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THE ROLE OF OXYGEN IN THERMO-PHOTODYNAMIC PROCESSES LEADING TO SUNSCALD-LIKE DAMAGES IN GREEN TISSUES

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Sunscald is a physiological disorder causing, in warm climates, severe damages to green tissues. Earlier studies established the essential role of heat, light and chlorophyll in the development of the disorder. In the present work, the role of oxygen in the development of the injury was investigated. Green cucumber fruit were exposed to heat and light under controlled atmosphere, and the development of the injury was followed. No damage occurred in the absence of either heat or light. Tissues exposed to heat, light and either nitrogen or air, suffered marked electrolyte leakage, indicative of membrane damage. Chlorophyll fluorescence values of cucumbers treated with high temperatures, oxygen and light, were considerably lower than those measured for the respective dark treated tissues. Irradiated cucumbers flushed with either 100% N_2 or < 2.5% O_2, lost about 50% of their chlorophyll. These tissues turned olive-green but did not bleach. At higher conc., oxygen treated tissues lost their plastid pigments and bleached within 7 hr of exposure to heat and light. A significant decrease in chlorophyll was evident also in atmosphere containing 1% CO_2 in nitrogen. Malondialdehyde conc was significantly higher in the presence of O_2 as compared with N_2 atmosphere. The results clearly show, that sunscald damage is a result of thermo-photooxidative processes.

KEY WORDS: Cucumber; carotenoids; high-temperrature; oxygen free radicals; photooxidation; sunburn; superoxide; superoxide dismutase.

INTRODUCTION

Sunscald is a physiological disorder of frequent occurrence in warm climates, which severely damages green tissues: stems, leaves and fruit. The injured tissues bleach and die. Earlier works established the essential role of heat, light and chlorophyll in the development of the disorder:[1-4] High temperatures above 40 to 42°C inhibit and interfere with a number of physiological systems, including respiration and photosynthesis, and in combination with light, photoinhibition and photodynamic processes are markedly enhanced.[5] These may result in the bleaching and death of green tissues.[1-3,6]

The role of oxygen in the development of sunscald damage has not yet been clarified: Anaerobic conditions, cause damage to the photosynthetic system, but chlorophyll destruction is not complete. In the dark, this damage is irreversible[7] and Lomagin and Antropova,[8] found no bleaching in leaves exposed to heat and light. On the other hand, Adegoroye and Jolliffe[9] reported sunscald damages in tomato fruit both at 0 and 100% oxygen atmosphere. Under ambient conditions, inhibition of CuZnSOD resulted in bleaching of naturally illuminated tomato leaves (unpublished)

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and positive correlation between superoxide dismutase (SOD) activity and tissue susceptibility to sunscald damage was demonstrated in tomatoes, peppers and cucumbers.[10-12] The latter indicating the role of oxygen free radicals in the development of sunscald damages.

In the present work, the role of oxygen in the development of sunscald damage and characterization of processes occurring during the development of this disorder, were investigated.

MATERIALS AND METHODS

Plant Material and General Experimental Procedures

Freshly harvested large-green[1] cucumber (*Cucumis sativus* L.), cvs. Kesem and Delila (Hazera Seed Company, Haifa, Israel), were used throughout. These were deconditioned at 20°C in the dark for 24 hr, prior to any treatment. Cylindrical slices, 2.5 cm long, were placed horizontally in 35 mm diameter test tubes sealed with silicon stoppers. The laboratory temperature was maintained at 20 ± 2°C, and fruit were flushed continuously with the desired mixture of gases, starting 2 hr before any other treatment was commenced. The heat source was an infrared heat lamp with a capacity of 1 KW. Pericarp temperature measurements were made with copper-constantan thermocouples of very fine gauge.[1] Fruit temperature was regulated by adjusting the distance between the lamp and the upper surface of the fruit. Two Dicrolite lamps, 500 W each were arranged so that the light intensity at the surface of the fruit was 550 to 600 $\mu Em^{-2} sec^{-1}$. Preliminary experiments under ambient atmosphere, showed typical sunscald symptoms and total bleaching already after 7 hr of exposure to heat (48°C) and light.

Damage Evaluation:

At the end of each experiment, fruit were graded, according to their appearance, as follows:

No injury. The fruit slices had normal appearance and could not be visually distinguished from the non-treated controls.

Heat injury. The tissue turned green-brown or olive-brown, and the upper surface became sunken and dry.

Sunscald injury. Bleaching and necrosis were the most common symptoms, and the upper surface became sunken and dry.

Electrolyte Leakage — EC Measurements

Electrical conductivity (EC) measurements and calculations were according to Marsh *et al.*,[13] with some modifications. At the end of the experiment, 1 mm thick skin disks (5 mm diameter) were thoroughly rinsed with distilled water, placed in test tubes containing 4 ml distilled water, and shaken for 1 hr in the dark. The initial electrical conductivity was measured using conductivity-meter model CDM 83 (Radiometer

A/S Copenhagen). The disks were then stored for 48 h at −70°C, then thawed and EC was read again. The latter reflecting the entire electrolyte content of the tissue. Leakage was calculated as the percentage of the initial value from the final value.

Chlorophyll Fluoresence from skin samples was measured immediately when light was turned on (F_0) and after 2 sec illumination (F_{max})[14]. F_{max} is the maximal value of the fluorescent signal emitted from illuminated chlorophyll. F_v is the difference between the two readings.

Plastid pigments conc was determined according to[15,16] and *lipid peroxidation* was assessed using the thiobarbituric acid (TBA) test.[17]

RESULTS

Green cucumber fruit suffered typical sunscald symptoms only when heat, light and oxygen were combined. Heat damage was observed when tissues were exposed to oxygen-free atmosphere, regardless of the illumination treatment. Chlorophyll content was reduced to ca 10 and 50%, respectively, and carotenoids bleached only in fruit exposed to heat, light and oxygen (Table I). The significant interactions between the three environmental factors on chlorophyll content in the cucumber skin, are detailed in Table II and the multiple range test is described in Figure 1. Heated tissues suffered a significant decrease in chlorophyll content only in the presence of light and oxygen (Figure 1a). The combined effect of heat and light is markedly dependent on the gaseous composition in the atmosphere: In the light, chlorophyll content of

TABLE I
Effect of sunscald treatment on chlorophyll and carotenoid pigments and on visible symptoms in cucumber fruit. Values are means ± SD, $n = 6$

Treatment			Chloroplast Pigments [mg/10 gr fresh weight]		External
Heat	Light	Oxygen	Chlorophyll	Carotenoids	appearance
+	+	+	5.7 ± 1.0	1.3 ± 0.5	Sunscald
+	+	−	34.4 ± 3.2	21.0 ± 2.7	Heat injury
+	−	+	73.4 ± 4.9	23.4 ± 4.7	No damage
+	−	−	38.0 ± 1.8	20.3 ± 1.8	Heat injury
−	+	+	52.0 ± 6.3	21.0 ± 5.2	No damage
−	+	−	76.4 ± 11.9	21.3 ± 3.0	No damage
−	−	+	65.0 ± 5.0	20.9 ± 4.3	No damage
−	−	−	59.3 ± 6.6	25.9 ± 3.8	No damage

TABLE II
Significance levels for main effects and interactions between heat, light and oxygen treatments

Source of variance	$F_{(1\ d.f.)}$	$P > F$
Heat	114.40	0.001
Oxygen	4.21	0.044
Light	2.38	0.013
Heat * Oxygen	13.32	0.001
Heat * light	4.49	0.037
Oxygen * Light	5.92	0.017
Heat * Oxygen * Light	7.80	0.007

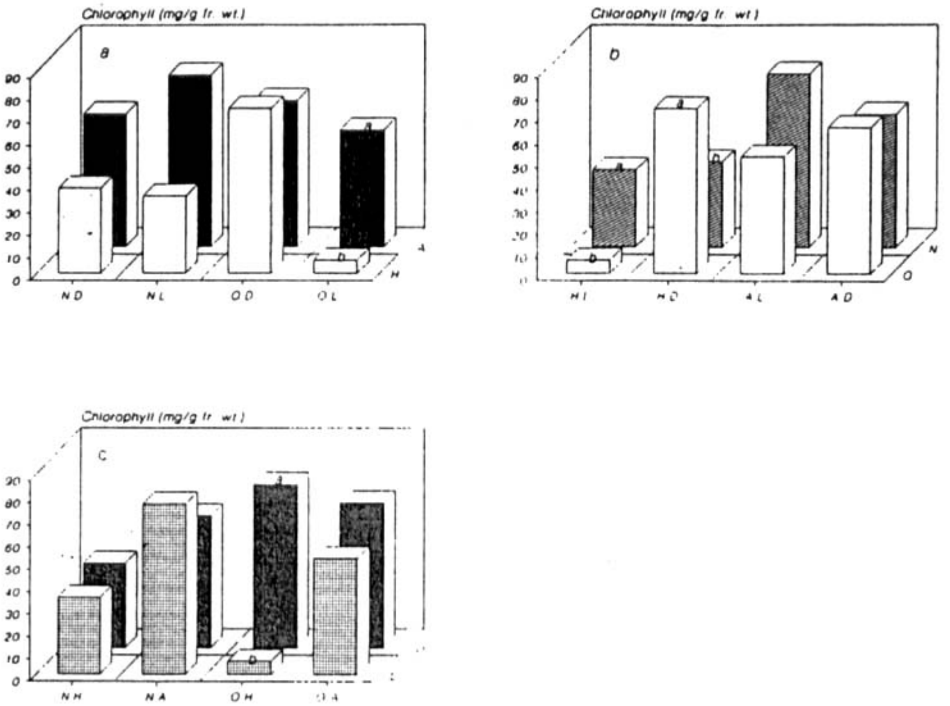

FIGURE 1 Effect of heat, light and oxygen on chlorophyll degradation in cucumber fruit. A) Effect of temperature; B) Effect of oxygen; C) Effect of light. Values are means of 9 replications. Means followed by the same letters are not significantly different at $P \leqslant 0.05$. Abbreviations; A = 20°C; H = 48°C; D = dark; L = light; O = 21% O_2 in ambient atmosphere; N = nitrogen atmosphere.

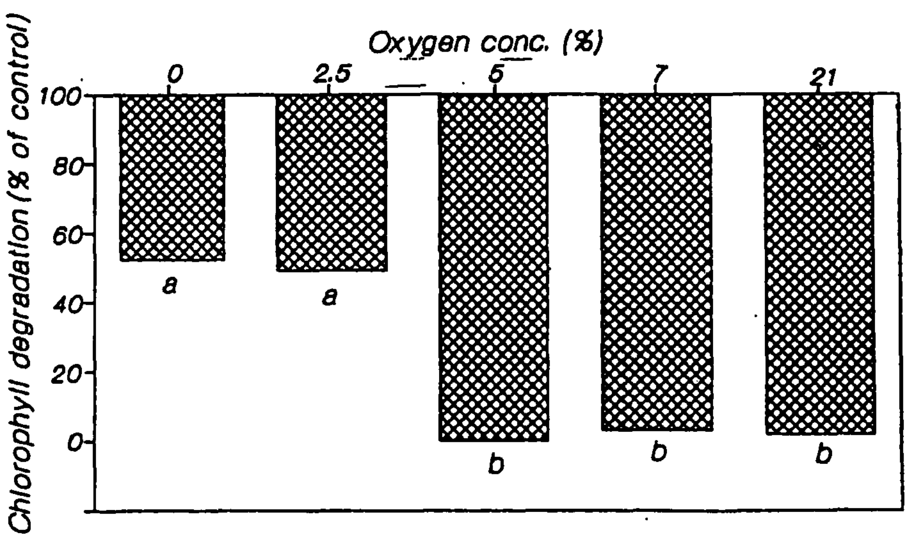

FIGURE 2 Effect of oxygen concentration on chlorophyll degradation in cucumber fruit exposed to heat and light. Values are means of 8 replications. Means followed by the same letters are not significantly different at $P \leqslant 0.05$.

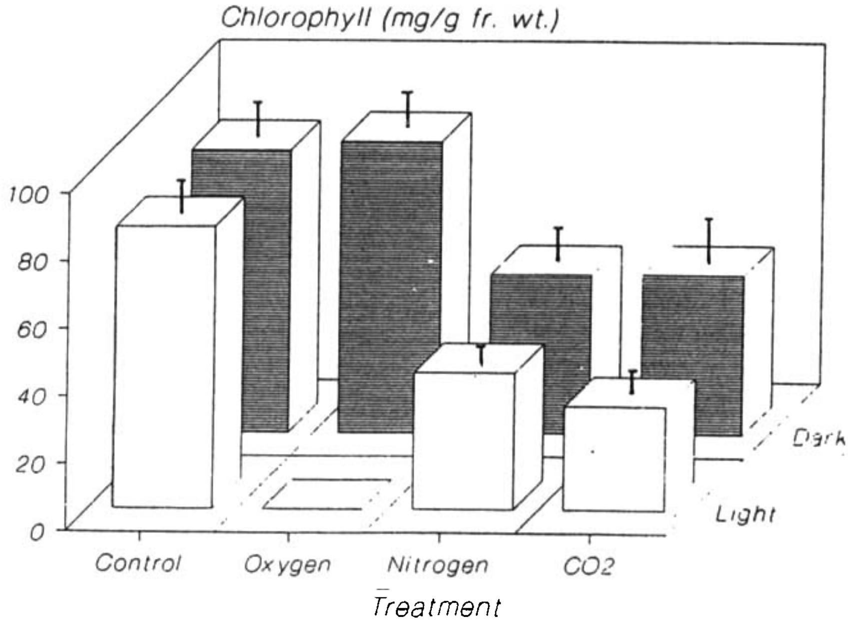

FIGURE 3 Effect of 1% CO_2 in nitrogen, heat and light on bleaching of cucumber fruit.

aerated cucumbers was significantly lower than that extracted from fruit flushed with nitrogen. In the dark, the former suffered no chlorophyll breakdown, the latter fruit contained only ca 50% chlorophyll of the control (Figure 1b). The damaging effect of light is significant only in fruit exposed to heat and O_2 (Figure 1c).

Typical sunscald damages were observed only in fruit exposed to oxygen at conc. of 5% and above. At lower levels, the visual symptoms resembled heat damage, and chlorophyll content was ca 50% of the unheated control (Figure 2).

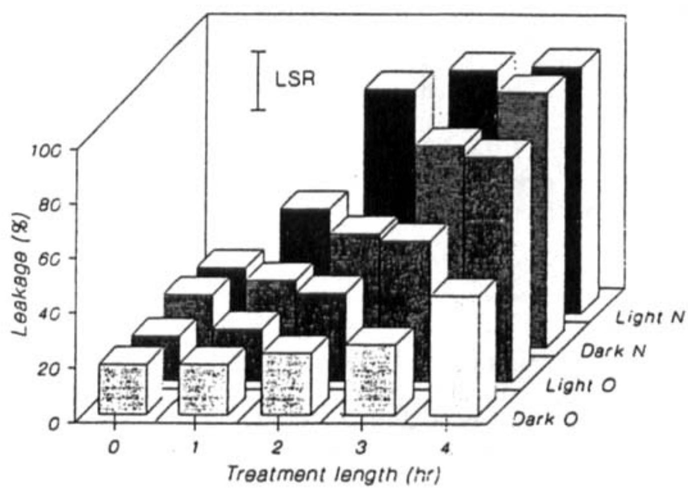

FIGURE 4 Effect of heat, light and oxygen on electrolyte leakage from cucumber fruit. Values are means of 4 replications.

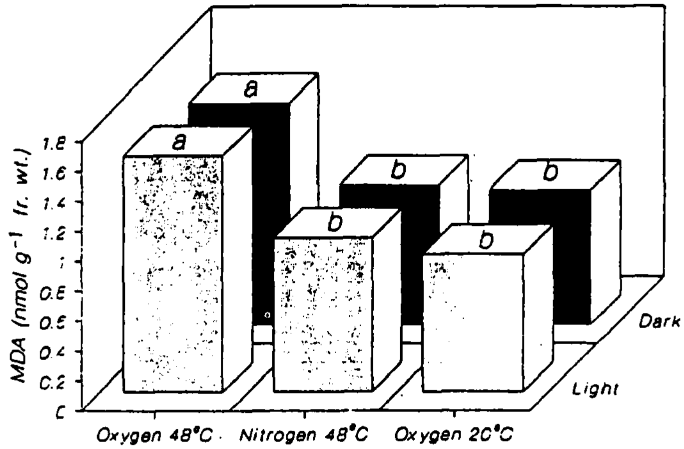

FIGURE 5 Effect of heat, light and oxygen on the production of malondialdehyde in cucumber fruit. Values are means of 5 replications. Means followed by the same letter are not significantly different at $P \leqslant 0.05$.

When compared to anaerobic atmosphere, 1% CO_2 in nitrogen caused a further decrease in chlorophyll content to heat and light treated tissues. In both treatments, however, the amount of extractable chlorophyll was considerably higher than that in cucumbers flushed with air (Figure 3).

The term "heat killing time" (HKT) was coined by Inaba and Crandall[18] to describe the effect of high temperatures on the time required for 50% electrolyte leakage. There was ca 20% electrolyte leakage from sliced untreated cucumbers. When exposed to heat, nitrogen and to light or dark, HKT values were 77 and 135 min, respectively. Heat, oxygen and light treatment yielded HKT values of 175 min. In the dark, however, only 40% leakage was obtained after 240 min treatment (Figure 4).

Lipid peroxidation, as measured by the TBA test, was significantly higher in heated tissues at ambient atmosphere than in both, heated cucumbers flushed with nitrogen or fruit at 20°C, and light had no effect (Figure 5).

Fluorescence emission from cucumber fruit treated with heat and oxygen in the dark was significantly higher than those measured at all other environmental combinations. These results indicate a more pronouned damage to the light harvesting system by the latter treatments as compared to the former (Figure 6).

DISCUSSION

The involvement of oxygen in the development of sunscald disorder has not yet been clarified. Adegoroye and Jolliffe[9] had not ruled out a role for oxygen in tomato sunscald but stated that the O_2 conc during radiation treatments had little influence on fruit response. In their experiments, exocarp at 0% O_2 became grayish rather than whitish as it did at higher O_2 conc. The former resembles our observations of heat damage rather than sunscald *per se*. On the other hand, tolerance to sunscald damage in tomatoes and peppers was correlated with the level of SOD activity[10-12], and leaf

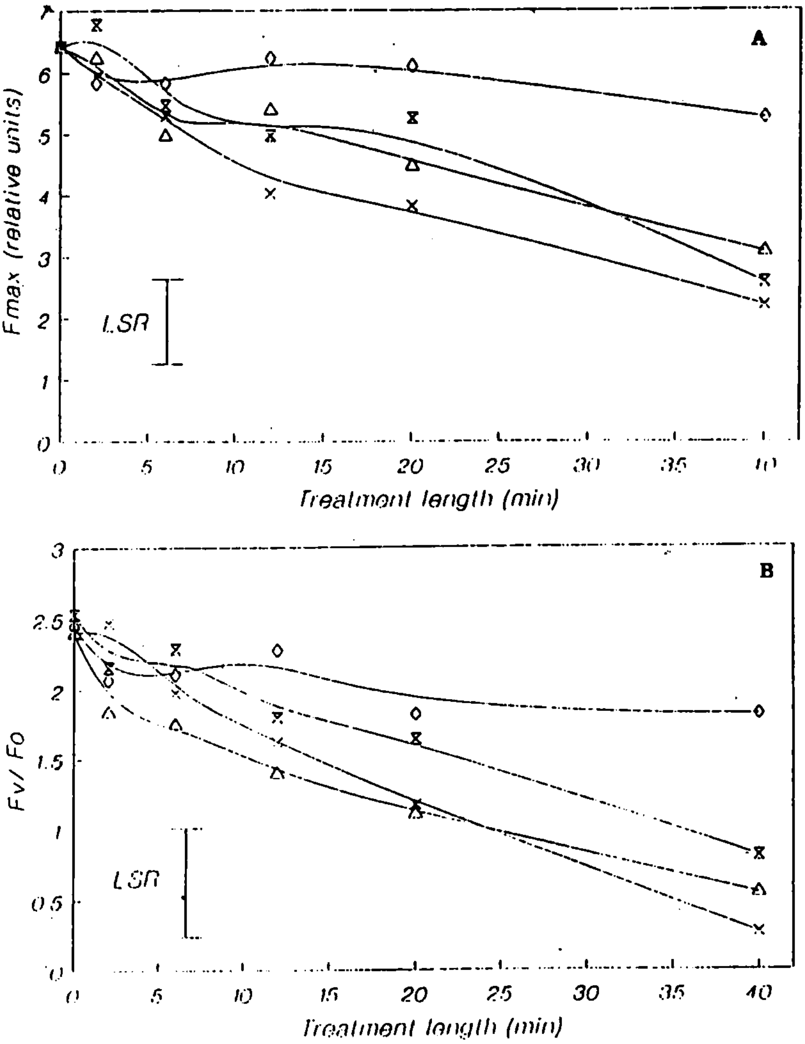

FIGURE 6 Effect of heat, light and oxygen on chlorophyll fluorescence of the green skin of cucumber fruit. A) Maximum fluorescence (F_{max}); B) Variable fluorescence/initial fluorescence (F_v/F_0). Values are means of 3 replications. Chlorophyll conc remained unchanged during the first 40 min of the experiment. The initial values ranged between 5 to 6 mg chlorophyll per 1 gr skin fresh weight.

bleaching caused by short exposure to high temperatures, occurred only in the presence of oxygen.[8] In the present experiments, we have provided unequivocal evidence that typical sunscald symptoms and destruction of the main plastid pigments occur only in tissues exposed to heat, to light and to oxygen at conc above a threshold of 2.5%. We therefore conclude that oxygen is essential for the development of sunscald in green tissues and that the disorder results from thermo-photooxidative processes.[19]

Chlorophyll fluorescence is used as an indicator of the soundness of the photsynthetic apparatus.[20] We have measured a significant decrease in fluorescence emission from cucumbers treated with heat, light and oxygen, as compared with the respective dark treated tissues. When the photosynthetic system is disturbed, electron leakage onto oxygen occurs, and superoxide and other oxygen free radicals are generated.[21-23] The lipids present in the chloroplast envelope, and the thylakoids which contain high percentage of polyunsaturated fatty acids, are very susceptible to attack by oxygen free radicals,[22] thus resulting in damaged membranes. Indeed, the level of malondialdehyde was found to be significantly higher in the presence of oxygen as compared with that measured under the respective anaerobic conditions. Anaerobic conditions, can also lead to membrane disruption.[7] Damage to membranes facilitates electrolyte leakage,[18] disturbs inner cell compartmentalization, and enhances the enzymic degredation of chlorophyll. We have measured a significant increase in electrolyte leakage both under aerobic conditions plus light and in nitrogen atmosphere. Chlorophyll degradation was observed in both cases, but total chlorophyll and carotenoids bleaching occurred only in the presence of oxygen and light, when oxygen free radicals are generated. Hence, our previous findings that SOD provides protection.[10-12]

The sequence of events leading to the development of sunscald damage can now be summarized as follows: When exposed to high temperature, above 40 to 42°C, to light and to oxygen, the photosynthetic system is disturbed and oxygen free radicals are generated. These interact with pigments, lipids and other cell components, thus causing bleaching and death of the treated tissue, the very symptoms occurring in tissues suffering from sunscald damage.

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