NOTAS CIENTÍFICAS

Optimal, lower and upper temperature thresholds for uredospore germination and germ tube growth of *Phakopsora pachyrhizi*

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ABSTRACT

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In laboratory experiments, the effects of temperatures and exposure times on uredospore germination and germ tube growth of *Phakopsora pachyrhizi* were measured. The study was conducted in biological oxygen demand (BOD) growth chambers, and the effects of the temperatures (factor 'a') 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 31, 32, 33, 34, 35 and 36ºC, combined with the exposure times (factor 'b') 3, 6, 12 and 24 hours, were tested in a randomized block design and four replicates. After each exposure time, germination and germ tube length were determined. The experiments were repeated twice and the data underwent regression analysis. The generated equations were used to calculate the lower and upper thermal thresholds and the optimal temperature, which were 6.5ºC, 34.2ºC and 22.3ºC, respectively, for spore germination, and 5.8ºC, 34.2ºC and 22.2ºC, respectively, for germ tube length. The temperature thresholds for spore germination are used to calculate the thermal constant K so that rust occurrence in an area cultivated with soybean can be forecasted based on the accumulated heat.

Keywords: Asian soybean rust, *Glycine max*, degree-day, thermal time.

RESUMO

Reis, E.M.; Carlini, R.C.; Zanatta, M. Temperatura ótima e limiares térmicos inferior e superior para a germinação de uredosporos e crescimento do tubo germinativo de *Phakopsora pachyrhizi.* ***Summa Phytopathologica,*** v.48, n.1, p.25-27, 2022.

Em experimentos conduzidos no laboratório, os efeitos de temperaturas e de tempos de exposição na germinação de uredosporos e no crescimento de tubo germinativo de *Phakopsora pachyrhizi* foram quantificados. O trabalho foi conduzido em câmaras de crescimento do tipo Biological Oxigen Demand (BOD) e avaliados os efeitos das temperaturas (fator 'a') 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 31, 32, 33, 34, 35 e 36ºC e, combinadas com (fator 'b') tempos exposição de 3, 6, 12 e 24 horas no delineamento experimental de blocos ao acaso com quatro repetições. Após cada tempo de exposição, foi determinada a germinação e o comprimento dos tubos germinativos. Os experimentos foram repetidos duas vezes e os dados submetidos a análise de regressão. Com as equações gerados foram calculados os limiares térmicos inferior de 6,5ºC, superior 34,2ºC e a temperatura ótima 22,3ºC para a germinação de esporos e 5,8ºC, 34,2ºC e ótima de 22,2ºC para o crescimento dos tubos germinativos. Os limiares térmicos para a germinação de esporos são utilizados no cálculo da constante térmica K para prever a ocorrência da ferrugem numa área cultivada com soja pelo calor acumulado.

Palavras-chave: ferrugem asiática de soja, *Glycine max,* graus-dia, tempo térmico.

Soybean [*Glycine max* (L.) Merr.] is the major plant species cultivated in Brazil. In the 2000/2001 season, a new disease emerged in South America: Asian soybean rust, caused by *Phakopsora pachyrhizi* Sydow & Sydow. (12). Since then, the most frequently used control strategy has been spraying of fungicides on above-ground organs (9).

Decision making to time the first fungicide spraying for effective rust control has motivated long debates among scientists and farm advisers, but no consensus exists. One of the few scientific options is to perform the first spraying before the disease intensity reaches the economic damage threshold (5).

Research efforts have been intensified to find solutions for a sustainable chemical management of soybean rust. Thus, more precise early-warning systems need to be developed to detect the disease onset and consequently indicate the most appropriate time for starting fungicide spraying to maintain the crop economic and environmental sustainability (14).

Catching air-borne spores with air traps has been an attempt, but there are some difficulties since it is time consuming and *P. pachyrhizi* hyaline uredospores do not ensure reliable identification under a microscope (10). Furthermore, the disease onset not only depends on the presence of inoculum but also on favorable environment after its deposition. To improve early-warning systems, degree-days or the accumulated heat principle can be introduced considering the fungal development since they have been originally used in modeling plants, insect pests (1, 6, 7, 13) and, to a lesser extent, fungi (4, 6). Lower and upper temperature thresholds are basic tools to model the pathogen development based on accumulated degree-days and to calculate the thermal constant (K). Rust should be detected when leaflet incidence is less than 5% in the following season, when K is obtained (8).

Therefore, thermal thresholds are important tools but have not been determined yet to calculate K for *P. pachyrhizi.* The thermal time should be determined because the degree-days strategy is a low-cost, accurate and useful tool to help detect rust onset in a soybean-growing region.

The objectives of the present study were to identify the lower and upper temperature for uredospore germination and germ tube growth of *P. pachyrhizi* so that the data can be used in a system to calculate accumulated degree-days (ºC) and K.

Fungal inoculum production and maintenance. *Phakopsora pachyrhizi* inoculum was obtained from naturally infected soybean leaves collected from the experimental field of University of Passo Fundo, Rio Grande do Sul State, Brazil, in the 2007 growing season. The inoculum was maintained and increased by periodic inoculations of potted-grown soybean plants, cultivar 'Coodetec 214 RR', in a growth chamber, at 22ºC and 14h light and 10h dark photoperiod cycle.

Substrates for spore germination

Soybean leaf extract agar (2.5 g soybean leaf extract, 14g agar and 1000 mL water) was selected from previous experiments and used as substrate for spore germination. After autoclaving for 20 minutes, the medium was poured into 6.0-cm sterile plastic Petri dishes.

Spore germination and germ tube length

One day after the medium preparation, a spore suspension containing 2,000 spores/mL was obtained by adding leaflets to a plastic bottle with distilled water and a Tween 20 drop to 100-mL water, which was agitated for spore removal. Four drops of 0.01mL were pipetted on a microscope slide to determine the spore concentration under the microscope. After adjusting the concentration, 1.0 mL was poured into each Petri plate, evenly spread on the agar surface and taken to biological oxygen demand (BOD) chambers.

Plates were kept in BOD chambers, at the different studied temperatures and exposure times, under continuous dark.

The experiment was conducted in a factorial design. The factor 'a' temperature: 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 31, 32, 33, 34, 35 and 36°C, and the factor 'b' exposure time: 3, 6, 12 and 24 hours for each temperature were studied in a randomized block design with four replicates.

After each exposure time, the plates were removed from the BOD chamber and received four drops of acetone (100%) plus cotton blue each to stop germination and germ tube growth, as well as to stain the structures.

Uredospore germination was evaluated under a light microscope by scanning the plate surface, examining 100 uredospores per replicate. Uredospores with a germ tube longer than their largest

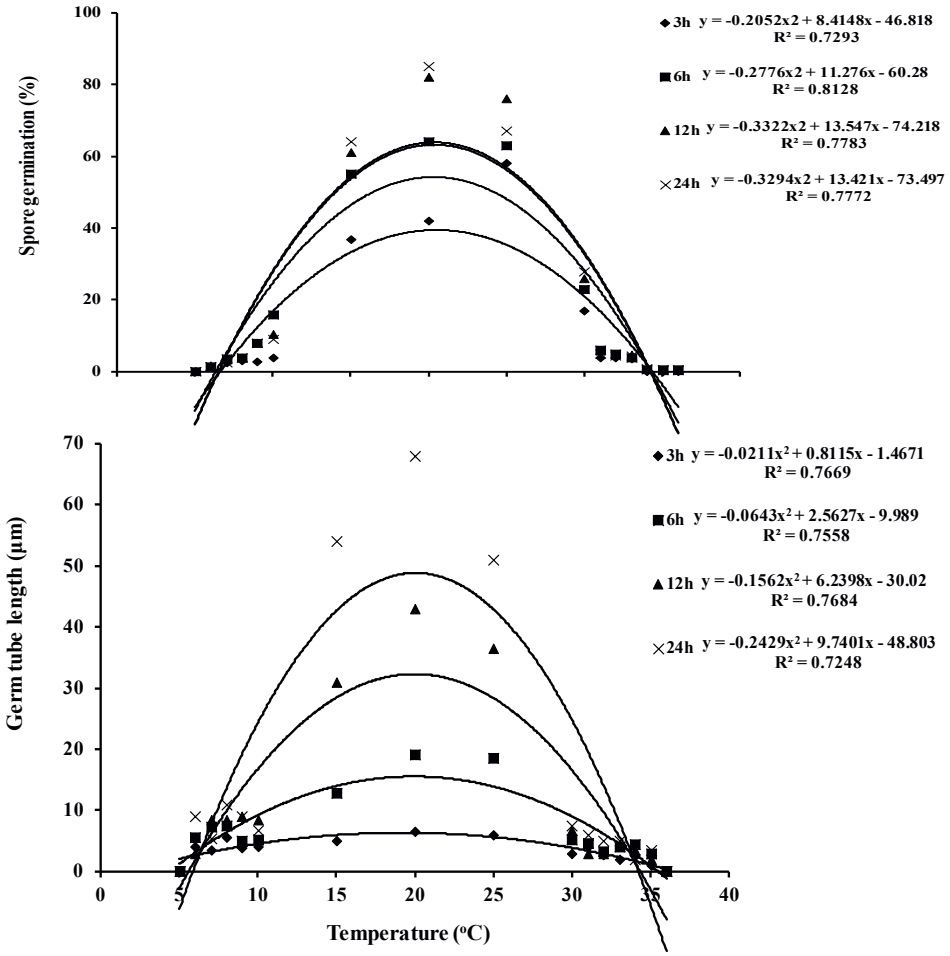

Figure 1. Effects of temperatures (ºC) and exposure times (h) on spore germination and germ tube length (µm) of *Phakopsora pachyrhizi* on soybean leaf extract agar in the dark.

diameter were considered germinated (15) and were measured with a micrometer eyepiece adapted to the microscope (Zeiss West Germany 100/100 ± 5 mm).

The experimental unit consisted of a plastic disposable Petri dish, 6.0cm diameter, and four replicates in a completely randomized block design. Experiments were repeated twice. Data underwent regression analysis and were expressed as germination percentage (%) and pro-mycelium length (µm).

The lower and the upper temperature threshold for spore germination after 24h exposure time was 6.52ºC and 34.25ºC, respectively, while the optimal temperature was 22.3°C (Figure 1). Such results are similar to those reported by Bonde et al. (3), who tested temperatures between 7 and 28°C for uredospore germination and concluded that the optimal temperature for *P. pachyrhizi* spore germination was in the range of 15 to 25°C. Blum et al. (2) found that, for *P. pachyrhizi*, the optimal temperature for uredospore germination was at 21.8 and 22.3°C and maximum germ tube growth was at 21.4 and 22.1°C, which are similar to the present data.

Some researchers, when referring to the optimal temperature, adopt the term "*in a range*"; however, to be more precise, only one optimal temperature value must be identified for each fungal development phase.

The lower temperature threshold, or basal temperature, was 5.86°C, at which the smallest pro-mycelium length was obtained. Below this temperature, there were no germination and germ-tube growth. The optimal temperature was 22.2ºC, at which the pro-mycelium reached the longest length. At the upper temperature threshold, 34.2ºC, pro-mycelium growth stopped (Figure 1).

The basal temperature, or the lower threshold, determined in the current study is used to calculate degree-days and K in the development of soybean rust warning systems based on heat units. Reis et al. (8) calculated the thermal constant K by using the thresholds reported in the present study.

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