Fruiting of some light-requiring fungi as influenced by cellophane

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Abstract. Cellophane film overlying agar media enabled dark fruiting in two fungi (Phoma sp. and Ascochya pisi Lib.), fruiting in light on Czapek's agar in two other fungi [Phoma sorghina (Sacc.) Boerema et al and Leptosphaerulina crassiasca (Sechet) (Jackson and Bell)] and increased fruiting in all the four fungi. The fungal hyphae branched more profusely on cellophane. The role of cellophane in the induction of fruiting in these fungi is discussed.

Keywords. Fruiting in fungi; cellophane.

1. Introduction

It was reported from this laboratory that an isolate of Phoma (Phoma sp.) and one of Ascochya pisi which require light for formation of pycnidia could fruit even in darkness when grown on a disc of cellophane or filter paper overlying agar media (Swamy and Govindaraghavan 1972). Another isolate of Phoma (P. sorghina) and an isolate of Leptosphaerulina crassiasca could form spores and fertile perithecia, respectively, on Czapek's agar in light only if a disc of cellophane was present over the agar (Suryanarayanan and Swamy 1977). In this paper we report some further investigations on the role of cellophane in the fruiting of these four fungi.

2. Materials and methods

The fungal isolates used were those used in our earlier work (Swamy and Govindaraghavan 1972; Suryanarayanan and Swamy 1977).

Petri dishes containing potato dextrose agar or Czapek's agar were inoculated by transferring to the centre a 7 mm diameter plug of growth cut out from the growing margin of a 5-day old dark-grown culture. The plug was placed mycelium face-down. The inoculum was placed either directly over the agar surface

or on a disc of cellophane overlying the agar medium. The cellophane disc had been sterilized by autoclaving at 1.05 kg/cm² for 15 min before being laid on the medium.

Inoculated plates were incubated for 5 days in continuous darkness or in light-dark cycles (12 hr light : 12 hr darkness) in an air-conditioned room where the temperature was 23 ± 2°C. The light source consisted of two 40 W Philips daylight fluorescent lamps and one Sylvania BL 40 W fluorescent lamp placed 45 cm above the cultures.

On the 6th day, the aerial hyphae were collapsed by flooding the plates with alcohol and the number of fruit bodies present in an area covered by the low power field of a microscope was determined (three or four areas in three different plates were examined). From this the number of fruit bodies in a 1 cm² area was calculated. Statistical analyses were carried out using the Student's 't' test.

3. Results

As seen from table 1, Phoma sp. and Ascochyta pisi formed pycnidia even in the dark when cellophane was present. This confirms our earlier observations (Swamy and Govindaraghavan 1972). Phoma sorghina and L. crassiasca could form fertile fruit bodies on Czapek’s medium only when light and cellophane were provided. On both the media, the number of fruit bodies formed was increased by the presence of cellophane.

Table 1. Effect of light and cellophane on formation of fruit bodies in two isolates of Phoma (Phoma sp. and P. sorghina), Ascochyta pisi and Leptosphaerulina crassiasca on potato dextrose and Czapek’s agar media.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Treatment</th>
<th>No. of fruit bodies/cm²</th>
<th>Phoma sp.</th>
<th>P. sorghina</th>
<th>A. pisi</th>
<th>L. crassiasca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato dextrose</td>
<td>-D</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>540</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+D</td>
<td>360</td>
<td>0</td>
<td>0</td>
<td>1140</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>-L</td>
<td>720</td>
<td>200</td>
<td>0</td>
<td>1560</td>
<td>2840</td>
</tr>
<tr>
<td></td>
<td>+L</td>
<td>1200</td>
<td>580</td>
<td>0</td>
<td>3220</td>
<td>3360</td>
</tr>
<tr>
<td>Czapek’s agar</td>
<td>-D</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+D</td>
<td>460</td>
<td>0</td>
<td>0</td>
<td>700</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>-L</td>
<td>600</td>
<td>0</td>
<td>0</td>
<td>1340</td>
<td>980b</td>
</tr>
<tr>
<td></td>
<td>+L</td>
<td>860</td>
<td>760</td>
<td>0</td>
<td>1360</td>
<td>2020</td>
</tr>
</tbody>
</table>

Critical Difference at 5% level 20 32 140 160

Notes: + and — indicate presence or absence of cellophane on the media. D = continuous darkness; L = 12 hr light : 12 hr dark cycles.

b sterile fruit bodies.
Figure 1. (Captions in P. 140).
Figure 1. (a) Hyphal tips of *Phoma* sp. growing on agar. (b) Hyphal tips of *Phoma* sp. growing on cellophane overlying agar (note the extensive branching). (c) Aggregation of hyphae of *Phoma* sp. growing on cellophane. The other three fungi also behaved similarly (the photographs were taken after 5 days' growth in darkness).
Cellophane and fruiting in fungi

Table 2. Numbers of hyphal tips in unit width of the colony margin in two isolates of Phoma (Phoma sp. and P. sorghina), Ascochyta pisi and Leptosphaerulina crassiasca while growing on Czapek's agar medium and on cellophane overlying the medium.

<table>
<thead>
<tr>
<th></th>
<th>Phoma sp.</th>
<th>P. sorghina</th>
<th>A. Pisi</th>
<th>L. crassiasca</th>
</tr>
</thead>
<tbody>
<tr>
<td>On agar</td>
<td>27</td>
<td>26</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>On cellophane</td>
<td>50</td>
<td>45</td>
<td>44</td>
<td>45</td>
</tr>
<tr>
<td>Critical Difference at 5% level</td>
<td>5</td>
<td>12</td>
<td>7</td>
<td>18</td>
</tr>
</tbody>
</table>

*per unit width (0.88 mm) of margin of colony after 5 days' growth in darkness.

The growth pattern of the fungi was altered by the presence of cellophane. On cellophane the growth was adpressed and with few aerial hyphae, while on agar the growth was fluffy with a lot of aerial hyphae. Hyphae showed a tendency to branch more profusely on cellophane. This was verified by determining the number of hyphal tips in unit width of the margin of the colony while growing on agar directly or on cellophane overlying the agar. The results presented in table 2 and figures 1a and 1b show that the number of hyphal tips on cellophane was significantly more than on agar in three of the fungi. There was also a tendency for the hyphae to aggregate while growing on cellophane (figure 1c).

4. Discussion

In an earlier report (Swamy and Govindaraghavan 1972) we had suggested that the cellulosic nature of cellophane had something to do with its ability to induce dark sporulation in Phoma sp. and A. pisi. However, the two fungi failed to sporulate in the dark on Czapek's medium with cellulose as carbon source (Suryanarayanan 1978). From this and our present results we are inclined to believe that the role of cellophane in the fruiting of the four fungi studied here is a physical one. The possibility that, on cellophane, availability of nutrients becomes limited because of a diffusion barrier and that the resulting starvation conditions encourage fruiting can be ruled out since, in all four fungi, dilution of the medium decreased the number of fruit bodies formed (Suryanarayanan 1978) whereas on cellophane the numbers increased (table 1). The better fruiting in light on cellophane could be a result of a more efficient absorption of light energy owing to the adpressed growth and lack of aerial hyphae to cut off radiation. As regards the induction of fruiting by cellophane in darkness, perhaps, the presence of cellophane somehow (by a contact stimulus?) alters the growth pattern of the fungi, as a consequence of which morphogenetic changes leading to fruiting occur. Fukuki and Aragaki
(1973) have reported the beneficial effect of dialysis membrane placed over agar on production of perithecia by *Cochliobolus heterosporus*. Recently, Zafar and Coletelo (1979) have been able to induce fruiting in darkness in *Plenodomus meliloti* by culturing the fungus in petri dishes containing glass beads and liquid medium. They believe that a substance necessary for fruiting was induced when growth of the fungus was arrested by a mechanical barrier or contact stimulus. Pillai *et al.* (1980), who could induce sporulation in *Helminthosporium gramineum* by using cellophane on agar medium, have also suggested that cellophane provided a contact stimulus. Hyphal walls and changes in them are of vital importance in many phases of fungal development (Morton 1967). We have seen that hyphal branching was increased and lateral adhesion of hyphae occurred on cellophane (figure 1). Germ tubes of *Puccinia coronata* uredospores which are normally straight and relatively unbranched have been observed to show considerable degree of branching when growing over nitrocellulose membranes (Dickinson 1970). According to Steele and Trinci (1975) differentiation in hyphae lead to their branching subapically. Recently, Dickinson (1977) has further shown that, in *P. coronata* germ tube growing on nitrocellulose membranes, there is a clear difference between the membrane-contact side and the air-contact side in the microfibrillar structure. This is a clear indication of the effect a membrane may have on the micromorphology of fungal hyphae. A similar effect due to cellophane seems plausible.

Acknowledgements

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References

Dickinson S 1970 Studies in the physiology of obligate parasitism. VII. The effect of a curved thigmotropic stimulus ; *Phytopathol. Z.* 69 115-124

Dickinson S 1977 Studies in the physiology of obligate parasitism. X. Induction of responses to a thigmotropic stimulus ; *Phytopathol. Z.* 89 97-115

Fukuki K A and Aragaki M 1973 Perithecial formation by *Cochliobolus heterosporus* on dialysing membrane ; *Mycologia* 65 705-709

Morton A G 1967 Morphogenesis in fungi ; *Sci. Prog. Lond.* 55 579-611

Pillai P K, Lal S P and Suryanarayana D 1980 Conidial production in *Helminthosporium gramineum* Rabb. in culture ; *Curr. Sci.* 49 114-115

Steele G C and Trinci A P J 1975 Morphology and growth kinetics of hyphae of undifferentiated and differentiated mycelia of *Neurospora crassa* ; *J. Gen. Microbiol.* 91 362-368


Swamy R N and Govindaraghavan B 1972 Induction of pycnidia by cellulosic substrates in two light-requiring fungi ; *Curr. Sci.* 41 750-751

Zafar S I and Coletelo N 1979 Replacement of light by depleting nutrient supply for pycnidium production by *Plenodomus meliloti* ; *Mycologia* 71 219-223