Blue-light Inhibition of Sporulation in *Botrytis cinerea*

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SUMMARY

Prolonged blue-light irradiation was necessary for almost total reversal of sporulation in *Botrytis cinerea* Pers. ex Fr. Studies of blue-light exposures of various durations during the dark period after photoinduction showed that there were two phases of maximum sensitivity to blue light, namely between 12 and 16 h, and between 20 and 24 h after the end of photoinduction. A brief exposure of 15 min to blue light ($250 \mu\text{W}/\text{cm}^2$) at the 12th hour after the end of photoinduction brought about 20% suppression of sporulation. The inhibition response was saturated at the 60% inhibition level with more than 6 h blue-light irradiation. Blue light arrested spore formation and then caused de-differentiation of conidiophores to sterile mycelia.

INTRODUCTION

Blue light induces sporulation in many fungal species, e.g. *Botrytis gladiolorum*, *Trichoderma* sp. (Bjornsson, 1959), *Trichoderma viride* (Gressel & Hartmann, 1968; Kumagai & Oda, 1969a; Jenssen, 1970), *Aspergillus ornatus* (Stallings, 1970), *Penicillium isariiforme* (Bennink, 1971) and *Phycomyces blakesleeana* (Bergman, 1972), but is ineffective in *Botrytis cinerea* (Tan & Epton, 1973). Suppression of sporulation by blue light after photoinduction has been shown for *Alternaria tomato* (Aragaki, 1962; Kumagai & Oda, 1969b), *Alternaria solani* (Lukens, 1963), *Helminthosporium oryzae* (Honda & Sakamoto, 1968), *Stemphylium botryosum* (Leach, 1968), *Stemphylium solani* (Sproston, 1971), and *B. cinerea* (Tan & Epton, 1974). This suppression of sporulation by blue light was in fact a reversal of the potentiated sporulation (Honda, Sakamoto & Oda, 1968; Kumagai & Oda, 1969b; Tan & Epton, 1974). This paper describes further studies of the blue-light reversal of near ultraviolet potentiated sporulation in *B. cinerea*.

METHODS

Botrytis cinerea Pers. ex Fr. was the isolate used by Tan & Epton (1973, 1974) who also described the cultural procedures.

Philips 'Black Light' tubes (TL 40W/08) were used as the source of near-ultraviolet radiation for inducing sporulation. Cultures were photoinduced for 12 h after dark incubation for $4\frac{1}{2}$ to 5 days before being irradiated with blue light. Atlas 40 W blue fluorescent tubes were used as the source of blue light and, unless otherwise stated, an Ilford filter No. 622 was used. The filters (4 in^2) were fitted to the lids of boxes which had been used to keep photographic plates, and these boxes were used to contain the fungal cultures for the irradiation experiments. Transfer of cultures to the boxes was always done in total darkness.

The irradiance of black light at culture level, measured by an Eppley thermopile in conjunction with a Keithley microvoltmeter (see Tan & Epton, 1973), was $151 \mu\text{W}/\text{cm}^2$. Unless

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Table 1. *Inhibition of sporulation in B. cinerea by blue light at two different irradiances*

Irradiation programme*	Spore no./0.1 mm ³ †	
	100 μ W/cm ²	250 μ W/cm ²
12 h BLB+12 h B+12 h D	45.6 $\pm$ 3.9	33.8 $\pm$ 3.4
12 h BLB+12 h D+12 h B	110.8 $\pm$ 4.0	91.8 $\pm$ 4.8
12 h BLB+24 h B	9.9 $\pm$ 1.5	0.9 $\pm$ 0.4
12 h BLB+24 h D	115.9 $\pm$ 3.7	109.2 $\pm$ 4.3

* BLB, Black light; B, blue light; D, dark.

† Average of three cultures, ten counts per culture, counts made immediately after completion of irradiation programme. Confidence limits at $P = 0.05$.

Fig. 1. Effect on sporulation of *B. cinerea* of duration of blue-light irradiation given immediately after photoinduction. O, Expt 1; Δ , Expt 2. Each point is the mean of two cultures, 10 counts/culture. Confidence limits at $P = 0.05$.

otherwise stated, the irradiance of light filtered through Ilford filter No. 622 at culture level was 250 μ W/cm².

The spores produced were counted 72 h after the start of photoinduction, unless otherwise stated, and were expressed as no./0.1 mm³ homogenate. For details of procedure, see Tan & Epton (1973). Percentage sporulation is:

$$\frac{\text{Mean spore no./0.1 mm}^3 \text{ homogenate of culture}}{\text{Mean spore no./0.1 mm}^3 \text{ homogenate of control culture}} \times 100,$$

where the control culture was kept in the dark for 60 h after 12 h black-light irradiation.

RESULTS

Inhibition of sporulation at two different blue-light irradiances

Cultures were irradiated with black light for 12 h and then subjected to various combinations of incubation in the dark and with blue light. Irradiance of blue light was approxi-

Fig. 2. *B. cinerea* cultures subjected to 12 h black light, (a) and (c), and 12 h black light followed by 24 h blue light, (b) and (d). Photographs taken 72 h after the start of black-light irradiation.

Fig. 3. Transmittance of Ilford filters 601 and 602.

Table 2. *Inhibition of sporulation in B. cinerea by blue light: studies using Ilford filters 601 and 602*

Irradiation programme*	Sporulation† (%)
12 h BLB + 12 h B(601) + 48 h D	89.1 ± 2.8
12 h BLB + 12 h B(602) + 48 h D	68.3 ± 2.3
12 h BLB + 60 h D	100.0 ± 2.8
12 h BLB + 24 h B(601)‡ + 36 h D	71.1 ± 2.7
12 h BLB + 24 h B(602)‡ + 36 h D	58.2 ± 2.5
12 h BLB + 60 h D	100.0 ± 3.6

* BLB, black light; B, blue light; D, dark. Numbers in parentheses refer to the filter used.

† Average of three cultures, 10 counts/culture. Confidence limits at $P = 0.05$.

‡ Irradiance: B(601), 111 $\mu\text{W}/\text{cm}^2$; B(602), 113 $\mu\text{W}/\text{cm}^2$.

mately 100 $\mu\text{W}/\text{cm}^2$ for one experiment and 250 $\mu\text{W}/\text{cm}^2$ for another. Spores were counted immediately after the experimental treatment, and the results (Table 1) showed that sporulation was suppressed more at the higher irradiance and that 12 h blue light was less effective than 24 h.

Effect of duration of blue-light irradiation given immediately after photoinduction

After 12 h black-light irradiation, cultures were placed under filtered blue light (irradiance 250 $\mu\text{W}/\text{cm}^2$) for various periods before returning to the dark. Those not subjected to blue light but placed in the dark after photoinduction constituted the control. The results (Fig. 1) showed that potentiated sporulation was almost completely reversed by continuous blue light of more than 36 h. Erect sterile hyphae were observed on all cultures irradiated for more than 24 h with blue light (Fig. 2).

Studies using Ilford filters 601 and 602

To study which region of the blue spectrum was most effective in the suppression of sporulation, Ilford filters 601 and 602 were used under Atlas blue-light tubes. The trans-

Fig. 4. Response of *B. cinerea* to blue light exposures of (a) 12 h, (b) 6 h, and (c) 4 h, during the dark period following photoinduction. Short horizontal lines denote the period of illumination and the mean percentage sporulation (average of three replicate cultures) at the end of 72 h from the start of black-light irradiation. $\circ$, $\triangle$, Percentage sporulation of individual cultures (10 counts/culture) subjected to light filtered through Ilford filters 622 and 602, respectively; $\bullet$, percentage sporulation of control cultures (cultures not subjected to blue light exposures after photoinduction). The bar at the base of each figure indicates the general irradiation schedule.

Fig. 5. Blue-light inhibition of sporulation in *B. cinerea* at the 12th hour (a), and 14th hour (b), after the end of photoinduction. Results of two experiments (○ and △) are given for each case. Each point is the mean of two cultures (10 counts/culture). Confidence limits at $P = 0.05$.

mittance of the filters is shown in Fig. 3. After 12 h black-light irradiation, cultures were subjected to 12 h or 24 h filtered blue light, and then replaced in the dark. The results (Table 2) showed that light transmitted by Ilford filter 602 (430 to 490 nm) was more effective in the suppression of sporulation.

Time of maximum sensitivity to blue light

Since there could be a difference in response if blue light was given at different times after photoinduction (Table 1), experiments were designed to determine the stage of maximum responsiveness to blue light (irradiance $250 \mu\text{W}/\text{cm}^2$).

After 12 h black-light irradiation, cultures were replaced in the dark. At various times during the dark period, 12 h exposures to blue light were given. The results (Fig. 4a) suggest that the fungus was most sensitive to blue light given between 24 h and 36 h after the start of irradiation. Similar results were obtained using Ilford filter 602. Fig. 4(b) and (c) shows the results of 6 h and 4 h blue-light exposures, respectively, at various times during the dark period after photoinduction. Two phases of maximum sensitivity were found, between 24 and 28 h and between 32 and 36 h after the start of black-light irradiation. A third phase

Fig. 6. Time course of sporulation in *B. cinerea* and the effect of blue-light interruption of (a) 4 h, (b) 8 h. Open symbols, sporulation of cultures exposed to blue light; closed symbols, sporulation of cultures in darkness after photoinduction. Results of two experiments (circles and triangles) are given for each case. Each point is the mean of two cultures (10 counts/culture). Confidence limits at $P = 0.05$. The duration of blue light is indicated by rectangles.

between 40 and 44 h was indicated, but this may not be significant ($P = 0.05$). Enhancement of sporulation by a short period of blue-light irradiation given immediately after photoinduction was also indicated.

For *Alternaria tomato* and *Helminthosporium oryzae*, sensitivity to blue light was maximal at the 6th hour after the end of photoinduction (Kumagai & Oda, 1969b; Honda *et al.* 1968). Lukens (1963) also found that for *A. solani*, the 6th hour is the time most sensitive to blue-light inhibition.

Duration of blue-light irradiation and inhibition of sporulation

Various periods of blue-light irradiation (irradiance $250 \mu\text{W}/\text{cm}^2$) were given 12 h and 14 h after the end of 12 h black-light irradiation. The results (Fig. 5) showed that blue light given at the 12th hour after photoinduction was more effective in the inhibition of sporulation. As brief an exposure as 15 min given during this period caused some 20 % inhibition of sporulation (Fig. 5). Irradiation of longer duration resulted in more inhibition.

In another series of experiments in which blue-irradiation periods longer than 4 h were given at the 12th hour after the end of photoinduction, it was found that the inhibition response was saturated with more than 6 h blue light. The inhibition response saturated at about the 60 % inhibition level because spore formation had already reached the 40 % sporulation level when blue light was given. The cultures subjected to more than 8 h blue-light irradiation again possessed abundant sterile erect mycelia.

In *Alternaria solani*, Lukens (1963) found that 30 min white-light irradiation at low intensity resulted in 80 % inhibition, and in *Helminthosporium oryzae*, Honda and co-workers found that 30 min interruption of darkness with blue light at an irradiance of $125 \mu\text{W}/\text{cm}^2$ could saturate the inhibition response (Honda & Sakamoto, 1968; Honda *et al.* 1968). Kumagai & Oda (1969*b*) showed that in *A. tomato*, sporulation could be inhibited 40 % by 30 min blue-light interruption (irradiance $170 \mu\text{W}/\text{cm}^2$) and that the inhibition response was saturated with more than 60 min interruption.

Time-course of sporulation and blue-light interruption

The formation of spores as a result of blue-light interruption was investigated. After photoinduction, cultures were replaced in the dark. About half of the total number of cultures was subjected to a 4 h blue-light interruption ($250 \mu\text{W}/\text{cm}^2$) of the darkness at the 12th hour after the end of photoinduction and then replaced in the dark. At various times, counts were made of the spores in cultures exposed to blue light and of those cultures kept in the dark after photoinduction. The results (Fig. 6*a*) showed that the rate of production of spores was greatly reduced after the blue-light interruption.

In another series of experiments, cultures were subjected to 8 h blue-light interruption at the same irradiance, and sporulation was found to be arrested after the blue-light interruption (Fig. 6*b*). Sterile erect hyphae were first observed after 36 h from the start of photoinduction (i.e. about 4 h after the end of the blue-light interruption), and these increased in abundance with time.

DISCUSSION

These results may give further insight into the action mechanism of the hypothetical receptor pigment, mycochrome (Honda, 1969; Kumagai & Oda, 1969*b*; Tan & Epton, 1974).

It has already been suggested that blue light converts the M_B form, which is effective for sporulation, to M_{NUV} , the form that is not active for sporulation (Tan & Epton, 1974). The fact that blue light must be applied continuously and for long duration to obtain almost total inhibition of sporulation suggests that: (i) the quantum efficiency for the conversion of M_B to M_{NUV} is lower than the reverse process; (ii) the conversion may involve more reaction steps, some of which may be time-dependent; and (iii) M_{NUV} or some intermediate(s) in the photo-transformation $M_B \rightarrow M_{\text{NUV}}$ may be unstable and revert back to M_B when light is removed, thus causing some spores to be formed.

It can also be argued that as the filtered blue light extends to the edge of the near-ultraviolet spectrum, light of some stimulatory wavelengths would also be absorbed. A situation might exist in which the processes $M_B \rightarrow M_{\text{NUV}}$ and $M_{\text{NUV}} \rightarrow M_B$ are occurring simultaneously. In view of the low proportion of near-ultraviolet light, the former reaction would predominate, but some M_B formed could still contribute to sporulation.

Sterile erect mycelia were formed in *Botrytis cinerea* cultures as a result of blue-light irradiation. This indicates that the M_{NUV} form was also active, and substantiates the postulation of Honda (1969) that M_{NUV} is active for the formation of sterile erect mycelia.

As was observed by Honda (1969) for *Helminthosporium oryzae*, potentially spore-forming conidiophores were de-differentiated to sterile mycelia in blue light. Another instance of de-differentiation of conidiophores was reported by Aragaki, Hylin & Maeda (1968) for *Alternaria tomato*. In this species, de-differentiation was induced by white light in the absence of ultraviolet. Thus blue light arrests spore formation and then causes de-differentiation of conidiophore to sterile mycelia.

The mechanism of mycochrome can thus be summarized as follows:

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