hepatoma (H-4-II-E-C3) and a mouse hepatoma (HEPA 1), as well as on primary rat hepatocyte cultures. At a 1:100 dilution of the 217c antibody, this binding was insignificant. Direct tissue binding experiments (Table 2) demonstrated that the 217c antibody binds to a C6 glioma cell pellet, as well as to transformed oligodendrocyte (TOP-ET-1) tumor homogenates, but does not bind to normal rat brain, thymus, or spleen. Some binding to liver tissue was observed, but the nature of the binding sites needs further study.

We selected a monoclonal antibody that demonstrates the existence of a cell surface antigen on transformed glial cells and glial tumor homogenates and its absence on nontransformed cells or tissue homogenates from most rat organs. Various investigators have previously referred to brain tumor–specific antigens (*9*), but these studies employed heterospecific antiserums that contain contaminating antibodies. The development of monoclonal antibody methodology (*10*) has permitted investigators to ascertain with greater specificity the existence of tumor-specific antigens on various neoplastic tissues (*11, 12*).

A monoclonal antibody against human glioma cell lines has been described recently (*13*), but these investigators did not demonstrate the presence of the antigen on tumor tissue. We have shown that this antigen is absent on the normal glial cell line but is expressed after these cells undergo neoplastic transformation. Therefore, the importance of a specific cell surface antigen during transformation can now be explained. This antibody also may have immunotherapeutic applications.

For example, Herlyn *et al.* (*12*) demonstrated the inhibition of colorectal carcinoma growth in nude mice by a monoclonal antibody against colorectal carcinoma cells. In addition, a new technique of conjugating diphtheria toxin A chain or ricin A chain to a specific antibody has been reported. The antibody will direct the action of A chain to its specific target cell and thus the conjugate becomes a very potent cell type–specific toxin (*14*). If conjugated ricin A–217c antibody can be developed, it might ultimately be used as a brain tumor–specific therapeutic agent.

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Delayed Light Imaging for the Early Detection of Plant Stress

Abstract. *Image-intensified photographs of delayed light emission (DLE) from soybean leaves exposed to sulfur dioxide showed evidence of the stress that developed during the exposure period. A comparison of DLE images taken during the fumigation with a conventional photograph taken 5 days later showed a clear correspondence between leaf areas that had the most diminished DLE intensity and those that showed the greatest visible injury. These results suggest that DLE imagery will be a useful tool in the investigation of the spatial distribution and temporal development of plant stress.*

Exposing plant tissue to an acute dose of SO_2 results in irreversible foliar injury most often characterized by interveinal chlorosis in broadleaf species (*1*). This example of spatially heterogeneous response to plant stress is apparently the result of irreversible perturbations in local physiological and metabolic phenomena. Heretofore, little use has been made of the spatial and temporal development of stress characteristics to elucidate plant response to stress. This deficiency is due largely to a lack of experimental techniques that can detail changes in physiological variables over small (~ 1 mm^2) areas of the whole leaf. Two recently developed methods should help eliminate these shortcomings.

One method, described by Omasa *et al.* (*2*), is a thermal scanning technique that permits spatial mapping of leaf surface temperatures. Since areas of higher or lower temperature correspond to areas of lower or higher transpiration (*2*), respectively, a spatial mapping of surface temperatures can be used to locate differences in transpirational activity and hence differences in gas exchange.

The second development is a method (*3, 4*) by which one can detect stress-induced biochemical or physiological changes in a leaf well before stress symptoms become apparent by measuring delayed light emission (DLE). The DLE phenomenon is universal in green plants and results when light-generated photosynthetic intermediates recombine in the dark to produce an electronically excited state of chlorophyll that gives rise to fluorescence (*5*). The emission originates from chlorophyll molecules associated with the photosystem II (oxygen-evolving) portion of the photosynthetic electron transport chain and is a very sensitive indicator of the status of photosynthetic electron transport (*5*).

Delayed light emission may only be observed in the dark after a period of illumination because its spectrum is identical to and its intensity is much less than that of light-generated chlorophyll fluorescence (*5*). Furthermore, the low intensity of DLE does not permit photography by conventional means (*3*). However, the relatively recent development of image intensifiers has provided a method for recording the spatial distribution of DLE in plants. Björn and Forsberg (*4*) used such a device to record the distribution of DLE from leaves that had been subjected to a number of physiological and pathological stresses. We report here the results of experiments in which this method was used to explore the

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temporal and spatial aspects of acute stress induced by SO_2 in soybean leaves (*Glycine max* L. cultivar Beeson) before foliar lesions developed.

The procedure for illuminating plant samples and recording DLE is similar to that described by Björn and Forsberg (*4*). In our system, however, we use a special two-compartment cuvette designed so that one half of the leaflet can be exposed to SO_2 and gas exchange can be monitored for either half (*6*).

In our procedure, a single terminal leaflet of an attached soybean trifoliolate leaf was enclosed in the cuvette. The DLE images of the soybean leaf in Fig. 1 were taken during the SO_2 fumigation sequence described in Fig. 2D. Two fumigations occurred, one lasting for 15 minutes followed by 50 minutes without SO_2 exposure and a second SO_2 fumigation that lasted for 170 minutes. The SO_2 concentration was 2.0 parts per million (ppm) (by volume) for both fumigations.

The sequence of photographs in Fig. 1 includes a series of six DLE images taken during the fumigation sequence (Fig. 1, a through f, taken at the times marked on Fig. 2A), one DLE image taken 18 hours after the fumigation event (Fig. 1g), and a conventional photograph showing visible injury taken 5 days after the fumigation (Fig. 1h). Measurements of CO_2, SO_2, and leaf resistance and an analog recording of total DLE intensity are shown in Fig. 2.

During the first exposure a nearly fivefold increase of total DLE (see Fig. 2A) occurred immediately in the half-leaf exposed to SO_2. This enhanced DLE was most likely due to the SO_2-induced inhibition of electron transport through photosystem II (*7*) and occurred over the entire leaf area exposed to SO_2, although to a lesser extent in the vascularized areas (Fig. 1b). There was a 60 percent decrease in CO_2 uptake during the first exposure (Fig. 2B); a 95 percent recovery of CO_2 uptake was eventually obtained over a 50-minute period without exposure. The total DLE returned to the prefumigation level during this interim (Fig. 2A), and there was also no obvious visible difference between the prefumigation DLE image (Fig. 1a) and the image taken just prior to the second fumigation (Fig. 1c). Leaf resistance (*8*), which had increased from 2.3 to 3.2 sec cm^{-1} within 25 minutes after the first exposure began, returned to the prefumigation value by the time of the second fumigation.

The second fumigation caused an even more rapid and dramatic increase in DLE and a more complete reduction in CO_2 uptake (Fig. 2, A and B). However, leaf resistance, which increased almost immediately upon SO_2 exposure as in the first fumigation, continued to decrease for about 10 minutes (to 2.2 sec cm^{-1}) before increasing again. The greater increase in DLE during the second fumigation is most likely due to the incomplete metabolic recovery of the leaf from the first fumigation. Such differences in DLE response may thus prove to be an effective measure of the degree to which one stress event is metabolically related to a second.

A very heterogeneous DLE intensity distribution also developed over the SO_2-exposed leaf surface during the second fumigation (Fig. 1, d through f).

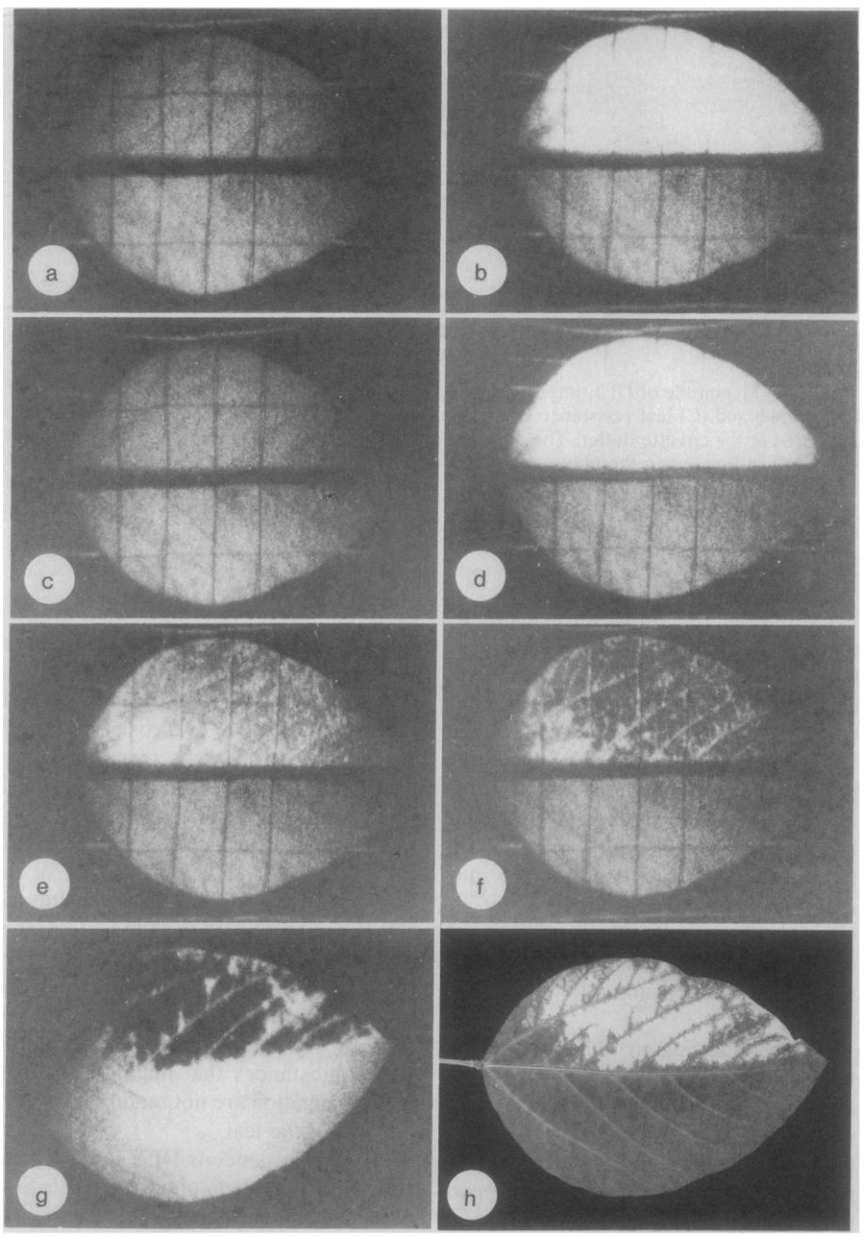

Fig. 1. (a) through (f) Delayed light emission (DLE) images taken during the course of an SO_2 fumigation event described in Fig. 2. Images were recorded 5 minutes prior to (a) and at 10 (b), 65 (c), 85 (d), 140 (e), and 280 (f) minutes from the beginning of the fumigation event (see Fig. 2A). (g) A DLE image taken 18 hours after the end of the fumigation period. A longer photographic exposure was used to record (g), accounting for the apparently higher level of emission from the unexposed half of the leaf as compared to (a) through (f). The thin horizontal and vertical lines in (a) through (f) are due to support wires within the cuvette; the thicker horizontal line is due to the cuvette partition. (h) Normal reflected-light photograph of the same leaf taken 5 days after the fumigation, by which time the visible injury was fully developed. Note the correspondence between areas still showing significant emission intensity in the fumigated part of the leaf, (f) and (g), and leaf areas still alive 5 days later (h). The leaf sample was the youngest fully expanded middle trifoliolate of a 4-week-old soybean plant. The leaf remained attached to the plant throughout the experiment. A rotating (40-Hz) sectored wheel allows DLE to be observed every 25 msec for a 10-msec period when the leaf is in the dark. Images were recorded over a 0.5-second period representing 20 separate cycles.

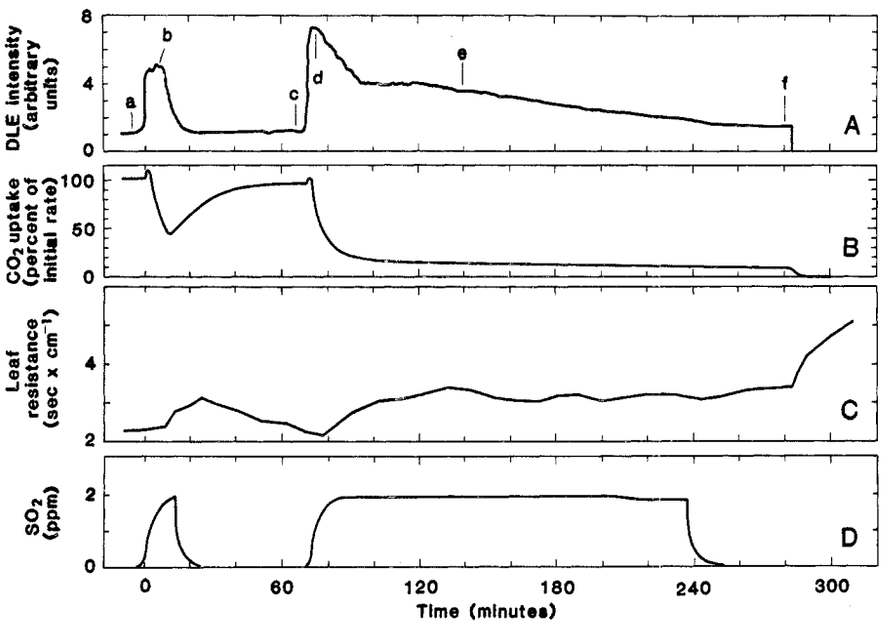

Fig. 2. (A) Magnitude of DLE intensity integrated over the half of the leaf exposed to SO_2; (B) CO_2 uptake and (C) leaf resistance of the same leaf half; (D) profile of the SO_2 concentration measured at the cuvette outlet. The pointers labeled *a* through *f* along the curve in (A) signify the points in time when the photographs in Fig. 1, a through f, were taken. The leaf had been exposed to an alternating (40-Hz) light-dark cycle for 2 hours prior to the first SO_2 exposure and had reached a steady-state CO_2 uptake rate of 13.5 mg dm^{-2} hour^{-1}. Average illumination over each cycle was approximately 600 μE m^{-2} sec^{-1}.

Areas that had begun to show distinctly diminished DLE intensity after 1 to 2 hours of exposure continued to show a decline in DLE intensity even after the exposure ended (not shown). However, there was no sign of injury to the leaf at the end of the fumigation. No recovery of CO_2 uptake or significant change in leaf resistance was observed when the fumigation was terminated (at 235 minutes). However, leaf resistance increased substantially when the light was extinguished (at 283 minutes), an indication of stomatal closure at that time (Fig. 2C).

A DLE image taken 18 hours after the fumigation (Fig. 1g) exhibited distinct areas of little or no DLE, which corresponded to water-soaked and chlorotic areas that had begun to appear by this time. The apparent high emission shown in the fumigated half of the leaf is a result of a longer photographic exposure, which provided greater detail of DLE features in the exposed half of the leaf. A visible-light photograph taken 5 days after the fumigation shows areas of fully developed necrosis resulting from the fumigation (Fig. 1h). There is a striking correspondence between areas that showed enhanced DLE in Fig. 1, f and g, and the areas still green 5 days later (Fig. 1h).

In these and other experiments, leaf areas that had developed noticeable SO_2-induced injury within 24 hours showed a time-dependent pattern of DLE that was characteristically different from that of areas of the same leaf that were less severely affected. Necrosis invariably was observed in areas that had shown the type of irreversible decline in DLE evidenced in Fig. 1, e through g. These areas tended to be interveinal. Acute injury failed to develop in areas of the leaf that did not show this transition in DLE by the end of the fumigation period. Moreover, we have never observed any evidence for injury spreading beyond the area of the leaf that was exposed; this finding suggests that soluble toxic substances that might result from SO_2 fumigation are not readily transported within the leaf.

The heterogeneous DLE intensity distribution that developed over the SO_2-exposed leaf surface is a feature that the integrated emission curve (Fig. 2A) totally fails to convey. Figure 2, A, B, and C, should thus be regarded with the primary limitation in mind that these curves reflect integrated values of phenomena that are inherently heterogeneous over the whole leaf and do not provide the more meaningful, localized detail.

Effects of DLE can also be observed at SO_2 concentrations that are considerably lower than 2 ppm. For instance, with SO_2 at a concentration of 0.5 ppm there was still a twofold increase in DLE (and a 30 percent reduction in CO_2 uptake) observed during a 15-minute fumigation. For any given concentration of SO_2, however, the magnitude of the initial DLE increase depends largely on the physiological state of the leaf. For instance, those leaves having relatively low leaf resistances have invariably shown the greatest initial increase in DLE, undoubtedly a manifestation of their greater rate of uptake of SO_2.

Although DLE effects are obviously very sensitive to the presence of SO_2, we do not claim that the DLE phenomenon is a more effective indicator of photosynthetic stress per se than CO_2 uptake or other conventional physiological measurements. The important consideration is that DLE imagery is capable of identifying where stress is occurring. Thus, DLE imagery reflects the true spatial distribution of irreversible stress-induced effects as evidenced by the subsequent development of foliar necrosis. One appealing aspect of these measurements is that, by observing changes in the intensity of DLE from specific areas of the leaf, one may begin to characterize these changes and determine at what point in a stress event those areas sustain irreversible damage. Then, by combining DLE results with more conventional gas exchange and biochemical techniques, one might determine the sequence of events that leads from initial photosynthetic stress to the detailed appearance of visible necrosis or chlorosis. This is a new capability in the area of plant stress analysis.

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