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Conidial germination in Claviceps fusiformis Lov. in relation to physical and ontogenic factors

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Influence of certain physical and ontogenic factors on germination of conidia of *Claviceps fusiformis* Lov., the incitant of pearl miller (*Pennisetum americanum* (L.) Leeke) ergot, was investigated. Maximum germination of conidia was recorded at 24°C and 100% RH. The germination was completely checked in washed conidia. Increasing dilution of honeydew with sterile water as well as drying of conidia for more than 30 minutes caused significant reduction in their germination. Conidia up to the age of 3 days gave significantly more germination than older conidia.

INTRODUCTION

Ergot (*Claviceps fusiformis* Lov.) is a serious disease of pearl millet (*Pennisetum americanum* (L.) Leeke) in India (Ramaswamy 1968; Nene and Singh 1976) and some countries in Africa (Saccas 1954; Loveless 1967; King 1975). Although role of conidia in disease epidemiology has been very well demonstrated (Reddy et al. 1969; Siddiqui and Khan 1973; Thakur and Williams 1980; Sharma and Chohan 1982), the study of various factors which adversely affect of favour the germination of conidia has not received much attention. The present communication deals with germination of conidia under the influence of physical and ontogenic factors.

MATERIAL AND METHODS

Unless mentioned otherwise, following procedrue was adopted to study the effest of various factors on conidial germination. Conidia were obtained from the honeydew state of SKR isolate maintained on earheads of pearl millet var. BJ 104 in a polythene house. The experiments were conducted by Slide germination test. Honewdew collected 3 days after its first appearance on the earhead was smeared on a clean glass slide. The smear was covered with a thin film of sterile water. Two smeared slides were kept on glass rods inside a humid chamber. A humid chamber was prepared by lining both bottom and cover of a Petri dish with double layers of moist filter paper. Each treatment had 4 slides. In each slide, conidia within 5 randomly selected microscopic fields were examined for recording per cent germination. Observations were recorded 24 h after in ubation at 25 $\pm$1°C.

The honeydew smeared slides were exposed to 10, 15, 20, 25, 30, 35, 40 and 45°C. Since germination was found to be more effective at 20, 25 and 30°C than at other temperatures, another experiment was laid to find out the optimum temperature more precisely. In this experiment, the slides were exposed to 20, 22, 24, 26, 28 and 30°C.

Slides smeared with honeydew were not covered with thin film of water. The slides were exposed to 50, 60, 70, 80, 90 and 100 per cent RH. Different RH levels were pr duced by mixing different proportions of sterilized water and concentrated sulphuric acid (Buxton and Mellanby 1934). Ten ml of the required mixture was placed in the bottom of a Petri dish in which the smeared slides were accommodated. The Petri dishes were then sealed with alkathene tape. The dishes were incubated at 25 $\pm$ 1°C for 24 h.

Honeydew collected 3 d after its first appe rance on the earhead was suspended in sterile water and shaken gently for 15 min. The suspension was centrifuged at 2000 r.p.m. for 10 min and the supernatant was poured-off. The process of centrifugation was repeated for 5 cycles. The spores were fina'ly resuspended in sterile water and a thin film of spore suspension was applied on a clean glass slide. The slides were placed in humid chambers and incubated at 25 $\pm$ 1°C for 24 h. The experiment was repeated 4 times. Proper control was maintained.

The washed conidia did not germinate. To investigate whether this was due to absence of honeydew, the following experiment was conducted. Honeydew was diluted in the following proportions. The smeared slides were placed in humid chambers and incubated at 25 $\pm$ 1°C for 24 h.

1 —	No dilution and without film cover of water	Honeydew used as such. The smear not covered with thin film of water.

2 –	No dilution and with film cover of water	Honeydew used as such. The smear covered with a thin film of water.
3 –	Dilution 1 : 1	Honeydew and sterile water mixed in equal proportions by volume and smeared on clean glass side.
4 –	Dilution 1 : 4	Honeydew diluted 4 times with sterile water and smeared on clean glass slide.
5 –	Dilution 1 : 10	Honeydew diluted 10 times with sterile water and smeared on clean glass slide.
6 –	Dilution 1 : 50	Honeydew diluted 50 times with sterile water and smeared on clean glass slide.
7 –	Dilution 1 : 100	Honeydew diluted 100 times with sterile water and smeared on clean glass slide.

The smeared slides were air-dried at room temperature (16.5-28°C) for different durations (0 min (no drying), 30 min, 1, 4, 8, 1 , 16, 20, 24, 28, 32 and 36 h). The smears were then covered with a thin film of sterile water. The slides were accommodated in humid chambers and incubated at 25 ±1°C for 24 h.

Conidia of different ages (honeydew at the time of its first appearance as whitish droplets was considered as O-d-old) were obtained from the infected earheads. The honeydew was smeared on the slides and the smear was covered with a thin film of sterile water. The smeared slides were placed in humid chambers and incubated at 25 ±1°C for 24 h. The spore ages utilized in the experiment were: 1, 3, 5, 8 and 20 d.

RESULTS

Germination was mostly from the centre of conidia. In most cases, a conidium produced single germ tube bearing a secondary conidium at the tip.

Germination (73.60%) was significantly more at 25°C. It was drastically reduced at temperatures below 20 and above 30°C and was completely checked at 45°C. In the subsequent experiment, maximum germination was recorded at 24°C but it was statistically at par with that at 2 and 26°C (Table 1).

Every 10% increase in RH caused significant increase in conidial germination except that germination at 80 and 90% RH was at par. Maximum germination was recorded at 100% RH (Table 2).

Germination was completely checked in washed conidia as against 69.60% germination in unwashed conidia.

Increasing dilutions of honeydew up to 1:50 caused significant reduction in conidial germination (Table 2). Germination declined sharply in honeydew diluted 10 times or more. Presence of thin film of water on undiluted honey caused slight increase in conidial germination.

Table 1

Effect of temperature /A/ and of narrow ranges of temperature /20 and 30 °C/ /B/ - on germination of conidia

	Temperature /°C/	Germination /%/
A	10	23.80 /29.02/
	15	34.42 /35.91/
	20	65.82 /54.23/
	25	73.60 /59.10/
	30	59.35 /50.68/
	35	32.35 /34.65/
	40	8.12 /16.49/
	45	0 / 0 /
	LSD /5 %/	3.17
B	20	69.75 /56.65/
	22	72.70 /58.51/
	24	73.22 /58.85/
	26	70.30 /56.95/
	28	60.15 /50.86/
	30	57.85 /49.40/
	LSD /5 %/	2.09

Figures in parentheeis are angular valuea

Table 2

Effect of RH /A/ and of dilution of honeydew /B/ - on germination of conidia

RH /%/	Germination /%/
A 50	3.18 /10.19/
60	5.01 /12.92/
70	35.20 /36.37/
80	65.62 /54.11/
90	68.53 /55.90/
100	76.30 /60.89/
LSD /5 %/	2.48
Dilution /honey:water/	
B	
No dilution and without cover of film of water	76.22 /60.88/
No dilution on and with cover of film of water	77.95 /62.05/
1:1	51.00 /45.57/
1:4	41.72 /40.23/
1:10	10.50 /20.95/
1:50	4.40 /11.96/
1:100	2.65 / 8.09/
LSD /5 %/	4.42

Figures in parenthesis are angular values

Table 3
Effect of spore drying /A/ and of conidial age /B/ - on germination of conidia

	Duration of drying /h/	Germination /%/
A		
	0.5	65.30 /53.95/
	1	62.31 /52.14/
	4	51.20 /45.69/
	8	42.55 /40.71/
	12	28.17 /32.03/
	16	14.97 /22.72/
	20	7.10 /15.28/
	24	2.92 / 7.26/
	28	2.20 / 7.17/
	32	0.75 / 3.75/
	36	0.75 / 2.92/
	0 /check/	71.95 /58.04/
	LSD /5 %/	5.07
	Age of conidia /days/	
B	1	77.45 /61.71/
	3	72.57 /58.31/
	5	55.65 /48.25/
	8	37.95 /38.01/
	20	15.22 /22.87/
	LSD /5 %/	3.77

Figures in parenthesis are angular values

Drying of conidia for more than 0.5 h caused significant decrease in germination (Table 3). Germination percentage did not differ significantly in spores dried for 24 to 36 h.

Maximum germination was recorded when conidia were 1-d-old and it was at par with the germination of 3-d-old conidia. There was significant reduction in germination of conidia that were more than 3-d-old (Table 3).

DISCUSSION

Spore germination was higly dependent upon temperature. Inability of the fungus to germinate at higher temperatures could account for absence of the disease in seasons having high temperatures during the crop flowering period. More severity of the disease during humid weather (Ramaswamy 1968; Siddiqui et Khan 1973; Gaur et al. 1975; King 1975; Saxena et al. 1978) may partly be due to the fact that conidia germinate well only when RH is higher than 70%. The poor secondary spread of the disease under dry conditions may also be due to reduced germinability of the conidia resulting from their desiccation for more than 0.5 h.

The washed conidia could not germinate. Either the washing damaged the spores or it removed germination stimulant(s) present in the honeydew. A third possibility is that nutrients were leached out of spores during washing process. Reduction of germination of conidia obtained from diluted honeydew also indicates the presence of some germination promoting factor(s) in undiluted honeydew. The physico-chemical factors and their interactions reducing the conidial germination in diluted honeydew deserve further investigation.

Germination percentage of conidia was such influenced by their age. This observation clearly warns that in investigations on conidial germination and studies concerning epidemiology and evaluation of host resistance, conidia of proper and same age must be employed. Since under natural conditions, the proportions of young and old conidia in a drop of honeydew may vary from time to time, the interactions of conidial age with host and environment should influence the disease spread. Studies on these aspects are needed to improve our understanding of anatomy of the ergot epidemic.

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