島根大農研報 (Bull. Fac. Agr. Shimane Univ.) 22 : 161−175, 1988

Factors Affecting Conidium Germination of *Septoria obesa*, Causal Fungus of Brown Spot of Cultivated Chrysanthemum

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キク褐斑病菌，*Septoria obesa* の分生胞子の発芽に影響を及ぼす要因

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Inoculum concentration, age of cultures from which conidia were taken and light conditions were examined as factors affecting condium germination of *S. obesa*. Conidia for inoculation were taken from cultures which were started by streaking the conidia to vegetable juice agar plates and incubated under continuous light. When the concentration of the inoculum was 10^6 conidia/ml (= 3500 conidia/cm^2 agar plate), germination of conidia was suppressed strongly even the conidia were taken from young cultures. At a lower concentration of 10^5 conidia/ml (= 350 conidia/cm^2 agar plate), conidia germinated well. Germination of conidia which were taken from cultures with one-week incubation under continuous irradiation of near ultraviolet radiation (NUV) was not suppressed by light during germination. However, conidia which were taken from cultures with 4-week incubation under continuous NUV irradiation were completely inhibited to germinate by light. Effective wavelengths for suppression of conidium germination were NUV region shorter than 357 nm. Near ultraviolet radiation also suppressed the growth of germ tubes. From these observations it will be concluded that viability of conidia of *S. obesa* decreased by continuous irradiation of NUV and germination of conidia is suppressed by the same wavelengths of radiation.

Introduction

The leaf-spot diseases of cultivated chrysanthemum, *Chrysanthemum morifolium* Ramat., caused by *Septoria* spp. are very common and destructive in Japan. The occurrence of the diseases in Japan had already been confirmed in Kagoshima to Hokkaido including Shimane prececture (Hemmi and Nakamura, 1927).

The names of the causal fungi of Septorioses of the cultivated chrysanthemum had been somewhat confused. However, two *Septoria* spp. have been identified as causal organisms so far and they seem to be generally accepted. One is *Septoria chrysanthemella* Sacc. for the black spot disease and the other is *S. obesa* Syd. for the brown spot disease (Hemmi and Nakamura, 1927; Punithalingam, 1967). These diseases are distinguished each other by the symptoms and the morphological characteristics of

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conidia of the causal fungi (Punithalingam, 1967).

Many factors are involved in the germination of fungal spores and self-inhibitors for spore germination have been demonstrated in many fungi (Sussman and Douthit, 1973). The presence of self-inhibitors may be suspected if high concentrations of spores germinate less well than lower ones (Boyd, 1952).

Cirrhus extract has profound effect on conidium germination in *Septoria nodorum* (Berk.) Berk. The highly concentrated aqueous extract of cirrhi inhibits conidium germination, whereas cirrhus extract stimulates it when diluted (Fournet, 1969). Brennan et al. (1986) confirmed that the presence of cirrhus extract stimulated germination of conidia of *S. nodorum* in suspension. The conidial matrix tended to promote the germination of conidia of *Phoma medicaginis* Malbr. & Roum. (Chung and Wilcoxson, 1969).

Light is another important factor influencing the germination and viability of spores. Exposure to sunlight decreases germination rate of conidia of *S. nodorum* (Fournet, 1969). The viability of conidia of the same fungus in aqueous suspension decreased more rapidly under daylight than in the dark (Brennan et al., 1986). About 20 hr exposure to sunlight on relatively clear days in mid summer in England reduced germinabilbty of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn. uredospores to 10%. Wavelengths within the 315-400 nm band were mainly responsible for this inactivation (Maddison and Manners, 1972). On the other hand, Calpouzos and Chang (1971) showed in *P. graminis* f. sp. *tritici* uredospores that a marked inhibition of germination occurred in two spectral regions, one with a peak in the blue around 419 to 425 nm and the other with a peak in the far red around 720 nm. Simultaneous irradiation with ineffective red (653 nm) and inhibitory far red light (720 nm) results in partial nullification of the inhibition brought about by far red light alone. This result would be consistent with the involvement of a photoreversible pigment system similar to phytochrome (Lucas et al., 1975).

In the disease cycle of Septorioses on chrysanthemum the pathogen overwinters in diseased leaves left on the ground in autumn. The conidia are said to be splashed from the diseased leaves up to a height of 8-16 inches and initiate the disease. Thereafter the conidia are disseminated by splashed or wind blown water or by mechanical transfer on workman's clothing, tools and the cloth covering plants when the foliage is wet (Punithalingam, 1967). All these observations indicate that viability and germinability of conidia are important rings in the infection chain of this disease.

In this paper we reported the effect of light on the viability and germinability of conidia of *S. obesa* in connection with conidium concentration, age of conidia and cirrhus extract.

Materials and methods

Organism: The causal fungus, *Septoria obesa* Syd., Se 80, of brown spot disease of cultivated chrysanthemum was isolated by mass of conidia oozing out from a pycnidium formed on a diseased leaf of *Chrysanthemum morifolim* Ramat. (cv. Seikou-

no-hikari). The cultures of the isolate have been kept on vegetable juice agar (VJA: Campbell's V-8 juice 200 ml, $CaCO_3$ 2 g, agar 17 g, distilled water 800 ml; pH 5.8) slants.

Cultivation: Spore horns were taken from a slant culture and the conidia were inoculated by streaking method to VJA (10ml/60mm diam. Pyrex Petri dish) plates. At first, the inoculated plates were incubated in the dark for 3-4 days to form mycelial mats, then irradiated continuously by two 20 W Black Light (BLB) fluorescent lamps (FL20S. BLB, Toshiba Corporation, Tokyo; 300-410 nm, Max. 360 nm) suspended 10 cm apart and 18 cm above the plates for 7 days at 20 C to form pycnidia. These cultures with abundant pycnidia were stored in a refrigerator at 4 C and conidia for the inoculations were taken from them for the following experiments.

Germination of conidia from cultures with differnt periods of irradiation: Cultures started with streak inoculation of conidia were incubated under continuous irradiation by two 20 W BLB fluorescent lamps with the light intensity of 370 $\mu W/cm^2$ for 1, 2, 3, or 4 weeks at 20 C. 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). After each incubation period cultures were flooded by certain amount of sterilized distilled water with 0.1% Tween #20 and conidia were dispersed by a L-shaped glass rod. Resulting conidium suspensions were further dispersed by a thermomixer (TM-100, Thermonics Co., Ltd., Tokyo) for 10 min, then filtered through two layers of cheese cloth. Concentrations of conidia were adjusted to 10^6 and 10^5 conidia/ml. An aliquot of 0.5 ml of conidium suspension was inoculated to plane water agar (agar 17 g, distilled water 1000 ml; pH 5.5: 10 ml/60 mm Pyrex Petri dish) plates and conidia were dispersed by a L-shaped glass rod. Surfaces of plane water agar plates were dried for two days in a clean room at ca. 20 C before inoculation. Inoculated plates were divided into two groups, one group was incubated under continuous light from two 20 W BLB fluorescent lamps (370 $\mu W/cm^2$), and another group was kept in the dark at 20 C. Conidium germination was observed at 12, 24, 36, 48 and 96 hr after inoculation. Percentage germination, types of germination and length of germ tubes were determined at each observation. Conidium germination was classified into three types: terminal type, a conidium germinates from one or both of terminal cell (s); intercalary type, a conidium germinates only from intercalary cell (s); mixed type, a conidium germinates from both terminal cell (s) and intercalary cell (s). If there were more than one germ tubes from a conidium, length of the longest one was measured.

Effect of light on the viability of spores in suspension: Conidium suspension of 10^6 conidia/ml was prepared as described above. It was dispensed 20 ml each to a 60 mm Pyrex Petri dish. These Petri dishes with conidium suspension were then divided into two groups, one group was irradiated continuously and the other was kept in the dark. Time of irradiation was 12, 24, 48 and 96 hr, respectively. During 96 hr experiment period no conidia germinated in the suspension. After each irra-

diation period an aliquot of conidium suspension was taken and diluted to 10^5 conidia/ml, and 0.5 ml each of the diluted conidium suspension was inoculated to a plane water agar plate. Inoculated plates were incubated in the dark at 20 C. Germination of conidia was observed at 12, 24, 36 and 48 hr after inoculation. Germination of conidia in suspension kept in the dark was observed the same way as for the irradiated ones.

Five combinations of light conditions for incubation of cultures for inoculum and irradiation conditions of conidium suspension were set as follows.

(1) One-week cultivation under continuous irradiation by two 20 W BLB fluorescent lamps (near ultraviolet radidtion = NUV, 370 $\mu W/cm^2$) for the inoculum, and also for irradiation of the conidium suspension.

(2) One-week cultivation under continuous irradiation (NUV, 370 $\mu W/cm^2$) for the inoculum, and five 20 W BLB fluorescent lamps (850 $\mu W/cm^2$) for irradiation of the conidium suspension.

(3) One-week cultivation under continuous irradiation (NUV, 370 $\mu W/cm^2$) for the inoculum, and five 20 W Cool White fluorescent lamps (FL 20 S. D, Toshiba Corporation, 350–730 nm, 1290 $\mu W/cm^2$) filtered through an ultraviolet-absorbing vinyl film (Hi-S; Nippon Carbide Co., Ltd., with a UV cut-off at 390 nm) for irradiation of the conidium suspension.

(4) Three-week cultivation under continuous irradiation (NUV, 370 $\mu W/cm^2$) for the inoculum, and two 20 W BLB fluorescent lamps (370 $\mu W/cm^2$) for irradiation of the conidium suspension.

(5) Three-week cultivation under continuous irradiation (NUV, 370 $\mu W/cm^2$) for the inoculum, and five 20 W BLB fluorescent lamps (850 $\mu W/cm^2$) for irradiation of the conidium suspension.

Effect of cirrhus extract on conidium germination: Conidium suspension was prepared from the cultures incubated under continuous irradiation of NUV (370 $\mu W/cm^2$) for one week at 20 C. By adding distilled water to cultures and scraping conidia with a L-shaped glass rod, conidium suspension was obtained. The conidium suspension was centrifuged at 3000 rpm for 10 min and supernatant water was discarded. Conidium pellet was resuspended and centrifuged again. Thus cirrhus extract was removed from the mass of conidia. Washed conidia were resuspended to distilled water and conidium concentrations were adjusted to 10^6 and 10^5 conidia/ml. Conidium suspensions of 0.5 ml from each concentration were inoculated to plane water agar plates (60 mm diam. Pyrex Petri dish). Conidium suspensions without washing were also prepared and the concentration of conidia was adjusted as same as the washed ones. These conidium suspensions were also inoculated to plane water agar plates. Of two inoculated plates of different concentrations of conidia and different conidium preparations, one plate was irradiated continuously by NUV (370 $\mu W/cm^2$), and the other was incubated in the dark. Germination of conidia was observed 12, 24, 36 and 48 hr after inoculation.

In order to avoid infiltration of cirrhus extract into an agar plate, germination of

conidia was observed in suspension with or without cirrhus extract at three different conidium concentrations of 10^6, 10^5 and 10^4 conidia/ml. Conidia washed twice with distilled water were resuspended into distilled water at three above mentioned concentrations. Three milliliteres of each conidium suspension were dispensed into a Pyrex Petri dish (60 mm diam.). The conidium suspension without washing was also dispensed as same as the washed one. Conidium germination was observed after 12, 24, 36, and 48 hr incubation under continuous irradiation of NUV (370 $\mu W/cm^2$) or in the dack.

Effect of light quality on spore germination : A conidium suspension was prepared from cultures incubated under continuous irradiation of NUV (370 $\mu W/cm^2$) for three weeks at 20 C. Conidia were suspended into sterilized distilled water with 0.1% Tween #20 and the concentration was adjusted to 10^5 conidia/ml. The conidium suspension of 0.5 ml each was inoculated to a plane water agar plate. Inoculated agar plates were incubated under continuous irradiation of monochromatic radiations

Table 1. Interference filters and intensities of monochromatic radiations

λ_{max}(nm)	T_{max}(%)[a]	$\Delta\lambda 1/2$(nm)[b]	Type[c]	Intensities[d] ($\mu W/cm^2$)
297	25	18	MIF-W	0.8
309	26	15	MIF-W	1.1
317	28	16	MIF-W	2.2
328	25	16	MIF-W	4.9
338	25	20	MIF-W	12.2
349	18	20	MIF-W	15.4
357	25	22	MIF-W	20.5
369	22	19	MIF-W	17.3
383	30	13	MIF-S	7.9
391	35	12	MIF-S	6.7
409	36	12	MIF-S	5.9
441	37	10	MIF-S	11.1
458	32	10	MIF-S	14.0
480	43	10	MIF-S	9.7
499	37	8	MIF-S	6.2
520	36	8	MIF-S	7.3
540	43	8	MIF-S	9.5
558	37	7	MIF-S	8.9
579	38	8	MIF-S	7.1
599	43	8	MIF-S	9.2
622	37	7	MIF-S	7.1
640	40	8	MIF-S	4.9
660	40	8	MIF-S	2.4
683	42	8	MIF-S	1.4
700	38	8	MIF-S	1.1

[a] Maximum transmittance (%) at the central wavelength (λ_{max}) of transmission.
[b] Band width at the half of maximum transmittance (=half band width).
[c] Metal interference filters combined with appropriate colored glass filters. S-type has narrow half band width with wide band width. W-type has wide half band width with rather narrow band width.
[d] Intensities of monochromatic radiations were measured by a theremopile coupled with a digital multimeter.

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 light from a 20 W BLB fluorescent lamp through interference filters. Daylight fluorescent lamps were used as light source combined with interference filters for the wavelengths between 440 nm and 700 nm. Intensities of monochromatic radiations were not adjusted to the same level and indicated in Table 1. Germination of conidia was observed after 24 hr incubation at 20 C.

Results

1. Effects of conidium concentration and irradiation of light on germination of conidia obtained from cultures with different incubation periods under continuous irradiation.

Light irradiation did not affect the germination of conidia from cultures with incubation period of one week under continuous irradiation of NUV. Instead conidium

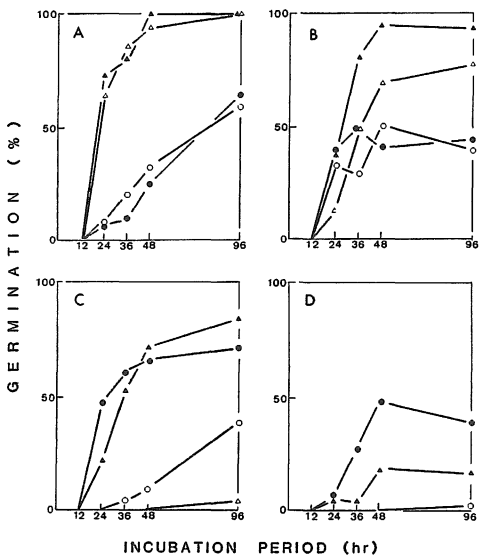

Fig. 1. Effect of light on germination of conidia at two concentrations taken from cultures with different incubation period under continuous light.
● ; Dark, 10^6 conidia/ml
▲ ; Dark, 10^5 conidia/ml
○ ; Light, 10^6 conidia/ml
△ ; Light, 10^5 conidia/ml
A : Conidia taken from one-week old cultures
B : Conidia taken from two-week old cultures
C : Conidia taken from three-week old cultures
D : Conidia taken from four-week old cultures

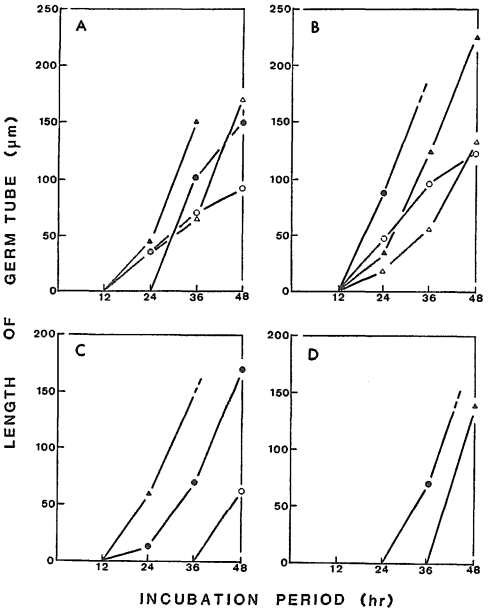

Fig. 2. Effect of light on growth of germ tubes of conidia at two concentrations taken from cultures with different incubation periods under continuous light.
● ; Dark, 10^6 conidia/ml
▲ ; Dark, 10^5 conidia/ml
○ ; Light, 10^6 conidia/ml
△ ; Light, 10^5 conidia/ml
A : Conidia taken from one-week old cultures
B : Conidia taken from two-week old cultures
C : Conidia taken from three-week old cultures
D : Conidia taken from four-week old cultures

concentration had profound effect on the germination of conidia (Fig. 1A). At higher concentration of conidia (10^6 conidia/ml = 3500 conidia/cm^2 agar plate) germination delayed and percentage germination attained at 96 hr incubation period was smaller compared with that of lower concentration (10^5 conidium/ml = 350 conidia/cm^2). On the other hand the concentration did not affect profoundly on the germination of conidia taken from 3- to 4-week old cultures. Instead light irradiation suppressed the germination of these conidia (Fig. 1C, D). Percentage germination decreased with incubation period, under continuous NUV irradiation, of cultures from which the conidium suspension was prepared. Conidia from 2-week old cultures showed intermediate characteristics of germination between conidia from one-week old and 3- or 4-week old cultures (Fig. 1B). That is, light exposure suppressed conidium germination and suppressive effect of higher concentration was also apparent. Conidia from 4-week old cultures germinated only in the dark irrespective of inoculum concentration, and percentage germination did not exceed 50%.

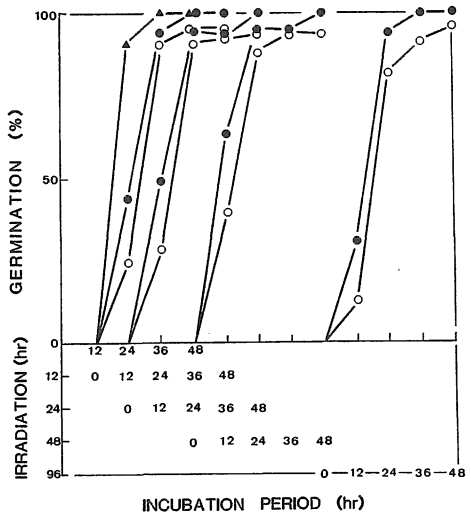

Fig. 3. Effect of near ultraviolet radiation on germination of conidia from one-week old cultures at low intensity.
Suspension of conidia taken from one-week old cultures was irradiated for 12 to 96 hr by two 20 W BLB fluorescent lamps (370μW/cm^2). After respective irradiation conidia were inoculated to water agar plates and incubated in the dark. Suspension of conidia for dark control was kept in the dark and conidia were inoculated as same as the light treatment. A water agar plate inoculated with conidia immediately after preparation of conidium suspension served as another control.
▲ ; Immediate inoculation control
● ; Dark control
○ ; Light treatment

Inoculum concentration influenced the germination types of conidia. At higher concentration (10^6 conidia/ml), more than 90% of conidia germinated from terminal cell (s) (terminal type germination). At decreased concentration (10^5 conidia/ml), percentage of conidia germinated from both terminal and intercalary cells (mixed type germination) increased along with incubation period, and it came up to more than 50% at 48 hr incubation when conidia were taken from one-week old cultures. In general, conidia of this fungus at first germinated from terminal cell (s), then the intercalary cell (s) resulting in the mixed type germination for the most of the conidia. Conidia which germinated only from intercalary cell (s) were in small number in the population. With the increase of incubation period of cultures for inoculum under continuous irradiation of NUV, percentage of conidia increased which germinated only from terminal cell (s). Fifty percent of conidia taken from one-week old cultures showed terminal type germination in the dark 36 hr after inoculation at concentration of 10^5 conidia/

ml, contrasting with the result that more than 90% of conidia showed terminal type germination after 48 hr incubation if the conidia were taken from 3-week old cultures.

Growth of germ tubes was suppressed by irradiation of conidia during germination (Fig. 2). In all treatments and through out whole incubation period for germination, length of germ tubes in the dark was longer than those under continuous irradiation. The longer the incubation period of cultures for inoculum, the stronger the suppressive effect of light on the growth of germ tubes, and conidia from 4-week old cultures did not germinated at all under continuous irradiation of NUV.

2. Effect of light on the viability of conidia in suspension.

Conidia taken from cultures with various incubation periods under continuous irradiation of NUV were irradiated continuously in the suspension at the concentration of 10^6 conidia/ml. At this concentration, conidia did not germinate in the suspension during 48 hr irradiation treatment.

(1) One-week old cultures under continuous irradiation for the inoculum, and two 20 W BLB fluorescent lamps (370 μW/cm^2) for irradiation of the conidium

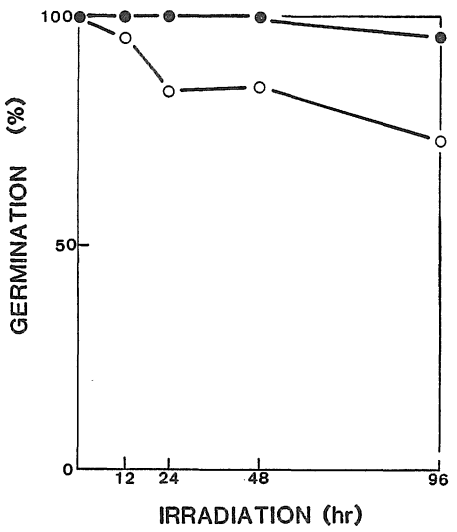

Fig. 4. Effect of near ultraviolet radiation on germination of conidia from one-week old cultures at high intensity.
Suspension of conidia taken from one-week old cultures was irradiated for 12 to 96 hr by five 20 W BLB fluorescent lamps (850μW/cm^2). After respective irradiation conidia were inoculated to water agar plates and percentage germination was examined after 48 hr incubation in the dark. Suspension of conidia for dark control was kept in the dark and conidia were inoculated as same as the light treatment.
● ; Dark control
○ ; Light treatment

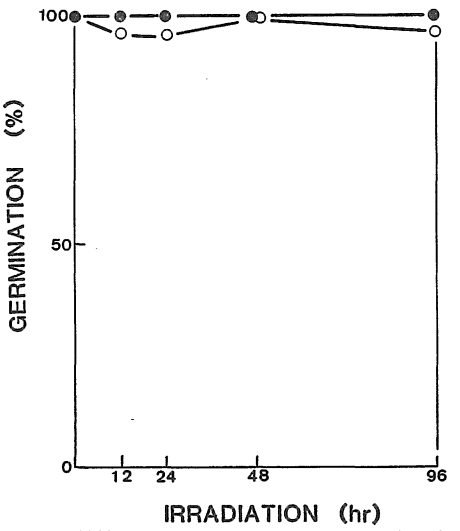

Fig. 5. Effect of white light on germination of conidia from one-week old cultures. Suspension of conidia taken from one-week old cultures was irradiated for 12 to 96 hr by five 20 W daylight fluorescent lamps (1290μW/cm^2) covered with UV absorbing vinyl film to exclude UV wavelengths. After respective irradiation conidia were inoculated to a water agar plate and percentage germination was examined after 48 hr incubation in the dark. Suspension of conidia for dark control was kept in the dark and conidia were inoculated as same as the light treatment.
● ; Dark control
○ ; Light treatment

suspension.

Almost all conidia germinated in the dark 48 hr after inoculation on plane water ager plates (Fig. 3). However, germination of conidia irradiated in the suspension delayed as compared with conidia which were kept in the dark in the suspension. Difference in the percentage germination between irradiated and nonirradiated conidia was most prominent at 12 hr after inoculation. In this experiment prolonged irradiation of conidia for 96 hr did not suppress the final percentage germination.

(2) One-week old cultures under continuous irradiation for inoculum, and five 20 W BLB fluorescent lamps (850 $\mu W/cm^2$) for irradiation of the conidium suspension.

Conidia irradiated continuously with NUV radiation of high intensity for 12 to 96 hr in the suspension was examined for their germination on a water agar plate after 48 hr incubation in the dark. Percentage germination of irradiated conidia decreased along with duration of irradiation, and germination of conidia irradiated for 96 hr decreased to 73% as compared with almost 100% germination of conidia which were kept in the dark (Fig. 4). Type of spore germination did not differ between irradiated and non-irradiated conidia, and 60 to 80% of conidia showed terminal type germination.

(3) One-week old cultures under continuous irradiation for the inoculum, and five 20 W daylight fluorescont lamps (1290 $\mu W/cm^2$) for irradiation of the conidium suspension.

To exclude ultraviolet (UV) wavelengths daylight fluorescent lamps were covered with UV absorbing vinyl film. Conidium germination was examined after 48 hr of incubation in the dark. The result showed that germination of conidia was not suppressed even by the prolonged irradiation (96 hr) of daylight fluorescent lamps without UV wavelengths at high intensity of 1290 $\mu W/cm^2$ in the conidium suspension, contrasting with decreased percentage germination of conidia irradiated by NUV with a high intensity of 850 $\mu W/cm^2$ for 96 hr (Fig. 5).

There was no difference in germination type between irradiated and non-irradiated conidia. Half of conidia showed terminal type germination and the rest proceeded to mixed type germination.

(4) Three-week old cultures under continuous irradiation for the inoculum, and two 20 W BLB fluorescent lamps (370 $\mu W/cm^2$) for irradiation of the conidium suspension.

Conidia from 3-week old cultures were used as inoculum and other conditions for the experiment were the same as the preceding experiment (1). Germination of conidia at 12 hr incubation period was significantly suppressed in irradiated conidia compared with non-irradiated conidia irrespective of the length of irradiation period (Fig. 6). However, percentage germination of irradiated conidia increased to almost the same level with the non-irradiated conidia after 24 hr incubation and there was no difference at 48 hr incubation. Maximum percentage germination slightly decreased with the time for which conidia were kept in suspension for both of irradiated and non-irradiated ones.

Terminal type germination was greater in number in the irradiated conidia than

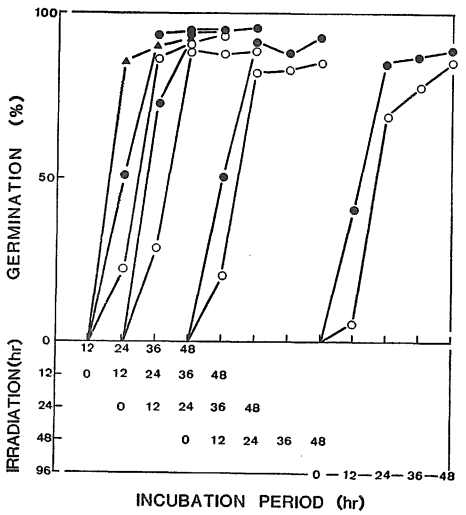

Fig. 6. Effect of near ultraviolet radiation on germination of conidia from 3-week old cultures at low intensity.
Suspension of conidia taken from 3-week old cultures irradiated for 12 to 96 hr by two 20 W BLB fluorescent lamps (370μW/cm^2). After respective irradiation conidia were inoculated to agar plates and incubated in the dark. Suspension of conidia for dark control was kept in the dark and conidia were inoculated as same as the light treatment. An agar plate inoculated with conidia immediately after preparation of conidium suspension served as another control.

▲ ; Immediate inoculation control
● ; Dark control
○ ; Light treatment

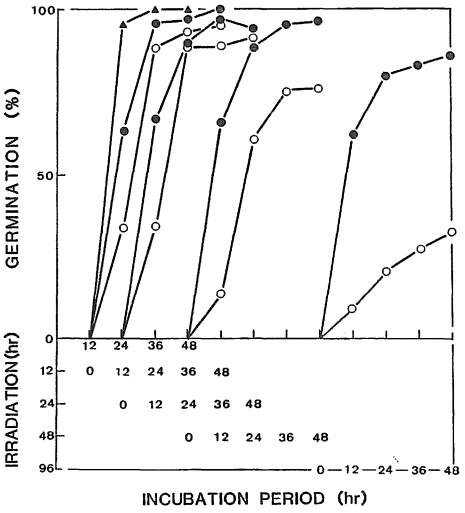

Fig. 7. Effect of near ultraviolet radiation on germination of conidia from 3-week old cultures at high intensity.
Suspension of conidia taken from 3-week old cultures irradiated for 12 to 96 hr by five 20 W BLB fluorescent lamps (850μW/cm^2). After respective irradiation conidia were inoculated to agar plates and incubated in the dark. Suspension of conidia for dark control was kept in the dark and conidia were inoculated as same as the light treatment. An agar plate inoculated with conidia immediately after preparation of conidium suspension served as another control.

▲ ; Immediate inoculation control
● ; Dark control
○ ; Light treatment

the non-irradiated ones. In contrast, mixed type germination was greater in number in the non-irradiated conidia.

(5) Three-week old cultures under continuous irradiation for the inoculum, and five 20 W BLB fluorescent lamps (850 μW/cm^2) for irradiation of the conidium suspension.

In this experiment intensity of NUV was increased to 850 μW/cm^2 by exploiting five 20 W BLB fluorescent lamps as light source. The final percentage germination at 48 hr incubation decreased with the increase of the irradiation period of the conidium suspension (Fig. 7). The conidia which were irradiated for 96 hr in suspension germinated only 33% at 48 hr incubation, contrasting with high percentage of germination (88%) of the non-irradiated conidia. Most of the irradiated conidia showed terminal type germination. On the other hand, only half of the non-irradiated conidia germinated terminally and the rest germinated through mixed type.

3. Effect of cirrhus extract on the conidium germination.

Conidia inoculated to the plane water agar plate with cirrhus extract (non-washed conidia) germinated as well as those inoculated without the cirrhus extract (washed conidia) in the dark. However, germination of conidia retarded under continuous irradiation of NUV, especially when the concentration was 10^6 conidia/ml, and the final percentage germination was around 60% (Fig. 8). Both washed and non-washed conidia germinated 100% after 36 hr incubation in the dark irrespective of the inoculum concentration.

Conidium germination in suspension was examined after 12 to 48 hr incubation in the dark. Conidia in the suspension with cirrhus extract germinated as well as those in the suspension without cirrhus extract at the concentrations of 10^5 and 10^4 conidia/ml, although the maximum germination of conidia at the concentration of 10^5 conidia/ml remained around 60%, significantly lower compared with around 90% germination of conidia with the concentration of 10^4 conidia/ml (Fig. 9). On the other hand,

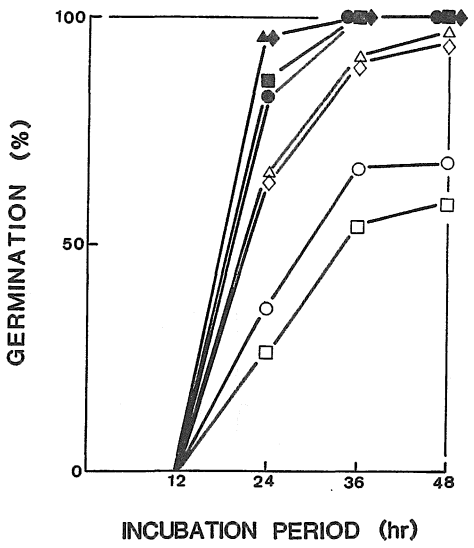

Fig. 8. Effects of light and cirrhus extract on the conidium germination on water agar plates.
Conidia were washed thrice to exclude cirrhus extract and inoculated to water agar plates at concentrations of 10^5 and 10^6 conidia/ml. Inoculated plates were incubated in the dark and the continuous light for 12 to 48 hr. Time course of germination was examined during incubation. Non-washed conidia served as controls.

● ; Non-Washed, 10^6 conidia/ml, Dark
▲ ; Non-Washed, 10^5 conidia/ml, Dark
○ ; Non-Washed, 10^6 conidia/ml, Light
△ ; Non-Washed, 10^5 conidia/ml, Light
■ ; Washed, 10^6 conidia/ml, Dark
◆ ; Washed, 10^5 conidia/ml, Dark
□ ; Washed, 10^6 conidia/ml, Light
◇ ; Washed, 10^5 conidia/ml, Light

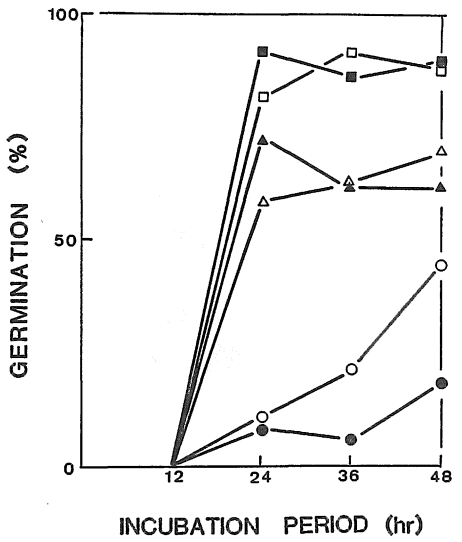

Fig. 9. Effect of cirrhus extract on the conidium germination in suspension.
Conidia were washed thrice to exclude cirrhus extract and resuspended in the distilled water at three concentrations (10^4, 10^5, 10^6 conidia/ml). These suspensions were incubated in the dark for 12 to 48 hr. Time course of germination was examined during incubation in the dark. Non-washed conidia served as controls.

● ; Non-Washed, 10^6 conidia/ml, Dark
▲ ; Non-Washed, 10^5 conidia/ml, Dark
■ ; Non-Washed, 10^4 conidia/ml, Dark
○ ; Washed, 10^6 conidia/ml, Dark
△ ; Washed, 10^5 conidia/ml, Dark
□ ; Washed, 10^4 conidia/ml, Dark

germination was significantly suppressed by the cirrhus extract at the concentration of 10^6 conidia/ml. Conidia in the suspension of 10^6/ml without cirrhus extract germinated slowly and attained 44% germination at 48 hr incubation in the dark, contrasting with 18% germination of the conidia with cirrhus extract. The final percentage germination decreased with the increase of inoculation concentration of conidia.

4. Effect of light quality on conidium germination.

Percentage germination was 75% in the conidia incubated in the dark for 24 hr. Continuous irradiation with panchromatic light suppressed the conidium germination and the final percentage germination remained at 35%. Under continuous irradiation of monochromatic radiations between 370 and 700 nm, conidia germinated as well as those kept in the dark. However, NUV radiations from around 300 to 360 nm suppressed conidium germination to about 50% (Fig. 10).

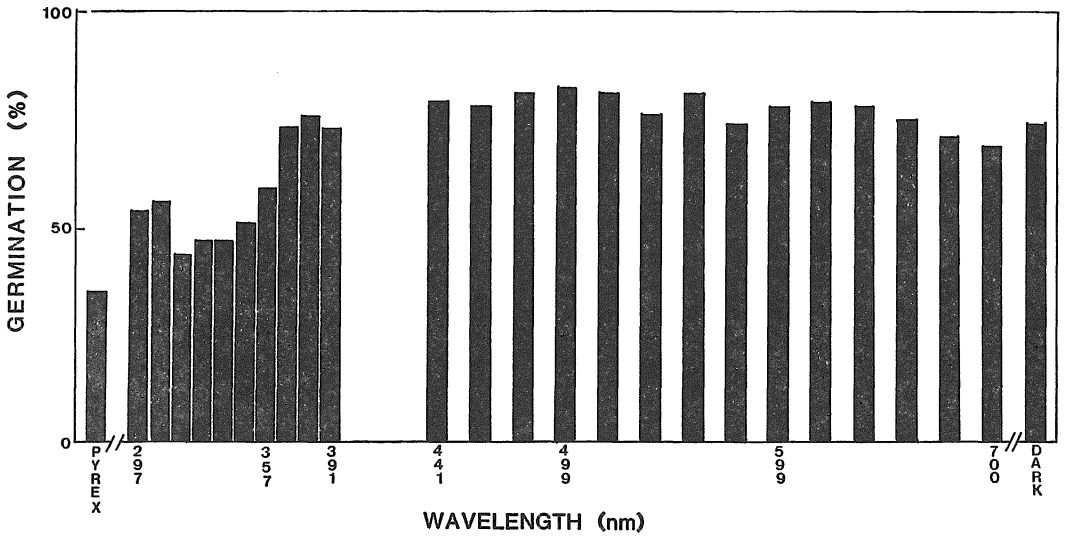

Fig. 10. Effect of monochromatic radiations on the conidium germination.
Conidium suspension (10^5 conidia/ml) was inoculated to water agar plates and irradiated continuously by monochromatic radiations through interference filters for 24 hr. Control plates (Pyrex) without interference filter were beside them. Water agar plates with conidia for dark control were kept in the dark and also beside other plates. Conidium germination was examined after 24 hr irradiation.

There was no obvious effect of monochromatic rddiation on the growth of germ tubes. Terminal type of conidium germination prevailed more than 80% under monochromatic radiations from 300 to 360 nm where percentage germination was low compared with the non-irradiated conidia. Mixed type of germination was less than 15% accompanied by intercalary type of germination of 5% or less in the conidia irradiated by monochromatic radiations of 300 to 360 nm. By contrast, more than 30% of conidia showed mixed type of germination under monochromatic radiations of 370 to 700 nm and in the dark, where percentage germination was high. At the

lowest percentage germination under continuous irradiation of non-filtered light (= Pyrex), more than 95% of germinated conidia were terminal type.

Discussion

The isolate used for this experiment was identified as *S. obesa* based on the morphological characteristics of conidia and vigorous growth at 20 C. The vigorous growth and abundant sporulation at 20 C were also reported for *Septoria nodorum* Berk. (Richards, 1951). In *S. chrysanthemella* Sacc., causal fungus of auother Septoria leaf-spot of cultivated chrysanthemum, black spot disease, the most vigorous growth was at about 28 and 24 C (Hemmi and Nakamura, 1927). Conidia of the test fungus are hyaline, whip-shaped, thick and round at the base, gradually tapering towards a round apex, 73-99 × 2.6-3.2 μm, 6-10 septate. These measurements and shape of conidia clearly differentiate this fungus from *S. chrysanthemella*, which has filiform conidia 36-65 μm long, 1.5-2.5 μm wide and 4-9 septate (Punithalingam, 1967).

Germination of conidia of *S. obesa* was inhibited by light, especially conidia obtained from cultures incubated under continuous NUV irradiation with high intensity for long period decreased their germinability. Effective wavelengths for germination inhibition were restricted to near ultraviolet region of 300 to 360 nm. Radiations longer than 370 nm were not inhibitory to the conidium germination. In uredospores of *Puccinia graminis* f. sp. *tritici*, effective wavelengths for reducing germinability are in 315-400 nm band, and NUV of 300 nm does not reduce the germinability (Maddison and Manners, 1972). Near ultraviolet radiation of 297 nm was effective for rdeucing germinability in *S. obesa*, even the intensity of the NUV was quite low compared with other wavelengths of radiation. The upper limit (357 nm) of inhibitive wavelengths was also different from that (400 nm) of *P. graminis* f. sp. *tritici*. From these results spectral dependence for photoinhibition of conidium germination in *S. obesa* seems to be different from that of *P. graminis* f. sp. *tritici*. Calpouzos and Chang (1971) reported blue (peak at around 419-425 nm) and far red (peak at around 720 nm) spectral regions were effective to inhibit uredospore germination in the same species of fungus. We could not confirm this effect in *S. obesa*, although percentage germination reduced a little under monochromatic radiations of 680 and 700 nm. This reduction in germination is not significant compared with the dark control, and blue light wavelengths did not show any inhibitive effect on germination in *S. obesa*. This observation will be enough to rule out the possibility in this fungus of involvement of phytochrome-like photoreversible pigment system in photoinhibition of conidium germination that Lucas et al. (1975) proposed for uredospore germination in *P. graminis* f. sp. *tritici*.

Light intensity of five 20 W BLB fluorescent lamps used for the irradiation experiment on the conidium germination in suspension was 850 $\mu W/cm^2$ and the conidium germination was significantly suppressed. Inhibitory effect of sunlight to conidium germination has been reported in *S. nodorum* (Fournet, 1969; Brennan et al., 1986) and *P. graminis* f. sp. *tritici* (Maddison and Manners, 1972) as well. The intensity of

radiation from BLB fluorescent lamps (emission range: 300–410 nm) roughly corresponds to one-third of 2600 $\mu W/cm^2$ (calculated from the data on spoctral energy distribution of sunlight in Honda and Yunoki, 1977) which is the intensity of near-ultraviolet region of 300–400 nm of direct sunlight at noon in summer time in Morioka, northern part of Japan Therefore, conidia exuded from pycnidia on leaves will be exposed to the sunlight and inhibited to germinate by ultraviolet wavelengths of radiation with quite higher intensity than the experimental condition if they were spread by rain splash or dew on the leaves. This suggests that certain duration of shady period is prerequisite to successful germination and infection for the conidia of *S. obesa*.

Germination was greatly affected by the concentration of conidia. Conidia inoculated at the concentration of 10^6/ml (= 3500 conidia/cm^2) always showed lower germination compared with the conidia of 10^5/ml. The decreased germination at higher concentration of conidia can be brought about by high concentration of cirrhus extract in the conidium suspension as reported by Fournet (1969) for conidium germihation of *S. nodorum* in the highly concentrated aqueous extract of cirrhi. It is partly the case for *S. obesa* because elimination of cirrhus extract from conidium suspension by repeated washing allowed conidia to germinate in the suspension even at the high conidium concentration (10^6 conidia/ml). However, in *S. nodorum* higher percentage of germination was obtained in the suspension with cirrhus extract when it was diluted (Fournet, 1969; Brennan et al., 1986). Promotive effect of the conidial matrix on conidium germination has also been reported for *Phoma medicaginis* (Chung and Wilcoxson, 1969). Concentration of the conidia in *S. nodorum* experiment was 3.25×10^5 conidia/ml. Contrary to this result, at the same concentration of conidia in *S. obesa* the cirrhus extract did not promote conidium germination. Thus, though conidium germination is suppressed by cirrhus extract, the suppression was not enough to account for decrease of spore germination at higher concentration of conidia. As Boyd (1952) pointed out, this result suggests the presence of self-inhibitor in the conidia of *S. obesa*. Although the nature of the self-inhibitor of *S. obesa* is still to be revealed, it may have the properties as a regulatory substance which prevents metabolic activities of conidia in high concentration from proceeding at more than a minimum rate as suggested by Gottlieb (1973). Inhibitory effect of cirrhus extract at high concentration may work additively to the effect of the self-inhibitor to prevent conidia from germinating in a spore horn or within a pycnidium.

Type of germination varied with concentration of conidia inoculated and the percentage germination. At higher concentration of conidia terminal type germination prevails the most of the germinated conidia with only a small portion of mixed type and intercalary type of germination. Almost the same result was obtaioned under conditions where the percentage germination was low. In general, the conidia with low germinability or activity germinate only terminally, and germination of the conidia with high germinability proceeds from terminal type to mixed type.

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摘　　要

キク褐斑病菌，*Septoria obesa* の分生胞子形成に及ぼす光の影響を解析する過程で，分生胞子の発芽が各種の要因に影響されることが判明したので，接種胞子濃度，培養期間及び光条件と分生胞子の発芽の関係について検討した．V-8 野菜ジュース寒天培地に分生胞子を画線接種し，ブラックライト螢光灯 (BLB) による近紫外線の連続照射下で培養した菌叢に形成された分生胞子を素寒天培地に接種して試験した．その結果，活性が高いと考えられる胞子でも胞子の接種濃度が 3500個/cm^2 と高い場合には，発芽が強く抑制されたが，350個/cm^2 ではよく発芽した．BLB 照射下での培養期間が短い分生胞子の発芽は光によって影響されないが，培養期間が長くなるにつれて発芽は光によって完全に阻害されるようになった．連続照射下での胞子発芽阻害光の有効波長域は 360nm 以下の近紫外線であった．また，光は発芽管の伸長を抑制した．休止状態にある分生胞子に照射される近紫外線にも弱いながら発芽阻害効果が認められた．以上の結果から，本菌の分生胞子は近紫外線の照射を受けることによって，生存力を徐々に失うと共に近紫外線によって強く発芽阻害を受けるようになることが明らかになった．