Sporulation of Pestalotia theae Sawada

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Pestalotia theae Sawada is one of the fungi investigated by the senior author with regard to its nutritional requirements. The results on carbon and nitrogen nutrition (Mandahar, 1966 a and b) and the micronutrient nutrition (Thind and Mandahar, 1968) have already been published. During the course of these studies, *P. theae* seemed to give interesting results about its sporulation. This aspect was investigated later and is reported here.

Materials and Methods

P. theae was isolated from leaves of *Thea sinensis* collected from Dehra Dun, India. Single spore isolates were prepared. The pathogen sporulates in abundance on PDA slants forming jelly-like, thick, jet black acervuli on surface of the medium. It was grown in 4″ diameter Petri dishes, each containing 20 ml PDA, wrapped in black paper, and incubated in darkness at 26° C for 8 days. Uniform discs of mycelium get from the edges of such a colony were used for inoculating media in these studies. All the studies were made in 4″ diameter Petri dishes, each containing 20 ml of PDA or basal medium (glucose 20 g; KNO_3 5.0 g; KH_2PO_4 5.0 g; $Mg\ SO_4$. $6H_2O$ 0.5 g; and distilled water to make 1000 ml.) solidified with 2% agar. Each petri dish was wrapped in black paper immediately after inoculation and incubated in darkness at 26° C. For exposure to light Petri dishes were always placed near the window of the laboratory receiving the maximum of daylight. An average of linear measurements of colony diameters in 5 petri dish cultures is taken as a measure of growth. Spores were counted in the following manner: A disc of 3 mm diameter was removed from each of the 5 replicates. Mycelium and spores from all the discs were removed and suspended in 3 ml of 5% glycerine. The spore suspension was shaken to evenly distribute the spores before the spore count was done with a hemaecytometer. A satisitcal mean of 5 such counts, divided by 5 to culculate the spores formed per disc, was always recorded.

Results

Effect of light: Preliminary experiments indicated that light is essential for sporulation of *P. theae*. Even a single exposure to light

during the routine laboratory work was enough to initiate sporulation. To find the duration of exposure necessary for sporulation, eight sets of 8-day old petri dish cultures on PDA were placed in light for varying periods of time. They were then wrapped in black papers and placed in incubator for 6 more days. Sporulation was recorded afterwards (Table 1).

Initiation of sporulation takes place after the minimum exposure of 5 minutes. However, an exposure of 30 minutes is the optimum after which the rate of sporulation levels off. Acervuli are formed in more or less regular zones, being greatest in number and bigger in size at places occupied by the margins of the mycelium at the time of exposure. They grow progressively less abundant and msaller in size towards the centre of the colony till in the innermost circle of 4 mm radius around the inoculum disc there is no sporulation at all. These data indicate that the intensity of sporulation of mycelium seems to depend upon its age at the time of exposure.

The last observation was investigated further. Three sets of 8-day old Petri dish cultures on PDA wrapped in black paper were taken. A horizontal strip $\frac{1}{2}'' \times \frac{1}{4}''$ was removed from the black wrapper on the upper side of each Petri dish so that only a particular portion of mycelium could be exposed to light, rest of the colony remaining in darkness. In the first set the strip was removed above the margins, i. e., above the young growing mycelium; in the second set it was removed $\frac{1}{2}''$ *internal to the margins*, i. e., above the 3—4 day old mycelium; and in the third set it was removed from the centre, i. e., above the 7—8 day old mycelium. All the sets were exposed to light for 30 minutes to 20 hours (with the help of three fluorescent tubes at a distance of 10 feet where necessary) and incubated for a further 6 days. The results are indicated in Table 2.

Two observations stand out clearly:

1. Photosensitivity of mycelium is clearly controlled by its age factor. The greater the age of mycelium at the time of exposure, the longer is the duration needed for sporulation. Sporulation in the 7—8 day old mycelium never reached the maximum level even after an exposure of 20 hours.

2. Acervuli were formed only in that region which received the photostimulus; the mycelium formed after or before exposure did not form any spores.

Effect of carbon and nitrogen: The effect of various carbon sources on growth and sporulation of *P. theae* is indicated in Table 3. In this experiment glucose in the basal medium was replaced by various carbon sources used singly and separately, each calculated to give the same amount of carbon as in 20 g glucose. Starch, however, was used at the rate of 20 g/l. All the Petri dish cultures in this and the following experiments were exposed to natural daylight for

30 minutes on every alternate day. The data were taken on the 14th day.

It is noteworthy that the maximum sporulation of *P. theae* occurred on starch. This could be explained on the basis that the total amount of glucose available to the fungus after hydrolysis of starch is far in excess of the amount of glucose (20 gm) present in the basal medium. An experiment was therefore set up to observe the effect of glucose concentrations on sporulation. Results are given in Table 4.

There is an increase in sporulation with increasing amount of glucose (up to) 30 g/l, after which the rate of sporulation falls progressively with greater increases in concentration of glucose. Vegetative growth of the fungus, however, attains its optimum level at 20 g/l glucose and thereafter remains almost constant. Poor sporulation with increase in glucose concentration, vegetative growth remaining almost constant, indicated a role of the C : N ratio in sporulation. Increasing carbon concentration may have tilted the C : N ratio rather unfavourably for sporulation. This was investigated further in a separate experiment. The results (indicated in Table 5) show that the C : N ratio does affect the rate of sporulation, the optimum C : N ratio being approximately 17 : 1. Sporulation increased even on the higher carbon concentrations when the amount of nitrogen was increased so as to bring the C : N ratio to about 17 : 1.

Discussion

Table 1 indicates that light is necessary for sporulation of *P. theae*. Similar results were also obtained by McLellan *et al.* (1955) on *Pestalotia* sp. In fact the total dependance of sporulation on light is rather a common phenomenon a among fungi (Hawker, 1957).

Table 2 indicates that the stimulus of light is completely local. The fact that the outer mycelium formed in dark ness after exposure to light, and also that the mycelium inner to the exposed strip remained vegetative, suggested that the photomorphogenic stimulus was not transported to the unilluminated mycelium on either side. Only the illuminated portion of mycelium produced spores. Fahim (1966) arrived at identical conclusions with respect to *Alternaria porri*.

One note worthy feature revealed by table 2 is that the youngest actively growing mycelium is highly photoresponseive and the older mycelium successively less and less so. The latter required more light energy for sporulation. The maximum number of spores were formed by the young growing mycelium after 30 minutes exposure, and longer duration of light made practically no difference; 3—4 day old mycelium, produced equally abundant sporulation only after 4 hours of illumination and then it levelled off; 7—8 day old mycelium never reached

the stage of maximum production of spores even after an exposure of 16 hours and longer. This dependance of degree of sporulation on the age of the mycelium may exist because of the differing rates of respiration of mycelium of different ages. This is supported by the work of Hawker and Hepden (1962) who observed that intensive respiration accompanied the production of spores by *Rhizopus sexualis* and that of McDowell and De Hertogh (1968) who observed more rapid oxidation of glucose by young sporulating culture than by equally aged mycelium of non-sporulating cultures of *Endothia parasitica*. Thus the oldest mycelium, which does not sporulate or sporulates extremely poorly, even during extended periods of irradiation, may never have that minimum intensity of respiration necessary for its optimum sporulation.

Different carbon sources have differing effects on growth and sporulation of *P. theae* (Table 3). This is quite normal in fungi (Hawker, 1957; Cochrane, 1958). Results on starch were striking. Starch supports maximum sporulation which may be due either to the greater amount of glucose made available to the pathogen after hydrolysis or to the increased osmotic pressure. Results on sporulation by the pathogen on different concentrations of glucose (Table 4) support the first idea rather than the second. Hendrix and Toussoun (1964) arrived at the same conclusions with *Fusarium solani* f. *phaseoli*, while Leach and Moore (1966) showed that the effects of sucrose and sodium nitrate on sporulation of *Botrytis fabae* were primarily due to their osmotic effects. It may also be observed from table 3 that optimum sporulation occurred over a narrower range of glucose concentrations (25—30 g/l) than optimum vegetative growth (20—60 g/l) thus showing that amount of sporulation is not related with growth. Kafi and Tarr (1966) also arrived at identical results with *Helminthosporium* species.

In many cases the C : N ratio, than only the increase or decrease in the carbon or nitrogen source, is preeminent in determining sporulation of fungi (Hawker, 1957). The results of table 5 indicate that the C : N ratio is more important for sporulation of *P. theae*. The C : N ratio most useful for sporulation is 17 : 1. Hendrix and Toussoun (1964) found 10 : 1 C : N ratio as optimum for sporulation of *Fusarium solani* p. *phaseoli*.

Summary

Pestalotia theae Sawada, causal agent of leaf blight of tea (*Thea sinenesis* L.) has an absolute requirement of light for sporulation. Thirty minutes exposure to daylight is the optimum exposure. Response of the pathogen to light is completely localized. Sporulation is greatly dependent upon the C : N ratio; a 17 : 1 ratio gives the best results. Different carbon sources have different effects on sporulation.

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Table 1. Sporulation of *P. theae* after exposure to varying durations of light

Period of exposure in minutes.	None	1	5	15	30	60	90	120	240
Number of spores produced per disc	0	0	4±3	16±4	36±5	38±4	37±5	37±4	36±6

Table 2. Age as a factor in photosensitivity of the mycelium

Period of exposure in hours	½	2	4	8	12	16	20
			Number of spores formed per disc (3 mm diam)				
Yound growing mycelium	38±7	39±6	37±8	36±4	35±8	39±6	37±6
3–4 days old mycelium	0	5±3	7±2	22±10	32±6	35±8	33±5
7–8 days old mycelium	0	0	0	4±2	16±6	15±5	15±8

Table 3. Effect of various carbon sources on growth and sporulation of *P. theae*

Name of carbon source added	Colony diameter (in mm)	Number of spores produced per disc (3 mm diam)
Control (No carbon except of agar)	10±3.16	00
Arabinose	80±4.56	20±5
Xylose	70±7.38	26±4
Rhamnose	82±5.61	30±6
Glucose	92±4.67	32±4
Fructose	90±7.79	26±3
Sorbose	90±8.45	15±2
Sucrose	98±6.61	25±4
Maltose	90±8.58	30±4
Starch	90±6.78	48±5

Table 4. Sporulation of *P. theae* on various concentrations of glucose

Glucose concentration in g/l	0	5	10	20	25
No. of spores produced per disc (3 mm diam)	0	6±2	16±3	32±4	42±4
Colony diameter in mm	10±3.6	50±7.8	72±6.1	95±6.5	98±7.6
Glucose concentration in g/l	30	40	50	60	
No. of spores produced per disc (3 mm diam)	59±5	40±5	20±3	12±3	
Colony diameter in mm	95±5.5	96±6.5	95±4.8	94±5	

Table 5. Effect of C : N ratio on sporulation of *P. theae*

Carbon in g/l	Nitrogen in g/l	Number of spores formed per disc (3 mm diam)	C : N ratio
8	0.69	30±5	11 : 1
10	0.69	41±4	14 : 1
12	0.69	55±5	17 : 1
16	0.69	31±6	23 : 1
20	0.69	18±5	32 : 1
24	0.69	12±6	34 : 1
16	1.03	48±5	16 : 1
20	1.20	57±5	17 : 1
24	1.38	52±4	18 : 1