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Short Communication

OPTIMIZED CONDITIONS FOR THE GERMINATION OF SUGARCANE SMUT SPORES-(*USTILAGO SCITAMINEA* SYDOW) *IN VITRO*- SPORE CONCENTRATION, TEMPERATURE AND PH

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ABSTRACT
In order to cause infection of sugarcane the smut spores must first germinate on the surface of the sugarcane bud. Thereafter, penetration of the bud occurs and infection is initiated. This research investigated the three major conditions necessary for the germination of smut spores=Spore Concentration, Temperature and pH. The optimum spore concentration for smut spore germination is 10^5 spores/ml. The optimum temperature for spore germination is 25^0C and the optimal pH is 7.0.

INTRODUCTION
Ustilago scitaminea Sydow is a basidiomycete of the order Ustilaginales or smut fungi and is found mainly in the tropics and sub tropics. The taxonomy of smuts is based largely on spore morphology with spore size, color, ornamentation, and shape being especially important. (Lee-Lovick, 1978). Mundkur (cited in Lee-Lovick, 1978) studied *U. scitaminea* in detail and divide it into *U. scitaminea* var sacchari-barberi and *U. scitaminea* var sacchari-officinarum. His basis for classification was size, color, and the pattern of the spore wall. Hirschhorn (1943) in Lee-Lovick, 1978, divided smuts in Argentina into six groups, one of which approximates Mundkur's var saccharum-officinarum. Whether or not a relationship exists between the morphological characteristics of the spores and the pathological is uncertain. Gillaspie and colleagues (1983) compared the pathogenecites of six different isolates of sugarcane smut from different geographical regions and decided each isolate represented a different race of U. scitaminea. However, a similar study by French workers failed to show any differences in germination, virulence, or electrophoretic patterns of three enzyme systems between smut isolates from different parts of the world. (Peros and Baudin, 1983)

Sexuality occurs in Smut with haploid compatible cells fusing to give a diploid condition (Alexander and Srinivasin, 1966). This same heterothallism is evident in isolates from different parts of the world (Peros and Baudin, 1983). The spores are spherical, smooth wall or slightly papillate, light brown to black in color and have a diameter of 5-10 um (Commonwealth Mycological Institute Descriptions of Pathogenic Fungi and Bacteria N0. 80). Smut spores have no dormancy and germinate readily over a wide range of temperatures, 5-40 ^{0}C, with an optimum between 25- 30 ^{0}C (Waller, 1969).

Before infection occurs the spores must germinate on the substratum. The germination time is about 6h (Bock, 1964; Waller, 1969; Trione, 1980). Once the germ tube has protruded, this promycelium becomes four-celled, having undergone meiosis (Bock, 1969; Waller, 1970).The type of germination which then ensues is of two types, hyphal or sporidial (Waller, 1970). For the purpose of this study smut spores from Barbados were used, and spores were counted as germinated when a germ tube has protruded.

MATERIALS AND METHODS
Methods for Optimizing Germination Conditions
1. The Effect of Smut Spore concentration on Spore Germination
Smut spores were harvested from recently produce whips on field grown sugarcane and stored in a dessicator at 25 ^{0}C until required. Spores were suspended in sterile distilled water containing Tween 80

(0.1%v/v) at spore suspension concentrations of 10^4, 10^5, 10^6, 10^7, and 10^8 spores per ml. Twenty- five ml of each suspension, in a flask covered with perforated foil, was incubated in a shaking water bath at 30^0 C for 6h., then scored for percentage germination using a haemocytometer and a light microscope. Spores were stained with aniline blue prior to counting. The experiment was repeated 10 times and then results pooled.

2. The Effect of Temperature on smut Spore Germination

A one hundred ml aliquot of a 10^5 spores per ml suspension (prepared as above) was maintained in a water bath at each of the following temperatures: 20, 25, 30, 35, and 40^0 C. A 0.1 ml sample was periodically examined using a haemocytometer and a light microscope, and scored for percent germination after staining with aniline blue. The experiment was repeated 10 times and the results pooled.

3. The Effect of pH on the Germination of Smut Spores

To 4.5 ml of buffers at different pH values (10mM acetate- pH 4 and 5; 10mM phosphate- pH 6, 7, and 8) was added 0.5 ml of a 10^5 spore per ml suspension as prepared above. The suspensions were incubated in a water bath for 6h at 25^0C, then scored for percentage germination using a haemocytometer and a light microscope after staining with aniline blue. The experiment was repeated 10 times and the results combined.

RESULTS

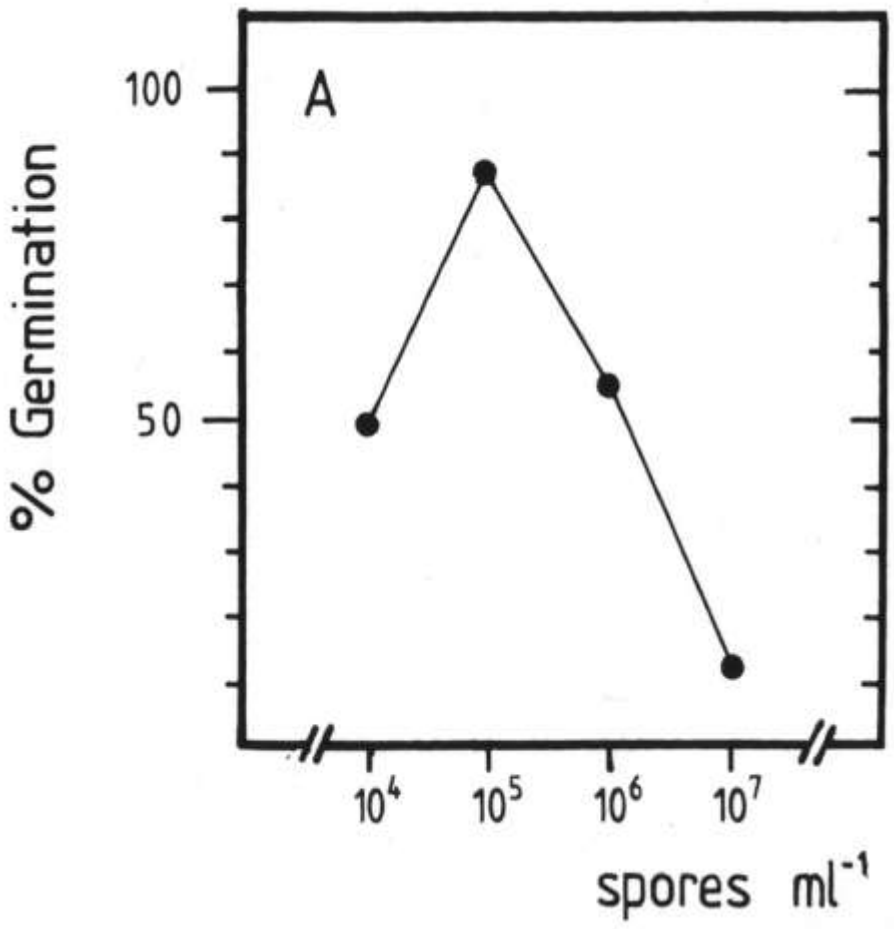

Figure 1: The Effect of Smut Spore concentration on Smut spore Germination

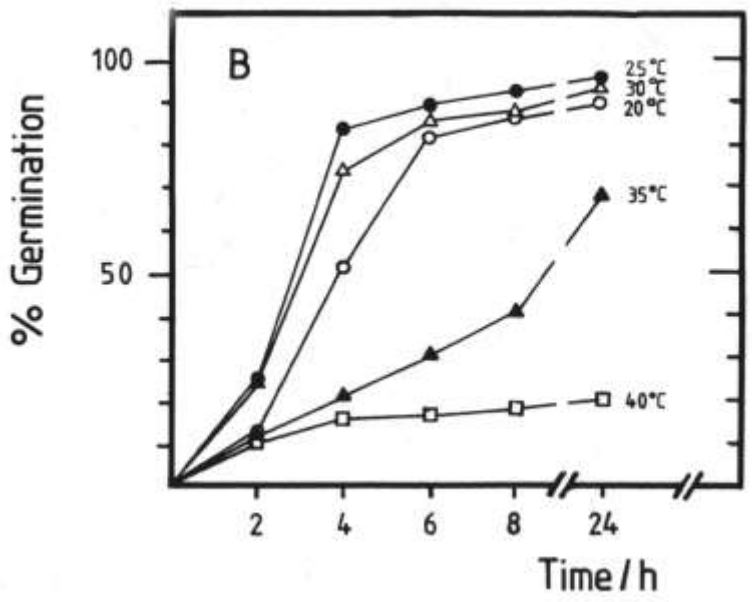

Figure 2: The Effect of Temperature on Smut spore Germination

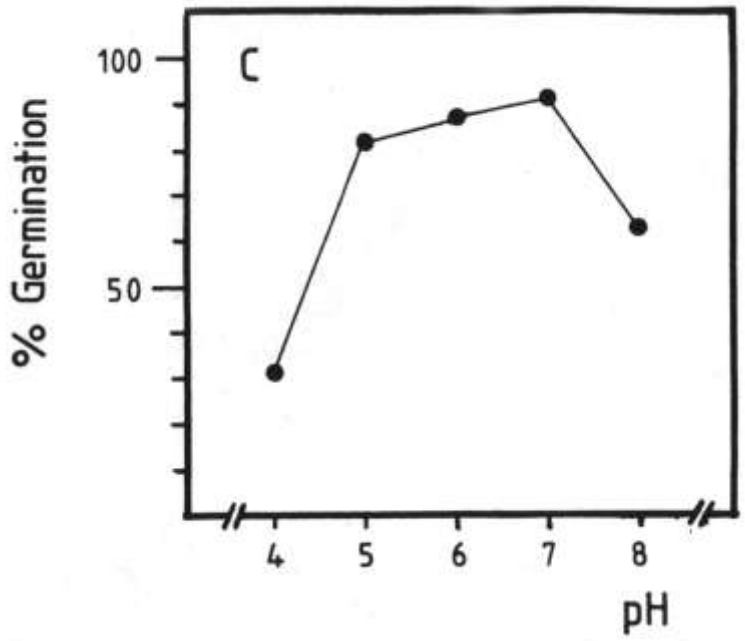

Figure 3: The Effect of pH on the Germination of Smut Spores

DISCUSSION AND CONCLUSION

Figure 1 shows the effect of spore suspension concentration on the germination of *Ustilago scitaminea* spores. There is a sharp optimum at a population density of 10^5 spores per ml, with germination falling off above and below this concentration. Spores with germ tubes protruding were scored as having germinated (Manners, 1966). The reduction of fungal spore germination at high spore concentrations has been known for a long time (Edgerton, 1910, in Allen, 1976). It has been attributed variously to starvation, oxygen deficiency, and the leaching of inhibitory substances (Allen, 1976). The starvation hypothesis can be ruled out since water was used as the germination medium in this case, and cannot be considered a nutrient. Doran (1922) in Allen (1976) suggested oxygen deficiency in dense populations as a factor inhibiting spore germination, but others have argued this only holds for very high spore concentrations of 10^6-10^7 spores per ml. (Fletcher and Morton, 1970; Steele, 1973). Nonetheless, percentage germination does not simply increase as spore concentration falls as would be expected if anaerobiosis was an over-riding factor. In fact, at the lowest concentration, 10^4 spores per ml, germination is severely depressed and an optimum is reached at 10^5 spores per ml. for many fungi, evidence shows that an important factor in the population effect is the inhibitory substances which leach from the spores. Benzaldehyde (an exogenous compound) nat a concentration of 1.5ppm was found to be at an optimum concentration for the germination of spores from *Ustilago maydis* (Noble, 1924, in Fisher and Holton, 1957). However, several factors may be involved as in the case of Microsporum gypseum where macro-conidial germination is promoted by the release of proteases into the medium as well as by calcium ions, but inhibited by phosphate ions. Self-inhibition may then be the result of a balance between stimulatory and inhibitory factors leached from the spores. For the germination of *Ustilago scitaminea* spores in vitro, nutrient limitation and oxygen deficiency appear to be minor factors in the reduction of spore germination at suspension concentrations of 10^4, 10^6, or 10^7 spores per ml. This reduction of germination is probably associated with inhibitory and/or stimulatory compounds-the nature of which is unknown. The possibility also exists that a particular regulatory substance has an optimal effect at a specific spore concentration. In this case, a spore density of 10^5 spores per ml seems to provide this optimal balance/concentration.

Figure 2 shows that over a 24h period spore germination can occur between 20 and 40^0C. Spore germination was observed as early as 2h after imbibition of water as has also been reported by Leu (1972) and Rampersad (1982). Spores germinated more quickly at temperatures of 25 and 30^0C than at 35 and 40^0C, with those at 20^0C exhibiting an intermediate germination rate. After 6h, the highest percentage germination occurred at a temperature of 25^0C. These results are in agreement with those of previous workers who have reported spore germination in this species as occurring between 5 and 40^0C with an optima at 25^0C and 30^0C at 100% humidity (Leu, 1972; Lee-Lovick, 1978; Singh and Agnihotri, 1978). Extending this to the situation in the field, Bock (1964) showed 31^0C to be the optimum temperature for the production of infection hyphae and that smut whips developed on the inoculated setts at a range of temperatures between 20^0C and 30^0C.

Figure 3 shows that the optimum pH for spore germination in *Ustilago scitaminea* is pH 7.0, though spores germinated at all pH levels tested from pH 4.0 to pH 8.0. The effect of acidity on spore germination in several smut fungi has been known in the literature for some time (Walker, 1923; Fisher and Holton, 1957). From the literature it seems that most fungi have a broad optimum, being able to tolerate a wide range of pH values. (Fisher and Holton, 1957). Germination of smut spores of other smut fungi, for example, Ustilago maydis, is affected by carbon dioxide levels and this is also considered to be a pH effect via dissolved carbon dioxide (Platz *et al.,* 1927, in Fisher and Holton, 1957). In terms of optimizing the spore germination conditions, buffering of the spore incubation medium was seen as being especially important as acidic or basic compounds added to the suspension might inhibit germination by simply altering the pH of the medium. The presence of a buffer in the medium would preclude such non-specific effects.

It should be noted that these optimized conditions might vary due to the race specific aspects of smut spores from different geographical locations. Optimized conditions are useful for the inoculation of sugarcane sets to determine resistant ratings. It can also be used to inoculate field grown sugarcane.

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