Control of Early Blight of Greenhouse Tomato, Caused by *Alternaria solani*, by Inhibiting Sporulation with Ultraviolet-Absorbing Vinyl Film

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ABSTRACT

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Fifty-two of 59 isolates of *Alternaria solani* from tomato (*Lycopersicon esculentum*) were light-dependent for sporulation in pure culture (effective wavelengths shorter than 360 nm), whereas seven isolates (approximately 12%) were light-independent. Tomato cv. Earlypak No. 7 was grown without fungicides during the 1984–1985 and 1985–1986 crop seasons in an experimental greenhouse covered with an ultraviolet-absorbing (UVA)-vinyl film (lower limit of transmission at 385 nm) and in a control greenhouse covered with a common agricultural (CA)-vinyl film (lower limit of transmission at 290 nm). At the end of the growing season, disease incidence in the UVA-vinyl greenhouse was reduced to less than 50% of that in the CA-vinyl greenhouse. At the same time, plant growth in the UVA-vinyl greenhouse was better than in the CA-vinyl greenhouse, when plant height, number of inflorescences, and yield were compared.

Alternaria solani Sorauer causes three important syndromes on tomato: collar rot, fruit rot, and early blight. The last one, which consists of leaf spotting leading to defoliation, is endemic in greenhouses in Crete, Greece, where the pathogen thrives throughout the growing season.

Sporulation of *A. solani* has been reported to be induced by light (3,10,13). Effective light quality for induction of conidiophore formation is confined to the ultraviolet (UV) region, with an upper limit of effectiveness at 331 nm (6). Because one of the requisites for an epidemic of *A. solani* is an abundance of spores, reduction of inoculum by controlling light quality should control the disease.

In the field, it is generally impractical for tomato growers to modify the quality of solar radiation absorbed by the plants. However, where tomatoes are grown commercially in greenhouses, it is possible to alter the quality of radiation received by plants. Honda and Yunoki (5) and Honda et al (4), recognizing the practical application of their laboratory findings that some fungal species are induced to sporulate by UV, constructed experimental greenhouses that filtered out UV wavelengths. This resulted in a significant reduction in the incidence of gray mold of cucumber and tomato caused by *Botrytis cinerea* Pers.:Fr. and white mold of eggplant and cucumber caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. These pioneering studies proved that the use of UV-absorbing (UVA) vinyl film may be a simple and economically feasible means of reducing losses attributable to certain diseases in greenhouse crops.

The purpose of the present study was to investigate the possibility of controlling tomato early blight in greenhouses by controlling light quality with UVA-vinyl films under the climatic conditions of the island of Crete.

MATERIALS AND METHODS

Effect of light quality on sporulation of several isolates of *A. solani*. Fifty-nine isolates of *A. solani* obtained from diseased tomato plants in greenhouses located in several areas in Crete between 1984 and 1987 were grown on V8 juice agar (VJA) (10% V8 juice, 0.2% $CaCO_3$, and 1.8% agar, final pH 6.2) plates (20 ml/90-mm petri plate).

Cultures were incubated either under a 12-hr photoperiod from a 20W Sylvania BLB fluorescent lamp (310–420 nm) and a 20W Osram daylight fluorescent lamp (340–750 nm) suspended 37 cm above the plates or in darkness for 7 days at 20 C. The radiation emitted from the BLB and daylight fluorescent lamps was filtered through a UVA-vinyl film (>385 nm), a common agricultural (CA)-vinyl film (>290 nm), and several combinations of color filters placed at the top of each plate. Each combination of filters allowed only the light of certain wavelengths to illuminate the fungal culture in the plate. The light qualities obtained by Kodak Wratten color filters were as follows: 1) 600 nm to infrared (filter 29), 2) 550–610 nm (filter 58 from 470 to 610 nm plus filter 22 from 550 nm to infrared), and 3) 390–530 nm (filter 47 from 360 to 530 nm plus filter 2B from 390 nm to infrared).

The light intensities under all three combinations of color filters and vinyl films were adjusted at 0.5 $\mu E \cdot m^{-2} \cdot s^{-1}$ with Kodak Wratten neutral density filters 96 and were measured by a Lambda LI-185 quantum radiometer photometer (LI-COR, Ltd., Lincoln, NE). A Toshiba UV-D_1A glass filter (300–400 nm) (Toshiba Machine Co., Ltd., Tokyo, Japan) was also used to give radiation from 310 to 400 nm. The intensity of near-UV radiation at the level of the petri plate was 0.30 $\mu E \cdot m^{-2} \cdot s^{-1}$ and was measured actinometrically with potassium ferrioxalate (1,2).

Five *A. solani* isolates from tomato leaf spots in the UVA-vinyl greenhouse were induced to sporulate by UV radiation, and 20 single-spore isolates of *A. solani* from them were examined for the capability to sporulate either in darkness or under CA-vinyl and UVA-vinyl filtered lights.

Conidia were collected from plates by gently rubbing the agar surface with a rubber policeman and repeatedly rinsing with 10-ml aliquots of sterile distilled water. Conidia collected were counted with a hemacytometer. The experiment was performed twice.

Effect of UVA-vinyl film vs. CA-vinyl film on disease development and plant growth. Two greenhouse experiments to determine the effectiveness of UVA-vinyl film vs. CA-vinyl film on disease development and plant growth were carried out. For this purpose, tomato (*Lycopersicon esculentum* Mill. 'Earlypak No. 7') plants were grown in a 5 × 8 m experimental greenhouse with UVA-vinyl film (UVA-vinyl; Mitsui Toatsu Chemicals Inc., Nagoya, Japan) with a lower limit of transmission at 385 nm. Another experimental greenhouse of the same size with CA-vinyl film (CA-vinyl; Mitsui Toatsu Chemicals Inc., Nagoya, Japan), with a lower limit of transmission at 290 nm, served as a control.

The greenhouses, constructed just before the experiments, were oriented in a similar direction (from north to south) and located 4 m from each other at the Katsambas area, Heraklio, Crete. Soil type in both greenhouses was silt loam with pH 7.4, total calcium carbonate 35%, and electrical conductivity of 2 mmhos/cm. Spectrophotometric transmission curves of the two films used are shown in Figure 1. The greenhouse entrance was installed with double doors and kept light-tight with respect to ultraviolet radiation. At temperatures above 25 C, forced ventilation was facilitated by two electric fans with outside ducts to reduce stray UV radiation. Air intake ducts made of the same material were also installed. Soil in both greenhouses

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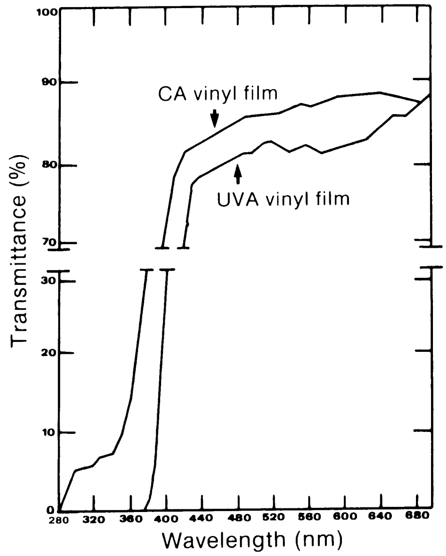

Fig. 1. Transmission spectra of an ultraviolet-absorbing (UVA) plastic film and a common agricultural (CA) plastic film measured by a Perkin-Elmer 55 1S UV/VIS spectrophotometer.

and experiments was sterilized similarly with methyl bromide; plants were fertilized in a similar fashion and grown according to local horticultural practices.

In the first experiment, seeds were sown in plastic pots on 26 September 1984. Seedlings were transplanted to each greenhouse in four rows with 11 plants per row on 24 October 1984. Disease ratings were taken on 12 March, 10 April, and 16 April 1985. In the second experiment, seeds were sown on 1 October 1985 and seedlings were transplanted to each greenhouse on 6 November 1985. Disease ratings were taken on 10 March, 3 April, and 24 April 1986. Because of the regular occurrence of early blight on greenhouse tomatoes at the Katsambas area, artificial inoculations were not made. Disease development was evaluated on a 0–5 visual rating scale, which corresponded to the percentage of leaf area damaged, where 0 = no disease, 1 = 1–9, 2 = 10–24, 3 = 25–49, 4 = 50–74, and 5 = 75–100% damaged or, essentially, death of the foliage. The numbers of rotted fruits and the production of healthy fruits were also recorded.

RESULTS

Effect of light quality on sporulation of several isolates of *A. solani*. Among 59 isolates of *A. solani* from infected leaves and fruits of greenhouse tomato plants, seven isolates sporulated abundantly under light filtered through the UVA- and CA-vinyl films as well as through all filter combinations (Table 1). These isolates also formed numerous spores under darkness. On the contrary, sporulation of the remaining 52 isolates of *A. solani* was closely associated with the lower limit of transmission of both filters and vinyl films. Sporulation did not occur with radiation passing through filters with lower limits of transmission longer than 260 nm (Table 1). When the experiment was repeated, it gave similar results.

Of 20 single-spore isolates of *A. solani* from leaf spots in the UVA-vinyl greenhouse, only one isolate sporulated moderately, and the rest failed to sporulate in darkness. This isolate also produced a small number of spores by UVA- and a larger number by CA-vinyl filtered light.

Effect of UVA-vinyl film vs. CA-vinyl film on disease development and plant growth. In the first experiment, spots on tomato leaves were first observed in the CA-vinyl greenhouse on 16 January 1985 and rotted fruits on 10 February 1985. In the UVA-vinyl greenhouse, the first observations of leaf spots and rotted fruits were made on 10 February and 3 March 1985, respectively, about 24 days later than in the CA-vinyl greenhouse. The number of affected leaves and fruits in the CA-vinyl greenhouse steadily increased compared with the UVA-vinyl greenhouse (Table 2). The disease index was 2.1 in the UVA-vinyl greenhouse at the end of the growing season, compared with 4.7 in the CA-vinyl greenhouse, or 45% of that in the CA-vinyl greenhouse. In the UVA-vinyl greenhouse, the number of diseased fruits was 20% of that in the CA-vinyl greenhouse. The production of fruits was 3.18 kg per plant in the UVA-vinyl greenhouse and 2.5 kg per plant in the CA-vinyl greenhouse.

In the second experiment, spots on tomato leaves were first observed in the

Table 1. Sporulation of isolates of *Alternaria solani* obtained from various locations on the island of Crete under two vinyl films and several combinations of color filters

Filter[a]	Range of transmission (nm)	Sporulating isolates[b] (no.)	Conidia/plate[c] (no.)
UV-D$_1$A	310–400	59	19.0
47 + 2B	360–530	7	13.7
58 + 22	550–610	7	11.7
29	600–IR	7	12.0
CA-vinyl film	>290	59	16.7
UVA-vinyl film	>385	7	11.4
Darkness	···	7	11.6

[a] UV-D$_1$A = 300–400 nm glass filter; 47, 2B, 58, 22, and 29 = Kodak Wratten color filters; CA-vinyl = common agricultural vinyl; and UVA-vinyl = ultraviolet-absorbing vinyl.

[b] Number sporulating out of 59 total tested.

[c] Mean number of conidia ($\times 10^5$) per plate produced by isolates that sporulated for each filter. Conidiation of each isolate was counted on six replicate plates.

Table 2. Effect of UV-absorbing (UVA)-vinyl film vs. common agricultural (CA)-vinyl film on tomato early blight, caused by *Alternaria solani*, and yield of tomatoes during the 1984–1985 season

Observation date	Disease index[a]		Total diseased fruits[b]		Total yield (kg/plant)	
	UVA	CA	UVA	CA	UVA	CA
12 March	0.3 ± 0.5	2.9 ± 0.9	1	10	1.14	0.91
10 April	1.2 ± 0.9	4.1 ± 0.6	2	30	2.50	2.16
16 May	2.1 ± 1.0	4.7 ± 0.5	8	40	3.18	2.50

[a] Mean ± standard deviation of 44 plants. Scale: 0 = no disease to 5 = 75–100% of leaf area damaged.

[b] Total diseased fruits of 44 plants.

Table 3. Effect of UV-absorbing (UVA)-vinyl film vs. common agricultural (CA)-vinyl film on tomato early blight, caused by *Alternaria solani*, and tomato growth and yield in greenhouses during the 1985–1986 growing season

Observation date	Disease index[a]		Total diseased fruits[b]		Fruits/plant (total no.)		Total yield (kg/plant)		Inflorescences/plant[a] (no.)		Plant height[a] (cm)	
	UVA	CA	UVA	CA	UVA	CA	UVA	CA	UVA	CA	UVA	CA
10 March	0.5 ± 0.8	2.6 ± 0.7	3	8	4.93	5.61	0.75	0.91	···	···	···	···
3 April	1.0 ± 0.9	3.8 ± 0.6	3	20	12.45	11.89	1.91	1.91	10.0 ± 1.9	8.8 ± 2.2	290 ± 40	250 ± 50
24 April	2.2 ± 1.0	4.9 ± 0.3	14	53	19.00	18.57	2.68	2.48	···	···	···	···
9 May	···	···	14	54	21.16	20.00	2.86	2.34	11.7 ± 2.6	9.8 ± 2.8	320 ± 60	260 ± 50

[a] Mean ± standard deviation of 44 plants. Scale: 0 = no disease to 5 = 75–100% of leaf area damaged.

[b] Total diseased fruits of 44 plants.

CA-vinyl greenhouse on 20 January 1986 and rotted fruits on 14 February 1986, 11 and 15 wk after transplanting, respectively. In the UVA-vinyl greenhouse, first observations of leaf spots and rotted fruits were made on 14 February and 7 March 1986, respectively, about 20 days later than in the CA-vinyl greenhouse. The number of diseased leaves and fruits in the CA-vinyl greenhouse steadily increased compared with the UVA-vinyl greenhouse (Table 3). The disease index was 2.2 in the UVA-vinyl greenhouse at the end of the growing season, compared with 4.9 in the CA-vinyl greenhouse, or 45% of that in the CA-vinyl greenhouse. In the UVA-vinyl greenhouse, the number of diseased fruits was 26% of that in the CA-vinyl greenhouse. Growth of tomato plants in the UVA-vinyl greenhouse was also better than in the CA-vinyl greenhouse; in the UVA-vinyl greenhouse, plant height and number of trusses exceeded those in the CA-vinyl greenhouse by 23 and 19%, respectively. The production of fruits was 2.86 kg per plant in the UVA-vinyl greenhouse and 2.34 kg per plant in the CA-vinyl greenhouse.

DISCUSSION

Fifty-two isolates of *A. solani* from greenhouse tomato plants taken from various locations on the island of Crete between 1984 and 1987 were found to have a light requirement for sporulation when they grew in pure culture, whereas seven sporulated abundantly under complete darkness. Studies with the 52 isolates showed that the effective wavelength was confined to the ultraviolet region shorter than 360 nm. This is common to many other fungi requiring light for sporulation (12). Honda and Yunoki (6) have defined a precise action spectrum for photosporogenesis in *A. solani* with a lower limit of effective wavelength at 331 nm and three peaks of effectiveness at 230, 270, and 285 nm.

Any attempt to relate the role of light to crop diseases cannot ignore other environmental factors (7,8,15). Temperature, for example, is particularly important in the sporulation process, and each fungus has its own unique optimum range. Humidity and leaf wetting are also important factors in the sporulation process (9). Thus, although light may be optimum for sporulation, if other factors are limiting, the influence of light can be nullified (9). Therefore, because of environmental interactions on fungal sporulation and the fact that all isolates of a single fungal species may not react identically, the value of UVA-vinyl films to control vegetable diseases caused by fungi belonging to the UV-induction group with respect to sporulation, such as tomato early blight, should be dependent on the climate conditions and the type of greenhouse constructions in the areas where the greenhouses are located.

Our results clearly demonstrate an effective control of early blight of greenhouse tomato under the natural conditions of Crete by inhibition of sporulation with light filtered by the UVA-vinyl film. These results are in agreement with those reported by Sasaki et al (11) in Japan for control of tomato early blight.

There are isolates of *A. solani* that can sporulate in the dark, but there are not many (about 12%) under natural conditions in Crete. Nevertheless, their existence in Crete, even in relatively low numbers, may indicate the possibility of selection under the UVA-vinyl film in the future, creating some problems in these greenhouses.

The pest management practice of inhibiting reproduction of *A. solani* with UVA-vinyl films, integrated with other control measures (such as decreasing relative humidity in greenhouses, removal of diseased fruits and leaves from the lower part of the plants, regular fertilizations, and a few sprays with effective fungicides, such as maneb, dichlofluanid, and chlorothalonil [14]), should effectively control tomato early blight in Crete. In addition, the better plant growth under the UVA-vinyl film should increase tomato yield.

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