Temperature Effects on the Aggressiveness of 
*Puccinia striiformis* f. sp. *tritici*, Stripe Rust of Wheat

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**Abstract:**

Stripe rust is a destructive foliar disease of wheat, caused by the obligate fungal pathogen, *Puccinia striiformis* f. sp. *tritici* (*Pst*). Stripe rust causes considerable yield reduction in wheat regions with cool temperatures and high humidity. It was recently suggested that the pathogen has adapted to warmer conditions it previously tolerated and this is responsible for the world-wide spread of stripe rust to regions where it previously was not observed. The present study has been conducted to investigate the influence of temperature on the aggressiveness of *Pst*. Fourteen isolates, 10 new (post 2000) and 4 old (previous to 2000) were examined for their ability to germinate on water agar plates at 5°C, 10°C, 15°C and 20°C. In addition, the influence of post inoculation temperatures (10°C, 15°C and 20°C) on latent period and area under the disease progress curve (AUDPC) was investigated on the susceptible cultivar AC Bellatrix. Germination rate of stripe rust was favored by cool temperatures (5°C, 10°C and 15°C compared with 20°C). At the warm temperature (20°C), new isolates had significant higher germination rates than old isolates. In addition, new isolates had shorter latent periods and higher AUDPC at 15°C and 20°C compared with old isolates.

**Introduction**

Stripe rust of wheat caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is an important disease of wheat worldwide. Stripe rust reduces grain quality and causes significant yield loss by reducing the area of photosynthesis on infected leaves, and yield losses could be 100% in vulnerable wheat host when infection started at early season (Chen 2005).

In the United States, stripe rust has been a problem in wheat growing areas under cool temperatures such as the Pacific Northwest and California but the pathogen was not able to cause infection in warmer areas, such as east of the Rocky Mountains or on the Great Plains (Milus et al., 2006). However, the occurrence of stripe rust was observed in areas under warm temperature in the late 1950s and early 1960s. Temperature adaptation of the pathogen was extensively studied, suggesting that the new isolates (post 2000) were better adapted to warm temperatures than old isolates (Milus et al., 2006) and thus this was suggested as the reason for wide spread and severe epidemics of stripe rust in south-central USA in 2000-2005.

In Canada, stripe rust of wheat was regularly observed in southern Alberta under irrigated systems compared to other wheat growing areas (Su et al., 2003). This disease was absent from the eastern Prairies (Saskatchewan and Manitoba) before the year 2000. Since 2000, stripe rust has occurred
every year in these two provinces and has caused epidemics in 2005 (McCallum et al., 2006) and 2011 (Kutcher et al., 2012).

Based on the importance of temperature in stripe rust infection and epidemics, the present study was carried out to determine the ability of pathogen isolates (mostly from western Canada) to cause disease under cool (5°C and 10°C) and warm (15°C and 20°C) temperature and to determine if the current isolates were more aggressive than previous isolates at warm temperature. The results of this study should be useful in disease forecasting and management.

**Materials and Methods**

*Stripe rust isolates and plant material*

Fourteen isolates tested in this study were chosen from various geographic locations and years, including 10 new isolates: 3 from Alberta (AB), 3 from Saskatchewan (SK), 2 from Manitoba (MB), 1 from British Columbia (BC), 1 from Arkansas (AR); and 4 old isolates (1 from AB, 1 from BC and 2 from Arkansas).

Latent period and area under the disease progress curve (AUDPC) were examined on the red winter wheat, stripe rust susceptible cultivar AC Bellatrix, a cultivar was released in 1998 by AAFC in Lethbridge, AB.

*Inoculum*

Stripe rust isolates collected from different years were stored at -80°C. These were refreshed under uniform environmental conditions. Urediniospores of each isolate were increased seedlings at the 2 leaf-stage on the susceptible cultivar Avocet. Before the inoculation, isolates were reactivated after retrieval from -80°C by heat shock at 45°C for 5 minutes in a water bath. The seedlings were inoculated with urediniospores suspended in Bayol mineral oil (5 mg of spores + 900 µl Bayol per 180 seedlings) by using an air compressor. Inoculated plants were placed in the humidity chamber at 10°C for 24 h at 100% humidity. After incubation plants were transferred to growth chambers set to 15°C days and 10°C nights with a 16 hour photoperiod. To avoid cross contamination each isolate was covered by plastic and only 5 to 6 isolates were increased at each time. Urediniospores were collected at 14 days after inoculation and thereafter; spores were kept in a desiccator at 4°C for 3 days before each experiment.

*In vitro germination test*

Approximately 100 µl of fresh spores suspended in Bayol (2 mg spores + 1 ml of Bayol) was spread on 1 day old water agar by air compressor. Inoculated plates were placed at 5°C, 10°C, 15°C and 20°C in darkness. At 24 hours post-inoculation, the germination process was stopped by adding a 10x dilution solution of lactophenol blue. The spore germination was examined under the light microscope at x100 magnification. Spores with length of germ tubes equal or longer than the diameter of spores were considered as germinated spores. The experiment was laid out in a randomized complete block design with 3 replicates. Germination rate (percentage) was transformed by arcsin and was subjected to analysis of variance (ANOVA) to determine differences between new and old isolates using the SAS program PROC GLM.
Latent period and AUDPC on AC Bellatrix at different post inoculation temperatures

Seedlings of AC Bellatrix were inoculated as described above but with greater spore concentrations: 10 mg spores suspended in 900 µl of Bayol oil was used for 64 seedlings to avoid disease escape. After an incubation period in the humidity chamber, inoculated seedlings were placed at 10°C, 15°C and 20°C. The experiment was conducted as split plot design with 4 replicates, with 16 seedlings evaluated at one temperature. The ANOVA of new versus old groups of isolates was analyzed following the SAS program PROC GLM.

Results and Discussions

In vitro germination test

In general, the percentage of germinated urediniospores were similar for both new and old isolates. Germination was greatest at 5°C, slightly reduced at 10°C followed by 15°C and dramatically decreased at 20°C (Figure 1). This result was found to be different with result reported by Milus et al. (2006), in which the authors found new isolates had a significantly higher germination percentage at 18°C compared to 12°C. The statistical analysis of new versus old isolates revealed no significant difference in germination rates at 5°C, 10°C and 15°C; however, new isolates had significant higher germination rate at 20°C compared with old isolates (P <0.04). The optimum temperature for urediniospores to germinate in vitro has been studied extensively. McCracken and Burleigh (1962) reported that urediniospores were able to germinate between 2°C and 20°C but the optimum temperature was 2-5°C, similar to our study. In addition, de Vallavieille-Pope et al. (1995) found that germination was greatest between 8 and 12°C and the upper limit was 20°C. In this study we found the optimum temperatures for urediniospore germination was 5, 10 and 15°C.

![Figure 1. Germination rate of new and old Puccinia striiformis f. sp. tritici isolates at 4 different temperatures at 24 hours post-inoculation. New isolates in red and old isolates in blue for all of figures in this paper.](image-url)
Latent period and AUDPC on AC Bellatrix at different post inoculation temperatures

Results of the latent period study (Figure 2) indicated that latent period was shorter at warmer temperatures than at cooler temperatures. Stripe rust sporulation was observed sooner at 20°C than at 15°C and sporulation was slowest at 10°C for all of the isolates evaluated. The effect of temperature on latent period was not different between new and old isolates on AC Bellatrix at 10°C. However, at 15°C and 20°C new isolates had an approximately 0.7 day shorter latent period (P < 0.0001; Figure 2, Table 1). A similar result was also reported by Milus et al. (2006) in which new isolates had a higher latent period at a warm temperature (18°C) compared to old isolates, suggesting that latent period is one of the basic criteria for aggressiveness Pst. However, Loladze et al. (2013) compared latent period of pre-2002 and post-2002 Australian isolates at 17°C and 23°C post inoculation and reported high temperature extended latent period of all pathotypes.

Table 1. Analysis of variance of new versus old Puccinia striiformis f. sp. tritici isolates on latent period and AUDPC.

| Isolate                  | Latent period (days) Estimate | Pr > |t|  | AUDPC Estimate | Pr > |t|  |
|--------------------------|-------------------------------|-----|---|----------------|-----|---|   |
| New vs old at 10°C       | 0.1063                        | 0.562 |    | 1.745          | 0.0892 |    |   |
| New vs old at 15°C       | -0.781                        | <.0001* |    | 4.6363         | <.0001* |    |   |
| New vs old at 20°C       | -0.746                        | <.0001* |    | 4.3838         | <.0001* |    |   |

Figure 2. Latent period of new and old Puccinia striiformis f. sp. tritici isolates at 10, 15 and 20°C at 24 hours post-inoculation.
Results of AUDPC of the 14 isolates tested are presented in Figure 3. Generally new isolates had similar AUDPC at 10°C, 15°C and 20°C. In contrast, most old isolates had higher AUDPC at 10°C and AUDPC was reduced at 15°C and 20°C. New isolates had higher AUDPC at all temperatures (Table 1 and Figure 3); however, analysis of variance of new versus old isolates indicated that only at 15°C and 20°C did new isolates have greater AUDPC (P < 0.0001) compared to old isolates.

The wider virulence spectrum of new isolates compared to old isolates (Brar 2015) and the adaption to warmer temperatures of the new stripe rust population may be important factors that increased the aggressiveness of stripe rust and the observation of this disease in the eastern Prairies.

![Figure 3. AUDPC of new and old Puccinia striiformis f. sp. tritici isolates at 10, 15 and 20°C at 24 hours post-inoculation.](image)

**Conclusion**

New and old *Puccinia striiformis* f. sp. *tritici* isolates had a similar germination rates of urediniospores at 5°C, 10°C and 15°C; however, new isolates had a higher germination rate at 20°C compared with old isolates. On AC Bellatrix there was no significant difference in latent period or AUDPC of new and old isolates at 10°C; however, new isolates had a shorter latent period and a higher AUDPC at 15°C and 20°C. The ability to adapt to a wide range of temperature (from cool to warm) of new isolates may pose a potential risk to wheat production in western Canada.
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References


