

SOME PHYSICAL FACTORS AFFECTING GERMINATION AND APPRE-
SSORIA FORMATION OF *COLLETOTRICHUM DEMATIUM* VAR
TRUNCATUM (PERS. EX. FR.) GROVE

BY

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The effects of some physical factors on the germination (G) and appressoria formation (A. F.) by *Colletotrichum dematium* var. *truncatum* (Pers. Ex. Fr.) Grove, a virulent pathogen of soybean, were studied *in vitro*. Results revealed that both G. and A. F. declined with age of culture, increased concentration of spores and also when spores were incubated under complete darkness. Spores germinated in a wide range of pH (4.0-8.0). Optimum was noted at 5.5. Low temperature (0 to -15°C) pretreatment for a period of 20 days significantly reduced germination (50%). Germination and appressoria formation also decreased with increase in pretreatment temperature from 40°C to 70°C for 10 min. Duration of high temperature pretreatment is more effective in inhibiting G and A. F. Only 7% germination and 50% appressoria formation of germinated spores were recorded when spores were pretreated for 40 min at 60°C.

INTRODUCTION

Colletotrichum dematium var. *truncatum* (Pers. Ex. Fr.) Grove, a causal agent of anthracnose disease of soybean, produces conidia, which on germination usually form appressoria. These structures are associated with infection because with a few exceptions, host penetration is preceded by appressoria formation (Purkayastha and Sengupta, 1973). Both germination and appressoria formation are influenced by certain physical factors, although probably, there are many factors making up the conducive environment for appressoria formation in a particular species (Emmett and Parbery, 1975). The purpose of this investigation is to gather information about the effects of some physical factors on the germination and production of appressoria by *C. dematium*.

MATERIALS AND METHODS

Culture of *Colletotrichum dematium* var. *truncatum* (Pers. Ex. Fr.) Grove was obtained from the Indian Agricultural Research Institute, New Delhi, maintained on potato dextrose agar medium. Conidia from 20-day-old cultures were suspended in sterile distilled water, centrifuged, resuspended, in sterile water, and concentration adjusted by haemocytometer counts. Conidial germination

tests were carried out as described by Purkayastha and Deverall (1965). Culture with conidia, or conidial suspension was pretreated, depending on the nature of the experiment.

RESULTS AND DISCUSSION

Effect of age of conidia—Conidial suspension was prepared separately from cultures of different ages (viz. 15, 30, 45, 60, 80-day-old) and incubated at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 h. Both germination and appressoria formation declined with age of culture. Only 30% conidia germinated from 80-day-old culture. Maximum germination occurred between 15-30-day-old culture, while the rate of production of appressoria remained fairly high up to 60 days.

Effect of spore concentration—Different concentrations (20×10^6 , 10×10^6 , 5×10^6 , 2.5×10^6 spores per ml.) of spore suspension were prepared and two drops (0.01 ml/drop) were placed on each glass slide, incubated at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 h and percentage germination and appressoria formation were calculated. Results (Table 1) revealed that the percentage of germination decreased significantly with increased concentration of conidia, while percentage of appressoria was more or less similar (80-90%) in a range of spore concentration (2.5×10^6 — 20×10^6 spores/ml).

TABLE 1. Effect of concentration of spores

Spores (conidia/ml)	* % germination with S. E.	* % appressoria formation of germinated spores with S. E.
20×10^6	5.14 ± 0.13	80.21 ± 0.66
10×10^6	32.61 ± 0.33	89.01 ± 0.97
5×10^6	88.53 ± 0.90	95.12 ± 0.40
2.5×10^6	90.17 ± 0.57	93.29 ± 0.54

* 400-500 spores counted.

Incubation time—24 hr.

Incubation temperature $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$

Effect of pH—Spores germinated in a wide range of pH (4.0-8.0). There was no significant difference in germination (80-90%) and appressoria formation. The maximum germ tube length ($147.26 \mu\text{m}$) was observed at pH 5.5.

Effect of light and dark—The spore (20-day-old) suspension of *C. dematium* was incubated under four conditions for 24 h: (i) spores in continuous light (435 lux), (ii) spores in complete darkness, (iii) spores exposed to light (435 lux) for first 8 h and then in complete darkness for 16 h (iv) reverse

of (iii). Results revealed that continuous dark slightly inhibited both germination and appressoria formation. In other cases, no significant difference was observed.

Effect of temperature pretreatment—Twenty-day-old cultures of *C. dematium* were subjected to low temperature pretreatment (-15°C , 0°C , 5°C) for a period of 1, 3, 7, 20 and 40 days separately. It is significant to note that no change in germination or appressoria formation was noted at 5°C , but percentage of germination significantly reduced (about 50% and 80%) after 20 and 40-day pretreatment at 0°C and -15°C .

Spores were also subjected to a range of higher temperatures (40° – 80°C) for a period of 10 minutes in each case. Results (Table 2) indicated that percentage germination decreased with increase in temperature up to 70°C . No germination was observed when pretreated for 10 minutes at 80°C . Considerable reduction in germination was noted at 50°C and appressoria formation at 70°C when treated for a similar period.

TABLE 2. *Effect of high temperature pretreatment (for 10 minutes in each case)*

Temperature Pretreatment	* % germination with S. E.	* % appressoria formation of germinated conidia with S. E.
Control (without Pretreatment)	79.51 ± 1.45	99.23 ± 0.37
40°C	65.47 ± 2.26	96.81 ± 0.72
50°C	46.67 ± 1.18	95.50 ± 1.24
60°C	28.26 ± 0.74	74.39 ± 1.40
70°C	10.46 ± 0.57	39.47 ± 0.94
80°C	0	0

* 400-500 spores counted
Incubation period—24 hr.
Incubation temperature $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Effect of duration of high temperature (60°C) pretreatment Spores suspended in sterile water were treated for different time periods (10-80 min.) at 60°C . The percentage of germination and appressoria formation were calculated after 24 h of incubation. Results are given in Table 3. Both percentage of germination and appressoria formation declined with increase in time of pretreatment. It is interesting to note that only 2-3% spores germinated when treated for 60 minutes at 60°C but percentage of appressoria formation was more than 40% of the germinated conidia. It suggests that duration of high temperature pretreatment is more effective in inhibiting germination, than appressoria formation.

TABLE 3. Effect of duration of high temperature pretreatment (60°C)

Pretreatment time (minutes)	* % germination with S. E.	* % of appressoria formation of germinated spores with S. E.
Control (without treatment)	88.46 ± 0.62	96.79 ± 0.38
10	28.93 ± 1.11	80.10 ± 1.64
20	14.84 ± 1.15	72.44 ± 1.46
30	10.77 ± 0.63	49.36 ± 1.46
40	7.65 ± 0.75	50.00 ± 2.88
60	2.88 ± 0.19	43.75 ± 3.82
80	0	0

* 400-500 spores counted

Incubation time—24 hr.

Incubation temperature 25°C ± 1°C.

Both germination and appressoria formation decreased significantly with age and higher concentration of spores of *C. dematium*. Similar results with other fungi were also reported by previous workers (Ray and Purkayastha, 1977; Bilgrami and Verma 1978). Failure of fungal spores to germinate when present in large number may be due to competition for space and nutrients.

Prolonged freezing (20 days or more) also significantly reduced both spore germination and appressoria formation. Treatment at very low temperature causes to form ice crystals in the cell or on the membrane, which in turn inactivate germination process. On the contrary, very high temperature treatments also had adverse effect on germination and appressoria formation. For example, conidia of *Neurospora tetrasperma* failed to germinate, when exposed to 60°C for 5 minutes (Lingappa and Sussman, 1959). Present results also sustain the theory that lethal action of higher temperature is usually due to denaturation of proteins as pointed out by Cochrane (1958). Tolerance of *C. dematium* to higher temperature (70°C) for 10 minutes is not uncommon in other fungi. For example, *C. lini* is known to survive 3-4 hr. at 55°C (Cochrane, 1958).

Response of fungi to light also varies considerably in some cases (Emmett and Parbery, 1975). Light stimulated germination and appressoria formation of *C. dematium*. This result supports the findings of other workers (Gassner and Nieman, 1954; Gould and Shaw, 1969; Ray and Purkayastha, 1977).

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