Action Spectrum of Photoinduced Conidiation in *Alternaria cichorii*

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An action spectrum for photoinduced conidiophore formation in the fungus *Alternaria cichorii* was determined by exposing colonies to monochromatic radiation obtained from a diffraction grating monochromator. The action spectrum showed a peak near 290 nm with a noticeable shoulder between 310 and 330 nm and another one between 260 and 265 nm. This action spectrum has many common characters with those for photoinduced conidiophore formation of *Helminthosporium oryzae* and *Alternaria tomato*, where the induction of conidiophore formation is controlled by a blue-light-inducible, near-UV-reversible, photoreaction.

INTRODUCTION

Sporulation in many fungi is induced by exposure to light. There seem to be two main categories of response: one group of light-sensitive fungi responds to wavelengths of 330–510 nm and the other to UV radiation of wavelengths less than 330 nm (Tan, 1978). Conidia are formed in colonies of *Alternaria cichorii* when exposed to UV radiation of wavelengths less than 340 nm but none are formed in colonies exposed to violet, blue-green, yellow, orange or red light (Vakalounakis, 1981). Sporulation in *A. cichorii* presents two distinct phases: an 'inductive phase' leading to formation of conidiophores and a 'terminal phase' leading to formation of conidia (Vakalounakis, 1981; Vakalounakis & Christias, 1986). The inductive phase is induced by near-UV radiation of wavelengths below 340 nm and proceeds most efficiently at high temperatures, while the terminal phase is inhibited by blue light (360–530 nm) and is operative at lower temperatures. The purpose of this study was to determine an action spectrum for photoinduced conidiophore formation in *A. cichorii* and reveal the probable absorption characteristics of the photoreceptor, and also to compare this action spectrum with those for photoinduced conidiation of *Helminthosporium oryzae* and *Alternaria tomato*, in which the induction of conidiophore formation is under control of a blue-light-inducible, near-UV-reversible photoreaction, regulated by the 'mycochrome' system.

METHODS

Strain and culture conditions. The isolate of *A. cichorii* used in this study has been described (Vakalounakis, 1981; Vakalounakis & Christias, 1981). It was grown at $25 \pm 0.5^\circ\text{C}$ on potato dextrose agar in standard 9 cm Petri dishes containing 15 ml medium with an initial pH of 6.3. Cultures, inoculated with agar plugs from the periphery of 5-d-old cultures grown in darkness at $25 \pm 0.5^\circ\text{C}$, were grown for 4 d in darkness, exposed to monochromatic radiation and then incubated for 48 h in darkness before being examined microscopically. During irradiation, the lids of the Petri dishes were removed and the dishes were transferred to an optical bench where they were held vertically under illumination from a dim red light with wavelengths longer than 600 nm.

Irradiation. Monochromatic radiation was obtained from a Bausch and Lomb 250 mm diffraction grating monochromator with a 900 W Xenon lamp (Engelhard Hanovia, Inc.). A Corning glass filter no. 0-53 was used to reduce stray light as well as to eliminate the overlapping second-order spectrum at the longer wavelengths (Table 1). Intensity of radiation was measured with a model E20 thermopile (Kipp and Zonen) and an A70 galvanometer (Kipp and Zonen). When necessary, intensity of radiation was adjusted by using suitable neutral-density filters (Vacuum Optics Corporation of Japan) at the exit slit. Cultures were irradiated from 250 to 340 nm at 10 nm

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Table 1. *Wavelengths, intensities and range of exposure times used in action spectrum study of A. cichorii*

Wavelength (nm)	Intensity (mW m ⁻²)	Filter*	Duration of irradiation†
250	803	None	a
260	852	None	b
270	881	None	b
280	895	None	b
290	509	None	b
300	506	None	b
310	366	None	a
320	372	0-53	a
330	1956	0-53	c
340	4118	0-53	d

* Filter no. 0-53 transmits wavelengths longer than 280 nm.

† a, 10, 30, 50, 70, 90, 110 and 130 s; b, 10, 20, 30, 40, 50 and 60 s; c, 10, 30, 50, 70, 90, 110, 130, 150 and 170 s; d, 100, 1000, 5000, 10000 and 50000 s.

intervals in regions of the spectrum in which preliminary studies had indicated the presence of peaks of activity. Half-band width was about 10 nm at all wavelengths. Intensity of radiation at each wavelength was kept constant while the duration of irradiation was varied (Table 1). This procedure is fully valid if the Bunsen-Roscoe Law (full reciprocity between intensity and duration of irradiation) can be shown to hold at the most effective wavelengths (Carlile, 1965). For this purpose two experiments were done with various time/intensity combinations at 290 nm and 320 nm.

Determination of conidiation response. The number of conidia produced, which under these experimental conditions was equal to the number of conidiophores, was counted *in situ* in a zone that corresponded to the growing periphery at the time of irradiation (Vakalounakis & Christias, 1981).

The standard effect selected was governed by various considerations. Vakalounakis (1981) showed that as doses at the shorter UV wavelengths were increased, so sporulation was inhibited. In addition, although radiation in the region 250–270 nm stimulated sporulation, the maximum density of spores formed at these wavelengths was generally lower than at the longer wavelengths. Another important practical consideration was the inability to measure the density of sporulation accurately when spores were produced very abundantly. Because of these considerations the standard effect selected was the production of 100 conidia mm⁻², low enough to enable accurate counting of conidia and to minimize possible inhibitory effects; this response also occurred within the linear portion of the dose-response curves. Dose-response curves were plotted for each monochromatic radiation used. The dose that induced the standard effect was calculated for each monochromatic radiation series by the following formula: dose (mJ m⁻²) = intensity (mW m⁻²) × duration of irradiation (s). The action spectrum was obtained by plotting the reciprocals of the standard effect dose against wavelength. Relative effectiveness was normalized at 290 nm.

For each treatment three colonies were irradiated, and 12 counts were made for each colony. Replicate experiments gave similar results.

RESULTS AND DISCUSSION

Dose-response curves (Fig. 1) and the action spectrum for light-induced sporulation of *Alternaria cichorii* (Fig. 2) showed a peak near 290 nm, with shoulders in the 315–320 nm and 260–265 nm regions. Reciprocity (equal response) between intensity and duration of irradiation was found to hold at 290 and 320 nm (Table 2). The action spectrum was obtained by plotting the reciprocals of the standard effect dose (Table 3) against wavelength. If these data were corrected for radiation in quantum energy at the different wavelengths and then plotted as reciprocals, the resultant action spectrum was still very similar to that shown in Fig. 2. This action spectrum resembles the absorption spectra for many substances, including proteins and aromatic amino acids (Harm, 1980). Long UV radiation (330 nm) only slightly stimulated conidiation. The lower limit of effective radiation was around 250 nm, but effectiveness of shorter-wavelength far-UV cannot be excluded since such irradiation was not tested in these experiments.

Fig. 1. Dose-response curves for induction of conidophore formation in *A. cichorii* from which standard effect doses were obtained: (a) 250 nm (●), 260 nm (○), 270 nm (▲), 280 nm (△), 290 nm (■); (b) 300 nm (●), 310 nm (○), 320 nm (▲), 330 nm (△). Each point is the mean of three replicate cultures with 12 counts per replicate, $\pm$ SD (vertical bars).

Fig. 2. Action spectrum for induction of conidiophore formation in *A. cichorii*. Relative effectiveness was normalized at 290 nm. D , dose needed to produce 100 conidia mm^{-2} .

The action spectrum of photosporogenesis in *A. cichorii* has similarities with action spectra of conidiation reported for *Ascochyta pisi* (Leach & Trione, 1965), for *Pleospora herbarum*, *Stemphylium botryosum* and *Alternaria dauci* (Leach & Trione, 1966), for *S. solani* (Sproston, 1971), for *Leptosphaerulina trifolii* (Leach, 1972), for *Helminthosporium oryzae* (Yamamura *et al.*, 1977) and for *Botrytis cinerea* (Honda & Yunoki, 1978). In each case the response included a main peak around 280–300 nm, a secondary peak at 260–270 nm, minimum stimulation around 250–260 nm and no stimulation by wavelengths longer than 340 nm. In this investigation additional stimulation was measured between 310 and 330 nm in the action spectrum of *A. cichorii*. Similar stimulation between 310 and 330 nm has also been detected in the action spectra for sporulation of *A. tomato* (Kumagai, 1983a), *H. oryzae* (Yamamura *et al.*, 1977) and *S. botryosum* (Leach & Trione, 1966). On the other hand, close to the stimulation between 310 and 330 nm was the small peak in activity at 303–310 nm which has been found in the action spectra for photoinduced conidiation of *B. cinerea* (Honda & Yunoki, 1978), *P. herbarum* (Leach & Trione, 1966) and *L. trifolii* (Leach, 1972). The increase in effectiveness at 230 nm observed in

Table 2. *Reciprocity of conidiation response at 290 nm and 320 nm*The results are means $\pm$ SD.

Wavelength (nm)	Intensity (mW m ⁻²)	Duration of irradiation (s)	Dose (mJ m ⁻²)	Conidiation (conidia mm ⁻²)
290	1350	10	13500	130 $\pm$ 20
	700	20	14000	126 $\pm$ 15
	350	40	14000	134 $\pm$ 25
	220	60	13200	108 $\pm$ 18
	160	80	12800	115 $\pm$ 20
320	1500	20	30000	112 $\pm$ 13
	750	40	30000	98 $\pm$ 10
	500	60	30000	110 $\pm$ 18
	400	80	32000	127 $\pm$ 30
	300	100	30000	115 $\pm$ 25
	250	120	30000	105 $\pm$ 20

Table 3. *Exposures at different wavelengths necessary to induce the formation of 100 conidia mm⁻² (standard effect)*

Wavelength (nm)	Intensity (mW m ⁻²)	Duration of irradiation (s)	Dose (mJ m ⁻²)
250	803	85.0	68255
260	852	44.5	37914
270	881	34.4	30306
280	895	20.9	18706
290	509	26.8	13641
300	506	33.7	17052
310	366	74.6	27304
320	372	81.5	30318
330	1956	139.6	273058

the action spectra of other fungi (Leach & Trione, 1965, 1966; Sproston, 1971; Honda & Yunoki, 1978) cannot be excluded from the action spectrum of *A. cichorii* since this wavelength was not tested in our experiments.

Conidial development in *A. cichorii* is controlled by a blue and near-UV reversible photoreaction regulated by the 'mycochrome' system at two developmental stages: the inductive stage of conidiophore formation (Kumagai, 1983*b*) and the maturation stage of conidiophore development (Vakalounakis, 1981; Vakalounakis & Christias, 1981, 1983, 1985; Vakalounakis *et al.*, 1983). Such a reversible photoreaction has also been found in the induction of conidiation in *A. tomato* (Kumagai, 1982) and *H. oryzae* (Kumagai, 1983*a*) and in the maturation of conidiophores in *A. tomato* (Kumagai & Oda, 1969), *H. oryzae* (Honda *et al.*, 1968), *B. cinerea* (Tan, 1974) and *S. botryosum* (Vakalounakis, 1982). Common characters in the action spectra for photoinduced sporulation among all the above-mentioned fungi suggest that conidiophore maturation in *S. solani* may be controlled by the blue and near-UV reversible photoreaction involved in the 'mycochrome' system and also that the blue and near-UV reversible photoreaction may control the inductive stage of conidiophore formation in *B. cinerea*, *S. botryosum* and *S. solani*.

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