THE EFFECT OF ENVIRONMENTAL CONDITIONS ON THE GERMINATION OF UREDOSPORES OF *PUCCINIA GRAMINIS-TRITICI* ERIKSS. AND E. HENN., RACE 56

By

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Abstract

Over a range of levels of certain environmental factors, the germination of uredospores of *Puccinia graminis-tritici*, Race 56, is characterized by qualitative as well as quantitative differences in response, as well as by pronounced variability between samples. Both of these features are probably dependent upon the level of a self-inhibitor evolved by these spores.

Evidence is presented for the existence of the following interactions:

a. In the presence of light, but not in its absence, the lowering of the carbon dioxide tension causes a reduction in percentage germination, and results in lysis of the germ tubes.

b. In terms of lower percentage germination, uredospores are more sensitive to the self-inhibitor at low carbon dioxide tension in the presence of light.

c. Uredospores are more sensitive to low oxygen tensions at low carbon dioxide tension, in the presence of light.

From the above considerations, it appeared likely that the effects of carbon dioxide, oxygen, and inhibitor concentrations were interrelated, and that the suppression of germination of submerged spores was probably associated with their sensitivity to the inhibitor.

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INTRODUCTION

At the present time, the major obstacle to the study of obligate parasitism in fungi, in this instance, Puccinia graminis-tritici Erikss. & E. Henn., Race 56, is that these organisms cannot be grown apart from their living hosts, their metabolic activities being intimately associated with, and hence obscured by, those of the host plant. There is, however, one relatively brief phase, the germination of fungal spores, which can proceed for a limited time independently of the host. For this reason, the physiology of spore germination has received considerable attention.

Apart from their value per se, studies on spore germination provide two means of revealing in greater detail the distinctive characteristics of obligately parasitic fungi: by comparing the germination behaviour of spores of the parasitic type with that of spores of normally saprophytic fungi, and by noting similarities and differences between the independent phase and the resulting parasitic one.

Unlike the majority of fungal spores, it was noted in this laboratory (Isaac), that Race 56 uredospores frequently did not germinate when covered with a thin layer of water.

Since this could conceivably be due to either an insufficient oxygen supply, or to an accumulation of an inhibitor known to be evolved by these spores, it was suggested that an enquiry into the relationships between the gaseous environment and germination, and also the role of the inhibitor, might be of some assistance in explaining this behaviour.

Before carrying out the main body of the investigation, certain inconsistencies in the germinability of the spore samples, involving storage conditions, required attention. These are dealt with in Part I. The effects of carbon dioxide concentration and oxygen supply, are treated in Parts II and III respectively. Finally, in Part IV, with an assessment of the information gathered from the previous sections, together with more direct evidence, an attempt is made to interpret the problem of why the germination of submerged spores is reduced.

METHODS AND MATERIALS

The Race 56 culture of uredospores of Puccinia graminis-tritici Erikss. & E. Henn. was originally obtained from a well-isolated pustule and grown continually on Little Club wheat (Triticum compactum Host.) under normal greenhouse conditions. The genetic stability of the rust collections was tested periodically on the standard set of twelve differential hosts. Both the rust material and the wheat seed were kindly provided by the Dominion Laboratory of Plant Pathology, Fort Garry, Manitoba.

Collection of the Spores.

The spores were collected daily with a cyclone harvester, which is shown diagramatically in Figure 1. It consisted of a 50 ml boiling-tube (A), a two-hole rubber stopper (B), and intake tube (C) with a 2 mm bore, and a 5 mm outlet tube (D), which was connected with rubber tubing to an aspirator pump. Tube (C) extended about halfway down the side of the boiling-tube, and was bent to an angle of about 45° (c_1), so that the spores were directed downwards in a circular direction as they were drawn into the boiling-tube.

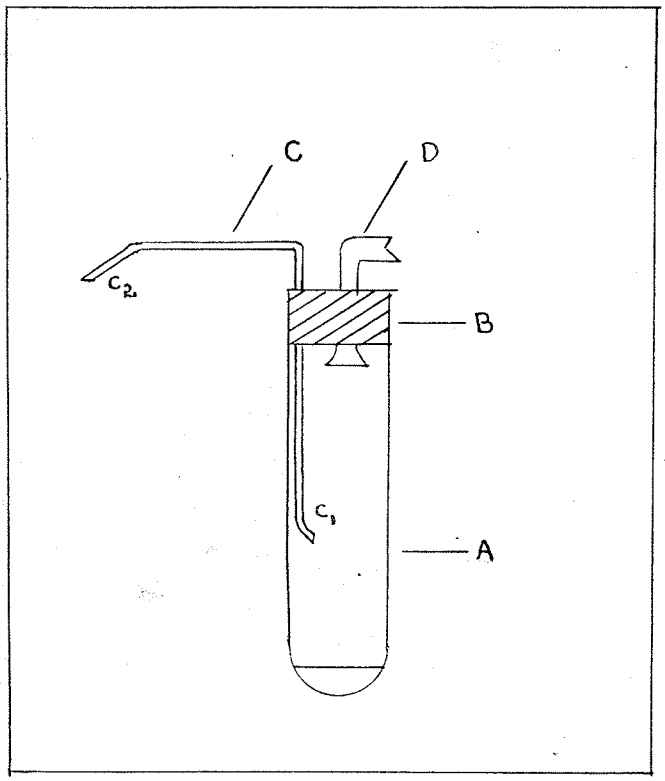


Fig 1. Diagram of apparatus used for the collection of uredospores. see text for details.

The tip (c_2) was slightly flattened horizontally. The outlet tube (D) did not extend as far into the boiling-tube as (C), and was flared at the inner end.

When the collector was attached to an aspirator pump, the velocity of the air passing through (C) was sufficient to carry the spores into the boiling-tube; here the drop in air velocity brought about through the increased cross-sectional area, caused the spores to be precipitated to the bottom of the tube.

Inoculation Technique.

The apparatus illustrated in Figure 2 assisted in dispersing the inoculum evenly over the surface of the medium, so that the spores were well separated from each other to facilitate counting. This was accomplished by vibrating the spores through a fine copper gauze screen and allowing the spores to settle onto a rotating petri dish.

A commercial vibrating tool (A) was fitted with a metal cylinder (B), to the bottom of which was soldered a circle of gauze screening. A turntable (C), such as that used for ringing microscope slides, was placed directly beneath the vibrator. When air currents caused the spores to drift, it

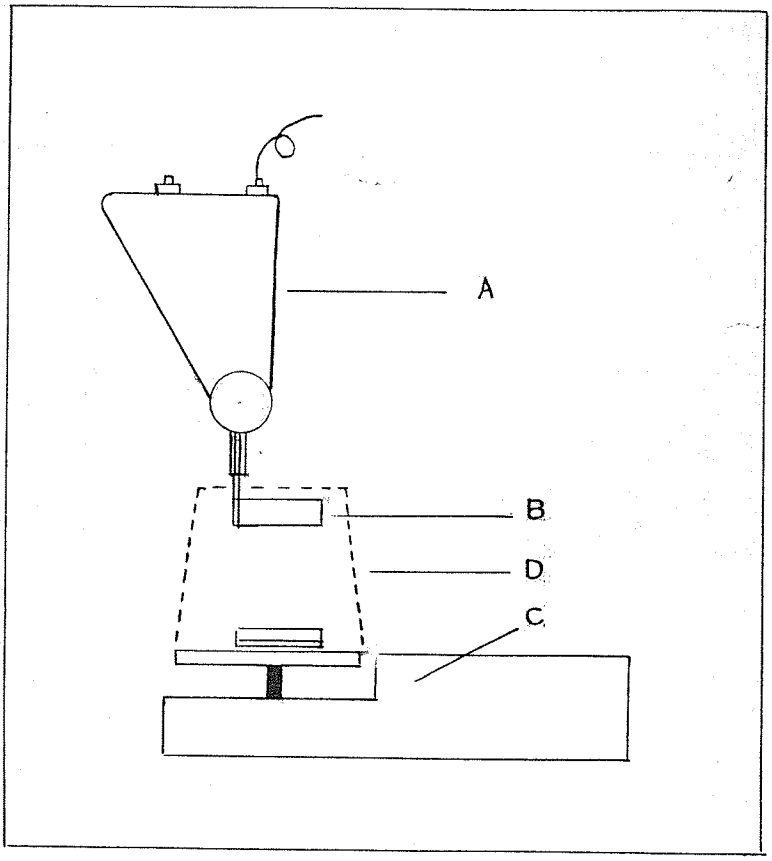


Fig 2. Diagram of apparatus used for the inoculation of uredospores. see text for details.

was sometimes found convenient to enclose the area above the petri dish with an inverted polythene container (D).

To inoculate the medium, the spores were placed in the container (B), the turntable and petri dish were set in motion, and the spores were subjected to vibration for five to fifteen seconds.

No significant difference in final percentage germination was found between spores "vibrated" in the above manner, and those dusted onto agar with a camel's hair brush.

Note.

Both pieces of apparatus described in this section were assembled in the Botany Department, U. of M.

Germination Conditions.

Many of the germination trials to be reported here were carried out in a growth-chamber which provided uniform conditions. These were:

Light ----- ca. 2400 foot-candles. When exclusion of light was desired, the germination vessels were wrapped in aluminum foil.

Temperature ----- 20° C $\pm$ 0.25

Humidity ----- 65% $\pm$ 2 Relative Humidity

Note.

By the following terms which appear throughout the work, the conditions implied are:

"Plain agar" --

Composition:

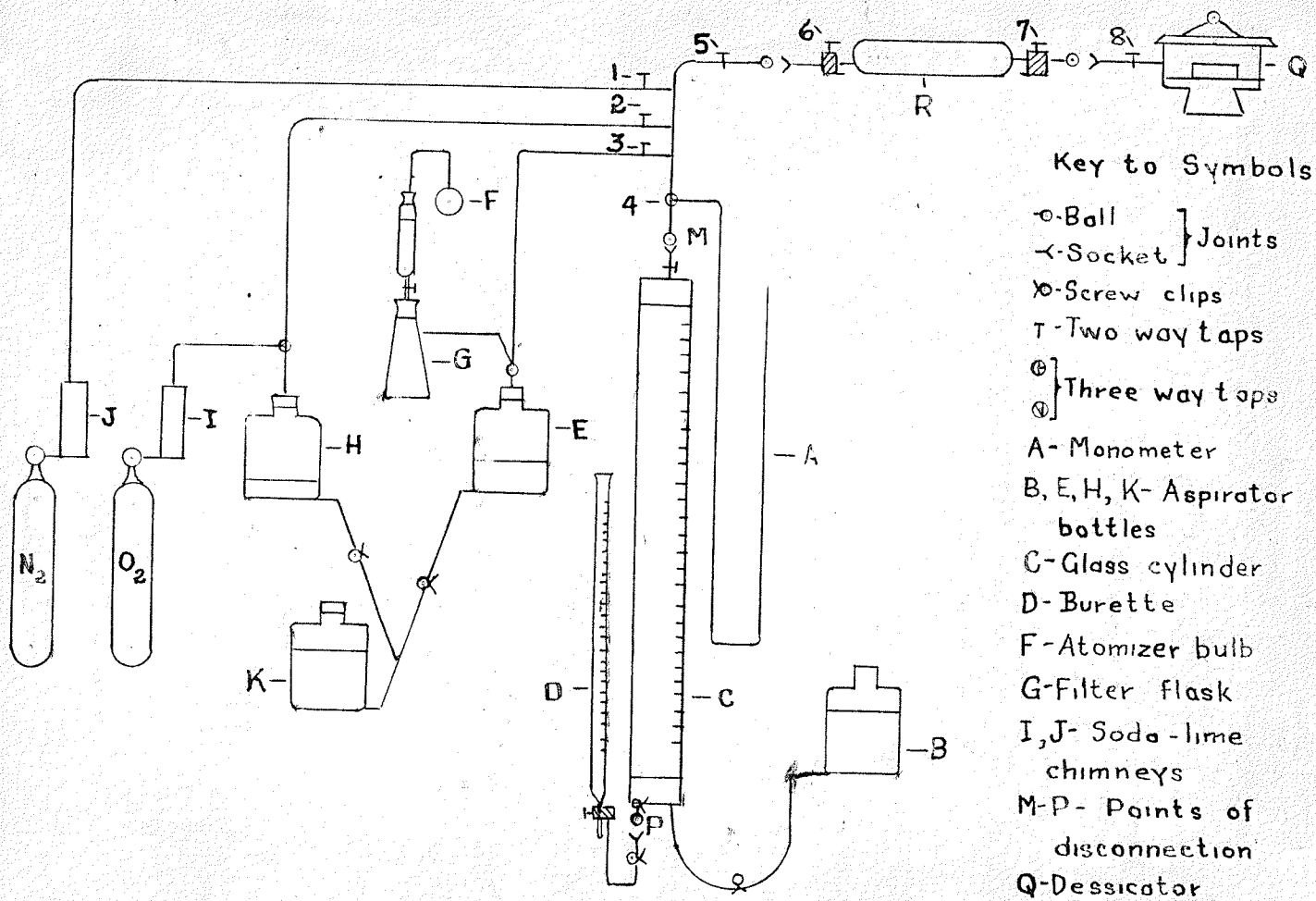
"Difco" agar	7.5 g
Distilled water	1000 ml

The agar was poured into 50 ml petri dishes, and used not more than four hours after preparation. In any experiment involving low carbon dioxide concentrations, the dishes were kept over 20% potassium hydroxide.

"fresh spores" -- spores that had been produced during the twenty-four hours previous to the experiment.

Apparatus for the Handling of Gas Mixtures.

The apparatus shown schematically in Figure 3 was designed to prepare mixtures of carbon dioxide, oxygen, and nitrogen of any desired combination. Essentially this is achieved by withdrawing a known volume of liquid from a graduated cylinder, creating a partial vacuum, then bringing the pressure back to atmospheric by adding the required gas to the cylinder.



Key to Symbols

- Ball, Socket } Joints
- Screw clips
- Two way taps
- Three way taps
- A- Monometer
- B, E, H, K- Aspirator bottles
- C- Glass cylinder
- D- Burette
- F- Atomizer bulb
- G- Filter flask
- I, J- Soda-lime chimneys
- M-P- Points of disconnection
- Q- Dessicator
- R- Gas sampling tube

Fig 3 - Diagram of apparatus for the preparing and handling of gas mixtures