New Phytologist

The development of a foliar fungal pathogen does react to leaf temperature!

Frédéric Bernard[1,2], Ivan Sache[3], Frédéric Suffert[3] and Michaël Chelle[1]

[1]INRA, UMR1091 EGC, F–78850, Thiverval-Grignon, France; [2]ARVALIS – Institut du Végétal, F–91405, Orsay, France; [3]INRA, UR1290 BIOGER, F–78850, Thiverval-Grignon, France

Author for correspondence:
Michaël Chelle
Tel: +33 1 30 81 55 31
Email: chelle@grignon.inra.fr

Received: *31 October 2012*
Accepted: *6 December 2012*

New Phytologist (2013) **198:** 232–240
doi: 10.1111/nph.12134

Key words: fungal pathogen, latent period, *Mycosphaerella graminicola* (*Zymoseptoria tritici*), phylloclimate, reaction norm, temperature, thermal performance curve, wheat (*Triticum aestivum*).

Summary

- The thermal performance curve is an ecological concept relating the phenotype of organisms and temperature. It requires characterization of the leaf temperature for foliar fungal pathogens. Epidemiologists, however, use air temperature to assess the impacts of temperature on such pathogens. Leaf temperature can differ greatly from air temperature, either in controlled or field conditions. This leads to a misunderstanding of such impacts.
- Experiments were carried out in controlled conditions on adult wheat plants to characterize the response of *Mycosphaerella graminicola* to a wide range of leaf temperatures. Three fungal isolates were used. Lesion development was assessed twice a week, whereas the temperature of each leaf was monitored continuously.
- Leaf temperature had an impact on disease dynamics. The latent period of *M. graminicola* was related to leaf temperature by a quadratic relationship. The establishment of thermal performance curves demonstrated differences among isolates as well as among leaf layers.
- For the first time, the thermal performance curve of a foliar fungal pathogen has been established using leaf temperature. The experimental setup we propose is applicable, and efficient, for other foliar fungal pathogens. Results have shown the necessity of such an approach, when studying the acclimatization of foliar fungal pathogens.

Introduction

Among climatic variables, temperature is a major driving force in physiological processes affecting the development of living organisms (Belehradek, 1926; Campbell & Norman, 1998). The biological processes involved in development are driven by the organism's temperature; in ecology this is termed 'body temperature' (Brown *et al.*, 2004; Huey & Kingsolver, 2011). Although widely used by ecologists in the study of ectotherm organisms, the concept of body temperature has been largely ignored by agronomists and epidemiologists. For fungal pathogens that develop mainly onto and into leaves, the body temperature is the leaf temperature (i.e. the temperature they actually perceive; Lovell *et al.*, 2004).

Plants attempt to maintain a leaf temperature equilibrium, which would make metabolic functions operate at nearly optimal temperatures (Helliker & Richter, 2008). For a leaf at equilibrium, the amount of energy that enters via solar radiation and ambient heat is equal to that which exits the leaf through heat loss, scattered light and transpired water. If the leaf is not at equilibrium with its environment, the leaf temperature will change until equilibrium is achieved. Equilibrium for leaves is usually attained in <1 min (Linacre, 1966; Kitano *et al.*, 1983). The energy budget equation of a single leaf (Gates, 1980) can be expanded to:

$$\text{Absorbed radiation} = \text{Re-radiation} + \text{Convection} + \text{Transpiration} \quad \text{Eqn 1}$$

where re-radiation depends on leaf temperature, convection depends on the temperature gradient between leaf and air, and transpiration depends on the gradient of vapor pressure between leaf and air, which in turn depends on the leaf and air temperatures.

In order to characterize the climate of a leaf within a plant population, Chelle (2005) defined the 'phylloclimate'. Phylloclimate is characterized by a high temporal and spatial variability owing heterogeneity of microclimatic conditions (wind, radiation, humidity and air temperature) mainly generated by the canopy architecture. Because of the leaf energy balance (Eqn 1), phylloclimate differs from local microclimate, which corresponds to the climate at the scale of a layer (canopy). Microclimate itself differs from the regional climate (mesoclimate) occurring around a given canopy (Lhomme, 1995), because of the complex interaction between the energy fluxes and the canopy structure. Often mesoclimatic variables used are those measured by the network of weather stations.

Moreover, the difference between air and leaf temperatures depends on the enclosure used for experiments (Linacre, 1964; Smith, 1978; Weng & Liao, 2001). For the same air

temperature, the difference in leaf temperature between field and controlled environment can be > 3°C (De Boeck *et al.*, 2012). In addition, the growing environment in a controlled growth chamber or a glasshouse usually has a nonuniform spatial pattern of air temperature (Potvin *et al.*, 1990; Demrati *et al.*, 2007) and of leaf temperature (Kichah *et al.*, 2012). These patterns consistently differ from the pattern in the field.

Thermal performance curves (TPCs), which comprise a special case of reaction norm for phenotypic traits related to fitness, are used to assess the response of organismal development to temperature (Kingsolver *et al.*, 2004; Schulte *et al.*, 2011). These curves have recently re-emerged as a central tool in ecological studies. Thermal performance curves provide objective estimates of optimal temperature, 'performance breadth' (the range of temperature with high performance; Huey & Stevenson, 1979; Gilchrist, 1995; Angilletta *et al.*, 2002) and 'tolerance zone' (the temperature range of survival of the organism; Jobling, 1981; Huey & Kingsolver, 1989). These curves are strongly nonlinear (Angilletta, 2006).

The difference between air and leaf temperatures combined with the nonlinearity of TPCs leads to errors when using air temperature to estimate the development of organisms onto or into leaves (Helmuth *et al.*, 2010; Pincebourde & Woods, 2012). This has already been reported for insects (Pincebourde *et al.*, 2007; Potter *et al.*, 2009; Park *et al.*, 2011) but not for foliar fungal pathogens. Such studies of such pathogens have only considered air temperature, regardless of the growing system (field, glasshouse, controlled growth chamber).

The aim of this study is to quantify the effect of leaf temperature on the development of a foliar fungal pathogen using a TPC-based approach. To this end, we grew adult wheat plants in a controlled growth chamber or in a glasshouse and inoculated their leaves with the fungus *Mycospaherella graminicola*. During the course of infection, we measured the temperature of each leaf and assessed disease on the same leaf. The data were used to establish the TPC for the latent period (generation time). The fungus *Mycosphaerella graminicola* is the causal agent of the septoria tritici blotch (STB) disease on wheat. Present wherever wheat is grown, and developing throughout the wheat growing season, this pathogen is exposed to a wide range of temperatures. Thermal adaptation, achieved through both phenotypic plasticity and genetic differentiation, has been demonstrated for *M. graminicola* grown on artificial media (Zhan & McDonald, 2011). The effect of air temperature on the development of *M. graminicola* on wheat leaves is well-known (Hess & Shaner, 1987; Shaw, 1990; Magboul *et al.*, 1992; Chungu *et al.*, 2001), but experiments addressing the effect of leaf temperature have not yet been reported.

Materials and Methods

Plant material

Wheat (*Triticum aestivum* L. cv Apache) seeds were sown in Jiffy peat pots (Jiffy Strip Planter; Stange, Norway). Two weeks after sowing, when coleoptiles emerged, plants were vernalized in a controlled growth chamber (Strader, Pellouailles-les-Vignes, France), equipped with HPI-T PLUS lamps (400 W; Philips Electronics NV, Amsterdam, the Netherlands) for 8 wk at 6°C with a 10-h light period and a 14-h dark period. Seedlings were subsequently transplanted into 1-l pots filled with commercial potting soil mixed with 5 g of fertilizer (Osmocote Exact; Scotts, Heerlen, Netherlands). Plants were sprayed with spiroxamine (Aquarelle SF at 2 ml l^{-1}; Bayer CropScience, Lyon, France) as a preventive measure to control powdery mildew (*Blumeria graminis* f. sp. *tritici*). Plants were then separated into two groups, placed either in a glasshouse or in a controlled growth chamber. Plants were grown under long-day conditions (15 h : 9 h, day : night). The average air temperature measured above plants was 11.4°C in the controlled growth chamber and 18.4°C in the glasshouse. Throughout the experiment, tillers were eliminated weekly to a final count of only three stems per plot.

Fungal material and leaf inoculation

Three isolates of *M. graminicola,* aggressive to wheat cv. Apache (Suffert *et al.*, 2013), were used: INRA08-FS0001 (isolate 1), INRA08-FS0002 (isolate 2) and INRA08-FS0003 (isolate 3). Isolates 1 and 2 were isolated in France in 2008 from a wheat field located in Grignon (48°50′43″ N, +1°56′45″E); isolate 3 was isolated the same year from a wheat field located in Le Rheu (+48°5′54″ N, −1°47′52″E). Grignon and Le Rheu are under mid-latitude continental and true temperate maritime climates, respectively. Inoculation was performed at growth stage 39 (Zadoks *et al.*, 1974) when the flag leaf was fully emerged, as described in detail by Suffert *et al.* (2013). Each leaf was inoculated with a single isolate. Conidia suspensions were prepared the day of inoculation by flooding the surface of 5-d-old culture on Petri dishes with water and then scraping the potato dextrose agar (PDA) surface with a glass rod to release conidia. Concentration was adjusted to 10^5 conidia ml^{-1}. Three drops (0.15 ml) of Tween 20 (Sigma-Aldrich) were added to 50 ml of inoculum solution to prevent drift of inoculum. The suspension was applied with a paintbrush over a length of 25 mm on penultimate (F2) and flag (F1) leaves of the main tiller. Inoculated leaves were enclosed in a transparent polyethylene bag for 72 h, which was moistened with distilled water to provide optimal humidity conditions for infection. Once infection was completed, to avoid lighting artifacts, inoculated leaves were maintained horizontally with nylon wires at the height of each leaf layer (Fig. 1). The number of inoculated leaves for each enclosure, leaf rank and isolate is given in Table 1.

Leaf temperature measurements

The temperature of each leaf was measured with thin T-type thermocouples (diameter 0.2 mm) positioned under the leaf in contact with the inoculated area. Each thermocouple was connected to a datalogger (Campbell Scientific, North Logan, UT, USA) that recorded leaf temperature every 20 s, averaged over 15 min. Owing to the high number of leaves, five dataloggers were used. The contact of thermocouples with leaves was checked

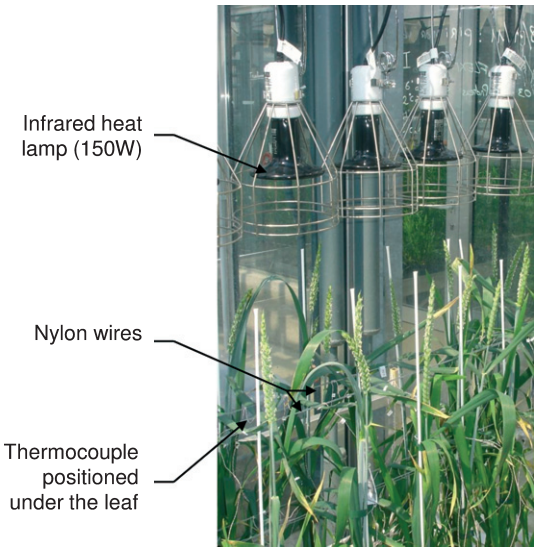

Fig. 1 Close-up of the experimental setup in the glasshouse. Wheat (*Triticum aestivum*) leaves (F1 (flag leaves) and F2 (second leaves) of the main tiller) inoculated with *Mycosphaerella graminicola* were maintained horizontally with nylon wires. The range of leaf temperature was increased using various densities of black infrared heat lamps (150 W) positioned 40 cm above the leaves. Leaf temperatures were measured by thermocouples positioned under the leaves in contact with the inoculated areas.

Table 1 Number of wheat (*Triticum aestivum*) leaves inoculated with three *Mycosphaerella graminicola* isolates

Location	Growth chamber		Glasshouse	
Leaf layer	F1	F2	F1	F2
INRA08-FS0001 (isolate 1)	17	19	12	20
INRA08-FS0002 (isolate 2)	18	16	8	20
INRA08-FS0003 (isolate 3)	14	20	9	18

F1, flag leaves; F2, second leaves.

three times a week. The thermocouples were calibrated before and after the experiment. To avoid bias from using multiple dataloggers, the temperature of a single brass block was measured continuously by each datalogger. Temperature data homogenization was performed based on brass block temperature measurements and on results of pre- and post-experiment calibrations. The range of leaf temperature was increased, without altering light incidence, using black infrared heat lamps (ceramic heat emitter 150 W; Hagen, Baie d'Urfé, QC, Canada) positioned in groups of three lamps above 33% of the leaves and five lamps above 33% of the leaves. No lamp was positioned above the remaining leaves.

Lesion development

From 12 d to 78 d post-inoculation (dpi), the development of lesions on each leaf was assessed every 2–4 d. The proportions of the inoculated area covered by chlorosis, necrosis and pycnidia (spore-bearing organs) were estimated visually by the same assessor. In the following text, we call the temporal progress of chlorotic and necrotic areas 'disease dynamics' and the temporal progress of the area covered by pycnidia 'sporulating area dynamics'. Disease assessment ended when the leaf apical senescent area coalesced with the diseased area.

Curve fitting and disease assessment

Lesion and sporulating areas We denoted the maximum percentage of the inoculated area covered by chlorosis and necrosis together as LES_{max} and the maximum percentage of the inoculated area covered by spores as SPO_{max}. For each leaf, a Gompertz growth curve (Hunt, 1982) was fitted to the sporulating area dynamics, SPO(t).

$$SPO\ (t) = SPO_{max} \times e^{(c \times e^{(d-t)})} \quad \text{Eqn 2}$$

In this equation c is the growth rate at the inflection point, and d is the time (in dpi) at inflection point.

Incubation and latent periods Incubation period, the time needed for lesions to appear, was defined as the time to reach 50% of leaves with visible lesions (Johnson, 1980; Shaw, 1986; Douaiher *et al.*, 2007). Latent period, LP, the time needed for a generation of the pathogen, was defined as the time elapsed from inoculation to 37% of SPO_{max}, assessed from the sporulation area fitted curve (Suffert *et al.*, 2013). The value of 37% corresponds to the ordinate at the point of inflection of Gompertz curve (Winsor, 1932). Incubation and latent period were expressed in dpi. Once the latent period had been determined for all leaves, a quadratic function (Eqn 3) was fitted to the relationship between leaf temperature and latent period:

$$LP(T_l) = LP_{min} + Curv \times (T_l - T_{l,opt})^2 \quad \text{Eqn 3}$$

LP_{min} is the minimal latent period at optimal leaf temperature ($T_{l,opt}$), $Curv$ is a shape parameter and T_l is the mean leaf temperature during the experiment.

Establishment of thermal performance curves As proposed by Shaw (1990), we expressed the performance as the inverse of the latent period. Eqn 4 corresponds to the TPC of *M. graminicola* latent period to leaf temperature.

$$TPC(T_l) = 1/(LP_{min} + Curv \times (T_l - T_{l,opt})^2) \quad \text{Eqn 4}$$

Analysis of leaf layer effect on latent period Because of the unequal distribution of mean leaf temperatures between flag leaves (F1) and second leaves (F2), the analysis of leaf layer effect was performed on the subsample that included all the leaves having a mean leaf temperature between 17.0°C and 17.5°C. For this temperature range, mean leaf temperature for F1 ($n = 12$) and F2 ($n = 12$) groups is equal ($P = 0.44$).

Statistical analysis

Disease curve fitting and subsequent statistical analysis were performed using R statistical software v. 2.12.1 (R Development Core Team, 2010). Mean leaf temperatures ranged from 10.1°C to 16.4°C in the controlled growth chamber and from 16.3°C to 20.9°C in the glasshouse. For the sake of clarity, the disease dynamics was analysed by grouping leaves in classes according to their mean leaf temperature. Hence, leaves were grouped in five classes of equal amplitude: C1 from 10.1°C to 12.3°C ($n = 83$), C2 from 12.3°C to 14.4°C ($n = 17$), C3 from 14.4°C to 16.6°C ($n = 12$), C4 from 16.6°C to 18.7°C ($n = 69$), and C5 from 18.7°C to 20.9°C ($n = 10$). Hereafter, $C1_L$, $C2_L$, $C3_L$, $C4_L$, and $C5_L$ will refer to leaves having mean leaf temperatures in classes C1, C2, C3, C4 and C5, respectively.

Results

Disease dynamics

Lesions appeared on all inoculated leaves except for five leaves, which were excluded from the dataset. Disease assessment was carried out until 61 dpi on $C3_L$, $C4_L$ and $C5_L$ and until 78 dpi on $C1_L$ and $C2_L$, depending on leaf senescence and disease dynamics. The first visible lesions (Fig. 2a) appeared 15 dpi on $C4_L$ and $C5_L$, with, respectively, 17% and 70% of the leaves having visible lesions (chlorosis and/or necrosis areas). The occurrence of the first lesions was delayed until 18 dpi for 13%, 71% and 67% of $C1_L$, $C2_L$ and $C3_L$, respectively (Fig. 2b). The incubation period lasted 15 d on $C5_L$, 18 d on $C2_L$, $C3_L$, and $C4_L$, and 25 d on $C1_L$. The incubation period was delayed by 10 dpi between leaves of the two extreme leaf temperature classes (C1 and C5).

Lesions developed more slowly on $C1_L$ than on other leaf classes (Fig. 2b). Differences between mean diseased area on $C1_L$ compared with $C3_L$, $C4_L$, and $C5_L$ remained highly significant from 15 dpi to 78 dpi. Lesions developed similarly on $C2_L$ and $C3_L$ until 27 dpi. From 29 dpi to the end of the disease assessment period, lesions on $C2_L$ developed more slowly to reach a disease level similar to $C1_L$. Disease dynamics was similar on $C3_L$ and $C4_L$ throughout most of the experiment. Confidence intervals of $C3_L$ were large because of the low number of leaves ($n = 12$), which prevented demonstration of differences between $C3_L$ and $C4_L$, except from 27–36 dpi, a time span that corresponds to lesion expansion. The mean area of lesions developed on the hottest leaves ($C5_L$) increased significantly more rapidly than on $C4_L$ from 15 dpi to 18 dpi (Fig. 2a). Lesions then developed similarly until 22 dpi. Finally, from 25 dpi to the end of the disease assessment period (61 dpi for both classes), lesion development was higher on $C4_L$ than on $C5_L$. The leaf temperature class C4, from 16.6°C to 18.7°C, appeared to be the optimal range for the development of *M. graminicola* in our experimental conditions.

We expressed the normalized disease dynamic as the increase in the lesion area (covered by chlorosis and necrosis) relative to the final lesion area (LES_{max}) (Fig. 3). The differences in normalized disease dynamics between leaf temperature classes were less marked (Fig. 3) than for absolute disease dynamics (Fig. 2). Lesions on $C5_L$ developed first and more rapidly than

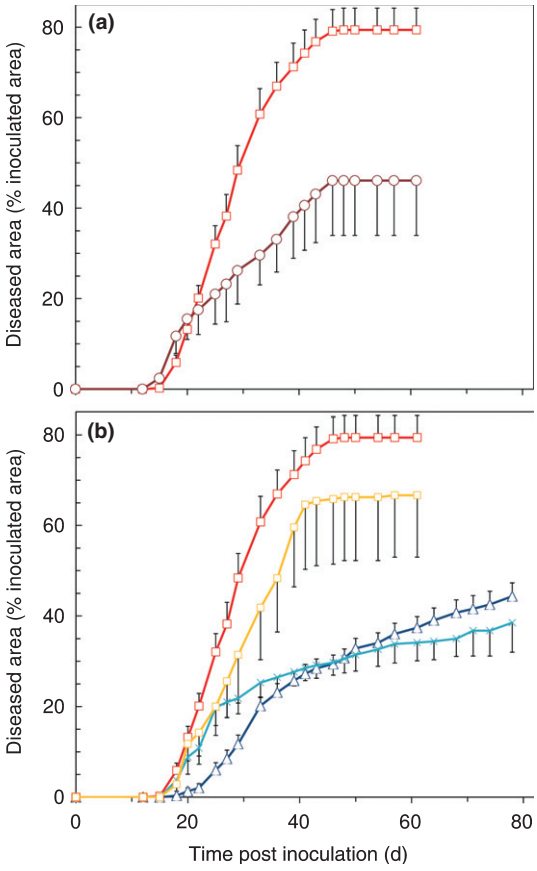

Fig. 2 Disease dynamics (percentage of the inoculated area) of *Mycosphaerella graminicola* on wheat (*Triticum aestivum*) leaves in five leaf temperature classes. (a) Optimal and supraoptimal leaf temperature classes C4 (squares) and C5 (circles). (b) Optimal and suboptimal leaf temperature classes C1 (triangles), C2 (crosses), C3 (yellow squares), and C4 (red squares). Error bars are confidence interval (95%), for the sake of clarity, only one-half of the error bars are shown.

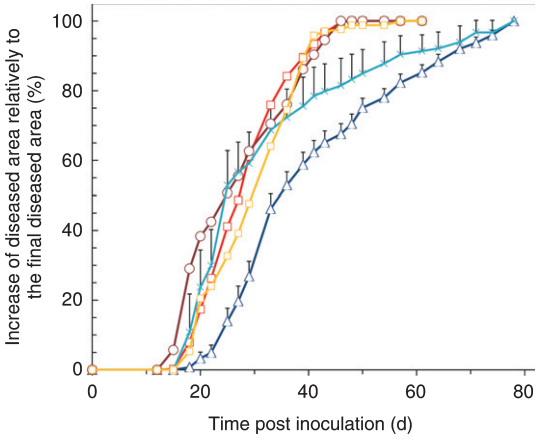

Fig. 3 Normalized disease dynamics of *Mycosphaerella graminicola* on wheat (*Triticum aestivum*) leaves in five leaf temperature classes: C1 (triangles), C2 (crosses), C3 (yellow squares), and C4 (red squares), and C5 (circles). Error bars are confidence intervals (95%); for the sake of clarity, only one-half of each error bar is shown.

on other classes ($P < 0.001$), then normalized disease dynamics were similar for $C3_L$ $C4_L$ and $C5_L$. Normalized disease dynamics on $C2_L$, $C3_L$ and $C4_L$ were similar until 36 dpi. From 39 dpi to 61 dpi, normalized disease dynamics were slower on $C2_L$ and similar on $C3_L$ and $C4_L$. Finally, from 64 dpi to 82 dpi, normalized disease dynamics were similar on $C1_L$ and $C2_L$. Before 64 dpi, normalized disease dynamics were slower on $C1_L$ than on other classes ($P < 0.03$).

The LES_{max} depended greatly on the leaf temperature classes (Fig. 4a). The leaves of the two lowest classes and the highest class (C1, C2, and C5) had the lowest LES_{max} (38.5% to 51.9% of the inoculated area). The LES_{max} was higher on $C4_L$ (79.4%) than on leaves of previously cited classes ($P < 0.001$). Class $C3_L$ had an intermediate final diseased area that was significantly different only from that of $C2_L$ ($P \leq 0.005$).

Differences in SPO_{max} between classes were even more marked than for LES_{max} (Fig. 4b). As for LES_{max}, SPO_{max} was also maximal on leaves in the optimal temperature class ($C4_L$: 68.4%, $P < 0.003$). Classes $C1_L$ (14.0%), $C2_L$ (9.2%) and $C5_L$ (24.6%) had the lowest SPO_{max} ($P < 0.034$). On $C3_L$, SPO_{max} was intermediate (47.1%) and significantly different from other classes. The ratio $SPO_{max} : LES_{max}$, which represents the proportion of the diseased area developing spores, was lower for $C1_L$ (0.27), $C2_L$ (0.24) and $C5_L$ (0.53) than for $C4_L$ (0.86) and $C3_L$ (0.71).

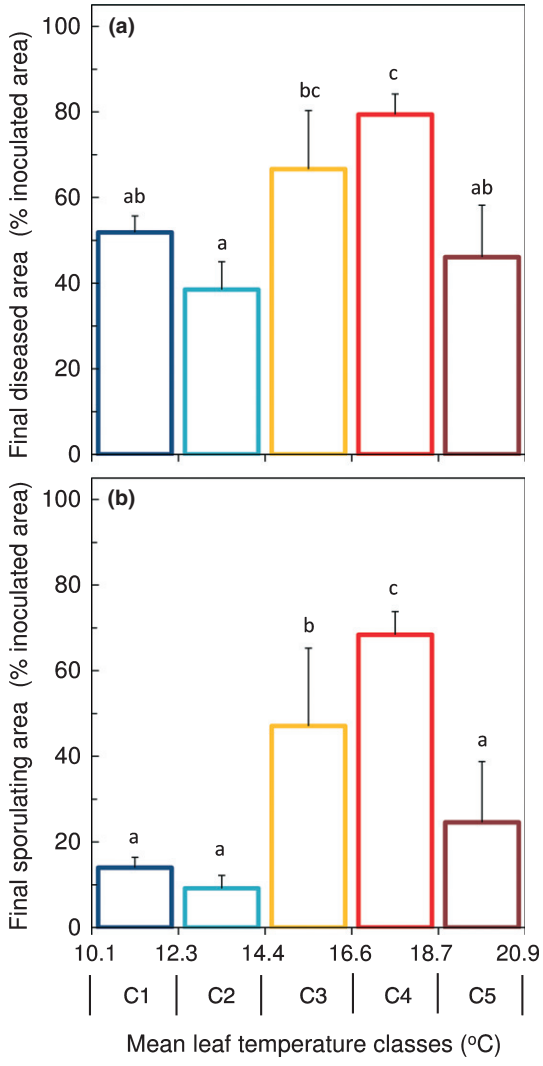

Fig. 4 (a) Final diseased area (LES_{max}) and (b) sporulating area (SPO_{max}) on wheat (*Triticum aestivum*) leaves infected by *Mycosphaerella graminicola* in five mean leaf temperature classes. Values are means ± confidence interval (95%); letters represent significant differences by Student's *t*-test ($P \leq 0.05$).

Latent period

Latent period ranged from 19 dpi to 102 dpi (Fig. 5): from 19 dpi to 90 dpi for isolate 1, from 24 dpi to 102 dpi for isolate 2 and from 25 dpi to 102 dpi for isolate 3. The variability of the response of the latent period to leaf temperature was higher at low leaf temperature: at leaf temperature between 10°C and 12°C, latent period varied between 51 dpi and 102 dpi while at leaf temperature from 17°C to 19°C, latent period varied between 23 dpi and 39 dpi. For the three isolates studied, the latent period decreased with increasing mean leaf temperature, reached a minimum and eventually increased slightly beyond 20°C. From Eqn 3, a minimal latent period of 28.8 dpi was reached for a temperature of 18.4°C.

Between-isolates variability

The TPCs of the three isolates tested suggested a differential response of *M. graminicola* isolates to leaf temperature (Fig. 6). The fit of Eqn 4 to the data, which describes the performance of latent period of *M. graminicola* depending on mean leaf temperature, was highly significant for the three isolates (R^2 from 0.83 to 0.89, Table 2). The values of parameter *Curv* for isolates 1 and 2 were not significantly different ($P = 0.35$) from each other but differed from the value for isolate 3 ($P < 0.001$). The values of the other two parameters were different for the three isolates ($P < 0.02$, Table 2). Consequently, each isolate has its own TPC. The optimal mean leaf temperatures calculated from the TPCs

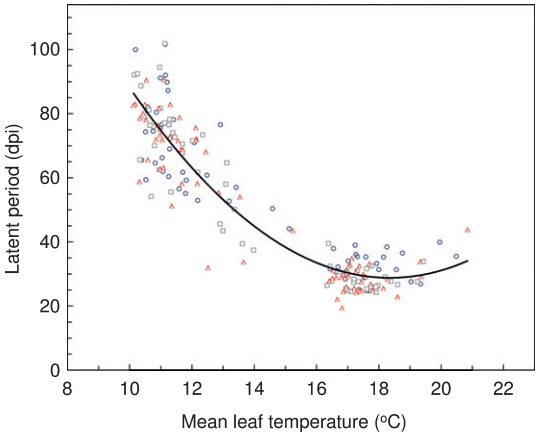

Fig. 5 Effect of mean temperature of wheat (*Triticum aestivum*) leaves on the latent period (number of days post-inoculation (dpi) to reach 37% of final sporulating area) of three isolates of *Mycosphaerella graminicola* (triangles) isolate 1, (squares) isolate 2, (circles) isolate 3. The curve represents the fitting of Eqn 2 to the data pooled for the three isolates.

Table 2 Fitting of Eqn 4 to thermal performance curves (TPCs) obtained for three isolates of *Mycosphaerella graminicola*

	Parameters				
Isolates	*n*	LP_{min} (dpi)	*Curv* (dpi °C^{-2})	$T_{l,opt}$ (°C)	R^2
Isolate 1	61	28.0 ± 1.6 *(a)*	0.90 ± 0.16 *(a)*	18.1 ± 0.7 *(a)*	0.88
Isolate 2	62	26.9 ± 2.2 *(b)*	0.86 ± 0.20 *(a)*	18.5 ± 0.9 *(b)*	0.89
Isolate 3	68	31.1 ± 2.5 *(c)*	0.72 ± 0.19 *(b)*	18.9 ± 1.2 *(c)*	0.83
Isolates pooled	191	28.8 ± 1.2	0.85 ± 0.11	18.4 ± 0.5	0.86

Eqn 4: $TPC(T_l) = 1/(LP_{min} + Curv \times (T_l - T_{l,opt})^2)$. LP, latent period (dpi); LP_{min}, minimum latent period at optimal temperature (dpi); T_l, mean wheat (*Triticum aestivum*) leaf temperature (°C); $T_{l,opt}$, optimal mean leaf temperature (°C); dpi, days post-inoculation. Values are means ± SD. Letters represent significant differences of parameter values between isolates by Student's *t*-test ($P \leq 0.05$).

were 18.1, 18.5 and 18.9°C for isolates 1, 2 and 3, respectively. The optima were significantly different between isolates ($P < 0.02$). Latent period at optimal mean leaf temperature was 28.0, 26.9 and 31.1 dpi for isolates 1, 2 and 3 respectively (Table 2).

Effect of leaf layer on latent period

The latent period was significantly longer on F1 leaves (31.9 ± 3.4 dpi) than on F2 leaves (27.1 ± 3.7 dpi) at mean leaf temperature between 17.0°C and 17.5°C ($P = 0.004$). The latent period was up to 4 d longer on F1 leaves than on F2 leaves.

Discussion

To the best of our knowledge, we have established the first thermal performance curve (TPC) of a foliar fungal pathogen using the temperature actually perceived by the fungus, which is the temperature of the infected leaf. The previously published TPCs (Maziero *et al.*, 2009; Milus *et al.*, 2009) were based on the air temperature, which is an unreliable indicator of the leaf temperature. This may lead to erroneous results and interpretations, as shown by ecologists studying trees (Helliker & Richter, 2008), insects (Potter *et al.*, 2009) and intertidal organisms (Helmuth *et al.*, 2010). In parallel to our study, Bonde *et al.* (2012) studied the effect of temperature fluctuations on a foliar fungal pathogen, measuring simultaneously leaf and air temperatures in controlled growth chambers. In their specific experiments, they have found that leaf and air temperatures in their controlled growth chambers were nearly equal, concluding that the use of air temperature was relevant. Their results were probably an exception, as it has been largely proved that uncontrolled environmental variations in controlled growth chambers and glasshouses generate an heterogeneous pattern of air temperature (Potvin *et al.*, 1990; Demrati *et al.*, 2007) and leaf temperature (Kichah *et al.*, 2012). To ensure that no effect of such heterogeneities occurs when establishing TPC, we propose the necessity of measuring the temperature actually perceived by foliar pathogens, that is, the temperature of each individual diseased leaf. In this study, we continuously measured the temperature of 191 wheat leaves to establish the TPC of *M. graminicola* characterizing its latent period.

The methodology we set up is relevant for studies on other foliar fungal pathogens. Wherever the experiment is carried out (controlled growth chamber, glasshouse or field) the temperature measured will be the body temperature of fungus. This setup has allowed the generation of TPCs for a wide range of leaf temperatures (from 10.1°C to 20.9°C) using infrared heat lamps. Achieving such a range of leaf temperature necessitated only two experiments in controlled growing environments, which were set up with two different air temperatures. To obtain the same results for air temperature would have required several experiments (one for each air temperature). Moreover, as the use of infrared heat lamps can modify the humidity and the vapor–air pressure gradient between substomatal cavities and atmosphere (Amthor *et al.*, 2010), we avoided possible artifacts by having well-watered plants during our experiments. This followed the conclusion of Wall *et al.* (2011), who found that no effect on gas exchange or water relations was associated with the infrared warming lamps when a supplemental irrigation minimized plant-to-air water vapor pressure differences between infrared-warmed and non-warmed plots. Such a methodology will have to be applied to field conditions to take into account its specificity regarding the leaf energy budget, especially the fluctuating feature of its terms (air temperature, radiation, wind, etc.).

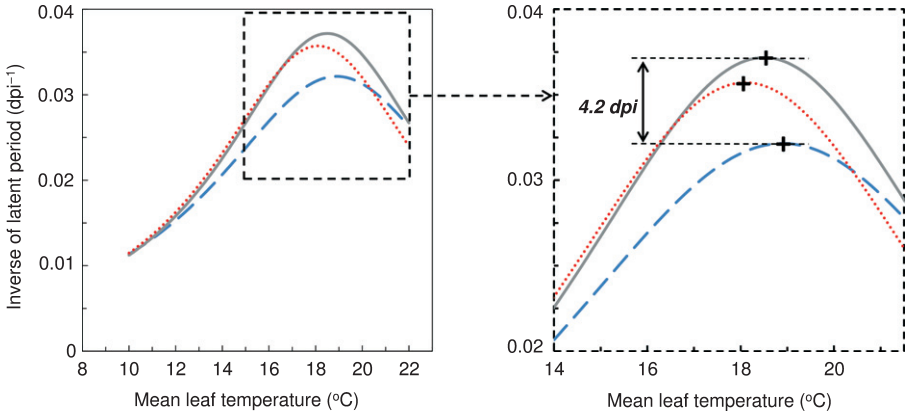

Fig. 6 Thermal performance curves (TPC) for latent period of three *Mycosphaerella graminicola* isolates infecting wheat (*Triticum aestivum*) leaves: (red dotted line) INRA08-FS0001 (isolate 1), (solid grey line) INRA08-FS0002 (isolate 2), and (dashed blue line) INRA08-FS0003 (isolate 3). Latent period is the number of dpi (days post-inoculation) to reach 37% of final sporulating area; +, optimal mean leaf temperatures determined for each isolate (see Table 2 for parameter values).

The optimal mean leaf temperature for the latent period of *M. graminicola* varied significantly between 18.1°C and 18.9°C, depending on isolates, in our experimental conditions (Fig. 6, Table 2). The reported optimal air temperature for the latent period of *M. graminicola* varies between 15°C and 25°C, both in field (Shaw, 1990; Lovell *et al.*, 2004) and controlled conditions (Hess & Shaner, 1987; Shaw, 1990; Magboul *et al.*, 1992). However, optimal temperatures obtained with air temperature and leaf temperature should not be compared directly, as the difference between the two temperatures varies, depending on the growing conditions through the leaf energy budget (Eqn 1). A similar increase in latent period as observed by us for supraoptimal temperatures has been previously described for *M. graminicola* (Shaw, 1990) and *Phytophthora infestans* (Andrade-Piedra *et al.*, 2005), but using air temperature.

Latent period was significantly longer on flag leaves (F1) than on second leaves (F2) at equal leaf temperature. By contrast, Shaw (1990), also experimenting in controlled growth chambers, found a longer latent period on F2. The difference between Shaw's result and ours illustrates the possible error in the interpretation of results when measuring air temperature instead of leaf temperature for foliar fungal epidemics studies. As F2 leaves are lower than F1 leaves inside the canopy, they are further from the light sources and can be shaded by F1 leaves and stems and thus receive less light irradiance. Thus, in a controlled growth chamber, leaf temperature is higher on F1 leaves than on F2 leaves for a given air temperature. As a consequence of the higher leaf temperature, latent period on F1 leaves, if deduced from air temperature measurements, is reduced. This may explain the results observed by Shaw. The difference of latent period we have observed between F1 and F2 leaves at equal leaf temperatures may be explained by other factors known to influence fungal development, such as morphology, age and physiology of the leaves (Lovell *et al.*, 2004). Further experiments are required, involving inoculation on other leaf layers submitted to temperature ranges consistent with the wheat growth stage, including for example the lowest leaf layers at seedling stage under winter conditions.

Formulating the TPC of latent period as a function of leaf temperature should improve the models of plant disease epidemics. Indeed, as pointed out by Shaw (1990), the relationship between temperature and latent period of *M. graminicola* is not linear (Fig. 5). Thus, regarding the range of temperature perceived by the fungal pathogen, latent period cannot be summarized as a single thermal time-value, although this is commonly done in experimental studies (Thomas *et al.*, 1989; Lovell *et al.*, 2004) and models (O'Callaghan *et al.*, 1994; Audsley *et al.*, 2005; Robert *et al.*, 2008). Moreover, leaf temperature instead of air temperature and related TPCs ought to be considered when simulating the interaction between canopy architecture and foliar fungal epidemics (Pangga *et al.*, 2011). Admittedly, leaf temperature may appear less easily accessible to measurement than air temperature. However, it can be indirectly estimated by providing soil–vegetation–atmosphere transfer (SVAT) models with data recorded by standard weather stations. These models, such as CUPID (Norman, 1979), dynamically simulate the temperature of each leaf layer. A similar approach allowed the simulation of the leaf wetness duration (LWD), another climatic variable that drives foliar fungal epidemics, whose estimation was preferred to direct measurement of air humidity that introduced some artifacts into epidemics models (Dalla Marta *et al.*, 2005).

The sensitivity of physiological and ecological traits of fungi to temperature has been widely studied (Dell *et al.*, 2011). Some studies have measured the TPCs of different fungi for a wide range of temperatures (Shaw, 1990; Fargues *et al.*, 1992; McLean *et al.*, 2005) but never for foliar pathogens while measuring the leaf temperature. We have found that the TPCs of *M. graminicola* for latent period based on leaf temperature varied significantly between the three isolates (Table 2). The identification of diverse TPCs for each isolate is a characterization of *M. graminicola* phenotypic plasticity. Measuring the growth on artificial media of *M. graminicola* isolates sampled from five host populations in four locations, Zhan & McDonald (2011) have shown a local adaptation of the fungus, concluding that temperature is a selective agent responsible for the adaptation of *M. graminicola*. In contrast to the method of Zhan & McDonald (2011), who studied the adaptation of the fungus to air temperature in agar medium, our device made it possible to study the effect of temperature on the fungus development while accounting for its interactions with the leaf physiology. To identify and quantify the thermal adaptation of *M. graminicola*, a greater number of isolates should be grown on living leaves. This should require the use of adult plant leaves to consider the physiological aspects of the plant–pathogen interaction (Wang *et al.*, 2009; Diamond & Kingsolver, 2012). Finally, TPCs are known to have great potential for predicting the responses of populations or species to climate change, as they can be incorporated into mechanistic models of response of organisms to climate change (Schulte *et al.*, 2011). Here, we have made a first step toward a new way for the study of the adaptation of *M. graminicola* (and other foliar fungal pathogens) to climate change.

Acknowledgements

We thank Fabrice Duhamel, Alain Fortineau and Nathalie Galet for technical assistance, Robert Faivre (INRA, Toulouse, France) for statistical advice and Sylvain Pincebourde (CNRS, Tours, France) for helpful discussions on physical ecology. We are also grateful to Prof. Vince P. Gutschick (New Mexico State University, Las Cruces, USA) for his critical reading of the manuscript. This study was funded by INRA ('Plant Health and Environment' and 'Environment and Agronomy' Divisions) and ARVALIS – Institut du Végétal.

References

Amthor JS, Hanson PJ, Norby RJ, Wullschleger SD. 2010. A comment on 'Appropriate experimental ecosystem warming methods by ecosystem, objective, and practicality' by Aronson and McNulty. *Agricultural and Forest Meteorology* **150**: 497–498.

Andrade-Piedra JL, Hijmans RJ, Forbes GA, Fry WE, Nelson RJ. 2005. Simulation of potato late blight in the Andes. I: Modification and parameterization of the LATEBLIGHT model. *Phytopathology* **95**: 1191–1199.

Angilletta MJ. 2006. Estimating and comparing thermal performance curves. *Journal of Thermal Biology* **31**: 541–545.

Angilletta MJ, Niewiarowski PH, Navas CA. 2002. The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* **27**: 249–268.

Audsley E, Milne A, Paveley N. 2005. A foliar disease model for use in wheat disease management decision support systems. *Annals of Applied Biology* **147**: 161–172.

Belehradek J. 1926. Influence of temperature on biological processes. *Nature* **118**: 117–118.

Bonde MR, Nester SE, Berner DK. 2012. Effects of daily temperature highs on development of *Phakopsora pachyrhizi* on soybean. *Phytopathology* **102**: 761–768.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. 2004. Toward a metabolic theory of ecology. *Ecology* **85**: 1771–1789.

Campbell GS, Norman JM. 1998. *An introduction to environmental biophysics, 2nd edn*. New York, NY, USA: Springer-Verlag.

Chelle M. 2005. Phylloclimate or the climate perceived by individual plant organs: what is it? How to model it? What for? *New Phytologist* **166**: 781–790.

Chungu C, Gilbert J, Townley-Smith F. 2001. *Septoria tritici* blotch development as affected by temperature, duration of leaf wetness, inoculum concentration, and host. *Plant Disease* **85**: 430–435.

Dalla Marta A, Magarey RD, Orlandini S. 2005. Modelling leaf wetness duration and downy mildew simulation on grapevine in Italy. *Agricultural and Forest Meteorology* **132**: 84–95.

De Boeck HJ, De Groote T, Nijs I. 2012. Leaf temperatures in glasshouses and open-top chambers. *New Phytologist* **194**: 1155–1164.

Dell AI, Pawar S, Savage VM. 2011. Systematic variation in the temperature dependence of physiological and ecological traits. *Proceedings of the National Academy of Sciences, USA* **108**: 10591–10596.

Demrati H, Boulard T, Fatnassi H, Bekkaoui A, Majdoubi H, Elattir H, Bouirden L. 2007. Microclimate and transpiration of a greenhouse banana crop. *Biosystems Engineering* **98**: 66–78.

Diamond SE, Kingsolver JG. 2012. Host plant adaptation and the evolution of thermal reaction norms. *Oecologia* **169**: 353–360.

Douaiher MN, Nowak E, Durand R, Halama P, Reignault P. 2007. Correlative analysis of *Mycosphaerella graminicola* pathogenicity and cell wall-degrading enzymes produced *in vitro*: the importance of xylanase and polygalacturonase. *Plant Pathology* **56**: 79–86.

Fargues J, Maniania NK, Delmas JC, Smits N. 1992. Influence of temperature on *in vitro* growth of entomopathogenic hyphomycetes. *Agronomie* **12**: 557–564.

Gates DM. 1980. *Biophysical ecology*. New York, NY, USA: Springer-Verlag.

Gilchrist GW. 1995. Specialists and generalists in changing environments – 1. Fitness landscapes of thermal sensitivity. *American Naturalist* **146**: 252–270.

Helliker BR, Richter SL. 2008. Subtropical to boreal convergence of tree-leaf temperatures. *Nature* **454**: 511–514.

Helmuth B, Broitman BR, Yamane L, Gilman SE, Mach K, Mislan KAS, Denny MW. 2010. Organismal climatology: analyzing environmental variability at scales relevant to physiological stress. *Journal of Experimental Biology* **213**: 995–1003.

Hess DE, Shaner G. 1987. Effect of moisture and temperature on development of septoria tritici blotch in wheat. *Phytopathology* **77**: 215–219.

Huey RB, Kingsolver JG. 1989. Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution* **4**: 131–135.

Huey RB, Kingsolver JG. 2011. Variation in universal temperature dependence of biological rates. *Proceedings of the National Academy of Sciences, USA* **108**: 10377–10378.

Huey RB, Stevenson RD. 1979. Integrating thermal physiology and ecology of ectotherms – discussion of approaches. *American Zoologist* **19**: 357–366.

Hunt R. 1982. *Plant growth curves the functional approach to plant growth analysis*. Cambridge, UK: Cambridge University Press.

Jobling M. 1981. Temperature tolerance and the final preferendum – rapid methods for the assessment of optimum growth temperatures. *Journal of Fish Biology* **19**: 439–455.

Johnson DA. 1980. Effect of low temperature on the latent period of slow and fast rusting water wheat *Triticum aestivum* genotypes. *Plant Disease* **64**: 1006–1008.

Kichah A, Bournet P-E, Migeon C, Boulard T. 2012. Measurement and CFD simulation of microclimate characteristics and transpiration of an *Impatiens* pot plant crop in a greenhouse. *Biosystems Engineering* **112**: 22–34.

Kingsolver JG, Izem R, Ragland GJ. 2004. Plasticity of size and growth in fluctuating thermal environments: comparing reaction norms and performance curves. *Integrative and Comparative Biology* **44**: 450–460.

Kitano M, Eguchi H, Matsui T. 1983. Analysis of heat balance of leaf with reference to stomatal responses to environmental factors. *Biotronics* **12**: 19–27.

Lhomme JP. 1995. Modélisation du microclimat végétal en relation avec l'épidémiologie. In: Savary S, ed. *Modélisation en protection des cultures*. Paris, France: ORSTOM, 73–80.

Linacre ET. 1964. A note on a feature of leaf and air temperatures. *Agricultural Meteorology* **1**: 66–72.

Linacre ET. 1966. *Resistances impeding the diffusion of water vapour from leaves and crops*. PhD thesis, London University, London, UK.

Lovell DJ, Hunter T, Powers SJ, Parker SR, Van den Bosch F. 2004. Effect of temperature on latent period of septoria leaf blotch on winter wheat under outdoor conditions. *Plant Pathology* **53**: 170–181.

Magboul AM, Geng S, Gilchrist DG, Jackson LF. 1992. Environmental influence on the infection of wheat by *Mycosphaerella graminicola*. *Phytopathology* **82**: 1407–1413.

Maziero JMN, Maffia LA, Mizubuti ESG. 2009. Effects of temperature on events in the infection cycle of two clonal lineages of *Phytophthora infestans* causing late blight on tomato and potato in Brazil. *Plant Disease* **93**: 459–466.

McLean MA, Angilletta MJ, Williams KS. 2005. If you can't stand the heat, stay out of the city: thermal reaction norms of chitinolytic fungi in an urban heat island. *Journal of Thermal Biology* **30**: 384–391.

Milus EA, Kristensen K, Hovmoller MS. 2009. Evidence for increased aggressiveness in a recent widespread strain of *Puccinia striiformis* f. sp *tritici* causing stripe rust of wheat. *Phytopathology* **99**: 89–94.

Norman JM. 1979. Modeling the complete crop canopy. In: Barfield BJ, Gerber JF, eds. *Modification of the aerial environment of plants*. St Joseph, MI, USA: American Society of Agricultural Engineers, 249–277.

O'Callaghan JR, Dahab MH, Hossain AHMS, Wyseure GCL. 1994. Simulation of *Septoria tritici*–winter wheat interactions. *Computers and Electronics in Agriculture* **11**: 309–321.

Pangga IB, Hanan J, Chakraborty S. 2011. Pathogen dynamics in a crop canopy and their evolution under changing climate. *Plant Pathology* **60**: 70–81.

Park J-J, Park KW, Shin K-I, Cho K. 2011. Evaluation and comparison of effects of air and tomato leaf temperatures on the population dynamics of greenhouse whitefly (*Trialeurodes vaporariorum*) in cherry tomato grown in greenhouses. *Korean Journal of Horticultural Science & Technology* **29**: 420–432.

Pincebourde S, Sinoquet H, Combes D, Casas J. 2007. Regional climate modulates the canopy mosaic of favourable and risky microclimates for insects. *Journal of Animal Ecology* **76**: 424–438.

Pincebourde S, Woods HA. 2012. Climate uncertainty on leaf surfaces: the biophysics of leaf microclimates and their consequences for leaf-dwelling organisms. *Functional Ecology* **26**: 844–853.

Potter K, Davidowitz G, Woods HA. 2009. Insect eggs protected from high temperatures by limited homeothermy of plant leaves. *Journal of Experimental Biology* **212**: 3448–3454.

Potvin C, Lechowicz MJ, Bell G, Schoen D. 1990. Spatial, temporal, and species-specific patterns of heterogeneity in growth chamber experiments. *Canadian Journal of Botany* **68**: 499–504.

R Development Core Team. 2010. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Robert C, Fournier C, Andrieu B, Ney B. 2008. Coupling a 3D virtual wheat (*Triticum aestivum*) plant model with a *Septoria tritici* epidemic model (Septo3D): a new approach to investigate plant–pathogen interactions linked to canopy architecture. *Functional Plant Biology* **35**: 997–1013.

Schulte PM, Healy TM, Fangue NA. 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology* **51**: 691–702.

Shaw MW. 1986. Effects of temperature and leaf wetness on *Pyrenophora teres* growing on barley cv. Sonja. *Plant Pathology* **35**: 294–309.
Shaw MW. 1990. Effects of temperature, leaf wetness and cultivar on the latent period of *Mycosphaerella graminicola* on winter wheat. *Plant Pathology* **39**: 255–268.
Smith WK. 1978. Temperatures of desert plants – another perspective on adaptability of leaf size. *Science* **201**: 614–616.
Suffert F, Sache I, Lannou C. 2013. Assessment of quantitative traits of aggressiveness in Mycosphaerella graminicola on adult wheat plants. *Plant Pathology*, in press.
Thomas MR, Cook RJ, King JE. 1989. Factors affecting development of *Septoria tritici* in winter wheat and its effect on yield. *Plant Pathology* **38**: 246–257.
Wall GW, Kimball BA, White JW, Ottman MJ. 2011. Gas exchange and water relations of spring wheat under full-season infrared warming. *Global Change Biology* **17**: 2113–2133.
Wang Y, Bao Z, Zhu Y, Hua J. 2009. Analysis of temperature modulation of plant defense against biotrophic microbes. *Molecular Plant–Microbe Interactions* **22**: 498–506.
Weng J, Liao T. 2001. Effect of microclimate on leaf temperature of two subalpine grassland plants in Tatachia area. *Journal of the Experimental Forest of National Taiwan University* **15**: 95–107.
Winsor CP. 1932. The Gompertz curve as a growth curve. *Proceedings of the National Academy of Sciences, USA* **18**: 1–8.
Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. *Weed Research* **14**: 415–421.
Zhan J, McDonald BA. 2011. Thermal adaptation in the fungal pathogen *Mycosphaerella graminicola*. *Molecular Ecology* **20**: 1689–1701.