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THE EFFECT OF TEMPERATURE ON SPORE GERMINATION, GROWTH AND APPRESSORIUM FORMATION OF *COLLETOTRICHUM GLOEOSPORIOIDES* AND *DOTHIORELLA AROMATICA*

F D N DENNER, J M KOTZÉ & J F PUTTERILL
DEPARTMENT MICROBIOLOGY AND PLANT PATHOLOGY, UNIVERSITY OF PRETORIA

SUMMARY

*Conidial germination of **Colletotrichum gloeosporioides and Dothiorella aromatica** was affected by temperature. The optimal temperature for germination was between 25 and 30°C. No germination occurred at 5 and at 40°C respectively. C. **gloeosporioides** produced appressoria **in vitro** while **D. aromatica** produced long germ tubes without appressoria. Appresorium formation was more sensitive to temperature than was germination. Appressoria were produced at temperatures ranging from 10 to 30°C. Temperature requirements for mycelial growth of **C. gloeosporioides** and of D. **aromatica** were similar to those for conidial germination. The information is useful for the prediction of infection periods.*

OPSOMMING

*Temperatuur het 'n invloed gehad op spoorontkieming van **Colletotrichum gloeosporioides en Dothiorella aromatica. Die** optimum temperatuur vir ontkieming was tussen 25 en 30°C. Geen ontkieming het plaasgevind by 5 en 40°C nie. C. **gloeosporioides** het appressoriums **in vitro** gevorm, terwyl **D. aromatica** lang kiembuise sonder appressoriums gevorm het. Appressoriumvorming was meer sensitief vir temperatuur as ontkieming. Temperature tussen 10 en 30°C was gunstig vir appressoriumvorming, maar infeksies is onwaarskynlik onder 15 en bo 28°C. Temperatuurbehoeftes van **C. gloeosporioides en D. aromatica** was bate dieselfde as die vir spoorontkieming. Die inligting is nuttig vir die voorspelling van infeksieperiodes.*

INTRODUCTION

Post-harvest diseases are a major factor hampering export of avocados from South Africa (Darvas, 1982). Apart from the stem-end rot complex, *Colletotrichum gloeosporioides* and *Dothiorella aromatica are* the most important pathogens causing fruit decay (Kotzé & Darvas, 1985).

Forecasting infection periods of *C. gloeosporioides* and *D. aromatica* may assist in better timing of fungicide applications. The meteorological factors which form the basis of such predictions usually are prevailing temperatures and wet periods (Tarr, 1972). Although it is known that temperature affects spore germination and infection, which in turn influence disease development, very little information is available on this aspect of the epidemiology (Young, Prescott and Saari, 1978). The period required for infection will be largely influenced by prevailing temperatures, provided wetting is unlimited. If the wetting period is short, infection will only be successful if the temperature is optimal.

The purpose of the present study was to evaluate the influence of temperature on germination of *C. gloeosporioides and D. aromatica for the* possible inclusion of those factors in a disease forecasting model.

MATERIALS AND METHODS

TABLE 1
Effect of temperature on the linear growth of *C. gloeosporioides* and *D. aromatica* over period of 72 h.

Pathogen	Temperature	Mean radial growth (mm)					
	°C	12 h	24 h	36 h	48 h	60 h	72 h
C. gloeo-sporioides	5	0	0	0	0	0	0 b
	10	0	0	9.5	10.1	10.5	11.3 b
	15	0	10.7	15.2	20.6	27.0	33.8 b
	20	11.6	23.6	39.3	50.8	63.3	76.5 a
	25	13.0	27.1	43.3	56.0	70.4	85.0 a
	28	15.6	30.7	47.9	60.8	76.2	85.0 a
	30	14.2	28.7	44.1	56.8	70.4	83.3 a
	35	10.5	12.5	15.3	19.3	24.4	27.7 b
	40	0	0	0	0	0	0
D. aromatica	5	0	0	0	0	0	0 b
	10	0	0	0.5	1.1	1.8	2 b
	15	0	1.0	2.2	3.2	5.9	8.8 b
	20	1.7	6.2	10.7	15.6	20.5	26.0 b
	25	3.5	16.6	25.9	35.6	43.2	49.5 a
	28	3.9	19.5	26.7	37.4	46.8	52.3 a
	30	3.5	18.8	29.4	39.3	46.2	51.9 a
	35	0.9	3.3	5.2	6.8	8.8	10.3 b
	40	0	0	0	0	0	0 b

Means followed by the same letter do not differ significantly according to Duncan's multiple range test (p=0.05).

2. Effect of temperature on germination of spores on glass slides

Conidia of *C. gloeosporioides* were harvested from cultures grown on PDA for 6 d. A spore suspension containing 7.8x 10^4 spores ml^{-1} was prepared in sterile distilled water. In the case of *D. aromatica*, it was necessary to culture the fungus aseptically on fruit segments under near UV-light to stimulate sporulation (Darvas, 1982). A spore suspension containing 4.7 x 10^4 spores ml^{-1} was prepared in distilled water. Glass slides and moist chambers were prepared by using the method of Felton and Walker (1946) and these were incubated at the same temperatures described for linear growth measurement. Ten μl of spore suspension of each fungus was placed at each end of a slide. A slide was removed form each moist chamber at 3, 4, 6, 8, 10, 14 and 24 h intervals respectively. Each drop was covered with a coverslip and examined under a light microscope. For quantitative studies 50 spores per drop were counted and considered as germinated if a germ tube half the length of the spore had formed. All treatments were replicated twice.

3. Germination of spores of *C. gloeosporioides* and *D. aromatica* on leaves for scanning electron microscope viewing

Avocado leaves were dipped in spore suspensions of 1 x 10^5 spores ml^{-1} of *C. gloeosporioides and D. aromatica.* The inoculated leaves were placed in a moist chamber at 28°C for 10 h to facilitate spore germination. The leaves were prepared for SEM viewing as previously described by Denner (1985).

RESULTS

1. Effect of temperature on linear growth of *C. gloeosporioides* and *D. aromatica*

The optimum temperature for growth of *C. gloeosporioides* and *D. aromatica* was 28°C (Table 1). Above 30 and below 10°C, growth was retarded and no growth occurred at 40 and at 5°C. The growth rate of *C. gloeosporioides was* greater than that of *D. aromatica.*

2. Effect of temperature on germination of spores on glass slides

a). *C. gloeosporioides*

Conidial germination of *C. gloeosporioides* occurred over a range between 10 and 35°C (Table 2). The optimum temperature for germination after 3 h was between 25 and 30°C. Germination ceased at

b). *D. aromatica.*

The optimum temperature for germination of *D. aromatica* was between 25 and 30°C (Table 4). Germination ceased at 5 and at 40°C. No appressorium formation was observed *in vitro.*

c). Germination of spores of *C. gloeosporioides* and *D. aromatica* on leaves for scanning electron microscope examination.

C. gloeosporioides produced appressoria, while *D. aromatica* produced long germ tubes. The appressoria attached to the leaf surface (Fig. 1 and 2).

TABLE 2

Effect of temperature on the conidial germination of *C. gloeosporioides* on glass slides over a period of 24 h.

Temperature °C	Percentage spore germination						
	3 h	4 h	6 h	8 h	10 h	14 h	24 h
5	0	0	0	0	0	0	0 c
10	0	0	0	0	0	2	19 c
15	0	0	1	4	26	65	59 b
20	2	28	58	91	82	84	84 a
25	58	65	56	66	74	69	81 a
28	28	60	62	71	86	76	77 a
30	10	23	46	63	81	88	89 a
35	3	23	66	84	80	87	83 a
40	0	0	0	0	0	0	0 c

Means followed by the same letter do not differ significantly according to Duncan's multiple range test (p=0.05).

TABLE 3

Effect of temperature on appressorium formation of *C. gloeosporioides* on glass slides over a period of 24 h.

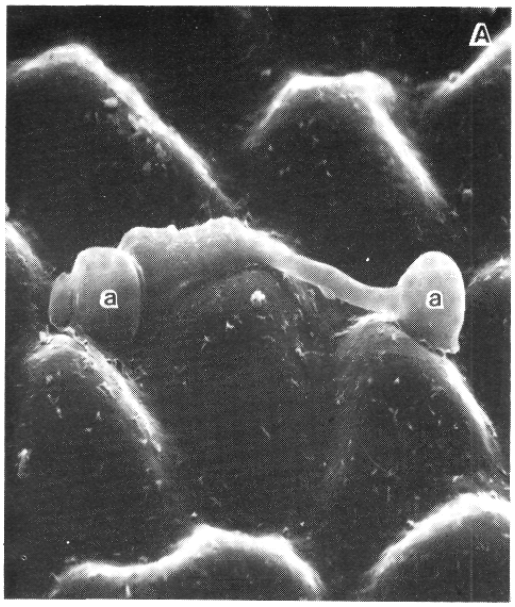
Temperature °C	Percentage appressoria formed						
	3 h	4 h	6 h	8 h	10 h	14 h	24 h
5	0	0	0	0	0	0	0 c
10	0	0	0	0	0	0	0 c
15	0	0	0	0	7	38	56 bc
20	0	1	5	24	67	76	81 a
25	0	0	10	21	39	51	71 ab
28	0	0	6	16	39	44	77 ab
30	0	0	0	0	0	0	12 c
35	0	0	0	0	0	0	0 c
40	0	0	0	0	0	0	0 c

Means followed by the same letter do not differ significantly according to Duncan's multiple range test (p = 0.05).

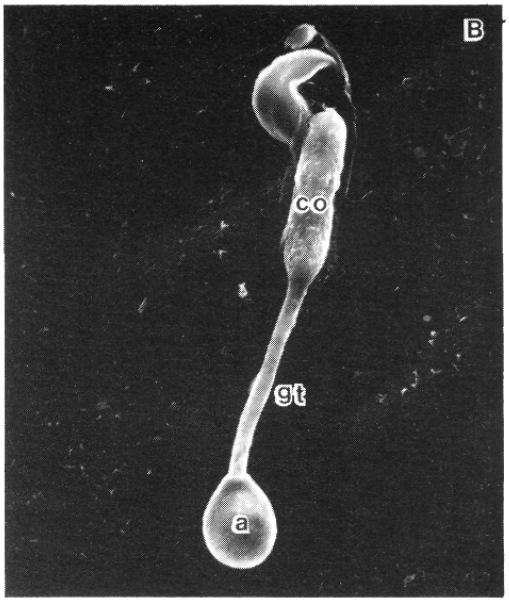
TABLE 4

Effect of temperature on conidial germination of *D. aromatica* on glass slides over a period of 24 h.

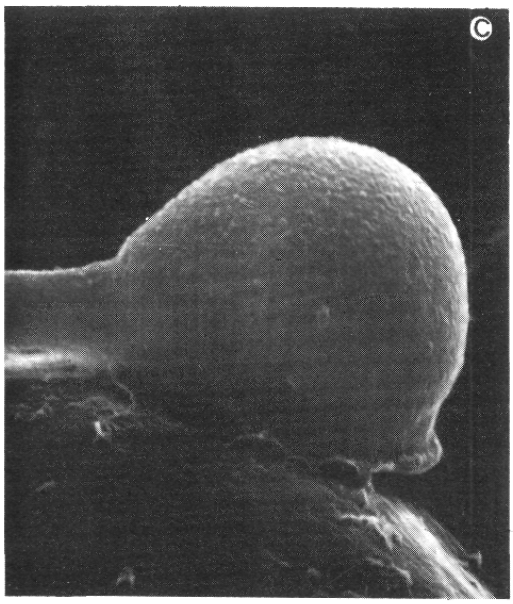
Temperature °C	Percentage spore germination						
	3 h	4 h	6 h	8 h	10 h	14 h	24 h
5	0	0	0	0	0	0	0 d
10	0	0	0	0	0	0	0 d
15	0	0	9	13	31	22	36 cd
20	31	10	13	27	24	65	89 bc
25	20	13	30	60	59	74	85 b
28	29	31	83	95	97	97	89 a
30	43	23	83	92	87	97	99 a
35	17	36	37	60	71	67	74 ab



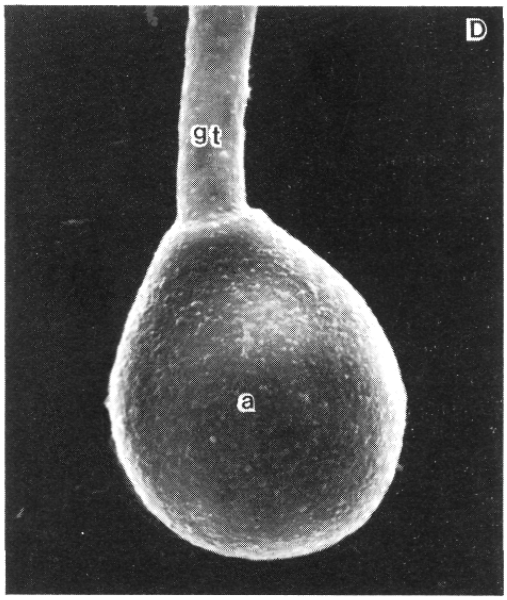
A:
Bipolar germination with two appressoria (a), one on each germ tube tip (1500x).



B:
Germ tube (gt) and appressorium (a) growing from a conidium (co) during monopolar germination (2000x).

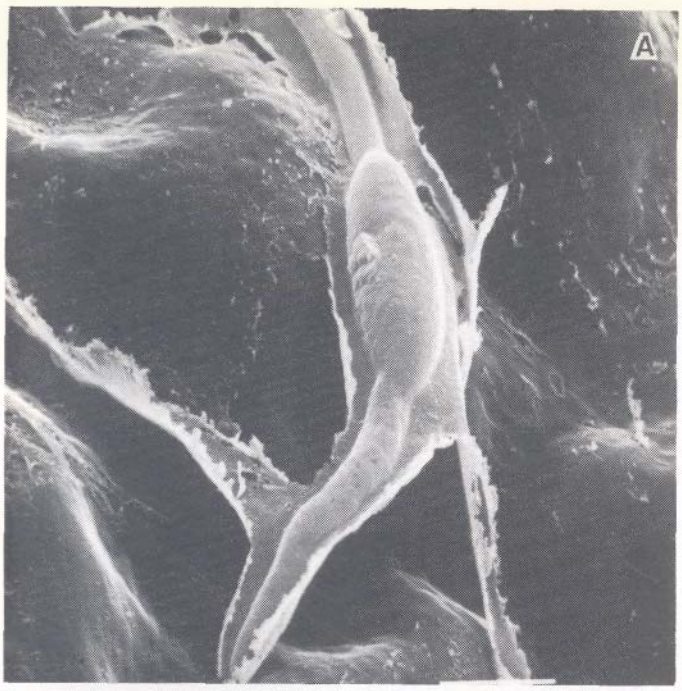


C:
Attachment of an appressorium on the leaf surface (8000x).



D:
Germ tube (gt) and appressorium (a) (8000x).

Fig. 1. Scanning electron photomicrographs of avocado leaves showing germination of and appressorium formation by conidia of *C. gloeosporioides*.



A: Bipolar germination (8000 x).

B: Germ tube (gt) growing from a conidium (co) during monopolar germination (1000 x).

Fig 2. Scanning electron photomicrographs of germinated conidia of *D. aromatica* on leaves.

DISCUSSION

The results obtained with the *in vitro* germination of *C. gloeosporioides* and *D. aromatica* clearly show how temperature affects spore germination. Germination of both pathogens occurred over a wide temperature range (10-35°C). Approximately 3 h were required for germination initiation to take place at 20-35°C. Temperatures below 20°C delayed spore germination. At 15°C both pathogens germinated after 6 h and at 10°C *C. gloeosporioides* germinated after 14 h and *D. aromatica* after 24

30°C. The optimum temperature range for conidial germination was broader than that for appressorium formation. Similar results were obtained for other *Colletotrichum* spp. (Ishida & Akai, 1969; Miele & Lukezic, 1972). Temperature required for optimal mycelial growth of *C. gloeosporioides* and *D. aromatica* were very similar to that for conidial germination.

From these studies it is apparent that infection will not take place below 10°C and that a period of at least 10 h of wetness is required at 15°C before infection is possible. As the infection process that follows after appressorium formation will take some hours, it appears as if temperatures below 15°C will prevent extensive infections. The same is true of temperatures above 28°C. Between 20°C and 25°C infections often occur in the higher rainfall regions of the Transvaal Lowveld and Natal during summer.

Results obtained with electron micrographs of the infection pattern of *D. aromatica* and *C. gloeosporioides* showed that *D. aromatica produced* no appressoria, whereas appressorium formation was very conspicuous in C. *gloeosporioides.* These results correlate very well with the temperature studies *in vitro,* where it was found that *D. aromatica* only produced long germ tubes without appressoria.

Besides temperature, other meteorological factors such as moisture need to be investigated under controlled conditions in the laboratory in order to formulate a disease predicting model. This model will have to be tested under field conditions to establish field data.

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