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Application of low intensity light pulses to delay postharvest senescence of *Ocimum basilicum* leaves

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ABSTRACT

Fresh basil (*Ocimum basilicum* L.) is a highly perishable leafy green vegetable with a storage life of 4–5 d at room temperature. Exposure of basil leaves to temperatures below 12 °C during storage results in chilling injury; therefore, refrigeration cannot be used to extend postharvest life of basil. Typically, leafy vegetables are stored in darkness or extremely low irradiance. Darkness is known to induce senescence, and the initial phase of senescence is reversible by exposure to light. In this work, we studied the effects of low-intensity white light pulses at room temperature on postharvest senescence of basil leaves. Daily exposure for 2 h to 30–37 μmol m^{-2} s^{-1} of light was effective to delay postharvest senescence of basil leaves. Chlorophyll and protein levels decreased, ammonium accumulated and leaves developed visual symptoms of deterioration (darkening) during storage in darkness. Light pulses reduced the intensity of these senescence symptoms. The photosynthesis light compensation point of basil leaves was 50 μmol m^{-2} s^{-1} i.e., higher than the intensity used in this study, and the effect of treatment with red light was the same as with white light, while far red light was ineffective. Light pulses exerted a local effect on chlorophyll loss, but the effect on protein degradation was systemic (i.e., spreading beyond the illuminated parts of the leaf blade). The results of this study indicate that daily treatment for 2 h with low intensity light (30–37 μmol m^{-2} s^{-1} every day) during storage at 20 °C is an effective treatment to delay postharvest senescence of basil leaves. The delay of postharvest senescence by low intensity light pulses seems to be mediated by phytochromes, and it is systemic for protein, and partially systemic for chlorophyll degradation.

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1. Introduction

Fresh basil (*Ocimum basilicum* L.) is a highly perishable leafy green vegetable, with a storage life of 4–5 d at room temperature (Cantwell and Reid, 2002). Postharvest senescence of green leaves is induced by detachment and exposure to darkness or very low light, probably as a consequence of the ensuing water and nutrient deficiencies, and lack of photosynthesis (Ella et al., 2003). Senescence involves enhanced chlorophyll and protein degradation, and accumulation of ammonium in detached leaves (Rolny et al., 2011; Chen et al., 1997; Clarke et al., 1994).

Postharvest senescence causes serious commercial losses due to rapid decline in basil leaf quality (Hassan and Mahfouz, 2010). One of the main goals of postharvest technology in green vegetables is to delay senescence symptoms (Page et al., 2001). Basil is a chilling susceptible plant of tropical origin, therefore exposure of leaves to temperatures below 12 °C during storage results in the development of chilling injury, which is manifest by the appearance of dark lesions on leaves, followed by decay (Lange and Cameron, 1994). Therefore, low temperature is not an option for postharvest handling of this vegetable, and it is important to find alternative technologies.

Darkness induces senescence in detached leaves of green vegetables. The initial phase of senescence is reversible, and it can be reversed by light (Zavaleta-Mancera et al., 1999). In the last few years, several publications have reported on the influence of light during storage on the quality of different vegetables. Lester et al. (2010) reported that spinach leaves exposed to continuous light (26.9 μmol m^{-2} s^{-1}) during storage had higher nutritional quality than leaves exposed to continuous darkness. Fluorescent light (21.8 μmol m^{-2} s^{-1}) has been described as promoting weight loss

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but preserving vitamin C of Chinese kale during storage (Noichinda et al., 2007). Light exposure during storage prevented senescence in cabbage (Perrin, 1982). Oms-Oliu et al. (2010) showed that the application of pulsed light at doses of 4.8 J cm^{-2} could extend the shelf life of fresh-cut mushrooms without dramatically affecting texture and antioxidant properties. Brief postharvest exposure to pulsed light stimulates coloration and anthocyanin accumulation in fig fruit, and this treatment seems to be a feasible means of compensating for insufficient sunlight stimulation of color development in figs and possibly other fruit as well (Rodov et al., 2012). The combinations of 24 $\mu mol\, m^{-2}\, s^{-1}$ light exposure with storage at low temperature delayed sensory quality deterioration and prolonged shelf-life of fresh-cut broccoli (Zhan et al., 2012).

However, a negative effect on the quality of different vegetables has been observed during storage under light due to an increase in physiological activity (Sanz et al., 2009). Some authors have reported that light increased respiration of freshly cut green vegetables, which can accelerate browning of the cut edges in leeks (Ayala et al., 2009), accelerated chlorophyll loss in broccoli, and increased transpiration and fermentation in cauliflower (Olarte et al., 2009).

Although several studies have shown that light delays senescence of leaves, the mechanism underlying this effect remains largely unknown. Light effects may be related to its role in photosynthesis and photo-assimilate availability, or light can interact with phytochromes that are involved in several development processes (Paul and Khurana, 2008; Casal et al., 1998). Phytochromes maximally absorb in the red and far-red region of the solar spectrum and play a key role in regulating plant growth and development. Phytochromes exist in two inter-convertible conformations with different absorption spectra: Pfr absorbs far red light and is generally the biologically active conformation, Pr absorbs red light. Absorption of red light converts Pr to Pfr while absorption of far red converts Pfr to Pr. Phytochrome responses are classically defined by their red/far red reversibility (Quail, 2002).

The aim of this paper was to assess the feasibility of using low intensity light pulses to delay postharvest senescence of *O. basilicum* leaves. The physiological basis of the effects of low intensity light on postharvest senescence is also discussed.

2. Materials and methods

2.1. Plant material and treatments

Basil (*O. basilicum* L.) leaves, harvested early in the morning, were obtained from local producers (La Plata, Argentina). Leaves were placed in trays (ten leaves per tray) and covered with a PVC film to decrease water loss. Trays were maintained at 20 °C in darkness, or given pulses of low intensity white light (30–37 $\mu mol\, m^{-2}\, s^{-1}$) provided by fluorescent lamps. To illuminate leaves with red and far red light, the respective LEE filters were placed between lamps and samples so that irradiance at leaf level reached 30–37 $\mu mol\, m^{-2}\, s^{-1}$. To examine if the response to low intensity light pulses is local or systemic, in one set of leaves one half (lengthwise) of the leaf lamina was covered with aluminum foil while the rest of the lamina was treated with low intensity white light pulses. Complete darkness below aluminum foil was confirmed through measurements with a photosynthetically active radiation quantum sensor (RADIAPAR, Cavadevices, Buenos Aires, Argentina). For analytical determinations in this experiment, each half of each leaf was considered as a different sample: A and B. After treatment with low intensity light for 2 h, all trays were returned to darkness. In all experiments the intensity of light was measured with a photosynthetically active radiation quantum sensor.

2.2. Chlorophyll and carotenoid content

Relative chlorophyll content per unit leaf area was determined non-destructively using a SPAD (Soil Plant Analysis Development) analyzer (SPAD-502 Chlorophyll Meter, Konica Minolta, Tokyo, Japan). Alternatively, pigment content was determined in samples of fresh leaves spectrophotometrically according to Lichtenthaler (1987). Five discs (1 cm diameter each) were ground in liquid nitrogen, extracted for 15 min with 1 mL of 95% ethanol (v/v), and then centrifuged at 3000 × *g* for 10 min. The supernatant was used to determine the absorbance (Abs) at 664.2 and 648.6 nm for chlorophylls and 470 nm for total carotenoid content. Total chlorophyll and carotenoid contents were calculated using Lichtenthaler's equations. Three replicates per treatment were analyzed.

2.3. Protein content

Two freshly cut leaf discs (1 cm diameter each) were homogenized in chilled buffer (50 mM Tris hydroxy-methyl aminomethane–HCl, pH 8. 5 mM EDTA, 1 mM PMSF, 1 μM cysteine and 5 nM Leupeptin) and centrifuged at 10,000 × *g* for 10 min at 4 °C. Proteins in the supernatant were determined according to Bradford (1976) with bovine serum albumin as standard. Three replicates per treatment were analyzed.

For SDS-PAGE analysis, one volume of the supernatant from protein extraction was mixed with one volume of 2× solubilization buffer (125 mM Tris pH 6.8; 4%, w/v, SDS; 10%, v/v, glycerol; 10%, v/v, β-mercaptoethanol), boiled for 5 min and separated in 1.5 mm thick, 12% acrylamide concentration minigels as in Laemmli (1970). Proteins were visualized by staining with Coomassie Brilliant Blue R-250. Gels were photographed with a digital camera, and the protein content was calculated by using the SIGMA gel analysis software. Different concentrations of bovine serum albumin were included in each gel to serve as standard. Three replicates per treatment were analyzed.

2.4. Ammonium content in leaves

Eight freshly cut leaf discs (1 cm diameter each) were homogenized with 1 mL of 0.3 mM H_2SO_4. Samples were centrifuged at 10,000 × *g* for 10 min at 4 °C and ammonium was determined with Indophenol blue according to Hung and Kao (2007). $(NH_4)_2SO_4$ was used as standard. Three replicates per treatment were analyzed.

2.5. Glutamine synthetase (GS) activity

Five freshly cut leaf discs (1 cm diameter each) were extracted in 1 mL of extraction buffer (pH 7.6) containing 100 mM HEPES, 1 mM EDTA, 10 mM $MgSO_4$, 5 mM glutamate, 10% (v/v) ethylene glycol, 10 μM leupeptin and 6 mM cysteine. The crude extract was centrifuged at 12,000 × *g* for 30 min at 4 °C. GS activity was measured using a synthetase assay based on the method described by Lea et al. (1990). 100 μL of crude leaf extract was added to 380 μL of assay mix, which consisted of 100 mM HEPES, 80 mM glutamate, 6 mM hydroxylamine HCl, 20 mM $MgSO_4$, 4 mM EDTA at pH 7.6. The reaction was started by the addition of 20 μL of 0.2 M ATP at pH 7.6. After 10 min of incubation at 30 °C, the reaction was stopped by the addition of 500 μL of ferric chloride reagent (0.24 M TCA, 0.1 M ferric chloride, 1.0 M HCl). Samples were then centrifuged at 10,000 × *g* for 5 min and absorbance was read at 505 nm. GS activity was expressed as the increase of $Abs_{505\,nm}\, s^{-1}\, m^{-2}$. Three replicates per treatment were analyzed.

Table 1
Selection of optimal duration of the low intensity light pulse treatment.

Duration of light pulses (30–37 μmol m^{-2} s^{-1})	0 min	30 min	60 min	90 min	120 min	180 min
SPAD D0	37.5 ± 2.2	35.8 ± 2.4	36.2 ± 2.5	38.0 ± 2.2	38.9 ± 2.1	37.4 ± 3.2
SPAD D5	31.7 ± 3.1	34.1 ± 2.2	32.4 ± 1.8	33.2 ± 2.1	37.5 ± 2.3	31.5 ± 3.2
δ SPAD (D0–D5)	5.8	1.7	3.8	4.8	1.4	5.8
Protein content D0 (mg m^{-2})	3.8 ± 0.4	4.1 ± 0.3	3.8 ± 0.3	3.9 ± 0.4	4.0 ± 0.3	3.9 ± 0.4
Protein content D5 (mg m^{-2})	1.2 ± 0.3	2.2 ± 0.3	2.3 ± 0.3	2.7 ± 0.3	3.3 ± 0.2	1.5 ± 0.3
% Protein content after 5 d	31.1	54.5	61.1	67.8	80.9	39.4

Changes in chlorophyll and protein content in basil leaves after 0 (D0) and 5 d (D5) at 20 °C. Leaves received low intensity light pulses for 30–180 min every day. Chlorophyll content was expressed as relative content per area unit (SPAD) and protein content was expressed as mg per area unit. Each SPAD data point represents the mean of 15 independent measurements and each protein data point represents the mean of three replicates.

2.6. Sugar content in leaves

Total and soluble sugar content was determined using the anthrone method (Yemm and Willis, 1954). For analysis of soluble sugars, four freshly cut leaf discs (1 cm diameter) were homogenized with 750 μL 95% (v/v) ethanol, the extract was centrifuged and the resulting pellet was washed again with 750 μL 95% (v/v) ethanol for 2 min and centrifuged. The pellet was stored for determination of ethanol-insoluble sugars (starch). The ethanol supernatants were combined and centrifuged at 9000 × g for 5 min at 4 °C to precipitate any remaining ethanol-insoluble material; the supernatant was diluted 2 times with distilled water. To measure the concentration of soluble sugars, the supernatant was mixed with 1.0 mL of 0.2 g L^{-1} anthrone in concentrated H_2SO_4. The ethanol-insoluble pellet containing the starch fraction was mixed directly with 2.0 mL of 0.2 g L^{-1} anthrone in concentrated H_2SO_4. Both anthrone mixtures were heated for 10 min at 100 °C. Samples were then cooled in ice-water and absorbance at 620 nm was measured. Glucose was used as standard, and total sugar content was calculated by adding up the soluble sugar and ethanol-insoluble (starch) fractions. Three replicates per treatment were analyzed.

2.7. Photosynthesis–light response curves and net CO_2 exchange measurement

An *i*nfrared *g*as *a*nalyser (IRGA, CIRAS II, PPSystems, UK) was used to measure net photosynthesis. The system allows for automated microclimate control in the leaf chamber. Before each measurement, leaves were adapted for 10 min to chamber conditions. CO_2 concentration was kept at 380 μmol mol^{-1}. To determine the response of net photosynthesis to light intensity, we varied irradiance from 0 to 800 μmol m^{-2} s^{-1} PAR (400–700 nm), at 25 °C air temperature and 50% relative humidity. Three curves were analyzed.

Net CO_2 exchange was measured in three leaves for each treatment (white light pulses and darkness) after 0, 3 and 5 d of storage. For leaves receiving daily light pulses, measurements were carried out both in darkness and at 37 μmol m^{-2} s^{-1} of white light.

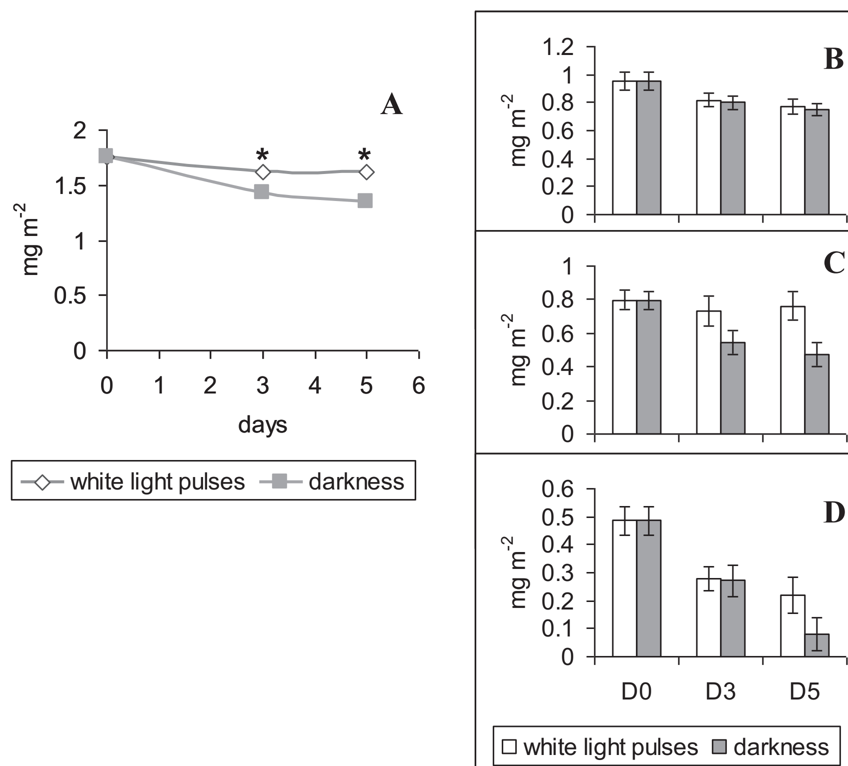


Fig. 1. Changes in the levels of total chlorophyll (A); chlorophyll a (B); chlorophyll b (C), and carotenoids (D) in basil leaves without light treatment (darkness) or given low intensity light pulses (30–37 μmol m^{-2} s^{-1}) for 2 h every day (white light pulses) and then stored at 20 °C for 0 (D0), 3 (D3) or 5 (D5) days in darkness. Pigment content is expressed on the basis of leaf area. Three independent extracts were made for each sampling date and treatment. Bars indicate standard deviation (B–D) and asterisks (A) indicate significant differences ($p < 0.05$) between treatments.

Table 2
Leaf appearance (darkening).

Treatment	Control			Low intensity light treatment		
	D0	D3	D5	D0	D3	D5
Total number of leaves	24	24	24	24	24	24
Number of darkened leaves	0	8 ± 2	16 ± 2	0	2 ± 2	7 ± 2
% darkened leaves	0	33	66	0	8	29

Visual appearance of leaves estimated from the proportion of darkened (damaged) leaves after 0 (D0), 3 (D3) and 5 (D5) days. Average ± standard error of the mean.

2.8. Statistical analysis

Each experiment was carried out twice. Data were analyzed by ANOVA, and the means were compared with Tukey's test at a significance level of 0.05.

3. Results

3.1. Selection of optimum low intensity white light pulse duration

Basil leaves were irradiated each day for different periods of time (0, 30, 60, 90, 120 and 180 min) with low intensity white light (30–37 μmol m^{-2} s^{-1}) and then returned to darkness. Chlorophyll and protein degradation were used as characteristic symptoms of leaf senescence. Chlorophyll content, estimated by SPAD, decreased after 5 d of storage (Table 1). Initial SPAD values decreased in all samples during storage, but the smallest change was found when white light pulses lasted for 30 and 120 min. Total protein content was analyzed using gel analysis software (SIGMA Gel) and we calculated percentage protein content after 5 d in darkness with respect to the initial values (Table 1). The highest retention of protein (80.9% of the initial value) was found in leaves exposed to 120 min of low intensity light every day. Based on protein and chlorophyll degradation, pulses of 120 min with low intensity white light were shown to be effective in delaying postharvest senescence of basil leaves, and were chosen to further analyze the effects of light.

3.2. Effects of light pulses on senescence of basil leaves

Basil leaves were treated with low intensity white light (30–37 μmol m^{-2} s^{-1}) for 120 min every day and senescence symptoms were evaluated during storage.

3.2.1. Pigment content and leaf visual appearance

Total chlorophyll content decreased slightly in both treatments (Fig. 1A). Control leaves lost less than 25% of chlorophyll after 5 d in darkness at 20 °C, while light-treated leaves lost only 10% of chlorophyll. Thus, the chlorophyll degradation rate was reduced by white light pulses. Chlorophyll a content decreased slightly after 3 d and then remained constant over time in both samples (darkness and light treatment). Chlorophyll b content showed a significant decrease in darkness, but light-pulses reduced this degradation (Fig. 1B and C). As in many vegetables, the level of carotenoids decreased during senescence at 20 °C (Fig. 1D). Low intensity light pulses did not have any effect on carotenoid degradation during the first 3 d of storage, however; after 5 d leaves given light pulses had a significantly higher carotenoid content than control leaves kept in darkness (Fig. 1D).

The visual appearance of leaves was analyzed by counting the number of darkened leaves (Table 2 and Fig. S1). White light pulses reduced this damage symptom, therefore control samples had more leaves affected by darkening than treated leaves at all times during storage.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2013.06.017.

3.3. Effect of low intensity white light pulses on protein degradation, ammonium accumulation and GS activity

The protein content of basil leaves decreased continuously during storage. Protein degradation was significantly retarded by white light pulses (Fig. 2A), with the greatest effect recorded on day 5. By the 5th day, the protein content of control leaves maintained in darkness was 50% of their initial value, whereas leaves exposed to light pulses retained 70%.

In addition, ammonium accumulation during senescence was analyzed (Fig. 2B). The ammonium content of control leaves increased during senescence, and after 5 d in darkness it was about 3 times higher than the initial values. The ammonium content under low intensity light pulses remained unchanged during senescence, and much lower than for leaves kept in darkness.

Glutamine synthetase (GS) functions as the main reassimilatory enzyme for ammonium, therefore GS activity and ammonium level were determined after 0 and 4 d of treatment (Fig. 3). Ammonium content increased fivefold in darkness, whereas it remained constant in light-treated leaves after 4 d. GS activity decreased by 50% after 4 d in darkness, while GS activity remained unchanged in leaves treated with white light pulses. Possibly, low intensity light treatments inhibited ammonium accumulation because of the retention of GS activity.

3.4. Photosynthesis–light response curves

To estimate the potential photosynthetic contribution of the light pulses employed in this work, the net photosynthesis–light response curve of basil leaves was analyzed (Fig. 4A). The response curve can be divided into two phases. Under low-light levels, the rate of net photosynthesis increased as the irradiance level increased; whereas above 400 μmol m^{-2} s^{-1} photosynthesis was saturated. The light compensation point is the light intensity where the rate of photosynthesis matches the rate of respiration, therefore net carbon dioxide assimilation is zero. The light compensation point for basil leaves was 50 μmol m^{-2} s^{-1}, while the intensity of light used in this paper was between 30 and 37 μmol m^{-2} s^{-1}; i.e., the light pulses were too low to cause net carbon dioxide fixation.

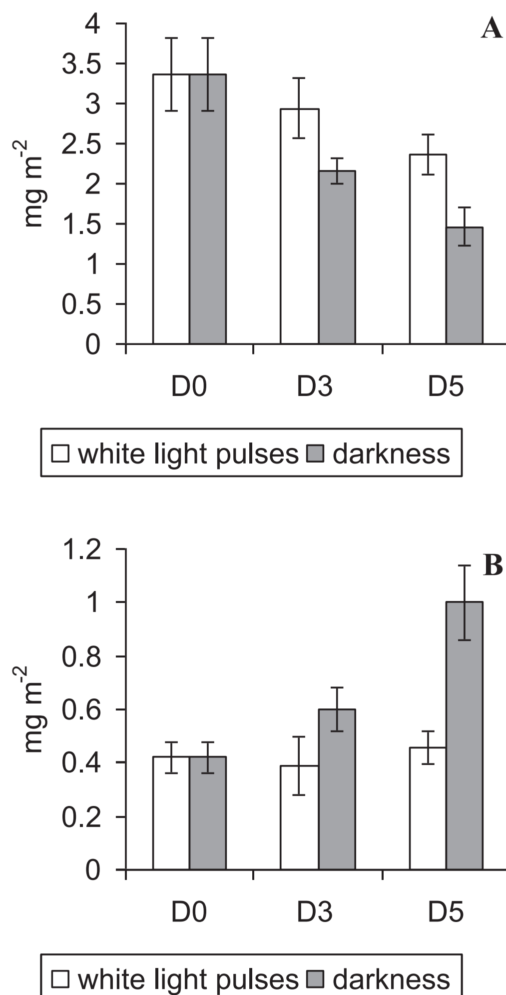


Fig. 2. Changes in total protein (A) and ammonium (B) content in basil leaves without light treatment (darkness) or given low intensity light pulses (30–37 μmol m^{-2} s^{-1}) for 2 h every day (white light pulses) and then stored in darkness for 0 (D0), 3 (D3) or 5 (D5) days at 20 °C. Protein and ammonium content are expressed on the basis of leaf area. Three independent extracts were made for each sampling date and treatment. Bars indicate standard deviation.

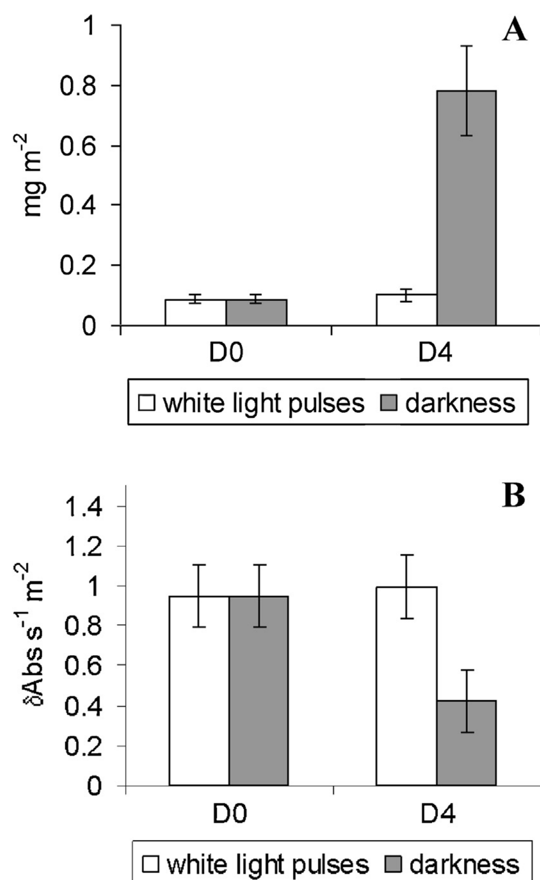


Fig. 3. Ammonium accumulation (A) and GS activity (B) in basil leaves without light treatment (darkness) or given low intensity light pulses (30–37 μmol m^{-2} s^{-1}) for 2 h every day (white light pulses) after 4 d of storage in darkness at 20 °C. Ammonium content and GS activity are expressed on the basis of leaf area. GS activity is expressed as the increase of Abs per second. Three independent extracts were made for each sampling date and treatment. Bars indicate standard deviation.

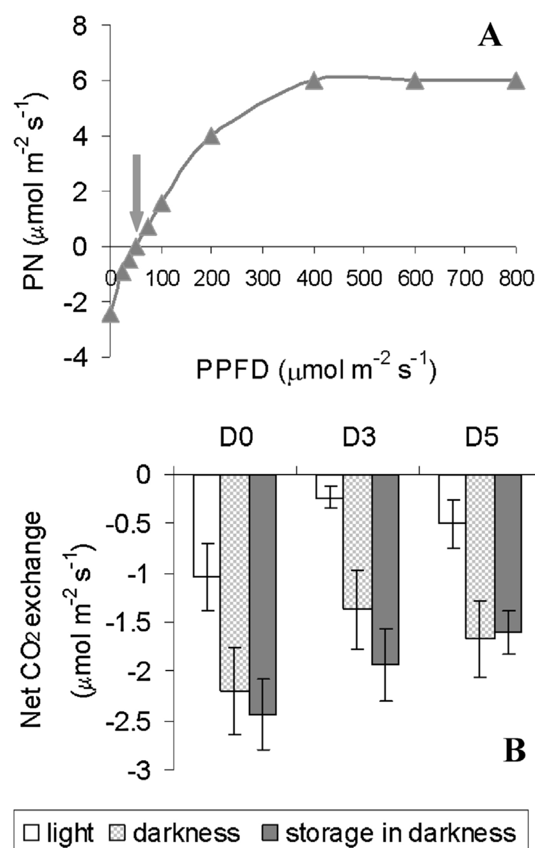


Fig. 4. (A) Photosynthesis–light response curve (A). PPDF: photosynthetic photon flux density; PN: net photosynthesis. The curve is the average of measurements on three leaves. The arrow shows the light compensation point of basil leaves. (B) Net CO_2 exchange during storage was measured in three leaves for each treatment (white light pulses and darkness, labeled "storage in darkness") after 0, 3 and 5 d of storage at 20 °C. For leaves treated with light pulses, measurements were carried out both in darkness (labeled "darkness") and under light (37 μmol m^{-2} s^{-1}, labeled "light"). Negative values represent net CO_2 emission.

It is important to note that net CO_2 exchange under light is the balance between respiration and photosynthesis, whereas it represents only respiration in darkness. Net CO_2 exchange of basil leaves was measured after 0, 3 and 5 d of storage for both treatments (white light pulses and continuous darkness). In the case of leaves given light pulses, net CO_2 exchange was measured under the light pulse (37 μmol m^{-2} s^{-1}) and under darkness. Net CO_2 exchange was always negative, with only slight differences between dark- and light-treated leaves when measurements were made in darkness (Fig. 4B). Respiration rate (i.e., net CO_2 exchange measured in darkness) showed a slight decrease during storage in both treatments. When measured under light in leaves given light pulses, net CO_2 exchange was a little higher, but still negative.

3.5. Effect of low intensity white light on sugar level

Soluble sugars represented a high proportion of total sugar content in basil leaves harvested early in the morning (i.e., after a whole night in darkness), at the beginning of experiment (D0) and 100% of total sugar after 5 d in both treatments (Fig. 5). Total sugar content was 1.4 g kg^{-1} at the beginning of the storage treatment and it remained constant after 5 d in light treated samples, while soluble sugar concentration increased after 3 d (Fig. 5). In leaves stored in darkness, total and soluble sugars decreased by about 40% between 3 and 5 d, whereas sugar content remained relatively unchanged in light treated leaves up to the end of the storage period.

3.6. Effect of low intensity red light on senescence of basil leaves

Additional experiments investigated if pulses of red light have the same effect as white light on basil leaf senescence. Basil leaves were irradiated each day for 2 h with low intensity red light (30–37 μmol m^{-2} s^{-1}) using a LEE red filter positioned between the light source and leaves. Chlorophyll, protein and ammonium content were measured after 0 and 5 d (Fig. 6) and compared with white light pulses (light control) and a dark control.

Chlorophyll content showed a slight decrease, control leaves in darkness lost 20% after 5 d while leaves treated with white or red light lost 10 and 8% respectively (Fig. 6A). As shown in Fig. 6B, protein content decreased after 5 d in all leaves, but both light treatments retained high levels of proteins, approximately 80% with respect to their initial values. Ammonium content increased 2.5 times during senescence in darkness, whereas in leaves treated with red or white light there was no ammonium accumulation after 5 d (Fig. 6C). Thus, pulses of red light were as effective as white light in retarding postharvest senescence of basil leaves.

To analyze if the effect of light is mediated by phytochromes, basil leaves were irradiated each day for 2 h with low intensity red light or far red light (30–37 μmol m^{-2} s^{-1}) using a LEE red filter and a LEE far red filter, respectively, positioned between the light source and leaves. The spectral energy distribution of light transmitted through the filter was measured using a spectroradiometer (Model SR., Instrumentation Specialties Co., Lincoln, NE, USA). The transmission spectra of these LEE filters were different, producing high and low Red/Far red ratios (Fig. 7). Compared to red light, far red light did not cause protein retention, or reduced ammonium accumulation, and GS activity decreased more under far red than under red light pulses (Fig. 8B–D). While red light pulses caused almost complete inhibition of chlorophyll degradation, 13% of the initial chlorophyll content was lost after 4 d of incubation in far red light treated leaves (Fig. 8A). After 5 d of storage, the appearance of darkened lesions in the leaves was lesser under red light pulses than under far-red light (Fig. S2).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2013.06.017.

3.7. Local or systemic effects of light?

To analyze if the effects of light pulses are local (i.e., restricted to the illuminated parts of the leaf) or systemic (i.e., spreading beyond illuminated areas), one half of each leaf was covered with aluminum foil placed parallel to the central vein, and treated leaves with low intensity light pulses. In this experiment each half leaf was a different sample: uncovered, illuminated by the light pulses (A), and covered, maintained in darkness (B). Leaves in darkness (dark control) and leaves uniformly exposed to white light pulses (light control) were included in the experiment, and

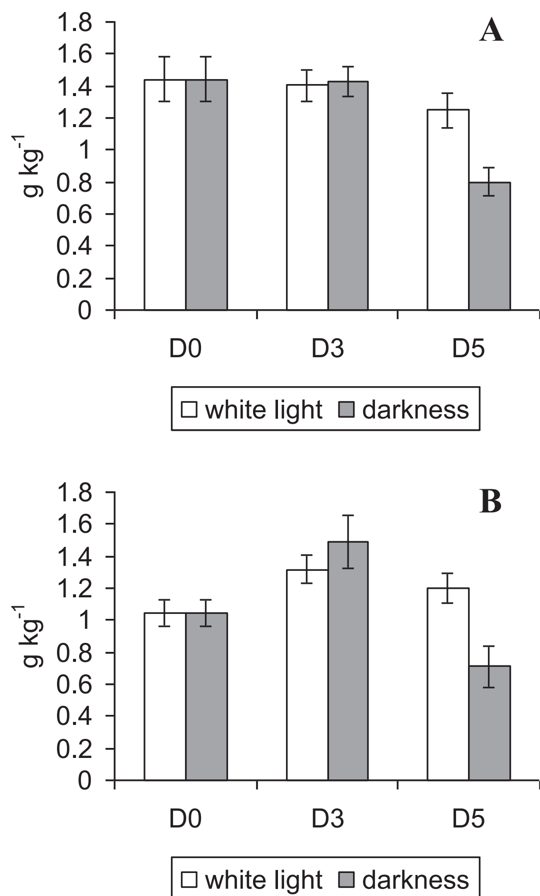


Fig. 5. Changes in total sugar (A) and soluble sugar (B) content in basil leaves without light treatment (darkness) or given low intensity light pulses (30–37 μmol m^{-2} s^{-1}) for 2 h every day (white light pulses) after 0 (D0), 3 (D3) and 5 (D5) days of storage in darkness at 20 °C. Sugar content is expressed on the basis of fresh weight. Three independent extracts were made for each sampling date and treatment. Bars indicate standard deviation.

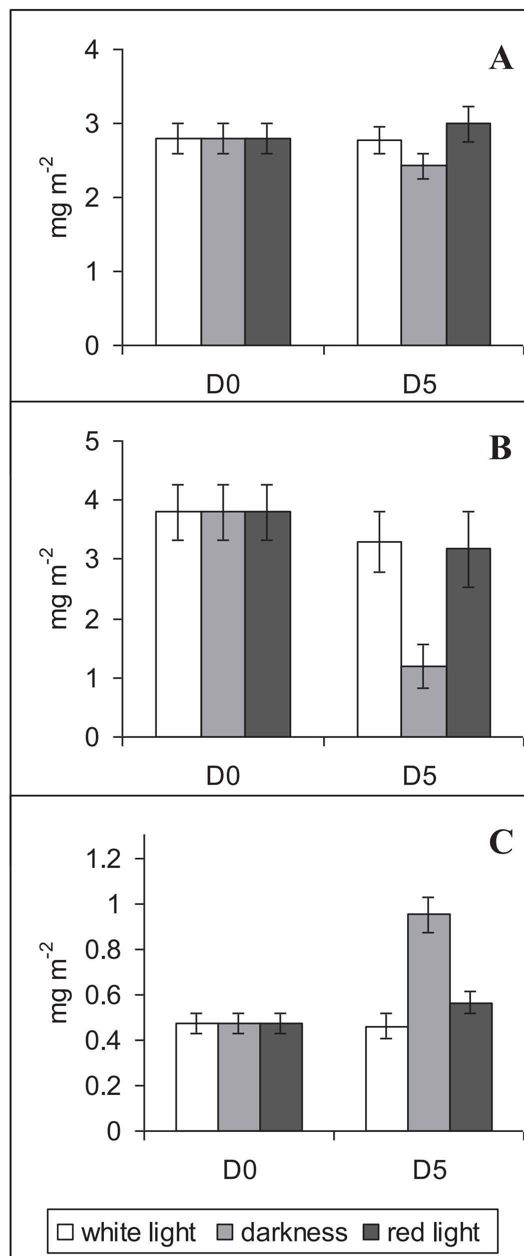


Fig. 6. Changes in the levels of chlorophyll (A), protein (B) and ammonium (C) in basil leaves without light treatment (darkness), given low intensity white light pulses (30–37 μmol m^{-2} s^{-1}) for 2 h every day (white light pulses) or given low intensity red light pulses (30–37 μmol m^{-2} s^{-1}) for 2 h every day (red light pulses) after 5 d of storage in darkness at 20 °C. Chlorophyll, protein and ammonium content are expressed on the basis of leaf area. Three independent extracts were made for each sampling date and treatment. Bars indicate the standard deviation.

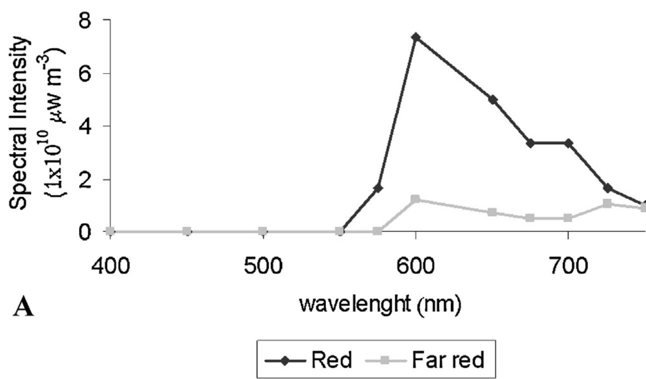
each half (A and B) of these leaves was taken as an individual sample too. Chlorophyll and protein levels between A and B halves of each leaf were compared after 5 d in storage. Chlorophyll content in darkness and in light control leaves was similar between A and B halves; the differences (A – B) between both halves were low and erratic since both halves were subjected to the same treatment, i.e., darkness or light pulses (Table 3). In leaves where half of the lamina was covered with aluminum foil, the illuminated part (A) showed higher chlorophyll content than the covered half (B). Chlorophyll content in the uncovered halves was similar to light controls (33.5 and 35.8 SPAD units, respectively) while in the covered half, chlorophyll content was only slightly higher than in the darkness control (31 and 29 SPAD units respectively). Thus, the effects of light on chlorophyll degradation were mostly local.

Protein content was analyzed in the same way. There were positive or negative differences in all cases, and the average value of the difference between both sides of the leaf was similar in all treatments (Table 4). After 5 d of storage, protein content was higher in the covered, non-illuminated half of otherwise illuminated leaves (2.9 mg m^{-2}) and in the light controls (2.6 mg m^{-2}) than in whole leaves kept in darkness (1.36 mg m^{-2}), implying that the effect of light was partly systemic, i.e., spreading from the illuminated to the non-illuminated half of the leaf.

4. Discussion

In recent years, a few studies have tested the use of light treatments during postharvest storage with different results. For example, continuous low intensity light (26–30 μmol m^{-2} s^{-1}) during storage prevented the loss of ascorbic acid in Chinese kale and spinach (Toledo et al., 2003; Noichinda et al., 2007). Brüchert et al. (2011) showed that storage under continuous low-intensity light (12 μmol m^{-2} s^{-1}) was an efficient and low-cost treatment to delay postharvest senescence in broccoli. Zhan et al. (2012) showed that low intensity white light during storage prolonged shelf-life of fresh-cut broccoli. In contrast, other studies (Sanz et al., 2007, 2009) showed that exposure to continuous low intensity light had a negative effect on quality parameters of cauliflower and asparagus, and caused a significant reduction in shelf life.

This paper examined the possible use of low intensity light pulses to delay postharvest senescence of basil leaves. Storage of excised leaves in darkness causes chlorophyll and protein breakdown, typical indicators of senescence progression in green tissues (Noodén et al., 1997). In darkness, chlorophyll levels decreased slightly during postharvest storage of basil leaves; however the decrease was less with daily 2 h light pulses (Table 1). Although all the light treatments reduced protein degradation, the highest protein content after 5 d was found with 2 h of treatment (Table 1).



Experimental Red (650 nm) / Far red (725 nm) ratio:

RED FILTER LEE: 3

B FAR RED FILTER LEE: 0.66

Fig. 7. (A) Spectral energy distribution of light transmitted through the LEE filters used. (B) Red/far-red ratio obtained with each LEE filter used.

The inhibitory effect of light on protein degradation increased with pulse duration up to an optimum, but longer pulses were not as effective. Possibly, longer pulses (e.g., 180 min) might cause a temporary stress in the tissues. This behavior, i.e., senescence retardation up to an optimum treatment dose, is similar to other postharvest treatments like hot air or UV-C treatments (Costa et al., 2005, 2006). The behavior of chlorophyll was different. The changes in chlorophyll content were similar to the response of protein content for light pulses from 60 to 180 min; however, unlike protein content, 30 min of low intensity white light reduced chlorophyll degradation. It is possible that there was a direct effect of light on chlorophyll content with a 30 min pulse, but that longer pulse durations counteracted this direct effect. The delay of chlorophyll degradation with longer pulses (120 min) may operate through an independent mechanism, possibly related with changes in metabolism.

A more detailed analysis of the effect of 2 h-light pulses showed that protein degradation preceded chlorophyll breakdown in control (dark stored) and light-treated samples. Chlorophyll decreased significantly after 5 d, but there was no difference in chlorophyll content between 0 and 3 d, whereas protein decreased significantly after 3 d of storage (Figs. 1A and 2A). Proteolysis generally precedes chlorophyll breakdown during senescence of green leafy vegetables (Paull, 1992; Hortensteiner and Matile, 2004).

Yellowing caused by degradation of chlorophylls does not seem to be a critical symptom of postharvest deterioration for basil, since chlorophyll degradation was evident only very late. Although chlorophyll content is routinely used to monitor senescence in many vegetables, senescence is also characterized by increased activity of peroxidases and polyphenoloxidases, which are responsible for tissue darkening (Underhill and Critchley, 1995). Leaf yellowing may not be the most obvious symptom of deterioration for basil, in which shelf life is eventually limited by the development of surface molds and/or darkened tissues (Lange and Cameron, 1994). The visual appearance of leaves and an estimation of tissue damage, made by counting the number of darkened leaves (Table 2

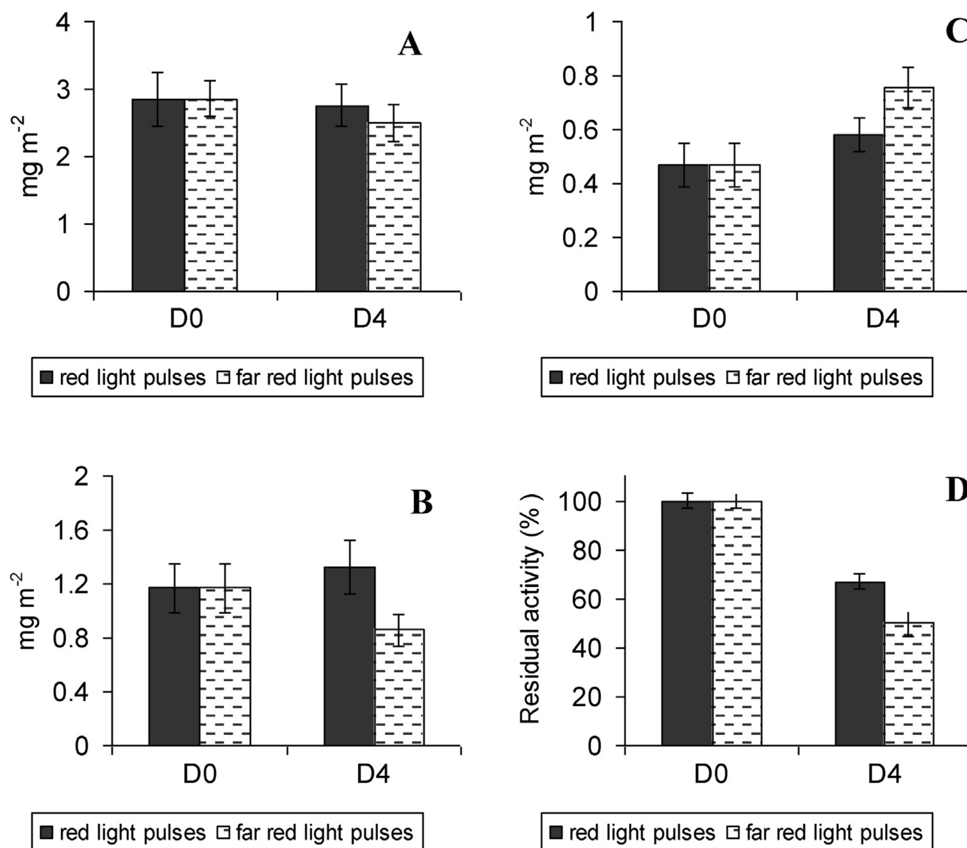
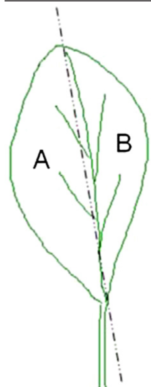


Fig. 8. Changes in the levels of chlorophyll (A), protein (B), ammonium (C) and GS activity in basil leaves given low intensity red light pulses (30–37 μmol m^{-2} s^{-1}) for 2 h every day (red light pulses) or low intensity far red light pulses (30–37 μmol m^{-2} s^{-1}) for 2 h every day (far red light pulses) and then stored in darkness at 20 °C for 4 d (D4). The parameters are expressed on the basis of leaf area. Three independent extracts were made for each sampling date and treatment. Bars indicate the standard deviation.

Table 3
Local vs. systemic effects of light pulses on chlorophyll loss.

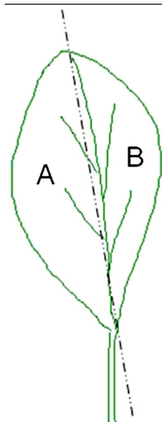
	Darkness control			Light control			Split treatment (A: light/B: darkness)		
	A	B	δ	A	B	δ	A	B	δ
Leaf 1	29.2	30.0	−0.8	34.5	33.2	1.3	30.2	28.3	1.9
Leaf 2	23.0	24.7	−1.7	32.0	33.0	−1.0	38.6	33.6	5.0
Leaf 3	35.0	32.0	3.0	36.9	35.5	1.4	31.5	27.5	4.0
Leaf 4	34.2	32.2	2.0	36.0	37.2	−1.2	34.4	34.4	0.0
Leaf 5	30.6	28.4	2.2	39.9	38.7	1.2	35.5	31.7	3.8
Leaf 6	26.3	28.0	−1.7	36.2	37.1	0.9	30.9	30.8	0.1
Average	*27*	29.2	1.9	35.9	35.8	1.2	33.5	31.0	2.47



Each half leaf was considered an individual sample, named A and B, as shown in the picture. Chlorophyll content was measured as SPAD units in each half leaf (A and B) of 6 leaves for each condition: darkness control, Light control and split treatment. In the darkness and light controls the whole leaf was under the same condition (darkness or daily low intensity light pulses respectively). In split treatment, one half of each leaf was covered lengthwise with aluminum foil to separate an illuminated half (A) and a non-illuminated half (B) in each individual leaf. δSPAD = SPAD in half A – SPAD in half B for each leaf after 5 d of storage. Data for individual leaves are included to facilitate pairwise comparison of the responses of each half of the leaf. SPAD unit is relative chlorophyll content per unit leaf area determined non-destructively using a SPAD (Soil Plant Analysis Development) analyzer (SPAD-502 Chlorophyll Meter, Konica Minolta, Tokyo, Japan). Average values for each column are shown in the bottom row.

Table 4
Local vs. systemic effects of light pulses on protein degradation.

	Darkness control			Light control			Split treatment (A: light/B: darkness)		
	A	B	δ	A	B	δ	A	B	δ
Leaf 1	1.20	1.00	0.2	2.45	30.3	−0.6	2.76	2.83	−0.07
Leaf 2	1.30	1.42	−0.1	2.20	23.7	0.17	3.34	3.36	−0.02
Leaf 3	1.55	1.25	0.3	2.62	25.3	0.09	2.96	2.75	0.21
Leaf 4	2.04	1.51	0.5	2.60	27.2	−0.12	2.42	3.02	−0.6
Leaf 5	1.06	1.81	−0.7	2.99	27.2	0.27	2.97	3.17	−0.20
Leaf 6	1.14	1.10	0.04	2.61	27.4	−0.13	2.79	2.68	0.11
Average	1.38	1.35	3.2	2.58	2.68	0.23	2.87	2.97	0.20



Each half leaf was considered individual samples, named A and B, as show in the picture. Protein content was measured as mg m^{-2} in each half leaf (A and B) of 6 leaves for each condition: darkness control, light control and split treatment. In the darkness and light controls the whole leaf was under the same condition (darkness or daily low intensity light pulses respectively). In split treatment, one half of each leaf was covered lengthwise with aluminum foil to separate an illuminated half (A) and a non-illuminated half (B) in each individual leaf. δ = protein content in half A – protein content in half B for each leaf after 5 d of storage. Data for individual leaves are included to facilitate pairwise comparison of the responses of each half of the leaf. Average values for each column are shown in the bottom row.

and Fig. S1) after 3 and 5 d of storage, indeed show that white light pulses also reduced this damage symptom.

Results in this study show clearly that low intensity white light pulses applied every day delay basil leaf senescence. Similar results were also found in fresh Chinese kale exposed to light (Noichinda et al., 2007) and cut-fresh broccoli where at the end of storage light exposure preserved chlorophyll, compared to darkness (Zhan et al., 2012). Chlorophyll a content, both in leaves exposed or not exposed to light, decreased after 3 d of storage and then remained constant over time. Chlorophyll b content showed significant degradation in control samples, but light pulses decreased this degradation (Fig. 1B and C). In addition, chlorophyll b declined more than chlorophyll a in leaves in darkness, which agrees with the fact that the first step of chlorophyll b degradation is conversion to chlorophyll a (Hortensteiner and Matile, 2004). Low intensity white light treatment delayed chlorophyll b degradation. Our results are similar to those of Zhan et al. (2012) in broccoli, but they detected an initial increase of chlorophyll a in light exposed samples. Carotenoids are secondary plant compounds that serve many functions like light harvesting, structure stabilization, excess energy dissipation and protection from free radicals such as triplet excited chlorophyll and singlet oxygen produced when light intensity exceeds photosynthetic capacity (Mortensen et al., 2001). Carotenoids decreased during postharvest senescence in darkness and in leaves given light pulses, and the effect of light treatment was evident only after 5 d (Fig. 1D). This result suggests that the light treatment delayed senescence through a mechanism independent from the role of carotenoids as essential components for the assembly of the photosynthetic apparatus and for the stability of chloroplasts (Sandmann et al., 1993)

In darkness, protein degradation during storage was accompanied by ammonium accumulation, but there was no accumulation of ammonium in light-treated leaves (Fig. 2). Recently it was shown that ammonium accumulation is responsible for increased electrolyte leakage during postharvest leaf senescence (Rolny et al., 2011), and Martínez-Sánchez et al. (2011) recently showed that electrolyte leakage of fresh-cut Romaine lettuce increased slightly during the first days of storage, and that samples stored in darkness had higher electrolyte leakage than those stored in light. These authors did not analyze protein degradation during senescence but it is possible that electrolyte leakage reflected accumulation of ammonium due to amino acid deamination, and their light treatment might have reduced ammonium content by delaying protein degradation and amino acid deamination.

Postharvest senescence in darkness could be the result of reduced photosynthesis due to light deprivation, or of low active/total phytochrome ratios (Simonovic and Anderson, 2008). In detached leaves, this may be exacerbated because cytokinin-producing roots are removed from the shoot, possibly reducing leaf cytokinin concentrations (Van Staden and Joughin, 1988; Fankhauser, 2002). Light can modulate gene expression directly, via activation of phytochromes and cryptochromes, and indirectly, by activation of photosynthesis followed by increased levels of carbon metabolites and other metabolic changes in chloroplasts.

Photosynthesis vs. light response curves determined a light compensation point (i.e., the irradiance where photosynthesis balances respiration) for harvested basil leaves of about 50 μmol m^{-2} s^{-1} (Fig. 4A). The light treatment used in this work was between 30 and 37 μmol m^{-2} s^{-1}, i.e., below the light compensation point. Thus, while there were no significant differences between net CO_2 exchange rates in darkness between leaves given light pulses and leaves kept in continuous darkness, net CO_2 exchange rate was higher (but still negative) for the duration of the white light pulses (Fig. 4B). However, because leaves were given light pulses for only 2 h every day (i.e., during less than 10% of the day) the possible positive impact of light pulses on carbon balance may have been quite small, and probably negligible. Consistent with this, results in this study showed that there was no net accumulation of sugars during postharvest of light-treated leaves (Fig. 5).

Büchi et al. (1998) showed that starch is the main storage carbohydrate in sweet basil leaves. These authors showed that starch content displayed distinct diurnal patterns with an increase during the day and a decrease during the night, however the levels of starch and soluble sugars were similar in leaves harvested early in the morning, after several hours of darkness. They used illumination at 200 μmol m^{-2} s^{-1} for 12 h every day, and high CO_2 levels during storage of harvested leaves. This treatment resulted in active photosynthesis and increased the concentrations of starch and soluble carbohydrates (mainly glucose and fructose) by 60- and 12-fold, respectively. Our results, unlike those of Büchi et al. (1998) showed that the level of total sugars decreased during senescence induced by harvest and darkness, but clearly the main difference between our experiments is the low intensity light used here. These results clearly suggest that the light intensity used in the light pulse treatment was not sufficient for net synthesis of sugars in basil leaves. Thus, it is unlikely that light has an important photosynthetic effect in these experiments; rather the light effect might occur via activation of phytochromes.

Recent studies revealed that phytochrome participates in controlling protein metabolism at different levels (Han et al., 2007). Studies in this laboratory found that red light pulses were as effective as white light to delay post-harvest senescence of basil leaves, in terms of chlorophyll and protein retention, and decreased ammonium accumulation. This is similar to the results reported by Lieburg et al. (1990) who showed that loss of quality during transport and storage of cut flowers in the dark may be due to short-term phytochrome-related processes. They found that flowers (*Alstroemeria pelegrina* cv. *Yellow King*) kept under red light for 9 d at 20 °C had higher total chlorophyll content than flowers in darkness.

White and red light pulses inhibited ammonium accumulation (Fig. 4B and C), which is an important component of decreased quality during storage. Ammonium probably accumulates in darkness because of depletion of available carbon skeletons (Paull, 1992), deamination of amino acids resulting from protein degradation, and reduced activity of plastidic glutamine synthetase (GS), a key enzyme in the assimilation of ammonium which is degraded during chloroplast breakdown. A cytosolic isoform of GS (GS1) assimilates ammonium generated by protein degradation during natural and postharvest senescence (Miflin and Lea, 1976; Downs et al., 1994). Several papers have shown that GS2 is induced by light (Hirel and Gadal, 1980; Sakakibara et al., 1992). The effect of light on GS2 expression has been shown to be, at least in part, mediated by phytochrome in pea (Edwards and Coruzzi, 1989; Tjaden et al., 1995), Arabidopsis (Oliveira and Coruzzi, 1999), and pine (Elmlinger et al., 1994).

After the demonstration of the spectral characteristics of purified phytochrome, red/far-red reversibility became a diagnostic characteristic of the action of phytochrome (Shinomura, 1997). Therefore, to check if the effects of light were mediated by phytochromes, the red/far-red response of postharvest senescence of basil leaves was analyzed and showed that far red light did not cause the same effects as red light on protein degradation and ammonium accumulation (Fig. 8). The effect of light treatment on chlorophyll degradation was not clear.

Finally, possible systemic response of basil leaves to local light treatment was studied. The results show that the effect of light pulses is mostly local for chlorophyll breakdown since non-illuminated areas of the leaf always had less chlorophyll than light-treated areas (Table 3). However, since chlorophyll content in the covered half of the leaf was higher than in the dark control, but lower than in the light control, there may be a small systemic

effect for chlorophyll retention. The level of protein was similar in both covered and uncovered halves of treated leaves, and this protein level was similar to light controls and higher than dark controls (Table 4). This clearly suggests systemic effects of low intensity light pulses on protein degradation.

5. Conclusions

Treatment of basil leaves for 2 h every day with low intensity (30–37 μmol m^{-2} s^{-1}) white light pulses delayed senescence. The light intensity used here was lower than the photosynthesis light compensation point, treatment with red light was sufficient to delay senescence, and these effects were not seen under far red light. Light effects during postharvest senescence of basil were at least partly systemic. This implies that even a treatment where light does not impinge uniformly over leaves, or where parts of the leaves are shaded by other leaves, might still be an effective postharvest treatment. These results suggest that it is possible to use a red light pulse treatment to delay postharvest senescence of vegetable leaves, which could be an inexpensive technology to preserve leafy vegetables.

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