STUDIES ON THE KINETICS OF FUNGICIDAL ACTION

PART II. THE EFFECT OF TEMPERATURE ON THE VIABILITY OF *PENICILLIUM NOTATUM* SPORES IN WATER AND SOLUTIONS OF PHENOL

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The mean Q_{10} value for the mortality of *Penicillium notatum* spores in water from 50 to 55° was 881. In 1 to 1·25 per cent solutions of phenol from 15 to 40° it was 3·4; higher values were obtained for 0·75 and 0·875 per cent phenol.

THE effect of temperature on the reaction velocity of fungicidal action has not been greatly studied, yet for the practical use of fungicidal solutions it is a parameter worthy of as much consideration as the concentration of the solutions.

This paper reports the effect of temperature on the viability of *Penicillium notatum* spores in aqueous suspensions with and without phenol.

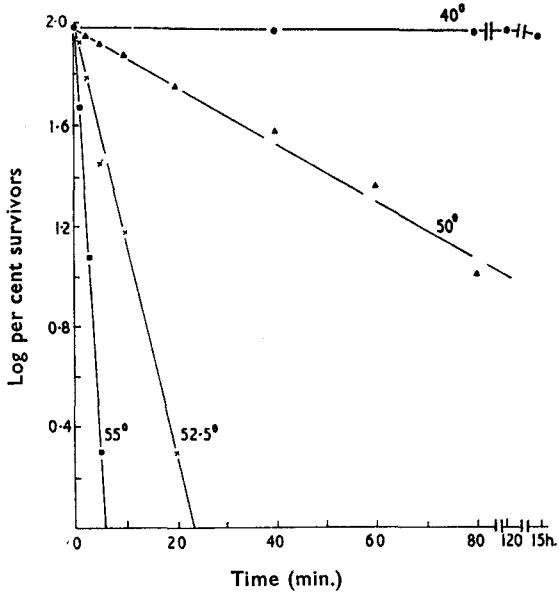

FIG. 1. Effect of temperature on the viability of *P. notatum* spores in water.

EXPERIMENTAL

The method of preparation of *P. notatum* spore suspensions and the slide-germination technique used were the same as those described previously (Chauhan and Walters, 1961).

The viability of spores in water was determined by adding 0·5 ml. of

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an aqueous suspension containing 10^{10} spores per ml. to 9·5 ml. of water at the required temperature. 1 ml. quantities were withdrawn at intervals and added to 9 ml. of water at 25° to stop the reaction. One drop aliquots were then mixed with equal volumes of double-strength Horowitz fluid medium. Counts were made after 12 hr. incubation and up to 48 hr. when there were few or no survivors. This was necessary since spores damaged by heat were found to germinate after longer lag phases than phenol treated spores.

To examine the effect of temperature on spores in solutions of phenol, 10 ml. of phenol solutions of twice the required concentration were pipetted into 10 ml. suspensions containing 10^8 spores per ml. The solutions and suspensions were previously equilibrated to the required temperature in the range 15 to 40° ± 0·1°. The reactions were allowed to proceed for 10 min. and in the case of 1·125 per cent phenol for various time intervals. Differential counts of 100 spores were made in duplicate afer 12 to 24 hr.

Results

The effect of temperature on the viability of spores in water from 40 to 55° is shown in Fig. 1. At 60° there were less than 1 per cent survivors after 30 sec., and after 60 sec., following incubation for 67 hr., only 3 spores in approximately 10^6 germinated.

The rate of germination and the percentage viability of spores exposed for 10 min. to 0·5 per cent phenol from 15 to 40° were the same as for spores in water whereas the activity of 0·75 to 1·25 per cent solutions increased markedly with temperature (Figs. 2, 3 and 4).

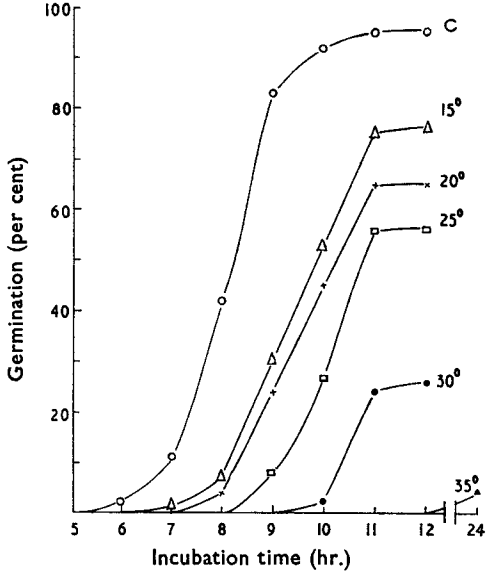

FIG. 2. Effect of temperature on the viability and rate of germination of *P. notatum* spores exposed to 1 per cent phenol for 10 min.

C = Control (spores in water at 25°).

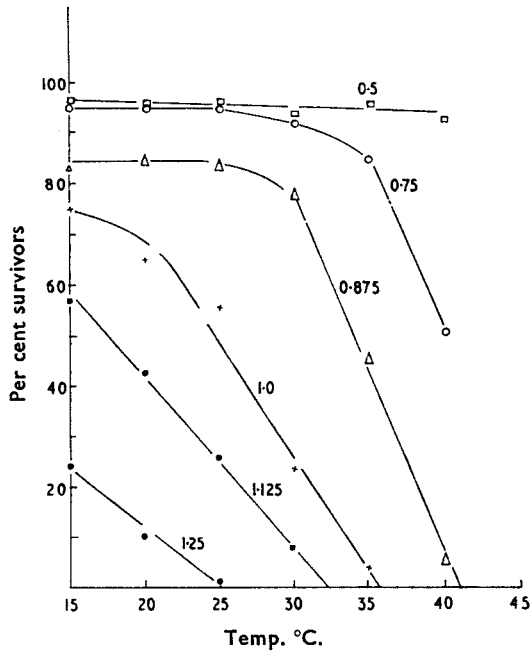

FIG. 3. Per cent survivor-temperature curves for *P. notatum* spores exposed to 0·5–1·25 per cent solutions of phenol for 10 min.

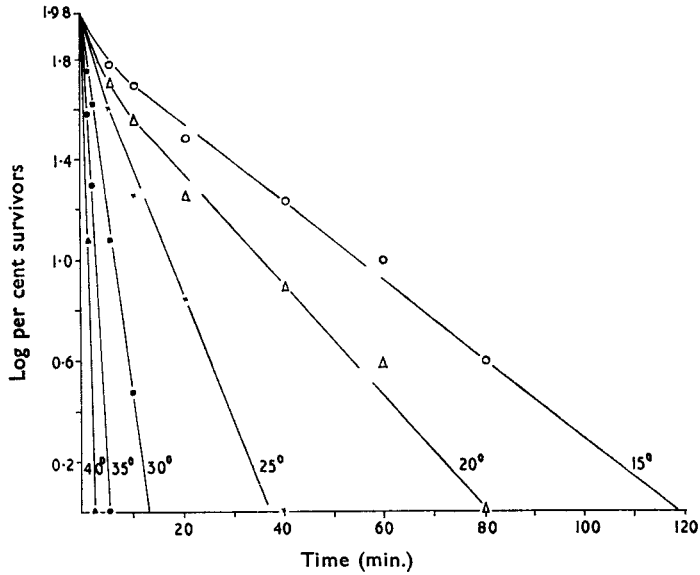

FIG. 4. Log per cent survivor-time curves for *P. notatum* spores in 1·125 per cent phenol. Viability before addition of phenol = 96 per cent.

Discussion

The destruction of *P. notatum* spores in 2 min. in water at 60° is typical of fungal spores. Relatively few species possess high resistance to moist heat, a notable example being the conidia of *Micromonospora vulgaris* which can withstand 100° for 45 min. (Cochrane, 1958; Erikson, 1952, 1955; Hull, 1939).

The times required for 50 per cent mortality of *P. notatum* spores in water (Fig. 1) were substituted in Phelps' (1911) equation to obtain the values of θ and Q_{10} given in Table I. These results show that when the sensitivity of an organism to a lethal agent is confined to a narrow temperature range, high Q_{10} values are obtained. High values were also obtained by Chick (1910) and Smith (1923).

TABLE I

TEMPERATURE COEFFICIENTS FOR THE MORTALITY OF *P. notatum* SPORES IN WATER

Temperature	θ	Q_{10}
50 –52·5°	2·24	3177
52·5–55°	1·74	254
50 –55°	1·97	881

TABLE II

TEMPERATURE COEFFICIENTS FOR THE MORTALITY OF *P. notatum* SPORES IN SOLUTIONS OF PHENOL

Phenol concentration (per cent)	Temperature (°C)	θ	Q_{10}
0·75	30–35	1·23	7·9
	35–40	1·39	27·0
0·875	25–30	1·09	2·4
	30–35	1·29	12·8
	35–40	1·30	13·8
1·0	15–20	1·10	2·6
	20–25	1·07	2·0
	25–30	1·21	6·7
	30–35	1·18	5·2
1·125	15–20	1·09	2·4
	20–25	1·10	2·6
	25–30	1·14	3·7
1·25	15–20	1·10	2·6
	20–25	1·15	4·0

The rate of death of *P. notatum* spores in water and 1·125 per cent phenol solution follows a first order reaction except for an initial higher death-rate in the phenol solution at lower temperatures (Figs. 1 and 4). These results differ from those of Smith (1921, 1923) who obtained mainly sigmoidal curves for *Botrytis cinerea* spores.

The temperature coefficients (Table II) calculated from reaction velocities (Fig. 3) are in good agreement for phenol concentrations of 1 to 1·25 per cent. Below 30° and 25° respectively, for 0·75 and 0·875 per cent phenol, the effect of temperature was negligible. Above these temperatures

the effect increased markedly, confirming the existence of a minimum temperature for the activity of low concentrations of phenol (Jordan and Jacobs, 1946b). Berry and Michaels (1950) also obtained higher Q_{10} values for the weaker concentrations of monoalkyl ethers of ethylene glycol examined. The values of n calculated from the results in Fig. 3 increased slightly with increase in temperature (Table III).

TABLE III

CONCENTRATION EXPONENTS FOR THE MORTALITY OF *P. notatum* SPORES IN SOLUTIONS OF PHENOL

Phenol concentration (per cent)	Temperature (°C)	n
1 to 1·25	15	7·9
1 to 1·25	20	8·0
1 to 1·25	25	9·6
0·875 to 1·125	30	9·9
0·75 to 1·0	35	11·3
0·75 to 0·875	40	9·8

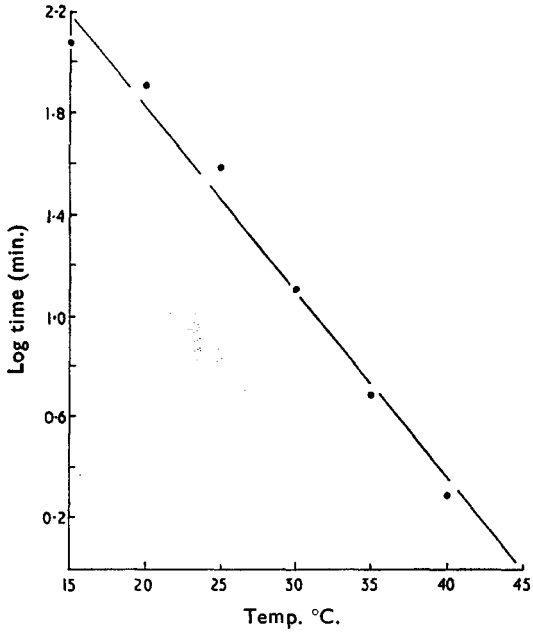

FIG. 5. Log time-temperature relationship for 99 per cent mortality of *P. notatum* spores in 1·125 per cent phenol.

The times required for 99 per cent mortality of *P. notatum* spores in 1·125 per cent phenol at temperatures of 15 to 40° were calculated from the results shown in Fig. 4. The calculated log. time-temperature regression for this percentage mortality was linear (Fig. 5) and its slope, log θ, gives mean values of 1·19 and 5·7 for θ and Q_{10}. These values compare favourably with those shown in Table II. Similar values of Q_{10} have

been obtained for phenol with bacteria (Chick, 1908; Jordan and Jacobs, 1946a; Tilley, 1942).

The marked difference in the Q_{10} values obtained for the death of *P. notatum* spores exposed to moist heat and phenol indicates an essential difference in the mode of action of these two lethal agents.

References

Berry, H. and Michaels, I. (1950). *J. Pharm. Pharmacol.*, **2**, 243–249.
Chauhan, N. M. and Walters, V. (1961). *Ibid.*, **13**, 470–478.
Chick, H. (1908). *J. Hyg., Camb.*, **8**, 92–158.
Chick, H. (1910). *Ibid.*, **10**, 237–286.
Cochrane, V. W. (1958). *Physiology of Fungi*, p. 424, London: Chapman and Hall Ltd.
Erikson, D. (1952). *J. gen. Microbiol.*, **6**, 286–294.
Erikson, D. (1955). *Ibid.*, **13**, 127–135.
Hull, R. (1939). *Ann. appl. Biol.*, **26**, 300–322.
Jordan, R. C. and Jacobs, S. E. (1946a). *J. Hyg., Camb.*, **44**, 243–248.
Jordan, R. C. and Jacobs, S. E. (1946b). *Ibid.*, **44**, 249–255.
Phelps, E. B. (1911). *J. infect. Dis.*, **8**, 27–38.
Smith, J. H. (1921). *Ann. appl. Biol.*, **8**, 27–50.
Smith, J. H. (1923). *Ibid.*, **10**, 335–347.
Tilley, F. W. (1942). *J. Bact.*, **43**, 521–525.