Inhibition of Fungal Sporulation by Ultraviolet-Absorbing Vinyl Film and Its Application to Disease Control

By YUICHI HONDA and TOSHIFUMI YUNOKI

Environment Division, Tohoku National Agricultural Experiment Stationt

Introduction

Many fungi sporulated well when illuminated but poorly or not at all when grown in total darkness. Light requirement for sporulation of fungi has been reported by many investigators during the past decades. A guide by Marsh et al.[12] to the extensive literature on reproduction and other phenomena indicates that blue and near-ultraviolet regions of spectrum are most effective to induce sporulation of fungi. Recent studies under precisely controlled conditions, however, revealed the importance of ultraviolet region shorter than 370 nm for many fungi[9,10].

The effect of light on sporulation has, so far, been investigated to obtain enough spores for inoculation and other purposes. The light requirement for sporulation, however, suggests the possibility that some diseases can be controlled by eliminating the effective ultraviolet wavelengths from sunlight by constructing a green house with ultraviolet-absorbing vinyl film. A similar suggestion has been made by Hite[2] for reducing the buildup of inoculum of gray mold in greenhouse. A field trial to control tomato early blight, caused by *Alternaria solani* Sorauer, was based on the inhibitive effect of light on sporulation by nocturnal illumination, but was unsuccessful due to interference of low temperature at night[11]. Low temperature at night allowed the pathogen to sporulate even when exposed to light during the phase sensitive to light inhibition of sporulation.

The present paper describes successful pest management by utilization of filtered light to prevent sporulation by *Sclerotinia sclerotiorum*[4] and *Botrytis cinerea*[5] and resulting control of Sclerotinia disease and gray mold.

Experimental results

1) *Light quality effect on sporulation of fungi*

a) *Sclerotinia sclerotiorum*

Sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Barry will germinate and produce stipes, but do not produce mature apothecia without light. In a preliminary experiment on light requirement for apothecium formation by *S. sclerotiorum* isolated from eggplant (*Solanum Melongena*) and cucumber (*Cucumis sativus*), it was shown that effective wavelengths of radiation were the same as those for *S. trifoliorum* Erik. isolated from clover.

Sclerotia of *S. trifoliorum* were irradiated by fluorescent lamps of daylight type (FL20S · D/NL, Toshiba Machine Co.) and black light type (FL20S · BLB, Toshima Machine Co.) through glass filters (Toshiba Machine Co.) with different cut-offs from 290 to 660 nm. After 3-week irradiation many mature apothecia were formed under filters of UV-29 with cut-off of 290 nm and UV-31 of 310 nm (Table 1)[3]. Under a filter of UV-35 cutting out less than 350 nm and a band pass filter of UV-D1A passing wavelengths from 320 to 410 nm, apothecia were formed less than under UV-29 and UV-31. None of the apothecia reached complete maturity under filters that cut the radiation shorter than 390 nm. These

Table 1. **Effect of lights filtered through glass filters with different cutoffs on maturation of apothecia in *Sclerotinia trifoliorum*[3)]**

Glass-filter	Immature apothecia	Mature apothecia	%of mature apothecia
UV-29	5	38	88
UV-31	3	28	74
UV-D1A	12	15	56
UV-35	23	16	41
UV-39	29	0	0
V-Y42	34	0	0
V-Y44	31	0	0
V-Y46	30	0	0
V-Y48	40	0	0
V-Y50	43	0	0
V-O52	46	0	0
V-O54	43	0	0
V-O56	44	0	0
V-O58	38	0	0
V-R60	30	0	0
V-R62	71	0	0
V-R64	55	0	0
V-R66	59	0	0
Dark	41	0	0

immature apothecia did not form hymenium and consisted entirely of sterile mycelia. This result shows that radiation longer than 390 nm is not effective for induction of ascospore formation in this fungus[3)].

b) Botrytis cinerea

Moreau[13)] first studied the effect of light quality on sporulation of *Botrytis cinerea* Pers. ex Fr. using a spectrograph. He reported that conidia formed exclusively in the blue-violet region of the spectrum but none were formed in colonies exposed to green, yellow, orange, or red rays. More recently Hite[2)] using glass and plastic filters with different UV cut-offs, demonstrated that near UV radiation induces sporulation but not longer wavelengths. We determined an action spectrum precisely for photo-induced conidium formation in *B. cinerea*[6)].

Cultures of the fungus isolated from diseased greenhouse cucumber were exposed to monochromatic radiation for 5 min to 8 hr depending on wavelength of radiation. Monochromatic radiation was obtained from a spectroirradiator (CRM-FC, Japan Spectroscopic Co., Ltd.). Spores were counted after 48 hr dark incubation following inductive irradiation and dose response curves were plotted for each monochromatic radiation series from 226 to 355 nm. An action spectrum (Fig. 1) calculated from the dose response curves showed that the most effective wavelength is around 231 nm and the limit of effective radiation is approximately 350 to 355 nm. Three prominent peaks are evident in the action spectrum, these are at 231, 268, and 283 nm, with a minor peak at about 303 nm.

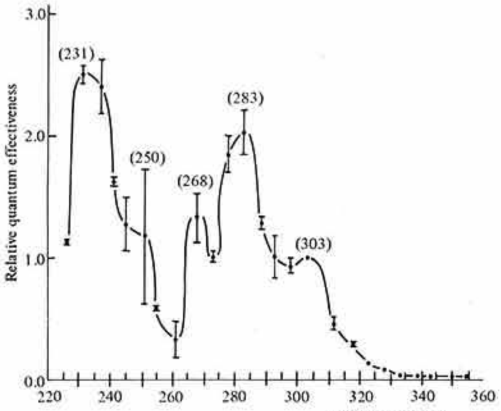

Fig. 1. Action spectrum for photosporogenesis in *B. cinerea*[6)]. Each point represents the mean relative quantum effectiveness against the 303 nm value which was derived from two independent dose response experiments. SD is indicated for each mean.

2) Control of Sclerotinia disease of greenhouse eggplant and cucumber by inhibiting apothecium formation

Sclerotinia disease of eggplant and cucumber, caused by *S. sclerotiorum*, appears as a stem blight or fruit rot to cause losses of 60–75%[7)]. Inocula of the disease consist of primarily ascospores discharged from fruit body, apothecium, at the soil surface.

Eggplants were grown in a green house with ultraviolet-absorbing vinyl film (UVA vinyl; Hi-S, Nippon Carbide Industries Co., Inc.) with a lower limit of transmission at 390 nm. As a control, eggplants were grown in a green house with common agricultural vinyl film (CA vinyl; Clean Ace, Mitsubishi Monsanto Co., Ltd.). With a lower limit of transmission of 300 nm, the CA vinyl allows formation of apothecia of *S. sclerotiorum*. Spectral energy distribution of sunlight filtered through each film was measured with an ultraviolet wavelength-distribution analyzer (UV-55, Japan Spectroscopic Co., Ltd.). The radiation through the CA vinyl was of longer wavelength than 300 nm through visible region, but the radiation through the UVA vinyl did not contain ultraviolet wavelengths shorter than 390 nm (Fig. 2)[4].

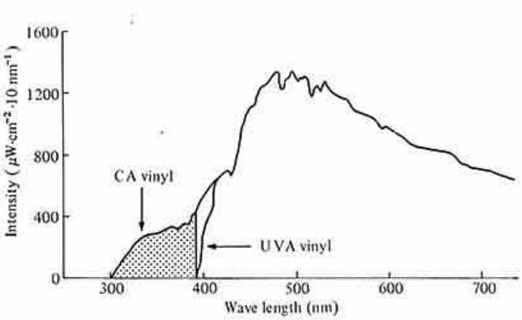

Fig. 2. Spectral energy distribution of sunlight filtered through ultraviotet absorbing vinyl film (UVA vinyl) and common agricultural vinyl film (CA vinyl), measured by an ultraviolet wavelength distribution analyzer for autumn noon sun in Morioka, Japan[4]

The number of mature apothecia with ascospores increased with time in the CA vinyl greenhouse; in the UVA vinyl greenhouse, however, mature apothecia were far less than in the CA vinyl greenhouse. The diameter of the apothecia formed in the, UVA vinyl greenhouse was smaller than those formed in the CA vinyl green house, with a reduced capability to produce ascospores.

These differences in formation of apothecia between two greenhouses were reflected in the substantial decrease of development of Sclerotinia disease in eggplant in the UVA greenhouse (Table 2)[4]. In the CA vinyl greenhouse many of the branches were killed and blackened, and lesions extended to the main stem at the soil line, resulting in girdling and stunting of plants in the final stage of the disease. On the other hand, eggplants in the UVA vinyl greenhouse retained many green and healthy leaves and stems, although there were one or two lesions on branches on each plant in the final stage of the study.

With completely closed chambers, there was a average of 3 stipes/pot, and these were without mature apothecia in the UVA vinyl chamber, and compared with 12 stipes/pot with 11 mature apothecia in the CA vinyl chamber. The total number of diseased cucumber fruits was 11.2 for each plant and

Table 2. **Effect of ultraviolet-absorbing vinyl film on development of Sclerotinia disease of eggplant caused by *Sclerotinia sclerotiorum*[4]**

Film[a]		Number of diseased flowers and fruits, and lesions on stems each date[b]				
		10/18	10/28	11/4	11/11	11/18
UVA	Diseased flowers and fruits	0/18[c]	7/31	12/56	24/61	27/86
	% infected	0	22	22	39	31
	Lesions on stems	0	0	0	5	13
CA	Diseased flowers and fruits	12/18	50/56	62/70	75/90	82/97
	% infected	67	89	89	83	85
	Lesions on stems	0	0	2	27	58

a: UVA; Ultraviolet-absorbing vinyl film, CA; Common agricultural vinyl film.

b: Total number on 12 plants.

c: Fractions indicate number of diseased flwers and fruits out of total of flowers and fruits.

12.4 lesions on the stem of each plant in the later stage of disease development in the CA vinyl chamber as compared with no disease in the UVA vinyl chamber.

3) Control of gray mold of greenhouse cucumber and tomato by inhibiting sporulation

Gray mold of cucumber and tomato (*Solanum Lycopersicum*) commonly occurs as blossom blight and fruit rot. Large number of spores form on rotted fruit, which serve as further inoculum. The disease has been difficult to control, particularly with recent development of chemical resistant strains of the causal fungus, *B. cinerea*[14]. Sporulation of more than 200 isolates of *B. cinerea* from different plants was closely associated with the cut-off wavelengths of films. Sporulation did not occur with radiation passing through films with the cut-offs longer than 345 nm[5].

Cucumber and tomato were grown in a greenhouse with the UVA vinyl. A greenhouse with the CA vinyl served as a control. The number of rotted fruits of tomato and cucumber increased rapidly in the CA vinyl greenhouse as compared with a sporadic occurrence of rotted fruits in the UVA vinyl greenhouse (Table 3). In cucumber, ingress of the pathogen was restricted to flower petals, followed by blossom end rot of fruit. There were 93 diseased fruits in the CA vinyl greenhouse as compared with 16 rotted fruits in the UVA vinyl greenhouse, or 17% of that in the CA vinyl greenhouse. In the UVA vinyl greenhouse plant growth was better than in the CA vinyl greenhouse by 34, 10, and 17% when plant height, number of leaves, and length of leaves, respectively, were compared[5].

In tomato, rotted fruit in the CA vinyl greenhouse steadily increased as compared with a few scattered diseased fruits in the UVA vinyl greenhouse. There was a total of 70 rotted fruits in the CA vinyl greenhouse at the end of the growing season, compared with only 5 rotted fruits in the UVA vinyl greenhouse. Growth of tomato in the UVA vinyl greenhouse was also better than in the CA vinyl greenhouse; in the UVA vinyl greenhouse plant height, number of leaves, and length of leaves exceeded those in the CA vinyl greenhouse at 7, 4, and 8%, respectively[5].

The production of cucumber and tomato fruits were 10.1 kg, and 3.5 kg per plant,

Table 3. Fruit rot of cucumber and tomato by gray mold in common agricultural vinyl film (CA) and ultraviolet-absorbing vinyl film (UVA) greenhouses[a, 5)]

Crop	Film[a]	Diseased fruits[b] 4/10	/20	/30	5/10	/20	/30	6/10	/20
Cucumber :									
	CA vinyl	8	29	48	58	68	81	91	93
	UVA vinyl	1	3	7	7	7	9	11	16
	Ratio of UVA to CA	13%	10%	15%	12%	10%	11%	12%	17%
Tomato :									
	CA vinyl	3	6	10	18	26	37	52	70
	UVA vinyl	0	0	1	2	2	5	5	5
	Ratio of UVA to CA	0%	0%	10%	11%	8%	14%	10%	7%

a: CA vinyl ; common agricultural vinyl film (Clean-Ace, Mitsubishi Monsanto Chemical Co., Ltd.), UVA vinyl ; ultraviolet absorbing vinyl film (Hi-S, Nippn Carbide Industries Co., Inc.)

b: Total diseased fruits of 56 plants of cucumber, and 52 plants of tomato respectively, in the UVA vinyl greenhouse as compared with 8.7 kg of cucumber and 2.6 kg of tomato in the CA vinyl greenhouse[5].

Discussion

Development of Sclerotinia disease and gray mold was greatly decreased in the UVA vinyl greenhouse. We are not aware of practical or experimental control of any plant disease by successful application of light effects on fungal reproduction. An attempt to control tomato early blight in the field by nocturnal illumination failed because low temperatures at night nullified inhibition of sporulation[11]. The control in the present study relied on the elimination of light required for sporulation. Light is necessary for formation of apothecia in *S. sclerotiorum* and conidia in *B. cinerea* even under conditions of low temperatures.

Some apothecia of *S. sclerotiorum* were formed, however, along with limited Sclerotinia disease development. Because of inhibition of development of apothecia in a small closed box and also in a closed chamber made of the UVA vinyl, the formation of apothecia which resulted in limited disease in the UVA vinyl greenhouse is attributed to stray light.

Because our basis for controlling Sclerotinia disease in the greenhouse is the filtering out of light that induces formation of apothecia, the extent of this light dependency in *S. sclerotinia* is important. Henson and Valleau[1] reported that in *S. sclerotiorum* and *S. trifoliorum*, only one abnormal apothecium was obtained from thousands of stipes produced and kept in the dark. Examination of more than 200 isolates from various localities of Japan showed none that were capable of formation of apothecia in the dark. Although the possibility that apothecia can develop in the dark cannot at present be excluded, observations thus far indicate that isolates of *S. sclerotiorum* capable of producing apothecia in the dark are rare.

There are isolates of *B. cinerea* which can sporulate in the dark, but there seem to be few in number under natural conditions, as indicated by the fact that none of more than 200 isolates from vegetables sporulated in the dark[5]. Two isolates from field strawberry and one from cucumber grown in the UVA vinyl greenhouse did sporulate in the dark, but the number of spores formed under darkness was far less than that formed under light. The isolation of *B. cinerea* which can sporulate in the dark may indicate the possibility of selection under the UVA vinyl filtered light creating some problems in these greenhouses. Inhibition of these isolates by the UVA filtered light, however, will keep fungal sporulation at a low level and make it possible to control by the UVA vinyl.

As observed in the UVA vinyl greenhouse, the primary source of infection with *B. cinerea* from outside of the greenhouse brought about scattered infection. Because there is no difference in an influx of primary source of infection between the two greenhouses, differences in disease incidence was attributed to secondary infection. Secondary infection from rotted fruits by gray mold in the greenhouse is important because in a commercial greenhouse it is impossible to remove all diseased fruits, which usually bear abundant spores over their entire surface.

These results clearly demonstrated the effectiveness of control of Sclerotinia disease and gray mold of greenhouse vegetables by inhibition of sporulation with light filtered through the UVA vinyl. Better growth of vegetables under the UVA vinyl should also encourage the use of the UVA vinyl for commercial greenhouses.

Although sporulation responses of fungi to light varies with species, many pathogenic fungi require ultraviolet radiation to sporulate. The possibility of controlling diseases caused by pathogenic fungi such as *Sclerotinia*, *Botrytis*, *Alternaria*[8], *Stemphylium*[9] in the greenhouse is attractive because dependence on pesticides is reduced.

This pest management practice of inhibiting reproduction of the causal fungus with the UVA vinyl, integrated with other control measures such as maintaining a dry greenhouse, and removal of diseased fruits and flowers from the greenhouse, will help to overcome such difficulties as increased resistance of fungi to agricultural chemicals.

References

1) Henson, L. & Valleau, W. D.: The production of apothecia of *Sclerotinia sclerotiorum* and *S. trifoliorum* in culture. *Phytopathology*, **30**, 869–873 (1940).
2) Hite, R. E.: The effect of irradiation on the growth and asexual reproduction of *Botrytis cinerea*. *Plant Dis. Reptr.*, **57**, 131–135 (1973).
3) Honda, Y. & Yunoki, T.: On spectral dependence for maturation of apothecia in *Sclerotinia trifoliorum* Erik. *Ann. Phytopathol. Soc. Japan*, **41**, 383–389 (1975).
4) Honda, Y. & Yunoki, T.: Control of Sclerotinia disease of greenhouse eggplant and cucumber by inhibition of development of apothecia. *Plant Dis. Reptr.*, **61**, 1036–1040 (1977).
5) Honda, Y., Toki, T. & Yunoki, T.: Control of gray mold of greenhouse cucumber and tomato by inhibiting sporulation. *Plant Dis. Reptr.*, **61**, 1041–1044 (1977).
6) Honda, Y. & Yunoki, T.: Action spectrum for photosporogenesis in *Botrytis cinerea* Pers. ex Fr. *Plant Physiol.*, **61**, 711–713 (1978).
7) Kadow, K. J., Anderson, H. W. & Hopperstead, S. L.: Control of Sclerotinia and Botrytis stem rots of greenhouse tomatoes and cucumbers. *Phytopathology*, **28**, 224–227 (1938).
8) Leach, C. M. & Trione, E. J.: Action spectra for light-induced sporulation of the fungi *Pleospora herbarum* and *Alternaria dauci*. *Photochem. Photobiol.*, **5**, 621–630 (1966).
9) Leach, C. M.: The light factor in the detection and identification of seed-borne fungi. *Proc. Int. Seed Test. Assoc.*, **32**, 565–589 (1967).
10) Leach, C. M. & Tulloch, M.: Induction of sporulation of fungi isolated from *Dactylis glomerata* seed by exposure to near-ultraviolet radiation. *Ann. Appl. Biol.*, **71**, 155–159 (1972).
11) Lukens, R. J.: Interference of low temperature with the control of tomato early blight through use of nocturnal illumination. *Phytopathology*, **56**, 1430–1431 (1966).
12) Marsh, P. B., Taylor, E. E. & Bassler, L. M.: A guide to the literature on certain effects of light on fungi: reproduction, morphology, pigmentation, and phototropic phenomena. *Plant Dis. Reptr. Suppl.*, **261**, 251–312 (1959).
13) Moreau, F.: Sur l'action des différentes radiations lumineuses sur formation des conidies du *Botrytis cinerea* Pers. *Bull. Soc. Bot. France*, **60**, 80–82 (1913).
14) Watson, A. G. & Koons, C. E.: Increased tolerance to benomyl in greenhouse populations of *Botrytis cinerea*. (Abstr.) *Phytopathology*, **63**, 1218–1219 (1973).