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Long-lasting effects of red and blue light exposure on the growth of soil fungi

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ABSTRACT The experimental assessment of inter-species difference in long lasting effects produced in fungi by a brief exposure to the monochromatic light was performed. 24-h cultures grown from 1 mm mycelium fragments of *Alternaria alternata*, *Aspergillus clavatus*, *Fusarium fujikuroi*, *Penicillium citrinum* and *Trichoderma viride* were exposed for 30 min to blue light (BL, 450 nm) or red light (RL, 660 nm) and cultured for the next 10 days. Radial growth rate, conidial yield and germination, contents of proteins and phenolsand fungal antibacterial activity were estimated. BL- or RL-exposure did not essentially affect the final size of colonies of *A. clavatus* but delayed the growth of *P. citrinum* and stimulated it in *A. alternata* and *F. fujikuroi*; these changes were more profound after BL, than after RL. In *T. viride* the BL exposure led to a remarkable delay of growth, whereas the RL significantly increased the growth rate. Photo-induced changes in the conidial yield, conidial germination, contents of proteins and phenols also were dependent on the light wavelength and showed strong inter-species heterogeneity. Fungal antibacterial activity in exposed cultures was similar to the unexposed control. The observed effects are indicative targets for future research of possible molecular regulatory mechanisms underlying the photobiology in different fungal taxons.
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Introduction

Illumination is an important environmental factor for soil microscopic fungi and has an essential impact on their survival. By contrast to phototrophs, fungi use the light not as a source of energy, but as a trigger or regulatory signal which influences their growth and behavior. In ecological context, the light helps the mycelium to discriminate between different habitats, thus increasing their competitiveness against other soil microbiota (Fuller et al. 2015). Mechanisms of sensing and further transmission of the light signals in fungi includes complex molecular network, which ends up in differential gene expression and metabolic changes (Idnurm and Heitman 2005; Purschwitz et al. 2006; Corrochano 2007, 2011, 2019; Yu and Fischer 2019). A lot of data has been accumulated regarding the effects produced by the ultraviolet (UV), blue, green or red light in fungal species belonging to different systematic groups and their respective molecular machinery of the light reception has been extensively explored (Laringuet and Dunand 2005; Purschwitz et al. 2006, 2009; Herrera-Estrella and Horwitz 2007; Kritsky et al. 2010; Tisch and Schmoll 2010; Corrochano 2011).

Reports and reviews cited above shows that physi-

ological responses of fungi to the light exposure comprise changes in their growth, conidiation, sexual development, spore release, phototropism and shifts in their circadian clock. A number of photo-induced effects were detected at subcellular and biochemical levels, e.g. hyperpolarization of the cell membrane, alterations in intracellular levels of energy carriers, changes in the oxygen consumption rate, carbon and sulphur metabolism, modulations of enzyme activity and biosynthesis of various products, and response to oxidative stress. At the genomic level hundreds of light-regulated genes were identified in fungi, and many of these genes are related to stress responses (Herrera-Estrella and Horwitz 2007; Schmoll et al. 2010; Lokhadwala et al. 2015; Brancini et al. 2019).

Mechanisms of photoreception and responses to the light exposure were extensively explored in different taxons of Kingdom Fungi. There are plenty of publications presenting data on light-induced effects and light sensors in one particular fungal genera, e.g. *Aspergillus* (Hill 1976; Kato et al. 2003; Sprote and Brakhage 2007; Purschwitz et al. 2009; Bayram et al 2010), *Alternaria* (Soderhall et al. 1978; Igbalajobi et al. 2019), *Botrytis* (Canessa et al. 2013; Schumaher 2017), *Fusarium* (Myung et al. 2009; Avalos and Estrada 2010), *Metarhizium* (Rangel et al. 2011, 2015; Brancini et al. 2016, 2018a; Oliveira et al. 2018; Dias et al.

2020), *Trichoderma* (Betina and Zajacova 1978; Strigácová et al. 2001; Pokorný et al. 2005; Schmoll et al. 2010), or broader systematic groups, like *Zygomycota* (Corrochano and Garre 2010). The photoresponsive proteins appeared to be highly conserved across the entire fungal kingdom, but different taxons may potentially show very divergent growth or biochemical responses to the same light signal. However, there is a lack of reports in modern literature containing a direct comparison of photoreactions assessed in two or more fungal species within one experiment.

Also, in a number of studies, photoinduced responses were measured after constant or protracted exposure of fungi to the light of various wavelengths. By contrast, much less data are available on the long lasting or delayed responses occurring after brief exposure, while such 'memory'-based effects in fungi has been known from classic studies (Zalokar 1955; Betina and Zajacova 1978; Schrott 1980) and confirmed in more recent experiments (Schmoll et al. 2010; Brancini et al. 2016, 2018a, 2019). And again, there is a gap in current knowledge regarding the species-related peculiarities of gross photoinduced reactions in such experimental conditions.

Therefore, the aim of the present research was to assess long lasting responses, including changes in the growth rate and basic biochemical and physiological characteristics, in soil microscopic fungi from different taxonomic groups after a relatively brief exposure to the light of different wavelengths.

Materials and methods

Light exposure and culturing of fungi

The strains of microorganisms for this experiment were obtained from the microbiological collection hold at the Department of Physiology and Biochemistry of Plants and Microorganisms of the School of Biology of V. N. Karazin Kharkiv National University (Kharkiv, Ukraine). Five fungal species – *Aspergillus clavatus* Desm., *Alternaria alternata* (Fr.) Keissl., *Fusarium fujikuroi* Nirenberg, *Penicillium citrinum* Thom and *Trichoderma viride* Pers., and two bacterial species – *Escherichia coli* and *Bacillus subtilis* were used in the study.

Microscopic fungi were grown on Petri dishes on the solid medium comprising non-boiled wort with 1.5% agar. Cultures were initiated by placing 1 mm fragment of mycelium into the center of Petri dish with 20 mL of solid medium and incubated in darkness at 22 ± 1 °C for 24 h. After that cultures were exposed for 30 min to the monochromatic light generated by the light diode matrix array (Korobov and Korobov 2012). The matrix array had dimensions 190 × 98 mm and contains 24 light diodes of total power 120 MW, providing the output light flux

density of 6444 MW×m^{-2}. The distance from the light matrix to culture surface was 15 cm. Two arrays of diodes emitting different wavelengths were used: Red light (RL) of 660 nm, or blue light (BL) of 450 nm. Control cultures were sham exposed; for that cultures were kept wrapped in a foil and light source was not turned on. The day of exposure was set as day 0 in the experimental time scale. After the light exposure or sham exposure, cultures were returned to the incubator and kept in darkness at 22 ± 1 °C for the next 10 days.

Cultures on liquid medium were set up from exposed or sham exposed fungi grown on solid medium, when their colony diameter reached 20 mm (3 to 5 days of culturing). A round shape fragment of 10 mm diameter was cut out from the center of the fungal lawn, placed into a glass flask with 200 mL non-boiled wort supplemented with 4% sugars and cultured in darkness at 22 ± 1 °C.

Measurements of physiological and biochemical parameters

The diameter of growing fungal colonies was measured periodically, with 24 h intervals for 10 days, starting from day 1 after the light exposure. The number and size of conidia were estimated at the stage of active sporogenesis, at 5 to 7 day of culturing depending on the strain (Bilay 1982). A round shape fragment of 10 mm diameter was cut from the solid lawn of the fungal culture, placed into a flask with 100 ml distilled water and shaken intensively for 15 min. A drop of the resultant suspension was analyzed in a cell-counting chamber (hemocytometer) using a light microscope. The number of conidia per 1 ml was estimated. Simultaneously, the linear size (diameter or length) was measured using eyepiece micrometer in 50 conidia randomly chosen in different fields of view. In *F. fujikuroi* the number (contents) and the length of macrconidia were assessed.

For conidium germination assay, 100 µl of conidiospore suspension (1 × 10^7 conidia × ml^{-1}) was inoculated evenly onto Petri dishes with solid medium and incubated in darkness at 22 ± 1 °C as described above. The number of colonies was counted on 2 to 4 days of culturing.

Samples from liquid cultures were taken at the stage of active sporogenesis and used for measurements of protein and polyphenol contents in the mycelium. For this, Lowry method (Waterborg and Matthews 1994) and Löwenthal method (Protsenko and Kostina 2015; Ma et al. 2019) were applied, respectively.

The antagonistic properties of microscopic fungi against bacteria were assessed using the technique of counter co-culture on agar plates (Bilay 1982). Fungal cultures were exposed to RL or BL in 24 h after their mycelium fragment inoculation, as described above. The inoculation of test bacteria *E. coli* or *B. subtilis* into counter

co-cultures was carried out by a needle-dash technique, taking samples from their cultures grown for 24 h on Nutrient Agar (Himedia Laboratories, India). Fungal antagonistic action towards bacteria was estimated by periodic visual examination of the cultures. The effects were assessed as:

- Total oppression (TO), if the growth of test bacteria was completely stopped;

- Active antagonism (AA), if test bacterial colonies displayed a remarkable shrinkage compared to control, and a fungal lawn proliferated over the bacterial side of Petri dish;

- Passive antagonism (PA), if a visible delay in bacterial growth occurred, but without hyperexpansion of fungi;

- Neutralism, if no remarkable impact on the test bacteria was observed.

Statistical analysis of the data

The experiment was performed thrice. In each independent repeat three identical replicates of fungal cultures on solid or liquid media were set up. All endpoints were measured in each of these replicates. The values of the measured parameters in three replicates within each experiment were pooled together and their mean was calculated. Means from three independent experiments were averaged, and the respective standard error (SE) for the overall mean was estimated.

For each fungal species the patterns occurred in the experimental series of RL- or BL-exposure were compared to that observed in sham exposed, control series. Means with associated SE were compared using Student's *t*-test, considering the difference significant at $p \leq 0.05$ for 4 degrees of freedom (d.f. $= n_1 + n_2 - 2 = 3 + 3 - 2 = 4$).

Also, for demonstration purposes, conidial yield, size of conidia and conidial germination data in RL- or BL-exposed fungal cultures were normalized as a percent of the respective control values in sham-exposed cultures. In this case, SE for the mean were calculated from the dispersion of the 'exposed to control' ratios in three repeats of the experiment (n = 3).

Regression analysis of the time-effect relationship for radial growth of fungal cultures from day 1 to day 10 after exposure was performed using the respective option of the Microsoft Excel ™ package. Fitting of the data to sigmoid curve included the intermediate step of double logarithming of the colony size values.

Results

The radial growth of different fungal species after the exposure to RL or BL is presented on Fig. 1 (A-E). The sizes of fungal colonies at the moment of their expo-

sure to the light (day 0) were too small for any accurate measurement. Starting from day 1 after exposure the changes of the colony diameter followed, as expected, a sigmoid curve with an elongated left part. The data on the colony growth between day 1 and day 10 were fitted to the equation:

$$S(t) = A \times e^{(1 - e^{b - c \times t})}$$

where *S* is the size of the colony (diameter in mm), *t* is time in days, *A* is the asymptote of the colony growth, *e* is the natural logarithm base, *b* and *c* are regression coefficients. In this formula the coefficient *c* corresponds to the growth rate and the coefficient *b* determines the virtual "starting constant" for the size of the colony, i.e. the overall vertical shift of the curve from the baseline zero value, which is intuitively understood and easy to compare between experimental series. The estimated growth parameters are given in Table 1.

The response to the light exposure appeared to be rather heterogeneous between species. *A. clavatus* after either RL or BL exposure started growth from smaller colonies in compare with control (Fig. 1A). The difference in colony diameter became statistically significant between BL-cultures and control on days 3 (t = 3,51; p<0,05) and 4 (t = 3,79; p<0,05). However, in the interval between days 4 and 6 the growth rate of the exposed colonies increased, and their diameter became similar to control values till the end of the culturing period.

The growth of *P. citrinum* initially was delayed in BL-cultures: on day 1 the difference with control was significant (t = 3,27; p<0,05) (Fig. 1B). Due to the similar growth rate the smaller colony size in BL-cultures persisted till the end of the experiment; the difference with control was significant on day 9 and day 10 (t = 2,81 and 2,91, respectively; p<0,05 in both cases). In RL-cultures the starting size was similar to that in control; however, the growth rate was lower, therefore colonies had smaller diameter on day 5 and later on; on day 10 the difference between RL-cultures and control became meaningful (t = 2,91; p<0,05).

The remarkable delay at the start of growth was found in BL-cultures of *T. viride*: for the colony size on day 1 *versus* control t = 4,62 (p<0,01) (Fig. 1C). The growth rate was not affected by BL exposure, therefore the diameter of colonies in BL-cultures remained significantly smaller than in control during all the experiment, e.g., t =4,59 (p<0,05) on day 2, t = 5,68 (p<0,01) on day 4, t = 3,24 (p<0,05) on day 10. By contrast to that, RL produced no effect on the starting size but enhanced a growth rate of *T. viride*, so that RL-cultures had continually larger colonies than control cultures, especially in the second half of the
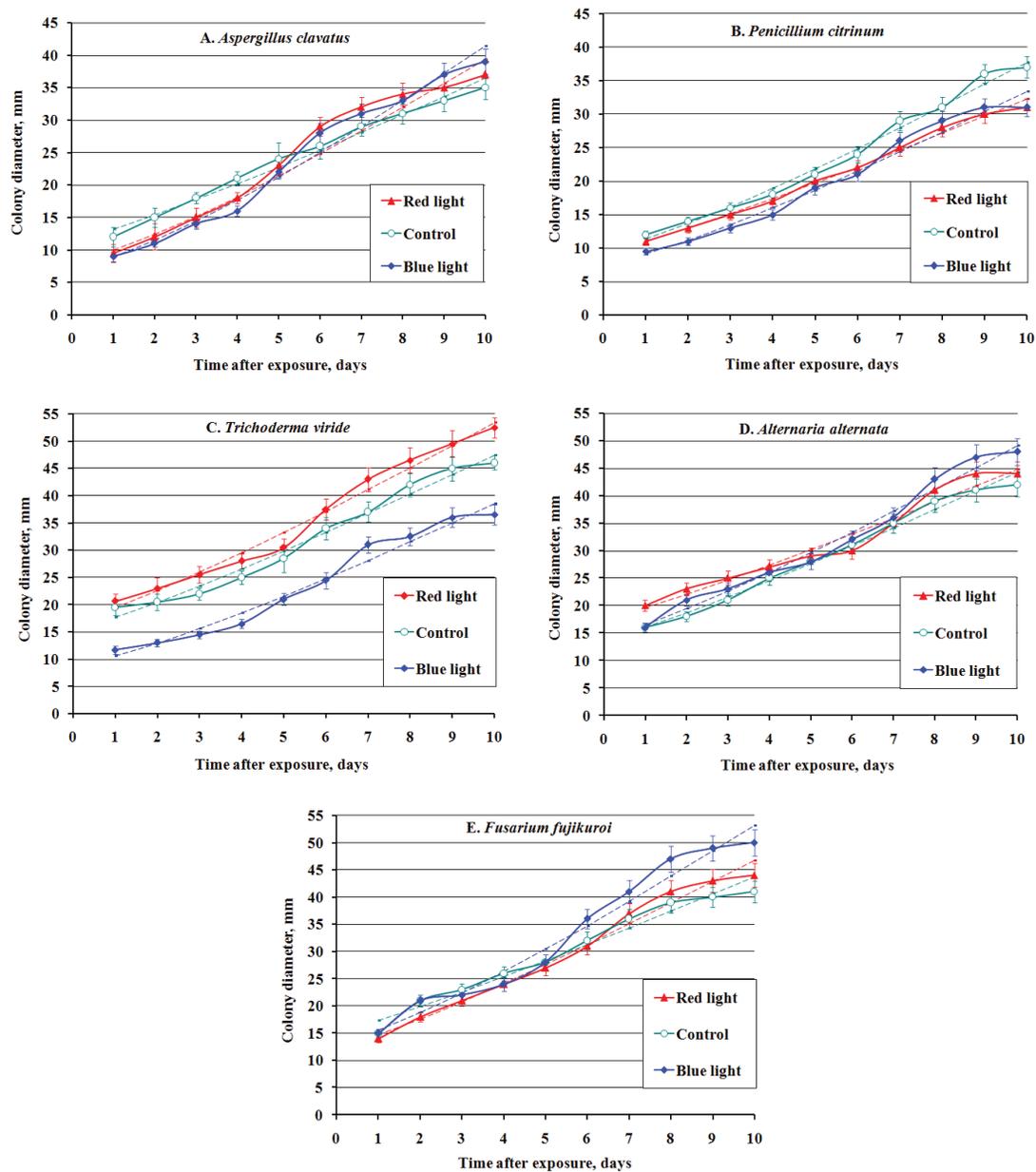
Figure 1. Time course of radial growth of different fungi species in cultures grown after brief exposure to red or blue monochromatic light. A: *Aspergillus clavatus*; B: *Penicillium citrinum*; C: *Trichoderma viride*; D: *Alternaria alternata*; E: *Fusarium fujikuroi*. Filled triangles: cultures exposed to red light. Filled diamonds: cultures exposed to blue light. Open circles: sham-exposed, control cultures. Dashed lines: growth predicted by the model (Equation 1 and estimated coefficients are given in Table 1).

experiment; on day 10 this difference became significant (t = 2,90; p<0,05). The size of colonies in RL-cultures remarkably exceeded that of in BL-cultures either on day 1 (t = 5,76; p<0,01) or day 10 (t = 5,00; p<0,01).

In *A. alternata* the size of colonies in BL-cultures was statistically indistinguishable from that in control, including the last day (t = 1,88; p>0,05) (Fig. 1d). RL-cultures had a noticeable increase of the starting size in compare

to control (t = 3,12 p<0,05) on day 1. However, the growth rate of RL-cultures was slightly lower, therefore at the end of the observation period the colony size difference with the control diminished.

The dynamics of culture growth in RL-cultures of *F. fujikuroi* overlapped with that in control cultures (Fig. 1e). The growth of BL-cultures had a starting index similar to the control value, but the higher rate, that lead

Table 1. Growth parameters of fungi cultured after exposure to the light of different wavelength.

Exposure conditions	*A. clavatus*	*P. citrinum*	*T. viride*	*A. alternata*	*F. fujikuroi*
Regression free coefficient (b*)					
Control	0.759	0.868	0.749	0.754	0.697
Red light	0.929	0.790	0.769	0.662	0.826
Blue light	1.012	0.896	0.903	0.811	0.868
Growth rate coefficient (c*)					
Control	0.080	0.089	0.078	0.081	0.076
Red light	0.103	0.083	0.079	0.068	0.089
Blue light	0.107	0.097	0.096	0.083	0.,093
Asymptote (A*)					
Control	35	37	46	42	41
Red light	37	31	52	44	44
Blue light	39	31	37	48	50
Modeled, virtual "starting constant" of the colony size (mm**)					
Control	11.24	9.28	15.09	13.63	12.11
Red light	8.04	9.31	16.51	17.22	12.57
Blue light	6.78	7.28	8.53	13.77	12.18

* = coefficients are estimated for the fungal colony growth formula $S(t) = A \times e^{(1-e^{-b-c \times t})}$, where S is the size of the colony (diameter in mm), t is time in days, A is the asymptote of the colony growth, e is the natural logarithm base, b and c are regression coefficients.
** = virtual "starting constant" of the colony size is calculated for $t = 0$.

to the meaningful increase in colony size at the end of the experiment (t = 3,08 on day 9 and t = 2,88 on day 10, p<0,05 in both cases).

Mean conidial yield, size and germination rate in RL and BL experimental series were normalized against the respective control values (Fig. 2). Conidial yield appeared to be lower in *A. clavatus* RL-cultures and BL-cultures than in control; t = 3,55 (p<0,05), and t = 6,17 (p<0,01), respectively (Fig. 2A). The decrease of conidial yield in RL-cultures of *P. citrinum* was insignificant (t = 1,77; p>0,05). *T. viride* showed a nearly two-fold decline of conidial counts in RL-cultures (t = 4,58; p<0,05) and three-fold – in BL-cultures (t = 7,45; p<0,01); the difference between RL- and BL-exposed cultures was also significant (t = 4,08; p<0,05). In *A. alternata* conidial yields increased in RL- and BL-cultures *versus* control cultures (t = 3,65 and t = 3,02, respectively; p<0,05 in both cases). In *F. fujikuroi* no light-induced changes were observed for this parameter.

No effect of RL or BL exposure on the size of conidia in studied fungal species was observed (Fig. 2B). The germination of conidia collected in RL-cultures was indistinguishable from that in control cultures of *A. alternata* and *F. fujikuroi* and slightly suppressed in other three microscopic fungi (Fig. 2C). Some decline of conidial germination was found in BL-exposed cultures of all five fungi species, and for *F. fujikuroi* a difference with control was significant (t = 3,14; p<0,05).

Biochemical parameters measured in RL- or BL-

exposed fungal cultures are given in Table 2. RL exposure led to some elevation of protein contents in *A. clavatus*, *T. viride* and *A. alternata* (p>0,05 for all three species) and to significant decrease in *P. citrinum* (t = 4,67; p<0,01) and *F. fujikuroi* (t = 6,83; p<0,01). Also, protein contents were lower in BL-cultures of *P. citrinum* (t = 3,18; p<0,05) and *F. fujikuroi* (t = 7,7; p<0,01), than in their controls. Due to the opposite directions of the changes of protein contents in RL- and BL-cultures of *T. viride* the meaningful difference occurred between these experimental series (t = 3,13; p<0,05).

A tendency of increased phenolic content occurred in the exposed cultures of all studied species, but the difference in comparison to the control didn't reach a statistical significance even for the 20% increase in RL-cultures of *A. alternata* (t = 1,54; p>0,05).

The assessment of fungal antagonistic properties gave results as follows. *A. clavatus* caused TO of both test bacteria species. *P. citrinum* showed TO of *E. coli* and AA against *B. subtilis*. *F. fujikuroi* provided AA against *E. coli* and PA towards *B. subtilis*. *T. viride* and *A. alternata* appeared to be neutral towards both the tested bacterial species. These patterns occurred in all experimental series of a particular fungal species; i.e. RL- or BL-exposure didn't influence the antagonistic properties of the studied microscopic fungi.
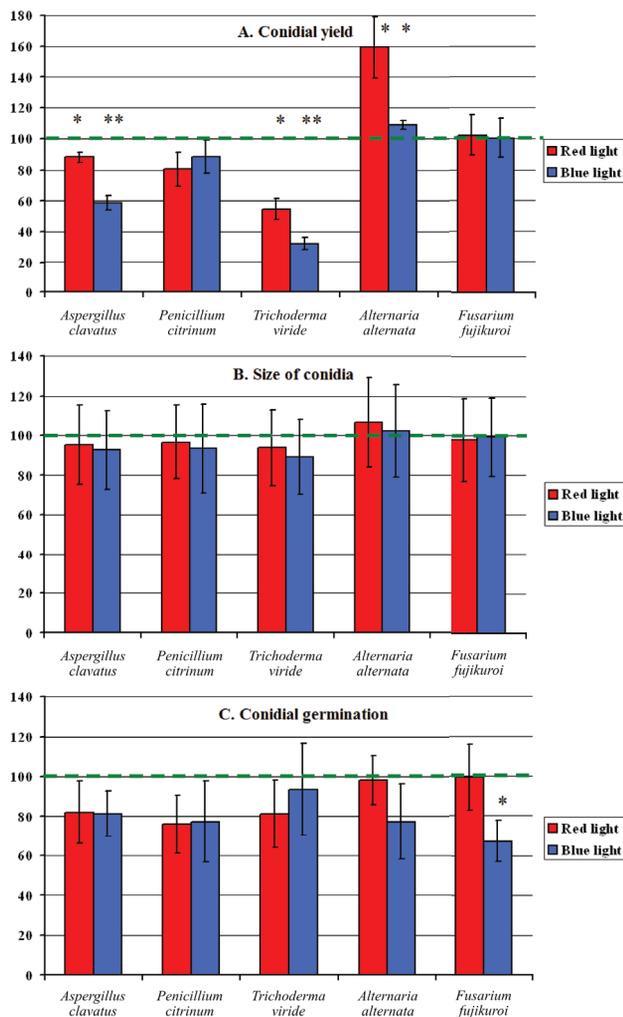
Figure 2. Conidial production in cultures of different species of soil microscopic fungi grown after brief exposure to red or blue monochromatic light. A: Conidial yield; B: Size of conidia; C: Conidial germination rate. Data are presented as a percent of the respective control values (the latter is indicated by the dashed line). Statistically significant changes of measured parameters in light exposed cultures in comparison to the control are indicated by the asterisk: * = p<0,05; ** = p<0,01.

Discussion

The responses to the light signal are very diverse between fungal species and can be either up- or down-regulative, depending on duration, intensity and spectral characteristics of the light fluence (Tisch and Schmoll 2010; Fuller et al. 2015; Corrochano 2019). It is still impossible to predict a total range of photoinduced effects for a certain fungal genus, or family, or even class. The results of our study confirm this opinion. Each of five soil fungal species used in present experiment displayed its own, unique pattern of growth reactions and biochemical changes caused by brief exposure of the 24-h culture, initiated from a small piece of mycelium, to monochromatic blue or red light.

Comparison of the end-sizes of colonies (day 10) in the exposed and unexposed series revealed that RL illumination resulted in growth reduction in *P. citrinum* (16%) and increase of growth in other species (5-13%), while BL exposure reduced the growth of *P. citrinum* and *T. viride* (16 and 20%, respectively) but increased the growth of the other three species (6-22%). However, such variations actually resulted from a combination of an early growth response and more prolonged growth rate reaction. BL caused a growth delay at the starting phase in *A. clavatus*, *P. citrinum* and *T. viride* and speeded up of the subsequent growth in *A. clavatus*, *P. citrinum*, *T. viride* and *F. fujikuroi*, as estimated from changes of c coefficient of the growth equation (Table 1). RL effects included a delay of initial growth phase in *A. clavatus* and its remarkable stimulation in *A. alternata*, while growth rate was increased in *A. clavatus* and *F. fujikuroi* but reduced in *A. alternata*. The overall changes of the fungal growth rate, either stimulation or reduction, appeared to be somewhat more prominent after BL exposure, than in RL-treated cultures. That agrees with findings of other researchers, who concluded stronger effect of BL, than of other wavelengths, on fungal morphogenesis, metabolic activity, development and behavior (Corrochano 2007, 2011; Herrera-Estrella and Horwitz 2007; Tisch and Schmoll 2010).

The radial growth is widely accepted as the integral parameter of physiological status of microscopic fungi, but growth reactions, probably, are the most complex phenomena in fungal photobiology (Moore 1998; Kotov and Reshetnikov 1990; Kotov et al. 2005; Karpenko 2010). In our study, the exposure of *T. viride* to RL led to a remarkable intensification of the radial growth, meanwhile there are reported data that RL provoked reduction in mycelial growth in *Trichoderma* (Schmoll et al. 2010). This is a good example of the intra-species and inter-experiment heterogeneity in fungal photobiology.

A photoinduced decline in mycelial growth was observed for numerous fungal species, and the mechanism appeared to be based on the activation of oxidative stress (Fuller et al. 2015). That was proved by the experiment, in which a white light-dependent reduction in growth rate of *Botritis cinerea* was abolished simply by an addition of the antioxidant to the medium (Canessa et al. 2013). The phenomenon of the regulation of fungal growth response to the light by the contents of culture medium had been known for decades (Tisch and Schmoll 2010). This underlines the importance of keeping the culture conditions identical for all species used in such comparative experiments.

Conidial yield and conidial germination are very important characteristics in fungal photobiology, and

Table 2. Changes of biochemical parameters measured in fungal cultures grown after their exposure to the light of different wavelength (mean ± SE).

Exposure conditions	*A. clavatus*	*P. citrinum*	*T. viride*	*A. alternata*	*F. fujikuroi*
	Protein contents (mg/g of raw mass)				
Control	311 ± 27	746 ± 65	280 ± 24	498 ± 43	639 ± 55
Red light	361 ± 31	40 1± 35**	356 ± 31	632 ± 55	237 ± 21**
Blue light	371 ± 32	498 ± 43*	239 ± 21#	558 ± 48	194 ± 17**
	Phenol contents (% of mycelium mass)				
Control	1,05 ± 0,15	1,03 ± 0,19	1,13 ± 0,17	4,50 ± 0,39	0,91 ± 0,14
Red light	1,22 ± 0,18	1,35 ± 0,20	1,40 ± 0,21	5,44 ± 0,47	1,12 ± 0,19
Blue light	1,13 ± 0,17	1,24 ± 0,16	1,21 ± 0,22	4,93 ± 0,43	1,23 ± 0,18

Difference is statistically significant: * = versus control at $p<0,05$; ** = versus control at $p<0,01$; # = between RL- and BL-cultures at $p<0,05$.

high heterogeneity of the photo-induced changes of these parameters arises in classic or recent reports (Calpouzous and Chang 1971; Hill 1976; Betina and Zajacova 1978; Pokorný et al. 2005; Schmoll et al. 2010; Corrochano and Garre 2010; Rangel et al. 2011; Oliveira et al. 2018). In our study the size of conidia was not affected by either BL or RL (Fig. 2). However, the exposure of the starting culture to RL led to the decrease of conidia numbers and their reduced germination in *A. clavatus, P. citrinum* and *T. viride*. By contrast, RL-treated cultures of *A. alternata* showed an elevated conidial yield. The exposure to BL produced a negative effect on the conidial yield again in *A. clavatus, P. citrinum* and *T. viride*, while a decrease in the germination rate did not occur in the latter species but was noticeable in four other species.

Such a diversity of the positive or negative effects of the monochromatic light on conidiation is not surprising (Schumaher 2017). It was regularly shown that blue and/or red light can repress or stimulate the asexual development in fungal species of the same genera. At one hand, in many fungi the conidiogenesis requires a light pulse to be initiated (Tisch and Schmoll 2010). A near-UV light, alone or combined with cool-white light, enhances conidiation in *Fusarium* and many other fungi (Avalos and Estrada 2010). On another hand, a negative influence of light signal on the conidiation is also well known. In *Fusarium graminearum* a significant decrease of the viability of conidia under BL exposure was shown (De Lucca et al. 2012), and in *Alternaria* a photo-induced downregulation of the conidial production can be effectively blocked by riboflavin (Soderhall et al. 1978; Kritsky et al. 2010). Experiments on *Aspergillus nidulans* showed that light-regulated morphogenetic pathways integrate blue and red-light action, and full stimulation of conidial production was only achieved with a combination of red and blue light (Bayram et al. 2010).

The results of our study support the opinion that fungi perceive a brief light exposure, especially to BL,

primarily as a stress signal and due to this conidiation and/or germination become repressed in favor of the induction of stress resistance pathways (Fuller et al. 2015). This hypothesis is partially confirmed by 'no negative effect' on the conidiation in RL-exposed cultures of both pigmented species – *A. alternata* and *F. fujikuroi*, for which RL probably is not that effective stress messenger, because these fungi are evolutionary better prepared to protect themselves against light-induced damage, e.g. by the increase of carotenoid content (Stahl and Sies 2005; Avalos and Estrada 2010).

The assessment of potential changes in protein content and antimicrobial activity seems to be a reasonable part of our study in view of the biotechnological usage of many fungal species. The effects of light exposure on growth, metabolism and productivity were investigated in microscopic fungi, which can produce biologically active secondary metabolites, including carotenoids and antibiotics (Kato et al. 2003; Sprote and Brakhage 2007; Myung et al. 2009; Tisch and Schmoll 2010; Avalos and Estrada 2010; Corrochano and Garre 2010). The idea of the intentional stimulation of protein production or enhancement of bacteriostatic properties by a simple procedure of brief light exposure is very attractive. However, in our experiment neither BL, nor RL exposure of microscopic fungi changed their species-specific antagonistic activity towards bacteria. The fluctuations in protein content corresponded to the respective growth reactions in all studied species except *F. fujikuroi*, in which the decrease in protein contents in exposed cultures contrasted to the elevation of colony size; a divergence between growth and biochemical parameters was especially apparent in BL series (Table 2). A possible reason for this finding needs to be further explored.

Inter-taxons differences in the changes of the fungal protein metabolism in response to light exposure were already described in the literature (Hill 1976; Strigácová et al. 2001; Pokorný et al. 2005; Tisch and Schmoll 2010).

Several reports with transcriptomic data showed that fungal photoreactions require new proteins in sufficient quantities. However, the underlying molecular mechanisms are also rather diverse. The well studied fungal blue light photoreceptor class, the White Collar complex (WCC), works as a general transcriptional activator in *Neurospora*, but it is thought to play repressive roles in other species, including *Aspergillus*, *Alternaria*, *Trichoderma*, *Fusarium* and *Cercospora* (Fuller et al. 2015).

The moderate elevation of phenol content in BL- or RL-exposed cultures appeared to be a common trait for all five fungal species in present work (Table 2). That can be an adaptive reaction, aimed at the development of tolerance to possible further damaging impact of light via the regulation of pigment synthesis (Corrochano and Garre 2010; Karpenko 2010; Schumacher 2017). The modulation of the UV stress response by a preliminary light exposure is a well known phenomenon, e.g. observed in *Metarhizium acridum*, and the respective molecular machinery of the signaling cascade leading to the up-regulation of certain genes involved in the UV protection has been already described (Rangel et al. 2015; Brancini et al. 2016, 2018a, 2019; Dias et al. 2020). Experiments with testing different fungal species to UV stress may clarify this hypothesis. Also, it seems reasonable to check whether a brief exposure to the monochromatic light may affect the differential susceptibility of microscopic fungi to antifungal agents, similarly to what has been done using *Metarhizium* species (Brancini et al. 2018b), as this characteristic can be an important species-specific trait in different ecological niches.

It should be underlined that the design of present experiment included a relatively brief exposure of the starting fungal culture. Such a methodology had been applied earlier in classic studies; e.g. there were reports that just 1-min illumination exposure of *Neurospora crassa* mycelia resulted in a minor but significant induction of colored carotenoid biosynthesis (Zalokar 1955; Schrott 1980). The exposure to the light lasted for 2 seconds appeared to be enough to induce detectable induction of sporulation in *T. viride* (Betina and Zajacova 1978). Moreover, the photoinduction of conidiation in *Trichoderma* can be 'remembered' while the culture is maintained in conditions that do not allow cellular growth (e.g., low temperature or hypoxia); as soon as growth is resumed, under optimal conditions, the colony conidiates (Schmoll et al. 2010).

Obviously, our results can be hardly compared to published datasets and conclusions, which were obtained using protracted exposure of fungal mycelium or conidia. The experiments involving a longer term, continuous or fractionated light exposure suit better for revealing complex adaptive reactions, which are based on the feed-back regulation, e.g. phototropism, circadian rhythm or changes in sporulation or sexual development. Under the prolonged illumination the photoinduced gene expression is increased or decreased according to positive or negative feedbacks, respectively (Schumaher 2017). In contrast to that, a single brief action of the monochromatic light induces longer term or delayed responses via one-way triggering mechanism, which probably involves a quick alteration of several key global metabolic regulators, like photo-induced fungal protein velvet A (Kato et al. 2003; Sprote and Brakhage 2007; Purschwitz et al. 2009; Tisch and Schmoll 2010).

The starting stage of the photosensing in fungi is already quite well understood; it is mediated by highly sophisticated signaling machinery, which may contain at least eleven potential photoreceptors (Schumaher 2017). The set of photoresponsive proteins in microscopic fungi is evolutionary conserved and include orthologs of White Collar proteins (in *Trichoderma* it is "Blue Light-Receptors" BLR1 and BLR2), cryptochromes, photolyases, phytochromes and photoactive opsins (Avalos and Estrada 2010; Bayram et al. 2010; Igbalajobi et al. 2019; Schmoll et al. 2010; Schumaher 2017). Such a complex fungal 'eye' allows it to react to a broad spectrum of wavelengths, integrating (near)-UV, blue, green, red and far-red light signals. Photoreceptors typically consist of an apoprotein and a chromophore that reacts by isomerization or reduction to light of a certain wavelength through absorption of a photon with a defined amount of energy. The associated structural changes in the chromophore induce conformational changes in the apoprotein leading to signal transduction via the output domains – if present – or via protein-protein interactions (Bayram et al. 2010).

The early phase of the photoinduced gene expression in fungi is very eventful; in *N. crassa* new transcripts were detectable as early as 2 min after the BL signal and within 30 min from 60 to 80 genes were regulated (Sommer et al. 1989). It seems possible, that the subsequent downstream chain of events includes the occurrence of a self-sustaining cascade or cycle of signals. However, the exact ontogenetic links between the elements of such a regulatory network that finally ends up in protracted metabolic changes and physiological reactions still need to be elucidated.

In general, our study showed that photo-induced effects in soil microscopic fungi depend on the light wavelength and had a remarkable inter-species heterogeneity. Gross, long lasting physiological and biochemical responses occurring in microscopic fungi after brief light exposure point at the directions of future studies that might be aimed at the discovering of the underlying regulatory mechanisms, signaling cascades and transcriptional changes in fungal species from different taxons. It is

particularly desirable to revise the photo-induced fungal reactions in the experiments involving various stress impacts and non-optimal growth conditions, which might occur in natural habitats. That might contribute to better understanding of fungal biology with regard to their role in various ecological niches.

Conclusions

A relatively brief (30 min) exposure of 24-h cultures of soil microscopic fungi to monochromatic blue or red light is sufficient to induce long-lasting effects observed during their long-term culture. The responses to the light signal appeared to be species-specific and dependent on the wavelength.

Overall radial growth was not remarkably affected by any of the two light wavelengths in *A. clavatus* but delayed in *P. citrinum* and stimulated in *A. alternata* and *F. fujikuroi*, and these changes were more pronounced after BL, than after RL exposure. The BL illumination of *T. viride* culture led to its growth delay, whereas the RL caused the enhancement of the growth rate. Protein content followed the patterns of radial growth changes in all species except *Fusarium,* in which it was decreased after both BL and RL exposure. The moderate elevation of phenol content in BL- or RL-exposed cultures was noted in all five fungal species and probably was an adoptive reaction aimed to help their resistance against possible further light exposure. Conidial yield and conidial germination were reduced in RL-exposed cultures of *Aspergillus, Penicillium* and *Trichoderma*. By contrast, RL-treated *Alternaria* cultures showed a significantly elevated conidial yield and normal conidial germination. The exposure to BL negatively affected both conidial yield and germination in *A. clavatus* and *P. citrinum*, while *T. viride* showed a reduced conidial yield only, and BL-exposed *A. alternata* and *F. fujikuroi* had a decreased conidial germination rate. The size of conidia and fungal antagonism towards test bacteria showed no changes in response to the light signal.

Future research, particularly using transcriptomic and proteomic methods, must reveal the molecular machinery and signaling pathways underlying long-lasting effects induced in fungi by a brief light exposure.

Acknowledgement

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