Comparative Effects of Temperature and Interrupted Wet Periods on Germination, Penetration, and Infection of *Puccinia recondita* f. sp. *tritici* and *P. striiformis* on Wheat Seedlings

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ABSTRACT

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Under optimal temperature and nonlimiting wetness duration, the infection efficiency (defined as the proportion of inoculated urediniospores causing lesions on wheat seedling leaves) was 12 times greater for *Puccinia recondita* f. sp. *tritici* than for *P. striiformis*. Penetration of both species, however, was similarly affected by a 1-h dry period interrupting a 24-h wet period 1, 2, 4, 6, 8, or 16 h after inoculation at several temperatures between 5 and 30 C. Appressoria from germinated *P. r. tritici* urediniospores prior to penetration were unable to survive the 1-h dry period. An interruption of the wet period by a dry period did not affect ungerminated urediniospores, which were able to infect leaves during a subsequent dew period. The minimal continuous dew period necessary for infection increased from 4 to 6 h at optimal temperatures (8 C for *P. striiformis*, 15 C for *P. r. tritici*) to at least 16 h at suboptimal temperatures. Infection by *P. r. tritici* occurred over a wide range of temperatures (5–25 C), whereas infection by *P. striiformis* was restricted to a narrower range (5–12 C). Percentage of infection as a function of the duration of the continuous dew period was described by a Richards's function with temperature-dependent parameters. For a dry period interrupting the 24-h dew period before the minimal continuous dew period necessary for infection, percentage of infection at specific temperatures was fitted by a negative exponential function of time of interruption. If the dry period occurred after the minimal dew duration for infection, percentage of infection was the same as with a continuous wet period.

Additional keywords: comparative epidemiology, leaf brown rust, stripe yellow rust.

Cereal rusts develop in specific weather conditions; in some temperate regions, wheat (*Triticum aestivum* L.) stripe and leaf rust epidemics usually occur in succession during cool and humid periods and warm and dry periods, respectively. *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* Eriks & E. Henn. D. M. Henderson, the leaf rust pathogen, is adapted to a higher range of temperatures than is *P. striiformis* Westend., the stripe rust pathogen. Sporulation and infection were analyzed in a recent study of *P. r. tritici* and *P. striiformis* (24). Under optimal conditions, *P. striiformis* produces a greater number of spores but has a lower infection efficiency than *P. r. tritici*, and the lesion surface area is much greater for stripe rust than for leaf rust. Therefore, the severity of a leaf rust epidemic may be more dependent on the infection efficiency of individual spores of *P. r. tritici* than it is for stripe rust. Among the environmental conditions favorable for germination and penetration of the fungal spores, temperature and free moisture on plants are the most important (23). For most fungal pathogens, successful infection depends mostly on a minimal duration of wetness, which varies as a function of temperature (19). A dry period interrupting leaf wetness can kill germinating spores of *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (7) and *P. r. tritici* (27), and the time at which the spores encounter the dry period, rather than the period's duration, appears to be important. Eversmeyer et al (17) suggested that penetration can occur after a dry period, provided that appressoria have been formed previously. Clifford and Harris (13) confirmed that dew is not required for substomatal vesicle formation.

More studies to assess the effects of wetness interruption are needed to better characterize the biology of these pathogens. For *P. striiformis* in particular, appressoria are not produced before penetration, and this factor might affect its ability to survive a dry period. These studies should have useful applications because of the increasing number of weather stations measuring temperature and wetness on a short time scale (1 h or less). The main purpose of this study was to compare successful infection of *P. r. tritici* and *P. striiformis* under different combinations of temperature, wetness duration, and interrupting dry periods. To make the comparison more general, an analytical model based on biologically meaningful parameters was used. The modeling objective was to extend an existing approach applied in the case of continuous wetness (8) to the case of discontinuous wetness.

MATERIALS AND METHODS

Plants, fungi, and inoculation. Seedlings of wheat cv. Michigan Amber, highly susceptible to French races of stripe and leaf rusts, were grown to the two-leaf stage (about 10-day-old, stage 12 in Zadoks scale [29]) in square pots (7 × 7 × 8 cm, 10 seeds per pot along one side) placed in an air-filtered chamber inside a greenhouse maintained at 15–20 C. Single urediniospore isolates collected in France were used (race 45E140 of *P. striiformis* and coded isolate B8620-3 of *P. r. tritici*). Spores were produced in a growth chamber at 14 C during an 8-h dark period and 17 C during a 16-h light period with a light intensity of 300 μE m^{-2} s^{-1}. Urediniospores from plants inoculated 11 and 15 days earlier with *P. r. tritici* and *P. striiformis*, respectively, were harvested 1 day before use and placed in a desiccator filled with glycerol for 24 h at 5 C to avoid agglomeration of spores.

On each of six plants per pot, the second leaf from the top of the plant was removed and first leaves were attached horizontally with their adaxial side up on a plexiglass sheet with plasticine (Ulmann, Paris, France). Plants in 10 pots were inoculated together in a settling tower (18) with either 1 mg of

P. r. tritici or 1.5 mg of *P. striiformis* urediniospores, corresponding to 115 ± 10 urediniospores/cm^2.

Temperature and wetness conditions. The temperatures tested were 5, 10, 15, 20, 25, 30, and 35 C for *P. r. tritici*, and 5, 8, 12, 16, 20, and 24 C for *P. striiformis*. The initial dew periods (DP_1s) tested were 0, 1, 2, 4, 6, 8, and 16 h. Each experimental treatment corresponding to the combination of one temperature and one initial dew period DP_1, consisted of 10 pots of six plants and was repeated four times. After inoculation, the 10 pots were partitioned into three groups. A group of three pots was put under DP_1 followed by a 1-h dry period and a subsequent dew period (DP_2), with $DP_1 + DP_2 = 24$ h. A second group of three pots stayed in the dew chamber only during DP_1 and did not receive DP_2. The four remaining pots, used as a reference for maximum infection, stayed under a 24-h continuous dew period. The dry period was imposed by placing the pots under a fume exhaust hood (30–40% RH, 20 C) where free water on the leaves evaporated within 5 min. After the dew period treatments, the pots were transferred into a growth chamber with conditions set as described above until lesions developed.

Experimental conditions were reproducible by using a dew chamber adapted from Clifford (12). A 12 × 20 cm trap was added in the middle of the plexiglass door so that plants could be removed during experiments without greatly modifying environmental conditions. Plants were placed on a grid 75 cm above the water surface. Evaporation of water placed in a 0.01 m^3 reservoir heated by an electric resistance (200 W) at the bottom of the chamber provided a saturated atmosphere in the dew chamber. The dew chamber was installed in a dark, temperature-regulated growth chamber.

A preliminary calibration experiment showed that a temperature difference of 1.5 C between growth chamber and leaf temperatures was necessary to obtain a similar dew formation at different temperatures (condensation rate was approximately 0.1 mg h^{-1} cm^{-2} in the dew chamber). Leaf temperature (± 0.1 C) was monitored with an electronic circuit and a data logger (Campbell Scientific 21X, Shepshed, Leicestershire, England) that regulated the water reservoir temperature as a function of leaf temperature. By activating or interrupting the electrical resistance placed in the water reservoir, the data logger maintained the leaf temperature between two values (e.g., 14.9 C and 15.1 C for a desired temperature of 15 C). The temperature measurements were obtained as 15-min averages using copper-constantan thermocouples.

Germination and penetration. Germination was studied at 7 and 6 temperatures for *P. r. tritici* and *P. striiformis*, respectively, whereas penetration was studied only at optimal temperatures: 15 C for *P. r. tritici* (13,17) and 8 C for *P. striiformis* (14,22). Germination and penetration were assessed on one leaf per pot and infection on the five remaining leaves. When chlorotic flecks indicative of infection began to appear (6 and 5 days after inoculation for leaf and stripe rust, respectively), one leaf per pot was cut into three 1.5-cm-long pieces and placed with its adaxial side up on a filter paper impregnated with lactophenol for clearing in a petri dish. After at least 5 days, the leaf pieces were mounted in lactophenol-trypan blue-acid fuschin (6:1:2, v/w/w) (3). The number of germinated spores and the number of leaf rust appressoria were assessed and expressed as percentages. A spore was considered to have germinated when the germ tube length was at least half the spore diameter. Penetration by *P. r. tritici* spores was evaluated by counting numbers of empty appressoria with blue-stained cell walls but no stained cytoplasm that had already passed into the substomatal vesicle and was no longer visible (11). Before penetration, the cytoplasm of an appressorium stained red. Penetration by *P. striiformis* was assessed by counting substomatal vesicles because of the absence of appressoria. The number of spores germinated and penetrated was assessed microscopically from 100 fields (about 200 spores) per treatment for leaf rust and from 250 fields (about 400 spores) for stripe rust.

Infection. Infection by *P. r. tritici* was assessed 11 days after inoculation by recording the number of pustules on a 1-cm^2 area in the middle of five leaves in each pot. Because of the semisystemic development of *P. striiformis*, stripe rust infection was estimated by counting numbers of chlorotic spots 8 days after inoculation. In nonlimiting wetness conditions, an infection efficiency was assessed equal to the number of lesions per deposited urediniospore ×100. At optimal temperature, each stage (germination, penetration, and infection) was assessed and infection efficiency was calculated as the product of three ratios (germination, penetration/germination, and infection/penetration). Relative infection was calculated as percentage of infection divided by maximal percentage of infection at optimal temperature and under nonlimiting wetness condition ×100.

Statistical analyses. Data were analyzed using the general linear models procedure of the Statistical Analysis System (25) by analysis of variance (ANOVA) combined with orthogonal contrasts after appropriate transformation to homogenize the variances among treatments. The transformations used are cited in the figure captions. Comparison between the two rusts at optimal temperature for germination, penetration, and infection was conducted with ANOVAs with one factor (the pathogen). Germinated and penetrated spores were analyzed using leaf fragments as individuals. Infection, first analyzed with data classified per pot, showed no significant difference between pots; therefore ANOVAs were conducted using plants as individuals. The two factors, dew duration and occurrence of a dry period, were tested for germinated spores and spores causing infection at optimal temperature with data for each pathogen analyzed separately. Appressorium data were analyzed with a three-factor ANOVA (penetration, dew duration, and occurrence of a dry period). Comparison of the two rusts for germination and infection efficiency at different temperatures and nonlimiting wetness condition was made with ANOVAs with temperature and pathogen as factors.

Model. An equation based on five parameters was developed to describe the influence of temperature, dew duration and wetness interruption on leaf and stripe rust infection. The influence of temperature on maximal infection ($\imath_{max}(T)$) was described using a beta function (2,8) by:

$$\imath_{max}(T) = p\,((T - T_{min})/(T_{max} - T_{min}))^n\,((T_{max} - T)/(T_{max} - T_{min}))^m \quad (1)$$

Minimum (T_{min}) and maximum (T_{max}) temperatures were fixed equal to observed values: 2 C and 30 C for *P. r. tritici* (10,28), and 0 C and 18 C for *P. striiformis* (14), respectively. As shown by Analytis (2), the parameter p can be expressed as a function of n and m: $p = (n + m)^{n+m}/(n^n\, m^m)$. The adjustment was made by minimizing the mean square error between observed and simulated maximal infection for varying values of n and m.

The influence of continuous dew duration on relative infection ($\imath_{cont}(DP_1,T)$) was described as the product of maximal infection $\imath_{max}(T)$ and a Richards's function of dew duration with temperature-dependent parameters (26):

$$\imath_{cont}(DP_1,T) = \imath_{max}(T)\,(1 - \exp(-b(DP_1 - DP_{min}))) \quad \text{for } DP_1 \geq DP_{min} \quad (2)$$

where DP_1 is the duration of the initial dew period, DP_{min} is the minimal dew period necessary for infection and b is the initial infection rate. Using infection data measured in continuous wetness conditions and values simulated by equation 2, DP_{min} and b were estimated by minimizing the mean square error between measured and simulated values of $\imath_{cont}(DP_1,T)$ for varying values of b and DP_{min} at each temperature.

To account for the influence of wetness interruption, a negative exponential term was added to equation 2.

$$\imath_{int}(DP_1,T) = \imath_{cont}(DP_1,T) + \imath_{max}(T)\exp(-(DP_1/\tau)) \quad (3)$$

where $\imath_{int}(DP_1,T)$ is the relative infection after 24 h of dew when a 1-h dry period occurs after DP_1 h of dew. This equation consists of two terms: the first term corresponds to infection if a dry period occurs after the minimal wetness duration; the second term corresponds to infection if a dry period occurs before DP_{min}.

This second term includes the time constant (τ), which is the time when a dry period leads to an infection equal to maximal infection divided by a factor e (base of natural logarithm). Using the infection data obtained in conditions of interrupted wetness, the parameter τ was fitted with parameters b and DP_{min} previously calculated using results of experiments with continuous wetness.

RESULTS

Germination at optimal temperatures. The maximum proportions of *P. r. tritici* and *P. striiformis* spores that germinated were 76 and 56%, respectively (Table 1). Initiation of germination began after 1 and 2 h of dew for *P. r. tritici* and *P. striiformis*, respectively (Fig. 1A and B). After 4 h of dew, germination of urediniospores reached 92% of its maximum for *P. r. tritici*; after 6 h of dew, the corresponding percentage was 93% for *P. striiformis*.

TABLE 1. Comparison of *Puccinia recondita* f. sp. *tritici* and *P. striiformis* for germination, penetration, and infection at optimal temperatures and continuous 24-h dew period

	P. r. tritici	*P. striiformis*	Ratio of *P. r. tritici* to *P. striiformis*
Germination (%)[a]	75.9	56.5	1.3
Penetration (%)[a,b]	90.3	7.1	12.7
Infection (%)[a,c]	63.6	88.8	0.7
Infection efficiency (%)	43.6	3.6	12.1

[a]Three separate ANOVAs were performed for germination, penetration, and infection. The probability of having a greater F value for the difference between the two rusts was 0.0001 for all three cases.
[b]Penetration was calculated as percentage of germinating spores.
[c]Infection was calculated as percentage of spore penetration.

In experiments with a dry period interrupting a 24-h dew period at the end of DP_1, germinated urediniospores of both pathogens were divided into two categories: spores with short, thick germ tubes (<75 μm) and spores with long, thin germ tubes (>150 μm) (Table 2). There were no spores with germ tubes in the 75–150 μm length range. After DP_1 (1, 2, or 4 h), all germinated spores produced only short germ tubes. After DP_2, the proportions of spores with short germ tubes were equal to the proportions of spores germinated during DP_1. Therefore, the short germ tubes obtained during $DP_1 \leq 4$ h were not able to grow further during DP_2 after a dry period had occurred. The dry period definitively blocked the germination process previously initiated during DP_1.

Infection at optimal temperatures. Penetration by *P. r. tritici* was more efficient than by *P. striiformis* (Table 1) but *P. striiformis* was more efficient for infection. Overall, *P. r. tritici* urediniospores were approximately 12 times more successful than those of *P. striiformis* in infecting leaves at optimal temperature and wetness conditions.

For both rusts, infection was initiated after a minimal dew period of 4 h (Fig. 1C and D). The maximum number of infections

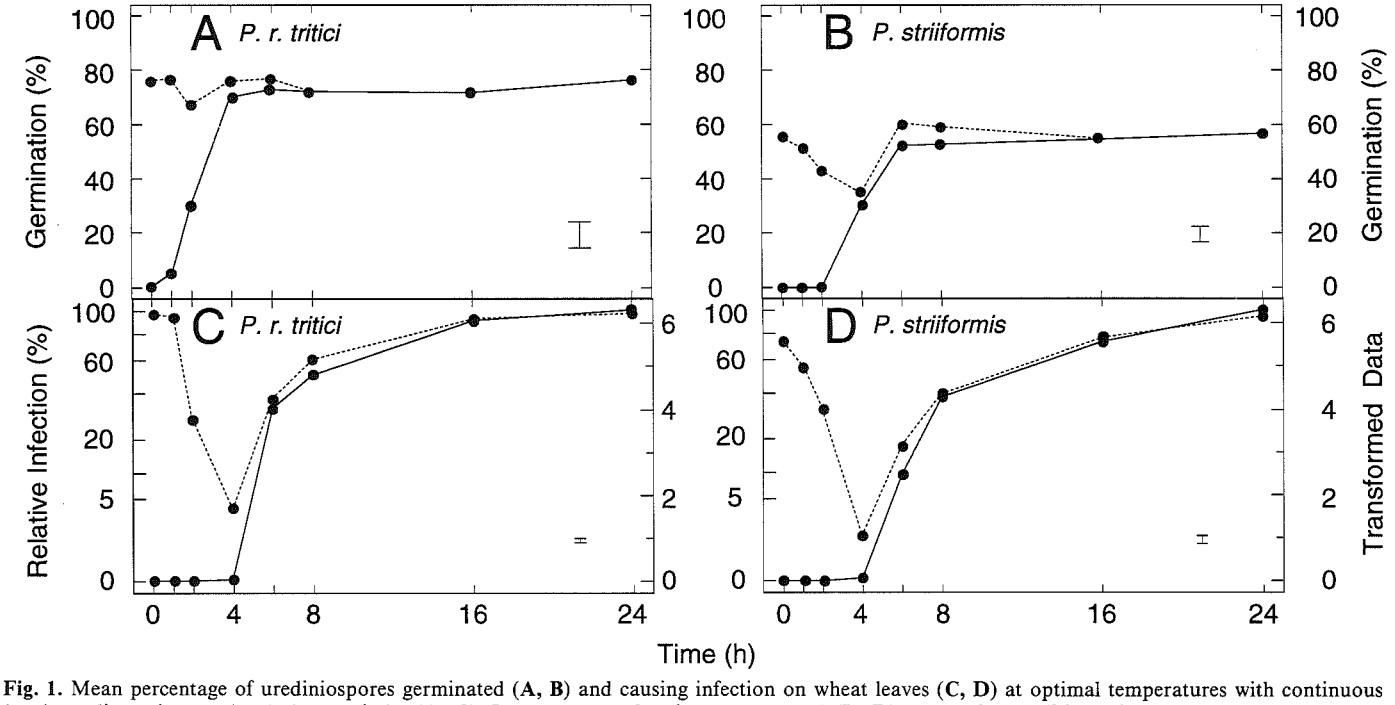

Fig. 1. Mean percentage of urediniospores germinated (**A, B**) and causing infection on wheat leaves (**C, D**) at optimal temperatures with continuous (——) or discontinuous (·····) dew periods, (**A, C**) *Puccinia recondita* f. sp. *tritici* and (**B, D**) *P. striiformis*. Discontinuous 24-h dew treatments were interrupted for 1 h at 0, 1, 2, 4, 6, 8, and 16 h. Infection percentages were calculated relative to maximal infection with a continuous 24-h dew period. Percentage of spores causing infection (y) (**C, D**) were transformed by $y^{0.4}$. The bars represent the standard error of the difference.

TABLE 2. Effect of a 1-h interruption of the dew period on germination of *Puccinia recondita* f. sp. *tritici* and *P. striiformis* urediniospores

Time of dew period interruption[a] (h)	Percentage of *P. r. tritici* spores with germ tubes			Percentage of *P. striiformis* spores with germ tubes		
	DP_1[b] only (h)	$DP_1 + DP_2$[c]		DP_1 only	$DP_1 + DP_2$	
	Short[d]	Short	Long[d]	Short	Short	Long
1	5.3[e] (±1.9)	5.2 (±2.6)	71.4 (±11.1)	0	0	51.6 (±12.9)
2	29.8 (±9.1)	34.8 (±7.3)	31.8 (±6.5)	0.2 (±0.5)	3.4 (±2.1)	39.5 (±6.3)
4[f]	...	...	...	30.3 (±8.9)	33.2 (±8.2)	1.4 (±2.2)

[a]To interrupt the dew period, plants were removed from the dew chamber and dried under a hood.
[b]DP_1 is the length of initial dew period up to the time of interruption.
[c]DP_2 is the length of the dew period after the interruption ($DP_2 = 24$ h $- DP_1$).
[d]Short germ tubes were <75 μm and long germ tubes were >150 μm.
[e]Means (± standard error) were calculated for a minimum of 200 spores for *P. r. tritici* and 400 spores for *P. striiformis* for each treatment.
[f]For *P. r. tritici*, after 4 h of dew, short and long germ tubes were not distinguishable.

occurred faster with *P. r. tritici* than with *P. striiformis*. Dry periods imposed after 4 h of continuous dew caused infection curves to be similar to those with continuous wetness. Interruption of wetness duration before 4 h greatly decreased the percentages of spores causing successful infection. Infection by *P. r. tritici* was not affected (no significant difference at $P = 0.05$) by a dry period at 0 or 1 h of wetness duration (Fig. 1C) whereas *P. striiformis* infection progressively decreased when dry periods occurred at 0, 1, 2, and 4 h after inoculation (Fig. 1D). For *P. striiformis* spores exposed to a dry period before continuous dew ($DP_1 = 0$ h, $DP_2 = 24$ h), the percentage of infection was 81% of the maximum observed. When DP_1 was 1, 2, and 4 h, relative infection decreased to 57, 39, and 4%, respectively. For both rusts, the minimum percentage of infection observed (approximately 5%) was at $DP_1 = 4$ h.

Penetration at optimal temperatures. Curve shapes of *P. r. tritici* spores that successfully penetrated in different dew period treatments, without or with interruption by a dry period (Fig. 2A and B), were similar to those of spores that caused infection on leaves (Fig. 1C). After 6 h of continuous dew, the ratio of infection to penetration was almost one, i.e., each penetrated spore produced a pustule (data not shown). The ratio was 0.64 after 24 h of continuous dew indicating that several penetrated spores could result in a single pustule (Table 1). For *P. striiformis*, the ratio of infection to penetration was close to one for a continuous dew duration of 4–24 h.

Appressoria in *P. r. tritici* were not observed until 2 h of continuous dew, and their formation reached a maximum after 8 h (Fig. 2A). After 4 h of continuous dew, substomatal vesicles were observed and penetration had occurred. Penetration was near-complete after 16 h. Whether a dry period occurred or not for $DP_1 = 4$–24 h, the number of appressoria with red-staining cytoplasm (before penetration) was the same (orthogonal contrasts, $P = 0.52$) and the number of appressoria from which leaves were penetrated was the same ($P = 0.80$; Fig. 2A and B). Therefore, when appressoria were developed but the leaves were not penetrated before the dry period occurred, penetration would not happen during DP_2. For discontinuous wetness conditions ($DP_1 = 1$–4 h, $DP_2 = 23$–20 h), appressoria development led to penetration. Because no appressoria were observed during continuous $DP_1 = 1$–4 h, it can be inferred that differentiation of appressoria and penetration occurred during $DP_2 = 23$–20 h.

Effect of temperature on germination. Maximum spore germination with continuous 24 h dew occurred over a wider temperature range in *P. r. tritici* (5–25 C) than in *P. striiformis* (5–16 C). The majority of *P. r. tritici* spores were killed at 30 C, and no germination occurred at 35 C. The maximum number of *P. striiformis* urediniospores germinated between 8–12 C, and no germination occurred above 20 C (Fig. 3A).

In *P. r. tritici*, the time to germination decreased with increasing temperature from 5–25 C (data not shown). At 15 C and above, maximum percentage of germination was observed within 4 h, while it took 6 h at 10 C and more than 8 h at 5 C. In *P. striiformis*, initial germination rates were identical at 8 and 12 C and maximum percentage was observed within 6 h. At 5, 16, and 20 C, germination was slower, and it took 8 h for the maximum percentage of urediniospores to germinate.

Effect of temperature on infection. For both rusts, infection efficiency decreased at less favorable temperatures. For *P. r. tritici*, infection efficiency was 23% or higher at temperatures between 5 and 25 C and no infection occurred at 30 C. In contrast, for *P. striiformis*, infection efficiency was always low over a narrow range of temperatures (5–12 C) and no infection occurred above 15 C (Fig. 3B).

The shape of relative infection curves was similar for both rusts at their respective minimal and optimal temperatures, and at the

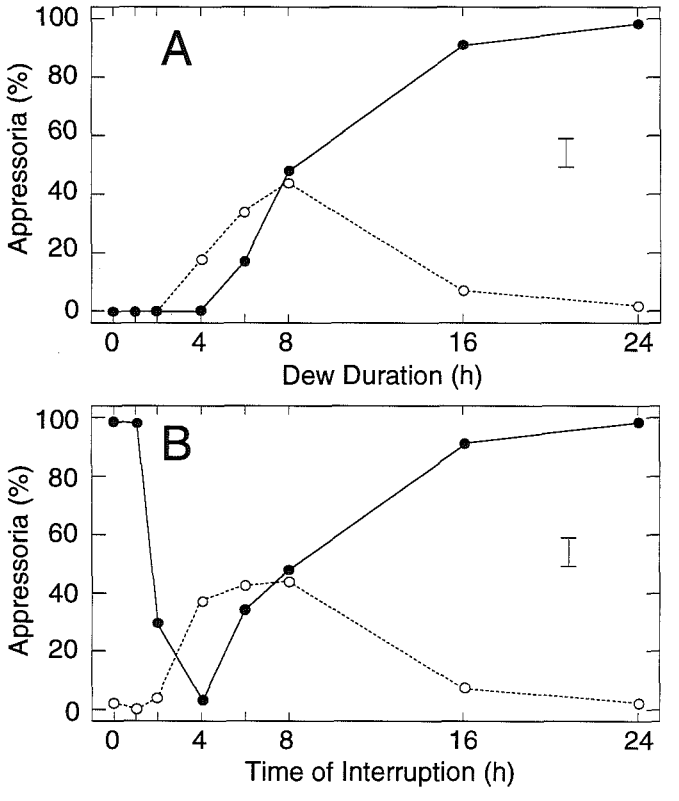

Fig. 2. Mean percentage of *Puccinia recondita* f. sp. *tritici* urediniospores that formed appressoria on wheat leaves at optimal temperature under continuous (**A**) and interrupted (**B**) dew periods. The time scale for **B** was the time at which a 1-h dry period interrupted the 24-h dew period. A continuous 24-h dew period was used as a reference to calculate appressoria percentage. ·····: Appressoria with red staining cytoplasm (before penetration). ——: Appressoria with blue walls (after penetration). Each point was an average of a minimum of 200 spores. The bars represent the standard error of the mean.

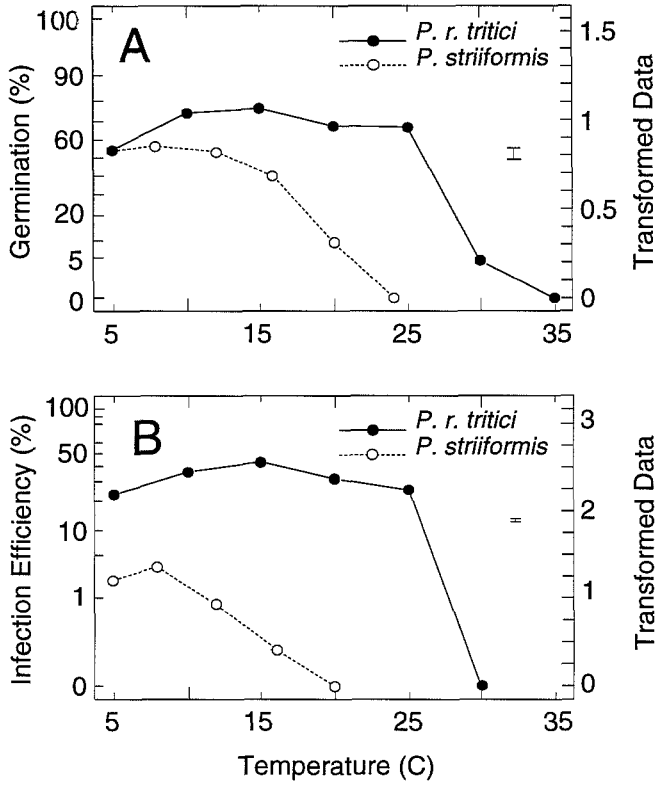

Fig. 3. Mean percentage of germination and infection efficiency of urediniospores at different temperatures from 5–35 C. For *Puccinia recondita* f. sp. *tritici* and *P. striiformis*, percentage of infection was assessed using numbers of pustule and chlorotic areas, respectively. Data transformation was the arcsin square root for germination (**A**) and a fourth-root for infection (**B**). The bars represent the standard error of the mean.

highest temperatures tested, but infection efficiency was greater for *P. r. tritici* (Figs. 4 and 5). Infection was minimal at optimal temperatures when the dry period occurred after about 4 h of dew. This minimum percentage of infection was reached for *P. r. tritici* after the minimal wetness duration for infection equal to 2 h at 25 C, 2–4 h at 20 C, 6 h at 10 C, and 6–8 h at 5 C (Fig. 4A), and for *P. striiformis* after 2–4 h at 12 C, and 6 h at 5 C (Fig. 5A). After the minimal wetness duration, infection increase was faster at temperatures above optimum than at temperatures below optimum.

Model. The parameters n and m were fitted using observed maximal infection values at temperature T (Fig. 6A and B). Estimated values of n and m were equal to 0.75 and 0.9 for *P. r. tritici*, and 5 and 7 for *P. striiformis*, respectively. The corresponding values of p are 3.12 and 3,484. Equation 1 describes the maximal infection capacity of *P. r. tritici* for a wide range of temperatures and the specific requirements of *P. striiformis*.

For both rusts, DP_{min}, b, and τ varied with temperature (Fig. 7A and B). At the highest temperature tested for each pathogen, the estimated values of DP_{min} were similar (about 3.5 h) for *P. r. tritici* and *P. striiformis*. At their respective optimal temperatures, the estimated values of DP_{min} (4.1 h for *P. r. tritici* and 5.3 for *P. striiformis*) were similar to the observed values. The initial infection rate (b) was comparable in range for both rusts between 5 and 12 C. Above 15 C, only *P. r. tritici* was able to develop and values of b were much higher than at lower temperatures. The range of τ was similar for both rusts but the slopes of the curves of τ versus temperature showed that the response time of infection reduction when a dry period occurs decreases faster for *P. striiformis* than for *P. r. tritici*. The effects of dew period on relative infection at different temperatures for *P. r. tritici* (Fig. 4) and for *P. striiformis* (Fig. 5) were well summarized by this analytical model.

DISCUSSION

Comparisons between leaf and stripe rust development in controlled conditions at the monocyclic level showed that leaf rust was 12 times more efficient than stripe rust for infection

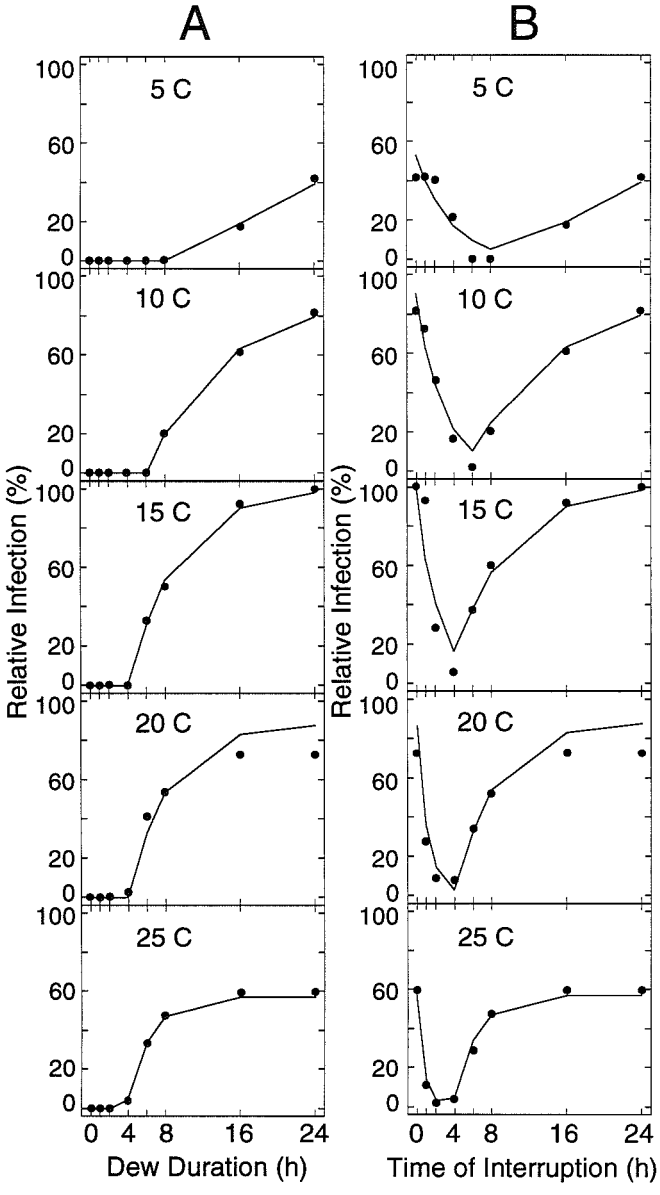

Fig. 4. Effects of temperature and wetness duration on relative infection by *Puccinia recondita* f. sp. *tritici* without (**A**) or with (**B**) a dry period interrupting the dew period. The points are means of four replicates. The solid lines were generated by equations 2 and 3 (see text). Infection data were expressed relative to maximal infection at optimal temperature.

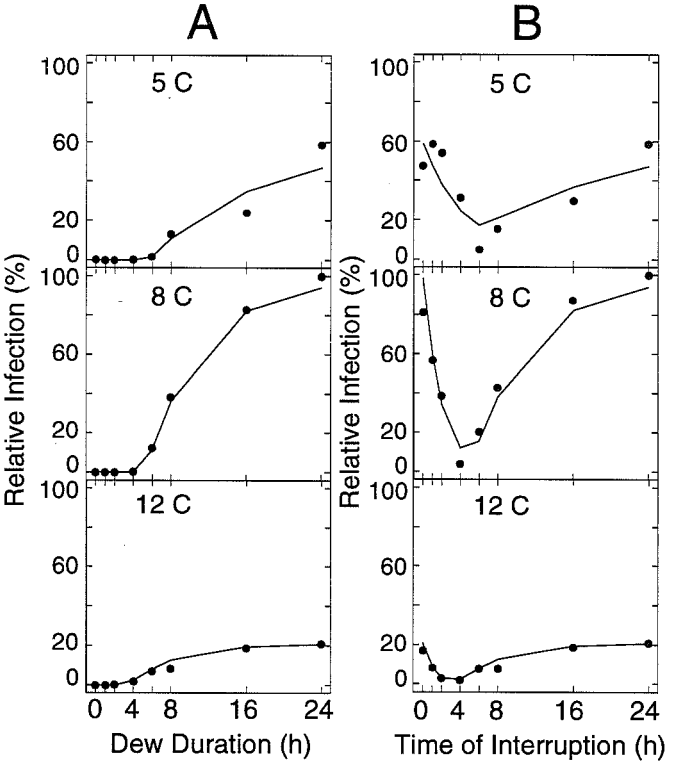

Fig. 5. Effects of temperature and wetness duration on relative infection by *Puccinia striiformis* without (**A**) or with (**B**) a dry period interrupting the dew period. The points are means of four replicates. The solid lines were generated by equations 2 and 3 (see text). Infection data were expressed relative to maximal infection at optimal temperature.

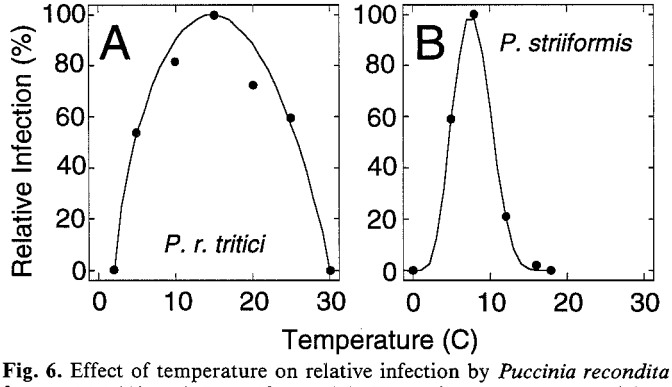

Fig. 6. Effect of temperature on relative infection by *Puccinia recondita* f. sp. *tritici* (**A**) and *P. striiformis* (**B**). The points are averages of four replicates of an experiment. The solid lines were generated by equation 1 (see text). Infection data were expressed relative to maximal infection at optimal temperature.

success in optimal conditions, and also adapted to a wider range of temperatures. The comparison of relative infection of both rusts showed that they were similarly affected by a dry period occurring after urediniospores had initiated germination and before substomatal vesicles had formed. When the initial wet period (DP_1) was shorter than the minimal wetness duration (DP_{min}), the spores that had begun to germinate were killed, and only those that had not begun to germinate were able to infect during the second wet period (DP_2); thus, infection efficiency decreased as a function of time of dry period occurrence. Because germination was slower at low temperatures, the pathogens were less affected by a dry period after a short wetness period at low temperatures than at high temperatures. However, the inoculum loss due to a dry period is much higher for leaf rust than stripe rust, given their very different infection capacities. For initial wet periods longer than the minimal wetness duration, infection was always observed, and increased over time. For both rusts, a similar pattern in the response of relative infection to changing wetness conditions means that maximal infection explains most of the difference between the two rusts in limiting wetness conditions. Therefore, temperature that governs maximal infection appears to be the variable largely responsible for the differential infection response of the two rusts over the range of wetness conditions studied.

At temperatures from 12–20 C, the observed percentage of germination, percentage of appressoria from which leaves were penetrated, and percentage of infection for *P. r. tritici* compared well with previous results (13,17). Results obtained by Dennis (14) on *P. striiformis* were similar to the infection data reported here. Both percentage of germination and percentage of infection were higher for *P. r. tritici* over a wide range of temperatures than for *P. striiformis* over a narrow range. These findings can be correlated with their respective geographic distributions. The adaptation of urediniospore infection to a wide range of temperatures partially explains the worldwide distribution of *P. r. tritici*. In contrast, *P. striiformis* is adapted to relatively cool conditions because of the comparatively narrow range of low temperatures favorable to penetration (6).

When dry periods occurred after 1, 2, or 4 h of wetness, the proportion of appressoria from which leaves were penetrated after DP_2 was the same as the proportion of spores that had initiated germination during DP_2. The dry period had definitively blocked the germinating spores and therefore decreased the proportion of appressoria from which leaves were penetrated. The evolution of the number of appressoria was similar to those of germinating spores with a 2–4 h delay at optimal temperature. When appressoria were observed but no substomatal vesicles had formed, the pathogen was sensitive to wetness interruption. When substomatal

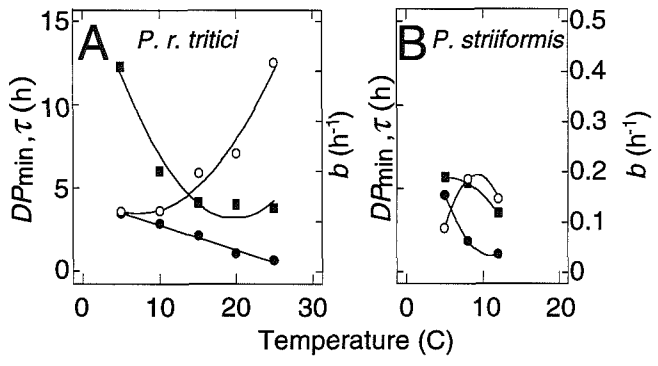

Fig. 7. Variation of parameters DP_{min} (■), τ (●) and b (○) with temperature, obtained by fitting data from **Figs. 4** and **5** using non-linear regression for *Puccinia recondita* f. sp. *tritici* (**A**) and *P. striiformis* (**B**). Experimental data points represent means of four replicates. Regression equations obtained were: $b = 0.1648 - 1.3269e^{-2}T + 9.1429e^{-4}T^2$, $R^2 = 0.97$; $DP_{min} = 18.74 - 1.58T + 4e^{-2}T^2$, $R^2 = 0.96$; $\tau = 4.25 - 0.146T$, $R^2 = 0.98$ for *P. recondita* (**A**) and $b = -0.3163 + 0.111T - 6.0238e^{-3}T^2$; $DP_{min} = 4.5571 + 0.4548T - 4.5238e^{-2}T^2$; $\tau = 13.1 - 2.2T + 0.1T^2$ for *P. striiformis* (**B**). R^2 values for *P. striiformis* were high but had low significance because of the limited number of temperatures.

vesicles were observed, the pathogen became independent of wetness conditions on the leaf surface. Both rusts were unable to survive and infect wheat seedlings if a dry period occurred between urediniospore germination and penetration. A similar decrease in infection has been observed with other diseases such as soybean rust (21), onion Botrytis leaf blight (1), cherry leaf spot (15), and Stylosanthes anthracnose (9). However, germ tube growth was not studied in these pathosystems and the biological mechanism affected by an interrupted wet period was only hypothesized. In contrast, in some pathogens adapted either to semi-arid habitats (such as *Stemphylium botryosum* f. sp. *lycopersici* Rotem, Cohen & Wahl. on tomato) (5), or to tropical humid climates (such as *Mycosphaerella fijiensis* Morelet var. *difformis* Mulder and Stover on banana) (20), germ tubes survive dry intervals between moisture periods.

Under similar cumulative wetness duration but with differences in temperature and the timing of the dry period, different levels of infection were observed. Dry periods that occurred after the minimal wetness duration did not reduce infection levels, which remained equal to those reached with continuous wetness conditions. When the dry period occurred before the minimal wetness duration, the closer the occurrence was to this minimal value, the higher was the infection reduction. A dry period at maximal urediniospore germination resulted in the greatest inoculum destruction. Because *P. r. tritici* spores germinate over a wide range of relatively high temperatures, the magnitude of negative effect caused by wetness interruption is greater compared with *P. striiformis*, which can germinate at lower temperatures and, therefore, at higher relative humidity favorable to germ tube growth and penetration. The narrower range of temperatures favorable to stripe rust was compensated for by the higher quantity of inoculum stored as ungerminated urediniospores, able to complete infection when suitable conditions returned. Therefore *P. striiformis* can be considered less vulnerable than *P. r. tritici* to discontinuous wetness conditions. However, *P. striiformis* spores lost viability if a dry period occurred before the 24-h wet period, whereas *P. r. tritici* spores were not affected by this treatment.

Appressoria play a critical role in the survival of pathogens subjected to adverse environmental conditions (16), and it was hypothesized that *P. r. tritici* appressoria may remain viable after a dry period (17). Our results showed that only appressoria that had completed penetration were able to continue infection after a desiccation period. Transfer of cytoplasm from appressoria into substomatal vesicles forming "ghost" appressoria (13) render the pathogens independent of free water on the plant's surface. The differentiation of appressoria by *P. r. tritici* did not provide a significant advantage for infection against dry weather conditions compared with *P. striiformis*, which does not produce appressoria.

The modeling procedure consisted of three steps (maximal infection versus temperature, infection versus time in continuous wetness conditions, and infection versus time in discontinuous wetness conditions). The step by step procedure allowed us to summarize the different environmental conditions. The Richards's function, including two parameters (initial infection rate, minimal wetness duration), seems to provide a versatile solution for describing infection by several rusts (8). This model was modified to take into account the discontinuous wetness situation and its interest could be further tested with other pathogens. To describe the influence of wetness interruption on percentage of infection, the addition to the Richards's function of a negative exponential term including only one parameter, a time constant, was sufficient. As we can expect from the decreasing time to germination for increasing temperature, the time constant is a decreasing function of temperature. The slope of decrease is higher for *P. striiformis* than for *P. r. tritici* because of the lower range of favorable temperatures for *P. striiformis*.

Quantitative comparison of the number of spores produced and infection efficiency revealed contrasting characteristics in the two pathogens. Relatively low spore production by leaf rust was compensated for by the high infection efficiency of its urediniospores, whereas the very low infection efficiency of stripe rust urediniospores was compensated for by high spore production

(24). A favorable level of one factor (e.g., penetration rate for *P. r. tritici*) compensating for deficiency in another (e.g., survivability of germinated spores after a dry period) has been observed in other plant pathogens (4,5). For example, *S. b. lycopersici* requires long wet periods to penetrate but succeeds in semi-arid climates because its germinating spores survive dry periods (5). Another example is *Phytophthora infestans* (Mont.) de Bary, the sporangia of which have poor tolerance to dry periods but which succeeds in semi-arid climates because of their high germination and penetration rates (5). Comparison for climate adaptation showed another contrasting characteristics in the two pathogens; *P. r. tritici* was tolerant over a wide range of environmental conditions whereas *P. striiformis* compensated for a low infection efficiency with systemic growth within the leaf.

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