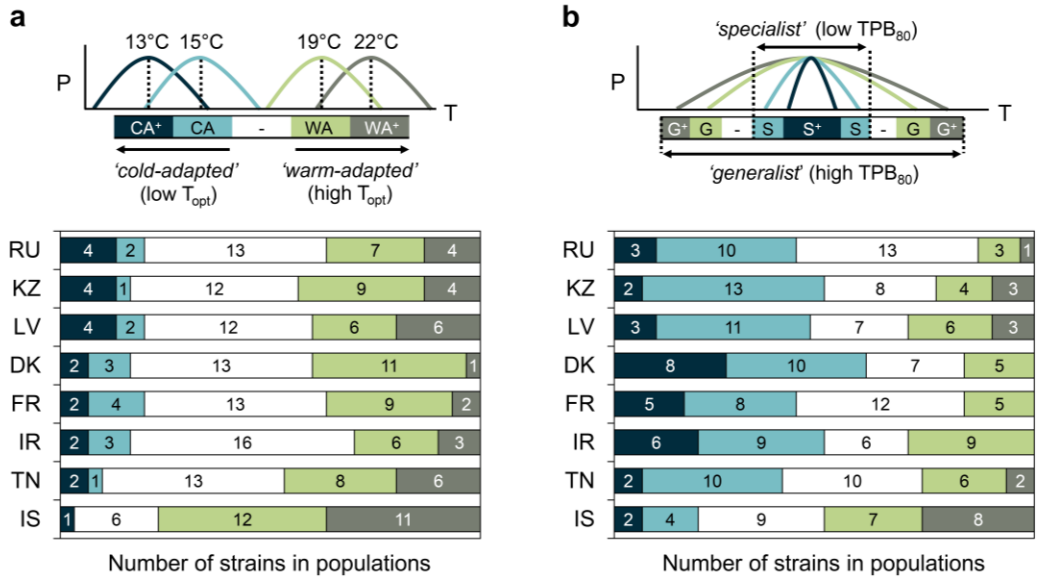


Fig. 4 Thermal differentiation in the functional composition of the 8 geographic Zymoseptoria tritici populations. The functional composition of these populations is displayed according to two complementary reading grids relating to: (a) optimal temperature, with the relative proportions (x-axis) and corresponding number of individuals (bar values) of highly cold-adapted (CA^+), cold-adapted (CA), intermediate (- in white), warm-adapted (WA), and highly warm-adapted (WA^+) thermotypes within each population; (b) thermal breadth with the relative proportions (x -axis) and corresponding number of individuals (bar values) of high (S) and very high (S^+) specialist (mean TPB_{80} of 10.8°C) vs. high (G) and very high (G^+) generalist (mean TPB_{80} of 23.6°C) thermotypes Populations were sampled in RU (Russia), KZ (Kazakhstan), LV (Latvia), DK (Denmark), FR (France), IR (Ireland), TN (Tunisia), and IS (Israel).

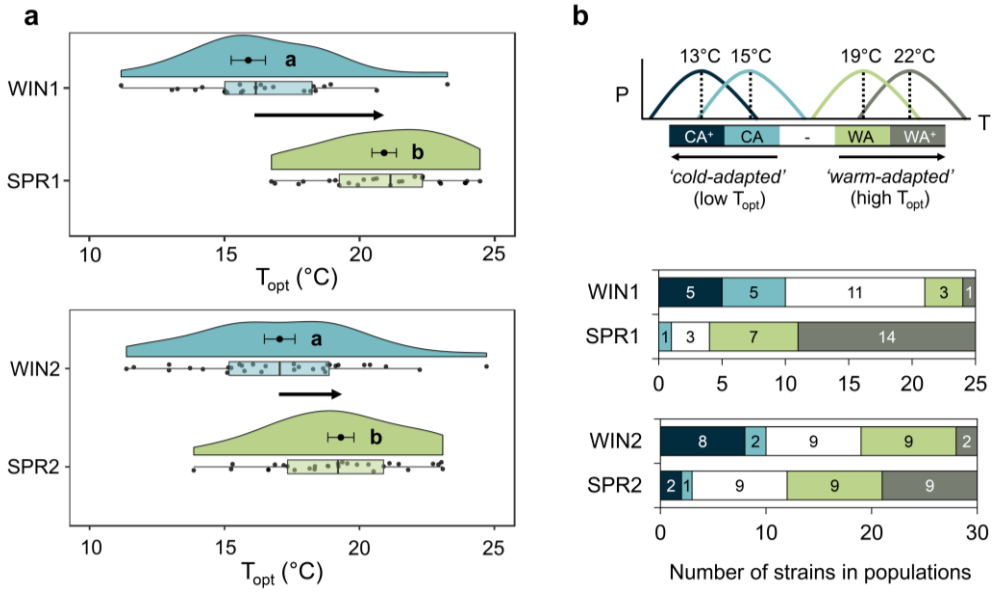


Fig. 5 Individual differentiation in the thermal optimum of Zymoseptoria tritici strains between French winter and spring subpopulations. (a) The population-level thermal optima (means ± SEM) are presented together with the distribution of individual T_{opt} within populations (associated raw data points, boxplots and split-half violins). A significant shift in T_{opt} distribution along the temperature-axis was detected between winter (WIN1 and WIN2) and spring (SPR1 and SPR2) subpopulations sampled from two local neighbouring fields (annotated 1 and 2). The letters indicate the output of paired Student's t-tests with $P < 0.05$. (b) Functional thermotype composition within winter and spring subpopulations is displayed as relative proportions (x-axis) and corresponding numbers of individuals (bar values) for highly cold-adapted (CA^+), cold-adapted (CA), intermediate (- in white), warm-adapted (WA), and highly warm-adapted (WA^+) thermotypes.

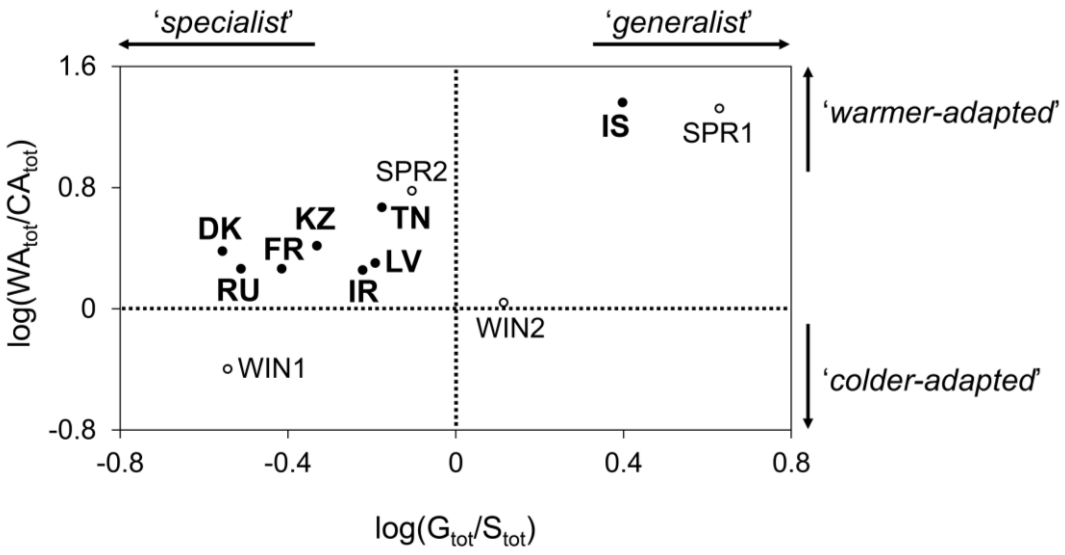


Fig. 6 Functional diversity in thermal responses between the 12 Z. tritici populations. Geographic (in bold) and seasonal (in standard text) populations are situated along: (i) a scale of increasing degree of adaptation to warm conditions (y-axis) discriminating colder- and warmer-adapted populations (logarithm of the ratio of the total of warm-adapted individuals – WA and WA^+ – to the total of cold-adapted individuals – CA and CA^+); (ii) a scale of thermal breadth continuum (x-axis) discriminating more specialist and more generalist populations (logarithm of the ratio of the total number of generalist individuals – G and G^+ - to the total number of specialist individuals – S and S^+).

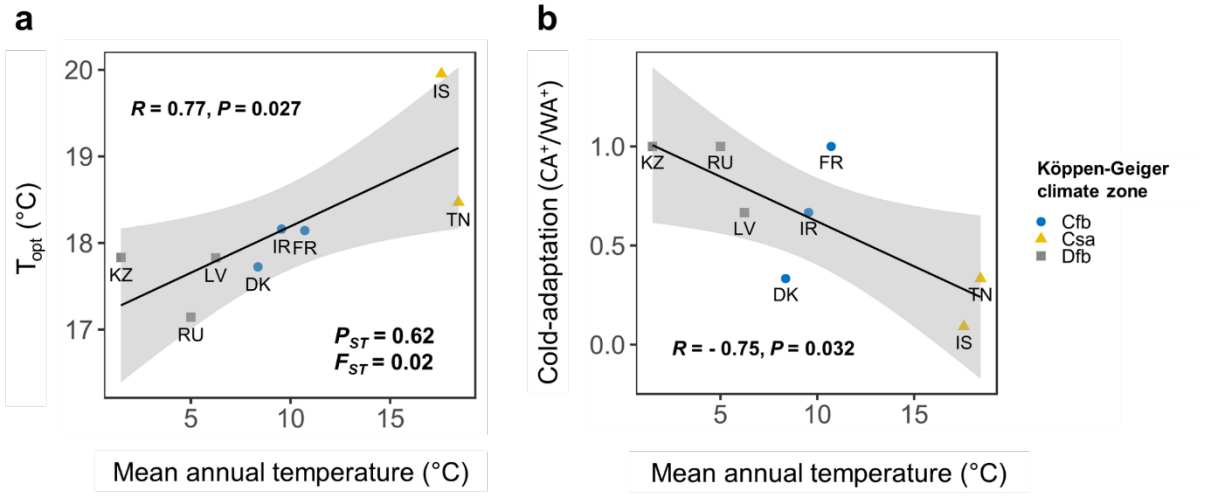


Fig. 7 Signatures of Zymoseptoria tritici adaptation to the mean annual temperature of the local environment in the 8 geographic populations. (a) Relationship between population thermal optimum (T_{opt}) and the mean annual temperature of the sampling sites (monthly averaged values of 1961-1990 climate normals, themselves positively correlated with the monthly averaged values over the sampling year; Pearson's correlation coefficient: R = 0.98, P < 0.01). Population differentiation in T_{opt} relative to neutral genetic differentiation is indicated by P_{ST} and F_{ST} values. (b) Relationship between cold-adaptation level, defined as CA^+ / WA^+ (ratio of the number of highly cold-adapted to highly warm-adapted thermotypes), and the mean annual temperature at the sampling site. Linear dependence between these pairs of variables is indicated by the regression line (solid line and its 95% confidence interval, shown as a shaded area), Pearson's correlation coefficient R and its associated p-value P (see Fig. S1 for a description of the three Köppen-Geiger climate zones, Cfb, Csa and Dfb).

DISCUSSION

Spatiotemporal diversity and distribution of Z. tritici thermal responses

By characterizing thermal performance curves of Z. tritici strains collected over different spatiotemporal scales, we were able to develop a fine description of the extensive interindividual variation in thermal sensitivity: maximum performance (P_{max}), thermal optimum (T_{opt}), and thermal performance breadth (TPB_{80}). This detailed characterisation was made possible by the large range of temperatures and the high resolution of this experimental study (12 temperatures, ranging from 6.5 to 33.5 °C), the extensive sampling strategy (350 strains from 12 populations collected within the Euro-Mediterranean region) and the use of a dedicated and previously validated experimental framework based on turbidity measurements (Boixel et al., 2019). It is important to bear in mind that these turbidity measurements may not reflect the sole growth multiplication rate via yeast-like budding but more precisely quantify the total fungal biomass that could be affected by the pleomorphic nature of some strains of Z. tritici under some environmental stimuli (e.g. partial transition to pseudohyphae or induction of a few chlamydospores, a very recently highlighted form at high temperatures; Francisco et al., 2019). Precautions were taken to work under culture conditions limiting morphological transitions in the four-day time window of the experiments: very few hyphae were observed at 96 h when validating the method (see ESM1-3 in Boixel et al., 2019), and no chlamydospore has so far been described in the literature before 96 h in liquid medium (Francisco et al., 2019). As such, this framework enables to detect differences in thermal sensitivity between isolates (whatever the physiological bases that underpin these differences) and to go beyond the usual tests of 'thermal sensitivity' based on two temperatures, which can be misleading due to the non-linearity of reaction norms (Angiletta, 2009). The individual variation of thermal traits was conserved across populations (similar variance within populations) but was generally more marked over the seasonal scale (for a similar average metapopulation-level response between seasonal and geographic scales). These findings are particularly striking because the choice of geographic populations made it possible to cover three contrasting Köppen-Geiger climatic zones (Fig. S1).

Singular geographic patterns of Z. tritici population adaptation to local conditions

The geographic variation of TPCs provides evidence of thermal adaptation to local conditions in Z. tritici, with: (i) an increase in the mean thermal optimum of a given population with the annual mean temperature of its location of origin; (ii) a particularly marked adaptation to high temperatures of the population sampled in Israel, consistent with the results obtained for another Israeli population investigated by Zhan & McDonald (2011); (iii) differences in the level of specialisation of individuals between populations with higher proportions of specialist individuals in the Cfb (climatic zone with lower annual temperature range) than in the Dfb (climatic zone with higher annual temperature range) populations, consistent with the assumption that thermal generalists are favoured in more variable environments. By contrast, over a smaller geographic scale (France), using the same experimental method, we detected: (i) high levels of local diversity but no structuration of thermal responses between spring populations sampled along a gradient of increasing mean annual temperature; (ii) a marked difference between post-winter populations sampled along a gradient of increasing annual temperature range: presence of thermal generalists in the population exposed to the largest

annual temperature range (19.9°C) vs. the complete absence of such generalists in the population exposed to the smallest annual temperature range (11.9°C; Boixel et al., 2019). The phenotypic differentiation of thermal responses at the population level probably results from local short-term selection of the fittest strains over the course of an annual epidemic. We investigated the adaptation to the location of origin of populations with respect to mesoclimatic temperature conditions. The patterns of adaptation detected may have have been blurred by a non-optimal descriptive resolution of the thermal niche. Indeed, the microenvironment actually perceived by organisms can diverge from the surrounding macroenvironment due to complex biophysical filters across scales (here phylloclimate vs. mesoclimate; Chelle, 2005). In a second approach, scaling the actual climate perceived by Z. tritici populations down to the phylloclimate would help refining the definition of a thermal niche for each population (Pincebourde & Woods, 2012; Pincebourde & Casas, 2019). Such an approach might provide deeper insight into the maintenance of high levels of diversity and some degree of maladaptation in individual thermal responses within each population.

Seasonal dynamics of thermal responses in two local Z. tritici populations

Sampling over the geographic scale occurred during spring, between the two time points investigated at the seasonal scale (i.e. post-winter and post-spring conditions). These seasonal samplings highlighted a marked seasonal shift of TPCs towards higher temperatures and changes in the thermotype composition of two local Z. tritici populations. This result is consistent with previous observations of seasonal short-term selection on aggressiveness traits (Suffert et al., 2015). This study thus reveals a two-tier thermal adaptation, with seasonal dynamics nested within and potentially occurring in each geographic local adaptation over annual epidemics. This key finding shows that adaptive patterns are 'eco-evolutionary snapshots' that should be interpreted with caution, to such an extent that certain evolutionary dynamics of microbial populations can be of one type over a very short time scale and another type over longer time scales. Indeed, adaptive dynamics may differ with the time scale investigated (annual or pluriannual), particularly for annual crop pathogens with both sexual and asexual reproduction cycles, such as Z. tritici (Suffert et al., 2018). Our findings could be summarised by the counterintuive statement 'local seasonal adaptation is stronger but more fleeting than geographic adaptation' although we could expect that regions with lower seasonal contrasts in temperature (e.g. with mild winters) will exert weaker selective pressure. The use of sequential temporal sampling would make it possible to capture shifts in thermal adaptation over and between wheat-growing seasons and to detect potential trade-offs between aggressiveness and survival over winter (e.g. Montarry et al., 2007).

From adaptation patterns to eco-evolutionary processes

Consistent with previous studies, our findings highlight the existence of high levels of genetic diversity and an absence of its structuration across Z. tritici populations collected from local wheat fields (Zhan et al., 2001) up to the regional and continental scales (Schnieder et al., 2001; Linde et al., 2002) or over the course of an epidemic cycle (Chen et al., 1994; Morais et al., 2019).

The high level of gene flow suggested by this low level of genetic differentiation between populations may partly explain the maintenance of some degree of maladaptation to local conditions (e.g. the detection of three CA+ individuals in the IS population). More generally,

we observed almost all the T_{opt}-adapted thermotypes (CA+, CA, WA, WA+) in each phenotyped population (except that CA individuals were absent from the IS population and CA+ individuals were absent from the SPR1 population), despite the clear patterns of adaptation observed for T_{opt} and the large differences in environmental temperatures. This maintenance of diversity suggests that Z. tritici is highly tolerant to thermal variations (high probability that environmental conditions are favourable to the development of at least some individuals in a given local population). One possible explanation for this finding is that the substantial adaptation of populations to their environments (e.g. only warm-adapted individuals under a warm environment) is hindered by a balance between gene flow and local selection (Ronce & Kirkpatrick, 2001). It also raises the issues of the occurrence of counter-selection during the interepidemic period that might explains how local populations shift in thermotype structure to reestablish similar structures between years through heritability and genetic reassortment during sexual reproduction which is driven by antagonistic density-dependent mechanisms (Lendenmann et al., 2016; Suffert et al., 2019). Further studies are required to determine the extent to which the detected pattern of geographic adaptation is driven by the thermal conditions of the environment. For this, the potential counteracting effects of selection, gene flow, random genetic drift, mutation and recombination on the increase or decrease in genetic variation would need to be assessed (Hanson et al., 2012). In particular, the combination of the high diversity of thermal responses in Z. tritici highlighted here, their heritability (Lendenmann et al., 2016) and the high level of local heterogeneity within wheat canopies (Chelle, 2005) suggest that local thermal conditions probably exert strong selection pressure on thermal sensitivity (for which TPCs are probably the best proxy as they may themselves be direct targets of selection; Scheiner, 1993; Via, 1993), even in the presence of high gene flow.

Functional group composition: a browsing pattern to investigate population dynamics

Our study illustrates how the functional classification of TPCs into thermotypes with multivariate statistical procedures can provide a complementary means of deciphering diversity patterns in the biological responses quantified in reaction norms. In particular, it constitutes an operational tool for assessing functional similarity at the individual level (i.e. whether the apparent variation observed in thermal parameters is functionally significant; Fig. 8a and 8b) and at the population level (i.e. whether the thermotypes constituting a population are more or less well-differentiated within the whole functional space; Fig. 8c). However, caution will be required in the extension of this approach to comparisons over multiple data sets, through either: (i) the development of comparable classification systems, taking into account the variation of the classification with the populations sampled by explicitly stating which ranges of trait values are hidden behind a given group description (e.g. affixing levels of adaptation: very low, low, high, very high); or (ii) the validation of group delineations between experiments, by combining a priori and a posteriori methodologies. This description of populations in terms of functional groups makes it possible to move from a description of phenotypic patterns and shifts in population composition to an inference process. This process may, for example, be based on comparisons of the competitive advantage of thermotypes under given thermal scenarios: e.g. ‘do more variable environments favour thermal generalists?’ or ‘is there a shift in the optimal range of thermal responses with mean temperature conditions?’ (Fig. 8d). This classification into thermotypes enabled here to go

beyond a purely descriptive framework and future investigations will need to be undertaken to tackle the physiological bases of these differentiations in thermal responses. The thought-provoking results of Francisco et al. (2019) could be used to test whether several strains belonging to those thermotypes also correspond to specific or main morphotypes that would increase their tolerance under some environmental conditions (e.g. if warm-adapted individuals exhibit higher proportions of stress tolerant growth forms such as chlamydospores under warmer temperatures). All in all, this functional approach lays the foundations for future studies of the potential of pathogen populations to adapt to changes in their environment, from seasonal changes in the short term, to global warming in the long term. In particular, it will prove useful in gaining a fuller understanding of how new aggressive fungal strains may emerge and expand into previously unfavourable environments (Milus et al., 2009; Mboup et al., 2012; Stefansson et al., 2013). This is a crucial area of investigation that is all too often overlooked in models for predicting plant disease epidemics in conditions of climate change (West et al., 2012).

CONCLUDING REMARKS

The detailed characterisation of a microbial phenotype as a profile rather than a mean allowed for analyses that accounted for the range of sensitivies of individual strains rather than solely their mean sensitivity. This gave insight into the high level of functional divergence in the plasticity and variation of individual thermal responses over geographic and seasonal scales, highlighting the occurrence of a two-tier dynamics in thermal adaptation. These findings raise intriguing questions regarding the mode of selection operating on these functional groups of individuals with similar competitive advantages in given thermal conditions. Deciphering the mechanisms underlying this maintenance of diversity in population phenotypic composition will prove useful for expanding our understanding of eco-evolutionary responses and the potential of populations, species and communities to adapt to environmental change.

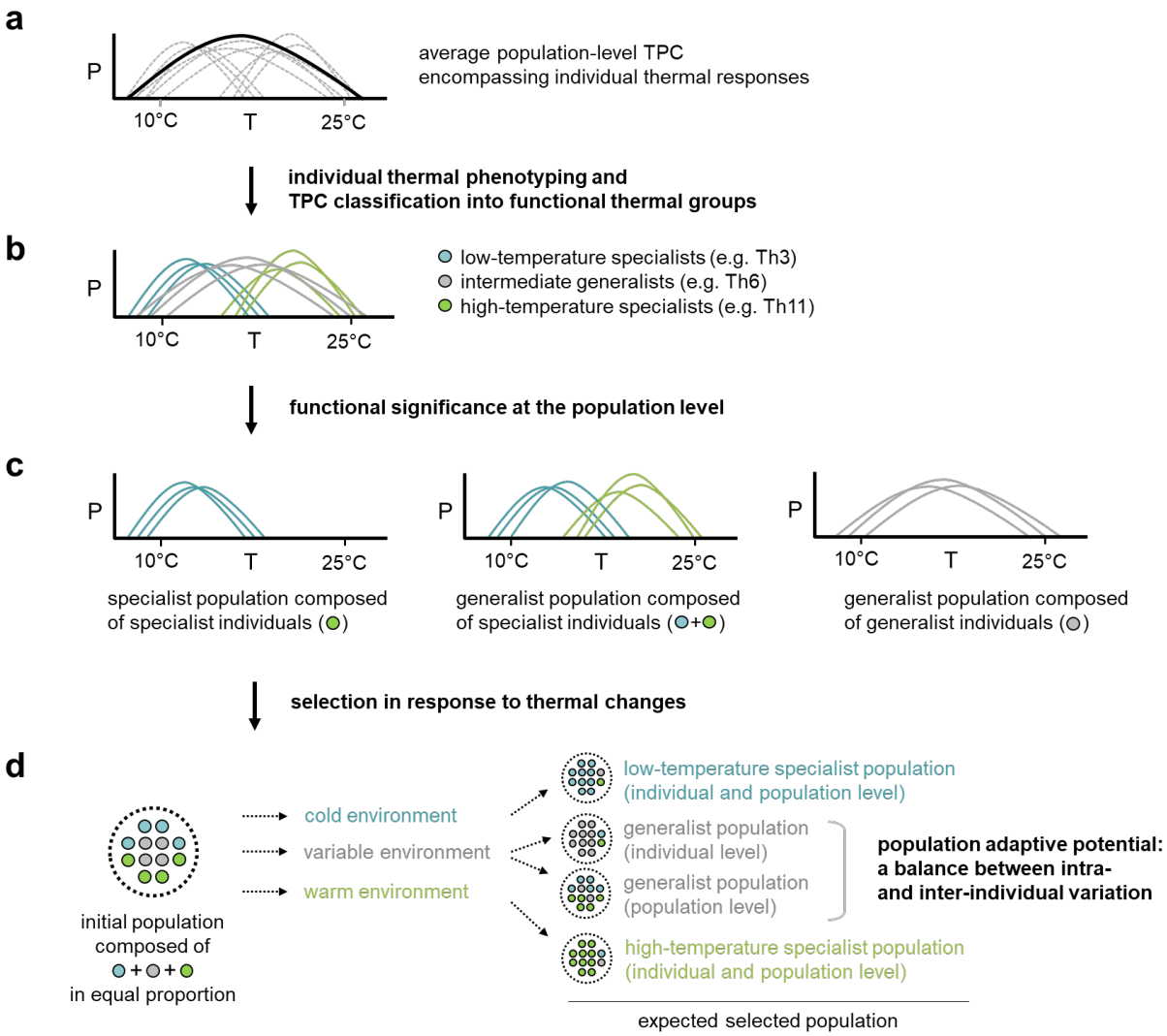


Fig. 8 Summary of the way categorisation into thermotypes (functional thermal groups) sheds light on the translation of population diversity patterns into selection dynamics in response to climate conditions. (a) Average population-level TPC (solid line) concealing a set of varied individual TPCs (dashed lines); (b) Breakdown of the variation in individual TPCs based on their classification into thermotypes and screening for a functional significance of variation at the individual level (given example of three thermotypes within which individuals are considered functionally redundant: low-temperature specialists, intermediate generalists and high-temperature specialists). (c) Categorisation tackling functional redundancy at the population level (i.e. whether the thermotypes composing a population are more or less well-differentiated within the whole functional space). The three populations presented here demonstrate the relevance of considering functional redundancy vs. vacant functional space when assessing emergent properties of populations such as a generalist nature at population level (e.g. a generalist population can be composed of specialist individuals with narrow individual TPB_{80} distributed over the functional space, resulting in broad TPB_{80} population coverage). (d) The translation of populations into functional groups makes it possible to investigate selection, testing for general assumptions of adaptation to given environments (e.g. competitive advantage of low-temperature specialists in cold environments, generalists in variable environments, high-temperature specialists in warm environments) providing insight into the potential of populations to adapt to changes in their environment (a subtle balance between diversity levels for intra- and inter-individual variation in thermal responses).

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURES

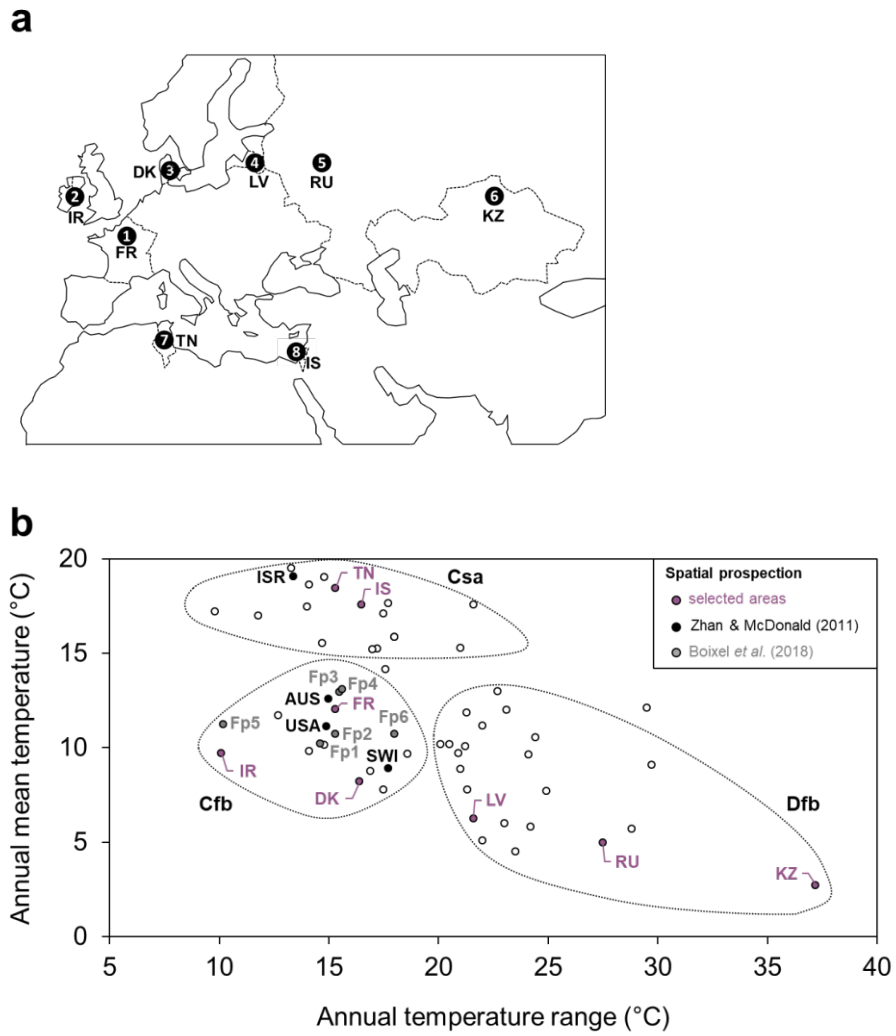


Fig. S1 Selection of sampling sites in the Euro-Mediterranean wheat-growing area. (a) Location of the eight fields from which the Euro-Mediterranean populations of Zymoseptoria tritici were collected: FR (Thiverval-Grignon, France), IR (Carlow, Ireland), DK (Flakkebjerg, Denmark), LV (Jelgava, Latvia), RU (Moscow, Russia), KZ (Penkovo, Kazakhstan), TN (Manouba, Tunisia), IS (Kiryat-Tivon, Israel). Dotted lines correspond to the national borders of the countries from which the populations were collected. (b) Representative panel of climatic conditions encountered at the Euro-Mediterranean scale, for classification of the diversity of thermal responses in Z. tritici populations. Sampling locations (in purple) were chosen on the basis of 1961-1990 climate normals to maximize environmental heterogeneity at a level much greater than in two previous studies on thermal adaptation in Z. tritici (Zhan & McDonald, 2011; Boixel et al., 2019). The scatter plot depicts the annual mean temperature and temperature range (Norwegian Meteorological Institute, 2019) of each country location in the Euro-Mediterranean zone and in the two aforementioned studies (see open, grey and black points, respectively). The various sampling sites were classified according to their predominant Köppen-Geiger climate types (Köppen, 1936; Peel et al., 2007): Cfb (temperate oceanic climate), Csa (hot-summer Mediterranean climate), Dfb (warm-summer humid continental climate). AUS (Wagga Wagga, Australia), ISR (Nahal Oz, Israel), SWI (Berga Irchel, Switzerland) and USA (Corvallis, United States) correspond to the sampling locations of the five Z. tritici populations investigated in Zhan & McDonald (2011). Fp1 (Villers-lès-Cagnicourt), Fp2 (Versailles), Fp3 (Bergerac), Fp4 (Lectoure), Fp5 (Ploudaniel) and Fp6 (Bretenière) correspond to the sampling locations of the six French Z. tritici populations investigated in Boixel et al. (2019).

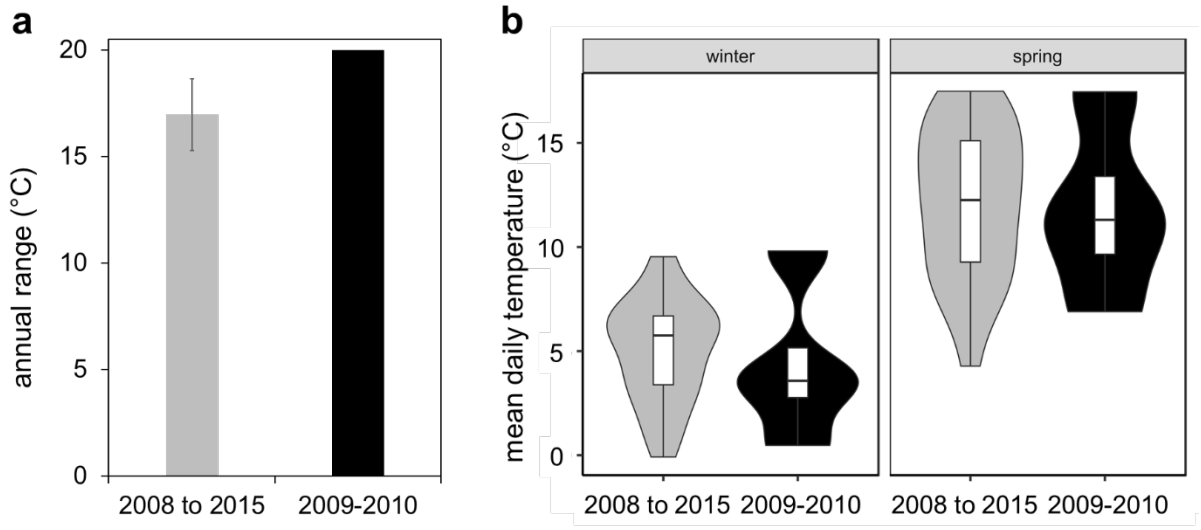


Fig. S2 Selection of the seasonal subpopulations. Analysis of the climatic data for Grignon from 2008 to 2015 (INRAE AgroClim, 2019) used to select the most contrasting wheat growing season to be investigated from those for which samples were available from the INRAE BIOGER Z. tritici collection. Seven pairs of subpopulations were sampled from two neighbouring field plots during a long-term population survey (partially presented in Suffert et al., 2018) over seven wheat growing seasons (from 2008-2009 to 2014-2015): one post-winter subpopulation (sampled at the beginning of March) and one post-spring subpopulation (sampled at the end of June). We investigated seasonal effects in the 2009-2010 subpopulations, which had (a) the highest annual amplitude (20°C, vs. 16.9°C ± 1.7°C in the other seasons) and (b) the greatest contrast between 'winter' (November – February: tighter distribution of mean daily temperatures) and 'spring' (March – June: shortened low end of the distribution tail of mean daily temperatures) temperature conditions.

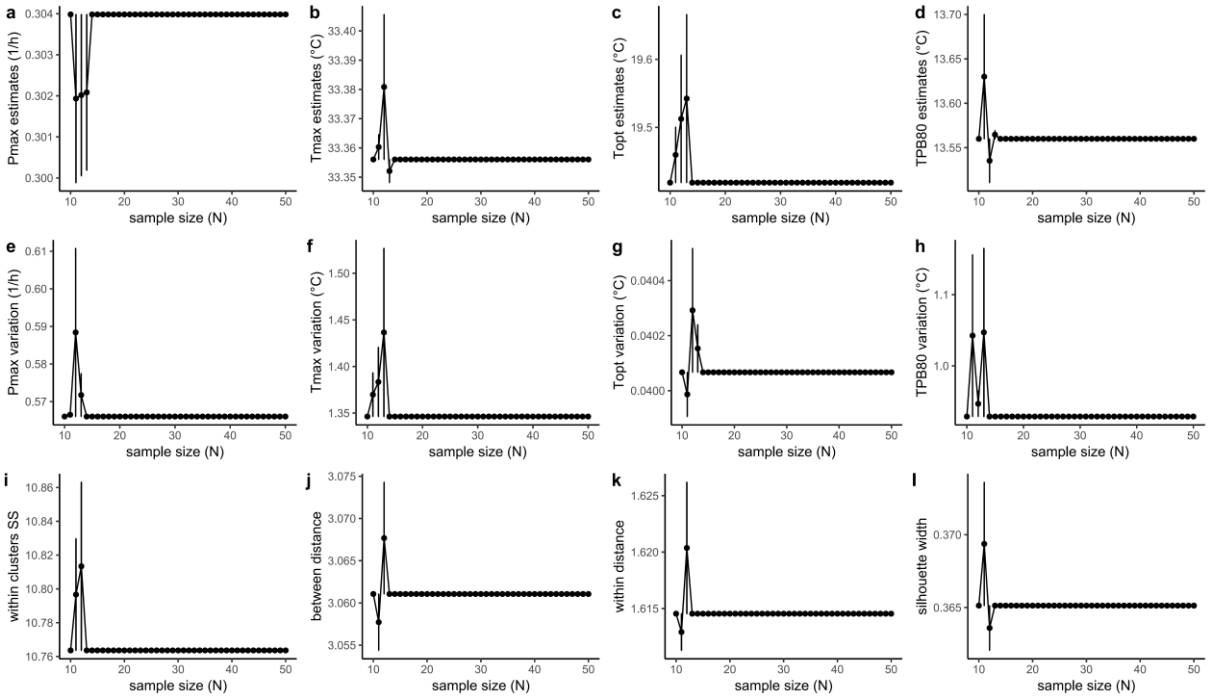


Fig. S3 Appropriate sample size for estimating diversity in TPCs within Z. tritici populations. For the definition of a target sample size per population, we performed a rarefaction analysis on a set of 66 Z. tritici strains that had undergone preliminary phenotyping for their responses to temperature (see Boixel et al., 2019). Rarefaction curves of expected diversity for key thermal parameters (a: mean maximum performance P_{max}; b: mean maximum temperature T_{max}; c: mean thermal optimum T_{opt}; d: mean 80% thermal performance breadth TPB_{80}; e: P_{max} standard deviation; f: T_{max} standard deviation; g: T_{opt} standard deviation; h: TPB_{80} standard deviation) and in the typology of thermal responses based on HCPC clusters (i: within-thermotype sum of squares; j: mean distance between clusters; k: mean distance within clusters; l: mean silhouette width providing information about the compactness, separation, and connectivity of the cluster partitions) were obtained for 41 levels of sampling depth (mean ± resampling standard error; n = 15 subsampling repetitions per sampling depth) ranging from 10 to 50 individuals per subsample (sample size N; x-axis). Similar results were obtained for samples of more than 15 strains. We chose to phenotype and genotype 30 of the 50 strains that we isolated in total for each population (INRAE BIOGER collection), to ensure a sufficiently high statistical power and precision and to ensure that we could estimate allele frequencies and gene diversity in a population (Dale & Fortin, 2014).

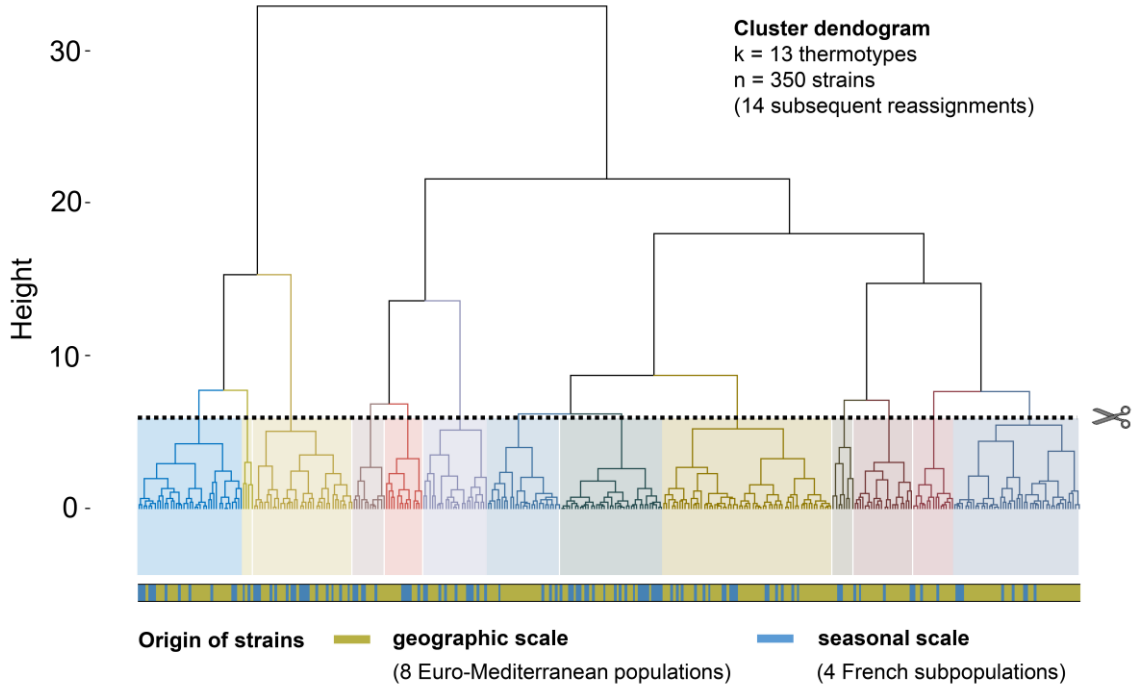


Fig. S4 Clustering of Z. tritici strains into 13 thermotypes. (a) The dendogram (distance-based tree) illustrates the hierarchical organisation of the TPCs of the 350 Z. tritici strains (seasonal and geographic scales) in terms of functional thermal responses. The distance between strains in the hierarchical cluster tree was calculated based on Euclidean distances (the greater the difference in height, the greater the dissimilarity). We identified 13 thermotypes, colour-coded in the figure, with the procedure presented in Methods S2. Each thermotype includes strains from both data sets (seasonal: blue segments; geographic: yellow segments in the horizontal bar below the tree). These results demonstrate the relevance of establishing a unique typology to compare the TPCs of all 350 strains. The quality of the clustering was assessed by silhouette analysis (Rousseeuw, 1987), based on the mean distance between clusters. 14 individuals were placed in the wrong cluster (negative silhouette width) and were therefore reassigned to the closest neighboring cluster before assessing the distribution and features (compactness, separation, connectivity) of each thermotype.

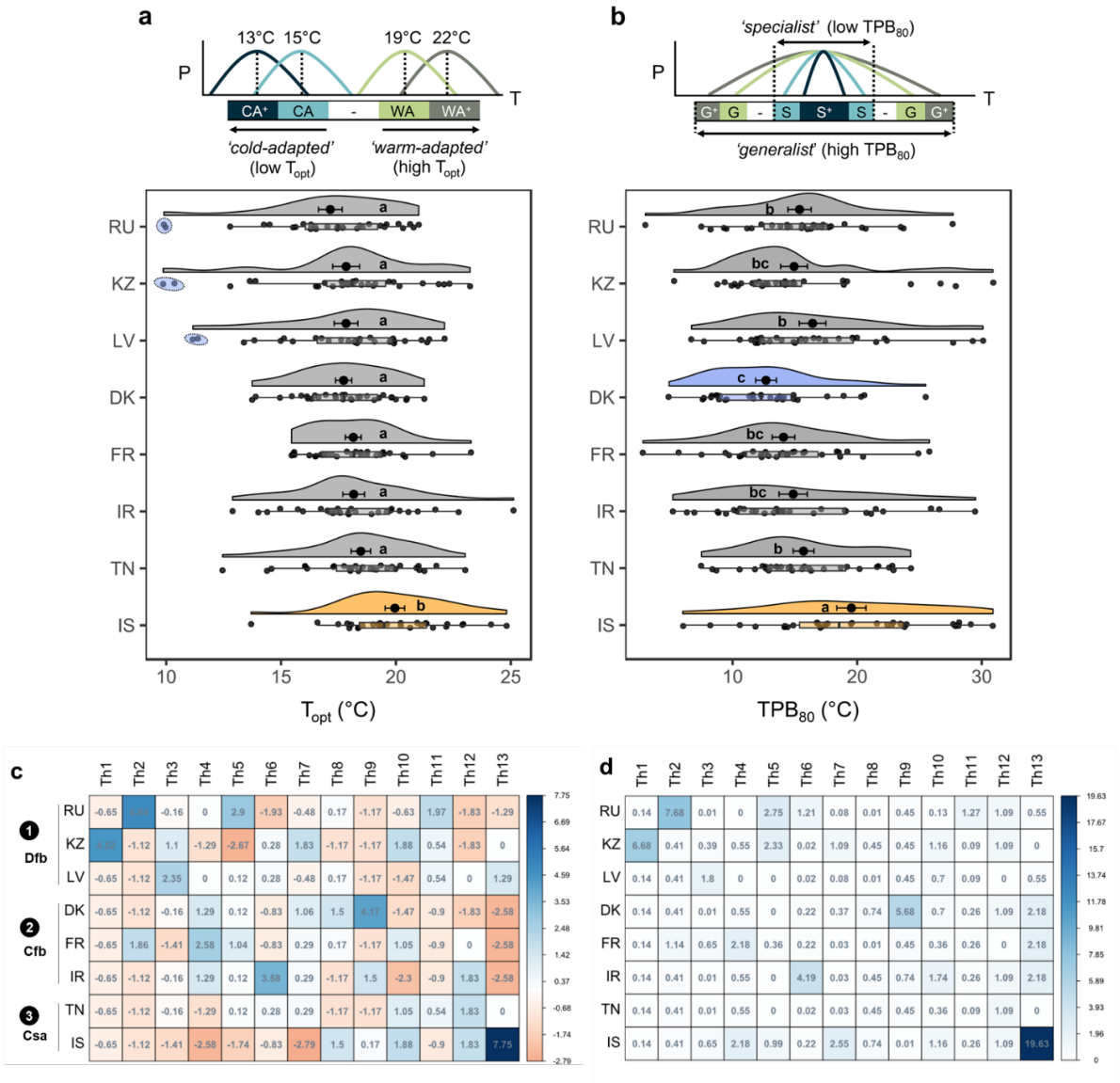


Fig. S5 Distribution of thermotypes across the 8 Euro-Mediterranean Z. tritici populations. (a) Population-level thermal optima (means ± SEM) are presented, together with the distribution of individual T_{opt} values within populations (associated raw data points, boxplots and split-half violins). A significant shift in T_{opt} distribution towards higher temperature was detected for the Israeli population (IS); the letters indicate the outputs of Kruskal-Wallis post-hoc pairwise comparisons, with $P < 0.05$. (b) Population-level thermal breadth (means ± SEM) values are presented together with the variation in that response among individuals (associated raw data points, boxplots and split-half violins). The letters indicate the outputs of Kruskal-Wallis post-hoc pairwise comparisons, with $P < 0.05$. (c) Contingency table for the Chi-squared test of independence. Each row represents a population and each column represents a thermotype. Associations between rows and columns are displayed for each cell as Pearson residuals. Positive values in blue indicate an attraction and negative values in red indicate a repulsion. This table reveals that: (1) the most strongly cold-adapted thermotypes (CA^+, Th1-Th2-Th3) are preferentially found in Dfb populations (RU-KZ-LV); (2) individuals with the greatest thermal breadth (G^+, Th1 and Th13) are less abundant in Cfb populations (DK-FR-IR), which are characterised by more highly specialist individuals (S^+, Th4 and Th9) than the average; (3) warm-adapted generalist individuals (WA^+-G/G^+, Th12,Th13) are found in higher proportions in the IS and, to a lesser extent, TN populations. (d) The relative contribution of each cell to the total Chi-squared score (darker cells are those corresponding to the bulk of the variability in thermotype distribution) indicates that the three aforementioned aspects account for (1) 17.7 %, (2) 17.1%, and (3) 21.8% of total phenotypic differentiation in thermal response between populations.

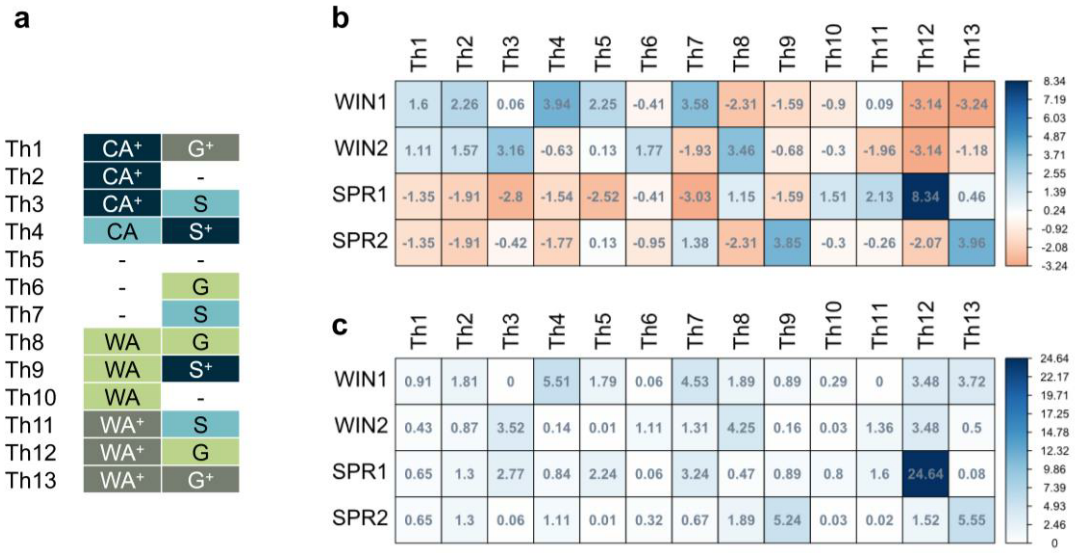
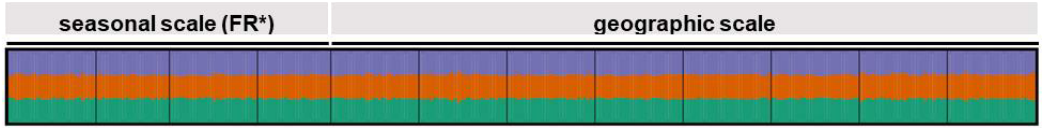


Fig. S6 Distribution of thermotypes across the four French seasonal Z. tritici subpopulations. (a) Stacked bar plots of functional thermotype composition across populations: highly cold-adapted (CA^+), cold-adapted (CA), intermediate (- in white), warm-adapted (WA), highly warm-adapted (WA^+) thermotypes. The relative abundance within populations of each thermotype is expressed as a single bar percentage on which the thermotype ID is displayed. (b) Contingency table of the Chi-squared test of independence. Each row represents a population and each column represents a thermotype. Associations between rows and columns are displayed for each cell as Pearson residuals. Positive values in blue indicate an attraction and negative values in red indicate a repulsion. These residuals show that winter (WIN1 and WIN2) and spring (SPR1 and SPR2) subpopulations fall into discrete clusters in terms of their composition in thermotypes highly adapted to cold (CA^+: positive association with winter populations and negative association with spring populations) and in thermotypes highly adapted to warm conditions (WA^+: negative association with winter populations and positive association with spring populations). (c) The relative contribution of each cell to the total Chi-squared score (darker cells are those corresponding to the bulk of the variability in thermotype distribution) indicates that the compositional dissimilarity in thermotypes between winter and spring populations can be accounted for mostly by the proportions of generalist warm-adapted individuals (Th12 and Th13; 43% of the total Chi-squared score).



Population	WIN2	WIN1	SPR2	SPR1	DK	FR	IR	IS	LV	KZ	RU	TN
MNA	4.17	3.67	3.92	4.00	3.83	3.83	3.67	3.33	3.83	3.42	4.33	3.92

Fig. S7 Genetic diversity and population structure of the 12 Z. tritici populations. The 350 Z. tritici strains were collected over a large spatial scale (8 geographic sites: DK: Denmark; FR: France; IR: Ireland; IS: Israel; KZ: Kazakhstan; LV: Latvia; RU: Russia; TN: Tunisia) and over a seasonal scale during another growing season in France (FR* with two pairs of post-winter (WIN1; WIN2) and post-spring (SPR1; SPR2) subpopulations). These strains were genotyped for 12 neutral microsatellite markers (SSRs) and assigned to different genetic clusters by a Bayesian cluster approach (see the detailed procedure in Methods S3). Each strain (one unique multilocus genotype) included in the analysis is displayed as a thin vertical line partitioned into colored segments representing its probabilities of assignment to genetically different clusters (cluster 1 in green, cluster 2 in orange and cluster 3 in purple). Each strain was affected to each of the three genetic structures with the same probability, indicating an absence of population structure. Genetic diversity is indicated for each population as the mean number of alleles observed over the 12 SSRs (MNA).

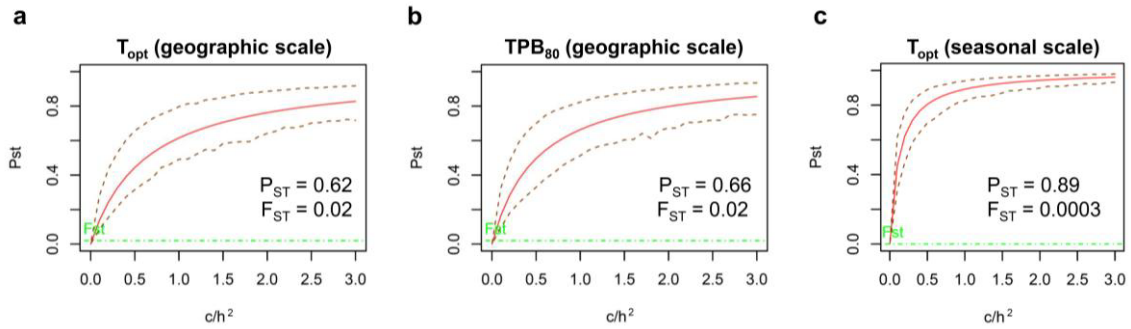


Fig. S8 Sensitivity analysis of the robustness of P_{ST}–F_{ST} comparisons for the 12 Z. tritici populations. The robustness of P_{ST}–F_{ST} comparisons was explored by studying variations of the c/h^2 ratio, which determines the accuracy of the approximation of Q_{ST} by P_{ST}. Phenotypic divergence in thermal sensitivity (P_{ST}) was investigated for: (a) the thermal optimum (T_{opt}) of the Euro-Mediterranean populations (geographic scale); (b) for the thermal breadth (TPB_{80}) of Euro-Mediterranean populations; (c) for the thermal optimum (T_{opt}) of the French seasonal subpopulations (seasonal scale). For each plot, estimates of P_{ST} (solid line in red, the P_{ST} value displayed being calculated at the critical c/h^2 ratio of 1), its lower and upper 95% confidence interval limits (dashed lines in red) and the upper confidence estimate of the neutral divergence (F_{ST}; dashed line in green) are plotted. For each trait, the 95% CI of P_{ST} at $c/h^2 = 1$ indicates the occurrence of strong phenotypic divergence and of a robust difference in P_{ST} and F_{ST}, as their confidence intervals only overlap ($P_{ST} > F_{ST}$) at low c/h^2 ratios (a: $c/h^2 = 0.02$; b: $c/h^2 = 0.02$; c: $c/h^2 < 0.005$).

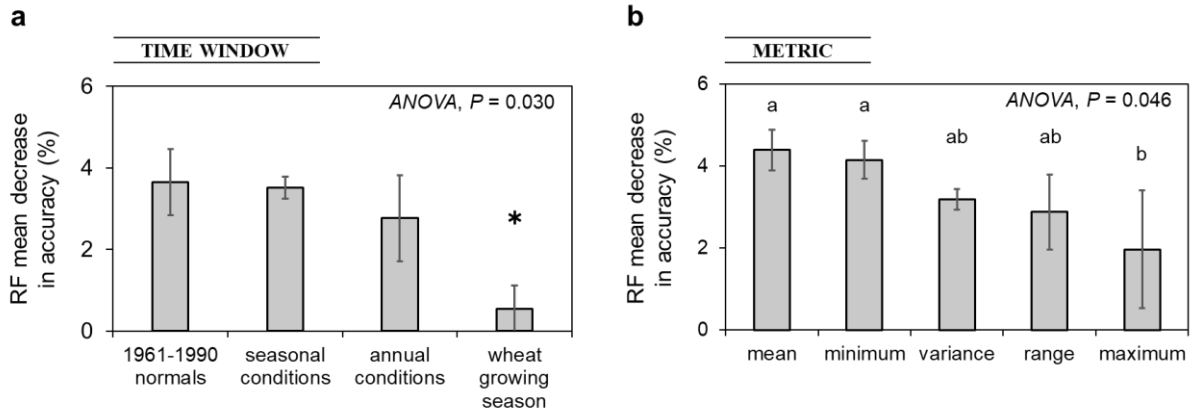


Fig. S9 Characterisation of the thermal niche at each sampling site. The mesoclimatic thermal environments of the 8 Euro-Mediterranean sampling locations were summarised (a) for four climatic time windows ('1961-1990 climate normals' and thermal conditions for the year of sampling averaged over the calendar year i.e. 'annual conditions', the 'wheat growing season' i.e. from late October to early July, the 'seasonal conditions' i.e. contrasts between the winter and spring periods) and (b) with five metrics (thermal mean, range, maximum, minimum and variance). The 20 thermal variables were ranked according to their contributions to overall variations in temperature between the three contrasting Köppen-Geiger climatic zones prospected. For the establishment of a thermal niche classification, the most discriminating variables were identified by applying a nonlinear and nonparametric random forest algorithm (RF; Breiman, 2001) with the 'randomForest' package of R (n_{tree} bootstrap samples = 1000; Liaw & Wiener, 2002). The importance of the variables was compared based on the RF mean decrease in accuracy, which provides a metric indicating the extent to which the exclusion or permutation of a variable reduces RF accuracy (classification error). Higher mean decreases in accuracy for a given variable indicate a greater importance of the variable concerned for the classification of climatic zones. One-way analyses of variance (ANOVA outputs displayed with letters indicating the results of Tukey post-hoc multiple pairwise-comparisons) indicate that (a) the climatic time window of the wheat growing season and (b) maximum temperature are significantly less informative for classifying the three climatic zones.

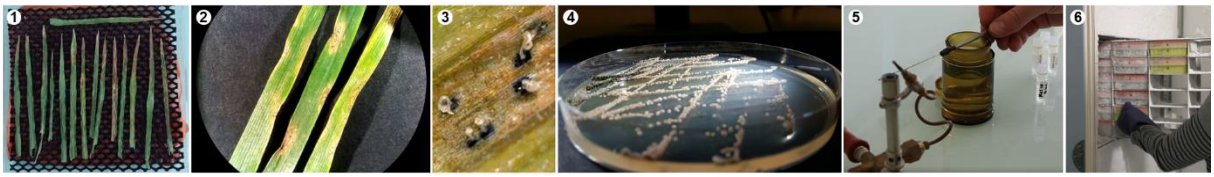
Table S1 Selection of a candidate mathematical model for establishing TPCs. Model equations relating performance (P) to temperature (T) by means of K parameters were harmonised with the following key thermal parameters, when relevant: P_{max} (maximum performance), T_{opt} (thermal optimum), T_{min} (minimum temperature), T_{max} (maximum temperature), Curv (shape parameter) and TPB (thermal performance breadth). A single model was retained to fit the TPC of each strain represented in our data set: the model most accurately and efficiently describing the overall growth data. Indeed, for the characterisation of individual variations, we tried to minimise the estimated error of the curve fitting algorithm. We thus assigned a greater weight to stabilising the systematic errors in performance prediction as a function of temperature (the goodness-of-fit of models varying with temperature on the x-axis of TPCs) than to obtaining better fitted absolute performance values for each strain × temperature combination (introduction of biases in the comparison of individual TPCs). The most appropriate model was selected on the basis of: (i) statistic metrics accounting for goodness-of-fit (information-theoretical approach; Burnham & Anderson, 2004): residual sum of squares (RSS); Akaike weight (w_{AIC}) and Schwarz weight (w_{BIC}); (ii) the similarity between equation predictions of thermal responses over the temperature range and the best-fitting model (prediction dissimilarity: significant differences in the quality of data adjustments between models; NS: not significant; ∗: $P < 0.05$) and their corresponding distribution of residuals along the temperature axis (with a view to maximising the accuracy of estimation for T_{opt} and the surrounding supra- and suboptimal estimates (i.e. mid-temperature range) over thermal extremes (T_{min} and T_{max}); (iii) model constraints potentially forcing the fitting process in a particular direction (see the detailed procedure in Boixel et al., 2019). Successive steps in the selection process are represented from the left to the right side of the table (black cells display discarded models). In this study of the thermal responses of Z. tritici over a Euro-Mediterranean spatial scale and a seasonal temporal scale, as in the initial study performed in France (Boixel et al., 2019), no significant difference in fit quality was observed between the quadratic and beta models for growth rate data over the entire range of temperatures investigated (from 6.5 to 33.5°C). However, residuals were not similarly distributed along the temperature-axis, leading to small differences between equations in estimation accuracy for performance at mid-temperature ranges relative to outer edges (see the column 'distribution of residuals' below and in ESM2 of Boixel et al., 2019). In both studies, we selected the model that best estimated performance over the mid-temperature range (here the quadratic model), as diversity was assessed on three thermal traits extracted from this range of the TPC (P_{max}, T_{opt}, TPB_{80}).

Mathematical models	Relationship between temperature (T) and performance (P)	K	Goodness of fit over the temperature range					Model constraints	Final choice
			RSS	w_{AICc}	w_{BIC}	Prediction dissimilarity	Distribution of residuals		
Kontodimas (modified Analytis equation)	$P(T) = a \times (T - T_{min})^2 \times (T_{max} - T)$	3	119.3	0.24	0.31	NS	Mid-range		
Quadratic	$P(T) = P_{max} + Curv(T - T_{opt})^2$	3	119.9	0.21	0.26	NS	Outer edge (inner boundary)	Symmetry	✓
Yan and Hunt (modified beta distribution)	$P(T) = P_{max}(\frac{T_{max} - T}{T_{max} - T_{opt}})(\frac{T}{T_{opt}})^{T_{opt}/(T_{max} - T_{opt})}$	3	119.7	0.22	0.27	NS	Outer edge (outer boundary)	T_{min}=0	
Baker *et al.*	$P(T) = ae^{bT}(T_{max} - T)(T - T_{min})$	4	119.5	0.07	0.03	∗			
Beta distribution	$P(T) = P_{max}((\frac{T_{max} - T}{T_{max} - T_{opt}})(\frac{T - T_{min}}{T_{opt} - T_{min}})^{\frac{(T_{opt} - T_{min})}{(T_{max} - T_{opt})}})$	4	119.7	0.07	0.03	NS	Mid-range upper limits		
Modified Gaussian	$P(T) = P_{max}e^{(-0.5(\frac{\lvert T - T_{opt}\rvert}{TPB})^d}$	4	118.3	0.10	0.05	∗			
Square-root model (Ratkowsky equation)	$P(T) = (a(T - T_{min})(1 - e^{b(T - T_{max})}))^2$	4	134.4	0.01	0.01	∗			
Third-order polynomial	$P(T) = a + bT + cT^2 + dT^3$	4	119.3	0.08	0.04	∗			

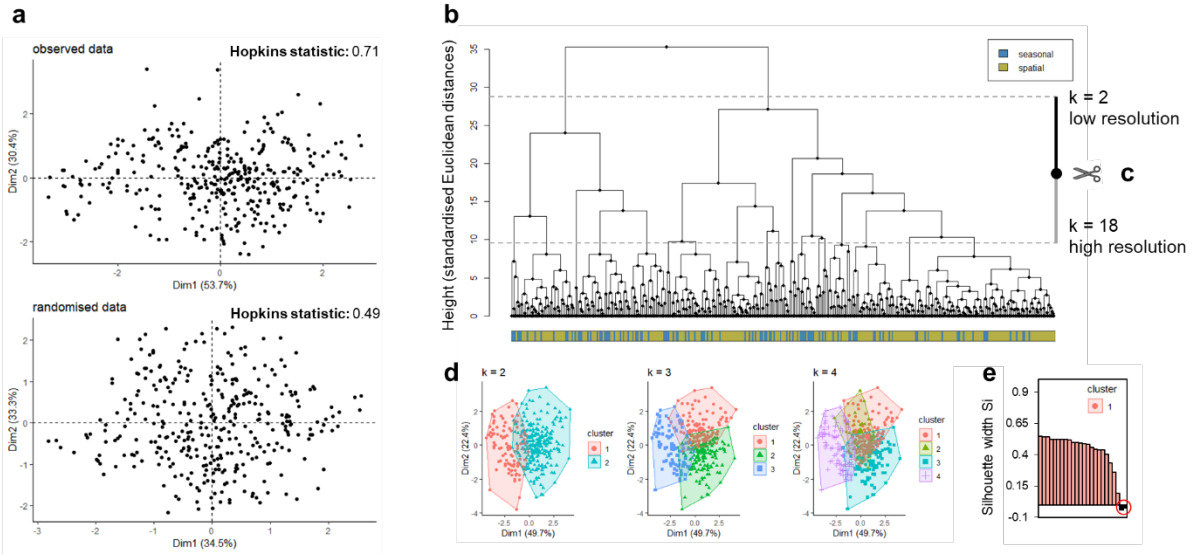
Table S2 Population-pairwise genetic distance (matrix of F_{ST} values). Pairwise comparisons between the 8 geographic Z. tritici populations (DK: Denmark; FR: France; IR: Ireland; IS: Israel; KZ: Kazakhstan; LV: Latvia; RU: Russia; TN: Tunisia) and 4 seasonal Z. tritici subpopulations (WIN1, WIN2: post-winter; SPR1, SPR2: post-spring). The matrix displays the estimation of pairwise F_{ST}-values for each combination of populations based on 12 neutral microsatellite markers. Values in italics indicate significant F_{ST} values ($P < 0.05$), as evaluated with random allelic permutation procedures (1,023 permutations). These low population pairwise F_{ST}-values and associated statistical analyses (conducted with the ARLEQUIN program; Methods S3) indicate an absence of genetic differentiation between populations based on neutral markers (exact global test of sample differentiation based on haplotype frequencies conducted with 100,000 Markov steps: $P = 0.75 \pm 0.11$).

	WIN2	WIN1	SPR2	SPR1	DK	FR	IR	IS	LV	KZ	RU	TN
WIN2	0											
WIN1	-0.013	0										
SPR2	-0.007	-0.008	0									
SPR1	-0.012	-0.014	*-0.002*	0								
DK	*-0.005*	*-0.005*	*0.001*	*0.000*	0							
FR	-0.006	*-0.004*	*-0.004*	*-0.005*	*-0.001*	0						
IR	-0.007	-0.006	*-0.006*	*-0.002*	-0.006	-0.006	0					
IS	*0.012*	*0.010*	*0.008*	*0.016*	*0.001*	*0.004*	*-0.000*	0				
LV	*0.024*	*0.022*	*0.031*	*0.021*	*0.021*	*0.021*	*0.023*	*0.033*	0			
KZ	-0.012	-0.010	*-0.002*	-0.009	*-0.002*	*-0.004*	*-0.005*	*0.019*	*0.018*	0		
RU	*0.004*	*0.006*	*0.009*	*0.005*	*0.010*	*0.006*	*0.008*	*0.024*	*-0.001*	*-0.001*	0	
TN	*0.014*	*0.014*	*0.015*	*0.010*	*0.022*	*0.012*	*0.017*	*0.020*	*0.033*	*0.015*	*0.015*	0

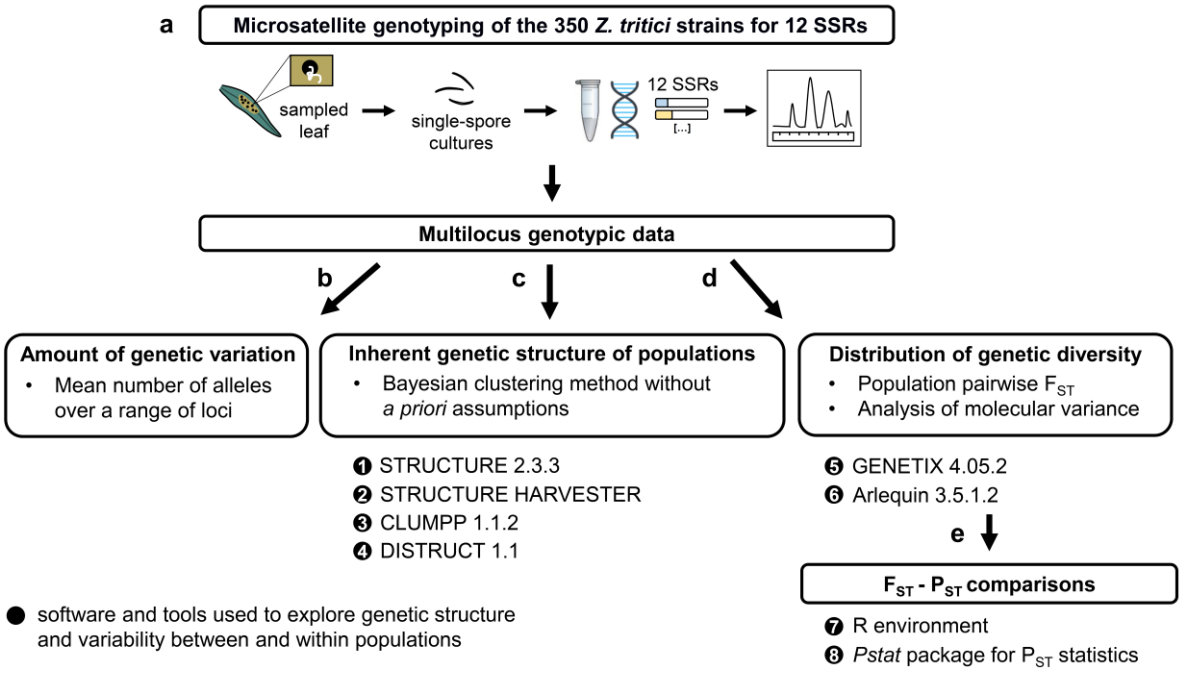
SUPPLEMENTARY METHODS



Methods S1 Procedure for the sampling, collection and recovery of Z. tritici strains. Leaves with Septoria tritici blotch lesions were sampled at the various field locations, dried and flattened naturally for 2-3 days between two pieces of blotting paper, (1) placed overnight at 18 °C in a humidity chamber to promote cirrhus exudation and then (2) observed with a binocular magnifier to visualise pycnidia. (3) On each leaf, one cirrhus from a single pycnidium was retrieved from a randomly sampled single lesion, for further isolation in pure culture through (4) subculturing a single colony on PDA (potato dextrose agar, 39 g L^{-1}) at 18°C in the dark. (5) After purification, Z. tritici spore suspensions were stored in stock tubes, in a 1:1 glycerol–water mixture, and were added to (6) the INRAE collection stored at − 80 °C.



Methods S2 Definition of Z. tritici 'thermotypes'. Thermotypes should be considered here as functional groups of thermal performance curves (TPCs) with similar thermal sensitivity features. Thermotype definition involves the establishment of a typology accounting for the diversity encountered in a given data set. This typology was built on the standardised phenotyping multidimensional data (including P_{max}, T_{opt} and TPB_{80} for both seasonal and geographic populations) in a five-step approach: (a) testing for uniformity in the data by calculating the Hopkins statistic (which measures spatial randomness and, thus, the tendency of a given data set to cluster; Lawson & Jurs, 1990) to determine whether a given data set can be divided into meaningful clusters; (b) calculating Euclidean distances between pairs of individual phenotypes and building the corresponding distance-based tree; (c) determining the optimal number of clusters (the cut-off being represented by the pair of scissors) by varying k from k = 2 (as we highlighted that there are clusters in the data set; see the Hopkins statistic in S2a) to k = 18 (to account for all combinations of variables: $P_{max} \times T_{opt} \times TPB_{80}$). We achieved this by data mining, by identifying the best clustering scheme using 30 clustering validity indices implemented in the 'NbClust' R package (Charrad et al., 2014); (d) K-means clustering, an iterative algorithm with the objective function of minimising the pooled mean distances within clusters. This approach identified the 13 clusters to be generated into which the data could be partitioned ('kmeans' function with 13 centers and 50 initial configurations); (e) assessing the quality of the clustering result, by determining how well observations clustered with the 'silhouette' function of the 'cluster' package of R (Maechler et al., 2018). Based on this silhouette analysis (Rousseeuw, 1987), outliers among the thermal responses clustered (i.e. wrongly assigned phenotypes with a negative silhouette width coefficient – circled in red in the figure) were reassigned to the neighbouring cluster. This final partitioning of data (clustering result with reassigned outliers) was used to compare the corresponding clusters (i.e. thermotypes) on the basis of their features (compactness, separation, connectivity within and between clusters), abundance and distribution pattern within and between the 12 Z. tritici populations.



Methods S3 Procedure for acquiring and analysing multilocus genotypic data. (a) Genetic variability, structure and the distribution of diversity between and within the 12 Z. tritici populations were assessed based on microsatellite genotyping data. The overall data set was acquired for 12 SSRs (ST1, ST2, ST3A, ST3B, ST3C, ST4, ST5, ST6, ST7, ST9, ST10, ST12 neutral microsatellites; Gautier et al., 2014) following: (i) DNA extraction from single-spore cultures with the DNeasy Plant Maxi Kit (QIAGEN); (ii) amplification and sequencing of the SSR markers in one multiplex PCR sample (Eurofins Analytics France); (iii) the determination and annotation of allele sizes through visual analysis of individual chromatograms with Peak Scanner (Applied Biosystems). (b) Genetic variation in allelic distributions was assessed manually by the direct counting of the mean number of alleles (MNA) observed over all loci. (c) The distribution of this genetic variation within and between populations (population structure) was inferred with the Bayesian clustering approach implemented in STRUCTURE (Pritchard et al., 2000) under the admixture and correlated allele frequencies model. The algorithm was run on the basis of 500,000 iterations of the Markov chain ('burn-in length') followed by a run phase of 1,000,000 iterations ('burn-in period') with 10 independent replicates for each tested number of clusters (range set from 1 to 10). The optimal number of inferred genetic clusters (K) was estimated by the Evanno method (Evanno et al., 2005) with STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Output data were then visualised with CLUMPP (Jakobsson & Rosenberg, 2007) and DISTRUCT (Rosenberg, 2003). (d) The genetic divergence between the sampled populations (genetic differentiation assessed on pairwise estimates of Weir and Cockerham's F-statistic: 1,000 randomisations) was calculated with GENETIX (Belkhir, 2004). Hierarchical analyses of molecular variance (AMOVA) were conducted with Arlequin (Excoffier & Lischer, 2010) to assess the contribution of sampling date and location to the differences and patterns of genetic variance detected. (e) For inferences of the contribution of genetic drift and natural selection to the variation in thermal responses between populations, P_{ST} values (phenotypic differentiation between populations) of P_{max} (maximum performance), T_{opt} (thermal optimum), TPB_{80} (thermal performance breadth) and their confidence intervals were calculated with the 'Pstat' package of R (using the 'TracePst' function under the arguments boot = 1000 and pe = 0.95; Da Silva & Da Silva, 2018). F_{ST}-P_{ST} comparisons were performed to test for patterns of local adaptation and to assess whether phenotypic differentiation between populations were greater or smaller than expected under the influence of genetic drift.

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Chapter 4

Fluctuations in population composition under thermal variations: evidence from selection experiments

Foreword. The seasonal shifts in functional composition of a local population highlighted in Chapter 3 suggest that populations may be locally structured by selection, which could contribute to shape or modify adaptation patterns. How does population composition shift under environmental variations? In this chapter, I present in vitro and in planta competition experiments between functional groups of thermal responses under increasingly complex selective environments (constant, diurnally fluctuating and field temperature regimes) to tackle progressively the multifold aspect of the selective pressures and parameters imposed on Z. tritici populations. This chapter therefore intends to decipher the adaptive dynamics of fungal populations to environmental short-term variations, both at the individual and population levels, and advance a more mechanistic understanding of population processes.

A manuscript derived from chapter 4 is in preparation under the heading: Short-term selection on phenotypic variation for thermal adaptation in plant pathogen populations

contents

abstract

Background and Aims

Plant pathogen populations display astounding levels of phenotypic variation, which may be structured to various degrees across time and space, depending on the traits considered. This is particularly true for thermal responses. Temperature-induced local adaptation patterns have been extensively documented in both wild and crop plant pathosystems. However, experimental evidence that such patterns result from selection on phenotypic variation between individuals and that the potential to adapt to environmental variations is enhanced by such selection is lacking.

Key Results

We performed competition experiments in vitro and in planta, with two thermotypes of a wheat fungal pathogen (Zymoseptoria tritici) in environments of various complexities (constant, diurnally fluctuating and field temperature regimes). We considered competitive ability and relative fitness at the individual and group levels, monitoring the population dynamics of replicate lines across multiple generations, to investigate the occurrence and strength of phenotypic selection for thermal adaptation. All competition in controlled conditions resulted in significant adaptive changes in the populations concerned, driven by thermal selection on individual phenotypes. By contrast, competition in field conditions favoured the stable maintenance of phenotypic diversity.

Conclusion

Together, these results indicate that, by altering strain and thermotype frequencies, temperature regimes can drive changes in population composition, increasing adaptation. This study thus provides insight into the mechanisms underlying population dynamics in response to thermal variations, which are essential for the prediction of population responses to changing environments.

Keywords: phenotypic selection, thermal responses, individual variation, environmental changes, adaptive potential

INTRODUCTION

The natural selection of phenotypes and their heritable transmission across generations govern evolution (Fisher, 1930). Phenotypic selection occurs when particular phenotypes offer a fitness advantage, resulting, for example, in the production of more offspring than other phenotypes under the same environmental conditions (Kingsolver & Pfennig, 2007). Determining how natural selection regulates adaptation to environmental conditions is critical to elucidate the mechanisms driving changes in ecological and evolutionary dynamics. This question has been studied in long-term selection laboratory experiments (Lenski & Travisano, 1994), involving perturbation of the selected traits (Mitchell-Olds & Shaw, 1987) or modulations of the agents responsible for selection (Wade & Kalisz, 1990). Phenotypic selection acting on a large number of traits has also been detected in hundreds of natural populations (Kingsolver et al., 2001; Siepielski et al., 2009). This selection for quantitative traits often changes in intensity and direction, due to the fluctuation of environmental conditions over different time scales, imposing strong directional selective pressure (Bell, 2010). The resulting fluctuating selection pressures have been shown to be related to continual changes in population composition, with remarkable patterns emerging in some cases (e.g. adaptive oscillations for balanced polymorphisms, repeated over seasons in Drosophila; Bergland et al., 2014). Large-scale meta-analyses of selection studies on field populations have suggested that strong directional selection is relatively uncommon, but most of the investigations considered focused on morphological, rather than physiological traits (Kingsolver et al., 2001, 2012).

Several ecological studies have recognised the crucial impact of variations of environmental conditions on physiological responses (Carlson & Quinn, 2007; Siepielski et al., 2009; Calsbeek & Cox, 2010). Fungal and bacterial taxa interacting with other living organisms, such as plant pathogens, display astounding levels of genetic diversity and phenotypic variation (Salvaudon et al., 2008). They are, therefore, particularly relevant study models for investigations of evolutionary dynamics in response to environmental changes. Interest in this area is growing, particularly as concerns the challenges facing agriculture in terms of the evolution and spread of plant pathogens resistant to antibiotics or xenobiotics used to limit the impact of plant disease epidemics on crop yields (e.g. Vogwill et al., 2012; Gutiérrez-Alonso et al., 2017). It has been clearly established, from various studies comparing the fitness of individuals sampled at the start and end of annual plant disease epidemics, that selection for quantitative phenotypic traits may operate over short time scales in pathogen populations. This selection process often results in an increase in aggressiveness over the growing season (Chin & Wolfe, 1984; Villaréal & Lannou, 2000; Pariaud et al., 2009).

Correlates of selection have also been investigated for climate variables, such as precipitation and temperature (Carlson & Quinn, 2007; Husby et al., 2011; MacColl, 2011). The study of phenotypic selection in response to thermal changes is particularly timely, given the shifting perspectives concerning the importance of interindividual variation (Bolnick et al., 2011) and plasticity and their contribution to the adaptive potential of individuals and populations (Wadgymar & Austen, 2019). Trait variation in response to temperature is commonly summarised on thermal performance curves depicting the variation of performance over a range of temperatures (Huey & Stevenson, 1979). These thermal performance curves can be

used to assess functional diversity at the individual and group level. They can also be used to investigate whether thermal sensitivity (e.g. being adapted to cold or warm temperatures) is a product or a target of selection, in experiments specifically developed for the study of phenotypic plasticity (Scheiner, 2002). Many signatures of temperature-mediated patterns of local adaptation have been identified in the wild, but there is still very little quantitative evidence for selection on thermal plasticity and very few experimental data (Arnold et al., 2019). A number of questions have thus remained unanswered. For example, how does such a phenotypic plasticity influence short-time eco-evolutionary trajectories and population dynamics? What are the relative contributions of the individual and functional group levels to selection by temperature?

In this study, we investigated the dynamics of phenotypic selection on thermal responses within fungal plant pathogen populations, which are usually subjected to a broad range of climate conditions, under different selective regimes (Suffert et al., 2015; Robin et al., 2017). We investigated the conditions in which thermal selection occurs, and its strength and speed over the course of an annual epidemic. We hypothesized that the selection of certain individuals or group of individuals in response to short-term selective pressures on life-history traits might lead to an adaptation of the population to the constraint concerned. We considered two situations in which groups competed as a function of their thermal responses: (i) cold- vs. warm-adapted groups of individuals, assuming that cold conditions would favour individuals performing better at lower temperatures, whereas warm environments would favour individuals performing better at higher temperatures; (ii) generalist vs. specialist groups of individuals, assuming that more variable environments would favor generalist individuals.

We chose to focus on the wheat fungal pathogen Zymoseptoria tritici (formerly Mycosphaerella graminicola, the causal agent of Septoria tritici blotch; Eyal, 1999; Quaedvlieg et al., 2011). The prevalence of this pathogen differs between contrasting climate zones, and its thermal biology has been investigated in recent studies. It is, therefore, a particularly relevant model system for addressing these research questions over short time scales. Indeed, several lines of evidence suggest that selection may occur: (i) the existence of phenotypic variation, with considerable variability in thermal responses across individuals (Boixel et al., 2019a,b); (ii) emerging evidence of selective adaptation patterns at annual (Suffert et al., 2015) and large geographic (Zhan & McDonald, 2011; Boixel et al., 2019a) scales; (iii) the heritability of phenotypes (Lendenmann et al., 2016); (iv) variation in competitive fitness between strains established in both the presence (in planta) and absence (in vitro) of interactions with plants (Bernard et al., 2013; Boixel et al., 2019b).

METHODS

Four competition experiments were conducted to evaluate selection on phenotypic variation itself and to investigate the effect of different thermal regimes on the frequency of different genotypes (individual-level) and thermotypes (group-level) across multiple generations of Z. tritici populations. These complementary experiments were conducted under three selective environments, from simplified to naturally complex settings in terms of selective pressures, summarised with eight parameters (Table 1).

Table 1 Design and specific features of the Zymoseptoria tritici two-thermotype competition experiments. A set of eight structural parameters summarises the differences between in vitro (A), and in planta growth chamber (B) or field (C_1-C_2) competition experiments: nature of resource competition (liquid artificial medium or plant tissues), duration of the experiment defining the number of generations monitored, thermal regimes (constant, diurnally fluctuating and natural conditions in the field), marking strategy for distinguishing strains and capturing changes in population composition (GFP or SSR neutral markers), population system (closed or open natural populations), number of strains for each competing thermotype (pairwise or sextet strain competition), number of competition cases (tracked starting experimental populations), number of temporal samplings (monitoring mesh).

	In vitro experiment (A)	Growth chamber experiment (B)	Field experiment (C_1-C_2)
Nature of resource competition	Liquid culture medium	Wheat seedlings	Wheat microplots
Duration of the experiment	72 h (in vitro multiplication) [1]	4 months (5 pathogen cycles) [2]	7 months (± 6 pathogen cycles) [3]
Thermal regimes	4 constant regimes	2 diurnally fluctuating regimes	1 field regime
Marking strategy	GFP	SSR neutral markers	SSR neutral markers
Population system	Closed	Open	Open
Strains per thermotype	1	3	3
Competition cases	3	1	2
Temporal samplings	1	5	3

[1] yeast-like replication by budding

[2] non-overlapping asexual infection cycles over successive batches of wheat plants

[3] asexual infection cycles estimated to have occurred over the complete field epidemic period

Joint workflow for monitoring population changes in the competition experiments

Defining experimental populations (Fig. 1.1) - Each experiment was performed by placing two contrasting thermotypes (i.e. groups of individuals with similar thermal responses as defined in Boixel et al., 2019a) in competition. The strains forming the initial populations were retrieved from a subset of the INRAE BIOGER Z. tritici collection sampled from the wheat cultivar 'Soissons' for which in vitro and in planta thermal responses had already been characterised (Boixel et al., 2019a ; 2019b). We chose strains so as to carry out competitions between cold- and warm-adapted (experiments A, B, C_1) or generalist and specialist (experiment C_2) strains, by focusing successively on: (i) their thermal responses, with the aim of minimising the differences within and maximising the difference between the two thermotypes (Table 2); (ii) minimising inter-strain differences in aggressiveness components (Fig. S1) contributing to overall fitness (Pariaud et al., 2009); (iii) the ease with which they could be distinguished on the basis of their genotypes; (iv) maximising the number of strains of similar mating type, to limit recombination between inoculant genotypes in the field experiment (Table S1).

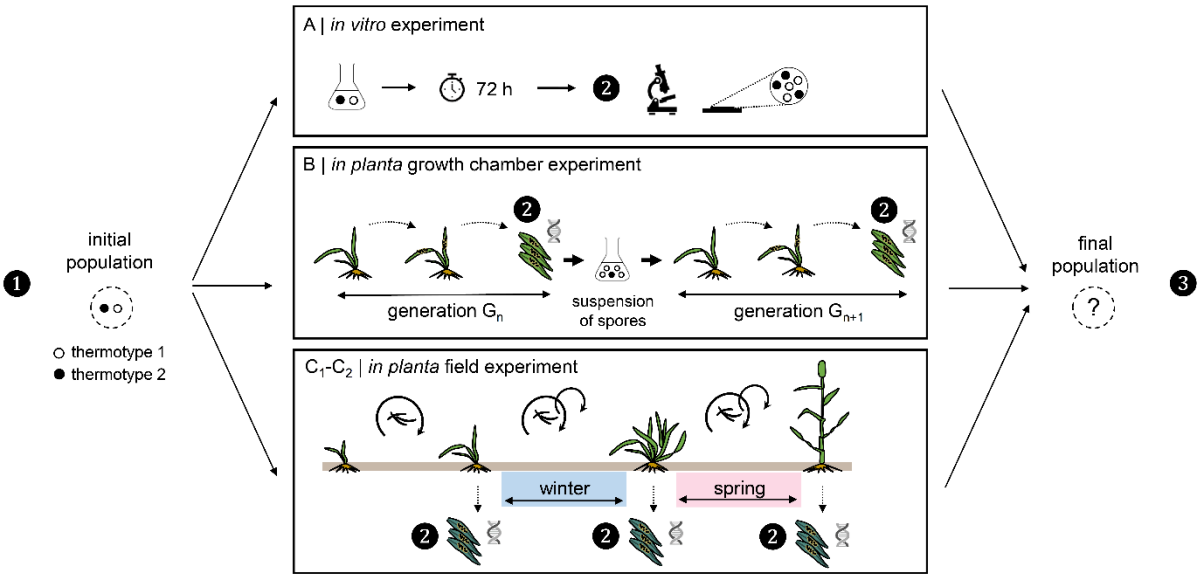

Fig. 1 Experimental design to investigate Zymoseptoria tritici thermal selection under in vitro (A) and in planta (B, C_1-C_2) conditions. Changes in strain frequencies are monitored through a mark-release-recapture strategy that can be divided into three distinct stages: (1) the choice of strains for pairwise and sextet competition on the basis of thermotype (either cold- vs. warm-adapted or generalist vs. specialist); (2) estimation of the frequency of each strain after the recovery of individuals from the sampling of pools of spores or diseased leaves at different time intervals (in vitro: 72 h after deposition; growth chamber: every pathogen generation; field: early winter, post-winter, post-spring) and the use of a GFP (A) or SSR (B, C_1-C_2) labelling strategy; (3) analyses of (mal-)adaptive dynamics and outcomes, considering selection pressures and induced changes in the phenotypic composition of populations.

Table 2 Thermal responses of the Zymoseptoria tritici strains belonging to the thermotypes placed in competition in vitro (A), and in planta in growth chambers (B) and in the field (C_1-C_2). Data are presented for each experiment, specifying the environmental conditions, the competing thermotypes and the parameters T_{opt} (thermal optimum) and TPB_{80} (80% thermal performance breadth) capturing the features of the thermal performance curves. The values of T_{opt} and TPB_{80} were averaged across the three strains of each thermotype in a given competition experiment (mean ± SD), except in experiment A, which consisted of separate pairwise cocultures of a single cold-adapted strain (* either CA_1 or CA_2 or CA_3) with a single warm-adapted strain, (** the GFP-expressing IPO323 transformant WA_1).

	Thermotype 1				Thermotype 2			
	Feature	Inoculant strains	T_{opt} (°C)	TPB_{80} (°C)	Feature	Inoculant strains	T_{opt} (°C)	TPB_{80} (°C)
A	Cold-adapted	CA_1 or CA_2 or CA_3 *	19.2 ± 0.1	13.9 ± 0.8	Warm-adapted	WA_1 **	25.8	11.0
B		CA_4 + CA_5 + CA_6	17.4 ± 0.4	12.6 ± 1.2		WA_4 + WA_5 + WA_6	19.3 ± 0.3	15.9 ± 0.8
C_1		CA_7 + CA_8 + CA_9	17.5 ± 0.3	12.8 ± 1.9		WA_7 + WA_8 + WA_9	19.7 ± 0.9	14.2 ± 1.1
C_2	Generalist	G_1 + G_2 + G_3	18.9 ± 1.0	17.9 ± 0.9	Specialist	S_1 + S_2 + S_3	18.1 ± 0.1	12.2 ± 1.1

Recovering pathogen populations and distinguishing between competing individuals (Fig. 1.2) – At the start of each competition experiment, two thermotypes were used, in equal proportions (to minimise within-host competition ; Fellous & Koella, 2009), for inoculation. Asexual progenies (i.e. spores resulting from the clonal multiplication of these two thermotypes), were sampled from liquid cultures or infected leaves at various time points, to constitute temporal snapshots of the composition of the population. The genotype of each strain was determined by a GFP (experiment A; Lorang et al., 2001) or SSR labelling strategy (experiments B, C_1-C_2; Ennos & McConnell, 1995; Zhan & McDonald, 2013). The sampling effort for estimating population composition was set to one third of the total volume for the in vitro experiment (50 µL sampled from each of the five cocultures for a given treatment; experiment A) and to 30 pure strains per time point for the in planta experiments (isolation from infected wheat leaves and culture of one cirrhus per fruiting body and per leaf lesion on PDA medium for each replicate line of a given treatment; experiments B, C_1-C_2). This in planta sampling effort was chosen to estimate allele frequencies and to optimise the potential recapture of each of the six genotypes used for inoculation (see Table 1 in Zhan & McDonald, 2013) and magnified to achieve the level of precision required to ensure sufficient statistical power (Dale & Fortin, 2014). Sampled individuals were genotyped for 12 neutral SSR markers (ST1, ST2, ST3A, ST3B, ST3C, ST4, ST5, ST6, ST7, ST9, ST10, ST12; Gautier et al., 2014). Genotyping was performed on DNA extracted (QIAGEN DNeasy Plant Maxi Kit) from 50 mg of fresh fungal material (from five-day-old cultures on PDA medium at 18°C in the dark), with SSR markers amplified and sequenced within one multiplex PCR sample (Eurofins France). The determination and annotation of allele sizes (visual analysis of individual chromatograms, Peak Scanner, Applied Biosystems) made it possible to determine whether the genotypes of the sampled strains were similar (parental allele combinations; sexual offspring or asexual progeny of strains investigated in the experiment) or different (novel alleles or allele combinations indicating the presence of potential immigrants, recombinants or mutants) to those of the genotypes used for inoculation.

Assessment of eco-evolutionary dynamics and experimental outcomes (Fig. 1.3) – For each competition experiment, population snapshots were supplemented by the collection of information about the selective environment (air temperatures over the course of the experiments) for further analyses of the impact of experimental design on the competitive fitness of strains and population demographic parameters.

Experiment A. In vitro experiment under five constant thermal regimes

The in vitro experiment was performed within a specifically designed framework (Boixel et al., 2019b). Three pairwise competition experiments were performed, between a warm-adapted strain (a GFP-expressing transformant, WA_1) and a cold-adapted strain (non-GFP-expressing strains similar in terms of their thermal responses, CA_1, CA_2 and CA_3, respectively), with five replicates each. Pairs of strains were cocultured in 150 µL of liquid glucose peptone medium (14.3 $g.L^{-1}$ dextrose, 7.1 $g.L^{-1}$ bactopeptone and 1.4 $g.L^{-1}$ yeast extract), with an initial total spore concentration of 2.5×10^5 spores mL^{-1}, under five constant-temperature regimes (7.0, 18.5, 21.0, 25.0 and 30.5°C; Fig. 2a). Changes in strain frequencies were assessed by fluorescence microscopy on 50 µL aliquots of each coculture replicate, once stationary phase had been reached (i.e. 72 h after inoculation). The final proportions of each strain were quantified on aliquots photographed under both white and fluorescent light under a

fluorescence microscope (Leica DM5500 B, Leica Microsystems), by analysis with ImageJ 1.48v software (further detailed in Methods S1).

Experiment B. In planta growth-chamber experiment under fluctuating thermal regimes
Six replicate wheat (cv. 'Soissons') populations, each consisting of 19 seedlings, were sown in pots and grown in a chamber at 18°C (day) / 16°C (night), with a 16-h light period (photosynthetically active radiation [PAR], 75 $\mu mol.m^{-2}.s^{-1}$). We separated these populations into two groups 15 days after sowing, and exposed the groups to two contrasting thermal regimes (Fig. 3a). Three wheat populations were transferred to another growth chamber set to 22°C (day) and 20°C (night) for acclimatisation to a warmer thermal regime (21 ± 1°C). The other three wheat populations were left in the initial growth chamber, at its initial, colder settings (17 ± 1°C). Seedlings were inoculated the following day, by spraying until run-off (ECOSPRAY micro-sprayer, Bluestar Forensic) with 12 mL of a suspension of spores from three cold-adapted (CA_4, CA_5, CA_6) and three warm-adapted individuals (WA_4, WA_5, WA_6) in equal proportions, at a total concentration of 10^6 spores.mL^{-1}. This high concentration was chosen to maximise infection and minimise founder effects. Plants were then enclosed in polythene bags for 72 h to keep humidity levels high and promote infection. The resulting pathogen populations were exposed to the two prevailing thermal regimes over five successive pathogen cycles in a serial-passage experiment, in which each population was regularly transferred by sampling and reinoculation onto a new batch of healthy 16-day-old wheat populations at the end of each generation cycle (i.e. after pathogen sporulation on the leaves). This made it possible to distinguish offspring produced under different generation cycles. Serial passages were conducted 20 days post-inoculation, after checking that all the parental strains were sporulating in each environment, to prevent the artefactual extinction of the less well-adapted strains (Fig. S3a). At that time point, all leaves presenting disease lesions were collected from each wheat population (thermal regime × replicate line × experimental time) and incubated overnight at high humidity to promote cirrhus exudation. We chose a subsample of 30 lesions from which we collected one cirrhus per fruiting body and per leaf lesion, to obtain individuals for genotyping for SSR markers. All leaves were then immersed in a volume of water, which was agited over the area covered by fruiting bodies, with a paintbrush swept over the surface to release and harvest spores. The inoculation conditions (concentration, volume sprayed, application procedure) used for the resulting spore suspensions (one per experimental line) were identical to those used for the inital inoculation at G0 (as described above), to minimise uncontrolled changes in selective pressure (Lanfear et al., 2014). We estimated the respective abilities of the various strains to contribute to the next generation (i.e. their selective value), for each pathogen cycle under the cold and warm regimes (detailed in Methods S2).

Experiment C_1-C_2. In planta experiment under field temperature regimes
A field trial was performed on nine microplots of wheat cv. 'Soissons' in 2016-2017 at the INRAE Grignon experimental station (Yvelines, France). Two treatments were studied in a randomised complete block design with three replicates each (one microplot of 21 m² with each block sown at a density of 220 seeds per m²): (i) competition between cold- and warm-adapted strains; (ii) competition between generalist and specialist strains.

Uninoculated control microplots were also included for the quantification of background contamination due to natural inoculum (see Methods S3). Experimental strains were used for

inoculation in the field at a concentration of 10^6 spores.mL^{-1}, by spraying until run-off (F2+ 1.5L manual sprayers, Berthoud), on two occasions, one week apart (6 and 13 December 2017) in early winter (wheat phenological growth stage: BBCH 12-13; Lancashire et al., 1991). The composition of the population in each microplot was described at three time points over the growing season: mid-winter to capture the effective population established after one cycle of asexual pathogen reproduction (February 1st); post-winter (April 11th); and post-spring (June 16th). At each time point, we collected 30 strains at random from each plot, from the most recent diseased leaves of 30 infected plants (same leaf layer and same infection cycle for a given sampling point). We thus obtained a sample of the active part of the population, i.e. strains representative of the last overt pathogen cycle, just below the emergent leaf layer and, therefore, primarily responsible for subsequent secondary infections. For the inference of competitive advantage, fitness assays were conducted on wheat seedlings and adult plants, to capture the effect of plant development and leaf age on the strains used for inoculation (Fig. S5; Develey-Rivière & Galiana, 2007).

Statistical analyses

Analyses were carried out with R statistical software version 3.6.1 (R Core Team, 2018). Proportions were compared between strains and thermotypes over the course of (temporal changes in composition) and within (between experimental lines for each generation, to assess the consistency of the response) a given competition experiment, in chi-squared tests (χ^2). Mean differences in fitness components between competitor strains (experiments B, C1-C2) were assessed by one-way analysis of variance (ANOVA) or its non-parametric alternative for non-normally distributed variables (Kruskal–Wallis). In experiment B, we were able to calculate the selection coefficient s of each strain under the two prevailing thermal regimes and over the five generations of Z. tritici, to detect and quantify phenotypic selection ($s = 1 – w$, where w is the relative fitness of a given thermotype under a given thermal regime). This coefficient quantifies the difference in overall fitness across the infection cycle between the best-adapted strain and the other competitor strains, based on their relative contributions to the gene pool of the next generation. We quantified the proportion of inoculant and non-inoculant genotypes recovered from the field experiment (C_1-C_2), to assess the effects of other evolutionary forces on dynamic outcomes. A coarse distinction was made between 'asexual progeny' (genotypes identical to the parental inoculant genotypes), 'immigrants' (genotypes carrying novel alleles), 'potential sexual recombinants' (genotypes carrying novel allele combinations but theoretically also potentially corresponding to immigrants or mutants) on the basis of SSR multilocus genotypes analyses, to assess the relative magnitude levels of these groups. The relative contribution of experimental factors to population dynamics and outcomes was assessed for each experiment (A, B, C_1-C_2). When appropriate, we tested for the effects of stochastic processes (e.g. random genetic drift; 'replicate lines'), generation ('time'), thermal responses ('thermotype'), strain genetic background ('strain | thermotype' nested factor), imposed thermal regime ('thermal conditions') and its dependence on time ('time × thermal conditions' interaction term) and thermotype ('thermotype × thermal conditions' interaction term) on the proportion of each strain over time, by multivariate analysis of variance (MANOVA; Table 3).

RESULTS

Temporal changes in composition, with an overall consistency of response in experimental lines - In all competition experiments, changes in population phenotypic composition occurred during the course of the experiment (chi-squared tests; $\chi^2_{B\ cold\ regime}$ = 29.99; $\chi^2_{B\ warm\ regime}$ = 27.04; χ^2_{C1} = 48.78; χ^2_{C2} = 18.02; P < 0.01). The changes in frequency of each competitor strain and thermotype followed similar trends in the independent replicates of experiments A, B and C_2 (MANOVA; P = 0.11, 0.68, 0.39, respectively; Table 3). This consistency of responses between experimental lines (n = 5 for in vitro experiments; n = 3 for in planta experiments) was confirmed by chi-squared tests (P > 0.05), but nevertheless concealed a certain degree of sampling variability between experimental lines. For instance, the most extreme case in experiment B concerned one replicate line under the cold regime at generation time G4, with the amount of CA_1 strains detected only half that for the other two cold-adapted strains. However, this difference was not significant in the corresponding chi-squared test ($\chi^2_{B\ cold\ regime}$ = 16.31, P = 0.09). The observed changes in population composition can, thus, be attributed to the effects of selection rather than differential founder effects and/or random genetic drift. Conversely, different trajectories were found between the three microplot populations of experiment C_1 at the post-winter and post-spring timepoints (F = 8.94; P < 0.01; Table 3), despite similar initial states for the established populations (chi-squared test for the mid-winter sampling; P = 0.38).

Table 3 Relative contribution of experimental factors to Zymoseptoria tritici population dynamics in terms of phenotypic composition. Variance components potentially affecting outcomes in terms of changes in phenotypic proportions are reported, with their variance ratio (F-statistic) and their statistical significance (p-value). For each competition experiment (A, B and C_1-C_2), the variance of strain frequencies was divided into sources attributable to the strain, thermotype (WA, CA, G, S), replicate, time, thermal conditions, interaction between time and thermal conditions and interaction between thermotype and thermal conditions when applicable. n/a, source of variation not applicable to the experimental design of the corresponding competition experiment; cfd, thermal conditions confounded with time in experiment C_1-C_2 implying that the sources of variation are non-computable. ⁜: P < 0.01.

Experiment	A		B		C_1		C_2	
Two-thermotype competition	WA vs. CA		WA vs. CA		WA vs. CA		G vs. S	
Source of variation	F	P	F	P	F	P	F	P
Replica lines [1]	1.95	0.11	0.39	0.68	8.94	⁜	0.98	0.39
Time [2]	n/a		16.45	⁜	5.52	⁜	12.07	⁜
Thermotype [3]	n/a		0.98	0.32	0.86	0.36	0.92	0.34
Strain × Thermotype [4]	1.66	0.18	5.20	⁜	0.48	0.75	10.02	⁜
Thermal conditions [5]	41.03	⁜	3.98	0.05	cfd		cfd	
Time × Thermal conditions	n/a		2.07	0.07	cfd		cfd	
Thermotype × Thermal conditions	n/a		143.46	⁜	1.23	0.30	2.39	0.10

[1] Replicate populations corresponding to parallel repetitions of the experiment
[2] Z. tritici generation (G_i in experiment A) or sampling events (S_i in experiments B and C_1-C_2)
[3] Groups of individuals with similar thermal responses (WA, CA, G, S)
[4] Effect of parental genotypes
[5] Cold (17.0 ± 1°C) or warm (21.1 ± 1°C) thermal regime, tested only under controlled conditions

Relative contributions of phenotypic means and variation to competitive outcomes – Belonging to a given thermotype (i.e. presenting a given differential thermal response) had different effects on prevalence within the population, depending on the thermal regime applied (significant 'thermotype × thermal conditions' interaction effect). This was particularly true for experiment B (MANOVA, $F = 143.46$, $P < 0.01$) and a similar but non-significant trend was observed for experiment C_2 (MANOVA, $F = 2.39$, $P = 0.10$) despite a significant strain effect detected in the main experiments ('Strain | Thermotype' nested effect, due, in particular, to the lesser mean fitness of WA_6 and G_1; Table 3).

Differences in thermal responses govern competitive advantages under constant thermal regimes in vitro (experiment A) – The proportion of warm-adapted strains in coculture with any one of the three cold-adapted strains increased significantly with the temperature of the constant-temperature regime applied (Kruskal-Wallis $\chi^2 = 62.60$; $P < 0.01$), although the proportion of cold-adapted strains was greater overall (minimum proportion of 0.63 recorded at T_{max}). The same trend was observed for the three cocultures, suggesting that the dissimilarity in thermal responses between the cold- and warm-adapted strains was responsible for the outcome of competition (chi-squared test; $P > 0.05$). No difference in the cold-adapted to warm-adapted strain ratio was observed under the two warmest thermal regimes, T_{optWA} and T_{max}, despite there being more than five degrees of difference between them (Fig. 2).

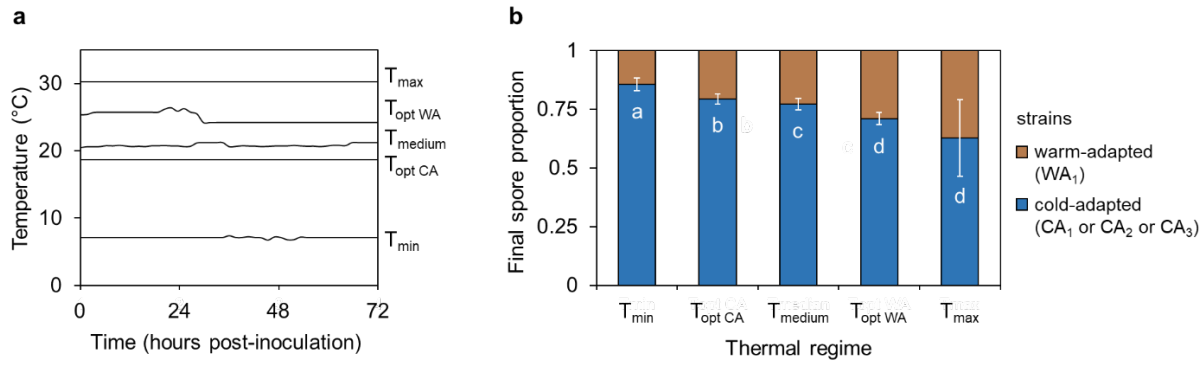

Fig. 2 Outcomes of in vitro pairwise competition experiments for cold- vs. warm-adapted Z. tritici strains under five constant thermal regimes (experiment A). (a) Constant thermal regimes to which three pairs of strains, used in equal proportions for inoculation, were exposed over three days post-inoculation in liquid culture medium: T_{min} (7.0°C), $T_{opt\ CA}$ (18.5°C), T_{medium} (21.0°C), $T_{opt\ WA}$ (25.0°C), T_{max} (30.5°C); (b) Proportion of cold-adapted (T_{opt} = 19.2 ± 0.1°C; blue bars) and warm-adapted (T_{opt} = 25.8°C; orange bars) strains in the cocultures after 72 h of growth. Experimental data are presented as means ± standard deviation based on competition in three pairs of strains averaged across 5 coculture replicates. Different letters below error bars indicate significant differences in proportions as a function of temperature (Kruskal-Wallis test, $P < 0.01$).

Short-term in planta selection for thermal responses under controlled thermal regimes (experiment B) – Over the five Z. tritici generations in the competition experiment, we observed rapid decreases in the frequency of the warm-adapted thermotype under the cold regime and the frequency of the cold-adapted thermotype under the warm regime (i.e. the less well-adapted strains under the two prevailing thermal regimes; Fig. 3).

These shifts in the phenotypic composition of the population became statistically significant from the second generation under the cold regime and the third generation under the warm

regime (test for deviation from equal proportions of strains; Table S3). Further support for differential selection under the two thermal regimes was provided by: (i) the significant interaction between thermal regime and thermotype in the MANOVA (Table 3); (ii) the strong, stable selection coefficients indicating the relative strength of selection acting against each thermotype: s_{CA} under the warm regime = 0.66 ± 0.03 (mean ± SEM) and s_{WA} under the cold regime = 0.79 ± 0.02 (mean ± SEM); (iii) the gradual increase for the WA thermotype and gradual decrease for the CA thermotype of Pearson's residuals in chi-squared tests comparing the proportions of each thermotype across generations under the warm regime (and vice versa under the cold regime, except for a pause in the selection gradient at G3; Table S4); (iv) the absence or very low frequency of the less well-adapted strains after the last experimental cycle G5 (Fig. 3), with no WA strain in the three experimental lines subjected to the cold regime and one CA_5 representative in one of the three experimental lines subjected to the warm regime (under which CA_5 has a higher selective value than CA_4 and CA_6; Table S2). The pause in the selection gradient at generation time G3 under the cold regime (Table S4) was not due to differences in population size (ANOVA, P = 0.67; Fig. S2) or prior composition (chi-squared test; $\chi 2$ = 6.41, P = 0.78). Instead, it probably reflects differences in the overall fitness of some competitor strains, as shown in the B_{2a} single-generation experiment (Methods S2). For instance, two strains presented differences in sporulation intensity at this generation time relative to other generation times, lower for CA_1 and higher for WA_3 (Kruskal-Wallis test, P = 0.02 and P < 0.01, respectively). This may have decreased the overall competitive advantage of the CA thermotype under the cold regime at this precise time point.

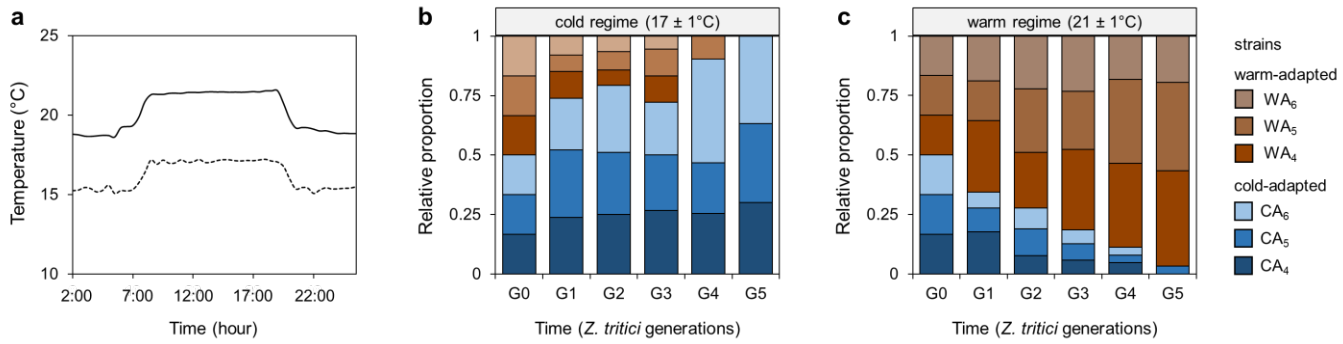

Fig. 3 Outcomes of the growth chamber competition experiment for cold- vs. warm-adapted Z. tritici strains under two diurnally fluctuating thermal regimes (experiment B). (a) Daily patterns of air temperature variation above plants were automatically recorded at one-minute intervals with a ventilated sensor in the two growth chambers. The records (here from 21 to 22 April 2017) illustrate the two diurnally fluctuating temperature regimes of similar amplitude but with different means (cold regime at 17 ± 1°C, dashed line; warm-regime at 21 ± 1°C, solid line) to which the sextet of strains was exposed in planta over four months (five Z. tritici generations). (b-c) Changes in the relative proportions of each of the inoculated strains within Z. tritici populations measured over consecutive generations (mean of the three replicate populations) under the (b) cold and (c) warm thermal regimes. The colour scheme indicates the thermotype (cold-adapted strains in blue and warm-adapted in orange), with the intensity of the hues (dark, intermediate, light) distinguishing strains. The bars for G0 correspond to the proportion of spores in the initial inoculant suspensions.

Overall maintenance of diversity in the field experiment despite a sharp decrease in the resolution acuity of recapture (C_1-C_2) – Novel alleles or new allelic combinations were

detected when the strains retrieved from the field trials were genotyped. This background noise can be attributed to immigration and recombination (Fig. S6). Consistently, the mid-winter samples, collected only one cycle after inoculation, were less strongly affected. These findings can be explained by the high concentration of the inoculum applied artificially relative to the small amounts of primary inoculum naturally present locally at the site (Fig. S4a). These conditions probably favoured the establishment of the inoculant strains, as highlighted by the significant difference in disease index between inoculated and uninoculated plots (Wilcoxon test, $V = 45$, $P < 0.01$; Fig. S4b). The gradual decrease in the proportion of inoculant genotypes in the post-winter and post-spring populations reveals a progressive loss of resolution in the description of their composition: the size of the inoculant population size retrieved had halved by the post-winter sampling and decreased by three quarters by the post-spring sampling (Fig. S6). This limits conclusions about the selective processes and outcomes at work in populations. Nevertheless, asexual progeny of the six competitor strains were still present at the end of both experiments (post-spring sampling in Fig. 4), highlighting a maintenance of diversity for thermal adaptation that may or may not be adaptive. Indeed, we found at least one representative of each inoculant strain in each of the three replicate lines, except for CA_7, for which only one representative was retrieved for the whole of experiment C_1, indicating that this strain had not established itself as well as the other competitor strains (consistent with its lower, albeit non-significantly so, sporulation intensity on wheat seedlings; Fig. S5).

Signs of winter selection during competition in the field between cold- and warm-adapted strains despite an artificial founder effect (experiment C_1) - Temporal changes in phenotypic composition were detected over the experiment ('Time' effect in MANOVA, $F = 5.52$, $P < 0.01$; Table 3), with non-inoculant strains accounting for 35.0% of the total variation (contribution to the chi-squared statistic; $\chi^2 = 48.78$, $P < 0.01$). We detected a founder effect expressed as a difference between the composition of the inoculated population and the actual composition of the population established in planta after one asexual cycle in Z. tritici (mid-winter sampling in Fig. 4). More precisely, warm-adapted strains were more frequent at the start of the experiment, after the initial inoculation. This experimental artefact, accounting for 31.3% of the total variation of the phenotypic composition of the population (Table S4), may be partly due to a lower, albeit non-significantly so, intrinsic fitness of CA_7 (Fig. S5). If we focus on changes in inoculant strain frequencies, the only deviation from equal proportions of competitor strains was detected at the mid-winter sampling ($\chi^2 = 17.20$, $P < 0.01$; Table S3). This highlights a re-equilibration of the proportions of warm-adapted and cold-adapted strains between the mid-winter and post-winter samplings (which was marked in only two of the three experimental lines), suggesting a potential selective advantage of the cold-adapted strains during the winter season (Fig. 4). Focusing on thermotypic frequencies, the increase in the proportion of CA strains between the mid- and the post-winter samplings accounted for 8.8% of the overall variation (Table S4).

Stable generalist-specialist balance over the growing season (experiment C_2) – Temporal changes in phenotypic composition were detected over the experiment ('Time' effect in MANOVA, $F = 12.07$, $P < 0.01$; Table 3), with non-inoculant strains accounting for 51% of the total variation (contribution to the chi-squared statistic; $\chi^2 = 18.02$; $P < 0.01$). Focusing on the inoculant strain frequencies, there was no significant deviation from equal proportions of the strains, with a frequency of 1/6 for each inoculant strain (chi-squared tests, $P > 0.05$; Table S3). Despite a slight decrease in the proportion of generalists between the early-winter (inoculation) and mid-winter samplings, and of specialists between the mid- and post-winter samplings

(Table S4), the initial balance between generalist and specialist strains was clearly preserved over the growing season (Fig. 4).

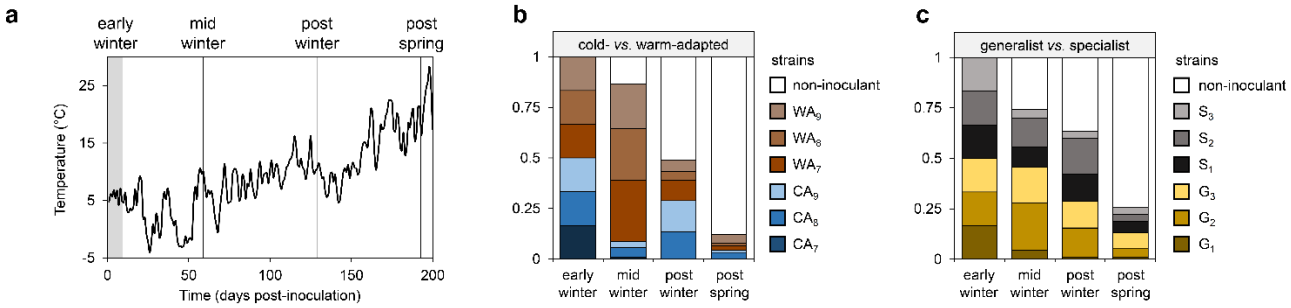

Fig. 4 Outcomes of the field competition experiments for cold- vs. warm-adapted strains and generalist vs. specialist strains under natural conditions (field experiment C_1-C_2). (a) Daily mean temperatures (from December 6, 2016 to June 23, 2017) retrieved from local meteorological records at the INRAE Grignon experimental station (Yvelines, France) for the 2016-2017 growing season. The grey section covers the early period, between the two inoculation events, whereas vertical lines indicate the timing of the three population samplings (mid-winter, post-winter, post-spring); (b-c) Changes in the relative proportions of each of the inoculated and non-inoculated strains (non-inoculant genotypes, i.e. potential immigrants or recombinants) averaged across the three replicate populations in the case of competition between (b) cold- (CA_7, CA_8, C_9) and warm-adapted strains (WA_7, WA_8, WA_9) and between (c) generalist (G_1, G_2, G_3) and specialist strains (S_1, S_2, S_3). The bars for early winter correspond to the proportion of spores in the initial inoculant suspensions.

DISCUSSION

Design and exposure to increasingly complex selective environments – The combination of competition experiments conducted in this study and their variation with respect to a range of factors (in vitro or in planta, nature of the thermal regimes, temperature-dependent only, or with multiple environmental stressors, openness to immigrants) enabled us to consider the complexity of the parameters and selective pressures imposed on Z. tritici populations in a progressive manner. These complementary experimental settings made it possible to quantify the occurrence and strength of thermal phenotypic selection. The first experiment (A) provided proof of concept that different thermal responses may lead to a differential competitive advantage. It showed that the responses of microbial populations to their environment, based on the traits of individuals and groups, was predictable under a simplified expression of selective regimes. The second experiment (B) demonstrated the occurrence of selection on phenotypic variation for thermal adaptation. It highlighted the adaptive consequences of imposing certain thermal regimes (with all other factors controlled or randomised between lines) in populations with phenotypic and group composition variations. The third experiment (C_1-C_2) investigated the translation of the results obtained in controlled conditions to field situations, in which highly diversified natural populations are subject to varying, and sometimes conflicting selective pressures. This experiment highlighted the overall maintenance of two thermotypes under natural selection, despite changes in their frequencies over the growing season, raising questions about the occurrence and importance of selection on thermal responses in the field (in both the presence and absence of balanced polymorphism and background selection).

This combination of experiments provided insight into how the diversity within a microbial population and the composition of that population can influence responses to environmental

change. The consistency of competitive outcomes shows that the maintenance of three replicate populations in each selection regime and the size of the sampling effort were sufficient for the detection and documentation of selective processes in closed systems. Unsurprisingly, the populations in field studies were subject to greater variability resulting in a progressive loss of detection acuity for the experimenter due to the increasing detection of non-inoculant genotypes over the growing season.

Experimental caveats and limitations on competition outcomes – The strength of selection detected in competition experiments of this type depends partly on the experimental design, which can affect the outcome of selection (Fuller, 2005; Kawecki et al., 2012; Van den Bergh et al., 2018). The experimental caveats are: population size per generation (Weber & Diggins, 1990), the occurrence of a bottleneck at transfer (Lanfear et al., 2014), the number of replicates required to separate selection from random genetic drift (Hill & Caballero, 1992), the duration of the experiment in terms of the number of generations (Papadopoulos et al., 1999), sampling effort, particularly in terms of the timing and precision of the snapshots of populations (Siepielski et al., 2009; Bell, 2010; Cornwallis & Uller, 2010), and the intensity of the selective regime (Huang et al., 2014). Here, we were more interested in documenting thermal selection than in comparing its strength under different temperature regimes. However, it is important to bear in mind that the time between two successive serial transfers (here fixed at 20 days) determines which competitors can contribute to the next generation, and the extent of this contrfibution (Fig. S3). One of the greatest technical difficulties encountered in this study concerned the correct tracking of changes in population composition such that we could distinguish between selective variation and sampling error (Hersch & Phillips, 2004). This was a particular concern in the field trial, in which immigration made a non-negligible contribution to the changes in populations. High levels of contamination with immigrants has already been reported in similar mark-release-recapture experiments focusing on Z. tritici, but with lower proportions of immigrants retrieved over the course of the experiments (at about 20% in Zhan et al., 1998 vs. 66 % in our study by the end of the growing season). This difference in the proportion of immigrants may partly reflect differences in the number of strains used for inoculation and in local primary inoculum pressure: the year of our field trial was characterised by a weak Septoria disease pressure and, as such, was particularly suitable for a field competition experiment with inoculant genotypes (Fig. S4a).

Thermal conditions drive short-term selection for fitness traits – Thermal selection may occur over short time-scales, and may act on differential phenotypes presenting variation in thermal sensitivity and, consequently, in their degree of adaptation to the environment. Indeed, the in planta experiment performed in growth chambers demonstrated that rapid adaptive changes can occur, over as little as five asexual generations in Z. tritici, with significant changes appearing from the second or third generation, for the cold and warm regimes, respectively. On average, six asexual generations (complete infection cycles) can theoretically occur during an annual Septoria tritici blotch epidemic, as estimated by calculating the number of successive latent periods allowed by the number of degree-days over multiple growing seasons (Lovell et al., 2004). This finding provides additional support for the detection of patterns of adaptation to host resistance or environmental conditions in cross-infection experiments studying the relative fitness of individuals and populations collected over a single epidemic (Chin & Wolfe, 1984; Villaréal & Lannou, 2000; Zhan et al., 2002; Suffert et al., 2015). The in planta experiment performed in growth chambers also highlighted the symmetry of responses for a particular trait (i.e. selection of the cold-adapted strains under the cold regime and of the warm-adapted strains under the warm regime (Fig. 2). Note that symmetry is not perfect, as the calculated

selection coefficients were lower under warmer temperatures, with more disadvantageous effects on all strains, as can be seen from the comparison of demographic rates between the two regimes (Fig. S2). This result echoes the higher vulnerability to thermal extremes found in the in vitro experiment, in which the two warmest thermal regimes (T_{optWA} and T_{max}) were not discriminant, despite differing by more than five degrees (Fig. 2). This result also elegantly corroborates the significant negative impact of thermal extremes on the fitness of species, consistent with the steeper slope of thermal performance curves under warm temperature (over the thermal optimum T_{opt}) than under cold temperatures (Ma et al., 2015a,b; Pincebourde & Casas, 2019). The development of a more detailed picture of thermal phenotypic selection will require additional studies to screen for (mal)adaptive consequences of a broader range of selective regimes, with the modulation of time, sequence, duration, temperature and their interaction (see the time series model in Woodin et al., 2013). This would make it possible to tackle the conceptual issues of prior thermal history, time scale and the duration of the response (order and changing rate of thermal exposure; Sinclair et al., 2016). These questions should be addressed through a combination of laboratory experiments, field experiments, and analyses of multi-year field observations, each of which provides important insights into physiological responses, but with different limitations. For instance, controlled conditions do not faithfully reproduce natural conditions and cannot capture the complexity of the temporal history of exposure to temperature fluctuations, whereas field studies are subject to the effects and interactions of multiple stressors (Crain et al., 2008).

Implications for climate change and plant disease – Our results show that selection on phenotypic variation for thermal adaptation may occur in the very short term in microbial populations under constant and diurnally fluctuating temperature regimes. This thermal selection may thus drive the environmental adaptation of plant pathogen populations. Our findings are consistent with those of previous studies highlighting changes in life history strategies over a single epidemic (Suffert et al., 2015). This adaptive potential should be taken into account when considering the responses of plant pathogen populations to climate change. It is generally overlooked in models of the effect of climate on plant disease (e.g. Gouache et al., 2013 in the case of Z. tritici), due particularly to the lack of laboratory, field, and mesocosm experimental data (Rohr et al., 2011). Other limitations include overly simple approaches to dealing with the thermal biology of plant pathogens and their diversity in terms of thermal sensitivity (e.g. one species-one TPC or rough pathogen classifications according to epidemic type, mode of dissemination and infection biology; West et al., 2012). In this respect, competition experiments, such as those conducted here, and experiments considering extreme temperatures and biological variation are particularly informative for decreasing the uncertainty relating to the impact of climate change on pathogen populations (Williams et al., 2016; Kingsolver & Buckley, 2017).

Do we now have a complete picture and, if not, what more do we need? – This study focused essentially on selective adaptation, with only occasional considerations of other evolutionary processes (drift, migration and recombination). Two points that could be considered in more detail emerge: (i) the interaction between these evolutionary forces, as they all affect population changes and responses to environmental change (Zhan & McDonald, 2004); (ii) the other eco-ecolutionary forces at work, including, in particular, phenotypic acclimatisation at the individual scale, which may have a major impact not investigated here (Lagerspetz, 2006; Malcolm et al., 2008).

The molecular changes occurring in Z. tritici in response to fluctuating selection, as studied by Jallet et al. (2019), also need to be taken into account in studies over longer time scales than single annual epidemics. The maintenance of thermotype diversity in natural populations, as demonstrated here in the field experiment, raises three other issues relating to the complexity of natural environments: (i) the importance of pleiotropic effects and other fitness costs in generating trade-offs between fitness traits and shaping the response to selection (as highlighted by Montarry et al., 2007; Laine & Barrès, 2013; Suffert et al., 2015, 2018); (ii), the importance of capturing the diversity of natural populations to minimise biases in selection estimates (Siepielski et al., 2009; Bell, 2010; Cornwallis & Uller, 2010), whether from a technical (going from population snapshots to exhaustive allele frequency estimation; e.g. via quantitative real-time PCR or pyrosequencing in Lavebratt et al., 2004; Zwart et al., 2008; Karisto et al., 2019) or paradigm (considering experimental populations with levels of diversity similar to those found in the field, going from pairwise to more than two-thermotype competition to tackle higher order interactions; e.g. Levine et al., 2017) standpoint; (iii) the difficulties involved in characterising thermal heterogeneities appropriately at scales relevant to Z. tritici. Indeed, the observed maintenance of diversity may reflect the existence of thermal microrefugia in wheat canopies (Chelle, 2005; Pincebourde & Woods, 2012; Potter et al., 2013). Another critical aspect concerns the uncertainties on the drivers of this observed thermal selection, particularly as concerns the biologically relevant time and spatial scale of thermal responses (Kingsolver & Woods, 2016; Dillon et al., 2016; Sinclair et al., 2016). The exploration of these aspects and the quantification of their effects on eco-evolutionary dynamics and outcomes will require modelling approaches, to disentangle the selective effects of environmental drivers and to clarify the results obtained. Following cross-infection experiments and a detailed characterisation of thermal heterogeneities within wheat canopies over space and time, this would make it possible to investigate (i) multiple solutions tackling these both aspects under a set of selective regimes; (ii) the processes and mechanisms underlying the role of spatial thermal heterogeneity in the maintenance of diversity in wheat canopies (Korona et al., 1994; Bell, 1997; Rainey & Travisano, 1998); (iii) the importance of the initial interindividual variation in the population, through the testing of larger numbers of individuals and groups (richness) and different initial proportion (evenness), with a view to representing the natural population more correctly and addressing the joint effects and relative importance of these factors for changes in the population (e.g. Maestre et al., 2012), density-dependent and frequency-dependent selection (Slatkin, 1979).

CONCLUDING REMARKS

The concept of 'thermotypes' (Boixel et al., 2019a), used to describe populations in terms of functional groups of thermal responses, makes it possible to move from patterns to processes and to tackle selective shifts in population composition in response to environmental change. This selection, which acts on genetic variability for fitness-related traits, affects population dynamics in ways that may lead to short-term adaptation at the population level. By deciphering the competitive advantage of these thermotypes under given thermal scenarios, it would be possible to test general assumptions currently accepted in thermal biology. Our work paves the way for further studies of the ecological effects of individual-level and group-level phenotypic variation in natural populations and their role in eco-evolutionary dynamics (Forsman, 2015; Wadgymar & Austen, 2019).

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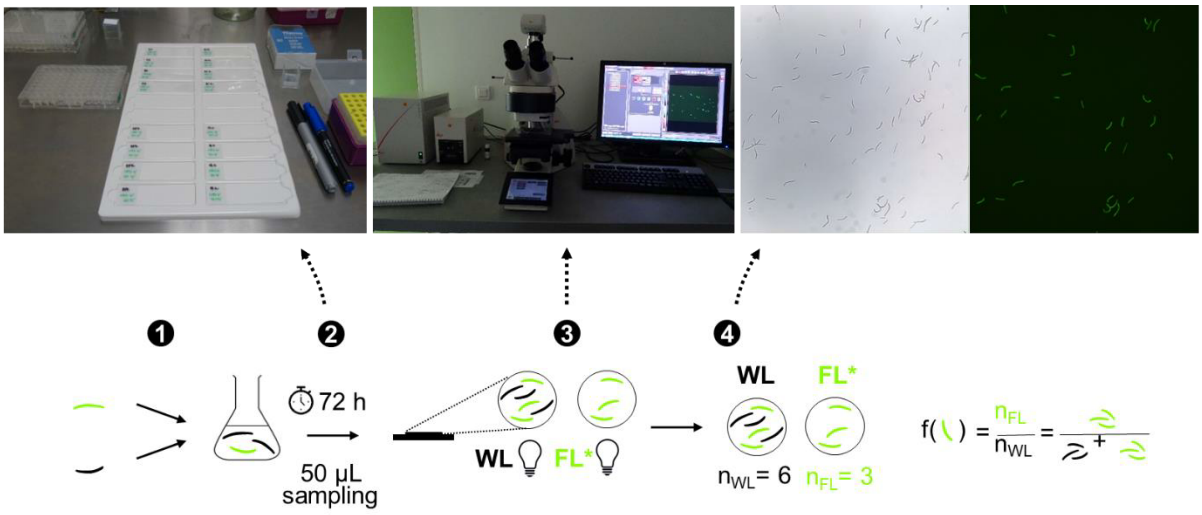
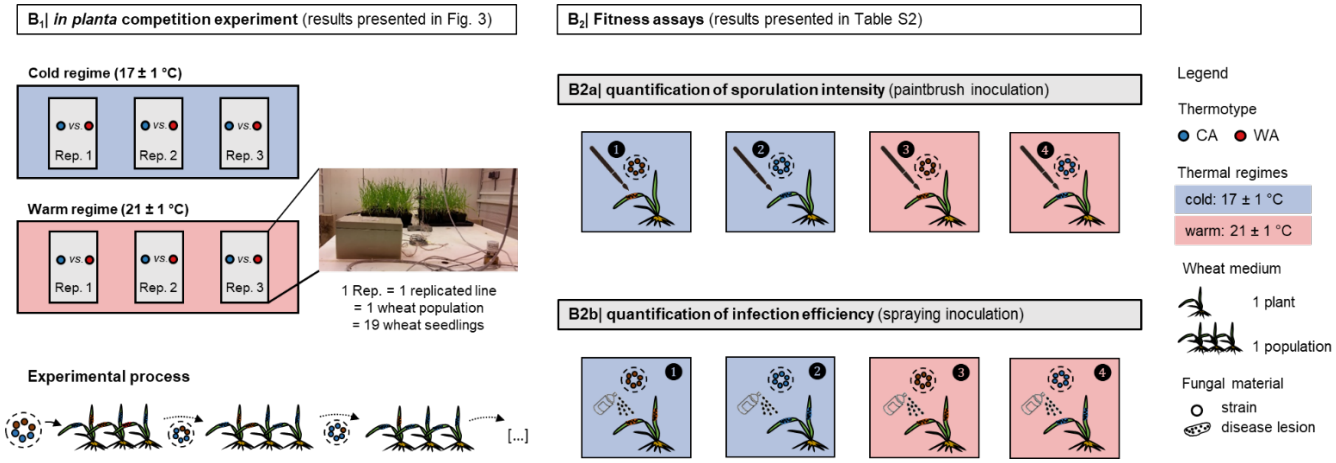
SUPPLEMENTARY MATERIAL

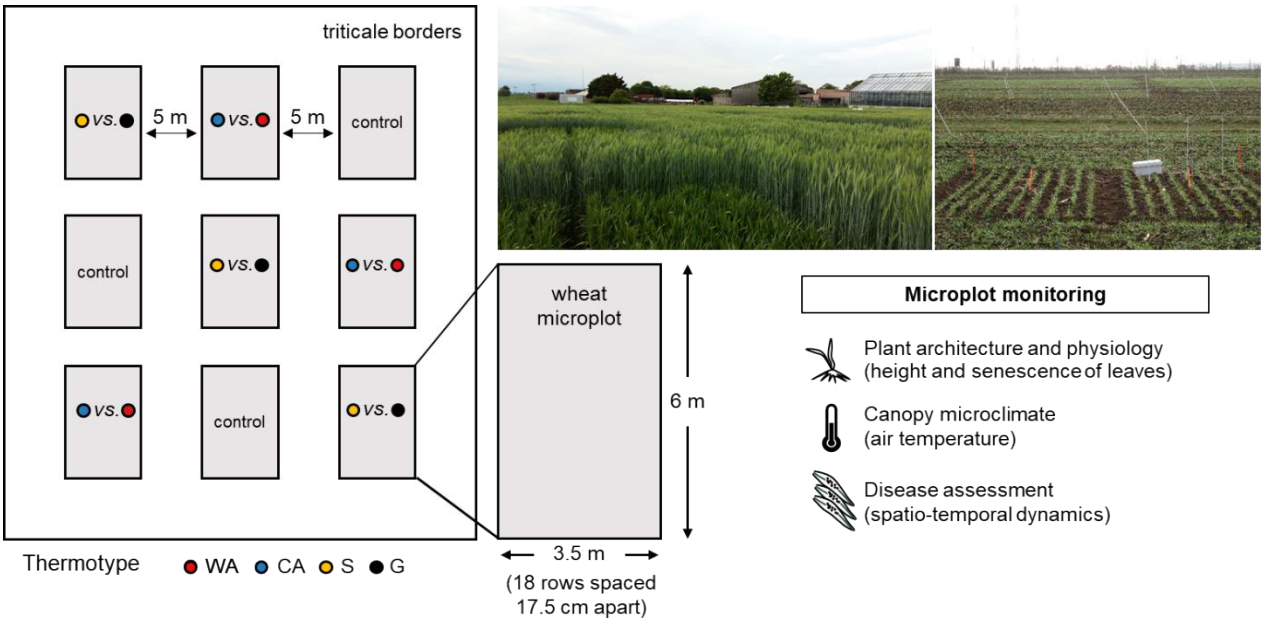
SUPPLEMENTARY METHODS



Methods S1 Overview of in vitro competition under constant thermal regimes (experiment A). (1) A cold-adapted (non-GFP-expressing; CA_1 or CA_2 or CA_3) and a warm-adapted (GFP-expressing; WA_1) strain were placed in competition, in equal proportions, in 150 µL of a liquid glucose peptone medium (14.3 $g.L^{-1}$ dextrose, 7.1 $g.L^{-1}$ bactopeptone and 1.4 $g.L^{-1}$ yeast extract) with an initial total spore concentration of 2.5×10^5 spores mL^{-1}. For each pairwise competition experiment, five replicates were cultured under five constant thermal regimes (7.0, 18.5, 21.0, 25.0 and 30.5°C) for 72 h. (2) At the end of the incubation period, 50 µL aliquots were sampled and deposited on a microscope slide for observation by fluorescence microscopy at ×10 magnification (Leica DM5500 B, Leica Microsystems). (3) Aliquots were photographed under white light, in conditions in which the spores of GFP-expressing and non-GFP-expressing strains were visible (n_{WL}: cold-adapted and warm-adapted spores), and fluorescent light, in conditions in which only the spores of GFP-expressing strains were visible (n_{FL}: warm-adapted spores). (4) All acquired images were analysed with ImageJ 1.48v software (built-in 'Subtract Background', 'Threshold' and 'Analyze Particles' functions) to count the spores of both strains and to calculate their relative proportions in the co-cultures (from Boixel et al., 2019b).



Methods S2 Overview of in planta competition under cold and warm diurnally fluctuating thermal regimes (experiment B). Polycyclic (B_1) vs. single-generation (B_2) experiments for investigation of the fitness of each strain with and without competition (observed vs. predicted), respectively. (B_1) Sextet competition between cold- (CA) and warm-adapted (WA) strains was studied simultaneously via successive serial transfers under a cold and a warm daily cyclic thermal regime with three replicated lines each. (B_2) Cross-infections were conducted to compare the fitness of inoculant strains (alongside each generation cycle of competition experiment B1, under the same thermal regimes (results summarised in Table S2). B2a: quantification of sporulation intensity (sporulating area and spore production) via separate inoculations of each (1) warm-adapted strain under the cold regime; (2) cold-adapted strain under the cold regime; (3) warm-adapted strain under the warm regime; (4) cold-adapted strain under the warm regime. Spore suspensions for each strain were used for inoculation with a paintbrush at a concentration of 1.7×10^5 spores.mL^{-1} (i.e., at 1/6 the concentration of the inoculant spore mixture to assess the actual within-host fitness response) on the median part of the first leaf (along a 75 mm-long section of the adaxial face) of 16-day-old wheat seedlings (n = 6 leaves per strain and per thermal regime). B2b: Single-spore suspensions were also sprayed until the point of run-off onto whole seedlings, to quantify the infection efficiency of each competing strain within the inoculant spore suspensions (n = 3 plants per strain and per thermal regime).



Methods S3 Overview of the microplot field trial (experiment C_1-C_2). Complete randomised block design with three replicates (wheat cv. Soissons). Cross-contamination between plots was limited by sowing borders, at least 1.75 m wide, of the tall triticale cultivar 'Vuka'. Two types of competition were investigated: (i) cold- (CA) vs. warm-adapted (WA) strains with a population used for inoculation in early winter for the tracking of changes under consecutive winter and spring field conditions; (ii) specialist (S) vs. generalist (G) strains with a population used for inoculation in early winter to track changes under consecutive winter and spring field conditions. Over the course of the wheat growing season, wheat architecture and physiology, canopy microclimate and the spatiotemporal progression of disease were regularly monitored in each microplot, for the reconstruction and interpretation of population dynamics. Control plots were not inoculated and were therefore exposed only to natural inoculum.

SUPPLEMENTARY FIGURES

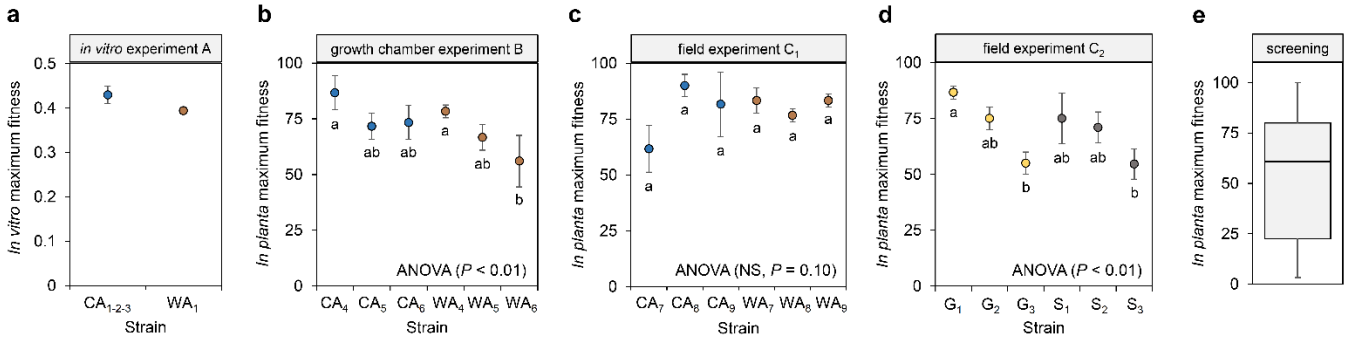


Fig. S1 Selective screening of Zymoseptoria tritici strains included in the experiments with respect to the tendency of in vitro and in planta fitness to homogeneity in terms of mean competitive ability. (a) Experiment A: in vitro maximum fitness corresponds to the maximum performance P_{max} of the thermal performance curve for growth rate (mean of 8 replicate microwell cultures and SD of the cold-adapted strains CA_1, CA_2 and CA_3; see Methods section in Boixel et al., 2019b). (b,c,d) in planta maximum fitness corresponds to the maximum sporulating area assessed on the first leaf of wheat seedlings at 21 DPI (n = 3 plants) under 20°C day/18°C night temperature regimes i.e. the median of the average thermal environment between $T_{opt, CA}$ (cold regime: 17 ± 1°C) and $T_{opt, WA}$ (warm regime: 21 ± 1°C). Strains belong to cold-adapted (CA), warm-adapted (WA), generalist (G) or specialist (S) thermotypes. (e) Competitor strains differ in their in planta performance, but this variation has been minimised, taking all other selection criteria (including the characteristics of their thermal responses) into account. To provide an idea of the variability of in planta performance (understood in the sense of maximum sporulating area) present in Z. tritici, the distribution of this variable obtained during a screening trial of 104 strains in which quantitative aggressiveness traits were assessed using the same experimental setting (i.e. on the first leaf of wheat seedlings at 21 DPI) is displayed in a boxplot.

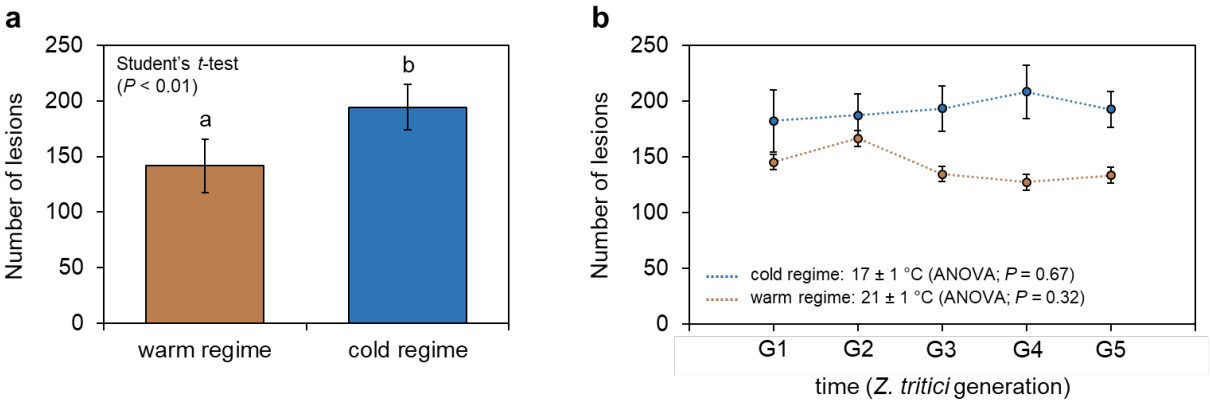


Fig. S2 Differences in the size of in planta Zymoseptoria tritici populations exposed to either a warm or a cold regime (experiment B). Population size corresponds to the number of disease lesions assessed in the wheat population averaged across the three replicate lines in each chamber (mean ± SD) for (a) the whole experiment or (b) detailed at each generation. Different letters above error bars indicate significant differences.

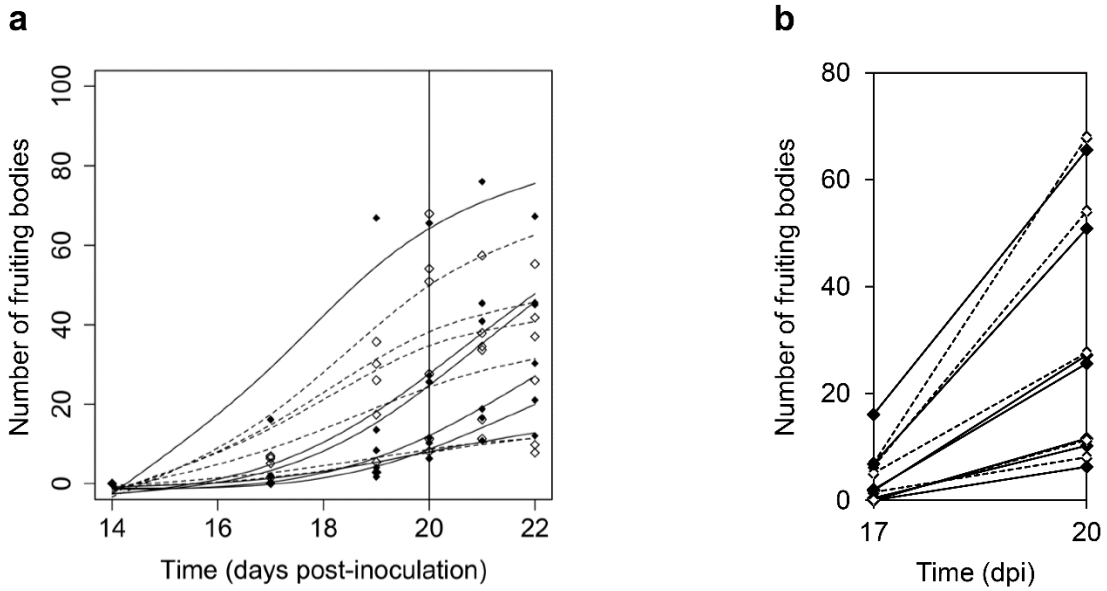


Fig. S3 Temporal sporulation dynamics of the six competing Zymoseptoria tritici strains under two diurnally fluctuating thermal regimes (experiment B; data arising from the single-generation experiments presented in Methods S2). (a) Dynamics of fruiting body production. Points correspond to the experimental data and lines to the fitted curves for the warm regime (solid lines and full diamonds; 21 ± 1°C) and the cold regime (dotted lines and empty diamonds; 17 ± 1°C). The vertical line at 20 dpi indicates the time for serial transfer chosen in the competition experiment as the earliest time point at which all strains were producing spores, to prevent the artificial extinction of a given strain due to the experimental design. (b) Focus on the fitness ranking of strains established between 17 and 20 days post-inoculation, highlighting the potentially strong impact of this set-up parameter on the outcomes of the competition experiment (widening gaps between strains). Lines link the values for a given strain between the two times under consideration for the warm regime (solid lines and full diamonds; 21 ± 1°C) and the cold regime (dotted lines and empty diamonds; 17 ± 1°C).

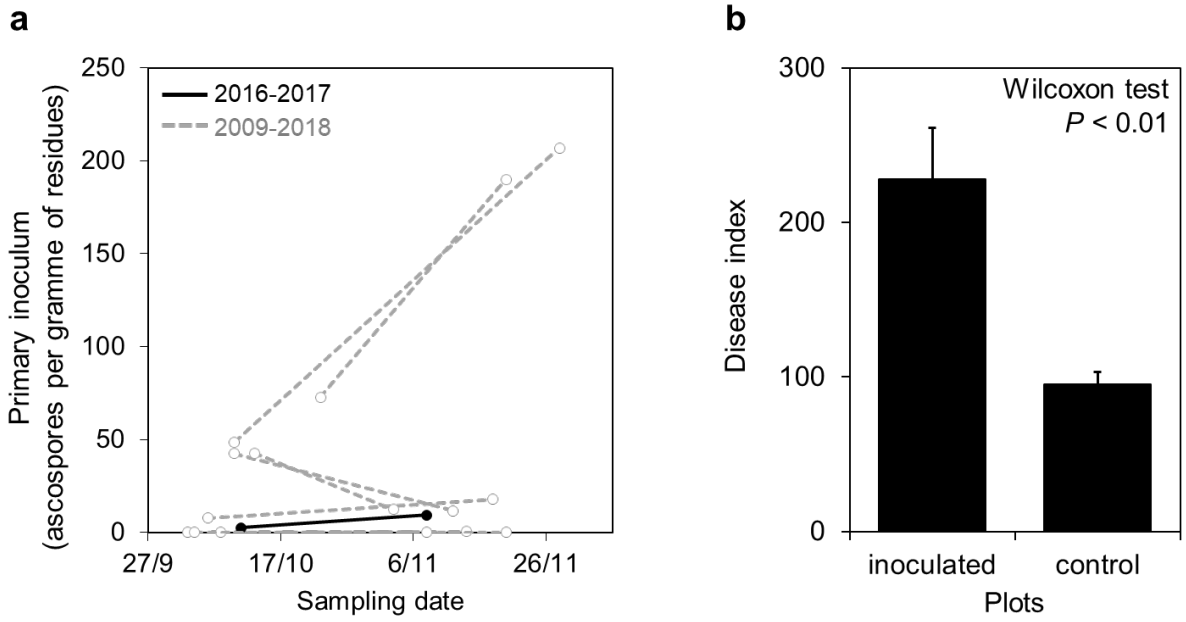


Fig. S4 Early epidemic conditions that could explain the role of immigrant Zymoseptoria tritici strains in the inoculated field microplots (experiment C_1-C_2). (a) Comparison of the amount of local primary inoculum (number of ascospores discharged per gramme of overwintered wheat residues) around the sowing period of the field trial in 2016-2017 (black solid line) and around the sowing period for nine contemporary growing seasons from 2009 to 2018 (grey dashed lines) in a sentinel plot located near the field experiment. (b) Difference in the mean ± SEM disease index, expressed as the number of lesions on 100 plants, between the inoculated (n = 6) and control (n = 3) microplots one month after inoculation. The difference between these two values should reflect differences in the numbers of lesions caused by the inoculant strains.

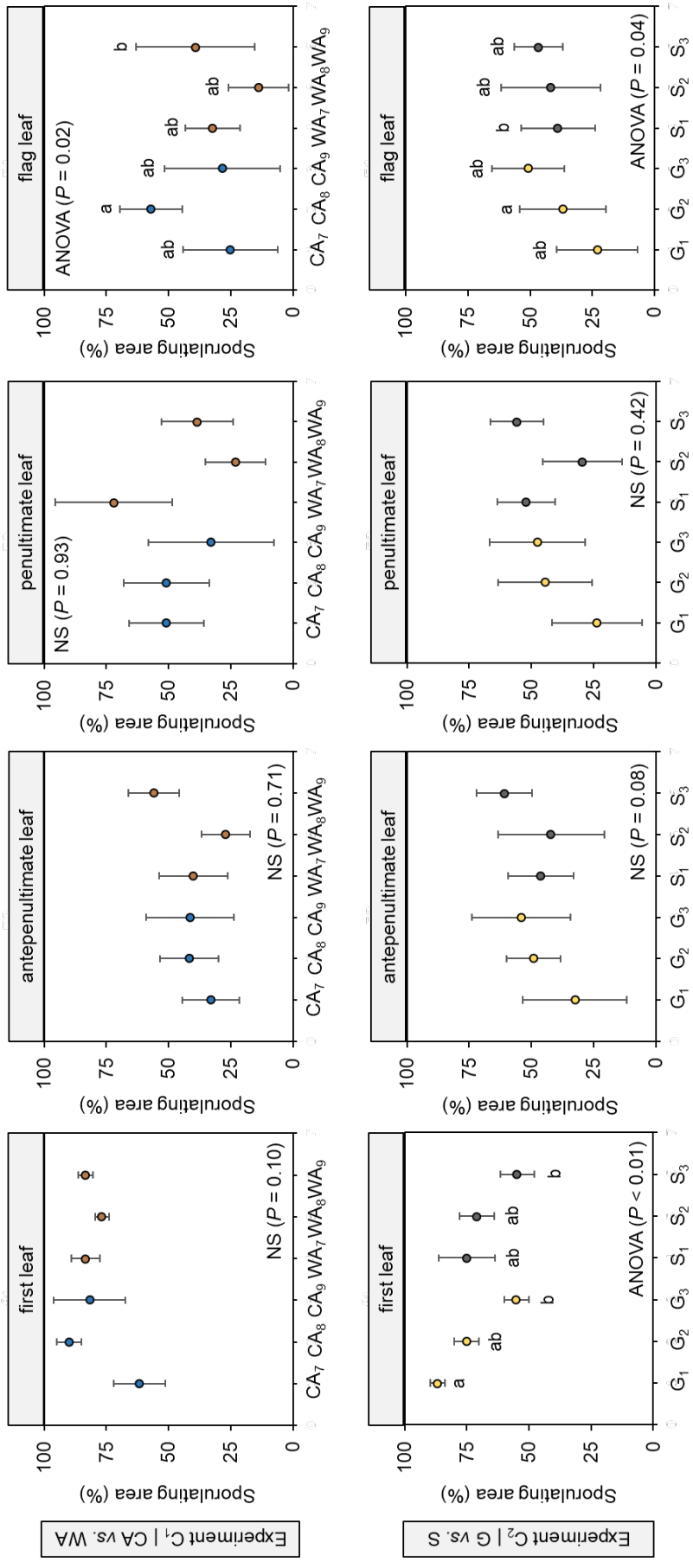


Fig. S5 Changes in Zymoseptoria tritici strain aggressiveness with wheat growth stage. Sporulating area (maximum percentage of the inoculated area covered by fruiting bodies), assessed on the first leaf of wheat seedlings and the upper three leaves of adult plants, was retained as a proxy for aggressiveness to capture changes in strain competitiveness.

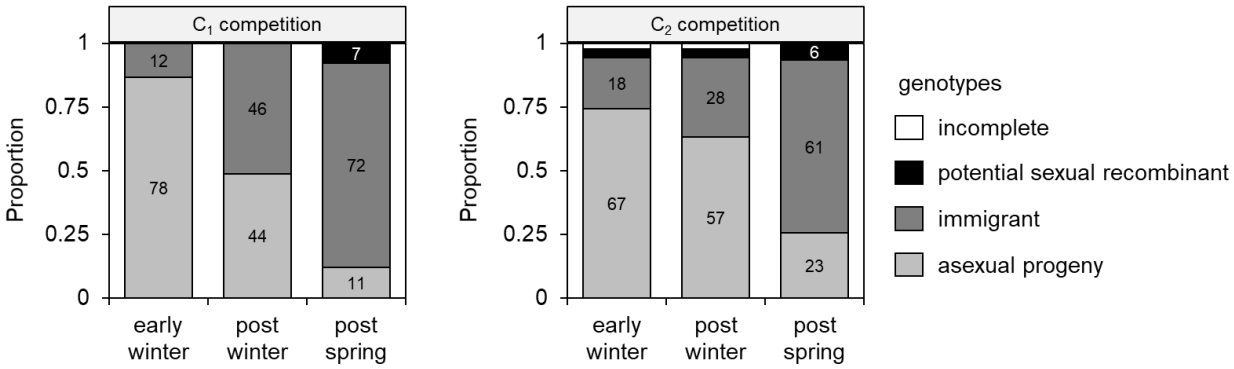


Fig. S6 Proportion of inoculant and non-inoculant genotypes recovered from the field (experiment C_1-C_2). Distinctions between 'asexual progeny' (genotypes identical to parental inoculant genotypes), 'immigrants' (genotypes carrying novel alleles), 'potential sexual recombinants' (genotypes carrying novel allele combinations that might also theoretically correspond to immigrants or mutants), 'incomplete' (uncertain genotypes due to missing allele values) were made on the basis of SSR multilocus genotype analyses of 2 × 90 strains per sampling period. Data are summarised separately for competition between warm- and cold-adapted strains (C_1) and between generalist and specialist strains (C_2).

SUPPLEMENTARY TABLES

Table S1 SSR multilocus genotypes and mating types of the cold- (CA) and warm-adapted (WA) or generalist (G) and specialist (S) strains used in the in planta two-thermotype competition experiments (B, C_1-C_2). We maximised the number of strains sharing a similar mating type to limit recombination between inoculant genotypes in the field experiment C_1-C_2. We favoured the MAT1–1 group, which has a higher reported pathogenicity than the MAT1-2 group, together with a high frequency in locally sampled populations (Zhan et al., 2007; Morais et al., 2019).

Strain	SSR multilocus genotype	Mating type
	ST4-ST3A-ST9-ST6-ST7-ST3C-ST2-ST1-ST5-ST10-ST12-ST3B*	
Experiment B: CA vs. WA		
CA_4	190-246-342-164-199-244-356-192-250-151-226-272	MAT1–1
CA_5	188-246-342-164-185-229-359-192-250-154-226-263	MAT1–1
CA_6	190-230-342-164-185-229-362-192-250-154-226-263	MAT1–2
WA_4	188-246-346-164-187-244-359-195-250-157-226-X	MAT1–2
WA_5	188-248-342-167-185-247-356-195-250-154-226-263	MAT1–1
WA_6	188-248-342-164-185-229-356-192-250-151-226-275	MAT1–2
Experiment C_1: CA vs. WA		
CA_7	188-246-342-164-199-244-359-195-250-139-226-263	MAT1–2
CA_8	188-246-342-164-185-229-359-192-250-154-226-263	MAT1–1
CA_9	190-246-342-167-185-229-359-195-250-154-226-263	MAT1–1
WA_7	188-246-346-164-187-244-359-195-250-157-226-X	MAT1–2
WA_8	188-230-342-164-185-229-356-195-250-148-226-263	MAT1–1
WA_9	188-246-346-164-199-229-356-195-250-154-226-263	MAT1–1
Experiment C_2: G vs. S		
G_1	188-234-342-164-187-229-353-195-250-157-232-272	MAT1–1
G_2	188-230-342-164-185-229-356-195-250-154-226-272	MAT1–1
G_3	192-246-342-164-185-229-353-195-250-151-226-263	MAT1–2
S_1	188-246-342-164-199-244-359-195-250-154-226-272	MAT1–1
S_2	188-248-346-164-199-229-353-195-250-151-226-275	MAT1–1
S_3	188-250-X-164-185-229-356-195-250-154-226-263	MAT1–2

* see Gautier et al. (2014) for details of the corresponding microsatellite PCR multiplexes

Table S2 In planta fitness of the six strains placed in competition under the two growth-chamber cycling thermal regimes (experiment B). Fitness is broken down here into three standard aggressiveness components: infection efficiency, sporulating area and spore production. Values averaged across the five generation cycles (mean ± SD) are given for each strain under the colder regime (17.0 ± 1°C) and the warmer regime (21.1 ± 1°C). Different letters in brackets indicate significant differences between strains (Kruskal-Wallis test followed by post-hoc pairwise comparisons, P <0.05).

Strains	Infection efficiency [1]		Sporulating area [2]		Spore production [3]	
	17.0 ± 1°C	21.1 ± 1°C	17.0 ± 1°C	21.1 ± 1°C	17.0 ± 1°C	21.1 ± 1°C
CA_4	1.82 (a)	1.32 (a)	21.4 ± 12.5 (a)	27.6 ± 18.7 (a)	33.3 ± 16.8 (a)	10.8 ± 4.5 (b)
CA_5	1.57 (ab)	1.18 (a)	11.4 ± 10.4 (bc)	14.3 ± 9.4 (b)	14.3 ± 9.9 (ab)	18.1 ± 12.0 (ab)
CA_6	1.35 (ab)	1.13 (a)	13.6 ± 5.9 (bc)	18.9 ± 12.1 (ab)	23.8 ± 19.8 (ab)	8.7 ± 9.3 (b)
WA_4	1.33 (ab)	1.32 (a)	17.5 ± 11.9 (ab)	17.9 ± 11.0 (ab)	17.9 ± 8.7 (ab)	25.8 ± 19.8 (ab)
WA_5	1.38 (ab)	1.15 (a)	10.4 ± 7.5 (c)	23.1 ± 12.6 (ab)	10.4 ± 11.0 (b)	29.5 ± 6.6 (a)
WA_6	7.58 (b)	1.38 (a)	9.1 ± 8.1 (c)	22.3 ± 12.6 (ab)	11.9 ± 8.2 (b)	36.4 ± 47.5 (ab)

[1] ratio between the number of lesions observed and the number of spores used to inoculate the leaves (x 10^{-5})

[2] expressed as the percentage of the inoculated leaf surface covered by fruiting bodies

[3] number of spores produced on a lesion over a cycle (expressed in x 10^5 spores.mL^{-1})

Table S3 Testing for equal distributions of competitor strains in the Zymoseptoria tritici populations across time and thermal regimes (in planta experiments B, C_1-C_2). For each competition experiment, a chi-squared test was conducted per Z. tritici generation or sampling period to determine whether there was any significant difference between the observed strain proportions in replicate lines and the state of perfectly equal proportions (1/6, 1/6, 1/6, 1/6, 1/6, 1/6) at each time point.

Experiment B					
Generation	G1	G2	G3	G4	G5
Cold regime	χ^2 = 8.36, P = 0.14	χ^2 = 11.84, P = 0.04	χ^2 = 6.49, P = 0.26	χ^2 = 31.27, P < 0.01	χ^2 = 30.40, P < 0.01
Warm regime	χ^2 = 5.91, P = 0.31	χ^2 = 6.23, P = 0.28	χ^2 = 12.45, P = 0.03	χ^2 = 25.25, P < 0.01	χ^2 = 33.33, P < 0.01

Experiment C_1-C_2			
Sampling period	mid-winter	post-winter	post-spring
Cold- vs. warm-adapted	χ^2 = 17.20, P < 0.01	χ^2 = 6.39, P = 0.27	χ^2 = 2.02, P = 0.85
Generalist vs. specialist	χ^2 = 6.98, P = 0.22	χ^2 = 6.37, P = 0.27	χ^2 = 1.81, P = 0.87

Table S4 Changes in the frequency of each competitor thermotype (in planta experiments B, C_1-C_2). Frequency variations are summarised on the basis of a statistical comparison of population composition in chi-squared tests: '–' no deviation from the status quo between the competitor strains (Table S3); '↗' large increase in proportion; '↗↗' larger increase in proportion; '↘' decrease in proportion; '↘↘' larger decrease in proportion. The values in brackets are Pearson's residuals for chi-squared tests on the two-way frequency table intersecting the proportions of thermotypes and generation time (those highlighted in bold account for most of the variance in population composition).

Experiment B - cold regime

Thermotype	Inoculation	G1	G2	G3	G4	G5
Cold-adapted (CA)	- (-1.80)	- (-0.22)	↗ (0.12)	- (-0.43)	↗↗ (1.06)	↗↗ (1.28)
Warm-adapted (WA)	- (3.51)	- (0.43)	↘ (-0.23)	- (0.84)	**↘↘** (-2.06)	**↘↘** (-2.50)

Experiment B - warm regime

Thermotype	Inoculation	G1	G2	G3	G4	G5
Cold-adapted (CA)	- (3.03)	- (1.26)	- (0.51)	↘ (-0.52)	**↘↘** (-1.76)	**↘↘** (-2.52)
Warm-adapted (WA)	- (-1.67)	- (-0.70)	- (-0.28)	↗ (0.29)	↗↗ (0.97)	↗↗ (1.39)

Experiment C_1

Thermotype	Inoculation	Mid-winter	Post-winter	Post-spring
Cold-adapted (CA)	- (0.93)	↘ (-0.80)	**↗↗** (2.08)	↘ (-1.44)
Warm-adapted (WA)	- (0.25)	**↗↗** (3.91)	↘ (-1.41)	↘↘ (-2.54)
Unknown*	- (-0.71)	↘↘ (-2.85)	- (0.07)	**↗↗** (2.91)

* non-inoculant strains

Experiment C_2

Thermotype	Inoculation	Mid-winter	Post-winter	Post-spring
Generalist (G)	- (0.38)	**↗** (1.62)	- (-0.06)	↘ (-1.63)
Specialist (S)	- (0.49)	- (0.37)	↗ (0.98)	↘ (-1.44)
Unknown*	- (-0.67)	↘ (-1.59)	- (-0.68)	**↗↗** (2.40)

* non-inoculant strains

Chapter 5

Fitness consequences for leaf microbial populations of temperature heterogeneity in crop canopies

Foreword. The maintenance of a high local diversity in individual thermal responses of populations sampled over contrasted climatic conditions was established in Chapter 3. The characterisation of thermal heterogeneities actually perceived by Z. tritici populations in their habitats, i.e. in wheat canopies, is necessary to investigate more closely the selective pressures exerted by local conditions. Indeed, my hypothesis was that local patterns can overwhelm those of large-scale climate leading to complex thermal mosaics of temperature rather than simple latitudinal gradients. What are the selective pressures exerted by phylloclimatic conditions? I present here a quantification of the extent of leaf temperature heterogeneity and variance that can be encountered in wheat canopies under field conditions and an assessment of how small-scale environmental heterogeneity can generate diverse thermal niches and refugia from unfavourable growth conditions for Z. tritici populations.

A manuscript derived from chapter 5 is in preparation under the heading: Thermal heterogeneity in crop canopies produces a broad fitness landscape for the foliar pathogen Zymoseptoria tritici

contents

abstract

Background and Aims

The leaf surface is a highly dynamic climatic micro-environment, due, in particular, to temperature variation over small spatiotemporal scales. Investigations of the role of leaf surface temperature in the ecology and evolution of plant-associated microbes require quantification of the thermal environment actually perceived by micro-organisms in the phyllosphere. Such quantification must be performed at a scale relevant to biological development, and has been attempted in only a few studies to date.

Key Results

By combining measurements of leaf temperature with fine and coarse spatio-temporal resolution over wheat canopies, we demonstrate here the heterogeneous nature of the leaf temperature distribution encountered in field crops. We also show how these heterogeneities might affect fungal development, using the Zymoseptoria tritici-wheat pathosystem and its growth rate during the latent period as a case study. Large differences (between -6°C and 8°C) were observed between leaf and air temperatures over a growing season. The heterogeneity in leaf temperatures was substantial locally: mean daily amplitudes of 10.2°C, mean intra-leaf heterogeneity of 4.6°C, mean between-leaf heterogeneity of 2.8°C. We evaluated the fitness consequences of this spatiotemporal heterogeneity of leaf temperature, and of the deviation of leaf temperature from air temperature, with a theoretical approach based on the thermal performance curves of Z. tritici. Taking leaf temperature into account rather than air temperature greatly affected estimates of the rate of fungal development, with differences of between 10 days less and 23 days more, depending on the excess leaf temperature.

Conclusion

Temperature heterogeneities in crop canopies might provide thermal refugia, contributing to the maintenance of microbial diversity within the phyllosphere, as different sites in the canopy provide differential competitive advantages between strains. Further investigations, with the screening of more environments and populations, would increase our understanding of the ways in which environmental conditions and phenotypic variation interact and influence the eco-evolutionary dynamics of foliar fungal pathogen populations.

Keywords: phyllosphere, leaf temperature, microbial ecology, thermal refugia, competitive ability, Zymoseptoria tritici

INTRODUCTION

The phyllosphere, the biome consisting of the aerial parts of plants (Ruinen, 1956), includes leaf surface habitats. It is one of the most important habitats for micro-organisms (Morris & Kinkel, 2002; Woodward & Lomas, 2004), and is home to a diverse array of bacteria, fungi and archaea (Lindow & Brandl, 2003; Vorholt, 2012; Peñuelas & Terradas, 2014). Some of these leaf-associated micro-organisms greatly affect plant health. This is the case for foliar plant pathogens on crops (e.g. Beattie & Lindow, 1995; Vorholt, 2012), which can cause epidemics on host plant leaves if the environmental conditions are suitable in terms of temperature, light, and humidity (Colhoun, 1973; e.g. Shaw, 1986, 1990, 1991). These meteorological factors often differ between the leaf surface and the atmosphere (Hirano & Upper, 2000; Whipps et al., 2008). The microclimate experienced by the leaf microbiota, which Chelle (2005) called the "phylloclimate", is continually changing, sometimes drastically, with effects of various magnitudes on the physiological performance of organisms. For example, leaf tissue temperature across apple tree canopies may be up to 12°C warmer than the air, with major consequences for the development of phytophagous insects (Pincebourde & Woods, 2012). The phylloclimate encompasses the microclimatic spatiotemporal heterogeneity inherent to plant organs, including leaf surfaces within a canopy in particular, at the scale at which these organisms live (Pincebourde & Woods, 2012; Potter et al., 2013).

Temperature is a crucial driver of many biological processes (Angilletta, 2009), including plant growth (Monteith, 1977), pathogen development (Lovell et al., 2004; Bernard et al., 2013), and the expression of host plant resistance to pathogens (Zhu et al., 2010). In particular, temperature is often a key input into epidemiological models and decision support tools simulating the development of plant disease epidemics and defining the most appropriate time windows for control measures (De Wolf & Isard, 2007). Weather-monitoring equipment is often cumbersome to install and maintain. Temperature values are therefore often retrieved from data routinely collected by meteorological stations, with measurements at a standard position (typically at 1.5 m above the ground, with low levels of vegetation cover, and with a time resolution of one hour; Gent & Schwartz, 2003). However, thermal conditions within plant canopies can differ considerably from the temperature measured by a meteorological station, and this may introduce biases into models of disease outcomes (Pfender et al., 2012). In particular, there is a difference between ambient air and leaf temperatures, the magnitude of which depends on climatic conditions (local energy balance equation between incoming and outgoing heat fluxes; Gates, 1968; Ehrler et al., 1978; Haseba & Iso, 1980) and leaf microtopography (Saudreau et al., 2017).

Phyllosphere micro-organisms display considerable inter- and intraspecific diversity (Redford et al., 2010), particularly as concerns their responses to temperature. For instance, the response to temperature of a given fitness trait may differ between individuals (e.g. Milus & Line, 1980; Milus et al., 2006) even within a single population, due to the coexistence of thermotypes (i.e. groups of individuals with similar thermal responses; Boixel et al., 2019a). This may lead to population- and community-level shifts, as selective pressures give rise to phenotypic selection, with certain phenotypes producing more offspring than others under the same conditions (Kingsolver & Pfennig, 2007). The large differences in temperature within plant

canopies (Boulard & Wang, 2002) occur over various spatiotemporal scales: over a single leaf (temperature colder at the edges; Stokes et al., 2006), but also between leaves within the same plant or same leaf layer (Chelle, 2005). We can consider these different zones of the canopy as "niches" (microhabitats offering specific climatic conditions; Orians & Jones, 2001; Scherrer & Körner, 2009) potentially contributing to the maintenance of leaf microbiota diversity. Characterisations of the thermal environment at a coarse scale (i.e. glossing over spatiotemporal variations occurring at the leaf and canopy levels) may therefore mask the heterogeneity governing the physiology, dynamics and distribution of phyllosphere micro-organisms and driving their thermal adaptation (Angilletta, 2009).

We focused on wheat canopies, due to its agronomic importance of this crop worldwide. We used a three-step procedure to test the hypothesis that environmental heterogeneity in these canopies generates contrasting thermal niches, and even thermal refugia from unfavourable growth conditions, potentially helping to maintain phyllosphere microbial diversity. In particular, leaves are warmer than the surrounding air, which may prove challenging for the development of cold-adapted individuals. We hypothesise that the temperature differences encountered within crop canopies may result in patches of colder temperature in which cold-adapted individuals can develop. We first recorded temperatures in growing canopies for (i) individual leaves on a given plant, (ii) leaves on different individual plants, and (iii) a whole-canopy subplot (thermal mapping). This made it possible to cover temporal variation under different conditions, including transient phenomena (e.g. sunflecks; Pearcy et al., 1990; Chelle, 2005). We then characterised the thermal environments experienced by the phyllosphere organisms (e.g. a given infection cycle in the case of plant pathogens). We also investigated the extent to which this spatiotemporal variation of phylloclimatic conditions was likely to affect the fitness landscape of phyllosphere micro-organisms, by considering a common fungal pathogen of wheat (Zymoseptoria tritici, the causal agent of Septoria leaf blotch) as a case study for evaluation of the ecological consequences of exposure to variations of leaf temperature. We calculated temperature-dependent differences in strain performance (Huey & Stevenson, 1979), based on the thermal sensitivity of strains, as determined from their thermal performance curves.

METHODS

Quantification of temperature heterogeneities in wheat canopies

We measured phylloclimatic (leaf surface) temperatures within wheat canopies to quantify the temperatures experienced by phyllosphere micro-organisms under field conditions, and their variation over time (from one hour to a whole growing season) and space (local, canopy, and leaf levels; Table 1). We simultaneously recorded mesoclimatic data from a meteorological station positioned 500 m from the field plot (INRAE Grignon experimental station, Yvelines, France; 48° 51' N, 1°58'E). This meteorological station measured air temperature at a height of 2 m above a grass canopy (model Enerco 516i, CIMEL Electronique, Paris, France; data available from the CLIMATIK platform: https://www6.paca.inrae.fr/agroclim/Les-outils). Phylloclimatic data were acquired in two complementary experiments during the 2011-2012 and 2014-2015 growing seasons, for the assessment of both inter-leaf (Experiment 1; Fig. 1) and within-leaf variation (Experiment 2; Fig. 2).

Table 1 Quantifying temperature variations in a wheat canopy at three complementary levels.

Measurement	Recording instrument	Temperature	Spatial resolution	Temporal resolution	Assets	Limitations
Local level (exp. 1-2)	Meteorological station[1]	Air temperature	Coarse (one regional value)	Hourly or daily	Routinely collected and readily available	Does not account for underlying heterogeneity[5]
Plot level (exp. 2)	IR camera[2]	Inter- and intra-leaf temperature	Two-dimensional (continuous thermal maps for each aerial plant organ of a given canopy)	Discrete[3]	Highly spatially precise	Cumbersome for more than a few hours
Leaf level (exp. 1)	Thermocouple[3]	Inter-leaf temperature	One-dimensional (discrete horizontal and vertical profile)	Continuous [4]	Precise real measurement	Captures only a very small portion of the spatial variability

[1] Ex situ: out-of-canopy and off-site location at a standard meteorological placement (nearest weather station measuring the mesoclimate occurring around a canopy)
[2] In situ: in-canopy and on-site location (field instrumentation to measure phylloclimatic conditions within the plot)
[3] In situ: thermal mapping recordings at every time point (here, one late sunny afternoon at flowering; BBCH 61)
[4] Continuous measurements and average recordings (here, from seedling to inflorescence emergence; BBCH 12-61)
[5] One value acquired at the meteorological station, masking inter- and intra-leaf temperature heterogeneity (Fig. 3)

Leaf temperature heterogeneity and variance during canopy growth (Exp. 1)

This experiment focused on the temporal dynamics of leaf temperatures during the growth of wheat canopies, as assessed with thermal sensors. Leaf temperature was measured in one field plot of cv. Tremie sown on October 26th 2011 at a density of 180 seeds.m^{-2} and a total of 65 kg.ha^{-1} nitrogen fertilizer applied in two lots, at tillering and booting. Leaf temperatures were recorded from the seedling stage until the start of flowering, on the three youngest fully expanded leaves on the main stems of nine wheat plants. These leaves display the greatest variation of disease levels, due to the vertical progression of Septoria leaf blotch (senescent leaves do not allow subsequent new Z. tritici infections). Thin copper–constantan thermocouples (T-type; diameter 0.2 mm), used as temperature sensors, were attached to the abaxial surface of the leaf with porous hypoallergenic adhesive tape. The thermocouples were calibrated before and after the field experiment, to take measurement errors and drifts into account, to achieve thermocouple calibration uncertainties of less than 1°C (thermal sensitivity < 0.1°C). The thermocouples were connected to a datalogger (Campbell Scientific CR10X, Logan, Utha, USA) to record leaf temperature every 20 seconds and average it at 15-minute intervals. Sensors were moved following the emergence of new leaves, to ensure that the temperature measured at each time point was that of the three youngest fully expanded leaves

on each main stem (Fig. 1). We checked that there was adequate contact between each leaf and each thermocouple, three times weekly.

Thermal heterogeneity was analysed on a three-frame temporal window: (i) daily, for which we performed principal component analysis (PCA) and hierarchical clustering (HCPC) on a 24-h leaf temperature time series (Gaudio et al., 2017) to establish a typology of daily leaf temperature patterns (Fig. S3); (ii) over three theoretical infection cycles on three leaf layers at different growth stages (Fig. S2); (iii) a complete leaf layer temperature time series (Fig. 5a).

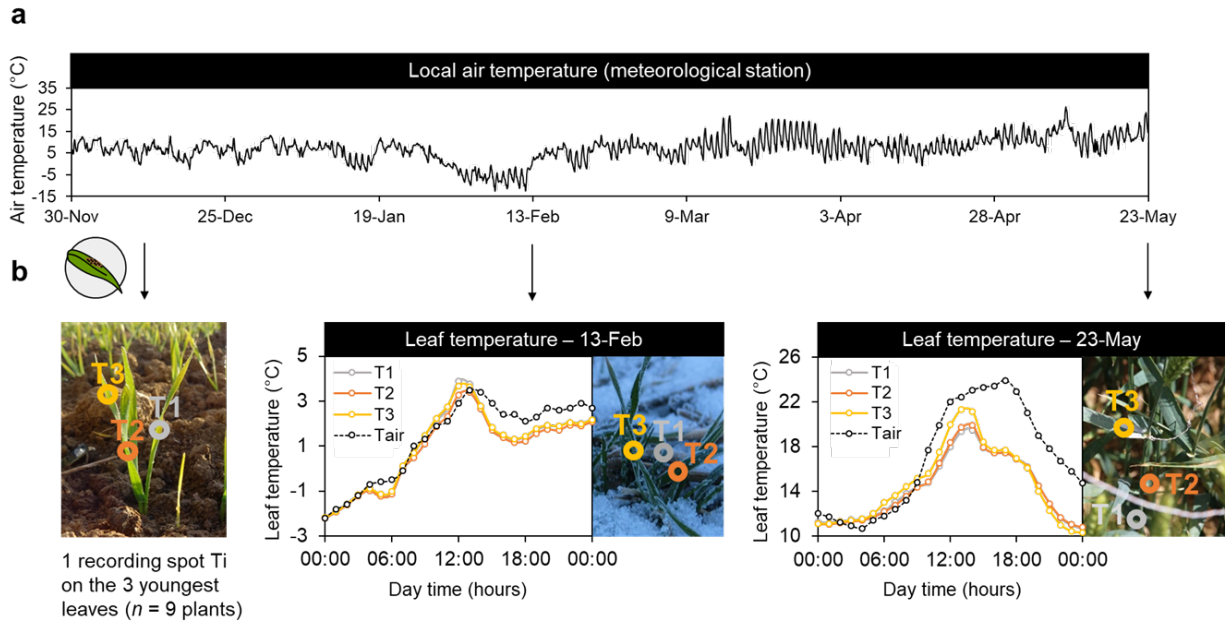


Fig. 1 Temporal exploration of leaf temperature heterogeneity during the growth of the wheat canopy (experiment 1). The thermal environment within and around a wheat canopy was quantified from November 30th 2011 to May 23th 2012, by recording (a) mesoclimatic air temperature at the standard height of 2 m at the nearby INRAE Grignon meteorological station; (b) leaf temperature of the three youngest leaves, measured locally with three thermal sensors (thin T-type thermocouples) T1, T2 and T3 positioned under the antepenultimate, penultimate, and final youngest fully expanded leaves, respectively (L3-L4-L5 on February 13th and L10-11-12 on May 23th).

Leaf temperature heterogeneity at a fine spatial scale in a fully developed canopy (Exp. 2)

This second experiment provided a detailed thermographic analysis of temperature heterogeneity at the leaf surface at a very fine spatial scale. Leaf temperature heterogeneity was assessed at the subplot scale, once the canopy had fully developed (i.e. after flowering stage had been reached, on a sunny afternoon, on June 4th 2015 from 4:02 to 4:12 pm, the time window used for the case study), on a single field plot of cv. Soissons sown on October 24th 2014 at a density of 180 seeds.m^{-2} and a total of 65 kg.ha^{-1} nitrogen fertilizer applied in two lots, at tillering and booting. A marked wheat canopy subplot was photographed every 30 s for 10 min with a FLIR E60 thermal infrared imaging camera with a spatial resolution of 320 × 240 pixels (thermal sensitivity < 0.05°C; FLIR Systems Inc., Wilsonville, USA). The camera was supported by a tripod at a 45° angle, 1.80 m above the ground, such that it spanned four wheat rows. Over the same period, we also recorded used thermocouples to record leaf

temperature on the uppermost three leaves (antepenultimate L10, penultimate L11, and ultimate flag L12 leaves) of three plants, to calibrate the infrared (IR) imaging camera (Fig. 2).

The digital and infrared images obtained were analysed by separating out vegetation (wheat plant material) and non-vegetation (soil surface, thermocouple instrumentation) fraction on the basis of thresholds of hue, saturation and brightness defined with ImageJ software (Abràmoff et al., 2004). The pixels corresponding to the plants (about 87.5% green leaf area in each image) were selected on each thermal image and retained for further extraction and analysis of the relevant distribution of canopy temperatures (Fig. S9). For the detection of influential and high-leverage data points, we calculated Cook's distances using the standard threshold (observations with a Cook's distance greater than four times the mean were classified as potential statistical outliers; Cook, 1977). Outlier pixels with extreme temperature values (0.4% of all initial pixels) were related to edge effects in the separation of the image elements or to the copper targets positioned in the canopy to obtain spatial references between photographs taken at different times; they were therefore excluded from the analysis (Fig. S10). Statistical analyses of thermal heterogeneity were conducted on the instantaneous temperature for each vegetation pixel and its variation over the 10-minute snapshot sequence.

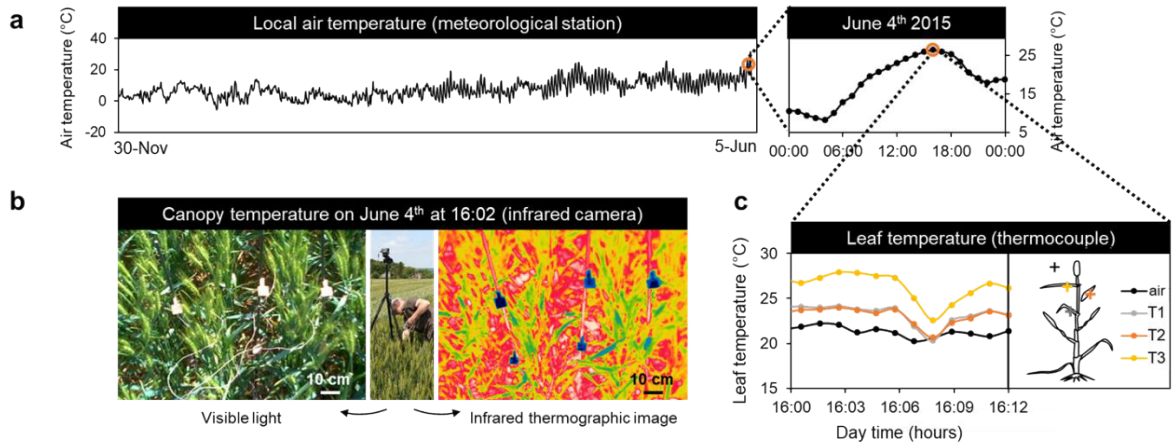


Fig. 2 Spatial exploration of leaf temperature heterogeneity in a fully developed wheat canopy at a fine spatial scale (experiment 2). (a) Temporal series of mesoclimatic air temperatures on a sunny afternoon at the end of the 2014-2015 growing season, measured at the standard height of 2 m and retrieved from the nearby INRAE Grignon meteorological station. (b,c) Leaf temperatures measured on a square microplot within a wheat field, on June 4th 2015 from 4:00 to 4:12 pm. Two resolutions were used: (b) a fine spatial resolution, with an infrared thermographic camera that took both digital (left) and infrared (right) images of the wheat canopy (here the photographs were taken at 4:02 pm); (c) a coarse spatial resolution comparing leaf temperature at one spot of each of the three youngest mainstem leaves of three plants. Thermal sensors (thin T-type thermocouples) T1, T2 and T3 were positioned on the antepenultimate (L10), penultimate (L11), and final flag (L12) leaves, respectively.

Characterisation of the thermal responses of a fungal wheat pathogen

We considered a natural Z. tritici population composed of nine existing strains, sampled in February 2013 at Bretenière, France. The in planta development rates of the nine strains had previously been characterised under five thermal regimes (Boixel et al., 2019b) to establish their

thermal performance curves (TPC; here describing the temperature dependency of the latent period).

Performance was evaluated on the basis of the rate of development, which was assessed by calculating the 'latent period' of each strain. The latent period is the time between host infection and the onset of pathogen sporulation, corresponding to the time required for the completion of one generation of the pathogen. We focused particularly on three of the nine Z. tritici strains representative of three thermotypes (Boixel et al., 2019a): a cold-adapted strain (CA; strain S1), a strain adapted to mild conditions (MA; strain S4), and a strain adapted to warm conditions (WA; strain S8) (see Fig. S1).

Exploration of the consequences of using air temperature as a proxy for leaf temperatures when estimating pathogen performance

We assessed the consequences of considering air temperature rather than leaf temperature to infer the performance of these nine Z. tritici strains from their TPC equation. We considered the observed hourly discrepancies between the air temperature recorded by the weather station and the leaf thermocouple measurements from the datasets for experiments 1 and 2 (Fig. 3). These discrepancies were transposed to about the thermal optimum for performance (T_{opt}) of the nine strains (i.e. matching with the T_{opt} of each strain was performed for T_{air} with a T_{leaf} distribution $\in [T_{opt}-6, T_{opt}+8]$; Fig. 3-4). We aimed to assess the error on inferences of the performance of phyllosphere micro-organisms based on air temperature rather than leaf temperature. By focusing the analysis to about the T_{opt}, we chose to quantify the minimum expected bias for this transposition. Indeed, temperature plasticity is lowest in the mid-temperature range (T_{opt}- and its surrounding supra- and suboptimal estimates), as shown by the derivative of the reaction norm quantifying thermal plasticity (Fig. S11). The observed discrepancies in performance, calculated with the TPC equations (application of Eq. A to the T_{leaf} distribution $\in [T_{opt}-6, T_{opt}+8]$ for each strain) therefore correspond to the minimum expected errors. A two-way ANOVA was performed for simultaneous evaluation of the effects of the intrinsic thermal response (TPC of the strains) and of the difference between air and leaf temperatures on performance values (Fig. 4).

$$P = LAT^{-1}(T_{leaf}) = P_{max} + Curv(T_{leaf} - T_{opt})^2 \quad \text{(Eq. A)}$$

Selective consequences of spatiotemporal heterogeneity in leaf temperatures for the leaf pathogen

We calculated the responses of the wheat pathogen population to the leaf temperature heterogeneity and variance recorded in the field in experiments 1 and 2, based on the TPC of the strains. In experiment 2, we took a snapshot of canopy temperature and therefore ended up with a single temperature value per canopy pixel. Performance was computed by directly applying the non-linear TPC equation for each strain to the single temperature value for each recorded spatial location in the wheat canopy (analyses conducted on the instantaneous performance values computed for each time step and each pixel with Eq. A). We used a different approach in experiment 1, taking temperature time series into account for each monitored spot. We therefore had to take leaf temperature variance into account, as

performance is altered differently under instantaneous or constant and variable conditions (Scherm & Van Bruggen, 1994).

Several different methods exist for taking into account and estimating the effects of temperature variance on the mean performance of an individual (here, a strain). We chose to estimate the integrated performance at each monitored spot of a leaf layer of the canopy after calculating the mean $\overline{T_{leaf}}$ and variance σ_{Tleaf} of leaf temperature at this spot over the time window studied (Eq. B, corresponding to the application of scale transition theory - Chesson, 2012 - to ecophysiology - Dowd et al., 2015; Koussoroplis et al., 2017). As we did not have access to the actual performance data for the nine strains under these recorded natural patterns in the field, we assessed the impact of three calculation methods (mean, integrated, summed performance, differing in their level of completeness in the consideration of thermal variation) and three temporal windows (hourly, daily, over an infection cycle) on the inference of differences in competition between strains and thermotypes (Fig. S7).

$P = LAT^{-1}(T_{leaf}) = P_{max} + Curv(T_{leaf} - T_{opt})^2$ (Eq. A applied in experiment 2)

$P = LAT^{-1}(\overline{T_{leaf}}) + \frac{1}{2}LAT^{-1}''(\overline{T_{leaf}})\sigma^2_{Tleaf}$ (Eq. B applied in experiment 1)

RESULTS

Air temperature is a poor proxy for the temperature experienced by the leaf microbiota – The difference between air and leaf temperatures (excess leaf temperature) can reach several degrees, even over time frames as short as an hour. Over six months of monitoring, from seedling to flowering (Experiment 1), the temperature of the uppermost three leaves deviated from air temperature by a mean of 0.6°C (± 1.7°C (SD)), the recorded differences lying within the range -4.6°C to 6.0°C (Fig. 3). This was also the case in the time window of less than one hour investigated in experiment 2, during which the thermal map of the canopy obtained with the infrared camera recorded an excess leaf temperature of -0.8°C to 8.3°C, over the same period (Fig. 3). The shift in the distribution of leaf temperatures to the right in experiment 2 (smaller negative deviation) is partly due to the difference in the timing and temporal grain of the measurements. Indeed, leaf temperature is higher than air temperature during the day (but lower than air temperature overnight; Fig. S2b). Furthermore, the two distributions are realigned if the leaf temperatures from experiment 1 are subsampled taking into account only hourly time windows corresponding to sunny afternoons. The higher mean deviation from air temperature in experiment 2 (2.4°C ± 0.8°C (SD)) can be explained by the incorporation into the thermal maps of all leaves, including senescent leaves, which are not epidemiologically "active" in the case of Z. tritici infection (no strain of this pathogen can develop on senescent leaves), rather than just the three youngest leaves as in experiment 1. A deviation of more than 1°C was reported for 52.8% and 97.4% of the temperature measurements in Exp. 1 and Exp. 2, respectively (Fig. 3).

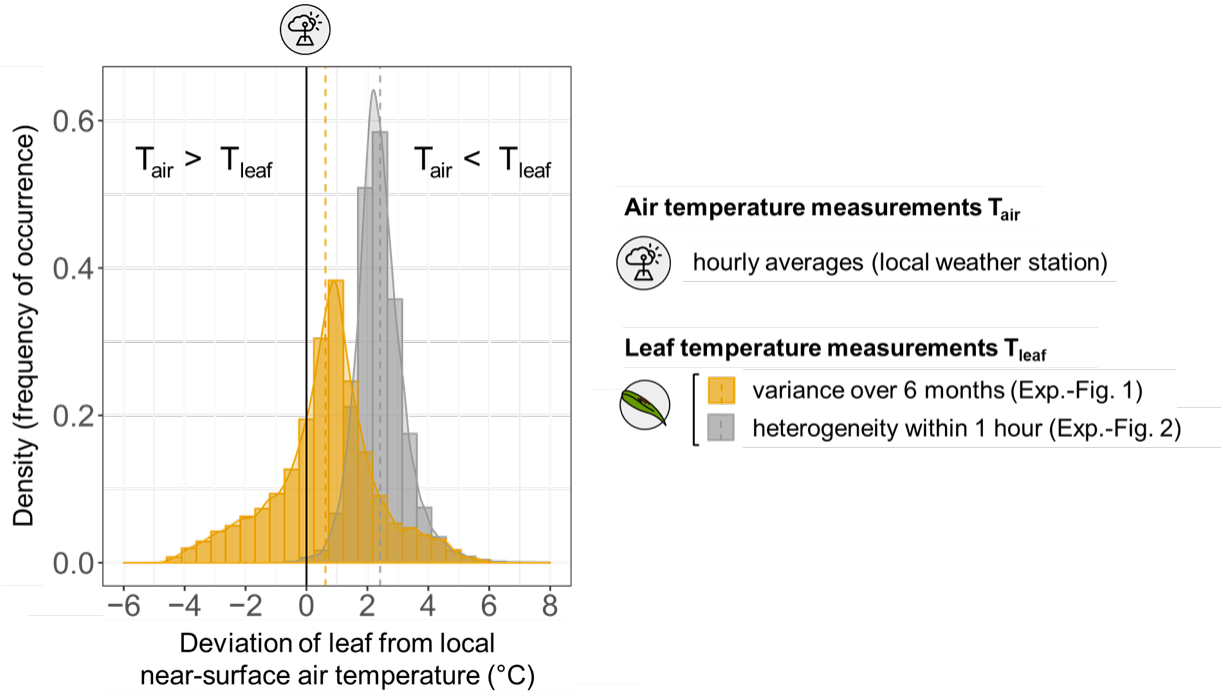


Fig. 3 Mesoclimatic air temperature conceals spatiotemporal variability in leaf temperatures, as shown by the absolute hourly temperature difference between two grains of thermal recording (experiments 1-2). The plot highlights the frequency distribution (density curves and histograms) of differences between the hourly averages of air temperature measured by a local weather station and the leaf temperatures recorded in experiments 1 (hourly leaf temperature variance of the three upper leaves over 6 months in orange; Fig. 1) and 2 (intra-hourly leaf temperature heterogeneity over a wheat canopy in grey; Fig. 2). These two distributions of Tleaf highlight the existence of deviations from Tair at these two different grains of temporal resolution. The black solid vertical line indicates no deviation from local air temperature. Dotted lines indicate the mean deviation from local air temperature for each experiment.

Wheat leaf temperature varies considerably over both time and space – Daily leaf temperature patterns over the wheat growing season (24-h leaf temperature profiles from Experiment 1; Fig. S3) can be grouped into three clusters (Hopkins' statistic, Fig. S4a): (i) days with low wind and moderate radiation levels, (ii) high wind and moderate radiation levels and (iii) high wind and high radiation levels (Fig. S4b), with mean daily thermal amplitudes of 10.2°C (± 5.3°C (SD)). In addition to radiation and wind levels, canopy structure, in terms of plant stage and leaf layers, was also found to structure the variation in daily leaf temperature patterns (Fig. S5). Indeed, the mean and range of leaf temperatures differed between leaf layers. The corresponding monitoring time window for each leaf layer highlights the difficulty dissociating spatial and temporal variability in such a temporally defined patch system. Very steep thermal gradients within a single plant were recorded, and both within- and between-leaf heterogeneities contributed to these gradients. Within-leaf variation ranged from 15 to 30°C, depending on leaf level; with a mean deviation of 4.6°C (± 0.9 (SD)). Significant differences in mean between-leaf heterogeneity were observed between leaf levels, with a mean difference of 2.8°C and a maximum difference of 6.4°C (ANOVA, F-value = 284.1, $P < 0.01$; see the letters indicating post-hoc Tukey HSD outputs in Fig. 5a); the thermal variance also differed by up to 2.9°C (Bartlett test of homogeneity of variances, $\chi^2 = 843.4$, $P < 0.01$; Fig. 5a).

Leaf temperature heterogeneity influences estimates of leaf pathogen performance - We considered the deviations of leaf temperature from air temperature measured in experiments 1 and 2, around the thermal optimum for performance, T_{opt}, of each strain (particularly for the contrasting strains S1, S4, S8) to highlight the consequences of this heterogeneity of leaf temperature for the leaf pathogen,. We took the temperature range [T_{opt} – 6°C, T_{opt} + 8°C] as a reference for estimating performance (latent period) because this is the temperature range over which plasticity is lowest (minimum impact assessment). Variations within this range lead to significant differences in performance, with an effect size proportional to the thermal plasticity of strains (two-way ANOVA, F-value = 6.59, P < 0.01). The estimation of performance with leaf temperature rather than mesoclimatic air temperature resulted in large differences in the latent period relative to that calculated with air temperature, of up to 23 dpi (+70%) for strain S1 and 10 dpi (+45% and +42%) for strains S4 and S8 (Fig. 4).

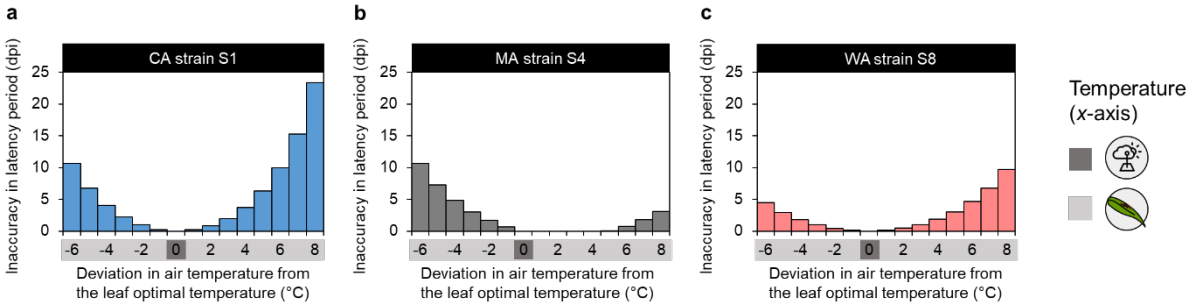


Fig. 4 Evaluating the effects of using air rather than leaf temperature to estimate the performance of leaf pathogen strains. We applied the distribution of range in the deviations of leaf (T_{leaf}) from air (T_{air}) temperatures (quantified in Fig. 3) to the thermal optimum for performance (T_{opt}) of each strain from the Z. tritici case study population. Matching with the T_{opt} of each strain was performed for T_{air} (T_{leaf} distribution ∈ [T_{opt}-6, T_{opt}+8]). Data are provided here for the (a) cold-adapted (CA) strain S1, (b) strain adapted to mild conditions (MA) S4, (c) strain adapted to warm conditions (WA) S8, corresponding to the three thermotypes found in the population. We computed the performance of each strain over the T_{leaf} distribution around T_{opt} and the extent of inaccuracy (error: $\Delta = LAT_{Tleaf}$-LAT_{Topt}) in the latent period in days post inoculation (dpi): (a) $\Delta \in$ [0, 23.3 dpi]; (b) $\Delta \in$ [0, 10.6 dpi]; (c) $\Delta \in$ [0, 9.7 dpi].

Differences in competitive advantage between thermotypes in a local Z. tritici population – The heterogeneity of leaf temperature patterns led to differences in competitive advantage between CA, MA and WA strains in the Z. tritici population (estimation of integrated performance over three contrasting temperature time series simulating three infection cycles). We detected significant differences in performance between thermotypes (ANOVA, F-value = 7.62, P < 0.01) and between the three infection cycles (Fig. 5b and S5; ANOVA, F-value = 87.34, P < 0.01), with the effect of temperature time series depending on the thermotype considered (temperature x thermotype interaction term, ANOVA, F-value = 2.84, P = 0.05). We detected temporal changes in the ranking of strains on the basis of competitiveness, with differences between infection cycles (Fig. 5b). For instance, only one strain (the cold-adapted strain, S1) was able to develop over infection cycle 1, whereas eight and nine strains developed over infection cycles 2 and 3, respectively. Competitiveness ranks differed between cycles (e.g. performance values: S7 < S3 < S8 < S2 < S9 < S6 < S5 < S1 in cycle 2, with differences of up

to 208 dpi, versus S4 < S6 < S3 < S8 < S5 < S9 < S7 < S1 < S2 in cycle 3, with differences of up to 12 dpi).

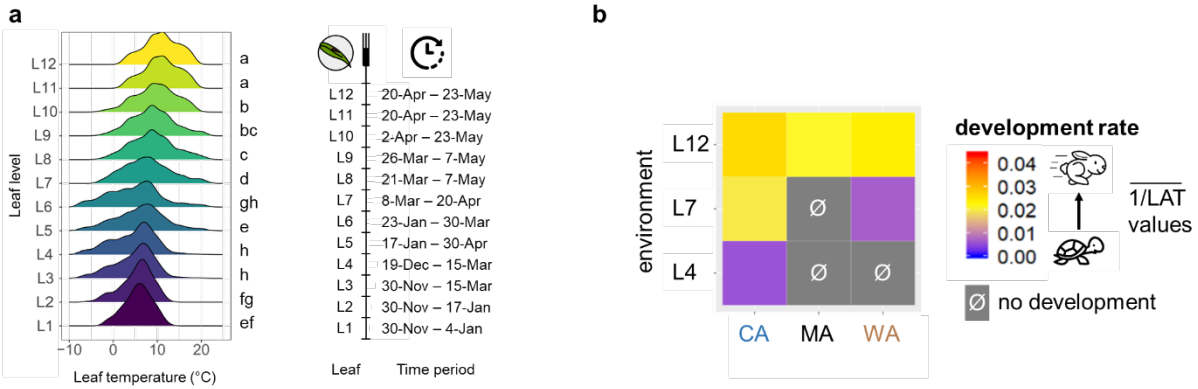


Fig. 5 Spatiotemporal differences in leaf temperature and competitive advantage between three strains forming a natural Z. tritici population over the wheat growing season (phylloclimatic conditions recorded in experiment 1). (a) Representative leaf temperature distributions were filtered for each leaf layer, to obtain the mean and range of thermal values recorded over its lifetime. (b) Performance of three Z. tritici thermotypes (strains adapted to cold, mild and warm conditions, in the CA, MA and WA columns, respectively) making up the population, in response to the temperature time series over the three 250 degree-day infection cycles on leaf layers L4, L7 and L12, in rows (from the coldest L4 to the warmest L12 environment; Fig. S5). Competitive advantage (coloured squares; see scale of development rate on the right) is expressed as the inverse of latent period 1/LAT, i.e. the inverse of the time interval between successive generations of pathogens, calculated from the TPC of each strain (values ranging from 0 for no development to higher values for fast development of the corresponding strain).

Thermally heterogeneous wheat canopies provide thermal refugia for cold-adapted strains of the leaf pathogen – The local heterogeneity of leaf temperatures allowed all strains to develop. Strains S1, S4 and S8 developed in 15.0%, 81.1%, and 99.9% of vegetation pixels, respectively (Fig. 6). Nevertheless, the mean performance of the various strains differed over the canopy subplot (Kruskal-Wallis test, $P < 0.01$) and performance distribution varied over the landscape (width of the density plots). The performance distribution was largest for strains S2, S3, S4, and S7 (which have a higher TPC curve coefficient i.e. a lower thermal breadth; Fig. S1). Thus, the fitness values of the nine strains differed between locations within the canopy in such a way that this heterogeneity and the differences in thermal adaptation generated very different fitness landscapes for the various strains (Fig. 6). For the population as a whole, we were able to identify favourable (in which all or most strains developed – here a total of 7 to 9 strains in 91.8% of the vegetation area) and risky (in which only a few strains developed – here a total of 4 to 6 strains in 8.2% of the vegetation area) spots within the canopy. There may be strong competition between spores, with a variation of thermal adaptation at a given site. For example, a difference of more than 50 dpi in the duration of the latent period was observed between the CA strain S1 and the WA strain S8 at all sites within the canopy tested in experiment 2 (Fig. 7).

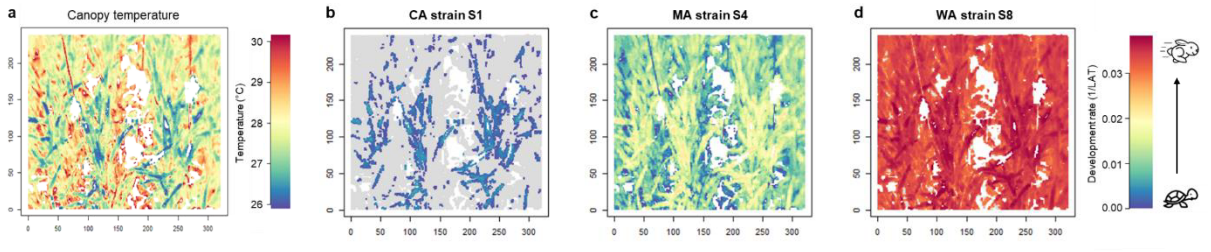


Fig. 6 Wheat canopies provide thermal refugia that could help to maintain diversity within microbial populations (phylloclimatic conditions recorded in experiment 2). (a) Temperature maps of leaf surfaces extracted from infrared images illustrating small-scale spatial heterogeneity. Instantaneous spatial performance at each location in the canopy for (b) the cold-adapted strain (CA) S1; (c) the strain adapted to mild conditions (MA) S4; (d) the strain adapted to warm conditions (WA) S8, in this thermally variable landscape. White areas correspond to non-plant pixels (e.g. soil, wires). Grey areas correspond to spots where strains were unable to develop over the duration of the infection cycle. Performance is expressed as the inverse of latent period 1/LAT with a colour scale distinguishing between slow (blue - turtle) and high (red - rabbit) rates of development.

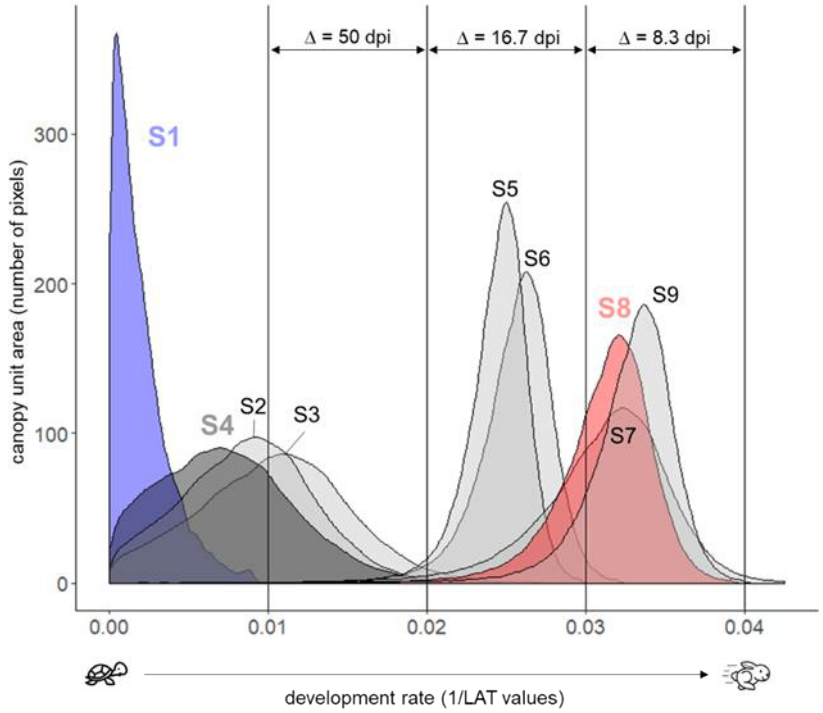


Fig. 7 Distribution of performance across nine strains making up a natural Z. tritici population in a wheat canopy characterised by small-scale leaf thermal heterogeneities (phylloclimatic conditions recorded in experiment 2). The performance of each strain (see Fig. S1) at each location of the canopy, characterised with an IR camera (320 × 240 pixels), was computed based on the application of each strain's TPC equation to each recorded temperature by pixel value. Three strains were identified as having particular patterns of thermal sensitivity: a cold-adapted strain S1 (in blue), a strain adapted to mild conditions S4 (in dark grey), a strain adapted to warm conditions S8 (in red). Performance is expressed as the inverse of latent period 1/LAT, describing slow (turtle) to fast (rabbit) development rate from lower to higher values. Delta correspondence in days post inoculation (dpi) between four 1/LAT values (Δ values between the vertical lines) is provided for an appreciation of the difference in competitive advantage between the strains in the wheat canopy. The wider the density plot, the more a strain presents different fitness values at different spots within the habitat. The higher the density plot, the larger the number of patches in the habitat in which the strain can develop at this rate.

DISCUSSION

If we are to understand the effects of temperature on the development of organisms and its ecological consequences for populations and communities, it must be measured at a scale relevant to biological development (Helmuth et al., 2010). For foliar pathogens and other leaf micro-organisms, this scale is the phyllosphere (Pincebourde & Casas, 2019). This approach has already been applied to other plant systems, such as tree crowns (e.g. leaf-dwelling insects on apple leaf surfaces; Pincebourde & Woods, 2012), but has only rarely been applied to crop canopies (e.g. corn fields; Norman et al., 1984; Holtzer et al., 1988). In these environments, plant temperature was mostly measured at the canopy scale (using thermography) rather than at the scale of the leaf organ itself, generally for the detection of plant water stress and assessment of yield performance (Pinter et al., 1990; Leinonen & Jones, 2004) or to screen for stress tolerance in wheat lines (Balota et al., 2008). We show here that the microbiome in the phyllosphere experiences thermal conditions very different from atmospheric temperature, and that wheat canopies generate a high degree of thermal heterogeneity that may contribute to the maintenance of high levels of diversity in the phyllosphere.

The importance of considering leaf temperatures

Consistent with previous studies, we found that standard air measurements from a local weather station and measurements conducted over leaf surfaces provided different information (Drake et al., 1970; Shaw & Decker, 1979; Chelle, 2005). Leaf temperatures deviated substantially from air temperatures, ranging from 6°C below to 8°C above air temperature (Fig. 3). As expected from previous results (Ehrler et al., 1978; Shaw & Decker, 1979; Haseba & Iso, 1980), we found that leaves were generally warmer than the air during the day and cooler than the air during the night, under clear sky conditions (Fig. S3b). These differences may affect the intensity of epidemics by modifying the latent period, which determines how many complete disease cycles can occur during an epidemic. In our experiment, we found a minimum of 10 to 23 days' difference in the estimated time required to complete one infection cycle for three contrasting thermotypes (Fig. 4). Biologically relevant time scales should capture the hidden and cryptic changes in temperature likely to have a strong impact on the response of species to thermal changes (Sheldon & Dillon, 2016). Over a six month period extending from the wheat seedling to flower stages, we recorded an intra-hour leaf temperature variance of 0.2°C on average, and of less than one degree for 98.2% of measurements (Fig. S6). The measuring device used here can detect temperature differences of less than 0.1°C. Our findings suggested that there is no need to have a finer time resolution than one hour for assessments of the biological impact of hidden changes. One hour may thus be considered a biologically relevant time scale for high-frequency temperature data (Fig. S6).

A high level of thermal heterogeneity over crop leaf surfaces in the field

We quantified the considerably leaf temperature heterogeneity and variance on crop leaf surfaces in the field. On average, we found: (i) a daily mean leaf temperature amplitude of 10.2°C; (ii) a mean within-leaf heterogeneity of 4.6°C and a between-leaf heterogeneity of 2.8°C

over the corresponding monitoring windows; (iii) a leaf temperature variance of more than 20°C over the three infection cycles of the temperature time series (Fig. S2). This spatiotemporal heterogeneity covers a large breadth of thermal performance curves (Fig. S1). It also raises questions about the impact of this heterogeneity on the physiology of phyllosphere micro-organisms and its consequences for population dynamics and distribution. The differences in leaf temperature found within local environments (e.g. a single leaf surface) were large, in some cases exceeding those found along biogeographic gradients. For instance, a difference in mean temperature of 5.5°C was recorded between French and Israeli locations from which Z. tritici populations were collected for thermal phenotyping (Fig. S1 in Boixel et al., 2019a), whereas we observed a difference of 6.1°C between leaf 6 and leaf 11 over the growing season. This finding is consistent with published findings suggesting a similar level of thermal heterogeneity at the micro-, local and landscape scales (Potter et al., 2013; Pincebourde et al., 2016). Our findings therefore support calls to consider microclimate diversity at finer scales (Woods et al., 2015; Pincebourde et al., 2016; Maclean et al., 2017).

Moving towards a finer description of thermal heterogeneity

For further characterisation and analysis of the heterogeneity of conditions around wheat leaf surfaces, we need to identify the factors affecting this microclimate variability (Valdés-Gómez et al., 2008; Saudreau et al., 2013). Analyses of daily leaf temperature patterns appear to be a promising way to shed light on the mechanisms at work (Gaudio et al., 2017). We used this method here (Fig. S3), to highlight the effects of radiation, wind and canopy structure on daily leaf temperature patterns (Fig. S5). A finer description of the thermal heterogeneities to which the leaf microbiota is exposed could be achieved by taking temperature measurements for a wider range of crop species, conditions and situations (e.g. plant density and structure, as canopy architecture modulates microclimate - Boudreau, 2013 – or environmental variability in light intensity, temperature, relative humidity, and wind speed, as leaf temperature varies with these meteorological variables - Ribeiro et al., 2005). For instance, the temporal window for monitoring in the thermal imaging (thermography) experiment (Fig. 2) was restricted to a 10-min snapshot in a day (high spatial resolution, but low temporal resolution). Improvements could be achieved by extending measurements to different times of day, environmental conditions and stages of the growing season (Costa et al., 2013). Interestingly, a laboratory experiment performed in parallel highlighted a disease-induced increase in leaf temperature from the appearance of the first symptoms to the general acceleration of senescence processes (Fig. S8). This increase in temperature is consistent with the results of previous thermography studies monitoring stress and health in crop canopies (Chaerle & Van Der Straeten, 2000), although it should be stressed that diseased leaf area has less of an effect on canopy temperature than other abiotic factors (Lenthe et al., 2007).

The dynamics of thermal patches within wheat canopies

This study adds to previous findings showing that the microenvironments available to micro-organisms are not well described by conventional meteorological data. This has direct consequences for estimates of the performance and fitness of phyllosphere micro-organisms,

with differences in estimates of the rate of Z. tritici strain development (latent periods) of +23 days and -10 days, taking T_{opt} as the reference point (Fig. 4). It is, thus, possible to quantify the selective pressures on the thermal sensitivities of phyllosphere individuals imposed by the patchiness of wheat canopies. The heterogeneity of leaf temperatures must be taken into account in models of the ecology of leaf microbiota. However, routine temperature monitoring at the leaf level in diverse experimental conditions is difficult, for two reasons: (i) the experimental characterisation of leaf temperature is cumbersome and difficult to implement in the field; (ii) field instrumentation may alter leaf temperatures and disturb plant growth, thereby modifying the canopy environment (Chelle, 2005). One way to solve this problem would be to use soil-vegetation-atmosphere transfer (SVAT) modelling approaches, which down-scale weather information from the global to the canopy scale (Seem, 2004). These models (e.g. SHAW, ISBA, and CUPID; Norman, 1979; Olioso et al., 2002) can simulate leaf temperatures within a wheat canopy from meteorological input variables and direct measurements of canopy structure (Xiao et al., 2006; Huang et al., 2011).

Inferring performance in heterogeneous environments: standards and challenges

The substantial temporal heterogeneity in leaf temperatures at different sites in the canopy raises questions about whether responses to constant or fluctuating temperatures should be estimated. We inferred the performance of Z. tritici strains from their TPC, a performance curve typically plotted from measurements made under constant conditions (Huey & Stevenson, 1979). However, biological performance under constant temperature is often different from that in conditions of fluctuating temperature (Worner, 1992; Scherm & Van Bruggen, 1994; Bernard, 2012) due to the Jensen's inequality between the mean of the function and the function of the mean (time-averaged performance differs from performance at the average temperature when temperature varies over time; Ruel & Ayres, 1999). Three main approaches have been proposed to extrapolate the results obtained under constant temperatures (TPC) to performance in a fluctuating environment (natural field conditions): (i) calculation of performance at the mean temperature (taking into account the mean value of a thermal environment; Schrodter, 1965); (ii) integrated performance (scale-transition theory calculation method taking into account the mean and variance of the environment; Dowd et al., 2015; Koussoroplis et al., 2017); (iii) cumulative performance over the time interval (calculation method taking into account the mean, variance, and, extremes of a thermal environment; Xu, 1996). In our simulations, the method retained for the calculation of performance did not significantly affect the mean difference in performance between strains (i.e. gap between the least and the most adapted strains; Fig. S7). This is not surprising, as we were working with high-frequency temperature data. We retained the integrated performance in our theoretical approaches as it gave a variance in outcomes intermediate between those of the other two methods (Fig. S7). This integrated method has the advantage of requiring smaller amounts of variance data than the cumulative performance approach, which requires the whole distribution.

Leaf thermal niches could contribute to the local maintenance of microbial diversity

Leaf temperature drives the development and adaptation of organisms living in the phyllosphere, like most microclimates studied to date over both spatial (Chelle, 2005; Helmuth et al., 2010) and temporal scales (Benedetti-Cecchi et al., 2006). As a result of the heterogeneity of leaf temperature, strains do not develop everywhere in the canopy at the same rate. All strains can develop somewhere in the canopy, given the degree of heterogeneity of the environment. In particular, our results show that the heterogeneity of foliar temperatures at a fine scale (canopy) provides thermal refuges with lower foliar temperatures, allowing the development of cold-adapted strains (Fig. 6b). However, this heterogeneity and the differences in thermal adaptation generate very different fitness landscapes for each strain. Indeed, the same spot in the canopy could be more or less favourable, depending on the thermal sensitivity of the individual and the phenotypic diversity of the microbial population to which it belongs (Fig. 6). In Z. tritici, the differential competitive advantage was as high as 50 dpi between the cold-adapted and warm-adapted thermotypes at all locations in the canopy (Fig. 7). This environmental heterogeneity would be expected to promote diversity (Stein et al., 2014) and to act as a buffer against climate change (Scherrer & Körner, 2011; Saudreau et al., 2013), leading to the emergence of a spatially structured population in mosaic canopy systems (Orians & Jones, 2001). This may explain why placing strains with contrasting thermal responses together in experiments favours the stable maintenance of phenotypic diversity within foliar pathogen populations in field (fluctuating) conditions, but not in laboratory experiments with constant-temperature regimes (Boixel et al., 2020). These results for the wheat-Z. tritici pathosystem can easily be extrapolated to other organisms colonising wheat leaves by routinely applying their TPC equations to the leaf temperature time/spatial series recorded during this study. Extrapolation to other crop species would require several precautions, as substantial variations in leaf temperatures have been reported for different plant species (Leuzinger & Körner, 2007).

CONCLUDING REMARKS

By performing a fine characterisation of leaf temperatures within wheat canopies, we were able to estimate fitness consequences for the development of a foliar fungal pathogen, used as a case study. In the case of latent period, the differences in one-trait performance between strains would be expected to lead to a differential competitive advantage that could result in changes to the phenotypic composition of populations in response to variable environments (Farine et al., 2015; Forsman, 2015). By combining demography, ecology, and evolution to address the complexities inherent to these two sources of variability — environmental (temperature) and biological (individual physiological responses) — this work paves the way for studies of the contributions of climatic heterogeneity and phenotypic diversity within populations to adaptive potential. Such studies will improve our understanding of the diversity of responses and eco-evolutionary dynamics. This will require the exploration of a large number of scales and fitness metrics, potentially through modelling approaches and in silico evolution experiments (Hindré et al., 2012).

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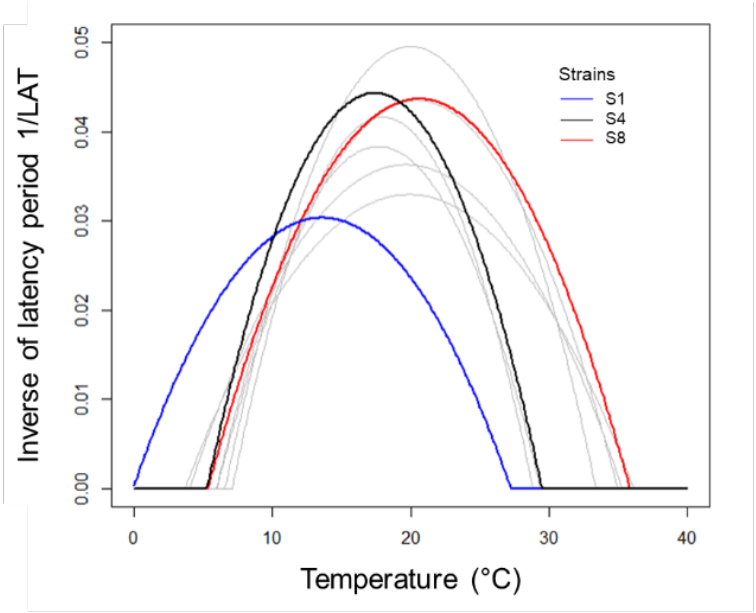
SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURES



Strain	Code	Thermotype	P_{max}	Curv	T_{opt}
S1	INRAE13-FS5021	CA	0.0303	-0.0002	13.58
S2	INRAE13-FS5055	MA	0.0443	-0.0003	17.38
S3	INRAE13-FS5038	MA	0.0383	-0.0003	17.66
S4	INRAE13-FS5028	MA	0.0416	-0.0004	17.96
S5	INRAE13-FS5011	WA	0.0363	-0.0001	19.65
S6	INRAE13-FS5072	WA	0.0330	-0.0001	19.90
S7	INRAE13-FS5035	WA	0.0495	-0.0003	19.97
S8	INRAE13-FS5006	WA	0.0435	-0.0002	20.46
S9	INRAE13-FS5000	WA	0.0436	-0.0002	20.58

CA, cold-adapted; MA, adapted to mild conditions; WA, adapted to warm conditions

Fig. S1 Typology of the strains used to infer Z. tritici performance in wheat canopies. These strains, belonging to a single French natural population, were previously characterised for in vitro and in planta thermal sensitivity (Boixel et al., 2019). The chosen fitness proxy for this investigation was development rate, calculated as the inverse of in planta latent period (1/LAT, where LAT is the number of days post-inoculation – dpi – required to reach 5% of the final sporulating area). (a) Thermal performance curves (TPC) highlighting the effect of mean leaf temperature on the 1/LAT values of the nine Z. tritici strains (S1 to S9) in the study population considered in experiments 1 and 2. The coloured curves shown are the TPC of the cold-adapted strain (S1; in blue), the strain adapted to mild conditions (S4; in black) and the strain adapted to warm conditions (S8; in red). (b) The thermal parameters P_{max}, Curv and T_{opt} of the TPCs for the latent period were obtained by fitting a quadratic function: $LAT^{-1}(T_{leaf}) = P_{max} + Curv(T_{leaf} - T_{opt})^2$.

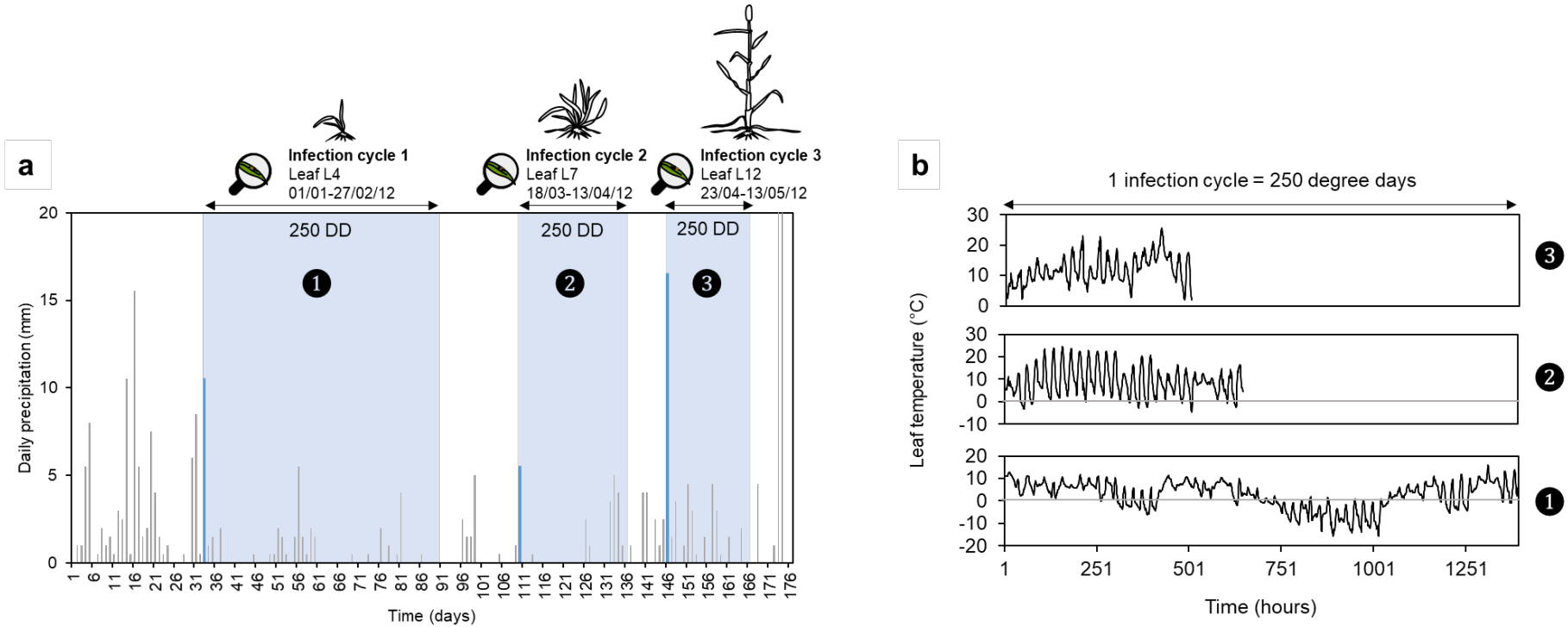


Fig. S2 Typology of the thermal environments used to investigate strain performance (expressed as differential competitive advantage over three theoretical Z. tritici infection cycles) within a natural pathogen population across phylloclimatic patterns in a wheat canopy. (a) Time windows were defined on the basis of the occurrence of a significant rain event (thick blue histogram bar) until the standard duration of the latent period was reached (250 degree-days above the base temperature -2.4 °C; Lovell et al., 2004). (b) The phylloclimatic temperature time series used to compute Z. tritici strain performance were measured on L4 for infection cycle 1, L7 for infection cycle 2, and L12 for infection cycle 3 (bottom-up numbering system from first leaf - L1, to flag - L12).

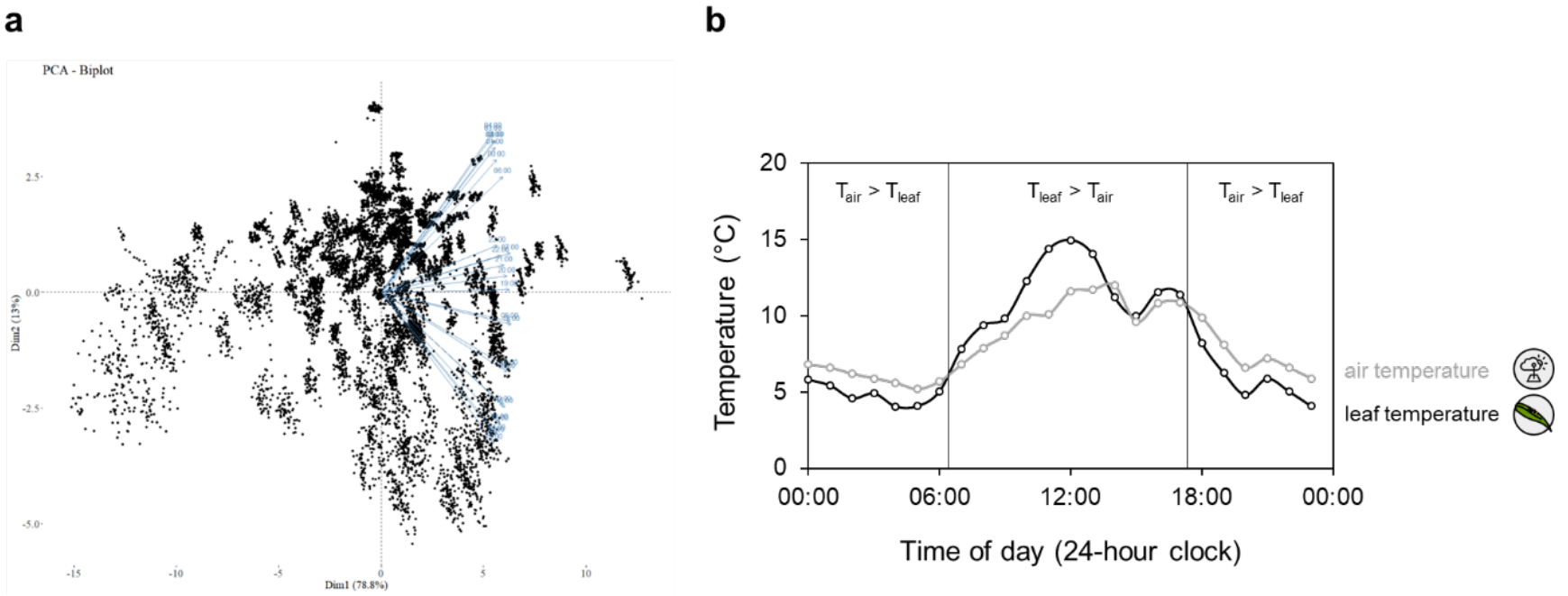


Fig. S3 Principal plane defined by the first two components of the principal component analysis (PCA) performed on all the 24-h leaf temperature curves. (a) The daily phylloclimatic patterns were recorded with thermocouples positioned on the three youngest leaves of nine wheat plants over the course of a wheat growing season (see Fig. 1). Each point on the principal plane describes a temperature curve over the course of one hour, around the daily mean. PCA variables (blue arrows) correspond to the temperatures recorded at each hour in the day (from 00:00 to 23:00). Differences in daily phylloclimatic thermal patterns were linked to vertical shifts (i.e. variation in the height of these thermal patterns or overall temperatures across all hour time steps; accounting for 91.8% of the total variance). (b) Illustration of one daily phylloclimatic pattern in black, corresponding to a single point projected on the principal component plane in Fig. S3a (April 21st 2012, Leaf 10).

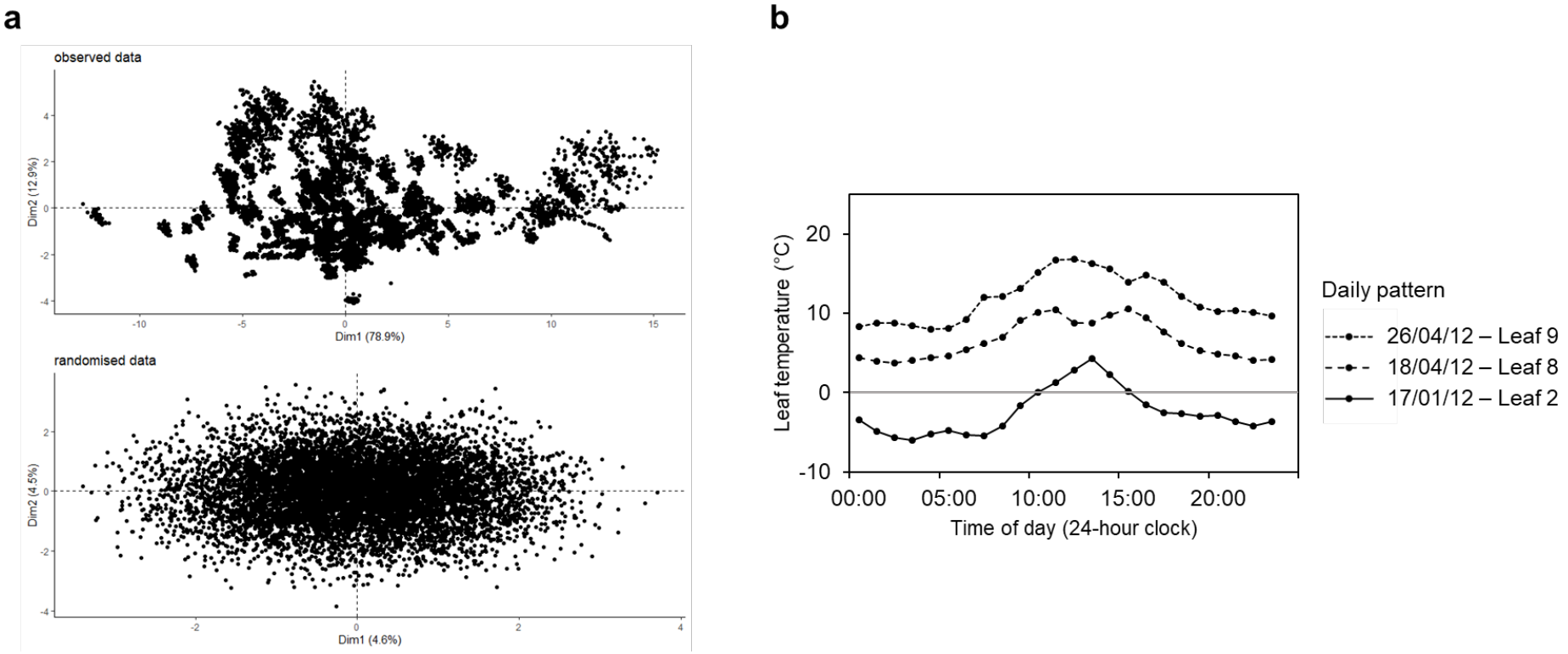


Fig. S4 Division of temporal variations of recorded daily phylloclimatic thermal patterns into representative 24-h temperature curves. (a) The tendency of patterns to cluster was assessed by measuring their spatial randomness on the principal component plane. The comparisons between observed and randomised data indicated a clustering of data, justifying the establishment of a typology of daily patterns. (b) Typology of daily leaf temperature profiles (hourly time step recordings corresponding to black data points). These profiles correspond to the paragons i.e. the most representative patterns of each HCPC cluster obtained on the principal component analysis of all monitored 24-h leaf temperature profiles (Fig. S3): April 26th 2012 (paragon of cluster 1: days with high levels of wind and radiation), April 18th 2012 (paragon of cluster 2: days with high levels of wind and intermediate levels of radiation), January 17th 2012 (paragon of cluster 3: low levels of wind and intermediate levels of radiation).

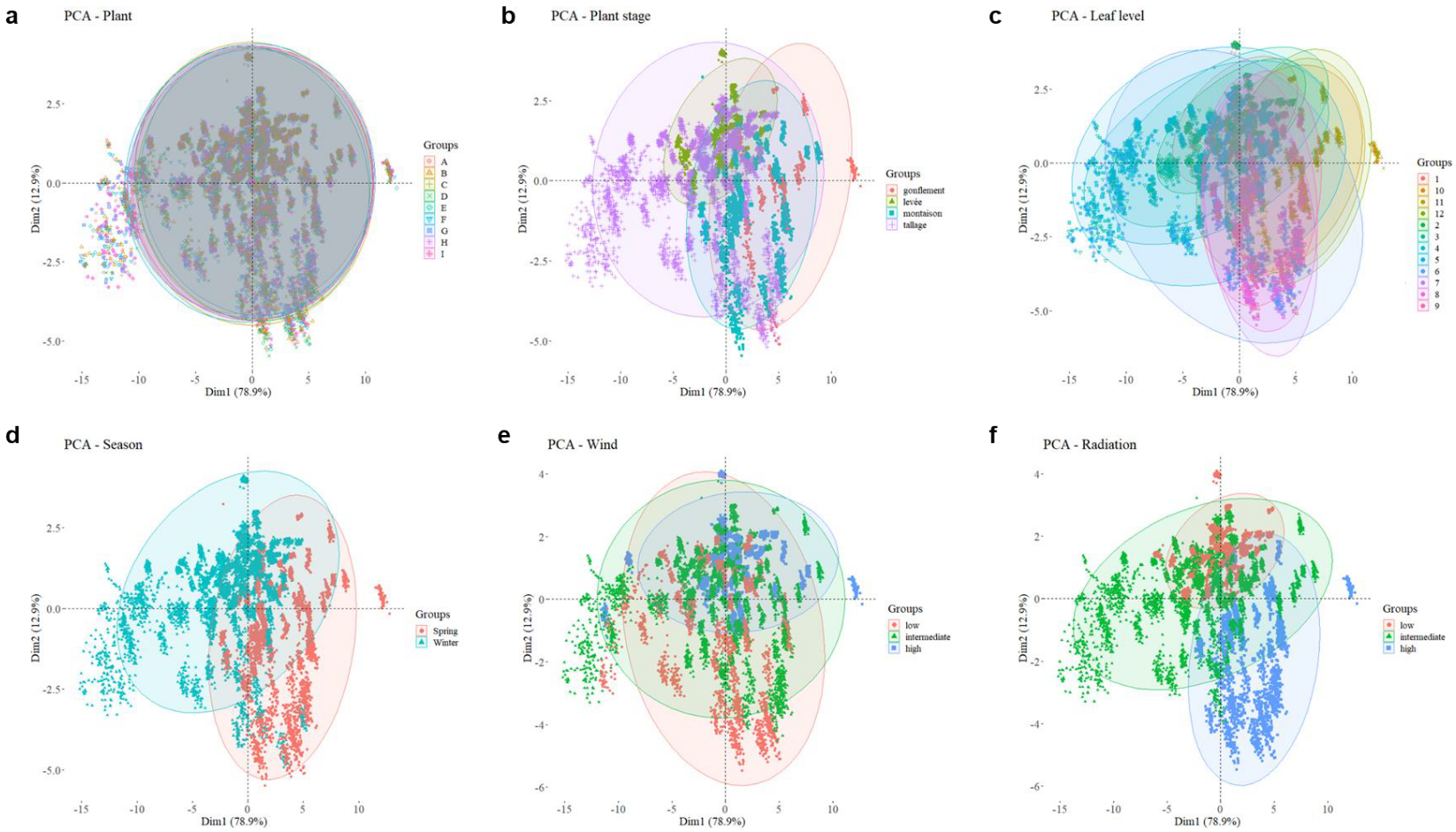


Fig. S5 Effect of canopy structure, wind and radiation factors on daily phylloclimatic thermal patterns. (a) Plant (n = 9 replicates; no structuring effect with overlapping of ellipses); (b) Plant stage (structuring effect, but confounded with leaf level and season factors); (c) Leaf level (structuring effect, but confounded with plant stage and season); (d) Season (structuring effect of this predefined time division, but confounded with plant stage and leaf level); (e) Wind (lower variation in daily phylloclimatic patterns under higher wind conditions); (f) radiation (lower variation in daily phylloclimatic patterns under low radiation conditions).

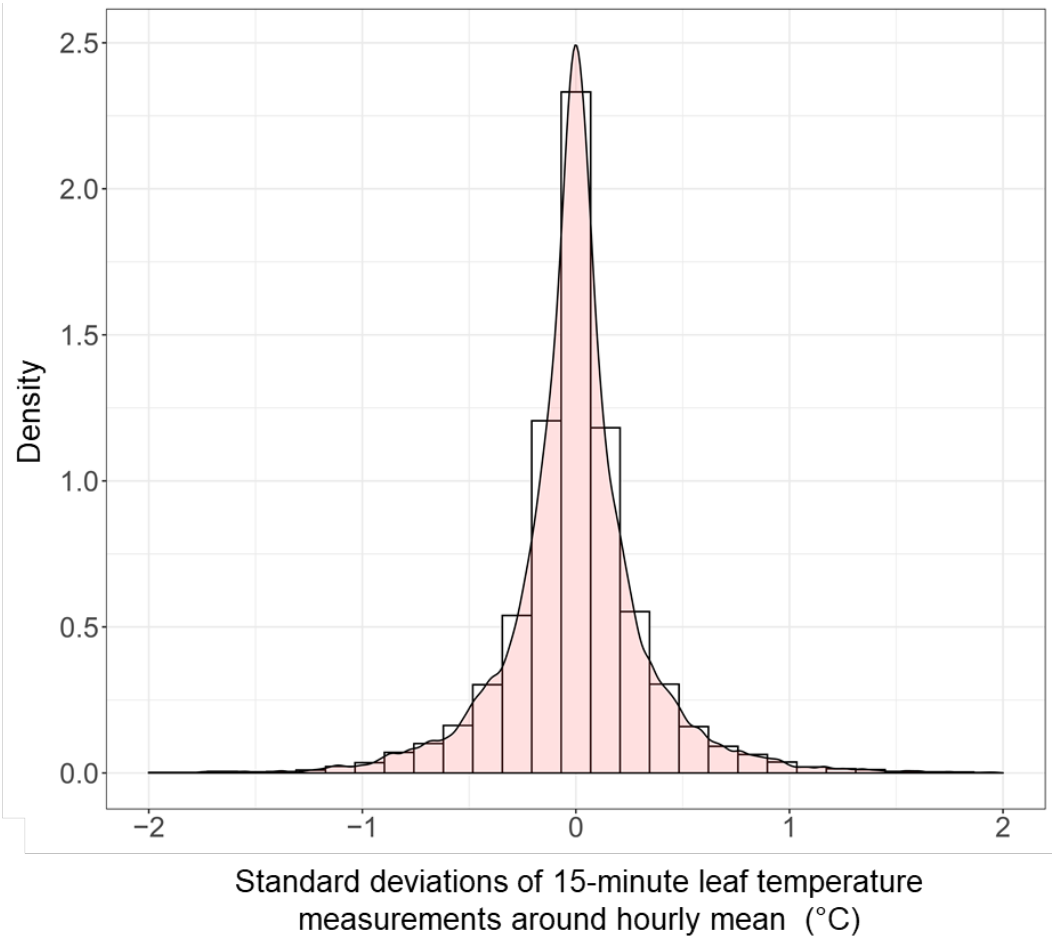
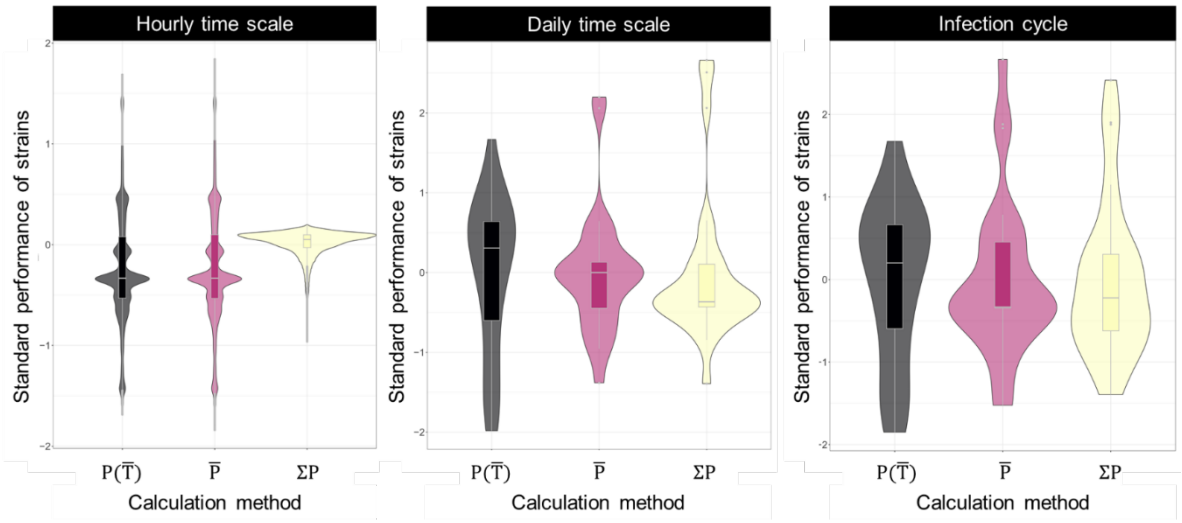


Fig. S6 Distribution of standard deviations of 15-minute leaf temperature measurements around the corresponding hourly mean over the entire duration of experiment 1. The probability density histogram displays intra-hour variability over six months, from the wheat seedling to flowering stages (mean absolute deviation within one hour of 0.22°C ± 0.26°C SD); 98.2% of recordings deviate by less than 1°C. The measuring device can measure temperature differences of less than 0.1°C.



Code	Name	Equation	Consideration of the thermal environment[1]
P(T̄)	mean performance	$P = P_{TPC} = LAT^{-1}(\overline{T_{leaf}})$	mean leaf temperature $\overline{T_{leaf}}$[2]
P̄	integrated performance	$P = \overline{P_{TPC}} = a + b = LAT^{-1}(\overline{T_{leaf}}) + \frac{1}{2}LAT^{-1''}(\overline{T_{leaf}})\sigma^2_{Tleaf}$	mean temperature $\overline{T_{leaf}}$ and its variance σ_{Tleaf}[3]
ΣP	cumulative performance	$P = \sum_t P_{TPC,t}$ when $P_{TPC,t} \geq 0$	sum of performance at each encountered T_{leaf}[4]

[1] with underlying assumptions about the effects of thermal fluctuations on the variance of performance (none, partial importance, total inclusion)
[2] thermal variations summarised in a single averaged value
[3] thermal variations summarised in two terms: the single averaged value and its variance over the time window considered
[4] thermal variations taken as a whole: cumulative constant-temperature development rates over the thermal regime

Fig. S7 Gap-widening estimation of differences in strain performance from TPC as a function of the selected calculation method and the time window for thermal variations. The impact of calculation method (mean, integrated or cumulative performance) and time window (sub-hourly time scale: 15-minute leaf temperature measurements; daily time scale: 24-h leaf temperature profiles, see Fig. S3b; infection cycle: 250 degree-days after the occurrence of a dispersal event, see Fig. S2) on scaled performance was assessed for nine strains (Fig. S1) with the leaf thermal variations recorded over experiment 1. No significant differences were observed between the three calculation methods for estimates of the mean competitive advantage of strains within the population (ANOVA, P = 0.17). However, integrated performance tended to be associated with a wider range of variation in competitiveness at the hourly time scale (as for mean performance) and a narrower range of variation at longer time scales (as for cumulative performance), depending on thermotypes (i.e. the thermal sensitivity of strains; ANOVA, P < 0.01).

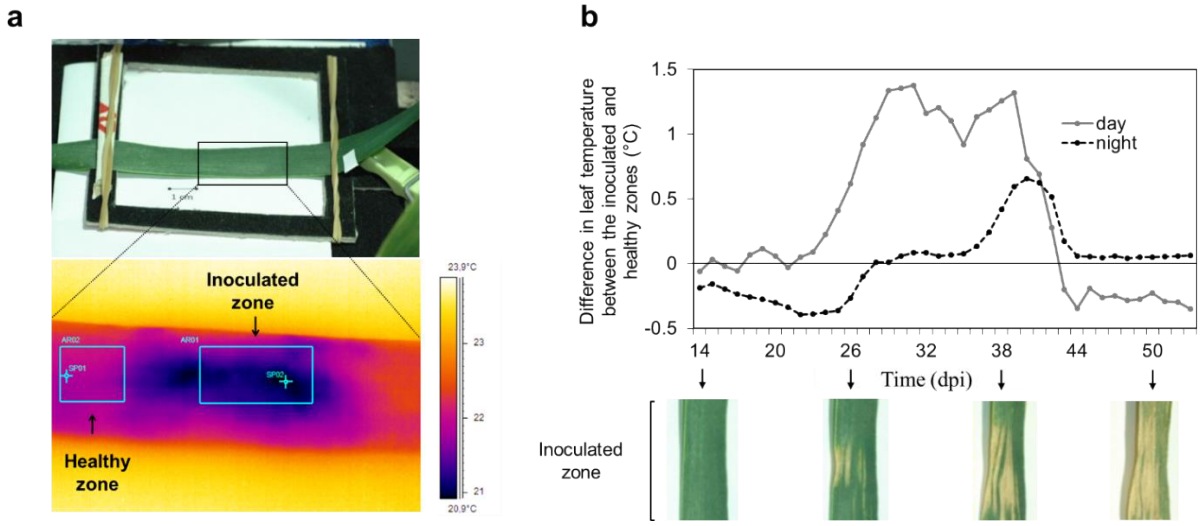


Fig. S8 Temporal changes in leaf temperature resulting from pathogen development. (a) Experimental design: we monitored leaf temperature over the development of a Septoria tritici blotch lesion (from 14 to 53 dpi, i.e. days post inoculation) within ("inoculated zone" AP01 with a focus on the spot SP02) and outside ("healthy zone" AP02 with a focus on the spot SP01) an inoculated area on a given wheat leaf. Thermographic measurements were conducted every 20 minutes over 40 days of monitoring beginning at 14 dpi. The single-timepoint thermal image illustrates the difference in leaf temperatures upon pathogen infection (SP01: 21.9°C within a [21.5, 22.2°C] range in the AR02 healthy zone; SP02: 21°C within a [20.9, 21.9°C] range in the AR01 inoculated zone). (b) The changes in temperature induced by disease are shown as the difference in temperature between the inoculated and healthy zones (averaged over the day – from 7 a.m. to 9 p.m. - and the night – from 9 p.m. to 7 a.m.) between 14 and 53 dpi (days post inoculation). Differences in leaf temperature were between -0.5°C and 1.5°C, with fungal infection associated with an overall increase in leaf temperature. Deviations were smaller during presymptomatic detection and were reduced by a general acceleration of senescence processes.

Fig. S9 Separation of vegetation (wheat plant material) from non-vegetation (soil surface, thermocouple instrumentation) pixels on digital photographs of the wheat canopy. We screened (a) all digital images obtained with infrared imaging cameras, based on thresholds defined by hue, saturation and brightness, in ImageJ software; (b) to identify non-vegetation pixels (c), which were then removed for analyses restricted to the corresponding plant pixels (around 87.5% green leaf area in each image) for extraction and analysis of the relevant distribution of canopy temperatures.

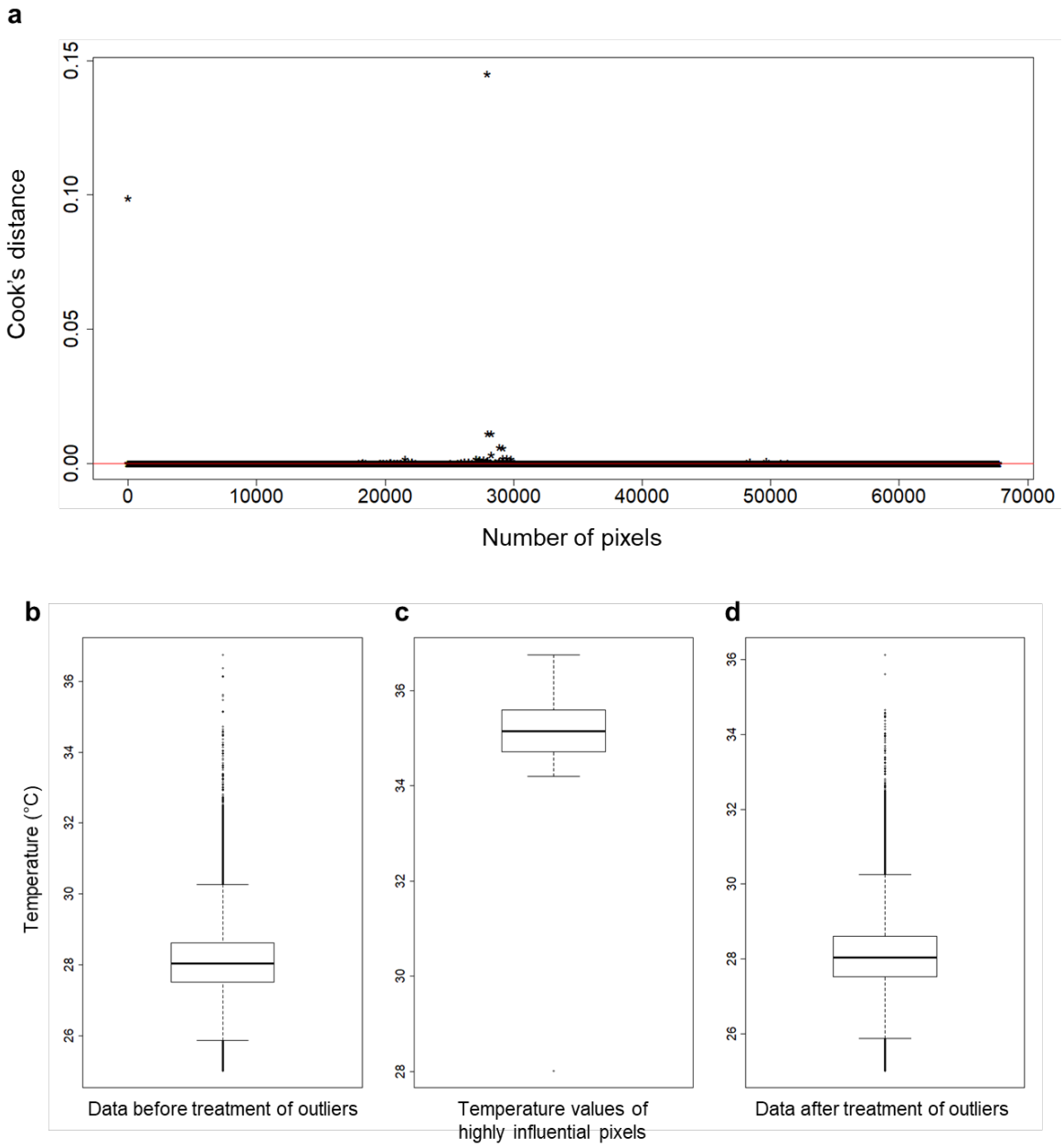
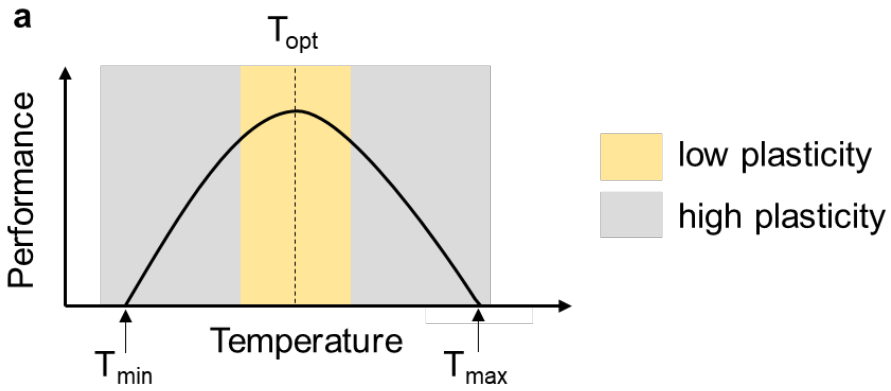


Fig. S10 Detection of outlier pixels in the thermal maps of wheat canopies illustrated for the canopy snapshot at 4:02 p.m. on June 4th 2015 (experiment 2). Cook's distance statistic, which indicates the extent of change in model estimates when a particular observation is omitted, was computed for each pixel in turn, to examine, in greater detail, highly influential data points for which it was particularly worthwhile checking for validity. Outlier pixels with extreme temperature values (0.4% of all initial pixels) were related to edge effects in the separation of the image elements, and, as such, were excluded from the analysis. (a) Cook's distance plot with a red line corresponding to the threshold value of four times the mean of the total observations. Boxplot distributions of: (b) all pixel temperatures before outlier detection and treatment; (c) temperature values of highly influential pixels subsequently removed from the analysis after identification; (d) temperatures of the pixels validated as plant material and retained in the analysis.

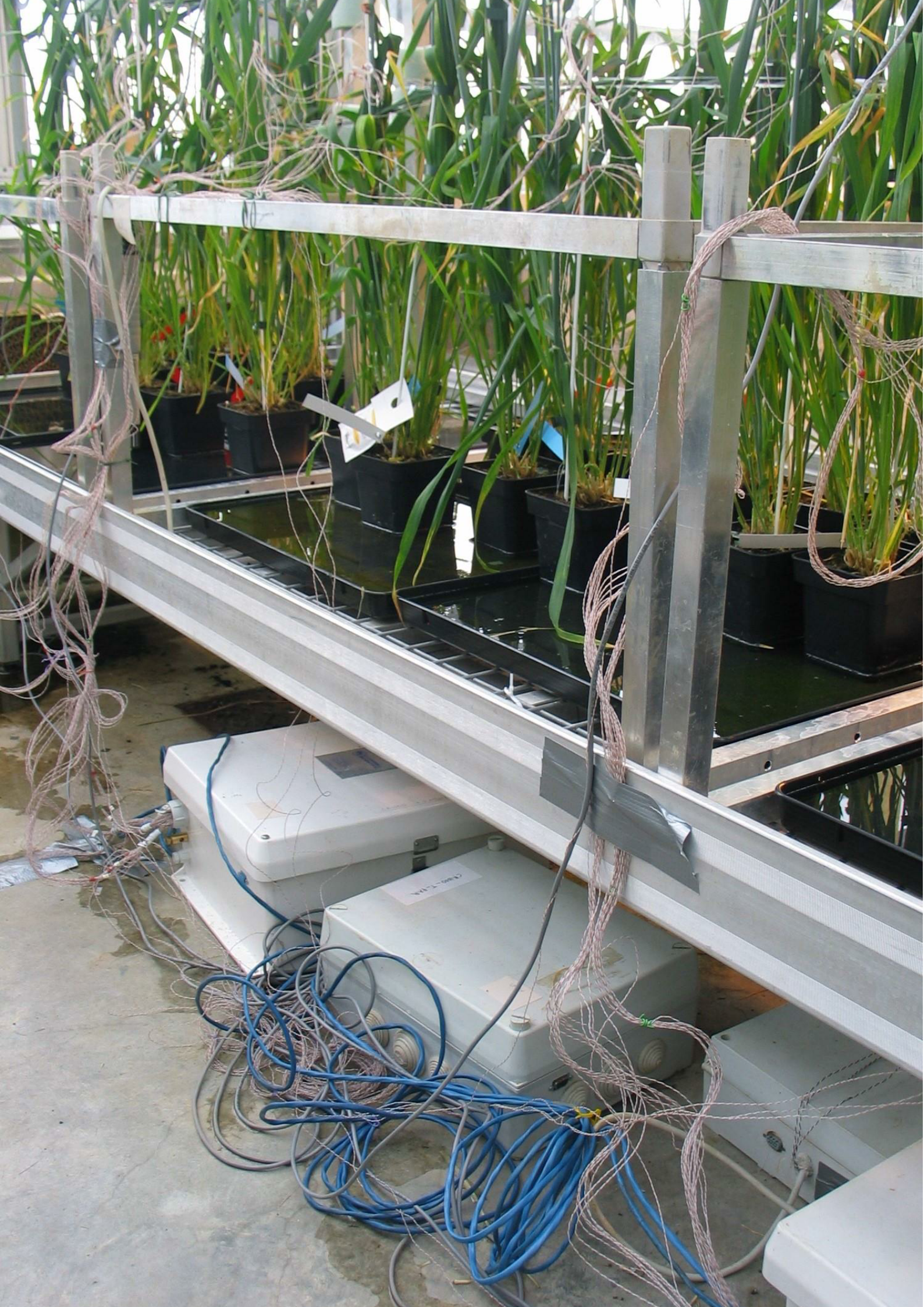
a



b

Temperature range	Range of performance values (1/LAT in dpi^{-1})		
	S1	S4	S8
$T \in [T_{opt}-1, T_{opt}+1]$	0.0002	0.0004	0.0002
$T \in [T_{min}, T_{min}+2]$	0.0082	0.0126	0.0112
$T \in [T_{max}-2, T_{max}]$	0.0081	0.0128	0.0104

Fig. S11 Rationale of conducting impact assessments in the mid-temperature range of the thermal performance curve around the thermal optimum for performance (T_{opt}). (a) The mid-temperature range around T_{opt} corresponds to the range in which we would expect the smallest variation in performance values, due to the lower thermal plasticity over this range. (b) We evaluated the discrepancies in thermal plasticity in more detail by quantifying the range of performance values obtained around T_{opt} ($T \in [T_{opt}-1, T_{opt}+1]$), T_{min} ($T \in [T_{min}, T_{min}+2]$) and T_{max} ($T \in [T_{max}-2, T_{max}]$) for the strains S1, S4 and S8 (Fig. S1). If discrepancies in performance are observed in the mid-temperature range when considering a given temperature variation or distribution, then an even greater impact may be anticipated elsewhere on the thermal performance curve.



Chapter

6

‘PhenoPatch’, a generic modelling framework to explore the ecological consequences of environmental heterogeneity

Foreword. Chapter 4 and Chapter 5 highlighted that phenotypic diversity of thermal responses within populations can lead to a short-term adaptation of these populations to their heterogeneous environments. This may foster both diversity maintenance and temporal changes, depending on the thermal environments in which the populations evolve. The last chapter of this thesis set out to advance a more mechanistic understanding of the processes that underpin population dynamics. To this end, I turned to a modelling approach involving an explicit description of the underlying interactions down to the individual level and to the environmental heterogeneity perceived by a population at a given time and location. The goal of this generic phenotype × patch-based model was to carry out fast-track in silico competition experiments in multiple context and under multiple conditions.

This work was initiated during a three-month stay in the ‘Evolution and Ecology of Phenotypes in Nature’ research group at Lund University in Sweden, working with Professor Erik I. Svensson.

contents

abstract

Background and Aims

Models focusing on the ecological consequences of inhabiting heterogeneous environments have mainly left out the existing intrinsic variability, both in terms of individuals and of their inhabiting environment. But, this variability considerably affects population processes and, as such, has motivated prompting calls to explicitly account for them when investigating ecological drivers of phenotypic selection.

Key Results

The generic phenotype × patch-based model 'PhenoPatch' was developed with this purpose in mind. It enables to simulate the changes in the phenotypic composition of a set of clonal populations inhabiting discrete patches that are environmentally heterogeneous both in space and time, and moving from patch to patch. This individual-based model operates on reaction norms and accounts for environmental variation and population demography. The model was applied to investigate phyllosphere microbial populations' responses to heterogeneous thermal environments, taking the wheat fungal pathogen Zymoseptoria tritici, that causes Septoria tritici blotch epidemics, as a case study. On this case study, we have conducted a first exploration of the mechanisms behind population responses to leaf temperature variations. The model behaviour was preliminarily evaluated using previously acquired empirical data from a competition experiment conducted under controlled conditions. To illustrate the potential of the model, we performed two numerical experiments based on the reconstruction of a natural plant disease epidemic. We first tested the impact of initial microbial population patterns, including its level of functional diversity and its spatial distribution, on competition outcomes. Then, we quantified the impact of considering different levels of thermal heterogeneities between patches. The outputs highlight that the model is effective for investigating population responses to variable environments.

Conclusion

As such, it could be used for defining the degree of heterogeneity to take into account when investigating ecological processes, especially in plant disease epidemiology. Furthermore, the outputs highlighted that glossing over the natural extent of individual variation in an initial population or over the local heterogeneity experienced in the field by crop phyllosphere populations lead to an estimated loss of maintenance of the actual diversity and balance between phenogroups over short-time scales. Further amendments and validation based on more detailed experimental data will improve 'PhenoPatch' relevance before considering applying it to a broader range of ecological situations.

Keywords: functional diversity, heterogeneous environment, competition experiment, ecological dynamics, environmental selection, adaptive potential

INTRODUCTION

Organisms inhabit a network of discrete, contiguous patches of habitat of varying size and differing in their attributes (e.g. physical environment, resource availability and quality; Girvetz & Greco, 2007). The notion of patch may be difficult to define. Indeed, for some systems, a habitat that seems prima facie homogeneous may be home to subpopulations that can be patchily distributed (Pannell & Obbard, 2003). This idea of a 'population of populations', first coined by Richard Levins (1969), has been considered in classical metapopulation or spatially structured approaches (Hanski, 1999; Fronhofer et al., 2012). It has motivated a number of theoretical studies on population responses to variable environments since theoreticians started to incorporate spatio-temporal variations in their models by the mid-1960s and the seminal studies of Levin and Wiens (1976).

This research topic has since been a central investigation in classical population genetics models (see Felsenstein, 1976 for review). Population-level models are of immense value to describe population changes in spatially homogeneous (commonly described by ordinary differential equations) or structured (commonly described by partial differential equations) environments. However, they become increasingly at odds when the heterogeneity in the environment and/or individual responses becomes too high to be neglected (Evans et al., 2013). One limitation of these population-level models is that they are built on qualitatively invariant variables only differing in temporal quantity, consequently considering that all individuals are the same and glossing over individual-environment interactions (Breckling, 2002).

These assumptions are problematic as there is now evidence that there may be striking phenotypic differences between individuals of a given population (Bolnick et al., 2011) as well as non-linearities in the responses of organisms to their environment (Wu & O'Malley, 1998). This is all the more important since variances and heterogeneities have been highlighted to be important drivers or indicators of ecological change (Dall et al., 2012). Yet, it is still unclear to what extent, in particular with respect to resilience and vulnerability of populations to environmental changes (Bolnick et al., 2003). Thus, there have been prompting calls to explicitly incorporate phenotypic variations at all the levels (individual, population, community; Violle et al., 2012; Forsman, 2015) and at the relevant spatio-temporal scales of environmental heterogeneity into predictions of population structure and dynamics (Potter et al., 2013).

This paradigm shift has been made possible by a number of approaches and advances allowing to address the complexities inherent to most natural populations and their environment:

- the emergence of individual-based models (IBM) enabling to consider aspects usually ignored in population-level models (Huston et al., 1988) as they represent individuals not only as countable entities, but as varying agents whose sets of traits affect population-level properties (DeAngelis & Mooij, 2005);
- the concept of reaction norms (RN) describing how individuals respond to their environment in any relevant situation it meets (de Jong, 1990; Pigliucci, 2001);
- the effect of the environmental variation and the time step of response on organism performance through the transposition of scale-transition theory to ecophysiology (Chesson, 2012; Dowd et al., 2015);
- proposal of more general theoretical frameworks to model selection in subdivided populations than strict hard and soft selection models (Ravigné et al., 2009; Débarre & Gandon, 2011).

Investigating population evolution in heterogeneous environments requires to analyse population characteristics at a high resolution to explicitely consider individual variation and the heterogeneity (space) and variability (time) of environments (Kreft et al., 2013). This research topic can be tackled through in silico experimental evolution (Hindré et al., 2012). Going from in vivo to in silico approaches offers a number of advantages. First, it makes it possible to explore the interplay of evolutionary processes and the spatio-temporal environment under a higher number of experimental conditions that are not possible to investigate empirically (e.g. natural environments that cannot be reproduced in the lab). Second, it enables a quantification of the weight of certain ecological parameters on outcome dynamics using sensitivity analyses that allow to manipulate selective pressures not only one at a time but also simultaneously. Third, it allows testing of ecological principles, evolutionary scenarios or the repeatability of evolution notably of atypical evolutionary trajectories. Finally, this offers the possibility to be both more precise since in silico experiments can be extended over as many generations and repeated as many times as desired, and exhaustive since every temporal change or event can be recorded.

Several existing computational platforms explore the interplay of evolutionary processes in heterogeneous habitats. However, these in silico evolution platforms (Adami, 2006) are based on quantitative genetic or genetically explicit niche-based algorithms (e.g. splatche, aevol, PhenoEcosim, Nemo, REvoSim; Currat et al., 2004; Batut et al., 2013; Ashander et al., 2016; Cotto et al., 2017; Garwood et al., 2019). They are therefore particularly suitable to investigate mutation-based evolution. However, such genetic simulations are not necessarily the most appropriate angle of approach for tackling some research questions. For instance, these approaches are not suitable in the following range of situations: (i) when there is unrevealed genetic architecture of the traits under consideration in the studied populations; (ii) when there is known phenotypic variability between identical genotypes; (iii) when focusing on non-heritable phenotypic variation; (iv) in the case of hidden reaction norms or cryptic genetic variation (Palinska et al., 1996; Robinson & Dukas, 1999; Schlichting, 2008). Furthermore, a phenotype-centred approach directly incorporating reaction norms is more appropriate to explore the consequences of patterns of phenotypic plasticity operating within and across generations on ecological time-scales (Ghalambor et al., 2007). Such an approach can inform the emergent properties that arise from individual variation on the population-level. It also provides better accountability of competitive ability between individuals in occupied patches by not aggregated biological information in the form of averages (Stearns, 1989; Farine et al., 2015; Forsman, 2015).

Studying population responses to heterogeneous and variable environments requires to bring the underlying interactions down to the individual level. It also requires to finely explore the environmental heterogeneity perceived by a population at a given time and location (Lomnicki, 1988; Rueffler et al., 2006). Both aspects are still given limited consideration in analytical models to date despite having been related to the intensity of competitive interindividual interactions (Bassar et al., 2016). We therefore developed a generic phenotype × patch-based modelling framework that explicitly incorporates and accounts for individual and environmental variations in ways that allow careful consideration of their consequences on population stucture and dynamics.

Our goal was to investigate the changes in phenotypic composition within a series of replicated populations studied across multiple generations and composed of phenotypes differently adapted to the patches a local habitat is made of. The responses of phyllosphere microbial populations to the leaf temperature heterogeneity and variance encountered within a crop canopy appeared as a relevant case study to apply and test such a model. Indeed, crop canopies are good examples of heterogeneous environments: they offer microclimates and buffering properties, with ranges of variations that can strongly impact phyllosphere microorganisms (Pincebourde & Woods, 2012; Boixel et al., 2020).

METHODS

The 'PhenoPatch' model simulates the ecological dynamics of spatially-structured populations inhabiting a spatio-temporally heterogeneous environment. We first present the general model layout, applicable to several living systems. 'PhenoPatch' allows to consider a population of individuals inhabiting a set of discrete and environmentally heterogeneous sites (Conceptual framework and Model description). Here, we conducted a case study ('PhenoPatch Zymo' model application and Model evaluation) to illustrate its potential on a preliminary exploration of the general mechanisms behind phyllosphere microbial populations' responses to the thermal variations occurring in a crop canopy (Numerical experiments).

Conceptual framework

The modelling framework 'PhenoPatch' (Variation in Phenotypic Composition within Patchy environments differing both in space and time) allows a general layout of organism – environment interactions of different living systems by relying on several key concepts. A 'patch' represents a spatial unit that is a physical entity characterised by a tangible boundary, differing from its neighbors, and supporting a given subpopulation. An 'environmental signal' corresponds to all the spatio-temporal changes in intensity or frequency of a local environmental variable. An 'individual' is characterised by its reproductive or non reproductive stage, a patch location, and a functional response described as a reaction norm. 'Functional groups of phenotypic responses' based for instance on a typology of reaction norms, thereafter called 'Phenogroup', enable to aggregate individuals presenting the same responses to the environmental signal under consideration into a group that can be viewed as a 'super individual' (Scheffer et al., 1995). This allows to reduce computational complexity of a 'local subpopulation', considered as a set of individuals that live in the same habitat patch and are subjected to a common environment. 'PhenoPatch' incorporates these generic concepts and can thus be applied to various cases of biological systems, life-cycle structures and sources of environmental heterogeneities (e.g. potential applications in thermal biology; Table 1). Furthermore, by operating on reaction norms and accounting for population structure and processes, the model focuses primarily on two evolutionary forces affecting the evolution of populations: natural selection and random genetic drift. This model framework is thus able to account for the effect of phenotypic selection in populations of asexual individuals (clonal reproduction) in which phenotypes depend on genotypes that are inherited. As such, it can be used to reconstruct in vivo or compute in silico competition experiments between different phenotypes inhabiting a same environment. To facilitate a broad use of this generic model, it was developed on the widely used R environment software.

Table 1 Examples of biological systems which may be studied by the 'PhenoPatch' model.

Organism	Habitat	Patch	Environment	Further references
dragonfly larvae	marshes and ponds	water-filled hole	water and air temperature	(Gresens et al., 1982; Suhling et al., 2015)
amphibians	wetland	stormwater sites	water temperature	(Lucas & Reynolds, 1967; Forrest & Schlaepfer, 2011)
intertidal organisms (e.g. algae, mussels or ochre sea stars)	intertidal zones	rocks	rock temperature	(Pincebourde et al., 2008; Pearson et al., 2009; Helmuth et al., 2010)
herbivorous insects (e.g. aphids, leaf miner)	apple orchards	apple leaf	leaf temperature	(Pincebourde et al., 2007)
	crop canopies	leaf	nutritional quality	(Müller et al., 2001)
phyllosphere microorganisms (e.g. foliar pathogens)	crop canopies	leaf layer	leaf temperature	(Boixel et al., 2020)
			UV-B radiation	(Paul, 2000)
predator-prey systems	large number of coupled patches	patch	local densities of heterospecifics	(Sjödin et al., 2013)

Model description

The model has been conceived to investigate how a given population performs in a habitat which is characterised by a dual dynamics in terms of patch configuration and environmental signal over time (Fig. 1). It operates on inputs describing specific demographic and connectivity information of a set of patches coupled with the characteristics of the initial population under investigation (Fig. 2). Notably, it includes one module that manage the virtual or experimental library of reaction norms and their typology (constitution of phenogroups based on a Hierarchical Clustering on Principal Components – HCPC - approach). The model operates on discrete time steps. Each time step represents a potential full life cycle. At each time step, a loop updates the system and the age of all the individuals to identify only the reproductive individuals. For these individuals, the loop goes successively through the "growth", "asexual reproduction", "release", and "survivorship" steps (Fig. 3). The 'performance' of each reproductive individual present in a patch is defined based on a three-step approach: (i) evaluation of individual experience of its environment within the patch based on the extraction of the temporal pattern of the variable of interest since the end of the last global timestep and division of this temporal pattern into computational time steps corresponding to the time scale of the individual response to environmental exposure; (ii) integration of the computation of individual performance for each of these computational time steps based on its reaction norm taking into account both the nonlinearity and the variance of the environmental signal

(computation under variable conditions using the static reaction norms established under constant conditions; Chesson, 2012); (iii) relation of this cumulative integrated performance value obtained over all computational time steps to fitness (offspring production) using a correspondence scale defined on a maximum performance and fitness reference values (Fig. S3). The model thus simulates changes in population composition based on a discrete update of variables (Table 2). Regarding temporal considerations, the simulated processes operate on different time scales that have to be defined prior to the simulation in an input file describing the characteristics of the living system under investigation. A scheduler will orchestrate their synchronization during the simulations. The time increment is calculated when the next update cycle takes place and the respective event is generated (these time steps can be of equal length or dependent on the environment; e.g. expressed in degree-days). Furthermore, we have paid attention to allow the possibility for modulating model features that have been shown to affect the outcomes of evolution in spatially heterogeneous environments and/or lead to different selective regimes for the evolution of local adaptation (see below and Johst & Brandl, 1997; Ronce & Kirkpatrick, 2001; Ravigné et al., 2004; Débarre, 2010; Massol et al., 2011).

Life cycles and the timing of dispersal – The temporal order of dispersal (i.e. the redistribution of offsprings between time step t and time step t+1) with respect to reproduction govern whether there is local, global or both density regulation. Density regulation may intervene before, during and after dispersal. This means that the order of events in the model life-cycle is easily alterable in the 'PhenoPatch' model allowing to tackle alternate life histories and selection regimes depending on the biological system under the study (e.g. the Levene soft selection, Dempster hard selection, Ravigné type 3 and Débarre & Gandon's regimes; see Fig. 1-2 in Débarre & Gandon, 2011).

Demography and relative population maladaptation – The model considers a metapopulation corresponding to a set of subpopulations present in a set of patches and connected in space and time through dispersal events. This framework allows to relax or not the assumption of a constant carrying capacity across sites (fixed and equal population sizes in all patches). The overall carrying capacities can be either described by an equation describing population growth (e.g. logistic or exponential) or by providing experimental data describing actual field dynamics. As such, the model adopts the option to block the demography before entering simulations, a common approach when modelling population genetics, for which a certain number of sites is opened at each generation and then filled stochastically. However, the model was conceived to allow the conduction of virtual experiments under an additional correction. When non-implemented, the number of open sites is independent of the genetic composition of the population. This means that population growth will be identical whether the population is globally made up of more or less well-adapted individuals to the simulated environment (soft selection regime which is expected to favour diversity). When implemented, this correction allows to modulate the metapopulation growth so that the number of open sites depends on the fitness of the population actualized at each time step. This means that population growth does not formally stick to input constraints (e.g. field data) as we allow that the population can be invaded very quickly by a single genotype or conversely very slowly (hard selection regime which is expected to counter-select diversity). We allow the operator to set an absolute fitness (mal)adaptation defining a threshold serving as a quantitative absolute reference point (global fitness optimum). Then, we can correct population growth depending on population mean absolute fitness.

Spatial configuration – Dispersal is the process that binds the subpopulations of a metapopulation together. In the model, the operator is able to control whether the initial distribution of the functional diversity in the different patches would be done (i) at random; (ii) in a balanced (equiproportion of each phenogroup in each patch); (iii) in a clustered way (presence of a phenogroup in an exclusive group of patches).

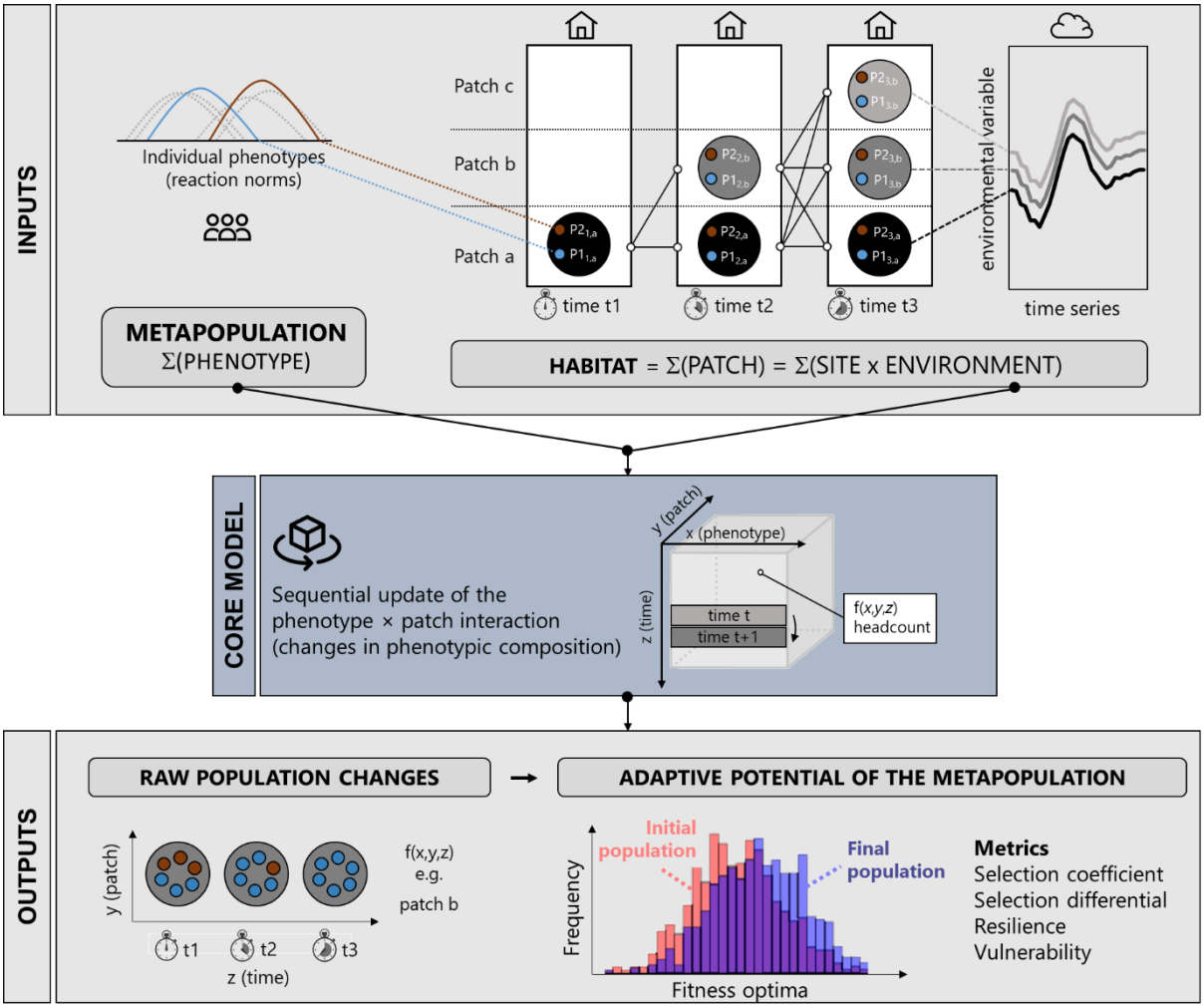


Fig. 1 Structure and components of the 'PhenoPatch' model. This phenotype × patch-based model enables to monitor the temporal changes in the composition of a closed local metapopulation evolving in a habitat divided into patches whose environmental heterogeneity is accurately characterised. The model operates on input-driven data describing the initial phenotypic composition of the metapopulation and the properties of the patches. Patches are characterised by their spatial position (e.g. architecture of a crop canopy; Fig. 4), an environmental signal (e.g. temperature), and an occupancy rate (e.g. size of phyllosphere microbial population). There is a successive update of the system (metapopulation × patch × environmental signal) at discrete time steps incremented using an algorithm describing the generation cycle and applied sequentially to each individual (Fig. 3). This allows the computation of the changes in the number of individuals per phenotype (x), per patch (y) and across time steps (z; e.g. from time t to time t+1). The comparisons of initial and final metapopulations lead to compute four metrics (selection differential, selection coefficient, resilience and vulnerability; Table 1) used to characterise the adaptive potential of an initial population in a given environment at a given time.

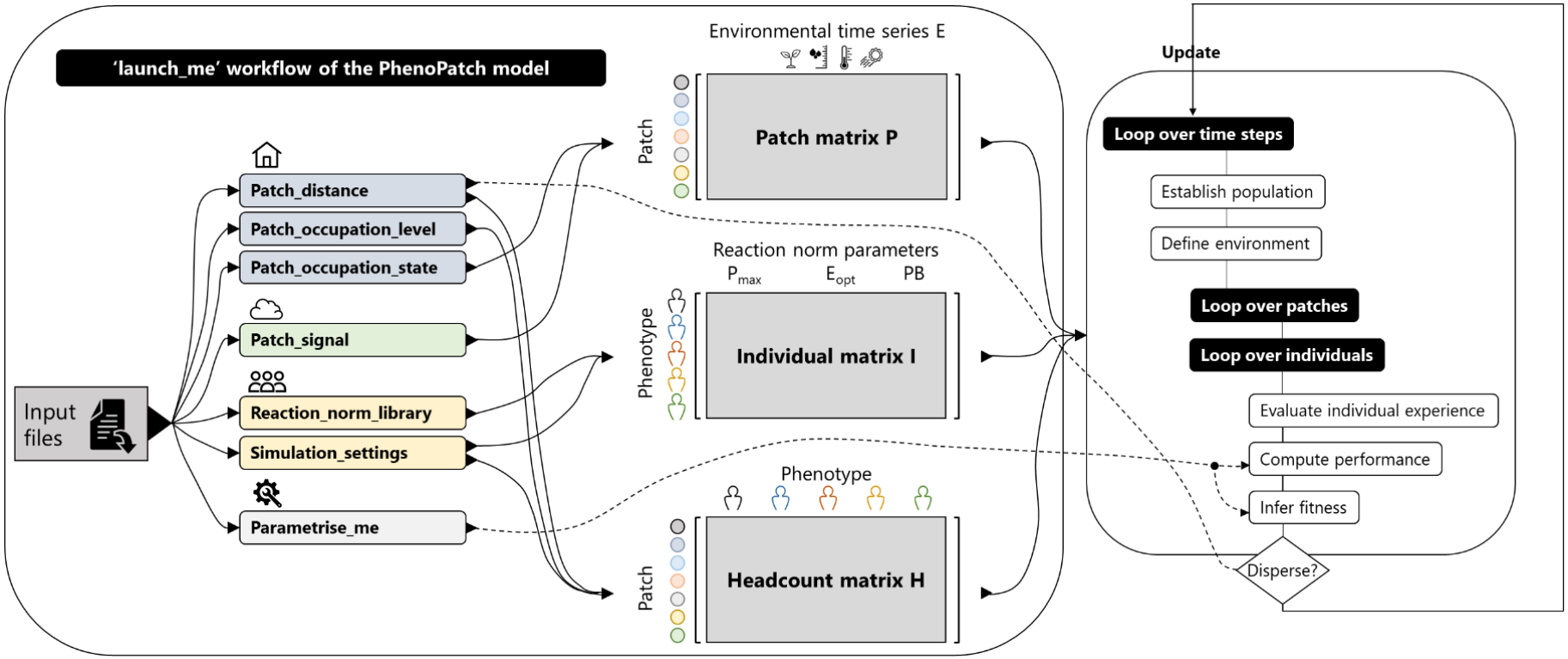


Fig. 2 Raw data and groundwork matrices on which the 'PhenoPatch' model operates. The 'launch_me' workflow load all supplied files containing information defining occurrence in a set of temporal and/or spatial samples (Patch_occupation_level, Patch_occupation_state files), environmental (Patch_signal file), trait (Reaction_norm_library), diversity (Simulation_settings defining how the meta-/sub-populations and phenotypes therein are going to be drawn from the Reaction_norm_library) and system specificities data (e.g. population regulation and demography, dispersal processes between patches, computation of performance and fitness) measured over the considered spatio-temporal sampling units. This information is used to link them to and populate the Patch, Individual and Headcount matrices allowing the subsequent computation of fitness for each phenotype in each patch at each snapshot computational time step. This is the combination of this information on the selective values of each individual and on demographic stochastisticity (effects of ecological processes on offspring establishment success) that allows to inform and update the 'PhenoPatch' dynamics over the simulations.

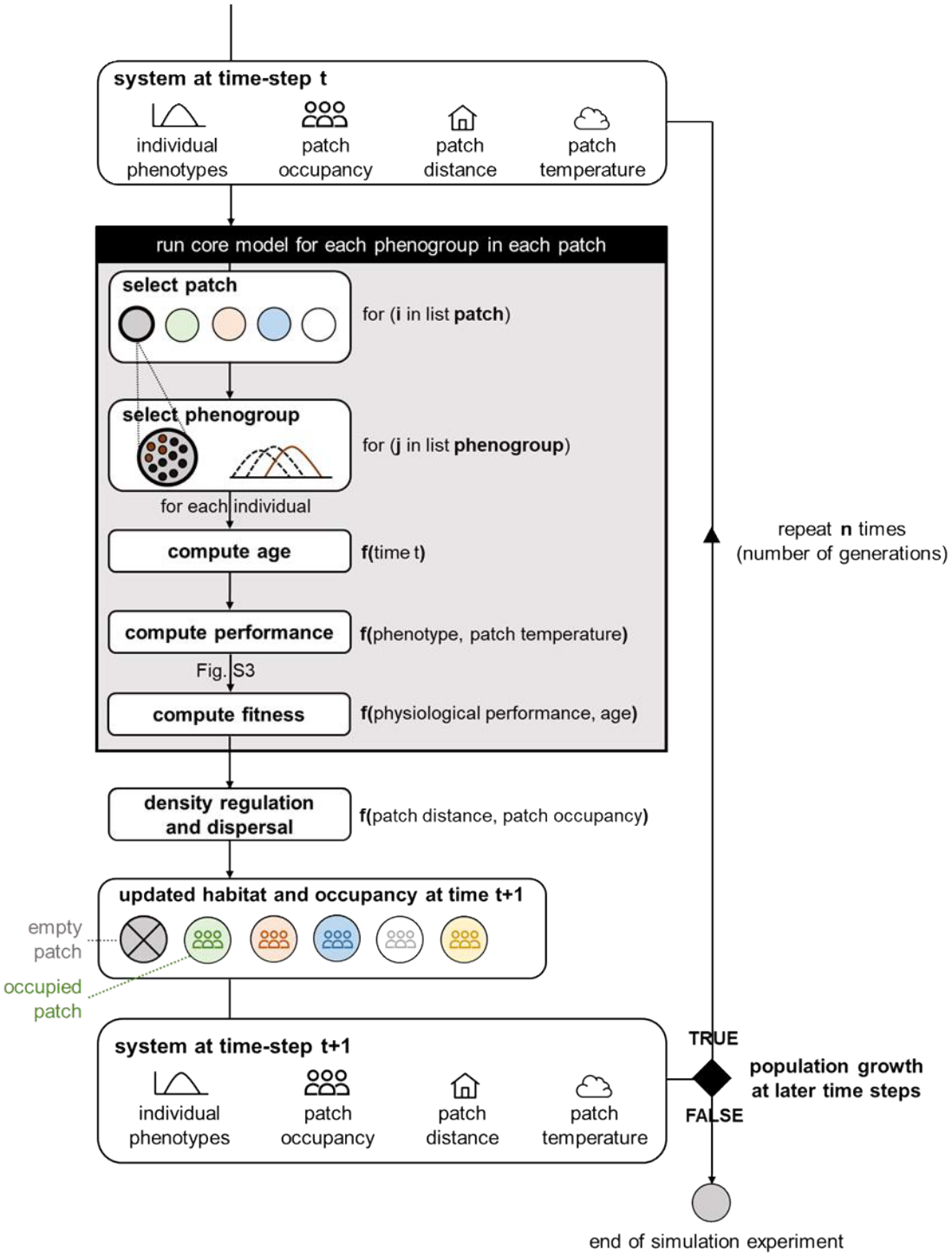


Fig. 3 Time-step incremental algorithm allowing the update of the 'PhenoPatch' model dynamics. At each time step, the model runs sequentially through all patches to compute the age, performance and fecundity of each individual in each phenogroup (individuals sharing the same reaction norm). After computing the offspring production of each individual in the different patches of the habitat, the model proceeds to density regulation and dispersal to determine which offsprings effectively contribute to the next generation. Each generation cycle is only applied to the active part of the population (i.e. reproductive individuals). In the case of the wheat phyllosphere microorganism Z. tritici, it concerns only sporulating lesions and not latent or empty lesions. The updated system at time t+1 becomes the future system t except when simulations are stopped due to population extinction or on the instruction of the operator.

Table 2 Definition of model input and output variables categorized according to their hierarchical levels (Phenotype, Population, Habitat, and Environment).

INPUTS	
Phenotype	
Group of responses	typology of reaction norms (RN, e.g. thermal performance curves)
Maximum performance	fitness optima of a given individual (RN height)
Environmental optimum	environmental value at which individual fitness is maximum (RN position)
Performance breadth	generalist-specialist variation in environmental sensitivity (RN width)
Population	
Initial size	number of individuals composing the initial population
Richness	number of and individual variation within thermotypes
Evenness	frequency of thermotypes in population
Spatial distribution	random, regular, or clumped distribution of thermotypes in the environmental patches
Habitat	
Patch connectivity	inter-patch matrix through which dispersers move
Patch occupancy	within-patch dynamics in terms of number of individuals
Environment	
Patch heterogeneity	temporal variation in resource or environmental signal (e.g. temperature) in each patch
OUTPUTS	
Raw headcount	number of individuals per patch, per phenotype and per time
Selection coefficient	average growth rate of the selective value of the individuals making up the investigated population
Selection differential	difference in mean phenotype[1] before and after selection
Resilience	maintenance of initial phenotypic diversity[2]
Vulnerability	deviation of mean population phenotype from overall phenotypic optima[3]

[1] the value of the population mean for the given trait

[2] the ability of the population to absorb disruptions and reorganize itself in such a way as it maintains the same level of diversity as the initial state, based on the quantification of deviation from initial indices of richness, evenness, differentiation, pattern.

[3] we computed the performance of the 7983 individuals composing the virtual performance curve library over all recorded leaf temperature series of theoretical infection cycles in the 2016-2017 experiment. The 90th percentile of these computed integrated performances was used to define the overall phenotypic optima. We defined vulnerability referring to this standard value. Indeed, the more the average value of the integrated performance of individuals over the duration of the numerical simulation deviates from it, the more the population can be expected to be less aggressive.

'PhenoPatch Zymo' model application

The 'PhenoPatch' model was tailored to investigate phyllosphere microbial population responses to heterogeneous and variable thermal environments using the wheat fungal pathogen Z. tritici as a case study. To make it easier to read and link the common concepts between the generic 'PhenoPatch' model and this application, called 'PhenoPatch Zymo', one can refer to Table 3. Z. tritici populations are restricted to discrete patches of habitat corresponding to different horizontal vegetation ranks of a wheat canopy characterised by a single thermal signal (Fig. 4). This subdivision in leaf layers generates a dynamic and structured collection of sites that has already been adopted in previous crop pathosystem models (Milne et al., 2003, 2007; Audsley et al., 2005). In such systems, an individual corresponds to a lesion (i.e. a unit of disease that is considered as genetically homogeneous). A lesion, resulting from a single spore infection, can generate several new lesions by secondary infections after it ultimately produces spores itself. At every cycle, only a proportion of spores produced by the lesions will land on new sites and initiate new lesions following spore losses occurring before (wash-off effects), during (non-interception by canopy healthy sites) and after (failing of establishment and/or development) dispersal. Another infection cycle is initiated and new individuals appear once a spore successfully infects a site (Fig. 5). Each individual exists in one of three states in the model: latent, infectious, dead (echoing susceptible-infectious-removed – SIR - epidemic models describing the number of hosts susceptible to, infected with and recovered from a disease). In the following numerical experiments, performance was linked to a single fitness component (spore production), disregarding differential effects on other traits such as development rates. Indeed, as a first approach, generation time (i.e. latent period) was set to be equal for all individuals. This duration was defined in degree days to account for the temperature-dependency of development rates. The sporulation date of individuals having a similar phenotype in a given cohort was thus modulated by local environmental conditions in the different patches. All model processes and parameters are summarised in Table 4 and Table 5 with their appropriate values and sources.

Table 3 Correspondence between ecological and epidemiological concepts implemented in the generic model 'PhenoPatch' and its derivative 'PhenoPatch Zymo'.

'PhenoPatch'	'PhenoPatch Zymo'
patch	crop canopy leaf layer
environment	leaf layer temperature
reaction norm	thermal performance curve
fecundity	sporulation capacity of an individual
fitness	offsprings actually contributing to the next generation
generation time	latent period
individual	lesion (disease unit)
dispersal unit	spore
migration	dispersal of individuals from inoculum sources to sink sites
selection	competitive ability

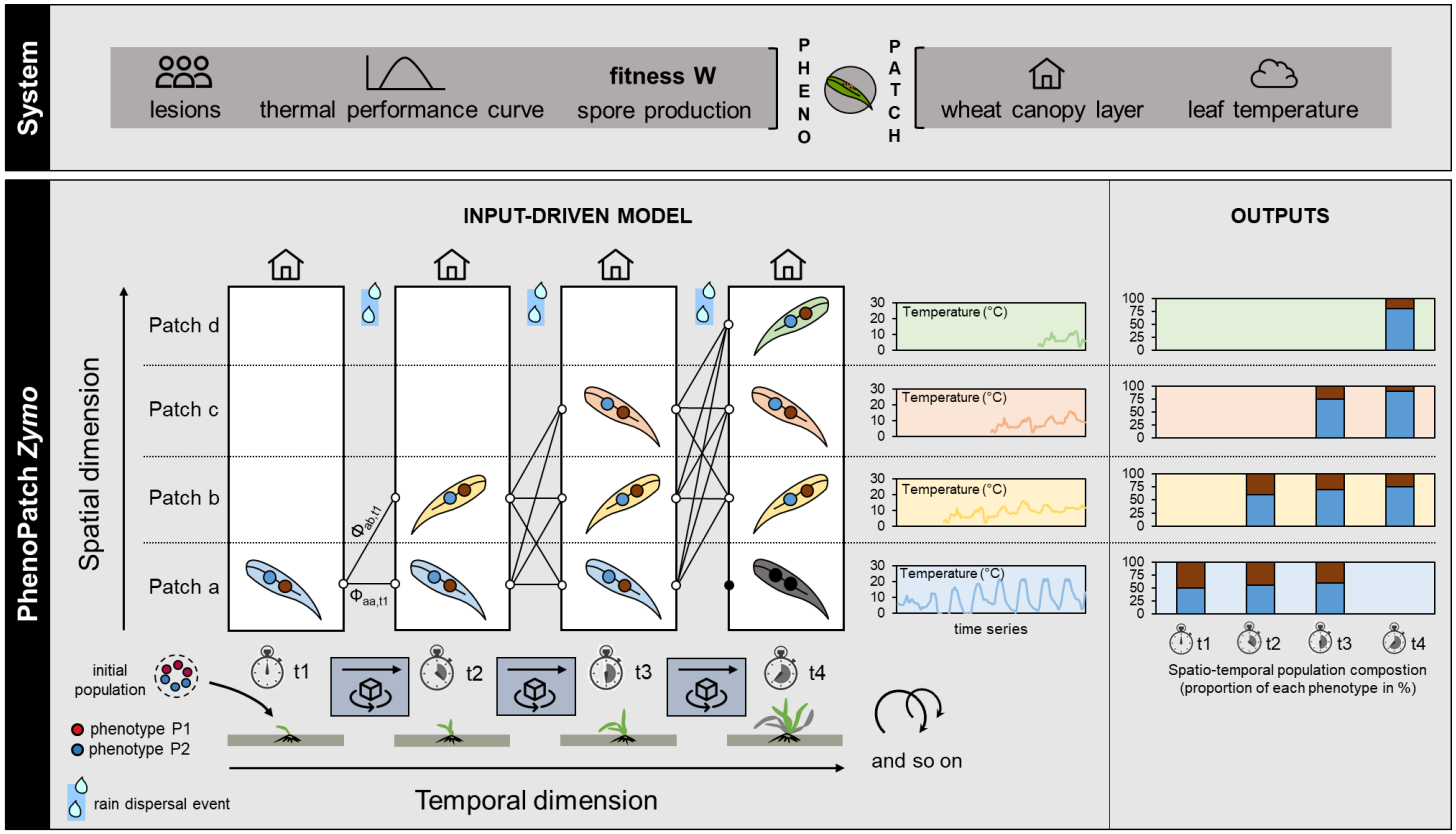


Fig. 4 Application of the 'PhenoPatch' model to the thermal ecology of the phyllosphere microorganism Zymoseptoria tritici. The 'Habitat' is divided into patches that correspond to horizontal vegetation ranks (here, a given wheat leaf layer in the canopy) as they constitute (i) an ontologically and trophically homogeneous unit; (ii) a thermally homogeneous unit, as vertical temperature profiles adequately reflect the spatial distribution of air temperature within a canopy (Stigter et al., 1976; Graser et al., 1987; Chelle & Cellier, 2009; Fig. S5 in Boixel et al., 2020). The 'Environment' corresponds to leaf temperature time series as this is the actual signal perceived by phyllosphere microorganisms. The 'Population' is composed of individuals defined as the smallest distinguishable groups of microorganisms (here, one individual disease unit is one Septoria tritici blotch lesion caused by a single Z. tritici genotype) that exhibit each a homogeneous thermal 'Phenotype' (characterised by a thermal performance curve from which we inferred its fitness, i.e production of offsprings, here asexual spores). Time steps are defined by the occurrence of dispersal events of the next generation of microorganisms (discrete rainy events allowing the redistribution of Z. tritici spores, between the leaf layers by rain splash both upward and downward; Shaw, 1987). The passage from one time step to another is done by applying the time-step incremental algorithm (Fig. 3) to each individual which allows to update the frequencies of each phenotype in each patch at each timestep, as illustrated with the raw barplot outputs.

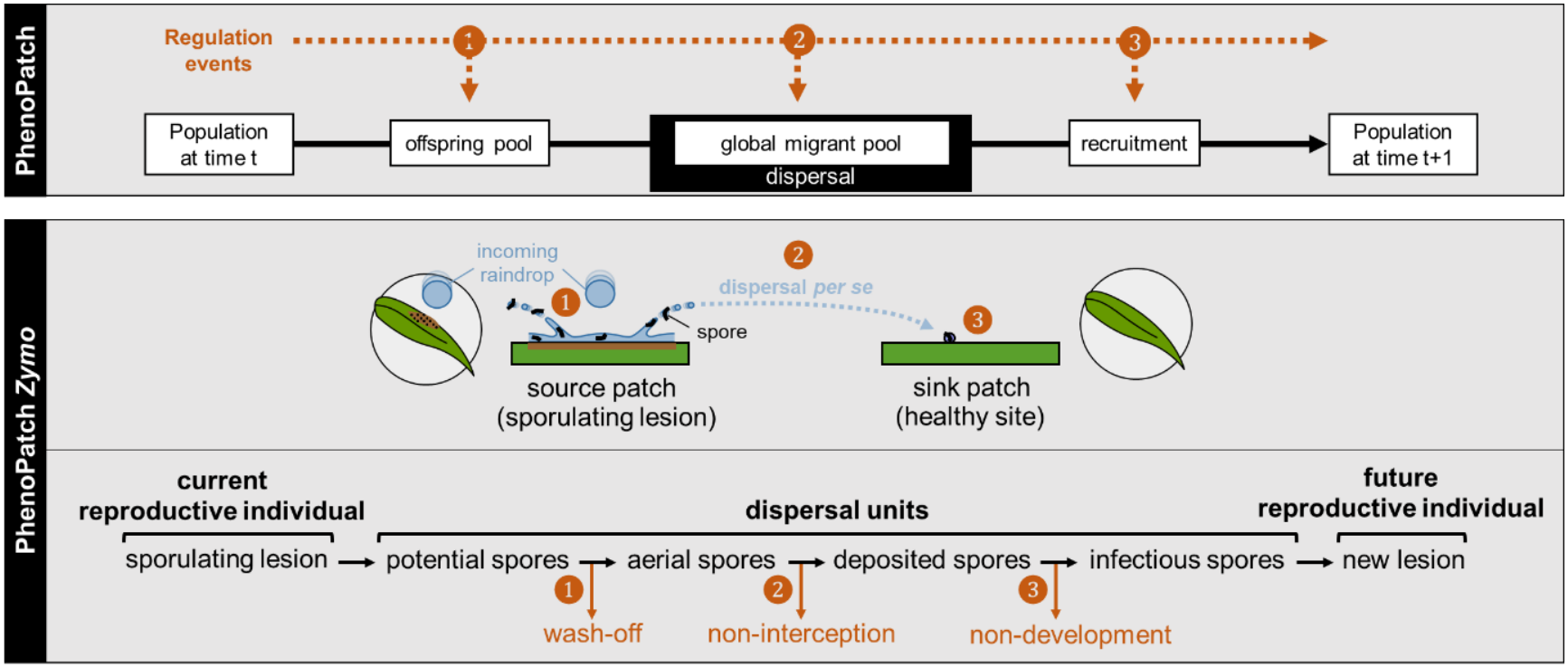


Fig. 5 Regulation of reproductive and dispersal events in the 'PhenoPatch' model exemplified with the fate of Z. tritici dispersal units (offsprings) within the crop canopy from 'source' to 'sink' patches. All offsprings (i.e Z. tritici spores) produced from a 'source' patch do not contribute to the next generation. Several of them exit from population dynamics during dispersal events. Regulation brings density back to carrying capacity with only a fraction of the spores produced by a lesion that will ultimately start a new lesion thus initiating a new infection cycle. Both local and global density regulation processes are incorporated in the model through spore "losses" due to: (1) wash-off randomly applied to the spore pool produced by each 'source' patch; (2) non-interception of spores by canopy elements during dispersal, as a function of the distance between 'source' and 'sink' patches applied after overall spore pooling; (3) non-development of spores (failing of germination, penetration or colonization) that land on the leaves forming a 'sink' patch.

Table 4 Mathematical representation of dynamic individual fitness and population processes for calibrating the model to the wheat phyllosphere microorganism Z. tritici.

Function	Description	Equation	Description
Phenotype performance and fitness			
Performance (Eq. 1)	sum of integrated performance at each computational time step μ (i.e. every 4 hour)	$\sum_{\mu} \bar{P} = \sum_{\mu} P(\overline{T_{leaf}}) + \frac{1}{2} P''(\overline{T_{leaf}}) \sigma^2_{Tleaf}$	scale-transition theory (Chesson, 2012; Dowd et al., 2015; Koussoroplis et al., 2017)
Fitness (Eq. 2)	relation between performance and fitness in the sense of selective value	$\Omega = \frac{\sum_{\mu} \bar{P}}{s_{max}} \times \Omega_{max}$	
Population processes			
Growth (Eq. 3)	dynamics of increase in the number of lesions on a wheat canopy: we assumed that every plant followed the same dynamics regarding the number of lesions developing on it. As such, the variable that will modulate pathogen population growth is the size of the host plant population or the size of the canopy plot defining the numerical environment and considered during the simulations.	$N(t) = n_{plant} \times \frac{\alpha}{1 + e^{\beta - \gamma \times t}} \times CT_{APP}$	logistic model (Van Der Plank, 1963)
Reproduction (Eq. 4)	temporal integration of spore production rate as a function of time since the iniation of infection ($time\ elapsed\ \tau$) s(t): number of spores available at the dispersal event t	$s(t) = \begin{cases} \int_0^t S(t) = \int_0^t \begin{cases} 0\ if\ \tau < \varphi \\ \Omega \frac{\lambda^{\omega} \times (\tau)^{\omega-1} \times e^{-\lambda(\tau)}}{\Gamma(\omega)}\ if\ \tau \geq \varphi \end{cases} & (\boldsymbol{a}) \\ \int_{t-1}^t S(t) = \int_{t-1}^t \begin{cases} 0\ if\ \tau < \varphi \\ \Omega \frac{\lambda^{\omega} \times (\tau)^{\omega-1} \times e^{-\lambda(\tau)}}{\Gamma(\omega)}\ if\ \tau \geq \varphi \end{cases} & (\boldsymbol{b}) \end{cases}$	cumulative response of delayed γ distribution for (a) the first and (b) subsequent dispersal events (Kermack & McKendrick, 1927)
Dispersal (Eq. 5)	change in the number of infections over distance d from an inoculum source a to a sink source b: we assumed that the number of spores reaching a target patch b decreases exponentially with the distance from the inoculum source (originating patch a). As the wheat model is operating on leaf layers, we only had to consider disease vertical gradients that were calculated from the height of the leaves.	$\Phi_{a,b} = A \times e^{-B d_{a,b}}$	simple exponential model (Kiyosawa & Shiyomi, 1972)

Table 5 Measured and estimated parameter values for tailoring the model framework to the wheat phyllosphere microorganism Z. tritici.

Parameter	Description	Value	Unit	Source
Population growth (number of lesions NL(t))				
α	continuous shape parameter	124.90	NA	Fig. S1
β	continuous scale parameter	5.54	NA	
γ	continuous location parameter	0.03	NA	
CT_{APP}	correction term taking into account the level of aggressiveness of the pathogen population under investigation	f(fitness population)		
Reproduction (individual production of spores s(t))				
Ω	total number of spores produced per lesion during its entire lifetime	[50; 216,000]	spores	(Suffert et al., 2013)
λ	parameters determining the shape of the sporulation curve during the infectious period	0.33	NA	Fig. S2
ω		12.42		
φ_w	latent infection or threshold to be exceeded for sporulation (winter period)[1]	327.5	degree-day	(Suffert et al., 2015)
φ_s	latent infection or threshold to be exceeded for sporulation (spring period)[1]	418.6	degree-day	
SD	length of sporulation period	70	days	Fig. S2
s_{max}	Maximum cumulative performance[2]	293.8	NA	Fig. S3
Dispersal $\Phi_{a,b}$				
A	proportional to the amount of inoculum produced at a source location	100	NA	percentage driven reasoning
B	influences the steepness of the dispersal gradient	0.08	NA	Fig. S4
AC	autoinfection correction	5	NA	(Mundt, 2009)
RO[3]	local regulation due to runoff of water on leaves[4]	10	%	
IE[3]	infection efficiency[5]	8	%	(Sache & Vallavieille-Pope, 1995; Fones et al., 2015; Karisto et al., 2019)

[1] this corresponds to the individual generation time, i.e. the interval between infection and the onset of sporulation from that infection

[2] correspondence scale parameter relating cumulative integrated performance (computed on reaction norms) to fitness (offspring production)

[3] see Fig. S5 for details of timing of density regulation with respect to dispersal of local and global pools of Z. tritici spores

[4] the probability that a spore is washed off by incoming rain drops

[5] the probability that a spore landing on a healthy site succeeds in initiating a new infection

Model evaluation

To evaluate the model, we first confronted model predictions with experimental data acquired and analysed in a previous study (Experiment B in Chapter 4). Simulations were designed to mimic the experimental settings of this two-thermotype competition experiment between three cold- and three warm-adapted strains conducted under a cold (17 ± 1°C) and a warm (21 ± 1°C) regime (Table 6). To reach the same number of replicate lines and as such the same level of statistical power than this empirical experiment, this numerical experiment was conducted only three times (3 replicates lines). We modeled predictions against measurements of the changes in the proportion of the cold-adapted thermotype and strains using a chi-square test statistic.

Table 6 Input data for evaluating the model (calibration to the experiment B in Chapter 4)

	Simulation settings
Phenotypes	Thermal performance curve (TPC) of the three cold-adapted (CA_4, CA_5, CA_6) and the three warm-adapted (WA_4, WA_5, WA_6) individuals
Patch occupancy	Number of individuals (lesions) on each leaf during the competition experiment
Patch connectivity	Distance between the first three leaves of the wheat seedling populations used in the experiment
Patch temperature	Leaf temperature of the first leaves of wheat seedlings measured with thermocouples during one infection cycle
Individual state	Latent (non reproductive stage), Infectious (reproductive stage), Removed (no longer infectious lesion, equivalent to a “dead individual”)
Time window	time steps of equal length (every 20 days) over 5 non-overlapping Z. tritici generations

Numerical experiments

Model calibration on a natural field epidemic – ‘PhenoPatch Zymo’ was operated on field data inputs to reconstruct an actual Septoria tritici blotch epidemics occurring in a wheat canopy. Input data were collected over a field experiment conducted at the INRAE experimental station in Grignon (France) in the 2016-2017 growing season (experiments C_1-C_2 in Chapter 4). During this experiment, we retrieved measurements of three types (Fig. 6a). First, plant architecture was characterised: leaf layer height above ground was measured to infer patch connectivity (position of the secondary inoculum sources – i.e. infectious lesions - within the canopy; n = 5 plants monitored over the growing season). Second, leaf temperature was measured using thermocouples on the epidemiologically active leaf layers (i.e. those bearing infectious lesions) to have access to actual patch temperature (n = 6 plants per leaf layer). Finally, disease epidemic was monitored to characterise secondary inoculum location both in terms of lesion number and position to quantify patch occupancy. Patch occupancy or carrying capacity at each site corresponds to the mean number of lesions per leaf layer (n = 45 plants on 18 monitoring dates). In order to stick as closely as possible to the disease dynamics observed in the field, the discrete event system was set to sampling intervals determined by the occurrence of rainy dispersal events (in other words, we allowed for overlapping

generations to occur). Simulations were runned over the course of an annual epidemic (over which one can expect between 4 and 6 non-overlapping and complete disease cycles; Table S1).

Competition case study - For a given simulation of a pairwise competition experiment, the initial Z. tritici population consisted of two thermotypes (a cold-adapted one with a T_{opt} around 17°C and a warm-adapted one with a T_{opt} around 21°C). The initial size of the pathogen population was set to a reference value of 0.92 lesions per plant for the first cycle (3 uninoculated plots during the 2016-2017 field experiment) over a crop canopy of 10 m² with a wheat plant density of 220 plants per m². The number of opened sites at each time t was confined to the the population dynamics observed in the field with a correction term CT_{APP} that depended at each time t of the current fitness of the simulated population to take into account the effect of maladaptation on population size and density: N_{t+1}-N_t = f(mean population fitness). That is to say, a certain number of lesions are opened at each generation and are then filled stochastically. At each timestep, new patches corresponding to new leaves resulting from plant growth were created. These patches were considered as "opened" meaning that they could be infected. Meanwhile, old leaves were removed from the system after reaching senescence and corresponding patches were closed. A theoretical infection cycle was defined from the occurrence of a significant rain event until reaching a standard duration value of latent period (250 degree-days above the base temperature -2.4 °C; Lovell et al., 2004).

Numerical experiment 1 – We explored the joint effects and relative importance of composition in phenogroups (functional diversity in terms of richness, evenness and divergence; Mason et al., 2005) within a population with or without a clumped spatial pattern to investigate how and to what extent interindividual variation may affect population dynamics and the adaptive potential of a population in response to thermal changes. A series of numerical experiments was conducted by simultaneously manipulating phenogroup richness (1, 2, 25, 50, 75, 100 phenotypes per phenogroup), evenness (strong, slight or no imbalance between phenotypes), differentiation (strong, slight or no gap between the two extreme phenotypes of each phenogroup), and spatial pattern (clumped, random, regular initial affectation to patches). Their joint effects and relative importance were evaluated, both as drivers of changes in the proportion of phenogroups and in terms of the adaptive potential of population in response to the simulated environment (see illustration of the modulations of two functional diversity components in Fig. 6b).

Numerical experiment 2 – We explored how much of phenotypic diversity maintenance within a local population is due to the existence of local heterogeneity in the actual environment perceived by organisms, here expressed as local thermal patches in a crop canopy. This was done by modelling the changes in phenotypic composition of a Z. tritici population composed of a cold-adapted (T_{opt} = 17°C) and a warm-adapted (T_{opt} = 21°C) thermotype in equiproportion and evolving in a wheat canopy. In this canopy, the environmental conditions between patches were set to be either heterogeneous (unique temperature time series per leaf layer as recorded in the field by thermocouples) or homogeneous (same temperature time series for each patch as set to air temperature variation from weather station data) in terms of thermal signal (Fig. 6c).

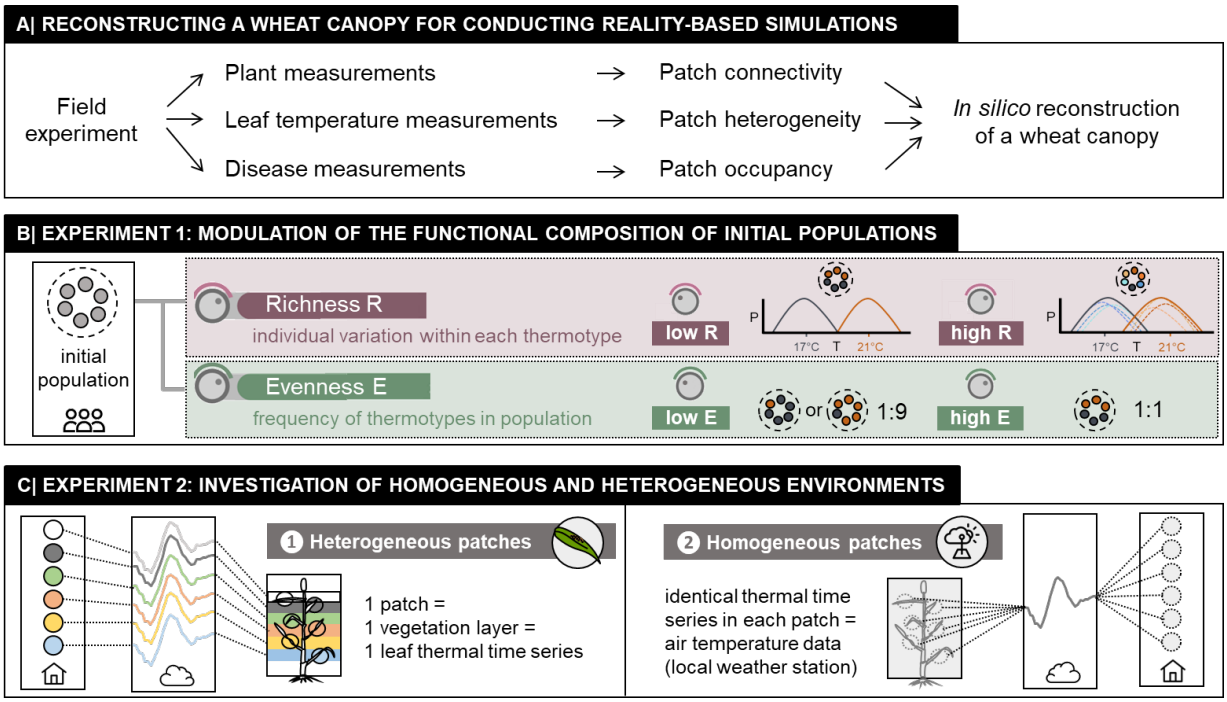


Fig. 6 Numerical experiment design. Simulations of 'PhenoPatch' applied to the Z. tritici case study were performed based on the virtual reconstruction of an actual field epidemics. (A) This required the provision of input data describing the corresponding specific demography and spatial distribution that were recorded in a 2016-2017 field experiment. Plant disease progress was reconstructed based on the position of Plant leaves (height above ground to retrieve vertical distance between them), recordings of Leaf temperature of epidemiologically active leaves (i.e. leaves with lesions) and measurements of Disease (lesion number and position). These experimental data helped to quantify patch connectivity, heterogeneity and occupancy, respectively. In this virtual 'PhenoPatch' simulation setting, two competition experiments between cold- and warm-adapted strains were performed to investigate: (B) the effects of initial population levels of functional diversity on the adaptive potential of the population (n = 5 repetitions per modelling scenario) varying initial: (i) richness (number of different thermal responses per thermogroup), (ii) evenness (from equi- to uneven proportions), (iii) differentiation (extent of intra- and inter-thermogroup functional differences), and (iv) spatial distribution (random, clumped, cluster distribution); (C) the effects of spatio-temporal heterogeneity between patches on population dynamics (n = 5 repetitions per modelling scenario) through comparisons of two competition cases of an initial population composed of 2 thermotypes in equiproportion randomly distributed in the different patches either subjected to (i) real leaf temperature time series (heterogeneous environment across patches) or (ii) local air temperature (homogeneous environment across patches).

Statistical analyses – The model was run 5 times per scenario. These repetitions, considered as equivalent to replicate lines in in vivo selection experiments, were required due to the random effects of population density regulation (e.g. founder effect or spore losses during dispersal). Statistical analyses were conducted on two types of outputs. The raw outputs correspond to changes in proportions per patch, phenotype and time step, and were analysed using chi-tests. The processed outputs correspond to the components of the adaptive potential defined for one given population in a given environment at a given time, and were analysed using mean and variance comparisons.

RESULTS

Model evaluation (Fig. 7) – Model errors in estimating the proportions of the warm-adapted thermotype in the numerical two-thermotype competition experiments are of 7.1 ± 2.7 % with a maximum recorded at 10.6% across the 18 cases (i.e. 3 repetitions × 3 generation times G1-G3-G5 × 2 thermal regimes). Overall, the model provided comparable predictions of the relative proportion of warm-adapted strains on this simplified case (R^2=0.77 under the cold regime; 0.99 under the warm regime). Model predictions also underline the difficulty of reaching an equilibrium state between the strains placed in competition (founder effect at G1). More deviations appeared in G3 than in G5, highlighting that the effect of stochastic processes may progressively be reduced due to fitness costs. Finally, the proportions of warm-adapted strains were overestimated under the cold regime in the model predictions and underestimated under the warm regime. This may be due to the inherent mathematical properties of asymmetrical thermal performance curves which dictate that warm temperature regimes impair performance more than cold temperature regimes do (Fig. S5).

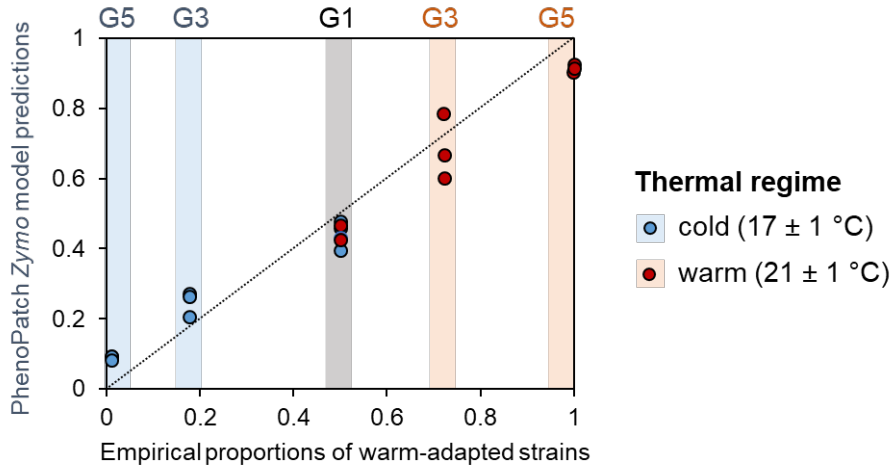


Fig. 7 Preliminary evaluation of the 'PhenoPatch Zymo' model on a simplified selection experiment. Predicted proportions of warm-adapted Z. tritici strains at the post-initial (G1), intermediate (G3) and final (G5) generation times were compared with the empirical ratio resulting from the corresponding two-thermotype competition experiment conducted under a cold (17 ± 1°C) and a warm (21 ± 1°C) regime (Boixel et al., 2020; experiment B, Fig. 3 – chapter 4). Experimental data and model predictions have been the subject of three repetitions (replicate lines and number of simulations per scenario, respectively). Fitness was predicted based on assessments of sporulation capacity (a component which is experimentally tractable) acquired in single-generation (monocyclic) experiments simultaneously to the course of the competition experiment.

Effect of initial individual variation and occurring environmental heterogeneity on competitive outcomes - Higher individual variation in the initial population is correlated with a better balance betwen cold- and warm-adapted thermotypes (Fig. 8). The comparisons of predicted proportions of each phenogroup under homogeneous and heterogeneous environments illustrate the importance of taking into account local heterogeneity between patches as it has a huge impact on the trajectory of populations (Fig. 9).

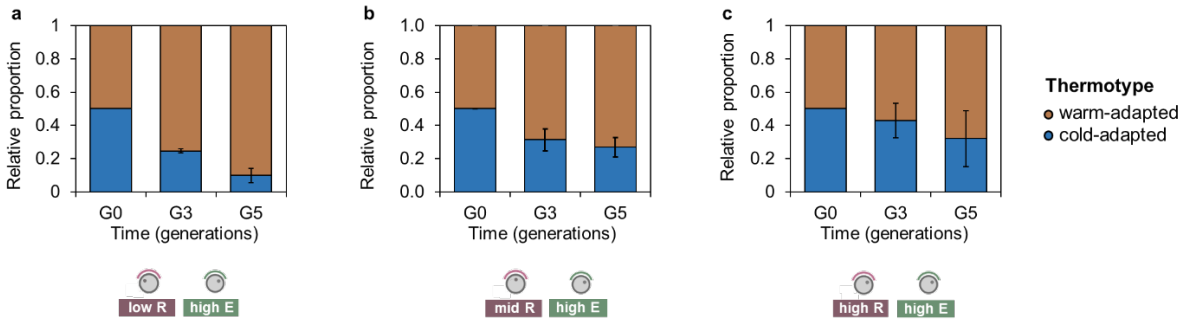


Fig. 8 Samples of simulation results: sensitivity of competition outcomes to the initial functional diversity in terms of richness of a Z. tritici population composed of cold- and warm-adapted individuals over five generations (numerical experiment 1; Fig. 6B). Outcomes display the relative proportion obtained for the post-initial (G0), intermediate (G3) and final (G5) generation times in the case of numerical competition experiments between cold- and warm-adapted Z. tritici strains under field leaf thermal regimes with initial populations of: (a) low richness and high evenness; (b) high richness and high evenness; (c) intermediate richness and low evenness in favour of the warm-adapted phenotype; (d) intermediate richness and high evenness (mean ± S.D.; n = 5 repetitions per modelling scenario).

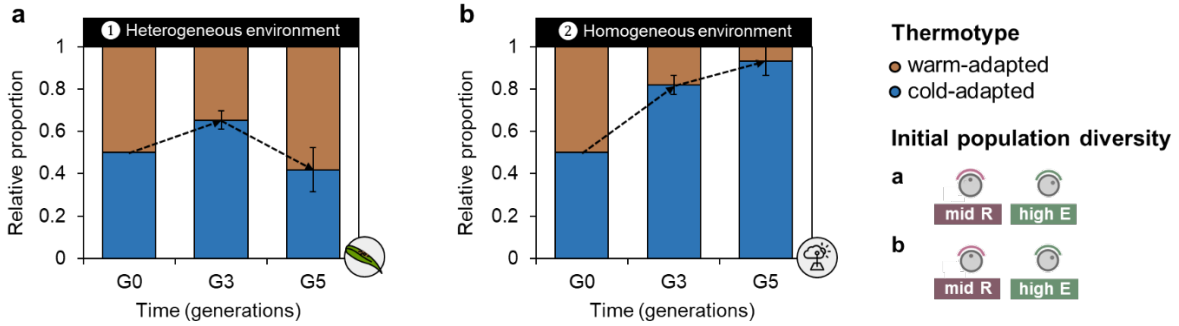


Fig. 9 Samples of simulation results: sensitivity of competition outcomes to local habitat thermal heterogeneity of a Z. tritici population composed of cold- and warm-adapted individuals over five generations (numerical experiment 2; Fig. 5C). Outcomes display the relative proportions of cold- and warm-adapted Z. tritici strains obtained for the post-initial (G0), intermediate (G3) and final (G5) generation times in the case of numerical competition experiments conducted in a: (a) heterogeneous environment in which all patches are subjected to its own temperature variations (leaf layer temperature time series at a given time t); (b) homogeneous environment in which all patches are subjected to the same local air temperature variations based on weather station data (mean ± S.D.; n = 5 repetitions per modelling scenario).

Effect of initial individual variation and occurring environmental heterogeneity on adaptive potential (Table 7) - We modulated the initial levels of functional diversity within populations and investigated their consequences on their adaptive potential to thermal variations (numerical experiment 1). The simulation outputs highlight a high importance of richness (inter-invidividual variation within phenogroups) on the four chosen components. To a lesser extent, they highlight an impact of evenness and pattern on the resilience metric, i.e. on the maintenance of initial phenotypic diversity. This latter impact is noticeable. It paves the way to explore the question of the existence of refugia inherent to spatially structured populations (as highlighted in Fig. 6 and Fig. 7 in Chapter 4). In the case of the investigation of the contribution of environmental heterogeneity to the adaptive potential of populations (numerical experiment 2), the further the environmental heterogeneity was summarised or averaged, the more selection coefficient and vulnerability were overestimated and resilience tended to be underestimated.

Table 7 Global effects of individual variation and environmental heterogeneity on the four components of adaptive potential. P-value: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' non significant 'NS'

	Richness[1]	Evenness[2]	Differentiation[3]	Distribution[4]	Patch heterogeneity[5]
Levels	1, 2, 25, 50, 75, 100	0[a] – 0.5[b]- 1[c]	0[d] – 0.5[e]- 1[f]	random[g] clumped[h] regular[i]	actual[j] neglected[k]
Selection differential	***	.	interaction with R **	NS	**
Selection coefficient	***	NS	main effect * interaction with R *	NS	**
Resilience	***	*	.	*	.
Vulnerability	***	NS	**	NS	NS

[1] number of phenogroups in a thermotype
[2] frequency of thermotypes in populations
[3] extent of intra- and inter-thermogroup functional differences in thermal performance curves
[4] random or constrained positioning of initial individuals (i.e. infections or lesions) within patches
[5] differentiation of patch based on their respective environmental signals
[a] strong imbalance between cold- and warm-adapted phenogroups
[b] slight imbalance between cold- and warm-adapted phenogroups
[c] no imbalance between cold- and warm-adapted phenogroups
[d] no differentiation between thermal performance curves of competing individuals
[e] slight differentiation between thermal performance curves of competing individuals
[f] strong differentiation between thermal performance curves of competing individuals
[g] all phenotypes are randomly distributed in the different patches
[h] similar phenotypes are aggregated in the same patches
[i] all phenotypes are equally distributed in the different patches
[j] each patch is characterised by its own leaf temperature time series (heterogeneous patches)
[k] all patches are subjected to the same thermal variations at a given time t (air temperature from weather station)

DISCUSSION

'PhenoPatch' provides an individual-based and spatially explicit description of discrete changes in phenotypic composition within a metapopulation exposed to an environment exerting selection on a quantitative trait. This conceptual framework accounts for spatial variation in the environmental conditions between patches, differences between individuals, and individual fitness. As such, it will prove useful in expanding our understanding of how environmental signals, inter-individual phenotypic variations and ecological processes interact and influence both population and evolutionary dynamics. This is particularly relevant in the case of studies where heterogeneity and variability are high and cannot be easily disentangle empirically.

Genericity of the conceptual framework - The modelling framework 'PhenoPatch' is generic as it operates on reaction norms with a flexible description and incorporation of life-cycle structures.

It is thus capable of handling different biological systems and accurate sources of environmental heterogeneities that actually influence population processes. The modelling framework has been tailored here for the foliar wheat pathogen Z. tritici but can be easily applied to other systems, in particular to other phyllosphere micro- or macroorganisms by making the necessary changes in terms of population demography, connectivity and diversity. The environmental conditions, already documented in the modelling framework, can be kept as it is or can be changed to take into account different temporal dynamics and spatial patterns. Such a quantitative information about inherent characteristics of other organisms and environmental conditions can be provided from the literature or from specific experiments. 'PhenoPatch' thus offers the possibility to answer specific questions or aid in the interpretation of experimental data, e.g. testing models against empirical data and limited ability to predict the response to selection in the wild (Pujol et al., 2018). Finally, this modelling framework provides new eco-evolutionary insights into a broad set of theoretical questions: it gives an integrated view of the potential of maintenance of phenotypic diversity in spatially heterogeneous and temporally variable environments, that can be further explored through running numerical experiments in multiple contexts.

Coupling of the conceptual framework with other programs - 'PhenoPatch' is a spatially-explicit model. So it can be extended by coupling it with other 3D approaches such as Functional-Structural Plant Growth (e.g. Adel-Wheat or Septo3D; Fournier et al., 2003; Robert et al., 2008) and Soil Vegetation Atmosphere Transfer (e.g. RATP; Sinoquet et al., 2001) modelling approaches in the case of plant disease epidemics developing in plant canopies, including those of cultivated crops, wild plant communities and forests. Such a coupling could be used to better understand: (i) a particular experimental case through the computing of the detailed descripton of plant and canopy growth and the associated micro-environmental variation in time and space; (ii) how different canopy structure and patterns of environmental conditions affect phyllosphere microbial populations. This can be investigated by performing numerical competition experiments under a wide range of experimental conditions allowing the extrapolation to a wider range of situations without resorting to heavy experiments.

Validation range of the 'PhenoPatch Zymo' model application - The results obtained with 'PhenoPatch Zymo' remain preliminary. Indeed, its validation has to go beyond a coarse coherence on a simplified experimental case. Before being broadly used, we have to check its robustness (e.g. assessing model coherence on comparisons of outcomes of competition experiments conducted in the field; experiment C1-C2 in Chapter 4) and to test the structural assumptions of the model using data acquired across a wider range of environmental conditions (e.g. impact of relaxing the assumption of non flexible latent and infectious periods in compartmental models). This could be done through sensitivity analyses intended to: (i) better understand the impact of certain model functions (e.g. context-dependency of maladaptation of populations; Brady et al., 2019) or modulating features (cf. 'Life cycles', 'Demography' and 'Spatial configuration' sections in Methods) on the outputs; (ii) identify any weak points in the model and suggest further modifications (e.g. accommodation of environmental covariances between traits and fitness; Rausher, 1992); (iii) broaden model usage (e.g. to test general ecological concepts such as the stabilizing role of high dispersal rates; Hastings, 1982). The results of sensitivity analysis runs could also be used to inform future experience-based research, for instance by highlighting the impact of data paucity and identifying the essential parameters and inputs to avoid the necessity to wedge the model on heavy or unnecessary measurements.

PhenoPatch limitations and extensions – The simplicity of 'PhenoPatch' makes it well suited for expansion and adaptation. Indeed, the model in its current state has several limitations. It does not consider:
- the balance of eco-evolutionary forces under fluctuating selection such as acclimation (Bowler, 2005; Lagerspetz, 2006) and derivation of allele frequencies under mutation–selection–drift balance (Taylor, 2008). This could be built in following an adaptive dynamics approach where a trait value is modified by a small amount at each mutation occurrence (Geritz et al., 1998; Dieckmann et al., 2006);
- a modulation of establishment or resource competition in patches accounting for the lottery representation of competition (Sale, 1977) or low density behavior with the incorporation of frequency dependence within patches (Comins & Noble, 1985; Day & Taylor, 1996; Ajar, 2003);
- sexual but asexual (clonal) reproduction which could influence in some cases the shape of within-patch selection (e.g. no counteraction of local extinctions of polymorphisms; Svardal et al., 2015) by overestimating the occurrence of disruptive selection (less overall maintenance of intermediate phenotypes under asexual mode which can emerge notably by recombination of diverging alleles at different loci; but see Rueffler et al., 2006).

Diversity maintenance and adaptive potential of populations - 'PhenoPatch' was applied here to explore the interplay between individual variation and environmental heterogeneity as a first approach based on a modulated factorial design. The first emerging trends from the simulations that were conducted resonate with a variety of existing models (e.g. Levene, 1953; Dempster, 1955; Deakin, 1966; Christiansen, 1974; Chesson & Warner, 1981). Indeed, the first oucomes clearly highlight the role of inter-individual variation and of the influence of spatio-temporal thermal heterogeneity on maintenance of phenotypic diversity in the different patches of an environment and impacted the metapopulation dynamics. Conditions occuring in patchy environments such as those encountered in crop canopies are so diverse, changing and unpredictable that the ranking of favoured individuals on the basis of competitiveness is continually altered from one time step to the other (Chapter 5). The preliminary outputs of 'PhenoPatch Zymo' suggest that this tends to facilitate the maintenance of increased levels of diversity in line with theoretical population biology (Svardal et al., 2015). In some cases however, the outputs showed that individuals maintain despite they are less adapted to all environmental conditions encountered in the different patches. It is not necessarily an aberration to have such a representation. Indeed, in the field, diversity would be all the more easily maintained as there is a strong negative genetic correlation between the ability to thrive under warm weather and survive under cold weather. This explanatory hypothesis is consistent with seasonal trade-offs highlighted in the fitness of individuals in numerous studies focusing on plant pathogens (Montarry et al., 2007; Hamelin et al., 2011), notably in the case of Z. tritici (Suffert et al., 2015, 2018). Furthermore, glossing over local heterogeneity led here to an estimated loss of balance between cold- and warm-adapted thermotypes over 5 Z. tritici generations (Fig. 7). In other words, downscaling the consideration of environmental signal to the physical environment actually perceived by organisms leads to underestimate the resilience of a local population, and thus its short-term adaptive potential: the further environmental heterogeneity is simplified or averaged, the more the selection coefficient is overestimated. Another noticeable point is the dependence of final competition outcomes to the modulation of the initial edge conditions of the system, in particular 'Evenness' and 'Distribution' (i.e. frequencies and spatial distributions of the different phenogroups composing the population into the starting patches; Table 4). The significant impact of these two components of functional diversity on resilience paves the way to explore the question of the existence of

refugia inherent to spatially structured populations. Concretely, depending on the spatial starting point of the system (e.g. founder effect related to primary inoculum in plant disease epidemiology), a population may evolve differently if the individuals of a phenogroup are all on a given patch or evenly distributed on all the patches present at the start. This is a future area of exploration, which might have theoretical interests in ecology, but also practical applications for those interested in the dynamics of emerging plant diseases as a result of transient or permanent changes in local climatic conditions.

In silico complementary players to establish a dialogue with in vivo evolution experiments - The 'PhenoPatch' modelling framework provides an in silico evolutionary experiment platform allowing to carry out fast-track experiments in multiple contexts and under multiple conditions (Hindré et al., 2012). Beyond the ability to conduct model-based virtual experiments, such a platform can be used to: (i) pre-run proposed experiments to verify if the competitive outcomes will be large enough to be characterised or fine tune experimental protocols so as to enhance their value; (ii) interpret experiments by enabling to understand the isolated effect of a given factor; (iii) test certain kind of hypotheses as well as the repeatability of evolution (quantified assessments of stochasticity under a higher number of replicates). This would allow to explore different parameter value of factors promoting or hindering adaptive diversification such as spatial aggregation of functional groups (that could prevent competitive exclusion in some cases; Kuno, 1988) or local density regulation after an abrupt environmental shift (Chevin & Lande, 2009). One important point to mention here is that when allowing generations to overlap, thermal niches or refugia can also appear within a spatially homogeneous patch due to temporal fluctuations ("storage effect" or "time dispersal" effect; Chesson & Warner, 1981; Chesson, 1984; Comins & Noble, 1985).

PROSPECTS

Following further evaluation of the model, we plan to conduct a set of in silico experiments putting into competition different phenogroups (e.g. specialist-generalist or faster-slower instead of hotter-colder) under different crop canopy environments (e.g. more or less dense wheat canopy) to tackle questions such as: Do the identified thermal niches still promote diversity in the case of specialist vs. generalist individuals? Do rare or specialist individuals accumulate in the patches where they have higher performance or are they counterselected compared to individuals that equally exploit all niches? To which extent the answer to these questions depends on trade-offs in performance between these specialists and generalists? These further simulations should take into account that competition between individuals can be: (i) affected by when is the competition between strains and the saturation of resources precisely occurring; (ii) stronger when their phenotypes are the most similar (Heinz et al., 2009). We will further analyse the adaptive potential of populations based on the context-dependent metrics we implemented here by comparing initial and final populations. These metrics cover the characterisation of adaptation as a response process (Table 2) to inform on the ability of the system to evolve in the short and/or long term to respond to changing environments (Lefèvre et al., 2015) based particularly on the ability of populations to maintain residual phenotypic variation in spite of the trend of optimal phenotypes selection (Jump et al., 2008). This maintenance of diversity is expected to provide a higher adaptive potential to populations (Houle, 1992; Walsh & Blows, 2009). This modelling approach is a proposed step in the endeavor to increase our understanding of spatio-temporally varying selective pressures and their implications for diversity.

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SUPPLEMENTARY MATERIAL

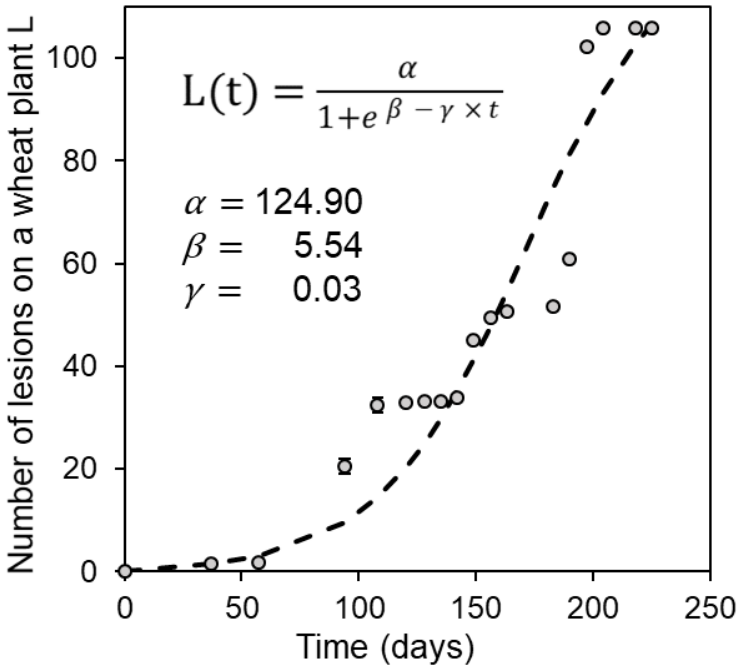
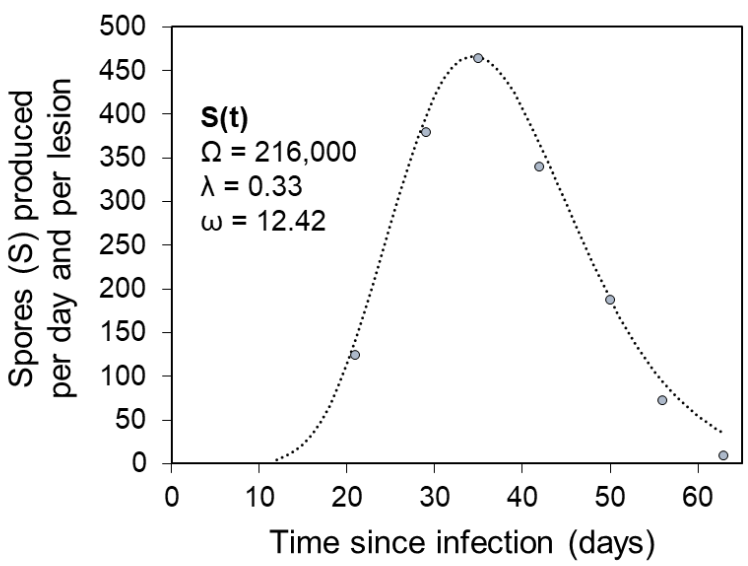


Fig. S1 Dynamics of Z. tritici lesion (L) appearance on a wheat plant. Three naturally infected field plots were monitored for disease severity expressed as a number of number of lesions (disease units) per plant over the 2016-2017 growing season (18 monitoring dates: n = 5 plants per plot except for the two first dates where n = 100 plants per plot). Pathogen population dynamics was described by a logistic growth. In the simulations, every plant followed the same dynamics regarding the number of lesions. This implies a homogeneous initialization and progression of disease in a wheat plot regardless of the position of plants with respect to inoculum location or architecture differences between plants. As such, the variable that modulates pathogen population growth is the size of the host plant population (i.e. the number of wheat plants in the system), which determines the initial size of the pathogen population with the reference value of 0.92 lesions per plant for the first cycle and the size of the population at time t: $P(t) = P(t-1) + (1-x) \times$ host population size $\times NL(\Delta t)$; x is a correction term to take into account the adaptation level of the individuals composing the population under investigation in relation to all possible individuals present in the virtual library.



$$S(t) = \begin{cases} 0 & if\ \tau < \varphi \\ \Omega \dfrac{\lambda^{\omega} \times (\tau)^{\omega-1} \times e^{-\lambda(\tau)}}{\Gamma(\omega)} & if\ \tau \geq \varphi \end{cases}$$

Ω	total number of spores produced per lesion during its entire lifetime
λ and ω	shape parameters of the sporulation curve
τ	time elapsed since the initiation of infection
φ	latent period (time threshold to be exceeded before sporulation occurs)
$\Gamma(\omega)$	the gamma function

Fig. S2 Dynamics of the spore (S) production by a single Z. tritici lesion on a wheat plant. Sporulation curve (delayed-gamma distribution) was fitted on experimental assessments of spore production (number of spores produced per day discharged from a disease unit as a function of time since infection). Spores produced by 8 lesions were collected in an aqueous solution on a weekly basis over 7 weeks from the beginning of sporulation until total leaf senescence. Spore concentration of the solution was estimated using a Malassez chamber (see detailed protocol in Suffert et al., 2013; 2009-2010 experiment investigating the aggressiveness components of four strains on wheat cv. Soissons). Total number of spores produced by a lesion was calculated by summing the number of spores produced weekly by one lesion from the first to the last collection event. The total number of spores produced per lesion during its entire lifetime Ω was set at the upper average spore production per lesion based on sporulation data of four strains inoculated on the two uppermost leaves of cv. Soissons (analyses of raw data from Suffert et al., 2013 and Morais et al., 2015).

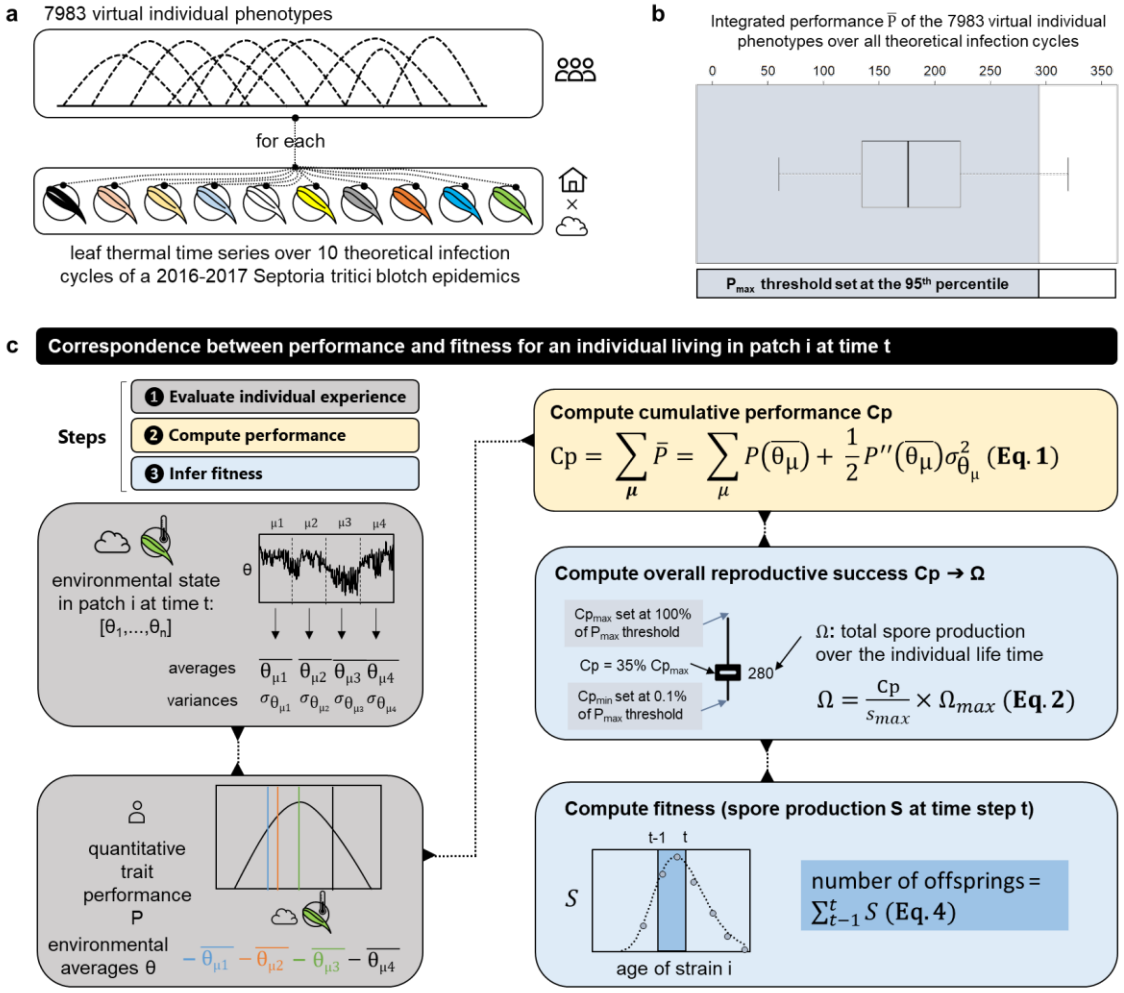
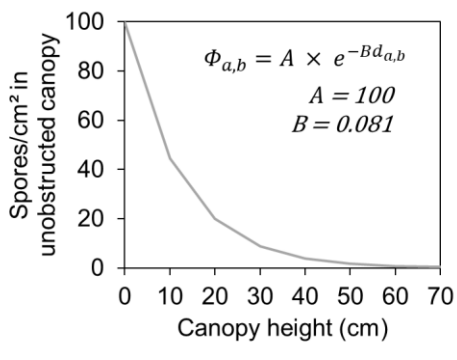


Fig. S3 Correspondence scale to relate the performance of an individual over a temperature time series (computed on reaction norms) to its fitness (offspring production, i.e. cumulative number of dispersal units produced by this individual between time steps t-1 and t). (a) A TPC virtual library of 7983 thermal phenotypes was computed using the value distribution of three key thermal parameters (T_{opt}, P_{max}, TPB) retrieved from the TPCs of the six French Z. tritici populations analysed in Boixel et al. (2019). We computed the integrated performance $\bar{P}$ of each individual TPC obtained over 10 leaf layer × temperature time series corresponding to the theoretical infection cycles. (b) The computation of the integrated performance of each TPC led to a distribution of integrated performance values across phenotypes and environmental conditions. The 95th percentile was retained as a standard threshold for the maximum cumulative performance when computing the selective value of an individual living in a patch i at time t. This allows to make a correspondence between Cp and Ω (the maximal number of offsprings was set at the same value for each individual). (c) We implemented a five-step approach to compute the expected number of offsprings produced by an individual with trait value y under the environmental conditions in patch i at time t (before any event of regulation): (i) compute environmental averages and variances over an environmental filter time step μ (physiological response time of the organism); (ii) computation of performance over these environmental averages; (iii) compute the cumulative performance Cp of the individual under these conditions; (iv) relation to the overall reproductive success (total spore production over the individual life time) so as to obtain the spore production curve S(t); (v) extract the number of offsprings produced between the last time step (t-1) and the current time step (t) that will join the local offspring pool of patch i at time t.



Dispersal function

$\Phi_{a,b}$: probability of deposition per unit of distance
A: number of spores deposited per amount of disease at the source
B: constant defining the decrease rate in spore deposition with distance
$d_{a,b}$: distance between a patch a and a patch b

Fig. S4 Spore dispersal as a function of vertical distance between patches of vegetation layer. The dispersal function was obtained by adjusting the simple exponential model of Kiyosawa & Shiyomi (1972) to experimental data highlighting a decrease of spore flux with height using spore traps placed at different distances from the inoculum source (Vidal et al., 2017).

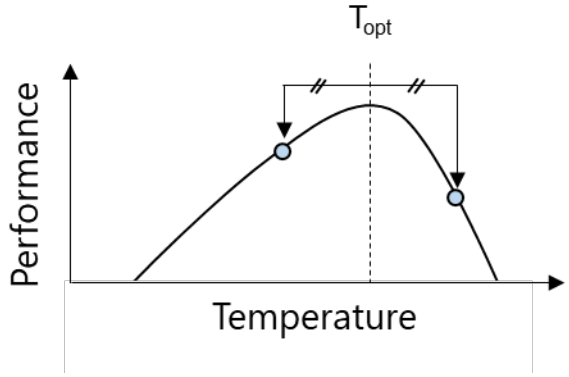


Fig. S5 Higher costs in performance of exceeding rather than receding thermal optima due to the asymmetry of TPC.

Table S1 - Number of complete monocycles (non-overlapping pathogen generations) that could theoretically occur during annual Septoria tritici blotch epidemics. This number was estimated in the case of the INRAE Grignon experimental station (France) for every wheat-growing season of the 2007-2017 period based the computation of thermal time on air temperature measured at a nearby standard meteorological station (250 degree-days above the base temperature -2.4 °C; Lovell et al., 2004). One infection cycle was assumed to be completed when a standard latent period duration of 328°C-days under winter conditions (seedlings, cold temperature regime) or of 419°C-days under spring conditions (adult plants, warm temperature regime) was reached (Suffert et al., 2015).

Growing season	Winter period	Spring period	Non-overlapping and complete pathogen generations (floor function)
2007-2008	2.5	3.6	5
2008-2009	1.8	3.8	4
2009-2010	2.1	3.7	5
2010-2011	2.0	4.1	6
2011-2012	2.8	3.4	5
2012-2013	1.9	3.3	4
2013-2014	2.8	3.6	5
2014-2015	2.3	3.8	5
2015-2016	2.9	3.5	5
2016-2017	2.6	3.9	5

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Chapter 7

Discussion of Findings

contents

LOOKING BACK ON THE RESEARCH STRATEGY

This thesis has explored the importance of phenotypic plasticity, individual variation, and environmental heterogeneity in driving population processes and dynamics. I have addressed this multifaceted issue through the lens of foliar pathogen populations inhabiting crop canopies which are temporally erratic and spatially heterogeneous thermal environments.

With this purpose in mind, I first characterised spatio-temporal patterns before going beyond 'description' considering what could be the 'processes' and 'mechanisms' underlying these patterns. This approach, commonly adopted in ecological research (see the a posteriori standpoint presented in Jeltsch et al., 1999; Fortin & Dale, 2005), allowed me (i) to propose hypothetical processes that may drive the observed adaptation patterns (Chapter 2-3) and (ii) to explore the actual impact of the most relevant hypotheses in situ, based on multi-generational experiments conducted both under controlled and field conditions (Chapter 4-5). As multiple processes may result in the same snapshot pattern, I supplemented my experimental work with a modelling approach (Chapter 6). As such, this work went from documenting microbial biogeographic patterns without a priori, subsequently going from the elaboration of hypotheses about the possible underlying processes to their validation, coupling an empirical approach of miniaturization with a more integrative modelling approach taking into account the whole system to validate the processes and their interactions (Fig. 1).

To address these challenges, a number of technical locks had to be lifted. First, I addressed what minimum set of information should be recorded in phenotyping experiments. This required the definition of standard protocols on which to assess thermal sensitivity using a functional trait-based approach to determine the relationships among the different traits and between traits and environment through the establishment of reaction norms (Keddy, 1992; Zanne et al., 2019). Second, I used calibration algorithms to determine the key properties of these resultant reaction norms (Bernard & Rémond, 2012). This led to the design and validation of a high-throughput and versatile experimental method to phenotype thermal responses (Chapter 2). Using this method, I highlighted a high level of diversity within each local population sampled at different spatio-temporal scales (Chapter 3). This raised the issue of which hypotheses could explain the maintenance of such high local diversity in individual thermal responses despite marked differences in environmental conditions. I investigated if this could conceal fluctuating population selection dynamics going on in the background over short-time scales (Chapter 4). Indeed, the seasonal shifts in functional composition of the populations suggested that they may be locally structured by selection. That is why, I assumed that the thermal heterogeneities may have been mainly observed at the wrong scales with respect to the question at hand, the relevant scale(s) being the one(s) at which the leaf microbiota fully perceived the environmental signals. Thus, I tested the hypothesis that foliar fungal populations might be exposed to significant small-scale thermal heterogeneities by characterising the environmental heterogeneity that may be encountered within a wheat canopy (Chapter 5). I further explored in silico the possible consequences of this cryptic environmental heterogeneity on population dynamics and competitive outcomes (Chapter 6).

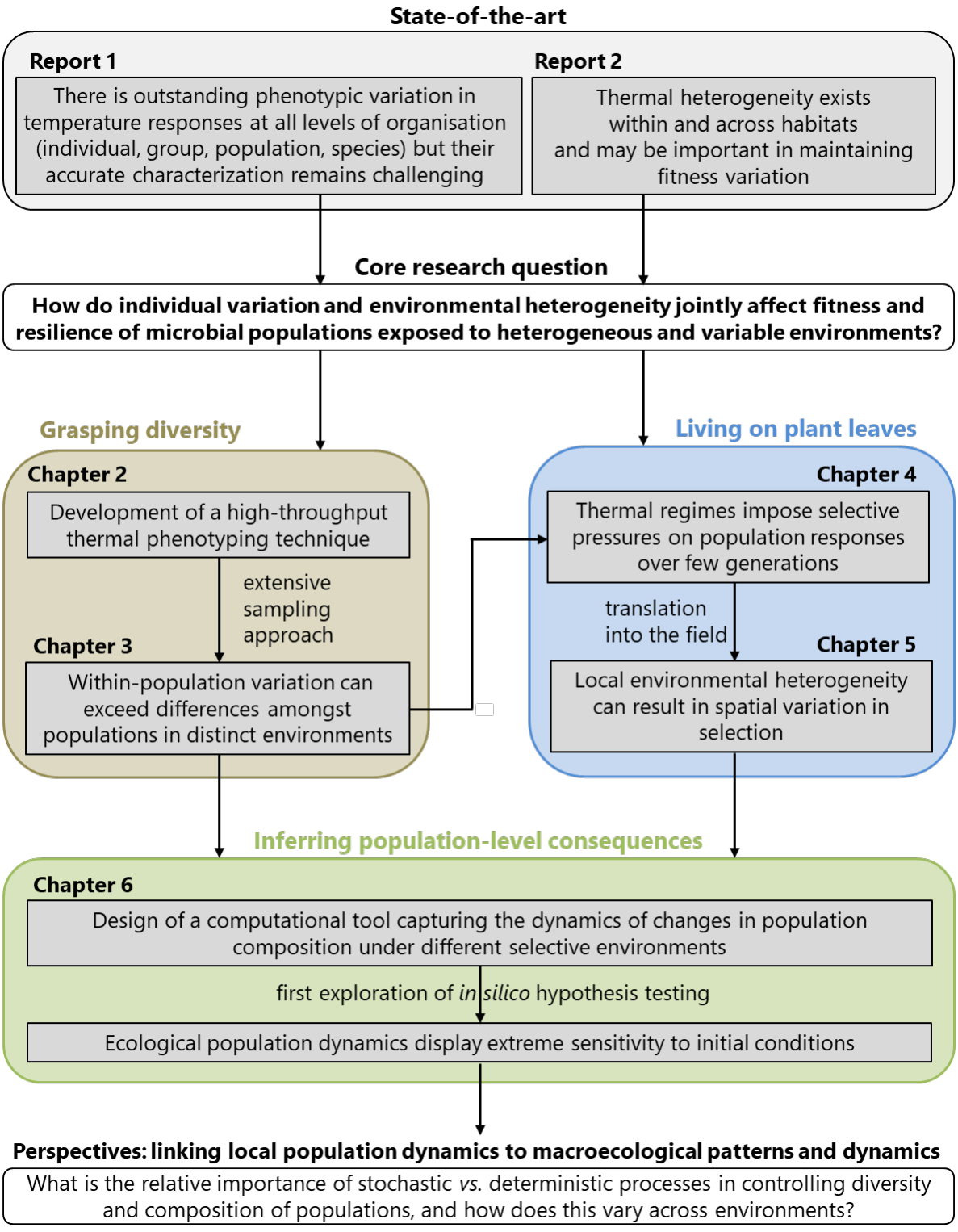

Fig. 1 Contributions of this thesis.

CONTRIBUTIONS AND PERSPECTIVES

How to examine phenotypes to infer temperature sensitivity?

In Chapter 2, I developed a generic experimental framework to accurately characterise the responses of yeast-like micro-organisms to their thermal environment. The corresponding conceptual scheme for this thermal phenotyping is presented in Fig. 2. This original framework addresses several technical challenges raised as causes of biases and non-comparability between assessments performed with current protocols. These challenges are multifold and were tackled by implementing several steps. First, I had to chose a relevant proxy for fitness (Zanne et al., 2019) considering both time-dependency effects inherent to labile traits (Wiser & Lenski, 2015) and variation in temperature dependence of biological traits (Dell et al., 2013). Second, I provisioned standard protocols allowing to isolate the impact of temperature and to provide high-resolution data (Boyd et al., 2013). Third, I built a more complete view of thermal responses considering the range in measurement temperatures. Finally, I calibrated an algorithm to fit a data set (Bernard & Rémond, 2012), that appropriately select candidate models and appreciate their inherent constraints on the estimation of key thermal parameters (Quinn, 2017; Low-Décarie et al., 2017).

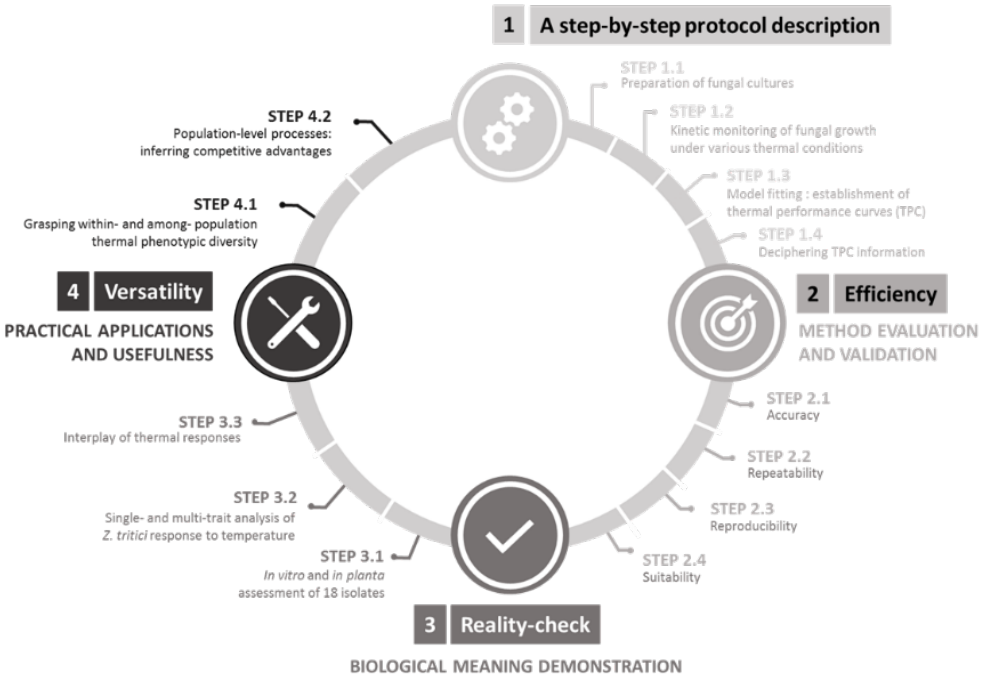

Fig. 2 Step-by-step directions of the in vitro approach for thermal phenotyping from individual-level to population-based studies.

As several other studies on thermal phenotyping (Paisley et al., 2005; Zhan & McDonald, 2011; Stefansson et al., 2013; Robin et al., 2017; Birgander et al., 2018), the retained high-throughput method for further application was a standardised in vitro approach. An advantage of this approach is that it enables large-scale investigations while alleviating major confounding factors (e.g. cross effect between host resistance and temperature adaptation; Pariaud et al., 2009). The trait-based screening revealed consistent thermal sensitivity rankings for T_{opt} of 18 strains in terms of growth whether estimated in vitro or in planta. The good correlation between in planta and the methodology for assessing in vitro fungal biomass was in line with other findings (Paisley et al., 2005; Zhan et al., 2016) and justify using the in vitro yeast-like growth as a proxy for the

temperature response experienced by Z. tritici to draw conclusions based on rank-based analyses of variance. Taking into account the complex interaction between fungus growth, plant growth, and temperature in natural conditions should be the next step for such large-scale phenotyping studies. This requires the development of new thermal phenotyping platforms, enabling to control the phylloclimate (Chelle, 2005), to be able to study extreme events (Gutschick & BassiriRad, 2003), and to avoid the biases pointed out by Poorter et al. (2016).

Phenotypic diversity in thermal responses was assessed by establishing an average TPC template and determining the extent to which it varied between strains through a typological comparison drawing upon the concept of 'functional groups'. This comparison was conducted without a priori assumption. More precisely, the classification is relative in contrast to previous methods that establish differences in mean and patterns based on predetermined modes of variation (Izem & Kingsolver, 2005). This clustering allowed to group together individual showing a similar response to temperature (e.g. cold-adapted thermal specialists). These functional thermal groups, that I referred to as 'thermotypes', were summarised in terms of compactness, separation, and connectivity (distance between cluster centres and between strains in different clusters; Rousseeuw, 1987). The variation of TPCs within and between populations was assessed by analysing both divergence in the means and variances of the key thermal parameters (P_{max} – T_{opt} – TPB_{80}) and in the composition patterns of thermotypes.

An issue that went beyond the scope of this thesis is whether there is a morphological basis of the differences in thermal responses. Indeed, it cannot be excluded that the thermotypes identified here could be linked to preferential morphotypes. Specific growth forms known for instance to be more tolerant to heat stress could have appeared. Z. tritici has at least three well characterised in vitro growth forms: blastospores (the 'yeast-like' form), hyphae, and the most recently described morphotype, chlamydospores (Francisco et al., 2019). Growth state transitions have already been highlighted in Z. tritici under different experimental conditions (nutrient limitation, inoculum size, oxidative stress, culture volume, temperature; Mehrabi et al., 2006; Motteram et al., 2011; Lendenmann et al., 2016; King et al., 2017). In some cases, this could have affected the nature of the information provided by OD measurements, giving access not solely to yeast-like budding growth rate but more generally to fungal biomass (Pijls et al., 1994; Fraaije et al., 2002; Stergiopoulos et al., 2003; Mavroeidi & Shaw, 2005; Siah et al., 2010; Hawkins et al., 2014). Although I did not specifically search for chlamydospores, I nonetheless considered the pleomorphic switch from yeast-like growth to pseudohyphal and hyphal growth during the validation of the phenotyping framework for two strains of reference (IPO323 and INRAE-FS0932) to limit the risks of form transitions bias (from yeast-like form to hyphal form).

The application of this framework to quantify individual and population phenotypic diversity of thermal responses has provided new opportunities in exploring the structure, causes, and consequences of this diversity in comparative population-based studies. A natural extension of this work is now to investigate the genetic determinants and heritability of thermal responses by going from phenotype to genotype (Perspective Box 1).

Perspective Box 1. Deciphering the molecular bases of thermal adaptation

One perspective of this work could be to investigate the molecular bases of thermal adaptation in Z. tritici. One way to proceed would be to carry out a genome-wide association study (GWAS) coupled with a conventional linkage mapping investigating the progeny of biparental crosses of a cold-adapted and warm-adapted strain (protocol detailed in Suffert et al., 2019) after selecting compatible strains based on the PCR amplification of the two mating-type idiomorphs (Waalwijk et al., 2002). Two biparental crosses have already been conducted in 2018-2019 with strains whose thermal optima were phenotyped at 14 and 20°C, respectively. 110 progenies per biparental cross were collected and stored for future investigations at -80 °C. Phenotyping the thermal responses of these progenies would also allow to address the issue of the heritability of thermal responses and thermotypes, drawing upon the work conducted by Lendenmann et al. (2016).

To what extent do individuals and populations vary in their thermal responses and to what extent does this variation reflect adaptation?

In Chapter 2 and 3, I examined the diversity and shifts in the thermal sensitivity of natural Z. tritici populations collected across time (local seasonal dynamics) and space (wheat growing areas). Over the 416 strains that have been phenotyped (Fig. 3), four main results emerged:

1 - There is a high degree of individual phenotypic plasticity and variation in sensitivity to temperature conditions across scales. This pattern provided new insight into the broad diversity and two-tier dynamics of thermal responses encountered within pathogen populations living on plant leaves, suggesting that populations have a high potential to adapt to climate fluctuations (Nussey, 2005; Baythavong & Stanton, 2010; Chevin et al., 2013). This also highlighted the maintenance of some maladaptation in the individual thermal responses within each population (Brady et al., 2019). This work paves the way to a better understanding of the potential of plant pathogen populations to adapt to changes in their environment. This is a timely subject, as this is a missing link in models for predicting plant disease epidemics and their impact on yield under climate change (Visser, 2008; Sutherst et al., 2011).

2 - Seasonal patterns overwhelm spatial variability (with the following ranking of scales in increasing order of diversity in thermal traits: Seasonal scale S1 > Climate zones S4 > French transects S2-S3; Fig. 3). This led me to wonder what could happen on the scale of an epidemic: could a local short-term selection for the fittest strains adapted to successive climatic conditions occurs over the course of an annual epidemic (in line with the findings of Chin & Wolfe, 1984; Villaréal & Lannou, 2000; Pariaud et al., 2009)? This hypothesis was firmly established in Chapter 4 and is consistent with earlier empirical findings concerning the possible selection on Z. tritici life-history traits in response to seasonal variations (Suffert et al., 2015). This is a call to move from the description of actual patterns to the experimental inestigation of eco-evolutionary processes to explore how thermal selection may drive population dynamics in response to seasonal variations (Farine et al., 2015; Bacigalupe et al., 2018).

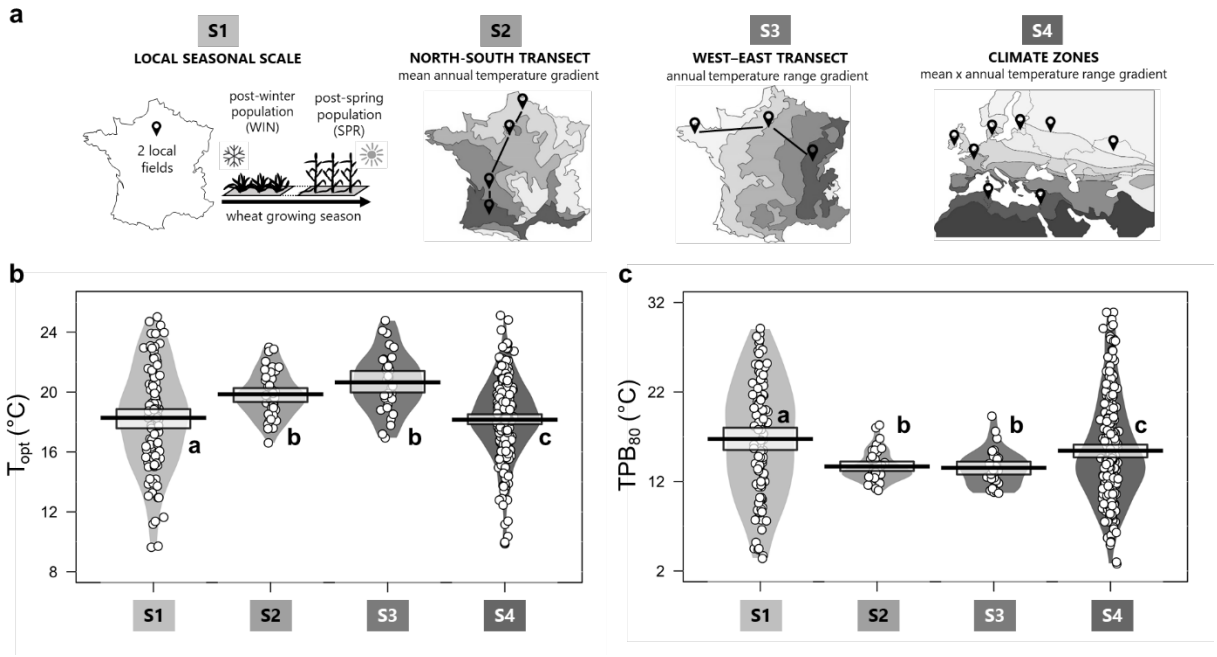

Fig. 3 Quantification of individual differences in thermal phenotypes over four scales of investigation. (a) Individuals (n = 416 strains) and populations (n = 18) were sampled over: (S1; Chapter 3) a local post-winter vs. post-spring seasonal scale; (S2-S3; Chapter 2) two French spatial gradients of increasing annual mean temperature and temperature range, respectively; (S4; Chapter 3) a Euro-Mediterranean climatic gradient representative of oceanic, continental and mediterranean conditions. RDI plots (Raw data, Descriptive and Inference statistics) show jittered points of raw (b) thermal optimum (T_{opt}) and (c) thermal performance breadth (TPB_{80}) values. Centre bars indicate the mean of the data, beans outline the smoothed density of the data, and inference bands show the Bayesian 95% High Density Interval inferential statistics for each group. Letters show statistical differences between groups in terms of individual variation (Levene's Test for Equality of Variance, a: F-value = 9.98, b: F-value = 22.0, $P < 0.05$).

3 - A dual signature of thermal adaptation at the most local (seasonal; S1) and widest spatial (continental; S4) scales (Fig. 3). These results expanded two studies which have shown that Z. tritici populations exhibit adaptation patterns in space (populations from 4 geographic locations investigated under two temperature regimes; Zhan & McDonald, 2011) and time (local field subpopulations investigated under winter and spring thermal conditions; Suffert et al., 2015). Remarkably, the high temperature adaptation of the Israeli populations to warm conditions revealed in Zhan & McDonald (2011) and Chapter 2 is an intriguing one which could be explored in further research.

4 - All the T_{opt}-adapted thermotypes were identified at almost all study sites despite the large differences between local thermal conditions. A possible explanation for these results may be the lack of adequate characterisation of thermal heterogeneities at scales relevant to Z. tritici. Local patterns could overwhelm those of large-scale climate leading to complex thermal mosaics of temperature rather than simple latitudinal gradients. This led me to investigate within site variation in abiotic conditions to decipher to what extent temperature heterogeneity may contribute to the conservation of thermotypes at the local scale, i.e. within a wheat canopy (Chapter 5).

Perspective Box 2. Refining the understanding of thermal adaptation patterns

A first step to refine our understanding of the detected adaptation patterns could be to consider biologically relevant within-site variation in abiotic conditions. In the case of temperature, this can be done by going from characterising the environments based on air temperature to the actual leaf temperature within a canopy based on a Soil-Vegetation-Atmosphere-Transfer model downscaling variability to statistical, extrapolated averaged leaf temperatures (e.g. CUPID model; Norman, 1979).

A second step could be to assess if the adaptation could result from a stable equilibrium in response to local climatic conditions. This could be achieved by using the 'PhenoPatch Zymo' model developed in Chapter 6 for conducting a virtual reciprocal transplant experiment ('home vs. away' and 'local vs. foreign' comparisons). Simulations could be perfomed for instance on the 8 Euro-Mediterranean populations under the regimes of the 8 zones (64 possible combinations). This would give insight into whether the climatic conditions that minimize the difference between initial and final populations over the course of an annual epidemic would be the place of origin (native environment).

A third step could be to consider adaptation to the other environmental factors than temperature that contribute to define the climate zones. This investigation has already started with the consideration of the differential tolerance to high moisture regimes during early infection stages of the Irish and Israeli Z. tritici populations (Fig. 4; see preliminary results in Boixel et al., 2019).

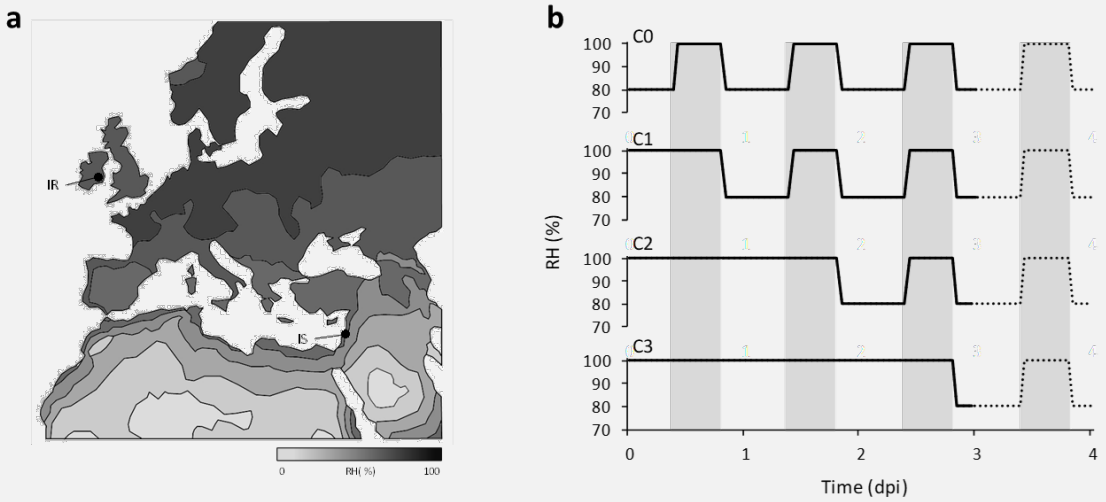

Fig. 4 Experimental design used to investigate the differential tolerance to changes in high moisture regime during early infection stages in two Euro-Mediterranean Z. tritici populations (from Boixel et al., 2019). (a) Average annual air moisture at the sites from which the Irish (IR) and Israeli (IS) Z. tritici populations were collected (Chapter 3); (b) Moisture regimes (C0-C3) investigated over the three first days post-inoculation (dpi). Solid (from 0 to 3 dpi) and dotted (after 3 dpi) lines depict the post-inoculation relative humidity (RH) to which the inoculated wheat seedlings were subjected.

A research avenue is to further characterise and decipher the dual impact of both geographical and seasonal dynamic population processes that occur along a continuum of space and time and lead to the adaptation patterns observed in the different Euro-Mediterranean areas (S4 scale in Fig. 3) This would allow to reduce possible mischaracterisation or oversimplification of the environmental clines as experienc ed by Z. tritici but would require to take into account the climatic conditions prevailing before the sampling date (Perspective Box 2).

Are there differential changes in the phenotypic composition of plant pathogen populations in response to thermal variations over the course of an annual epidemic?

In Chapter 4, I performed in vitro and in planta competition experiments between functional groups of Z. tritici strains (thermotypes defined in Chapter 2-3) under increasingly complex selective environments (constant, diurnally fluctuating and field temperature regimes). This experimental approach allowed to progressively tackle the multifold aspect of selective pressures imposed on fungal populations. Pathogen adaptation to environmental variation was thus deciphered at the population level, leading to a more mechanistic understanding of the epidemiological processes underlying population dynamics. In particular, when tailoring selective regimes to each thermotype, I found that thermal selection may occur over short time scales, corroborating previous findings (Suffert et al., 2015). In controlled conditions (Experiment B, Chapter 4), this short-term selection started from the second generation under the cold regime and from the third generation under the warm regime (i.e. less than the number of non-overlapping generations one can encounter over an annual plant disease epidemics; Table S1 in Chapter 6). These experimental conditions fostered both the maintenance of diversity and temporal changes, with differences in specificity as a function of prevailing thermal conditions.

The capture of population composition snapshots during the in planta competition experiments allowed to detect statistically significant population changes under controlled conditions. The field experiment was limited by high levels of immigration over the course of the experiment as well as by a strong founder effect. To gain deeper insight, it would be useful to turn to modelling approaches for replaying these in planta experiments in silico using the 'PhenoPatch' model developed in Chapter 6 (Perspective Box 3). This would allow to:

- develop a fuller picture of the contribution of selection on thermal sensitivities vs. demographic processes to the dynamic of population adaptation;
- assess how the experimental design, such as the timing of serial transfer, might have modulated the selective dynamics;
- reach a sufficient statistical power (both in terms of replicate lines and sampling effort) that enables to: (i) assess all possible evolutionary trajectories even in the least stressful environments; (ii) separate changes in phenotypic composition due to actual selective pressures from random sampling error (Hersch & Phillips, 2004).

Another form of adaptation to thermal heterogeneity that was not investigated in my thesis is acclimation, understood as a change in genotype and not with respect to the period of thermal exposure (Bowler, 2005; Lagerspetz, 2006). Brief exposure of organisms to extreme thermal conditions could enhance their tolerance to these conditions within hours (Hoffmann & Watson, 1993; Sinclair et al., 2003; Sinclair & Roberts, 2005). It cannot be excluded that acclimation has occured in such a way during the in planta competition experiments as temperature varied temporally within generations in those experiments (Chapter 4) and had an impact on the performance of certain strains, for instance enhancing their fitness. This possibility would have to be accounted for (Perspective Box 4).

Perspective Box 3. Replaying in silico the tape of the in vivo competition experiments

Running the 'PhenoPatch' modelling framework (Chapter 6) on the experimental data acquired in Chapter 4 would allow to monitor more finely the dynamics at play with a view to further explore the processes that may have led to the detected changes in phenotypic composition. Indeed, the advantage of in silico experiments is that they enable to reach perfectly controlled conditions, multiple repetitions, and exhaustive records (Hindré et al., 2012). It could also prove useful in informing the design of further experiments through detailed analyses of which and how key influence parameters of experimental conditions (reviewed in Van den Bergh et al., 2018) affect population-level outcomes.

Perspective Box 4. Acclimation, a missing player?

This question of how the phenotype of a strain may be preserved or 'deformed' when moving from the monocyclic to the polycyclic scale in variable thermal environments could be explored experimentally. Such investigations could be based on the comparisons of the thermal phenotypes (TPC) of each representative of each genotype collected and stored over the in planta competition experiments of Chapter 4 ('snapshot freezing' of the evolutionary process). This could help in answering whether there are physiological costs of adaptation to temperature and to what extent these possible physiological costs could label that the performance of a particular thermotype that is supposed to be performing well under given temperature conditions deviates from what one would expect solely based on their thermal performance curves.

To what extent does local temperature heterogeneity contribute to the maintenance of diversity in thermotypes in a wheat canopy?

Drawing upon studies highlighting the importance of accounting for microclimates (Potter et al., 2013), one can hypothesize that the favored phenotypes within a population may be continuously changing under this assumption of a high local heterogeneity and variance in environmental conditions. This is expected to maintain very diverse populations through the conservation of thermotypes at a very local scale, for instance within a wheat canopy.

In Chapter 5, I established that wheat canopies generate high levels of thermal heterogeneity and that a local foliar fungal population may experience thermal conditions very different from those in the atmosphere (Fig. 5). This environmental heterogeneity generated diverse thermal refugia (ecological niches resulting from spatial differences in selection) from unfavourable growth conditions for some individuals of the population, with the presence of colder leaf spots in the canopy that could allow the development and maintenance of individuals adapted to colder conditions. As such, the heterogeneities imposed differential selective pressures interacting with the thermal plasticity of the strains. This is in agreement with previous studies that highlighted the balancing effect of selection leading to a similar mean fitness of individuals over time (Via & Lande, 1985; Shuster & Wade, 1991; Greenfield & Rodriguez, 2004). This could explain why competition experiments between strains with contrasted thermal plasticity favoured the stable maintenance of phenotypic diversity within populations in the field under fluctuating conditions (Experiment C_1-C_2 in Chapter 4) in contrast with lab or growth-chamber experiments under constant temperature regimes.

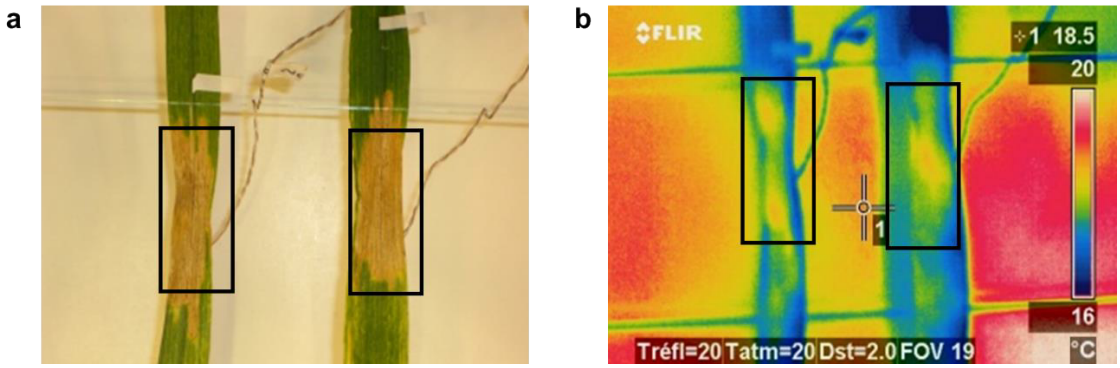

Fig. 5 Experimental design used in this thesis for measuring leaf temperatures. The measurements are centered on individual lesions in order to accurately represent the actual temperature perceived by Z. tritici.

How to model phenotypic selection in spatially and temporally variable environments? For which purpose?

I developed the 'PhenoPatch' model to simulate the phenotypic changes occuring in metapopulations inhabiting heterogeneous environments and to assess the consequences of such heterogeneity for population persistence and adaptive potential.

In Chapter 6, I started the exploration of three overarching questions taking the thermal ecology of Z. tritici as a case study:

1. How important is individual variation to population, community and ecosystem dynamics?
2. Which characteristics determine the resilience of natural populations to changes in thermal conditions?
3. How do spatial and temporal environmental heterogeneity influence diversity at different scales?

The preliminary results of simulations support the prediction that adaptive population divergence is less pronounced, and maladaptation more pervasive, in heterogeneous environments and with high initial functional diversity levels within local populations.

However, the overall picture is still very much incomplete as multiple processes can lead to the same patterns. For instance, there is abundant room for further exploration of the role of demographic stochasticity (which generally leads to a more or less strong difference between realized and expected fitness; Bürger & Lynch, 1995). A first step in this direction would be to conduct sensitivity analyses modulating the gradual intensity of population bottlenecks between serial transfers.

As it stands, the 'PhenoPatch' model currently assumes that migration, mutation and recombination are not occuring and thus do not impact genotype frequencies. However, as the mechanisms that underpin these adaptations can be quite diverse, it would be interesting to integrate them in the long term.

'PhenoPatch' could also be refined with respect to the different challenges and prospects regarding the prediction of competition from TPCs. In particular, three further possible direction of development could be tackled considering:

- the balance of selection acting through different fitness components. Evolution of traits is governed by a balance of conflicting fitness advantages, i.e. opposite selection or trade-offs between different components acting on fitness (e.g. fecundity, mating success, survival; Robinson et al., 2006). This could lead to contrasting or antagonistic selection pressures acting on the same genotype (Stearns, 1989; Roff, 2000; Roff et al., 2002; Pariaud et al., 2013). In Chapter 5, fitness was assessed solely on one fitness trait, namely latent period. It would be interesting to conduct the same investigation on another fitness trait, such as sporulation capacity. This may be important to incorporate for a more accurate characterisation of competitive ability (sequential fitness traits informing on development rate for latent period and reproduction for sporulation capacity). However, this raises the question of how to implement this temporal integration of reaction norms describing different life-history traits of a same genotype in modelling approaches;
- the time scales of exposure to environmental variation. How do organisms perceive the thermal heterogeneity of their environment? The response of an organism to temperature, in a broad sense, may be dependent on: (i) the prior thermal exposure (time history allowing for acclimation); (ii) the duration of the exposure; (iii) the delay between the perception of the index and the phenotypic response as thermal sensitivity (Schulte et al., 2011). To date, there is still very little information on these aspects in the literature. In the 'PhenoPatch' model, inferences of performance are based on instantaneous integrated performance over small time intervals of 4 hours (longer time steps appeared too wide for simulations attempted to account for temperature variance; Xu, 1996; Bernard, 2012) taking into account both the thermal averages and variances over these repeated time frames. A sensitivity analysis conducted on this parameter would help to determine how this may have impacted competition outcomes;
- the interaction between temperature and other environmental factors. Populations are exposed to multiple environmental conditions stressors (Darling & Côté, 2008). These factors may interact additively, antagonistically or synergistically with thermal responses of individuals and populations. Humidity (Colhoun, 1973; Shaw, 1990, 1991) and UV radiation (Colhoun, 1973), or the physiological aspects of the plant-pathogen interactions (host × temperature adaptation; Laine, 2008), could thus act as confounding factors altering the relationships between trait and fitness. Further research could examine more closely the links between these environmental stressors and their effect on TPC (Gunderson & Leal, 2016) and ecological shifts (Holmstrup et al., 2010).

This implies to go from single-factor to multi-stressor experiments that investigate simultaneously at least two variables (Todgham & Stillman, 2013). In the case of Z. tritici, further experiments screening for the responses of the most contrasted strains to differential moisture conditions (as mentioned above and illustrated in Fig. 4), host species or cultivar (e.g. bread vs. durum wheat) could shed more light on these critical variables of interest. To achieve simultaneous thermal performance assessments of strains either in vitro or in planta in different moisture conditions seem not reasonably feasible experimentally due to the heavy logistic constraints but modelling approaches could allow an initial exploration of these issues.

CONCLUSION

This thesis illustrates how classical approaches in plant disease epidemiology can benefit from concepts derived from population biology and functional ecology. The biological model Z. tritici was an excellent opportunity to explore such an issue through a diversity of approaches - in vitro, in planta, in natura, in silico - following a multidisciplinary and even transdisciplinary research. Indeed, drawing upon ecology to study agroecosystems such as a cultivated field cannot be reduced to the sole transposition of methods and concepts from one discipline to another, but requires their integration into the essence of agroecology (Dalgaard et al., 2003).

The research scope of my thesis is naturally transposable to the thermal ecology of crop phyllosphere microbial populations. Due to their inherent patchiness, plant canopies are particularly relevant to investigate the processes underlying population responses to heterogeneous environments over multiple timescales. Indeed, leaves of a crop canopy constitute a discrete, hierarchical, and dynamic habitat across space and time that make them particularly fascinating to test ecological principles (e.g. Redford & Fierer, 2009).

What is more, the fact that I tackle environmental heterogeneity from a thermal biology point of view allowed to mobilise integrating tools and concepts such as the one of thermal performance curve that constitutes the red thread of the thesis (Angilletta, 2009; Schulte et al., 2011). As such, this thesis exemplifies an approach that could be applied to other organisms for which the question of adaptation to temperature is relevant, particularly in the climate change and the 'One Heath' context. This work could be used for instance to complement previous research on the barley pathogen Rhynchosporium commune or the potato pathogen Phytophthora infestans (Stefansson et al., 2013; Yang et al., 2016; Mariette et al., 2016) or to other model organisms of interest for thermal biologists (Angilletta, 2009). This work could be also used to investigate population responses to other environmental variables, whether physical (e.g. radiation, humidity and wetness) or biological (e.g. host nutrients). The multidisciplinary approach adopted here makes this possible and, in this sense, results from a continuous search for genericity in the different choices made over the research process, whether in terms of concept (e.g. reaction norms), methodology (e.g. the thermal phenotyping framework proposed in Chapter 2), or modelling tools (e.g. the 'PhenoPatch' modelling framework proposed in Chapter 6).

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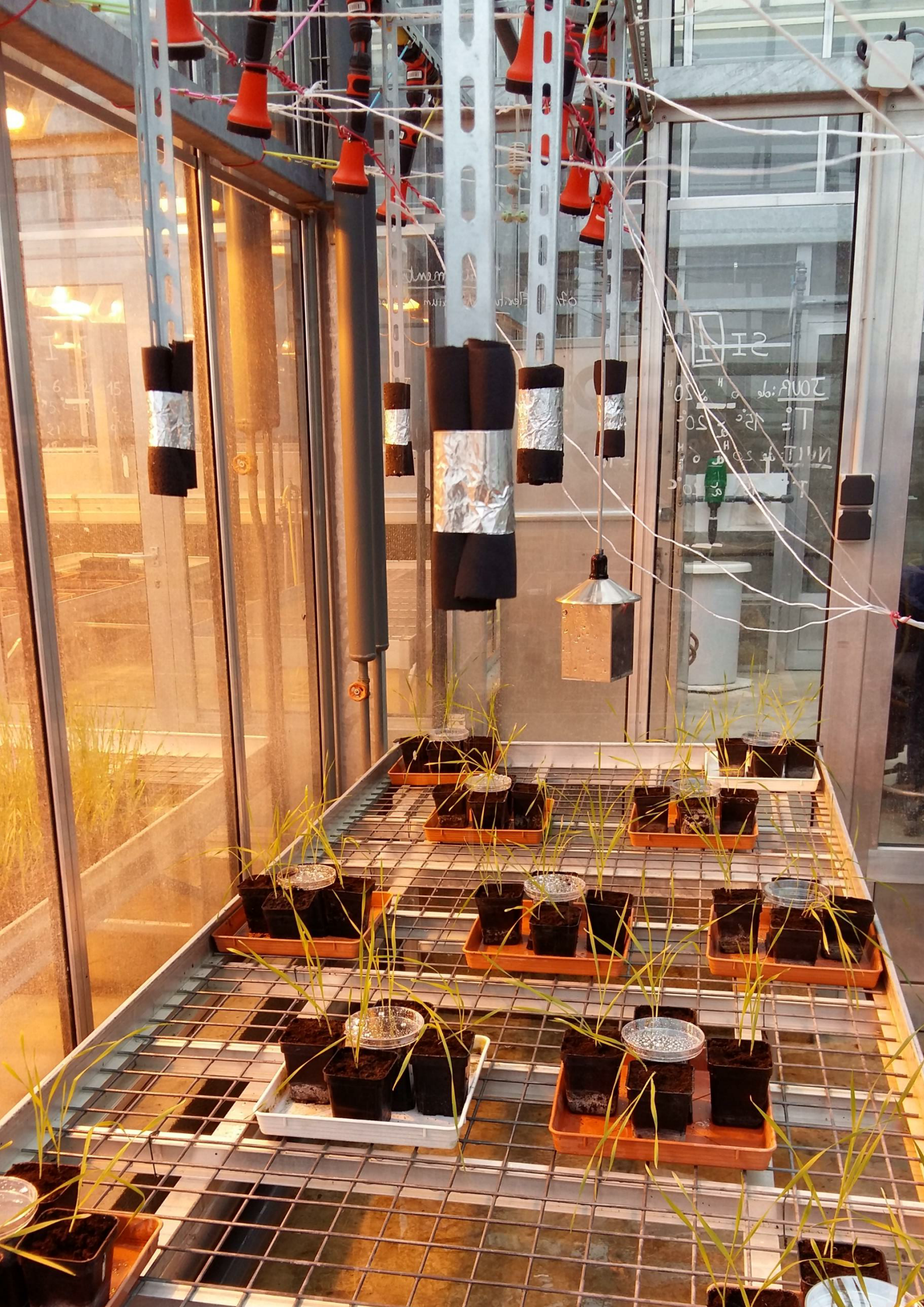
› And to you there, any reader of this thesis. I am happy you are stopping by to have a look at this research investigation, I hope that you plan to go beyond the acknowledgements section and that you will enjoy uncovering this work (if you are in a hurry, the figure 1 on page 238 gives a quick overview of the key contributions). ;-)

Thank you all!
Anne-Lise
Thiverval-Grignon
February 28, 2020

In the picture
Credits for heading photographs reproduced with permission

Contents	Diving into the world of foliar plant pathogens under binocular loupe magnification © F. Suffert
Chapter 1	Experimental design to study pathogen development as a function of leaf temperature © F. Bernard
Chapter 2	Setting up an in vitro phenotyping session under a Microbiological Safety Cabinet © A.-L. Boixel
Chapter 3	Kazakh leaves from which were isolated one natural population phenotyped in the thesis © A.-L. Boixel
Chapter 4	View of the greenhouse and microplot field trial in which strains were placed in competition © A.-L. Boixel
Chapter 5	Recording the temperature actually perceived by leaf foliar plant pathogens© F. Suffert
Chapter 6	Recording of input environmental and disease data for running the 'PhenoPatch' model © F. Suffert
Chapter 7	Wheat leaves covered by Septoria tritici blotch lesions © F. Suffert
Thank you!	Early winter morning in the greenhouse © A.-L. Boixel
Appendices	Setting up a device for characterizing the dispersal of fungal spores by rain-splash © A.-L. Boixel



Appendices

Recurring abbreviations

Acronym	Description
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AUDPC	Area Under the Disease Progress Curve
CA	Adapted to Cold conditions
DPI	Days Post-Inoculation
F_{ST}	Wright's fixation index
G	Generalist
GLM	General Linear Model
GFP	Green Fluorescent Protein
HCPC	Hierarchical Clustering on Principal Components
MA	Adapted to Mild conditions
OD	Optical Density
PCA	Principal Component Analysis
P_{max}	Maximum performance
Q_{ST}	Quantitative genetic analog of Wright's F_{ST}
RN	Reaction norm
S	Specialist
SSR	Simple Sequence Repeats
STB	Septoria tritici blotch
TPB	Thermal Performance Breadth
TPC	Thermal Performance Curve
T_{opt}	Thermal optimum
WA	Adapted to Warm conditions

Glossary of terms

Given the multiplicity of meanings and possible interpretations attached to specific terms, consensus definitions convenient for understanding the research covered in this thesis are provided below. The definitions are simplified as I intend them to be interpreted in the context of this thesis, consistently nevertheless with literature and disciplinary concepts.

Aggressiveness - quantitative variation in the ability of a plant pathogen to colonize and cause damage to a susceptible host plant, characterised by life history-traits such as infection efficiency, latent period, sporulating area

Adaptation - dynamic evolutionary process that fits a population to its environment, thus enhancing its evolutionary fitness; or phenotypic state reached by this population during that process

Adaptive potential - capacity of a population to respond to selection under novel environmental conditions by means of phenotypic change

Bioclimatology - study of the effects of climate on the biological processes of living organisms

Bottleneck - temporal constriction in population size

Cirrhus - mucus-bound ribbon-like mass of spores that is exuded from a fruiting body of a fungal pathogen

Competition - interaction in which the growth and/or survival of individuals are affected adversely and can lead to a change in their relative occurrence

Degree-day - departure of the average daily temperature from a defined base

Ecological niche - all of the biotic and abiotic environmental properties that determine where a population lives

Evenness - component of diversity based on the frequencies of phenotypes, maximal when all phenotypes occur at equal frequencies

Fitness – the expected number of offspring contributed by individuals with a particular phenotype to the gene pool of the next generation

Fluctuation - regular wave motion (e.g. a discontinuous thermal regime)

Founder event – effects of random genetic drift when a small number of individuals establish a new population

F_{ST} - measure of genetic differentiation among populations for neutral molecular markers

Gene **fl**ow - movement of alleles from one population to another through dispersal or migration

Generalist - individual or population that is able to thrive under a wide range of environmental conditions

Genetic drift – random change in allele frequencies due to chance factors

Genetic structure - the amount and distribution of genetic variation within and among populations

Heritability - the fraction of total phenotypic variance in a trait that is accounted for by additive genetic variance

Heterogeneity - difference or diversity of component elements or environmental properties in space

Hyphae - branching filamentous structures with rigid walls forming a network (mycelium)

In planta - in interaction with its host plant

In natura or in situ - in its original environment, i.e. exposed to non-artificial biotic (e.g. host plant) and/or abiotic (e.g. temperature) constraints

In vitro - in an artificial environment, i.e. exposed to constraints not necessarily encountered in natural conditions

Inoculum - pathogen part or propagule (e.g. spore) capable of infecting host tissues when transferred to a favourable location

Isolate - pure culture and derived subcultures resulting from the separation of a microorganism from host or substrate

Latent period - length of time between the start of the infection process by a unit of inoculum (e.g. spore deposition on host tissues) and the start of production of infectious units (e.g. appearance of sporulating lesions on host tissues)

Life cycle - the complete cyclical stages in the growth and development of an organism

Local adaptation - phenotypic state reached by an individual or a population that has evolved to be better suited to its local/immediate environment than other individuals or populations

Maladaptation - phenotype-environment mismatch

Natural selection - process of differential survival or reproduction when trait variation influences fitness

Neutral molecular markers - variable portions of the genome, which are not influenced by natural selection and are used to estimate the genetic divergence of populations

Patch - surface area differing from its surrounding in environmental character states

Pathogen - micro-organism capable of causing disease

Phenotype - all outward, observable characteristics of an individual that result from the interaction of its genes and the environment

Phenotypic plasticity - ability of a genotype to alter its phenotype in response to its environment

Phyllosphere - microenvironment extending from the leaf surface outward to the outer edge of the boundary layer surrounding the leaf and inward into the leaf tissues

Plant disease epidemiology - study of the temporal and spatial changes that occur during epidemics of plant diseases that are caused by populations of pathogens in populations of plants

Pleiotropy - phenomenon of a single gene influencing two or more distinct phenotypic traits

Population - complete group of individuals of the same species occupying a given geographic area at the same time

Population dynamics - variation in time and space in population size, density and composition

Population structure - patterns of diversity within and among populations

P_{ST} - phenotypic divergence in a trait across populations

Q_{ST} - measure of phenotypic differentiation among populations for a quantitative trait

Reaction norm - graphical depiction of phenotypic plasticity, i.e. pattern of phenotypic expression of a single genotype across a range of environments

Richness - component of diversity representing the number of phenotypes in a sample

Selection - process of changes in allele frequencies that occurs when heritable phenotypic variation has differential effects on fitness

Selection differential - difference between the average value of a phenotypic trait in a whole population and the average value of individuals that reproduce

Spore - unit of sexual or asexual reproduction (e.g. pycnidiospore or ascospores in Zymoseptoria tritici, respectively) adapted for dispersal and for survival, which are part of the life cycles of many microorganisms, especially fungal plant pathogens

Strain - a genetically unique individual (pure culture of an individual micro-organism of a single genotype)

Subpopulation - set of individuals sampled from a population that occupies discrete areas or periods of times

Thermal performance curve - continuous reaction norms that describe the relationship between the performance/fitness of an organism and temperature

Thermal response - effects of temperature on performance or fitness

Trade-off - a situation which arises when two traits cannot be simultaneously optimised i.e. when a change in one trait that increases fitness causes a change in the other trait that decreases fitness

Trait - any character of feature of an organism

Variation - a partial change in time or the property to vary from a form, position or state

French summary
Résumé substantiel en français

L'hétérogénéité environnementale, un moteur de l'adaptation à la température des populations d'agents phytopathogènes foliaires ?

Point de départ : formalisation de la problématique de recherche

Parmi les facteurs physiques affectant le développement des agents pathogènes foliaires, la température apparaît de premier ordre, car elle influence tous les processus du cycle de la maladie depuis l'infection jusqu'à la reproduction (production de spores). Ce facteur se caractérise par des variations environnementales qui se manifestent à différentes échelles spatio-temporelles, du méso- au micro-climat (Chelle, 2005; Pincebourde et al., 2007), et se répercutent sur la vitesse de développement des agents pathogènes, avec des conséquences en chaîne sur les dynamiques épidémiques (Scherm & Van Bruggen, 1994; Bernard et al., 2013). La réponse non-linéaire à la température des processus du cycle infectieux a pu être établie à l'échelle individuelle chez de nombreux agents pathogènes, notamment ceux du blé (e.g. Hess & Shaner, 1987; Shaw, 1990). Toutefois, la façon dont les agents pathogènes répondent et peuvent s'adapter aux variations de température reste insuffisamment caractérisée à l'échelle populationnelle (Garrett et al., 2006; Chakraborty, 2013). Les prédictions d'adaptation des agents pathogènes à leur environnement font généralement abstraction de la variabilité qui existe entre individus ou entre sous-groupes d'une même population (Bolnick et al., 2011). Cette variabilité devrait pourtant être prise en compte, tant à l'échelle individuelle (plasticité phénotypique) qu'à l'échelle populationnelle (variabilité entre les individus et dynamique temporelle en réponse aux processus de sélection ; Schulte et al., 2011), pour estimer cette capacité d'adaptation, a fortiori dans le contexte du changement climatique (Sutherst et al., 2011). L'objectif de ma thèse découle de ce constat : il consiste à évaluer le rôle de la variabilité spatio-temporelle ainsi que celui de l'hétérogénéité individuelle dans la réponse et l'adaptation locale de populations d'agents phytopathogènes aux variations de leur environnement.

Pour atteindre cet objectif, j'ai procédé en trois étapes : (i) en quantifiant la diversité phénotypique des réponses à la température au sein de populations naturelles soumises à des conditions climatiques contrastées et en mettant en évidence l'existence d'éventuels patrons d'adaptation à la température ; (ii) en caractérisant la façon dont la diversité de ces réponses au sein des populations contribue à leur potentiel adaptatif par la sélection des individus les plus performants sous les conditions thermiques environnantes ; (iii) en proposant un cadre formel de modélisation à l'échelle des sites d'interaction feuille-agent pathogène conçu pour évaluer l'impact de variations spatio-temporelles de température sur le développement des agents pathogènes (structure des populations) et ses conséquences épidémiologiques (intensité de maladie). Pour atteindre ces objectifs, j'ai mis en œuvre une approche translationnelle gravitant autour de trois disciplines (l'épidémiologie, l'écologie des populations et la biologie thermique) et d'un concept central (les courbes de réponses à la température ; TPC). Mes travaux, également à vocation appliquée, se sont focalisés sur un champignon pathogène du blé à fort impact agronomique, Zymoseptoria tritici (septoriose), retenu en raison de son exposition à une large gamme de température et de son potentiel adaptatif élevé (Linde et al., 2002).

Introduction - Les micro-organismes foliaires : un développement en environnement hétérogène et changeant (chapitre 1)

Les agents pathogènes foliaires se développent dans les tissus hôtes au sein d'un couvert végétal qui constitue un habitat singulier à l'échelle de ces micro-organismes. Un couvert végétal se distingue en effet par son microclimat foliaire (ou phylloclimat) souvent décrit comme extrême (Chelle, 2005 ; Vorholt, 2012 ; Gordon, 2020) en raison de son exposition aux rayonnements ultra-violets et à des fortes variations journalières de température. Par exemple, en élevant la température de surface de la feuille (Field et al., 1982), les radiations incidentes contribuent à des différences atteignant ponctuellement jusqu'à 10 °C entre la feuille et l'air ambiant (Cook et al., 1964 ; Pincebourde et al., 2007). Les hétérogénéités peuvent être fortes au sein d'un même couvert, voire à la surface d'une seule feuille (1°C à l'échelle d'une feuille de plant de tomate, 4°C dans le cas du soja et du haricot ; Cook et al., 1964 ; Jones, 1999 ; Aldea et al., 2005). La phyllosphère constitue donc un environnement à la fois hétérogène dans l'espace et changeant dans le temps. Les connaissances acquises sur d'autres systèmes d'études suggèrent que l'hétérogénéité de l'environnement modulent les processus de persistance, de compétition et d'évolution des populations d'agents pathogènes structurées spatialement et exposées à cette hétérogénéité. L'objectif fondamental de ma thèse était d'étudier les conséquences écologiques (à court terme) et évolutives (à plus long terme) de ces interactions.

Décrire les réponses à la température : vers du phénotypage à haut débit (chapitre 2)

La première étape de ma thèse a consisté à mettre au point une méthode de caractérisation standardisée de la réponse à la température de Z. tritici basée sur l'établissement et l'analyse d'un grand nombre de courbes de performance thermique. En effet, les méthodes de phénotypage actuellement employées ne prennent pas en compte de manière adéquate toutes les dimensions de l'expression du phénotype et de sa plasticité. Il existe en particulier des défis techniques concernant la robustesse, la reproductibilité et la quantification de ces réponses phénotypiques. Ces défis doivent être au préalable relevés afin de fournir des méthodes standardisées qui pourront ensuite être utilisées pour le phénotypage à grande échelle. J'ai ainsi conçu, développé et validé une méthode de phénotypage permettant de comparer les courbes de performance thermique, tant à l'échelle de l'individu (différences d'optima thermiques et plasticité) que de la population (polymorphisme intra-population). Sur quels traits doit-on mesurer ces réponses ? Comment quantifier la diversité phénotypique interindividuelle de ces réponses thermiques ? Quel aperçu nous en donne-t-il ? Telles sont les questions auxquelles j'ai apporté des éléments de réponse dans le cas particulier de Z. tritici. La méthode retenue est basée sur un suivi temporel du taux de multiplication cellulaire in vitro par mesure de densité optique en milieu liquide, sur microplaques, dans des conditions de laboratoire standardisées et miniaturisées (Figure 1). Elle s'accompagne d'une réflexion et d'une évaluation du sens biologique des courbes de performance thermique ainsi que d'une comparaison des réponses à la température de différents traits quantitatifs de la pathogénicité phénotypés in vitro et in planta pour 18 isolats qui viennent enrichir la caractérisation du phénotype.

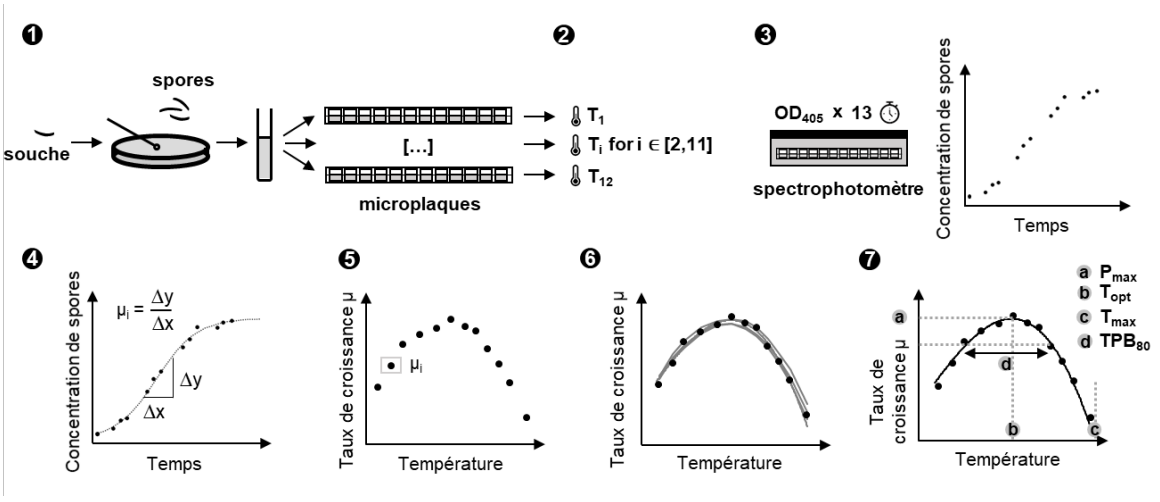


Figure 1 Méthode de phénotypage des réponses à la température chez Z. tritici

Caractérisation de patrons de diversité à quatre échelles spatio-temporelles (chapitre 3)

La seconde étape a consisté à analyser la variabilité de la réponse à la température des populations naturelles de Z. tritici en utilisant la méthode de phénotypage in vitro développée dans le chapitre précédent. Différents types d'échantillonnage ont permis de phénotyper des populations collectées à quatre échelles spatio-temporelles : (i) sur un même site géographique mais à différents stades de l'épidémie avec deux prélèvements au champ au début de l'hiver et à la fin du printemps ; (ii) le long d'un transect français Nord-Sud caractérisé par des températures annuelles moyennes croissantes mais des amplitudes été-hiver identiques ; (iii) le long d'un transect français Est-Ouest caractérisé par des températures moyennes identiques mais des amplitudes été-hiver croissantes ; (iv) à partir de populations issues de zones climatiques différentes à l'échelle euro-méditerranéenne. Par ces échantillonnages, il s'agissait en particulier de répondre à deux questions : dans quelle mesure les réponses thermiques sont-elles diverses et structurées entre les populations ? Peut-on détecter une adaptation locale aux conditions thermiques ? La comparaison de la réponse à la température de 18 sous-populations naturelles de Z. tritici (416 individus) a permis une analyse fine de la plasticité individuelle et des variabilités intra- et inter-populationnelles (Figure 2). Elle a mis en évidence un degré élevé de plasticité individuelle et de variation de la sensibilité aux conditions de température qui m'ont amenée à décrire ces populations en fonction de leur composition en "thermotypes" (groupes d'individus ayant des réponses thermiques similaires). Elle a également confirmé l'existence d'une dynamique d'adaptation à la température chez Z. tritici à deux vitesses suivant que l'on considère l'échelle de patrons saisonniers locaux (variation à une échelle temporelle) ou l'échelle régionale (variation à une échelle euro-méditerranéenne).

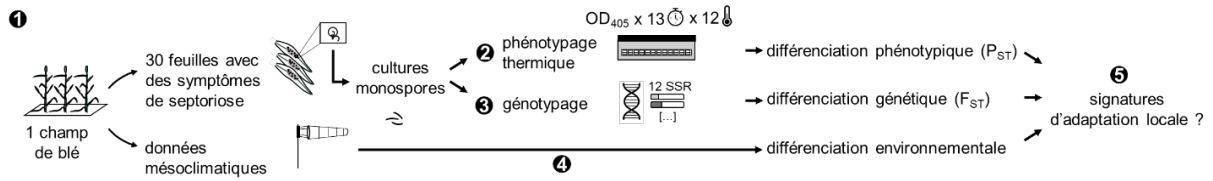


Figure 2 Caractérisation des patrons de diversité et d'adaptation locale des populations naturelles de Z. tritici à quatre échelles spatio-temporelles

Des patrons de diversité aux processus évolutifs (chapitre 4)

Les effets de la saisonnalité sur la composition fonctionnelle d'une population locale ainsi mis en évidence, plus prononcés semble-t-il que ceux de la géographie, suggèrent que les populations peuvent être structurées localement par une sélection opérant à court terme. J'ai exploré les conséquences de cette variation phénotypique inter-individuelle des réponses à la température sur la dynamique adaptative de populations à l'échelle d'une épidémie annuelle et au sein d'un peuplement hôte en cherchant à répondre à la question : Comment la composition de la population évolue-t-elle en fonction des variations environnementales ? J'ai postulé que la diversité des réponses individuelles à la température entre isolats de Z. tritici contribuait au potentiel adaptatif des populations via la sélection des individus les plus performants sous les conditions thermiques environnantes. J'ai examiné la multiplicité des pressions sélectives et des paramètres auxquels sont soumis les populations pathogènes (Figure 3). Pour cela, j'ai réalisé des expériences mettant en compétition des couples de Z. tritici appartenant à deux groupes fonctionnels de réponses thermiques (thermotypes) placés dans des environnements sélectifs de plus en plus complexes (in vitro, in planta et in natura ; régime thermique stationnaire ou fluctuant). Cette étape, conduite en partie à l'échelle polycyclique, a permis de déchiffrer la dynamique adaptative des populations fongiques aux variations environnementales à court terme, tant au niveau des individus que des populations, et a amélioré la représentation mécaniste que l'on peut avoir des processus populationnels. En conditions contrôlées, le suivi à l'échelle polycyclique de l'évolution des fréquences de différents thermotypes au sein de populations synthétiques, spécialement constituées pour tester des hypothèses spécifiques, a mis en évidence des processus de sélection naturelle basés sur l'adaptation thermique des différents isolats. En conditions naturelles, en revanche, il a été constaté un maintien de la diversité phénotypique qui pourrait être lié à des niveaux d'hétérogénéité thermique différents, selon l'échelle spatiale considérée, de la feuille à celle de la canopée.

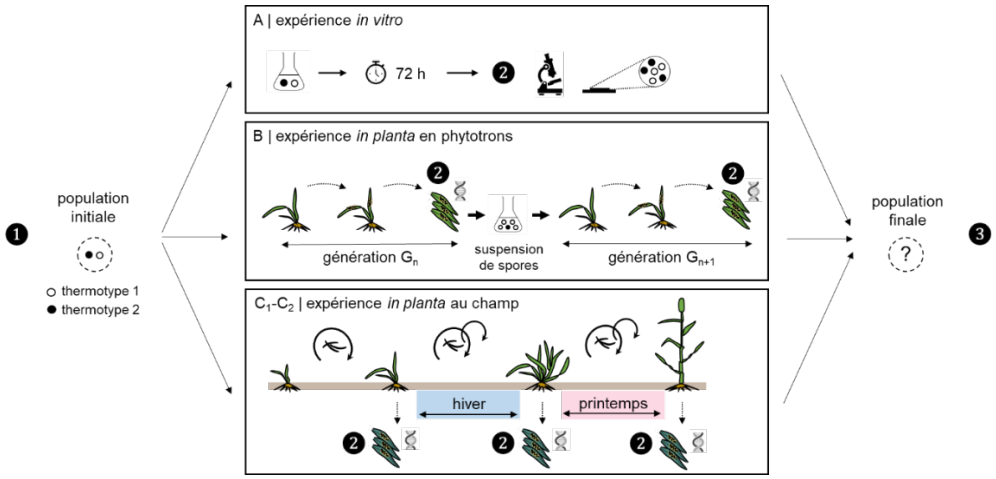


Figure 3 Mise en œuvre d'expériences de compétition de l'échelle monocyclique à l'échelle polycyclique pour caractériser les processus populationnels

Des patrons de diversité maintenus par l'existence de refuges thermiques (chapitre 5)

Le maintien local de la diversité phénotypique en conditions naturelles pourrait s'expliquer par le caractère hétérogène et dynamique des températures de l'habitat foliaire à des échelles spatio-temporelles fines. C'est pour tester la validité de cette hypothèse que j'ai évalué les hétérogénéités thermiques auxquelles sont effectivement soumises les populations de Z. tritici dans les couverts de blé afin de caractériser les pressions sélectives locales en fonction de l'échelle d'étude spatiale (de l'organe au couvert) mais aussi temporelle (pas de temps). Des dispositifs de mesures de température, déployés au champ, ont ainsi révélé à quel point la température des feuilles de blé varie à de très petites échelles d'espace et de temps. Cette hétérogénéité thermique locale du couvert entraîne une variabilité spatiale de la vitesse de développement de souches Z. tritici. Cela se traduit par des différences d'avantages compétitifs en différents points du couvert entre souches via l'existence de niches thermiques ou de zones refuges vis-à-vis de conditions de croissance qui seraient plus défavorables pour certains représentants du microbiote foliaire (Figure 4).

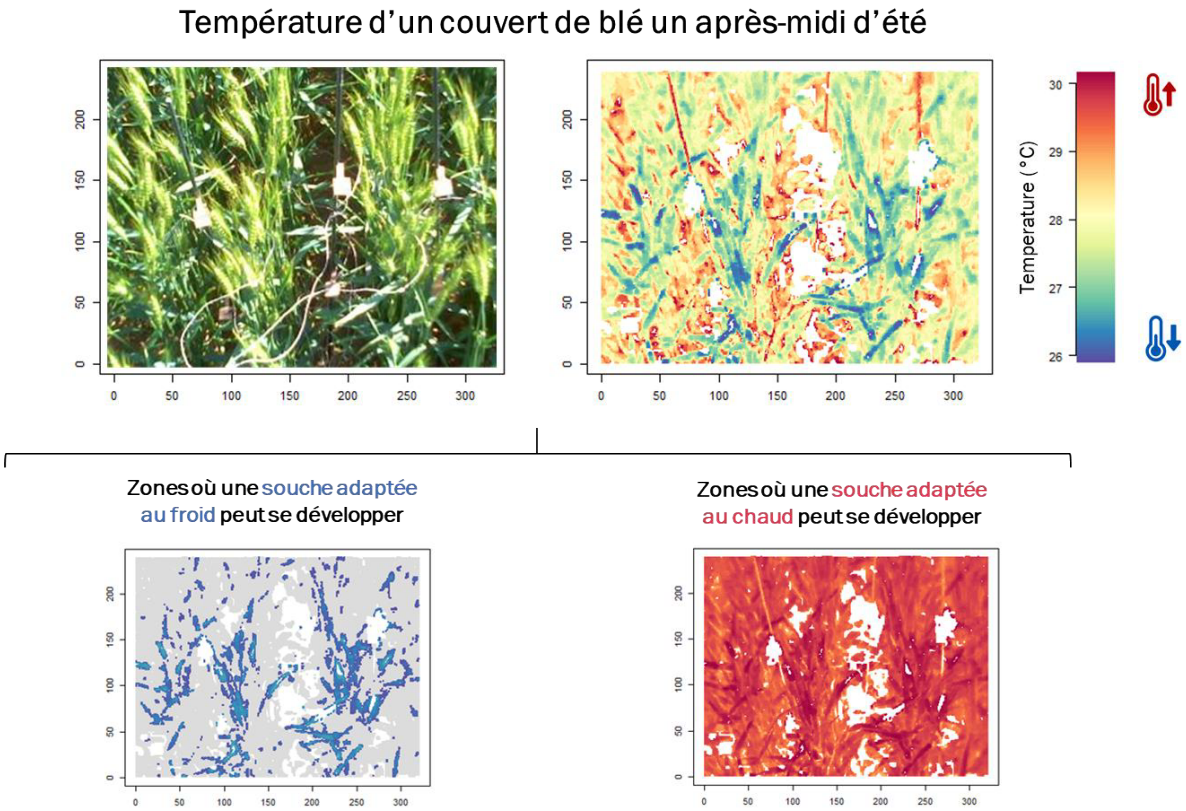


Figure 4 Hétérogénéité thermique d'une canopée de blé illustrant la diversification et à la stratification verticale de niches écologiques

Des données aux modèles, des modèles aux données (chapitre 6)

Dans une dernière étape, j'ai modélisé le lien entre la diversité phénotypique au sein d'une population et le potentiel adaptatif de cette population en réponse à une variable environnementale hétérogène à différentes échelles spatio-temporelles. Pour cela, j'ai cherché à établir la manière dont la sélection de groupes fonctionnels (englobés sous le terme de thermotypes précédemment définis) peut déterminer la dynamique et le potentiel d'adaptation des populations en réponse à l'hétérogénéité thermique. L'objectif recherché était d'une part, d'aboutir à une compréhension plus mécaniste des processus qui sous-tendent la dynamique des populations et, d'autre part, de quantifier l'impact des variations de réponses à la température entre individus sur la capacité d'une population à s'adapter à un environnement climatique fluctuant. Le modèle prend en paramètres d'entrée les caractéristiques des phénotypes de la population initiale et une description spatio-temporellement explicite de leur environnement thermique sur le nombre de cycles infectieux au cours d'une épidémie annuelle (Figure 5). Il rend compte de l'avantage compétitif que présente certains des phénotypes en réponse à la pression de sélection exercée par la température. Il permet, en tant qu'outil de recherche, d'amorcer la réflexion sur le couplage des processus ainsi que sur les différents points d'attention à considérer. Les premières simulations ont montré que s'affranchir de l'amplitude de la variation individuelle dans une population initiale ou de l'hétérogénéité thermique de la phyllosphère conduit à sous-estimer le maintien, à court terme, de la diversité et de l'équilibre dynamique entre thermotypes.

Des ajustements, s'appuyant sur des données expérimentales plus détaillées, pourront être apportées au modèle avant d'envisager de l'appliquer à un éventail plus large de situations écologiques. Le cadre conceptuel, développé sur la base des acquis des chapitres précédents et de cet outil numérique, permet d'ores-et-déjà d'étudier les processus de sélection en jeu, leur vitesse et leur ampleur en fonction de différents régimes de sélection de température.

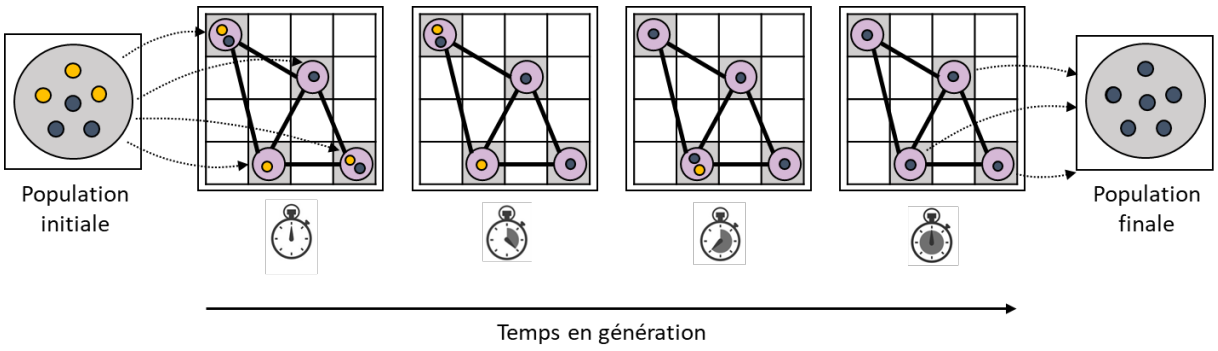


Figure 5 Modèle générique simulant la dynamique de populations spatialement structurées et évoluant en environnements hétérogènes

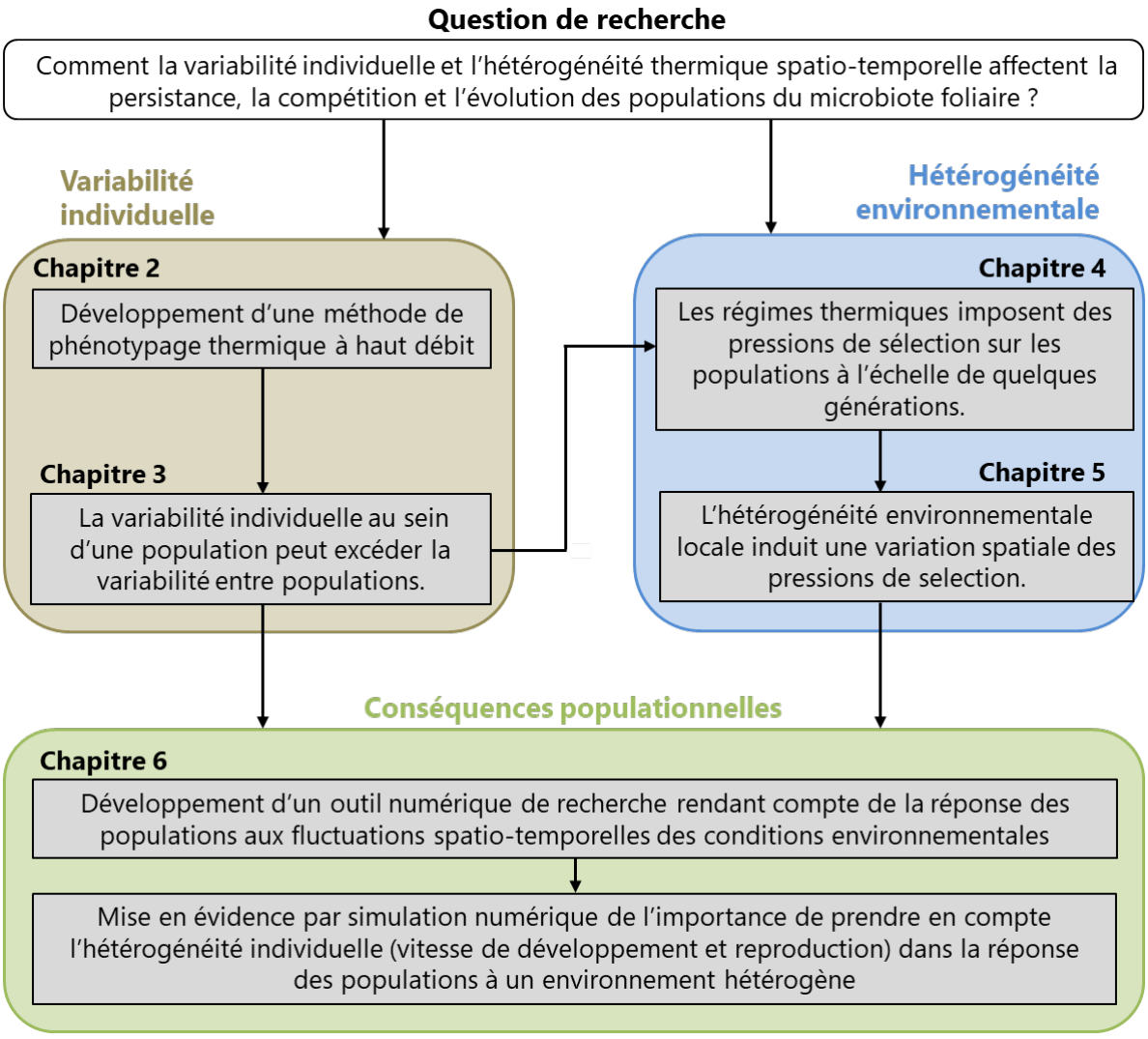


Figure 6 Articulation des résultats principaux de la thèse

Conclusions transversales et perspectives (chapitre 7)

L'objectif de ma thèse était d'étudier l'importance de la variation phénotypique individuelle et de l'hétérogénéité environnementale sur les processus écologiques et la dynamique des populations. J'ai exploré cette question, aux multiples facettes, à travers le prisme des populations d'agents pathogènes foliaires qui réalisent leurs cycles de développement dans les couverts végétaux, habitats thermiques temporellement erratiques et spatialement hétérogènes. L'intégration d'approches expérimentales et numériques pour étudier les patrons, les processus et les mécanismes d'adaptation locale à la température au sein de populations d'agents pathogènes ont souligné l'importance de prendre en compte l'hétérogénéité entre les individus dans la réponse des populations aux fluctuations des conditions (Figure 6). Ce travail de recherche sur les interactions plante-climat-pathogènes, intégrant l'environnement physique de l'organe comme composante fondamentale des interactions, constitue un cadre d'étude générique permettant d'évaluer le potentiel d'adaptation de populations d'agents pathogènes à des variations de température se manifestant à différentes échelles spatio-temporelles. Il s'agit d'une question pertinente et d'actualité qui s'insère dans un enjeu plus global : comprendre et anticiper le développement des épidémies végétales, leur gestion et leurs évolutions à des échelles de temps plus larges dans un contexte de bouleversements environnementaux et de changements climatiques (Chakraborty & Newton, 2011 ; Rohr et al., 2011 ; Chakraborty, 2013).

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Dissemination of Findings

PEER-REVIEWED PUBLICATIONS

› Boixel A.-L., Delestre G., Legeay J., Chelle M., Suffert F. 2019. Phenotyping thermal responses of yeasts and yeast-like microorganisms at the individual and population levels: proof-of-concept, development and application of an experimental framework to a plant pathogen. Microbial Ecology, 78: 42–56.

PREPRINTS

› Boixel A.-L., Chelle M., Suffert F. 2019. Patterns of thermal adaptation in a worldwide plant pathogen: local diversity and plasticity reveal two-tier dynamics. bioRxiv, https://doi.org/10.1101/2019.12.16.877696

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CONFERENCE PRESENTATIONS

Talks

› Boixel A.-L., Bernard F., Legeay J., Sache I., Chelle M., Suffert F. 2016. Response of French Zymoseptoria tritici populations to temperature at different spatio-temporal scales. Ninth International Symposium on Septoria Diseases of Cereals. Paris, France.

› Boixel A.-L., Chelle M. & Suffert F. 2017. Spatio-temporal diversity of thermal responses in populations of the wheat pathogen Zymoseptoria tritici. 12th EFPP (European Foundation for Plant Pathology) - 10th SFP (French Society for Plant Pathology) conference: Deepen knowledge in plant pathology for innovative agro-ecology. Dunkerque - Malo-les-Bains, France.

› Boixel A.-L., Svensson E., Chelle M. & Suffert F. 2018. Mechanisms behind population responses to variable thermal environments: experiments and model-based analyses of the role of intraspecific phenotypic variation. International Conference on Ecological Sciences (SFécologie 2018). Rennes, France.

› Boixel A.-L., Chelle M. & Suffert F. 2019. Hectic life on wheat leaves: dynamics of phenotypic selection within Zymoseptoria tritici populations facing microclimatic heterogeneities. International Symposium on Cereal Leaf Blights (ISCLB2019). Dublin, Ireland.

Posters

› Boixel A.-L., Chelle M., Suffert F. 2016. Impact of seasonal changes on Zymoseptoria tritici: can we detect thermal adaptation in local populations over the course of an annual epidemic? 8th Plant Health and Environment PhD students conference. Toulouse, France.

› Boixel A.-L., Chelle M. & Suffert F. 2019. Seasonal shifts and spatial variability of thermal adaptation in populations of Zymoseptoria tritici sampled over the Euro-Mediterranean region. International Symposium on Cereal Leaf Blights (ISCLB2019). Dublin, Ireland.

› Boixel A.-L., Gélisse S., Marcel T.C., Suffert F. 2019. First evidence of moisture adaptation in Zymoseptoria tritici. International Symposium on Cereal Leaf Blights (ISCLB2019). Dublin, Ireland.

INVITED ORAL COMMUNICATIONS

› Boixel A.-L. Adaptive responses of plant pathogen populations to spatio-temporal variations in temperature: an application to the case of the wheat foliar pathogen Zymoseptoria tritici. Scientific meeting of the 'Climate Change Impact on Fungal diseases' project. Rennes, France (December 2015).

› Boixel A.-L., Suffert F. Deciphering and predicting the adaptive potential of plant pathogen populations to global changes. Steering meeting of the 'Adaptation' group of the Biodiversity, Agro-ecosystems, Society and Climate Laboratory of Excellence. Gif-sur-Yvette, France (September 2017).

› Boixel A.-L. A journey into phenotypic diversity: the road taken and the road ahead. Evolutionary Ecology Unit, Department of Biology, Lund University. Lund, Sweden (April 2018).

SCIENTIFIC PROJECT RESTITUTION

› Boixel A.-L., Carpentier F., Chelle M., G Delestre, Fortineau A., Gélisse S., Goyeau H., Legeay J., Marcel T., Pincebourde S., Ravigné V., Retout N., Sache I., Valade R., Suffert F. 2017. SEPTOVAR project: responses of Z. tritici populations to spatial variations of temperature and host resistance and inference of their adaptive potential. Research funded by the French National Research Agency (LabEx BASC; ANR-11-LABX-0034).

- Contribution to the deliverables of the project: poster and final written project report [10 pages]
- In charge of the final oral restitution in 2017 at the Annual days of the Biodiversity, Agro-ecosystems, Society and Climate Laboratory of Excellence (BASC LabEx). Paris, France

université PARIS-SACLAY

ECOLE DOCTORALE N°581
Agriculture, alimentation, biologie, environnement et santé (ABIES)

Titre : L'hétérogénéité environnementale, un moteur de l'adaptation à la température des populations d'agents phytopathogènes foliaires ?

Mots clés : variation phénotypique, norme de réaction, diversité fonctionnelle, capacité compétitive, biologie thermique, *Z. tritici*

Résumé : Les facteurs environnementaux, au premier rang desquels la température, ont un impact sur la biologie des micro-organismes foliaires. Ils peuvent aussi modifier significativement leurs dynamiques populationnelles, voire leurs trajectoires évolutives. Classiquement, les modèles épidémiologiques, utilisés pour mieux gérer les maladies des plantes, intègrent l'influence des conditions météorologiques. Ils s'intéressent surtout à des réponses et des effets moyennés, ne tenant compte ni des variations des réponses individuelles, ni de l'hétérogénéité des changements environnementaux aux échelles réellement perçues par les agents pathogènes. Ces deux niveaux de simplification sont acceptables lorsque les états individuels et les variables continues qui leur sont associées, peu diversifiés, sont représentatifs de ceux de l'ensemble de la population. Il en va différemment lorsque les populations présentent des niveaux substantiels de variation individuelle susceptibles d'influencer leur capacité à s'adapter à leur environnement, et, par voie de conséquence, la dynamique des épidémies sous un climat fluctuant ou changeant. Pour mettre en évidence les conséquences de ces hypothèses réductrices, j'ai étudié comment la variation individuelle et l'hétérogénéité environnementale affectent simultanément la fitness, la composition phénotypique et la résilience des populations d'un agent pathogène foliaire (Zymoseptoria tritici) dans des couverts de blé. Trois étapes clés ont structuré l'exploration de ce cas d'étude. Tout d'abord, un protocole in vitro de phénotypage haut débit a été spécifiquement développé, validé et utilisé pour caractériser la diversité des réponses à la température de populations de Z. tritici échantillonnées à des échelles climatiques contrastées (variation spatiale et saisonnière) ainsi que leurs patrons d'adaptation. Les variations environnementales spatio-temporelles rencontrées dans les couverts de blé, considérées comme exerçant des pressions sélectives différentielles sur ces sensibilités thermiques individuelles, ont ensuite été examinées. Enfin, la façon dont la sélection de « thermotypes » (groupes fonctionnels rassemblant des individus présentant une même sensibilité thermique) détermine la dynamique adaptative des populations en réponse à l'hétérogénéité environnementale a été étudiée. Pour cela, des approches expérimentales (in vitro, in planta et in natura) et de modélisation (in silico) ont été couplées. Elles ont notamment porté sur plusieurs générations de populations placées dans des environnements sélectifs de plus en plus complexes. Ces travaux ont montré que le fait de négliger l'amplitude réelle de la variation phénotypique inter-individuelle d'une population microbienne et l'hétérogénéité des pressions de sélection, s'exerçant des échelles phyllo- à mésoclimatiques, conduit à sous-estimer la résilience de cette population, et donc son potentiel adaptatif. Les résultats de cette thèse, à l'interface entre épidémiologie, micrométéorologie et écologie, améliorent notre compréhension d'une part, de l'importance de la variation individuelle dans la dynamique adaptative des populations et, d'autre part, de la manière dont l'hétérogénéité environnementale permet de maintenir des populations globalement très diverses. Elle permet finalement d'expliquer l'existence de patrons d'adaptation, à la fois à des échelles locales et à des échelles très larges, par des dynamiques adaptatives « à deux vitesses ».

Title: Environmental heterogeneity, a driver of adaptation to temperature in foliar plant pathogen populations?

Keywords : phenotypic variation, reaction norm, functional diversity, competitive ability, thermal biology, *Z. tritici*

Abstract : Environmental drivers, most notably temperature, affect the biology of phyllosphere microorganisms but also induce changes in their population dynamics, even in their evolutionary trajectories. The impact of climate on foliar plant disease epidemics is usually considered in forecasting models to inform management strategies. Such models focus on averages of environmental drivers but disregard both individual variation within populations and the scale and extent of biologically relevant environmental changes. These simplifications are glossing over substantial levels of individual variation that may have important consequences on the capacity of a population to adapt to environmental changes, and thus on the dynamics of epidemics in a fluctuating or changing climate. To examine the range of validity and consequences of these simplifying assumptions, I investigated how individual variation and environmental heterogeneity jointly affect fitness, phenotypic composition and resilience of populations of a foliar pathogen (Zymoseptoria tritici) inhabiting wheat canopies. Three complementary ways of exploration were adopted in this case study. First, an in vitro high-throughput phenotyping framework was developed, validated, and used to characterise the diversity in patterns of thermal responses existing across Z. tritici populations that were sampled over contrasted scales (spatial and seasonal variation of temperature). Second, the spatio-temporal thermal variations encountered in a wheat canopy, considered as a habitat exerting fluctuating selective pressures on these differential thermal sensitivities of individuals, were investigated in depth. Third, the way selection of "thermotypes" (functional groups of individuals displaying a similar thermal sensitivity) occurs and drives dynamics of Z. tritici populations was examined. To this end, both empirical (in vitro, in planta and in natura) and theoretical (in silico) competition experiments were conducted under increasingly complex selective environments. This research work demonstrates that glossing over the natural extent of individual phenotypic diversity in a phyllosphere microbial population and over the heterogeneity of selective pressures – from phyllo- to mesoclimate – leads to underestimate the resilience of this population, and thus its adaptive potential to environmental variations. In doing so, the results of this thesis, at the interface between epidemiology, micrometeorology, and ecology, improve our understanding of how important is individual variation to population dynamics and how environmental heterogeneity allows to maintain population diversity. Finally, this thesis provides insight into how large-scale patterns and local population processes are interlinked and display a "two-tier" adaptive dynamics.

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