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Assessment of Urediniospore Germination of *Puccinia striiformis* at Various Temperatures on Agar and Detached Leaves of Wheat

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ABSTRACT

The percentage of urediniospore germination of two isolates of yellow rust *{Puccinia striiformis f. sp. tritici)* namely, WYR 69/10 |Race 104E 137(1)] and isolate WYR 79/4 |Race 41E 139 (4)], was studied at various temperatues between 5-20°C on agar and the adaxial surface of detached leaves on a layer of benzimidazole agar. Four replicates for five temperature treatments were arranged as a preliminary investigation to find the most appropriate temperature for the subsequent comparison of spore germination on the host material under investigation. Mean percentage values were analysed separately and the levels of urediniospore germination at different temperatures were compared using analysis of variance and Student-Newman-Keuls techniques. Spore germination of both isolates was high over the temperature range 5-10°C, but dropped significantly at 15-20°C . At the cooler temperatures 5° , 7.5° and 10°C , isolate WYR 69/10 consistently germinated at a higher level than isolate WYR 79/4 , whereas at 20°C, the latter isolate gave the highest figures. This observation indicates that both isolates germinated most effectively at 10°C on agar and detached leaves.

Keywords: Urediniospore germination ; *Puccinia striiformis* ; Wheat.

INTRODUCTION

Yellow rust of cereals caused by *Puccinia striiformis* westren / *sp. tritici* Erikes is potentialy a damaging disease in all cool temperature climates [16], although its range is now extending to warmer and more arid region such as Yugoslavia and Iran |3j.

The annual yield losses due to wheat yellow rust have been estimated up to 8-75% of total production [4]. In Iran, epidemies of cereal rusts occur every 3 or 4 years and Khazra & Bamdadian [10] estimated that overall losses in such years may be as high as 30 to 40 percent. These authors also reported that under favourable conditions in northen Iran, yellow rusts may cause a total loss of yield for susceptible cultivars. In 1993 in some parts of Iran yield losses due to yellow rust was estimated about 1.5 million tones [22].

Yellow rust is considerably more sensitive to environmental conditions than other cereal rusts [23]. Urediniospore germination is dependent on genetic constitution and on environmental condition **[14].** In the field, climatic condition during spore formation and spreading have a major effect on spore germination [19,8]. Consequently, environmental condition during culture maintenance and inoculation and infection of expermental

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materials must be rigidly standardised if reproducible results are to be achieved [18].

The optimum temperature for urediniospore germination of cereal rusts depends on the spicies, race and isolate studied. Urediniospores of *P.* ***graminis*** germinate within the optimal temperature range of 15-24°C [20] . Those of *P. coronata* germinate between 10-30°C [11] and 10- 20°C.

Ling [12] indicited that urediniospores were viable for two days at 36°C but could withstand a temperature of 45°C for only five minutes. Coplan and Manners [6| suggested that the temperature occurring during urediniospore production had an effect on their viability. Spores produced under low temperature regimes, germinating significantly better than those produced under higher temperatures. Urediniospores of P. *striiformis* require very high humidity, or the presence of free water, for good germination [19]. Germination is also generally higher on the adaxial leaf surface than on the abaxial surface [17].

Hassebrauk and Schroder [8] studied yellow rust urediniospore germination on an agar or gelatin-based artificial media and reported a relationship between agar quality and spore germination. Thus confirming the earlier findings of Manners **[13].** They observed that germination was very sensitive to the pH of the medium, noting that a minimum pH of 3.0, maximum of 11.2 and optimal germination pH respectivly was within the range 5-7.

MATERIALS AND METHODS

Two isolates of *Puccinia striiformis* Wes-tend / *sp. tritici* Eriks. Race 104E 137 [1] [isolate WYR 69/10] and Race 41E 139 [4] [Isolate WYR 79/4] were kindly supplied by Dr. R. Johnson of the plant Breeding Institute, Cambridge, England.

Isolates WYR 69/10 and WYR 79/4 of *puccinia striiformis* were maintained on seedlings of the wheat cvs Maris Beacon and Maris Templar, respectively. Approximately 20-30 seeds of both susceptible cultivars were sown in 12.5 cm. diameter pots containing John lnnes No. 1 or No. 2 potting compost and covered with transparent propagator tops (Stewart Plastics pic, Cat. No. 312).

The pots were then placed in a growth cabinet for 8-10 days. When the first leaf was fully expanded, the seedlings were transferred to the bench of a laminar flow cabinet for inoculation. Fresh urediniospores were collected, in a clean dry boiling tube, from a stock pathogen culture. These urediniospores were transferred to the surface of the first seedling leaves by means of a paint brush, previously sterilised with acetone or ethanol and dried in the sterile laminar airstrcam. The inoculated seedlings were then misted with distilled water using a Shandon Chromatography Spray Unit and the propagator lop replaced immediately.

The ventilation holes in the propagator were covered with masking tape set at *U)°C* for 24h. The pots of seedlings were then returned to the growth cabinet and the tape removed from the propagator ventilation holes. Four or five days later, the propagator tops were replaced by open-ended Perspex tubes which permitted the relative humidity to drop to a level nearer to that of the growth cabinet.

The growth cabinets used incorporated an air-circulation fan refrigeration unit and external light source (which was separated from the cabinet by a layer of Perpex) and they operated at a relative humidity within the range 50-75%. The refrigeration unit was set to maintain a temperature of 15±1°C . Light was supplied by a bank of 10 or 12x80W fluorescent tubes for 14 h daily. The tubes were a colour mixture of white and warm white producing a light intensity of approximately 8000 lux at seedling height.

Wherever possible, inoculum for experiments was collected from a freely sporing stock culture which had been inoculated approx-

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imately three weeks previously. In order to acquire fresh spores, cultures were vigorously shaken 48-72 h prior to collection. Spores were collected in dry, clean, boiling tubes and the appropriate amount for inoculation weighed out using a Mctller HK 60 microbalance. Such spores were always used within 2 h of their collection and were stored at approximately 4°C over thes period.

Working with agar, agar coated slides were prepared, under sterile conditions, by pipetting approximately 4 ml of 0.5% Oxoid agar No. 3 onto each slide. Four replicate slides for each germination temperature treatment, were arranged and inoculated on the inoculation table of a small settling tower adapted by Poyntz [15]. From a design by Eyal *et al.* [5], approximately 10 mg of fresh spores of isolates WYR 69/10 and WYR 79/4 were used as inoculum.

Following inoculation, the four replicate slides were allocated to each of the temperature treatments and placed in a Perspex box (Stewart Plastics pic, Code X 64). The bottom of the box was lined with a moist paper towel so that a relative humnidity of approximately 100% was rapidly achieved. The boxes were then incubated in the dark at 5° , 7.5° , 10° , 15° and 20°C . After 24 h of incubation the slides were removed from the boxes and fixed by placing them above a 40% solution of formaldehyde. Slides fixed in this manner, could be stored, in a refrigerator for several days without detectable deterioration.

For detached leaf work a similar experimental design was used. Segments of first seedling leaves of wheat cvs Maris beacon and Maris templar were used for isolated WYR 69/10 and WYR 79/4 , respectivly. These segments were placed adaxial surface up, in perspex boxes cotaining a layer of benzimidazole agar [0.04 g benzimidazole (BDH Chemicals Ltd.) per 200 ml of 0.5%] OXIDE agar No. 3 giving a benzimidazole concentration of 200 ppm. These leaf segments were then inoculated, and fixed in the manner described for agar slides. During this study, the agar slides and the detached leaves were inoculated simultaneously thus ensuring that similar spores and spore densities were used.

For the agar slides, germination was assessed by direct microscopic observation. The assessment of germination on detached leaves segments was performed as follows:

The segments were placed adaxial surface up on a card. Then both ends of each segment were secured to the card using masking tape so that a length of approximately 4 cm remained unobstructed. The segments were then coated with nitrate cellulose dope (Humbrol Ltd.) by spraying in a fume cupboard, with a 1:1 mixture of dope and cellulose thinners. The dope was allowed to dry naturally and then carefully peeled off using fine forceps. The dope strips were immediately mounted on a microscope slide in laciophenol trypan blue solution and covered with a covcrslip. These preparations could be stored in a refrigerator for several weeks without noticeable deterioration.

For both techniques, spore germination was assessed using a Swift S.R.L. microscope (Swift Instruments Inc.) at a magnification of x 100. For eash replicate, three samples of 100 spors were counted. All those spores having germ tubes at least as long as their width as germinated. The percentage for each sample was then transformed using the arcsin transformation and the mean value for each replicate and treatment then calculated. The data relating to observations on agar slides and on detached leaves were analysed separately and the levels of urediniospre germination at different temperature were compared using analysis of variance and the Student-Newinan-Keuls techniques [21].

RESULTS AND DISCUSSION

This experiment was performed as a

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preliminary investigation to find the most appropriate temperature. For the subsequent comparison of spor germination on the host material under investigation. Mean percent values for spore germination isolates WYR 69/10 and WYR 79/4 are presented in Table 1 and Table 2 for agar and detached leaves respectively, and compared in Figure 1.

The data presented in Table 1 showed that on agar, 10°C proved most suitable for urediniospore germination for both nearly 97% (arcsin 79.2) germination and isolate WYR 79/4 achiving 85% [arcsin 67.3 ; (Plate 1)]. Spore germination was high over the temperature range 5-10°C (93-97% for isolate WYR 69/10 , 80-85% for isolate WYR 79/4) but dropped significantly at 15°C (48% for WYR 69/10 ; 45% for WYR 79/4) and 20°C [41% for isolate WYR 79/4 ; 37% for isolate WYR 69/10 (Plate 2)].

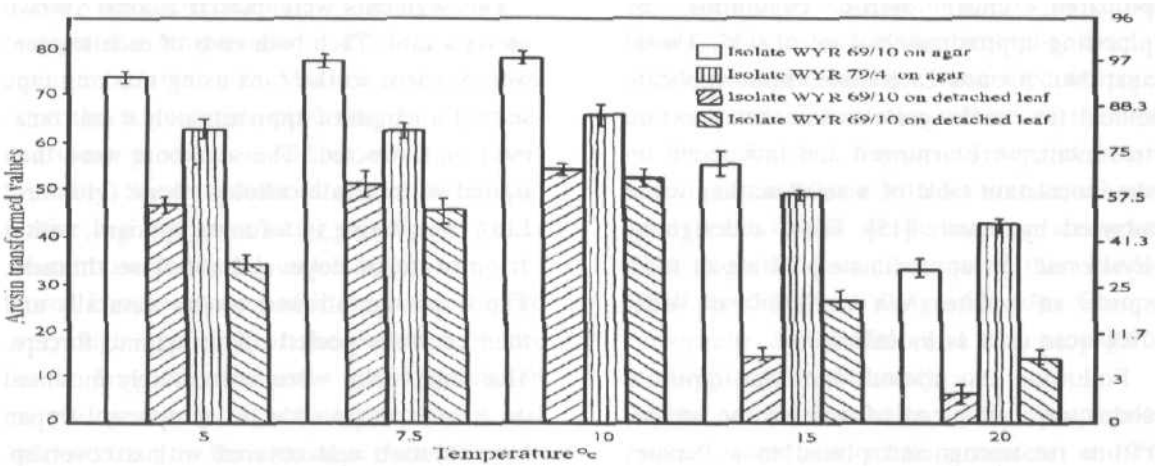

Figure 1. Comparison of spore germination rates on agar and detached leaves over a range of temperatures.

Table 1. Ranked mean percentage germination of *Puccmia striiformis* (arcsin transformed) on agar.

	Temperature °C									
	20	20	15	15	7.5	5	10	5	7.5	10
Isolate WYR	69/10	79/4	79/4	69/10	79/4	79/4	79/4	69/1	69/1	69/1
Germillation %	33.7	43.7	50.0	56.S	63.5	63.5	67.3	74.6	78.4	79.2

Values underlined with a common line do not differ singnificannlly at P = 0.025

Table 2. Ranked mean percentage germination of *Puccinia striiformis* (arcsin transformed) on detached leaves.

	Temperature °C									
	20	20	15	15	7.5	5	10	5	7.5	10
Isolate WYR	69/10	79/4	79/4	69/10	79/4	79/4	79/4	69/1	69/1	69/1
Germination%	6.6	14.3	14.7	26.9	34.8	46.4	47.7	51.8	53.6	55.3

Values underlined with a common line do not differ singnificanntly at P=0.025

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Urediniospore germination on detached leaves (Table 2) was again highest at 10°C where isolate WYR 69/10 achived approximately 68% [arcsin 55.3 (Plate 3)] and isolate WYR 79/4 achieved 65% (arcsin 53.6) germination. At cooler temperatures, **the** germination level dropped. Particularly for isolate WYR 79/4 and at higher temperatures, the rate fell markedly with isolate WYR 79/4, giving 21% and 6% and isolate WYR 69/10 giving 6% and 1% at 15°C and 20°C respectively (Plate 4). As a result of this study, 10°C was chosen as the temperature at which to investigate spore germination on the range of host cultivars under investigation.

The results of the investigation on agar and detached leaves suggest that both isolates germinated most effectively at 10°C (Tables 1 and 2). This is similar to the findings of Butler and Jones [2] Manners [13] Hassebrauk and Schroeder [8] and Osman-Ghani and Manners [14]. Germination decreased sharply at 15^{Q}C , as also reported by Gottlieb [7], and was intermediate at 5° and 7.5°C . At the cooler temperatures 10 , 7.5 and 5°C isolate WYR 69/10 consistently germinated at a higher level then isolate WYR 79/4. Whereas at 20°C the latter isolate gave the highest figures. This observation indicates that although the two isolates have a similar optimum temperature for germination, their performance over a range of temperatures may be inherently different.

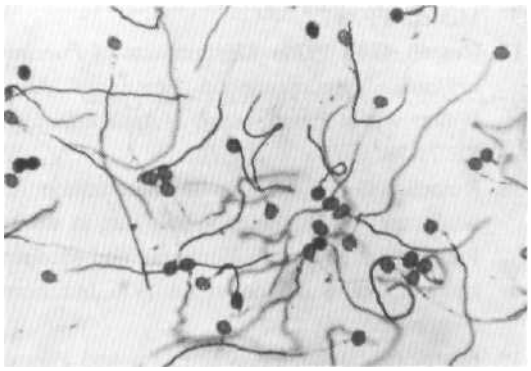

Plate 1. Urediniospore germination of isolate WYR 69/10 at 10°C on agar. 75*μ* ___

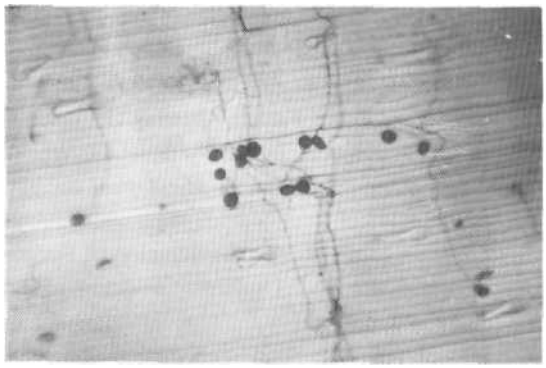

Plate 2. Urediniospore germination of isolate WYR 69/10 at 10°C on delached leaf. *15/H* ___

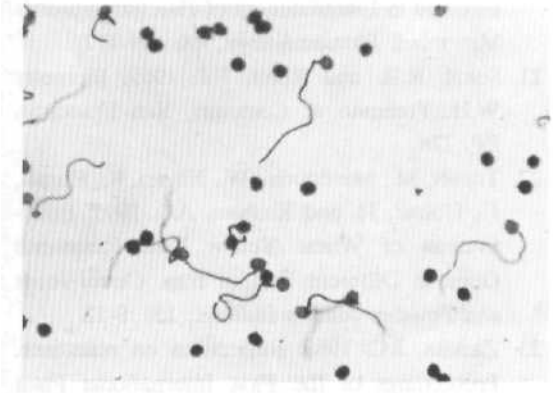

Plate 3. Urediniospore germination of isolate WYR 69/10 at 20°C on agar. *15ft*

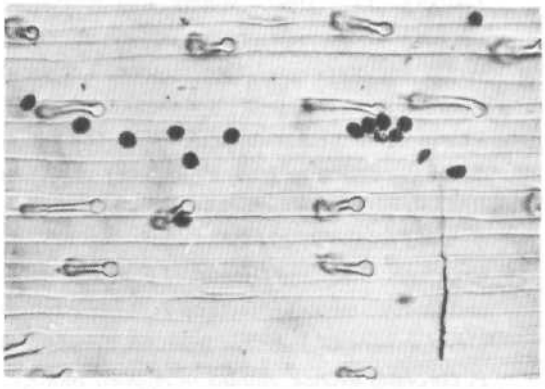

Plate 4. Urediniospore germination of isolate WYR 69/10 at 20°C on detached leaf. *75fi* ___

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بررسی جوانه‌زدن یوردینیوسپور *Puccinia striiformis* در دماهای مختلف روی آگار و برگهای جدا شده از گندم

چکیده

درصد جوانه زدن یوردینیوسپور دو جدایه از عامل بیماری زنگ زرد *Puccinia striiformis f.sp. tritici* به نامهای [WYR 96/10نژاد E(137) 104] و جدایه [WYR 79/4نژاد (4)139 E 41] در دماهای مختلف بین ۵ تا ۲۰ درجه سانتی‌گراد روی آگار و سطح زیرین برگ‌های گندم جدا شده از بوته روی یک لایه از بنزیمیدازول آگار مورد مطالعه قرار گرفتند. در این بررسی آزمایشی با ۵ تیمار برای پیدا کردن مناسب‌ترین دما جهت جوانه‌زدن اسپور، روی برگ‌های میزبان انجام شد. میانگین درصد جوانه زدن اسپورها در دماهای مختلف با استفاده از روش آماری Student-Newman Keuls مورد تجزه و تحلیل قرار گرفته و با هم مقایسه شدند. میزان جوانه زدن هر دو جدایه قارچ در حرارتهای بین ۵ تا ۱۰ درجه سانتیگراد بالا بود، اما در حرارت‌های بین ۱۵ تا ۲۰ درجه سانتیگراد کاهش معنی‌داری داشت. در حرارتهای ۵، ۷/۵ و ۱۰ درجه سانتیگراد جدایه WYR 69/10 در مقایسه با جدایه WTR 79/4 از جوانه‌زنی بالاتری برخوردار بود در حالی‌که در حرارت ۲۰ درجه سانتی‌گراد جدایه دوم بیشتر از جدایه اول جوانه‌زنی داشت. این بررسی نشان داد که مناسب‌ترین درجه حرارت برای جوانه زدن اسپور در روی آگار و برگهای جدا شده از بوته برای هر دو جدایه ۱۰ درجه سانتیگراد می‌باشد.

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