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# Effects of monochromatic radiations on pycnidium formation in *Septoria obesa*[1)]

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# キク褐斑病菌, *Septoria obesa* の分生子殻形成に及ぼす単色光の影響

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Effects of monochromatic radiations between 300 nm and 700 nm on pycnidium formation were examined. Primordia of pycnidia were formed under darkness and irradiation of light was required for the formation of mature pycnidia with spore horns. The effective wave region was near ultraviolet radiation shorter than 370 nm. Radiation of 300 nm was the most effective for stimulating spore horn formation. On the contrary visible radiations between 400 nm and 700 nm showed more or less suppressive effect on the formation of mature pycnidia.

## Introduction

Light exposure has profound effects on spore formation in fungi. Many members of Sphaeropsidales are induced to sporulate by light, especially by ultraviolet radiation (Marsh et al., 1953), and some other members are inhibited to sporulate by light (Maiello, 1977). However, the inhibition of sporulation by light is rare in the members of Sphaeropsidales. There are also some members which sporulate well either under dark or light (Marsh et al., 1959). The effect of light on pycnidium formation in *Septoria* species has been studied and ultraviolet radiation, in general, has been recognized to be most effective for inducing pycnidium formation (Templeton, 1963; Leach, 1962; Calpouzos et al., 1970; Schlosser, 1970). However, effect of monochromatic radiation on pycnidium formation in *Septoria* species has not yet been revealed. In this report we studied effects of radiation quality on pycnidium formation of *Septoria obesa* Syd., causal fungus of brown spot of cultivated chrysanthemum, *Chrysanthemum morifolium* Ramat.

## Materials and Methods

**Organism**: The isolate of *S. obesa* for this study is the same one used in the previous report (Honda and Miyawaki, 1988). The cultures of the isolate have been grown on vegetable juice agar (VJA: Campbell's V-8 juice 200 ml, $CaCO_3$ 2 g, agar

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17 g, distilled water 800 ml ; pH 5.8) slants.

**Irradiation through band-pass filters**: Spore horns were taken from a slant culture by a nichrome wire inoculating loop and the conidia in the mucilage were streaked on the VJA (10 ml/60 mm diam. Pyrex Petri dish) plates. The inoculated plates were incubated for 4 days in the dark to form mycelial mats, then they were irradiated continuously by a 20 W Black Light (BLB) Fluorescent lamp (FL 20S・BLB, Toshiba Corporation, Tokyo ; 300-400 nm, Max. 360 nm) and a 20 W daylight fluorescent lamp (FL 20S・D, Toshiba Corporation, Tokyo ; 350-730 nm) suspended 10 cm apart and 18 cm above the plates. Plates were kept in the boxes covered with band-pass filters during irradiation so that the colonies were irradiated by the radiation filtered through each band-pass filter. Each band-pass filter has specific wavelength of maximum transmission of 450, 500, 550 and 600 nm, respectively, as indicated in Table 1. Control colonies without filters or in light-tight boxes were adjacent to test colonies. Light intensities were measured by a thermopile having a quartz window (MIR-100 Q, Mitsubishi Yuka Co. Ltd., Yokkaichi, Japan) coupled with a digital multimeter (TR 6846, Advantest Co. Ltd., Tokyo, Japan). The intensity of the light at the surface of the plate without filters was 865 $\mu$W/cm$^2$. Intensities of light filtered through band-pass filters were not adjusted to the same level and they were also shown in Table 1.

Table 1. Band-pass filters[a] and light intensities filtered through each of them[b].

| Type[c] | $\lambda_{max}$(nm) | $T_{max}$(%) | $\Delta\lambda 1/2$(nm) | Light intensity($\mu$W/cm$^2$) |
|---|---|---|---|---|
| BPB-45 | 450 | 55 | 59 | 304 |
| BPB-50 | 500 | 54 | 52 | 249 |
| BPB-55 | 550 | 57 | 52 | 326 |
| BPB-60 | 603 | 47 | 69 | 300 |

[a] Specifications for each filter were adopted from data on Fuji Film Band-pass Filters shown in Fuji Film Photo Handbook.

[b] A 20 W BLB fluorescent lamp and a 20 W daylight fluorescent lamp were suspended 10 cm apart and 18 cm above the plate. Light intensities were measured by a thermopile having a quartz window.

[c] Type numbers for the plastic band-pass filters produced by Fuji Photo Film Co., Ltd., Tokyo, Japan.

After 7 days of incubation under continuous irradiation the number of spore horns in a microscopic field (86.5 mm$^2$) was counted under a stereoscopic dissecting microscope fitted with an eyepiece reticule. Ten counts were made for each plate and three replicate plates were averaged.

After counting the spore horns conidium suspension was made by adding certain amount of 0.05% Tween #20 solution to each plate (60 mm diam.) and gently scraping the whole surface of the colony with a L-shaped glass rod. Resulting conidium suspension was further dispersed by a thermomixer (TM-100, Thermonics Co. Ltd., Tokyo) for 10 min. Concentration of conidia was determined with a hemocytometer.

**Monochromatic irradiation through interference filters**: One milliliter of conidium suspension ($5\times10^4$ conidia/ml) was inoculated to each VJA plate (60 mm diam.) and

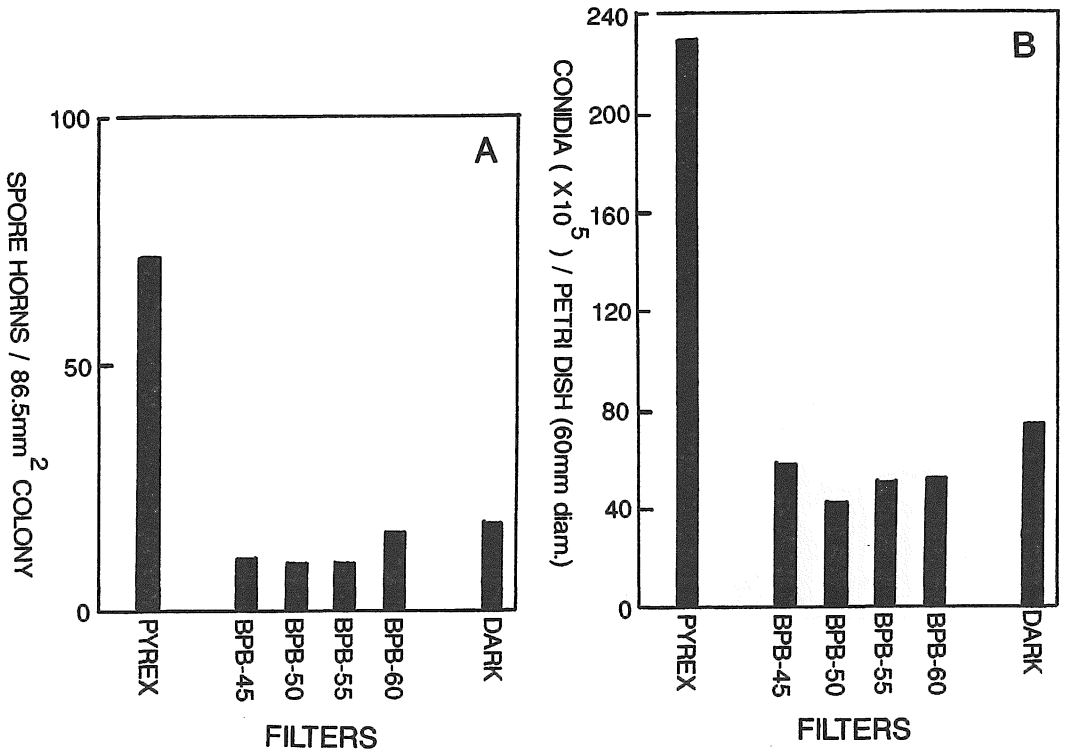


Fig. 1. Effect of light filtered through band-pass filters on sporulation of *Septoria obesa*. Colonies were irradiated continuously through band-pass filters for 7 days at 20 C. Colonies covered with only Pyrex petri dish lids served as a control. Dark control colonies were also included. Light source was comprised of a 20 W BLB fluorescent lamp and a 20 W daylight fluorescent lamp. The effect of light was determined by the numbers of spore horns (A) and conidia (B) formed in a unit area.

the inoculated plates were incubated for 4 days in the dark at 20 C to form mycelial mats. Then cultures were irradiated continuously under monochromatic radiations from 300 to 700 nm with approximately 10 nm intervals between 300 nm and 390 nm, and 20 nm intervals between 440 nm and 700 nm. Monochromatic radiations of 300 to 390 nm were obtained by filtering out the radiation from a 20 W BLB fluorescent lamp through interference filters for ultraviolet wave region. Two 20 W daylight fluorescent lamps and interference filters were exploited for the visible region of wavelengths between 440 nm and 700 nm. Intensities and other characteristics of monochromatic radiations were described in the previous report (Honda and Miyawaki, 1988). Counting of spore horns and conidia was made by the same way as described for the band-pass filter experiment.

## Results

**Effect of radiation filtered through band-pass filters on sporulation.**

Colonies of *S. obesa* initiated by streaking inoculation of conidia formed pycnidia

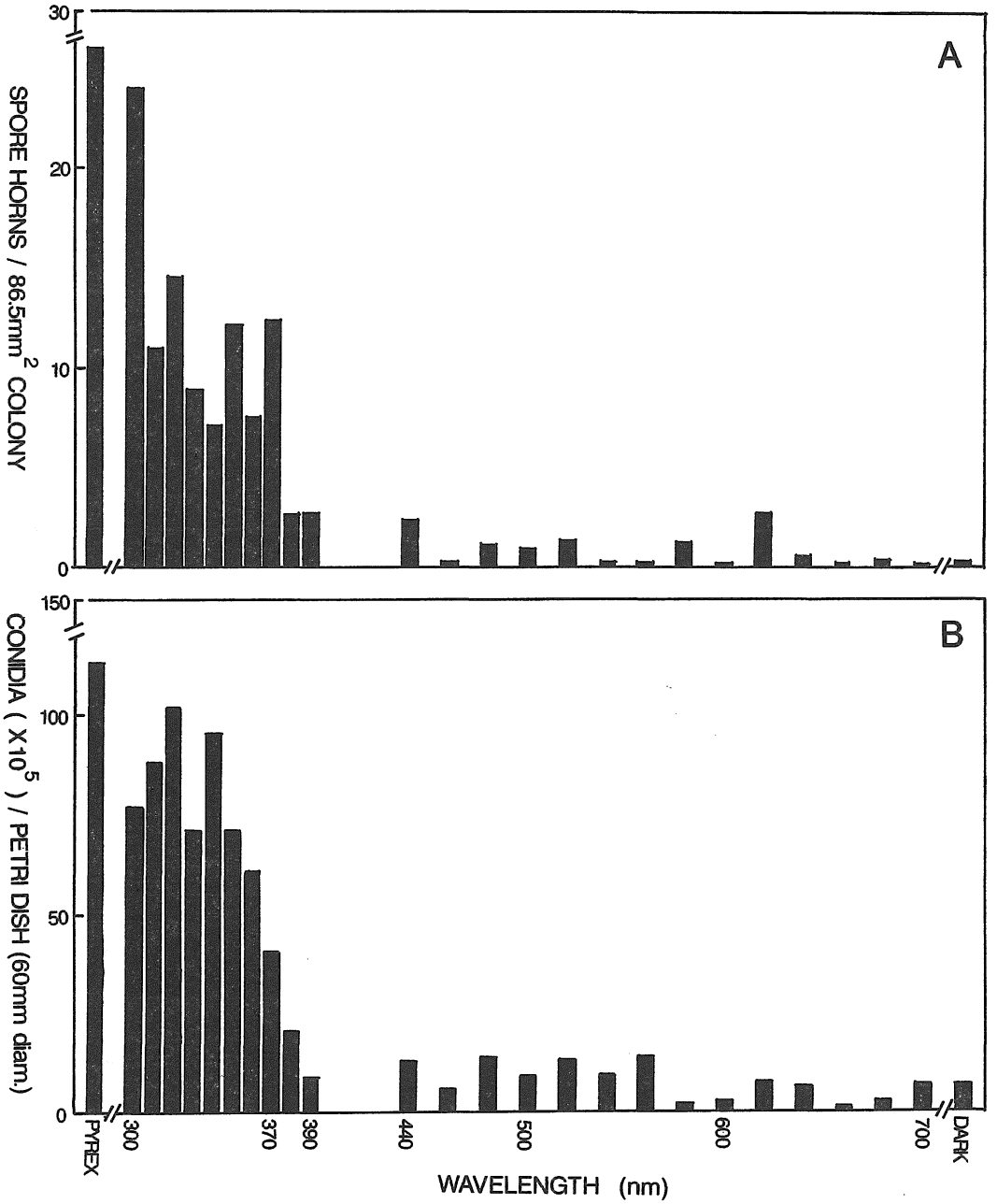


Fig. 2. Effect of monochromatic radiation on the sporulation of *Septoria obesa*. Monochromatic radiations from 300 to 700 nm were obtained by interference filters combined with a 20 W BLB fluorescent lamp and two 20 W daylight fluorescent lamps. Colonies were irradiated continuously for 7 days at 20 C. Intensities of the monochromatic radiations were not adjusted to the same level. Control colonies without filters or in light-tight boxes were adjacent to test colonies. The effect of light was determined by the numbers of spore horns (A) and conidia (B) formed in a unit area.

and exuded spore horns in the dark (Fig. 1). The number of pycnidia (Fig. 1A) and

conidia (Fig. 1B), however, was much smaller compared with colonies irradiated continuously by BLB and daylight fluorescent lamps without filters. Radiations filtered through band-pass filters with maximum transmission at 450, 500, 550, and 600 nm, respectively, were not effective to stimulate sporulation in the fungus. The numbers of spore horns and conidia formed under band-pass filters were smaller than the dark grown colonies, although the differences were not significant. Relative numbers of spore horns to conidia formed under different band-pass filters, without filters or darkness, respectively, did not differ significantly.

**Effect of monochromatic radiation on sporulation.**

In this experiment colonies were initiated by the whole surface inoculation with conidium suspension. The number of spore horns formed in the dark was small and it was less than one per 86.5 $mm^2$ colony on the average (Fig. 2A). Radiations shorter than 370 nm significantly increased the number of spore horns. Wavelength of 300 nm was the most effective to stimulate spore horn formation in spite of its lowest intensity among monochromatic radiations.

Almost the same result has been obtained for conidium formation (Fig. 2B). Wavelengths longer than 380 nm were not effective to stimulate sporulation. However, the relative numbers of conidia formed under radiations longer than 380 nm to those formed under 370 nm and shorter were larger compared to the same relative numbers of spore horns. Spore horns under radiation of 300 nm outnumbered those under other monochromatic radiations. But the number of conidia under radiation of 300 nm was smaller than those under some other effective monochromatic radiations as a result largely of smaller size of pycnidia.

Although many pycnidial primordia were formed in the dark or under continuous irradiation with monochromatic radiations longer than 380 nm, spore horns did not form under these conditions. The number of pycnidial primordia did not significantly differ between colonies irradiated continuously by non-filtered light and those kept in the dark, and also among colonies irradiated by monochromatic radiations irrespective of the effectiveness for spore horn formation.

## Discussion

Punithalingam (1966) reported that development of the pycnidium in *Septoria* proceeded from pycnidial primordium formation to formation of pycnidial cavity and pycnidial beak, followed by a rupturing of the beak to form ostiole through which conidia were exuded in a form of spore horns. Pycnidial primordia in *S. obesa* formed in the dark. However, light exposure was necessary for exudation of conidia as spore horns. Primarily, the effective wave region for spore horn formation was ultraviolet radiation shorter than 370 nm. In other species of *Septoria* effective wavelengths for mature pycnidium formation were reported to be almost same with *S. obesa*. Calpouzos et al. (1970) reported that only wavelengths in the near ultraviolet shorter than 350 nm induced pycnidium formation in *S. nodorum* Berk. For *S. tritici*

Rob. wavelengths of 310-400 nm were effective (Leach, 1962). Schlosser (1970) examined many species of fungi for their dependency on light exposure for sporulation and revealed that altogether 84% of the 417 strains of many species of fungi tested were stimulated by the "black light" and 54% of them were light dependent. *Septoria avenae* Frank, *S. passerinii* Sacc. and 16 isolates of *Septoria* spp. were involved in the light-dependent members. Sporulation response of *S. obesa* to light exposure was confirmed to be the same with those reported previously.

Honda and Aragaki (1978) reported a differential effect of light for inducing pycnidial stromata formation and exudation of conidia from pycnidia in *Botryodiplodia theobromae* Pat. Wavelengths shorter than 520 nm were effective for inducing the formation of columnar pycnidial stromata, in contrast to the ultraviolet radiation shorter than 330 nm effective for exudation of conidia. In *S. obesa* pycnidial primordia were formed abundantly in the dark, but exudation of conidia occurred only when cultures were exposed to radiations shorter than 370 nm. This result indicates that only certain stages of pycnidial development following primordial formation are light sensitive and other steps of the development are light independent.

Near ultraviolet radiation peaking at 366 nm inhibits pycnidium development in *Phyllosticta antirrhini* Syd. and the inhibition occurred sooner by increasing light intensity from 100 $\mu$W/cm$^2$ to 320 $\mu$W/cm$^2$ (Maiello, 1977). For *S. obesa* cultures were continuously irradiated to form mature pycnidia by two 20 W BLB fluorescent lamps with the maximum wavelength of 360 nm at the light intensity of 370 $\mu$W/cm$^2$. Any sign of inhibition of pycnidium formation could not perceived, rather this intensity of near ultraviolet radiation was suitable for induction of mature pycnidium formation in *S. obesa*.

Under complete darkness spore horns were formed in quite a few number, but these spore horns were bigger than those formed under stimulative radiations. The number of conidia per spore horn was larger in the dark or under radiation ineffective to stimulate spore horn formation than those formed under stimulative radiations. Radiation of 300 nm stimulated to form many spore horns, but total number of conidia were not proportional to that of spore horns. These results indicate that radiation around 300 nm is effective for formation of beaks and ostioles resulting in increased number of spore horns for pycnidium.

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## 摘　　要

キク褐斑病菌，*Septoria obesa* の分生子殻形成に及ぼす 300 nm から 700 nm の単色光の影響を観察した．分生子殻の原基は暗黒下でも形成されるが，胞子角を溢泌する成熟した分生子殻の形成には光照射が必要であった．その光の有効波長域は 370 nm 以下の近紫外線であった．波長が 300 nm の単色光は胞子角の形成誘導に最も有効であった．一方，波長が 400 nm から 700 nm の可視光線は成熟分生子殻の形成にやや抑制的に作用した．