NOTAS CIENTÍFICAS

Uredospore density of *Puccinia triticina* races on infection efficiency in wheat

Camila Turra[1,2], Erlei Melo Reis[2], Amarilis Labes Barcellos[1]

[1,3]OR Melhoramento de Sementes Ltda, Passo Fundo, RS. Email, [2]Universidade de Passo Fundo (UPF) - Passo Fundo, RS.
Autor para correspondência: Erlei Melo Reis (erleireis@upf.br)
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ABSTRACT

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Wheat leaf rust caused by the fungus *Puccinia triticina* may lead to damage of up to 62%. This study aimed to test the effect of different uredospore concentrations on the infectious process of four physiological races. The races MFJ-MN, MFT-MT 4002 S, TPT-HT and TDP-HT were inoculated, when the first leaf was expanded in the seedling stage, on cultivars Ônix, Abalone, Morocco and Quartzo, respectively. The tested concentrations were 0.0; 5 x 10^3; 10 x 10^3; 20 x 10^3 and 40 x 10^3 uredospores/mL mineral oil (Soltrol). After inoculation, seedlings were kept in a growth chamber at 20°C ± 2, near 100% humidity, in the dark, for 20 hours. Fifteen days after inoculation, the density of uredia/leaf was evaluated. The concentration of 40 x 10^3 uredospores/mL resulted in a disease intensity that allows safe differentiation between susceptible and resistant cultivars in the seedling stage, without causing leaf senescence due to high uredinium density.

Keywords: Inoculum concentration, leaf rust, physiologic races, cultivar reaction, *Triticum aestivum*.

RESUMO

Turra, C.; Erlei M. Reis, E. M.; Barcellos, A. L. Efeito da densidade de uredosporos de raças de *Puccinia triticina* na eficiência da infecção, em trigo. ***Summa Phytopathologica,*** v.43, n.1, p.46-48, 2017.

A ferrugem da folha do trigo causada pelo fungo *Puccinia triticina*, pode causar danos de até 62%. Esse trabalho objetivou testar o efeito de concentrações de uredosporos no processo infeccioso de quatro raças fisiológicas. As raças MFJ-MN, MFT-MT 4002 S, TPT-HT e TDP-HT foram inoculadas, na primeira folha expandida no estádio de plântula, respectivamente nas cultivares Ônix, Abalone, Morocco e Quartzo. Foram testadas as concentrações de 0,0; 5 x 10^3; 10 x 10^3; 20 x 10^3 e 40 x 10^3 uredosporos/mL de óleo mineral (Soltrol). As plântulas, após a inoculação, foram mantidas em câmara climatizada a 20 ± 2 ºC, umidade próxima a 100% e no escuro por 20 horas. Quinze dias após a inoculação foi avaliada a densidade de urédia/folha. A concentração de 40 x 10^3 uredosporos/mL resultou numa intensidade da doença que permite com segurança a diferenciação de cultivares suscetíveis e resistentes na fase de plântula, sem causar a senescência das folhas pela alta densidade de urédias.

Palavras-chave: Concentração de inóculo, ferrugem da folha, raças fisiológicas, reação de cultivares, *Triticum aestivum*.

Leaf rust caused by *Puccinia triticina* Eriks. is one of the major foliar diseases affecting wheat in Brazil (7). Damage caused by leaf rust to wheat can be obtained based on the normalized function Y = 1,000 - 6.4 I (Y = yield kg/ha and I = leaf incidence) (9).

In susceptible cultivars, control is mainly achieved through the use of fungicides. Lately, cross-sensitivity of *P. triticina* (*Pt*) to demethylation inhibitor fungicides (DMI) has been reported (1, 2, 10). New races identified in the seasons beyond 2008 have been shown to keep the reduction in sensitivity to the DMI fungicide group but are still sensitive to quinone outside inhibitors (QoI) (1, 2, 9).

Thus, in recent years, breeding programs focusing on the search for genetic resistance to leaf rust have been prioritized, but the pathogen virulence is constantly evolving due to the emergence of new races. Approximately one to three new races of the pathogen emerge annually, requiring constant monitoring of the pathogen population (3, 4, 5, 6).

Numerous studies have been developed in Brazil and other countries, assessing the reaction of wheat cultivars to races occurring annually in the field, postulating *Lr* genes (*Leaf rust*) in different genotypes and identifying resistance genes used in breeding programs, besides epidemiological studies monitoring fungal sensitivity to different fungicides, and several others (4). However, in the literature, there is lack of studies related to the effect of uredospore density of different races on infection efficiency and disease intensity.

Determining the suitable inoculum density on the infection efficiency is important to optimize and standardize artificial inoculations in breeding programs.

The aim of this study was to assess the effect of different concentrations of uredospores on the infection efficiency of races of *P. triticina*, the causal agent of wheat leaf rust, in wheat seedling inoculations.

Wheat seeds of Ônix, Abalone, Morocco and Quartzo cultivars were grown in polyethylene pots containing 200 mL soil amended with poultry litter. Approximately 10 seeds of each cultivar were sown per pot and, after emergence, seedlings were thinned to only five plants. Seedlings were kept in a growth chamber at 20ºC ± 2ºC and 12 h photoperiod.

The inoculum of *P. triticina* MFJ-MN, MFT-MT 4002S, TPT-HT, and TDP-HT races was provided by the Wheat Rust Laboratory of OR Seed Improvement Ltd, Passo Fundo, RS.

The first completely expanded leaves were inoculated with different uredospore concentrations: 0.0, 5 x 10^3, 10 x 10^3, 20 x 10^3 and 40 x 10^3 uredospores/mL light industrial oil (Soltrol). A 0.01-mL aliquot of the suspension was poured onto a microscope slide. Three drops were scanned under an optical microscope (100x magnification), and the spores were counted, from the highest concentration (40 x 10^3 uredospores/mL) and according to the yielded dilution 20 x 10^3, 10 x 10^3 and 5 x 10^3 uredospores/mL. For the zero concentration (0.0), only mineral oil was sprayed.

After inoculation, seedlings were kept in a dew chamber at 20 ± 2ºC, in the dark, relative humidity close to 100%, and covered with transparent plastic sheet for 24 hours. After 12-hour incubation, photoperiod was re-established for colonization. The plastic cover was maintained for further 72 hours, keeping the humidity high, which is required for the infectious process. At 4-6 days after inoculation, symptoms/signs could be noticed and at 15 days after inoculation evaluation was performed.

The spore germination potential was determined for each race in plastic Petri dish, 6.0 cm diameter, containing wheat leaf extract-agar (8). A spore suspension in distilled water, without adjuvant, at the concentration of 40 x 10^3 uredospores.mL^{-1}, was poured (1.0 ml) onto the agar medium in each plate. Petri dishes were kept in a BOD (Biological oxygen demand), at 20ºC, in the dark, for 24 hours. After this period, 0.5 ml acetone and blue dye (food coloring) was added to stop germination and stain the germinating uredospores. Three replicates were performed per race and uredospore germination was evaluated under a light microscope (100 x magnification) by scanning the plate, examining 100 urediniospores/replicate. The uredospore with the germ tube longer than its greatest diameter was considered

Table 1. Transformation of the proposed uredospore concentrations into the actual spore germination determined *in vitro*

Race	Germination (%)	Proposed concentration (no./mL water) 5,000	10,000	20,000	40,000
MFJ-MN	95	4,750	9,500	19,000	38,000
TPT-HT	88	4,400	8,800	17,600	35,200
TDP-HT	85	4,250	8,500	17,000	34,000
MFT-MT 4002S	84	4,200	8,400	16,800	33,600

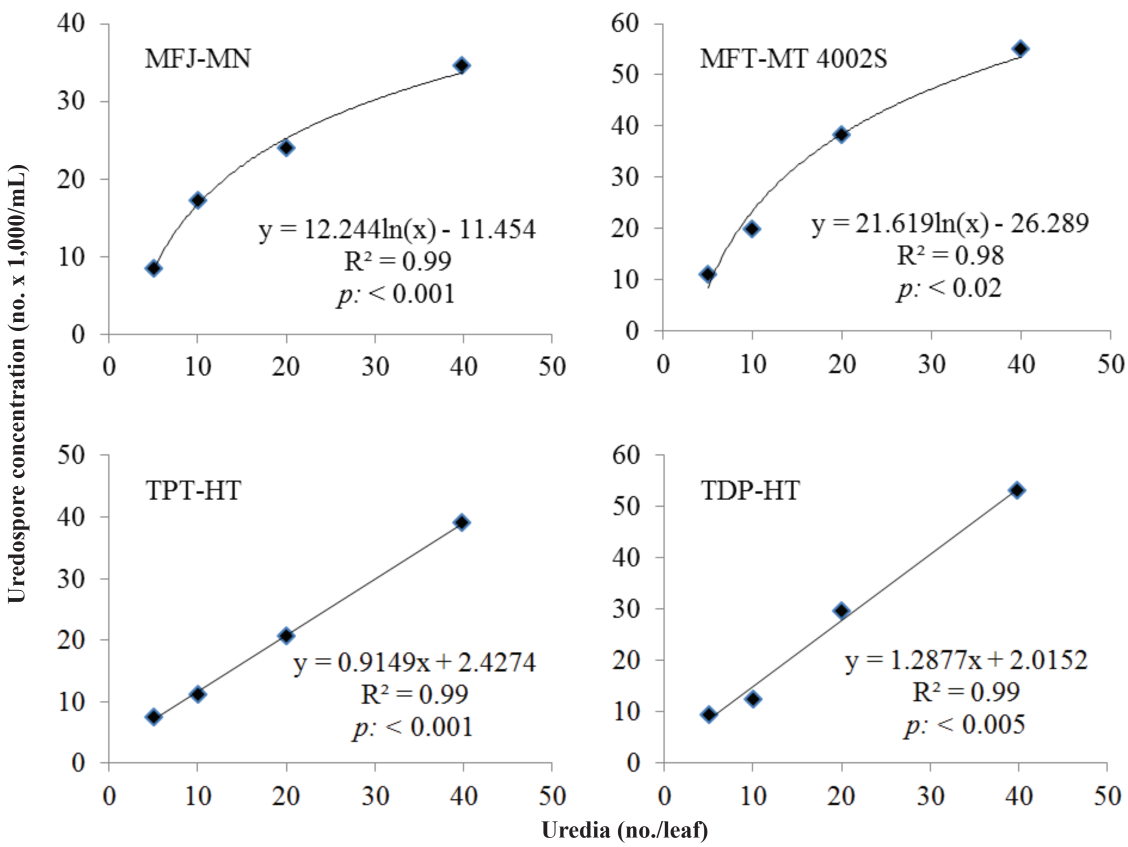

Figure 1. Relatioship between actual uredospore concentration and uredinium number/leaf for *Puccinia triticina* races MFJ-MN, MFT-MT S, TPT-HT and TDP-HT inoculated in Ônix, Abalone, Morocco and Quartzo cultivars, respectively.

germinated. Data were expressed as percentage of germination.

Seedling evaluation for genotypes and for races was performed at 15 days after inoculation by counting the number of uredia/leaf, under a stereomicroscope (50 x magnification). The first leaf area averaged 4,275 cm^2 for the three cultivars.

Experimental design was a randomized complete block design with four replicates. Plots were sown with seeds of the cultivar Marfim, the most widely grown cultivar which is susceptible to *P. triticina*. Data were subjected to analysis of variance and, when significant, subjected to regression analysis. The experiment was repeated twice with the same methodology.

The actual uredospore germination was determined *in vitro* and the theoretically proposed concentrations were adjusted to viable spores. Spore germination was 95% for MFJ-MN race, 84% for MFT-MT 4002S race, 88% for TPT-HT race, and 85% for TDP-HT race. Thus, the specific spore germination potential determined for each race was used to correct the actual viable spore concentration adopted for inoculation (Table 1).

There was an increase in the disease intensity (uredia/leaf) as the inoculum density increased. The lowest concentration, 5 x 10^3 urediniospores/mL, resulted in an average of 9.06 uredia/leaf and higher concentrations led to an increase in the number of uredia/leaf.

Disease intensity progressively increased with the four uredospore concentrations for the four races. The disease intensity was expected to stabilize or decrease with increasing concentrations of urediniospores, especially at concentrations greater than 38 x 10^3 urediniospores/mL, due to competition among the uredospores. Nevertheless, the maximum disease intensity was achieved with inoculation of 38 x 10^3 spores/mL, generating an average of 41.11 uredia/leaf.

Although working with other pathosystems, Carlini (5) and Zanatta & Reis (11) also observed that the highest inoculum concentration resulted in a corresponding increase in the disease intensity, also confirmed in our study.

In both experiments described by the above-mentioned authors, inoculation with 40 x 10^3 spores/mL generated high disease intensity, making difficult to quantify the disease and to determine the premature senescence of soybean leaflets. Moreover, inoculation with low concentrations generates individualized uredia, facilitating their quantification and extending the green leaf area duration. Zanatta & Reis (11) used the concentration of 40 x 10^3 spores/mL to maintain the *P. pachyrhizi* inoculum as the optimal concentration.

In our study, the highest tested concentration, 40 x 10^3 urediniospores/mL, resulted in greater disease severity, compared to the other concentrations (0, 5 x 10^3, 10 x 10^3, 20 x 10^3 urediniospores/mL) evaluated based on the number of uredia/leaf.

MFT-MT 4002S race had the lowest germination (84%) potential among races. However, the results obtained for urediniospore concentrations produced the largest number of uredia/leaf, characteristic of the race that has wide adaptation to temperature variations, high potential and increased sporulation efficiency in the germination of uredospores, which are determining factors in the epidemiological process of biotrophic fungi. These can be some of the factors to explain the prevalence of occurrence of this race in the 2005-2010 wheat growing seasons.

No studies were found on *P. triticina* infection efficiency, regarding inoculum density concentrations, for the tested races. Our results can be used as a reference for working with wheat inoculation to assess germplasm reaction to *Pt*.

Disease intensity was related to the uredospore density for the *Pt* races.

Artificial inoculations with 40 x 10^3 uredospores/mL generated disease intensity which allows rapid and accurate measurements, maintaining the leaf green area of wheat seedlings for a longer period.

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