Plant Physiol. (1987) 84, 748–752
0032-0889/87/84/0748/05/$01.00/0

Image Analysis of Chlorophyll Fluorescence Transients for Diagnosing the Photosynthetic System of Attached Leaves

Received for publication October 15, 1986 and in revised form March 19, 1987

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ABSTRACT

A new image instrumentation system for quantitative analysis of the rapid change in intensity of chlorophyll fluorescence during dark-light transition (CFI, chlorophyll fluorescence induction), which is a sensitive indicator of the various reactions of photosynthesis, was developed and its performance was evaluated. This system made it possible to resolve CFI at any small leaf area (about 1 square millimeter) of a whole leaf when the plant was illuminated by blue-green light at more than 50 micromoles photons per square meter per second. In order to test the usefulness of this system, we applied it to analyze the effect of SO_2 on photosynthetic apparatus in attached sunflower leaves. Dynamic CFI imaging over the whole single leaf, where there was no visible injury, indicated not only the local changes in photosynthetic activity but also the site of inhibition in photosynthetic electron transport system in chloroplasts. The new instrumentation system will be useful for the analytical diagnosis of various stress-actions on plants *in situ*.

Abiotic and biotic stresses such as air pollutants, water deficit, high or low temperature, and virus infection cause a spatially heterogeneous impairment in attached leaves. The heterogeneous impairment was indicated in stomatal responses and photosynthetic activity. For example, recent investigations employing thermal imaging methods (9, 19, 21, 22) and DLE[2] imaging methods (6, 7) have shown evidence of spatially different responses of stomata *in situ* to various stresses. The DLE imaging method, furthermore, clarified localized changes in photosynthetic activity induced by the stresses (2, 5). However, these techniques did not provide any information about the site of inhibition in the photosynthetic apparatus.

Rapid changes in intensity of Chl *a* fluorescence during dark-light transition (CFI) reflect the various reactions of photosynthesis (4, 11, 13, 18, 24), especially photosynthetic electron transport system. Therefore, the measurement and analysis of CFI in plant leaves *in situ* has been developed as a sensitive and nondestructive assay for the functional state of the photosynthetic apparatus (26, 29, 31–33). The conventional methods (27, 28) give only the spatially averaged CFI at a definite area because these use point sensors. The static imaging of Chl fluorescence such as photography (8, 15) reveals the location of impairment for the whole leaf in a similar manner as the DLE imaging method, but it does not give any information involving in CFI.

We therefore developed a new instrumentation system using a CCD image sensor for a quantitative analysis of CFI, the system of which would give the information not only on the localized differences in photosynthetic activity on whole leaf *in situ* but also on the inhibition site in the photosynthetic system. In order to test the usefulness of this system, we successfully applied it to analyze the stress induced by SO_2, a major phytotoxic air pollutant (10, 12, 17), on the photosynthetic apparatus of attached sunflower leaves.

MATERIALS AND METHODS

Plant Materials. Sunflower (*Helianthus annuus* L. cv Russian Mammoth) and cucumber (*Cucumis sativus* L. cv Natsusairaku 3) plants were grown in pots (10 cm in diameter and 20 cm in height) in a controlled-environment greenhouse (1) at 25/20°C day/night temperature and 70% RH under natural lighting for 4 to 5 weeks. The pots were filled with a 4:2:4:1 (v/v) mixture of vermiculite, perlite, peat moss, and fine gravel. The plants were watered daily and nutrient solution was supplied every other day. After the test plants were moved to a controlled-environment chamber designed for studies of air pollution effects on plants (1) and allowed to acclimate to the new environmental conditions for about 5 h, attached mature leaves of the intact plants were used in the experiments.

Image Instrumentation System for Measuring Chl Fluorescence Induction. The ordinary TV camera and recording systems are not suited for a quantitative analysis of CFI in attached leaves because of their low sensitivity, large afterimage, bad image quality, AGC function, and the indistinctness in timing of the playback image. The common tungsten and fluorescent lamps also cannot be used as light sources for CFI imaging because of the unevenness and fluctuation in light intensity. The new image instrumentation system (Fig. 1) was designed to overcome these problems. A highly sensitive CCD imager with the uniformity in sensitivity and the afterimage suppression was selected for a TV camera (SONY XC-47, improved type). The image quality was improved by the use of a computer-control VTR (SONY BVU-820) with a digital time base corrector (SONY BVT-800) and the preprocessing by using a high-speed TV image processor (KCR nexus 6400). The AGC function, which changes a rela-

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[2] Abbreviations: DLE, delayed light emission; CFI, chlorophyll fluorescence induction; TV, television; AGC, automatic gain control; CCD, charge-coupled device; VTR, video tape recorder; ND, neutral density; A/D, from analog signal to digital signal; I, D, P, S, M, and T, characteristic transient levels, where I is intermediary level, D is dip, P is peak, S is quasi-stationary level, M is second maximum, and T is terminal level; ID, DP, PS, and MT, major transient characteristics, where ID is amplitude after subtracting the intensity image at the I level from that at the D level (difference D − I), and DP, PS, and MT are amplitides determined in a similar manner.

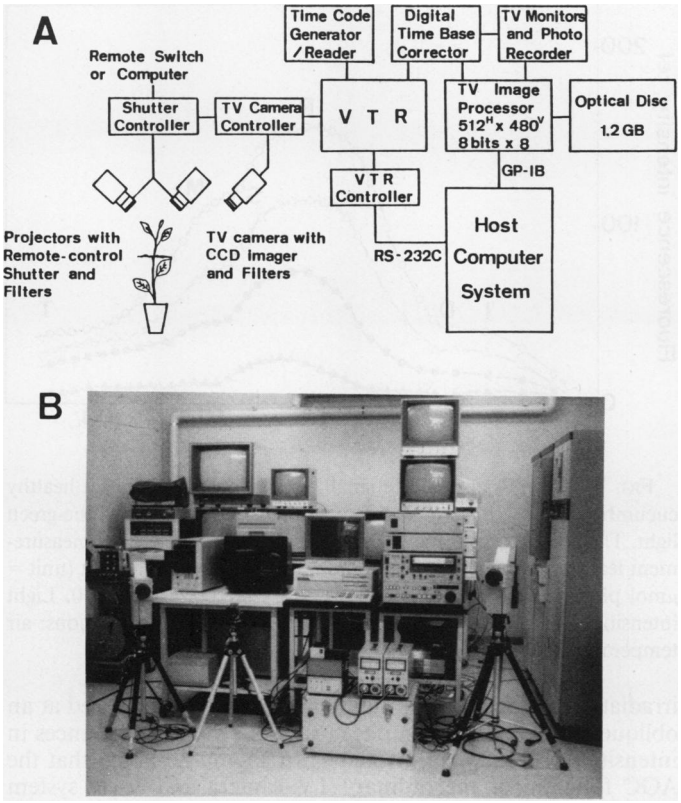

FIG. 1. Image instrumentation system for quantitative analysis of CFI in attached leaves. A, block diagram; B, photograph. This system is composed of (a) a highly sensitive CCD TV camera equipped with a red cut-off filter (Corning 2-64, >650 nm) and an interference filter (Vacuum optics co. Japan IF-W, 683 nm, half band width 10 nm); (b) two projectors of xenon lamps (Cermax LX-300F) with a remote-control shutter, a condensing lens, a blue-green filter (Corning 4-96, 380 to 620 nm), a special ND filter, and two heat-absorbing filters; (c) a computer-control VTR with a time code generator/reader and a digital time base corrector; (d) a TV image processor, which is capable of high-speed processing and 8-bit A/D conversion, with an optical disc (1.2 GB), a photorecorder and TV monitors; and (e) a host computer system.

tionship between fluorescence intensity and gray level of recorded image, was removed from the TV camera and the VTR. The timing of the playback image was exactly defined by the use of a shutter synchronized to the TV signal and by the time code recorded in each image. The uneveness in intensity of light source was improved by use of two projectors of xenon lamp (CERMAX LX-300F) attaching a special ND filter with different density in concentric circles.

The CFI was provoked by irradiation of the whole leaf with two beams of blue-green light (380–620 nm) from the projectors with band-pass filters via shutter opening after dark adaptation of the plant for 30 min. The fluorescence image was continuously measured at a TV field interval of 1/30 s by the TV camera equipped with an interference filter (683 nm; half-band width, 10 nm) and a red cut-off filter (>650 nm), and recorded with time code by the VTR. The VTR image was digitized by a video A/D converter after it was played back to a still image without guard band noise through the time base corrector. A series of the digitized images (512H × 480V 8 bits) was stored on an optical disc (National DU-15). A host computer system (MITSUBISHI MELCOM 70/40 and NEC PC9801 vm4) was used to control VTR and TV image processor.

The CFI curves, intensity images of characteristic transient levels (I, D, P, S, M, T) and amplitude images of major transient characteristics (ID, DP, PS, MT) were calculated by the TV image processor on the basis of a series of images after pre-processing for shading correction and noise removal. These were indicated by scales which correspond to the A/D conversion level.

Measurement of Chl Fluorescence Induction in SO_2-Injured Leaves. Sunflower plants were fumigated with 62.4 μmol SO_2 m^{-3} (1.5 μl l^{-1}) for 30 min in a growth chamber at 25.0°C air temperature, 70% RH, and 350 μmol photons m^{-2} s^{-1} light intensity. During the fumigation, one-half of a leaf blade was covered with a thin aluminum foil to prevent the SO_2 entrance into the plant tissue. This procedure allows the comparison between a fumigated area (F) and an unfumigated area (UF) of the same leaf (see Fig. 5). Immediately after the fumigation, the plant was kept in darkness in SO_2-free air for 30 min, then CFI images were measured. After that CFI from the same leaf was measured periodically every 60 min. The intensity of actinic blue-green light was maintained at 125 ± 6 μmol photons m^{-2} s^{-1} on the whole leaf surface unless otherwise stated. The light intensity was measured with a quantum sensor (LI-COR, Model LI-185).

RESULTS AND DISCUSSION

Performance of the Image Instrumentation System. Evaluation of the system was done first. Figure 2 shows the relationship between the intensity of artificial light in the likeness of fluorescence and the A/D conversion level of VTR image. The data indicated a linear correlation between the light intensity and the A/D conversion level. Since the artificial light was sufficiently diffused and the unevenness of the light intensity in the visual

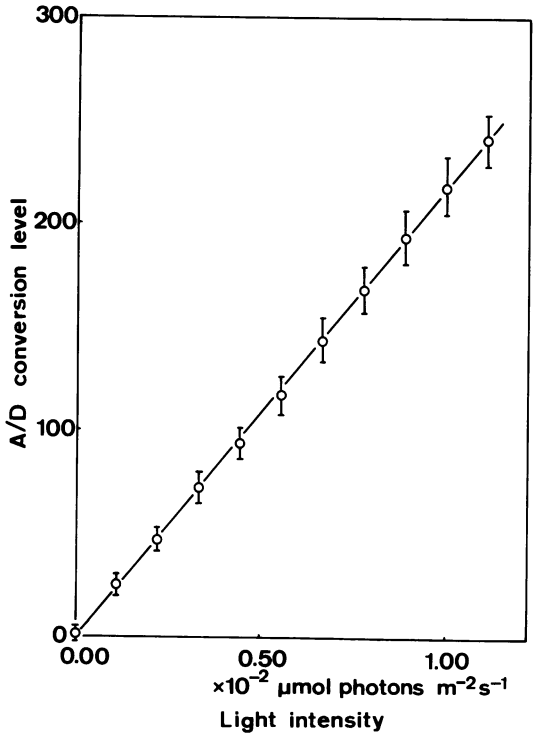

FIG. 2. Relationship between the intensity of artificial light in the likeness of fluorescence and the A/D conversion level of VTR image. Symbol O represents the mean value of A/D conversion levels of an image (512 × 480 pixels) measured at a light intensity and the vertical bar indicates ±SD. The artificial light was sufficiently diffused and the unevenness of the light intensity in the visual field of the TV camera was maintained within 0.1%. The light intensity was measured by a quantum sensor through two filters placed in front of the TV camera.

field of the TV camera was maintained within 0.1%, the standard deviation of 6.3% (maximum) in the A/D conversion level was due to image shading caused by unevenness in sensitivity of TV camera and any noise other than guard band noise of VTR. The image shading was corrected by calculating the ratio of an original image to a specific image (shading master) obtained by measuring a uniform light of definite intensity because the shading occurred mainly by lens and optical filters of TV camera. The noise was removed by the use of spatial smoothing filter and the averaging of images digitized from a still VTR image (Fig. 3). For example, the image quality could be improved to within 1% standard deviation by the shading correction and the smoothing of 3 × 3 pixels after averaging of 10 images, when the image resolution was 280 lines. The afterimage for the TV camera was about 4% at 30 ms after shutter opening and decreased to 0.3% at 50 ms.

The CFI from a defined part of the cucumber leaf *in situ* was measured by our system under different intensities of actinic blue-green light. The CFI curves were calculated on the basis of a series of fluorescence images (Fig. 4). Those clearly revealed the typical IDPSMT transients (14, 24) under light intensities from 50 to 200 μmol photons m^{-2} s^{-1} at leaf surface. In these intensity ranges of light, we could resolve IDPSMT transient from any part of leaf surface which has an area of at least 1 mm^2. Fluorescence intensities of I, D, P, S, M, and T, and rates in transients of DP, PS, and MT increased as the actinic light intensity increased. Appearance of peak P became faster with the increase of actinic light intensity.

Because the CFI is light intensity dependent as described above, it is important to illuminate the whole leaf with uniform light when we want to compare CFI curves derived from different areas (about 1 mm^2) of leaf with the single whole leaf. Combination of special ND filters and two projector lamps, which were placed at angles of 60° and 120° to the leaf surface, overcame this problem. The combination kept the spatial deviation of the intensity of actinic light within 5% over a flat surface of 20 cm diameter.

Ellenson (7) has reported a video recording system for DLE analysis but its performance was not characterized. In general, the quality of DLE image is worse than that of Chl fluorescence image because the image intensifier was used to image DLE. The

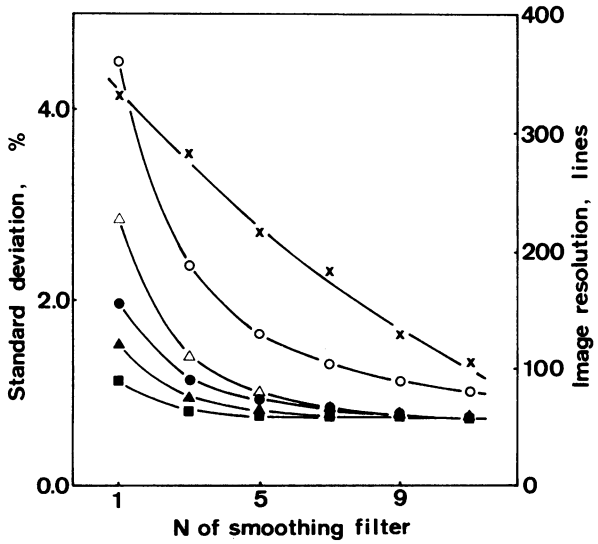

FIG. 3. Improvement in image quality by image processing. The image quality was improved by processing for shading correction and spatial smoothing after averaging of images digitized from a still VTR image. The number of images for averaging: (○) 1, (△) 2, (●) 5, (▲) 10, (■) 100, and (×) image resolution. The spatial smoothing was done by averaging of $N \times N$ pixels, where N is the size in matrix.

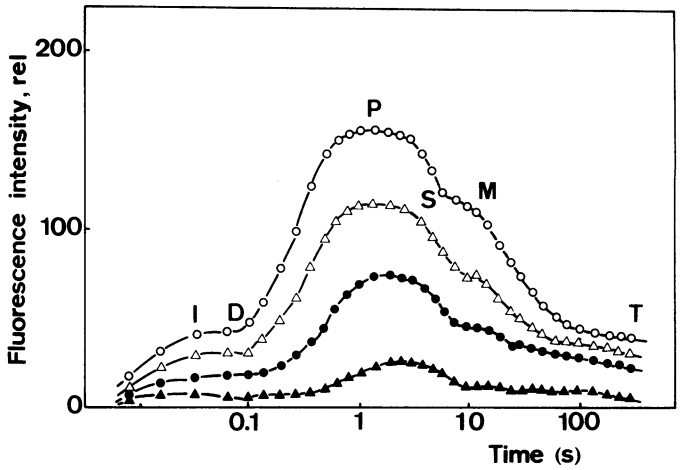

FIG. 4. The CFI curves of a small area (about 1 mm^2) of a healthy cucumber leaf *in situ* under different intensities of actinic blue-green light. The CFI was measured at the same definite area. Before measurement leaf was dark-adapted for 30 min. Intensity of actinic light (unit = μmol photons m^{-2} s^{-1}): (○) 200, (△) 150, (●) 100, and (▲) 50. Light intensities was varied using ND filters. Environmental conditions: air temperature, 25.0°C; RH, 70%.

irradiation of a leaf with a tungsten projector lamp placed at an oblique angle to the leaf surface (6) causes spatial differences in intensity of actinic light. Moreover, it should be noted that the AGC function of the ordinary TV camera and VTR system changes a linear correlation between the intensity of DLE or CFI and the A/D conversion level.

Effects of SO_2 on Photosynthetic System. Effects of SO_2 fumigation on CFI in attached sunflower leaves were analyzed by the image instrumentation system and were presented by the CFI curves and the images with gray scale (Fig. 5). In the unfumigated leaf area (UF), the CFI clearly showed the typical IDPSMT transients (14, 24) and almost identical transients at any location of leaf area. Since the CFI observed upon dark-light transition of the leaf reflects the partial reactions of photosynthesis, we can detect the alteration in photosynthetic apparatus by SO_2 from the changes in CFI curves. As shown in Figure 5, B and C, both intensity images of characteristic transient levels (I, P, M, T) and amplitude images of major transient characteristics (ID, DP, PS, MT) in fumigated counterpart (F) strikingly differed from those in unfumigated area. Fluorescence intensity at I was raised and at P reduced markedly, and that at T was increased in the fumigated area. Amplitude of fluorescence transients of DP-rise and PS- and MT-decline, indicating photosynthetic activity was reduced in the fumigated leaf. The changes in the intensity and amplitude varied with the location on the leaf surface; the effect of SO_2 was severe in locations of interveins and veinlets than in those near large veins. Contrary to the perturbation in photosynthetic apparatus shown above, there was no visible injury in the whole surface of leaf at the end of SO_2 treatment and 2 d later.

The significance of the changes in CFI induced by SO_2 fumigation was as follows. Because fluorescence intensity in the early induction phenomena is regulated by the redox state of Q, a primary electron acceptor of PSII (4, 18, 24), the elevated I level suggest that some portion of Q was brought to reduced state by the SO_2-fumigation. Since the DP rise in CFI reflects photoreduction of Q through reductant from H_2O (24, 26), a diminished rise of DP was consistent with the inactivation of the water-splitting enzyme system (29). Since PS decline involves energy-dependent quenching (13), the suppression of PS decline suggested the depression of formation of *trans*-thylakoid proton gradient probably due to the inactivation of the water-splitting

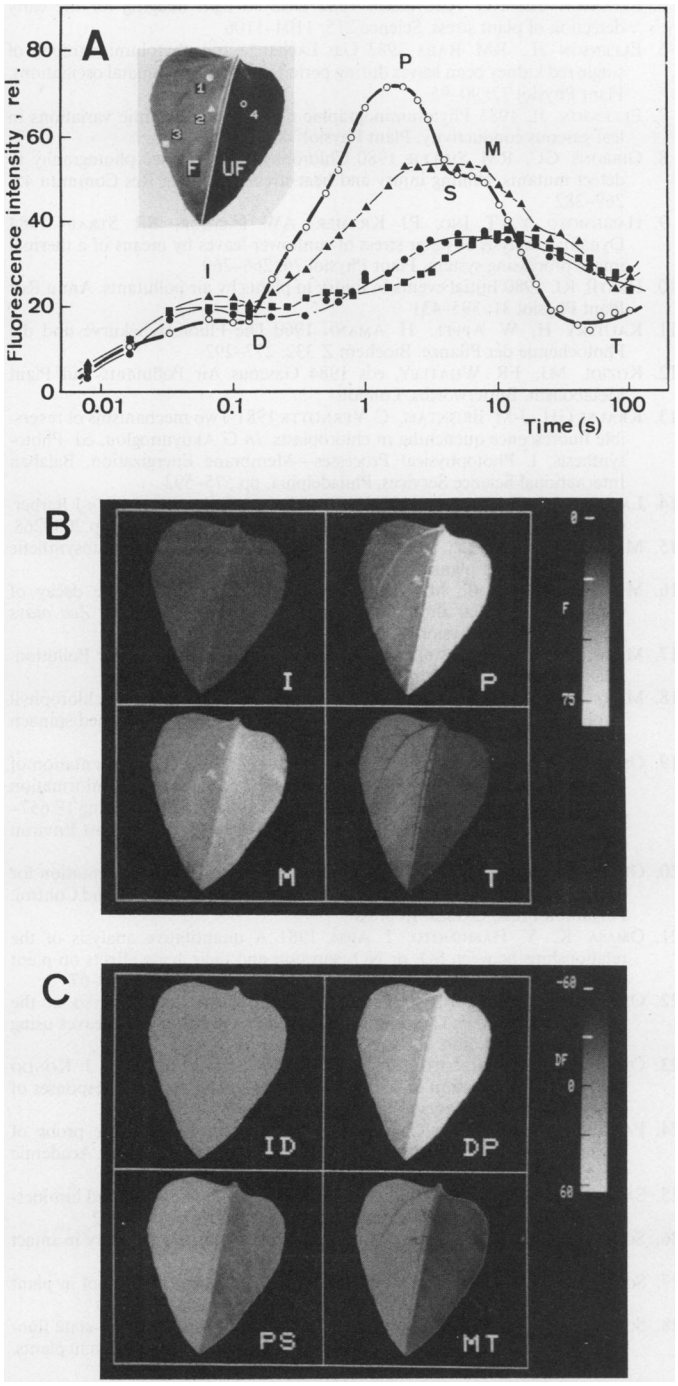

FIG. 5. Effect of SO_2 on CFI in an attached sunflower leaf. Sunflower plant was fumigated with 62.4 μmol SO_2 m^{-3} (1.5 μl l^{-1}) at 25.0°C air temperature, 70% RH, and 350 μmol photons m^{-2} s^{-1} light intensity for 30 min. After dark adaptation for 30 min, CFI of an attached leaf was measured under 125 μmol photons m^{-2} s^{-1} actinic light. A, CFI curves at different sites in fumigated area (F: ●, interveinal site 1; ▲, site 2 near a large vein; ■, site 3 near a veinlet) and unfumigated area (UF: ○, interveinal site 4). The vein and corresponding sites (1 to 4) are denoted in a photograph of the whole leaf. B, intensity images of characteristic transient levels (I, P, M, T). C, amplitude images of major transient characteristics (ID, DP, PS, MT).

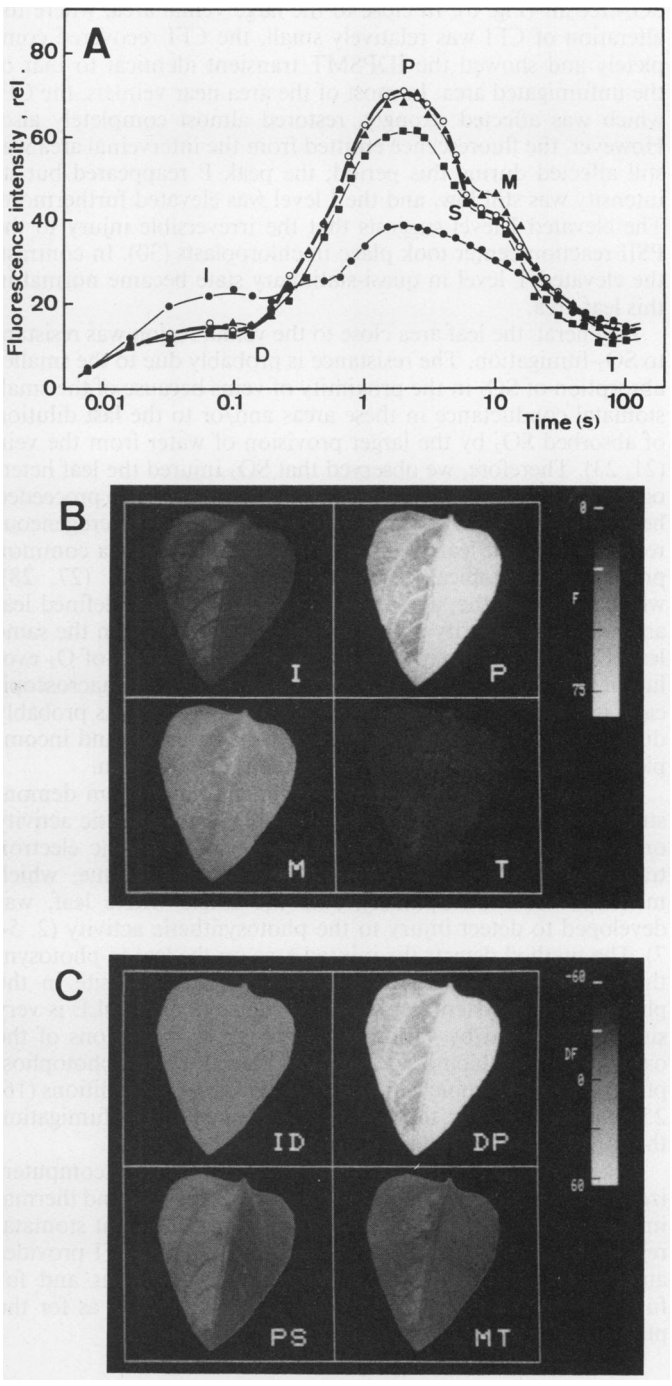

FIG. 6. Recovery of CFI from disturbances by SO_2. After the measurement described in Figure 5, the same plant was kept at 25.0°C air temperature and 70% RH in darkness and SO_2-free air. Then measurement of CFI was repeated for the same leaf every 60 min. The CFI measured at 6 h after the initiation of SO_2 fumigation was presented. A, CFI curves at leaf sites marked in Figure 5A. B, intensity images of characteristic transient levels. C, amplitude images of major transient characteristics. Symbols are the same as those in Figure 5.

enzyme system. However, the possibility that the PS decline was affected by the inhibition of electron flow from Q to PSI cannot be excluded because PS decline partly reflects the oxidation of Q by PSI (3). Suppression of MT decline was probably due to the inhibition of the *trans*-thylakoid proton gradient formation in addition to unidentified reactions in chloroplasts (3, 24). Although the extent of the SO_2-effect on CFI differed from area to area on a single leaf, the mode of SO_2 action was essentially the same.

The recovery from perturbation by SO_2 becomes evident by monitoring the CFI when the fumigated plants were allowed in

SO_2-free air (Fig. 6). In close to the large veinal area, where the alteration of CFI was relatively small, the CFI recovered completely and showed the IDPSMT transient identical to that of the unfumigated area. In most of the area near veinlets, the CFI which was affected strongly, restored almost completely also. However, the fluorescence emitted from the interveinal area was still affected during this period; the peak P reappeared but its intensity was still low, and the I level was elevated furthermore. The elevated I level suggests that the irreversible injury to the PSII reaction center took place in chloroplasts (30). In contrast, the elevated T level in quasi-stationary state became normal in this leaf area.

In general, the leaf area close to the veinal region was resistant to SO_2-fumigation. The resistance is probably due to the smaller absorption of SO_2 in the proximity of veins because of the small stomatal conductance in these areas and/or to the fast dilution of absorbed SO_2 by the larger provision of water from the vein (21, 23). Therefore, we observed that SO_2 injured the leaf heterogeneously and the recovery from the SO_2 injury also proceeded heterogeneously on the same leaf. Although the heterogeneous response of plant leaf to the environmental stress is a common process microscopically, the conventional method (27, 28), which measures the 'averaged' CFI derived from a defined leaf area, could not clarify a spatially different response in the same leaf. For example, we described the partial recovery of O_2 evolution and CFI from the SO_2 injury in spinach leaf macroscopically in previous work (29). Such a partial recovery is probably due to the combination ('average') of the complete and incomplete recovery as indicated in the present investigation.

As shown above, our image instrumentation system demonstrated not only the affected location in photosynthetic activity on leaf but also the inhibition site in photosynthetic electron transport system. The phytoluminographic technique, which measures the DLE spatially over the single whole leaf, was developed to detect injury to the photosynthetic activity (2, 5–7). The method detects the injured area on the leaf in photosynthetic activity but does not clarify the inhibition site in the photosynthetic system because the intensity of the DLE is very similarly reduced by various factors such as inhibitions of the oxidizing and reducing sides of PSII, uncoupling of photophosphorylation, anaerobic conditions, and high CO_2 conditions (16, 25). On the contrary, the CFI imaging points to SO_2 fumigation that inhibits the oxidizing side of PSII.

Together with previously developed techniques of computerized image processing, such as DLE imaging (2, 5–7) and thermal imaging (9, 19, 20), which provide information about stomatal response and gas exchange, the image analysis of CFI provides an integrative approach for early warning diagnosis and for functional analysis of disorders during stress as well as for the plant's capacity to recover.

LITERATURE CITED

1. AIGA I, K OMASA, S MATSUMOTO 1984 Phytotrons in the National Institute for Environmental Studies. Res Rep Natl Inst Environ Stud Jpn 66: 133–155
2. BJORN LO, AS FORSBERG 1979 Imaging by delayed light emission (photoluminography) as a method for detecting damage to the photosynthetic system. Physiol Plant 47: 215–222
3. BRADBURY M, NR BAKER 1981 Analysis of the slow phases of the *in vivo* chlorophyll fluorescence induction curve. Changes in the redox state of photosystem II electron acceptors and fluorescence emission from photosystems I and II. Biochim Biophys Acta 635: 542–551
4. DUYSENS LNM, HE SWEERS 1963 Mechanism of two photochemical reactions in algae as studied by means of fluorescence. *In* Japanese Society of Plant Physiologists, ed, Microalgae and Photosynthetic Bacteria. University of Tokyo Press, Tokyo, pp 353–372
5. ELLENSON JL, RG AMUNDSON 1982 Delayed light imaging for the early detection of plant stress. Science 215: 1104–1106
6. ELLENSON JL, RM RABA 1983 Gas Exchange and phytoluminography of single red kidney bean leaves during periods of induced stomatal oscillations. Plant Physiol 72: 90–95
7. ELLENSON JL 1985 Phytoluminographic detection of dynamic variations in leaf gaseous conductivity. Plant Physiol 78: 904–908
8. GIBBONS GC, RM SMILLIE 1980 Chlorophyll fluorescence photography to detect mutants, chilling injury and heat stress. Carlsberg Res Commun 45: 269–282
9. HASHIMOTO Y, T INO, PJ KRAMER, AW NAYLOR, BR STRAIN 1984 Dynamic analysis of water stress of sunflower leaves by means of a thermal image processing system. Plant Physiol 76: 266–269
10. HEATH RL 1980 Initial events in injury to plants by air pollutants. Annu Rev Plant Physiol 31: 395–431
11. KAUTSKY H, W APPEL, H AMANN 1960 Die Fluoreszenzkurve und die Photochemie der Pflanze. Biochem Z 332: 277–292
12. KOZIOL MJ, FR WHATLEY, eds 1984 Gaseous Air Pollutants and Plant Metabolism. Butterworths, London
13. KRAUSE GH, J-M BRISNTAIS, C VERNOTTE 1981 Two mechanisms of reversible fluorescence quenching in chloroplasts. *In* G Akoyunoglou, ed, Photosynthesis. I. Photophysical Processes—Membrane Energization. Balaban International Science Services, Philadelphia, pp 575–593
14. LAVOREL J, A-L ETIENNE 1977 *In vivo* chlorophyll fluorescence. *In* J Barber, ed, Primary Processes of Photosynthesis. Elsevier, Amsterdam, pp 203–268
15. MILES CD, DJ DANIEL 1973 A rapid screening technique for photosynthetic mutants of higher plants. Plant Sci Lett 1: 237–240
16. MORITA S, S ITOH, M NISHIMURA 1981 Acceleration of the decay of membrane potential after energization of chloroplasts in intact *Zea mays* leaves. Plant Cell Physiol 22: 205–214
17. MUDD JB, TT KOZLOWSKI, eds 1975 Responses of Plants to Air Pollution. Academic Press, New York
18. MURATA N, M NISHIMURA, A TAKAMIYA 1966 Fluorescence of chlorophyll in photosynthetic systems. II. Induction of fluorescence in isolated spinach chloroplasts. Biochim Biophys Acta 120: 23–33
19. OMASA K, F ABO, I AIGA, Y HASHIMOTO 1981 Image instrumentation of plants exposed to air pollutants—quantification of physiological information included in thermal infrared images. Trans Soc Instrum Control Eng 17:657–663 (in Japanese and English summary) also in Res Rep Natl Inst Environ Stud Jpn 66: 69–79 (1984) (in English translation).
20. OMASA K, I AIGA Environmental measurement: image instrumentation for evaluating plant stress. *In* M Singh, ed, Encyclopedia of Systems and Control. Pergamon Press, Oxford. In press
21. OMASA K, Y HASHIMOTO, I AIGA 1981 A quantitative analysis of the relationships between SO_2 or NO_2 sorption and their acute effects on plant leaves using image instrumentation. Environ Control Biol 19: 59–67
22. OMASA K, Y HASHIMOTO, I AIGA 1981 A quantitative analysis of the relationships between O_3 sorption and its acute effects on plant leaves using image instrumentation. Environ Control Biol 19: 85–92
23. OMASA K, Y HASHIMOTO, PJ KRAMER, BR STRAIN, I AIGA, J KONDO 1985 Direct observation of reversible and irreversible stomatal responses of attached sunflower leaves to SO_2. Plant Physiol 79: 153–158
24. PAPAGEORGIOU G. 1975 Chlorophyll Fluorescence: An intrinsic probe of photosynthesis. *In* Govindjee, ed, Bioenergetics of Photosynthesis. Academic Press, New York, pp 319–371
25. SATOH K, S KATOH 1983 Induction kinetics of millisecond-delayed luminescence intact *Bryopsis* chloroplasts. Plant Cell Physiol 24: 953–962
26. SCHREIBER U 1978 Chlorophyll fluorescence assay for ozone injury in intact plants. Plant Physiol 61: 80–84
27. SCHREIBER U 1983 Chlorophyll fluorescence yield changes as a tool in plant physiology. I. The measuring system. Photosynth Res 4: 361–373
28. SCHREIBER U, L GROBERMAN, W VIDAVER 1975 Portable, solid-state fluorometer for the measurement of chlorophyll fluorescence induction in plants. Rev Sci Instrum 46: 538–542
29. SHIMAZAKI K, K ITO, N KONDO, K SUGAHARA 1984 Reversible inhibition of the photosynthetic water-splitting enzyme system by SO_2-fumigation assayed by chlorophyll fluorescence and EPR signal *in vivo*. Plant Cell Physiol 25: 795–803
30. SHIMAZAKI K, K SUGAHARA 1980 Inhibition site of the electron transport system in lettuce chloroplasts by fumigation of leaves with SO_2. Plant Cell Physiol 21: 125–135
31. SIVAK MN, DA WALKER 1985 Chlorophyll *a* fluorescence: can it shed light on fundamental questions in photosynthetic carbon dioxide fixation? Plant Cell Environ 8: 439–448
32. SMILLIE RM, SE HETHERINGTON 1983 Stress tolerance and stress-induced injury in crop plants measured by chlorophyll fluorescence *in vivo*. Plant Physiol 72: 1043–1050
33. VERTUCCI CW, JL ELLENSON, AC LEOPOLD 1985 Chlorophyll fluorescence characteristics associated with hydration level in pea cotyledons. Plant Physiol 79: 248–252