

## Light-regulated asexual reproduction in *Paecilomyces fumosoroseus*

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The entomopathogenic fungus *Paecilomyces fumosoroseus* has been successfully used in the control of several insect pests. Asexually produced spores (conidia) are the means for dispersal and transmission of the entomopathogen; upon contact with the insect cuticle they germinate and penetrate the host. In model fungal systems it has been found that phototropism, resetting of the circadian rhythm, the induction of carotenogenesis and the development of reproductive structures are controlled by blue light. The effect of light quality on conidial yield of *P. fumosoroseus* was investigated. Incubation in total darkness resulted in continued vegetative growth and lack of reproductive structures. In contrast, growth of the fungus in continuous illumination or under a night–day regime resulted in prolific formation of conidiophores bearing abundant mature conidia. Conidiation was photoinduced in competent mycelia by a single pulse of blue light and colonies were competent only after they had grown at least 72 h under total darkness. The fluence–response curves generated with blue light indicated that the minimal fluence required for the photomorphogenetic response was  $180 \mu\text{mol m}^{-2}$  and the half-maximal response was at  $400 \mu\text{mol m}^{-2}$ . A fluence of  $540 \mu\text{mol m}^{-2}$  was enough to saturate the system, inducing the maximum production of  $2 \cdot 12 \times 10^8$  conidia per colony. Higher light intensities markedly decreased conidiation, suggesting the occurrence of a process of adaptation. The authors propose the existence of a dual light-perception system with at least two photoreceptors in *P. fumosoroseus*, one promoting and one inhibiting conidiation.

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## INTRODUCTION

The soilborne filamentous fungus *Paecilomyces fumosoroseus* is a common insect pathogen that has been isolated throughout the world. Because of its potential to cause epizootics naturally, *P. fumosoroseus* is used for microbial control of insect pests (Jackson *et al.*, 1997). Conidia are often the means for dispersal and transmission of entomopathogenic fungi; they must come into contact with, germinate on, and then penetrate the insect cuticle (James, 2001). However, there is very limited information about conidiation in *P. fumosoroseus*. Understanding the signals and mechanism that determine conidiation of this fungus will also be useful for its production and commercialization as a bioinsecticide. Previously our group reported that conidiation of *P. fumosoroseus* could be promoted by light (De la Torre & Cárdenas-Cota, 1996).

Physiological responses to blue light have been studied in a wide variety of organisms from microbes to animals. Examples include phototaxis in *Euglena* (Kuhnel-Kratz *et al.*, 1993), phototropism in *Avena* coleoptiles (Galland, 2002), induction of carotenoid synthesis in *Neurospora* (Rau & Mitzka-Schnabel, 1985) and entrainment of circadian pupal eclosion in *Drosophila* (Qiu & Hardin, 1996).

Blue light influences fungi in many ways. It can affect metabolism, growth, sexual and asexual development, pigment formation, and tropism, among other phenomena. Photoinduced conidiation (asexual reproduction) of fungi provides an interesting model for biochemical, physiological and morphological studies on differentiation since a relatively simple and natural external stimulus, light, is used to initiate a sequence of molecular events, which ultimately lead to conidiation (Lauter, 1996; Linden *et al.*, 1997).

*Phycomyces blakesleeanus* is best known for its sensitive and precise responses to light (Cerdá-Olmedo & Corrochano, 2001). In this fungus blue light controls sporangiophorogenesis, repressing the production of microsporangiophores and enhancing the development of macrosporangiophores (Maier *et al.*, 2001). White light produces changes in the concentration of some metabolites and alters enzyme activities of the citric-acid cycle and related pathways (Rua *et al.*, 1987). Another well-investigated photoregulated pathway is the accumulation of the yellow pigment  $\beta$ -carotene, which is increased by blue light (Arrach *et al.*, 2001). Photocarotenogenesis does not occur during mycelial growth, but is restricted to a period of competence: the age at which the mycelium is reactive to the light stimulus. The period of competence for carotenoid accumulation and photophorogenesis roughly coincide under the same culture conditions (Corrochano & Cerdá-Olmedo, 1991).

The ascomycete *Neurospora crassa* is considered a paradigm for biochemical, genetic and molecular studies of light responses. Several developmental and morphological processes of *Neurospora* are regulated by blue light (Linden *et al.*, 1997). Light perception in *Neurospora* occurs in the UV–blue light range and induces a wide variety of responses. Early blue light effects include the hyperpolarization of the cell membrane, changes of electrical input resistance (Potapova *et al.*, 1984), and the induction of mycelial carotenoid biosynthesis (Schrott, 1980). Light stimulation of the formation of protoperithecia and macroconidia (photoconidiation; Lauter *et al.*, 1997), phototropism of the perithecial beaks and conidiophores (Harding & Melles, 1983) and light effects on the circadian rhythm (Dunlap, 1999) can be regarded as late light responses, which occur only several hours after light induction (Linden *et al.*, 1997). Recently, He *et al.* (2002) demonstrated that the flavoprotein White Collar 1 (WC-1) is the blue light photoreceptor participating in the control of the circadian clock and other light responses in *Neurospora*.

Another fungal photomorphogenetic model is *Trichoderma atroviride*, in which conidiation is regulated by light (Betina, 1995). Indeed, a brief pulse of blue light given to a round colony of *T. atroviride* induces the formation of a ring of mycelia bearing dark green conidia at what was the colony perimeter at the time of exposure to light (Betina, 1984). Additionally, induction of photolyase gene expression is observed (Berrocal-Tito *et al.*, 1999).

Although the effect of light on conidiation has been documented in a wide variety of fungal species (Lauter, 1996; Linden *et al.*, 1997), there is extremely limited information about light-mediated developmental or other light-regulated physiological responses in *P. fumosoroseus*. We investigated the morphogenetic responses induced by light in this organism. In this study, we report that conidiation in *P. fumosoroseus* is photoinduced by a single pulse of blue light and that the photomorphogenetic response requires a competence period.

## METHODS

**Strain and growth conditions.** *Paecilomyces fumosoroseus* PfrD, obtained from the culture collection of the Centro Nacional de Referencia de Control Biológico de la DGSV-SAGARPA, México, was grown at 27 °C on complete PDYCA medium (per litre: 24 g potato dextrose agar, 2 g yeast extract and 1.2 g Casamino acids; all from Difco). Petri dishes containing 10 ml PDYCA were inoculated in the centre with a 5  $\mu$ l conidial suspension ( $5 \times 10^5$  conidia), wrapped in aluminium foil and incubated at 27 °C for 72 h. Plugs from the growing edge of a colony were then used to inoculate the centre of Petri dishes containing 10 ml fresh PDYCA medium. The plates were wrapped in aluminium foil and incubated at 27 °C until the colonies were competent for photoinduction.

**Photoinduction.** Colonies grown under continuous illumination were incubated at 27 °C in an incubator equipped with GE 20 W fluorescent bulbs positioned 20 cm from the agar surface, where the intensity of the light was about  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The blue light source used consisted of two cool-white fluorescent tubes and a blue filter (Lee 183), with transmission between 400 and 550 nm, peaking at 450 nm, and an intensity of  $3.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Green light was obtained by using a green filter (Lee 124) with transmission between 450 and 600 nm, and the light intensity obtained was  $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ . A red filter (Lee 019) with transmission of 600 and 700 nm was used to obtain red light with an intensity of  $2.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

For light-pulse experiments, cultures were grown for 72 h (unless indicated otherwise) in total darkness at 27 °C. Colonies were then exposed to the indicated light treatments. Plates were opened and the colonies photoinduced. Blue light with a fluence rate of  $12 \mu\text{mol m}^{-2} \text{s}^{-1}$  was obtained with two cool-white fluorescent tubes filtering through a blue filter (Lee 183). Green light with a fluence rate of  $8.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  was obtained using a green filter (Lee 124). A red filter (Lee 019) was used to obtain red light with a fluence rate of  $7.8 \mu\text{mol m}^{-2} \text{s}^{-1}$  and a violet filter (Lee B6) was used for violet light with a fluence rate of  $9.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Total light fluences were 0, 180, 540, 1440, 2880 and  $5760 \mu\text{mol m}^{-2}$ . In all cases, when the light pulses were given temperature was controlled at 27 °C. After light exposure, plates were incubated in the dark for 5 days. Control cultures remained in the dark and all operations requiring darkness were performed under a red safelight ( $0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Radiation intensities (fluence rates) were measured using a Li-Cor LI-189 photometer with a quantum sensor. Four replicate cultures were used for each experiment and each experiment was repeated three times.

**Quantification of conidial yield.** At the indicated times, conidia were scraped from the agar surface and vortexed in sterile 0.05% (v/v) Tween 80 and washed by filtration through cheesecloth and glass wool to separate them from mycelial debris. The number of conidia produced was determined by counting in a haemocytometer using a Leica microscope.

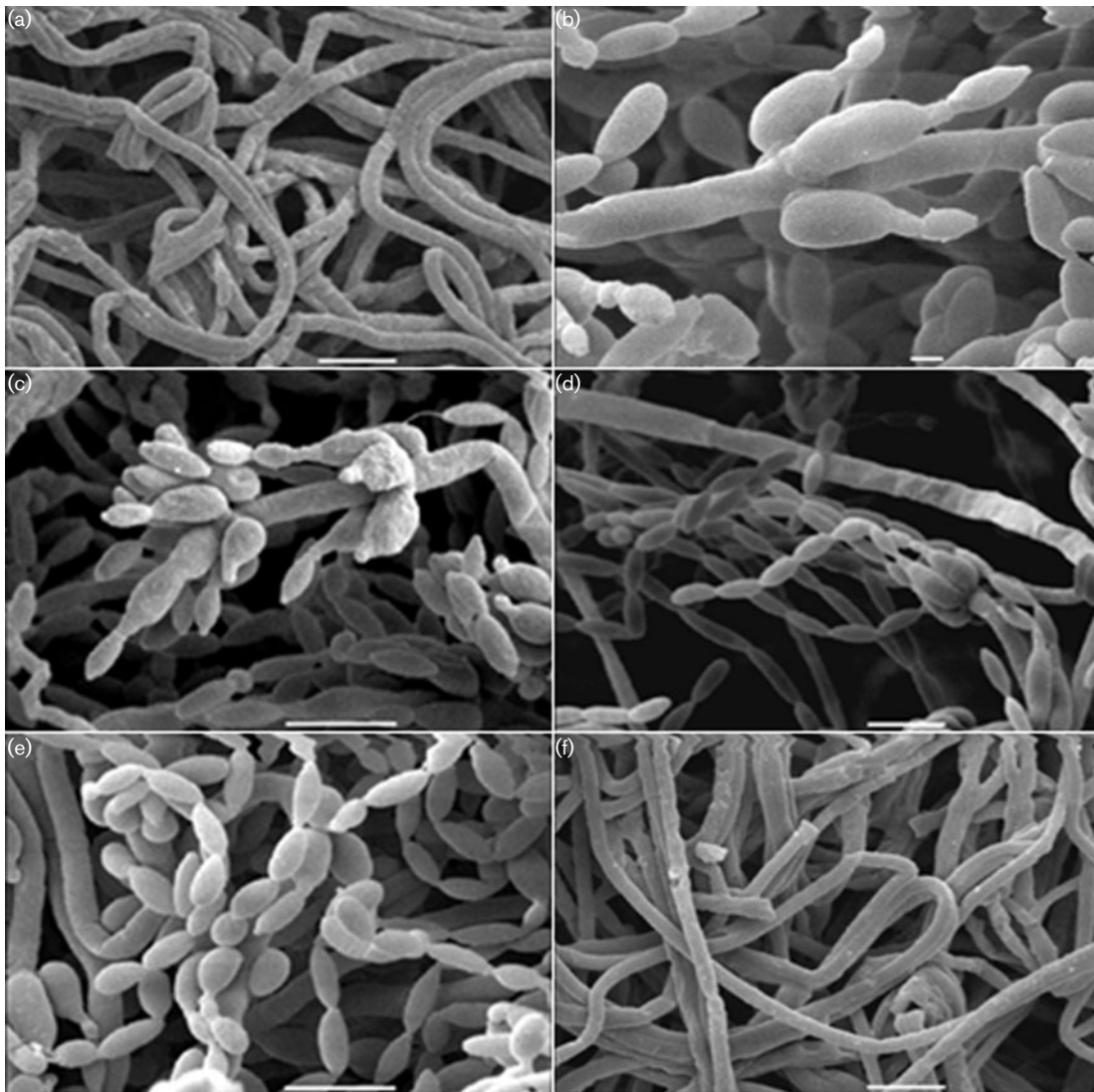
**Scanning electron microscopy.** Microcultures of the fungus were prepared for electron microscopy by growing them on  $1 \text{ cm}^2$  blocks of PDYCA on glass slides and incubated in continuous white light at 27 °C. After 60, 72, 84, 96 and 108 h samples were fixed in 3% glutaraldehyde in 0.1 M potassium phosphate buffer for 2 h at 4 °C. Samples were then rinsed with fresh phosphate buffer, postfixed with 1% OsO<sub>4</sub> in buffer for 2 h, and dehydrated stepwise using increasing concentrations of ethanol (30, 50, 70, 95 and 100%, 15 min per step). The samples were completely air-dried in a hood. Blocks of samples including agar were cut away, mounted on metal stubs, coated with gold and viewed with a JEOL JSM-5410LV scanning electron microscope.

## RESULTS

### Effects of continuous illumination on growth and conidiation of *P. fumosoroseus*

Preliminary experiments suggested that conidiation of *P. fumosoroseus* might be influenced by light. Thus, we designed a series of experiments to determine if indeed light had an effect on conidiation and whether it might also influence growth. Cultures were grown under continuous illumination in an incubator equipped with GE 20 W fluorescent bulbs positioned at a distance of 20 cm from the agar surface, giving a light intensity of  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Controls were grown in the same conditions except that they were kept under total darkness.

Growth of *P. fumosoroseus* under continuous white light resulted in compact and flat colonies with prolific formation of conidiophores bearing abundant and mature conidia. In contrast, incubation under total darkness resulted in profuse growth of aerial hyphae, giving a fluffy appearance to the colonies. This morphology was the result of continued vegetative growth and lack of conidiophores and conidia. The radial growth rate of the colonies exposed to continuous light was about  $0.077 \text{ mm h}^{-1}$ , 7.5% higher than that of the cultures kept in complete darkness. Light-grown colonies were examined by scanning electron microscopy and different stages of development were observed (Fig. 1). Vegetative growth in *P. fumosoroseus* began with the germination of a spore, which led to the formation of a tubular hypha that grew by apical extension and branching



**Fig. 1.** Scanning electron micrographs of developing conidiophores in *P. fumosoroseus*, taken at different times after exposure to light. (a) 60 h; (b) 72 h; (c) 84 h; (d) 96 h; (e) 108 h; (f) Mycelium in total darkness, 108 h. Scale bars: a, c, d, e and f, 5  $\mu\text{m}$ ; b, 1  $\mu\text{m}$ .

to form a mycelial network (Fig. 1a). Under these conditions, certain hyphal elements differentiated and produced aerial conidiophore stalks. After 72 h the stalks had grown away and produced a set of three to four phialides at a given point (Fig. 1b). Conidiophores continued to grow and developed new sets of phialides in a pattern repeated several times along the hypha (Fig. 1c). Apparently conidiophore stalks can form up to three sets of phialides. After 96 h long chains of conidia were produced following multiple mitotic divisions (Fig. 1d). By 108 h the cultures had developed abundant conidiophores and profuse mature conidia (Fig. 1e). In contrast, cultures grown in total darkness did not form reproductive structures even after 108 h of incubation (Fig. 1f). These results show that light has a determinant effect on *P. fumosoroseus* conidiation without greatly affecting vegetative growth.

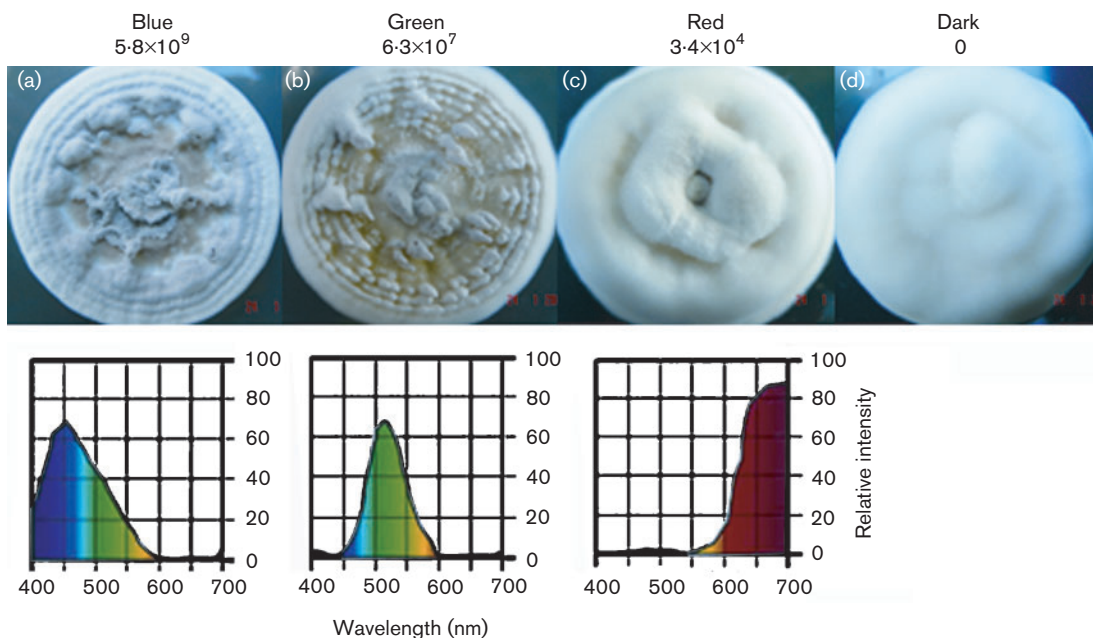
### Conidiation in *P. fumosoroseus* is induced by blue light

Having shown that light triggers conidiation in *P. fumosoroseus*, we investigated the wavelength dependence of this response. *P. fumosoroseus* was cultured under different conditions of illumination, using blue ( $3.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), green ( $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or red ( $2.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) light (Fig. 2). The effects of light quality on the cultures were assessed by recording the visible phenotype observed in the plates after 7 days of incubation and by measuring conidial yield. Similarly to the effect of white light, when blue light was used flat colonies were observed, which

developed abundant conidiophores and produced  $5.8 \times 10^9$  conidia per colony (Fig. 2a). When green light was used the flat colonies formed showed no significant differences in appearance from those grown under blue light (Fig. 2b). Nevertheless, the number of conidia produced in colonies grown under continuous green light was  $6.3 \times 10^7$  conidia per colony, two orders of magnitude less than the number of conidia formed under blue light. Under continuous red light colonies with profuse growth of aerial mycelium were formed and some mycelia clustered in the centre of the colony forming a structure resembling the stem of a tree (Fig. 2c). Under this light condition  $3.4 \times 10^4$  conidia per colony were formed, five orders of magnitude less than the number of conidia produced with blue light. Finally, colonies grown under total darkness showed profuse growth of aerial hyphae, giving a fluffy appearance to the colonies, and lacked conidiophores and conidia (Fig. 2d). Conidiophore development was similar when blue or white light were used. These data suggested that blue light was most effective in inducing conidiation of *P. fumosoroseus*.

### *P. fumosoroseus* responds to light at a specific period of growth

Here we have shown that blue light induces conidiation in *P. fumosoroseus*. However, in other systems the effect of light can be observed only when applied at certain stages of growth. In *T. atroviride*, for example, the capacity to perform the inductive transition from vegetative growth to

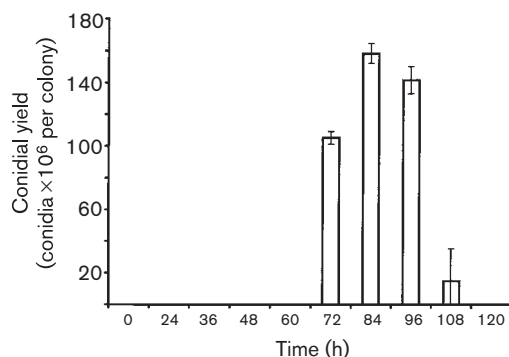


**Fig. 2.** Phenotypes of blue-, green-, red- and dark-grown cultures of *P. fumosoroseus*. (a) Blue light; (b) green light; (c) red light; (d) dark control. Cultures were assayed for conidial yield per colony at 7 days after inoculation. Conidial yield, indicated above the photographs, represents the mean of five independent trials. The transmission spectra of the filters are shown in the bottom panels.

sporulation seems to be restricted to a certain state of maturity of the fungal colony. Gressel & Galun (1967) reported that the fungus was capable of responses to light induction only after it had grown for over 16 h. On the other hand, photomorphogenesis in *Phycomyces* requires a competence period. Corrochano & Cerdá-Olmedo (1990) observed that the maximal response was found when a culture was exposed to a light pulse after 48 h of growth; however, the cultures exhibited some competence to respond to light over a period of about 35 h. Thus, experiments were carried out to determine if such a period of 'competence' exists in *P. fumosoroseus* and if a light pulse would be sufficient to induce sporulation.

In order to standardize the inductive system, colonies were exposed to 5 min blue light pulses at 0, 24, 48, 60, 72, 84, 96, 108 and 120 h of growth, at a fluence rate of  $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Before and after the light pulse colonies were kept under total darkness.

The critical period during which *P. fumosoroseus* must be exposed to the light pulse to induce conidiation is shown in Fig. 3. A pulse of blue light given to mycelia at any time between 72 and 108 h of growth resulted in a high production of conidia, reaching approximately  $10^8$  per colony. In contrast, light pulses given before 60 or after 120 h of growth failed to induce conidiation. Under those conditions, the number of conidia formed was several orders of magnitude lower or undetectable and the colony phenotype was disperse and fluffy, as in cultures grown under total darkness. When the colonies were exposed to the light pulse after 60 h,  $3 \times 10^3$  conidia per colony were produced. Thus, continuous exposure to light is not required, and at least 72 h of growth is required before a colony is fully competent to respond to the light stimulus. Development of conidiophores after light induction was



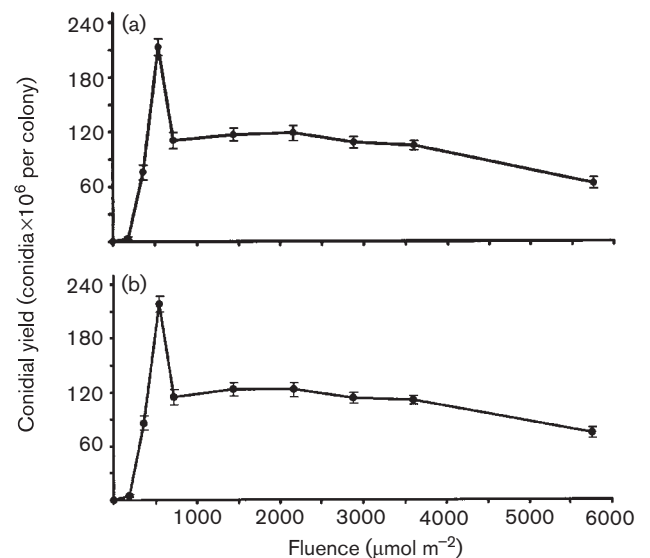
**Fig. 3.** Determination of the critical period for *P. fumosoroseus* light responsiveness. Colonies grown under total darkness for the indicated times were exposed to a 5 min broad-spectrum blue light pulse at different times after incubation, as indicated. Colonies were returned to the dark for 5 days and used to determine conidial yield. Each point is the mean of three experiments with four replicates. Bars indicate SD.

morphologically and chronologically similar to that of cultures grown under continuous illumination or light-dark photoperiods. The initial conidiophore-specific structures could be observed microscopically about 72 h after photoinduction with a light pulse and mature conidia appeared approximately 108 h later. Thus, in all further experiments cultures were photoinduced after 72 h of growth.

### Fluence required for photomorphogenesis in *P. fumosoroseus*

Because a pulse of light was sufficient to induce conidiation in competent mycelia, it was important to determine the qualitative and quantitative characteristics of the signal required to trigger such a photomorphogenetic response.

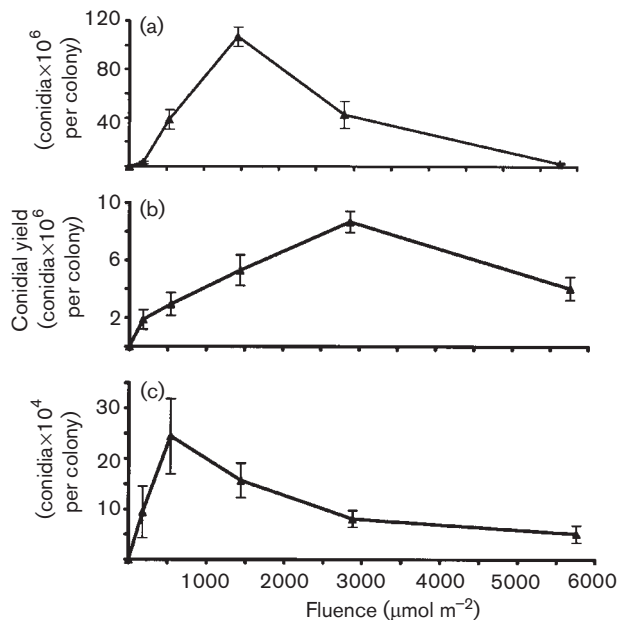
To determine the minimum light fluence exposure required for inducing conidiation, competent colonies of *P. fumosoroseus* grown for 72 h under total darkness were photoinduced with broad-spectrum blue light pulses of different duration. Exposure to fluences of  $360\text{--}720 \mu\text{mol m}^{-2}$  induced conidiation (Fig. 4a). With these blue light pulses the expected photomorphogenetic response was observed: branching of aerial hyphae was evident at 72 h after light exposure, and the hyphae eventually differentiated to conidiophores and produced mature conidia. Colonies that received a blue light pulse of  $540 \mu\text{mol m}^{-2}$  produced the maximum conidial yield under our experimental conditions ( $2.12 \times 10^8$  conidia per colony), as compared with those receiving 180 or  $360 \mu\text{mol m}^{-2}$  ( $3.6 \times 10^6$  and



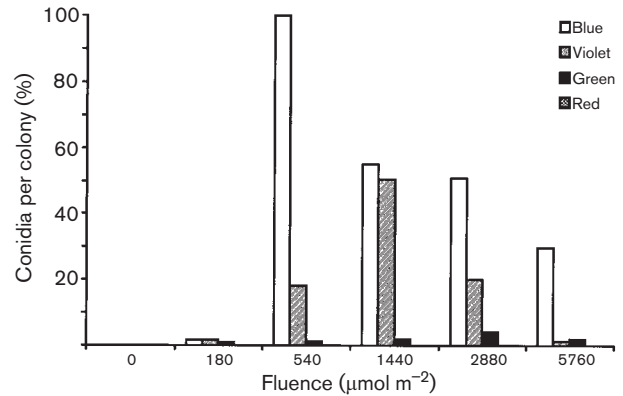
**Fig. 4.** Fluence–response curve of blue or white light in *P. fumosoroseus*. Colonies were exposed to pulses of broad-spectrum (a) blue or (b) white light for different times to obtain different fluences and assayed for conidial yield per colony 5 days after illumination. Each point is the mean of three experiments with four replicates. Bars indicate SD.

$7.6 \times 10^7$  conidia per colony, respectively). Although the initial developmental stage observed after 72 h was about the same using low or high fluence pulses, 5 days after the light pulse, the colonies receiving saturating light had produced far more conidia than those exposed to 180 or  $360 \mu\text{mol m}^{-2}$  (Fig. 4). Increasing the light fluence to  $720 \mu\text{mol m}^{-2}$  or above reduced conidiation, resulting in conidial yields per colony of about 40% of the maximum obtained. No obvious differences were observed if white light of the same intensity was used instead of blue light (Fig. 4b).

Competent mycelia were subjected to different treatments using blue ( $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), green ( $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), red ( $7.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or violet ( $9.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) light to obtain a preliminary, broad-band action spectrum for photoinduced conidiation. A collection of fluence–response data from experiments using the different filters at fluences of 0, 180, 540, 1440, 2880 and  $5760 \mu\text{mol m}^{-2}$ , with subsequent 5 day incubation under total darkness are shown in Figs 5 and 6. As described above, when a blue light pulse was used (Fig. 4) the maximum response was obtained with a fluence of  $540 \mu\text{mol m}^{-2}$ , with  $2.12 \times 10^8$  conidia per colony produced. When violet light pulses were used, the maximum response was obtained with a fluence of  $1440 \mu\text{mol m}^{-2}$ , producing  $1 \times 10^8$  conidia per colony (Fig. 5a), about 50% of the maximum response obtained with blue light (Fig. 6). With green light pulses, the maximum yield of conidia observed was  $8.6 \times 10^6$  conidia



**Fig. 5.** *P. fumosoroseus* fluence–response curves using different types of light. Colonies were irradiated by broad-spectrum (a) violet (b) green or (c) red light at different intervals of fluence. Each point is the mean of three experiments with four replicates. Bars indicate SD.



**Fig. 6.** Comparison of maximum conidial yield per colony using different types of light in *P. fumosoroseus*. The graph shows conidial yield relative to the maximum obtained using blue light. Each point is the mean of three experiments with four replicates. The values for red light are too low to be visible on the scale plotted.

per colony using a fluence of  $2880 \mu\text{mol m}^{-2}$  (Fig. 5b), corresponding to only 4% of the maximum obtained with the blue light pulses (Fig. 6). Finally, pulses of red light resulted in about  $2 \times 10^5$  conidia per colony (Fig. 5c), corresponding to only 0.11% of that induced by blue light (Fig. 6). As in the case of blue light, exposure to fluences above the value where the maximum response was obtained resulted in reduced conidiation for all types of light used.

## DISCUSSION

*Paecilomyces fumosoroseus* is a promising microbial alternative to chemical insecticides, but further investigations are needed to understand its reproductive life in order to design improved production strategies. The studies described here clearly demonstrate that conidiation in *P. fumosoroseus* is highly light dependent. Kumagai (1978) classified fungi into three types based on the type of light–dark cycles necessary for conidiation. The first type is characterized by its requirement of light for conidiophore induction and the suppression of conidial development by light. In the second type, light is not required for induction of conidiophore formation but conidial development is suppressed by blue light. In the third type, light is required for the induction of conidiophores but conidial development is not suppressed by light. *P. fumosoroseus* also required light for production of conidiophores and conidial development is not suppressed by low light intensities, but it is suppressed by high light intensities, suggesting that *P. fumosoroseus* might represent a novel fourth type of fungus.

We have shown, as has been reported for *N. crassa* (Lauter & Russo, 1991; Linden *et al.*, 1997) and *T. atroviride* (Kumagai & Oda, 1969; Betina, 1995; Sulová & Farkas, 1991), that conidiation in *P. fumosoroseus* is photoinduced by blue

light. A brief pulse of blue light was sufficient to induce conidiation. The developmental process of conidiation starts with the formation of aerial hyphae, which were microscopically detectable 72 h after photoinduction, and mature conidia appear 108 h after light exposure. The effectiveness of blue light in the photoresponse in *P. fumosoroseus* is not surprising. The vast majority of photoresponses, from growth responses to phototropism, studied in fungi are mediated by photoreceptors that absorb blue light (Kumagai, 1988; Lauter, 1996).

Colonies grown under green and red light produced conidia; this could be explained by postulating the existence of photoreceptors responsive to green and red light in this fungus. Ninnemann (1991) suggested that various photoresponses might be mediated by multiple photoreceptors in *Neurospora*. Indeed, WC-1 was shown to be the first fungal blue light photoreceptor (He *et al.*, 2002), and recently Schwerdtfeger & Linden (2003) showed that VIVID is a second blue light photoreceptor which enables *Neurospora* to perceive and respond to daily changes in light intensity. Furthermore, analysis of the gene set obtained from the genome sequence of *Neurospora* allowed the identification of genes potentially associated with red light perception (Galagan *et al.*, 2003). In addition, conidiation in the fungus *Aspergillus nidulans* is induced by red light and suppressed by an immediate shift to far red light, where a red light photoreceptor whose properties are reminiscent of phytochrome would be involved in these responses (Mooney & Yager, 1990). In *P. fumosoroseus* the fluence–response curves obtained with blue and white light were nearly identical (Fig. 4) and conidiation induced by either red or green light reached much lower levels. Thus, we conclude that light-induced conidiation is mediated by a blue light receptor.

In several fungi, light regulates conidiation in a circadian fashion. By definition a rhythm is circadian if it is maintained under constant environmental conditions and has a period of approximately 1 day. In addition, the period of a circadian rhythm remains unchanged over a broad, physiologically appropriate, temperature range. The circadian clock has been intensively studied in *N. crassa* (Lakin-Tomas *et al.*, 1990; Dunlap, 1999; Loros & Dunlap, 2001). During vegetative growth on an agar surface the clock initiates macroconidiation in mid-subjective-night, budding and giving rise to conidiophores. Some time later, during the subjective day, this developmental cycle is turned off and fungal growth continues as undifferentiated vegetative hyphae. The cycle persists, with 22 h periodicity in constant dark at 24 °C (Pittendrigh *et al.*, 1959). In *P. fumosoroseus* we observed concentric circles of conidia during its growth on an agar surface even under constant light exposure; this phenotype suggests that conidiation could have circadian characteristics, although our data are not sufficient to confirm this hypothesis. However, it is clear that conidiation can be induced and synchronized by a light pulse. The possibility of synchronizing the

development of conidiation by a light pulse may be of significance for the study of biochemical reactions inducing asexual differentiation.

As previously shown for *Phycomyces* (Corrochano & Cerdá-Olmedo, 1990) and *T. atroviride* (Gressel & Galun, 1967), the mycelium of *Paecilomyces fumosoroseus* requires a certain growth period to reach photocompetence in terms of conidiation. While *Trichoderma* responds to light after only 16 h of growth and *Phycomyces* presented the maximal responses when cultures were exposed to a light pulse after 48 h of growth, *P. fumosoroseus* required longer growth periods (72 h) to respond. Our data indicate the existence of a developmental window for the production of conidia in response to light, in which the ability to respond to light is apparently restricted to a certain state of maturity of the colony. A similar situation has been reported for *Trichoderma*, where the existence of such a developmental window has been postulated to correlate with the accumulation of metabolites of an as yet undetermined nature (Gressel & Galun, 1967).

The fluence–response curves generated with blue light indicated that the minimal fluence required for the photomorphogenetic response in *P. fumosoroseus* was  $180 \mu\text{mol m}^{-2}$  and the half-maximal response was  $400 \mu\text{mol m}^{-2}$ . A fluence of  $540 \mu\text{mol m}^{-2}$  was enough to saturate the system. This value is comparable with conidiation of *Trichoderma*, which requires  $480 \mu\text{mol m}^{-2}$  (Horwitz, 1984), and photocarotenogenesis in *N. crassa* (Schrott, 1980). No further increase of conidiation was induced when illumination was increased up to  $720 \mu\text{mol m}^{-2}$ , and at higher fluence rates conidiation markedly decreased. In *T. atroviride* 1 min (fluence of  $600 \mu\text{mol m}^{-2}$ ) with daylight was sufficient to induce the maximum production of conidia but prolonged irradiation (above  $3600 \mu\text{mol m}^{-2}$ ) also inhibited conidiation (Betina, 1984). The cause for this inhibition is unknown, but it could be related to other known effects of near-UV light on fungi, such as inhibition of protein synthesis. However, as shown in Fig. 5, the same phenomenon is observed even with red light, suggesting that inhibition of conidiation is not a consequence of exposure to near-UV light, although it is likely that such phenomena occur in *P. fumosoroseus*. Based on the observation that the relative effectiveness of the different wavelengths is fluence dependent (Fig. 6), even below saturation, we favour the occurrence of a process of adaptation taking place at high light intensities. Such an adaptation mechanism has been described for sporangiophore movement in *Phycomyces* (Bergman *et al.*, 1969; Galland & Lipson, 1987; Corrochano *et al.*, 1988) and explained by the presence of multiple photoreceptors. Similarly, in *N. crassa* the photoreceptor VIVID is not required initially for light perception, but plays a crucial role in the light response to increasing light intensities following a primary light response (Schwerdtfeger & Linden, 2003). We thus suggest the existence of a dual light-perception system with at least two photoreceptors in

*P. fumosoroseus*, with one promoting and one inhibiting conidiation.

The relative effectiveness observed with the different wavelengths used, together with the data available on other blue light sensors (Lauter, 1996; Linden *et al.*, 1997; Briggs & Christie, 2002; Gyula *et al.*, 2003), including the *N. crassa* WC-1, involved in the control of the circadian clock and other light responses (He *et al.*, 2002), allows us to suggest that photoconidiation in *P. fumosoroseus* is mediated by a flavin-binding protein. In this context, it is worth mentioning that we have recently cloned a gene fragment encoding a deduced protein sequence with high similarity to WC-1, but further studies are needed to determine if this protein is the light receptor involved in induced photoconidiation in *P. fumosoroseus*.

In nature, successful dissemination requires the production of abundant reproductive structures upon exposure of the organism to advantageous environmental conditions. Here we have shown that light plays a critical role in regulating asexual reproduction of *P. fumosoroseus*. It is therefore not surprising that exposure to light, signalling exit from the soil and entry into the air, induces asexual development.

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