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Regulation of Periodicity and Intensity of Photo-Induced Conidiation of *Trichoderma viride*

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ABSTRACT. After a short irradiation at 366 nm with 200 lx, the intensity of conidiation of *Trichoderma viride* colonies grown in the dark increased for the first 10 s proportionally with time. The increase slowed down after 10 s—5 min of exposure and after 10—60 min of irradiation the conidiation intensity began to decrease. When photo-induced by daylight, the conidiation started at a high rate after 25 h and persisted even after 48 h. The conidiation had no circadian character and its periodicity depended on the periodicity of photo-induction. Its intensity was also influenced by the carbon sources used, a maximum being reached with glucose (1—2 %). Higher glucose concentrations inhibited conidiation but had no influence on growth of colonies.

Ever-increasing attention is being paid at present to the effects of light on the reproduction of fungi (cf. *e.g.* Durand 1976). Certain fungal species react to exposure to far-UV radiation (200—300 nm), other species respond to the exposure to near-UV radiation (300—380 nm) and, at the same time, to blue light (≦520 nm). The imperfect fungus *T. viride* belongs to the latter class. According to Bjornsson (1959), sporulation of *T. viride* is induced by radiation at wavelengths from 430 to 490 nm. According to Gressel and Hartmann (1968) two efficiency maxima exist at 380 nm and 440 nm whereas radiation at wavelengths above 525 nm induces no sporulation. Kumagai and Oda (1969) found two action maxima in the near-UV range at 320 nm and 380 nm and two in the visible range at 430 nm and 480 nm.

The photo-induced sporulation of *T. viride* can be achieved either by short irradiation of colonies grown in the dark before and after induction (Galun and Gressel 1966) or by an analogous irradiation of mycelium grown in the dark which, after filtration and irradiation in Petri dishes, was further cultivated in the dark (Betina and Spišiaková 1976).

Since many fungi are characterized by an approximately circadian periodical activity of metabolic and morphogenetic processes (*e.g.*, synthesis of macromolecular substances, enzyme activity, conidiation, *etc.*) we tried to find the existence of a similar periodicity in the sporulation of *T. viride*. We also studied the dependence of intensity of sporulation on the carbon sources used, on the wavelengths of radiation, its intensity, and lengths of exposure.

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MATERIALS AND METHODS

Organism. The strain *Trichoderma* sf. *viride* Pers. ex S. F. Gray (strain with smooth conidia) 8-7 from the collection of this institute was used. The strain was maintained on wort agar and cultivated at 26 °C.

Cultivation media. Potato-dextrose agar (PDA), Czapek-Dox agar (both media according to Booth, 1971, but with 1.5 % agar) and corn-meal agar (Oxoid) were used. In some experiments 0.5 % yeast autolysate (Imuna, Šarišské Michaľany, Czechoslovakia) was added to the medium.

Experiments with carbon sources were performed on Czapek-Dox agar with sucrose replaced with the carbon source under study.

Photo-induction. Colonies cultivated in the dark on filter paper discs for 36 – 40 h (Betina and Spišiaková 1976) were placed on fresh media in Petri dishes (the procedure was performed in red light) and left in the dark at room temperature for 30 min. The colonies were then irradiated with near-UV light (366 nm, UVIS lamp, Desaga, Heidelberg) or white light (Tesla fluorescent tube). The irradiation intensity was measured at the level of Petri dishes with a PU 150 luxmeter (Metra, Blansko, Czechoslovakia). Incubation continued in the dark after the irradiation for another 24 to 40 h.

Counting of conidia. The colonies on paper discs were transferred from the agar plates to dry beakers washed with a standard volume of 10 ml water with 0.1 % Tween 80, and the conidia were released by means of a rubber-coated glass rod. The homogenized suspensions were filtered through four layers of gauze in order to collect mycelial fragments. Five ml of the filtered spore suspension were transferred into a centrifuge tube, centrifuged and resuspended in 5 ml of water with 0.1 % Tween 80. Absorbance at 650 nm was measured in 1 cm cuvettes in a SPECOL spectrophotometer (Zeiss, Jena). The concentration of conidia was determined from a calibration curve constructed for each experiment and relating absorbance to the number of conidia determined in a haemocytometer.

In Experiment 1 *T. viride* colonies grown in the dark for 40 h were irradiated (366 nm, 200 lx) for various time intervals ranging from 2 s to 5 min and than grown in the dark for further 30 h.

In Experiment 2 the effect of prolonged irradiation (366 nm, 200 lx) on conidiation intensity was studied. The exposure times were 1, 10, 30 and 60 min.

In Experiment 3 the colonies were irradiated with white light from a Tesla tube for equal time intervals but at various distances from the light source. The illumination intensities thus achieved were 0.5, 1, 2, 3, 4 and 5 klx.

Experiment 4 included a long-term observation of conidiation. Petri dishes (d = = 170 mm) with 50 ml of agar-containing medium were used. A sterile disc of Whatman No. 3 filter paper (d = 150 mm) with the central part (d = 20 mm) cut out and the rest divided into 8 equal segments was placed on the medium surface in each dish. Agar blocks cut out of the margin of the growing *T. viride* colony and functioning as inoculum were transfered into these central openings. The colonies cultivated in the dark for 36 h were irradiated once by daylight (3 min, 2.5 klx) and grown in the dark again. In order to determine the number of conidia the segments were collected at given intervals, the manipulation being carried out in red light.

Experiment 5 concerned a repeated photoinduction at various dark and light intervals. Petri dishes (d = 170 mm, 50 ml of medium) without filter papers were used. Inoculation with agar blocks was the same as in Experiment 4.

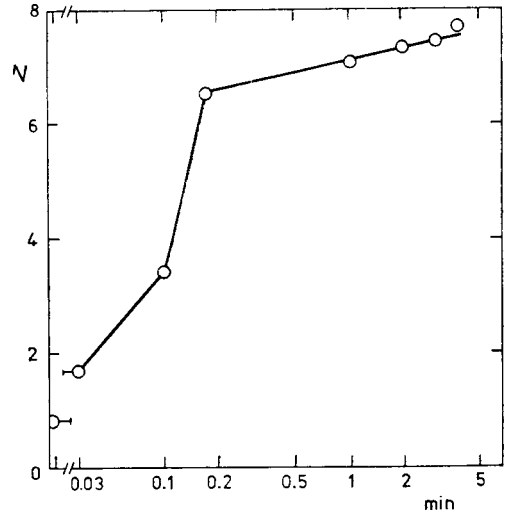

FIG. 1. Effect of legth of exposure to near-UV radiation (366 nm) on conidiation intensity of *T. viride* colonies; *N* conidia per colony $\times 10^{-7}$.

The growth and conidiation was studied under the conditions of day-and-night rotation (Experiment 6) in a glass tube (d = 30 mm, l = 500 mm) with both ends bent upwards and sealed with cotton stoppers. The sterile tube was filled with 80 ml of corn-meal agar with 0.5 % of yeast autolyzate and inoculated in one tube end with an agar block which was cut out of the perifery of a growing colony. The tube was then left under laboratory conditions out of the direct light for 16 days.

In Experiment 7 colonies of *T. viride*, inoculated in large-size Petri dishes with agar blocks were cultivated for 30 h in the dark. Every colony was then induced by day-light of equal intensity (2 klx) and exposure lengths (3 min) but at various intervals (8, 16, 24, 48 h), the first irradiation of all colonies being done simultaneously.

The effect of carbon sources on induced sporulation was studied in Experiment 8. Sucrose in Czapek-Dox agar was substituted by glucose, glycerol, or sodium acetate, or the carbon source was omitted altogether. Colonies grown in the dark on Czapek-Dox agar with sucrose were transferred from filter paper discs on plates with the above carbon sources and after 30 min of adaptation induced by daylight for 5 min.

RESULTS

Effect of white light and near-UV radiation on conidiation

The results of Experiment 1 are shown in Fig. 1. Sporulation already is induced by 2 s of irradiation with light of 366 nm and 200 lx and its intensity increases

TABLE I. Effect of the length of irradiation of *T. viride* colonies (UV-radiation, 366 nm) on conidiation

Exposure min	Number of spores in colony $\times 10^{-6}$
1	380
10	340
30	300
60	290

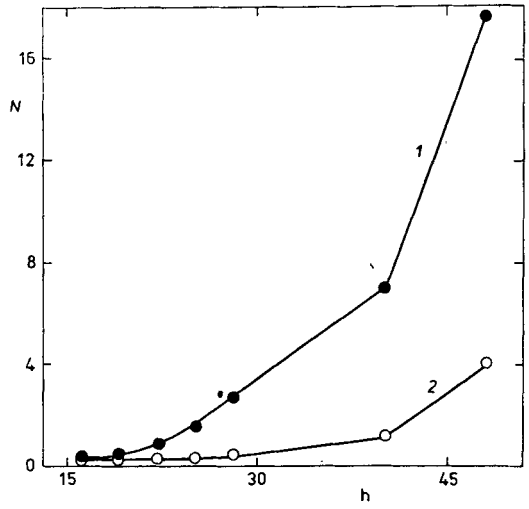

FIG. 2. Time course of photo-induced conidiation of *T. viride* colonies; *1* illuminated colonies, *2* control sample in the dark; *N* conidia $\times 10^{-7}$.

rapidly until the 20 s limit. Further increase of sporulation intensity after 5 min of irradiation is slower. Irradiation for 10—60 min (Experiment 2) results in a slight decrease of sporulation intensity compared to the 1-min irradiation (Table I). When different intensities of white light (Experiment 3) were used no differences in sporulation intensities were noticed (macroscopic observations).

Course of conidiation

In Experiment 4, where the intensity of sporulation of colonies was studied at various time intervals after irradiation, generation of nonpigmented conidia was observed as early as 16 h after the photo-induction, but a perceptible increase in conidiation and pigmentation began only after 25 h and lasted approximately until the 40th h. After this period the conidiation and pigmentation increase was even more striking (Fig. 2). Sporulation took place in a narrow ring (about 10 nm wide) in the region which the margin of the growing colony had reached during the photo-induction. The mycelium which was further cultivated in the dark was colourless in contrast with the deep-green colour of the ring. Colonies continuously cultivated in the dark had, on the other hand, in their older regions irregularly spaced small sporulation loci.

Sporulation periodicity

By means of repeated alternation of light and dark periods, we attempted to resolve the question of the circadian character of sporulation of *T. viride*. The results of Experiment 5 are demonstrated in Plate 1. Interval alternations: continuous exposure to white light for 8 h and incubation in the dark for 16 h resulted in alternation of sporulating and nonsporulating areas, whose widths were related to the duration of growth in the light or dark (Plate 1a). An analogous effect was achieved by irradiating growing colonies for 5 min every 24 h (Plate 1b). Colonies growing in dishes kept under normal laboratory conditions (day-and-night cycle) had rotating sporulating and nonsporulating areas, the former being wider than the latter (Plate 1c). Colonies growing for the first 30 h in the dark and continuously irradiated later had only one sharply defined sporulation area which corresponded to the

TABLE II. Effect of carbon sources on photo-induced sporulation of *T. viride* colonies

Carbon source added	Sporulation		Growth	
	number of spores in colony		increase of radius	
	$\times 10^{-6}$	%	mm	%
2 % sucrose	143	100	18.0	100
2 % glucose	223	150	20.0	106
2 % glycerol	146	102	16.5	89
40 mM sodium acetate	62	43	0.0	0
—	138	97	17.5	93

beginning of exposure. Later, during continuous cultivation in the light, the sharply outlined sporulation area disappeared (Plate 1d). Colonies growing in the dark continuously for 5 d had a vague central zone with sporulating projections radiating towards the margin of the colony, where they merged with the hyaline nonsporulating mycelium (Plate 1e). Culture growing in the long tube (Experiment 6) and subjected to 16 d of day-and-night rotation had a periodically alternating sporulating and nonsporulating areas, the former ones corresponding to the days, the latter to the nights (Plate 1 f).

In Experiment 7, one set of dishes was kept in the dark until the termination of the experiment; the result was similar to that shown in Plate 1e. Segments from the four differently induced colonies in Experiment 7 (Plate 2) provided evidence that the sporulation areas originated in different intervals depending on the frequency of irradiation of the corresponding colony. From this result the conclusion follows that the sporulation of *T. viride* has no circadian character but can be induced and synchronized by irradiation.

Effects of carbon sources on photo-induced spoluration

In Experiment 8 with different carbon sources the intensity of sporulation was not constant (Table II). The most conspicious sporulation intensity occured in the presence of glucose. Sodium acetate made further growth of colonies impossible and sporulation was about half that found in its absence. Sporulation in the presence of glycerol was practically the same as that in the control sample and was only slightly lower on agar without any carbon source. The effect of various concentrations of glucose in the Czapek-Dox agar on the sporulation of *T. viride* colonies is documented in Fig. 3. While the growth of colonies was not markedly affected by glucose concentration betwen 1 and 10 %, its effect on sporulation intensity was substantial. Sporulation was stimulated by lower concentrations while higher concentrations (5 and 10 %) inhibited sporulation almost to the control level.

DISCUSSION

The course of photo-induced *T. viride* sporulation can be divided into the following stages:

1) intensive vertical growth of short aerial white mycelial in a 3—4 mm wide ring on the colony margin which can be observed even macroscopically (4 h after photo-induction);
2) branching of growths on the aerial mycelium (after 8 h);
3) formation of phialides from the branches (after 12 h);

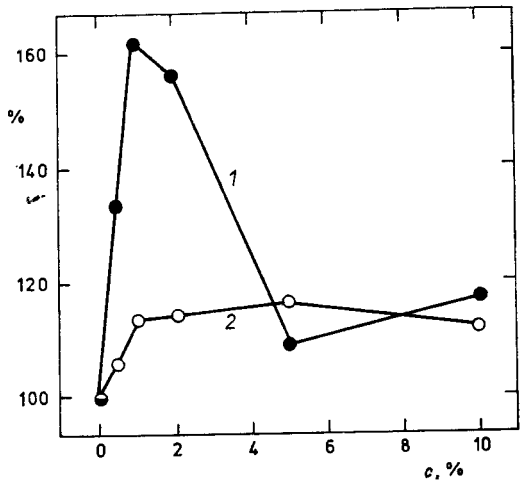

FIG. 3. Effect of glucose concentration in Czapek-Dox agar (*c*, %) on the intensity of photo-induced conidiation and growth of *T. viride* colonies (%); *1* conidia, *2* growth.

4) beginning of formation of nonpigmented conidia (after 16 h);
5) intensive conidiation and accumulation of deep-green pigments in the conidia (after 25 h).

Sporulation intensity can be regulated through the length of exposure at 366 nm (Fig. 1). The inhibition of colony sporulation by long-term irradiation (Table I) was not as marked as that observed in a mycelium grown under submerged conditions and filtered shortly before the irradiation (Betina and Spišiaková 1976).

With respect to the age of the growth regions the observed sporulation is synchronized in the sense that light induces the generation of spores in the youngest region but not in older regions. This effect differs from the photoinduced generation of macroconidia in *Neurospora crassa* where the conidiation was observed to begin at the mycelium periphery and advance towards the centre of the colony (Siegel *et al.* 1968). From the point of view of quantity of conidia generated the photo-induced sporulation of *T. viride* is not synchronized as the number of conidia per unit area of the sporulating region increases with time (Fig. 2, Table II). The photo-induced and synchronized conidiation of *Botrytis cinerea* colonies irradiated with near-UV light is, on the other hand, characterized by a sudden increase in the number of conidia which lasts for about 4 h and after which the number of conidia does not change (Tan 1976). The limited sporulation of nonilluminated colonies of *T. viride* is delayed as compared with the photo-induced sporulation and this fact could be related to the exhaustion of nutrients from the medium. There is a number of instances in the literature showing that nutrient limitation especially by the carbon and nitrogen sources leads to fungal sporulation (Smith and Galbraith 1971; Lowett 1975).

The insight into the photo-induced sporulation of fungi led many authors to speculate about possible photoreceptors and their character. According to one hypothesis the photoreceptors could be of the flavin type, according to another they could be carotenoid (Durand 1976). Gressel and Hartmann (1968) tested the effects of lyxoflavin (inhibitor of flavin synthesis) and diphenylamine (inhibitor of carotenoid synthesis) on *T. viride*. They found that both of these inhibitors suppressed colony growth to a greater extent than sporulation and the question of the *T. viride* photoreceptor seems thus to remain unanswered. Kumagi and Oda (1969) found two

action maxima of photo-induced sporulation of *T. viride* in the near-UV range of radiation. Especially the second maximum (320 nm) was very close to the absorption maximum (310 nm) of the recently discovered mycosporins 1 and 2 which were isolated from the basidiomycete *Stereum hirsutum* (Arpin *et al.* 1977*a*) or from the deuteromycete *Botrytis cinerea* after photo-induction (Arpin *et al.* 1977*b*).

Sporulation and growth on agar without any carbon source differed only slightly from that of the control sample with sucrose. This result is not surprising if we take into consideration the fact that the colonies grew on a filter paper and that *T. viride* is known to produce cellulases. Besides these facts also the agar polysaccharides could function as carbon sources of fungi (Payton *et al.* 1976).

The intensity of sporulation could be regulated by glucose concentration in the medium (Fig. 3). (Glucose is of course known to have catabolite effects on various metabolic processes and the manner of its utilization can be regulated by its concentration.) Concentration of glucose exceeding 2 % had in our experiments a negative effect on the photoinduced sporulation but the colony growth was similar to that found with lower concentration.

The plates will be found at the end of the issue.

REFERENCES

ARPIN N., THIEVEND S., FAVRE-BONVIN J.: Substances photoabsorbantes de *Stereum hirsutum* (Wild ex Fr.) Fr.; isolement, purification et caracteristique de P 310 (mycosporine 1). *Bull. Soc. Mycol. France* **93**, 39 (1977*a*).

ARPIN N., FAVRE-BONVIN J., THIVEND S.: Structure de la mycosporine 2, nouvelle molécule, isolée de *Botrytis cinerea*. *Tetrahedron Letters* 819 (1977*b*).

BETINA V., SPIŠIAKOVÁ J.: Suppression of photo-induced sporulation in *Trichoderma viride* by inhibitors. *Folia Microbiol.* **21**, 362 (1976).

BJORNSSON I. P.: Responses of certain fungi particularly *Trichoderma* sp. to light. *J. Washington Acad. Sci.* **49**, 317 (1959).

BOOTH C.: Fungal culture media, p. 49 in C. Booth (Ed.): *Methods in Microbiology*, Vol. 4. Academic Press, London—New York 1971.

DURAND R.: Influence des radiations lumineuses sur les processus de reproduction des champignons; hypothéses sur l'identité des photorécepteurs. Revue bibligraphique. *Mycopathologia* **60**, 3 (1976).

GALUN E., GRESSEL J.: Morphogenesis in *Trichoderma*: suppression of photoinduction by 5-fluorouracil. *Science* **151**, 696 (1966).

GRESSEL J. B., HARTMANN K. M.: Morphogenesis in *Trichoderma*: action spectrum of photoinduced sporulation. *Planta* (Berlin) **79**, 271 (1968).

KUMAGAI T., ODA Y.: An action spectrum for photoinduced sporulation in the fungus *Trichoderma viride*. *Plant Cell. Physiol.* **10**, 387 (1969).

LOWETT J. S.: Growth and differentiation of the water mold *Blactocladiella emersoni*: cytodifferentiation and the role of ribonucleic acid and protein synthesis. *Bacteriol. Rev.* **39**, 345 (1975).

PAYTON M., MCCULLOUGH W., ROBERTS C. F.: Agar as a carbon source and its effect on the utilization of other carbon sources by acetate nonutilizing (*acu*) mutants of *Aspergillus nidulans*. *J. Gen. Microbiol.* **94**, 228 (1976).

SIEGEL R. W., MATSUYAMA S. S., UREY J. C.: Induced macroconidia formation in *Neurospora crassa*. *Experientia* **24**, 1179 (1968).

SMITH J. E., GALBRAITH J. C.: Biochemical and physiological aspects of differentiation in the fungi. *Adv. Microbiol. Physiol.* **5**, 45 (1971).

TAN K. T.: Light-induced synchronous conidiation in the fungus *Botrytis cinerea*. *J. Gen. Microbiol.* **93**, 278 (1976).

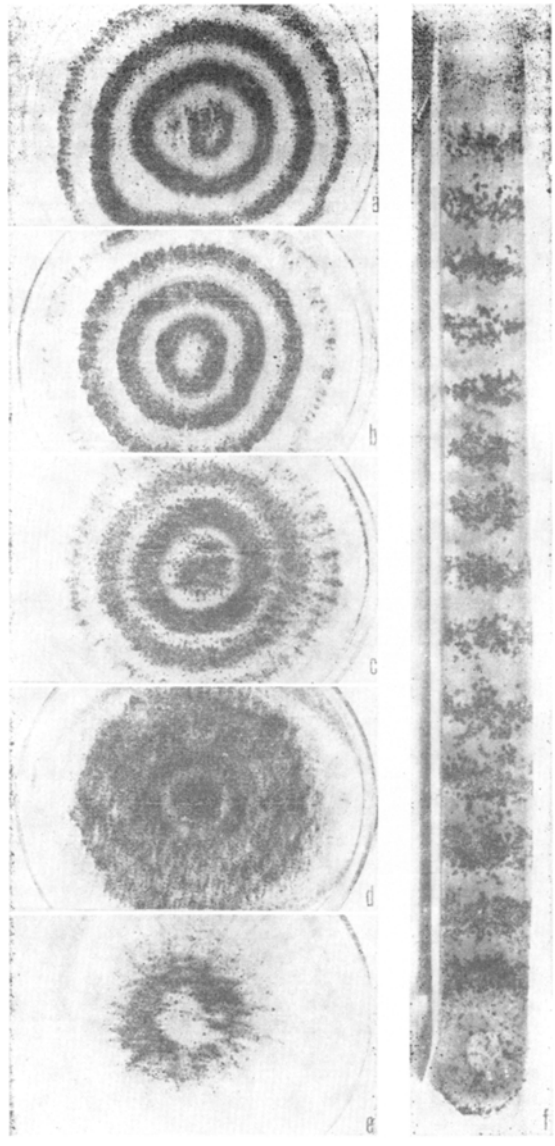

PLATE 1. Photo-induced conidiation of *T. viride*; **a** rotation of intervals of 8 h illumination and 16 h dark, **b** colony grown in the dark and illuminated for 5 min by daylight once in 24 h, **c** colony subjected to normal day-and-night regime, **d** colony grown under continuous irradiation, **e** colony grown continuously in the dark; **a**—**e** 5 d of cultivation, **f** 16 d of cultivation in the laboratory under a normal day-and-night regime.