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The Phenomenon of Photoinhibition of Photosynthesis and Its Importance in Reforestation

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I. Abstract

Photoinhibition, defined as the inhibition of photosynthesis caused by excessive radiance, affects field production to a great extent. This phenomenon is particularly relevant in reforestation practices, when one deals with forests of rapid growth such as *Eucalyptus*. The imposition of additional stress factors during exposure to high radiance increases the potential for photoinhibitory effects, so the inhibition of photosynthesis indicates that the plant is submitted to stressful conditions. Photoinhibition can be reversible, playing a protective role for the photosynthetic systems, but it can also reflect damage that has already occurred in the photosynthetic apparatus, being irreversible in this case. In this review, we present the physiological and molecular mechanisms of photoinhibition and discuss the interaction between light and other stress factors and its effects on plants destined for reforestation. In addition, the present work analyzes some of the features and strategies that help plants avoid or restrict the occurrence of photoinhibition. For instance, pigments and enzymes which naturally occur in plants can prevent photoinhibition, while preadaptation to nonideal conditions can enhance tolerance to a certain stress factor. Most of these morphological, metabolic, and biochemical mechanisms of defense are related to the dissipation of excessive energy such as heat. Understanding these mechanisms can help improve cultivation procedures, avoid the plants' death, and increase productivity in the field.

II. Introduction

The growing need for wood and cellulose to meet the demand of the world market increases society's pressure on the forest sector. This is expected, for most wood is extracted from native forest reserves. Unplanned extraction of the forest cover in these areas may put the regional balance at risk. Hence, Poggiani (1989) concluded that the only way to preserve and maintain the ecosystems is to intensify the implantation of homogeneous forests of rapid growth, mainly with the species *Pinus* and *Eucalyptus*.

According to the Forest Resources Assessment homepage of the Food and Agriculture Organization of the United Nations, the world forest cover is about 3.85 billion ha (2000 estimate), producing nearly 1.7 billion cubic meters of round wood. Conifers are approximately 1.14 billion cubic meters; the remainder are leafy (FAO, 1981). In 1987, the total area planted with eucalyptus was estimated at 6 million ha (Eldridge & Cromer, 1987).

The most important species of eucalyptus in forest plantations in the world, from a total of 244 species, include, in terms of average annual increment of wood: *Eucalyptus grandis, E. camaldulensis, E. tereticornis, E. globulus, E. viminalis, E. saligna, E. urophylla, E. deglupta, E. exserta, E. citriodora, E. paniculata,* and *E. robusta* (Eldridge & Cromer, 1987). All these species belong to the subgenus Symphyomyrtus, except for *E. citriodora,* which belongs to the subgenus Corymbia. Recently, hybrids (especially *E. urograndis,* the *E. grandis* x *E. urophylla* hybrid) have received special attention from reforestation companies.

Eucalyptus plantations have been created from seedlings produced in seedbeds, as direct sowing in the field is subject to special weather conditions and soil preparation (Aguiar & Mello, 1974). Hence, seeds are sown in special containers, normally small plastic tubes, in which the resultant seedlings are cultivated until they are ready for transplanting. Some seedlings are obtained from vegetative propagation techniques, mainly from stake rooting and micropropagation.

Seedlings are considered in proper condition to be planted when they are healthy and resistant enough to survive adverse environmental conditions (Reis et al., 1988). Usually, the selection of seedlings is made by biometric characteristics (height, number of leaves and branches,

and stem diameter) and by the color of the leaves (green or red). Nowadays, great value is placed on the photosynthetic efficiency of the seedling, which shows its ability to regain growth after transplantation and to maintain it when adult (Inoue & Oda, 1988; Barja et al., 2001).

Cultivation of eucalyptus comprises an initial growing of seedlings under shade conditions, and to this purpose one generally uses black polyolephin screens (shading screen) with different levels of sunlight blocking. Some years ago this shading lasted for about 70–80 days after sowing, with alternating periods of coverage when it was hotter or colder (Gomes et al., 1979). At present this coverage period is restricted to the first 40 days after cultivation, using screens with lower interception (30–50%) and keeping the crop rotation in the coverage periods. After this phase, seedlings are kept in full sunlight. The main functions of such shading screens are the maintenance of the humidity in the substratum and protection against external agents, such as birds, insects, wind, or hail (Pereira et al., 1980).

In the growing phase of the seedlings in the seedbed, besides the reduced volume of substratum (50 ml), manipulation of the material may cause stresses (as water and nutritional deficiencies) to the plants. Water stress is clearly observable when the shading screens are removed and seedlings are exposed to full sunlight until transplant time (Reis et al., 1988). On the other hand, 70 days after sowing, daily fertilization (with a nutritive solution) is interrupted, leading the plants to nutritional stress. These procedures are designed to harden the seedlings, but they may increase photoinhibition (Smith, 1982; Offler et al., 1983).

III. The Phenomenon of Photoinhibition of Photosynthesis

Photoinhibition has been defined as the inhibition of photosynthesis caused by excessive radiance; it may damage the photosynthetic apparatus, causing the (photo)destruction of the photosynthesizing pigments (Powles, 1984). However, recently, the term photoinhibition has been also used to define a slow and reversible reduction of the photosynthetic efficiency that depends on the irradiation and leads to a partial loss of capacity to convert radiant energy into dry material and, consequently, into growth (Long et al., 1994; Krause et al., 1995; Laing et al., 1995). Osmond (1994) named the first type "chronic photoinhibition" and the latter, "dynamic photoinhibition."

The natural evolution of plants has followed a path between maximizing the capture of light to enhance photosynthesis and minimizing the potential damage that results from excess light in the photosynthetic apparatus (Long et al., 1994). Light differs from other climatic elements in nature by the amplitude and rate of its variation. During the day, plants face many alterations in the quality and quantity of the radiation received. Plants can respond to low-frequency variations in quality and quantity of light by adapting their photosynthetic ability to disperse the intercepted radiation (Anderson et al., 1988; Osmond & Chow, 1988). When high-frequency variations occur, or when plants are not able to adapt themselves to the prevailing light conditions, there may be a surplus of stimulation in the photosynthetic apparatus, which can lead to photoinhibition (Long et al., 1994). In nature, plants have different responses to light surplus, which can be ranked between damage and nondamage, and these responses appear in different time scales (Osmond & Chow, 1988; Osmond, 1994).

Photoinhibition can be caused by ultraviolet light (UV), by visible light (V) and by the interaction UV–V (Powles, 1984). The use of the quantum absorbed by the leaf results in a hyperbolic response of photosynthesis to light (Farquhar et al., 1980). Under low-intensity light (less than 100 μmol.m^{-2}s^{-1}), more than 80% of the absorbed quantum can be used in photosynthesis, according to the maximum quantum efficiency in releasing O_2 (Björkman & Demmig-Adams, 1987); when light intensity approaches 1000 μmol.m^{-2}s^{-1} (50% of the full

sunlight value), less than 25% of the absorbed quantum is used; and, under full sunlight, utilization decreases to 10% (Long et al., 1994).

Several terms have been used to designate the reduction of photosynthetic capacity induced by the exposure of organisms, structures, or leaves to an excess of visible light. Some of these terms are: "photoinhibition," "photooxidation," "photoinactivation," "photolability," "solarization," and "photodynamic reactions" (Powles, 1984; Krause, 1988). With the relevance that the study of this phenomenon has acquired in the last 20 years, the term "photoinhibition" has been used more frequently (Krause, 1988). The same term has also been used as a synonym for the damage caused to photosystem II (PS II), but we should emphasize that this damage to PS II is considered photoinhibition when there is a decrease in the whole photosynthetic capacity and not in just one component of the photosynthetic apparatus (Long et al., 1994).

In practice, the consequences of photoinhibition are: reduced maximum quantum efficiency for CO_2 absorption (Φ) and release of O_2 (Φ_0); decreased convexity (θ) in the response curve of photosynthesis to light; reduced photochemical activity of PS II (Fv/Fm, ratio of fluorescence of chlorophyll *a*); and, with long exposure to excess light, decreased maximum photosynthetic rate (PS_{max}) (Boese & Huner, 1992; Long et al., 1994). The decreases of Φ, Φ_0, and Fv/Fm were noticed preceding reduction in PS_{max}; actually, they can occur without causing any alteration in PS_{max} (Long et al., 1994).

A prolonged exposure of plants or organisms to excessive radiation may result in the photodestruction of the photosynthetic pigments, since the discoloration (bleaching) of these pigments depends on oxygen and light; this phenomenon is normally called "photooxidation," and it may cause the death of the cell or the organism (Powles, 1984; Hendrey et al., 1987).

In most cases, photooxidation is a secondary phenomenon, occurring after a slow phase during which there is already a decrease of the photosynthetic activity dependent on light intensity and exposure time, but without any changes in the pigment pool (Powles, 1984; Long et al., 1994). Therefore, photoinhibition of photosynthesis does not appear after the destruction of the pool of pigments; on the contrary, the bleaching of pigments occurs when a certain degree of photoinhibition has already occurred (Hendrey et al., 1987).

As a rule, plants adapted to full sunlight are able to acclimate and grow in shady conditions, whereas shade-grown plants may not bear full sunlight (Smith, 1982). Furthermore, sun-acclimated plants show a higher capacity not only for the use of light in photosynthesis but also for xanthophyll cycle–dependent energy dissipation (Demmig-Adams et al., 1995). The transfer of a sunlight plant cultivated under low radiance conditions to high radiance produces an enhancement in the photosynthetic capacity as the plant adapts itself to the increase of irradiance. However, leaves from these plants may show photoinhibition, with a decline in photosynthetic activity and in the quantum yield, if this transfer is abrupt. In this case, mature leaves that at first suffered photoinhibition may suffer discoloration of the photosynthesizing pigments, leading to cellular death. Young leaves, developed after transfer to high irradiance, do not exhibit photoinhibition. Plants acclimated to low irradiance and exposed to high irradiance are more severely photoinhibited than are those primarily adapted to high irradiance (Long et al., 1994). This difference shows that the photosynthetic capacity influences susceptibility to photoinhibition.

The light-harvesting complexes (antennae) must have pigments of such shape and size that they can transfer energy to reaction centers efficiently. Thus, when the plant develops in shade, there is an increase in the ratio between antenna pigments and reaction centers (Anderson & Osmond, 1987; Osmond & Chow, 1988; Horton & Ruban, 1992). The result of this adaptation is that photosynthesis saturates at low irradiances. As a consequence, under high irradiance, the absorption rate exceeds the rate that can be used for photosynthesis, predisposing the plant

to damage induced by the excessive radiation (Horton & Ruban, 1992). Cleland and Melis (1987) proved that a mutant of *Secale cereale* without the light-harvesting chlorophyll complex *a/b* was less affected by high irradiance than was the wild variety.

Under normal conditions, a considerable amount of photons is intercepted by the photosynthetic apparatus, funnel shaped to the reaction centers and transferred, via electron transport chain, to production of $NADPH_2$ and ATP (Powles, 1984). According to the accepted electron-transport scheme, there should be 8 mols of photons for a reduction of 2 mols of $NADPH^+$, which is linked to the synthesis of 2.66 mols of ATP. In C3 plants, 2 NADPH and 3 ATP are necessary to assimilate one CO_2 in carbohydrate (Krause, 1988). The main drains of this chemical energy are the cycles of photosynthetic reduction of CO_2 (PCR) and the photorespiratory carbon oxidation (PCO). In this way, a large fraction of intercepted photons is transferred to propel carbon metabolism. When this metabolism is lacking, the use of excitation energy is insignificant, even though radiation absorption remains constant; this can result in photoinhibition (Powles, 1984). Actually, photoinhibition also depends on the rate of light absorption through the leaf (Anderson & Osmond, 1987).

IV. The Mechanism of Photoinhibition

Since 1956, when Kok published his work (mentioned by Long et al., 1994), it has been proposed that the primary site of damage of photoinhibition is the reaction center of PS II. Thereafter, decreases in Φ and Φ_o have been correlated with reductions in the mutable fluorescence of PS II in vivo, usually distinguished by the relation Fv/Fm of photochemical conversion of PS II (Krause, 1988; Ögren, 1991).

There are currently two hypotheses concerning the primary site of damage of photoinhibition of PS II: the first is related to the reaction center; and the latter, to protein D1. In the first hypothesis, according to Powles (1984), transportation of electrons through PS II is inhibited when photoinhibition is induced by a surplus of light or by illumination without the recycling of carbon. Observation that the action spectrum for photoinhibition follows the action spectrum of photosynthesis, and that the initial symptom of photoinhibition is the decreased photochemical efficiency of PS II, supports the hypothesis that photoinhibition is a result of energy absorbed by photosynthetic pigments and funneled to the reaction center of PS II, modifying it (Havaux & Davaud, 1994; Long et al., 1994). This modified reaction center captures radiant energy efficiently, but it converts this captured energy into heat (Krause, 1988). The second hypothesis, which says that the initial site of damage of photoinhibition is the D1 protein, has been studied in recent years and is well accepted (Richter et al., 1990a).

Actually, research in the last 15 years has established the role of the D1 protein in the photoinhibition phenomenon. Hundal et al. (1990) verified inactivation and/or debasement of this protein of PS II associated with photoinhibition. Greer et al. (1986) and Leitsch et al. (1994) confirmed the blockage in recovery from photoinhibition by chloramphenical, an inhibitor of the D1 protein synthesis. Kuhn and Böger (1990) and Fuerst and Norman (1991) verified that herbicides that cling to the linking site of Qb in protein D1 provide protection against photoinhibition. Closure of D1 with powerful oxidizing radicals can clarify the vulnerability of this protein (Richter et al., 1990b). Working with mutants of the D1 polypeptide of *Synechocystis,* Mäenpää et al. (1995) suggested that a modification in the structure of the D-de loop of D1 could affect the mechanism of recovery from photoinhibition. Ji and Jiao (2000) showed that PS II photochemical efficiency (Fv/Fm) decreased and that nonphotochemical quenching (qN) increased in rice leaves when synthesis of the D1 protein was inhibited. In DTT(Dithiothreitol)-pretreated leaves, when xanthophyll cycle was inhibited, there was a de-

crease in qN and, consequently, more loss of the D1 protein (associated with a large decrease in Fv/Fm). They concluded that the turnover capacity for the D1 protein is an important physiological basis for tolerance of photoinhibition.

V. Photoinhibition by Interaction between Light and Other Stress Factors

Because photoinhibition involves photochemical inactivation mainly of PS II, all photosynthesizing organisms are potentially susceptible to damage under some radiation incidence. However, as mentioned above, the degree of susceptibility is influenced by several types of factors: environmental (light, temperature, water, CO_2, O_2, and soil fertility), genotypical (sun or shade plants), phenotypical (bent of the leaf), and physiological (carbon metabolism). The imposition of additional stress factors during exposure to high radiance exacerbates the adverse effects (Krause, 1988; Long et al., 1994).

A. INTERACTION BETWEEN LIGHT AND LOW CO_2

Photoinhibition is not caused by a limitation of CO_2 diffusion through the stomata, since it was verified experimentally that the intercellular CO_2 partial pressure after the photoinhibitory treatment was similar or even larger than that observed before the treatment (Powles, 1984). Wong et al. (1985) noticed that after leaves of *Eucalyptus pauciflora* had been exposed to high irradiance for up to seven hours (2000 μmol.m^{-2}s^{-1}) in a CO_2-free environment with 10 mBar O_2, at 30°C, CO_2 assimilation taxes and leaf conductance decreased in similar proportions, while intercellular CO_2 partial pressure remained constant. Finally, after working with *Glycine max,* Griffin & Luo (1999) concluded that the photosynthetic sensitivity to ambient CO_2 partial pressure was consistent with the sensitivity to intercellular CO_2 partial pressure, indicating little effect of stomatal conductance on photosynthetic sensitivity.

According to Powles and Osmond (1978), lack of CO_2 and low CO_2 partial pressure caused photoinhibition in C3 plants, depending on intensity and length of light exposure. Maintenance of a minimal partial pressure of CO_2 during the treatment prevented photoinhibition. These authors also observed that photoinhibition could be totally or greatly prevented by a certain partial pressure of O_2, but only in C3 plants. As O_2 participates of the carbon metabolism through the PCO cycle, O_2 as well as CO_2 can prevent photoinhibition, since both allow the maintenance of a low rate of carbon metabolism during the period of exposure to light (Powles, 1984).

After exposure of *Phaseolus vulgaris* leaves to low partial pressures of CO_2 (6 Pa) for 24 hours, Ishibashi et al. (1997) observed a reduction in the carbon exchange rate. According to these authors, one site of inhibition in such leaves appeared to be the intersystem electron-transport chain, since there were no significant changes in the activities of photosystem I (PS I) and PS II.

B. INTERACTION BETWEEN LIGHT AND LOW–TEMPERATURE STRESS

Exposure of several plants to cool (0–12°C) or freezing (<0°C) temperatures produces adverse effects on their metabolic functions. Photosynthesis is one of the first processes to suffer these effects. A remarkable characteristic is that symptoms of low-temperature damage in the photosynthetic apparatus are particularly sharp when substantial light intensity follows exposure to low temperature. Long-term treatment results in photooxidation of the pigments (Hendrey et al., 1987).

There is a clear distinction between the response of plants to cool temperature in darkness and under light (Long, 1983). Cool temperatures in darkness have little effect on photosynthesis, especially in the PS II complex (Yakir et al., 1985; Öquist & Huner, 1991; Ottander et al., 1993).

Recent research indicates that, in chill-sensitive plants, the main site of photoinhibition at low temperatures is PS I (Sonoike, 1999). Hydroxyl-radical, formed by reaction between hydrogen peroxide and reduced FeS centers, seems to destroy the FeS centers and a PS I reaction center subunit, PsaB protein (Terashima et al., 1998).

Usually, species that are sensitive to cool temperatures have a tendency to exhibit photochemical damage (photoinhibition of photosynthesis) when they are exposed to high radiance under low temperatures (Greer & Laing, 1992). On the other hand, species that accept the cooling process are apparently much less sensitive (Saltveit, 1991; Ögren & Evans, 1992; Krause, 1994; Long et al., 1994). Photoinhibition of Φ_o and Fv/Fm occurred under freezing temperatures in natural stands of *Eucalyptus pauciflora* seedlings (Ball et al., 1991) and in *Ilex aquifolium* trees (Groom et al., 1991).

Alves (1998) asserted that *Eucalyptus grandis* showed a larger photosynthetic rate than *E. urophylla* when exposed to irradiances of 500–1000 μmol.m^{-2}s^{-1}. However, both species were unable to recover their normal photosynthetic rate after being exposed for 15 minutes to irradiances above 2000 μmol.m^{-2}s^{-1}, thus showing photoinhibition. This phenomenon was irreversible up to two hours after the photoinhibitory treatment and was more evident in *E. urophylla,* but there was no differentiation among plants preadapted at 10°C and 25°C.

Besides the difference of sensitivity to photoinhibition between species, differences among hybrids of an equal species have also been reported. Greer and Hardacre (1989) noticed that the CT hybrid of *Zea mays* was more susceptible to photoinhibition than the CBD hybrid, but the dependence of photoinhibition on temperature was stronger in the latter.

The temperature at which the plant was preadapted (thermal report) affects its susceptibility to a subsequent cooling. Therefore, the growth and development of plants in low temperatures gives them better tolerance of photoinhibition in low temperatures (Greer & Hardacre, 1989; Somersalo & Krause, 1989; Saltveit, 1991; Boese & Huner, 1992). Boese and Huner (1992) mentioned that, besides the thermal report of plants, the developing phase of leaves contributes to the response of *Spinacia oleracea* in vivo to photoinhibition.

The thermal report commands changes in thylakoids during the growing and developing phase of plants in low temperatures. Huner et al. (1987, 1989) and Krupa et al. (1987) mentioned that the development of *Secale cereale* at a low hardening temperature resulted in specific organizational changes in the light-harvesting complex of PS II (LHC II). Electronic microscopy in situ with cold severance showed that development at a low temperature resulted in a smaller stack of *grana* and in a reduction in the size of PS II antenna particles.

Most photoinhibition studies use high radiance rates for a short exposure period. Up to now, the interactive effect of cool temperatures and moderate irradiances in the photosynthetic activity has not been fully elucidated. Yakir et al. (1985) verified that cool temperatures of 5°C and 10°C during five days under moderate radiation (400 μmol.m^{-2}s^{-1}) resulted in an expressive decrease in Fv/Fm, Φ_o and in the total amount of energy chemically stored, without affecting the concentration of RubisCO in plants of *Lycopersicon esculentum*. Venema et al. (2000) subjected two *Lycopersicon* species to 1000 μmol m^{-2} s^{-1} at 5°C for two days. They reported that degradation of leaf pigments was slower in plants grown at suboptimal temperatures than in plants grown at optimal temperatures. Nonphotochemical quenching of Chl fluorescence was higher in leaves of suboptimally grown plants, which presented a larger pool of xanthophyll cycle pigments. These results show that acclimation to suboptimal temperature increased the capacity to resist chill-induced photodamage.

According to Ottander et al. (1993), there are four hypotheses to explain why plants become much more sensitive to photoinhibition at low temperatures: low temperatures can reduce photosynthetic capacity and therefore increase the probability of a surplus in stimulus of

PS II; the restoration capacity of PS II is reduced at low temperatures (degradation and synthesis of protein D1 in the reaction centers seem to diminish); the capacity of the oxygen seizers, which can provide protection against photoinhibition, decreases at low temperatures; and the ability to create zeaxanthin, which is capable of dissipating the excessive energy in the LHC II, may be inhibited at low temperatures. We emphasize that these hypotheses should not be considered mutually exclusive.

C. INTERACTION BETWEEN LIGHT AND HIGH–TEMPERATURE STRESS

The combination of high temperature and full sunlight is an ordinary occurrence that characterizes summer conditions in most parts of the world. Photoinhibition of photosynthesis in situ may happen in full sunlight, even in the absence of any other stressing factor, despite the occurrence of a high leaf temperature (Powles, 1984). When the temperature rises above the optimum level, photosynthesis begins to decrease. At first the decline is gradual and reversible, but after a critical temperature is reached it becomes slow and irreversible (Berry & Björkman, 1980). According to these authors, reversible inactivation of photosynthesis reflects damage to the chloroplasts that persists for some time after the plant comes back to favorable temperature conditions.

Ögren (1988) and Ögren and Rosenquist (1992) verified the occurrence of photoinhibition under full sunlight in the leaves of *Salix* sp. Adams (1988) verified it for several CAM plants; and Laing et al. (1995), for *Phaseolus vulgaris*. These authors noticed an inhibition of 25% in the vegetative growth rate of *P. vulgaris* plants acclimated to 25°C at 1300 μmol.m^{-2}s^{-1}; the inhibitory effect was stronger when these plants were compared with plants developed at (or transferred to) 10°C. Ögren and Evans (1992) observed a reduction of 30% in the Fv/Fm of six species of eucalyptus (*Eucalyptus camaldulenses, E. camphora, E. globulus, E. largiflores, E. melliodora,* and *E. sidiroxylon*) in the field, under high light and high temperature conditions. This effect was more evident on horizontally disposed leaves (40% of inhibition).

Little is known about photoinhibition of photosynthesis in the rain forest. In this case, leaves are supposed to dissipate excess energy under full sunlight; on the other hand, they must be able to photosynthesize efficiently under low intensity of light during long stretches of shade when it is cloudy. Krause et al. (1995) studied the effect of photoinhibition of Fv/Fm and Φ_0 in both young and mature leaves of six species of plants developed in a rain forest in Panama. They noticed a high degree of photoinhibition in the young leaves. On the other hand, these leaves recovered better from photoinhibition because of their larger content of α-carotene and zeaxanthin (to a smaller content of total chlorophyll).

Contrary to what happens at low temperatures, exposure to light during a short treatment at high temperature is beneficial to plants, diminishing the damage to the photosynthetic apparatus. Schreiber & Berry (1977) mentioned that this protective effect depended on the intensity and spectral characteristics of light, being saturated at low light levels (about 125 μmol.m^{-2}s^{-1}). Havaux et al. (1991) verified that previous exposure of *Pisum sativum* leaves at 40°C for a short period, in darkness, inhibited photosynthetic O_2 evolution and PS II fluorescence. However, no alteration was observed in these parameters when the plant was illuminated (30 W.m^{-2}) during the thermal treatment. This protective effect induced by a moderate irradiance was also observed for *Solanum tuberosum* leaves (Havaux, 1994).

Inside the photosynthetic apparatus, PS II seems to be more sensible to heat stress than PS I, as long as the activities of PS I, the stroma enzymes, and the chloroplast envelope are more thermostable (Havaux et al., 1991). Thus, the increase in temperature may initially cause a blockage in the reaction centers of PS II, followed by the dissociation of the complex protein

pigments of the antenna nucleus in the LHC II (Armond et al., 1978). According to Gounaris et al. (1983), this dissociation can be related to the disconnection of lipids phases that create a simple layer in the thylakoid membranes, or it can be a consequence of the reactions between O_2 and H_2O_2 derived from oxidative stress, which cause enzyme inactivation, pigment discoloration, lipid peroxidation, and proteolysis (Pastori & Trippi, 1993).

Similar to what happens in low-temperature stress, the thermal conditions preceding exposure of the plant to high temperatures influence the photoinhibitory effects. Smillie and Hetherington (1983) verified that plants with decreasing degrees of adaptation to cold, such as *Pisum sativum, Cajanus cajan, Triticum aestivum, Arachis hypogea, Pennisetum* sp., and *Carica papaya* showed, in this same order, a gradual increase of tolerance to the treatment of 44°C for 10 minutes. Tolerance was evaluated by the fluorescence of chlorophyll *a* in vivo. Havaux and Tardy (1996) noticed (using photoacoustic spectroscopy and fluorometry) that previous treatment of *Lycopersicon esculentum* leaves at 35°C for two hours induced a rapid increase in heat and tolerance of PS II to light and, also, to temperatures above 40°C. These authors confirmed that a short exposure of *L. esculentum* leaves to a radiance of 1000 μmol.m^{-2}s^{-1} during four minutes significantly increased the thermostability of PS II, as evaluated by subsequent measurements of fluorescence as a function of temperature.

D. INTERACTION BETWEEN LIGHT AND WATER STRESS (DROUGHT)

Besides being often associated with heat stress, high light conditions are frequently combined with water deficit in the field; actually, water stress is generally connected with heat stress. Water stress usually affects both stomatal conductance and photosynthetic activity in the leaf (Taiz & Zeiger, 1998). Therefore, the effect of water stress on photosynthesis has a stomatic component (restricted availability of CO_2) as well as a nonstomatic component (direct inhibition of photosynthesis). As water stress frequently occurs during summer conditions of light and temperature, the potential occurrence of photoinhibition and photooxidation is particularly evident in this season (Powles, 1984).

Several works demonstrate the photoinhibitory effect of the interaction between light and drought. Working with ecotypes of sun and shade of *Solanum dulcamara,* Gauhl (1979) noticed that some shade ecotypes grew and photosynthesized very well under conditions of full sun when they were normally irrigated, but with a simultaneous imposition of moderate water stress they quickly showed signs of photoinhibition. For other ecotypes, which suffered photoinhibition after being transferred to full sun, imposition of water stress emphasized the adverse effects of light. According to Osmond (1983), these results may have been consequence of nutritional stress, which interacted with the water stress.

Wong et al. (1985) pointed out that water stress and photoinhibitory treatment reduced the photosynthetic metabolism of *Eucalyptus pauciflora* leaves, with significant alterations in the partial pressure of intercellular CO_2. Munne-Bosch and Alegre (2000) showed that the efficiency of PS II photochemistry decreased to approximately 65% in plants exposed to the interaction of high light and drought. On the other hand, Havaux (1992) observed, for several plant species, that imposition of water stress enhanced the resistance of PS II to otherwise photoinhibitory conditions, such as heat or high light.

E. PHOTOINHIBITORY PRINCIPLES OF HERBICIDES

As previously mentioned, environmental stress factors affect plants mainly by altering the susceptibility of their photosynthetic apparatus to photoinhibition or photooxidation. The use of several herbicides causes direct inhibition of the photosynthesis and, when plants are ex-

posed to light, there is the photooxidative destruction of the photosynthetic pigments. Three groups of herbicides act directly on photosynthesis: inhibitors of electron transport, with active principles related to the reaction centers of PS II; blockers of electron transport before the acceptor of PS II, promoting the production of oxygen in the excited singlet state (O_2^-), with its consequent effects on PS I; and inhibitors of the synthesis of carotenoids inside the photosynthetic apparatus in motion, thus canceling its protective effect (Powles, 1984). The presence of oxygen is fundamental to the occurrence of photochemical damage induced by the herbicides of these three groups (Kenyon & Duke, 1985).

VI. Recovery from Photoinhibition of PS II

Studies on the recovery from photoinhibition established the role of the loss of the D1 protein in photoinhibition. These works showed that it was necessary to resynthesize the D1 protein in chloroplasts to achieve restoration of PS II activity (Greer et al., 1986; Aro et al., 1993; Leitsch et al., 1994).

Several works report that photoinhibition of PS II is inversely proportional to the phosphorilation level of PS II proteins. Giardi et al. (1994) verified that phosphorilation of the light-harvesting complex of *Spinacea oleracea* protected it against photoinhibition damage. While phosphorilation of polipeptides accelerated the fall of the electron transfer during high-radiance treatment, their dephosphorilation increased the susceptibility of the thylakoids to the photoinhibitory treatment. Rintamaki et al. (1995) confirmed that the degradation of protein D1 of *Cucurbita pepo* subjected to photoinhibitory treatment was followed by the accumulation of D1 in the phosphorilated configuration. This led the authors to suggest the involvement of phosphorilation in the regulation of protein D1 degradation, and therefore, in the whole redress cycle of PS II. The maximum rate of restoration occurs after the transition from high to low radiance. From this procedure and using inhibitors of protein synthesis, the conclusion is that the redressing of PS II by resynthesis of D1 occurs intermittently also during the photoinhibitory treatment, and that photoinhibition only happens when the inactivation rate or degradation of protein exceeds its restoration rate (Krause, 1988) and not only by a decrease in the consumption of photosynthetic energy (Greer et al., 1986).

VII. Mechanisms for Avoiding or Tolerating Photoinhibition

A. BIOPHYSICAL MECHANISMS

The excess energy of stimulation in the reaction center can be dispersed by various means. A fraction of the energy can be dispersed nondestructively by fluorescence or by heat emission; as fluorescence is very limited as a quantitative disperser, heat emission can dissipate a considerable surplus of energy (Krause, 1988).

Apart from photosynthesis, fluorescence, and heat emission, Butler (1978) presents in his revision alternative ways for dispersion and distribution of energy inside the photosynthetic apparatus. These include the potential for spillover of the stimulus energy of PS I, and transitions condition 2–condition 3, in which the fluorescence of PS II decreases without any concomitant increase of the fluorescence of PS I, showing a low transference of energy from the antenna to the reaction center.

Carotenoids observed in the thylakoidal membranes act as a protective system, since they abduct or prevent the formation of radicals or other reactive molecular species, especially those derived from oxygen (Demmig-Adams et al., 1987; Krause, 1988). Moreover, these pigments annul the chlorophyll triplet, stimulate the transition of oxygen singlets into triplets,

and disperse the absorbed energy as heat (Powles, 1984; Adams et al., 1990; Long et al., 1994; Verhoeven et al., 1996).

Analyzing a wild-type strain and two xanthophyll-cycle mutants of *Chlamydomonas reinhardtii* after illumination in the presence of chloramphenicol, Jahns et al. (2000) showed that PS II inactivation was less pronounced in the mutant, which was unable to form violaxanthin from zeaxanthin and thus contained high amounts of zeaxanthin even in low light. This was paralleled by much slower degradation of the D1 protein, supporting a protective role for zeaxanthin against photoinhibition.

B. METABOLIC MECHANISMS

The fastest response to a light surplus is the increase in the thermal dissipation in the antenna and/or the reaction center of PS II, associated with the development of a transthylakoidal ΔpH (Walters & Horton, 1993). This alteration in ΔpH may cause aggregation of the protein/chlorophyll molecules that constitute the LHC II, leading to an increased thermal dissipation in the antenna. In addition, alteration of ΔpH can result in a reversible inactivation of the reaction center of PS II by loss of Ca^{+2} and can increase the thermal dissipation at the reaction-center level (Long et al., 1994).

Oxygen can be reduced by Mehler reaction to produce hydrogen peroxide. Molecular oxygen can accept electrons in the photosynthetic electron transport, generating deleterious radicals by the monovalent reduction, which can occur in the reductor side of PS I (Powles, 1984; Richter et al., 1990b). This capture of electrons by oxygen is favored under high radiance, when the reduction rate of $NADP^+$ highly exceeds the demand of NADP for fixation of CO_2, thus leading to an accumulation of reduced components in the electron-transport chain (Richter et al., 1990b). It was confirmed that the limited regeneration of $NADP^+$ under lack of CO_2 increased photoinhibition (Krause et al., 1978). This concomitant increase of photoinhibition and of the activation rate of oxygen leads to the conclusion that the latter may be involved in the photoinhibition mechanism.

The activated oxygen, under conditions of free radicals (O_2), superoxides (H_2O_2), and hydroxyl radicals (OH), can be neutralized by the action of superoxide dismutase associated with the peroxidase ascorbat system (Krause, 1988; Long et al., 1994). We can still associate with them catalase and glutationa; both enzymes and antioxidizers occur in plants spontaneously (Powles, 1984; Richter et al., 1990b; Pastori & Trippi, 1993). Damage from these oxidizing agents can be prevented or minimized by the presence of carotenoids, but the capacity to prevent these reactions and/or antioxidizing compounds can be annulled as a result of photoinhibition, as verified by Havaux and Davaud (1994) for the activity of 50D in *Solanum tuberosum* exposed to 3700 μmol.$m^{-2}s^{-1}$ at a temperature of 3°C.

Although Mehler reaction plays a negative role in easing the reduction of oxygen, it can act as a dissipating energy mechanism, since it allows the maintenance of electron flow (Powles, 1984) and keeps the transthylakoidal ΔpH (Richter et al., 1990b).

Maintenance of a minimum rate of photosynthetic metabolism of carbon is essential to prevent or minimize photoinhibition (Powles, 1984; Krause, 1988; Long et al., 1994). A feasible explanation for this protective mechanism is the fact that carbon metabolism consumes ATP and $NADPH_2$, keeping the electrons flowing through the photosynthetic chain (Powles & Osmond, 1978; Powles, 1984). The quantitative consumption of energy by photosynthesis in itself does not clarify the prevention of photoinhibition (Krause, 1988). The specific rate of carbon metabolism needed to avoid photoinhibition will vary according to radiance maintained during the photoinhibitory treatment and the radiance under which the plant developed,

as well as other genotypical, phenotypical, and physiological characteristics of the plant (Powles, 1984; Krause, 1988).

According to Krause (1988), photorespiration, because it consumes oxygen, acts as a protective mechanism against photoinhibition. Long et al. (1994) report that the photosynthetic metabolism of hydrogen can consume up to about one-tenth of the total amount of quantum used in the photosynthetic metabolism of carbon, and it is likely to take part in the protective mechanism.

The rapid turnover of protein D1 in plants exposed to sunlight has been suggested as an effective protection against photoinhibition (Aro et al., 1993; Leitsch et al., 1994). Acclimation of plants to low radiance results in deceleration of the restoration cycle of PS II, and the same phenomenon occurs in cool temperatures (Leitsch et al., 1994; Tyystjärvi et al., 1994).

C. MORPHOLOGICAL MECHANISMS

One of the mechanisms for reducing the potential for photoinhibition is reduction of the total superficial area of chloroplasts exposed to high radiance. This reduction can be achieved by a rapid motion and aggregation of chloroplasts inside the cells (Anderson & Aro, 1994; Long et al., 1994) or by total motion of the leaves changing the angle of interception of light and, as a consequence, its absorption (Powles, 1984; Long et al., 1994). Some higher plants, such as *Cotyeedon orbicula,* can prevent excess light by forming a highly reflexive cuticle (Robinson et al., 1993). Other plants, like *Spartina anglica,* a C4 species tolerant of cool temperatures, normally show an accumulation of antocyanins on the adaxial surface in spring, when the clear sky predisposes it to photoinhibition (Long, 1983; Rabino & Mancinelli, 1986).

D. BIOCHEMICAL MECHANISMS

Recently, Shiraishi et al. (2000) found that the application of potassium formate (2 mM) before photoinhibitory treatment protected the photosynthesis of rice leaves from photoinhibition. Formate is possibly involved in endogenous radical scavenging and/or in the supply of CO_2 (derived from the formate), thereby reducing oxidative damage to the photosystems under photoinhibitory conditions.

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