Morphological changes of the Urediniospore of *Puccinia kuehnii* germ tube in function of temperature

Claudinei Antonio Minchio[1], Lucas Henrique Fantin[1], José Aparecido Rocha[1] and Marcelo Giovanetti Canteri[1,*]

[1]Universidade Estadual de Londrina, Centro de Ciências Agrárias, Departamento de Agronomia, Rod. Celso Garcia Cid, PR 445, Km 380, Cx. Postal 6001, 86051-990, Londrina, PR, Brazil. *Corresponding author, E-mail: canteri@uel.br

ABSTRACT

The aim of this study was to evaluate changes in the morfology and length of the germ tube of *Puccinia kuehnii*, under the influence of temperature and incubation time. The development of the germ tube was asssesed for 24 hours. The concentration of uredospores was 2x105 esporos.ml^{-1} calibrated by a Neubauer chamber and plated in a 0.1 ml aliquot in water – agar (1.5%), placed in BOD and regulated at temperatures of 10, 20 and 30 ˚C. Readings were performed after 1, 3, 6, 12, 18 and 24 hours, with 5 replications. At the end of each period, germination was stopped by adding 0.1 ml of lactophenol. The length and morphology of 10 germinated spores per plate were evaluated with the help of a Motic Images MCCamera – Plus 2.0 ML software. Data were subjected to analysis of variance in a 3x6 factorial design with an interaction in orthogonal polynomials. The model estimated that a temperature of 20 ˚C, at 24 hours of incubation, was required to reach the maximum length. Germ tubes morphological changes were observed at incubation temperatures of 10, 20 and 30 ˚C showing zigzag, rectilinear and branched forms, respectively.

Key words: Epidemiology, *Saccharum officinalis*, germination, sugarcane orange rust.

INTRODUCTION

Sugarcane Orange Rust caused by the *Puccinia kuehnii* fungus (W. Krüger) E.J. Butler is found in several producing regions around the world (Magarey 2000; Huang 2004; Comstock et al., 2008; Infante et al., 2009). The first report on its occurrence in the American Continent was in the state of Florida, in 2007, and later in other producing countries such as Mexico, El Salvador, Nicaragua, Panama, Costa Rica, Guatemala, Venezuela and Cuba (Ovalle 2008; Infante et al., 2009; Flores et al., 2009). In Brazil, the disease was diagnosed in 2009, in the state of São Paulo, in the region of Araraquara (Barbasso et al., 2010; Ministério da Agricultura Pecuária e Abastecimento 2010).

Environment conditions that favor the intensity and severity of the disease are high air relative humidity and high temperatures (Magarey 2000; Ovalle 2008). Fungus dissemination is done through the air and urediniospores transport that can germinate and develop apressoria at temperatures of 5 to 30 ˚C. According to Infante et al. (2009) the temperature interval for germination, both for the brown rust , caused by *Puccinia melanocephala* Syd. & P. Syd, and te orange rust, vary from 10 to 34 ˚C, reaching an optimal temperature between 22 and 26 ˚C. Minchio et al. (2011) reported on a greater germ tube of *Puccinia kuehnii* growth rate when the urediniospores were exposed to 21 ˚C for 14 hours.

It is known that the fungal germ tube may be influenced by growth temperature. Pfister et al. (2004) working with Rhodo*dendron cv*. White Ligths, verified that the length of the germ tube of *Thekopsora minima* (Arthur) P. Syd. & Syd reached maximum values of 122 µm and 118 µm at temperatures of 20 ˚C and 25 ˚C and shorter lengths when submitted to temperatures of 10 ˚C and 30 ˚C with 58 µm and 45 µm, respectively. Arauz and Sutton (1989) working with *Botryosphaeria obtusa* (Schwein.) Shoemaker concluded that the germ tube length increased whenevr incubated at temperatures of 24 to 28 ˚C and decreased as temperatures went over 32 ˚C.

Changes in the length and morphology of the germ tube were studied by Jong et al. (1987) in *Hemileia vastatrix* Berk. & Broome. When submitted to temperatures of 13 to 19 ˚C, the urediniospores showed apressora similar to rounded torpedoes and irregular format wen submitted o higher temperatures. According to Kramer and Eversmeyer (1992) the germination of *Puccinia recondita* Roberge ex Desm. and *Puccinia graminis* Pers. under temperatures above 30 ˚C shows greater germ tube lysis percentage. Such changes in the morphology of the germ tubes may compromise the processes relatd to the host pathogen interactions , affecting the evolution of the disease in the field.

Therefore, the objective of the present study was to evaluate the morphology and length of the germ tube of urediniospores of *Puccnia kuehnii* in function of temperature and incubation time.

MATERIALS AND METHODS

The study was conducted at the Londrina State University Phytopatology Laboratory, Paraná State, Brazil. urediniospore were collected from commercial sugar cane crops affected by the *Puccinia kuehnii* fungus, variety SP 891115, USIBAN Mill, in the county of Andirá-PR. The urediniospore were extracted through the scanning of infected leaves with the help of a paintbrush, transferred to a plastic container covered with aluminum foil. The collected sample was sieved through a 200 mash and transferred to a eppendorf. A urediniospore suspension (2x105

spores.ml^{-1}) was prepared with the help of a Neubauer chamber, in distilled water solution, with two drops of Twenn 20 for each liter of water. An aliquot of 0.1 ml was plated in plates containing water-agar medium (1.5%) (Garcia 2006).

The experimental design was entirely randomized in a 3x6 factorial scheme, with a three level temperature factor (10, 20 and 30 °C), and the incubation time factor (1, 3, 6, 12, 18, 24 hours). Each treatment included 5 replications, with one plate per replication. Plates with fungus isolates were packed in BODs regulated at the following temperatures: 10, 20 and 30 °C and spores germination assessment was carried out in six incubation periods: 1, 3, 6, 12, 18, 24 hours. At the end of each incubation period, germination was interruped through the addition of 0.1 ml of lactophenol on the surface of the water-agar medium.

The study evaluated 10 urediniospores germinated by plate. With the help of an optical microscope, they were increased 10 times. Spores were considered germinted when the germinative s reached greater or similar diameter of the urediniospore. Evaluations and measurements were carried out with the help of the Motic MCCamera-Motic Images Plus 2.0 ML software.

Results were expressed in germ tube length (µm) and submitted to an analysis of variance and F test at the 5 % level of significance. Based on the significant interaction ($p<0.05$) between the factors, treatments were unfolded and the means compared by the Tukey test ($p< 0.05$). Data were also submitted a linear regression analysis of the second order. Morphological analyses of the germ tube The morphological analyses of the germ tube followed a descriptive analysis, taking as standard the format of the germ tubes submitted to 20°C and 12 hours, defined by Minchio et al. (2011) as optimal for the development of the germ tube of urediniospores of *P. kuehnii*.

RESULTS AND DISCUSSION

All assumptions for the normality and homocedasticity analysis of variance were met. The F test of the analysis of variance showed significance ($p<0.05$) for the interaction effect between temperature and incubation time for the germ tube of *P. kuehnii* length. There was urediniospore germination for the three temperatures, according to Infante et al. (2009) and Minchio et al. (2011). Germ tube greater average length was verified for the temperature at 20 °C and the lower development at 30 °C (Table 1). These results are in agreement with those found by Jong et al. (1987) and Blum et al. (2015), who worked with the rust causing fungi *Thekopsora minima* and *Puccinia graminis*, respectively.

Table 1. Germ tube length mean values (µm) of urediniospore of Puccinia kuehnii in function of temperature (°C) and incubation period (h).

Temperature	Period of incubation (hours)					
°C	**1**	**3**	**6**	**12**	**18**	**24**
10	73.3 ±9.4 a[1]	78.0±7.7 a	147.3±14.7 c	389.3±59.6 b	499.7±39.8 b	546.0±86.9 b
20	90.3±16.0 a	222.9±45.2 a	404.8±21.5 a	887.1±37.0 a	972.4±96.0 a	1,106.4±43.0 a
30	82.9±9.3 a	170.3±38.4 b	201.5±42.7 b	143.2±35.3 c	147.0±38.3 c	134.2±27.2c
Mean	82.2±8.5	157.1±73.4	251.2±135.8	473.2±379.0	540.0±413.8	595.58±488.0

[1]Values followed by the same letter vertically do not differ statistically among them by the Tukey test at 5% of probability.

On the other hand, germ tube length of the fungi *Botryosphaeria obtusa* (Arauz and Sutton1989) and *Mycosphaerella fijiensis* var *difformis* (Jacome et al., 1991) showed maximum lengths at temperatures 27.1 °C and 30 °C, respectively, varying for *Spilocaea oleagina*, with maximum length at 15 °C (Obanor et al., 2008).

The regression analysis for the germ tube length of the urediniospore (dependent variable) and incubation temperature (independent variable) showed high correlation for temperature levels at 10 °C (R^2= 0.97) and 20 °C (R^2 = 0.99). The linear regression model developed for the urediniospore submitted to 30 °C showed low correlation (R^2= 0.26) (Table 2).

Regression parameters for the second-order adjustment were tested by the F test of the analysis of variance and showed significance at the 5% level. Results indicate that the model that explains the growth behavior of the germ tube of *P. kuehni* in function of incubation time is of a quadratic order. Equations for temperatures at 10, 20 and 30 were germ tube growth (µm) = -0.5831* (incubation time at 10 °C)^2 + 37.565* (incubation time at 10 °C) – 5.8238; Germ tube growth (µm) = -1.9004 (incubation growth at 20 °C)^2 + 9.309 (incubation time at 20 °C) – 25.406 and

Table 2. Math models obtained after the analysis of the data analysis of the germ tube length (µm) (Y) of P. kuehnii, in function of the incubation time variable (X) at temperatures of 10, 20 and 30oC.

Temperature (°C)	Model for several incubation times (hour)	R^2	PR>Fc
10	Y= -0.5831X2 + 37.565X – 5.8238	0.97	0.0005**
20	Y= -1.9004X2 + 92.309X – 25.406	0.99	0.0000**
30	Y= -0.3652X2 + 9.0093X + 116.89	0.26	0.0022**

germ tube growth (µm) = -0.3652 (incubation time at 30 °C)^2 + 9.0093(incubation time at 30 °C)^2 + 116.89, respectively.

Math models determined germ tube maximum growth points for the urediniospore of *P. kuehni*. Under the average temperature of 10 °C, maximum germ tube growth occurred after 32 hours of incubation. Urediniospore submitted to 20 °C showed maximum size after 24 hours and at 30 °C maximum growth occurred after 12 hours, being 599, 1.095 and 172 µm, respectively, the maximum length for these periods (Figure 1).

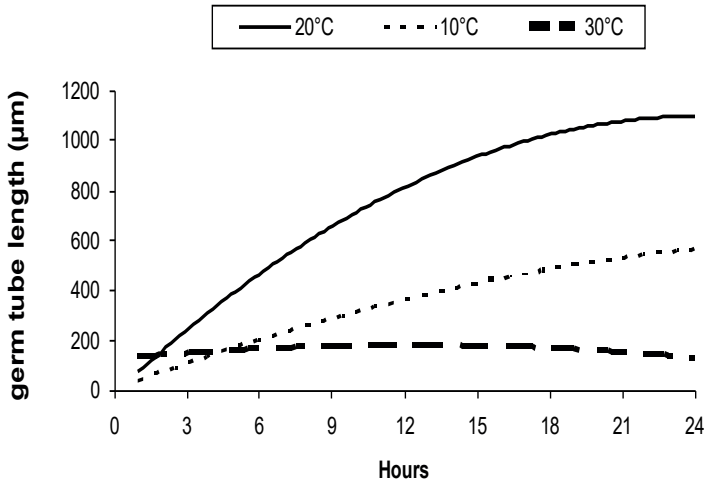

Figure 1. Temperature effect (°C) and incubation period (h) in the germ tube (µm) of urediniospore of *Puccinia kuehnii*.

Urediniospore growth curve showed no significant difference (p>0.05) between temperatures at the first hour of incubation. After three hours of incubation, the urediniospore submitted to a temperature of 10 °C showed shorter germ tube length and longer for those submitted to 20 °C. After six hours of incubation, treated germ tube development submitted to 30 °C was interrupted, remaining stable until the end of the evaluated period (Figure 1).

Germ tube format differentiation was observed for the three temperatures assessed from te third hour of incubation, remaining constant until the period of 24 hours of incubation. Germ tube format varied according to temperature and time of incubation. At 10 °C, the germ tube showed a spiral wave format, not ramified, visible after three hours of incubation. At 20 ° C, the germ tube became unidirectional in all periods of incubation studied, remaining without ramifications. At 30 °C, the germ tube showed an irregular behavior, with short ramifications, visible after 6 hours of incubation (Figure 2).

Germ tube format differentiation was observed when submitted to different temperatures as observed by Jong et al. (1987), who studied the *Hemileia vastatrix* fungus and reported that for high temperatures the format of the germ tube was irregular, being verified only germ tube ramification when submitted to 30 °C. According to Kramer and Eversmeyer (1992), germination of *Puccinia recondita* and *Puccinia graminis* for treatments submitted to temperatures over 30 °C went under greater percentage of lysis of the germ tube. According to Minchio et al. (2011), urediniospore of *P. kuehnii*, when submitted to temperatures over 30°C, shows lysing of germ tube differences resulting in lower disease development rates. Such changes may alter epidemic parameters, affecting directly the disease evolution and severity.

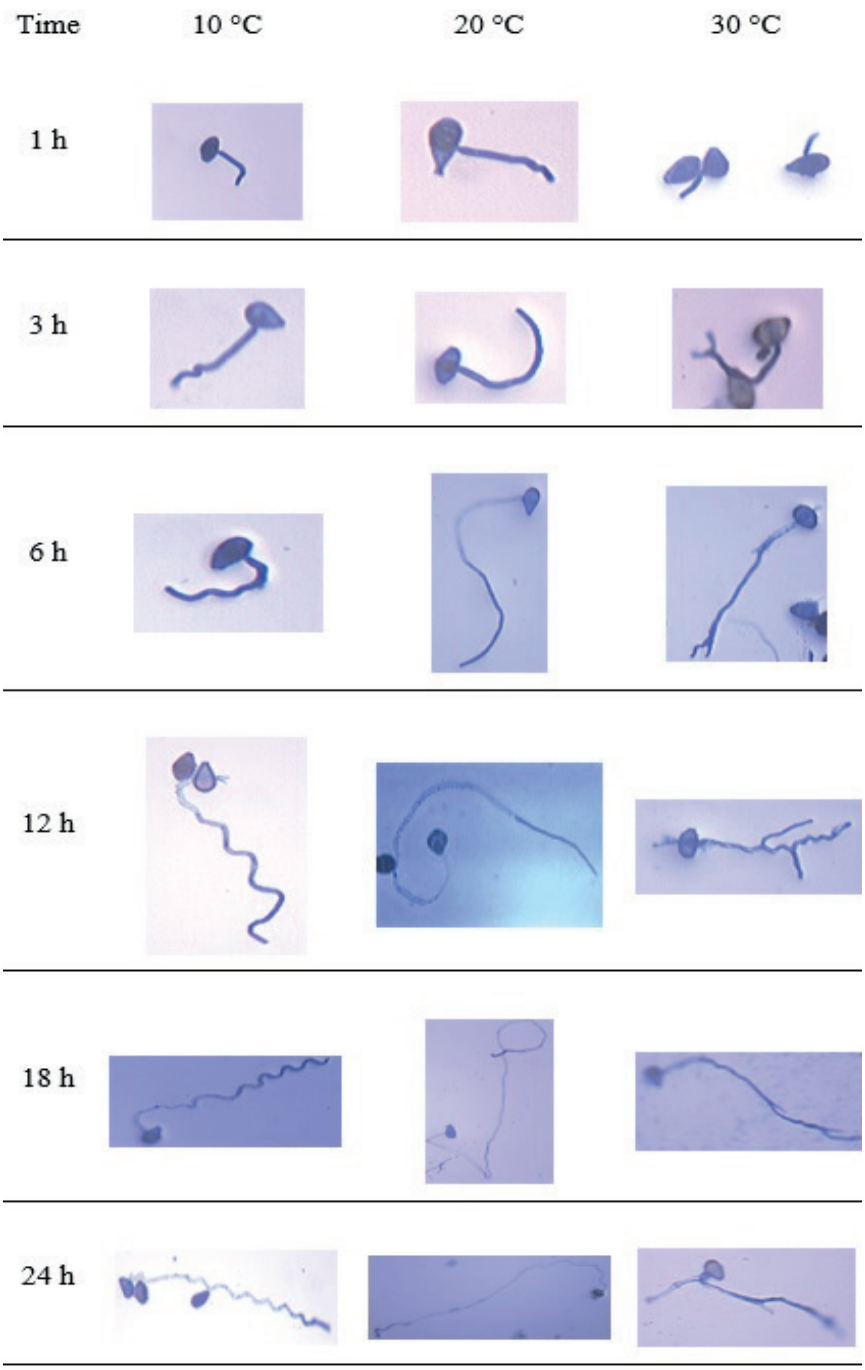

Figure 2. Temperature effect (°C) and incubation period (h) in the morphology of the germ tube of urediniospore of *Puccinia kuehnii* submitted to temperatures of 10, 20 and 30°C (vertical), during incubtion times of 1, 3, 6, 12, 18 and 24 hours (horizontal).

When studying the reaction of different hybrids and the progress of P. kuehnii, Chapola et al. (2016) observed a smaller disease progress curve during the warmer months, with an increase in intensity in the months of November and severity peaks in March and April, periods in which the average temperatures vary from 23 to 25 °C. Martins et al. (2010) noticed an increase in disease symptoms and severity at temperatures between 20 and 25 °C, reducing as the temperature increased. Similarly, the deleterious effect was also observed in the infection rate reduction, lesion expansion rate, sporulation rate and also an increase in the latent period of urediniospore of *P. kuehnii* (Cheng et al., 2014) and spore germination, germ tube growth and development of oidium in tomato plants caused by *Leveillula taurica* (Lév.) G. Arnaudin temperatures over 30°C (Guzman-Plazola et., 2003). This way, the smaller development of the disease may contribute to the control management (Tibolla et al. 2013) reducing yield losses caused by the disease (Zhao et al., 2011).

CONCLUSION

The format of the germ tube of the urediniospore of *Puccinia kuehnii* was modified at temperatures 10, 20 and 30 °C, showing a "zigzag", unidirectional and ramified development, respectively.

REFERENCES

Arauz LF, and Sutton TB (1989) Influence of temperature and moisture on germination of ascospores and conidia of Botryosphaeria obtusa. Phytopathology 79: 667-674.

Barbasso D, Jordão H, Maccheroni W, Boldini J and Sanguino A (2010) First report of *Puccinia kuehnii*, causal agente of orange rust of sugarcane, in Brazil. Plant Disease 94: 1170.

Blum MMC, Reis EM, Franciei TV and Carlini R (2015) In vitro efeito de substratos, temperatura e fotoperíodo na germinação e crescimento do tubo germinativo de uredosporos de *Phakopsora pachyrhizi*. Summa Phytopathologica 41: 101-106.

Cheng P, Ma Z, Wang X, Wang C, Li Y, Wang S and Wang H (2014) Impact of UV-B radiation on aspects of germination and epidemiological components of three major physiological races of *Puccinia striiformis* f. sp. tritici. Crop Protection, 65: 6–14.

Comstock JC, Sood SG, and Glynn NC (2008) First report of *Puccinia kuehnii*, causal aente of orange rust of sugarcane, in the United States and western hemisphere. Plant Disease 92: 175.

Flores RC, Loyo JR, Ojeda RA and Rangel OCA (2009) First report of orange rust of sugarcane caused by *Puccinia kuehnii* in Mexico, El Salvador, and Panama. Plant Disease 93: 1347.

Garcia EO, Casagrande MV, Rago AM and Massola JR NS (2006) Preservação de urediniósporos de *Puccinia melanocephala*, agente causal de ferrugem em cana-de-açúcar. Summa Phytopathologica 33: 1-9.

Guzman-Plazola RA, Davis RM and Marois JJ (2003) Effects of relative humidity and high temperature on spore germination and development of tomato powdery mildew (*Leveillula taurica*). Crop Protection 22: 1157-1168.

Huang S (2004) Progress of sugarcane disease research in China: Recent developments. Sugarcane Agriculture 6: 261-265.

Infante D, Martinez B, Gonzalez E and Gonzalez N (2009) *Puccinia kuehnii* (Kruger) Butler y *Puccinia melanocephala* H. Sydow y P Sydow. en el cultivo de la caña de azúcar. Revista de Protección Vegetal 24: 22-28.

Jacome LH, Schuh W and Stevenson RE (1991) Effect of temperature and relative humidity on germination and germ tube development of *Mycosphaerella fijiensis* var. difformis. Phytopathology 81: 1480-1485.

Jong EJ, Eskes AB, Hoogstraten JGJ and Zadoks JC (1987) Temperature requirements for germination, germ tube growth and appressorium formation of urediospores of *Hemileia vastatrix*. Netherlands Journal of Plant Pathology 93: 61-71.

Kramer CL and Eversmeyer MG (1992) Effect of temperature on germination and germ-tube development of *Puccinia recondita* and *P. graminis* urediniospores. Mycological Research 96: 689-693.

Magarey RC (2000) Orange rust. In: Root P, Bailey RA, Saumtally AS (Eds.) A guide to Sugarcane Diseases. CIRAD and ISSCT, Rotterdam, pp. 121-125.

Martins TD, Raid RN, Burnquist WL, Urashima AS, Bergamin Filho A and Comstock J (2010) Influence of temperature and leaf wetness duration on orange rust of sugarcane. Phytopathology 100: 78.

Minchio CA, Canteri MG and Rocha JA (2011) Germinação de uredósporos de *Puccinia kuehnii* submetidos a diferentes temperaturas e tempos de incubação. Summa Phytopathologica 37: 211-214.

Ministério da Agricultura Pecuária e Abastecimento (2010) A ferrugem alaranjada (*Puccinia kuehnii*). 2010. http: agricultura.gov.br/pls/portal/docs/PAGE/MAPA/PRINCIPAL/DESTAQUES. Acesso em 6 maio 2015.

Obanor FO, Walter M, Jones EE and Jaspers MV (2008) Effects of temperature, relative humidity, leaf wetness and leaf age on Spilocaea oleagina germination on olive leaves. European Journal of Plant Pathology 120: 211-222. Ovalle W(2008) First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane, in Guatemala. Plant Disease 92: 973.

Pfister SE, Halik S and Bergdhal DR (2004) Effect of temperature on *Thekopsora minima* urediniospores and uredinia. Plant Disease 88: 359-362.

Tibolla F, Sumida CH, Peitl DC, Canteri MG and Conte AM (2013) Viabilidade de Urediniósporos de *Puccinia Kuehnii* e sua germinaçao influenciada por extratos aquosos de folhas de cana-de-açucar. Semina Ciências Agrárias 34: 2239–2246.

Zhao D, Glynn NC, Glaz B, Comstock JC and Sood SG (2011) Orange rust effects on leaf photosynthesis and related characters of sugarcane. Plant Disease 95: 640–647.

Received: May 23, 2016.
Accepted: August 18, 2016.
Published: February 08, 2017.