LIGHT-INDUCED CONIDIATION IN AN UNDESCRIBED SPECIES OF *CYLINDROCARPON*

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Submitted to

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Master of Science

by

Barbara Alexander McMillan

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Note to the Reader

Dispersed throughout the text, in the figure captions, and in the tables the letter "u" has been used to represent the symbol for micron (μ), e.g. "um" = μm.

ABSTRACT

Light-induced conidiation in an undescribed Cylindrocarpon sp. and the influence of climatic and edaphic factors on this induced morphological change were investigated.

Monochromatic and polychromatic sources of radiation were utilized in the preparation of a response spectrum in order to study the wavelength response characteristics of photoinduction. The role of nucleic acid and protein synthesis during sporogenesis was investigated with the aid of synthetic inhibitors.

Both the qualitative and quantitative nature of the sporulation response were found to be dependent upon a variety of variables. These were: age of the culture when exposed, temperature of incubation, irradiation dosage, period of darkness following photoinduction, wavelength of light, surface moisture, intensity of radiant energy intercepted by the thallus, and composition of the medium.

Conidia failed to form in cultures held under continuous darkness from the time of inoculation, but were produced in abundance in periods of both continuous and intermittent irradiation. In light-induced, 12-day-old, dark-reared cultures the ability to respond to morphogenetically active radiation was restricted to hyphae less than 80-hours-old in the outermost 6.0 mm if the thallus in existence at the beginning of the illumination period.

In the response spectrum there was no evidence of any significant stimulation by radiation extending from 522 nm to 580 nm nor at wave-

lengths above 600 nm. Only cultures which received blue filtered light (430 nm to 490 nm) or were irradiated with black-light-blue lamps (peak emission at 356 nm) developed conidia. This type of spectrum is typical of NUV-blue sensitive fungi.

Neither 2'-deoxyadenosine (10^{-1} to 10^{-5} M), nor mitomycin C (10^{-3} to 10^{-5} M), nor puromycin diHCl (10^{-3} to 10^{-5} M) had an appreciable effect on sporulation. Actinomycin C (10^{-3} M), 8-azaguanine (10^{-1} to 10^{-5} M), cycloheximide (10^{-1} to 10^{-4} M), emetine HCl (10^{-1} to 10^{-2} M), and 5-fluorouracil (10^{-1} to 10^{-5} M) all inhibited, in part or in whole, the amount of sporulation in response to light. These experiments led to the conclusion that both protein synthesis and nucleic acid synthesis may be involved in light-induced conidiation in Cylindrocarpon sp.

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LITERATURE REVIEW

Although fungi do not normally require light for basic physiological processes, vegetative growth and differentiation in a large number of isolates belonging to major fungal taxa are affected by radiant energy. While these effects are varied, Page (166) has divided them into two categories: nonmorphogenetic effects in which light influences the rate or direction of movement or growth of a structure or the synthesis of a compound; and morphogenetic effects in which light induces or inhibits a process or the formation of a structure, e.g. germination, sclerotium formation, and reproduction (asexual and sexual). Nonmorphogenetic responses may be oriented, the response bearing a spatial relationship to the source of illumination, e.g. phototaxis and phototropism; or nonoriented with the response being manifest as either a stimulation or inhibition of the rate of growth or synthesis of a compound, e.g. carotenogenesis.

The present review describes the role of visible light and ultraviolet radiation in growth and development of several representative species of fungi. Literature related to photoinduction, photoenhancement, and photoinhibition of reproduction, photoreception, the mechanism of light action in induced morphogenesis, light mediated growth rhythms, and the consequence of illumination on vegetative growth is emphasized. Other morphogenetic and nonmorphogenetic photoresponses reviewed by Batra (12), Carlile (34, 35, 36), Chet and Henis (41), Curry and Thimann (49), Ingold (95), Page (166), and Thimann (218) are not considered.

RADIATION AND REPRODUCTION

The onset of reproduction, both sexual and asexual, in fungi is influenced by endogenous, climatic, and edaphic factors. While these factors also affect vegetative growth, the genetic and environmental conditions required for successive stages in the initiation, development, and maturation of particular spore types and spore bearing structures are usually more precise or more restricted, i.e. sporogenesis is initiated only if the sum of influences is favorable (44, 83, 85, 133, 147, 150).

The effects of visible and other radiations on fungal reproduction are extremely varied and complex. Indeed, photobiological literature not only includes reports of stimulation, indifference, and inhibition, but it is often contradictory and difficult to evaluate. Hawker (84) attributes this predicament to the complexity of the influences and the interactions between the external stimuli and the fungus' unique set of genetic factors:

> Both the nature of the response and the stage of development at which the fungus is light sensitive vary with the quality and intensity of the light, with the duration of the exposure, with the species of fungus and with changes in other environmental factors.

Unfortunately, most reports are of a decidedly qualitative nature concerning a particular light response, without reference to other possible endogenous and exogenous influences. Smith (194) made note of this situation as early as 1935, when she stated:

> For the most part, when monochromatic light was used, the intensity was not measured, and when the intensity was measured, the quality of the light used

> was not accurately determined; or the intensity was not kept constant when the wavelength was changed. Throughout most of the work there has been inadequate control of environmental conditions. These uncontrolled factors have often led to an unfair interpretation of results, and this fact must be given due weight in any attempt to estimate what is known about the effect of radiation on fungi.

Since that time, Lilly and Barnett (133), Ingold (95), Grover and Karvé (75), Carlile (34, 35), Leach (125), and others have made similar observations.

However, Smith (194) considered that fungi could be divided into four distinct groups with respect to their ability to fruit under different light conditions:

> (a) those in which fruiting is independent of light; (b) those which will fruit only in the light; (c) those in which light is necessary only to produce the fundamentals of fruiting; and (d) those in which light is not necessary to produce fundamentals but is necessary for the further development of fruiting structures.

In response to countless reports on photosporogenesis and the relationship of light to both sexual and asexual reporduction, this system may be expanded to include the following categories: (1) fungi which are able to produce viable spores in complete darkness and in which spore production is decreased or prevented by irradiation at some time during development; (2) fungi which are able to produce viable spores in complete darkness and which do not show increased or decreased spore production when illuminated; (3) fungi which are able to produce viable spores in complete darkness yet do so more freely when illuminated at some time during development; (4) fungi which are

able to produce rudimentary spore-bearing structures in complete darkness yet require irradiation for development and maturation of viable spores; (5) fungi in which irradiation is not required for the initiation of spore-bearing structures but continuous illumination suppresses development of viable spores; (6) fungi in which irradiation is required for the initiation of spore-bearing structures yet continued development and maturation of viable spores is suppressed by further illumination; (7) fungi in which irradiation is required for the initiation of spore-bearing structures after which further development and maturation proceeds independently of illumination; and (8) fungi in which irradiation is required both for the initiation of spore-bearing structures and the maturation of viable spores (83, 84, 109, 147).

Bjornsson (17) subdivided the fungi of groups 4, 6, and 7, in which both irradiation and a dark period are necessary for the reproductive process, as follows:

> those requiring a long duration of high intensity light; those requiring a short duration of relatively low or high intensity; those requiring a dark period prior to a relatively long duration of high intensity light; those conditioned by a fixed dark period prior to a short light exposure; and those responses requiring more than one cycle of darkness and light of a certain duration.

Factors other than light may cause a fungus to fit into more than one category, e.g. sporulation in Alternaria kikuchiana Tanaka is indifferent to light when growing on dry apricot juice medium, but is stimulated by V-8 juice, pear leaf juice, and dry apricot-V-8 juice media (156). However, it is possible to classify isolates given the parameters of the experimental design within which the research was conducted.

Group 1 examples are Coprinus sterquilinus Fr., in which further development of basidiocarp rudiments less than 1.0 mm in height at the time of illumination is inhibited (23); Sclerotinia fructicola (Wint.) Rehm, in which diffuse daylight suppresses the intense production of conidia occurring in total darkness (77); and numerous species of Phytophthora whose sexual reproduction is inhibited by light (132). Photoinhibition of sporulation has also been reported in several Oomycetes (125) and in isolates of Cephalothecium roseum Cda. (86) and Phyllosticta antirrhini Syd. (139, 140).

Ascochyta rabiei (Pass.) Labrousse (78), Coprinus lagopus Fr. (137), Endothia parasitica (Murr.) Anders. (131), and Helminthosporium sativum Pam. et al. (116) represent group 2, consistently demonstrating an independence of illumination in culture.

Many Fungi Imperfecti belong to group 3, sporulating freely in darkness but being further stimulated by light; e.g. Aspergillus ornatus Raper et al. (88, 199), Botryodiplodia theobromae Pat. (91), Colletotrichum phomoides (Sacc.) Chester (94), certain Fusarium species (7, 8, 186, 196), Septoria nodorum Berk. (46), Septoria tritici Rob. (46), Trichoderma viride Pers. ex Fr. (76), and Verticillium agaricinum (Lk.) Cda. (160).

Group 4 organisms, those which require light for the maturation of either sporangium or pileus, are Pilobolus crystallinus (Wigg.) Tode, Pilobolus umbonatus Buller (35), Polyporus brumalis Pers. ex Fr. (173), and six agarics discussed by Plunkett (172).

A group 5 example was recently confirmed by Yamamura, as reported by Kumagai (109), in the H_1 isolate of Helminthosporium oryzae B. de Haan; conidiophores developed independently of light, but conidial development was suppressed by blue radiation.

Group 6 fungi, those unable to fruit unless an exposure to light is followed by a certain period of darkness, have been labeled "diurnal sporulators" (122). This was initially observed in Choanephora cucurbitarum (Berk. and Rav.) Thaxt. which requires irradiation exclusively for the induction of conidiophores, the terminal phase of conidium formation being strongly inhibited by light (10, 42). Similar requirements exist for Alternaria dauci (Kuhn) Groves and Skolko (122, 128, 233), Alternaria tomato (Cke.) Web. (2, 4), Alternaria solani (Ell. and Martin) Jones and Grout (134), Botrytis cinerea Pers. ex Fr. (109, 206, 207, 217), Drechslera turcica (Pass.) Subram. and Jain (129), Exserohilum rostratum (Drechsl.) Leonard and Suggs emend. Leonard (90), Helminthosporium gramineum Rab. (93), Helminthosporium oryzae (92, 115), Helminthosporium teres Sacc. (159), Pyronema domesticum (Sow. ex Fr.) Sacc. (148), and Stemphylium botryosum Wallr. (122).

A short exposure to light is essential for the initiation of pycnidia in Plenodomus fuscomaculans (Sacc.) Coons (47) and Diaporthe soyae Lehman (77), acervuli in Pestalotia theae Sawada (75), and conidia in Helminthosporium catenarium Drechsl. and Cercosporella herpotrichoides Fron (122). In these group 7 fungi, photoinduced initials develop equally well in darkness or light.

Sphaerobolus stellatus Tode ex Pers. (1), Pilobolus kleinii van Tiegh. (164), and Septoria nodorum (25) belong to group 8. Culture irradiation with wavelengths of light less than 500 nm was necessary to initiate sporophores in S. stellatus; subsequent exposure to red light (640 or 720 nm) was required for their maturation. Page showed that to sporulate, P. kleinii required three periods of illumination, each preceded by a dark period, ie. light was required to induce trophocyst formation, sporangiophore emergence and elongation, and sporangium development. In S. nodorum, Calpouzos and Lapis found that light was needed not only to initiate the formation of pycnidia, but an additional dose, at a slightly later stage in pycnidial development, was needed to initiate spore production.

PHOTORESPONSES AND THE RECEPTORS INVOLVED

Only light which is absorbed by a molecule is effective in producing a photochemical change in that molecule. Thus, a photoreceptor is a particular compound absorbing light of specific wavelengths effective in inducing a response. Three approaches which have been adopted in an effort to determine the chemical identity of photoreceptor molecules mediating photoresponses in fungi are: (a) determination of the action spectrum of the response; (b) establishment of a correlation between the distribution or concentration of a potential photoreceptor and photosensitivity; and (c) demonstration that photosensitivity is influenced by compounds known to inhibit the synthesis or probable mode of action of a potential photoreceptor compound (34).

The action spectra for light-conditioned or light-stimulated pro-

cesses in fungi, in addition to polychromatic and monochromatic irradiation studies, illustrate the effectiveness of wavelengths from the ultraviolet to the red end of the electromagnetic spectrum. Comparisons of the available data, however, reveal four distinct photoabsorbing systems: a red absorbing system limited to wavelengths longer than 520 nm; a far ultraviolet absorbing system in the region of 230 nm to 330 nm; a near ultraviolet and blue reversible photosystem for which detailed action spectra studies have not been published; and a near ultraviolet and blue absorbing system in which wavelengths less than 520 nm and greater than 330 nm are effective.

Prior to 1961, there was little convincing evidence for the occurrence of red photoreceptors in fungi. Etzold (63) considered that responses to the red end of the spectrum were stimulated by a low blue threshold, coupled with blue contamination of red light. However, Page and Brungard (167) showed that phototropic sensitivity in Conidiobolus villosus Martin extended to 650 nm. Lukens (135) reported reversal of blue (450 nm) light inhibition of the terminal phase of sporulation in Alternaria solani by subsequent exposures to red light, ie. peak activity at 600 nm in the wild type and at 575 nm and 650 to 675 nm in a mutant. Ingold and Nawaz (96) found that yellow-red light was effective in accelerating fruiting in Sphaerobolus stellatus during the second half of the developmental process. Brook (22) demonstrated spore discharge in Venturia inaequalis (Cke.) Wint. could be promoted by far red light and suppressed by subsequent red light irradiation. Far red light (approximately 720 nm) resulted in a marked inhibition of uredospore germination in Puccinia graminis f.sp. tritici (Eriks.

and Henn.) Guyot (24). In Botrytis cinerea it has been found that the inhibitory effect of blue light on black light potentiation can be reversed by far red radiation (208, 211) and,further, this effect of far red light can then be reversed by exposure to red light (209, 210). Photoresponse to wavelengths longer than 520 nm has also been reported in several Myxomycetes (154, 178).

Lukens (135) speculated that semiquinones and phytochrome are involved in the response effected by light of wavelengths 550 to 600 nm and 625 to 650 nm, respectively. Calpouzos and Chang (24) attributed the results with P. graminis f. sp. tritici uredospores to the comparable "high energy reactions in higher plants" which some workers relate to phytochrome presence. "Until phytochrome is detected spectrophotometrically in fungi or isolated in fungi," Tan (215) emphasizes, "it can not be said for certain that this is involved in fungal 'red' photoresponses."

Early polychromatic irradiation studies with Hanova quartz, Cooper-Hewitt quartz, and General Electric capillary mercury arc and vapor lamps indicate the effectiveness of ultraviolet (UV) radiation in stimulating sporulation in a number of fungi (8, 40, 57, 94, 100, 177, 179, 202, 203, 204).

More precise investigations utilizing monochromatic radiation of various wavelengths and intensities have shown that far UV radiation is also responsible for photomorphogenesis in several Ascomycetes and Fungi Imperfecti. Conidium development in Alternaria chrysanthemi Schmidt, Helminthosporium dematioideum Bubak and Wrobl. (119), and Alternaria dauci (128) as well as pycnidium formation in Ascochyta pisi

Lib. (127) were induced by wavelengths of monochromatic UV from 230 to 360 nm. Leach and Trione (128) noted radiation between 230 and 370 nm and 230 and 390 nm induced conidial and perithecial formation, respectively, in Pleospora herbarum (Pers.) Rabenh. (Imperfect state = Stemphylium botryosum). Only UV wavelengths shorter than 350 nm induced pycnidia in Septoria nodorum (25). Sproston (197) determined the action spectrum for conidiation in the diurnal sporulator Stemphylium solani Web. and reported that the effective wavelengths were from 230 to 320 nm. Radiation between 340 and 365 nm was most effective in inducing sporulation in Alternaria kikuchiana (156) and Pyricularia oryzae Cav. (157). Results of extensive monochromatic radiation studies conducted on the Loculoascomycete Leptosphaerulina trifolii (Rost.) Petr. indicated that only wavelengths less than 370 nm stimulate perithecium formation (126). Onesirosan and Banttari (159) reported that conidiophore formation in three isolates of Helminthosporium teres required UV radiation in the region between 310 and 355 nm. Light of the same wavelength stimulated sporulation in Cercospora beticola Sacc. (26).

The chemical identity of the photoreceptor responsible for capturing light energy in this region of the spectrum is obscure. Alcohol extraction of a substance designated as P 310, which had a maximum absorption between 260 and 340 nm (peak 310 nm), from UV irradiated cultures of various fungi suggested a photoreceptor compound had been isolated (120, 127). This was later considered improbable as P 310 could be extracted from sporulating thalli of Ascochyta pisi grown in continuous darkness, but could not be extracted from nonsporulating

thalli grown under similar conditions (120, 223). Action spectra obtained for photoresponses in Ascochyta pisi, Alternaria dauci, and Stemphylium botryosum prompted Leach (123) to suggest that a protein or nucleoprotein might be involved, and Carlile (32) recommended investigation of pteridine, the bicyclic nitrogenous parent compound of the pterins. Owing to the strong absorption of UV radiation by organic compounds in vivo, it may be some time before the technology is available to distinguish such a photoreceptor, if indeed the far UV photoreceptor meets these specifications.

In Alternaria tomato (111), Botrytis cinerea (206, 207, 208, 217), and Helminthosporium oryzae HA_2 (92) conidiophores are induced by near ultraviolet (NUV) irradiation, and conidia develop only when such radiation is followed by a dark period. Brief illumination with blue light at a definite time during the dark period, i.e. between 6 and 8 hours after the beginning of the dark period in A. tomato (111) and H. oryzae (92) and between 12 and 16 hours and 20 and 24 hours in B. cinerea (208), suppresses the sporulation response potentiated by NUV, and developing conidiophores redifferentiate into sterile hyphae. If this blue exposure is immediately followed by NUV irradiation, the suppression of conidial development is reversed, and sporulation proceeds normally. However, further exposure to blue light immediately following NUV irradiation again aborts the sporulation process. Thus, the nature of the photomorphogenetic response depends upon the quality of the final dose of radiation.

A reversible NUV-blue photoreaction, inexplicable by the action of phytochrome, suggests the involvement of a new pigment system. Such

an interconvertible photoreceptor pigment, based on the compound mycochrome, was proposed by Kumagai and Oda (111). Although the chemical nature of mycochrome is ill-defined, it has been detected spectrophotometrically in an intracellular particulate fraction of A. tomato (112). Two layer sucrose density gradient centrifugation indicates that the NUV-form is either soluble in the cytoplasm or very loosely bound to the particulate fraction, and that the blue-form is tightly bound to the particulate fraction, probably a membranous structure (113). Kumagai (109), Kumagai and Oda (111, 112), and Tan (208, 210, 215) suggest possible mechanisms of mycochrome action.

The first mycologist to investigate the influence of wavelength on spore formation was Hedgcock (86). He observed that cultures of Cephalothecium roseum, Mucor sp., and Penicillium sp. grown in ordinary light or light passed through a blue solution of ammonium and copper carbonate produced fewer spores than cultures grown under red (a cochineal solution) or orange (a potassium bichromate solution) light or darkness. Daylight, in particular the blue wavelengths, inhibited sporogenesis.

The mycological literature pertaining to photobiologic phenomena clearly indicates the effectiveness of wavelengths from the NUV (320 nm) to the blue (550 nm) region of the electromagnetic spectrum in mediating fungal photoresponses (34, 35, 95, 125, 194, 215). In addition to nonmorphogenetic effects and the aforementioned inhibition of sporulation, NUV-blue photoresponses include: (a) induction of sporulation (73, 75, 91, 110, 181, 199); (b) enhancement of sporulation (10, 48, 56, 64, 97, 145, 160); (c) initiation of sporangiophores (13);

(d) coremium formation (215); (e) induction of perithecia (51, 230); (f) formation of ascospores (102); (g) acceleration and stimulation of fruiting (137); (h) sporophore initiation (1, 10, 104, 169, 188); and (i) inhibition of conidiation in diurnal sporulators (3, 5, 92, 111, 124, 134, 156, 197, 208, 217).

The chemical nature of the photoreceptor molecule in this region of the electromagnetic spectrum is controversial.. Although action spectra for NUV-blue mediated biological responses in many different organisms have suggested a common photosensitive mechanism (35, 51, 71, 81), they have been interpreted as resembling the absorption spectra of various compounds including beta-carotene (14), cis-carotenoid (51, 71, 219), gamma-carotene, lycopene (192), retinal attached to a protein (14), riboflavin (95), a flavin-nucleotide (134), flavoprotein (14, 31, 34, 35, 81, 108, 152, 153, 165, 171, 182), and cytochrome coupled to a flavin (71).

Riboflavin and cis-carotenoid, on the basis of action spectra profiles, are currently favored as the active pigment. However, similarity of absorption spectra coupled with the fact that modification by specific protein association and local environment may occur *in vivo* rule out spectroscopic discrimination of the two compounds and consequent identification of which is the NUV-blue receptor pigment.

Correlation between the distribution of carotenoids in photosensitive organs and photosensitivity, and the scarcity of direct evidence for the existence of riboflavin or other flavins or flavoproteins in fungi have been used to support a carotenoid photoreceptor hypothesis (32, 34, 35, 95, 190). However, scientists who believe a

flavoprotein is the active pigment cite the continued photosensitivity of albino strains (32, 37, 192, 199) and wild type cultures poisoned with diphenylamine, an antimetabolite of carotene synthesis (164, 199, 222).

Discovery of several pigment mutants of Phycomyces blakesleeanus Burgeff which contain less than one thousandth the beta-carotene of the wild type yet are fully photosensitive demonstrates that the bulk of beta-carotene is not the effective pigment, and that "carotenoid-less mutants" contain carotenoid pigments (14).

Inhibitor studies using phenylacetic acid, a compound responsible for photodestruction of flavin (110); atebrin, a flavin antagonist (51, 110); lyxoflavin, a riboflavin analog (73, 165); as well as diphenylamine (51, 73, 110, 163) in an attempt to ascertain which system is involved, leave the photoreceptor problem unsolved. Lyxoflavin and phenylacetic acid have been found to be relatively nonspecific enzyme inhibitors (215), while atebrin binds with protein in general (87) and may act through impairment of DNA (43). Similarly, the inhibitive action of diphenylamine on carotene synthesis is not specific since it is known to combine with DNA (193).

Arguments against the carotenoid hypothesis and against a flavoprotein as the active pigment for NUV-blue mediated photoresponses are summarized by Hartmann and Unser (81). Here, in an attempt to interpret the "low irradiance movement" of the Mougeotia chloroplast, they propose that the problems of action dichroism and all postulated blue transitions can be fully explained on the basis of phytochrome. Moreover, they discuss data which indicate the P_{fr} is an effector mole-

cule for "blue-uv" mediated photoresponses.

Evidence indicating that an electron transfer between a flavoprotein receptor pigment and a beta-type cytochrome may be connected with early processes in blue light induced physiological responses in Dictyostelium discoideum Raper (174, 175), Phycomyces blakesleeanus (174), and Neurospora crassa Shear and Dodge (152, 153) prompted the suggestion that the photoreceptor compound consists of a cytochrome b_2-like pigment with physically associated flavin and haem prosthetic groups.

A recent study with a Neurospora poky mutant deficient in cytochrome b, which showed both decreased sensitivity to light and greatly decreased light-induced absorbance changes of cytochromes, also suggests a correlation between absorbance changes and changes in physiology (105). In this connection, however, Klemm and Ninnemann caution that "the possibility exists that absorbance changes are only accompanying the photoact in the flavin without participating directly in the transduction chain to the physiological response" (105).

MECHANISM OF LIGHT ACTION

Stallings (199) has divided photoinduced morphogenetic changes into three categories: (a) stimulus detection, i.e. absorption of radiant energy by a specific receptor pigment, activation of the pigment molecules, and their participation in a photochemical reaction which ultimately results in the formation of a primary photoproduct; (b) induction, i.e. the photoproduct activates, or engages in, cell processes which subsequently lead to the "triggering" of differentiation; and

(c) differentiation, i.e. mobilization of synthetic and energy supplying capacities required in the synthesis of new structures recognizable as morphologic changes (199).

Attempts have been made to identify both the photochemical reaction(s) responsible for the synthesis of the primary photoproduct and the physiological change(s) thought to occur during the induction phase.

There is little information on chemical reactions occurring immediately after light absorption. Cohen (45) showed that a one minute irradiation of a Phycomyces decreased its level of adenosine-3', 5'-cyclic monophosphate (cAMP). In Neurospora crassa a photoinduced redox change appears to be the primary step in the blue light response (152). An activated receptor pigment is thought to catalyze the photo-oxidation of a compound, as yet unidentified, in the photoinduction of carotenoids (151). Light enhancement of protein synthetic activity, accompanied by a transformation of cytoplasmic monoribosomes to polyribosomes, in dark grown cultures of Verticillium agaricinum may involve a modification of the complement of ribosomal proteins (227). Carlile (35), Klemm and Ninnemann (105), and Stallings (199) have suggested that direct photochemical activation or alteration of certain enzymes may be responsible for photoresponses in fungi. Light may also act directly on the flavin moiety initiating an alternate path of electron transfer (33); experimental evidence is, however, lacking.

As one would anticipate differences in metabolite populations during morphogenesis when obvious changes in structure and function are occurring, it has been considered that differential gene activation is

likely to be involved (59, 158, 195). In fungi, developmental changes are thought to be accompanied by the induction of changes in enzyme synthesis and activity which result not only from internal alterations in metabolite concentration, substrate and primer availability, inhibitor disappearance, permeability, protein hydration, enzyme unmasking, etc., but also environmental factors (149, 231).

Hill (88) proposed that a light-induced, low molecular weight inhibitor in the hyphal tips of Aspergillus ornatus blocked the phosphorylation of glucose which led to a reduction in growth and the subsequent formation of conidia.

Uno and co-workers (224, 225) discovered that cAMP levels in Coprinus macrorhizus Rea f. microsporus Hongo increased during light-induced fruiting. They suggested that cAMP may play important roles in inducing sporophores and in controlling inducible enzyme synthesis.

Studies using inhibitors of nucleic acid metabolism have demonstrated a requirement for ribonucleic acid (RNA) synthesis during light-induced conidiation in Botrytis cinerea (212, 214), Neurospora crassa (221), and Trichoderma viride (68, 72), and during light-induced carotenogenesis in Verticillium agaricinum (151). However, Stavy et al. (200) were unable to find differences in transcription of RNA species following induced differentiation in T. viride using DNA/RNA hybridization techniques.

Conidium production in Botrytis cinerea (212, 214) and Neurospora crassa (221, 226) and carotenoid biosynthesis in Fusarium aquaeductum Lagerh. (183), Neurospora crassa (79,185), and Verticillium agaricinum (151) were arrested by various protein synthesis inhibitors, impli-

cating involvement of translation processes.

Neither nucleic acid metabolism nor protein synthesis was found to be specifically associated with photo-induced sporulation in Aspergillus ornatus (199). Instead of the synthesis of new enzyme molecule(s) to perform different function(s), Stallings suggested that small, qualitative and quantitative changes in pre-existing molecules may be sufficient to "trigger" the light controlled dichotomy between vegetative mycelium and conidiating mycelium in this fungus.

Additional evidence in favor of differential gene action during photomorphogenesis can be found in the published work on light-induced circadian rhythms of conidiation (21, 55, 89, 142).

LIGHT MEDIATED GROWTH RHYTHMS

Numerous fungi cultured on artifical substrates and growing in nature on plant material produce alternating zones of differentially pigmented mycelia, fertile and nonfertile hyphae, profusely developed and meagerly developed aerial hyphae, or aerial and superficial hyphae which give the appearance of concentric rings in Petri dish cultures, regularly spaced bands in horizontal growth tubes, and concentric rings on pods, leaflets, leaf stalks, etc. in vivo.

Aschoff (6) attributes the formation of regular differences in growth type to both endogenous and exogenous components. If zonation arises and persists in the absence of environmental fluctuations, or cannot be correlated with external physical conditions which fluctuate periodically, then physiological clocks and internal calendars are implicated as causal mechanisms and the rhythm is considered to be en-

dogenous. If, on the other hand, zonation is induced by a periodicity in the environment, ceases under uniform conditions, and can be correlated with the periodic fluctuation of one or more external environmental variables such as light, temperature, and humidity, then the rhythm is considered to be exogenous.

Endogenous rhythms in which bands of sporing mycelium alternate with bands which are sterile, or spore less profusely, have been observed in Hypocrea spp. (86), Sclerotinia fructigena Schroet. (Monilia fructigena Pers.) (77), Neurospora crassa strain 21863 (18), and nuerous other fungi (34, 99, 136).

Munk, as reported by Hall (77), found that fluctuations in humidity caused zonation of the sporulation type in Aspergillus niger van Tiegh., Aspergillus cinnabarina = A. niger mut. cinnamomeus (Schieman) T. and R., Aspergillus ochraceus Wilhlem, Cephalothecium roseum, and a species of Penicillium.

Alternations of temperature capable of evoking zonations of mycelial and sporulation types have been reported for cultures of Fusarium discolor Apple and Wollenw. var. sulphureum (Schlecht.) Apple and Wollenw. (16), Fusarium fructigenum Fr. (77), Fusarium rigidiusculum (Brick) Snyder and Hansen, Fusarium roseum Schwabe, Fusarium solani (Mart.) Appel and Wollenw. (232), Penicillium sp. (191), Pleospora herbarum (62), and Trichothecium roseum (Pers.) Lk. (99)

A study of the literature shows that rhythmic processes in fungi are more commonly influenced by light. Formation of successive zones under conditions of diurnal periodicity or artificially arranged photoperiods may be a consequence of either photostimulation or photoin-

hibition of aerial growth (100), photoinduction of carotenogenesis (151, 184, 234), or inductive, stimulative, or inhibitive effects on the formation of resting (162) as well as reproductive structures (16, 57, 75, 77, 84, 86, 88, 97, 100, 110, 116, 117, 125, 140, 145, 181, 186, 196, 232).

Whether light is essential for sporogenesis, is stimulatory, or is inhibitory, if the primary photoproduct is localized in directly irradiated, dark-grown mycelium or hyphae generated during the photoperiod and is not translocated within the hyphal elements to other parts of the thallus, then alternation of light and darkness will lead to periodicity in spore production. As a consequence, zonations of the sporulation type, which are produced by fungi in culture and which are virtually indistinguishable in gross morphology, have three very distinct origins, i.e. photoinduction, photoenhancement, and photoinhibition of sporogenesis.

In fungi such as Phyllosticta antirrhini (139, 140) and Sclerotinia fructicola (77) which sporulate only in darkness, zonation is unique in that it results from light inhibition of the reproductive process, i.e. rapidly advancing hyphae generated during irradiation are sterile.

Light stimulation of reproductive structures by daily periods of illumination usually results in alternating zones of intense and sparse sporulation; if the structure is light induced, then periodic exposure to light produces zones of intense sporulation which are separated by sterile hyphae. Unlike the aforementioned situation in which morphogenetically active wavelengths affect the differentiation

of light grown hyphae, these zones of intensely sporulating mycelia need not correspond with the advancing margin of mycelium produced during irradiation. Diurnal illumination may cause heavy sporulation on that region of the thallus extending back from the position of the growing margin when the transfer from dark to light was made, i.e. the region over which the fungus grew during the dark period immediately preceding illumination, or the sensitive region may extend both inward and outward from what had been the thallus margin at the time of exposure. Trichoderma viride, a group 3 fungus (110) and Pestalotia theae, a group 7 fungus (75) typify the former, while Sclerotinia fructigena (77) and Ascochyta pisi (117), with sporulation responses referable to either group 3 or group 7 as a consequence of the isolate studied, exemplify the latter.

The amount of light necessary to induce or stimulate the production of a zone of mycelium bearing conidia depends upon the intensity of the beam at the surface of the culture and the organism. Bisby (16) found that an exposure of approximately six minutes was required to produce a definite ring in Fusarium discolor var. sulphureum cultures placed at a distance of one meter from a carbon filament lamp standardized to give 25 candlepower. Irradiation for ten seconds was sufficient to induce zone formation in Trichoderma lignorum (145) and Verticillium lateritium Rabenh. (97) at a light intensity of 2 ft-c and 1500 lux, respectively. At an intensity of 4.04×10^3 to 4.31×10^3 lux, a minimum exposure of three hours resulted in the development of a ring of conidia in Aspergillus ornatus (88). One minute of exposure to a Phillips 40 watt fluorescent lamp at a height of one foot

from the culture dish was sufficient to induce sporulation in dark-grown mycelia of Botryodiplodia theobromae (180), while cultures of Pestalotia theae exposed for one hour to light emitted by three comparable lamps produced annular zones of acervuli during the subsequent period of darkness (75).

RADIATION AND VEGETATIVE GROWTH

Light-induced differentiation in fungi has been attributed to biological oxidation (47), hormonal systems (19), transcription from unique genetic loci, i.e. synthesis of ribosomal RNA, messenger RNA, and inducible enzymes (68, 72, 151, 188), and an inhibition of vegetative growth (44, 195).

According to Cochrane (44) any hypothesis attempting to explain the effects of light on the reproductive process in fungi "should take as its principal postulate that light is inhibitory to one or more regulatory systems which in the normal culture prevent or retard reproduction and favor continued vegetative growth." Consequently, he suggests that there is "no such thing as stimulation by light in the induction of reproductive activity; instead, growth is checked and the chain of events so initiated leads to reproduction if other factors are not limiting".

In a recent review of asexual reproduction in filamentous fungi, Smith (195) presents evidence in support of Cochrane's theory and, in conclusion, states:

> Sporulation in filamentous fungi can be considered as an aspect of differentiation that results from specific types of limitation to the normal unlimited

> pattern of vegetative growth. Although nutrient limitation is one of the most obvious means of limiting growth of a fungus other environmental factors such as light, temperature, etc. which have been shown to influence sporulation may do so indirectly by influencing essential growth limiting reactions. Thus perhaps growth rate is the central factor determining the nature and extent of sporulation in filamentous fungi.

Observations on a large number of fungi suggest that the most widespread effect of visible and ultraviolet radiation on hyphae is to check and/or suppress elongation (18, 47, 52, 61, 66, 80, 88, 94, 98, 100, 101, 148, 160, 166, 179, 186, 187, 194, 196, 216, 232). However, few of these investigations attempted to determine the effects of light on both growth and sporulation simultaneously.

A correlation between light-conditioned-sporulation and decreased growth is not apparent in fungi whose growth rates and yields are indifferent to, or stimulated by, radiant energy.

Growing hyphal tips of many species, including Botryodiplodia theobromae (180), Choanephora cucurbitarum (9, 42), Coprinus lagopus (137), Drechslera turcica (129), Pestalotia theae (75), Phycomyces blakesleeanus (14), Phyllosticta antirrhini (140), Trichoderma viride (76), and Verticillium lateritium (97), apparently are not influenced by light. Linear expansion on solid substrates occurs equally well in darkness, alternating light and darkness, or continuous illumination.

Examples of light stimulated hyphal growth are rarer, but Goldstein (70) reported that the vegetative growth of Thraustochytrium roseum Goldstein in aerated culture was positively influenced by light during the early stages of logarithmic increase. Leach (115)

found that the thalli of Helminthosporium oryzae harvested from nutrient broth cultures continuously exposed to NUV radiation showed a significant increase in dry weight over cultures grown in the dark; confirming measurements of superficial spread on agar plates under similar conditions. Enhancement of vegetative growth has also been reported for liquid cultures of Blastocladiella emersonii Cantino and Hyatt (27, 28, 29), Karlingea rosea (de Bary and Woron.) Haskins and Weston (82), and Verticillium albo-atrum Reinke and Berth. (125) incubated in the light. However, the actual effect of radiation on dry weight gain in these fungi is difficult to ascertain.

Results of light studies on the growth of K. rosea were significantly affected by the carbon source. Liquid glucose-nitrate cultures produced twice the amount of mycelial dry weight when incubated in the light as compared with cultures incubated in darkness, while more growth resulted in cultures maintained in total darkness, as opposed to light incubated cultures, when a liquid cellobiose-nitrate medium was utilized. Similarly, higher growth rates in irradiated cultures of B. emersonii, on complex or synthetic media, depended on the composition of the medium. Light-stimulated growth did not occur in the absence of carbon dioxide or bicarbonate and appeared to be associated with increased CO_2 fixation, nucleic acid synthesis, and stimulation of the succinate-alpha-ketoglutarate-isocitrate cycle. The effect of NUV on V. albo-atrum dry weight gain was also judged to be influenced by experimental conditions. In shake cultures, growth during the log phase was stimulated by irradiation. In still cultures,

radial growth of illuminated thalli was less than that which simultaneously occurred in dark-reared cultures.

Analogous data published on Briarea sp., Penicillium glaucum Bref. (133), Penicillium clavigerum Demelius (38), Pilobolus kleinii van Tiegh. (163, 164, 166), Poria ambigua Bres. (188), Rhizoctonia solani Kuehn (60), and Sclerotinia fructigena (77) in which the nature of the medium, i.e. pH, carbon source, iron source, presence or absence of a gelling agent, etc., determined whether radiation stimulated or depressed growth rate have been interpreted by Carlile (34) to mean that light has a depressant or stimulative effect when nutrition is suboptimal. He suggests "that under optimal conditions in many species, 'light' metabolism and 'dark' metabolism are approximately of equal effectiveness in supporting growth, and that departures from optimal conditions affect the processes differently."

This theory, however, would not explain Borriss' data, as interpreted by Page (166), on the light inhibition of mycelial growth in Coprinus lagopus. Haplophasic hyphae exposed to light continued to elongate at the normal rate for only five to ten minutes after which the growth rate fell rapidly with cessation twenty-five to thirty-five minutes following initiation of exposure. When the thallus was returned to darkness, growth resumed.

MATERIALS AND METHODS

A. THE ORGANISM.

A single conidial isolate (P_1-II^S_3-34) of an undescribed Cylindrocarpon sp., obtained from infected roots of Phragmites communis Trin., was utilized in this study. This fungus was selected for use because a morphological rhythm, represented by concentric rings of macroconidia, had been consistently observed in cultures of the original isolate.

B. CULTIVATION TECHNIQUES.

1. Media.

Initially, cultures were grown exclusively on PDA (potato dextrose agar, B.B.L.). However, when it became apparent that the extent and pattern of sporulation in illuminated cultures varied with the medium in relation to the manufacturer's lot number, a semi-synthetic agar containing medium which could be duplicated as required was sought. A modification of Robinson's (189) complete nutrient medium (MRM) for Aspergillus niger, consisting of (g/l distilled H_2O): KNO_3 (Baker Analyzed Reagent), 1.2500; KH_2PO_4 (Matheson, Coleman, and Bell), 0.6250; $MgSO_4 \cdot 7H_2O$ (Fisher), 0.3125; dextrose (Baker Analyzed Reagent), 1.2500; agar (Difco-Bacto), 20.0 was found to give abundant sporulation without excessive mycelial growth. This medium had an unadjusted pH of 6.0 prior to steam sterilization at 15 psi for 20 minutes. In contrast to PDA upon which sporulation frequently occurred around the inoculum plugs from which the hyphae developed, no conidia were produced by thalli on MRM incubated in the absence of light. MRM was used, with two exceptions, whenever a solid medium was required during experimental procedures.

The liquid medium (MRL) used in the study of the effects of metabolic inhibitors on vegetative growth and light-induced conidiation contained the aforementioned ingredients with the exception of agar. Sterilization was as above.

Conidia, to be used as inocula, were germinated on a sterile, non-nutritive medium containing 2% (w/v distilled H_2O) Difco-Bacto Agar (WA).

MRM plates were prepared as follows: 25 ml aliquots of molten medium were dispensed aseptically into each 15x100 mm polystyrene Petri dish (Fisher Brand) using a Zippette pipetter (Canlab). When the medium had solidified, the Petri dishes were stored, aseptically, at 4^{o} C until required. Water agar (WA) plates were similarly prepared, although no attempt was made to standardize the amount of medium per Petri dish.

Ten to 11 ml of molten PDA, prepared according to the manufacturer's instructions, were dispensed into 16x150 mm Pyrex culture tubes and capped with 16 mm polypropylene closures (Bacti-Capall). The tubes were then sterilized by autoclaving for 20 minutes at 15 psi and slanted. Following solidification of the agar slants, the culture tubes were stored, aseptically, at 4^{o} C until needed.

2. Cultural conditions.

The study necessitated culturing the fungus in several ways, i.e. on agar slants, directly on agar plates, and on membrane filters.

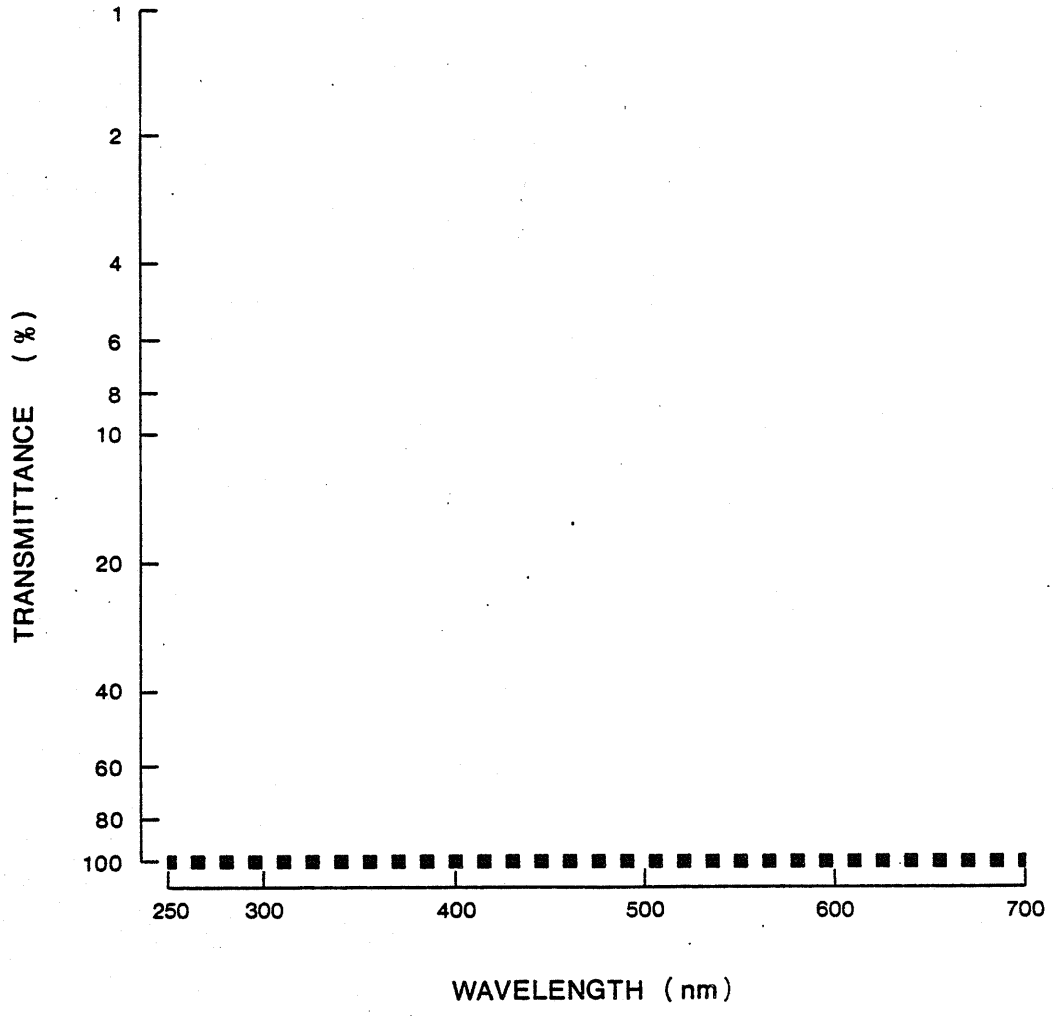
A continuous supply of 0 to 10-day-old cultures was generated according to the following procedure. From the original stock cul-

ture, on ten successive days, three monoconidial isolates were transferred to PDA slants. Inoculated tubes were then placed either in a refrigerator incubator (Controlled Environments Ltd.) illuminated twelve hours per day with Vita-Lite fluorescent lamps (Duro-Test) at 25± 1.0° C or on a laboratory bench in diffuse daylight. After the tenth day, successive 10-day-old stocks were subcultured in triplicate, i.e. three new slants were generated each day, and these subcultures were incubated as above. This procedure insured that in every twenty four hour period a complete set of 0 to 10-day-old actively growing cultures, in triplicate, was available. After the 10-day-old cultures had been subcultured and used as an inoculum source, all three tubes were stored at 4° C in darkness.

Thalli for descriptive and physiological assays were grown on MRM in Petri dishes which transmitted wavelengths from 250 nm to the red end of the visible spectrum (Figure 1). Preliminary experiments tested other variations of Robinson's A. niger medium. Potato dextrose agar (PDA) was the medium utilized in the investigation of the relation between temperature and radial growth in darkness.

Thalli to be used in the study of the effect of various inhibitors of nucleic acid and protein synthesis on light-induced conidiation were grown on MRM in Petri dishes overlaid with a steam sterilized cellulose mitrate membrane filter (47 mm diameter, Sartorius) prior to inoculation with a single germinated spore. Thalli were grown on membrane filters so that they could be quickly and cleanly separated from the agar. Preliminary trials with single

Figure 1. Transmittance of a Fisher Brand polystyrene Petri dish cover.


TRANSMITTANCE (%)
1
2
4
6
8
10
20
40
60
80
100
250
300
400
500
600
700
WAVELENGTH (nm)

and double layers of dialysis tubing (#8-6673 Dialyzer Tubing, Fisher; Spectra/Por 1 Dialysis Membrane, Spectra Medical Industries, Inc.) and filter papers (#541 Hardened Ashless, Whatman; #1 Medium Porosity, Whatman; GF/A Glass Microfiber, Whatman) suggested disintegration and/or digestion by the fungus since clean separation from the agar was not possible.

To mitigate potential problems associated with cell wall permeability, the rate of diffusion of the inhibitors through the membrane filter, and the retention of the inhibitors by the cellulose nitrate, two pore sizes were utilized: 0.2 um which restricted mycelial growth to two dimensions; and 5.0 um which, by allowing the penetration of hyphal elements, ultimately enabled a direct contact between the inhibitor solution and the plasmalemma of the damaged hyphae.

3. Inoculation.

Inoculation procedures were carried out in air-conditioned rooms equipped with ultraviolet germicidal lamps.

With the exception of the experiment in which inoculum age was the variable parameter, single spores, isolated from 10-day-old stock cultures and allowed sufficient time to put out primary germ tubes (approximately 12 to 16 hours), were used as inoculum in each experiment.

4. Incubation.

Following inoculation, each Petri dish culture was sealed along its circumference with masking tape to avoid accidental opening or contamination and to minimize potential differences in

aeration and relative humidity between replicate plates and treatments. Except for the experiments in which inoculated Petri dishes were immediately exposed to Vita-Lite and near ultraviolet radiation, agar and membrane cultures were completely wrapped in aluminum foil, fewer than ten dishes per package, and incubated in an unlit, light-sealed incubator (Coldstream, Fleming-Pedlar, Ltd.) at 25± 0.5° C until required.

For the temperature/growth rate experiment, cultures were foil wrapped in groups of three and incubated in the dark at 10, 15, 20, 25, and 30± 0.5° C.

Inoculated slant cultures were appropriately labeled and placed in an epoxy coated test tube rack on the floor of the refrigerator incubator.

C. LIGHT SOURCES AND MEASUREMENT OF PHOTON FLUX DENSITY.

Five types of lamps were used as sources of polychromatic radiation: (1) 20-watt Duro-Test Vita-Lite fluorescent lamps (spectral emission shown in Figure 2); (2) Sylvania F48T12/VHO Cool White fluorescent lamps (spectral emission shown in Figure 3); (3) 40-watt Sylvania F20T12-BLB fluorescent black light lamps equipped with a dark blue tube which absorbs almost all visible light (spectral emission shown in Figure 4); (4) a variable focus, variable intensity illuminator (Olympus Model LSD with transformer); and (5) 100-watt General Electric Shadow Ban incandescent bulbs.

Monochromatic radiation was obtained by using a set of com-

Figure 2. Emission spectrum of a Duro-Test 20 watt Vita-Lite fluorescent lamp (curve is normalized to 100 at λ = 560 nm). Duro-Test Horticultural Engineering Bulletin: Lighting for the Laboratory Animal Facility.

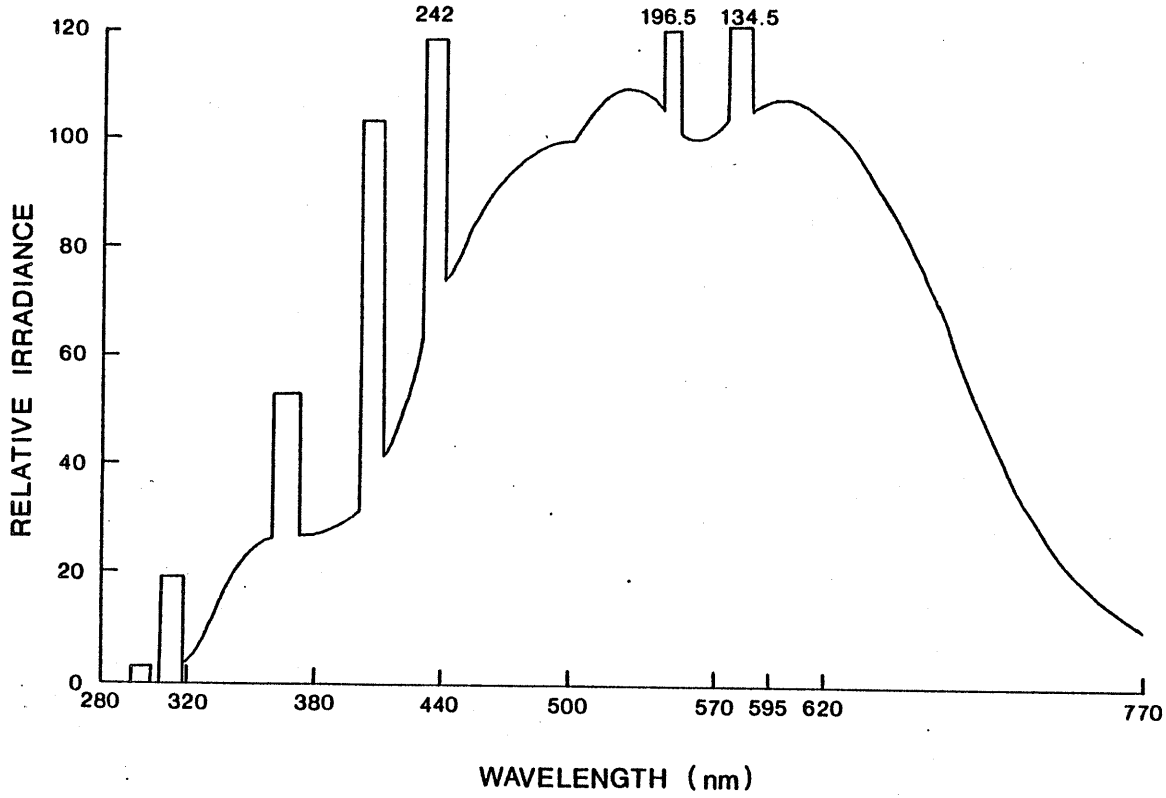

242
196.5
134.5
120
100
80
60
40
20
0
RELATIVE IRRADIANCE
280
320
380
440
500
570
595
620
770
WAVELENGTH (nm)

Figure 3. Spectral energy distribution of a Sylvania F48T12 Cool White fluorescent lamp. Sylvania Electric (Canada) Ltd. Engineering Bulletin 0-341, Fluorescent Lamps.

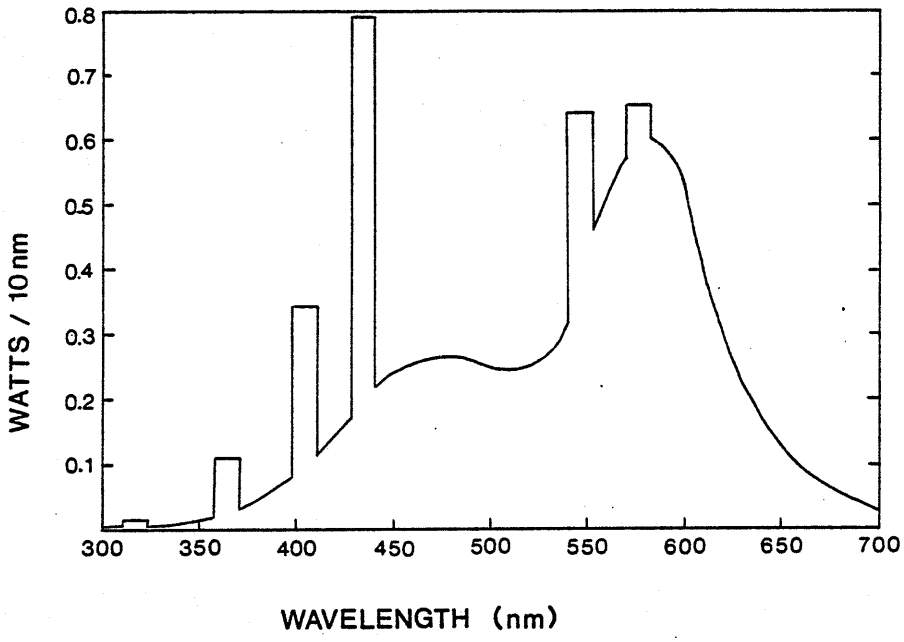
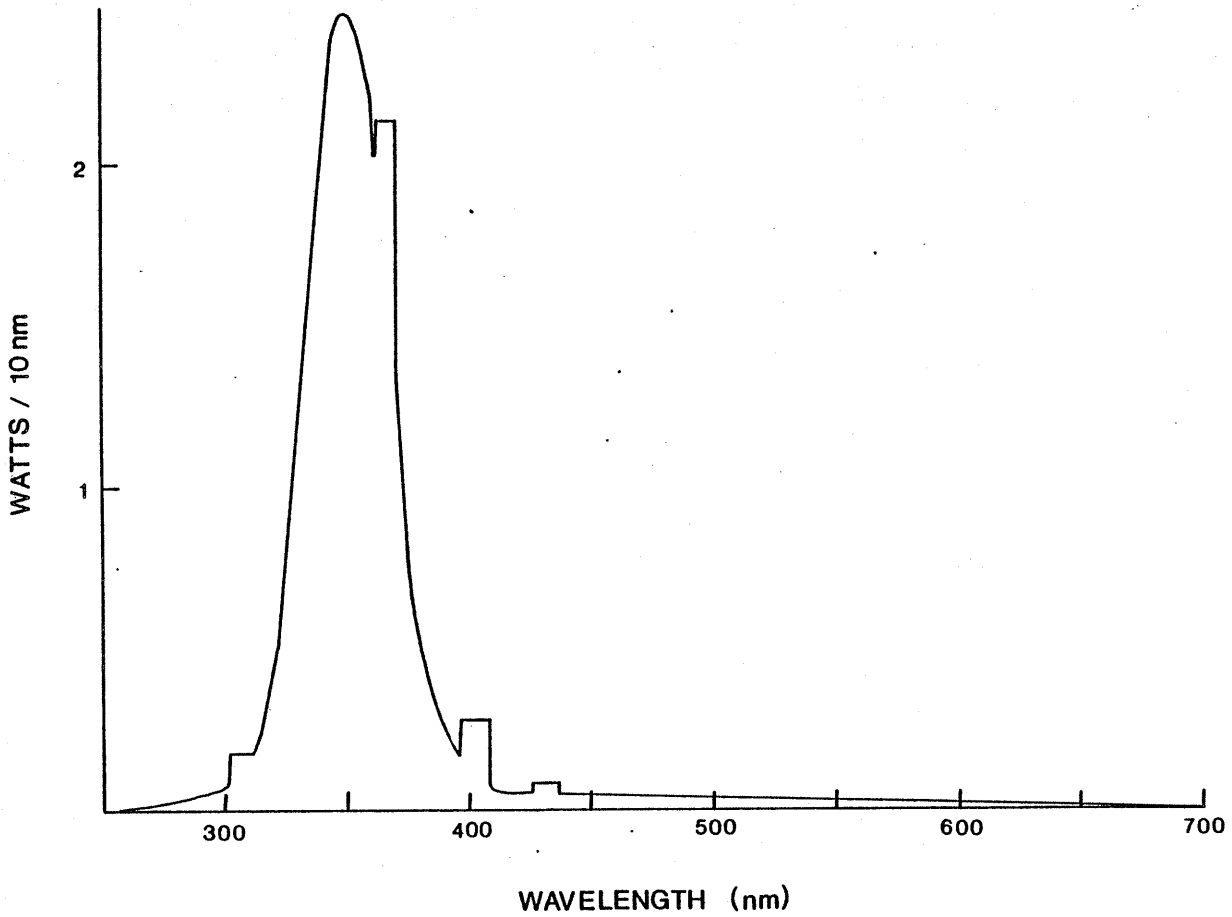

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
WATTS / 10nm
300
350
400
450
500
550
600
650
700
WAVELENGTH (nm)

Figure 4. Spectral energy distribution of a Sylvania F20T12 Black Light Blue fluorescent lamp. Sylvania Electric (Canada) Ltd. Engineering Bulletin 0-306 C, Black Light Radiation.


WATTS / 10 nm
2
1
300
400
500
600
700
WAVELENGTH (nm)

pound filters, i.e. #450 Blue, #545 Green, #650 Red, #750 Far Red (Carolina Biological Supply), each of which transmitted a band of monochromatic light rather than a single wavelength (Figure 5). Blue, green, and red filters were used separetely with a bank of five Vita-Lite fluorescent lamps, while far red filters were used to filter the light emitted by two incandescent bulbs. Test filters were placed directly on the lids of the Perti dishes containing the cultures to be exposed, and aluminum foil was wrapped around the base and circumference of the dishes, overlapping the periphery of the filter, to restrict stray light.

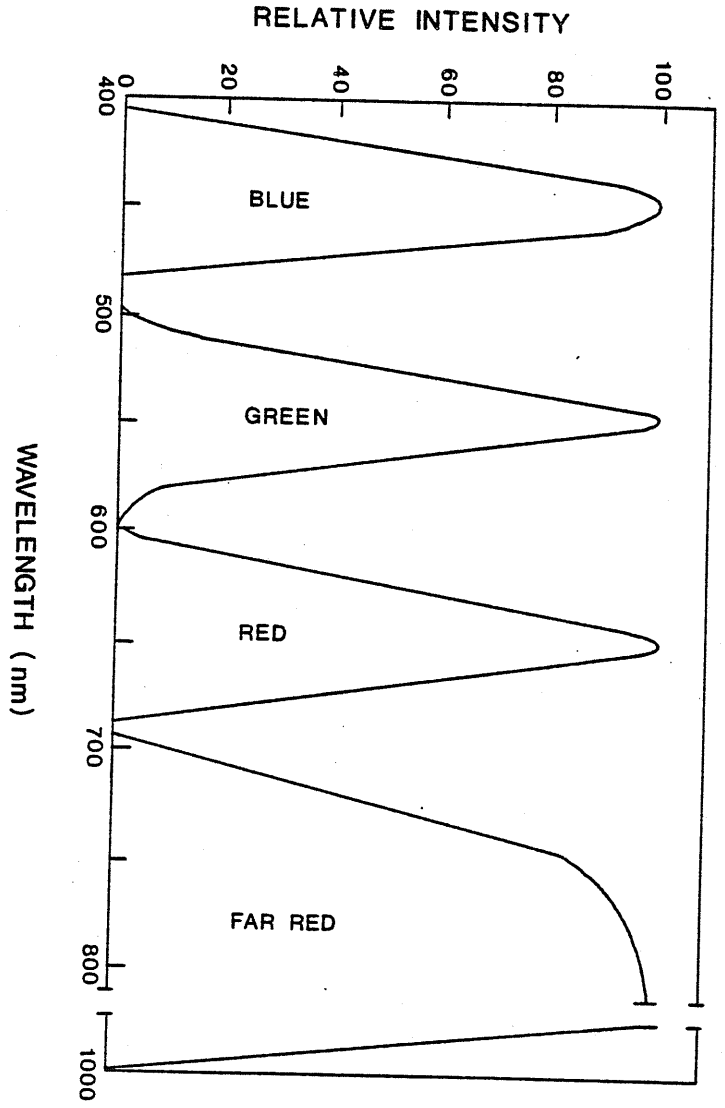
Measurements of polychromatic and monochromatic photon flux density, the radiant energy intercepted per unit area per unit of time (15), were made with a Lambda LI-185 Quantum/Radiometer/Photometer (Lambda Instrument Corporation, Lincoln, Nebraska). Readings taken with the quantum sensor at the culture level are given in Watts per square meter (Wm^{-2}). Corrections for absorption or scattering of radiation by the lids of polystyrene culture dishes were not made.

D. PHOTOINDUCTION AND LIGHT TREATMENTS.

With one exception, all descriptive and physiological experiments were carried out in two refrigerator-incubator chambers (Controlled Environments Ltd.) which could be programmed for temperature and illumination. Both chambers were located in a passageway which could be made relatively light tight.

Irradiation intensity was controlled by varying the number

Figure 5. Spectral transmission curves of Carolina combination filters #450 Blue, #545 Green, #650 Red, and #750 Far Red. Carolina Biological Supply Company.


RELATIVE INTENSITY
0
20
40
60
80
100
400
500
600
700
800
1000
BLUE
GREEN
RED
FAR RED
WAVELENGTH (nm)

of lamps in the chambers or the distance of the light source from the substrate surface. Immediately prior to exposing test plates, the photon flux density was measured and shelf/light source wattage adjusted to give the desired level. The quantum sensor, inclosed in the chamber in such a way as to exclude extraneous radiation, was used to find a sufficient number of shelf sites so that each thallus could be exposed to the same level of incident light intensity. Such sites were marked for later positioning of plates. Initially, a second measurement was made at the termination of each exposure period. This was eventually suspended when repeated trials did not reveal fluctuations in lamp output during the course of experiments.

Illumination experiments were carried out at 25° C, except when temperature was the variable parameter. Following irradiation intensity measurements and a warm-up period of at least thirty minutes for the lamps employed, dry bulb readings of ambient temperature were taken from two locations directly under the light source at the level of the culture dishes. The temperature was then adjusted by the appropriate controls to the desired level, allowed to equilibrate, and checked to insure the appropriate value had been reached. During light cycles, dry bulb readings were taken three times daily to verify temperature accuracy. Since air circulation fans in the chambers minimized possible localization, over test cultures, of radiant heat emanating from ballasts and

light sources, temperature fluctuations were generally ± 1.0° C. Dissipation of incident heat energy resulting from light striking the thallus and surface of the agar medium was not controlled, i.e. Petri dish covers were neither propped-up nor removed during irradiation.

No attempt was made to measure or control humidity.

Light programs were manually controlled by removing the foil wrapping as the cultures were positioned in illuminated chambers, and replacing the foil immediately after the exposure period. Petri plate cultures to be exposed to filtered light had monochromatic filters positioned, and the plate base and circumference as well as the filter periphery covered with foil in darkness, prior to irradiation.

Periodic light-dark cycles were regulated by the incubators which automatically cycled both temperature and illumination according to the individual programming of the chamber.

Except where stated otherwise, cultures were illuminated 12 days after inoculation by which time approximately half of the agar surface was covered by mycelium. After exposure to the specific periods of appropriate radiation, test cultures were incubated in darkness at 25± 0.5° C until termination of the experiment, i.e. 18 days after inoculation: preliminary trials had shown that conidia would mature in this time.

Studies to determine whether conidiation was restricted to an irradiated area of the thallus were performed in a photographic darkroom. The illuminators employed were three variable

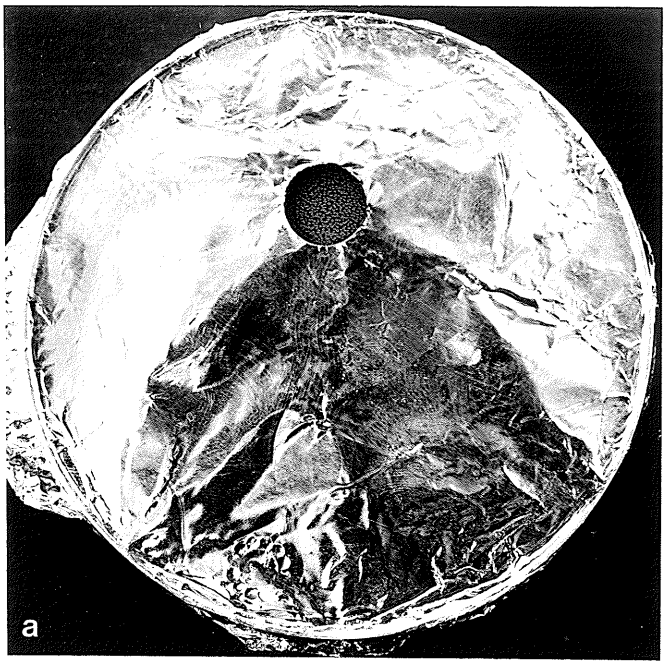
focus, variable intensity external illuminators clamped to standard support rods. Irradiation intensity was controlled by varying the distance of the bulb from the thallus and adjusting the beam intensity by means of the transformer. A variable speed fan circulating air directly over the culture dishes was used to dissipate radiant heat emitted from the light sources. To check on a potential heat effect, thermometers were placed on the test plates directly in the light beam. Dry bulb readings were taken six to ten times during 24 hour irradiation periods.

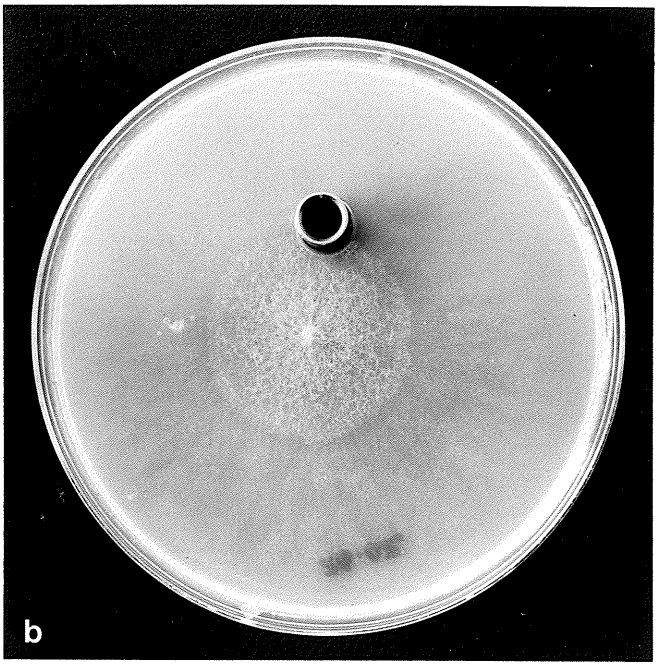
Attempts to expose a small portion of the thallus margin involved two procedures. (1) A cork borer (1.1 cm in diameter) was used to remove a disc of foil from 12-day-old Petri dish cultures which had been individually wrapped in aluminum foil following inoculation. The aperture, so created, permitted direct irradiation of the peripheral mycelium with which it was aligned (Figure 6a). (2) Foil wrapped plate cultures, incubated in darkness for twelve days following inoculation, were aseptically fitted with new covers in which a stainless steel penicillin assay cylinder had been inserted (Figure 6b). Plates were rewrapped in aluminum foil leaving only that portion of the thallus within the circumference of the cylinder exposed to light.

E. METABOLIC INHIBITORS.

Actinomycin D (Grade 1), 8-azaguanine, cycloheximide (actidione), emetine hydrochloride, 5-fluorouracil, mitomycin C, and puromycin dihydrochloride were obtained from Sigma Chemical Company,

Figure 6. Methods used to illuminate a restricted area of the thallus periphery: (a) 1.1 cm aperture in aluminum foil wrapping; (b) penicillin assay cylinder with aluminum foil wrapping removed.


a


b

St. Louis, Missouri. Chemato-pure 2'-deoxyadenosine was purchased from P.L. Biochemicals, Inc., Milwaukee, Wisconsin.

The 8-azaguanine and 5-fluorouracil were stored in their original brown glass containers at 25° C; actinomycin D, 2'-deoxyadenosine, cycloheximide, emetine HCl, and mitomycin C were stored in a dessicator at 4° C; and puromycin diHCl was frozen at -10.0° C.

Preparation and application of metabolic inhibitors were carried out in an Envirco air flow cabinet illuminated with cool white fluorescent lights.

Immediately prior to use, water soluble inhibitors were dissolved in sterile MRL to the appropriate concentration. The actinomycin D, soluble in alcohol, and 8-azaguanine, soluble in weak acid, were dissolved in absolute ethanol and 0.1 N hydrochloric acid, respectively; these solutions were then mixed, separately, with a predetermined volume of cooled, autoclaved medium to yield the desired molarity.

No attempt was made to sterilize any of these concentrated inhibitor solutions by membrane filtration, and each was immediately used in the establishment of a series of concentrations by dilution with appropriate volumes of MRL.

Inhibitors at various concentrations were administered to dark grown membrane filter cultures as follows. Sterile discs of Whatman Glass Microfiber paper (GF/A, 47 mm in diameter) were soaked in medium containing the required concentration of inhibitor. After fifteen minutes, the discs were aseptically transferred to 60x15 mm polystyrene Petri dishes (Falcon). Membrane filters on which the

fungus was growing were quickly separated from the agar and placed on the GF/A inhibitor-saturated pads. These plates were then sealed with masking tape, and three of each concentration of the inhibitor tested were irradiated at 25± 1.0° C in one of two growth chambers equipped with four Sylvania F48T12/VHO cool white fluorescent lamps.

After 24 hours of exposure, membrane filters were lifted off the glass fiber pads, placed on fresh MRM filled Petri plates, and incubated in the dark at 25± 0.5° C. Observations were made five days later.

F. REPLICATION AND CONTROLS.

In investigating the effect of temperature on radial growth rate, each treatment was replicated three times. Since cultures taken out of the dark for measurement every 24 hours were discarded after each observation, a total of 24 plates were inoculated for each of the five temperatures studied. Three replicates were used in assays attempting to elucidate the effects of radiation on hyphal tip growth, the age at which cultures could be induced to sporulate, and the translocation of a photomorphogenetic stimulus. Six replicate cultures were exposed to each concentration of a particular inhibitor. Similarly, six replicates were exposed to monochromatic or polychromatic radiations while studying the effectiveness of specific wavelengths in inducing conidiation. In investigating the effect of inoculum age on sporulation capacity, seven replicate cultures were initiated with spores isolated from 5, 10,

15, 20, 40, 60, or 80-day-old stock cultures. Each treatment in the examination of succeeding dark days necessary for spore development and maturation was replicated eight times. Nine replications were utilized in preliminary experiments investigating the effects of temperature, photon flux density, and duration of the irradiation period on photosporogenesis.

Three cultures exposed to Vita-Lite radiation (photon flux density = 12.0 Wm^{-2}) following twelve days of dark incubation were used as controls in the experiments investigating the quantitative relation of various wavelengths to conidiation. The sporulation response of these control plates enabled continuous monitoring of the isolate as well as experimental conditions, since any appreciable abnormality, e.g. reduction in the intensity of conidium production, morphological modifications, shifts in the position of the sporulation zone, etc., resulted in the test plates being discarded and the treatment being repeated with spores isolated from a different 10-day-old stock culture. Six membrane filter cultures exposed to GF/A pads soaked in MRL were used as controls for each inhibitor assay. Three foil wrapped plate cultures served as controls for stimulus translocation investigations.

With the exception of the inhibitor assays, in which equal numbers of similarly treated cultures were simultaneously exposed in two growth chambers, and investigations of the effect of moisture, inoculum age, and post-irradiation incubation on sporulation, each experiment was repeated at least once.

G. DETERMINATION OF GROWTH AND SPORULATION.

Assessment of fungal growth in these experiments was based upon the increase in radial diameter of the thallus during the course of an experiment. Under 31.5 X magnification, or with the unaided eye, the advancing edge of the mycelium was marked on two diameters, at right angles to each other, on the underside of the Petri dish. The mean diameter of the thallus measured between these marks was recorded. For the majority of experiments, the diameter of the thallus was either marked every 24 hours during the light cycle, before and after the photoinduction period, and/or during routine observation prior to quantitative spore determination.

The extent and pattern of sporulation in cultures placed under either continuous or intermittent illumination was assessed only at the termination of the experiment, i.e. 18 days after inoculation. Breadth of the annular zone of confluent sporodochia and/or conidia-laden conidiophores was marked by cutting the agar along the outer and inner diameter under 31.5 X magnification. The location of this zone with respect to the growing margin of the thallus, its spatial relation to the diameter of the thallus at photoinduction, as well as its breadth were subsequently recorded.

The influence of illumination, and other factors, on sporogenesis was determined quantitatively. Following visual examination of photoinduced thalli, conidia were harvested from agar and membrane cultures and counted. This was accomplished by flooding

each plate of a series with 20 ml of an electrolyte solution (0.25% NaCl w/v distilled H_2O) containing 0.025% Tween 20 (polyoxyethlene-20-sorbitan monolaurate, J.T. Baker Chemical Co.) and gently rubbing the thallus with a burnished glass rod. The resulting suspension was filtered through six layers of cheesecloth to remove as many hyphal fragments as possible. This procedure was repeated using a stainless steel spatula to dislodge the conidia. Then the combined filtrates, totaling 40 ml in volume, were collected in a 100 ml Pyrex beaker and gently agitated to disperse conidia prior to yield determination. Aliqouts of 0.5 ml were counted in a Coulter Counter with a 70 um aperture (amplification 2, aperture current 1). Four counts were made of each culture suspension, except in the inhibitor assays when each replicate was counted five times. Routine microscopic examination of filtrates was undertaken to reveal the presence or absence of asexual spores since the Coulter Counter was not capable of distinguishing between conidia and hyphal fragments.

Results are reported as either (1) conidia per thallus, where "conidia" represents the mean propagule count derived from the average count of 0.5 ml aliquots of 40.0 ml spore suspensions harvested from all test plates of a given treatment multiplied by the dilution factor, i.e.

$$\text{conidia} = \text{average replicate count } \left(\frac{\text{40 ml spore suspension}}{\text{0.5 ml aliquot}}\right);$$

or (2) the common logarithm of the mean propagule count.

H. SPECTROPHOTOMETRIC ANALYSIS.

A Unicam Ultraviolet Spectrophotometer, Model Sp. 800 was used for measuring the transmittance of polystyrene covers of Fisher Brand Petri dishes, and for determining the absorption spectra of living hyphae.

In light absorption studies, a rectangular piece of mycelium growing on either single ply dialysis tubing or nutrient agar (MRM) was cut from the periphery of a dark reared thallus, inserted into a spectrophotometer cuvette (Blue Label Herasil^{R} Silica, range 200 to 2500 nm, Canlab), and examined spectrophotometrically in a manner identical to that described by Leach (121).

I. ANALYSIS OF DATA.

Statistical analysis of data was computer assisted. The computer program employed, General Linear Models (GLM), was a 1976 Statistical Analysis System Package compiled by Barr et al. (11) of the S.A.S. Institute Inc., Raleigh, North Carolina. This particular program was used to enable an objective estimate of the variance due to treatment. With the exception of the experiment investigating the effect of moisture on sporulation, the common logarithm of sporulation measurements was utilized as this reduced the inherent population variance.

J. PHOTOGRAPHY.

Photographs were taken with Ilford Pan F black and while film using an Olympus OM-1 35 mm camera with diffuse illumination from below and/or direct illumination from above.

EXPERIMENTAL RESULTS

In the course of culturing the original isolate of Cylindrocarpon sp. it was observed that conidial development was always concentrated in concentric zones. Examination of this phenomenon indicated that the principal environmental factor affecting sporulation was light (Reid and Pearn, unpublished data). Results of subsequent investigations undertaken to study the response of this organism to various irradiation treatments and the nature of some of the physiological and biochemical changes responsible for morphogenesis and differentiation associated with photosporogenesis are reported.

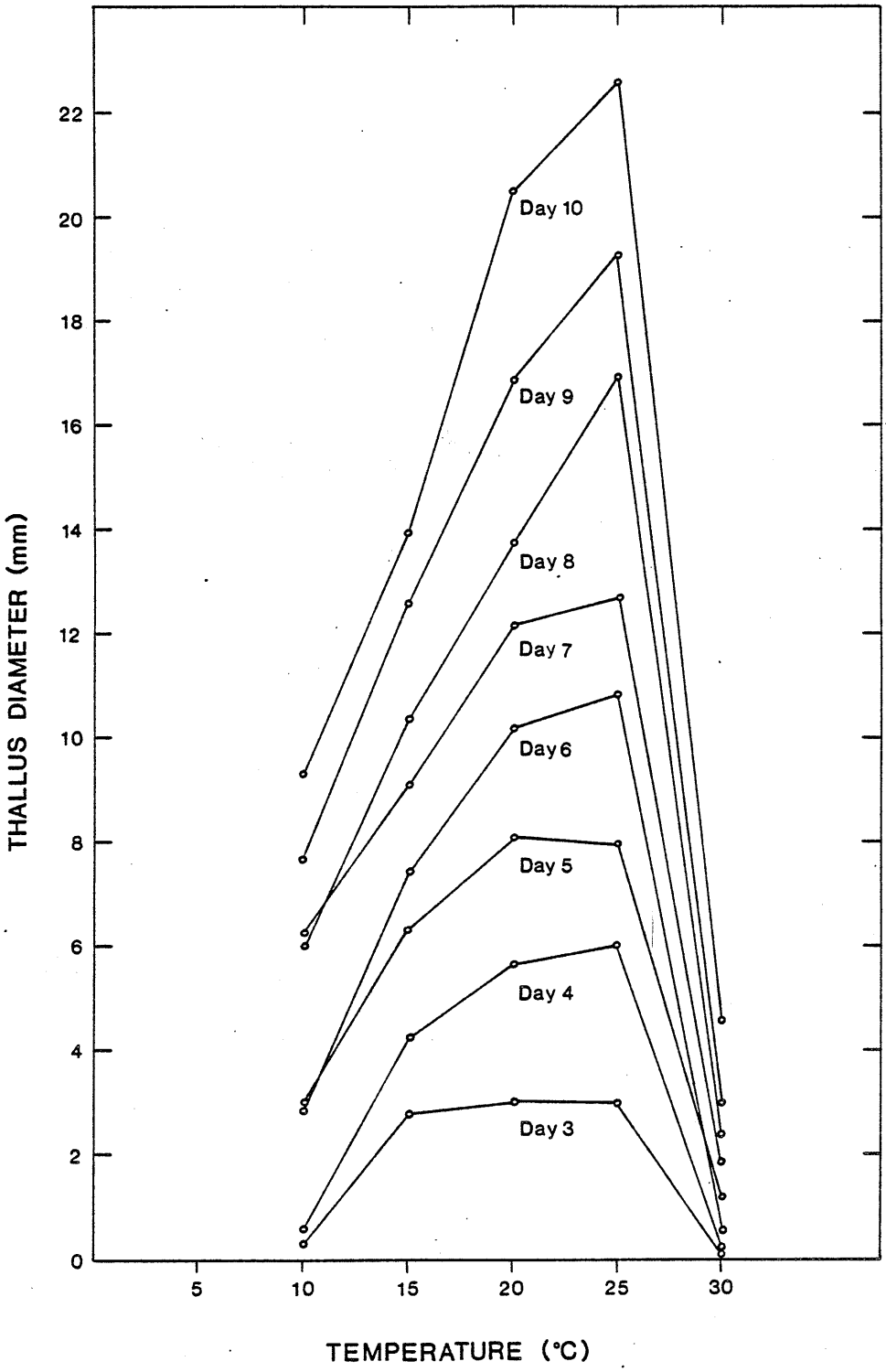
A. STANDARDIZATION OF CULTURING PROCEDURES.

As a prelude to descriptive and physiological investigations, various factors known to affect growth rate and sporulation response in fungi, i.e. incubation temperature, inoculum age, onset of photosensitivity, post-irradiation incubation period, and duration of irradiation necessary to stimulate or induce sporulation, were examined with respect to the test organism to standardize culturing procedures.

1. Effect of incubation temperature on vegetative growth.

Figure 7 depicts the nature of the temperature-growth curves of Cylindrocarpon sp. following incubation periods in total darkness of 3 to 10 days from the time of inoculation at 10, 15, 20, 25, or 30± 0.5° C. There is the characteristic linear portion in which growth appears to increase directly with temperature, an

Figure 7. Effect of temperature on radial growth in PDA cultures of Cylindrocarpon sp. incubated in darkness for 3, 4, 5, 6, 7, 8, 9, and 10 days following inoculation. Each point represents the mean of measurements on 3 replicate cultures, 2 measurements per thallus.


THALLUS DIAMETER (mm)
0
2
4
6
8
10
12
14
16
18
20
22
5
10
15
20
25
30
TEMPERATURE (°C)
Day 10
Day 9
Day 8
Day 7
Day 6
Day 5
Day 4
Day 3

optimum or optimum range in which growth rate is more or less steady, and a descending limb in which growth rate declines as temperature becomes too high for metabolic processes.

No attempt was made to determine the lower and upper limits which permitted growth.

It should be noted that the relation between temperature and radial growth is affected by the duration of the treatment period (Table 1), i.e. the range of temperature for optimum growth is broad initially, e.g. 15-25° C, Day 3 (P=0.05); and becomes progressively narrower with increase in dark incubation, e.g. 20-25° C, Day 6 (P=0.05); 25° C, Day 9 (P=0.05) (Table 2).

On the basis of this information, cultures generated for subsequent experiments were maintained at a temperature of 25± 0.5° C, a range which falls within the optimum for growth of this fungus irrespective of the incubation period.

2. Effect of inoculum age on vegetative growth and sporulation.

Conidia, obtained from sporulating stock cultures grown in illuminated incubation chambers for 5, 10, 15, 20, 40, 60, or 80 days, were germinated on 2% agar medium (WA) and used in the inoculation of plates containing MRM. Since spore age was assessed by counting from the day stock slants were inoculated, conidia produced on and around the original inoculum plug, rather than the periphery of the slant, were used in preparing spore suspensions. Plate cultures were incubated in the dark for 12 days at 25± 0.5° C and then exposed to radiation from 5 Vita-Lite fluorescent lamps (12.0 Wm^{-2}). Radial growth of the thalli was measured at the be-

Table 1

Analysis of Variance for the Effect of Temperature on Radial Growth of Cylindrocarpon sp. During Incubation Periods in Total Darkness of 3 to 10 Days From the Time of Inoculation

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	F	
Temperature	4	822.4160	205.6040	320.09	2.5174	S
Day	7	2552.6076	364.6582	567.72	2.1585	S
Temperature*Day	21	402.1140	19.1483	29.81	1.7265	S
Error	66	42.3993	0.6423			

F_o - observed test statistic = $\frac{SS\ (Treatment)/DF}{SS\ (Error)/\ DF}$, using Replication (Temperature*Day) as an error term

F - F statistic, 5 percent points

S - significant treatment difference at 0.05 level

Table 2

Analysis of Variance and Multiple Comparison (L.S.D.) Tests
on Mean Thallus Diameters for Various Incubation Periods and Incubation Temperatures

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o	$\lvert\bar{X} - \bar{X}\rvert$ 10° 15°	$\lvert\bar{X} - \bar{X}\rvert$ 15° 20°	$\lvert\bar{X} - \bar{X}\rvert$ 20° 25°	$\lvert\bar{X} - \bar{X}\rvert$ 25° 30°
Day 3										
Treatment	4	34.800	8.700	43.50	0.0001	0.575	S	NS	NS	S
Error	10	2.000	0.200							
Day 6										
Treatment	4	410.801	102.700	214.56	0.0001	2.228	S	S	NS	S
Error	10	4.787	0.479							
Day 9										
Treatment	4	1131.102	282.776	259.27	0.0001	1.343	S	S	S	S
Error	10	10.907	1.091							

F_o - observed test statistic for analysis of variance = $\frac{SS\ (Treatment)/DF}{SS(Error)/DF}$, using Replication (Treatment) as an error term

t_o - observed test statistic for L.S.D. multiple comparison procedure = $t_{\frac{\alpha}{2}, df\ (MSE)} \sqrt{\frac{2MSE}{n_i}}$

S - significant treatment difference between paired mean values at the 0.05 level

NS - no significant treatment difference between paired mean values at the 0.05 level

ginning of the 24 hour light treatment. Following irradiation, cultures were incubated for a further 120 hours in darkness and then examined quantitatively for spore production. Analyses of the data indicate that the age of conidia used as inoculum has no significant effect on either growth (Table 3) or sporulation intensity (Figure 8, Table 4). However, these results do not demonstrate that age is without an effect. Although cultures were always initiated with conidia which had germinated in the typical bipolar fashion, the percentage of conidia in the population which would germinate declined as the age of the stock culture increased, and those conidia capable of germinating often produced a solitary germ tube. Furthermore, changes in spore morphology, e.g. swelling of terminal cells, reduction in spore size and volume, granulation, etc., appeared to be associated with aging. As a consequence, only conidia isolated from 10-day-old cultures were used in later experiments.

3. Thallus age and sensitivity to morphogenetically active radiation.

The age at which the fungal thallus became light sensitive with respect to sporogenesis was qualitatively assessed. Fifteen cultures on MRM were incubated at 25± 0.05° C in darkness, three being removed every 24 hours and exposed to white fluorescent light (12.0 Wm^{-2}). After 24 hours of continuous irradiation, cultures were returned to darkness. Observations of thalli, exposed at ages up to 5 days, were made on Day 6.

Table 3

Analysis of Variance for the Effect of Inoculum Age on Radial Growth (mm) in Cultures Incubated at 25± 0.5° C in Darkness from the Time of Inoculation

Replicate Plate	Inoculum Age (Days) 5	10	15	20	40	60	80
1	38.50	37.25	36.25	34.25	35.40	36.00	33.90
2	39.10	37.25	35.90	34.70	35.00	35.60	34.25
3	38.00	38.00	36.25	36.90	35.00	34.75	34.50
4	38.25	38.00	36.25	36.15	35.25	33.25	33.75
5	40.00	35.50	35.75	37.25	34.75	35.40	34.75
6	39.50	38.00	38.25	36.95	35.10	35.05	35.00
7	38.50	38.00	37.50	38.15	34.60	34.30	36.20
Treatment Total	271.85	262.00	256.15	254.35	254.10	244.35	242.35
Treatment Mean	38.84	37.43	36.59	36.34	35.01	34.91	34.62

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	F	
Treatment	42	99.277	2.3637	0.4078	2. 3277	NS
Error	6	34.780	5.7967			
Total	48	134.057				

F_o - observed test statistic = $\frac{SS\ (Treatment)/DF}{SS\ (Error)/\ DF}$, using variation among experimental units treated alike as an error term

F - F statistic, 5 percent points

NS - no significant treatment difference at the 0.05 level

It was found that sensitivity to light developed within 24 hours of inoculation, i.e. cultures transferred immediately to illuminated chambers formed conidia on the inoculum plug. However, the formation of a distinct ring of mycelium bearing conidia only occurred on those thalli transferred to the light 96 hours after their growth had been initiated by single spore inoculation. By this time, hyphal tips had grown out from the inoculum plug over the agar. Thalli exposed prior to this time had a central region bearing scattered clumps of macroconidia. A "carry over effect", associated with a carry over of stimulus or primary photoproduct from the light-induced inoculum, was dismissed as the probable explanation for the production of this localized area of sporulation when it was determined that macroconidia were not produced on the inoculum plugs of thalli exposed 96 and 120 hours after their initiation or dark controls.

In subsequent experiments, although the fungus could be induced to sporulate in less than 24 hours of growth, cultures were irradiated 12 days after inoculation. By then slightly less than half the agar surface was covered by mycelium, and, unlike fungi in which photosensitivity has been reported to decrease directly with the age of the mycelium (25, 117, 216), the sporulation response of this isolate was not affected by the maturity of actively growing thalli.

Figure 8. Effect of inoculum age on conidium production in 12-day-old, dark-reared cultures of Cylindrocarpon sp. exposed to 24 hours of Vita-Lite fluorescent radiation (12.0 Wm^{-2}) at 25± 1.0° C. Each point represents the mean spore yield of 7 replicate cultures, 4 counts per culture. Vertical lines with limits denote variation among experimental units under similar treatment.

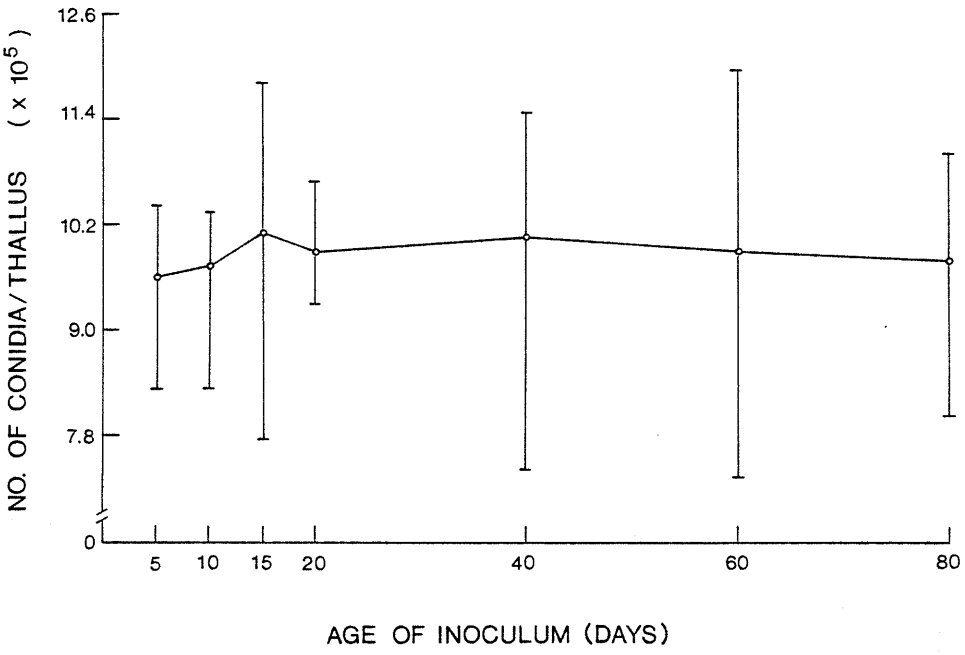

NO. OF CONIDIA/THALLUS (x 10^5)
12.6
11.4
10.2
9.0
7.8
0
5
10
15
20
40
60
80
AGE OF INOCULUM (DAYS)

Table 4

Analysis of Variance for the Effect of Inoculum Age on Conidium Production in Cylindrocarpon sp.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	F	
Age	6	0.1176	0.0196	0.36	2.345	NS
Error	39	2.1516	0.0552			

F_o - observed test statistic = $\frac{SS(Treatment)/DF}{SS(Error)/DF}$, using Plate (Age) as an error term

F - F statistic, 5 percent points

NS - no significant treatment difference at the 0.05 level

4. Post-irradiation incubation period.

Since light was known to stimulate or induce macrospore production, it seemed possible that the yield of conidia might be influenced by the length of the dark period between the time of exposure and the quantitative measurement of sporulation. Thus, the minimum dark period necessary for complete macrospore development, following irradiation, was investigated.

Sets of 12-day-old, dark-grown cultures were exposed for 24 hours to white fluorescent light (12.0 Wm^{-2}) and then returned to darkness for one of five different time periods, i.e. 24, 48, 72, 96, and 120 hours. Control plates were assessed for sporulation immediately following the irradiation period. The mean yields of these treatments are given in Figure 9.

Hyaline conidia were observed to be produced on all thalli during the latter half of the light exposure period. Although this observation demonstrated that a period of darkness following irradiation was not required for conidium development, fewer conidia were produced in those cultures either examined immediately after irradiation or incubated in darkness for 24 hours than in cultures receiving longer post-illumination dark treatments. However, in cultures receiving 48 hours of darkness, sporulation was not significantly different from that of cultures receiving 72, 96, and 120 hours (Table 5). These results indicate that under similar experimental conditions a dark period of not less than 48 hours must follow irradiation for the maximum production of macroconidia. In all subsequent experiments a post-irradiation incubation period

Figure 9. Effect of post-irradiation incubation period on conidium production in 12-day-old, dark-reared cultures of Cylindrocarpon sp. exposed to 24 hours of Vita-Lite fluorescent radiation (12.0 Wm^{-2}) at 25± 1.0° C. Each point represents the mean spore yield of 8 replicate cultures, 4 counts per culture. Vertical lines with limits denote variation among experimental units under similar treatment.

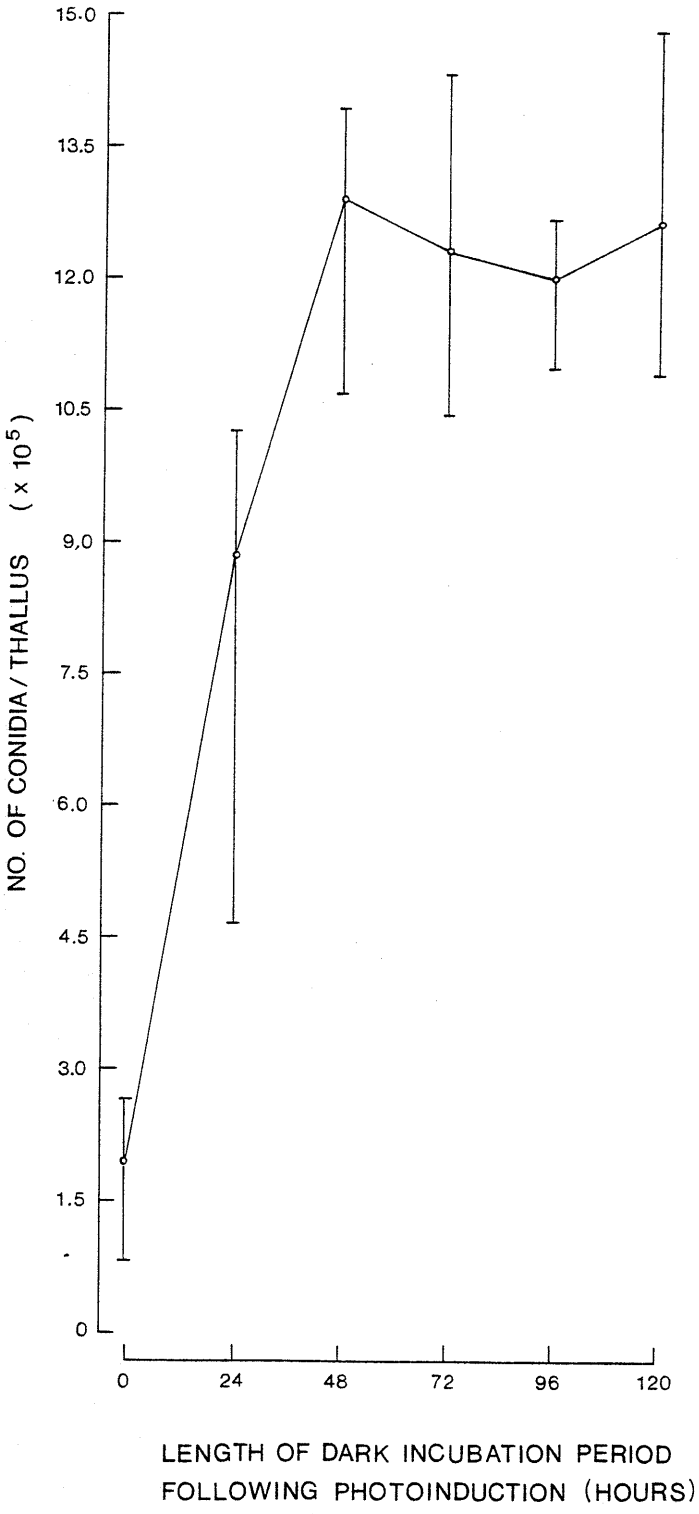

15.0
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0
NO. OF CONIDIA / THALLUS (x 10^5)
0
24
48
72
96
120
LENGTH OF DARK INCUBATION PERIOD
FOLLOWING PHOTOINDUCTION (HOURS)

Table 5

Analysis of Variance and Multiple Comparison (L.S.D.) Tests for the Effect of Post Irradiation Incubation Period on Conidium Production in Cylindrocarpon sp.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o	$\lvert\bar{X}_0 - \bar{X}_{24}\rvert$	$\lvert\bar{X}_{24} - \bar{X}_{48}\rvert$	$\lvert\bar{X}_{48} - \bar{X}_{72}\rvert$	$\lvert\bar{X}_{72} - \bar{X}_{96}\rvert$	$\lvert\bar{X}_{96} - \bar{X}_{120}\rvert$
Treatment	5	92.9168	18.5833	15.135	0.0001	0.2045	S	S	NS	NS	NS
Error	38	4.6658	0.1228								

F_o - observed test statistic for analysis of variance = $\frac{SS\ (Treatment)/DF}{SS\ (Error)/DF}$, using Plate (Treatment) as an error term

t_o - observed test statistic for L.S.D. multiple comparison procedure = $t_{\frac{\alpha}{2},\ df\ (MSE)}\sqrt{\frac{2MSE}{n_i}}$

S - significant treatment difference between paired mean values at the 0.05 level

NS - no significant treatment difference between paired mean values at the 0.05 level

of 120 hours was used, unless otherwise stated.

5. Irradiation period necessary to stimulate or induce sporulation.

In an attempt to determine the shortest period of exposure to radiation necessary to enhance or induce the production of macroconidia, 12-day-old, dark-grown cultures were exposed to radiation from 5 Vita-Lite fluorescent lamps (12.0 Wm^{-2}) for various periods ranging from a minimum of 3 hours to a maximum or 24 hours and then returned to darkness. Conidial production was quantitatively determined 18 days after inoculation. Results are presented in Figure 10.

Sporulation in cultures exposed for periods of 3, 6, and 9 hours was not significantly greater than that of control cultures kept in constant darkness. However, in comparison with nonirradiated cultures, the mean yield of conidia was significantly increased by the 12 hour light exposure, and continuous irradiation for 24 hours had a further significant effect (table 6). As a consequence, all succeeding experiments were run at or beyond this minimum effective exposure of 12 hours regardless of the source of radiation and the photon flux density.

B. EFFECT OF VARIOUS IRRADIATION TREATMENTS ON THE LOCATION OF CONIDIOPHORES.

The following investigations were undertaken to study where, topographically, conidia would be produced on actively growing thalli in response to various irradiation treatments. All experiments were carried out in triplicate with monoconidial isolates of

Figure 10. The length of exposure to Vita-Lite fluorescent radiation (12.0 Wm^{-2}) necessary to induce conidiogenesis in 12-day-old, dark-reared cultures of Cylindrocarpon sp. Each point represents the mean spore yield of 18 replicate cultures, 4 counts per culture. Vertical lines with limits denote variation among experimental units under similar treatment.

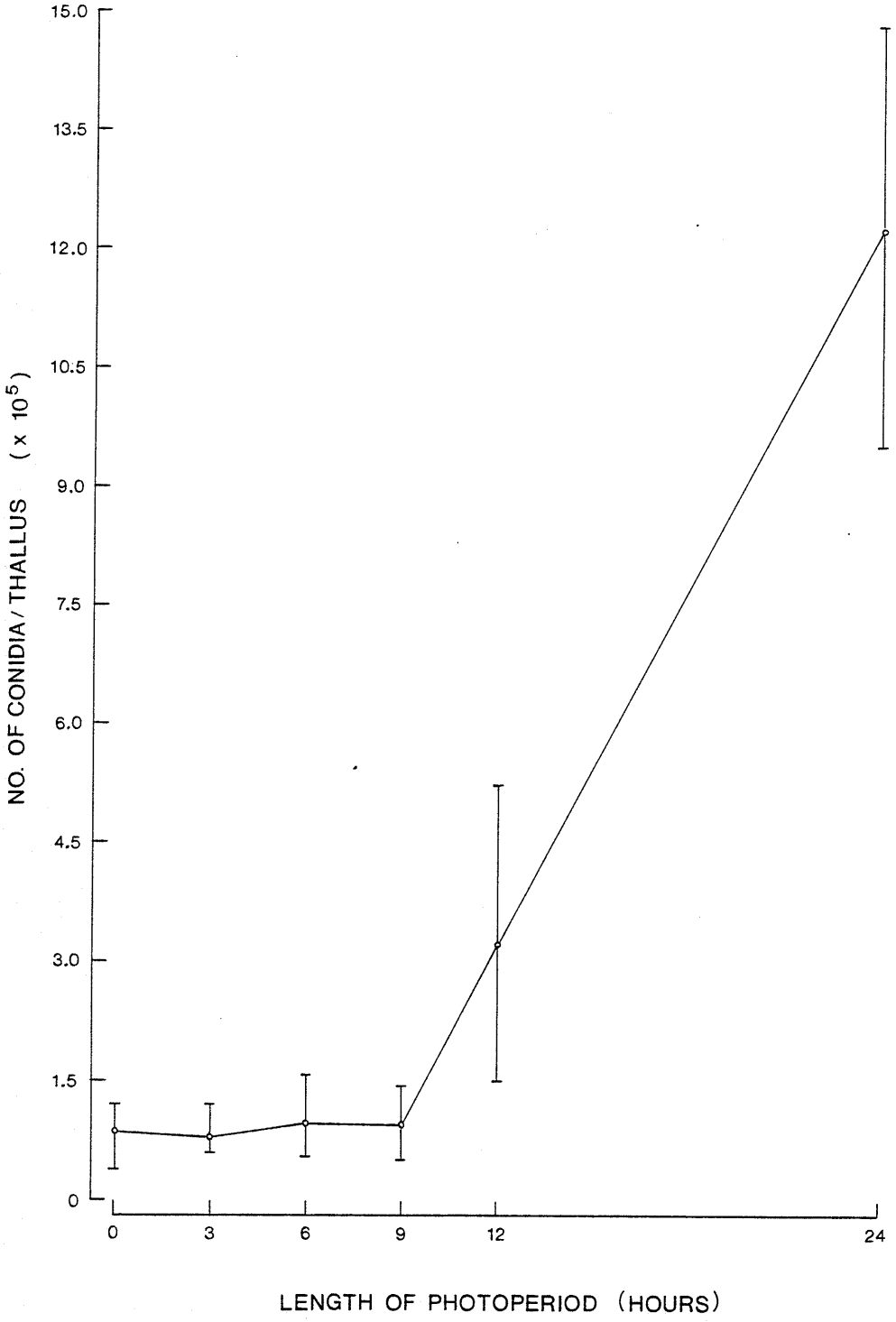

15.0
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0
NO. OF CONIDIA / THALLUS (x 10^5)
0
3
6
9
12
24
LENGTH OF PHOTOPERIOD (HOURS)

Table 6

Analysis of Variance and Multiple Comparison (L.S.D.) Tests on the Length of Exposure to Vita-Lite Fluorescent Radiation Necessary to Induce Sporulation in Cylindrocarpon sp.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o	$\lvert\bar{X}_0 - \bar{X}_3\rvert$	$\lvert\bar{X}_3 - \bar{X}_6\rvert$	$\lvert\bar{X}_6 - \bar{X}_9\rvert$	$\lvert\bar{X}_9 - \bar{X}_{12}\rvert$	$\lvert\bar{X}_{12} - \bar{X}_{24}\rvert$
Treatment	5	389.9581	77.9916	92.49	0.0001	0.3745	NS	NS	NS	S	S
Error	6	5.0593	0.8432								

F_o - observed test statistic for analysis of variance = $\frac{SS\ (Treatment)/DF}{SS\ (Error)/DF}$, using Experiment Replication (Treatment), i.e. Run (Exposure), as an error term

t_o - observed test statistic for L.S.D. multiple comparison procedure = $t_{\frac{\alpha}{2},\ df\ (MSE)}\sqrt{\frac{2MSE}{n_i}}$

S - significant treatment difference between paired mean values at the 0.05 level

NS - no significant treatment difference between paired mean values at the 0.05 level

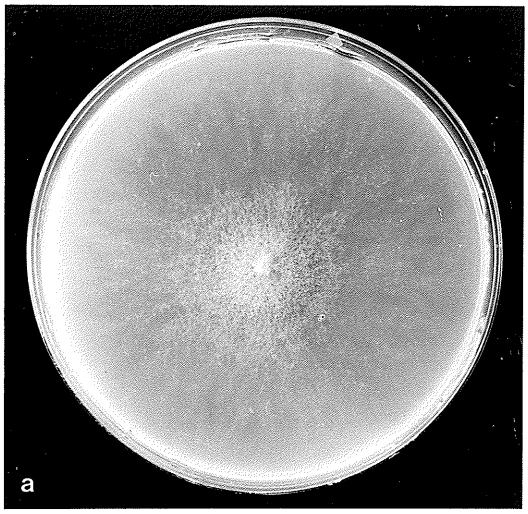
the fungus cultured on MRM at 25± 1.0° C. A bank of 5, 20 watt Vita-Lite fluorescent lamps served as the radiation source. Radiant energy intercepted by the thallus of each culture was 12.0 Wm^{-2} as measured with a quantum sensor.

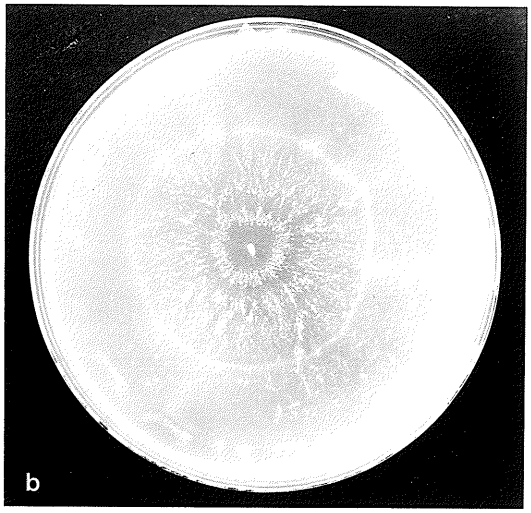
1. The effects of continuous irradiation and continuous darkness.

Freshly inoculated plates containing MRM were either placed under continuous fluorescent light or kept in total darkness. After 12 days of exposure to these conditions, both sets of cultures were examined and then incubated for an additional 5 days in the absence of light.

It was seen that sporulation did not occur in any of the plates incubated in complete darkness (Figure 11a). However, thalli grown in continuous light were covered to within approximately 1.25 mm of their margin by sporodochia and conidia-bearing conidiophores. The youngest portion of the thallus did not form spores and did not attain the ability to produce spores upon incubation in the dark (Figure 11b). In addition to the effect on sporulation, the presence or absence of light had a general effect upon culture morphology. Dark-grown cultures produced abundant white, fluffy aerial hyphae, while the mycelium of continuously illuminated cultures was prostrate to immersed. Radial extension of the thalli in the irradiated series was also markedly reduced. This, coupled with the reduced production of aerial hyphae, indicated a marked

Figure 11. Response of Cylindrocarpon sp. to various phototreatments and darkness: (a) 20-day-old thallus incubated in continuous darkness at 25± 0.5° C following inoculation; (b) 20-day-old thallus incubated under continuous Vita-Lite fluorescent radiation (12.0 Wm^{-2}) at 25± 1.0° C following inoculation; (c) 20-day-old thallus exposed at 25± 1.0° C to 12 hours of Vita-Lite fluorescent radiation (12.0 Wm^{-2}) in twelve, 24 hour cycles following inoculation and then incubated in darkness at 25± 1.0° C (arrow indicates the position of the thallus margin at the time of the final light cycle); and (d) 18-day-old thallus exposed at 25± 1.0° C to 24 hours of Vita-Lite fluorescent radiation (12.0 Wm^{-2}) following 12 days of dark incubation at 25± 0.5° C (single arrow indicates the position of the thallus margin just prior to irradiation as well as the outer diameter of the sporulation zone that formed along the hyphal front in response to irradiation, double arrow indicates the position of the thallus margin at the termination of the photoinduction period).


a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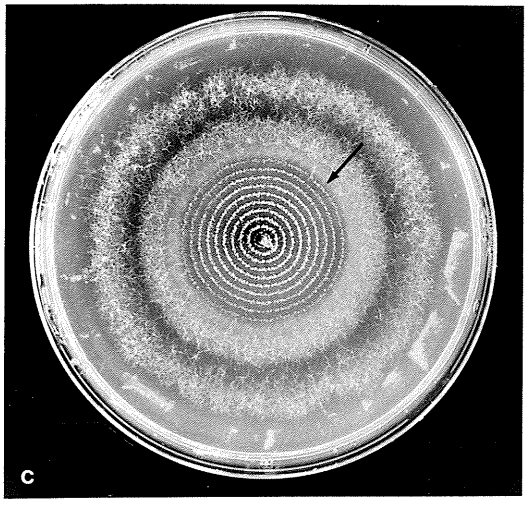
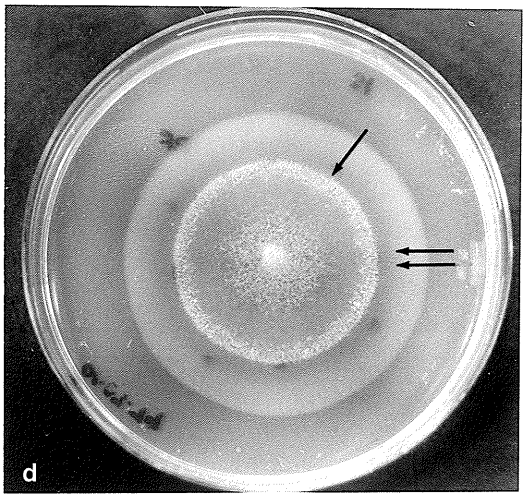

c


d

reduction in vegetative growth in constant light.

2. The effect of alternating cycles of irradiation and darkness.

To study the effect of alternating light-dark cycles on gross morphology and sporulation an experiment was carried out in which both freshly inoculated and 4-day-old, dark-reared cultures were grown in constant temperature chambers which were alternately illuminated and darkened for 12 hour periods, the first being one of light. Results recorded at the conclusion of the dark cycle on Day 12 showed the target board pattern of pronounced zonation described for many diverse species of fungi (Figure 11c). Concentric zonation, in this particular instance, was attributable to successive rings of confluent sporodochia separated by narrow regions of sterile, appressed hyphae. The number of rings formed by thalli initially incubated in darkness corresponded to the number of times the cultures had been illuminated, i.e. no subsidiary zones were noted. In addition, the width of the ring of spores and the distance between the rings were constant across the diameter of the thalli. In contrast, the initial lag in growth rate, characteristic of this fungus, resulted in the fusion of the spore rings formed during the first 72 hours of treatment in cultures exposed to the periodic alternation of light and darkness immediately following inoculation. Consequently, the number of distinct concentric rings produced by these thalli did not correspond to the number of light cycles, but was short by three.

3. The effect of continuous darkness interrupted by a period of irradiation.

The previous experiments had demonstrated that light induced, rather than stimulated, sporogenesis in cultures of Cylindrocarpon sp. grown in MRM, and suggested that conidium production was limited to that mycelium which had received light during its initial development. In an attempt to determine whether mycelium synthesized in the dark would generally produce macroconidia upon exposure, or if conidium production was, in fact, restricted to that portion of the thallus generated during the irradiation period, cultures were grown in total darkness for 12 days after inoculation, transferred to an illuminated chamber for one of six time periods (24, 48, 72, 96, 120, or 144 hours), and then returned to darkness for five days prior to examination. Control cultures were not irradiated.

It was found that all exposures of less than 120 hours brought about the formation of a distinct sporulation ring in the 6.0 mm region of the thallus which had been produced prior to irradiation. The outer circumference of the ring coincided with the position of the mycelial front upon first exposure to light. Although there was an absence of conidia anywhere but in the ring itself, the intensity of sporulation was not uniform across the width of the ring but declined dramatically upon inward progression from the periphery as sporodochia were replaced by scattered conidiophores. With longer periods of exposure, i.e. 120 to 144 hours, it was discover-

ed that sporulation was not confined to the peripheral region of the thallus since conidia were consistently observed in the newly formed mycelium generated during the irradiation period. However, sporulation in this region was patchy and never as profuse as in the peripheral zone. This indicated that the hyphal tips or the hyphae immediately behind them, rather than older mycelium or hyphae actively growing in the light, were more sensitive to the effect of radiation and the most highly photoresponsive (Figure 11d).

C. PHOTOBIOLOGY.

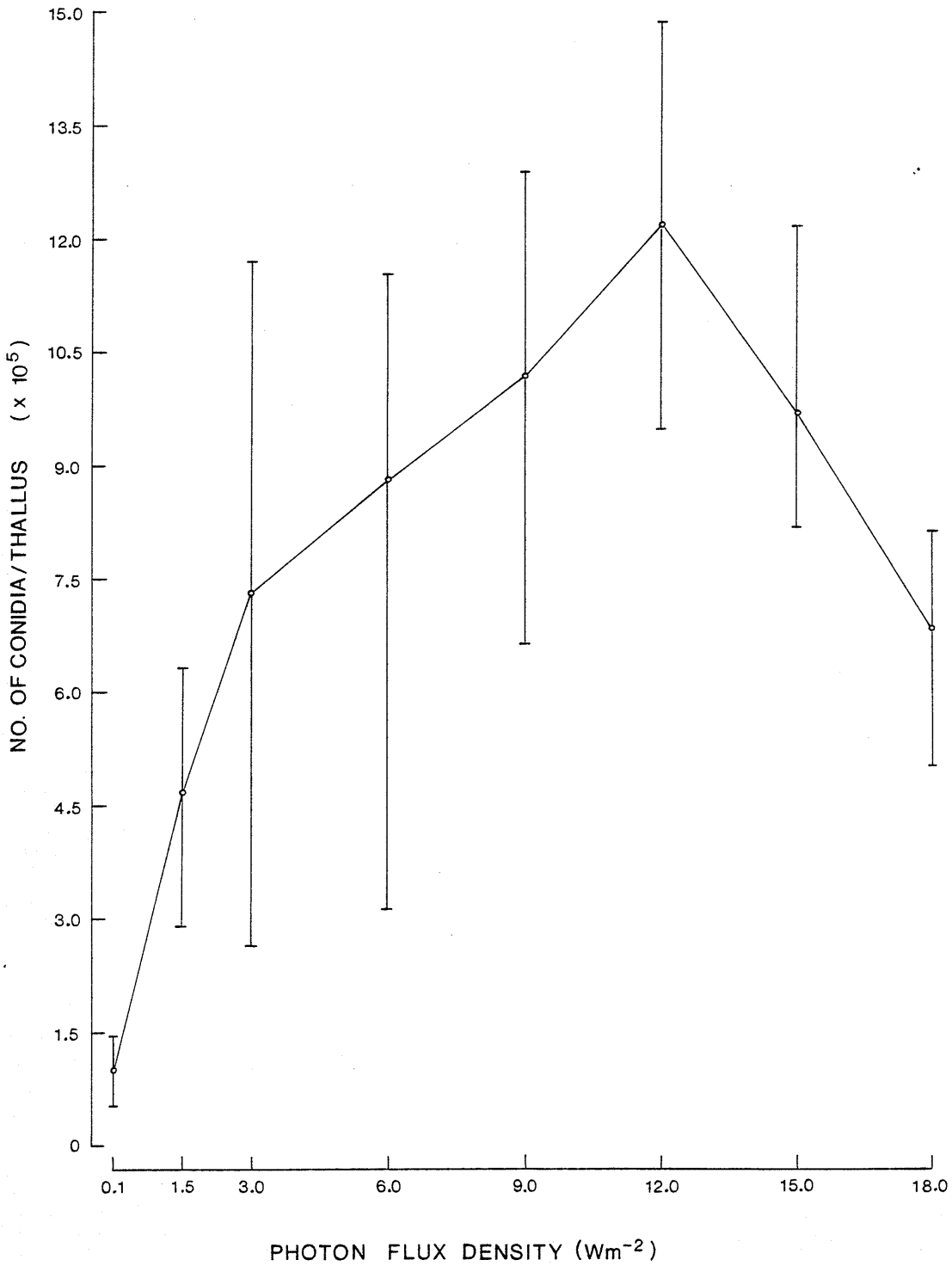
These investigations were undertaken to determine more precisely the interrelation of light and induced sporogenesis in Cylindrocarpon sp., and for the purpose of comparing the qualitative and quantitative light requirements of this species with those of other light sensitive fungi.

1. The effect of differing incident light intensities on sporulation.

To study the effect of intensity of incident light, a bank of from 1 to 5 Vita-Lite fluorescent lamps positioned in growth chambers at varying distances above the fungal thalli was used to obtain irradiance levels ranging from 0.1 to 18.0 Wm^{-2}. Twelve-day-old, dark-grown cultures were irradiated at selected intensities for 24 hours at 25± 1.0° C, and then returned to the 25± 0.5° C dark incubator. Sporulation was quantitatively measured five days after the irradiation period.

Results presented in Figure 12 indicate that conidium produc-

Figure 12. Effect of irradiance level on conidium production in 12-day-old, dark-reared cultures of Cylindrocarpón sp. exposed to 24 hours of Vita-Lite fluorescent radiation at 25± 1.0° C. Each point represents the mean spore yield of 18 replicate cultures, 4 counts per culture. Vertical lines with limits denote variance among experimental units under similar treatment.


15.0
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0
NO. OF CONIDIA/THALLUS (x 10^5)
0.1
1.5
3.0
6.0
9.0
12.0
15.0
18.0
PHOTON FLUX DENSITY (Wm^-2)

tion increased with irradiance up to 12.0 Wm^{-2}, and that higher levels of irradiation intensity, when compared with the 12.0 Wm^{-2} treatment, had an inhibitory effect. However, the number of conidia produced in cultures receiving 12.0 Wm^{-2} was not significantly greater than the mean yields of cultures exposed at irradiance levels of 9.0 Wm^{-2} and 15.0 Wm^{-2} (Table 7). Consequently, the depressive effect on sporulation occurred only at the maximum irradiation intensity used. Exposures at 18.0 Wm^{-2} resulted in a mean spore yield not significantly different from that of the 3.0 Wm^{-2} treatment.

2. The effect of the duration of the irradiation period on sporulation.

Preliminary experiments with cultures grown on MRM (p.72) demonstrated that an exposure of at least 12 hours was required to induce sporogenesis in this fungus, and that irradiation for 24 hours significantly increased the mean yield of conidia as compared with the 12 hour treatment. To determine the effect of extended periods of continuous illumination, 12-day-old, dark-grown cultures were exposed to Vita-Lite fluorescent radiation (12.0 Wm^{-2}) for intervals up to 144 hours, and then returned to darkness. Plates illuminated for less than 120 hours were examined six days from the start of the irradiation periods, while those exposed for 120 and 144 hours were left until Day 8. Although the treatments were evaluated at 48 to 132 hours after irradiation, post-irradiation periods greater than 48 hours had been shown to cause no signifi-

Table 7

Analysis of Variance and Multiple Comparison (L.S.D.) Tests for The Effect of Photon Flux Density on Photoinduced Sporulation

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o
Treatment	7	261.3216	37.3316	82.50	0.0001	0.3174
Error	8	3.6175	0.4522			

$\lvert\bar{X} - \bar{X}\rvert$	$\lvert\bar{X} - \bar{X}\rvert$	$\lvert\bar{X} - \bar{X}\rvert$	$\lvert\bar{X} - \bar{X}\rvert$	$\lvert\bar{X} - \bar{X}\rvert$
0.1 1.5	1.5 3.0	3.0 6.0	3.0 9.0	3.0 18.0
S	S	NS	S	NS
$\lvert\bar{X} - \bar{X}\rvert$	$\lvert\bar{X} - \bar{X}\rvert$	$\lvert\bar{X} - \bar{X}\rvert$	$\lvert\bar{X} - \bar{X}\rvert$	$\lvert\bar{X} - \bar{X}\rvert$
6.0 9.0	6.0 12.0	9.0 12.0	12.0 15.0	15.0 18.0
NS	S	NS	NS	S

F_o - observed test statistic for analysis of variance = $\frac{SS\ (Treatment)/DF}{SS\ (Error)/DF}$, using Experiment Replication (Treatment), i.e. Run (Photon Flux Density) as an error term

t_o - observed test statistic for L.S.D. multiple comparison procedure = $t_{\frac{\alpha}{2},\ df\ (MSE)}\sqrt{\frac{2MSE}{n_i}}$

S - significant treatment difference between paired mean values at the 0.05 level

NS - no significant treatment difference between paired mean values at the 0.05 level

cant increase in mean spore yield (p. 68).

The data presented in Figure 13 indicate that sporulation was enhanced by increased doses of irradiation over the entire range of exposures tested. However, analyses of the data (Table 8) show that longer photoinduction periods did not necessarily result in the production of significantly greater yields of conidia (P=0.05). While mean counts of sporulation increased linearly with exposure to 12, 24 and 48 hours of light, continuous illumination for more than 72 hours caused no net increase in mean spore yield. Significant treatment difference existed only between exposures of 12 hours and 24+ hours, 24 hours and 48+ hours, 48 hours and 96+ hours, and 72 hours and 144 hours.

3. The effect of temperature on sporulation.

Although irradiation experiments described up to this point had been carried out in chambers maintained at 25± 1.0° C, the possibility that photoinduction of conidiation might be affected by temperature was considered. Cultures grown in 25± 0.5° C dark incubators for 12 days were exposed for 24 hours to radiation from Vita-Lite fluorescent lamps (12.0 Wm^{-2}) at temperatures ranging from 10° C to 35° C. Following irradiation, the cultures were incubated for a further 120 hours in darkness at 25± 0.5° C and then examined. Results are given in Figure 14.

Conidia were formed in the peripheral zones of all photoinduced cultures at temperatures of 10, 15, 20, 25, 30, and 35° C. Sporulation was sparse at 10° C and practically absent at 35° C. Maximum numbers were harvested from thalli exposed at 25° C.

Figure 13. Effect of photoinduction period on conidium production in 12-day-old, dark-reared cultures of Cylindrocarpon sp. exposed to Vita-Lite fluorescent radiation (12.0 Wm^{-2}) at 25± 1.0° C. Each point represents the mean spore yield of 18 replicate cultures, 4 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.

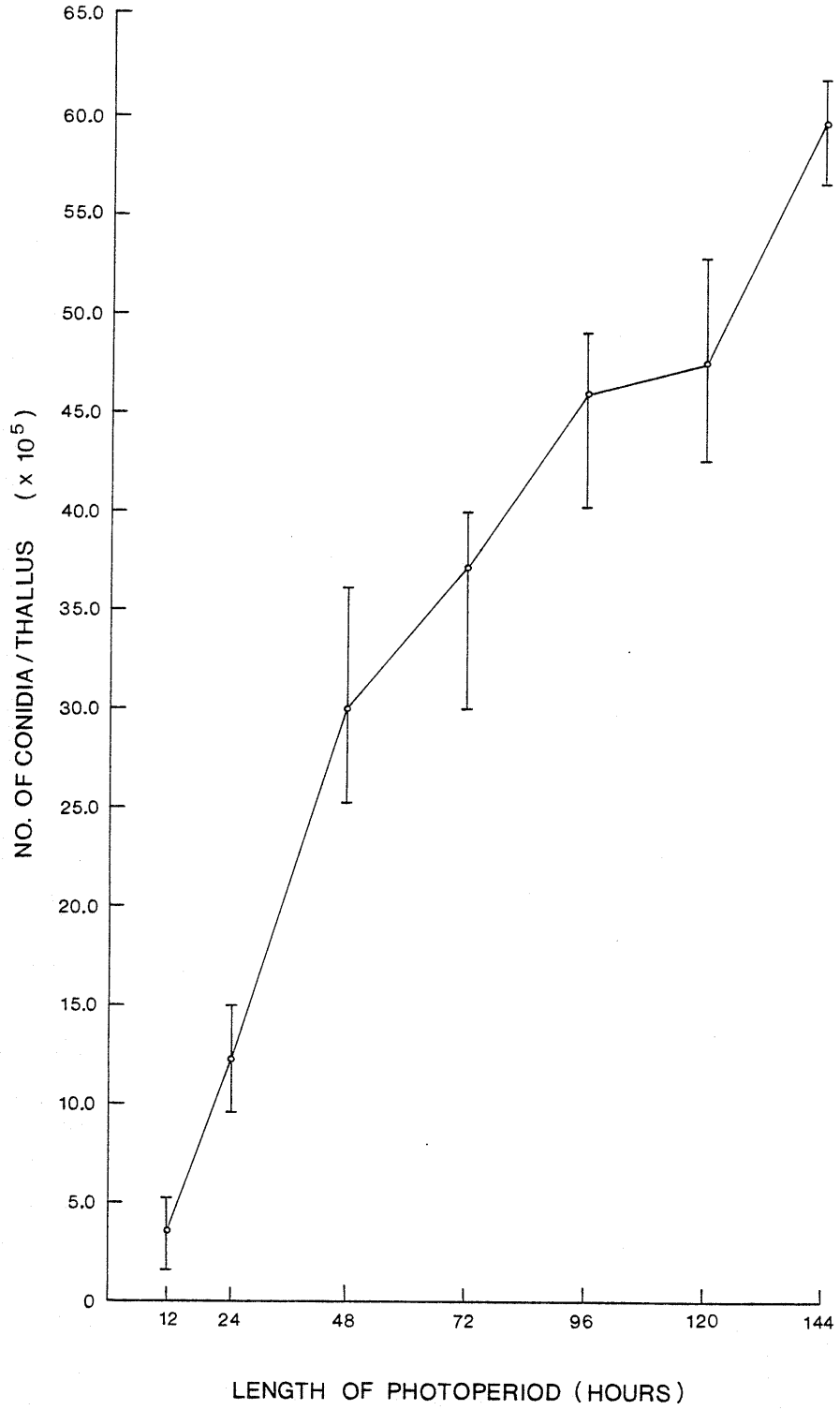

65.0
60.0
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0
NO. OF CONIDIA / THALLUS (x 10^5)
12
24
48
72
96
120
144
LENGTH OF PHOTOPERIOD (HOURS)

Table 8

Analysis of Variance and Multiple Comparison (L.S.D.) Tests for the Effect of Irradiation Period on Photoinduced Sporulation

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o
Treatment	6	417.6583	69.6097	105.52	0.0001	0.3210
Error	7	4.6178	0.6597			

$\lvert\bar{X}_{12} - \bar{X}_{24}\rvert$	$\lvert\bar{X}_{24} - \bar{X}_{48}\rvert$	$\lvert\bar{X}_{48} - \bar{X}_{72}\rvert$	$\lvert\bar{X}_{48} - \bar{X}_{96}\rvert$	$\lvert\bar{X}_{72} - \bar{X}_{96}\rvert$
S	S	NS	S	NS

$\lvert\bar{X}_{72} - \bar{X}_{120}\rvert$	$\lvert\bar{X}_{72} - \bar{X}_{144}\rvert$	$\lvert\bar{X}_{96} - \bar{X}_{120}\rvert$	$\lvert\bar{X}_{96} - \bar{X}_{144}\rvert$	$\lvert\bar{X}_{120} - \bar{X}_{144}\rvert$
NS	S	NS	NS	NS

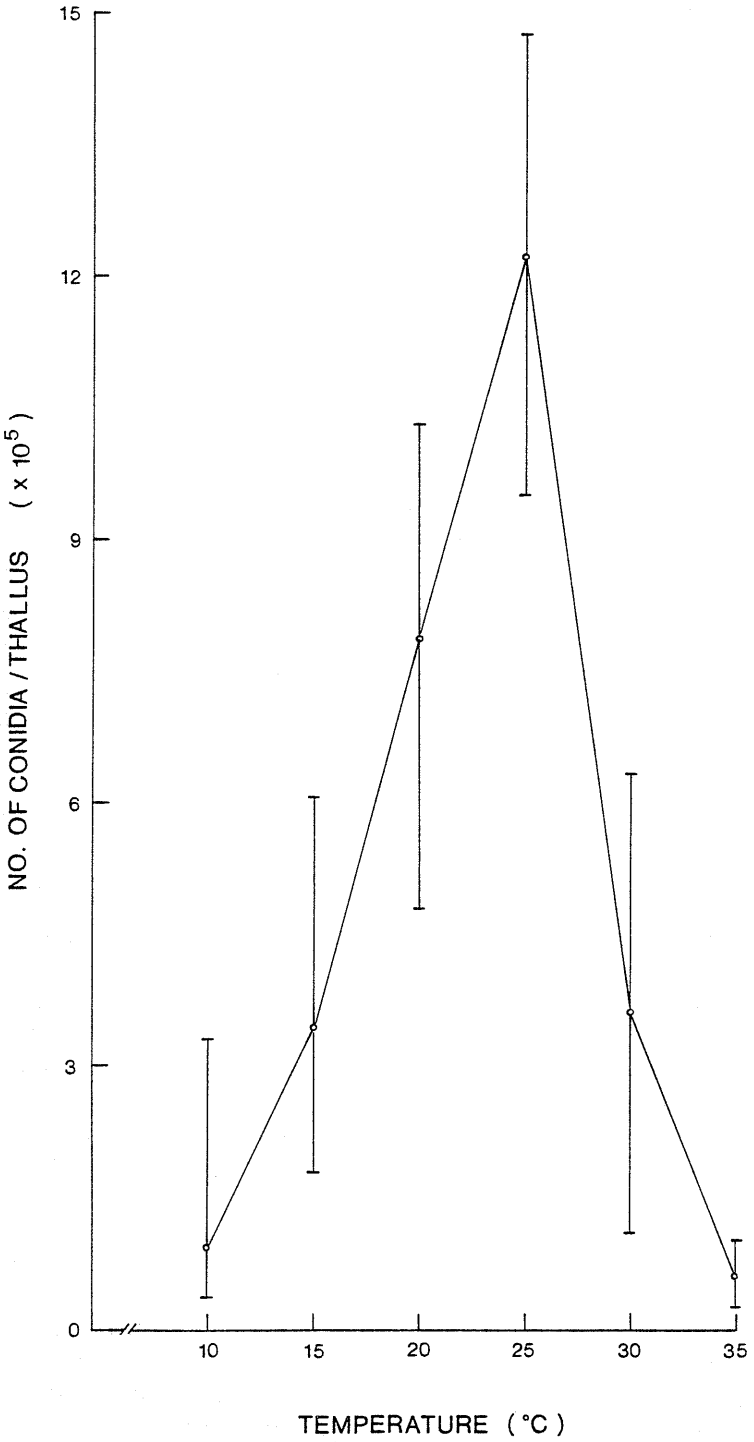
F_o - observed test statistic for analysis of variance = $\frac{SS\ (Treatment)/DF}{SS(Error)/DF}$, using Experimental Replication (Treatment), i.e. Run (Irradiation Period), as an error term

t_o - observed test statistic for L.S.D. multiple comparison procedure = $t_{\frac{\alpha}{2},\ df\ (MSE)}\sqrt{\frac{2MSE}{n_i}}$

S - significant treatment difference between paired mean values at the 0.05 level

NS - no significant treatment difference between paired mean values at the 0.05 level

Figure 14. Effect of temperature on conidium production in 12-day-old, dark-reared cultures of Cylindrocarpon sp. exposed to 24 hours of Vita-Lite fluorescent radiation (12.0 Wm^{-2}). Each point represents the mean spore yield of 18 replicate cultures, 4 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.


15
12
9
6
3
0
NO. OF CONIDIA / THALLUS (x 10^5)
10
15
20
25
30
35
TEMPERATURE (°C)

However, the response at this temperature was not significantly greater than the effect of treatment at 20° C (Table 9).

4. The effect of localized light exposure on mycelium grown in darkness.

Since it had been shown that conidiophores bearing conidia were only produced, as a result of light exposure, on the young tissue which existed around the edge of the thallus at the time of irradiation (p. 80), experiments were conducted to investigate whether there was any translocation of the light effect between irradiated and nonirradiated peripheral hyphae. Details of the procedures for irradiation have been described in the methodology section.

It was observed that a small section of thallus margin illuminated continuously for 24 hours as well as the nonexposed peripheral region produced conidia. Sporulation was not confined to directly irradiated tissue and photomorphogenetic materials were, presumably, transferred. However, similarly treated control plates of agar overlaid with single weight Ilford Ilfobrom (Contrast Grade 0) photographic paper demonstrated, upon development, that localized irradiation for 1 hour was sufficient to blacken every square millimeter of the paper surface. Owing to the complications of reflected incident radiation and the scattering of transmitted radiation, no inferences can be drawn.

Comparable investigations of fungi other than Cylindrocarpon sp. suggest that the primary product of light perception may either

Table 9

Analysis of Variance and Multiple Comparison (L.S.D.) Tests

for the Effect of Temperature on the Photoinduction of Sporulation in Cylindrocarpon sp.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o	$\lvert\bar{X} - \bar{X}\rvert$ 10° 15°	$\lvert\bar{X} - \bar{X}\rvert$ 15° 20°	$\lvert\bar{X} - \bar{X}\rvert$ 20° 25°	$\lvert\bar{X} - \bar{X}\rvert$ 25° 30°	$\lvert\bar{X} - \bar{X}\rvert$ 30° 35°
Treatment	5	494.8456	98.9691	123.00	0.0001	0.4439	S	S	NS	S	S
Error	6	4.8276	0.8046								

F_0 - observed test statistic for analysis of variance = $\frac{SS\ (Treatment)/DF}{SS\ (Error)/DF}$, using Experiment Replication (Treatment), i.e. Run (Temperature), as an error term

t_o - observed test statistic for L.S.D. multiple comparison procedure = $t_{\frac{\alpha}{2},\ df\ (MSE)}\sqrt{\frac{2MSE}{n_i}}$

S - significant treatment difference between paired mean values at the 0.05 level

NS - no significant treatment difference between paired mean values at the 0.05 level

be localized or translocated, eg. irradiation of a restricted part of the thalli of Coprinus lagopus (137), Pestalotia theae (75), and Physalospora obtusa (Schw.) Cke. (64) resulted in the production of reproductive structures only in areas directly or indirectly illuminated with the rest of the mycelium remaining vegetative, while narrow beam illumination of restricted areas of Trichoderma viride thalli indicated "some kind of joint perception of photoinduction" among adjacent mycelial fields (67) and a hypothesized "photoactivated sporulation precursor" responsible for the zone of Ascochyta pisi mycelium sensitive to radiation was presumed to move over short distances from irradiated to nonirradiated, newly formed mycelium (117).

5. The effect of moisture on sporulation.

During this study it was observed that the presence of a moisture film over the surface of thalli at the time of irradiation often resulted in a marked variation in the sporulation intensity between these and non-moistened thalli in various replicate sets. To investigate whether such variation could be due to the presence of a moisture film, four replicate cultures, established and incubated in the prescribed manner, were either flooded with 6.0 ml of sterile distilled water and drained prior to irradiation, or left dry. Following exposure to white fluorescent light (12.0 Wm^{-2}) for 24 hours, cultures were incubated in darkness for a further 5 days and then examined quantitatively for spore production.

Table 10 shows that the intensity of the sporulation response was affected by surface moisture. A significant inhibition occurred

Table 10

Analysis of Variance for the Effect of Surface Moisture on Photoinduced Sporulation in Cylindrocarpon sp.

Treatment	Replicate	Counts (No. Spores/Thallus) 1	2	3	4
Control	1	1 476 448	1 460 368	1 457 248	1 469 728
	2	1 551 008	1 565 088	1 531 728	1 551 248
Test	1	1 194 048	1 185 168	1 192 848	1 206 288
	2	1 195 328	1 165 008	1 196 368	1 203 568

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	F	
Treatment	1	0.2198	0.2198	70.80	18.51	S
Error	2	0.0062	0.0031			

F_o - observed test statistic = $\frac{SS\ (Treatment)/DF}{SS\ (Error)/DF}$, using Replicate (Treatment) as an error term

F - test statistic, 5 percent points

S - significant treatment difference at the 0.05 level

in those cultures treated with distilled water.

6. The effect of light quality and length of exposure on sporulation.

Preliminary tests with Vita-Lite fluorescent lamps indicated that radiation in the visible or near ultraviolet regions of the electromagnetic spectrum was responsible for inducing sporogenesis in Cylindrocarpon sp. Using monochromatic filters, the effectiveness of various wavelengths of the fluorescent light spectrum during 12, 24, and 48 hour photoperiods was investigated in two experiments in which radiant energies of the filtered and unfiltered light sources were either unadjusted or equalized at 0.1 Wm^{-2}. As sporulation had been shown to vary with intensity under the full fluorescent light spectrum, it was necessary, in order to make valid comparisons between results, to investigate the effect of a standard level of incident light energy, regardless of the different wavelengths to be tested. This was accomplished by adjusting the irradiance at the level of the culture surface to 0.1 Wm^{-2}, a value equivalent to the highest obtainable photometer reading through the densest filter.

The radiation sources as well as the transmittance of the filters employed are shown in Table 11.

In this experiment cultures were grown in light sealed incubators at 25± 0.5° C for twelve days. Test plates were then exposed, at 25± 1.0° C, for periods of either 12, 24, or 48 hours to the transmitted radiation of either 5 Vita-Lite fluorescent lamps, 5 Sylvania F20T12-BLB lamps, #450 Blue monochromatic filters, #545

Table 11

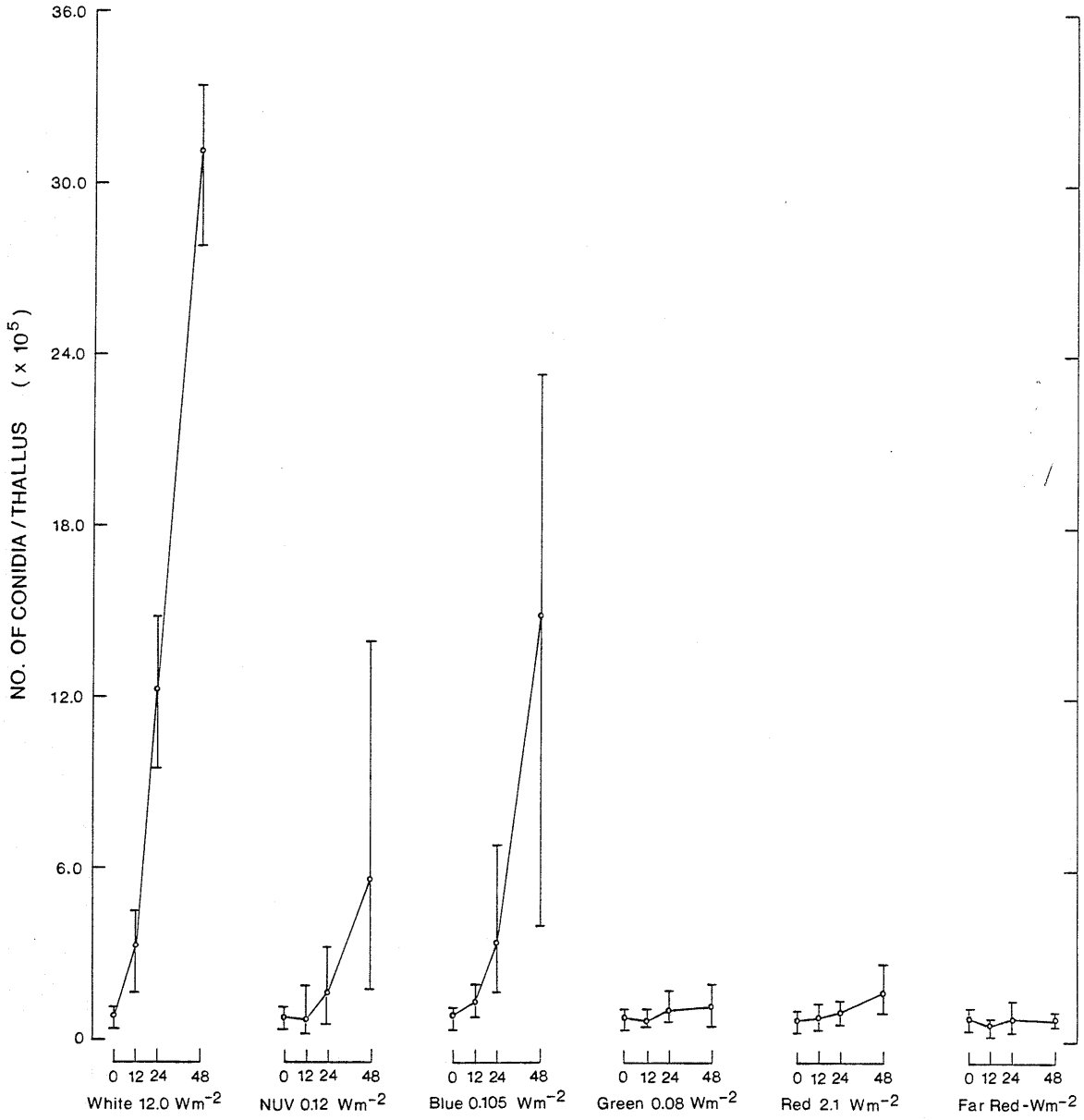
Light Sources, The Filters Used, and Their Transmittance Values

Light Source	Carolina Monochromatic Filters	Transmittance (nm)	
Near Ultraviolet (5 Sylvania Black Light Blue F20T12-BLB Lamps)	— — —	430-490	356.0 Peak
White (Duro-Test 20 Watt Vita-Lite Fluorescent Lamps)	#450 Blue	430-490	455.0 Peak
	#545 Green	520-580	545.0 Peak
	#650 Red	600-690	650.0 Peak
Infrared (2, General Electric 100 Watt Incandescent Bulbs)	#750 Far Red	700 and above	~850.0 Peak

Green monochromatic filters, #650 Red monochromatic filters, or #750 Far Red monochromatic filters. Following irradiation cultures were returned to darkness. Sporulation response was qualitatively and quantitatively assessed five days later.

Results obtained from the exposures to radiation of known wavelengths at dissimilar irradiance levels, ie. white (12.0 Wm^{-2}), NUV (0.12 Wm^{-2}), blue (0.105 Wm^{-2}), green (0.08 Wm^{-2}), red (2.1 Wm^{-2}), and far red (not measureable with sensors available), are presented in Figure 15. The only cultures in which appreciable sporulation occurred were those that received Vita-Lite fluorescent radiation (290 nm to 770 nm) and light of wavelengths 430 nm to 490 nm (#450 Blue filter) and 300 nm to 400 nm (Sylvania F20T12-BLB fluorescent radiation). However, at the applied intensities, neither blue nor NUV were as effective as white light during comparable photoperiods, although blue was more effective than NUV. Far red radiation (#750 Far Red filter) had no measureable affect on sporulation: over the complete range of exposures, counts were similar to those of controls which had been maintained in complete darkness. Cultures irradiated under the transmitted green light (#545 Green filter) and red light (#650 Red filter) produced a modest number of conidia. However, at these wavelengths, i.e. 520 nm to 580 nm and 600 nm to 690 nm, and at the wavelengths emitted by black light blue fluorescent lamps a longer exposure was necessary to induce sporulation than was required with white light and wavelengths at the blue end of the visible spectrum.

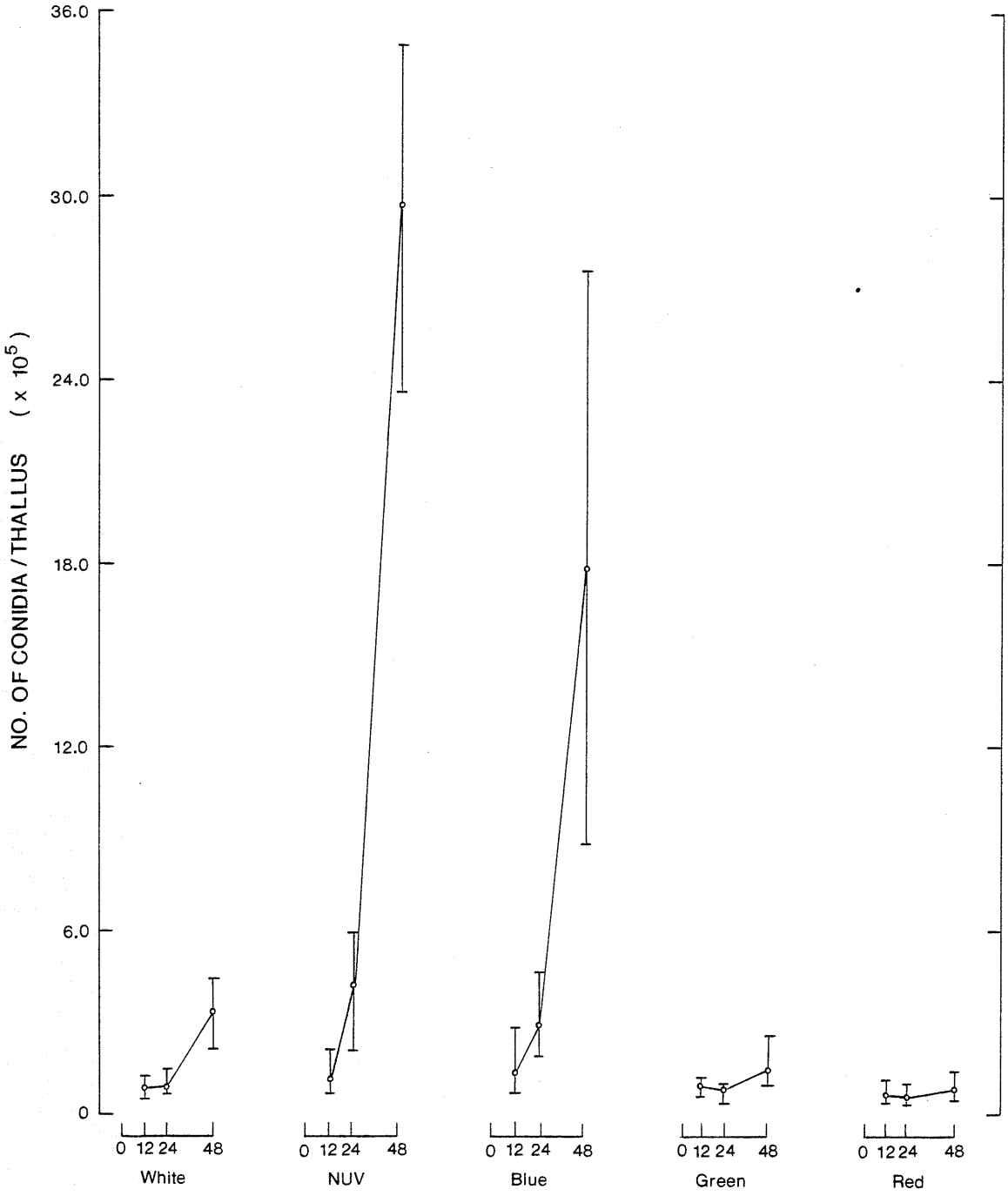
Figure 15. Effect of polychromatic and monochromatic radiation (of dissimilar photon flux density) and exposure period on conidium production in 12-day-old, dark-reared cultures of Cylindrocarpon sp. incubated at 25± 1.0° C. Each point represents the mean spore yield of 12 replicate cultures, 4 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.



HOURS OF EXPOSURE TO VARIOUS REGIONS OF THE ELECTROMAGNETIC SPECTRUM

Results of experiments with the photon flux density of the different light sources equalized at 0.10 Wm^{-2} show that the capacity to induce sporulation was confined to the NUV and blue wavelengths of the electromagnetic spectrum (Figure 16, Table 12). There was no significant difference in mean conidium yield between cultures receiving light of wavelengths 430 nm to 409 nm (#450 Blue filter) and those illuminated with Sylvania T20T12-BLB lamps at comparable photoperiods over the complete range of exposures. Radiation of wavelengths longer than 520 nm had no appreciable affect on sporulation. However, thalli treated with transmitted green light formed a ring of tightly interwoven hyphae in the peripheral region normally occupied by the ring of conidiophores and confluent sporodochia. Under red light, thalli exposed for 12 and 24 hours were not perceptibly different from those of controls maintained in complete darkness, but cultures treated for 48 hours produced a faint zone similar to the abnormal growth described for mycelium irradiated with green light. This morphological response of thalli to electromagnetic energy in the green region of the spectrum and relatively long exposures to electromagnetic energy in the red region of the spectrum suggests that these wavelengths may be affecting physiological reactions which ultimately lead to the differentiation of vegetative hyphae and the production of conidiophores. Since the initiation of asexual reproduction and the genesis of the conidiophore and conidium involve complex, multi-step processes, such an influence would certainly be feasible. However, it is unlikely that green and red radiations are either detected by the

Figure 16. Effect of polychromatic and monochromatic radiation (photon flux density 0.10 Wm^{-2}) and exposure period on conidium production in 12-day-old, dark-reared cultures of Cylindrocarpon sp. incubated at 25± 1.0° C. Each point represents the mean spore yield of 12 replicate cultures, 4 counts per culture. Vertical lines with limits denote vatiation among experimental units of similar treatment.



HOURS OF EXPOSURE TO VARIOUS REGIONS OF THE ELECTROMAGNETIC SPECTRUM (PHOTON FLUX DENSITY = 0.10 Wm^{-2})

Table 12

Analysis of Variance and Multiple Comparison (L.S.D.) Tests for the Effect of Polychromatic and Monochromatic Radiation ($0.10\ Wm^{-2}$) and Exposure Period on Photoinduced Sporulation

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	F	t_o
Treatment (12 Hour)	4	11.8400	2.9600	1.44	5.1922	*
Error	5	10.2570	2.0514			
Treatment (24 Hour)	4	131.9241	32.9810	21.87	5.1922	0.6445
Error	5	7.5412	1.5082			
Treatment	4	480.4704	120.1176	50.08	5.1992	0.8128
Error	5	11.9923	2.3984			

	$\lvert\bar{X}$ White $-\bar{X}\rvert$ NUV	$\lvert\bar{X}$ White $-\bar{X}\rvert$ Blue	$\lvert\bar{X}$ White $-\bar{X}\rvert$ Green	$\lvert\bar{X}$ White $-\bar{X}\rvert$ Red	$\lvert\bar{X}$ NUV $-\bar{X}\rvert$ Blue
12 Hr.	—	—	—	—	—
24 Hr.	S	S	NS	NS	NS
48 Hr.	S	S	S	S	NS

	$\lvert\bar{X}$ NUV $-\bar{X}\rvert$ Green	$\lvert\bar{X}$ NUV $-\bar{X}\rvert$ Red	$\lvert\bar{X}$ Blue $-\bar{X}\rvert$ Green	$\lvert\bar{X}$ Blue $-\bar{X}\rvert$ Red	$\lvert\bar{X}$ Green $-\bar{X}\rvert$ Red
12 Hr.	—	—	—	—	—
24 Hr.	S	S	S	S	NS
48 Hr.	S	S	S	S	NS

F - test statistic, 5 percent points

* - no significant treatment difference at the 0.05 level

F_o - observed test statistic for analysis of variance = $\frac{SS\ (Treatment)/DF}{SS\ (Error)/DF}$, using Experiment Replication (Treatment), i.e. Run (Wavelength), as an error term

t_o - observed test statistic for L.S.D. multiple comparison procedure = $t_{\frac{\alpha}{2},\ df\ (MSE)}\sqrt{\frac{2MSE}{n_i}}$

S - significant treatment difference between paired mean values at the 0.05 level (NS= no significant treatment difference . . .0.05 level)

NUV-blue photoreceptor or responsible for the generation of a comparable NUV-blue primary photoproduct, since their reception results only in the proliferation of hyphae, not the formation of conidia.

It should also be noted that there was no significant difference in the effectiveness of the various wavelengths tested in activating the asexual reproductive process during the 12 hour irradiation period. In contrast with the minimum effective exposure of 12 hours to Vita-Lite fluorescent radiation at 12.0 Wm^{-2}, these results suggest that a longer period of illumination was necessary to induce sporogenesis at this particular level of irradiance.

7. Spectrophotometric analysis of living mycelium.

The objective of this investigation was to determine whether there were any NUV-blue absorbing compounds in living, undamaged mycelium, and to dectet absorbance differences, in the range 200 nm to 450 nm, which might exist between mature and actively growing hyphae, and induced and noninduced peripheral mycelium.

In repeated experiments with cultures grown directly on MRM and MRM overlaid with dialysis tubing, the difference spectra obtained by comparing the light absorption of irradiated against dark-grown mycelium, old against young hyphae, and treated tissue against its MRM or dialysis tubing substrate were not sufficiently different from spectra obtained by comparing substrate (sample cell) against substrate (reference cell) to draw any conclusions. Since Leach (121) was able to obtain measurable differences between the irradiated and nonirradiated mycelia of Pyronema omphalodes (Bull.

ex St. Amans) Fuckel and Ascochyta pisi using a quartz plate-cellophane method as opposed to a filter paper method, the ineffectiveness of both the dialysis tubing and agar gel modifications of these procedures employed in this experiment suggests that the method itself was not sensitive enough to detect differences in the light absorption of Cylindrocarpon sp. mycelia. This situation may have arisen as a consequence of either the reflection and scattering of radiation or the high optical densities encountered and, thus, the relative sensitivity of the Unicam UV Spectrophotometer utilized.

8. The effect of intensity of near ultraviolet radiation on sporulation.

Since NUV light was active in the induction of sporulation, the response of Cylindrocarpon sp. to different irradiance levels of NUV light was studied. Twelve-day-old, dark grown cultures were exposed for 24 hours to the radiation of Sylvania F20T12-BLB fluorescent lamps at an irradiation intensity of either 0.02 Wm^{-2}, 0.04 Wm^{-2}, 0.06 Wm^{-2}, 0.08 Wm^{-2}, 0.10 Wm^{-2}, or 0.12 Wm^{-2} and then returned to darkness. Five days later, the response of each culture was measured quantitatively.

Mean yields of these treatments show that the photoreceptive mechanism operative in this organism is sensitive to very low levels of photon flux density (Figure 17). An irradiance of 0.02 Wm^{-2} was sufficient to induce the asexual reproductive process; irradiation intensities of 0.04 Wm^{-2} and 0.06 Wm^{-2} had no significant further effect (Table 13). The data also suggest that a depressive

Figure 17. Effect of irradiance level on conidium production in 12-day-old, dark-reared cultures of Cylindrocarpon sp. exposed to 24 hours of Sylvania F20T12-BLB fluorescent radiation (0.12 Wm^{-2}) at 25± 1.0° C. Each point represents the mean spore yield of 12 replicate cultures, 4 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.

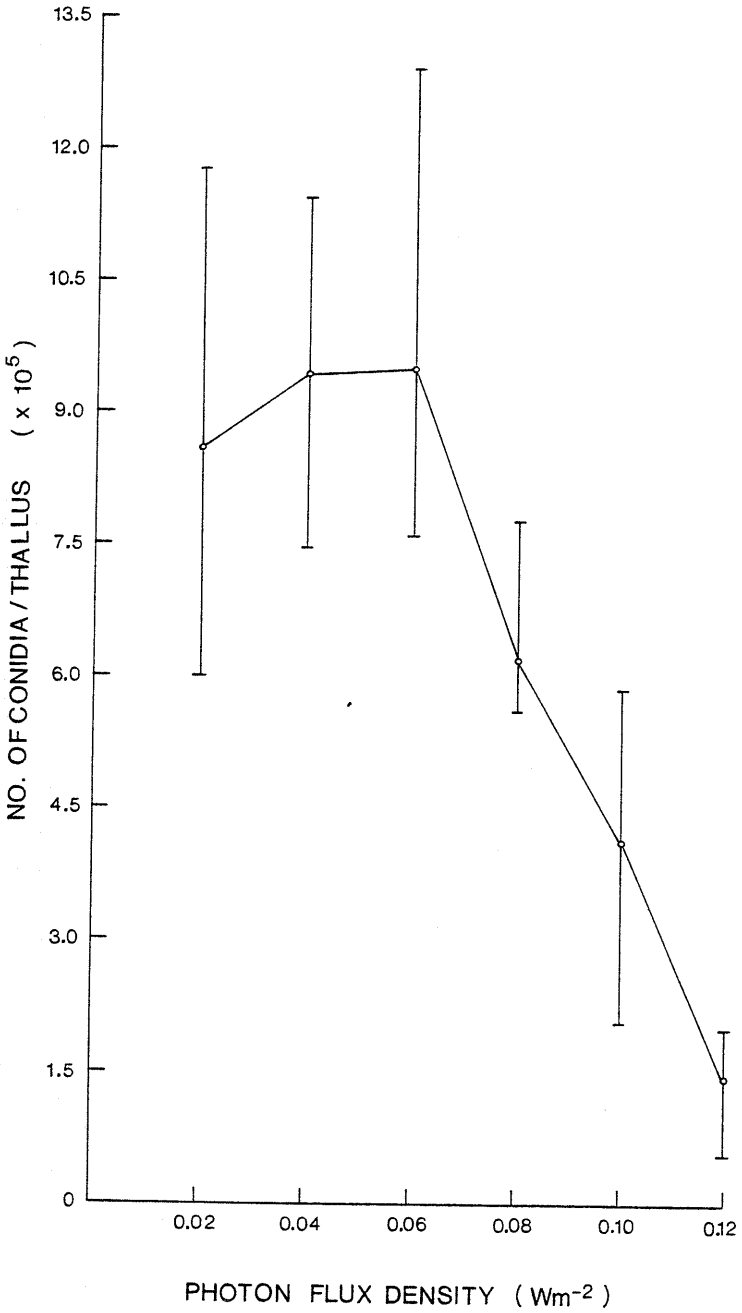

13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0
NO. OF CONIDIA / THALLUS (x 10^5)
0.02
0.04
0.06
0.08
0.10
0.12
PHOTON FLUX DENSITY (Wm^-2)

Table 13

Analysis of Variance and Multiple Comparison (L.S.D.) Tests for the Effect of Photon Flux Density on Photoinduced Sporulation

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o
Treatment	5	101.3151	20.2630	38.40	0.0002	0.4402
Error	6	3.1663	0.5277			

$\lvert\bar{X} - \bar{X}\rvert$		$\lvert\bar{X} - \bar{X}\rvert$		$\lvert\bar{X} - \bar{X}\rvert$		$\lvert\bar{X} - \bar{X}\rvert$		$\lvert\bar{X} - \bar{X}\rvert$		$\lvert\bar{X} - \bar{X}\rvert$	
0.02	0.04	0.04	0.06	0.06	0.08	0.06	0.10	0.08	0.10	0.10	0.12
NS		NS		NS		S		NS		S	

F_o - observed test statistic for analysis of variance = $\frac{\text{SS (Treatment)/DF}}{\text{SS (Error)/DF}}$, using Experiment Replication (Treatment), i.e. Run (Photon Flux Density), as an error term

t_o - observed test statistic for L.S.D. multiple comparison procedure = $t_{\frac{\alpha}{2},\ df\ (MSE)}\sqrt{\frac{2MSE}{n_i}}$

S - significant treatment difference between paired mean values at the 0.05 level

NS - no significant treatment difference between paired mean values at the 0.05 level

effect on sporulation occurred at irradiance levels of 0.08 Wm^{-2}, 0.10 Wm^{-2}, and 0.12 Wm^{-2}. However, the inhibitive effect at 0.08 Wm^{-2} was not significant. Thus, it would appear that inhibition only started at an intensity above 0.08 Wm^{-2}.

9. The effect of near ultraviolet radiation and white fluorescent light on radial growth.

A commonly observed phenomenon in cultures exposed to white fluorescent light at an irradiance of 12.0 Wm^{-2} and NUV at an irradiance of 0.12 Wm^{-2} was that actively growing hyphal tips penetrated the medium during the irradiation period resulting in a zone outside the region of sporulation in which there was scarcely any mycelium on the agar surface. In view of this behavior, comparisons were made between growth under white fluorescent light, black light blue fluorescent light, and in the absence of light.

MRM plates inoculated with a single germinated conidium were incubated at 25± 1.0° C in darkness as well as in chambers illuminated with either 5 Sylvania F20T12-BLB lamps (0.12 Wm^{-2}) or 5 Duro-Test Vita-Lite fluorescent lamps (12.0 Wm^{-2}). Treatments were replicated three times, and the diameter measurements of individual thalli were recorded at 24 hour intervals for the duration of the experiment. It was found that radial growth was delayed in white light and completely inhibited by NUV radiation (Figure 18).

In a subsequent experiment, test plates were placed in darkness following exposures of 48 and 96 hours to Sylvania F20T12-BLB fluorescent light (0.12 Wm^{-2}). Results presented in Figure 19

Figure 18. Effect of irradiation with 5 Vita-Lite white fluorescent lamps (12.0 Wm^{-2}) and irradiation with 5 Sylvania F20T12-Black-Light-Blue fluorescent lamps (0.12 Wm^{-2}) and darkness upon radial growth of freshly inoculated cultures of Cylindrocarpon sp. incubated at 25± 1.0° C. Each point represents the mean of measurements on 3 replicate cultures, 2 measurements per thallus.

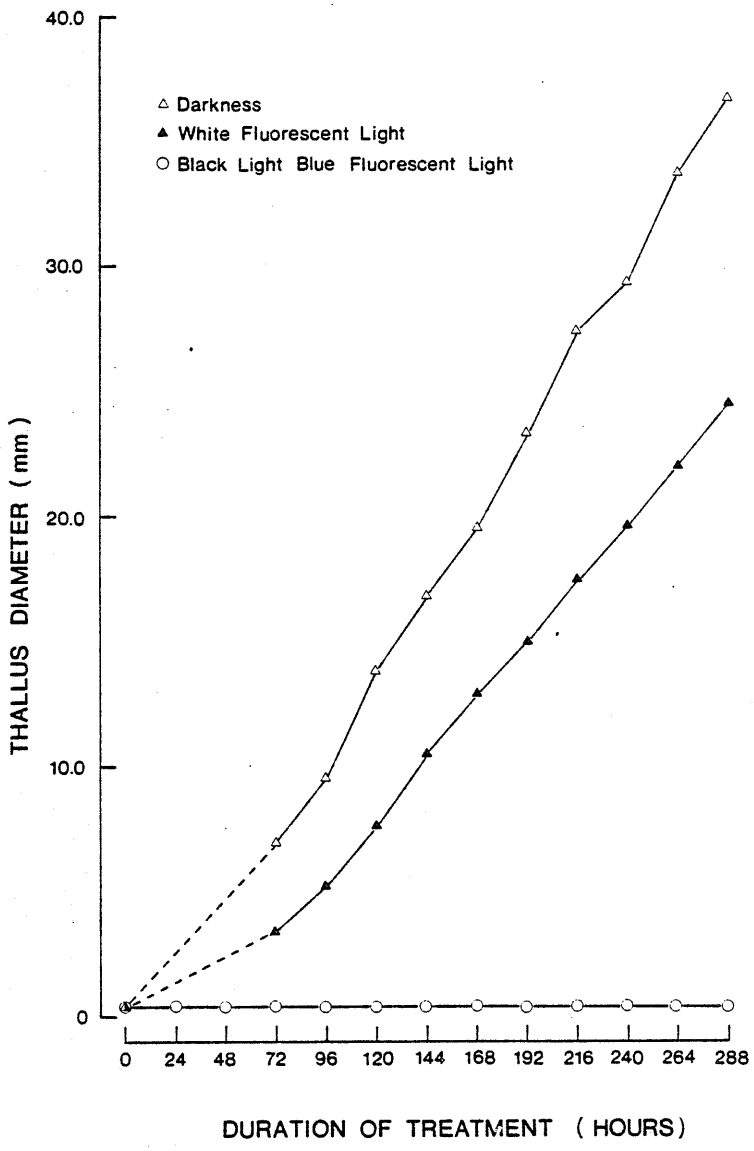
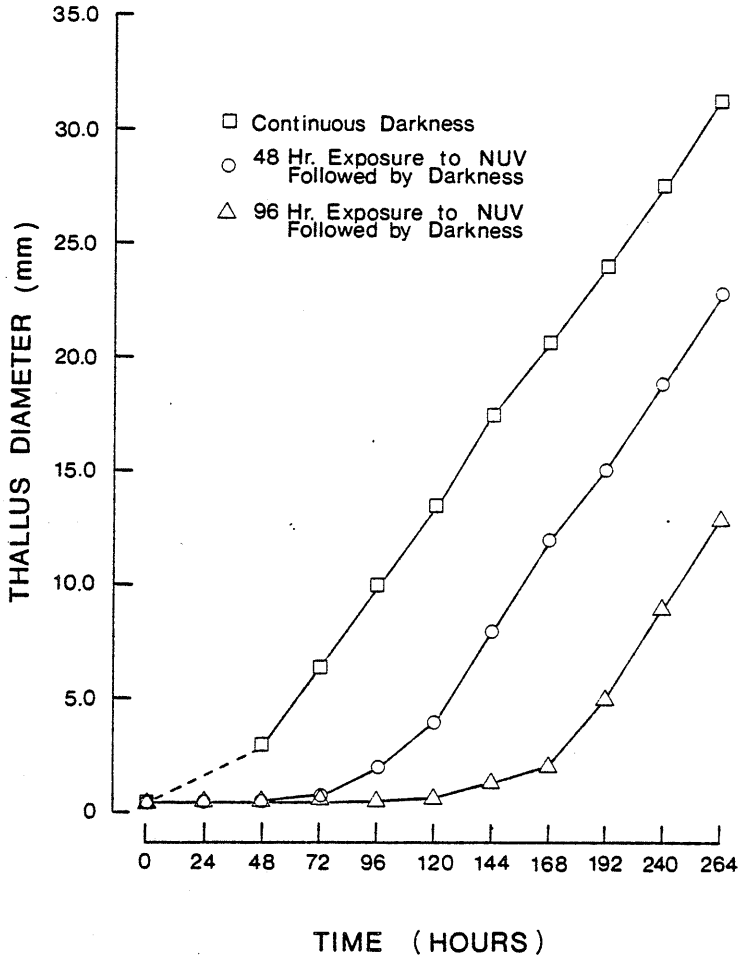

40.0
30.0
20.0
10.0
0
△ Darkness
▲ White Fluorescent Light
○ Black Light Blue Fluorescent Light
THALLUS DIAMETER (mm)
0 24 48 72 96 120 144 168 192 216 240 264 288
DURATION OF TREATMENT (HOURS)

Figure 19. Effect of near ultraviolet (NUV) radiation on subsequent radial growth under darkness in cultures of Cylindrocarpon sp. exposed at 25± 1.0° C to 48 and 96 hours of Sylvania F20T12-BLB fluorescent radiation (0.12 Wm^{-2}) immediately after inoculation. Each point represents the mean of measurements on 3 replicate cultures, 2 measurements per thallus.


35.0
30.0
25.0
20.0
15.0
10.0
5.0
0
THALLUS DIAMETER (mm)
Continuous Darkness
48 Hr. Exposure to NUV Followed by Darkness
96 Hr. Exposure to NUV Followed by Darkness
0
24
48
72
96
120
144
168
192
240
264
TIME (HOURS)

show that NUV radiation was not lethal to hyphae emanating from the germinated spores, but that growth had been checked and then rendered dormant. Upon transfer to darkness, the cultures ultimately recovered and equaled the control in growth rate.

Figure 20 gives the results of a similar experiment in which plate cultures were incubated in the absence of light for various periods prior to irradiation. Again growth rate was affected, but the inhibitive effect was considerably less in cultures preconditioned in darkness for periods of 3 to 9 days than in those exposed on Day 1 and Day 2.

D. THE ROLE OF NUCLEIC ACID AND PROTEIN SYNTHESIS IN INDUCED MORPHOGENESIS.

In this series of experiments an attempt was made to acquire some information on the possible biochemical basis of light-induced conidiation in Cylindrocarpon sp. Eight antimetabolites known to have specific biochemical effects on the synthesis of DNA, RNA, and protein in other eukaryotic systems were used to investigate at which stage, i.e. replication, translation, or transcription, light affects sporogenesis (Figure 21). The mechanism by which wavelengths in the NUV-blue region of the electromagnetic spectrum regulate the processes leading to differentiation was not studied.

Figure 20. Effect of interrupting light periods on radial growth in 1, 2, 3, 4, 5, 6, and 7-day-old, dark-reared cultures of Cylindrocarpon sp. exposed at 25± 1.0° C to Sylvania F20T12-BLB fluorescent radiation (0.12 Wm^{-2}) for 8, 7, 6, 5, 4, 3, and 2 days, respectively. Each point represents the mean of measurements on 3 replicate cultures, 2 measurements per thallus.

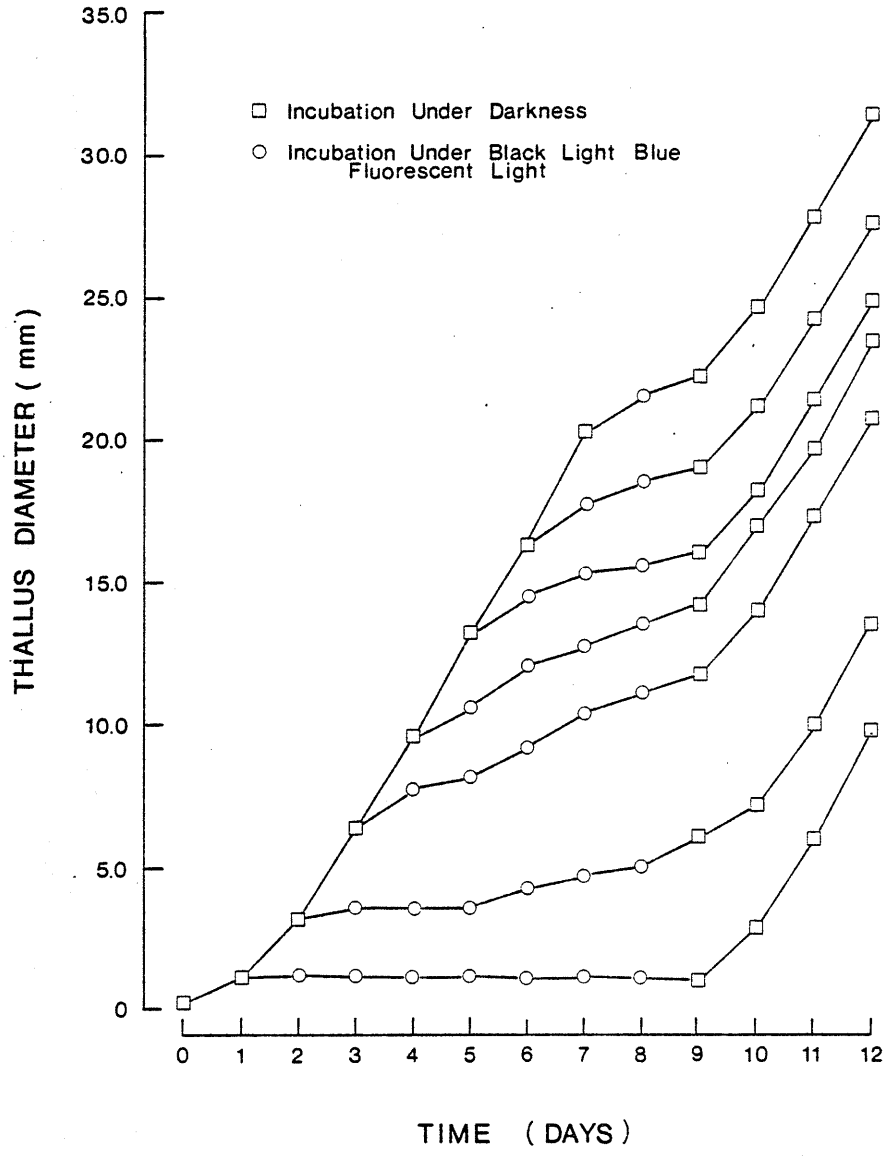
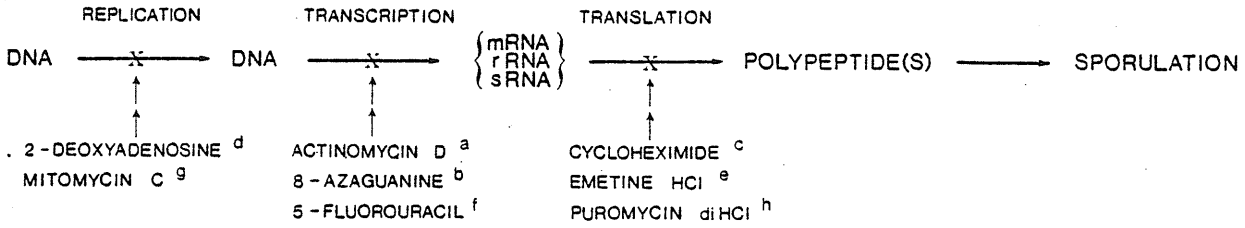

35.0
30.0
25.0
20.0
15.0
10.0
5.0
0
THALLUS DIAMETER (mm)
Incubation Under Darkness
Incubation Under Black Light Blue Fluorescent Light
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME (DAYS)

Figure 21. Site and mode of inhibition by actinomycin D, 8-azaguanine, cycloheximide, 2'-deoxyadenosine, emetine HCl, 5-fluorouracil, mitomycin C, and puromycin diHCl.



a. Actinomycin D selectively inhibits DNA dependent RNA synthesis by intercalating into a duplex at a guanine-cytosine base pair and binding, noncovalently, to the deoxyguanosine residue in such a fashion as to interfere with translocation of RNA polymerase down a guanine containing template. This interference ultimately leads to a marked decrease in the average chain length of RNA products. As a consequence of complex formation with double helical DNA, DNA dependent DNA synthetis with DNA polymerase I enzyme is also affected by actinomycin D, but to a lesser extent (69, 103, 107, 130, 138, 155, 205).

b. 8-Azaguanine is a purine analogue whose biological effect is due to its action as an antimetabolite in nucleic acid metabolism after its incorporation into DNA and RNA in place of guanine (107, 138, 141).

c. Cycloheximide inhibits the synthesis of proteins in the 80S ribosomes of eukaryotes. Although little is known about its site and mode of action, cycloheximide has been postulated as an inhibitor of (1) polysome breakdown and reassembly; (2) ribosome subunit reassociation; (3) transfer of aminoacyl-sRNA to ribosomes; (4) peptidyl-tRNA translocation; (5) peptide bond formation; (6) release of deacylated tRNA from the ribosome; and (7) chain termination. Apart from protein synthesis, cycloheximide has also been found to inhibit RNA polymerase I in some fungi as well as the synthesis of DNA and RNA in certain intact cells (39, 107, 130, 138, 155, 170, 228).

d. 2-Deoxyadenosine is an inhibitor of DNA synthesis whose effect, whether partially or exclusively indirect, is correlated to the accumulation of deoxy-ATP which interferes with the mechanism for regulation of deoxyribonucleotide biosynthesis, ie. deoxy-ATP acts as a feedback inhibitor for the reduction of all ribonucleoside 5' diphosphates (106, 114, 130, 161, 229).

e. Emetine HCl inhibits protein synthesis by 80S type ribosomes and has been observed to inhibit the synthesis of DNA in HeLa cells. Although its exact site and mode of action have not been determined, emetine HCl has been reported to inhibit binding of aminoacyl-tRNA as well as the aminoacyl-sRNA transfer reaction (74, 130, 170, 228).

f. 5-Fluorouracil is a synthetic analogue which is incorporated into both soluble and ribosomal ribonucleic acids in place of uracil. It is thought to interfere with new protein synthesis through miscoding and by decreasing the availability of functional ribosomes. Metabolized by-products of 5-fluorouracil, eg. fluorodeoxyuridylate, are also known to inhibit thymidylate synthetase, thus blocking deoxyribonucleic acid synthesis (20, 53, 68, 107, 143).

g. Mitomycin C has been reported to inhibit the synthesis of RNA, to induce the progressive and extensive degradation of DNA to acid soluble fragments, and to inhibit DNA synthesis by alkylating and cross-linking guanine residues on opposing helices so that separation of the two strands of the DNA molecule during the replication process is prevented (103, 107, 138, 155, 205).

h. Puromycin is a structural analogue of the aminoacyl adenylyl portion of aminoacyl-tRNA, ie. it lacks the normal ester linkage between the 2'- or 3'- hydroxyl group of the ribose moiety and the carboxyl group of the amino acid. This obvious, but altered, structural resemblance enables puromycin to compete for the A-site on 50S subunits of ribosomes, to accept nascent peptides from peptidyl tRNAs bound to ribosomal P-sites, and to cause the premature release of incomplete polypeptide chains (39, 130, 138, 170, 228).

Monoconidial cultures of the fungus initiated on 0.2 um and 5.0 um membrane filters were subjected to treatment with actinomycin D, 8-azaguanine, cycloheximide, 2'-deoxyadenosine, emetine HCl, 5-fluorouracil, mitomycin C, and puromycin diHCl during a 24 hour exposure to Sylvania F48T12/VHO Cool White fluorescent radiation (12.0 Wm^{-2}). Following irradiation, membrane cultures were transferred to Petri dishes containing MRM and returned to darkness. Conidiation was quantitatively assessed five days after the termination of the irradiation period by counting spore yields. The effects of inhibitors on vegetative growth were recorded concurrently with observations on sporulation by measuring the diameter of thalli. The procedures for inoculation, incubation, and irradiation have been described in the methodology section.

Actinomycin D concentrations of 10^{-3} M, 10^{-4} M, $5x10^{-5}$ M, 10^{-5} M, and $5x10^{-6}$ M were tested (Figure 22). When thalli were grown on 0.2 um membrane filters over MRM, actinomycin D at 10^{-3} M significantly reduced the amount of sporulation as compared with all lower concentrations and untreated controls (Table 14). However, no inhibition of vegetative growth was observed at this or any of the other concentrations tested (Table 15). In contrast, concentrations of 10^{-4} M and 10^{-5} M significantly decreased photoinduced conidiation in 5.0 um membrane cultures, and complete inhibition was observed at 10^{-3} M. Radial growth in cultures treated with 10^{-3} M actinomycin D was also less than that which occurred at all lower concentrations and in control plates (P=0.05).

Figure 22. Effect of actinomycin D on radial growth and photoinduced conidiation in 12-day-old, dark-reared, 0.2 um and 5.0 um membrane filter cultures of Cylindrocarpon sp. exposed to 24 hours of Sylvania F48T12/VHO Cool White fluorescent radiation at 25± 1.0° C (actinomycin D applied for 24.5 hours beginning 15 minutes before transfer to illuminated chambers and ending 15 minutes after transfer to fresh MRM and darkness). Each point representing thallus diameter is the mean of measurements on 12 replicate cultures, 2 measurements per thallus. Each point representing conidium production is the Log_{10} mean spore yield of 12 replicate cultures, 5 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.

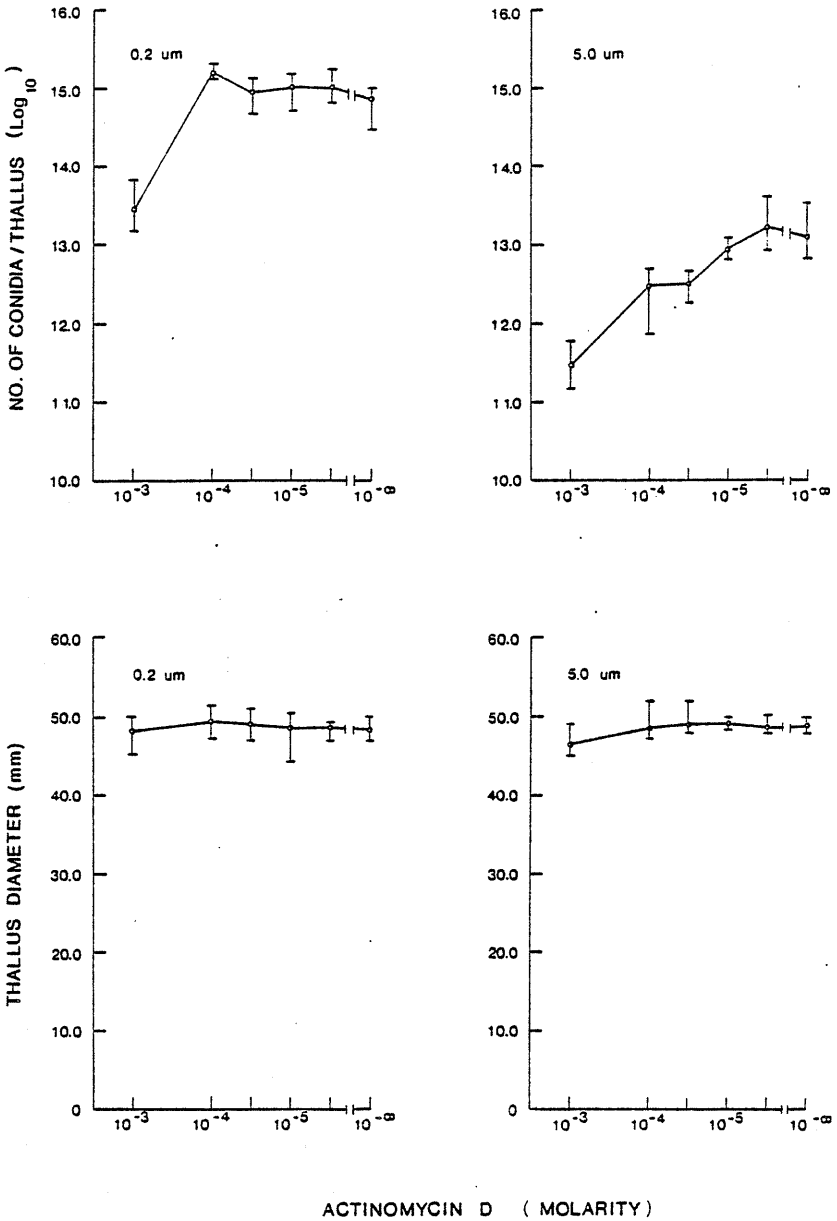

NO. OF CONIDIA / THALLUS (Log 10)
0.2 um
5.0 um
16.0
15.0
14.0
13.0
12.0
11.0
10.0
THALLUS DIAMETER (mm)
60.0
50.0
40.0
30.0
20.0
10.0
0
10-3
10-4
10-5
10-∞
ACTINOMYCIN D (MOLARITY)

Table 14

Analysis of Variance and Multiple Comparison (L.S.D.) Tests for the Effect of Metabolic Inhibitors on Photoinduced Sporulation in 12-Day-Old, Dark-Reared, 0.2 um and 5.0 um Membrane Filter Cultures of Cylindrocarpon sp.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o	$\lvert\bar{X} - \bar{X}\rvert$ 10^{-1}	10^{-2}	10^{-2}	10^{-3}	10^{-3}	10^{-4}	10^{-4}	10^{-5}	10^{-5}	$10^{-\infty}$	10^{-1}	$10^{-\infty}$
(8-Azag) 0.2 um	5	119.8555	23.9711	148.84	0.0001	0.1773		S		S		S		NS				S
Error	24	3.8652	0.1610															
(8-Azag) 5.0 um	5	17.3212	3.4642	11.49	0.0001	0.2425		NS		NS		S		NS				S
Error	24	7.2329	0.3414															
(Cycloh) 0.2 um	5	301.0127	60.2025	109.71	0.0001	0.3272		NS		S		S		S		N		S
Error	24	13.1696	0.5484															
(Cycloh) 5.0 um	5	81.3068	16.2614	59.18	0.0001	0.2324		S		NS		NS		S		N		S
Error	24	6.0447	0.2748															
(2-Dxad) 0.2 um	5	2.9539	0.5907	5.26	0.0021	0.1480		NS		NS		NS		NS				NS
Error	24	2.6963	0.1123															
(2-Dxad) 5.0 um	5	2.3405	0.4681	2.81	0.0389	0.1802		NS		NS		S		NS		NS		NS
Error	24	3.9949	0.1664															
(Emetin) 0.2 um	5	65.8733	13.1747	20.82	0.0001	0.3514		NS		S		NS		NS		N		S
Error	24	15.1897	0.6329															
(Emetin) 5.0 um	4	13.6508	3.4127	24.05	0.0001	0.1678		-		S		S		NS		N		-
Error	19	2.6853	0.1413															
(5-Fluo) 0.2 um	5	289.2658	57.8532	230.16	0.0001	0.2215		S		NS		NS		S		S		S
Error	24	6.0327	0.2514															
(5-Fluo) 5.0 um	5	115.0284	23.0057	48.30	0.0001	0.3048		NS		NS		S		NS		S		S
Error	24	11.4302	0.4762															

							$\lvert\bar{X} - \bar{X}\rvert$ 10^{-3}	$5x10^{-4}$	$5x10^{-4}$	10^{-4}	10^{-4}	$5x10^{-5}$	$5x10^{-5}$	10^{-5}	10^{-5}	$10^{-\infty}$	10^{-3}	$10^{-\infty}$
(Mito C) 0.2 um	5	1.7384	0.3477	1.22	0.3319	0.2362		NS		NS		NS		S		NS		NS
Error	24	6.8622	0.2859															
(Mito C) 5.0 um	5	2.7532	0.5506	2.72	0.0451	0.2025		NS		NS		NS		NS		NS		NS
Error	24	4.8144	0.2093															
(Puromy) 0.2 um	5	1.6978	0.3396	3.20	0.0236	0.1439		NS		NS		NS		NS		S		S
Error	24	2.5455	0.1061															
(Puromy) 5.0 um	5	3.7346	0.7469	1.56	0.2085	0.3054		NS		NS		NS		NS		NS		S
Error	24	11.4716	0.4780															

							$\lvert\bar{X} - \bar{X}\rvert$ 10^{-3}	10^{-4}	10^{-4}	$5x10^{-5}$	$5x10^{-5}$	10^{-5}	10^{-5}	$5x10^{-6}$	$5x10^{-6}$	$10^{-\infty}$	10^{-3}	$10^{-\infty}$
(Actm D) 0.2 um	5	65.0242	13.0048	91.64	0.0001	0.1664		S		NS		NS		NS		NS		S
Error	24	3.4059	0.1419															
(Actm D) 5.0 um	5	62.3908	12.4782	77.72	0.0001	0.1770		S		NS		S		S		NS		S
Error	24	3.8535	0.1606															

F_o - observed test statistic for analysis of variance = $\frac{SS\ (Treatment)/DF}{SS(Error)/DF}$, using Replication (Concentration*Chamber) as an error term

t_o - observed test statistic for L.S.D. multiple comparison procedure = $t_{\frac{\alpha}{2},\ df\ (MSE)} \sqrt{\frac{2MSE}{n_1}}$

S - significant treatment difference between paired mean values at the 0.05 level

NS - no significant treatment difference between paired mean values at the 0.05 level

8-Azag = 8-Azaguanine, Cycloh = Cycloheximide, 2-Dxad = 2-Deoxyadenosine, Emetin = Emetine HCl, 5-Fluo = 5-Fluorouracil, Mito C = Mitomycin C, Puromy = Puromycin diHCl, Actm D = Actinomycin D

Table 15

Analysis of Variance and Multiple Comparison (L.S.D.) Tests for the Effect of Metabolic Inhibitors on Radial Growth in 12-Day-Old, Dark-Reared, 0.2 um and 5.0 um Membrane Filter Cultures of Cylindrocarpon sp.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o	$\lvert\bar{X}_{10^{-1}} - \bar{X}_{10^{-2}}\rvert$	$\lvert\bar{X}_{10^{-2}} - \bar{X}_{10^{-3}}\rvert$	$\lvert\bar{X}_{10^{-3}} - \bar{X}_{10^{-4}}\rvert$	$\lvert\bar{X}_{10^{-4}} - \bar{X}_{10^{-5}}\rvert$	$\lvert\bar{X}_{10^{-5}} - \bar{X}_{10^{-\infty}}\rvert$	$\lvert\bar{X}_{10^{-1}} - \bar{X}_{10^{-\infty}}\rvert$
(8-Azag) 0.2 um	5	938.4894	187.7699	68.74	0.0001	1.3924	S	NS	S	S	NS	S
Error	24	65.5333	2.7306									
(8-Azag) 5.0 um	5	2482.9017	496.5803	329.29	0.0001	0.8578	S	S	S	S	S	S
Error	24	36.1933	1.5080									
(Cycloh) 0.2 um	5	882.2117	176.4423	170.45	0.0001	0.7107	S	S	S	S	NS	S
Error	24	24.8433	1.0351									
(Cycloh) 5.0 um	5	2435.3672	487.0734	218.46	0.0001	1.0467	S	S	S	S	NS	S
Error	24	49.0517	2.2296									
(2-Dxad) 0.2 um	5	31.8044	6.3609	2.44	0.1240	1.1610	NS	NS	NS	NS	NS	NS
Error	24	62.4900	2.6038									
(2-Dxad) 5.0 um	5	938.4849	187.6979	68.74	0.0001	1.1542	S	NS	S	S	NS	S
Error	24	65.5333	2.7306									
(Emetin) 0.2 um	5	1118.0117	223.6023	60.61	0.0001	1.3416	S	S	NS	NS	NS	S
Error	24	88.5367	3.6890									
(Emetin) 5.0 um	4	91.7015	22.9254	7.96	0.0007	1.2011	-	S	NS	NS	NS	-
Error	18	51.8167	2.8787									
(5-Fluo) 0.2 um	5	2951.3528	590.2706	352.09	0.0001	0.9044	NS	S	S	NS	S	S
Error	24	40.2353	1.6765									
(5-Fluo) 5.0 um	5	2477.2361	495.4472	442.75	0.0001	0.7389	NS	NS	S	S	S	S
Error	24	26.8567	1.1190									

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o	$\lvert\bar{X}_{10^{-3}} - \bar{X}_{5\times10^{-4}}\rvert$	$\lvert\bar{X}_{5\times10^{-4}} - \bar{X}_{10^{-4}}\rvert$	$\lvert\bar{X}_{10^{-4}} - \bar{X}_{5\times10^{-5}}\rvert$	$\lvert\bar{X}_{5\times10^{-5}} - \bar{X}_{10^{-5}}\rvert$	$\lvert\bar{X}_{10^{-5}} - \bar{X}_{10^{-\infty}}\rvert$	$\lvert\bar{X}_{10^{-3}} - \bar{X}_{10^{-\infty}}\rvert$
(Mito C) 0.2 um	5	11.2840	2.2568	1.57	0.2065	0.8375	NS	NS	NS	NS	NS	NS
Error	24	34.5033	1.4376									
(Mito C) 5.0 um	5	3.9494	0.7899	0.35	0.8766	1.0500	NS	NS	NS	NS	NS	NS
Error	24	51.8258	2.2533									
(Puromy) 0.2 um	5	31.8044	6.3609	2.44	0.0633	1.1271	S	NS	NS	NS	NS	S
Error	24	62.4900	2.6038									
(Puromy) 5.0 um	5	52.7711	10.5542	3.07	0.0279	1.2955	S	NS	NS	NS	NS	NS
Error	24	82.5500	3.4396									

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F_o	Pr>F	t_o	$\lvert\bar{X}_{10^{-3}} - \bar{X}_{10^{-4}}\rvert$	$\lvert\bar{X}_{10^{-4}} - \bar{X}_{5\times10^{-5}}\rvert$	$\lvert\bar{X}_{5\times10^{-5}} - \bar{X}_{10^{-5}}\rvert$	$\lvert\bar{X}_{10^{-5}} - \bar{X}_{5\times10^{-6}}\rvert$	$\lvert\bar{X}_{5\times10^{-6}} - \bar{X}_{10^{-\infty}}\rvert$	$\lvert\bar{X}_{10^{-3}} - \bar{X}_{10^{-\infty}}\rvert$
(Actm D) 0.2 um	5	16.5074	3.3015	0.91	0.4903	1.3296	S	NS	NS	NS	NS	NS
Error	24	86.9533	3.6230									
(Actm D) 5.0 um	5	63.1867	12.6373	5.64	0.0014	1.045	S	NS	NS	NS	NS	S
Error	24	57.7333	2.2389									

F_o - observed test statistic for analysis of variance = $\frac{SS\ (Treatment)/DF}{SS\ (Error)/DF}$, using Replication (Concentration*Chamber) as an error term

t_o - observed test statistic for L.S.D. multiple comparison procedure = $t_{\frac{\alpha}{2},\ df\ (MSE)} \sqrt{\frac{2MSE}{n_1}}$

S - significant treatment difference between paired mean values at the 0.05 level

NS - no significant treatment difference between paired mean values at the 0.05 level

8-Azag = 8-Azaguanine, Cycloh = Cycloheximide, 2-Dxad = 2-Deoxyadenosine, Emetin = Emetine HCl, 5-Fluo = 5-Fluorouracil, Mito C = Mitomycin C, Puromy = Puromycin diHCl, Actm D = Actinomycin D

The 10^{-1} M, 10^{-2} M, 10^{-3} M, 10^{-4} M, and 10^{-5} M concentrations of 8-azaguanine tested significantly reduced the amount of sporulation in response to light in both 0.2 um and 5.0 um membrane cultures (P=0.05), with almost complete suppression being observed microscopically at 10^{-1} M (Figure 23, Table 14). At concentrations greater than 10^{-4} M 8-azaguanine significantly decreased the radial expansion of thalli of 0.2 um membrane cultures as compared with untreated controls (Table 15). Reduction in the vegetative growth of thalli on 5.0 um membrane filters was also observed in plates exposed to 10^{-4} M and 10^{-5} M 8-azaguanine (P=0.05).

At concentrations of 10^{-1} M, 10^{-2} M, 10^{-3} M, and 10^{-4} M cycloheximide significantly decreased both the radial growth of thalli and the mean spore yield as compared with control cultures and thalli treated at a concentration of 10^{-5} M (Figure 24, Tables 14 and 15). Few conidia were observed at 10^{-1} M cycloheximide. The 0.2 um and 5.0 um membrane cultures were similarly affected.

When thalli were cultured on 5.0 um membrane filters, 2′-deoxyadenosine had no effect on light induced sporulation at the concentrations tested (P=0.05) (Figure 25, Table 14). However, the mean diameter was significantly less in plates exposed to inhibitor concentrations of 10^{-1} M, 10^{-2} M, and 10^{-3} M than in controls and plates treated with 10^{-4} M and 10^{-5} M 2′-deoxyadenosine (Table 15). In contrast, exposure to 2′-deoxyadenosine did not inhibit the vegetative growth of 0.2 um membrane cultures (P=0.05), but caused a slight, significant decrease in mean spore yield at all treatment concentrations as compared with control cultures.

Figure 23. Effect of 8-azaguanine on radial growth and photoinduced conidiation in 12-day-old, dark-reared, 0.2 um and 5.0 um membrane filter cultures of Cylindrocarpon sp. exposed to 24 hours of Sylvania F48T12/VHO Cool White fluorescent radiation at 25± 1.0° C (8-azaguanine applied for 24.5 hours beginning 15 minutes before transfer to illuminated chambers and ending 15 minutes after transfer to fresh MRM and darkness). Each point representing thallus diameter is the mean of measurements on 12 replicate cultures, 2 measurements per thallus. Each point representing conidium production is the Log_{10} mean spore yield of 12 replicate cultures, 5 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.

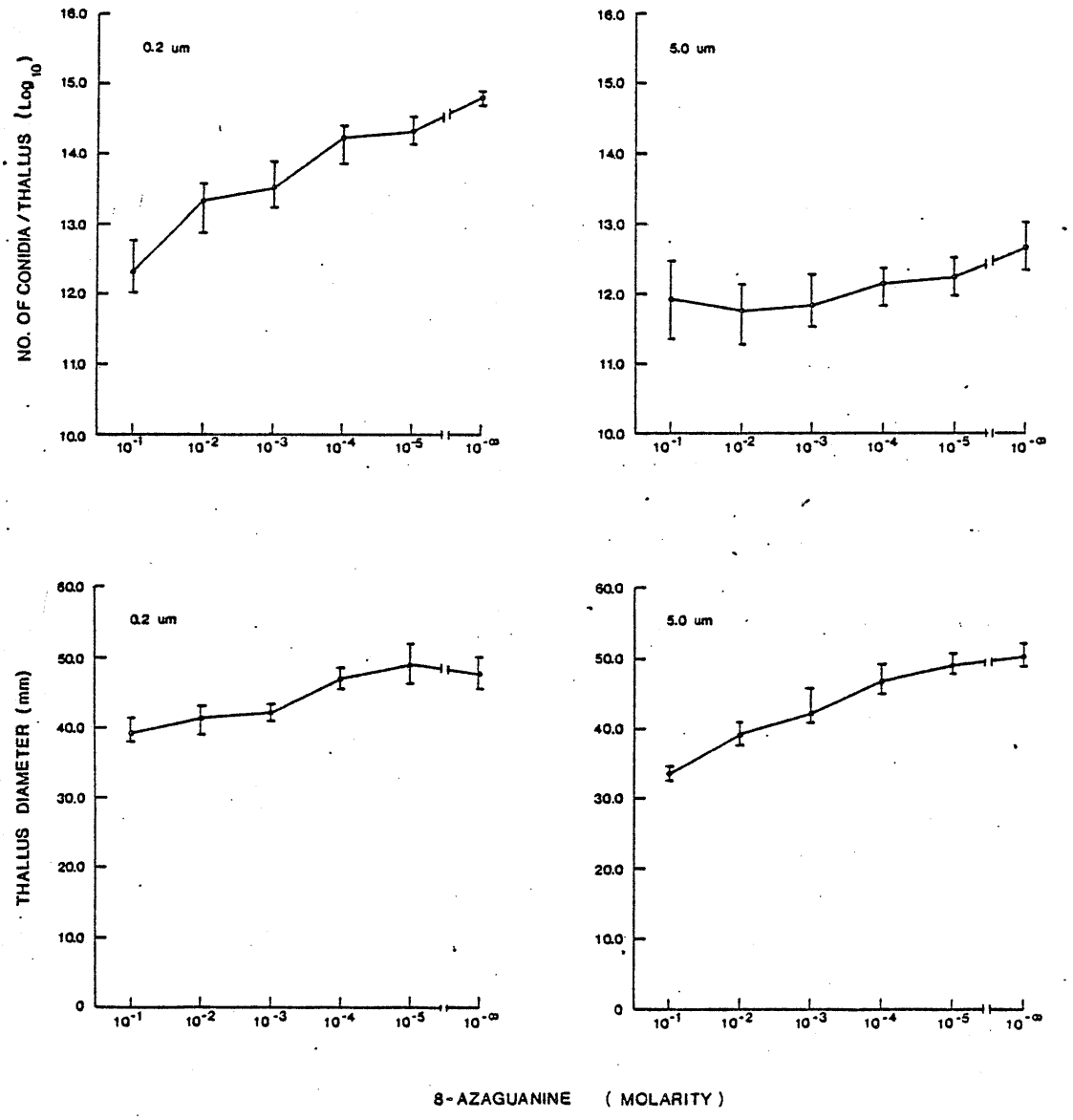
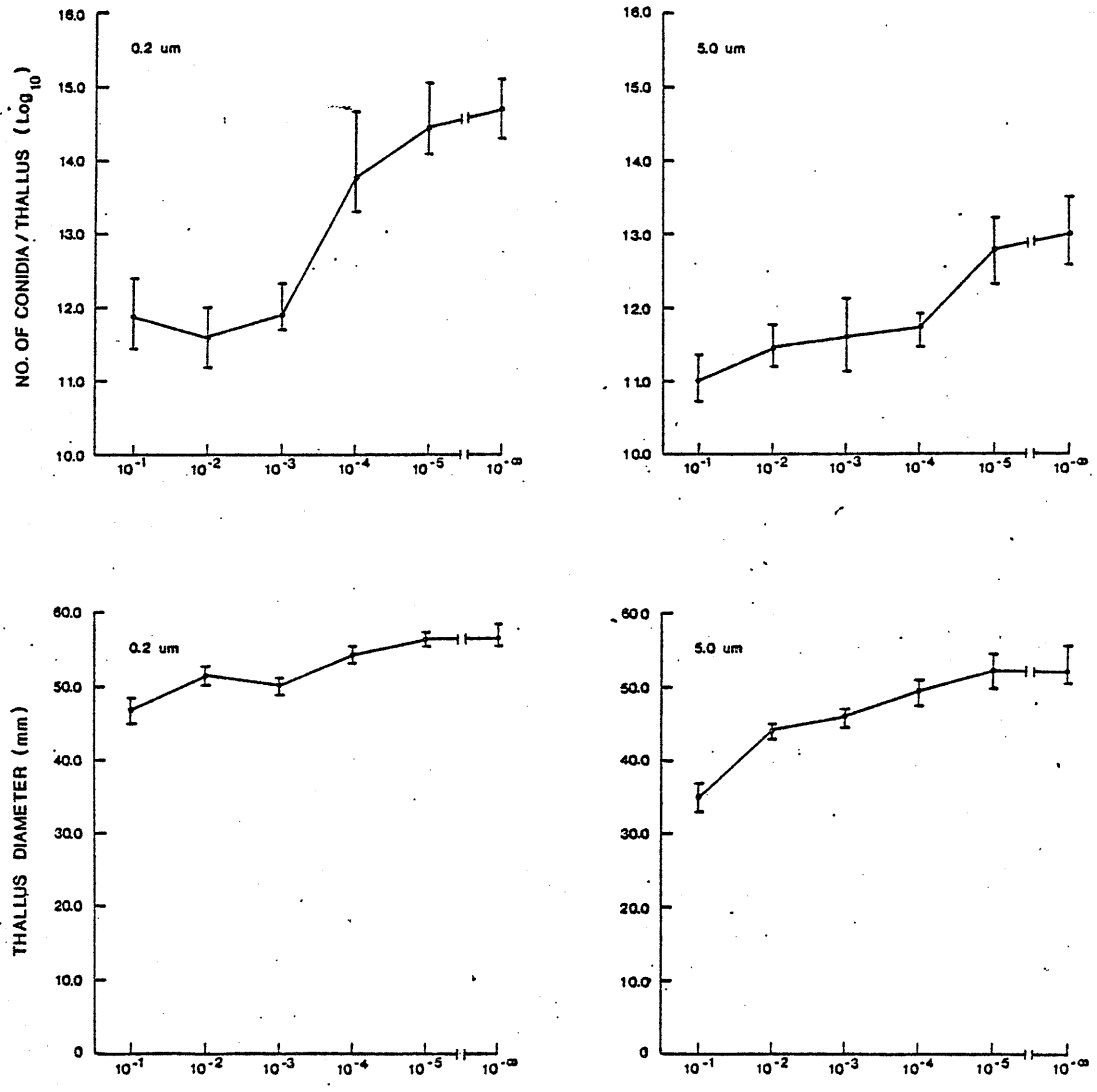
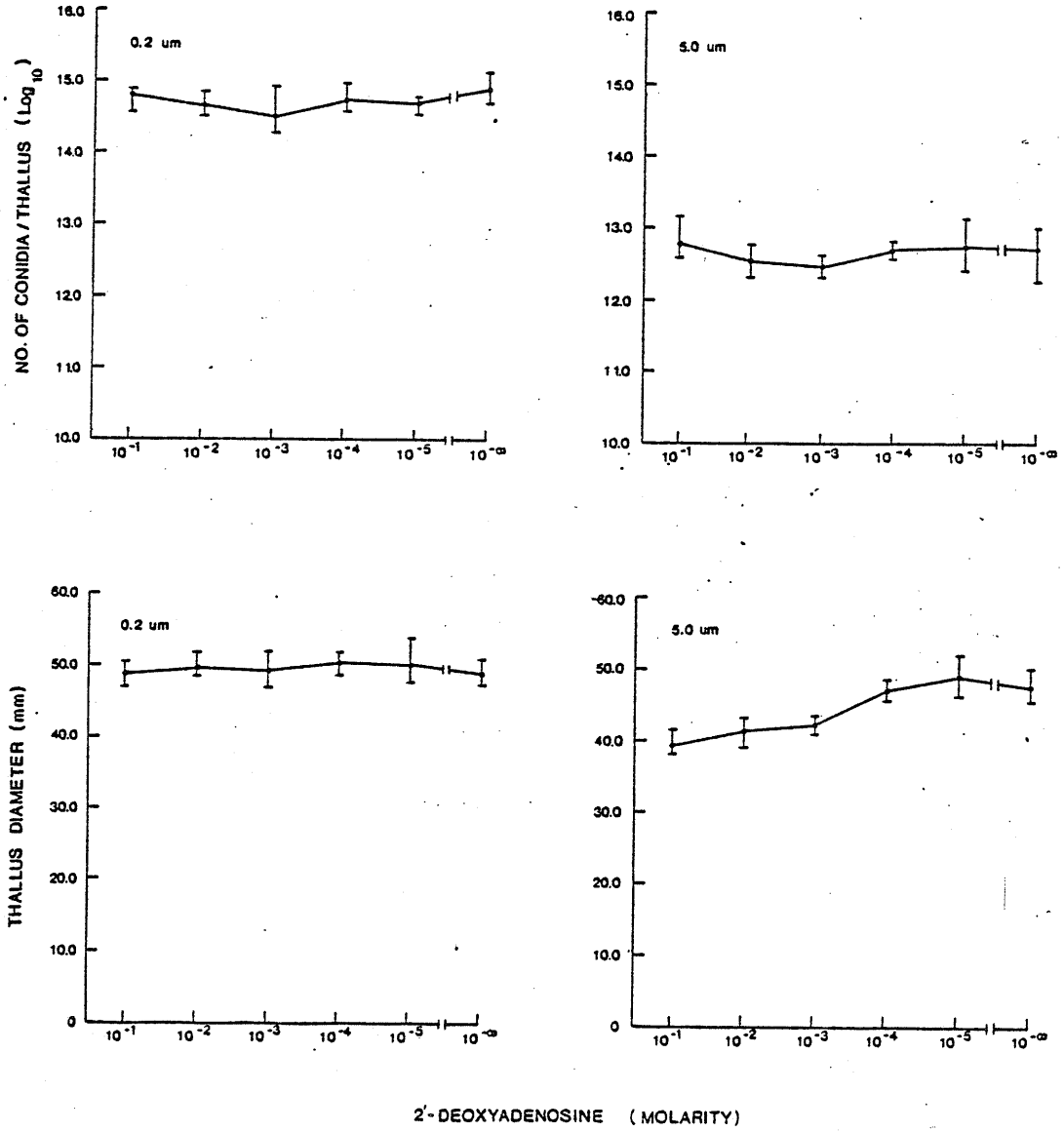

NO. OF CONIDIA / THALLUS (Log10)
THALLUS DIAMETER (mm)
0.2 um
5.0 um
16.0
15.0
14.0
13.0
12.0
11.0
10.0
60.0
50.0
40.0
30.0
20.0
10.0
0
10-1
10-2
10-3
10-4
10-5
10-∞
8-AZAGUANINE (MOLARITY)

Figure 24. Effect of cycloheximide on radial growth and photoinduced conidiation in 12-day-old, dark-reared, 0.2 um and 5.0 um membrane filter cultures of Cylindrocarpon sp. exposed to 24 hours of Sylvania F48T12/VHO Cool White fluorescent radiation at 25± 1.0° C (cycloheximide applied for 24.5 hours beginning 15 minutes before transfer to illuminated chambers and ending 15 minutes after transfer to fresh MRM and darkness). Each point representing thallus diameter is the mean of measurements on 12 replicate cultures, 2 measurements per thallus. Each point representing conidium production is the Log_{10} mean spore yield of 12 replicate cultures, 5 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.


NO. OF CONIDIA / THALLUS (Log10)
0.2 um
5.0 um
THALLUS DIAMETER (mm)
0.2 um
5.0 um
16.0
15.0
14.0
13.0
12.0
11.0
10.0
60.0
50.0
40.0
30.0
20.0
10.0
0
10-1
10-2
10-3
10-4
10-5
10-∞
CYCLOHEXIMIDE (MOLARITY)


0.2 um
5.0 um
NO. OF CONIDIA / THALLUS (Log_{10})
THALLUS DIAMETER (mm)
16.0
15.0
14.0
13.0
12.0
11.0
10.0
60.0
50.0
40.0
30.0
20.0
0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
$10^{-\infty}$
2'-DEOXYADENOSINE (MOLARITY)

Growth and conidiation in response to emetine HCl concentrations of 10^{-3} M, 10^{-4} M, and 10^{-5} M did not differ significantly from that recorded for controls (Figure 26, Tables 14 and 15). However, more concentrated solutions of the inhibitor resulted in a significant decrease in growth (P=0.05) and complete inhibition of light-induced sporulation. Cultures on both pore size membrane filters were similarly affected.

Treatment with 5-fluorouracil, irrespective of the concentration, significantly reduced the mean thallus diameter and the mean spore yield of membrane cultures as compared with untreated controls (Figure 27, Tables 14 and 15). Microscopic examination of thalli grown on 0.2 um membrane filters indicated that photoinduced sporulation had been completely suppressed at concentrations of 10^{-1} M, 10^{-2} M, 10^{-3} M, and 10^{-4} M, and that 5-fluorouracil was only slightly less effective at 10^{-5} M. In contrast, numerous conidia were observed in 5.0 um membrane cultures treated at 10^{-4} M as well as 10^{-5} M.

Mitomycin C and puromycin diHCl concentrations of 10^{-3} M, $5x10^{-4}$ M, 10^{-4} M, $5x10^{-5}$ M, and 10^{-5} M were also tested. The effect of mitomycin C on vegetative growth and sporulation did not differ significantly from that of control cultures (Figure 28, Tables 14 and 15). When thalli grown on 0.2 um membrane filters were treated with puromycin diHCl there was a slight, but significant decrease in the amount of sporulation in response to light at all concentrations (Figure 29, Table 14). Exposure of 0.2 um and 5.0 um membrane cultures to the highest concentration of puromycin

Figure 26. Effect of emetine HCl on radial growth and photo-induced conidiation in 12-day-old, dark-reared, 0.2 um and 5.0 um membrane filter cultures of Cylindrocarpon sp. exposed to 24 hours of Sylvania F48T12/VHO Cool White fluorescent radiation at 25± 1.0° C (emetine HCl applied for 24.5 hours beginning 15 minutes before transfer to illuminated chambers and ending 15 minutes after transfer to fresh MRM and darkness). Each point representing thallus diameter is the mean of measurements on 12 replicate cultures, 2 measurements per thallus. Each point representing conidium production is the Log_{10} mean spore yield of 12 replicate cultures, 5 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.

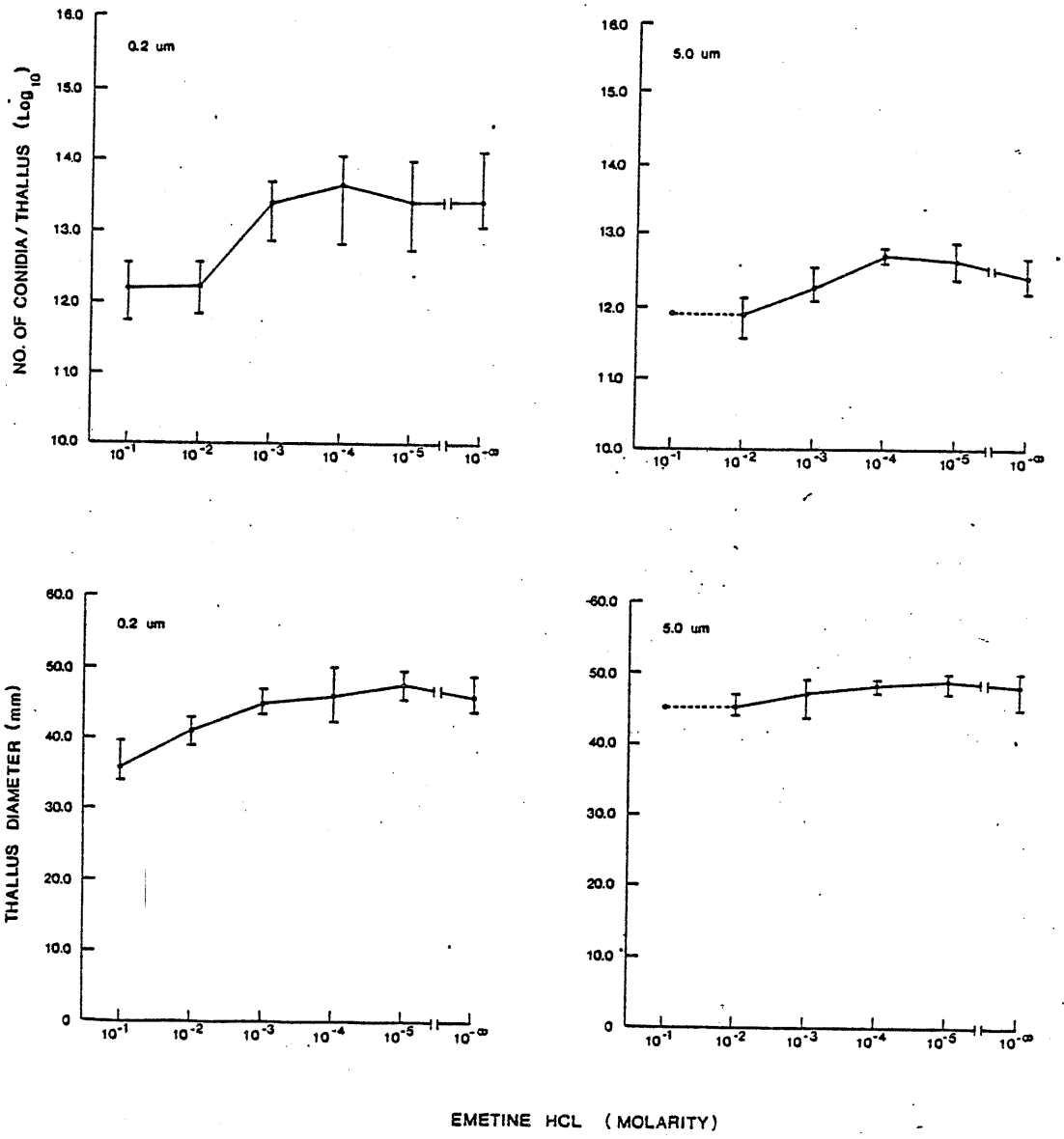

0.2 um
5.0 um
NO. OF CONIDIA / THALLUS (Log10)
THALLUS DIAMETER (mm)
EMETINE HCL (MOLARITY)

Figure 27. Effect of 5-fluorouracil on radial growth and photoinduced conidiation in 12-day-old, dark-reared, 0.2 um and 5.0 um membrane filter cultures of Cylindrocarpon sp. exposed to 24 hours of Sylvania F48T12/VHO Cool White fluorescent radiation at 25± 1.0° C (5-fluorouracil applied for 24.5 hours beginning 15 minutes before transfer to illuminated chambers and ending 15 minutes after transfer to fresh MRM and darkness). Each point representing thallus diameter is the mean of measurements on 12 replicate cultures, 2 measurements per thallus. Each point representing conidium production is the Log_{10} mean spore yield of 12 replicate cultures, 5 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.

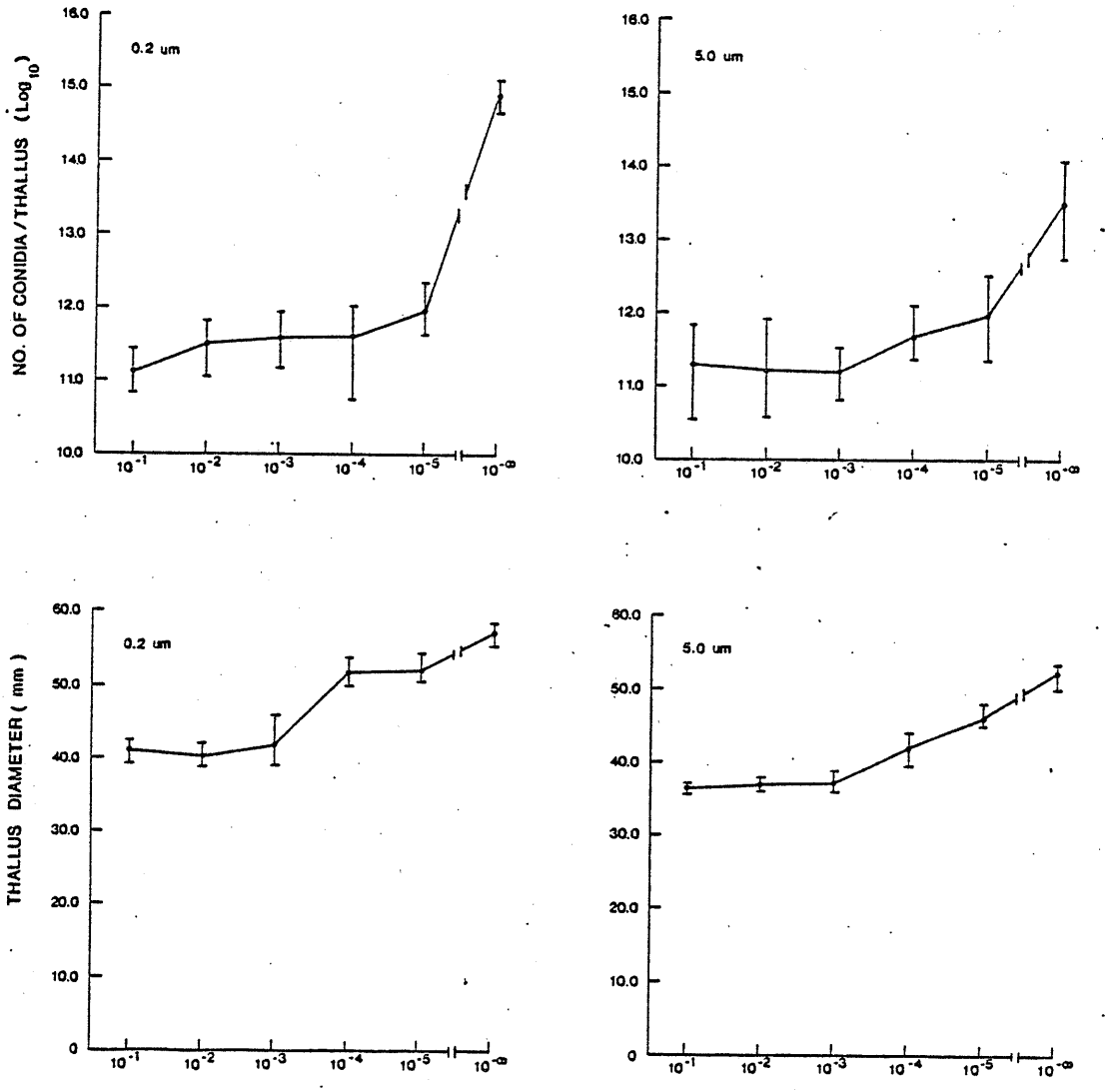

NO. OF CONIDIA / THALLUS (Log10)
0.2 um
5.0 um
THALLUS DIAMETER (mm)
0.2 um
5.0 um
16.0
15.0
14.0
13.0
12.0
11.0
10.0
60.0
50.0
40.0
30.0
20.0
10.0
0
10-1
10-2
10-3
10-4
10-5
10-∞
5-FLUOROURACIL (MOLARITY)

Figure 28. Effect of mitomycin C on radial growth and photo-induced conidiation in 12-day-old, dark-reared, 0.2 um and 5.0 um membrane filter cultures of Cylindrocarpon sp. exposed to 24 hours of Sylvania F48T12/VHO Cool White fluorescent radiation at 25± 1.0° C (mitomycin C applied for 24.5 hours beginning 15 minutes before transfer to illuminated chambers and ending 15 minutes after transfer to fresh MRM and darkness). Each point representing thallus diameter is the mean of measurements on 12 replicate cultures, 2 measurements per thallus. Each point representing conidium production is the Log_{10} mean spore yield of 12 replicate cultures, 5 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.

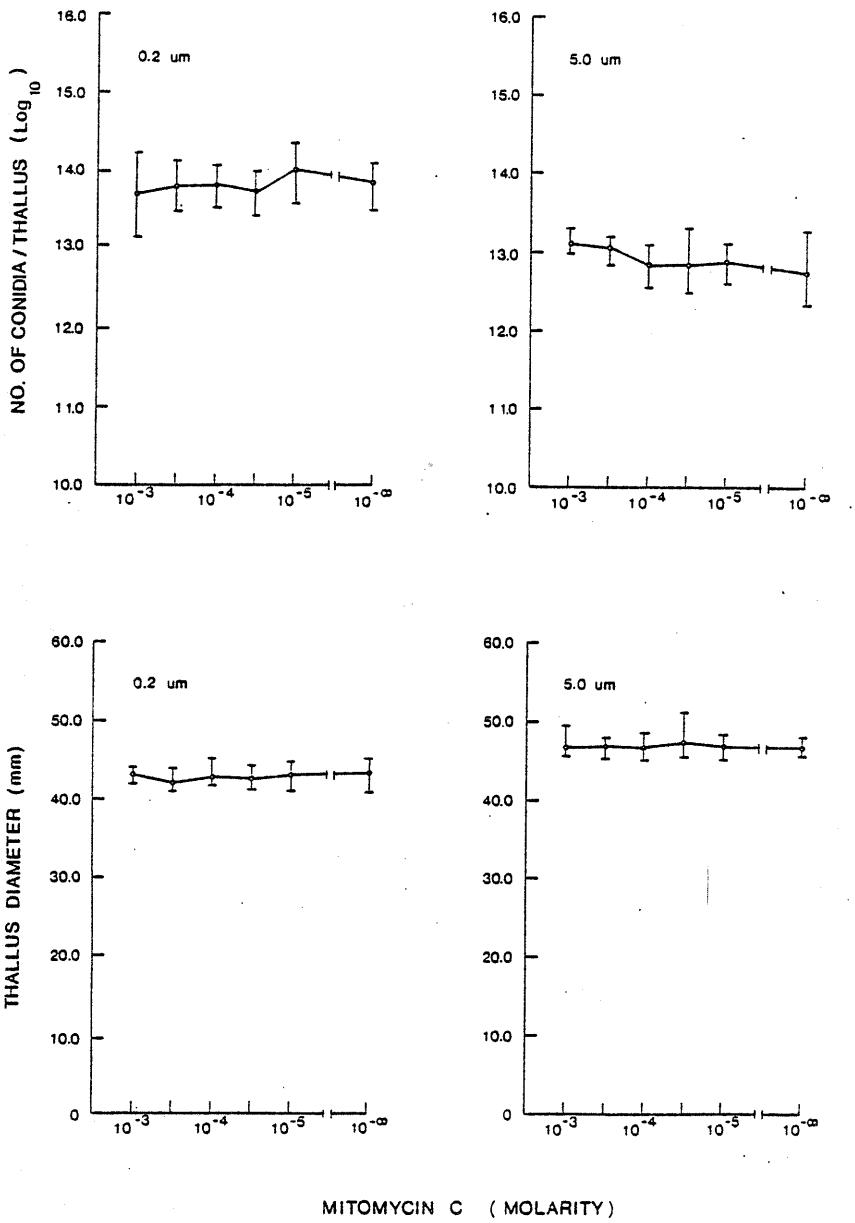
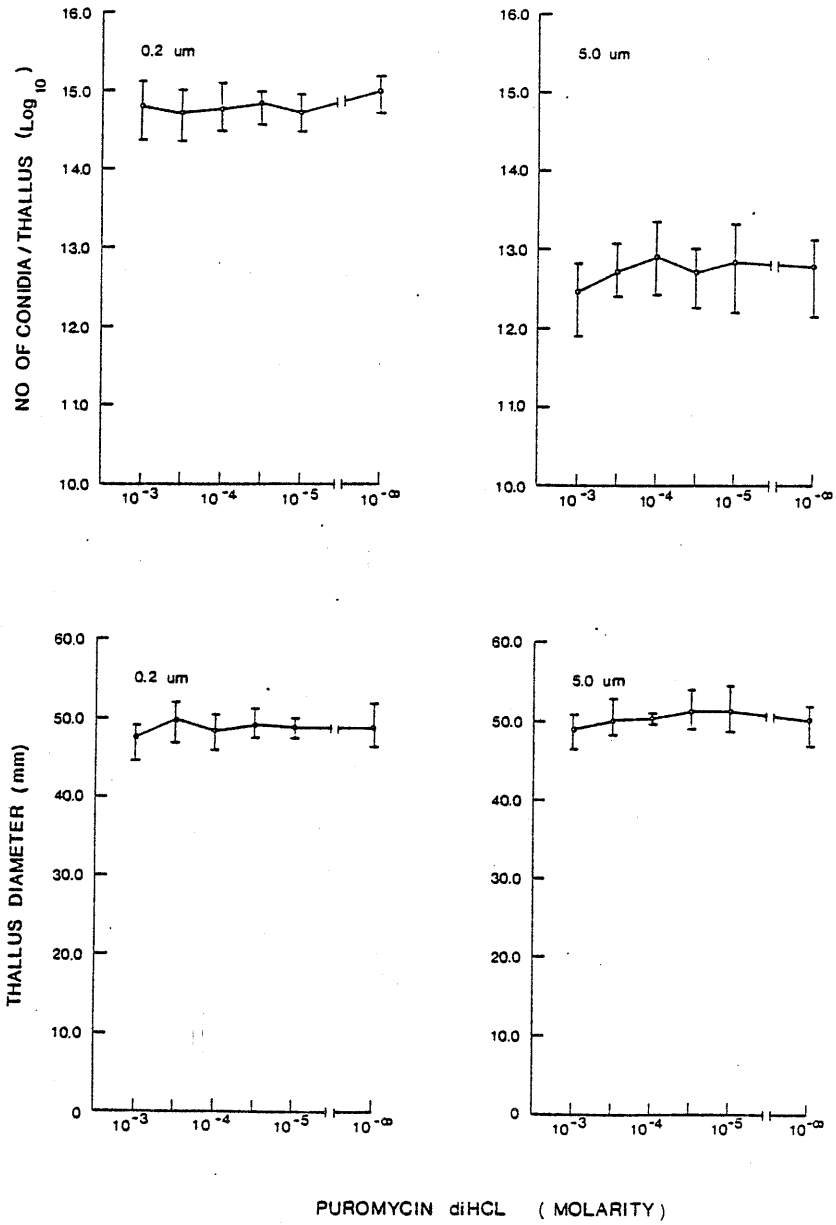

0.2 um
5.0 um
NO. OF CONIDIA / THALLUS (Log10)
THALLUS DIAMETER (mm)
16.0
15.0
14.0
13.0
12.0
11.0
10.0
60.0
50.0
40.0
30.0
20.0
10.0
0
10-3
10-4
10-5
10-∞
MITOMYCIN C (MOLARITY)

Figure 29. Effect of puromycin diHCl on radial growth and photoinduced conidiation in 12-day-old, dark-reared, 0.2 um and 5.0 um membrane filter cultures of Cylindrocarpon sp. exposed to 24 hours of Sylvania F48T12/VHO Cool White fluorescent radiation at 25± 1.0° C (puromycin diHCl applied for 24.5 hours beginning 15 minutes before transfer to illuminated chambers and ending 15 minutes after transfer to fresh MRM and darkness). Each point representing thallus diameter is the mean of measurements on 12 replicate cultures, 2 measurements per thallus. Each point representing conidium production is the Log_{10} mean spore yield of 12 replicate cultures, 5 counts per culture. Vertical lines with limits denote variation among experimental units of similar treatment.


NO OF CONIDIA / THALLUS (Log 10)
16.0
15.0
14.0
13.0
12.0
11.0
10.0
0.2 um
5.0 um
THALLUS DIAMETER (mm)
60.0
50.0
40.0
30.0
20.0
10.0
0
10-3
10-4
10-5
10-∞
PUROMYCIN diHCL (MOLARITY)

diHCl, ie. 10^{-3} M, also resulted in a significant reduction in vegetative growth as compared with other treatment concentrations and control plates (Table 15).

DISCUSSION

An investigation of the effect of light on this undescribed Cylindrocarpon species revealed that vegetative growth and sporulation are markedly influenced by electromagnetic radiation.

Cultures of the isolate incubated in darkness exhibited profuse production of fluffy, loose aerial hyphae as compared with the meager, prostrate mycelium of cultures incubated under continuous Vita-Lite radiation. Growth, as evidenced by radial expansion, was significantly greater when the thallus was not illuminated. Comparable effects were observed on certain Coelomycetes (47, 177, 187), Hymenomycetes (188), Hyphomycetes (38, 56, 66, 88, 160), Pyrenomycetes (18), and Zygomycetes (98). Such effects have been seen in cultures of closely related Fusarium spp. (8, 80, 101, 196, 232).

The inhibitory effect of light on hyphal tip growth of this Cylindrocarpon sp. was especially marked when Sylvania F20T12-BLB fluorescent lamps, which transmit near ultraviolet wavelengths, were used. With these lamps an irradiance of 0.12 Wm^{-2} was sufficient to arrest germ tube development, and an exposure of 12 hours produced a zone in which aerial hyphae were absent and growth was confined to a few submersed hyphae. In addition, as the irradiation dosage increased, those phialides (conidiogenous cells) which developed in the sporulation zone adjacent to this region of submerged hyphae were differentiated from hyphal elements growing in the agar medium and, consequently, the conidia they produced were also submerged.

Analogous responses have been described for several other fungi. An exposure of 15 minutes to wavelengths between 300 nm and 375 nm

virtually eliminated the aerial mycelium of Choanephora cucurbitarum and caused the hyphae to grow in the agar substratum (42). Dillon Weston and Halnan (58) working with Mucor mucedo, Sclerotinia trifoliorum, Stereum purpureum, and a species of Fusarium reported that growth was reduced to the minimum upon irradiation with 50 - 70 percent of the ultraviolet light from a quartz mercury vapor lamp and that "the mycelium grew well down into the medium away from the surfaces." Collybia dryophila, Sclerotium bataticola, and Fusarium batatatis showed a similar intolerence to light emanating from a Hanovia Universal Laboratory Model ultraviolet lamp in that a 5 minute exposure on each of five successive days was sufficient to cause a noticeable suppression of aerial growth and a tendency for the mycelium to grow deeper into the agar (100). Stevens (203) found that both perithecia of Glomerella cingulata and pycnidia of a Coniothyrium sp. gradually changed from being produced superficially to developing in a submerged position in the agar medium with increased dosages of irradiation from a Cooper-Hewitt quartz mercury arc. The location of pycnidia in cultures of Ascochyta pisi (117) and perithecia in cultures of Leptosphaerulina trifolii (126) and Pleospora herbarum (118), relative to the surface of the medium, was also reported to be directly correlated with dosage, intensity, and wavelength of radiation: those dosages which induced formation of submerged reproductive structures were least at the shorter wavelengths when intensities were high.

In each instance it was assumed that the medium acted either as a filter absorbing ultraviolet radiation or as a screen reducing ir-

radiation intensity; either would enable the fungus to develop at depths where energy in the ultraviolet region of the spectrum and/or incident ultraviolet intensity had been reduced below the inhibition threshold. Such observations were in keeping with the suggestion that growing mycelium, more easily killed by ultraviolet radiation than ungerminated spores, may penetrate a substance that is opaque to the rays and, thus, escape the killing effect (65).

It would appear the the response of Cylindrocarpon sp. to radiation emitted from both Vita-Lite and black-light-blue fluorescent lamps is partly in adaptation to the injurious effect of the ultraviolet wavelengths. The fact that conidia irradiation did not affect germination, but checked subsequent germ tube development and then rendered it dormant, indicates that vegetative hyphae are more sensitive to the action of the rays. This interpretation may also explain (a) the diminished effectiveness of ultraviolet radiation in retarding the growth rate of cultures predisposed for longer periods in darkness; (b) the propensity of growing hyphae at the periphery of the thallus to develop deeper in the medium during exposure; and (c) the synthesis of submerged conidiophores. Since both the growth and differentiation of hyphae at the surface of the medium had been suppressed by radiant energy, the inhibitive effect on synthetic processes would be most pronounced in immature cultures characterized by an essentially superficial mycelium and least obvious in those cultures which had been given sufficient time in the absence of light to develop an extensive network of hyphae beneath the surface of the agar. The observation that superficial hyphae at the periphery of the thallus when

the transfer from dark to light was made did not resume growth upon return to darkness suggests either that the hyphae were no longer viable or that the growing tips were negatively phototrophic to ultraviolet light and had penetrated the agar medium at a rate sufficient to reach depths where the inhibitive effect of these wavelengths had been mitigated. Since, in contrast to submerged hyphae, the aerial mycelium of the sporulation zone only produced conidia following photoinduction periods of 24 hours and less, the fungicidal action of ultraviolet radiation can not be ruled out: a brief exposure being capable of terminating apical growth but a much longer exposure being required to abort either the photochemical reaction or induction mechanism.

The effect of radiation on asexual reproduction in Cylindrocarpon sp. is much more striking than the effect on mycelial growth. Under the experimantal conditions, light was demonstrated to be an absolute requirement for sporogenesis.

Conidia fail to form in cultures held under continuous darkness from the time of inoculation, but are produced in abundance under periods of both continuous and intermittent irradiation. These observations are consistent with the view that at least two events are involved in the asexual reproductive process of this organism. The first of these must be based on a photochemical event and is necessary for the induction of conidiophores, and the second, essential for conidial development, proceeds whether or not irradiation is followed by a period of darkness. Thus, the relationship of light to sporulation for the test organism is analogous to that described for group 7 fungi (p. 5).

Both the qualitative and quantitative nature of the sporulation response were found to be dependent upon a variety of variables. These were: (a) the age of the culture when exposed; (b) temperature of incubation; (c) the period of darkness following photoinduction; (d) irradiation dosage; (e) wavelength of light; (f) surface moisture; (g) intensity of radiant energy intercepted by the thallus; and (h) the composition of the medium.

Preliminary attempts to standardize the inductive system demonstrated that single spore isolates of the fungus were physiologically competent to be induced, i.e. they were responsive to morphogenetically active radiation, within 24 hours of their germination. This is in contrast to both Botrytis cinerea (216) and Septoria nodorum (25) which could be induced to sporulate only after 3 days and 2 days of growth, respectively. In the context of Kleb's "laws" on reproduction in fungi as cited in Lilly and Barnett (133), it would appear that the period of assimilation accompanying the vegetative phase that is a prerequisite for reproduction need not be lengthy. In a report on an isolate of Trichoderma viride "capable of 'accepting' the induction" following 16 hours of growth (72), it was suggested that the response to light is conditioned neither by age nor size of thallus but rather by "metabolic changes related to the 'maturation' of the colony." A comparable situation may well be operative in the regulation of developmental competence in Cylindrocarpon sp.

Nonirradiated cultures of the test organism failed to sporulate over a range of temperatures from 10° C to 30° C. With exposure to white fluorescent light at adequate irradiation intensities, conidia

formed abundantly in the peripheral zones of thalli, in existence at the initiation of the illumination period, at test temperatures between 10° C and 35° C, inclusive, with maximum numbers of conidia being produced from approximately 20° C to 25° C. Temperatures either below 20° C or above 25° C resulted in a reduced mean spore yield with very few conidia being harvested from cultures exposed at either extreme. Thus irradiation not only induces sporulation over a considerable temperature range, but the temperatures favoring maximum conidial production correspond to those in the range for optimum hyphal growth. This influence of temperature on sporulation in fungi is not unique. Various investigators have found that the temperature range permitting sporulation is often narrower than that permitting growth. They have also observed that there is both an optimum temperature for sporulation as well as an optimum temperature for growth, and that these optima may or may not differ (9, 44, 64, 97, 117, 133, 137, 147, 150, 159, 168, 187).

Sporulation in this fungus occurred on mycelium produced prior to irradiation and, as a consequence, was not directly affected by a temperature dependent reduction in the growth rate. Further, since standardization of the incident light intensity at 12.0 Wm^{-2} compensated for the potential fluctuation in lumen output of the fluorescent lamps as the ambient temperature was increased above and decreased below the 25° C optimum, the reduced response of Cylindrocarpon sp. to light administered at 10° C and 35° C implies that the mycelium either lost its sensitivity to radiation or, alternatively, that the mechanism of induction following stimulus detection is either linked to normal met-

abolic processes which are adversely affected by extreme exogenous factors, or is itself hypersensitive to temperatures near the upper and lower limits of those which normally allow vegetative growth. This latter inference fits the hypothesis that the induction processes of photosporogenesis involve biochemical reactions and are, consequently, metabolic events.

Mean spore yields of photoinduced cultures which had been returned to darkness for periods ranging from 0 to 120 hours indicate that 48 hours are necessary for the completion of conidiation in Cylindrocarpon sp. following 24 hours exposure to white fluorescent light at 12.0 Wm^{-2}. A similar period was required by Fusarium oxysporum f. sp. gladioli for complete macrospore development following 60 minutes exposure to a 400 watt MA/V mercury vapor lamp (30), while sporulation in Alternaria tomato (111), Botrytis cinerea (213), and Trichoderma viride (72) was completed in 16, 12, and 24 hours, respectively, after the beginning of the dark period following photoinduction. These experiments demonstrated that while the action of electromagnetic radiation need not be continuous, the photoproduct is consumed over a period of hours in the production of a finite number of conidia. This accounts for the observation that post-irradiation incubation for periods of 48 hours and longer do not produce significantly different yields of conidia in Cylindrocarpon sp.

The minimum exposure necessary to induce sporulation in this fungus was approximately 12 hours under the conditions employed, i.e. Vita-Lite fluorescent radiation (12.0 Wm^{-2}) at 25° C. This is in contrast to the single exposure of black light for 1 minute at 151 uW/cm^2

which induced sporulation in Botrytis cinerea (216), the 1 minute exposure to a light source of 1 foot-candle intensity which was sufficient to induce sporulation in Trichoderma viride (76), the 4 hour exposure to a 15 watt daylight fluorescent lamp at 60 foot-candle which induced conidiation in Choanephora cucurbitarum (9), and the exposures less than 4 hours in duration which resulted in the production of reproductive structures in Ascochyta pisi (117), Aspergillus ornatus (88), Botryodiplodia theobromae (180), Fusarium discolor sulphureum (16), Helminthosporium oryzae (115), Pestalotia theae (75), Trichoderma lignorum (145), and Verticillium lateritium (97). Since the mean yield of conidia from cultures of Cylindrocarpon sp. exposed to irradiation peiods of 3, 6, and 9 hours did not differ significantly from unexposed controls, it appears that the photoreceptor system in this fungus is not particularly sensitive to light. The subsequent observation that conidiation occurred in increasing quantities with increasing irradiation dosage up to 72 hours, but that spore yields were not significantly increased with longer periods of photoinduction, suggests that 72 hours of continuous irradiation may have saturated the photoreceptor. Comparable results, irrespective of the saturation dosage, were reported for Botrytis cinerea where maximum sporulation occurred in cultures exposed to black light for 12 hours at 151 uW/cm^2 (216) and Trichoderma viride in which the response to light did not change after exposures of 1 to 5 seconds at 550 uW/cm^2 (Jensen 1970, as cited in Tan and Epton 217). In contrast, Zimmer and McKeen (233) concluded from the quantitative results of a similar study of Alternaria dauci

that spore production increased rapidly with exposure for the first 3 minutes, leveled off, and then gradually increased for the next 57 minutes of the assay. However, the fact that the conidial counts of the various treatments were not analyzed statistically cautions against such an interpretation. Especially since a similar conclusion, based solely on the graphical presentation of data, could easily have been made for Cylindrocarpon sp. during this study (see Figure 11).

The sporulation variability exhibited as a quantitative difference in mean spore yield at several wavelengths under conditions of equal intensity (0.10 Wm^{-2}) represents the response spectrum for light induced conidiation of Cylindrocarpon sp. Although this particular spectrum does not yield information about minor peaks of photoinductive activity, the experimental data obtained with the monochromatic filters and light sources tested indicates major spectral regions of activity.

There was no evidence of any significant stimulation by radiation extending from 522 nm to 580 nm nor at wavelengths above 600 nm. Only cultures which received blue filtered light (430 nm to 490 nm) or were irradiated with near ultraviolet emitting black-light-blue lamps (peak emission at 356 nm) developed conidia. Such responses at the standard energy level demonstrate that photoinductive activity is limited to radiant energy in the electromagnetic spectrum of less than 522 nm wavelength. Furthermore, output in this particular region of the spectrum accounts for the effectiveness of polychromatic Vita-Lite fluorescent lamps in inducing conidiophore differentiation.

While the results of these experiments do not exclude the possibility of a peak of photoinductive activity around 280 nm - 290 nm, a

region which is important in the action spectrum of ultraviolet-induced fungi, it is clear that this particular organism has the wavelength characteristics typical of the NUV-blue sensitive fungi: no response to radiation longer than 520 nm; and a peak of maximum effectiveness around 450 nm with subsidiary peaks near 430 nm and 480 nm; and a peak in the near ultraviolet at approximately 370 nm. Unfortunately, the precise relationship of electromagnetic energy to induction of asexual reproduction in Cylindrocarpon sp. awaits construction of an action spectrum for the photomorphogenetic response over the ultraviolet and visible range. Such detailed information on the relative quantum efficiency of each wavelength would also clarify whether photoinduction of sporulation in this fungus is confined to detection of a NUV-blue light stimulus, by providing additional data at the 280 nm - 290 nm wavelengths. As it now stands, any postulation on the nature of the photoreceptor can only take into account the sensitivity to wavelengths from the near ultraviolet, longer than 300 nm, to the blue region of the spectrum.

Experiments designed to investigate the influence of moisture on stimulus detection and conidium production in cultures of Cylindrocarpon sp. suggest that a percentage of the morphogenetically active radiation transmitted by the light source is lost in passage through the water film covering the thallus either by reflection, or absorption, or both. In Alternaria solani, a diurnal sporulator sensitive to near ultraviolet and blue light, residual surface moisture left after flooding and draining the cultures before photoinduction caused a decrease in sporulation (40). The film of water was thought to act

as a "partial barrier" to the radiation striking the thallus and thereby reduce the sporulation response.

Polychromatic radiation studies demonstrated a very definite relationship between the incident light intensity and the number of reproductive units induced in Cylindrocarpon sp. Under Vita-Lite fluorescent lamps, an increase in irradiation intensity was concomitant with an increase in the mean conidium yield up to an optimum range of approximately 9.0 Wm^{-2} to 15.0 Wm^{-2}; beyond this spore production was noticeably reduced. The general effect of varying the energy of the near ultraviolet radiation intercepted by the thallus is similar in that there was evidence of an optimum range, i.e. 0.02 Wm^{-2} to 0.08 Wm^{-2}, and suppression of conidiation at higher intensities, i.e. 0.10 Wm^{-2} to 0.12 Wm^{-2}. However, under black-light-blue fluorescent lamps, much lower levels of incident light intensity, as compared with Vita-Lite irradiation intensities, not only induced sporogenesis, but were also sufficient to depress or inhibit the morphogenetic response. A similar effect of irradiance on the formation of reproductive structures has been reported for Alternaria solani, Curvularia lunata, Helminthosporium sativum, and Piricularia oryzae (101), Fusarium cepae and Macrosporium tomato (179), three strains of Hypomyces solani (50), and Stemphylium solani (198). However, the mechanism by which morphogenetically active radiation inhibits or completely suppresses conidial formation at high energy levels is unknown. Curtis (50) has discussed the possibility of high light intensities creating inhibitors of perithecium formation in Hypomyces solani through photo-oxidation of com-

pounds in either the organism or medium. An alternative explanation, based on the work of Pomper (176) who has reviewed many of the biochemical and physiological changes known to occur in fungi exposed to ultraviolet, visible, infrared, ionizing, and cosmic radiations, is that radiation which is innocuous or beneficial at low energy levels becomes extremely damaging to genetic and metabolic processes at high levels.

The compensation between the length of exposure and irradiation intensity described for many light sensitive fungi, including Alternaria chrysanthemi (119), Ascochyta pisi (117), Pestalotia theae (75), Pleospora herbarum (118), and Trichoderma viride (67, 76), was not obvious in the experiments with Cylindrocarpon sp. Whether the law of reciprocity holds true for this photoinduced reaction must await further investigation.

The composition of the nutrient medium has been found to influence the results of illumination experiments (9, 42, 46, 83, 101, 150, 156, 157, 181, 187, 188, 220). In this study, an absolute light requirement for sporogenesis in cultures grown on MRM has been demonstrated. However, it was possible to induce conidiation in darkness by altering the composition of the nutrient medium. When this fungus was grown on PDA it sporulated abundantly in the absence of light. It has been suggested that the specific additives or combinations of compounds cause dark sporulation by "leading to metabolic changes which mimic the changes caused by light" (199). Although these compounds may not be normal metabolites of light-induced processes, their identity, as well as the metabolic changes they may initiate, deserve further study.

When dark reared cultures of Cylindrocarpon sp. were irradiated, the response to light was extremely localized. Irradiation of the entire thallus resulted in the formation of a dense ring of confluent sporodochia and mycelium bearing conidia, which corresponded to the peripheral 6.0 mm of hyphae that existed at the start of the illumination period. Since the linear growth rate of this fungus is approximately 0.075 mm/hour in darkness, all conidiophores were differentiated from pseudocells not more than 80 hours old.

The pronounced receptivity of hyphae at or near the growing front of light sensitive fungi has been repeatedly demonstrated. However, interpretation as the the exact location of the sporulation site is often hindered by an inadequate examination of this portion of the mycelium or by the omission of precise descriptive statements. Consequently, there are numerous reports of reproductive structures being produced by "the young tissue around the edge of the colony" (40), "at the tips of hyphae" receiving a short light stimulus (16), at the periphery (92, 150), in "actively growing peripheral mycelium" (126), etc. Such descriptions usually provide little supplementary information as to whether sporulation occurred on new hyphae produced during the photoinduction period, in the region occupied by the growing edge just prior to illumination, or both. Thus, the pattern of conidiation in Cylindrocarpon sp. is analogous only to that described for Alternaria tomato (111), Aspergillus ornatus (181, 199), Botrytis cinerea (216), Fusarium fructigenum (77), Fusarium oxysporum f. sp. gladioli (30), Pestalotia theae (75), Schizophyllum commune (168), Trichoderma lignorum (145), and Trichoderma viride (68, 110, 201). However, of these

organisms only A. tomato and P. theae are induced by light. In the others sporulation is merely enhanced, but, unlike Cylindrocarpon sp., A. tomato is a diurnal sporulator, and the breadth of the spore zone in cultures of P. theae is dependent upon the total light energy supplied to the thallus. Furthermore, only hyphal elements not more than 15 hours old and between 2 and 26 hours in age responded to light in A. ornatus (199) and S. commune (168), respectively. This suggests that the hyphae of Cylindrocarpon sp. are either highly photoresponsive or nonphotoresponsive, i.e. there is no intermediacy as in P. theae, and that the receptive hyphae behind the actively growing margin retain their sensitivity to light for a long period of time relative to other photoinduced species.

Alternating periods of light and darkness are essential for the formation of concentric rings of conidia in agar cultures of Cylindrocarpon sp. Each sporing zone is related to the time of exposure to light and develops inwardly from the position occupied by the growing edge of the thallus when exposure is initiated. In contrast to the breadth of the spore ring formed by dark grown cultures in response to a single 12 hour exposure, i.e. 6.0 mm, each successive fertile zone coincided closely with the mycelium laid down during the preceding 12 hour dark period, i.e. approximately 0.9 mm. However, on PDA slightly less than half of this peripheral region gave rise to conidia. This observation suggests that either the periodic fluctuation in light responsible for the exogenous rhythm or the duration of the dark period prior to exposure is affecting the length of time hyphal elements retain their sensitivity to morphogenetically active radiation.

The ability of eight antimetabolites to inhibit the conidiation of Cylindrocarpon sp. was investigated to assess nucleic acid and protein involvement in the induction and differentiation phases of light-induced morphogenesis. The results obtained show that 5-fluorouracil, a purine antagonist, and 8-azaguanine, an analogue of guanine, were the most potent inhibitors of sporogenesis. Concentrations of cycloheximide, an inhibitor of cytoplasmic protein synthesis, greater than 10^{-5}M, and high molar concentrations of emetine HCl (10^{-1}M and 10^{-2}M), an inhibitor of protein synthesis by 80S ribosomes, and actinomycin D (10^{-3}M with 0.2 um membrane filter cultures), an inhibitor of DNA dependent RNA synthesis, were also very effective. However, the high level of actinomycin D required for the inhibition of conidium formation makes it difficult to state categorically that the measured reduction in mean spore yield is a consequence of the specific effect of the inhibitor. In contrast, neither inhibitor of DNA synthesis, 2'-deoxyadenosine nor mitomycin C, had an appreciable effect on the sporulation response over the range of concentrations tested. The observation that the vegetative growth of thalli on 5.0 um membrane filters was inhibited by 2'-deoxyadenosine concentrations of from 10^{-1}M to 10^{-3}M, and the fact that mitomycin C (MW = 334.3) is a smaller molecule than either emetine HCl (MW = 553.6), puromycin diHCl (MW = 544.5), or actinomycin D (MW = 1255.5) suggest that the ineffectiveness of these compounds is not a consequence of either diffusion or permeability, given a comparable net charge. As far as the effect of puromycin diHCl is concerned, the weak inhibition of sporulation independent of concentration, suggests that if the compound interrupts peptide chain elongation in Cylindro-

carpon sp. it is not interfering with a process upon which light-induced sporulation depends.

On the basis of this information, both protein synthesis and nucleic acid synthesis would appear to be necessary requirements for conidiation in Cylindrocarpon sp. Speculation as to whether this entails the activation of parts of the genome resulting in the synthesis of mRNA, rRNA, or both and the subsequent quantitative and/or qualitative changes in the rate(s) of enzyme synthesis is unwarranted at this time. Investigations concerned with (a) the uptake of RNA and protein precursors in the presence and absence of inhibitors, (b) the variation of the rate of RNA and protein synthesis in light and dark treated cultures, and (c) differences in the transcribed RNA species during vegetative growth and immediately following the induction of conidiation are required if the mechanism mediating this light activating phenomenon is to be better understood.

It was found that the photoinduced sporulation of Trichoderma viride could be prevented by concentrations of 5-fluorouracil which did not suppress growth, i.e. 10^{-4}M to $7x10^{-6}$M (68). Since 5-fluorouracil could be shown to reduce the incorporation of radioactive uracil into RNA without affecting the ratio of uridylic to cytidylic acid (68), and illumination caused no detectable variation in the level of protein synthesis (201), it was suggested that the process of induced morphogenesis in this fungus is mediated by de novo synthesis of RNA. However, by means of DNA/RNA hybridization techniques, it was not possible to detect differences in RNA transcription between photoinduced and noninduced thalli (200).

Stallings (199) found no evidence of a role for either protein or RNA synthesis during the induction phase of morphogenesis in Aspergillus ornatus. Actinomycin D, chloramphenicol, cycloheximide, and 5-fluorouracil had no effect on light-induced sporulation in this fungus other than a non-specific retardation of the rate of conidiophore differentiation by cycloheximide and 5-fluorouracil. As morphogenesis did not appear to be directly controlled by differential gene activation, she suggested that conidiation in A. ornatus was most likely a reflection of irradiance dependent changes in preexisting molecules, e.g. alteration of substrate specificity and availability, modulation in activity, flux in the levels and flow among the various cellular pools, shifts in the rates of catabolism and anabolism, transformation, etc.

Tan (214) reported that light-induced conidiation in Botrytis cinerea requires both DNA and RNA synthesis as well as protein synthesis. Sporulation was completely inhibited by cycloheximide at 10^{-4}M and almost complete suppression was observed with both 10^{-3}M 5-fluorouracil and 10^{-1}M 8-azaguanine, while neither actinomycin D nor puromycin at molar concentrations of 10^{-4}, 10^{-5}, and 10^{-6}M had any appreciable effect. In addition, each of the following: cycloheximide, 5-fluorouracil, 5-fluoro-2-deoxyuridine, and miracil D, substantially reduced the incorporation of nucleic acid and protein precursors into perchloric acid/ethanol soluble material.

Since both the radial growth of hyphae and the differentiation of conidia in Cylindrocarpon sp. were inhibited by 8-azaguanine, cycloheximide, 5-fluorouracil, and emetine HCl in a concentration dependent manner, it would appear that the sporulation phenomenon is directly

influenced photochemically rather than being indirectly induced through the influence of light on essential growth limiting reactions. In an attempt to induce sporogenesis in the dark with the use of inhibitors such as cycloheximide and 5-fluorouracil, Hill (88) found that the growth of Aspergillus ornatus could be inhibited as much as 50 percent without the formation of conidia and suggested that "these events are not necessarily related." However, it is likely that the synthetic processes necessary for vegetative growth are also required for conidiation. The relationship between growth limitation and sporulation in light sensitive fungi clearly requires further study.

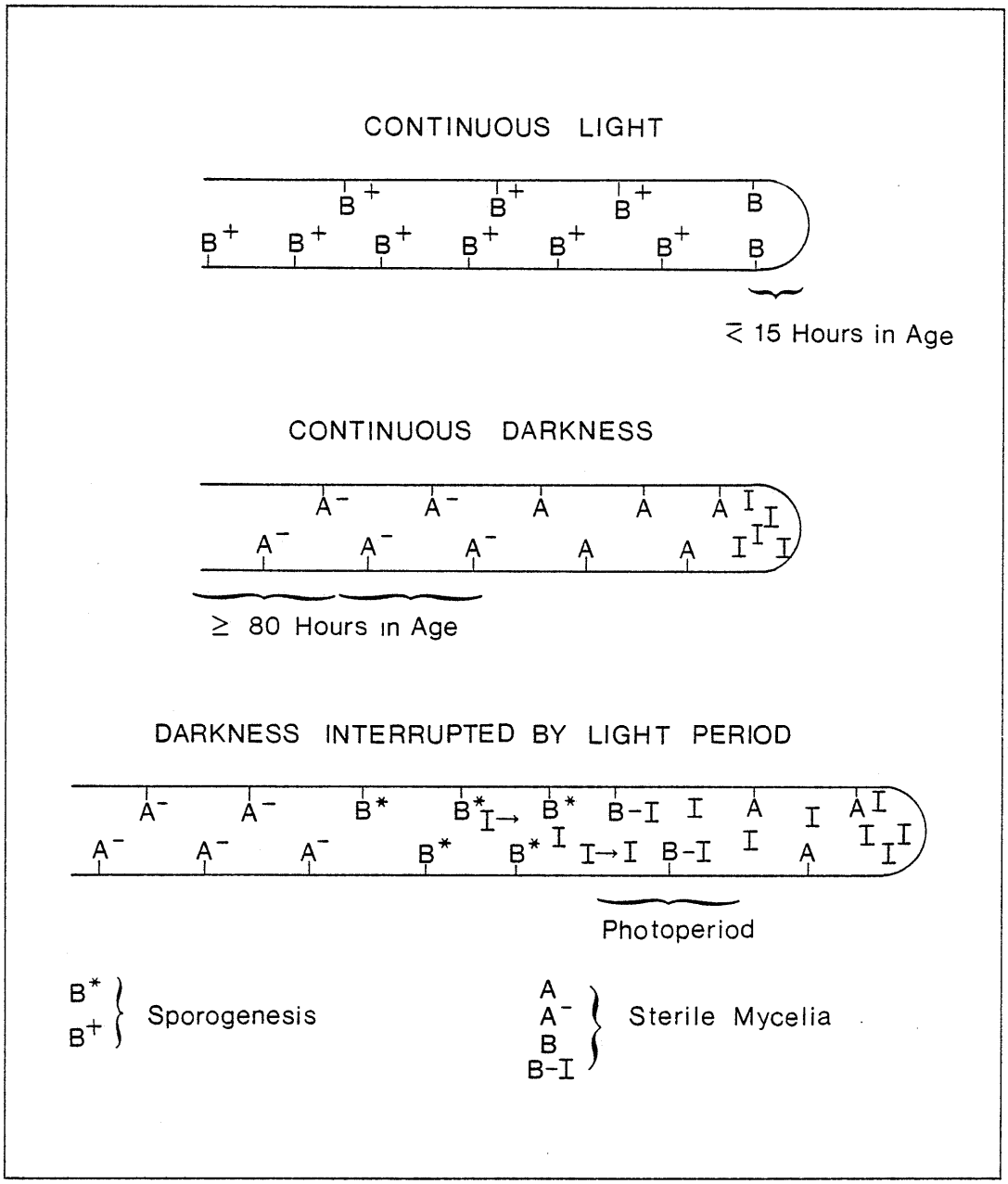
Any theory which attempts to explain light-induced conidiation in in Cylindrocarpon sp. must account for the following responses to phototreatments: (a) failure of conidia to form in cultures grown in continuous darkness from the time of inoculation; (b) the production of conidia from the point of inoculation to within approximately 1.25 mm of the thallus margin in cultures grown in continuous light from the time of inoculation; (c) the development, in response to irradiation periods of less than 120 hours, of a ring of confluent sporodochia and mycelium bearing conidia which corresponds to the peripheral 6.0 mm of hyphae, at the time of irradiation, produced by cultures incubated in darkness for 12 days from the time of inoculation; (d) the development, in response to irradiation periods of 120 hours and 144 hours, of scattered sporodochia on the mycelium generated during the photoinduction period as well as the aforementioned peripheral ring of conidia by cultures incubated in darkness for 12 days from the time of inocula-

tion; and (e) the production, in concentric zones, of mycelia bearing conidia which correspond to the radial growth of the dark period preceding illumination and alternate with rings of sterile, appressed hyphae which correspond to the radial growth in the light in response to repeating cycles of 12 hours of light and 12 hours of darkness.

The simplest explanation for most of these phenomena would be either the formation or destruction during the light period of a metabolite which was either required for, or was inhibitive to, the subsequent biochemical steps which result in the production of conidia. Although transcriptional and translational events would be necessary for synthesizing either a metabolite required for sporulation, or an enzyme responsible for inactivating an inhibitor of sporulation, it is likely that more than one compound is involved. A single compound could hardly account for the observations that hyphae several millimeters behind the growing front lose sensitivity to light, that the light receptive capacity of peripheral hyphae is modified either by prolonged incubation in the absence of light or periodic exposure to light, and that numerous conidiophores bearing conidia are produced by the hyphae of thalli maintained in continuous light, but not by the hyphae generated during photoperiods of less than 120 hours in cultures initially grown in darkness.

Based on hypotheses put forward by Rakoczy, as reported by Rao and Singhal (180), for photoinduced sporulation in Physarum nudum Macbr., by Brandt (18) for zonation in Neurospora crassa strain 21863,

and by Rao and Singhal (180) for photoinduced sporulation in Botryodiplodia theobromae, two hypothetical substances, A and I are proposed as initiators of the biochemical steps culminating in conidiation. Substance A is presumed to possess the following properties: (a) it is bound to the plasmalemma; (b) it is produced in the absence of light during the vegetative phase of growth; (c) upon exposure to light it is converted to B^* and in this form is capable of initiating specific changes which ultimately lead to the development of conidia at the end of the differentiation phase; (d) it undergoes slow degradation in the prolonged absence of light and loses its sensitivity to morphogenetically active radiation during the initial stages of the process (A^- will indicate this form of substance A); and (e) it is synthesized in a modified form, i.e. B, during growth in the light, but substance B is equally sensitive to radiation and is converted in approximately 15 hours to B^+, a form which also initiates the changes ultimately leading to the development of conidia. Substance I is presumed to possess the following properties: (a) it is cytoplasmic, i.e. not membrane bound but translocatable; (b) it is produced in the absence of light during the vegetative phase of growth and is rapidly inactivated; (c) its production is restricted to the apices of hyphae at the periphery of the thallus; (d) it is capable of binding to substance B, forming a B-I complex which is not sensitive to morphogenetically active radiation; and (e) it is not capable of interacting with either substance A, substance A^-, substance B^*, or substance B^+.



Thus in continuous darkness both substance A and substance I are produced, A slowly degrades to A^- as the plasmalemma to which it is bound ages, but prior to degradation maintains the capacity of conversion to B^*, as a consequence of a direct or indirect photochemical reaction, for approximately 80 hours from the time of synthesis.

In continuous light only substance B is produced, which is converted in approximately 15 hours to B^{+} as a consequence of a direct or indirect photochemical reaction.

In cultures incubated in darkness prior to irradiation substance A and substance I would be produced in that portion of the thallus generated in darkness, while substance B would be produced in place of substance A in hyphal elements generated during the photoinduction period. Substance A, bound to the membrane of dark grown hyphae less than 80 hours old, is converted to B^{*} upon illumination. Substance I, translocated from dark grown hyphae to pseudocells developing in the light, interacts with substance B, forming the B-I complex. As a consequence, conidia only develop in the peripheral region of thalli where the substance B^{*} is localized. In cultures exposed to 12 hour cycles of light and darkness, this would correspond to those hyphal elements produced during the 12 hour dark period. Since substance I is synthesized only in the dark and is rapidly inactivated, its concentration as a result of the light interruption would be finite. As a result, during extended periods of irradiation, ie. 120 to 144 hours, minute quantities of substance B could easily escape the interaction of substance I, be converted to B^{+}, and initiate the production of conidia on conidiophores differentiated from hyphae generated during the photoinduction period.

Whether or not this hypothesized mechanism for sporogenesis in Cylindrocarpon sp. is correct, the evolutionary significance of the complex control system which must regulate the asexual reproductive

process in this soil borne fungus is intelligible when the ecological value of producing spores, which serve as agents of dispersal, only in response to visible and near ultraviolet radiation is considered.

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