

# Photobiological Control of Crop Production and Plant Diseases

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Plants, as well as fungi, use ambient sunlight as information to regulate photomorphogenic processes. The photobiological control of this information showed that the development of photobiological greenhouse plastic covers simulates a photonic information that leads to a physiological enhancement of plant productivity and fungal disease control, thus minimizing the need for the use of agrochemicals. The main characteristics of these photobiological greenhouse plastic covers are the high transmission of photosynthetically active radiation (PAR, 400–700 nm) combined with an increase of the factor  $\zeta = \text{RL}_{(655-665 \text{ nm})} / \text{FRL}_{(725-735 \text{ nm})}$ , which affects the cellular phytochromic equilibrium  $\Phi = \text{Pfr} / (\text{Pfr} + \text{Pr})$  and regulates the photosynthetic activity and therefore the plant productivity. Additionally, increase of the spectral ratios from the transmitted light:  $\text{BL}_{(420-500 \text{ nm})} / \text{nearUV}_{(290-370 \text{ nm})}$  and  $\text{BL}_{(420-500 \text{ nm})} / \text{FRL}_{(725-735 \text{ nm})}$ , cause mainly the induction of biochemical, physiological and morphological responses, regulated by cryptochromes in plants (e.g. inflorescence and infructescence) and mycochrome in fungi (e.g. inhibition of sporulation). In the present work, comparative studies with randomly selected greenhouse plastics showed that small changes in the above-mentioned “photobiological” parameters raise the productivity of tomato plants and inhibit the sporulation of several isolates of the fungal pathogen *Botrytis cinerea*. Thus, a model for the photoregulation of these two phenomena in greenhouses is proposed.

**Key words:** Fungal Sporulation, Photobiological Greenhouse Covers, Plant Production

## Introduction

The sunlight reaching the biosphere consists of a broad spectrum of wavelengths. Organisms have evolved to exploit certain parts of the sunlight spectrum. The action spectrum for the photosynthetic activity in green plants shows that only a region between 400–700 nm is photosynthetically exploitable by the photosynthetic apparatus (PAR, photosynthetically active radiation) (Balegh and Biddulph, 1970). The photosynthetic apparatus, residing in the thylakoid membranes, consists of three main photosynthetic pigment/protein complexes (PSII/LHCII, Cytb<sub>6</sub>f and PSI) (Kaplan and Arntzen, 1982), and has the ability to adapt its structure and function towards the modifications of light conditions (Kotzabasis *et al.*, 1999). Adaptation in low light conditions changes the structure and function of the photosynthetic apparatus and leads to a much lower photosynthetic activity than in plants growing in high light conditions, a fact thus greatly affecting plant productivity (Berry, 1975). The sensing (*via* phytochrome) of the spectral ratio red light (RL)/far red light (FRL) regu-

lates the photoadaptation of the photosynthetic apparatus. Low values simulate low light conditions and high values high light conditions with the corresponding changes in the photosynthetic apparatus (Buschmann *et al.*, 1978).

Plants are also able to monitor the light environment and perceive light signals that modulate growth and development. Seed germination, de-etiolation, organ orientation and flowering are some of the processes profoundly affected by the light environment via nonphotosynthetic light signals. The signals for all these photomorphogenic responses are perceived by a number of photoreceptors (phytochromes and cryptochromes) (Casal, 2000). The best characterized of the major plant photoreceptors is phytochrome. The phytochrome photoreceptor family is very likely solely responsible for the detection of RL and FRL (Batschauer, 1998). Phytochrome exists in two forms: the active, FRL-absorbing form (Pfr,  $\lambda_{\text{max}} = 730 \text{ nm}$ ), and the inactive, RL-absorbing form (Pr,  $\lambda_{\text{max}} = 660 \text{ nm}$ ). The two forms are photointerconvertible to each other by shuttling between the two

absorption maxima (Pr: 660 nm; Pfr: 730 nm) (Quail *et al.*, 1983). Activated phytochrome triggers multiple biochemical processes, significant for plant growth and morphogenesis. Most of these processes are constantly adjusted according to the ratio between the active and the inactive form of phytochrome, the so-termed “phytochromic equilibrium,”  $\Phi$ , emerging from the equation  $\Phi = \text{Pfr} / (\text{Pfr} + \text{Pr})$ . This ratio is in turn dependent on the ratio of the RL versus the FRL, namely  $\zeta = \text{RL} / \text{FRL}$  (Smith, 1994). The importance of the  $\zeta$  factor in determining the  $\Phi$  value and concomitantly regulating phytochromic responses arises from the fact that phytochromic responses mainly depend on the ratio between RL and FRL without being affected by intensity shifts of both irradiances (Kendrick and Kronenberg, 1994).

Another family of photoreceptors are the cryptochromes. Cryptochromes are the blue light (BL) receptors. Two members of this family, Cry1 and Cry2, have been identified in *Arabidopsis thaliana* (L.) Heynh. (Ahmad and Cashmore, 1996; Lin *et al.*, 1998). Blue light, *via* cryptochromes, affects important functions such as growth of hypocotyls, stem and leaves, stomatal opening, phototropism and flowering (Briggs and Liscum, 1997).

Sunlight also affects a number of plant disease responses caused by fungal pathogens. It can influence both pathogen and host and cause indirect effects on plant disease (Leach and Anderson, 1982). The major light-driven morphogenic reactions in fungi involve reproductive processes (both sexual and asexual), spore germination and also changes in spore formation. Their photoreceptors, responsible for the photomorphogenic processes, almost exclusively absorb blue light and/or near-ultraviolet (near-UV) radiation (Vakalounakis, 1987). Despite the fact that in fungi not any mechanism involving phytochrome has been recognized, various responses affected by RL have been observed (Lukens, 1965; Ingold and Nawaz, 1967; Brook, 1969; Calpouzos and Chang, 1971; Chang and Calpouzos, 1971).

In several fungal pathogens that attack the above-ground part of the plants, such as *Alternaria tomato* (Cooke) Weber, *A. cichorii* Nattrass, *Bipolaris oryzae* (Breda de Haan) Shoem, and *Botrytis cinerea* Pers.: Fr., it has been shown that sporulation is regulated by a reciprocal photoreaction involving BL and near-UV radiation (Vakalounakis and Christias, 1981; Kihara *et al.*, 1997). These responses are triggered by a photoreceptor called

“mycochrome” (Honda, 1969). Mycochrome is activated, and induces sporulation, upon receiving near-UV radiation, whereas it is reversed to the inactivated form and inhibits sporulation, upon receiving BL illumination. Since one of the requisites for an epidemic of these fungal pathogens is the abundance of spores, reduction of inoculum by preventing near-UV radiation or increasing the ratio of BL/near-UV reaching the plants should control diseases. In the case of *B. cinerea* it has been shown that the inhibiting action of BL on sporulation in pure cultures can be reversed by FRL irradiation, and again this effect can be restored by either RL or BL irradiation (Tan, 1974, 1975).

Over the last decade, the technology of greenhouse plastic covers has been promoted producing a wide range of different kinds of plastics based on different materials (*e.g.* PVC, PET, PVDF, LDPE) along with the addition of certain stabilizers and other agents to retard the degradation of films by UV light and alter their photoselectivity in light transmittance (Li *et al.*, 2000). Photoselective greenhouse plastic films permit disproportional transmission of different wavelengths.

In the present study we investigated the possibility of controlling gray mold (*B. cinerea*) and increasing the tomato yield in greenhouses by controlling the photonic information of the transmitted light. Our purpose was to test the feasibility of developing “photobiological” greenhouse plastic films, which will improve the plant yield and control fungal diseases, thus minimizing the need for administration of chemicals.

## Materials and Methods

### Polyethylene (PET) films

A number of commercially provided photoselective greenhouse plastic (PET) films were randomly selected to be subjected to comparative analyses in order to evaluate their photobiological characteristics. These films, manufactured by Plastika Kritis S. A. (Heraklio, Crete, Greece), are designated in this study as A, B, C, D, E, F, and G.

### Spectral analyses and transmittance measurements

Spectral analyses were performed by using a hypersensitive radiometer system (International Light, Newburyport, MA) consisting of a control box (IL1700), a photomultiplier power supply (IL760) and a photomultiplier (IL780).

The direct measurements of sunlight spectra were performed on a day with average sunlight (middle of May) in an open field in Crete. The sunlight spectrum was thus estimated three times that day: early in the morning (7:45–8:15), in the noon (12:00–13:00), and late in the afternoon (18:00–18:30).

The spectral characteristics of the sunlight penetrating the filters were calculated in the morning, noon and afternoon. The transmittance (%) of each separate greenhouse plastic cover was estimated at intervals of 5 nm by covering the radiometer sensor with the respective plastic sheet and recording again the sunlight intensity with the radiometer.

Further analyses of the spectra (RL: 655–665 nm, FRL: 725–735 nm, BL: 420–500 nm, near-UV: 290–370 nm) allowed us to estimate a series of photobiological factors (PAR, RL/FRL, BL/UV, BL/FRL).

#### *Greenhouse crop*

To determine the effectiveness of the plastic film on the plant growth and productivity, a greenhouse experiment was carried out during the crop season. For this purpose, tomato (*Lycopersicon esculentum* Mill.) plants cv. Rentita [Heirloom seeds (P.O. Box 245, W. Elizabeth, PA 15088-0245)] were grown in 2 m × 4 m experimental greenhouses covered with the selected film B (for the selection see “Results”). Another series of experimental greenhouses of the same size, covered with the film A, served as a control. The greenhouses were oriented in a similar direction (from north to south) and located 6 m from each other at the Knossos area in Heraklio, Crete, Greece. In each greenhouse, 20 tomato plants were grown according to local horticultural practices. This type of small greenhouses was preferred to avoid any self-shading effect of plants that leads to changes in the molecular structure and function of the photosynthetic apparatus and therefore of plant production. By the end of the experimental period the fruit number per plant was recorded.

#### *Photosynthetic and respiration rate measurements*

Photosynthetic and respiration rates were measured polarographically as oxygen evolution and consumption, respectively, with a Clark-type electrode system (Hansatech Instruments, Kings' Lynn, Norfolk, UK) at 25 °C according to the

method of Walker (1988). Leaf disks (3.5 cm in diameter) were exposed to saturated actinic light ( $450 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) for maximal photosynthetic rate measurements. The light intensity was achieved with two ENX360W/82V (General Electric, Nela Park, Cleveland, OH) lamps. The light intensity was measured with a sensitive photoradiometer (International Light) consisting of a control box (IL1700), a power supply (IL760) and a photomultiplier (IL780). The infrared part of the applied irradiation was filtered off by inserting a 2%  $\text{CuSO}_4$ -containing cuvette (4 cm path length) into the light beam.

#### *Extraction and estimation of chlorophyll amount*

Photosynthetic pigments were exhaustively extracted from each leaf disk with hot methanol, and ultimately the total chlorophyll concentration was calculated spectrophotometrically according to the method of Holden (1965).

#### *Effect of light quality on sporulation of several isolates of Botrytis cinerea*

Ten isolates of *B. cinerea*, obtained by D. J. Vakilounakis [Plant Protection Institute, National Agricultural Research Foundation (N.A.G.R.E.F.), 71003 Heraklio, Greece] from diseased tomato plants in greenhouses located in several areas of Crete, were grown on potato dextrose agar (PDA) plates (20 ml/90 mm petri plate). Cultures were incubated either under a 12 h photoperiod by a 20 W Sylvania BLB fluorescent lamp (310–420 nm) and a 20 W Osram daylight fluorescent lamp (340–750 nm) suspended 37 cm above the plates or in the darkness for 7 d at 20 °C. The radiation emitted from the BLB and daylight fluorescent lamps was filtered through various combinations of colour filters as well as the common agricultural polyethylene films. Each combination of filters allowed only the light of certain wavelengths to illuminate the fungal culture in the plates. The light quality obtained by Kodak Wratten colour filters was as follows: 1) 390–530 nm (filter 47 from 360 to 530 nm plus filter 2B from 390 nm to infrared); 2) 550–610 nm (filter 58 from 470 to 610 nm plus filter 22 from 550 nm to infrared); and 3) 600 nm to infrared (filter 29). The light intensity under all three combinations of colour filters was adjusted at  $1.5 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  with Kodak Wratten neutral density filters 96 and were measured by a Lambda LI-185 quantum radiome-

ter photometer (LI-COR, Ltd., Lincoln, NE). A Toshiba UV-D1A glass filter (310–400 nm, Toshiba Machine Co., Ltd., Tokyo, Japan) was also used to give radiation from 310–400 nm. The intensity of near-UV radiation at the level of the petri plate was  $0.30 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  and was measured actinometrically with potassium ferrioxalate (Baxendale and Bridge, 1955; Calvert and Pitts, 1966). 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.

### Statistics

Photosynthetic and respiration rate analyses were performed regularly throughout the three months time of the experiment. Each time, ten out of the twenty plants in each greenhouse were chosen randomly and from each of these plants 3 leaves were cut and processed for photosynthetic and respiration rate measurements. The results represent the average of the obtained values. The standard deviations were also calculated. Statistics were always done by performing a two-independent-samples t-test analysis by the use of SPSS 14.0 software. Levene's test was also performed to check for equality between variances. Crop production measurements were also done, by the time of fruit development until the end of the experiment, by recording the number of fruits from each one out of the twenty plants in every greenhouse. Statistics were also performed for these data as described above (two-independent-samples t-test analysis).

### Results

The aim of the present contribution was to investigate the possibility of controlling gray mold (*B. cinerea*) and increasing the tomato yield in greenhouses by controlling the photonic information of the transmitted sunlight. Our purpose was to test the feasibility of developing "photobiological" greenhouse plastic films, which will improve the plant yield and control fungal diseases, thus minimizing the need for administration of chemicals.

### Spectral analysis of transmitted light through greenhouse covers

Spectral analysis of the transmitted light through the photosensitive greenhouse plastic covers A, B, C, D, E, F, and G showed that filter B exhibited the least transmittance in the region of near-UV radiation (~7% maximal variation at 360 nm), and increased the transmittance in the region of PAR radiation (~3.5% maximal variation at 520 nm) (Fig. 1). However, since the factors affecting plant photomorphogenesis and fungal sporulation ( $\zeta = \text{RL/FRL}$ ,  $\text{BL/UV}$ ,  $\text{BL/FRL}$ ) are dependent on the ambient sunlight, which varies during the day, direct measurements of sunlight spectra were also performed. As it is shown in Fig. 2, both the quality and intensity of sunlight reaching the ground changed greatly during the day and so did also the above-mentioned photobiological parameters presented in Table I. Notably the  $\zeta$  factor that regulates the phytochromic equilibrium  $\Phi$ , as well as the ratio  $\text{BL/FRL}$  were lower at noon compared to the morning and afternoon measurements. In contrast, the ratio values  $\text{BL/UV}$  were higher during noon, than the corresponding values in the morning and in the afternoon (Table I).

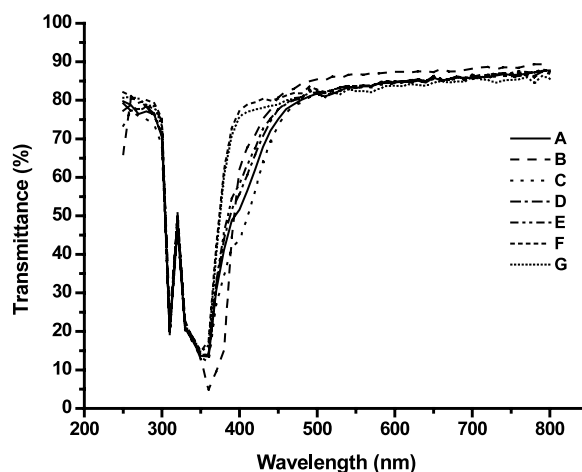


Fig. 1. Transmittance (%) of seven different greenhouse plastic sheets (A–G), measured with a hypersensitive photoradiometer system [International Light, Newburyport, MA, consisting of a control box (IL1700), a photomultiplier power supply (IL760) and a photomultiplier (IL780)] against the sunlight. The transparency of each separate greenhouse plastic cover was estimated by covering the radiometer sensor with the respective plastic sheet and recording again the light intensity with the radiometer.

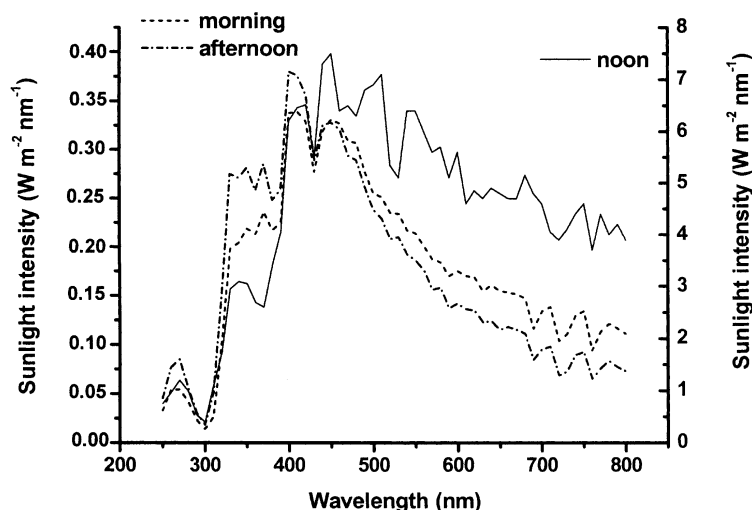


Fig. 2. Sunlight spectra (250–800 nm) in the morning (7:45–8:15), noon (12:00–13:00) and afternoon (18:00–18:30), measured in an open field in Crete (Heraklio) in the middle of May. The left y-axis represents the values of the morning and afternoon spectra and the right y-axis the values of the noon spectrum.

Table I. Spectral characteristics of the sunlight in an open field in Crete. The sunlight spectrum was estimated three times in the middle of May: early in the morning (7:45–8:15), in the noon (12:00–13:00), and late in the afternoon (18:00–18:30).

Parameter <sup>a</sup>	Light intensity [ $\text{W m}^{-2}$ ]		
	Morning	Noon	Afternoon
PAR (400–700 nm)	69.8	1785.2	64.0
RL (655–665 nm)	1.531	46.98	1.179
BL (420–500 nm)	24.390	530.10	24.040
Near-UV (290–370 nm)	11.075	165.75	14.683
FRL (725–735 nm)	1.108	41.00	0.727
$\zeta = \text{RL/FRL}$	1.382	1.146	1.623
BL/UV	2.202	3.198	1.637
BL/FRL	22.013	12.929	33.067

<sup>a</sup> PAR, photosynthetically active radiation; RL, red light; BL, blue light; near-UV, near-ultraviolet light; FRL, far red light.

The spectral characteristics (PAR,  $\zeta = \text{RL/FRL}$ , BL/UV, BL/FRL) of the sunlight penetrating each filter in the morning, noon and afternoon, showed that filter B exhibited the highest sunlight transmittance (83.8%). Filter F also gave a high value (82.2%), while the lowest values were those of filters C (78.6%) and A (79.7%) (Fig. 3, Table II).

The comparison of the values for the photobiological spectral ratios RL/FRL, BL/UV and BL/FRL among the sun and the seven filters produced significant findings. The  $\zeta$  parameter itself (RL/

FRL) revealed practically only minor differences (Table II). The parameter that showed the most prominent differences is the BL/UV ratio. All seven filters produced significantly higher BL/UV values compared to sunlight at all daytimes (~250% higher than the corresponding in the plain sunlight), but among them filter B stood out (~300% higher). The filters exhibiting the lowest BL/UV values were filters F and G (approximately 220% higher than sunlight) (Table II). In the case of the BL/FRL ratio, all seven filters gave values lower than ambient sunlight, although their mean difference from the corresponding sunlight value was quite small (~10% lower). Among them, filters G and F showed the closest to sunlight value, while filter B also followed (Table II).

Taking into consideration that the crop production depends on the photosynthetic rate and that the sensing (*via* phytochrome) of the RL/FRL ratio regulates the photoadaptation of the photosynthetic apparatus (low RL/FRL values simulate low light conditions, while high RL/FRL values simulate high light conditions) and also that the control of sporulation for several fungal pathogens is regulated by reciprocal photoreactions involving BL (inhibition of sporulation) and near-UV radiation (enhance of sporulation), one easily comes to the conclusion that the above presented data indicated filter B as the most suitable to accomplish the enhancement of crop production along with the re-

Table II. Spectral characteristics of the sunlight filtered through each of the seven greenhouse plastic films (A–G), estimated for three times a day (morning, noon and afternoon).

	Day time	Sun	A	B	C	D	E	F	G
Transmission of PAR (%)		100	79.71	83.76	78.59	80.73	81.04	83.21	81.97
$\zeta$ = RL/FRL	morning	1.382	1.361	1.385	1.362	1.361	1.358	1.362	1.388
	noon	1.146	1.129	1.149	1.130	1.129	1.126	1.130	1.152
	afternoon	1.623	1.599	1.627	1.600	1.599	1.595	1.599	1.631
BL/UV	morning	2.202	7.549	9.465	7.738	7.571	7.556	7.123	7.188
	noon	3.198	10.445	12.290	10.655	10.501	10.485	10.016	10.080
	afternoon	1.637	5.500	6.732	5.617	5.524	5.516	5.239	5.279
BL/FRL	morning	22.013	19.236	20.184	18.863	19.632	19.661	20.655	20.757
	noon	12.929	11.331	11.878	11.120	11.559	11.574	12.139	12.203
	afternoon	33.067	28.804	30.252	28.217	29.412	29.467	31.010	31.160

PAR, photosynthetically active radiation; RL, red light; BL, blue light; UV, near-ultraviolet light; FRL, far red light.

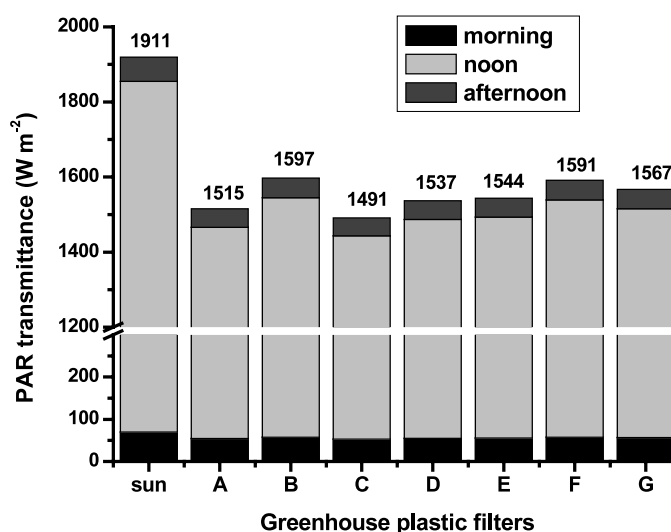


Fig. 3. Estimation of PAR (400–700 nm) intensity as a sum of the intensity measured in the morning (7:45–8:15), noon (12:00–13:00) and afternoon (18:00–18:30), in the sunlight and under each of the seven plastic sheets. The spectral analyses were performed by recording the light intensity (in  $\text{W} \cdot \text{m}^{-2}$ ) every 5 nm wavelength region.

duction of fungal proliferation. On the other hand, according to the same criteria, filter A revealed to be the less suitable of all the seven filters and therefore served as a control (reference filter). Thus, these two filters were chosen to be subjected to further experimentation under field conditions.

#### *Spectral changes through photobiological greenhouse covers adjust the photosynthetic rate and plant productivity*

Through the whole period of cultivation, plants grown in both greenhouse categories with filters A and B showed similar morphometric characters,

such as the stem elongating rate (measured as plant height) and leaf number and morphology (data not shown). On the other hand, maximal photosynthetic and respiration rates were found to be significantly elevated at all time points in plants of the greenhouses B, compared to the plants of the greenhouses A. Fig. 4 (a, b) depicts the differences in photosynthetic and respiration rates between the two types of greenhouses, as these were developed by the end of the experimental period.

Increased photosynthetic rate leads to increased total metabolic rates and ultimately to increased

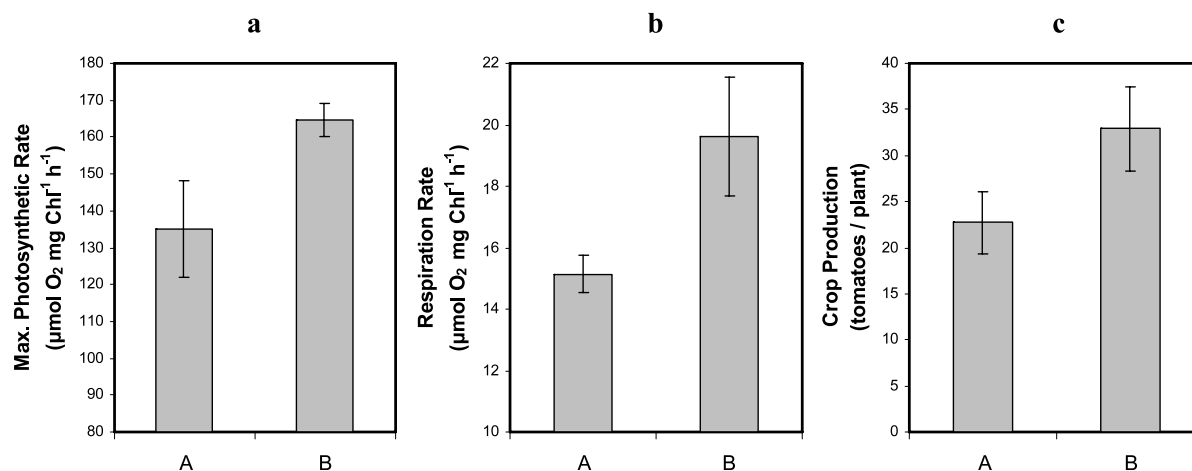


Fig. 4. (a) Photosynthetic and (b) respiration rates measured as oxygen evolution and consumption, respectively, in leaf disks of standard diameter, during the cultivation of tomato plants in greenhouses covered with A and B plastic sheets. All differences are statistically significant at the  $p < 0.05$  level (two-independent-samples t-test analyses; equal variances not assumed). (c) Crop production as tomato fruits per plant in greenhouses covered with A and B plastic filters. These differences are statistically significant at the  $p < 0.01$  level (two-independent-samples t-test analyses; equal variances not assumed).

productivity. Plant productivity can be more accurately represented by fruit development, whereas floral induction comprises the first step in the reproductive development (Levy and Dean, 1998). Fig. 4c summarizes the results provided by the recordings of the tomato fruits per plant, in the two greenhouse categories (A and B). The plants in greenhouse B exhibited a significantly higher number of fruits per plant (approximately 45% higher than in greenhouse A).

#### *Influence of photobiological greenhouse covers on the sporulation of Botrytis cinerea*

Among 10 isolates of *B. cinerea*, in nine no spores were detected and one sporulated least under light filtered through the greenhouse B film. The same result was also produced when the *B. cinerea* cultures were exposed to actinic BL (filter 47+2B) (Table III). However, most of these isolates sporulated moderately under darkness. Also, six of these isolates did not sporulate at all and four sporulated moderately under light filtered through the greenhouse A film (Table III). On the contrary, radiation with FRL allowed sporulation in 6 isolates, while sporulation of all ten isolates was profuse under UV radiation (Table III). When the experiment was repeated it gave similar results.

Table III. Sporulation of isolates of *Botrytis cinerea* obtained from various locations in the island of Crete under various combinations of colour filters and two greenhouse plastic films.

Filter <sup>a</sup>	Range of transmission [nm]	Sporulating isolates <sup>b</sup> (no.)	Conidia/plate <sup>c</sup> (no.)
UV-D1A	310–400	10	573
47 + 2B	360–530	1	516
58 + 22	550–610	5	124
29	600 – infrared	7	216
A film	PAR <sup>d</sup>	4	88
B film	PAR <sup>d</sup>	1	3
Darkness (control)	–	8	156

<sup>a</sup> UV-D1A, 300–400 nm glass filter; 47, 2B, 58, 22, 29, Kodak Wratten colour filters; A, B, commercial agricultural plastic films.

<sup>b</sup> Number sporulating out of 10 total tested.

<sup>c</sup> Mean number of conidia ( $\cdot 10^4$ ) per plate produced by isolates that sporulated for each filter. Conidiation of each isolate was counted on four replicate plates.

<sup>d</sup> Transmission spectra of the plastic greenhouse films A and B are presented in Fig. 1.

## Discussion

The spectral analysis of the transmitted light through the photoselective greenhouse plastic covers showed that filter B exhibited the least trans-

mittance in the region of near-UV radiation and increased transmittance in the region of PAR irradiation.

Important results also arose when the photobiological spectral ratios:  $\zeta = \text{RL/FRL}$ ,  $\text{BL/UV}$  and  $\text{BL/FRL}$  were taken into account (Table II). The  $\zeta$  parameter ( $\text{RL/FRL}$ ) itself showed practically only minor differences among the sunlight and the seven filters. However, as it is widely known, minor changes of the ratio  $\text{RL/FRL}$  can greatly affect the so-termed “phytochrome photoequilibrium, “ $\Phi$ ”, which in turn defines the direction of phytochromic responses. Under this point of view, we can presently consider filters B and G as advantageous, since they gave  $\zeta$  values somewhat higher than sunlight, while the rest of the filters exhibited  $\zeta$  values lower than the corresponding sunlight value (Table II).

Concerning the  $\text{BL/UV}$  ratio, although all seven filters produced significantly higher  $\text{BL/UV}$  values compared to sunlight, filter B again exhibited the most prominent difference. Although filter A, together with filters C–G, produced a higher  $\text{BL/UV}$  value than sunlight, these were all quite lower than for filter B. These results serve the purpose of our study, since a higher  $\text{BL/UV}$  value greatly inhibits fungal sporulation to benefit plant production. Thus, so far, concerning fungal proliferation, filter B comprised the most advantageous candidate to promote inhibition.

On the other hand, since the inhibiting action of BL on fungal sporulation can be compensated by FRL, an overview of the  $\text{BL/FRL}$  ratio can also produce significant information towards the evaluation of the greenhouse covers. Although all seven filters exhibited lower  $\text{BL/FRL}$  values than ambient sunlight, their difference from the sunlight value was quite small. Indeed, this result is not by any means a major drawback concerning fungal sporulation inhibition, since it can only attenuate at a small degree the prevailing inhibition caused by the putative vast increase of the  $\text{BL/UV}$  ratio.

It is obvious so far that in these series of analyses none of those seven photoselective filters exhibited optimal differences in either of the parameters discussed. This was rather expected, since they all were randomly selected, out of a number of commercially produced photoselective plastics that are manufactured on the basis of their physical tolerance and also inexpensiveness. However, taken together, the above presented data indicated filter B as the most suitable of all the filters tested

to proceed with further experimentation, meaning to test its properties on optimizing plant productivity in field conditions. In order to evaluate the contribution of its special characteristics (higher PAR transmission, higher  $\text{RL/FRL}$ ,  $\text{BL/UV}$  and  $\text{BL/FRL}$  ratios) and concomitantly its potential advantages towards plant productivity, we also selected filter A as the less suitable of all the seven filters, which therefore served as a control (reference filter) although the differences between A and B filters were really small.

The cultivation of tomato plants in the experimentally designed greenhouses lasted around three months. Both maximal photosynthetic and respiration rates were significantly elevated in plants grown under filter B (Figs. 4a, b). This effect suggests that plants in greenhouse B experience higher light intensity conditions relatively to plants in greenhouse A. Such a result was rather expected since the  $\text{RL/FRL}$  ratio as well as total PAR irradiation transmittance were both higher in the case of filter B. Higher plants interpret an elevated  $\text{RL/FRL}$  ratio as higher light intensity and they adapt to it by increasing their photosynthetic and respiration rates (Kendrick and Kronenberg, 1994). Additionally, the increased ratio  $\text{BL/FRL}$  contributes to this response. It is known that the monochromatic BL radiation simulates an adaptation of the photosynthetic apparatus to high light conditions, and the monochromatic FRL induces changes in the structure and function of the photosynthetic apparatus similar to the corresponding one of plants adapted to low light conditions (Kendrick and Kronenberg, 1994). In our case, the value of the  $\zeta$  parameter produced under the filter B was always somewhat higher than the corresponding value under filter A (Table II). Although this difference in the  $\zeta$  values between the A and B greenhouses may seem negligible, one must consider the asymptotic nature of the relationship between  $\text{RL/FRL}$  and  $\Phi$ , which means that small changes in  $\text{RL/FRL}$  elicit relatively large changes in  $\Phi$ . Thus, the combination of a higher PAR irradiation together with the higher  $\text{RL/FRL}$  and  $\text{BL/FRL}$  ratios experienced by plants in the greenhouse covered with the plastic sheet B promoted their adaptation to higher light conditions compared to the case of the A plastic sheet.

Photosynthetic rate is proportionally correlated with plant productivity. Thus, since plant productivity is ultimately reflected in crop production, greenhouse B is expected to display a higher rate



in fruit production. Blossoms appeared almost synchronously in plants of the A and B greenhouses (data not shown), indicating that the differences in light conditions between the two greenhouses did not produce any stress to promote floral induction. Indeed, a plant mechanism to overcome stress conditions is by promoting floral induction, which comprises the first step in seed development thus serving the species sustenance (Levy and Dean, 1998). On the other hand, fruit development was clearly favoured in the case of filter B, since the number of tomato fruits showed a significant increase. The estimated raise in plant productivity (tomatoes/plant) with filter B was in overall 45% higher than with filter A (calculated on the basis of total crop production in the A and B greenhouses, at the end of the experimental cultivation period).

Floral induction, which marks the onset of crop production, is under the control of a complex in-

teraction of various photoreceptors, both phytochromic and cryptochromic. Our results are in agreement to the presented results of Casal (2000), where phytochrome A (phyA) and cryptochromes (Cry1 and Cry2) act separately to induce flowering, whereas FRL via phyB inhibits flowering. Moreover, Cry2 antagonizes the inhibiting action of phyB.

The present results can be well explained on the basis of the above model. Indeed, plastic filter B exhibited a lower FRL irradiation level (increased RL/FRL ratio) compared to filter A, thus removing its blocking effect on floral induction. Concomitantly the increased RL/FRL ratio as well as BL/FRL ratio, as exhibited by filter B compared to filter A (Table II), favour the activation of phyA and cryptochromes (Cry1, Cry2), respectively, thus promoting flowering according to the model previously discussed. The above results show that even minor differences concerning the

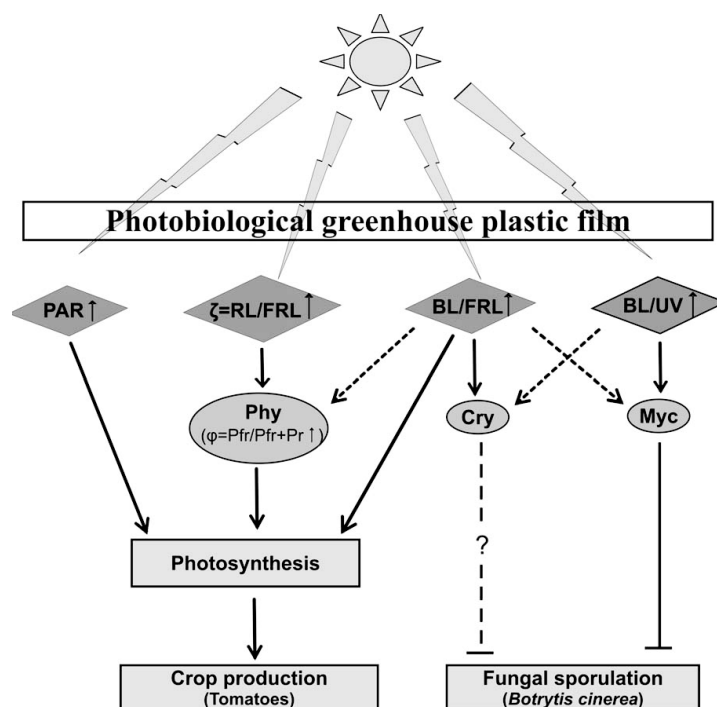


Fig. 5. Schematic outline of the potential advantages for plant cultivations deriving from the use of greenhouse plastic covers with special photobiological properties, that influence phytochromic (Phy), cryptochromic (Cry) and mycochromic (Myc) responses, thus leading to enhanced crop production and inhibition of fungal sporulation. Rhombuses represent spectral parameters of the transmitted sunlight, circles the photoreceptors and rectangles the photoresponses. Arrows represent induction and the symbol  $\perp$  inhibition. Dashed lines represent the possibility (under specific conditions) to influence directly or indirectly photoreceptors and photoresponses. PAR, photosynthetically active radiation; RL, red light; BL, blue light; UV, near-ultraviolet light; FRL, far red light.

light transmittance properties of the greenhouse filters prove efficient enough to cause notable differences in plant development and production.

The ten isolates of *B. cinerea* taken from greenhouse tomato plants from various locations in the island of Crete were found to sporulate abundantly under UV radiation shorter than 360 nm, and eight of them moderately under complete darkness. On the other hand, nine of these isolates did not sporulate at all under blue light. This is common for many other fungi with light-affected sporulation (Tan, 1978). Honda and Yunoki (1978) have defined a precise action spectrum for photosporogenesis in *B. cinerea* with a lower limit of effective wavelength at 355 nm and three major peaks of effectiveness at about 231, 268 and 283 nm and a minor peak at about 303 nm. Any attempt to correlate the role of light with vegetable diseases should not ignore other environmental factors (Leach, 1962, 1971; Vakalounakis *et al.*, 1983). Humidity and leaf wetting as well as temperature are important factors in the sporulation process (Tan, 1978).

Our results clearly show the possibility of preventing sporulation of *B. cinerea* by inhibition of sporulation with light filtered through the B film. The pest management practice of inhibiting sporu-

lation of *B. cinerea* with plastic films having a high BL/UV ratio of transmitted light integrated with other control measures, such as decreasing relative humidity in greenhouses, removal of diseased fruits and leaves from infected plants, regular fertilizations, and few sprays with effective fungicides, should effectively control *B. cinerea* in Greece and other countries.

Conclusively, our results suggest that a new generation of "photobiological" greenhouse plastic covers with improved photobiological properties (increase of PAR, BL/UV, RL/FRL and BL/FRL) may be developed, which would simulate a photonic information that would lead to a physiological enhancement of plant productivity and disease control through the spectral changes of the sunlight (see in Fig. 5a scheme of the proposed mechanism based on our results), thus decreasing the need for chemical control.

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