GERMINATION OF *PERONOSPORA TABACINA*: EFFECT OF TEMPERATURE

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Summary

In an investigation into the effect of temperature on the germination and germ-tube length of *Peronospora tabacina* Adam using an *in vitro* technique the following results were obtained:

(i) Some germination occurred after incubation for 1 hr over the temperature range 20–30°C, while after 12 hr there was no significant difference in germination response over the range 8–27°C.

(ii) Germ-tube growth was initially most rapid at the temperatures 24–27°C; however, after 12 hr, greatest growth had occurred over the temperature range 15–17·8°C.

(iii) Multispore isolates of *P. tabacina* from Canberra and Parada, N. Qld., could be distinguished from an isolate from Manjimup, W.A., on the basis of differences in their percentage germination and germ-tube length after incubation for 5 hr.

The significance of temperature as a factor effecting germination and its relationship to the epidemiology of blue mould (*P. tabacina*) under field conditions is briefly discussed.

I. INTRODUCTION

Angell and Hill (1931, 1932) reported that the germination of freshly detached conidia of *Peronospora tabacina* Adam in water on glass slides incubated at 16–18°C for 24 hr was very uneven. This variability in germination in water of *P. tabacina* has been confirmed by several workers, especially Clayton and Gaines (1945). Wolf *et al.* (1934), Armstrong and Sumner (1935), and Clayton and Gaines (loc. cit.) make reference to the effect of temperature on the germination of North American isolates of *P. tabacina* but made no detailed studies in this field. No quantitative temperature studies in relationship to the germination of Australian isolates of this fungus have been reported.

In this communication is described a study of progressive germination and germ-tube growth of one isolate and the comparative germination and germ-tube growth of three Australian isolates over the temperature range 0–35°C.

II. MATERIALS AND METHODS

Preliminary experiments indicated that germination of *P. tabacina* was stimulated by drops of water which had been in contact with tobacco leaves for several hours (cf. Brown 1922). An aqueous solution of this unknown factor was obtained by placing drops of sterile water onto leaves of young tobacco plants in a humidity cabinet at 20°C. After 24 hr the drops were removed, bulked, and centrifuged to remove any solid matter. The supernatant was stored at —15°C.

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Conidia to be tested for germination were prepared by removing non-sporulating infected leaf areas from plants and incubating them overnight under optimal conditions for sporulation (Cruickshank 1958). Conidial suspensions were prepared by shaking the sporulating leaf tissues in sterile water and filtering them through sterile muslin. The suspensions were then centrifuged at 2000 r.p.m. for 2 min and the supernatants decanted. The conidia were resuspended in sterile water and the concentrations adjusted to *c.* 50,000 conidia per ml. Test tubes of melted "Bacto" prune agar (6 ml) were seeded with 1·5 ml of conidial suspension in a water-bath at 40°C. The seeded agar was held in the bath for 30 sec and then poured into 9-cm dia. petri dishes. Aliquots of 0·5 ml of the germination stimulant, and five blocks (5 by 5 by 1 mm) of the seeded agar were dispensed into watch-glasses (4-cm dia.). Each watch-glass was placed in an individual small petri dish. In order to prevent germination prior to the initiation of treatments all preparative operations were carried out at 4°C and the conidia suspensions and seeded agar were stored at 0°C when not actually in use.

Petri dish units containing watch-glass, seeded agar blocks, and ambient solution represented the basic test unit in the experiments described in the next section. Incubators ranging from 1·5 to 34±0·5°C were used for the temperature treatments. At the cessation of the exposure periods the conidia were killed with 1% formalin and stained with 1% cotton blue in aqueous solution. The agar blocks were then examined microscopically. Percentage germination was determined on a quantal basis by recording the number of spores germinated in the first 100 spores counted per agar block. Germ-tube length was determined by classifying the first 10 germ tubes from each agar block on the basis of their lengths into five classes with the aid of an eyepiece graticule calibrated in microns (1 division = 108 μ).

The germination data was analysed using the angular transformation. The square-root transformation was used for the analysis of the germ-tube length data.

III. Experimental

(*a*) *Effect of Temperature and Time Combinations on Germination Response and Germ-tube Length*

Conidia of the Canberra isolate of *P. tabacina* were used in this experiment. Petri dish units were prepared as described above and seven were placed in incubators at each of the following temperatures: 1·9, 4·0, 8·6, 15·2, 17·8, 20·4, 23·9, 27·2, and 30·6°C. They were withdrawn individually from each incubator in the series after 1, 2, 4, 6, 8, 10, and 12 hr and the spores immediately killed. Results of this time course study are graphically illustrated in Figures 1 and 2. The three-dimensional diagrams represent the change in percentage germination and germ-tube length as temperature is increased over the range 1·9–30·6°C and the times of exposure to the temperatures within the range are increased from 1 to 12 hr.

Examination of the germination data showed that after 1 hr some germination had occurred at temperatures between 20·4 and 30·6°C. The percentage at 27·2°C was, however, significantly greater ($P<0·001$) than that at any other temperature.

After 2 hr the percentage at 27·2°C was still significantly greater ($P<0·01$) than that for any other treatment. At temperatures below 20°C and above 30°C there was at this time less than 50% germination while no germination had occurred at 1·9 or 4°C. The responses at the end of the 6-hr exposure could be grouped into five classes, viz.

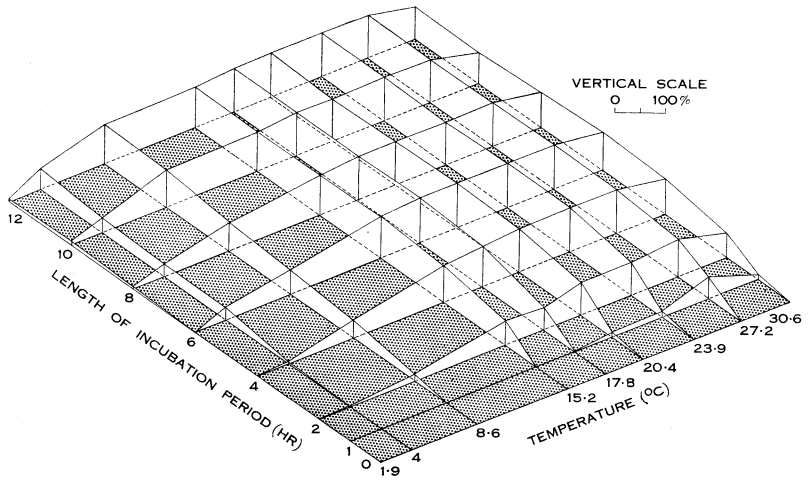

Fig. 1.—Relationship between mean germination response, temperature, and time.

1·7°C (zero germination); 4°C (28·4%); 8°C (50%); 15·2–27·2°C (88% mean value); 30·6°C (70·4% germination). The differences between classes were significant ($P<0·001$). At the end of the experiment (12 hr) the range over which no significant difference ($P<0·01$) in response occurred extended from 8–27°C.

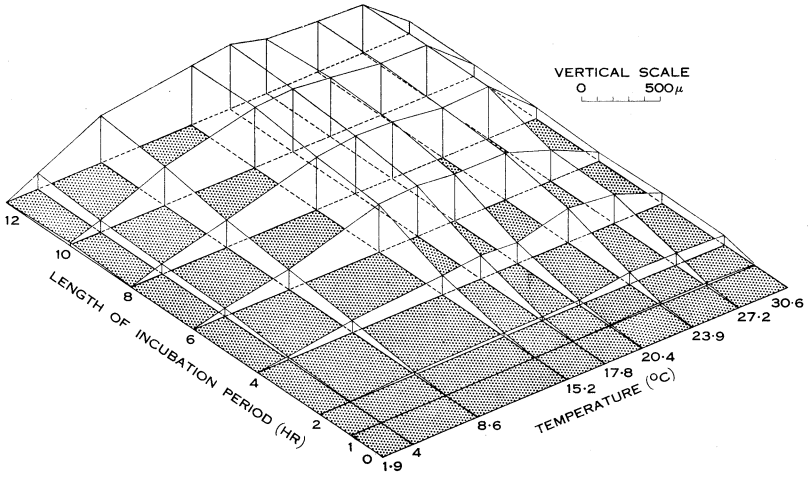

Fig. 2.—Relationship between mean germ-tube length, temperature, and time.

The analysis of germ-tube length data showed many similarities to the germination data but also some important differences as can be seen from a comparison of Figures 1 and 2. In the first hour greatest growth occurred at 23·9–27·2°C. Some growth, however, occurred over the whole 20·4–30·6°C temperature range.

After incubation for 2 hr germ-tube length had increased with temperature over the range 8·6–27·2°C. At the end of 4 hr, germ-tube length at 30·6°C had not significantly increased beyond growth at 2 hr while maximum growth had occurred at 23·9°C. The growth pattern after 6 hr showed that after this period of time germ-tube length at the lower temperatures, 15·2–20·4°C, had increased disproportionally to that of germ-tubes at 23·9°C or higher temperatures, with the result that a broad optimal temperature range 15·2–23·9°C for germ-tube growth was well defined. With increasing incubation time the optimal temperature range for growth fell and became less broad. At the completion of the experiment (12 hr) the optimal temperature range for growth was 15·2–17·8°C. Satisfactory growth, however, occurred over the much wider range of 8·6–27·2°C.

(*b*) *Comparison of the Effect of Temperature on Percentage Germination and Tube Length on Three Isolates of* P. tabacina

Multiconidia isolates of *P. tabacina* from Canberra (originally from Ovens Valley, Vic.), Parada, N. Qld., and Manjimup, W.A., were grown over two generations under standardized environmental conditions in the glass-house on similar plants of *Nicotiana tabacum* L. cv. Virginia Gold. Cross-contamination was prevented by use of separate humidity cabinets at the time of inoculation and by the maintenance of humidities unfavourable for sporulation (Cruickshank 1958) over the incubation period in the glass-house.

From the results in Section III(*a*) it was apparent that given a long enough incubation period, a very wide range of temperature was satisfactory for germination. In order to sharpen the comparison between isolates an incubation period of 5 hr was chosen in this experiment. Results are presented in Figures 3 and 4 where the mean percentage germination and the mean germ-tube length are plotted against temperature respectively. Hand-fitted curves were drawn with due allowance given to the statistical analysis of the data.

Figures 3 and 4 show the similar behaviour of the conidial isolates from Canberra and Parada and the general similarity in the shape of the response curves of all three collections in terms of both germination and germ-tube length. In terms of the temperature levels used in this experiment the lower limits of the optimal temperature ranges were the same for all isolates. The upper limit of the ranges of the Manjimup collection, however, sharply distinguished this isolate from the other two. For germination, the optimal temperature range for conidial isolates from Canberra and Parada was 14·5–27·2°C while that from Manjimup was 14·5–23·9°C. For germ-tube length the optimal temperature range for the isolates from Canberra and Parada was 17·8–23·9°C and that for the Manjimup isolate 17·8–20·6°C. Differences in the levels of maximum germination and germ-tube length between the Manjimup and the other two collections were highly significant. The behaviour of the Manjimup isolate was confirmed in a completely independent subsequent experiment.

(*c*) *Reliability of Experimental Techniques*

(i) *Biological.*—In temperature studies of this type where germination must be prevented during the preparatory period by working at low temperature there

is necessarily a time lag between the initial temperature and the test temperature. In the present studies precautions were taken to reduce this time lag but it was

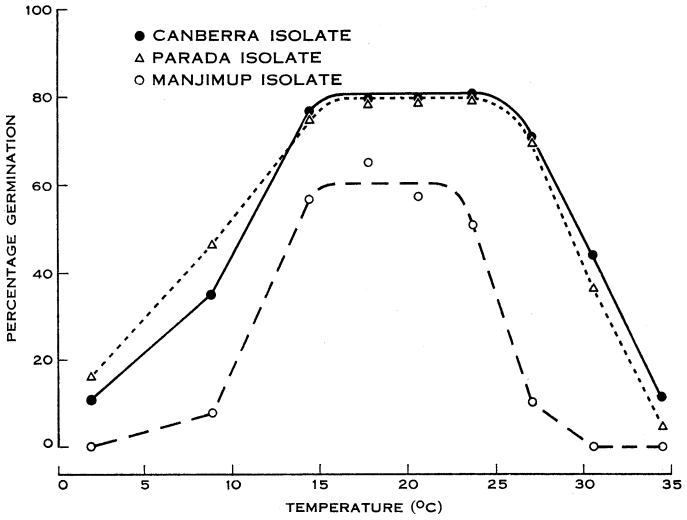

Fig. 3.—Germination response comparisons of three conidial isolates of *P. tabacina*.

not completely prevented. Measurements of the time necessary for the air within closed containers to come to equilibrium with test temperatures showed that the

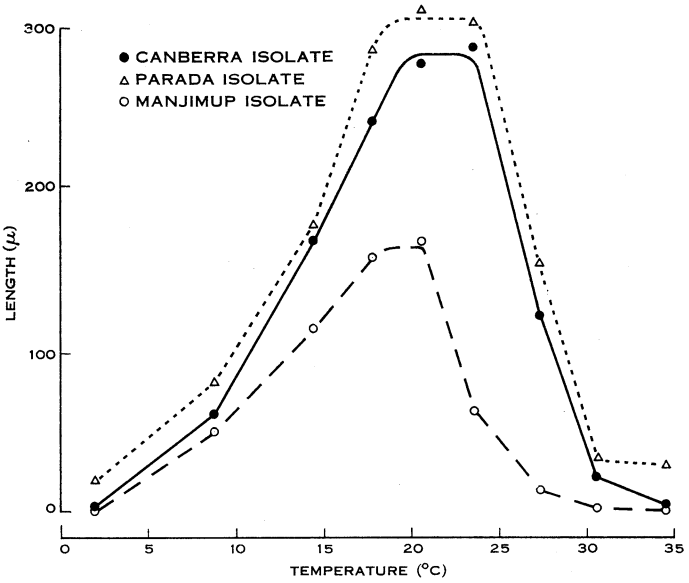

Fig. 4.—Germ-tube length comparisons of three conidial isolates of *P. tabacina*.

temperature adjustment followed a normal hysteresis curve. The time necessary for equilibrium was of the order of 10–15 min. The period at an intermediate temperature may have influenced the results in the shortest exposure (1 hr). It is

unlikely to have significantly affected the responses recorded after longer intervals of time.

(ii) *Statistical.*—For the germination data, the variability is most effectively expressed in terms of the binomial variation one would expect if all spores germinated separately instead of in groups in agar cubes. The data were analysed using the angular transformation, and the error variances for 100 spores per replicate were 17·17 and 12·82 in the two germination experiments in contrast to the expected value of 8·21. This indicates that the replicate variance for the 100-spore unit is 2·09 and 1·56 times greater than one would expect from chance alone and would imply the following replicate standard deviations for treatments:

Treatment Mean Germination	Replicate Standard Deviation	
	Expt. A	Expt. B
30%	6·63	5·72
50%	7·23	6·25
70%	6·63	5·72
90%	4·32	3·75

The variability in tube length increased with the mean. A square-root transformation was satisfactory in making replicate variation within treatments uniform. From the analysis one can estimate variances in the original units. Coefficients of variation between mean tube lengths of 10 tubes per replicate were:

Mean Tube Length (μ)	Coefficient of Variation
108	14·2%
216	11·9%
324	8·2%

IV. Discussion

On account of the *in vitro* character of the germination test described above the germination response and germ-tube lengths measured cannot be considered identical with the germination behaviour *in vivo* of *P. tabacina*. It is assumed, however, in this discussion that the results in relationship to temperature closely approximate to the *in vivo* behaviour as all other environmental and nutritional factors were held constant. Variation in replicate behaviour in the present technique did not exceed that normally associated with biological material.

Wolf *et al.* (1934) demonstrated that some germination was accomplished at 7·2–15·5°C within 2 hr and that at 21°C 50–59% germination occurred after 5 hr. Conidia maintained for 22 hr at 26°C did not germinate. Armstrong and Sumner (1935) using incubation periods of 22 and 48 hr reported the optimum germination range of *P. tabacina* to be 15–23°C. No germination was reported at temperatures higher than 29°C. Clayton and Gaines (1945) conducted a series of germination tests over undefined incubation periods on several isolates of *P. tabacina*. They stated that the germination response of their isolates fell into two distinct classes which had optimal temperature ranges of either 1·5–10°C or 17·7–26·1°C.

The points of major importance that became apparent from the time course analyses were, firstly, the rapidity with which germination occurred under favour-

able conditions and, secondly, the role of the time factor in determing the shape of the consecutive temperature response curves (see Figs. 1 and 2). Initially germination occurred most rapidly at 27·2°C. However, after 6 hr, there was no difference in germination over the range 15·2–27·2°C while after 12 hr the range had increased still further from 8·6 to 27·2°C. Initially germ-tube growth occurred fastest at the higher temperatures of the range studied. The advantage gained was not, however, maintained over longer periods, thus indicating that these temperatures were not the true optima for growth. The optimal temperature range for growth after 12 hr was in fact 15·2–17·8°C—approximately 10 degrees lower than the temperatures which initially most rapidly stimulated it.

The optimal temperature range for germ-tube length over shorter periods is more narrowly defined than that for germination over the same periods. This is well illustrated in Figures 3 and 4. After 12 hr, however, although the optimal temperature range for germ-tube growth is very narrow, the range over which satisfactory growth occurs more nearly coincides with the optimal temperature range for germination.

Figures 3 and 4 show that the three Australian isolates of *P. tabacina*, representing isolates of this plant pathogen from three widely geographically separated areas, can be placed into two classes on the basis of germination and germ-tube length responses. In terms of the temperature levels used in these experiments the actual values for germination are 14·5–27·2°C and 14·5–23·9°C while those for germ-tube length are 17·9–23·9°C and 17·9–20·2°C. Although the ranges overlap, the very sharp fall in response at the end of the optimal ranges clearly indicates the biological reality of the two classes. Wark *et al.* (1960) have recently shown the presence of distinct ecotypes within the Australian *P. tabacina* population. Clayton and Gaines' results and those reported here could be explained on this basis.

The optimal temperature range or ranges for germination of *P. tabacina* are only of importance in terms of epidemiology when the time factor is also considered. Thus a longer period at some of the lower temperatures studied was equally as satisfactory as short periods at the higher temperatures. Conversely short periods at low temperatures were unsatisfactory. Germination under field conditions is complicated by the necessity of visible moisture on the leaves (Hill 1957). Temperature conditions necessary for germination in the field must coincide with adequate moisture conditions.

Our knowledge of the wide optimal temperature range for germination would suggest that the duration of optimal moisture conditions for germination may be a more important limiting factor than temperature. According to unpublished observations by the author in the Ovens Valley, Vic., during the 1959–60 tobacco-growing season, moisture conditions favouring germination were of the order of 5–6 hr in duration on cool clear nights. Temperature records over the same period showed that minimum night temperatures were frequently close to the lower limits of the optimal temperature range for germination after 6 hr, namely 10–15°C. Under these conditions temperature was unlikely to be a serious limiting factor either for germination or germ-tube growth.

In this discussion environmental conditions at night have been emphasized as this is the period of dew formation on leaf surfaces. Showery periods during daylight hours associated with cloudy conditions may, however, also provide moisture conditions favourable for germination. Under these conditions when temperatures are higher (>20°C) germination occurs very rapidly as can be seen from Figure 1. Thus day termperatures as well as night temperatures may play a role in the germination of *P. tabacina* and consequently in the epidemiology of blue mould of tobacco.

Wark *et al.* (loc. cit.) have shown that isolates of *P. tabacina* vary in their sporulation capacity. The work reported here shows that germination and germ-tube growth are two further physiological characters which may be used to differentiate between strains of this fungus. The ecological advantage due to the differences demonstrated is not apparent in terms of long-term mean temperatures of the districts of origin of the isolates. There may, however, be factors not accounted for in normal meteorological records which have influenced the evolution of these differences.

V. Acknowledgments

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