ENVIRONMENT AND SPORULATION IN PHYTOPATHOGENIC FUNGI

II. CONIDIA FORMATION IN PERONOSPORA TABACINA ADAM AS A FUNCTION OF TEMPERATURE

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Summary

Using a leaf-disk technique an analysis was carried out to study the effect of environmental temperature on the length of the incubation period and sporulation intensity of *P. tabacina*. An analysis was also made of the sporulation response to temperature treatments prior to and during sporulation. The following points were demonstrated:

(1) Infected tobacco leaf is potentially capable of some sporulation from the 4th to the 17th days after infection. Maximum sporulation occurs 7–8 days after infection.

(2) Sporulation of *P. tabacina* is characterized by an optimal temperature range of 15–23°C, a minimum temperature of 1–2°C, and a maximum temperature of 30°C.

(3) The shape of the response curve is a characteristic of the fungus and independent of the condition of growth of the host plant.

(4) The intensity of sporulation is dependent on the physiological state of the host plant, the stage of the incubation phase at which it occurs, and the isolate of *P. tabacina*.

(5) The interaction between presporulation temperature and time affects sporulation intensity. Significant reduction in response occurs at high or low temperatures when the exposure times exceed 6–8 hr.

These results are discussed in relation to the epidemiology of blue mould.

I. INTRODUCTION

Clayton and Gaines (1933, 1945), Armstrong and Sumner (1935), and Dixon, McLean, and Wolf (1936), using plants grown under seed-bed, glass-house, and field conditions respectively, made observations on the effect of temperature on sporulation of *Peronospora tabacina* Adam. However, no systematic studies were carried out and no attempt was made to put the observations on a quantitative basis. Their values for the minimum, optimum, and maximum temperatures for sporulation appear to vary according to the locality, the isolate of the fungus, or the conditions under which the observations were made. Clayton and Gaines (1945) also observed and in part measured the effect of high day temperature on sporulation intensity but no detailed analysis of the response was attempted.

Since the overseas reports on the optimal temperature for sporulation of this fungus varied, and no Australian records were available, it was considered of interest to examine this and associated effects of temperature on sporulation, which might be

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of value in explaining some aspects of this phenomenon under field conditions. In this paper is presented a quantitative analysis of sporulation *in vivo* as a function of temperature. Firstly, the effect of the temperature factor in three environmental regimes on the length of the incubation phase of infection, and the intensity of sporulation with time, is studied; secondly, the effect of constant leaf-temperature levels during both presporulation and sporulation is reported; finally an analysis is presented of the effect on sporulation intensity of varying day and night leaf temperatures.

II. Materials and Methods

An isolate of *P. tabacina* originally from the Ovens River Valley, Vic., and subsequently described as the Canberra isolate, was used in all experiments unless otherwise stated.

The host plant material (*Nicotiana tabacum* L. cv. Virginia Gold) and its preparation prior to sporulation studies were each similar to that described previously (Cruickshank 1958). The infected leaf-disk technique of Cruickshank and Müller (1957) was used to measure the sporulation intensity per unit area of infected leaf. A water–water system replaced the manitol–glycerol–water system to ensure that the diffusion pressure deficit and the relative humidity levels were constant and within the optimal range for maximum sporulation, irrespective of the temperature level. Six or eight leaf disk replications were used in all experiments to measure sporulation intensity and the results were statistically analysed after square-root transformation of the data. Within experiments, for ease of comparison, counts were converted to a 0–100 scale and the values termed the sporulation intensity index.

III. Experimental

(a) *Effect of Environmental Regime on Length of the Incubation Phase of Infection and the Intensity of Sporulation with Time*

Groups of six tobacco plants were conditioned for 2 weeks to each of the following environmental regimes:

Regime	Day Temp. (°C) (0830–1630 hr)	Night Temp. (°C) (1630–0830 hr)	Relative Humidity (%) (day)	(night)
I	20	15	*c.* 48	50–70
II	25	18	*c.* 35	35–150
III	28	24	*c.* 40	42–50

The plants were removed for inoculation and after 24 hr returned to their respective environmental regimes. Leaf disk samples for sporulation tests were taken daily from each plant at 1700 hr and incubated overnight at 20°C as described above.

The results of this time course experiment, presented in Figure 1, show that the ability of *P. tabacina* to sporulate rose from zero on the 3rd–5th day to a maximum on the 7th–8th day after inoculation. Its subsequent capacity to sporulate fell off with time. Under each of the three environments used the observations showed that while

green or chlorotic leaf areas remained, *P. tabacina* retained some capacity to sporulate. In this series of determinations the level did not fall below approximately 25% of the maximum level.

The individual sporulation capacities were determined by the environmental regimes under which the host plants were growing. The first signs of sporulation occurred under regime III on the 4th day after inoculation, and on the 6th day under regimes I and II. There was, however, a significant difference ($P<0\cdot001$) in sporulation level between the latter two regimes at this stage. Sporulation reached its maximum level in regime III on the 7th day. At this time there was a significant difference ($P<0\cdot001$) between the sporulation responses under regimes I and II and under regime III. Eight days were required for maximum sporulation under regimes I and II and, at this stage, when maximum sporulation was occurring under all three environments, there was a significant difference ($P<0\cdot001$) in sporulation intensity between regimes I and III but not between regimes II and III. This latter relationship was maintained throughout the decline in sporulation capacity over the balance of the experiment.

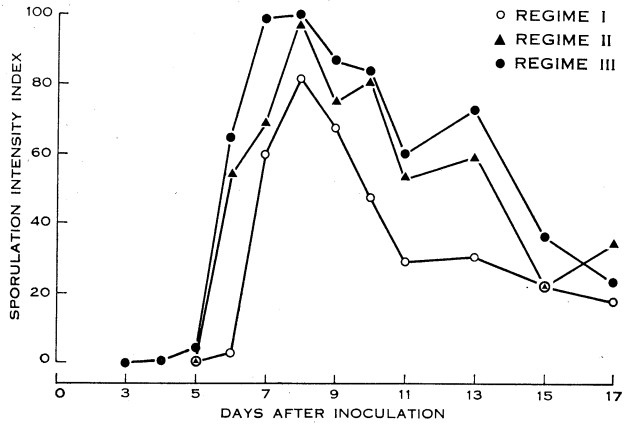

Fig. 1.—Time course of sporulation intensity of *P. tabacina* in leaf disks.

(*b*) *Effect of Leaf Temperature on Sporulation Response*

In this group of experiments the change in sporulation intensity with temperature was studied on two sets of material. In the first experiment (b,i) the fungal isolate was held constant. The responses of *P. tabacina*, infecting tobacco leaves grown in each of the three controlled environmental regimes, were compared. In the second experiment (b,ii) the environment was held constant (regime II) and the sporulation response of multispore isolates of *P. tabacina* from Parada, N.Qld., Canberra (originally Ovens River Valley, Vic.), and Manjimup, W.A., were compared. All experiments were repeated, and sporulation patterns confirmed.

In each experiment the leaf disk samples were cut from plant replicates in each treatment 8 days after inoculation. They were incubated in a series of unlighted

incubators set at temperatures ranging from 1 to 30°C. After 24 hr the disks were harvested and the sporulation intensity measured. Results of experiments (b,i) and (b,ii) are presented in Tables 1 and 2 respectively.

TABLE 1

COMPARISON OF SPORULATION OF P. TABACINA ON LEAF DISKS FROM TOBACCO PLANTS GROWN IN THREE ENVIRONMENTS

Incubation Temp. (°C)	Mean Sporulation Intensity Index		
	Regime I*	Regime II	Regime III
1·5	0·05	0·01	0·00
4·0	0·39	0·39	0·32
8·1	6·72	6·89	11·6
15·0	26·62	36·82	71·19
17·0	32·04	33·62	81·74
19·6	29·69	56·94	100·00
22·9	28·05	40·02	88·25
26·4	0·38	0·57	1·18
29·7	0·00	0·00	0·06

* Environmental regimes given on p. 199.

An analysis of the sporulation data from both experiments gave a unimodal response curve strongly skewed to the right if increasing temperature is plotted as the

TABLE 2

COMPARISON OF SPORULATION OF THREE ISOLATES OF P. TABACINA ON LEAF DISKS FROM TOBACCO PLANTS GROWN IN THE SAME ENVIRONMENT (REGIME II)

Incubation Temp. (°C)	Mean Sporulation Intensity Index		
	Parada Isolate	Canberra Isolate	Manjimup Isolate
1·7	0·09	0·15	0·44
4·0	1·03	6·76	1·84
8·5	21·14	35·16	16·00
15·0	38·95	85·12	48·22
18·5	39·04	95·87	52·55
20·2	44·23	100·00	54·96
23·6	36·90	80·61	45·82
26·9	0·00	1·61	0·02
30·2	0·00	0·00	0·00

horizontal ordinate (cf. Cochrane 1958). The minimum, optimal, and maximum temperatures were, 1–2, 15–23, and 30°C respectively.

Sporulation intensities of *P. tabacina* growing in leaves of plants grown under three environmental regimes are given in Table 1. Over the temperature range 15–22·9°C the intensities increase from regimes I through II to III, all differences at the same temperature being significant ($P<0·05$ at least) except between regimes I and II at 17°C. Reference to Figure 1, which shows the relative daily sporulation patterns of the Canberra isolate of *P. tabacina* at 20°C, confirms the above relationship between the sporulation intensity indices of *P. tabacina* under regimes I and III over the major portion of the period the sporulation responses were tested. The situation in relation to *P. tabacina* in regime II is less clear. An intermediate sporulation intensity is maintained but the level of significant difference from the other two regimes is variable.

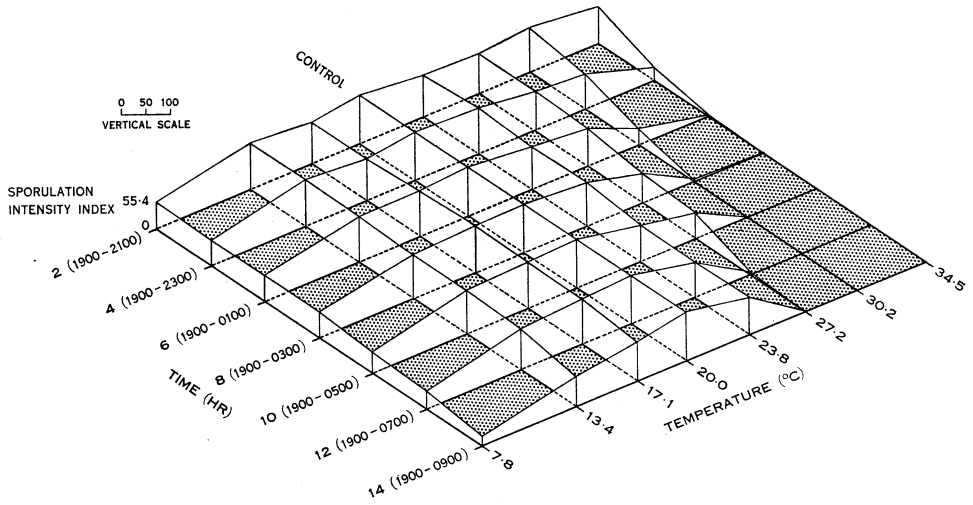

Fig. 2.—Relationship between night temperature, its duration, and sporulation intensity (night temperature constant, *c.* 20°C).

The comparison of the three isolates of *P. tabacina* showed that the Canberra isolate sporulated with approximately twice the intensity of the isolates from either Manjimup or Parada. A subsequent comparison of the Canberra isolate with a recent isolate from the Ovens River Valley has shown no difference in sporulation behaviour. It is concluded from these experiments that although the pattern of change in sporulation response with temperature was independent of the conditions of growth or the geographical source of the pathogen, the actual levels of sporulation intensity at any particular temperature appear to be closely related to these factors.

(c) *Effect of Various Temperature and Time Combinations on Sporulation Response*

(i) *Day Temperature Constant and Night Temperature Varied.*—Leaf disks were cut from infected host tissues which had been growing under regime I (day temperature 20°C) and incubated at each of the following temperatures: 7·8, 13·4, 17·1, 23·8, 27·2, 30·2, and 34·5°C. In the first study (results shown in Fig. 2) the treatments

were initiated at 1900 hr. Disk samples from each temperature were removed after 2, 4, 6, 8, 10, 12, and 14 hr and transferred to an incubator at 20°C until 0900 hr.

In the second experiment (illustrated in Fig. 3) the same temperature levels were used but the reverse time procedure was followed, viz. all leaf disks were placed in the 20°C incubator at 1900 hr and progressively transferred at 2-hourly intervals to the above temperature conditions where they remained until 0900 hr the following morning. In each series, the disks were killed at 0900 hr by addition of a drop of 1% formalin and then transferred to 50% ethanol as described in the technique referred to above.

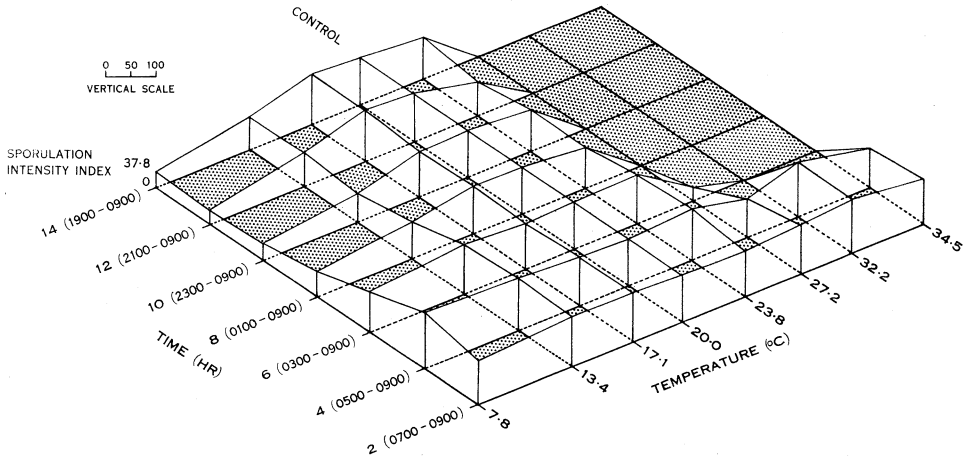

Fig. 3.—Relationship between night temperature, its duration, and sporulation intensity (night temperature constant, *c.* 20°C).

(ii) *Night Temperature Constant and Day Temperature Varied.*—In this experiment (results shown in Fig. 4) leaf disks were incubated at 8·5, 13·8, 17·6, 24·3, 27·8, 31·3, and 35·1°C for 2, 4, 6, 8, and 10 hr, starting from 0930 hr. After exposure the disks were transferred to a common incubator at 20°C for the balance of the 24-hr experimental period. The disks were then harvested and the sporulation intensities measured.

In each of the three experiments in this section one complete set of disks (times × replicates) were incubated at 20°C over the full experimental period to serve as controls.

The three-dimensional diagrams (Figs. 2, 3, and 4) represent in each case essentially the effect of superimposing fairly small temperature treatments for either increasing or decreasing intervals of time on a basic 20°C grid. In the two experiments where the night temperature treatments were changing with time, the times included the normal sporulation period—in this case 0300–0500 hr. On the other hand, in the experiment in which night temperature was held constant, the effect of day temperature was reflected in the sporulation response independently of the specific sporulation period.

Taking the response at 20°C as control and comparing other responses with it the following points can be demonstrated. In the first experiment (Fig. 2) temperatures 7·8 and 34·5°C significantly ($P<0·01$) depressed the sporulation response over the whole time range. The effect of high temperature was, however, much greater than that of low temperature; for example, the effect of the 13·4°C treatment only reached significance ($P<0·01$) after 14 hr exposure, viz. from 1900 to 0900 hr, while the effect of the 27·2 and 30·2°C treatments, on the other hand, significantly depressed sporulation in all but the 2-hr exposures. In the second experiment (Fig. 3) the 2-hr exposure occurred between 0700 and 0900 hr. At this time of day neither the lowest two (7·8 and 13·4°C) temperature treatments nor the highest three

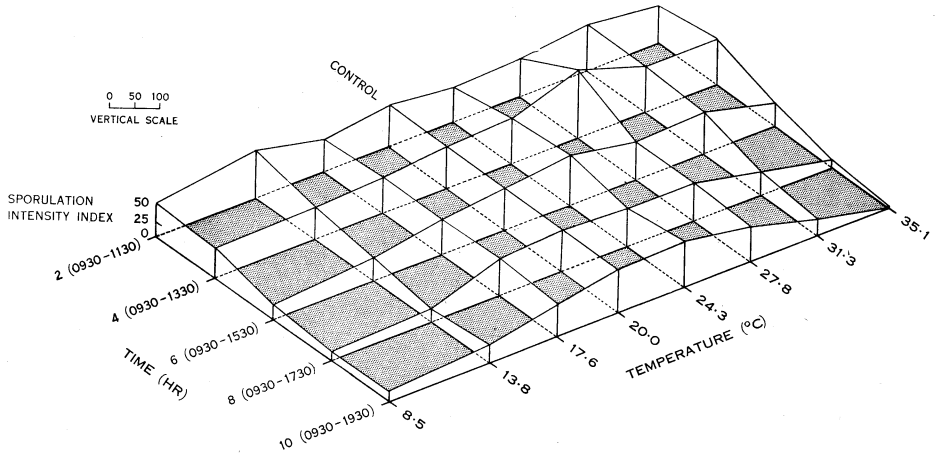

Fig. 4.—Relationship between day temperature, its duration, and sporulation intensity (night temperature constant, 20°C).

temperature treatments (27·2, 30·2, and 34·5°C) had any effect on sporulation. As the time of initiation of the lower treatments, however, receded from 0500 to 1900 hr the sporulation response was progressively reduced at these temperatures. The effect at the higher temperatures was more rapid. Sporulation dropped to zero where the highest temperature treatments (32·2–34·5°C) were initiated at 0300 hr. The same effect was produced at 27·2°C when the treatment was initiated at 0100 hr. Over the intermediate temperature range 17·1–23·8°C in both experiments treatment had no effect on the sporulation response.

In the third experiment (Fig. 4) no treatment of 2 hr significantly affected the sporulation rate; however, the 8·5 and 13·8°C treatments after 4 hr significantly ($P<0·01$) reduced sporulation intensity and this effect increased with time of exposure to the treatment. The 17·6 and 24·3°C treatments did not significantly effect the sporulation response. At 27·8°C there was a significant ($P<0·001$) increase in intensity after 4 hr, but with further increase in time of exposure the response fell and became not significantly different from the control. Treatments for 8 and 6 hr at 31·3 and 35·1°C, respectively, reduced the sporulation response significantly ($P<0·001$) and this effect increased with time of exposure.

(d) Comparison of Monthly Mean Temperatures in Tobacco-growing Districts

Data on the mean maximum and minimum temperatures for several tobacco-growing districts in Australia were obtained. However, only in two of the recording stations were the records available over long periods (30 years). These data are plotted in Figure 5 alongside two similar sets of data relating to two tobacco-growing districts in the United States (McGrath and Miller 1958). These data alone do not, in terms of the experimentally determined temperature responses, suggest differences between tobacco districts in Australia and the United States sufficient to explain the differences in incidence and severity of blue mould which occur between these two countries.

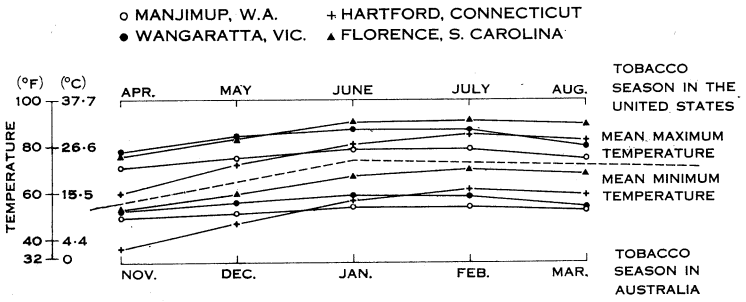

Fig. 5.—Comparison of long-term mean maximum and minimum temperatures between two tobacco-growing districts in the United States and Australia.

IV. Discussion

Sporulation as a function of temperature has been studied extensively *in vitro* and has been recently reviewed by Hawker (1957). *In vivo* studies of the sporulation response of phytopathogenic fungi to individual factors in the environment have received much less attention. Most *in vivo* studies have been limited to field studies in which semiquantitative observational methods have been employed.

Previous studies of this problem in relation to *P. tabacina* have been concerned primarily with devising control measures rather than with the behaviour of *P. tabacina*. The leaf-disk technique used here has enabled detailed *in vivo* studies to be carried out quantitatively. It has also allowed not only the effect of the ambient or environmental temperatures on sporulation to be considered but also the measurement of the effect of actual leaf temperature.

Several authors have studied the sporulation of *P. tabacina* under constant temperature conditions. Reported optimum temperatures range from 13·3 (Dixon, McLean, and Wolf 1936) to 21°C (Armstrong and Sumner 1935). In the present studies in which glass-house grown leaf material was used as the host substrate there was no significant difference in sporulation intensity over the temperature range 15–23°C. The host material studied here represents plants grown under different temperature regimes. The isolates of *P. tabacina* studied represent isolates of the fungus from widely separated geographical regions. The similarity of the sporulation response with increase in temperature suggests that *in vivo*, as *in vitro* in the case of facultative fungi,

the slope of the response curve is a characteristic of the fungus species and is independent of the host substrate. The intensity level of sporulation at any given temperature is, however, a function of the physiology of the host and the strain of the fungus. Although the Canberra isolate of *P. tabacina* was readily distinguished from those from Parada and Manjimup on the basis of its greater sporulation capacity it could not be distinguished from a recent isolate from Ovens River Valley. It thus appears that several years' (1954–1960) isolation and experimental use have not been sufficient to modify this characteristic.

Temperature affects both the length of the presporulation phase of vegetative growth of *P. tabacina* in the host tissues and the intensity of the sporulation response. Both these factors may be of considerable significance in terms of the epidemiology of blue mould. On account of their low night humidities the conditions of the regimes studied were unfavourable for sporulation. They were, however, satisfactory for vegetative growth. Potentially, the fungus was able, under these conditions, to sporulate on the 4th–6th day after inoculation, while maximum sporulation was possible after 7–8 days. Sporulation could be inhibited by high temperatures or low night humidities (Cruickshank 1958). The inhibition was, however, only temporary as the fungus remained potentially capable of sporulation for at least 10 days under all three environmental regimes. Thus, although the maximum sporulation for a particular lesion may not occur due to favourable conditions not coinciding with the 7th–8th day after infection, the lesion nevertheless is capable of producing conidia in considerable quantities providing favourable conditions occur some time from the 6th to at least the 17th day after infection. Under conditions of less severe infection than those used in the experiments described in this paper, the period over which sporulation could occur may be considerably longer than 10 days.

Under field conditions temperatures rarely remain constant for long periods of time. Although it is of interest to know the cardinal temperatures for sporulation, it is much more important from the epidemiological point of view to know the expected response for given time and temperature combinations. The situations chosen here were very simplified in relation to the fluctuating natural environment. They do, however, emphasize that the duration of exposure to a given temperature, and not temperature alone, decides the final sporulation level. This data, particularly in relation to high day temperatures, could possibly be of use in blue mould forecasting.

In nature the following generalized environments are possible in terms of temperature: cold days and cold nights; warm days and cool nights; hot days and cool nights; hot days and hot nights. In terms of the time and temperature combinations reported above hot days followed by either hot nights or cool nights would be least favourable for sporulation. Cold days and cold nights would also depress spore production, while warm days and cool nights, on the other hand, would favour maximum levels of sporulation. The data shown in Figure 5 do not indicate large differences in the mean temperatures between tobacco districts in Australia and the United States. The mean minimum temperatures (and therefore night temperatures) in general fall almost within the optimal temperature range for sporulation. The mean maximum temperatures, on the other hand, would inhibit sporulation only if maintained over extended daily periods. In Australia rapid changes in temperature frequently occur even in the

summer months. In the United States uniformly high summer temperatures are normally experienced. The dependence of sporulation not only on temperature but also its duration could be of significance in explaining why blue mould is more serious as a field disease in Australia than in the United States.

Within Australia differentiation with respect to pathogenicity and reproductive capacity between isolates of *P. tabacina* has been reported by Wark *et al.* (1960). Similar differences to those demonstrated may be true of ecotypes of *P. tabacina* in different countries. These differences may also be important in influencing the epidemiology of blue mould.

V. Acknowledgment

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VI. References

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