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PHOTO-OXIDATION OF LEAF PIGMENTS IN CUCUMIS LEAF DISCS DURING CHILLING

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SUMMARY

The effect of light on *Cucumis* leaf pigments at 1 °C has been studied. Pigment degradation is a photo-oxidative process. Kinetics of pigment degradation show a slow degradation rate (lag phase) followed by a fast one. The sensitivity to photo-oxidation corresponds to the length of the lag phase. Carotene has the shortest lag phase and is most sensitive to photo-oxidation, followed by xanthophyll, chlorophyll *a*, and chlorophyll *b*.

The lag phase decreases at 100% oxygen and disappears after boiling. Some possible factors causing the biphasic nature of chlorophyll degradation are discussed. It is suggested that carotene plays no primary role in the protection against photo-oxidation during chilling.

1. INTRODUCTION

Light combined with suboptimal growth temperatures causes a decrease in pigment concentration, especially in thermophilic plants, FARIS (1926); SIRONVAL (1957); MCWILLIAM & NAYLOR (1967); ALBERDA (1969).

High light intensity causes photo-oxidative pigment bleaching (solarization), FRANCK & FRENCH (1941); HAGER (1957); ZURZYCKI (1957).

SIRONVAL & KANDLER (1958), working with a light intensity of 100.000 lux, found a negligible effect of temperature on the photo-bleaching of *Chlorella* between 18 °C and 38 °C. Other authors found that lower temperatures enhance photo-bleaching caused by high light intensity (SIRONVAL 1957; HAGER 1957).

In studying the damaging effects of low temperature above freezing point (chilling) on thermophilic plants we observed that chilling in the light caused more injury to cucumber leaf discs than chilling in the dark.

At the same time degradation of leaf pigments took place in the light. To gain more insight in the damaging effect of light during chilling the degradation of leaf pigments was studied.

2. MATERIAL AND METHODS

Cucumis plants (cv. Kleine Groene Scherpe) were grown in pots in a greenhouse at a minimum day temperature of 25 °C and a minimum night temperature of 20 °C. Discs of 7 mm diameter were punched between the largest veins of the first leaves of ± 14 days old plants. Samples of discs were floated on 25 ml 1% sucrose solution in special P.V.C. dishes with an air-tight perspex screw-on lid.

Eight dishes were placed in a circle in a tray. The dishes stood in a 30% ethanol-water mixture kept at the desired temperature by circulating it through a cooling thermostat.

The dishes were filled with a gas mixture by flushing them three times. During the experiment gases were streamed through the dishes. Gases were humidified by bubbling them through washing bottles with water. N_2 was freed from traces of oxygen by conducting it through an alkaline pyrogallol solution.

A light intensity of 20.000 lux was obtained by placing the dishes under a 400 W. mercury lamp (type H.P.L.R.; Philips) in a glass bottomed metal container lined with silver paper. The container was filled with 10 cm distilled water to absorb most of the infrared radiation. The whole was placed in a cool room (2 °C ± 1 °C).

At different times samples of 40 discs were taken out of the dishes and dried between filter paper.

Leaf pigments were extracted with 100% acetone in a Mickle glass-pearl homogenizer for 5 minutes. Concentrations of chlorophyll *a* and *b* were determined spectrophotometrically and calculated as described by BRUINSMA (1963).

Carotene and xanthophyll concentrations (no distinction was made between the different xanthophylls) were determined as follows. A petroleum-ether extract was obtained by washing a mixture of 10 ml of the 100% acetone extract and 5 ml light petroleum (bp. 60–80 °C) with 500 ml of distilled water using an automatic method as described by GOODWIN (1955). The water layer was separated from the light petroleum extract and the extract was adsorbed into a cellulose column (Whatman C. F. 12) with a 1 cm layer of anhydrous sodium sulphate above the cellulose to absorb traces of water.

Elution of the pigments was carried out as described by LEDERER & LEDERER (1957). Carotene concentration was measured at 450 nm, xanthophyll concentration at 440 nm in a Zeiss spectrophotometer. All operations were carried out in the dark or in weak green light.

3. RESULTS

3.1. The effect of light

Fig. 1 shows that light during chilling causes degradation of chlorophyll and carotene in *Cucumis* leaf discs. No degradation is observed in the dark. During the first 16 hours the decrease in chlorophyll concentration is relatively slow and only after this lag phase the maximum degradation rate (fast phase) is attained.

The lag phase as well as the fast phase appear to proceed linearly. There is a sudden transition from the lag phase to the fast phase. Carotene degradation appears to proceed without lag phase.

3.2. The effect of an N_2 atmosphere

The decrease of the concentration of chlorophyll and carotene in air and in a nitrogen atmosphere was compared. During the first 24 hours there was no pigment degradation in an N_2 atmosphere. After 24 hours, however, there was some degradation, especially of carotene (*fig. 2*). So, oxygen and light are required for degradation of leaf pigments, at least during the first 24 hours.

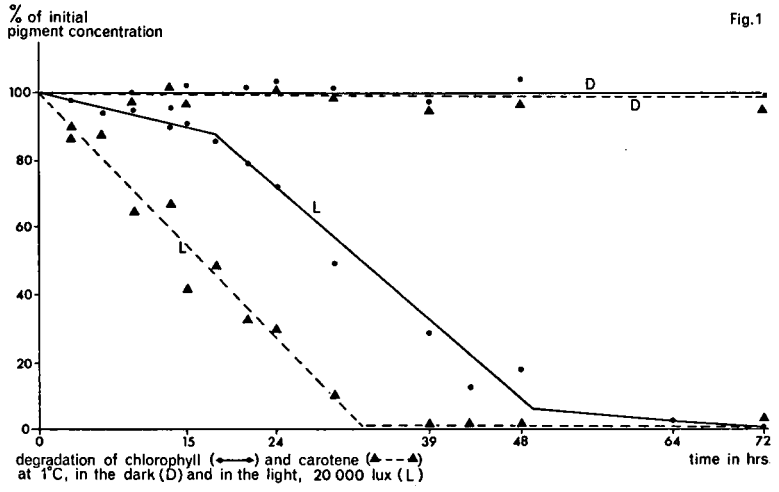

Fig. 1. Relation between the duration of the chilling treatment and the concentration of carotene and chlorophyll in the dark and in the light.

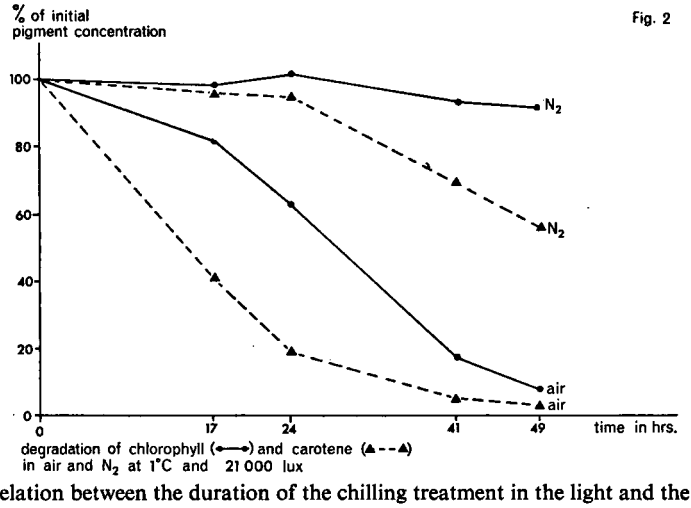

Fig. 2. Relation between the duration of the chilling treatment in the light and the concentration of carotene and chlorophyll in air and in an atmosphere of pure nitrogen.

We may conclude that light induced pigment degradation in *Cucumis* leaf discs, during chilling, is mainly due to an aerobic photo-bleaching process.

3.3. The effect of different oxygen concentrations

Fig. 3 shows that a higher oxygen concentration causes a shorter lag phase in the degradation of chlorophyll and of carotene. During the fast phase there is little difference between the degradation rate at 21% and 100% oxygen. However, oxygen still enhances pigment degradation during the fast phase:

Fig. 3

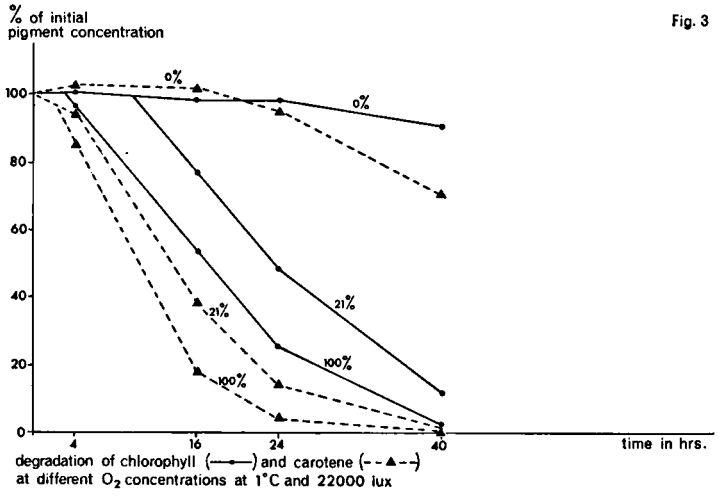

Fig. 3. Relation between the duration of the chilling treatment in the light and the concentration of carotene and chlorophyll in an atmosphere of 0%, 21% and 100% oxygen.

after 18 hours photo-oxidation in air bleaching of chlorophyll and carotene is inhibited in an atmosphere of pure N_2 (*table 1*). At 100% oxygen, the fast phase of chlorophyll degradation starts at a carotene concentration of 80–90% while at 21% oxygen the fast phase of chlorophyll degradation starts at a carotene concentration of 50–60%.

Table 1. The effect of an N_2 atmosphere during the fast degradation phase of chlorophyll and carotene at 1°C and 20000 lux. The pigment concentration is expressed in % of the initial concentration.

treatment	18 hrs air	42 hrs air	18 hrs air + 24 hrs N_2
chlorophyll	71%	10%	57%
carotene	32%	0%	20%

3.4. The sensitivity of different pigments to photo-oxidation

The sensitivity of different leaf pigments to photo-oxidation is shown in *fig. 4a* and *4b*. Carotene is the most sensitive pigment, followed by xanthophyll, then chlorophyll *a* and chlorophyll *b*.

The different sensitivity of the pigments to photo-oxidation is mainly due to the different length of the lag phase. Carotene has the shortest lag phase while chlorophyll *b* has the longest lag phase. When 20–30% of the initial pigment concentration is left, the rate of degradation diminishes.

Xanthophyll and chlorophyll *b*, which contain more oxygen than the corresponding forms carotene and chlorophyll *a*, are less sensitive to photo-oxidation.

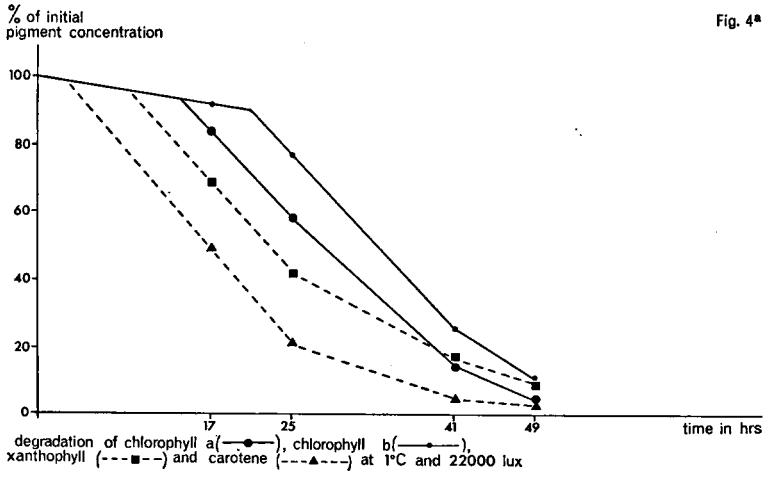

Fig. 4a. Relation between the duration of the chilling treatment in the light and the concentration of carotene, xanthophyll, chlorophyll *a* and chlorophyll *b*.

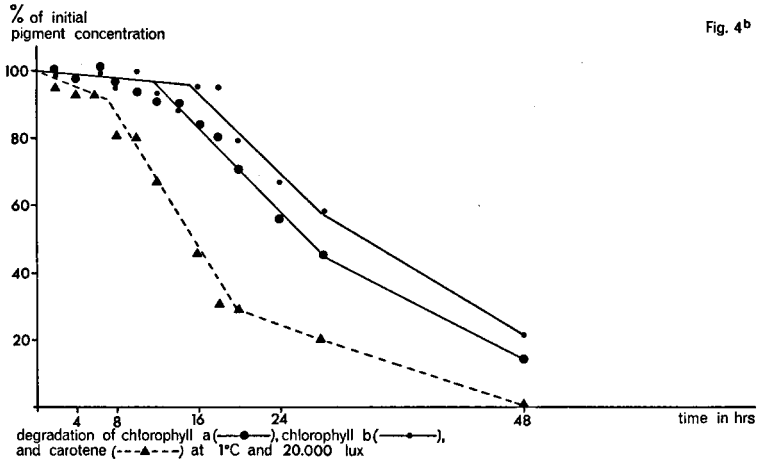

Fig. 4b. Relation between the duration of the chilling treatment in the light and the concentration of carotene, chlorophyll *a* and chlorophyll *b*.

3.5. The influence of light intensity

When the light intensity is reduced to one half (by means of a cheese cloth) the degradation rate during both the lag phase and the fast phase decreases (*fig. 5*). Therefore it can be concluded that light intensity is a limiting factor during both the lag phase and the fast phase of pigment degradation.

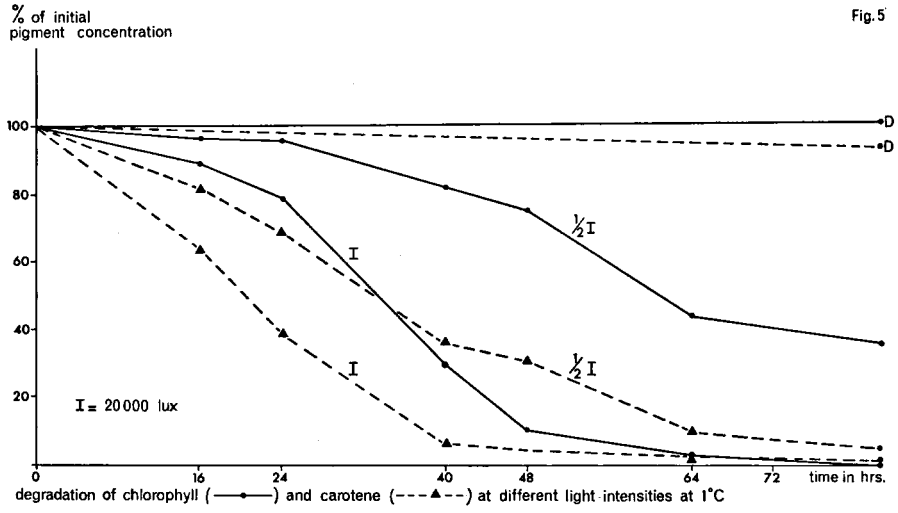

Fig. 5. Relation between the duration of the chilling treatment and the concentration of carotene and chlorophyll in the dark (D) and at a light intensity of 10000 lux and 20000 lux.

3.6. The effect of low temperature pretreatment on the lag phase

A better understanding of the factors protecting plant cells from photo-oxidation would be gained if more insight concerning the mechanisms involved in the lag phase were available.

We first considered the possibility of an induction of the fast phase by a slow, cold-induced alteration during the lag phase. *Fig. 6* shows that a pretreat-

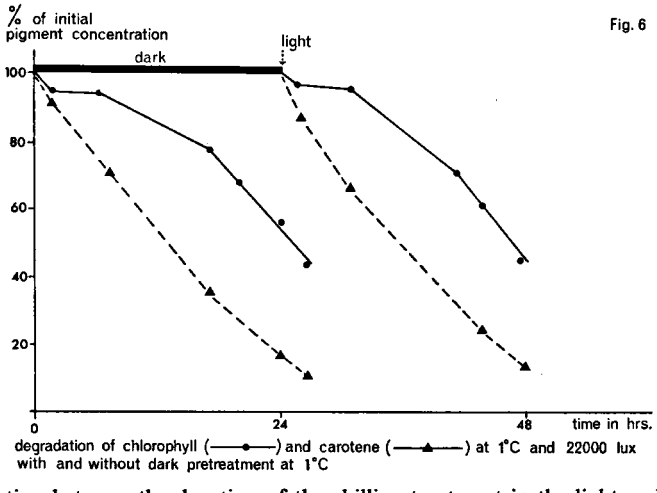

Fig. 6. Relation between the duration of the chilling treatment in the light and the concentration of carotene and chlorophyll with and without a pretreatment of chilling in the dark.

ment of the discs at 2 °C in the dark has no effect on photo-oxidation.

The lag phase is still present. Low temperature per se is therefore not responsible for the occurrence of the lag phase.

3.7. The effect of boiling

As demonstrated in *fig. 7* the lag phase is completely absent in discs boiled for 10 minutes. It can therefore be concluded that the lag phase is bound to the properties of the living cell.

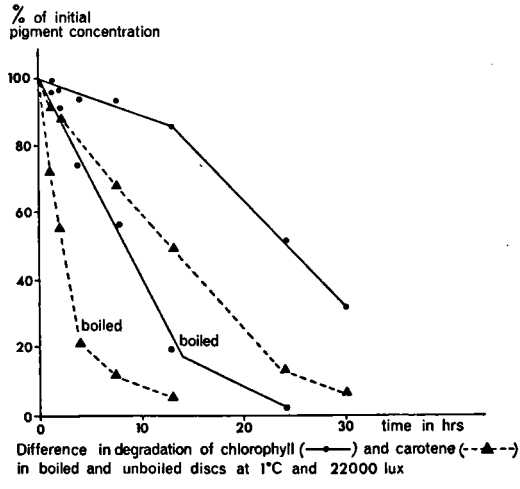

Fig. 7. Relation between the duration of the chilling treatment in the light and the concentration of carotene and chlorophyll in boiled and unboiled leaf discs.

4. DISCUSSION

Oxygen is needed for chlorophyll bleaching and enhances carotene degradation in the light during chilling. Therefore light-induced pigment degradation can be considered, to a large extent, to be a photo-oxidative process, in which O_2 serves as an electron acceptor. The light-induced carotene degradation beginning in an N_2-atmosphere after 24 hours may be regarded as a photo-oxidative process in which other cell substances, rather than oxygen, are the electron acceptors. Another explanation could be that oxygen set free by the photo-oxidation of H_2O serves as an electron acceptor. Preliminary experiments, however, on the effect of hydroxylamine, an inhibitor of O_2-yielding reactions, do not support this last explanation.

SIRONVAL & KANDLER (1958) concluded that xanthophyll is less sensitive than chlorophyll *a*, but almost as sensitive as chlorophyll *b* to photo-bleaching in *Chlorella*. These authors, however, determined pigment degradation only at the end of the bleaching period. *Fig. 4a* shows that the general kinetics of pigment degradation should be taken into account.

The observed differences in the length of the lag phase between the various leaf pigments during the first period of photo-oxidation (*fig. 4*) can be attributed to the different abilities of the pigments to quench harmful light energy. Carotene, the most sensitive pigment, may protect chlorophyll and other substances against photo-oxidation by its very efficient quenching of the triplet energy of chlorophyll (CLAES & NAKAYAMA 1959).

In addition the carotenoids could serve as a buffering system, which is able to accept the excess electrons from the photosynthetic mechanism (DONOHUE *et al.* 1967; KRINSKY 1968). These authors suggest a rapid enzymatic reduction of photo-oxidized carotenoids.

At 1 °C, however, enzymatic reactions are inhibited. As a consequence the CO_2 assimilation comes to a stop. The electron flow of the photosynthetic mechanism will be deflected towards carotene, which is oxidized to carotene epoxide. The enzymatic reduction of the epoxides however is inhibited by the low temperature. Therefore the carotene concentration rapidly decreases in the light during chilling.

On the other hand a primary role of carotene in the inhibition of photo-oxidation of chlorophyll during the lag phase is not in agreement with the variable amount of carotene at the beginning of the fast phase of bleaching of chlorophyll at different oxygen concentrations (*fig. 3*).

In addition, no alteration in the degradation rate of carotene is observed at the beginning of the fast phase of chlorophyll degradation. Therefore, it is unlikely that carotene as such plays a primary role in the inhibition of photo-oxidation during the lag phase of the other pigments.

The oxygen-containing pigments chlorophyll *b* and xanthophyll are less sensitive to photo-oxidation than the corresponding pigments chlorophyll *a* and carotene. This is in agreement with the results of HAGER (1957) and SIRONVAL & KANDLER (1958). The possibility of a photo-oxidative transformation of chlorophyll *a* into chlorophyll *b* and a transformation of carotene into xanthophyll can not be ruled out.

HAGER (1957) stated that in *Avena* shoots after two days at 50.000 lux (room temperature) an increase of xanthophyll concentration and a decrease of carotene concentration occur at the same time. In preliminary experiments we did not find such a correlation between the concentrations of the yellow pigments. This discrepancy may be due to a low enzymatic activity caused by the low temperature (1 °C) used in our experiments.

The lower rate of degradation of the yellow pigments after 24 hours might be attributed to a lower efficiency of the pigment-pigment energy transfer, caused by photo-oxidative damage to the chloroplast membrane system. Another explanation could be that a part of the pigment is localized inside the membrane and consequently is better protected against photo-oxidation.

The fact that a slow phase of pigment degradation precedes a fast phase during photo-oxidation is in accordance with results of SIRONVAL & KANDLER (1958). They found a similar lag phase in the case of *Chlorella* photo-bleaching. THOMAS & NIJHUIS (1968) describe a similar lag phase in the case of aerobic

photo-bleaching of chlorophyll-protein complexes in isolated *Aspidistra* chloroplasts.

When we consider that a higher oxygen concentration as well as a higher light intensity reduce the lag phase, we can conclude that (the origin of) the lag phase is due to the inhibition of damaging photo-oxidative processes. This inhibition might be produced by substances protecting leaf pigments against photo-oxidation. These substances could quench harmful light energy and trap oxygen. They could also serve as a buffering system draining electrons from the pigments.

As discussed before, it is unlikely that carotene as such plays a primary role as an inhibitor of photo-oxidation in the lag phase during chilling.

A more plausible explanation for the occurrence of a lag phase preceding a fast phase during photo-oxidation of leaf pigments is that the electron transport system remains intact during the lag phase, while during the fast phase the electron transport is disturbed. An intact electron transport system might deflect electrons, which otherwise would induce bleaching of pigments, to protecting substances.

The different length of the lag phase of the pigments might correspond to the photo-oxidative destruction of different factors needed for electron transport.

It seems likely that these factors are localized in the grana lamellae which consist of 50% proteins and 50% lipids (WEIER & BENSON 1966). They might well be lamellar proteins or bound to these proteins, since boiling of leaf discs causes complete disappearance of the lag phase (*fig.* 7). Several authors assume in addition that the primary site of photo-oxidative damage is localized in the cell membrane (KRINSKY 1968).

Further experiments to elucidate the origin of the lag phase during chlorophyll photo-oxidation are in progress.

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