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ABSTRACT

Thermal ecology studies on the ecophysiological responses of organisms to temperature involve two paradigms: physiological rates are driven by body temperature and not directly by the environmental temperature, and they are largely influenced not only by its mean but also its variance. These paradigms together have been largely applied to macro invertebrates and vertebrates but rarely to microorganisms. According to these paradigms, foliar fungal pathogens are expected to respond directly to the fluctuations in leaf temperature, rather than in air temperature. We determined experimentally the impact of two patterns of leaf temperature variation of equal mean temperature, but differing in their daily amplitude, on the development of *Zymoseptoria tritici*, a fungus infecting wheat leaves. The highest daily thermal amplitude resulted in two detrimental effects for the pathogen fitness: an increase in the length of the latent period, i.e. the 'generation time' of the fungus when infecting its host plant, and a decrease in the density of fruiting bodies on the leaves. We discussed these empirical results, mainly the impact of both the daily thermal amplitude and the fluctuation frequency on the pathogen development *in planta*, in the light of the mathematical effect of the integration of non-linear functions. We concluded that it is necessary to take into account daily leaf temperature amplitudes to improve our understanding and prediction of the development of foliar fungal pathogens and other micro-organisms living in the phyllosphere in the climate change context.

Keywords: daily thermal amplitude, leaf temperature, reaction norm, leaf pathogen, *Zymoseptoria tritici*, variance

1. INTRODUCTION

Most organisms grow and evolve in fluctuating environments (Du and Ji 2006). This is particularly true regarding temperature, a factor that affects many, if not all, physiological processes involved in growth and development (Angilletta 2009). Temperature fluctuates at various time scales, from minute to years, with periodicity over daily and yearly periods, and each time scale can matter for different physiological processes from thermal tolerance (small time scales) to diapause/quiescence strategies (long time scales) (Dillon et al. 2016). The exchange of energy (radiation, convection, conduction, latent heat) between an organism and its environment generates temperature deviations between the body of organisms and their surrounding air. In ectotherms, the body temperature, subjected to fluctuating solar radiation and wind, and to night sky radiation cooling, is expected to fluctuate more intensively and rapidly than air temperature (Gates 1980). This paradigm also applies to plant surfaces, which generate a particular thermal environment for the large variety of organisms living on them, from phytophagous arthropods to bacteria and fungal pathogens.

Leaf dwelling organisms experience variations in the temperature of the leaf surface rather than in ambient air (Pincebourde and Woods 2012; Pincebourde et al. 2021; Scherm and van Bruggen 1993). Temperature heterogeneity in space and time over leaf surface can depart largely from ambient air, with deviation of up to 20°C between a specific leaf area and ambient air (Saudreau et al. 2017). The potential effects of large thermal amplitude on biological processes within the leaf envelope have been considered in studies on the effect of climate variations on arthropod development (Bradshaw et al. 2000; Kingsolver 1979; Pincebourde and Casas 2006; Potter et al. 2009 ; Pincebourde et al. 2016). The influence of the 'phylloclimate' (the microclimatic conditions occurring in the phyllosphere; Chelle 2005) is presumed to be high on the whole leaf microbiota (Pincebourde and Woods 2012; Vacher et al. 2016). Many studies focused on the impact of (constant) temperature on plant disease

64 cycles (incubation, latent period, senescence, etc.), both by experimental and modeling
65 approaches (de Wolf & Isard, 2007), but the impact of the fluctuation of leaf temperature on
66 the development of leaf fungal pathogens has never been studied, except partly by Bonde et
67 al. (2012). Some studies focused on entire living plants or detached leaves (e.g. Scherm and
68 van Bruggen 1994a; Xu 1996; Shakya et al., 2015) but were based on air temperature
69 fluctuations, while some others were carried out on artificial media instead of leaf (e.g. Zhan
70 and McDonald 2011; Boixel et al., 2018). This last experimental approach has two main
71 drawbacks: (i) a Petri dish or wells of a microplate does not have the same energy budget as a
72 living leaf; (ii) only the direct effect of temperature on the fungus can be observed, while the
73 complex interrelationship between environmental temperature, leaf temperature, and the foliar
74 and fungal responses are ignored. Therefore, the use of artificial media limits our ability to
75 apply growth predictions to more natural situations.

76 Mean temperature can be an uninformative, even misleading, descriptor of a fluctuating
77 thermal environment (Kingsolver et al. 2004). Thermal fluctuations likely matter for the
78 growth of fungal pathogens. Scherm & van Bruggen (1994b) were the first to demonstrate
79 theoretically that the difference between growth of plant fungal pathogens at constant versus
80 fluctuating temperatures is maximized when the mean temperature is close to one of the three
81 cardinal temperatures (minimal, optimal and maximal temperatures) and/or when the
82 temperature range over which the growth response is approximately linear is narrow. As an
83 example, the use of daily mean temperature to predict the incubation period (for plant
84 pathologists, the time needed for the first symptoms to appear) and the latent period (the
85 'generation time', i.e. the duration between inoculation and the appearance of fruiting bodies
86 releasing contaminating spores) of a fungal pathogen under field conditions may result in
87 errors when the underlying rate function is non-linear (Xu 1996). A higher resolution in
88 temperatures is therefore required, and several studies showed that the hourly time step

89 increased the accuracy of predictions (Narouei-Khandan et al. 2020; Salotti & Rossi 2021).
90 The model developed by Narouei-Khandan et al. (2020) to simulate effects of daily
91 amplitudes on the development of late blight highlighted a significant interaction between
92 average air temperature and amplitude in their effects on the area under the disease progress
93 curve (AUDPC) as predicted from growth chamber data on a single infection cycle. Greater
94 effects of amplitudes were observed at the extreme temperatures (including the optimal
95 temperature), and no amplitude effect at the inflection point of the optimal temperature curve.
96 The importance of daily temperature fluctuations was also demonstrated by Salotti & Rossi
97 (2021) for the development of Ascochyta blight on chickpeas. Environmental sampling rate,
98 such as the frequency of temperature recording (duration of time step), relative to the
99 frequency of leaf temperature changes, is therefore crucial when predicting organism fitness,
100 yet few studies have quantified its importance.

101 The objective of this study was to assess the relevance of using leaf temperature rather than
102 air temperature as climatic driver by (i) comparing daily amplitudes of leaf and air
103 temperatures in field conditions, and (ii) comparing the *in planta* development of a foliar
104 fungal pathogen under two leaf temperature regimes of equal mean but differing in their daily
105 leaf thermal amplitude (DLTA). As a case study, we used the fungus *Zymoseptoria tritici*
106 (formerly *Mycosphaerella graminicola*), the causal agent of Septoria tritici blotch disease on
107 wheat. Present wherever wheat is grown and developing throughout the wheat growing season
108 (Suffert and Sache 2011), the pathogen is exposed to a wide range of mean and amplitude
109 temperatures across its geographical distribution (Suffert et al. 2015). Finally, we used a
110 simple mathematical model based on a non-linear relationship of fungal performance to
111 temperature (see Supplementary Materials) to provide additional support for discussing on the
112 importance of daily temperature amplitude relative to both the shape of the nonlinear growth
113 curve and the frequency of temperature recordings.

2. MATERIAL AND METHODS

2.1. Comparison between leaf and air temperatures in field conditions

2.1.1. Study site

Field experiments were conducted on a winter wheat (*Triticum aestivum*, cv Tremie) plot established on a deep silt loam soil, at INRAE Thiverval-Grignon, France (48° 50' 43" N, 1° 56' 45" E). The crop was conducted as a conventional crop with high sowing density (250 grains.m^{-2}; sowing date 25 October 2011) and nitrogen supply (210 kg.ha^{-1}). No irrigation was supplied.

2.1.2. Temperature measurements

During the development of the flag leaf (from 14 May 2012 to 11 July 2012), the environmental temperature was estimated from the air temperature (TairWS) measured by a weather station (WS). TairWS was measured at 2 m height above a grass canopy at an hourly time step by a standardized weather station (model Enerco 516i, CIMEL Electronique, Paris, France) located 200 m from the field experiment plot without any topographical discontinuity between them. During the same period, the temperature of nine flag leaves (Tleaf) was measured with thin T-type thermocouples (diameter 0.2 mm) in contact with the abaxial surface of the flag leaf (so, the thermocouples were always under the leaf shade). The contact of thermocouples with leaves was checked three times a week. The thermocouples were connected to data-loggers (CR10 and CR1000, Campbell Scientific, North Logan, UT, USA), using multiplexers (AM25T and AM32, Campbell Scientific), retrieving the temperature every 20 s. The thermocouples and data-loggers were calibrated before and after the experiment.

2.2. Effect of the daily leaf temperature amplitude (DLTA) on the development of *Z. tritici* in growth chamber experiments

The experimental study was designed in growth chamber to quantify the effect of daily leaf thermal amplitude (DLTA) on the development of *Z. tritici*. The fungal development, here lesion development on plants, was estimated by three components of fitness (also considered as pathogenicity or aggressiveness components by plant pathologists): (i) the incubation period, (ii) the latent period, and (iii) the density of asexual fruiting bodies (pycnidia) on lesions.

2.2.1. Plant material

Seeds of wheat (*Triticum aestivum* L., cv Apache) were sown in Jiffy peat pots (Jiffy Strip Planter, Stange, Norway). Two weeks after seeding, when coleoptiles emerged, plants were vernalized in two controlled growth chambers (Strader, Pellouailles-les-Vignes, France), equipped with HPI-T PLUS lamps (400W; Philips Electronics NV, Amsterdam, the Netherlands) for eight weeks at 5°C with a 10 h light period and a 14 h dark period. Seedlings were subsequently transplanted into 1-liter pots filled with commercial potting soil mixed with 5 g of fertilizer (Osmocote Exact, Scotts, Heerlen, Netherlands) and placed in a controlled growth chamber at 16°C with a 14 h light period and a 9 h dark period. Plants were sprayed with Spiroxamine (Aquarelle SF at 2 $ml.l^{-1}$, Bayer CropScience, Lyon, France) to prevent infection by powdery mildew (*Blumeria graminis* f. sp. *tritici*). Before inoculation, plants were separated into two groups and placed in two identical growth chambers. Throughout the experiment, tillers were eliminated weekly to a final count of only three stems per pot.

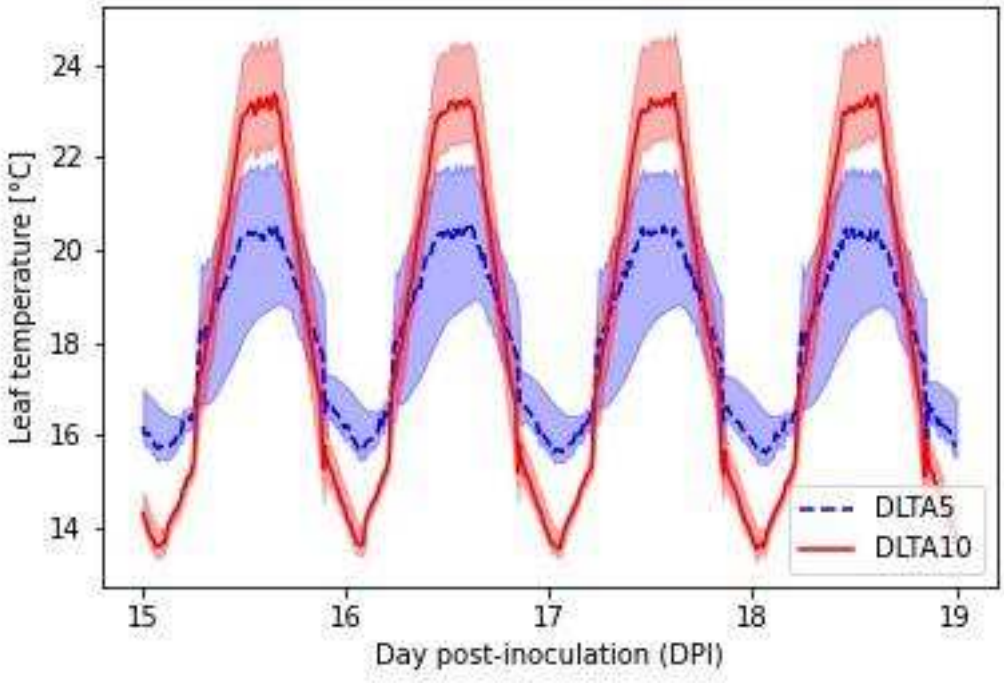
159 *2.2.2. Fungal material and leaf inoculation*

160 Three isolates of *Z. tritici* were used: INRA08-FS0001 (hereafter, isolate 1), INRA08-
161 FS0002 (isolate 2) and INRA08-FS0003 (isolate 3) (Suffert et al. 2013). Isolates 1 and 2 were
162 collected in 2008 from a wheat field located in Grignon (France); isolate 3 was collected the
163 same year from a wheat field located in Le Rheu (West part of France). In each growth
164 chamber, 144 leaves were inoculated with a single isolate. Three blastospore suspensions
165 were prepared the day of inoculation by flooding with water the surface of 5-day-old culture
166 on Petri dishes and then scraping the potato dextrose agar surface with a glass rod to release
167 blastospore. Concentration was adjusted to 10^5 blastospore ml^{-1} and three drops of Tween 20
168 (Sigma-Aldrich, St. Louis, MO, USA) were added to the suspensions to prevent the drift of
169 inoculum when applied on the leaves. The suspensions were applied with a paint brush over a
170 length of 25 mm on penultimate (rank F2) and flag (rank F1) leaves of the main tiller at
171 growth stage 39 when the flag leaf was fully emerged (Suffert et al. 2013). Inoculated leaves
172 were enclosed for 72 h in a transparent polyethylene bag moistened with water to provide
173 wetness requirement for infection. In the growth chamber, the light regime consisted of a 10-h
174 light period and a 14-h dark period. Once the infection was completed, to avoid artifacts
175 related to variation in exposure to light, inoculated leaves were maintained horizontally with
176 nylon wires at the height of each leaf layer, as described by Bernard et al. (2013).

177 *2.2.3. Daily leaf thermal amplitude (DLTA) patterns*

178 During the first 72 h after inoculation, plants were maintained at a similar thermal regime
179 (18 ± 2°C) in two identical growth chambers (same dimensions, same thermal regulation
180 system, same lighting system; Strader, Pellouailles-les-Vignes, France), to ensure comparable
181 optimal conditions for infection. Then, at 3 days post-inoculation (dpi) and throughout the
182 experiment, the two growth chambers were set up differently to generate a different DLTA ,
183 namely ± 2.5°C (DLTA5) and ± 5°C (DLTA10) (Fig. 1), while maintaining the daily mean

leaf temperature near an optimal temperature of about 18°C for both in order to maximize the effect of DLTA, as proposed by Scherm and van Bruggen (1994b). Importantly, these two contrasting leaf temperature regimes were obtained by playing on the different components of the leaf energy balance (air temperature, PAR and NIR lightning) at the level of each individual leaf. On the one hand, this mean temperature (18°C) matched with the optimal temperature of isolates 1, 2 and 3 (18.1°C, 18.5°C and 18.9°C, respectively, so 18.4°C in average; Bernard et al., 2013) and, more generally, with the average optimal temperature (18.3°C) estimated *in planta* using 110 *Z. tritici* isolates collected in the field at our study site (Boixel et al., 2022). On the other hand, these DLTAs are consistent with the daily temperature fluctuations which are recorded in winter (DLTA5) and spring (DLTA10) in Western Europe (Klein Tank et al. 2002). Moreover, the lighting systems in the two growth chambers were identical, with irradiance at the height of plant pots at different locations in the growth chamber varying from 238 to 353 $\mu mol.s^{-1}.m^{-2}$ with an average of 307 $\mu mol.s^{-1}.m^{-2}$. The relative humidity (RH), measured every 15 min, varied correlatively ($RH_{DTLA10} = 1.005 \times RH_{DTLA5}$; $r^2 = 0.998$) in the two growth chambers, with mean(RH) = 75.6 % and 76.0 %, max(RH) = 95.3 % and 96.3 %, and min(RH) = 56.0 % and 53.3 % for DLTA10 and DLTA5, respectively.



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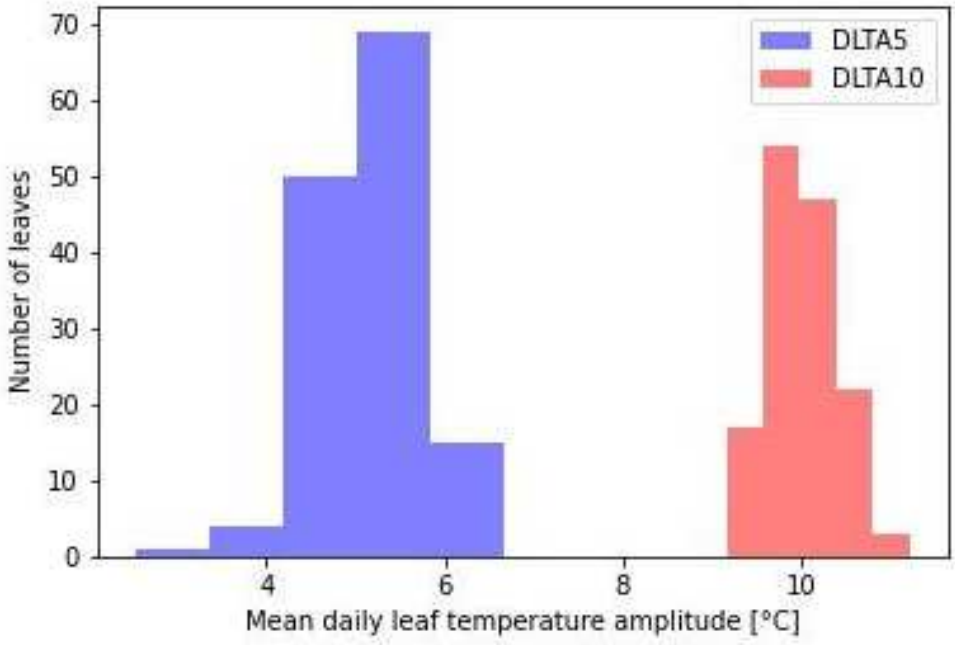


202

203 **Fig. 1.** (a) Leaf temperatures for the two treatments recorded on a four-day period; leaves
204 experiencing the same mean leaf temperature (18.2°C and 18.3°C, respectively) but distinct
205 daily leaf thermal amplitude (DLTA): $\underline{x} = 5.1$°C (DLTA5, dashed blue line) and $\underline{x} = 10.0$°C
206 (DLTA10, solid red line). The same pattern of leaf thermal amplitude was repeated

throughout the experiment (61 days). (b) Histogram of the leaf DLTAs averaged over the experiment duration for the two treatments: DTLA5 = 5.1 ± 0.3°C (blue bars); DLTA10 = 10.0 ± 0.2°C (orange bars).

2.2.4. Leaf temperature measurement

The temperature of each inoculated leaf was continuously measured with thin T-type thermocouples (diameter 0.2 mm) positioned under the leaf in contact with the inoculated area (Fig. 2; Bernard et al. 2013). Each thermocouple was connected to a data-logger, retrieving leaf temperature every 20 s. This allowed us to calculate temperature averages at increasing time steps, from 15 min to 24 h (0.25, 1, 2, 6 and 24 h), and to test the effect of temporal sampling temperature when modeling lesion development (see Supplementary Material S1). Due to the high number of leaves (N = 288), four data-loggers (CR10 and CR1000, Campbell Scientific) using multiplexers (AM25T and AM32, Campbell Scientific) were used. The contact of thermocouples with leaves was checked three times a week. The thermocouples were calibrated before and after the experiment. To avoid bias from using multiple data-loggers, the temperature of a single brass block was continuously measured by each data-logger. Temperature data homogenization was performed based on brass block temperature measurements and on results of pre and post experiment calibrations. The analysis of the two sets of leaf temperature measured every 15 minutes showed that the set-up of the two chambers did generate distinct distributions of the daily leaf temperature amplitude (Fig. 1), whose mean value was significantly different (Welch Two Sample t-test, p-value < 2.2e-16).

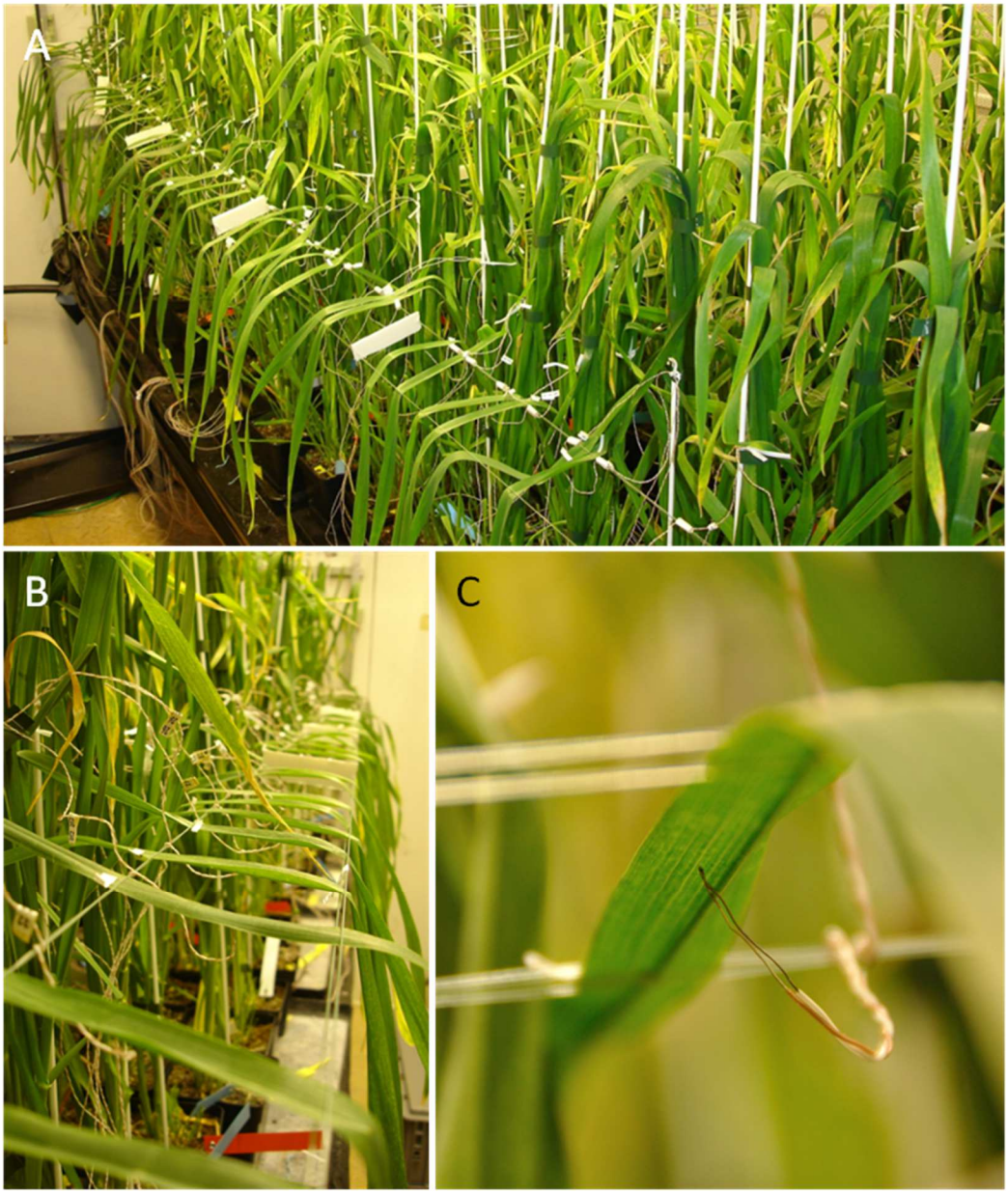


Fig. 2. (A) Wheat plants in one of the two growth chambers. (B) Inoculated leaves held in a horizontal position between two nylon wires. (C) T-type thermocouples positioned under the leaf in contact with the inoculated area.

2.2.5. Assessment of lesion development and pycnidia density

Starting 11 dpi, the development of each lesion was assessed 16 times, every 2 to 4 days. The respective percentage of the inoculated area covered by chlorosis, necrosis, and pycnidia (0, 1, 2, 3 and 5%, then increments of 5% up to 100%) was estimated visually by the same

236 assessor throughout the experiment (more details on methodology in Suffert et al. 2013).
237 Disease assessment ended 61 dpi when the leaf apical senescent area coalesced with the
238 diseased area. Finally, the number of pycnidia was counted by eyes on digitized images (1200
239 × 1200 PPI) of the adaxial side of each leaf. The density of pycnidia was obtained by dividing
240 the number of pycnidia by the inoculated area.

241 *2.2.6. Estimation of incubation period and latent period*

242 Incubation period was estimated for each leaf by the time elapsed from inoculation to the
243 first day with visible chlorosis. Latent period was estimated by the time elapsed from
244 inoculation to 37% of the maximum sporulating area, assessed by fitting a Gompertz model to
245 the area covered by pycnidia (Bernard et al. 2013; Suffert et al. 2013). The value 37%
246 corresponds to the ordinate at the inflection point of a Gompertz curve (Winsor 1932).
247 Incubation period and latent period were expressed in dpi. Disease curve fitting was
248 performed using R software v. 3.4.2 (R Core Team, 2013).

249 2.3 Statistical analysis

250 Leaf and air temperature metrics under field conditions were compared performing
251 Pearson correlations using the R software v. 3.4.2 (R Core Team, 2013). The influence of
252 daily leaf temperature amplitude (DLTA) on the development of *Z. tritici* was analyzed using
253 a Repeated Measure ANOVA (RM-ANOVA, under SYSTAT 13.1 software, SYSTAT Inc.)
254 to include the property that each dependent variable (respective percentage of the inoculated
255 leaf covered by chlorosis, necrosis, and pycnidia) was measured repetitively through time on
256 the same leaves. In this RM-ANOVA, both the DLTA (DLTA5 and DLTA10) and the
257 identity of the isolate were designed as factors. The rank of a given leaf for each individual
258 plant (penultimate (F2) and flag (F1) leaves) was included as a covariate to remove the
259 variability induced by a potentially different response between these two categories of leaves.

In the repeated measure procedure, the dpi was used to include the factor time and to estimate the within-subject variability (the identity of individual leaves). This statistical approach allowed us to analyze the interaction terms between time (dpi) and all factors (DLTA and isolate) on within-subject effect sizes. The between-subject analysis also included the interaction term between DLTA and isolate identity. Finally, we analyzed the latent and the incubation periods with a classic ANOVA since these variables were unique values for each individual leaf. A Tukey's Honestly-Significant-Difference Test was used to run pair-wise comparisons whenever this was needed. The conditions required to run an analysis of variance were checked for all variables using a Shapiro-Wilk Test (for normality) and a Levene test (for homogeneity of variances based on the mean or median). Statistical significance was estimated at a threshold of 0.05.

3. RESULTS

3.1. Comparison between leaf and air temperatures in field conditions

The daily mean leaf temperature was very similar to ($P = 0.88$), and correlated to the daily mean air temperature ($R^2 = 0.98$; Fig. 3a). In 90% of cases, the difference between the two mean temperatures was below 0.7°C. In contrast, the daily amplitude was higher for leaf temperature than for air temperature (Fig. 3b; $P < 0.001$). On average, the daily amplitude was 5.8°C higher for the leaf temperature (14.1 ± 3.8°C) than for the air temperature (8.3 ± 2.9°C). We refined these differences in amplitude by analyzing the daily minimum and maximum of these two temperature metrics. The daily minimum temperature of air from the weather station and of leaves were correlated ($R^2 = 0.91$); leaves were generally cooler than air, which could be explained by the radiative heat loss during nighttime in the case of clear

skies (Tleaf_min = 1.07; Tairws_min - 2.8 °C). The daily maximum temperature of air from weather station and of leaves were less correlated (R^2 = 0.79); leaves were generally warmer than ambient air, which could be explained by the radiative forcing (Tleaf_max = 0.94; Tairws_min + 4.6 °C). For a day randomly chosen during this period (7 July 2010), we present the corresponding range of development rate of *Z. tritici* depending on whether the leaf temperature or the air temperature is considered (Bernard et al. 2013) (Fig. 4c). This comparison illustrates the extent to which daily leaf temperature fluctuation may push the pathogen toward lower developmental rate, close to the upper temperature limit.

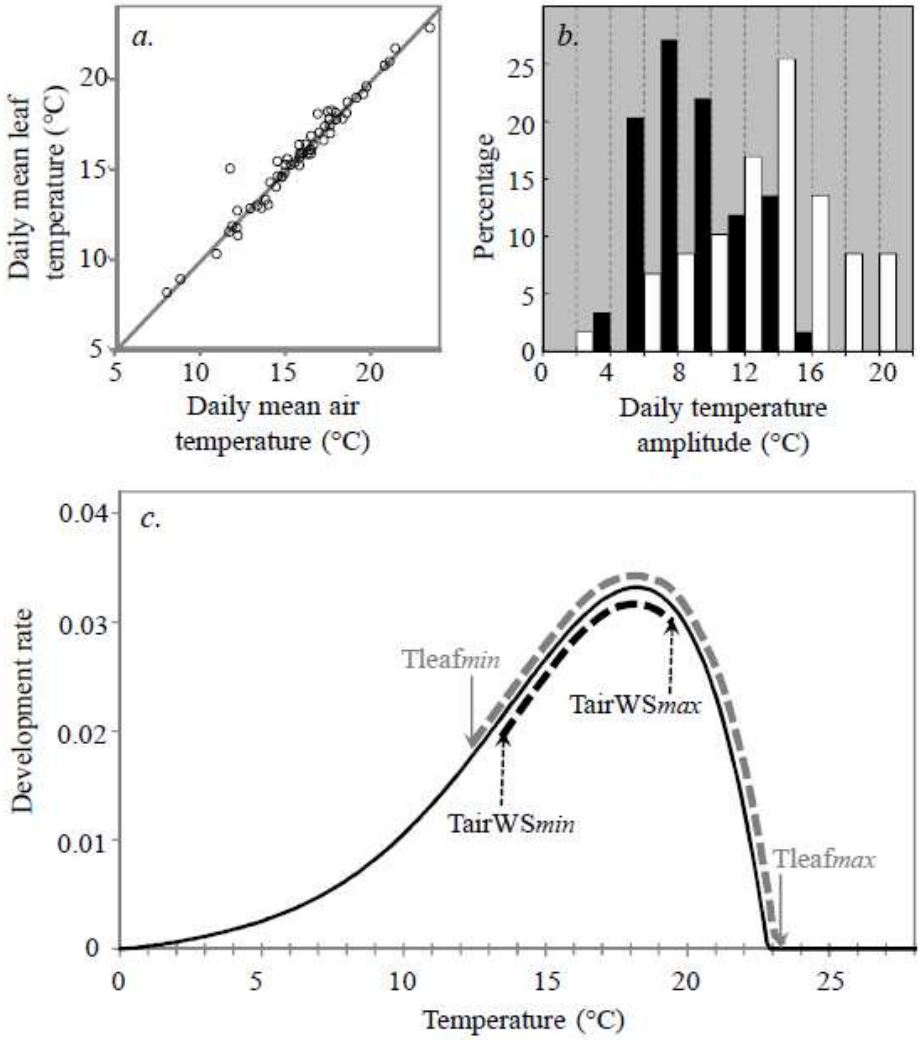


Fig. 3. (a) Relationship between daily mean leaf temperature and daily mean air temperature measured from 14 May to 11 July 2012. Leaf temperature corresponds to the mean

294 temperature of upper leaves measured by a thermocouple in a wheat plot (Thiverval-Grignon,
295 France). The air temperature corresponds to the temperature measured by a weather station
296 located ~200 m from the plot. (b) Frequency of daily leaf (white bars) and daily air (black
297 bars) temperature amplitudes (daily Tmax – Tmin) measured from 14 May to 11 July 2012.
298 (c) Example of the ranges of air (Tairws) and leaf temperatures (Tleaf) measured during a
299 single day (10 July 2012) and the corresponding ranges of development rate of *Zymoseptoria*
300 *tritici*, visualized here using an asymmetric reaction norm from Bernard et al. 2013 (S1.2).

301 ## 3.2. Effect of the daily leaf temperature amplitude (DLTA) on the
302 ## development of *Z. tritici* in growth chamber conditions

303 ### *3.2.1. Effect of DLTA on lesion development*

304 The development of necrotic area differed according to the daily leaf temperature
305 amplitudes (marginally) and according to the three isolates (Table 1; Fig. 4a-c). The absence
306 of interactive effect of DLTA and isolate indicates that all isolates responded similarly to
307 DLTA (Table 1). Necrosis displayed a strong temporal dynamics and the significant
308 interaction terms of the RM-ANOVA (dpi × DLTA, dpi × isolates) indicated that the temporal
309 dynamics differed according to the DLTA and to the isolate (Table 1). Necrosis appeared first
310 at 20 dpi on 27.8% and 15.6% of the leaves (all isolates together) under DLTA5 and
311 DLTA10, respectively. At 20 dpi, the mean necrotic area was smaller for DLTA10 than for
312 DLTA5 for the three isolates. Final mean necrotic area under the two DLTAs was similar for
313 all isolates, reaching more than 98% of the inoculated area.

314

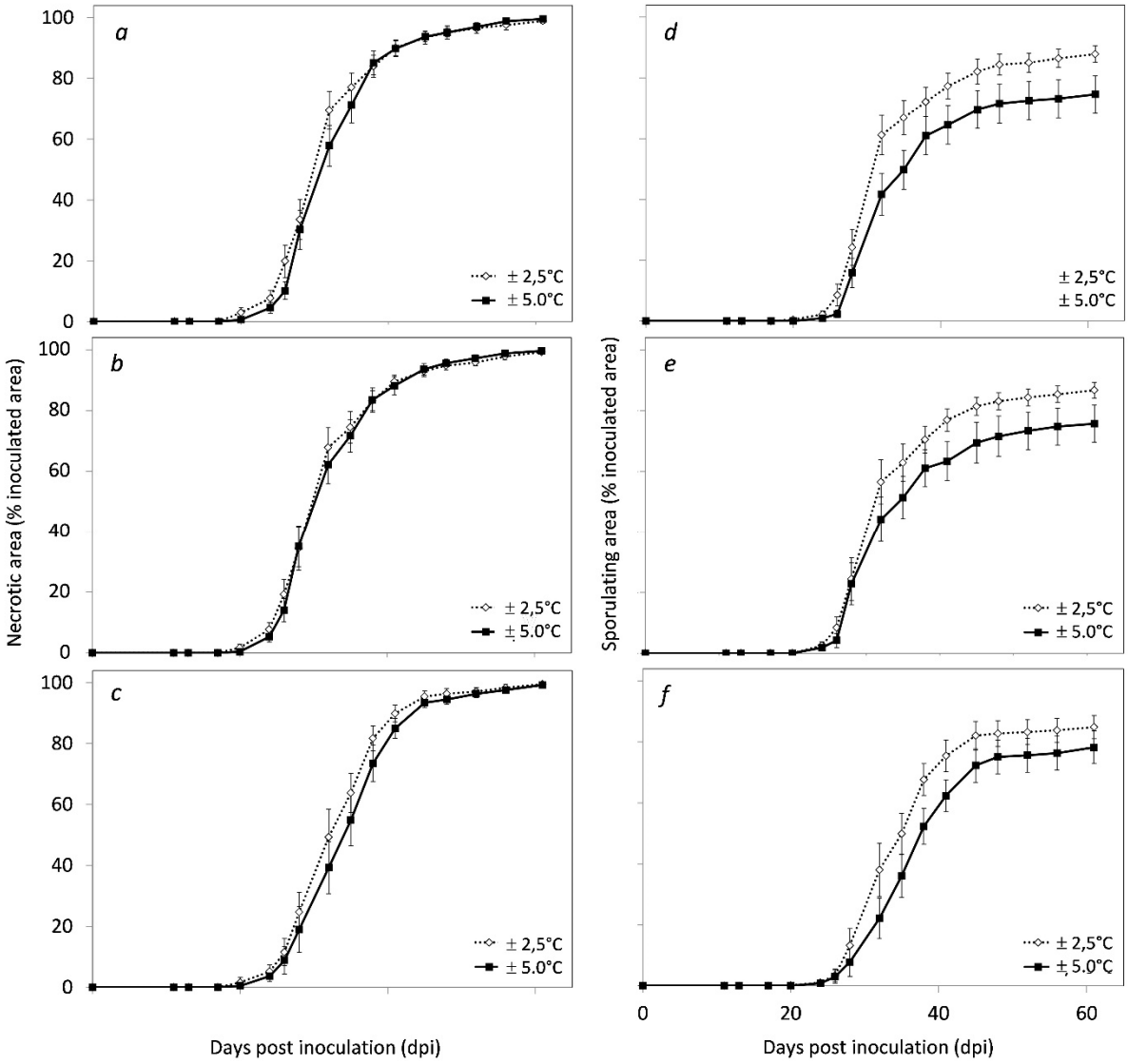
315 Table 1. Statistical report of the RM-ANOVA on the effects of DLTA (daily leaf temperature
316 amplitude, 2 levels), isolate (3 levels), the rank of the leaf (2 levels, defined as a covariate),
317 time (dpi: days post inoculation) and all interactions on the respective percentage of the

inoculated leaf covered by chlorosis, necrosis, and sporulating. Significant P-values are indicated in bold.

Variable	Level	Source	df	Mean square	F-ratio	P-value
Chlorosis	Between-subject	DLTA	1	2.492	0.083	0.774
		Isolate	2	716.526	23.861	< 0.001
		DLTA × isolate	2	19.446	0.648	0.524
		Rank	1	36.061	1.201	0.274
		Error	278	30.03		
	Within-subject	Dpi (time)	16	453.014	30.822	< 0.001
		Dpi × DLTA	16	72.983	4.966	< 0.001
		Dpi × isolate	32	169.722	11.548	< 0.001
		Dpi × DLTA × isolate	32	18.059	1.229	0.176
		Dpi × rank	16	68.442	4.657	< 0.001
		Error	4 448	14.698		
Necrosis	Between-subject	DLTA	1	5 306.765	5.191	0.023
		Isolate	2	8 687.176	8.498	< 0.001
		DLTA × isolate	2	518.625	0.507	0.603
		Rank	1	4 213.683	4.122	0.043
		Error	278	1 022.294		
	Within-subject	Dpi (time)	16	50 290.751	471.633	< 0.001
		Dpi × DLTA	16	521.837	4.894	< 0.001
		Dpi × isolate	32	1 174.404	11.014	< 0.001
		Dpi × DLTA × isolate	32	97.077	0.91	0.612
		Dpi × rank	16	532.962	4.998	< 0.001
		Error	4 448	106.631		
Sporulation	Between-subject	DLTA	1	78 429.364	49.22	< 0.001
		Isolate	2	7 718.209	4.844	0.009
		DLTA x isolate	2	557.922	0.35	0.705
		Rank	1	125 619.850	78.835	< 0.001
		Error	278	1 218.520		
	Within-subject	Dpi (time)	16	13 002.747	100.073	< 0.001
		Dpi × DLTA	16	2 548.793	19.616	< 0.001
		Dpi × isolate	32	1 297.766	9.988	< 0.001
		Dpi × DLTA × isolate	32	85.636	0.659	0.929

Dpi × rank	16	6 742.437	51.892	< 0.001
Error	4 448	129.932		

The development of sporulating area differed between the daily leaf temperature amplitudes ± 2.5°C (DLTA5) and ± 5°C (DLTA10), in a similar way for all isolates (Table 1). Again, interaction terms showed that the temporal dynamics of the sporulating area varied according to the temperature treatment and the isolate (Table 1). Pycnidia appeared at 20 dpi on 3.5% of the leaves under DLTA5 and 24 dpi on 19.1% of the leaves under DLTA10 (Fig. 5d-f). From 24 to 61 dpi, the mean sporulating area was higher under DLTA5 than under DLTA10. For the three isolates, the final sporulating area was significantly higher under DLTA5. Sporulating area was 18%, 15%, and 9% larger on leaves under DLTA5 than on leaves under DLTA10 for isolates 1, 2, and 3, respectively.



331

332 **Fig. 4.** Growth of necrotic (a-c) and sporulating area (d-f) for isolates 1 (a, d), 2 (b, e), and 3
333 (c, f) of *Zymoseptoria tritici*, for two daily leaf thermal amplitudes (DLTA): ± 2.5°C
334 (DLTA5, dashed lines) and ± 5°C (DLTA10, solid lines). Error bars are confidence interval
335 (95%).

336 *3.2.2. Effect of DLTA on components of fitness*

337 Overall, the mean incubation period was shorter under DLTA5 than under DLTA10 for all
338 isolates (Table 2; Fig. 5a-d). The pair-wise comparisons, however, indicated that this
339 difference was significant for isolates 1 ($P = 0.005$) and 3 ($P < 0.001$) and not for isolate 2 (P

340 = 0.369; Fig. 5). Under DLTA10, the incubation period was increased by 1.4, 0.8, and 1.8 dpi
341 on average compared to DLTA5, for isolates 1, 2 and 3, respectively, and by 1.3 dpi when
342 considering all isolates together.

343

344 **Table 2.** Statistical summary of the ANOVA on the effects of DLTA (daily leaf temperature
345 amplitude, 2 levels), isolate (3 levels), the rank of the leaf (2 levels, defined as a covariate)
346 and all interactions on the incubation period, latent period and density of pycnidia. P-values
347 indicated in bold are significant.

Variable	Source	df	Mean square	F-ratio	P-value
Incubation period	DLTA	1	119.371	31.51	**< 0.001**
	Isolate	2	3.213	0.848	0.429
	DLTA × isolate	2	5.187	1.369	0.256
	Rank	1	65.318	17.242	**< 0.001**
	Error	278	3.788		
Latent period	DLTA	1	87.964	8.613	**0.004**
	Isolate	2	185.136	18.127	**< 0.001**
	DLTA × isolate	2	3.034	0.297	0.743
	Rank	1	6.229	0.61	0.435
	Error	270	10.213		
Density of pycnidia	DLTA	1	4 654.721	32.52	**< 0.001**
	Isolate	2	936.062	6.54	**0.002**
	DLTA × isolate	2	397.127	2.775	0.064
	Rank	1	46 169.564	322.565	**< 0.001**
	Error	278	143.132		

348

349 The mean latent period was also shorter under DLTA5 than under DLT10 globally (Table
350 2; Fig. 5e-h), but this effect may be marginal given that the pair-wise comparison was unable
351 to retrieve significant differences between the two temperature treatments for each isolate ($P <$
352 0.05 when considering all isolates together). Under DLTA10, the latent period was increased
353 by 1.3, 0.7, and 1.4 dpi on average for isolates 1, 2 and 3, respectively, and by 1.2 dpi when
354 considering all isolates together.

The density of pycnidia, also influenced by the DLTA overall (Fig. 5i-l), was significantly different for isolates 1 ($P < 0.001$) and 3 ($P = 0.004$) but not for isolate 2 ($P = 0.682$). The density of pycnidia was on average 32% higher under DLTA5 (32 pycnidia.cm^{-2}) than under DLTA10 (24 pycnidia.cm^{-2}) (Fig. 5l). Under DLTA10, the density of pycnidia decreased by 37%, 11% and 25% on average for isolates 1, 2 and 3, respectively, and by 24% when considering all isolates together.

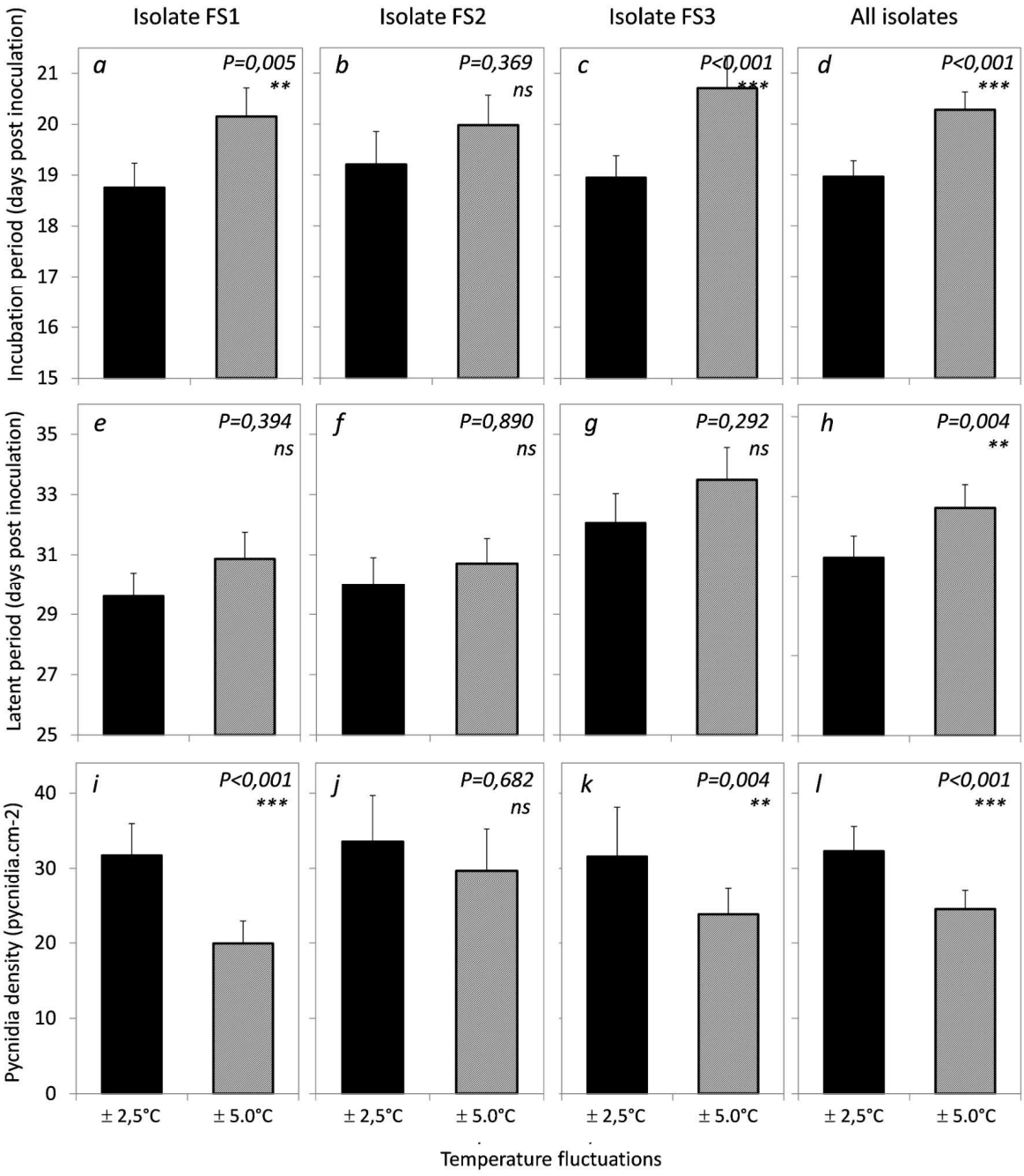


Fig. 5. Effect of two daily leaf temperature amplitudes (DLTA): ± 2.5°C (DLTA5; black bars) and ± 5°C (DLTA10; hatched bars) on incubation period (a-d), latent period (e-h), and density of pycnidia (i-l) for *Zymoseptoria tritici* isolates 1 (a, e, i), 2 (b, f, j), 3 (c, g, k), and all isolates together (d, h, l). Values are means. Error bars are confidence interval (95 %). *P*-values (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns for not significant) were determined from the ANOVA (Table 2).

4. DISCUSSION

We established in field conditions that the daily amplitude can be highly dependent on the type of temperature even if the average remains the same: air commonly measured by a weather station vs leaf, i.e., in more general terms ‘environmental’ vs ‘body’ temperature. Concretely, the temperature range of a wheat flag leaf is greater than that of air temperature. This can be explained by two mechanisms. During the day, solar radiation hits the leaf, increasing its surface temperature relative to the air. During clear-sky nights, the leaf loses energy due to thermal radiation, and its surface becomes cooler than air. Moreover, the microclimate at the weather station, even if placed near the field plot, may differ from the plant canopy microclimate (evapotranspiration, turbulent boundary layer, advection, slope, etc.). Therefore, the use of air temperature from weather stations, as commonly done, seems inappropriate, as highlighted empirically by Bernard et al. (2013) for *Z. tritici*. This leads to erroneous interpretation of the effect of temperature on the development of foliar fungal pathogens, which depends non-linearly on the amplitude of the temperature (see Fig. 3c). To our knowledge, Bonde et al. (2012) were the first to investigate the temperature amplitude effect on a foliar fungal pathogen (*Phakopsora pachyrhizi*, the causal agent of Asian soybean rust) by simultaneously measuring leaf and air temperatures. However, they concluded that air and leaf temperature were nearly equal. These contrasting results may come from their particular experimental set-up in growth chambers with light systems that did not induce temperature excess in soybean leaves. Moreover, their conclusion relates more to the impact of the variation in temperature patterns representative of the different locations throughout the growing season than to the impact of the diurnal temperature amplitude.

Our experimental results suggest that a higher daily leaf temperature amplitude (despite similar mean temperature) resulted in two detrimental effects for the pathogen: an increase in the length of the latent and incubation periods and a decrease in the density of fruiting bodies

394 (pycnidia). The effect size differed between these variables however. More precisely, while
395 the growth of the necrotic area (Fig. 4a-b) was marginally affected, which can be viewed as
396 the expression of damage, the growth of the sporulating area (Fig. 4d-f) and the three
397 components of fitness, which are strong drivers for a polycyclic plant pathogen, were much
398 more impacted (Fig. 5). Overall, the higher amplitude (± 5°C) resulted in lower pathogen
399 performance. The growth of the sporulating area was slowed down and the final area was
400 reduced, the incubation period and latent period were lengthened and, more strikingly, the
401 density of pycnidia was reduced. These results obtained with three isolates from two different
402 climatic areas will have to be extended to populations acclimated to various climatic regimes
403 differing both in terms of temperature average and variance, but we currently lack field data
404 to investigate this relationship at the biogeographical level.

405 Our study has inherent limitations that need to be discussed. The laboratory experiment
406 was not repeated sensu stricto contrary to what Shakya et al. (2015) did, in the sense that each
407 treatment (DLTA5 and DLTA10) was not replicated in each of the two growth chambers.
408 However, as mentioned in the Material and Methods section, the growth chambers were twin
409 (same model) and we verified by dedicated physical measurements that light and RH
410 conditions were similar. This point is crucial considering the potential impact of several
411 abiotic factors on the development of Septoria tritici blotch (Benedict 1971; Shaw 1991;
412 Boixel et al. 2022). Furthermore, our experimental design was relevant from a biological
413 point of view as the 'replicates' were not at the level of the chamber but at the scale of the
414 individual leaf: we measured independently the temperature for each inoculated leaf section,
415 i.e. the temperature really perceived by the pathogen, even if from a purely statistical point of
416 view all replicated leaves within a growth chamber appeared as 'pseudoreplicates' (Colegrave
417 & Ruxton, 2018). The statistical disadvantage of this approach was compensated by the
418 technical advantage of having several independent temperature measurements at the

individual leaf level. The leaf temperature is not spatially and temporally homogeneous, even in the case of true replicates. This was not the case in the experimental study of Shakya et al. (2015), for instance.

In addition, all plants in our experiment were maintained at a similar thermal regime during the first 72 h after spore deposition on the leaves to facilitate the start of the disease (Fantozzi et al. 2021). We cannot exclude however that the DLTA could also influence the start of the disease development. The impact of moisture, which is a parameter difficult to manage as it depends on temperature, is also known to be significant in *Z. tritici* (Boixel et al. 2022). The temperature-moisture interaction poses experimental problems in many other fungal plant pathogens and for this reason it is rarely investigated during the early stages of infection (e.g. during the first 24 h post-infection, when testing the impact of temperature on infection efficiency in *Puccinia striiformis* f. sp. *tritici*; de Vallavieille-Pope et al. 2018). Nevertheless, it should be acknowledged that spore germination and hyphal growth on the leaves are crucial steps in many pathosystems and models need to integrate the corresponding epidemiological components (de Wolf & Scott 2007; Chaloner et al. 2021).

The difference in disease development observed under two thermal amplitudes is partly due to the 'rate summation' effect, also called Kaufmann effect, which explains the differences in the growth of organisms under constant and various levels of fluctuating environments (Cossins and Bowler 1987; Scherm and van Bruggen, 1994b). We verified this effect for the studied host-pathogen interaction, using a simple modelling approach (see Supplementary Material S1); the model allowed us to test this effect on a wider range of DLTA than in our experimental approach (Fig. S1.4). Not related to a particular biological process, the Kaufmann effect is the mathematical consequence of the nonlinear shape of many biological functions together with the amplitude inherent in many environmental factors (Bozinovic et al. 2011; Ruel and Ayres 1999; Scherm and van Bruggen 1994a). This raises

444 the difficult question of the choice of the function to use for a given TPC (symmetric vs
445 asymmetric, number of parameters, etc.; Angilletta, 2006; Shi & Ge, 2010) (Fig. S1.1, S1.3).
446 This mathematical effect, called the Jensen's inequality (Jensen, 1906), expresses the fact that
447 the value of a non-linear function of an integral differs from the integral of the non-linear
448 function. We compared development near the optimal mean leaf temperature to maximize the
449 effect of temperature fluctuations on pathogen development (Scherm and van Bruggen,
450 1994b). The high leaf temperature obtained under the highest amplitude slowed down, and
451 probably even stopped the development of the fungus for several hours each day. In addition,
452 during the night, when temperatures were the lowest, the pathogen development was slower
453 under the highest amplitude. As a mathematical consequence of non-linear thermal reaction
454 norms, the latent period was longer under the highest amplitude (Fig. 5h).

455 How the temperature fluctuates during a day (frequency) has an impact on the dynamics of
456 the biological responses to temperature. However, the physiological inertia of each biological
457 process involved in these responses is still poorly understood. As this issue is difficult to
458 study experimentally, we relied on additional simulations (Supplementary Material S1) to
459 quantify the influence of the fluctuation regime on the Kauffman effect, by transforming the
460 sinusoidal daily variation of temperature into different step-functions corresponding to a
461 temperature sampling at different time-step. We observed that when the sampling time step
462 increased (from 0.25 to 6 h), the estimation of latent period slightly decreased, but it was
463 always higher that when estimated using daily mean temperature (Fig S1.4). A similar trend
464 was observed by Niehaus et al. (2012) on embryos and larvae of anurans for which growth
465 and development proceeded more rapidly than expected in variable environments. Xu (1996)
466 suggested that a time step up to 4 h is short enough to account for the diurnal fluctuating
467 temperatures. Our experimental design in growth chambers, which results in a steady
468 fluctuation during the day, could not generate short-time temperature extremes (e.g. during

sunflecks) that can also influence the development of the fungal pathogen (Bonde et al. 2012). Accounting for temperature extremes necessitates decreasing the time step of temperature used for simulations down to below 15 min to ensure that short-term extremes are captured (Gutschick and BassiriRad 2003). A time step of 1 h however proved to be sufficiently short for simulation of late blight, a fast epidemic, when temperatures remain close to the optimum (Narouei-Khandan et al. 2020).

In addition to the Kaufmann effect, physiological mechanisms may lead to an acceleration or – most likely – a retardation of the development under fluctuating temperature conditions, as previously suggested for insects (Worner, 1992). According to Niehaus et al. (2012), two biological phenomena can generate a mismatch between the predicted and actual fitness in fluctuating environments. Chronic exposure to an extreme temperature can have a deleterious effect on fitness, referred to as thermal stress (e.g. Pincebourde and Casas, 2019) and/or can trigger a beneficial response, referred to as thermal acclimation (e.g. Stillman and Somero, 1996). The development of a fungal pathogen in plant tissues might be also accompanied by overall 'homeostatic' and 'compensatory' effects. This hypothesis could be confirmed by comparing the impact of similar DLTA on the fungal growth *in planta* and *in vitro*, following the methodology developed by Boixel et al. (2019), for instance. Moreover, this leads us to analyze our results with caution as seasonal fluctuations in field conditions, at much larger time steps, was shown to drive the thermal adaptation in *Z. tritici* populations (Suffert et al. 2015). Given the population diversity in *Z. tritici* (Boixel et al., 2019), it is likely that intra-day thermal fluctuations studied here could have an impact on the adaptive dynamic of a local population. This could explain for instance how it adapts to the most stressful thermal conditions in certain geographic areas.

5. CONCLUSION

Incorporating microclimatic conditions ('phylloclimate') and the thermal reaction norm of plant pathogens when studying their interaction with plant hosts is a convincing way to develop future disease management in the frame of agroecology (Nicholls and Altieri 2007). Our study suggested the importance of considering daily leaf temperature amplitudes – and not only the average leaf temperature or daily air temperature amplitudes – when investigating the development of foliar fungal pathogens. Interestingly, our results parallel the conclusions of Paaijmans et al. (2010) who found that it is necessary to consider daily fluctuations in water temperature to predict mosquito development and the epidemiological dynamics of malaria. The dynamic of a polycyclic epidemic is characterized by several embedded infection cycles of the pathogen. Selective dynamics within a pathogen population can be amplified by a high number of these cycles, a high diversity in the thermal responses of the individuals, and the effective thermal amplitudes (Suffert et al., 2015). Small variations in temperature conditions can increase the variability in the responses, but at some points in the infection cycle, synchronization can occur, leading again to a more uniform reaction (Fantozzi et al. 2021). The phenomenon of alternating phases of variability and synchronization was already mentioned in the epidemiology book by Zadoks and Schein (1979). Our results contribute to define the adequate amplitude and time step that matter for these epidemiological processes. Therefore, differences in latent period under the two amplitudes that we highlighted at the scale of a single infection cycle are expected to be magnified over the course of the epidemic. Our results also have two major implications for foliar fungal pathogen studies: (i) leaf temperature amplitude has to be considered to study the acclimation of pathogens, and (ii) epidemiological models need to keep a high temporal resolution as the choice of the time step is crucial to obtain accurate forecasts. These models would also greatly benefit from integrating the spatial heterogeneity of leaf temperature within canopies as the

518 thermal amplitude can differ according to the leaf micro-environment. This point is
519 particularly critical when using models for disease forecasting and climate change
520 assessments (Garcia-Carreras and Reuman 2013; Paaijmans et al. 2010). Leaf temperature
521 should be characterized experimentally on very small plots, due to its high spatial and
522 temporal variability and its dependence on crop architecture. For larger-scale studies, the most
523 pragmatic way is to use microclimatic models (e.g. Berry et al., 1991) – or even
524 phylloclimatic models for a finer consideration of canopy architecture (Chelle, 2005) – to
525 simulate leaf temperatures from air temperatures and other climatic variables (radiation, wind,
526 humidity) from weather stations as it is now done for ectotherm organisms (Bramer et al.
527 2018).

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