EFFECT OF SOME ENVIRONMENTAL FACTORS ON THE *IN VITRO* GERMINATION AND GERM TUBE GROWTH OF UREDOSPORES OF *UROMYCES FABAE*

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Abstract

Uredospores of ***Uromyces fabae*** gave maximum germination and germ tube growth when they were young with low density at 21°C in light with an exogenous supply of nutrients.

Introduction

Uromyces fabae, a rust fungus of broad beans (***Vicia faba*** L.) is one of the Uredinales. It occurs on many leguminous plants such as peas, broad beans and vetches, and occurs in almost every part of the world, wherever these hosts are grown. Rust ***U. fabae*** uredospores are termed as dry spores and usually dispersed by wind and rain. It is generally believed that the percentage germination of rust uredospores on the surfaces of leaves is greatly affected by the number of spores in the inoculum ((Manner, 1981). Extensive work has been reported on the relationship between spore density and percentage germination on artificial surfaces such as agar or glass or floating on water.It is generally believed that rust spores on leaves behave similarly as on artificial surfaces (Manner, 1981).

In case of ***U. fabae***, germination of uredospores on leaves of broad bean (***V. faba*** L.) was slightly less than on glass surfaces. Numerous works have been devoted to self-inhibitors of rust uredospores (Allen, 1968; Bell and Dally, 1962; woodbury and Stahmann, 1970; Mortie; 1971) but little is yet known about the nature, origin and mode of action of substances responsible for this phenomenon. Rust uredspores including other fungal spores have an optimum density for spore germination. In case of ***U. fabae*** the optimum germination of uredospores lies between 10,000 to 25,000 spores per ml. It is generally believed that germination of ***U. fabae*** uredospores is greatly affected by environmental factors such as temperature, light and humidity. For successful uredospres

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germination and germ tube growth suitable temperature is very essential. Optimum temperature for ***U. fabae*** uredospores germination lies between 18° to 22° C (Channar, 1982). The presence of free water is essential for fungal spore germination, hence infection of leaves by rust uredospores only occurs when the surface of the plant is wet for several hours. Sharp **et al**, (1958) have confirmed that uredospores of ***Puccinia graminis*** require liquid water for spore germination and this is probably true of other rust spores. Uredospres of ***U. fabae*** germinated on broad bean leaves when there was 100% relative humidity (Channar, 1982). The uredospores of
U. fabae germinated better when they were placed in light than darkness (Buxton **et al**, 1957). Burrage (1970) claimed that light has little effect on spore germination in relation to temperature.

Little information is available on chemical factors affecting germination and germ tube growth of rust uredospores on leaf surfaces.It is generally held that germination and germ tube growth is stimulated when exogenous nutrients are applied. There is evidence to show that germination and germ tube growth of rust uredospores ***in vitro*** is enhanced after addition of nutrients including sugars, yeast extract and some mineral salts (Wilhelm 1931; Straib, 1940).

There is an extensive literature available on rust uredospores of different species, but little information is available on uredospores of ***U. fabae***. The present investigation was undertaken to determine the optimum conditions for ***U. fabae*** uredospores germination ***in vitro***; the effect of nutrients on uredospores germination and germ tube growth has also been studied.

Materials and Methods

Rust uredospores cultures were maintained on leaves of 4 to 5 weeks-old broad bean plants. The leaves were inoculated by brushing both sides of the leaf with dry uredospores. Then plants were covered with polythene bags for 24th to provide 100% relative humidity for infection (Plate 1). Uredospores were collected from newly developed pustules (Plate 2) 13 to 15 days after inoculation by brushing with a soft hair brush into a petridish and subsequently transferred to a Universal bottle. The suspensions from 11 to 17 days – old uredospores were prepared separately in a 10ml volume (1 drop "Tween" 20 in 50ml, sterile water). ***U. fabae*** were made up at concentrations ranging from 5×10^3 to 4×10^4 ml^{-1}. Nutrient solutions, one of glucose-glutamine (50 μg-0.1 μM/100 ml.) and the other yeast extract (0.01g/100 ml.) were prepared separately in sterile water. Nutrient solutions were sterilized

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prior to use by passing through an oxide "nuflow" cellulose acetate membrane (0.45 μm pore size).

Glass slides were cleaned with ethanol and flamed dry prior to use. Vaseline rings were made on each slide (2 rings per slide) to prevent the spread of spore inoculum. Droplets (20 μl) containing ***U. fabae*** uredospores were placed on glass slides and then they were kept in plastic petridishes lined with moist filter paper. The glass slides were immediately transferred to growth room at a temperature ranging from 15° to 25°C. After 24 th incubation the slides were removed from petridishes and transferred to a hot plate to dry. Microscopic examination of germinated uredospores was made after staining with phenolic aniline blue. Assessment of germination was based on count of 200 spores and germ tube lengths were measured from a sample of 50 germinated spores. Percentage germination of spores was calculated using an Arcsine Tranformation to give 95% confidence limits (Sokal and Rohlf, 1969; Blakeman, 1975). A t - test was applied to data for measurements of germ tube length.

Results and Discussion

Effect of Spore Age

Uredospores of different age were used in experiments to observe optimum spore age for germination and germ tube growth of ***U. fabae***. Uredospores (13 to 15-days-old) gave better germination and germ tube growth in distilled water than old uredospores (17-days-old). The optimum age of uredospores germination was found to be between 13 to 15- days after inoculation of plants (Fig. 1). Results suggest that age of uredospores has considerable effect on percentage germination and germ tube growth of ***U. fabae***. The age of uredospores also affected the amount of nutrients lost by hydration. Young uredospores may loose compounds if their wall has not matured. Similarly, old spores may loose compounds if the wall has deteriorated and this partly account for the decrease in germination of ***U. fabae*** old uredospores (Parker, 1982).

Effect of Spore Number

Different densities of uredospores were tested in experiments to determine the optimum spore density for germination and germ tube growth of ***U. fabae***. The optimum density of uredospores for germination and germ tube growth was found to be 2×10^4 ml^{-1} (Fig. 2). Evidence found in this investigation suggests that inhibition of uredcspores at higher densities may be related to self-inhibitors of germination or to interference with respiration among crowded spores. In common with other fungal spores, ***U. fabae*** uredospores did

not germinate well when they were crowded together. Last (1960) has found that conidia *Botrytis fabae* at a concentration of 1×10^5 ml^{-1} or more retarded percentage germinatio

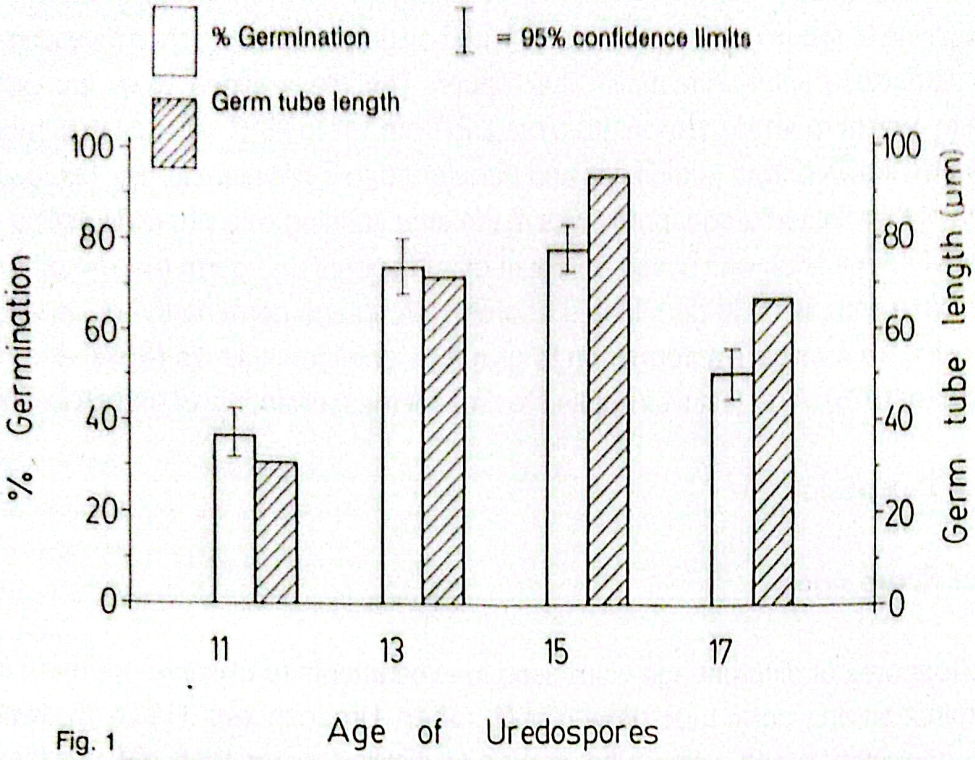


Fig. 1 Effect of spore age germination and germ tube growth of *Uromyces fabae* uredospores on glass.

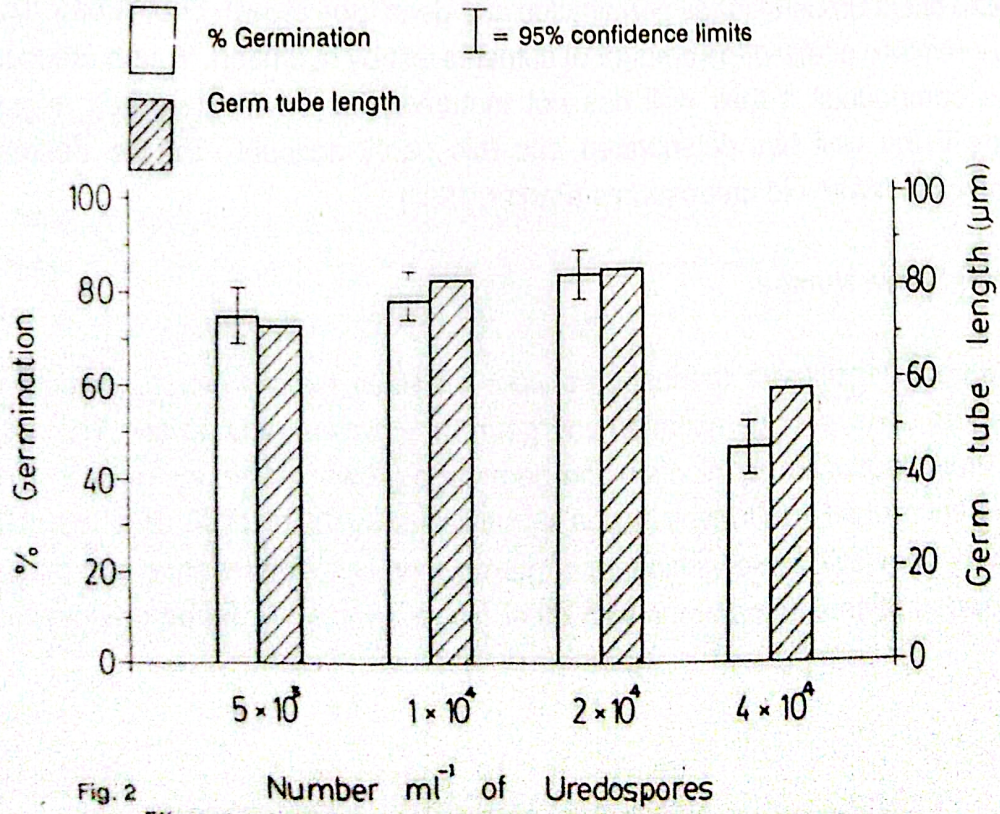


Fig. 2 Effect of spore number on germination and germ tube growth of *U. fabae* on glass.

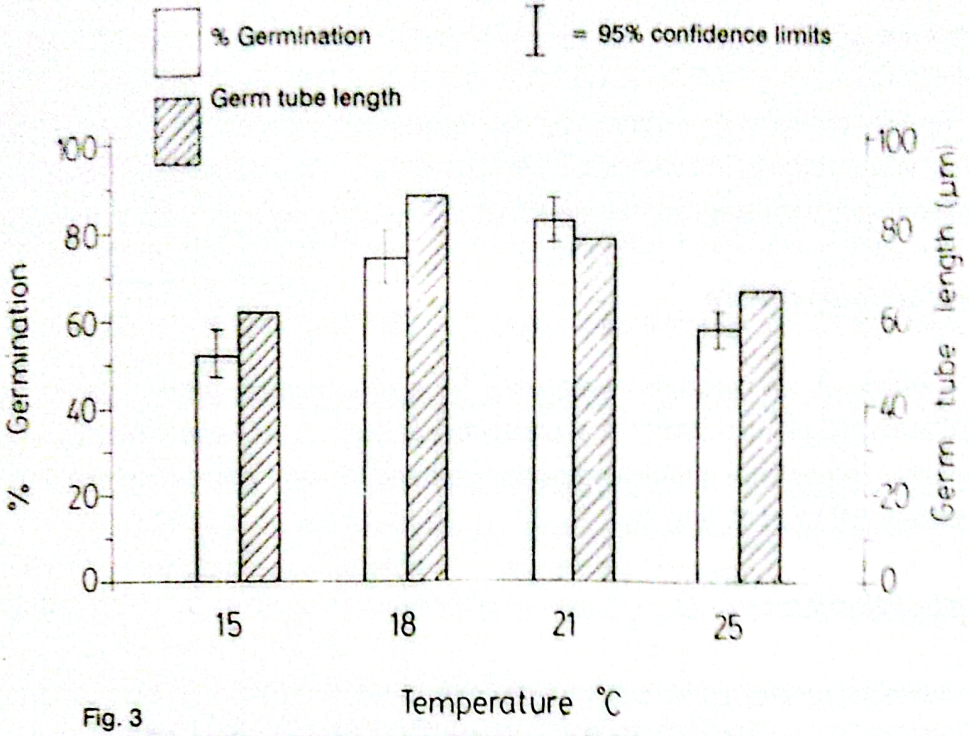
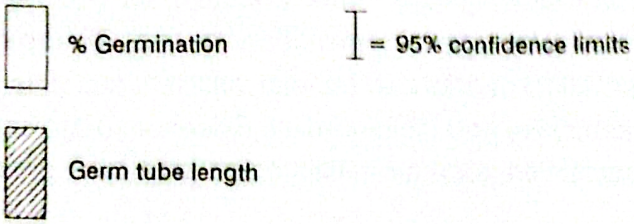


Fig. 3
Effect of temperature on germination of *U. fabae* on glass.



a p = 0.01 when compared with water control.
b p = 0.001 when compared with water control.

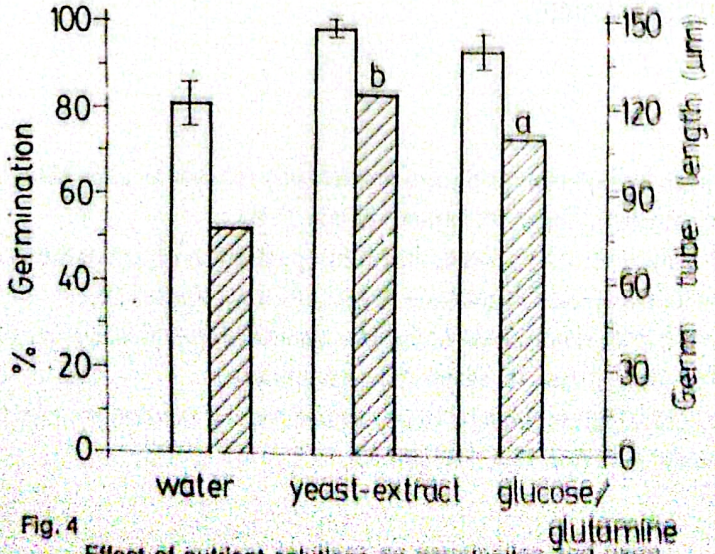


Fig. 4
Effect of nutrient solutions on germination and germ tube length of *U. fabae* on glass.

Effect of Light

Results of this study suggest that ***U. fabae*** uredospores placed in light germinate better than spores placed in darkness. Buxton ***et al***, (1957) have also shown that uredospores of ***U. fabae*** and ***Erysiphe graminis*** germinated better in day light than in darkness.

Effect of Temperature

A range of temperatures were used for a uredospore germination study. Results found in this investigation revealed that uredospores germinated less well at lower and higher temperatures. The optimum temperature for germination and germ tube growth was found to be between 18° to 21°C (Fig. 3)

Effect of Nutrients

Germination and germ tube growth of ***U. fabae*** uredospores increased significantly when uredospores were suspended in nutrient solutions of yeast extract (0.01g/100 ml), glucose and glutamine (50 μg-0, 1 μM/100 ml,); this effect appeared more noticeably with the former than the latter (Fig.4). Rust uredospores including ***U. fabae*** are nutrient independent in relation to spore germination. In fact, ***U. fabae*** uredospores are large compared to other fungal spores and contain sufficient nutrients for germination and germ tube growth on both glass and leaf surfaces. Evidence found in this investigation suggest that uredospores achieved good germination and germ tube growth in distilled water.

However, addition of nutrients such as yeast extract and glucose and glutamine together enhanced ***U. fabae*** uredospores germination and germ tube growth ***in vitro***. Similar effect of nutrients on rust ***Puccinia glumarum*** uredospores ***in vitro*** have been reported by Wilhelm (1931) and Straib (1940).

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