Control of Certain Diseases of Greenhouse Vegetables with Ultraviolet-Absorbing Vinyl Film

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ABSTRACT

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Certain pathogenic fungi require ultraviolet (UV) radiation for sporulation. The use of UV-absorbing vinyl film for controlling diseases caused by *Alternaria dauci, A. porri, A. solani, Botrytis squamosa* (UV-induction group) was attributed to elimination of inductive UV radiation. Disease control for *Stemphylium botryosum* and *A. brassicae* (light-inhibition group) was accomplished by filtering out UV radiation that would otherwise nullify blue light inhibition.

Light is one of several important factors that may influence plant disease (2). Lesion development in cucumber downy mildew is suppressed by limiting light exposure through decreasing photosynthetic activity of the host plant (9).

Ultraviolet (UV)-absorbing vinyl film was shown to reduce the incidence of stem rot of eggplant (*Solanum melongena* L.) and cucumber (*Cucumis sativus* L.) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary (8) and gray mold of tomato (*Lycopersicon esculentum* Mill.) and cucumber caused by *Botrytis cinerea* Pers. ex Fr. (7) by filtering out wavelengths shorter than 390 nm.

In this report, we extended these studies to diseases of other greenhouse-grown plants. To determine which diseases may be reduced by controlling light quality, we examined sporulation response to monochromatic light of such fungi as *Alternaria dauci* (Kühn) Groves & Skolko (leaf blight of carrot (*Daucus carota* L.)), *A. porri* (Ellis) Cif. (Alternaria leaf blight of welsh onion (*Allium fistulosum* L.)), *A. solani* (Ellis & G. Martin) Sorauer (early blight of tomato and leaf blight of pepper (*Capsicum annuum* L.)), *Botrytis squamosa* Walker (leaf blight of chinese chive (*A. tuberosum* Rotter)), *Stemphylium botryosum* Wallr. (Stemphylium leaf spot of asparagus (*Asparagus officinalis* L.)), and *A. brassicae* (leaf spot of chinese mustard (*Brassica campestris* L. chinensis group) and komatsuna (*B. campestris* L. rapifera group)).

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MATERIALS AND METHODS

Organisms. More than 20 single-spore isolates of each fungus were grown on vegetable juice agar (VJA) (10% Campbell's V-8 juice, 0.2% $CaCO_3$, 1.7% agar, final pH 6.2) plates (9 ml/60-mm Pyrex petri dish) under alternation of 12 hr of darkness and 12 hr of light from two 20W black-light fluorescent lamps (FL20S·BLB, Toshiba Co., Tokyo) suspended 15 cm apart and 30 cm above VJA plates or in darkness for 7 days at 25 C. All isolates of four species of fungi other than *A. brassicae* and *S. botryosum* sporulated well under alternating light and darkness but failed to sporulate in continuous darkness. The latter species sporulated moderately in darkness but sporulated profusely under alternating light and darkness.

Monochromatic irradiation. A series of interference filters (300–700 nm) combined with selected glass filters (with lower limits of transmission 15–20 nm shorter than the wavelengths of maximum transmission of interference filters) was used to obtain monochromatic radiation with two 20W black-light and four 20W daylight fluorescent lamps (FL20S·D N/L, Toshiba Co., Tokyo) as light sources. These lamps were suspended 12 cm apart and 20 cm above the colonies. Interference filters from 300 to 405 nm at about 10-nm intervals were placed directly under the black-light lamps, and those from 440 to 700 nm at about 20-nm intervals were placed under the daylight fluorescent lamps. Specifications of glass filters and interference filters were described previously (4). Intensity of light filtered through each interference filter was measured with a radiometer (RMA-8, Japan Spectroscopic Co., Ltd., Tokyo) and was of the same order as reported previously (4). Intensity of filtered light was not adjusted to the same level. Control colonies without filters (white-light treatment) and in lightproof boxes (darkness treatment) were adjacent to test colonies. Cultures were irradiated continuously through Pyrex petri dish covers, which effectively transmitted wavelengths longer than 280 nm. Cultures were incubated for 8–15 days, depending on the growth rate of each fungal species. Incubation temperature was 25 C except for *A. porri* and *A. brassicae*, where incubation temperature was decreased to 20 C because the inhibitory effect of continous light was so high that sporulation was almost completely inhibited under all monochromatic radiation shorter than 520 nm at 25 C.

Spore suspensions were made by adding 5 ml of a 50% sucrose-0.05% Tween 20 solution to each plate and gently scraping the whole surface of the colony with a rubber spatula. Spore concentrations were determined with a hemacytometer. The experiments were repeated twice.

Greenhouse experiments. Carrot cultivar M. S. Yonsun, welsh onion cultivar Kinchyo, tomato cultivar Oogata Fukuju, red pepper cultivar Suiko, sweet pepper cultivar Ace, chinese chive cultivar Green Belt, and asparagus cultivar Mary Washington were grown in a greenhouse (5.4 × 15 m) constructed with the UV-absorbing vinyl film (UVA-vinyl; Hi-S, Nippon Carbide Industries Co., Inc., Tokyo) with a lower limit of transmission of 390 nm. A greenhouse with the common agricultural vinyl film (CA-vinyl; Clean-Ace, Mitsubishi Monsanto Chemical Co., Ltd., Tokyo) with a lower limit of transmission of 300 nm served as a control. Spectral distribution of sunlight filtered through each film has been shown in a previous paper (8). The greenhouse entrance was installed with double doors and kept lightproof with respect to UV radiation. When temperatures rose higher than 25 C, the greenhouse was cooled by forced ventilation with an electric fan housed in a shelter of the UVA-vinyl to reduce stray UV radiation. An inside shelter of the same material with covering was also installed at an air intake. For chinese mustard cultivar Yukijiro and komatsuna cultivar Misugi, another set of two greenhouses (4.5 × 9.0 m) with UVA-vinyl and CA-vinyl, respectively, was used.

Sprouts from second-year plants were used for perennial plants like chinese chive and asparagus. Welsh onion, tomato, red pepper, and sweet pepper were transplanted from pots (11 cm in diameter). Carrot, chinese mustard, and komatsuna were sown directly in the greenhouse.

Immediately after transplanting on 18 May, inoculation with *A. solani* was made by spraying a spore suspension to single plants at the center of each row of tomato, red pepper, and sweet pepper, respectively, as the primary infection source. One month after sowing chinese mustard and komatsuna, one plant with many lesions of Alternaria leaf spot was transplanted to the center of each row of healthy plants on 16 October. Transplanting of welsh onion into the greenhouse on 6 August was accompanied by introduction of two plants with two large lesions per plant of Alternaria leaf blight to the center of a row of healthy plants. These treatments were identical for both greenhouses. Because of the regular occurrence of leaf blight of chinese chive and carrot and Stemphylium leaf spot of asparagus in greenhouse cultivation, artificial inoculations were not made for these diseases. Development of each disease was recorded by counting lesions, diseased leaves, and diseased stems or by estimating disease severity during the growing season.

RESULTS

Sporulation responses to monochromatic radiation placed *A. dauci, A. porri, A. solani*, and *B. squamosa* in the UV-induction group, although *B. squamosa* sporulated well under continuous white-light irradiation (Fig. 1). These four species of fungi required light for sporulation, and the effective wavelengths were restricted to UV radiation shorter than 340 nm with minor differences in longer limits of effective wavelengths.

In *B. squamosa*, sclerotium formation was also influenced by light. Sclerotia on agar were formed in darkness, but the numbers of sclerotia increased with decreased wavelengths of UV radiation shorter than 330 nm and reached a maximum at 300 nm. Monochromatic radiation between 340 and 520 nm suppressed sclerotium formation. There was no conidiophore formation under monochromatic radiation of 350, 380, 410, and 440 nm. Most conidiophores formed directly on sclerotia.

Sporulation inhibition by continuous white light increased at temperatures higher than 20 C for *Alternaria* species. Sporulation of *A. porri* was completely inhibited by continuous white light as well as UV radiation at 25 C. Sporulation response of *A. porri* to monochromatic radiation (Fig. 1) was observed at 20 C, and continuous UV irradiation induced abundant sporulation at this temperature. These three species of *Alternaria* sporulated during 24 hr of darkness following continuous irradiation with white light during which sporulation had been inhibited.

Development of diseases caused by the six fungi was effectively suppressed in the UVA-vinyl greenhouse compared with the CA-vinyl greenhouse. Leaf blight of carrot was first observed as brown to dark brown spots on leaves on 7 July, 68 days after sowing in the CA-vinyl greenhouse. The spots enlarged to form irregular lesions. The percentage of infected leaves increased to 50% by 11 September. In contrast, leaf blight was not observed throughout the entire growing period in the UVA-vinyl greenhouse.

Alternaria leaf blight of welsh onion was first observed on 29 August, 23 days after transplanting in both greenhouses. In the CA-vinyl greenhouse, the number of lesions increased rapidly; this was accompanied by enlargement of each lesion, which resulted in death of the distal portion of the leaf. The number of lesions did not increase in the UVA-vinyl greenhouse, and the ratio of lesions in the UVA- to CA-vinyl greenhouses decreased to 0.05 at the final growth stages. The number of functional leaves, plant height, and fresh weight of welsh onion from the UVA-vinyl greenhouse were 6.2, 89.2 cm, and 222 g, respectively, compared with 2.6, 69.7 cm, and 87 g from the CA-vinyl greenhouse on 14 October.

Tomato early blight was first observed on 9 June, 21 days after transplanting in the CA-vinyl greenhouse. The number of

lesions increased rapidly after 29 June, which coincided with growth of tomato fruits, and reached 477.4 per leaf on 22 July. In the UVA-vinyl greenhouse, first observation of lesions of the disease was 7 July, 1 mo later than in the CA-vinyl greenhouse, and the number of lesions per leaf was less than 0.5% of the CA-vinyl greenhouse through the growing season. The average production of fruit per plant for 15 plants was 3.3 kg in the UVA-vinyl greenhouse compared with 2.5 kg in the CA-vinyl greenhouse.

Similar results were obtained with leaf blight of red pepper and sweet pepper. First observation of lesions in the UVA-vinyl greenhouse was delayed 1 mo in red pepper and 1.5 mo in sweet pepper compared with occurrence in the CA-vinyl greenhouse. In the CA-vinyl greenhouse, the disease developed rapidly after 29 June. In contrast, the number of lesions per plant in the UVA-vinyl greenhouse remained less than 0.5% of that in the CA-vinyl greenhouse at the later growing stages. The production of red pepper and sweet pepper fruits were 1,098 and 783 g per plant, respectively, in the UVA-vinyl greenhouse compared with 545 and 510 g, respectively, in the CA-vinyl greenhouse.

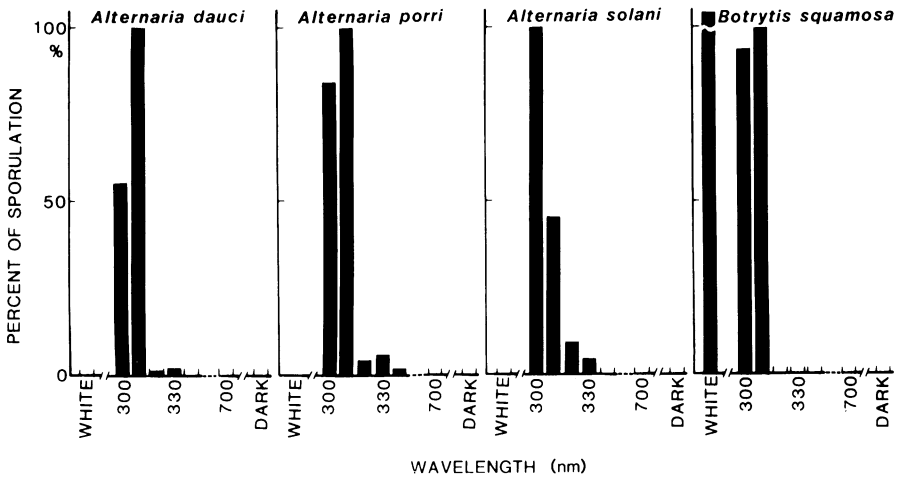

Fig. 1. Effect of monochromatic light on sporulation in the ultraviolet-induction group of fungi. Colonies were irradiated continuously under different interference filters (300–700 nm) with suitable glass filters to cut out high-order transmission at 25 C, except for *Alternaria porri*, which was irradiated at 20 C. One culture was used for each treatment.

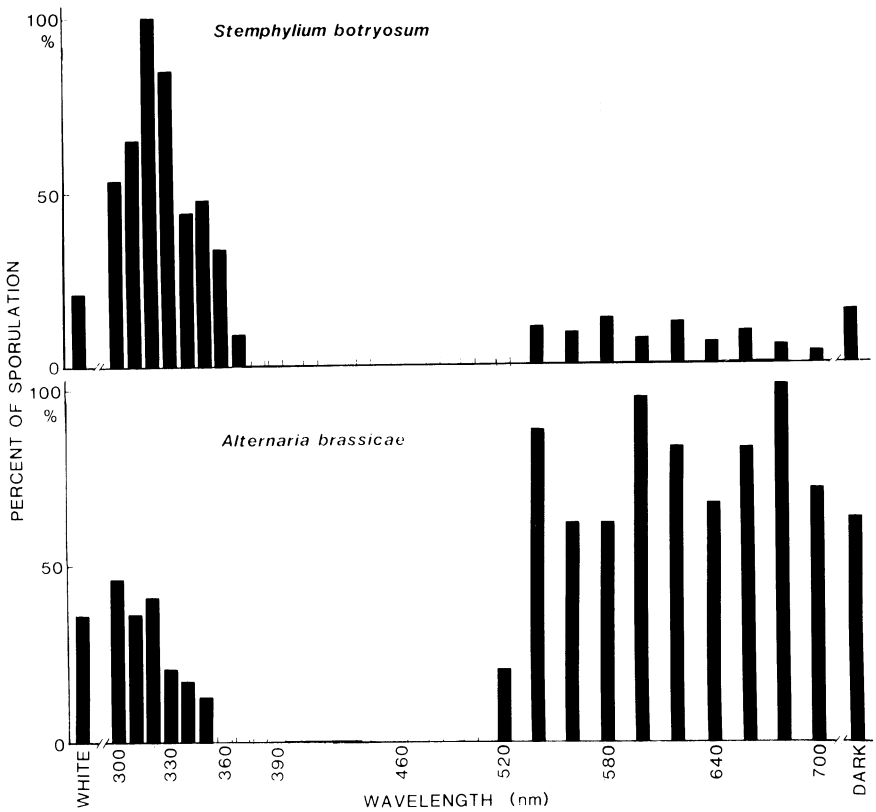

Fig. 2. Effect of monochromatic light on sporulation in the light-inhibition group of fungi. Colonies were irradiated continuously under different interference filters (300–700 nm) with suitable glass filters to cut out high-order transmission at 25 C for *Stemphylium botryosum* and at 20 C for *Alternaria brassicae*. One culture was used for each treatment.

Leaf blight of chinese chive was first observed on 14 May in the CA-vinyl greenhouse, with a peak in June and again in October. With a decrease of temperature in autumn, the disease became destructive and almost all plants were blighted by 1 December in the CA-vinyl greenhouse. In the UVA-vinyl greenhouse, the first occurrence of the disease was delayed 19 days compared with the CA-vinyl greenhouse and the severity remained low with less than 5% diseased leaves. Furthermore, lesions were observed in only 2 mo of growing season of chinese chive in the UVA-vinyl greenhouse.

Chinese chive was harvested 10 times from April through December by cutting all leaves at the soil line. The total production in a growing season for five plants and the average plant height at harvest were 152.8 g and 37.2 cm, respectively, in the UVA-vinyl greenhouse compared with 98.9 g and 31.5 cm, respectively, in the CA-vinyl greenhouse. Large white lesions were clearly distinct from the healthy tissues of the leaves and provided a useful means to assess market quality of the plant.

Sporulation responses of *S. botryosum* and *A. brassicae* to radiation differed from those of preceding fungi. Sporulation was inhibited by monochromatic radiation from 360 to 520 nm for *S. botryosum* (Fig. 2) as well as by continuous irradiation with the UVA-vinyl-filtered light, which did not involve UV radiation shorter than 390 nm. UV radiation shorter than 360 nm increased sporulation significantly in *S. botryosum*. Sporulation of *A. brassicae* was inhibited by monochromatic radiation between 350 and 520 nm and reduced by continuous irradiation of UV radiation shorter than 350 nm (Fig. 2). The UVA-filtered light inhibited sporulation of *A. brassicae* not only under continuous irradiation but also under alternating 8 hr of light and 16 hr of darkness.

Stemphylium leaf spot caused by *S. botryosum* (17) appeared as brown to gray spots on stems and cladophylla of second-year asparagus plants on 13 May in the CA-vinyl greenhouse. The number of stems infected increased rapidly and 68% of the stems were diseased 1 mo after first observation of lesions. Infection decreased as new stems emerged. In the UVA-vinyl greenhouse, the disease did not occur throughout the season.

Alternaria leaf spot of chinese mustard and komatsuna appeared on healthy plants 7 days after introduction of

diseased plants at the center of the row. The number of lesions at the first stage did not differ significantly between the UVA-vinyl greenhouse and the CA-vinyl greenhouse. After the primary infection from the introduced plants, the number of lesions increased rapidly in the CA-vinyl greenhouse contrasted with slow development in the UVA-vinyl greenhouse. The ratio of lesions in the UVA-vinyl greenhouse to those in the CA-vinyl greenhouse decreased from 0.81 for chinese mustard and 1.33 for komatsuna at the first stage to 0.19 for both plants at the final growth stages.

DISCUSSION

Of four fungal species in the UV-induction group with respect to sporulation response to monochromatic radiation, three species of *Alternaria* did not sporulate under continuous white light. Several reports have indicated that continuous blue light inhibits sporulation of fungi that require light to sporulate (1,5,13,19). Under continuous white light, the inhibitory effect of blue light may nullify the inductive effect of UV radiation. It has been reported that the effect of inhibitory blue light and inductive UV radiation are opposing reactions in conidiophore induction (10) and also in the terminal phase of sporulation (6). This mechanism may operate in sporulation inhibition by continuous white light in *A. dauci, A. porri*, and *A. solani*.

The same mechanism may also be involved in sporulation inhibition by UVA-filtered light in *S. botryosum* and *A. brassicae*, which do not require UV radiation for sporulation. Under continuous white light, including inhibitory wavelengths of 360–520 nm, sporulation proceeds moderately. However, upon removal of inductive UV radiation from white light by UVA-vinyl, only the inhibitory effect of blue light may be exerted, which suggests that UV radiation nullifies the inhibitory effect of blue light in such fungi as *S. botryosum* and *A. brassicae*.

Sporulation of *B. squamosa*, which is dependent on the UV radiation, however, was not inhibited by continuous white light, indicating absence of sporulation inhibition by blue light.

Sclerotium formation in *B. squamosa* was reported to be inhibited by a high-intensity incandescent-fluorescent radiation of 5×10^5 mW m^{-2} (16). In our studies, continuous monochromatic radiation between 340 and 520 nm at 10–30 mW m^{-2} effectively inhibited sclerotium formation in *B. squamosa*, similar to an observation in *B. cinerea*, in which blue light for 12–24 hr inhibited sclerotium formation (18). High intensities are probably not required to inhibit sclerotium formation if radiation is limited to the effective wavelengths.

The UVA-filtered light in continuous irradiation inhibited not only sclerotium formation but also conidium formation in *B. squamosa*. Sclerotia play an important role in the disease cycle as the survival structure and the primary infection source in spring. By reducing both sclerotium and conidium formation, the UVA-vinyl controlled leaf blight of chinese chive caused by *B. squamosa*.

Control of certain diseases caused by *Alternaria* spp. in the UVA-vinyl greenhouse conforms with the control of *Sclerotinia* disease and gray mold of cucumber and tomato as previously reported (7,8).

Falloon et al (3) suggested that *Stemphylium vesicarium* (Wallr.) Simmons (11), the causal organism of Stemphylium leaf spot, also causes purple spot in asparagus. Our isolate of *Stemphylium* sp., however, was identified as *S. botryosum* on the basis of conidial average of 2.6 transverse and 1.5 longitudinal septa, a single median constriction (15), and the ratio of length to width of 1.1–1.9 (1.5 average). These conidial characteristics conform to *S. botryosum* as provided by Suzui (17).

Some isolates of *S. botryosum* differ in their dependence on light exposure for sporulation (12). Leach and Trione (14) reported that the fungus required light for sporulation and also provided the action spectrum for photosporogenesis. An isolate we obtained from asparagus sporulated moderately in darkness and was inhibited from sporulating by monochromatic radiation between 370 and 520 nm. UV shorter than 350 nm exerted a stimulatory effect on the sporulation of this isolate. Sporulation was completely inhibited under UVA-filtered white light, probably the basis for the suppression of Stemphylium leaf spot of asparagus in the UVA-vinyl greenhouse. Sporulation of *A. brassicae* proceeds normally in darkness but is inhibited by monochromatic radiation from 360 to 500 nm, which could account for the suppression of Alternaria leaf blight of chinese mustard and komatsuna caused by *A. brassicae*.

Successful control of the diseases caused by *S. botryosum* and *A. brassicae* with UVA-vinyl suggested that light inhibitory to sporulation would control the disease in the greenhouse. The effect of UVA-vinyl on disease control was ascribed to accentuation of the inhibitory blue light effect by filtering out UV radiation from sunlight.

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LITERATURE CITED

1. Aragaki, M. 1962. Quality of radiation inhibitory to sporulation of *Alternaria tomato*. Phytopathology 52:1227-1228.
2. Colhoun, J. 1973. Effects of environmental factors on plant disease. Annu. Rev. Phytopathol. 11:343-364.
3. Falloon, P. G., Falloon, L. M., and Grogan, R. G. 1984. Purple spot and Stemphylium leaf spot of asparagus. Calif. Agric. 38:21.
4. Honda, Y., and Aragaki, M. 1978. Photosporogenesis in *Exserohilum rostratum*: Light quality effects on sporulation and spore morphology. Trans. Mycol. Soc. Jpn. 19:23-31.
5. Honda, Y., and Sakamoto, M. 1968. On the blue light inhibition of sporulation in *Helminthosporium oryzae*. Ann. Phytopathol. Soc. Jpn. 34:328-335.
6. Honda, Y., Sakamoto, M., and Oda, Y. 1968. Blue and near ultraviolet reversible photoreaction on the sporulation of *Helminthosporium oryzae*. Plant Cell Physiol. 9:603-607.
7. Honda, Y., Toki, T., and Yunoki, T. 1977. Control of gray mold of greenhouse cucumber and tomato by inhibiting sporulation. Plant Dis. Rep. 61:1041-1044.
8. Honda, Y., and Yunoki, T. 1977. Control of *Sclerotinia* disease of greenhouse eggplant and cucumber by inhibition of development of apothecia. Plant Dis. Rep. 61:1036-1040.
9. Inaba, T., and Kajiwara, T. 1971. Effect of light on lesion development in cucumber downy mildew. Ann. Phytopathol. Soc. Jpn. 37:340-347.
10. Kumagai, T. 1982. Blue and near ultraviolet reversible photoreaction in the induction of fungal conidiation. Photochem. Photobiol. 35:123-125.
11. Lacy, M. L. 1982. Purple spot: A new disease of young asparagus spears caused by *Stemphylium vesicarium*. Plant Dis. 66:1198-1200.
12. Leach, C. M. 1962. Sporulation of diverse species of fungi under near-ultraviolet radiation. Can. J. Bot. 40:151-161.
13. Leach, C. M. 1968. An action spectrum for light inhibition of the "terminal phase" of photosporogenesis in the fungus *Stemphylium botryosum*. Mycologia 60:532-546.
14. Leach, C. M., and Trione, E. J. 1966. Action spectra for light-induced sporulation of the fungi *Pleospora herbarum* and *Alternaria dauci*. Photochem. Photobiol. 5:621-630.
15. Miller, M. E., Taber, R. A., and Amador, J. M. 1978. Stemphylium blight of onion in South Texas. Plant Dis. Rep. 62:851-853.
16. Page, O. T. 1956. The influence of light and other environmental factors on mycelial growth and sclerotial production by *Botrytis squamosa*. Can. J. Bot. 34:881-890.
17. Suzui, T. 1973. Stemphylium leaf spot (*Stemphylium botryosum* Wallr.) on asparagus plants. Ann. Phytopathol. Soc. Jpn. 39:364-366.
18. Suzuki, Y., and Oda, Y. 1979. Inhibitory loci of both blue and near ultraviolet lights on lateral-type sclerotial development in *Botrytis cinerea*. Ann. Phytopathol. Soc. Jpn. 45:54-61.
19. Vakalounakis, D. J., and Christias, C. 1981. Sporulation in *Alternaria cichorii* is controlled by a blue and near ultraviolet reversible photoreaction. Can. J. Bot. 59:626-628.