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The induction of sporulation in Sclerotinia fructicola and some other fungi and the production of "P_{310}"

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Abstract

Evidence is presented suggesting that the sporulation of *S. fructicola* probably proceeds by several phases. Light stimulates development of primordia but inhibits conidia formation.

"P_{310}", for the first time mentioned by Leach, does not have a sporogenic character. It is, however, a photo product of a metabolic substance formed by the fungus. The production of "P_{310}" is much higher in mycelium cultivated in light of short wave-lengths and increases with the intensity of the light.

The "P_{310}"-production is not common for all fungi. It is possible that the precursor for the photochemical reaction is missing in the mycelium of fungi, which do not produce the factor.

Introduction

The effect of external circumstances (light, temperature, carbon dioxide, humidity etc.) on the sporulation of fungi has been studied by numerous authors. Until the present, it has been possible to infer from the many results a general principle which is responsible for the initiation and regulation of sporulation.

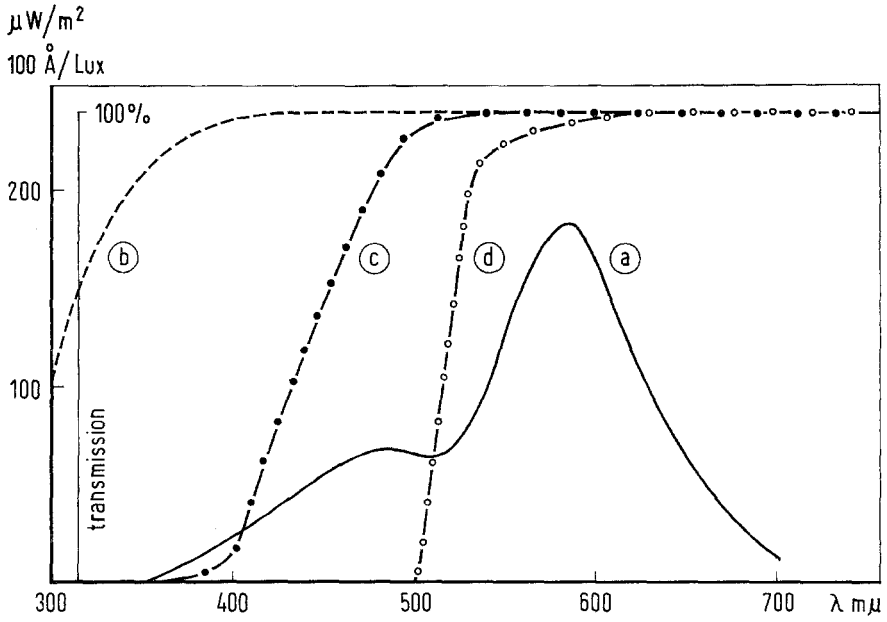
It is clear that light plays an important role in the sporulation of many fungi (Aragaki, 1961, 1962; Leach, 1961, 1962a, b, 1963; Gressel and Galun, 1967). A carotenoid should, therefore, be essential in the photo-induction (Gardner, 1955). The experiments of Carlile (1965), however, do not confirm this. Lukens (1965) has reported an inhibition of sporulation by blue light. The inhibiting factor had an absorption spectrum similar to that of riboflavine-5-phosphate-mononucleotide (FMN). His opinion was that semiquinones should inhibit a flavin dependent enzyme, necessary for sporulation. Trione et al. (1966) have found a sporogenic substance with a maximum absorption at 310 nm (P_{310}). This has not been proved conclusively.

The purpose of the present investigation was to determine whether there is, in fact, a relation between the so-called P_{310} and the induction of sporulation.

Materials and methods

The following fungi were used: (1) *Sclerotinia fructicola* (Wint.) Rehm, CBS 350.49, (2) *Alternaria tenuis* Nees, (3) *Alternaria porri* (Ellis) Sawada, CBS 107.61, (4) *Trichoderma viride* Pers. ex Fr., and (5) *Choanephora cucurbitarum* (Berk. & Rav.) Thaxter, CBS 120.25. The fungi 1, 3 and 5 were obtained from the "Centraalbureau voor Schimmelcultures", Baarn. Number 4 from the Weizmann Institute of Science, Rehovoth and 2 was isolated from plant tissue. The stock cultures 1, 2 and 3 were

Fig. 1. Spectral energy distribution of the light and the transmission curves.



a = absolute spectral energy distribution of the TL-tube color 333. Transmission of the light:
b = by the lid of a petri dish
c = by filter GG7
d = by filter OG1

Fig. 1. Spectrale energieverdeling van het licht en de doorlaatbaarheidskrommen van de gebruikte filters.

maintained on cherry agar and 4 and 5 on potato dextrose agar. All experiments were performed with one-spore cultures of the above mentioned fungi.

The cultivation for the experiments was carried out on Knop agar, to which was added 1 g of asparagine and 32 μg of aneurine per liter. *S. fructicola* was also cultivated on cherry agar.

The experiments were carried out in 9 cm petri dishes as follows:

a. The fungi were cultivated in a constant temperature room at 25 °C, with illumination by Philips fluorescent tubes (colour no. 33). The relative humidity was maintained at about 70%. For growth in the dark, the petri dishes were wrapped in paper which was black on one side and covered on the other side with stanniol. Fig. 1 shows the spectral energy distribution of the fluorescent lamps. "Light" will refer to the light of these tubes, unless otherwise mentioned.

b. Cultures were also grown in a water cooled stove, at about 19 °C, with illumination of the same type as above. The heat effect of the lamps was eliminated by a waterfilm placed between the source of light and the cultures. Some experiments were carried out with the Scott colour filters GG7 and OG1. The lid of the petri dish was replaced by this filter. The effect of both filters and of an ordinary lid is shown in Fig. 1. The light intensities were measured by a Luxmeter placed under the lid of the petri dish or under the filter.

S. fructicola, with 2-days-old mycelium, was transfered by means of small agar blocks punched out by a cork borer. The linear growth of the mycelium was determined with the "diameter method" described by Hawker (1950).

The sporulation was determined in two different ways:

a. The culture was covered with 10 ml of 0.5% Tween-80 solution and the spores were freed from the conidiophores by gently drawing the transfer needle over the mycelium. The spore suspension was counted by means of a counting chamber.

b. If there were clear transitions in the mycelium, then four pieces of about 0.65 cm^2 were punched out from one zone. The pieces were then taken up in 2 ml 0.5% Tween-80 solution to produce a spore suspension.

Samples from every spore suspension (0.1 mm^3) were counted nine times, so that the figures in the tables refer to the number of spores per 0.1 mm^3. Each figure is the average of the spore suspensions from two petri dishes.

The extraction method for P_{310} was the same as used by Leach (1965) as was the purification of the extracts. To compare the concentrations of P_{310} in the different extracts the volumes were equalized, and the absorption spectra were measured with a Zeiss PMQ II spectrofotometer or with a Bausch & Lomb Spectronic 505.

Experiments and results

The growth of S. fructicola and the influence of cultivation in continuous light and dark

The growth stages of *S. fructicola* were determined on cultures grown in petri dishes. The growth in darkness proceeds from the build up of a mycelium mat, followed by the development of primordia upon this mat. "Primordia" refers to that part of the

Fig. 2. The growth rate of the mycelium of *Sclerotinia fructicola* in continuous light and in dark; A: at 20 °C; B: at 25 °C.

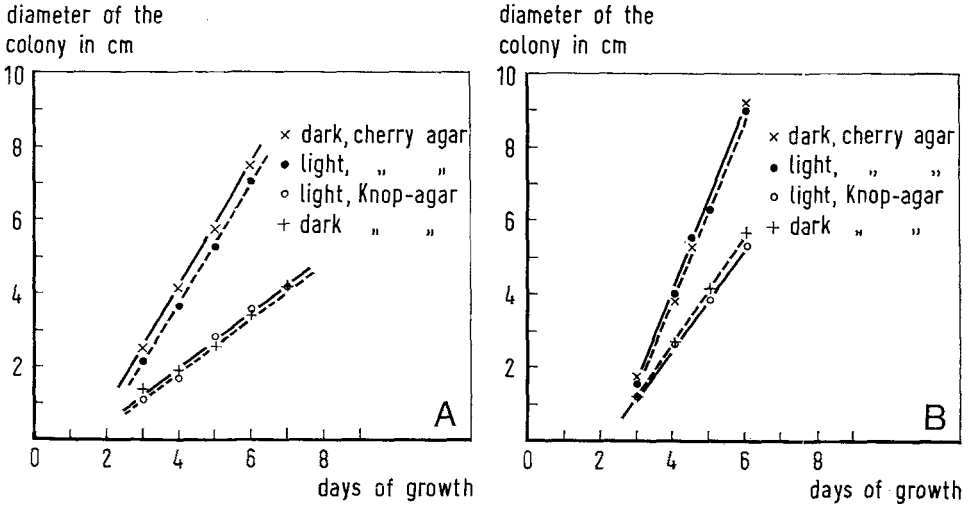


Fig. 2. De groeisnelheid van het mycelium van Sclerotinia fructicola in continu licht en in het donker; A: bij 20 °C; B: bij 25 °C.

hyphae which grow out with the tips above the agar. From these the conidiophores are developed, the latter branching abundantly. The development of the spores then begins and this forms a cover upon the agar. If the fungus is cultivated in the light at 4800 Lux, the growth stops after development of the primordia. Higher light intensities give the same picture as described for 4800 Lux. At lower light intensities, development of the conidiophores takes place, but their structure is more erect and little branching is observed. Development of the mycelium was examined in continuous light and dark because this can be of influence upon the sporulation. The growth rate of the hyphae proved to be the same in both cases (Fig. 2).

If a culture growing under light conditions with no sporulation is transfered to the dark at the same temperature, a sharp transition can be expected to sporulating mycelium. Table 1 shows that a period of continuous light is of no influence upon sporulation if the subsequent period of darkness is longer than 48 h. If this period is shorter, then previously developed mycelium will lag behind in sporulation, compared to mycelium developed in the dark.

From the foregoing results we conclude that the light has an inhibiting effect upon the sporulation. To determine if this is the only effect of light, a period of continuous light was followed by a short period of darkness (15 h). Table 2 demonstrates that sporulation in the youngest mycelium was stimulated, possibly through stimulated development of primordia and/or conidiophores. This effect was also reported by Aragaki (1961) for *Alternaria tomato*, and it is also implicit in experiments of Sagromsky (1959) with *Sclerotinia fructicola*.

The production of P_{310}, its sporogenic and photochemical character

Leach (1965) extracted a compound from mycelium of several fungi which were cultivated in near ultraviolet light (NUV). The compound had a maximum in the absorption spectrum at 310 nm and therefore was named P_{310}. Mycelium cultivated in continuous darkness did not contain such a substance. Leach was studying strains of fungi which sporulate when cultivated in the light. Therefore he concluded that P_{310}

Table 1. The influence of a light period preceding sporulation in the darkness.

Series	Conditions during incubation period	Spores (number/0.1 mm³)	
		old mycelium	young mycelium
1	0L–8D	124	141
2	3L–5D	117	110
3	4L–4D	134	138
4	5L–3D	151	110
5	6L–2D	21	121
		33	142
6	7L–1D	30	141
		18	135
7	8L–0D	0	0

4L–4D = 4 days of continuous light followed by 4 days of continuous dark at 25 °C and 4800 Lux.

Tabel 1. Invloed van een lichtperiode op de daaropvolgende sporulatie in het donker.

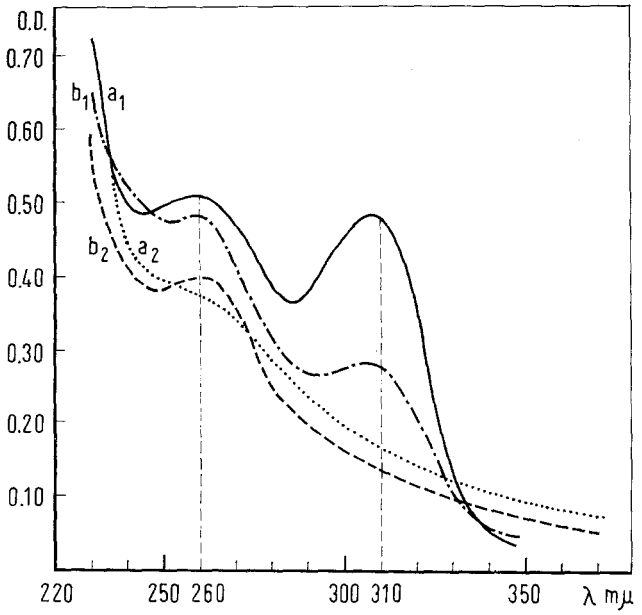
had a sporogenic character, which he attempted to confirm (Trione et al., 1966). It is quite possible, however, that the crude extracts used still contained substances other than P_{310}. The sporulation pattern reported by these workers also showed inhibition of mycelium growth, suggesting accumulation of substances. This effect is perhaps comparable to that found in the margins of petri dishes, where increased sporulation may occur because of accumulating of phosphate (Cochrane, 1958). We also applied the extraction used by Leach to sporulating mycelium (SM, cultivated in continuous darkness), and to non-sporulating mycelium (NSM, cultivated in continuous light).

Table 2. The influence of light on the initiation of sporulation in the dark at 25 °C and 9000 Lux.

Conditions during incubation period	Number of spores per 0.1 mm³ on the youngest mycelium	
Light, 6 days + 15 h dark	43.4	40.9
Continuous dark, 6 days + 15 h	27.1	28.0

Tabel 2. De invloed van het licht op de initiëring van de sporulatie.

Fig. 3. Absorption spectra of ethanol extracts of non-sporulating mycelium (a_1 and b_1) and sporulating mycelium (a_2 and b_2).



Curve a_1 irradiated at 6100 Lux and 25 °C.
Curve b_1 irradiated at 4800 Lux and 25 °C.
Curves a_2 and b_2 kept in dark at 25 °C.

Fig. 3. Absorptiespectra van aethanolextracten van niet-sporulerend mycelium (a_1 en b_1) en sporulerend mycelium (a_2 en b_2) gekweekt bij 25 °C.

Table 3. The influence of light intensity and wave-length on P_{310} production.

Series	Filter	Light intensity in Lux	Number of spores/0.1 mm³	Opt. density of mycelium extracts at 310 nm
1	lid of	8500	0	0.525
2	petri	6100	0	0.355
3	dish	4800	0	0.225
4	GG7	8200	0	0.135
5	GG7	5700	79	0.035
6	OG1	7000	0	0.095
7	OG1	4700	141	0.005

GG7 absorbs wave-lengths shorter than 400 nm.
OG1 absorbs wave-lengths shorter than 500 nm.
Tabel 3. Invloed van lichtintensiteit en golflengte op de vorming van P_{310}.

The NSM clearly contained P_{310}, but we were unable to detect the presence of P_{310} in the SM extracts (Fig. 3). These results do not confirm the conclusion of Leach that P_{310} has a sporogenic character.

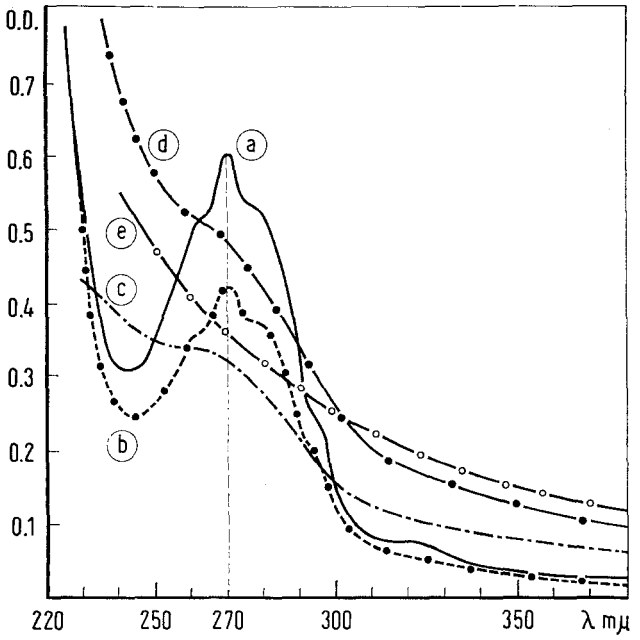
As P_{310} was found only in non sporulating hyphae, it is possible that this compound is transportable, thereby passing from the mycelium to the conidiophores where it is used in the development of the spores. Extractions from conidiophores and from conidia (2.25×10^7), however, showed that there were no demonstrable quantities of P_{310} in either the conidiophores or the conidia.

Because the light intensity influenced the sporulation, we also studied its effect upon the P_{310}-production, as well as the effect of wave-length. It is clear from the results shown in Table 3 that the amount of P_{310} formed is correlated with the given light intensity. The influence of wave-length, however, is much stronger. The series 1 and 4 differ only 3.5% in intensity, but more than 280% in P_{310}-production. The wave lengths smaller than 500 nm prove to be of great influence on the P_{310}-production.

The presence of P_{310} in some other fungi

Leach (1965) demonstrated the presence of P_{310} in five species of fungi, cultivated in the light. We studied in addition *Alternaria tenuis, A. porri, Trichoderma viride* and *Choanephora cucurbitarum. A. tenuis* produces much aerial mycelium but only a few conidia in the darkness, whereas in the light the fungus does not make aerial mycelium but many conidia. P_{310} proved to be present in the mycelia and the spores cultivated under light conditions, but was hardly demonstrable in mycelium formed in the darkness. P_{310} was also found in the mycelium of a sporulating culture of *A. porri*, grown under common daylight conditions at room temperature. *T. viride* produces much aerial and a slight sporulation in the dark. In the light (4800 Lux), however, this fungus shows good uniform sporulation. The absorption spectra of the mycelium extracts showed a high absorption between 240–300 nm. The substances measured at these wave-lengths appeared to be soluble in ether and disappeared during purification. P_{310} was found neither in the mycelium cultivated in the dark, nor in the light. *C. curbitarum* showed the same absorption spectrum of the mycelium extracts as *T. viride*.

Fig. 4. Absorption spectra of extracts of mycelium of *Trichoderma viride;* mycelium cultivated at 24 °C.



a = in the dark (ethanol extract)
b = in the light at 4800 Lux (ethanol extract)
c = in the dark (purification with ether)
d = in the lightfat 4800 Lux (purification with ether)
e = in the light at 4800 Lux (purification over Dowex 2 column)

Fig. 4. Absorptiespectra van mycelium-extracten uit Trichoderma viride, gekweekt bij 24 °C.

Cultivation in the light at 4800 Lux produced sporulation, and no P_{310} proved to be present.

Discussion

The inhibiting effect of light upon sporulation explains why Hall (1933) was unable to observe conidia in continuous light, whereas Sagromsky (1959) found sporulation under different conditions.

Since light also stimulates primordia development, it seems plausible that there are several phases in the sporulation mechanism, namely the stimulation of the development of primordia and/or conidiophores by light and the phase of conidia development, which is inhibited by light. Another argument for the theory that there are several phases is the formation of zones. Jerebzoff (1956) and Sagromsky (1959) have demonstrated that zonation is caused mainly by light, which stimulates conidiophore development. This is in agreement with the results of our experiments at low light intensities (< 4800 Lux), which are more in agreement with the natural situation for the fungus than is exposure to high light intensities.

The sporulation found in cultures exposed to high light intensities, but with a GG7

or an OG1 filter, can be explained by the fact that the wave lengths removed are just those responsible for the inhibition of the development of primordia or conidiophores.

The result that light of shorter wave-lengths is more effective for the production of P_{310}, correlated with the fact that high light intensities give the highest P_{310}-production, is in agreement with the report of Leach (1965) that velocity of P_{310}-production in irradiated cultures increases with the dose.

The sporogenic effect of P_{310} already discussed, could not be confirmed. As P_{310} is nearly always present in mycelia of irradiated cultures, both in these experiments and those of Leach, we believe that P_{310} is a photo product of a compound synthesized by the fungus. This precursor is probably not formed by fungi (e.g. *T. viride*) which do not produce P_{310} upon irradiation. If the precursor of P_{310} is an important compound in the metabolism of the fungus exposure to light may affect sporulation because the precursor must be produced continuously.

Samenvatting

De inductie van de sporulatie bij Sclerotinia fructicola en enige andere schimmels en de vorming van "P_{310}"

De groeistadia van *Sclerotinia fructicola* werden bij het kweken in licht en donker bestudeerd. De groei begint met de vorming van hyfen in de agar, daarna treden de toppen van bepaalde hyfen boven de agar uit (primordia) en vormen conidioforen en conidiën. In continu donker verloopt dit proces zoals boven weergegeven, doch in continu licht (4800 Lux of hoger) wordt wel het mycelium in de agar gevormd en de primordia aangelegd, doch dan stopt het groeiproces. Bij lichtintensiteiten lager dan 4800 Lux vindt wel de vorming van conidioforen plaats. Deze verheffen zich boven de agar en zijn spaarzaam vertakt in tegenstelling tot de conidioforen, die in het donker gevormd worden. Deze zijn rijkelijk vertakt en vormen als het ware een mat op de agar.

De groeisnelheid van het mycelium is in licht en donker even groot.

Gaat aan de donkerperiode een lichtperiode vooraf, dan is deze van geen invloed op de sporulatie, mits de donkerperiode langer dan 48 uur duurt. Is deze korter, dan blijft het mycelium dat vóór de donkerperiode gevormd is, achter in sporulatie.

Licht voorafgaande aan een donkerperiode van 15 uur, stimuleert in het jongste mycelium de sporulatie. Het licht heeft zowel een stimulerende als een remmende invloed.

P_{310}, voor het eerst door Leach vermeld, bleek geen sporegene stof; het is echter een stof, die onder invloed van licht ontstaat uit een stofwisselingsprodukt van de schimmel.

Bij de vorming van P_{310} is licht van kortere golflengte van groter invloed dan licht met langere golflengte en de P_{310}-productie neemt toe met de intensiteit van het gebruikte licht.

De vorming van P_{310} komt niet voor bij alle fungi. Het is mogelijk dat de precursor voor de fotochemische reactie ontbreekt in het mycelium van deze schimmels.

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