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Light-induced Fruiting in *Leptosphaerulina crassiasca* (Sechet) Jackson and Bell as Influenced by Carbon and Nitrogen Sources*

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On Czapek's agar, light-induced formation of fertile perithecia in *Leptosphaerulina crassiasca* only when a cellophane disc was present over the agar medium. When sucrose in the medium was replaced by starch or cellulose, the fungus formed fertile perithecia even without the cellophane. Similar results were obtained when nitrate was replaced by glycine or tyrosine. On glycine, the development of fruit bodies was more rapid than on nitrate. On the latter, fertile fruit bodies were formed only under black light (near-U V) lamps. On glycine, however, this occurred even under visible light.

Key Words: *Leptosphaerulina crassiasca*, **Carbon and Nitrogen sources, Light-induced fruiting**

Introduction

Leptosphaerulina crassiasca (Sechet) Jackson and Bell (=*L. arachidicola* Yen, & Chen Huang) causes pepper spot and leaf scorch on groundnut leaves. On natural media like potato dextrose agar, cultures of this fungus freely produce fertile perithecia on exposure to light. However, on a synthetic medium like Czapek's agar, fertile perithecia are formed only if the fungus is raised either on cellophane overlying the agar medium or by spreading a mycelial suspension on the agar medium (Suryanarayanan & Swamy 1977). In this paper, we report some further findings on the light-induced fruiting of this fungus on a synthetic medium as influenced by carbon and nitrogen sources.

Materials and Methods

A single ascospore isolate of the fungus was employed in the studies. Czapek's medium was used throughout. Unless otherwise stated the medium contained also 0.1% yeast extract. The *p*H of the medium was adjusted to 6.5 before autoclaving.

Media in petri dishes were inoculated by transferring to the centre, a plug of growth cut out from the growing margin of a five day old dark-grown culture.

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The plug was placed mycelium face down. Unless otherwise stated, cultures were exposed to light from two 40 W Sylvania BL black light lamps through the lids of petri dishes at a distance of 45 cm.

To determine the number of fruit bodies formed, the aerial hyphae were collapsed by flooding the culture with ethanol and the fruit bodies present in the area covered by the low power field of a microscope counted. (At least, three such areas from three different plates were examined in each case). The number of fruit bodies present in unit area (1 cm^2) was then calculated.

Experimental

The effect of glucose, sucrose, starch and cellulose as carbon source was tested since the fungus, as stated earlier, formed fertile fruit bodies in light on potato dextrose agar but not on Czapek's unless a cellophane disc was present on the latter. The effect of different nitrogen sources like sodium nitrate, ammonium tartarate, glycine, glutamic acid, asparagine and tyrosine was also tested. The carbon sources other than starch and cellulose were supplied at a level equivalent to 3% sucrose. Starch and cellulose (powder) were added to give 1% by weight.

The nitrogen sources were supplied to give 330 mg N/l (equivalent to the N provided by the $NaNO_3$ in Czapek's medium).

In the experiment with carbon sources, cultures were exposed to 12hr : 12hr light/dark cycles for 5 days and fruit bodies counted on the 6th day. In experiments with nitrogen sources, 5 day old dark-grown cultures were given a single induction period of 24 hr under the light source and fruit bodies counted after another 48 hr of incubation in darkness. Here, cultures were raised on agar media directly or on cellophane overlying the agar, and yeast extract was not included in the medium.

Carbon sources (table 1)

Fertile fruit bodies were formed on starch and cellulose. On sucrose, sterile fruit bodies were seen, and even these were not formed on glucose.

Table 1 *Effect of different carbon sources on light-induced fruiting in* Leptosphaerulina crassiasca

Carbon Source	No. of perithecia/cm^2
Glucose	0
Sucrose	385*
Starch	2759
Cellulose	2092
C.D at 5% level	47

*Sterile perithecia

Nitrogen sources (table 2)

In the absence of cellophane, fertile fruit bodies were formed only on glycine and tyrosine. In the presence of cellophane, the medium containing no nitrogen and that with nitrate also enabled formation of fertile fruit bodies. Ammonium tartarate did not allow formation of even sterile fruit bodies with or without cellophane.

Another interesting observation was that on glycine, the development of fruit bodies was more rapid than on nitrate (with cellophane) and even discharge of spores took place. The development on glycine was always ahead of that on nitrate by 6-12 hr (figure 1).

Table 2 *Effect of different nitrogen sources on light-induced fruiting in* Leptosphaerulina crassiasca

Nitrogen source	No. of perithecia/cm²	
	No Cellophane	Cellophane present
Nil	620*	880
Nitrate	0	1760
Ammonium	0	0
Glycine	320@	740@
Glutamic acid	0	1560*
Asparagine	0	3000*
Tyrosine	1740	2960
C.D. at 5% level	300	

*Sterile fruit bodies
@Ascopores discharges

Fruiting under visible light (table 3)

It had been observed by us that *L. crassiasca* produces only sterile fruit bodies under daylight fluorescent lamps on Czapek's agar medium overlaid with cellophane. In the light of the observation that glycine enabled a rapid development of fruit bodies it was of interest to see if, in the presence of glycine, fertile fruit bodies would be formed under daylight fluorescent lamps.

Czapek's agar medium containing either nitrate or glycine as the nitrogen source and overlaid with a cellophane disc was inoculated with the fungus. Inoculated plates were covered with a single layer of clear, blue, green, yellow or red cellophane and incubated under four daylight fluorescent lamps for

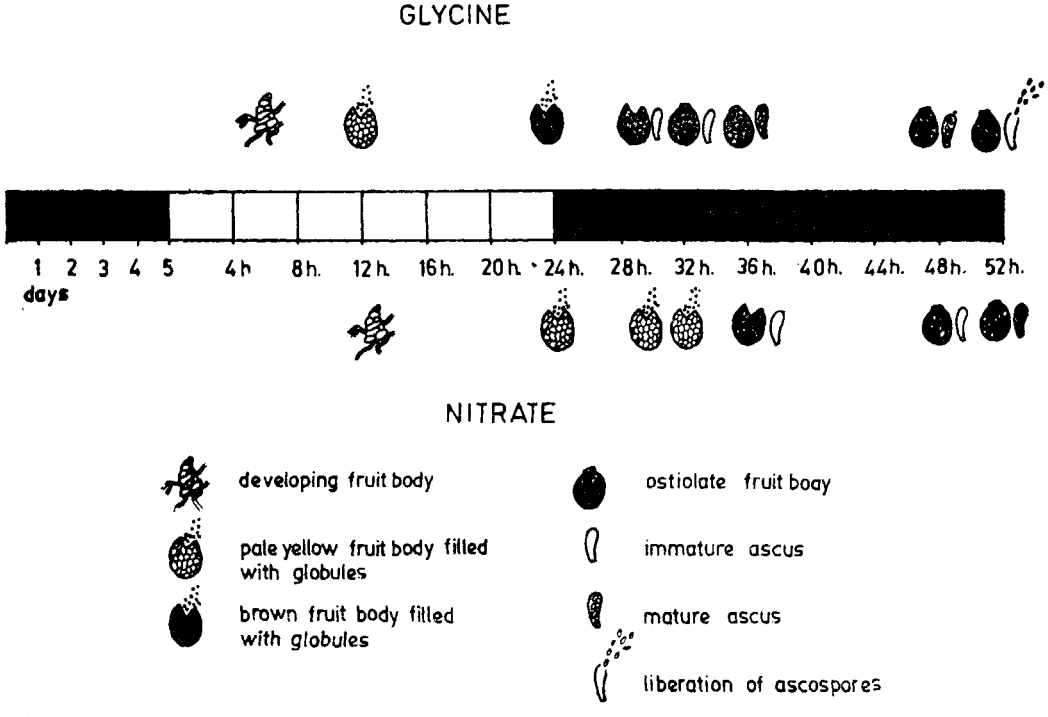

Figure 1 Sequence of development of fruit bodies of *Leptosphaerulina crassiasca* on Czapek's agar with nitrate or glycine as nitrogen source and a disc of cellophane overlying the agar medium. Cultures were exposed to light for 24 hr on the 6th day and incubated in darkness prior to and after the light treatment

Table 3 *Effect of nitrogen sources on fruiting of* Leptosphaerulina crassiasca *under different colour filters and day light fluorescent lamps*

Cellophane filter used (wavelength transmitted within brackets)	No. of perithecia/cm²	
	Nitrate	Glycine
Nil	4040*	2300
Clear cellophane	3333*	1880
Blue (mostly 350-400 nm)	3518*	1380*
Green (450-575 nm)	2352*	2060
Yellow (525-625 nm)	1555*	1340
Red (600-700 nm)	1574*	1640*
C.D. at 5% level	328	700

*Sterile fruit bodies

5 days (12 hr : 12 hr light-dark cycles). The plates were placed at appropriate distances from the lamps so as to receive through the filters a uniform intensity of light of 3000 lux as measured with a light meter. The numbers of fruit bodies were determined on the 6th day.

The results (table 3) show that on nitrate, only sterile fruit bodies were formed. Asci without spores were present in all cases except yellow and red filters. Under the latter, even asci were missing. On glycine, fruit bodies were fertile in all cases except blue and red filters. Ascospores were shot off from all fertile fruit bodies. Under blue filter, sterile asci were present. Asci and ostioles were lacking under red.

Discussion

The failure of the fungus to form fertile perithecia on Czapek's medium is partially explained by the results. Starch and cellulose seem to be better sources of carbon for fruiting than sucrose. Cellophane, probably, enables formation of fertile perithecia on nitrate medium by serving as a source of cellulose. The better fruiting on starch and cellulose could not have been a result of the lower concentration (1%) at which they were added since diluting the medium was detrimental to fruiting (Suryanarayanan 1978). The formation of fertile perithecia, even in the absence of cellophane, on some amino acids like glycine and tyrosine, suggests a further reason for fruiting without need for cellophane on potato dextrose agar where the nitrogen source would be the amino acids in the potato extract. The reason why glycine was taken up for more detailed investigation, even though fruiting was more abundant on tyrosine (table 2), was that discharge of ascospores occurred only on glycine in that experiment suggesting a more rapid development of the fungus on glycine compared to the other nitrogen sources. Normally, only cultures exposed to light for 30 hr or more will discharge spores. Glycine has been reported to be beneficial for sporulation in several fungi (Cantino 1959, Turian 1964, Manibhushanrao 1971, Bhama & Swamy 1973). It has been observed in this laboratory (Swamy & Mani 1978) that glycine not only enhances light-induced sporulation in *Cercospora personata* but also increases the sensitivity of the fungus to light as indicated by the fact that a higher percentage of colonies sporulate with a shorter duration of light compared to what obtains on nitrate. (This observation was the reason for giving only a single inductive light treatment in the experiments with nitrogen sources.) The results with *Cercospora*, referred to above, suggested the possibility that *L. crassiasca* which normally forms fertile perithecia only under black light might

be induced to form fertile fruit bodies even under visible light if glycine were the nitrogen source. The results (table 3) show that this was indeed the case. Relevant in this context is the observation of Pandey and Wilcoxon (1967) that in *L. briosiana*, formation of asci and ascospores and ejection of the latter are dependent on the source of nitrogen. In *L. trifolii* and *L. australis* also, perithecial formation under visible light has been reported (Kilpatrick 1962, Thomas & Halpin 1964). These fungi had been raised on V-8 juice agar and it is likely that the medium had an influence on the response to light.

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