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Effect of different temperature levels and time intervals on germination of uredospores of *Puccinia sorghi*

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In plant disease development, temperature is a critical factor. To determine how specific environmental variables affect corn rust, we determined temperature effects on urediniospore germination. Spore germination was maximum within 24 h of incubation, in the temperature range of 20-30°C. The mean maximum spore germination was recorded at 25°C (66.16% germination) which is statistically on par at 30°C (optimum range). The germination percentage declined gradually at temperatures above and below 25°C. The maximum reduction in germination was recorded at 30°C and 2 h time interval. Thus, the cardinal temperatures for uredospore germination on water agar are 5, 25 and 35°C for 24 h of incubation, respectively. At the maximum and minimum temperature levels, the minimum incubation period extended beyond two hours.

Key words: Effect, temperature, time intervals, *Puccinia sorghi*, maize, common rust.

INTRODUCTION

Maize (*Zea mays* L.) is a graminecious plant domesticated by indigenous people in Mesoamerica in pre-historic times. In Indian agriculture, maize occupies an important place. It is not only utilized as a staple food by the lower income groups, but it is also a crop par excellence for industrial use. Human food accounts for about 35% of the produce, while the balance finds use in industry and poultry feed. Maize is cultivated under diverse environmental conditions. Among the cereals, the maize is the fifth largest in area, third largest in output and yield. Maize is attacked by many diseases in *kharif*, *rabi* and summer seasons which are responsible for severe reduction in yield (Ali and Yan, 2012; Dey et al., 2012). The common rust of maize caused by *Puccinia sorghi* Schw is a severe disease on maize among all foliar diseases. An outbreak of rust was observed in 1966 in breeding nurseries and in fields of corn in the province of Quebec (Brawn, 1966). As in the corn belt in 1950 and 1951, the outbreak in Quebec Ln1966 appeared to be attributable to weather conditions being more favorable than usual. In 1950, rust became epiphytotic, and because of its potential threat to the leading crop in the

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USA, caused considerable alarm there (Semeniuk and Vestal, 1952; Wallin, 1951) and in Canada (McKeen, 1951) that year, again in 1951, and to a lesser extent in 1958 (Ullstrup and Laviolette, 1959). In recent years, common rust caused by *Puccinia sorghi* Schw. was observed in several districts in Northern Karnataka and few other places. According to LeRoux and Dickson (1957) the optimum temperature requirement for spore germination in *P. sorghi* varies with the medium used and the container in which the tests are made. Mederick and Sackston in 1972 noticed that the cardinal temperatures for germination of uredospores of *P. sorghi* on water agar were 2-5, 10-25 and 30-35°C. Losses in grain yields due to common rust disease ranging from 11.2 to 33.6% have been reported from different parts of the country (Gupta, 1981). Common rust of maize caused by *P. sorghi* whch is far more important than *Puccinia polyispora* Underw. Considering the importance of the disease which causes more economic losses to the crop, the present investigation was undertaken.

MATERIALS AND METHODS

An experiment was conducted at College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India during *Kharif*, 2010-11 to know the effect of different temperature levels and time intervals on germination of uredospores of *P. sorghi.*

Uredospore germination on water agar

A thin layer of 1.5% agar was applied on a clean microscopic slide. The mature uredospores of *P. sorghi* were collected by placing a drop of water on a pustule and then lifting the floating ones with a needle. A suspension of these uredospores was atomized on the agar film on the glass slides, which were kept in an inverted position in closed Petri dishes lined with moist cotton. A piece of moist cotton was placed on the upper surface of the slide to promote moisture condensation on the lower side. The slides were then incubated at various temperatures for different intervals of time. Care was taken to see that not more than 10-15 min elapsed between the time the spore suspension was made and the slides were brought into the incubator, to minimize the error that may be induced by increased germination. There were three replications for each treatment. Spores with the germ tubes longer than their diameter were considered as germinated, and the percent germination was based on a count of 800 spores for each treatment. The experiment was repeated twice. Percent uredospore germination was calculated by following the formula.

$$\text{Per cent spore germination (PG)} = \frac{A \times 100}{B}$$

Where, A = No. of uredospores germinated; B = No. of uredospores observed.

RESULTS AND DISCUSSION

The results (Table 1) of studies on the effect of temperature on germination of uredospores of *P. sorghi* on water agar indicated that germination varies both in percentage and rate depending on temperature and time interval. Spore germination was maximum within 24 h of incubation, in the temperature range of 20-30°C. The mean maximum spore germination was recorded at 25°C (66.16% germination) which is statistically on par at 30°C (optimum range). No germination occurred below 5°C, at which temperatures there were relatively low percentages of germination only after 8 and 4 h, respectively (Figure 1). Thus, the cardinal temperatures for uredospore germination on water agar are 5, 25 and 35°C, and the minimum incubation period extended beyond two hours only at the maximum and the minimum temperature levels. However, slight increase in germination percentage recorded at all temperature levels as the incubation period extended. Studies indicated that the optimum temperature for uredospore germination is 20-25°C (Plate 1).

The minimum temperature for germination under the conditions used was between 0 and 5°C, the optimum between 10 and 25°C, and the maximum between 30 and 35°C. Essentially, similar values were reported by Weber (1922), who found the minimum for germination to be 4°C, optimum 17°C, and maximum 32°C. Weber (1922) found the minimum temperature for infection of corn by rust to be below 8°C, the optimum about 18°C, and the maximum below 32°C. In *P. graminis*, germination counts made at 2 h gave the best correlation with infection (Sharp et al., 1958). If a similar correlation holds for *P. sorghi*, our results at 2 h may be at least indicative of the effect of temperature on infection.

Our results indicate that, although there is a wide temperature optimum for germination, the speed of germination shows a marked response to relatively small temperature differences. Similar results have been reported for other rusts (Chester, 1964; Shands and Schein, 1962; Sharp et al., 1958). We made our first counts of germination on spores exposed for 2 h. We might have obtained slightly different values if our counts had been made after 3 h, an interval used in various reports on *P. graminis* fsp. *tritici*, but which we used in only one experiment. Germ tubes had emerged from rehydrated urediospores of *P. graminis* fsp*. tritici* after 40-60 min under optimum conditions (Maheshwari and Sussman, 1970). As the usual criterion for germination is the production of a germ tube at least equal in length to the width of the urediospore, we did not think it useful to determine percentage germination after exposures shorter than 2 h. The very low germination rate of urediospores at low temperatures explains the slow rust development observed on plants in inoculated field plots at College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka in the season of 2010, when dew periods only 2 h long coincided with temperatures of 10-13°C (Mederick and Sackston, 1972).

Table 1. Effect of different temperature levels and time intervals on germination of uredospores of *P. sorghi*.

Temperature (°C)	Uredospore germination (%)						
	2 h	**4 h**	**8 h**	**16 h**	**20 h**	**24 h**	**Mean**
0	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00* (00.00)**
5	17.33 (8.88)	20.44 (12.22)	23.94 (16.48)	28.31 (22.51)	32.19 (28.40)	35.93 (34.47)	26.36 (20.49)
10	17.56 (9.12)	30.38 (25.60)	35.85 (34.33)	37.93 (37.82)	41.77 (44.40)	47.15 (53.77)	35.11 (34.17)
15	21.35 (13.27)	32.19 (28.40)	37.18 (36.54)	38.48 (38.74)	47.65 (54.64)	56.09 (68.91)	38.82 (40.08)
20	35.67 (34.02)	47.04 (53.59)	58.84 (73.26)	62.39 (78.55)	66.47 (84.09)	74.38 (92.76)	57.47 (69.38)
25	52.79 (63.47)	60.73 (76.13)	67.47 (85.34)	70.08 (88.42)	73.25 (91.53)	80.39 (97.22)	67.45 (83.69)
30	35.10 (33.09)	46.45 (52.57)	57.94 (71.85)	61.82 (77.73)	64.29 (81.21)	71.95 (90.42)	56.26 (67.81)
35	32.19 (28.40)	42.09 (44.96)	46.73 (53.05)	52.79 (63.47)	56.12 (68.96)	60.93 (76.41)	48.48 (55.88)
40	21.93 (13.96)	27.69 (21.61)	35.46 (33.68)	40.64 (42.45)	46.90 (53.35)	52.44 (62.88)	37.51 (37.99)
45	00.00 (00.00)	14.97 (6.68)	20.44 (12.22)	23.73 (16.21)	26.40 (19.79)	27.04 (20.68)	18.76 (12.60)
50	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
Mean	21.27 (18.57)	33.25 (29.27)	34.89 (37.89)	37.83 (42.35)	41.37 (47.85)	46.03 (54.32)	35.11 (38.37)
Comparing the means of		SEm±		CD at 1%			
Treatment (A)		0.15		0.54			
Concentration (B)		0.11		0.42			
A X B		0.36		1.32			
CV%				1.53			

* Arcsine transformed values; **Data in parenthesis are original values.

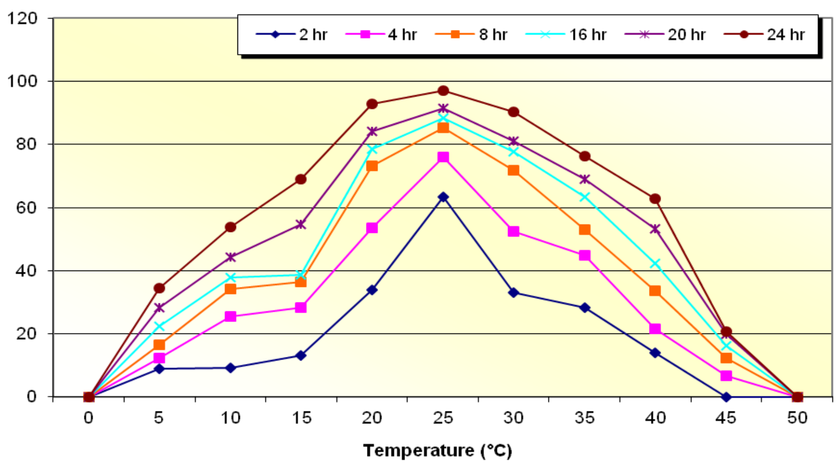

Figure 1. *In vitro* evaluation of different temperature and time intervals on germination of uredospores of *P. sorghi.*

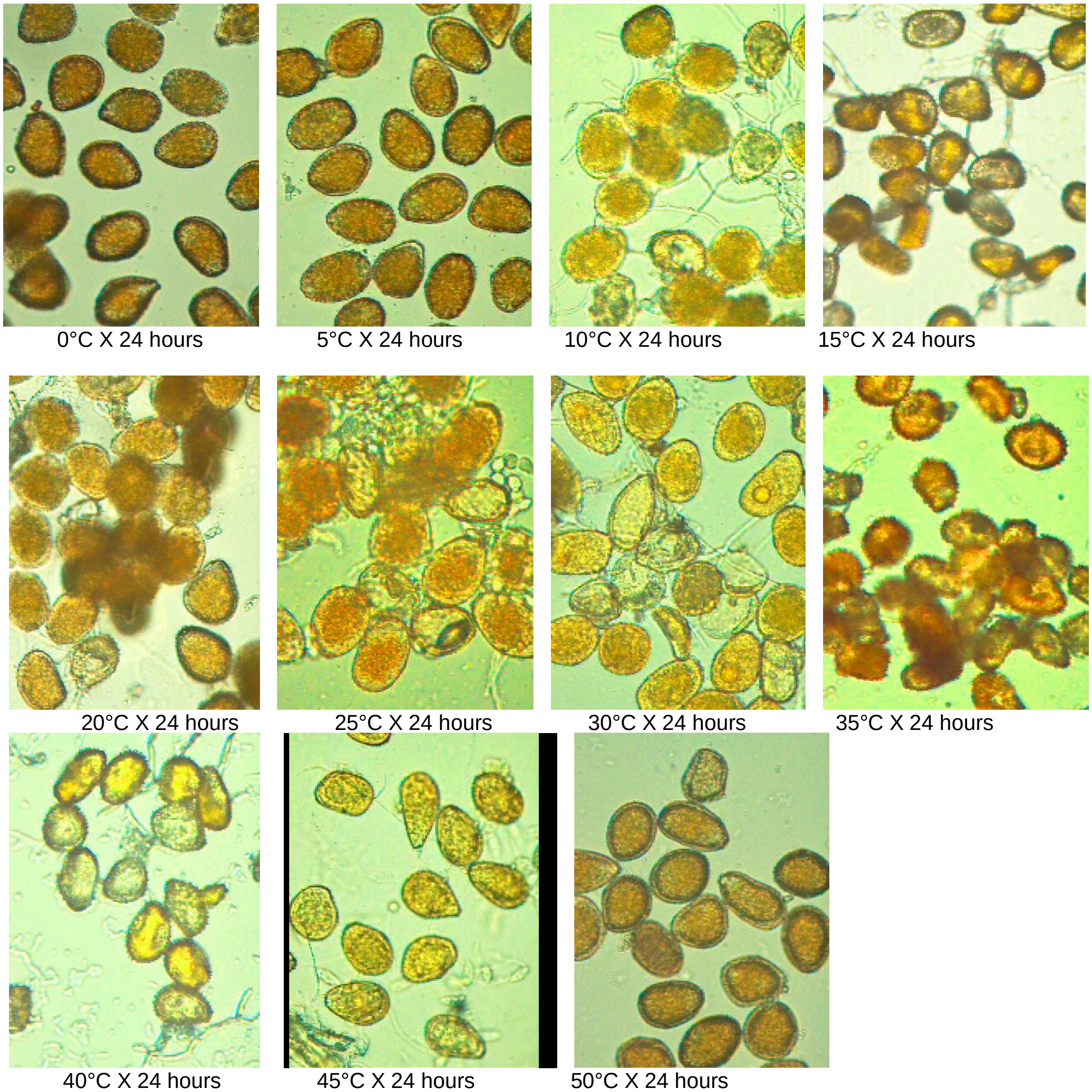

Plate 1. Effect of different temperature and time intervals on germination of uredospores of *P. sorghi.*

The effect of temperature on uredospore germination revealed that, 25°C is the optimum temperature for maximum spore germination (83.70%). The uredospore germination was also influenced by the time interval. At 2-4 h, lower uredospore germination was recorded, intervals between 8-16 h found moderate and 20-24 h recorded maximum germination irrespective of temperature levels. Thus indicates that, a minimum time interval of 20-24 h is required for better uredospore germination and highest being at 25°C. Uredospore germination drastically reduced at 40°C and at above temperature level, indicates unfavorable.

The cardinal temperatures for uredospore germination in the rust fungus vary widely among different species. According to Johnson (1912) the optimum temperature requirement for uredospore germination in the cereal rusts ranges from 7 to 25°C. The cardinal temperature range of 5 to 45°C established for *Puccinia sorghi* is wider than that reported by Weber (1922). On the other hand, it is narrower than that reported by LeRoux and Dickson (1957). The lack of agreement in these reports on cardinal temperatures for uredospore germination in *P. sorghi* may be due to difference in the methods used, conditions under which the spores are produced and stored (Smith, 1926), difference in the medium and it's container and the time lapse between the preparation of spore suspension and the start of the treatment (LeRoux and Dickson, 1957).

Temperature, humidity, solar irradiation, and other factors can affect spore survival in the atmosphere (Bernd et al., 1987). The first step in infection is urediniospore germination, which is influenced by environmental factors that include temperature (Bonde et al., 2007; Joseph and Hering, 1997; Tapsoba and Wilson, 1997) and duration of leaf surface wetness (Joseph and Hering, 1997). Most spores are extremely sensitive to even a few hours of intense sunlight (Maddison and Manners, 1972). Germination of spores of the common rust fungus may occur over a wide range of relatively cool temperatures (approximately 54 to 82°F) and requires nearly 100% humidity for several hours (Headrick and Pataky, 1987). Although, uredospores in groups were observed to germinate readily, the numbers of spores within groups were not determined. Germ tube elongation of all fungi was negatively affected by increased length of exposure to fluorescent light (Buck et al., 2010). However, the germ tubes from these groups were numerous. Germ tube lengths varied considerably at each temperature and time intervals.

This study will help us to describe and better understand the kinetics of spore germination and how this is influenced by different environmental factors such as temperature and other factors that influence spore germination. Even though spore germination and sporeling growth can occur under a broad set of conditions, optimal responses occur within narrow ranges. Knowledge of the abiotic influence on spore development should improve survival of the early microscopic stages and may help in cultivation trials based on spore shedding methods.

Conflict of interests

The authors did not declare any conflict of interest.

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