

# Effect of controlled fluctuating low temperatures on survival of *Puccinia striiformis* f. sp. *tritici*

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**Abstract** Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is an important disease of wheat worldwide. Understanding the survival of *Pst* during the winter is critical for predicting *Pst* epidemics in the spring. We used a real-time quantitative PCR (qPCR) method to quantify *Pst* CYR32 biomass in infected wheat seedlings under several fluctuating temperature regimes (three average temperatures 0, –5 and –10 °C, each with two daily fluctuating amplitudes 8 and 13 °C). The survival of *Pst* CYR32 increased with increasing average temperature but also varied greatly with the amplitude – larger amplitude led to lower survival, particularly at 0 and –5 °C. Nevertheless the survival at both amplitudes was still significantly greater than under the corresponding constant temperatures. There were small, albeit statistically significant, differences between the two cultivars (Xiaoyan 22, low winter-hardiness; Lantian 15, high winter-hardiness) in *Pst* CYR32 survival. This study indicated potential errors that could result from using daily

average temperatures to predict *Pst* survival during the winter.

**Keywords** *Puccinia striiformis* f. sp. *tritici* · Survival · Constant and fluctuating temperature

## Introduction

Wheat stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*), is an important disease of wheat worldwide (Chen 2005). Annual cycles of stripe rust on winter wheat can be divided into four stages: oversummering, infection of seedlings in autumn, overwintering, and spring epidemic. *Pst* has to overwinter in living host tissues (Li and Zeng 2002; Sharp and Hehn 1963) and its survival during the winter is a key determinant of spring epidemics that directly cause crop losses (Gladders et al. 2007; Li and Zeng 2002; Sharma-Poudyal and Chen 2010).

The low limit of air temperature for *Pst* overwintering (November to February in China and North America) is believed to be around –6 to –7 °C (monthly mean) (Sharma-Poudyal et al. 2014; Shen and Wang 1962; Zeng and Luo 2006). Wheat-growing regions in China are divided into overwintering and non-overwintering regions for *Pst* based on winter temperatures (Shen and Wang 1962; Shi and Ma 2005). However, these conclusions were all drawn from field observations based on average temperature. It is well known that using average temperatures (ignoring large fluctuations in temperatures within a day or between days) to predict biological

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processes will lead to considerable errors if the underlying relationship between temperature and the biological processes are nonlinear (Scherin and Vanbruggen 1994; Xu 1996). Recently we showed that the relationship of *Pst* survival with temperature is indeed nonlinear (Ma et al. 2015). Recent field observations suggest that the overwintering boundary may have been gradually pushed towards the northwest (Pan et al. 2011; Yao et al. 2014), which may partially result from climate changes in recent times (Fang et al. 2012).

We conducted a further study to understand the magnitude of potential errors in using average temperature to predict *Pst* survival under several controlled fluctuating temperature regimes. The experimental protocol followed the one we used previously to study the effect of constant temperature on *Pst* survival (Ma et al. 2015), except that fluctuating temperature regimes were used (Table 1). Briefly, (1) a *Pst* race, CYR32, was multiplied on 10-day-old seedlings of cultivar Mingxian 169 in pots of 10-cm in diameter and 12-cm in depth, containing cow dung compost and soil (1:2, V/V); (2) 10-day-old seedlings in pots of two cultivars (Xiaoyan 22 - XY22, low winter-hardiness; Lantian 15 - LT15, high winter-hardiness) were inoculated with urediniospores of CYR32 from Mingxian 169; (3) When symptoms of stripe rust were first observed (ca. 12 days after inoculation), seedlings with initial *Pst* symptoms were moved to a low-temperature-freezing cabinet (THD-3010, Tianheng, China) set to one of the six temperature regimes (Table 1); (4) three wheat leaves (one from each plant) were cut off at each of the five time points (Table 1) and immediately stored in liquid nitrogen; and (5) RNA was extracted (Ma et al. 2016) separately from each leaf for all samples and quantified. This experiment was done three times. Standards for RNA (cDNA) quantification were included in every plate in order to reduce batch-to-batch variability in quantification. Coefficients of determination of the standard curves were >0.99 in all quantification runs.

*Pst* CYR32 survival at a specific low temperature treatment was calculated as its biomass at a specific time as the percentage of the biomass at time zero [i.e. not subjected to cold treatment]. Repeated measurement ANOVA was used (Genstat - version 13, VSN International Ltd., England) to assess *Pst* CYR32 survival under different treatments over time, in which replicate experiments were used as a block factor. Treatment factors included average temperature,

amplitude of temperature fluctuation, variety and assessment time. Percentage data were angular-transformed to ensure that the residuals better fit normal distributions as well as reduced heterogeneity.

Percentage of viable *Pst* CYR32 biomass decreased steeply within the first 24 h, with the steepness increasing with decreasing average temperature (Fig. 1). The differences between four treatments also decreased with decreasing temperatures. As expected, the main effects of both average temperature and assessment time accounted for most of the observed variance, explaining ca. 54 % and 21 % of the observed variance, respectively. Reducing average temperature and incubation for longer time under low temperatures greatly reduced the survival of *Pst* CYR32 ( $P < 0.001$ ). Overall *Pst* CYR32 survival was 6 %, 18 % and 33 % for average temperature of  $-10$  °C,  $-5$  °C and  $0$  °C, respectively; it was 28 % at 24 h and reduced to 9 % at 96 h. The main effect of amplitude of fluctuating temperature was also highly significant ( $P < 0.001$ ), accounting for nearly 8 % of the total variability; average *Pst* CYR32 survival over the four assessment times were 15 % and 24 % for the amplitude of  $13$  °C and  $8$  °C, respectively. The results demonstrate that using average temperatures to predict *Pst* survival would result in significant errors. The larger mortality associated with the  $13$  °C amplitude, relative to the  $8$  °C amplitude, is probably due to the fact the reduced mortality at the upper temperatures (16 h in

**Table 1** Summary of temperature treatments to study the effects of fluctuating temperatures on the survival of *Pst* on infected seedlings of two cultivars (XY22 and LT15); seedlings were sampled at 0, 24, 48, 72, and 96 h

Mean temperature (°C)	Amplitude (°C)	Temperature range* (°C)
0	8	2.7 (16 h day) ~ -5.3 (8 h night)
	13	4.3 ~ -8.7
-5	8	-2.33 ~ -10.33
	13	-0.66 ~ -13.66
-10	8	-7.33 ~ -15.33
	13	-5.66 ~ -18.66

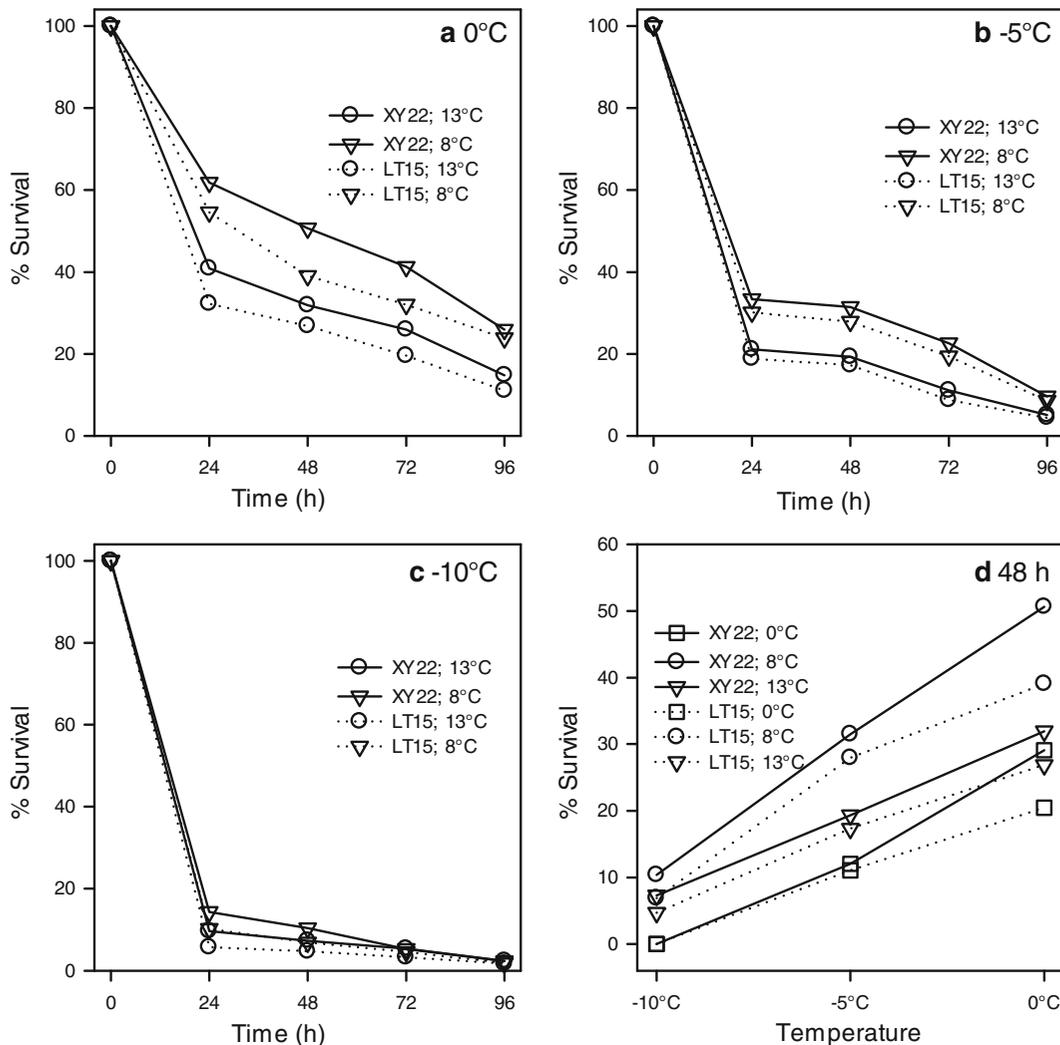
\*For each cycle (24 h), 16 h (with light) were in a constant “day” temperature and 8 h (dark) in the constant “night” temperature. To reduce potential injuries to seedlings from sudden temperature changes, temperature changes were completed within 30 min with the temperature increasing/decreasing gradually at three equally-spaced temperature steps (seedlings kept at each of the intermediate temperature for 10 min)

daytime) is far less than the increased mortality at the lower temperatures during the night.

*Pst* CYR32 survival on the two varieties also differed ( $P < 0.01$ ) but only accounted for 2 % of the total variation. There were only two significant ( $P < 0.05$ ) interactions: amplitude with average temperature, and assessment time with average temperature, both accounting for 2 % of the total variation. The reduction in *Pst* CYR32 survival from 0 °C to –10 °C was much greater from the amplitude of 8 °C (from 41 % to 7 %) than 13 °C (25 % to 5 %) – because of greater survival with the lower amplitude at 0 °C and –5 °C. The differences in *Pst* CYR32 survival between three

temperatures decreased with increasing assessment time (Fig. 1a, b, c and d).

In our recent publication (Ma et al. 2015), we conducted the study on *Pst* survival under constant temperatures, including *Pst* survival on XY22 and LT15 assessed at 48 h under 0 °C, –5 °C or –10 °C. ANOVA was applied to all the *Pst* survival data at 48 h for both constant and fluctuating temperature studies. *Pst* survival was greater under fluctuating temperature regimes than under constant temperatures, with the differences depending on average temperature and the extent of fluctuation. Again, the temperature treatment accounted for most of the observed variation (ca. 64 %).



**Fig. 1** a-c *Pst* biomass (quantified by a qRT-PCR method) expressed as percentage of the *Pst* biomass at time 0 (not subjected to any cold treatment) for *Pst* on wheat seedlings of two cultivars (XY22 and LT15) subjected to six temperature regimes (three

average temperature each at two fluctuating amplitudes); **d** *Pst* data at 48 h including data from a previous study under constant temperatures (Ma et al. 2015)

The differences in *Pst* CYR32 survival between three amplitudes (0 °C, 8 °C and 13 °C) also accounted for 20 % of the total variation ( $P < 0.001$ ) (Fig. 1d). Average survival was 12 %, 28 % and 18 % for the amplitude of 0 °C, 8 °C and 13 °C, respectively. The greater survival under fluctuating conditions at average 0 °C might also partly result from the fact that *Pst* biomass could increase when temperature  $> 0$  °C during daytime.

Models have been developed to describe the relationship between winter temperature and stripe rust epidemics based on field observations (Coakley et al. 1982; Coakley and Line 1981; Coakley et al. 1988) and further revised (Sharma-Poudyal and Chen 2010) to predict the spring epidemic. Given the nature of non-linear relationship between temperature and *Pst* survival (Ma et al. 2015), the present study demonstrated that using the field data based on average (daily or even monthly) temperatures to predict *Pst* overwintering potential would lead to considerable prediction errors purely as a consequence of nonlinearity. Of course, other factors may contribute to the prediction errors, such as the effects due to wind chill. In order to accurately understand the magnitude of nonlinearity, we need to better understand the nonlinear relationship between temperatures and *Pst* survival. Although further research is still needed to develop an accurate model relating *Pst* survival to temperature, this study clearly showed that using field average temperatures to predict *Pst* overwintering could lead to considerable inaccuracies.

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