A Latent Period Duration Model for Wheat Stem Rust

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Abstract

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Wheat stem rust (WSR), caused by *Puccinia graminis* subsp. *graminis* Pers., is a highly destructive disease of wheat and several other small grains. The discovery of *P. graminis* subsp. *graminis* race Ug99, which overcomes previously effective resistance in wheat, raises concerns for global wheat production and food security. There is currently no mathematical model that describes the duration of the WSR latent period based on temperature or heat units. A study using *P. graminis* subsp. *graminis* race GFCDC in 'Stephens' wheat was conducted at a range of temperatures (from 4.7 to 33.4°C), to determine their effect on latent period duration. There were significant differences in latent period duration among temperatures, and temperatures above 30°C generally were not conducive for pustule development. A mathematical model to predict latent period duration based on temperature was formulated; the model can be applied to data consisting of varying temperature readings measured at any desired time increment. The model was

validated in outdoor experiments under natural conditions on Stephens and 'McNair' wheat. In field and outdoor experiments, the latent period durations predicted with the model were within 16 h of the observed latent period, and most fell into the 99% confidence interval of the observations. For experiments conducted on field-grown plants, no significant differences were found between predicted and observed latent period duration. Factorial experiments conducted under growth chamber conditions, using four wheat cultivars (Stephens, McNair 701, 'Scout 66', and 'Kingbird') and four *P. graminis* subsp. *graminis* races (GCCNC, GCCSC, QFCSC, and GFCDC) at three temperatures (5, 15 and 30°C) showed significant differences among cultivars at each temperature. A quantitative model for latent period duration could help in estimating epidemic development, and also in improving our understanding of WSR epidemiology.

Wheat stem rust (WSR), or black rust, caused by *Puccinia graminis* subsp. *graminis* Pers. is an ancient and highly destructive disease of wheat (*Triticum aestivum* L. (1753)) and several other small grains. Wheat, the second-largest small-grain crop worldwide (11), provides approximately 55% of the carbohydrates and 20% of the calories for humankind (15). Recently, WSR has acquired renewed importance with the discovery of race Ug99 and associated races (hereafter referred to as Ug99) that overcome resistance to previously durable genes (*Sr31* and *Sr38*, among others), causing severe yield loss and menacing global wheat production (1,4,35,37). It is estimated that approximately 90% of wheat produced in the world is susceptible to Ug99 (1,35). Since its discovery in Uganda in 1999, this new race has spread to other countries in Africa, the Middle East, and Asia (1,35), generating fears of dispersion to all wheat production regions around the world.

In the effort to control WSR, the primary strategies have been wheat breeding and eradication of the alternate host barberry (*Berberis vulgaris* L.). Resistance breeding has been largely successful but the pathogen's plasticity challenges these efforts constantly, as illustrated by the emergence of race Ug99 (3,21,29). One tool in the breeding effort is the use of cultivars with adult plant resistance (APR), which is based on the combination of several minor, slow-rusting genes (35). The APR gene complex *Sr2* confers slow rusting to WSR Ug99 and is present in several wheat cultivars, including 'Kingbird' (35,36).

Given the success of the barberry eradication program in the United States from 1918 until the 1980s, sexual recombination is relatively rare in most U.S. populations of the pathogen. Urediniospores, the asexual stage of the pathogen, are thus of primary importance in maintaining its population and play the main role in epidemics (3,23,24,29,30,33). Therefore, understanding the duration of the WSR latent period (defined as the time elapsed between infection initiation and eruption of the sporulating pustule) in the uredinial cycle, and quantifying it as a mathematical model, can be a useful tool in predicting epidemic dynamics (7).

Temperature has been shown to have an effect on latent period duration in various rust pathosystems. For example, *P. hordei* G. H. Oth. on barley (*Hordeum vulgare* L.) (39) and *P. graminis* f. sp. *avenae* Erikss. & Henning on *Avena sativa* L. and *A. sterilis* L. (17) have been reported to have an inverse relationship of latent period duration with temperatures below approximately 25°C. Likewise, the latent period of *P. menthae* Pers. on peppermint decreased with increasing mean air temperature between 6.9°C (40 days) and 20°C (12 days), then increased at temperatures above 20°C (2). For *P. jaceae* var. *solstitialis* Oth., a pathogen of star-thistle (*Centaurea solstitialis* L.), greenhouse and field experiments showed that temperatures between 12 and 20°C produced a latent period of 11 days (264 h), whereas colder temperatures (5 to 15 or 2 to 12°C) produced a longer latent period of 17 or 24 days, respectively (408 or 576 h) (10). Latent period duration modeled for stem rust of perennial ryegrass and tall fescue had a similar relationship to temperature (25). In *P. recondita* Dietel & Holw., higher temperatures resulted in shorter latent periods than did cooler temperatures (41). *P. recondita* on wheat seedlings has a latent period of 291 h at 10°C, and its shortest latent period duration (157 h) at 26.5°C (9). At increasing temperatures above 26.5°C, an increase in latent period duration for this pathogen was observed up to the maximum permissive temperature of 32.2°C, at which plants senesced before urediniospore formation (9).

Similar to these observations of other rust diseases, for which latent period duration decreases inversely with temperature for moderate temperatures, the reported latent period in *P. graminis* subsp. *graminis* was 4 days longer at 15 than at 20°C in two wheat cultivars (16). That study also demonstrated differences among *P.*

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graminis subsp. *graminis* races: latent period for race 56 was 2 days shorter than for race 15B-1 (16).

Latent period is one of the most important variables affecting the final disease level in epidemics of WSR (7). Eversmeyer et al. (8) reported on WSR severity development in the field but did not establish a model for latent period. There is currently no quantitative model to calculate the duration of WSR latent period based on temperature variation. Such a mathematical model, if combined with information about infection events, could help to better predict the time when an epidemic may erupt locally, and also to understand and improve our knowledge of WSR epidemiology. As a component in a quantitative model for WSR epidemic development, a latent period model could improve the effectiveness of disease management practices based on better prediction of epidemic increase.

The objectives of the present work were to (i) identify experimentally the latent period duration of WSR for several wheat cultivars and pathogen isolates across a range of temperatures typical of agronomic situations, (ii) develop a mathematical algorithm to predict WSR latent period from naturally variable temperature data, and (iii) validate the model with field experiments.

Materials and Methods

Inoculation of plants. Single seed of 'Stephens' wheat were planted in plastic pots ("Cone-tainer", 3.8 by 21.0 cm; Stuewe & Sons Inc.) using soilless potting mix Sunshine LA4 P (Sunshine Growing Mix; Sun Gro Horticulture Canada Ltd.). Seed were kept constantly moist by enclosing the pots under plastic wrap until the tip of the first leaf was visible (Zadoks growth stage Z10) (42). Thereafter, pots were watered twice per week and fertilized once with approximately 10 ml of a solution consisting of 1.5 mg of N, 1.35 mg of P_2O_5 , and 1.5 mg of K_2O per liter of water. Plants were grown on a greenhouse bench for 2 weeks until they had two fully expanded leaves and one partially expanded leaf (Zadoks growth stage Z12) (42). Greenhouse temperature, measured with an automated datalogger at 30-min intervals, ranged from $15 \pm 2^\circ\text{C}$ (night) to $20 \pm 3^\circ\text{C}$ (day), with a 12-h day length. We used plants of seedling age because they are easier to handle experimentally and have better uniformity in size and condition than adult plants.

P. graminis subsp. *graminis* race GFCDC (isolated in Oregon in 2010) was cultured on wheat plants in the greenhouse. Spores were collected from open pustules, dried at 30% relative humidity overnight, stored at -60°C , and heat shocked at 46°C for 3.0 min before use (26,31). For experimental treatments, a urediniospore suspension of 1 mg/ml (approximately 5×10^5 spores/ml) was prepared in light mineral oil (Isopar M; Exxon Mobile Chemical Co.). A volume of this suspension at 50 μl /plant was sprayed onto the leaves using a handheld spore inoculator (31) propelling the suspension with an air pump (Gast Manufacturing) operating at 5 psi. The suspension was agitated immediately before being applied onto at least 10 plants/treatment, inside an inoculation chamber. The oil on the plants was allowed to dry for at least 30 min. Subsequently, plants were exposed to 19 h of mist in a mist chamber (Model I-35DL; Percival Mfg. Co.) at $18 \pm 2^\circ\text{C}$ as follows: 15 h of darkness immediately followed by a 4-h light period (25). This temperature is within the previously reported optimal range (15 to 24°C) for germination and appressorium formation of *P. graminis* (31). Mist was provided for 15 min at 2-h intervals during the dark period and continuously during the light period, to keep the leaves consistently covered with a fine layer of free water. At the time of inoculation, spore viability was checked to ascertain that all trials had comparably germinable inoculum. A 50- μl sample of the spore suspension was spread onto a water-agar plate and incubated in the dark at $23 \pm 2^\circ\text{C}$. Spore germination percentage was determined after 24 h by counting three sets of 100 spores randomly located in each plate. A spore was considered to be germinated if the germ tube was longer than the spore width.

Effect of temperature on latent period duration. Following the 4-h light period, plants were removed from the dew chamber and placed into controlled-temperature growth chambers (Model

E30BH0; Percival Scientific, Inc., and Model E15; Conviron Controlled Environments Ltd.) at constant temperatures, 10 plants per temperature. Because of a limited number of growth chambers, only four different temperature treatments could be run simultaneously. Target temperatures were 5, 10, 15, 24, 29, 30, and 32°C . Actual mean temperature in each chamber for each trial was computed from temperatures recorded at 15-min intervals with a leaf wetness and temperature logger (Watchdog; Spectrum Technologies, Inc.). As positive checks to verify that inoculation and disease development were normal, treatments at 10 or 15°C were included in each of the runs (13).

Chambers were set at 14-h day length and temperature was recorded every 15 min with a leaf wetness and temperature logger (Watchdog; Spectrum Technologies, Inc.). Temperature settings of the chambers were adjusted to produce temperatures that fluctuated approximately $\pm 1^\circ\text{C}$. Relative humidity ranged from 65 to 95%. Plants were kept watered by placing pots in trays of water. This watering method kept leaves dry, thereby avoiding subsequent infections that would interfere with observation of the latent period from the controlled inoculation event (25). Each plant was examined individually at least once per day with a $\times 30$ handheld lens, by removing plants briefly from the growth chamber. The number of open pustules on each plant was recorded at each examination time. A pustule was considered open when the plant epidermis over it was cracked enough to expose the urediniospores (25).

Model development. Temperature treatments were repeated in at least three different trials (5, 9, 15, and 16°C , three trials; 23, 29, 30°C , and above, four trials). The total number of pustules on the 10 plants in each temperature of each trial was plotted against elapsed time (number of hours since plants were removed from dew chamber). The number of pustules on each plant typically reached a maximum before adjacent pustules merged, reducing the count (Fig. 1). Data points, for observations from the first-erupting pustule to the time of the maximum number, were fit to a three-parameter logistic model in SigmaPlot Software (Systat Software, Inc. 2008), which uses the Marquardt-Levenberg algorithm to find the parameter values for best fit to the data. From each fitted equation we calculated Lp_{50} , the duration between time of infection (defined as the time when plants were removed from the dew chamber) and the time when 50% of the maximum number of pustules had erupted. The Lp_{50} rate (proportion of the Lp_{50} attained per unit of time) is the reciprocal of the time required to reach Lp_{50} . From these Lp_{50} rates, a temperature-response curve (Lp_{50} rate as a function of temperature) was constructed (25). The latent period duration model was constructed as an equation to fit this temperature response curve. We chose a simple model having a linear increase in rate with temperature from the minimum threshold temperature up to the optimum temperature, followed by an exponential decline in rate to zero at the maximum permissive

Fig. 1. Curves fit to data for appearance of erupting pustules (total per five plants) as a function of time since initiation of infection for three constant-temperature trials. The short, horizontal line bisects each curve at 50% of the maximum number of pustules, and the dashed line shows schematically the derivation of the duration of time from infection to 50% erupting pustules for that trial.

temperature: $\text{Rate} = (a)(t) \times ([T - T_{\min}] \times [1 - e^{-(T - T_{\max})}])$, for T between $T_{\min}$ and $T_{\max}$, where Rate = proportion of a complete Lp_{50} attained per time interval at temperature (T), a is a constant with units of $Lp_{50}/h/^\circ C$, t is time measurement interval in hours, $T_{\min}$ is minimum threshold temperature for activity, and $T_{\max}$ is maximum temperature for activity. Parameters of the equation were estimated by minimizing the sum of squares difference between data points and the model equation. For these calculations, we used an iterative solver routine in Microsoft Excel software.

Latent period duration for different cultivars and isolates. A factorial experiment was conducted to test effects of cultivar and isolate on latent period duration. We used four races of the WSR pathogen (GCCNC, GCCSC, QFCSC, and GFCDC) and four different wheat cultivars—soft white winter wheat Stephens (18), soft red winter wheat ‘McNair 701’ (22), hard red winter wheat ‘Scout 66’ (34), and spring wheat ‘Kingbird’ (5,36)—that differ in level of resistance to the isolates we used. Kingbird is the most resistant (infection type [IT] = ;1), Scout 66 is moderately susceptible (IT = 2) and Stephens and McNair 701 are susceptible (IT = 2+ and 3, respectively). The experimental design was a split-plot with blocks (trial), where main plot was *P. graminis* subsp. *graminis* isolate and split-plot was cultivar, with individual plants as subsamples within each subplot (12). Experimental unit was a single plant. The purpose of the experiment was to test for differences in latent period due to cultivar or isolate. The experiment was conducted at three temperatures (5, 15, and 30°C) but the experiment was not designed to test effect of temperature on the cultivar or isolate effects; therefore, data analyses were conducted separately for data from the three temperature conditions. Five plants per cultivar were inoculated with each *P. graminis* subsp. *graminis* race at Zadoks growth stage Z12 (42). To avoid cross contamination among pathogens in the inoculation chamber, once an isolate was applied, the plants were removed from the chamber and any remaining spores were washed out of the air column using a fine mist of water from a handheld sprayer for 2 min (30,32); the chamber was purged with the filtered air for 20 min before inoculating with the next isolate. As a way to verify the absence of contaminant spores from previous inoculations before the next inoculation, Stephens wheat plants were included as negative controls, by exposing them in the chamber for 2 min to verify that spores from the previous inoculations were no longer present. No infections were detected in these controls. After inoculation, plants were removed to a growth chamber and kept watered as previously described to avoid uncontrolled additional infections during the postinoculation incubation. The number of open pustules on each plant and time elapsed were observed at least once per day with a $\times 30$ handheld lens and recorded. The experiment was carried out twice.

Fig. 2. Curve fit to the observed latent period (time from infection to 50% erumpent pustules [Lp_{50}]) of wheat stem rust (*Puccinia graminis* subsp. *graminis*) in ‘Stephens’ wheat at various constant temperatures under controlled conditions. Plants that were incubated at 32.6 or 33.4°C (not graphed) produced no erumpent pustules.

Outdoors test of the model. Two latent period experiments were conducted outdoors to validate the model that was developed using temperature data from growth chamber experiments. In each outdoor experiment, 10 plants each of Stephens and McNair 701 were grown and inoculated as described above. Once removed from the inoculation chamber, plants in their pots were placed outdoors near a greenhouse facility in Corvallis, OR. Irrigation was supplied by setting pots in a pan of water, so that leaves could be kept dry thereby avoiding any additional infection events. Records of temperature, leaf wetness, number of pustules, and time elapsed were taken as described previously. Each plant was considered an experimental unit. Temperatures ranged from 3.2 to 35.3°C among the outdoor trials, which were conducted twice, in May and June of 2012.

Field test of model. A latent period experiment was conducted twice in the field at the Oregon State University Hyslop Field Research Laboratory, Schmidt Farm unit (44°38′ N, 123°11′ W) during October to December 2012. A single plot (82.0 by 2.1 m) with seven rows of Stephens wheat was planted on 11 October 2012 using a grain drill type planter at the rate of 112 kg/ha. Diuron (1.3 kg/ha) was applied 1 day after planting to control weeds, and no irrigation was used. When plants were at Zadoks growth stage Z13 (42), they were inoculated. One plant per row was randomly selected and inoculated. Seven plants total were inoculated for the first experiment (30 October 2012) and seven other plants for the second experiment (15 November 2012). Spores of *P. graminis* subsp. *graminis* race GFCDC were heat shocked at 43°C for 1.5 min before they were applied with a fine brush (number 000) to one leaf of each subject plant. In the first experiment, each plant was covered with a 0.5-liter glass jar overnight following inoculation, to maintain leaf moisture for infection. Jars were removed the next day, approximately 4.5 h after sunrise. In the second experiment, plants were inoculated in the morning and covered with a light-proof tub to maintain darkness and adequate temperature for initiating infection. The tubs also contained a 350-ml Styrofoam cup full of hot water placed next to the plants to act as a moisture source for condensation onto the plant leaves. After 6 h of this artificial darkness, tubs were removed and replaced with glass containers while there was still 4 h of daylight to promote completion of the infection process. Temperatures, recorded with a leaf wetness and temperature logger (Spectrum Technologies Inc.) inside the field inoculation chamber, ranged from 8.4 to 15.8°C during the infection process. Procedures for determining spore viability and for observing number of pustules per plant (the experimental unit), time elapsed, and air temperatures during the latent period were as described above. Rainfall measurements were not taken.

Data analysis. The GLIMMIX procedure of SAS (version 9.22; SAS Institute) was used to analyze data from the experiment comparing cultivars and isolates for latent period duration. Analysis of variance was used to test for differences among cultivars, isolates, or their interaction at each temperature. Fisher’s least significant difference ($P = 0.05$) test was used to compare mean differences (6). Comparison of expected (modeled) versus observed latent periods in the field and outdoors experiments was done with the Proc t test procedure. Confidence intervals (CIs) at 95 and 99% were calculated for the observed latent period durations.

Results

Effect of temperature on latent period duration and rate. In all, 24 constant-temperature treatments ranging from 4.7 to 33.4°C were executed, and the Lp_{50} was calculated from the graph of pustule number versus time (Fig. 1) for each temperature. The Lp_{50} values for each temperature were then used to construct the latent period temperature-response curve (Fig. 2). The duration of the latent period was longest at 4.7°C (1,109 h) and shortest at 29°C (128 h). At temperatures above 29.1°C, latent period duration increased steadily with increasing temperature up to 30.8°C. However, the length of Lp_{50} at 30.8°C was 396 h (i.e., not as long as it was at 4.7°C; Fig. 2). At 32.6 and 33.4°C, no erumpent pustules

were produced on Stephens. There was a positive linear increase in latent period rate as a function of temperature from 4.7 to 29°C, and a very sharp decline from 29 to 33.4°C (Fig. 3). At high temperatures (between 29.8 and 32.6°C), very few pustules survived to become erumpent (fewer than 0.4 pustules/plant) compared with the number of lesions at lower temperatures (3 to 88 pustules/plant at temperatures between 6 and 29°C).

Latent period model. The parameters for the model equation whose general form is given in the Methods section were determined to be $T_{min} = 1.8$, $T_{max} = 30.9^{\circ}\text{C}$, and $a = 2.9 \times 10^{-4}$. The proportion of the Lp_{50} completed during any time increment (in hours) of a given average temperature (T) can thus be calculated as $\text{Proportion of } Lp_{50} \text{ completed} = \text{hours} \times (2.9 \times 10^{-4}) \times (T - 1.8) \times [1 - e^{(T - 30.9)}]$, for values of T between 1.8 and 30.9°C.

For weather data with 1-h time resolution (Fig. 3), the proportion of the latent period completed during each 1-h time increment is obtained by using the hourly temperature reading and setting the value of hours in the equation to 1.0; for 15-min data frequency, the value of hours in the equation would be set to 0.25 and each 15-min average T would be used. These proportions can be cumulated to model the latent period duration under conditions of fluctuating temperature, using the average temperature within each time increment. For example, because a 1-h period at 10°C provides the heat units to complete 0.0024 of a latent period (Fig. 3), an infection that occurs (begins its latent period) when the temperature is

Fig. 3. Wheat stem rust (WSR) latent period completion rate as a function of temperature. Rate is the proportion of the time from infection to 50% erumpent pustules (Lp_{50}) that is completed in 1 h at the corresponding temperature. Data points are calculated from constant-temperature trials. Curve was fit to data by least-squares method, based on an equation describing linear increase followed by exponential decline. Temperature maximum was determined experimentally. The equation for Lp_{50} rate, between the permissive temperature limits of 1.8 to 30.9°C, is $Lp_{50} \text{ rate (proportion of latent period per unit time increment)} = (2.9 \times 10^{-4}) \times (\text{h}) \times [(T - T_{min}) \times [1 - \exp^{(T - T_{max})}]]$, where T = temperature ($^{\circ}\text{C}$), $T_{min} = 1.8$, $T_{max} = 30.9$, and h = length of time increment in hours.

10°C would, in the first 1.5 h of its existence, complete 0.0036 (1.5 $\times$ 0.0024) of its latent period. If the temperature were then to increase 0.5°C each half-hour, the next four 30-min time increments would provide, as calculated by the equation, 0.00126, 0.00133, 0.00141, and 0.00148, respectively, of the heat units needed for the latent period. Summing these proportions gives 0.00905 of the latent period completed in these first 3.5 h of the infection's existence. Continuing through time, the proportions of the latent period completed for each time increment (based on its temperature) are cumulated until 1.0 is reached, indicating the completion of the modeled latent period.

Field and outdoor tests of model for latent period. In the field experiments (conducted with plants in a field planting), differences between observed and predicted latent periods were less than a day (16.4 and 9.9 h in first and second experiment, respectively) and were not statistically significant ($P > 0.05$) (Table 1). The predicted latent periods fell within the 95% CI for the two field tests (Table 1). For the outdoors experiments (potted plants), the differences between observed and predicted latent period for Stephens were in a similar range as for the field experiments (6.5 and 13.6 h for first and second outdoors experiments, respectively), falling within the 99% CI of the observed values (Table 1). However, these differences were statistically significant ($0.0326 \leq P \leq 0.0367$). For McNair 701 (Table 1), differences between observed and modeled latent period duration were 3.7 h (nonsignificant, $P > 0.05$) and 32.7 h (significant, $P = 0.0018$) in the first and second outdoors experiments, respectively. Modeled latent period for McNair 701 in the first experiment fell into the 95% CI but the other was beyond the 99% CI (Table 1).

Effect of temperature on latent period duration and cultivar-isolate comparison. Significant differences ($P \leq 0.0396$) in latent period duration among four cultivars were found at each temperature tested (5, 15, and 30°C). The F values for isolate and for isolate-cultivar interaction were nonsignificant within each of the three temperatures (Table 2). A comparison of means among cultivars at 15°C showed a significantly longer latent period for Stephens and Kingbird (269 and 254 h, respectively) than for Scout 66 and McNair 701 (231 and 220 h, respectively) (Fig. 4). At 5 and 30°C, Kingbird had the longest latent period among the cultivars (1,063 and 219 h, respectively), followed by Stephens (977 and 186 h), Scout 66 (967 and 185 h), and McNair 701 (957 and 162 h) (Fig. 4).

Discussion

In this study, we developed a mathematical model to quantify the relationship of latent period duration to temperature. The optimum temperature for latent period (shortest duration) for WSR in our experiments was between 24 and 29°C. From other experiments on germination of *P. graminis* subsp. *graminis* uredini-

Table 1. Modeled and observed latent period duration of wheat stem rust (WSR), expressed as time in hours from infection to 50% of pustules erumpent (Lp_{50}), in experiments conducted in field plantings and in outdoor potted-plant experiments^a

	Field		Outdoors			
	Experiment 1	Experiment 2	Experiment 1		Experiment 2	
Cultivar ^b	Stephens	Stephens	Stephens	McNair 701	Stephens	McNair 701
Predicted ^c	449	667	267.2	267.2	254.8	254.8
Observed ^d	432.6	657.1	273.7	263.6	241.2	222.1
Difference ^e	16.4	9.9	6.5	3.7	13.6	32.7
95% CI ^f	377.7–487.5	624.9–689.3	268.1–279.2	247–280.2	229–253.4	209.7–234.4
99% CI ^g	341.5–523.7	603.6–710.6	264.5–282.9	236–291.1	220.9–261.4	201.6–242.6

^a Model for latent period duration was developed from data on 'Stephens' wheat: $\text{Proportion of } Lp_{50} \text{ completed} = \text{hours} \times (2.9 \times 10^{-4}) \times (T - 1.8) \times [1 - e^{(T - 30.9)}]$, for values of temperature (T) between 1.8 and 30.9°C.

^b Four cultivars were tested: soft white winter wheat Stephens (WSR susceptible), soft red winter wheat 'McNair 701' (WSR susceptible), hard red winter wheat 'Scout 66' (resistant), and spring wheat 'Kingbird' (WSR resistant).

^c Lp_{50} predicted from the mathematical model, using temperatures recorded every 15 min during exposure.

^d Lp_{50} mean from field ($n = 7$) and outdoors ($n = 10$) experiments under natural conditions at Corvallis, OR in 2012.

^e Difference in hours between predicted and observed Lp_{50} (absolute values).

^f Lower and upper limits of the 95% confidence intervals (CIs) for the Lp_{50} observed values.

^g Lower and upper limits of the 99% CIs for the Lp_{50} observed values.

ospores (*unpublished*), we estimate that the minimum temperature allowing biological activity for this fungus is 1.8°C. We have no direct observation for latent period at this low temperature but our fitted curve for latent period rate (Fig. 2) is consistent, with a lower limit of approximately 2°C. At temperatures of 29 to 31°C, WSR latent periods were longer than at the optimum temperature but not as long as at the coldest permissive temperatures. The highest permissive temperature we observed for WSR pustule development was approximately 31°C.

Other reports of temperature effects on latent period duration in grass rusts are broadly similar to ours—for example, for *P. recondita* (9,14,19,28,41), *P. hordei* (39), *P. avenae* (17), and *P. graminis* subsp. *graminis* (16,27,40) and *graminicola* (25)—but with some differences in details. These rust fungi all have similar minimum and maximum temperatures for uredinial growth. The high-temperature limitation on rust development is likely a consequence of the temperature tolerance of the host plant; Eversmeyer et al. (9) noted that wheat plants inoculated with *P. recondita* and held at 32.2°C senesced before pustules formed, and we also saw accelerated senescence of leaves at temperatures above 30°C. Despite these cardinal-temperature similarities among rust fungi, the latent period duration at optimum temperatures varies. Whereas we observed the minimum latent period duration for *P. graminis* subsp. *graminis* to be approximately 139 h, others report minimum durations shorter (127 h for *P. hordei*; 39) or longer (199 h for *P. recondita*; 41). These differences highlight the need for parameterizing latent period duration models individually for different rust fungus species. We note also that latent period differs somewhat among host and pathogen genotypes within a pathosystem (14,28). Cultivar-related differences in L_{p50} may need to be accounted for when using this model for slow-rusting cultivars. In our experiment, L_{p50}

was significantly longer in the resistant Kingbird than in the other cultivars used (Stephens, Scout 66, and McNair 701) at the three temperatures tested (5, 15, and 30°C).

The model presented here was created from data obtained under different regimes of constant temperatures in controlled conditions. The significance of latent period duration at constant temperatures is indirect, because temperatures normally fluctuate under field conditions. Plants are, however, commonly exposed to fluctuating temperatures that may sometimes be near the upper or lower limits for pustule development. In actual use, the model can be applied to temperature data that vary continuously over time, by accumulating latent period effects of temperature over successive small time increments. When the latent period accumulated total reaches 1.0, the model predicts the completion of the latent period, as in the example described in the Results section. The capability of the model for use with data of naturally occurring, variable temperature is an essential aspect for estimating actual latent period duration and, thus, epidemic progress under agronomic conditions. We compared latent period predicted from our model with results from experiments carried out under naturally variable temperatures occurring in a field planting, and found that the model-predicted values of L_{p50} in the two field experiments had only 16.4 and 9.9 h of difference with that observed experimentally.

The model might be improved by incorporating variability in latent period due to plant age. In our experiments, we determined latent period duration of WSR in seedling plants, and our proposed model is based on these results. Other researchers have observed latent periods of some rust pathogens to be 2 to 6% shorter in seedlings than in plants inoculated at anthesis (38,41) and 20% shorter than in plants that are heading (41). The possibility that latent period is extended somewhat in older plants could be important if the

Table 2. Analysis of variance for effects of wheat stem rust isolate, wheat cultivar, and their interaction on latent period duration at three different temperatures in growth chambers

Effects ^b	Latent period ^a								
	5°C			15°C			30°C		
	df	F	Pr > F	df	F	Pr > F	df	F	Pr > F
Isolate	3	1.00	0.5016	3	2.52	0.2332	3	1.07	0.4747
Cultivar	3	3.80	0.0396	3	10.68	0.0010	3	5.21	0.0254
Isolate × cultivar	9	1.47	0.2628	9	0.54	0.8200	9	0.77	0.6504

^a Latent period is the time in hours from infection to the time when 50% of the maximum number of pustules were open.

^b Isolates used were races GCCNC, GCCSC, QFCSC, and GFCDC. Four wheat cultivars were tested: soft white winter wheat ‘Stephens’, soft red winter wheat ‘McNair 701’, hard red winter wheat ‘Scout 66’, and spring wheat ‘Kingbird’.

Fig. 4. Latent period (time from infection to 50% eruptant pustules [L_{p50}]), expressed in degree-hours, of wheat stem rust on four wheat cultivars at three temperatures. **A**, L_{p50} of cultivars across all isolates at 15°C; **B**, L_{p50} of cultivars across all isolates at 5°C; and **C**, L_{p50} of cultivars across all isolates at 30°C. There was no statistically significant effect for isolate or cultivar–isolate interaction. Bars with the same letter within a temperature are not significantly different according to the least significant difference test at $P = 0.05$.

latent period model were to be used in a context where the need for late-season management inputs is assessed. We note that the age-related differences in reported latent period duration (38,41) are small for plants before the emergence of the flag leaf, whose protection is typically the goal of a fungicide program.

Duration of latent period in field experiments is one among several important variables affecting epidemic development and, thus, final infection levels (7,20). Ability to calculate latent period duration can be useful to predict disease development. If an infection event is known to have occurred at a particular time, the latent period model can predict the appearance of the disease. More generally, if the latent period model is combined with other quantifiable processes, it can be an essential component in a general model for epidemic development and management decision-making. Also, the latent period duration model as a stand-alone tool could be useful for comparisons among pathogen isolates after testing in controlled-environment experiments. We found no significant difference in Lp_{50} at 5, 15, and 30°C among four isolates of *P. graminis* subsp. *graminis* on four wheat cultivars. The existence of variants that might be more aggressive epidemiologically would be indicated by the observation of isolates and newly emerging pathotypes of the WSR pathogen with a latent period that is more rapid, or that occurs across a broader range of temperatures, than we observed in the representative isolates we tested.

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