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Heat-Induced Changes of Chlorophyll Fluorescence in Intact Leaves Correlated with Damage of the Photosynthetic Apparatus*

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Abstract. Methods were developed to measure chlorophyll fluorescence yield of intact leaf tissue during heat treatment under varying conditions of light intensity and photosynthetic activity. Fluorescence yield of a dark-adapted leaf increases by 2- to 3-fold with an increase of temperature into the region where heat-damage occurs. The temperatures of the fluorescence transition correlate well with the temperatures where quantum yield of CO_2 fixation is irreversibly depressed. Fluorescence-temperature (F-T) curves allow ranking of different species according to their heat sensitivity. Within a single species acclimation to different growth temperatures is reflected by shifts of the transition temperatures in the F-T curves. When F-T curves are recorded in the steady light states at increasing light intensities, substantial shifts (up to 6° C) of transition temperatures to higher values are observed. Quantum yield measurements of CO_2 fixation confirm that high-light conditions protect from heat-damage. It is suggested that chlorophyll acts as an intrinsic fluorescence probe of the thylakoid membrane and responds to the same changes which cause irreversible denaturation of photosynthetic enzymes.

Key words: Chlorophyll fluorescence – Heat damage – Temperature sensitivity.

Introduction

The thermal environment in which a plant is grown markedly affects the temperature dependency of photosynthesis (Björkman et al., 1975). Different species show differences in the genetically determined range of environmentally induced photosynthetic adaptation to temperature. This is an important factor determining the survival of plants in hot climates, as heat damage of the photosynthetic apparatus occurs before other symptoms of high-temperature injury can be detected (Björkman, 1975). There is a specific effect of heat causing a decay of the apparent efficiency of the photoacts as judged by the quantum yield for CO_2 fixation (Björkman, 1975). An inhibition of enzyme reactions should not affect the quantum yield when measured under strictly light-limiting conditions. From this and the work of Santarius (1974) it was suggested that one of the first components of the photosynthetic apparatus to be damaged by heat is the thylakoid membrane. Reactions depending on the intactness of this membrane are particularly water-splitting and photophosphorylation.

Chlorophyll is embedded in the thylakoid membrane and chlorophyll fluorescence yield is a sensitive indicator for the state of this membrane (for a review, see Papageorgiou, 1975). Heat damage is reflected in drastic changes of the light-induced fluorescence characteristics (Schreiber, 1971; Berry et al., 1975; Krause and Santarius, 1975; Pearcy et al., 1977). In general the changes can be correlated with inhibition at or close to Photosystem II reaction centers.

Besides the effect of heat damage on light-induced fluorescence transients, measured at room temperature, there are characteristic changes of fluorescence yield with heating both in the dark- and in the light-adapted states (Lavorel, 1969; Schreiber et al., 1975, 1976; Berry et al., 1975). The temperatures at which drastic fluorescence changes are observed depend on plant species and growth temperatures.

While most of the previous work relating fluorescence changes and heat damage was done with unicellular algae or isolated chloroplasts. the present study focuses on heat-induced fluorescence changes in intact

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leaves of *Tidestromia oblongifolia* and three species of *Atriplex*, the photosynthetic and thermal stability characteristics of which have been extensively studied in this laboratory (Björkman et al., 1974, 1975, 1976; Berry et al., 1975). It will be shown that a good correlation exists between the temperature at which quantum efficiency of CO_2 fixation declines, and the temperatures where fluorescence emission is stimulated. This provides a rapid, easy and valuable assay for heat damage in intact tissues, and gives some additional insight into the mechanism of thermal damage.

Materials and Methods

Tidestromia oblongifolia, Atriplex hymenelytra, A. glabriuscula and *A. sabulosa* were grown in controlled-growth facilities using natural sunlight (see Björkman et al., 1975). Before an experiment whole plants were kept in the dark for at least 45 min. Fluorescence was measured from detached, intact leaves.

The apparatus for measuring heat-induced fluorescence changes was essentially as previously described (Schreiber et al., 1976). The cuvette design was modified for measurements on intact leaf tissue. To insure rapid heat exchange and efficient oxygen exchange between sample and water, the sample was directly exposed to the water flow. Figure 1 shows a schematic cross-section of the cuvette. Connection to the fiber optics is achieved by a plexiglass rod, which also assures homogenization of the incident light. An excised disc of leaf tissue is gently pressed onto a heat-insulating polyurethane plug by transparent, extensible nylon net. The polyurethane plug is at the top of an O-ringed piston which pushes the sample close to the plexiglass rod and exposes it to the water flow. A copper-constantan thermocouple is in contact with the sample. The thermovoltage is either read off a digital voltmeter, or after appropriate amplification (2470A amplifier, Hewlett-Packard, Palo Alto, Cal., USA) is plotted versus fluorescence yield on an X-Y recorder (Plotamatic 705, M.F.E. Corporation, Salem, N.H., USA) Temperature of the water circulating through the cuvette was either increased slowly (1° C/min) by heating, or sudenly by using solenoid valves (J4–23, Jackes-Evans Mfg. Co., St. Louis, Mo., USA) to direct water from either of two controlled temperature baths to the cuvette. With a switch of the valve control system from a cold (20° C) to a hot (50° C) water flow through the cuvette, the thermocouple response was completed in 5 s, the response time being determined by the heat-capacity of the thermocouple. The temperature jump in the leaf can be expected to be substantially faster. When the temperature change was monitored via the fluorescence yield of a plastic filter disc, simulating a leaf, 92% of the change occurred in 1 s and 99% in 2 s.

If not stated otherwise fluorescence was excited by extremely weak, blue light (Corning 4–96) with an intensity of 0.3 erg cm^{-2} s^{-1}, and fluorescence was measured at wavelengths longer than 660 nm (Corning 2–64).

Measurements of the quantum yield for CO_2 fixation were as described by Ehleringer and Björkman (1976). The linear portion of the light-dependence curve at low intensities was taken as a relative measure of quantum yield. For C_4-species (*T. oblongifolia* and *A. sabulosa*) gas exchange was measured in air, 325 µbar CO_2 partial pressure. Quantum yield for *A. glabriuscula* (C_3-species) was determined in 2% O_2, 450 µbar CO_2.

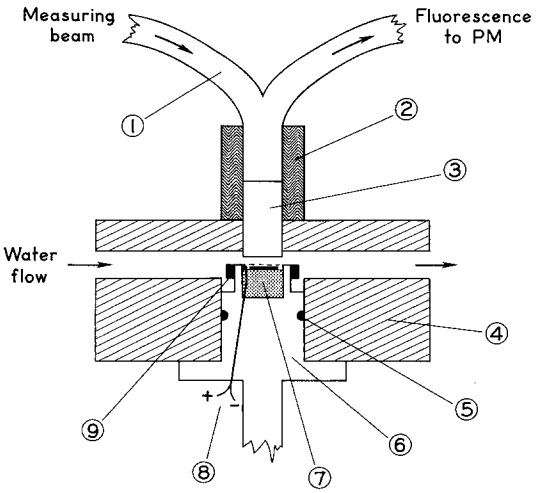

Fig. 1. Cuvette for measurement of heat-induced fluorescence changes in intact leave discs. (*1*) Bifurcated fiberoptics; (*2*) adaptor tube; (*3*) plexiglass rod as light guide; (*4*) black lucite housing; (*5*) O-ring; (*6*) black lucite piston which positions sample in water flow facing light guide; (*7*) polyurethane plug on top of which the leaf disc and the junction of a thermocouple (*8*) of which the leaf disc and the junction of a thermocouple (*8*) are held down by an elastic nylon net, tightly stretched by the lucite ring (*9*)

Results and Discussion

F-T and T-jump curves

Figure 2 shows the heat-induced increase in chlorophyll *a* fluorescence yield in *Tidestromia*. Fluores-

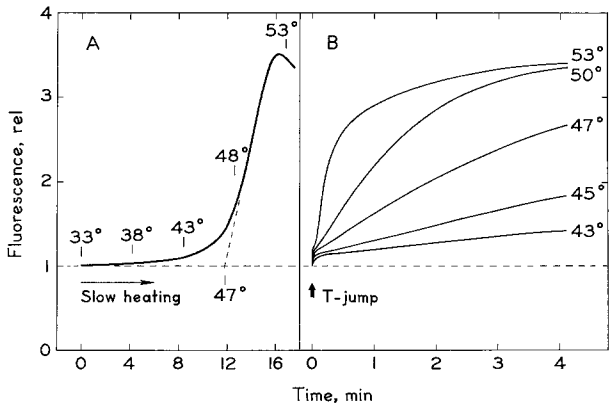

Fig. 2 A and B. Heat-induced changes in fluorescence yield in *Tidestromia oblongifolia*. **A** Slow heating curve: a leaf disc is heated by ca. 1° C/min. **B** T-jump curve: temperature is jumped from 35° C to the indicated values within ca. 1 s. Fluorescence is monitored with an extremely weak measuring beam (see Materials and Methods). A relative fluorescence unit of 1 corresponds to the F_o-level at room temperature. Note the difference in time units for **A** and **B**. *Tidestromia* was grown on a 35° C day, 20° night regime

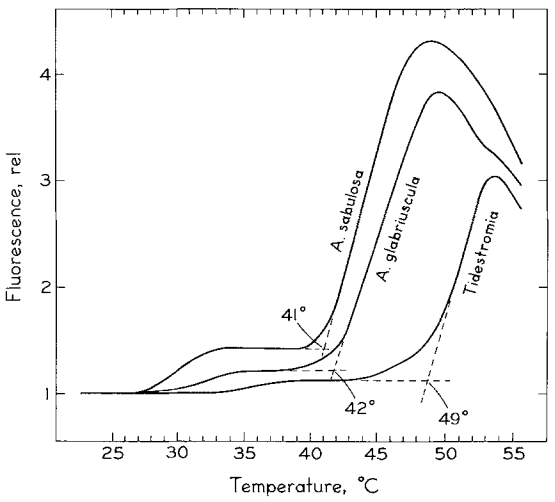

Fig. 3. Comparison of slow-heating F_o-curves in leaves of species differing in heat sensitivity. Curves normalized with $F_o = 1$ relative unit at 25° C. *A. sabulosa* and *A. glabriuscula* were grown at 20° C day, 15° C night. *Tidestromia* was grown at 45° C day, 31° C night

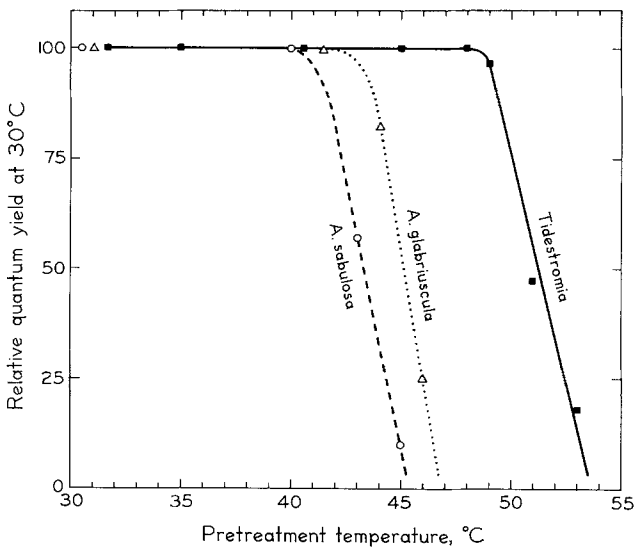

Fig. 4. Effect of pretreatment temperature on the subsequent quantum yield for CO_2-fixation by intact leaves at 30° C. Plant material as in Figure 3. Attached leaves were exposed to the indicated temperatures for 15 min. Time between pretreatment and measurement of quantum yield ca. 30 min

cence is measured with a weak excitation beam which by itself does not cause any change in fluorescence yield. Thus all through the experiment fluorescence of the dark-adapted sample is monitored; this is referred to as Fo (initial fluorescence yield in a dark-light induction curve). Heating is performed in two different ways: a) Slowly by about 1° C/min, resulting in a fluorescence temperature curve (F-T curve); b) rapidly within about a second, yielding temperature jump fluorescence induction curves (T-jump curves).

Similar results are obtained with either approach. When leaves are heated to temperatures in excess of about 45° C in the example of Figure 2, there is a substantial increase in the fluorescence yield. The final extent of the fluorescence increase is similar within the temperature range 47° C to 53° C, indicating a change from one fluorescent state to another. The temperature jump technique provides an assay of the kinetics and extent of this transition (Schreiber et al., 1976). The slow heating technique gives a sensitive assay for the threshold temperature at which fluorescence begins to increase. In our experience this temperature is reproducible from sample to sample of the same plant and differs predictably between plants of different species or previous history. Experiments not reported here indicate that this fluorescence transition is probably fundamentally the same as the discontinuity of fluorescence yield observed in the presence of 3-(3, 4)-dichlorophenyl-1,1-dimethylurea DCMU; Berry et al., 1975). The experimental approach employed here has the advantage that use of DCMU and possible modifications of the fluorescence yield by the redox state of the quencher Q are avoided (see Schreiber, 1976).

In Figures 3 and 4, F-T curves for *A. sabulosa, A. glabriuscula* and *Tidestromia* are compared with the thermal inactivation characteristics of photosynthesis as derived from quantum yield measurements of CO_2-fixation. It appear that the threshold for the fluorescence yield change in the F-T curves indicates quite accurately the threshold of thermal instability of these plants. Heat damage in *Tidestromia* (grown at 45° C) occurs at temperatures higher than 48° C, which corresponds well with the beginning of the fluorescence increase in the F-T curve. In the two *Atriplex* species (grown at 20° C) the quantum yield start to decline at substantially lower temperatures. In *A. glabriuscula* heat inactivation sets in at ca. 42° C and in *A. sabulosa* at ca. 41° C.

In comparing the data in Figures 3 and 4 it is important to note that the heat treatments in the two types of experiments are quite different. For the quantum-yield measurements samples were preheated for 15 min at the indicated temperatures and 30 min recovery time was given before the actual measurement of heat damage. On the other hand, the F-T curves reflect the immediate effect of heat on the fluorescence yield; the samples are only transiently exposed to a certain temperature as heating occurs at ca. 1° C/min. T-jump fluorescence-induction curves show that fluorescence responds to an increase in temperature with a rise in yield extending over many minutes (see Fig. 2). Also, upon re-cooling, fluorescence yield falls again; the extent of re-

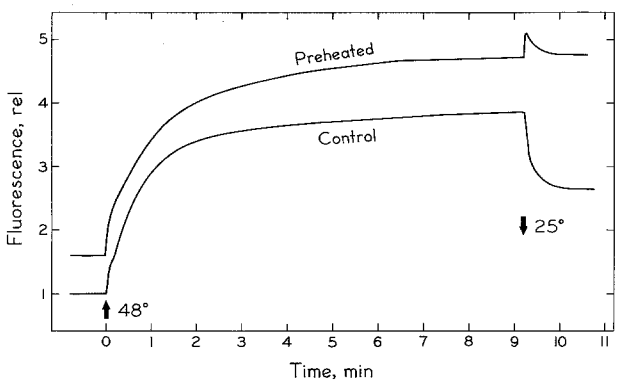

Fig. 5. Effect of preheating on T-jump fluorescence induction. Plant material, *A. glabriuscula* (20° C day, 15° C night). Preheating at 46° C for 5 min; sample subsequently stored for 1 h on ice before measurement; the same sample was completely heat-damaged in terms of CO_2-fixation. One relative fluorescence unit corresponds to the yield of the control at 25° C. Equal size leaf discs of control and preheated sample were used and fluorescence measured at identical sensitivity

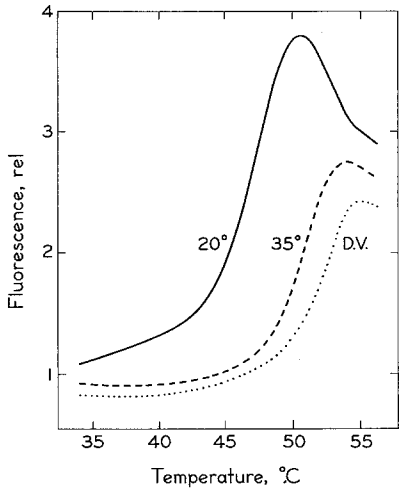

Fig. 6. Effect of growth temperature on heat-induced fluorescence rise in *Atriplex hymenelytra*. Plants were grown at 20° C and 35° C in controlled-growth facilities or collected from Death Valley (D.V.)

versibility is dependent upon the heating temperature. On the other hand, the changes in photosynthetic capacity are essentially irreversible. In view of these results it may be surprising that there is such a close correlation between F-T curves and heat-damage expressed as decline in quantum yield. The results presented in Figure 5 help to clarify this point. It is shown that a sample which has been pre-heated so that CO_2-fixation is completely and irreversibly blocked will still exhibit a heat-induced fluorescence rise. This finding indicates that the fluorescence yield is not a direct indicator of heat damage. It rather appears that at high temperatures the thylakoid membrane reaches a state which on one hand causes chlorophyll fluorescence to increase and on the other hand leads to destruction of certain photosynthetic enzymes. While the fluorescence yield change may be reversible to the extent that irreversbile damage of the membrane does not occur, the destruction of the enzymes appears to be permanent. On this basis the chlose correlation between the heat-induced fluorescence changes and heat-inactivation indicated by quantum yield measurements (compare Fig. 3 and 4) becomes understandable despite the fundamental differences in the heat treatment and the reversibility characteristics.

Atriplex hymenelytra is capable of a wide range of photosynthetic adaptation to different temperature regimes. It is native to a hot desert climate, but also survives well in a cool coastal environment (Björkman et al., 1974; Björkman, 1975). This species was chosen to demonstrate within a single species the effect of acclimation to different growth temperatures on the F-T curve characteristics. *A. hymenelytra* was collected from its natural environment in Death Valley (California, USA) where the maximum daily temperature was about 45° C. Plants were also grown at 20° C and 35° C in controlled-growth facilities at the laboratory. Figure 6 shows F-T curves for plants grown under these different temperature regimes. The critical temperatures where the fluorescence transitions occur are shifted for plants from the three different regimes. The shift amounts to about 4° C when comparing 20° C and 35° C plants, and to about 2° C when comparing 35° C and Death-Valley-grown plants. The lower limit of heat damage appears to be at 43° C, 47° C and 49° C for the 20° C 35° C and Death-Valley-grown plants, respectively. We have compared F-T curves for a number of other plants of a single species grown at different temperatures. In all cases a difference in growth temperature was reflected by a corresponding difference in the critical temperatures of the fluorescence transition. It can be concluded that the fluosescence method reflects closely changes of the photosynthetic apparatus which are induced by temperature acclimation of a plant.

Effect of Light Intensity on Apparent Thermal Stability

The preceding fluorescence studies were conducted at very low light intensities (0.3 erg cm^{-2} s). The use of higher light intensities would have the advantage that less sensitive measuring equipment could be used and that fewer precautions would be required to prevent the tissue from becoming anaerobic (see Schreiber and Vidaver, 1974; Schreiber et al., 1976;

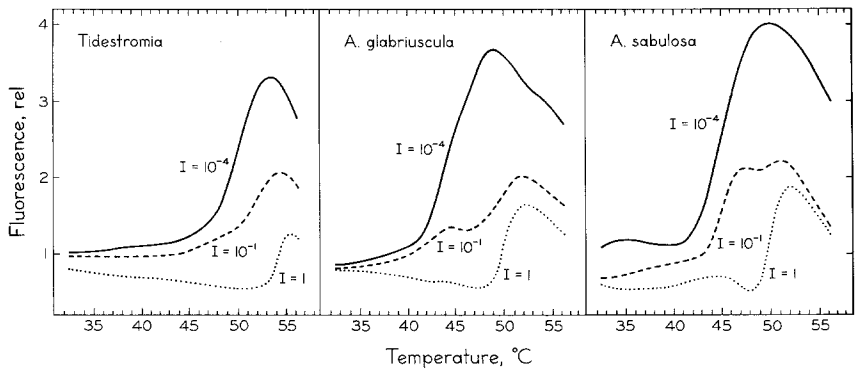

Fig. 7. Effect of light intensity on the temperature dependency of steady state fluorescence yield. I = 1 corresponds to a light intensity of 3×10^4 erg cm^{-2} s^{-1}. Curves were normalized for equal steady state fluorescence signal (F_s) at 25° C. In all cases F_s was close to F_o, as monitored via oscilloscope. No light-induced fluorescence change was observed at $I = 10^{-4}$

Schreiber, 1976). However, because of partial reduction of the quencher Q at higher light intensities the fluorescence yield assumes a steady state value, F_s, which is somewhat higher than F_o. While more complex patterns of F-T curves at increased light intensity might be expected, a rather clear-cut pattern was observed. Figure 7 shows the effect of light intensity on F_s-T curves for *Tidestromia, A. glabriuscula* and *A. sabulosa*. For these plants, and for a number of other species (not shown in the Figure) the following pattern was observed: (1) The threshold temperature for the fluorescence transition is shifted to higher temperatures with higher light intensity. (2) The amplitude of the heat-induced fluorescence transition is substantially less at higher intensities. (3) At intermediate light intensities there are indications of a separation of the fluorescence transition into two phases. Phenomenologically, the change of the F_s-T curves with increasing light intensities appears to involve a conversion of the transition observed with F_o-conditions into a transition occurring at ca. 6° C higher temperatures.

It seems unlikely that these differences between the F_o-T and F_s-T curves can be attributed primarily to changes in the redox state of Q with increasing light intensity. In far-red light (700 nm), which is efficient in keeping Q oxidized, the threshold temperature is also shifted to higher values at higher intensity (not shown in the Figures). It therefore seems likely that the F_s-T curves reflect similar changes in the chloroplast membranes as those shown in the F_o-T curves. And it appears that the shift in apparent thermal stability with increasing light intensity is based on some protective effect conditioned by the increased light intensity.

The question is whether the shift in the fluorescence transition with light intensity reflects a true change of thermal stability, or whether it only indicates some intrinsic property of the fluorescence assay. To resolve this question, leaves of *Tidestromia* were given a standard heat pretreatment at different light intensities. Heat damage was subsequently assayed by measurements of quantum yield for CO_2-fixation. As shown in Figure 8 the degree of heat damage does indeed depend on light conditions during heating. High light intensity can completely protect a plant from damage, at a temperature which causes a 50% loss of activity in darkness. This constitutes another argument for the reliability of the F-T assay in measuring heat damage in plants. In addition it appears that F_s-curves are suitable for monitoring heat deactivation; this should facilitate the use of the method with less sensitive equipment.

That light raises the heat tolerance of the photosynthetic apparatus may not be surprising, since peak leaf temperatures occur, in nature, in leaves exposed to strong light. At present it is not clear what causes the light-dependent change in heat sensitivity. One may assume that in the light the chloroplast reaches a state which is characterized by reduced susceptibility to heat. Protection could be caused by a high stroma pH or a low intra-thylakoid pH. Certain photosyn-

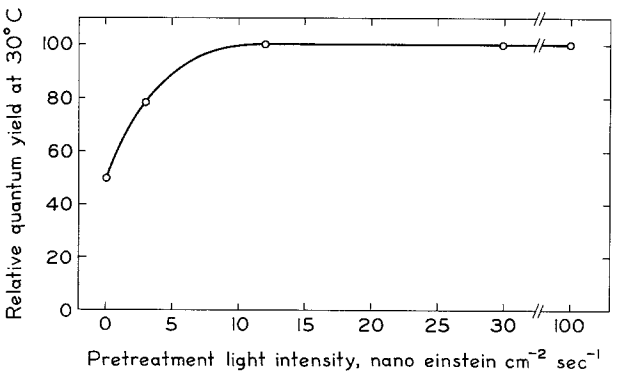

Fig. 8. Effect of light intensity during heat pretreatment upon the subsequent quantum yield for CO_2 fixation by leaves of *Tidestromia*. Pretreatment temperature, 49–50° C; pretreatment light, white from xenon-arc lamp. Conditions of measurement as in Fig. 4. *Tidestromia* grown at 35° C day, 20° C night

thetic enzymes may show different heat-deactivation kinetics at different pH values. Another possible mechanism could be related to a particular conformational state of the thylakoid membrane in strong light. The membrane may be more or less heat-susceptible in the dark or light states.

Conclusions

It appears that measurements of heat-induced fluorescence yield changes, both under F_o and F_s conditions, provide a useful tool for the study of heat-induced damage of intact leaft tissue. The method allows ranking of plant species in terms of their heat stability. Within a single species, F-T curves can distinguish between differences in the thermal environment in which the plants have been grown.

The method provides a rapid and valuable assay for the study of the heat stability of the photosynthetic apparatus and may also help elucidate some of the mechanisms involved. Since the increase in fluorescence yield is related to an increasing degree of heat damage, the kinetics of heat deactivation should be represented by T-jump induction curves (see Figs. 2 and 5). Further investigation of this aspect and also, in particular, of the light-intensity effect can be expected to tell us more about the primary sites of heat damage, the deactivation mechanisms, and possible protective mechanisms. Since chlorophyll *a* fluorescence yield responds readily to primary heat damage and chlorophyll *a*, embedded in the thylakoid membrane, serves as an intrinsic probe, one can conclude that at least some aspects of the primary damage are related to properties of this membrane. Furthermore, the above data indicate that membrane properties can change when the growth temperature of a plant is changed, presumably by adjustment of the membrane composition. It appears unlikely that primary damage is identical to irreversible membrane damage because heat-induced fluorescence changes can show a high degree of reversiblity when the quantum yield has declined irreversibly (see Fig. 5). It is more likely that some heat-induced change of membrane properties causes both the fluorescence yield increase and the denaturation of certain enzymes. Upon recooling, membrane properties and with it chlorophyll *a* fluorescence yield appear to be mostly reversible, whereas the enzymes remain denatured.

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