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https://doi.org/10.1017/S155192950005642X Published online by Cambridge University Press

Endogenous Fluorescence Identifies Dead Cells In Plants

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Various fluorescent stains and vital dyes have been used to identify dead cells in animal tissues and cell lines.[1,2] In plants, fluorescein diacetate and propidium iodide have been used to label nuclei and to identify necrotic cells in plant protoplasts[3,4] and 4,6-diamidino-2-phenylindole (DAPI) has been used to mark senescing cells in sections of roots.[5] However, these dyes may be problematic when used with intact plant tissue with well-developed cells walls which may impede dye penetration. Endogenous fluorescence has been used to identify dead cells in intact and sectioned plant tissues.[6,7,8,9] Published procedures typically employ ultraviolet (UV) excitation wavelengths of 340-380 nm and emission wavelengths of 400-425 nm,[8] thus requiring a UV filter set. The tissue may also require processing prior to evaluation in the UV fluorescence microscope.[7]

We report here a simpler approach for identifying dead and damaged cells in plant tissue using endogenous fluorescence. We use a standard Zeiss fluorescein filter set with excitation wavelength of 450-490 nm and emission wavelength of 515-565 nm. Our method does not require a UV fluorescence microscope or any special tissue processing. We visualize the endogenous fluorescence with a low magnification 6.3 X objective on a Zeiss research light microscope equipped with a fluorescein filter set and a mercury vapor lamp. Our procedure does not require any special fixation or mounting fluids; the freshly dissected tissue is simply held flat between two glass slides and viewed.

Using these conditions, we have observed endogenous fluorescence in situations in which cell death would be anticipated in plant leaves. For example, when a wound is made in a leaf (we used leaves from the velvetleaf plant, Abutilon theophrasti) by poking a hole in the leaf blade with forceps tips, the dead cells around the edge of the wound fluoresce (Figure IA). In addition, fluorescence can be used to identify dead or dying cells which occur as part of the aging process in plants. Figure IB illustrates fluorescent aggregates of senescent cells near the edge of a velvetleaf plant leaf. Plants with leaf mottling caused by environmental stresses such as dehydration or abrasion exhibit fluorescence in the mottled areas. We have observed endogenous fluorescence marking damaged cells in leaves from a variety of dicotyledons and monocotyledons, however the method works best for plants without thick or waxy leaves. Endogenous fluorescence is also a useful marker in evaluating cell viability in plant tissues following experimental treatments. The inset in Figure IC shows a discolored patch of damaged tissue on the leaf of a velvetleaf plant following exposure to a drop of glyphosate herbicide diluted in tap water. The herbicide itself does not fluoresce and tap water by itself on the leaf does not cause fluorescence but the herbicide treated area, as shown in Figure IC, fluoreses brightly.

Light and scanning electron microscopy have been employed to show that fluorescent regions consist of dead or damaged cells. By marking the boundaries of fluorescence in environmentally stressed or glyphosate-treated leaves with pin-pricks immediately prior to fixation for microscopy and then using the pin-pricks to mark the fluorescent areas, we have observed in both thick and thin plastic sections that these regions contain necrotic tissue, and that the necrotic areas match the fluorescence areas. By scanning electron and light microscopy the surface of a velvetleaf plant leaf has a cobblestone appearance (asterisk in Figure 2B), and the upper epidermis (UE in Figures 2B,D) consists of intact cells with large central vacuoles. However, in the damaged (fluorescent) regions of a herbicide-treated leaf, the upper epidermis has collapsed, the cell walls have become thin and attenuated, and cellular debris is present in the central vacuoles (Figures 2A,C). The pallisade and spongy mesophyll cells (PM and SM) and, to a lesser extent, the lower epidermis (LE) also become disrupted and exhibit cytolysis and pycnosis (Figures 2A,C). In contrast, the leaf cells from control plants treated with tap water alone are intact with large central vacuoles (Figures 2B,D). Leaves from environmentally stressed plants (*i.e*, plants suffering from dehydration, abrasion or poor soil) also exhibit cytolysis and pycnosis in the fluorescent regions. We have confirmed by microscopy that small fluorescing spots in a herbicide-treated leaf exhibit cytolysis and vacuolar collapse in the area of fluorescence and therefore endogenous fluorescence appears to be a sensitive method to identify individual or small groups of dead or damaged cells in plant leaves.

The cytological/biochemical basis for endogenous fluorescence in dead and damaged leaf cells is not known. Of the major plant pigments (carotenoids, chlorophyll a, chlorophyll b, and phycocyanin), both carotenoids and chlorophyll b absorb in the excitation wavelength of the fluorescein filter;[10] however, whether these pigments are involved is unknown.

Leaves were not processed prior to looking for fluorescence. Processing for light and scanning electron microscopy employed standard procedures. Micrographs were taken from freshly dissected leaf whole mounts, with the upper surface uppermost and the leaf held flat between two glass slides, using a low (6.3x) magnification objective and a standard light microscope equipped with a fluorescein filter set as described above. For light microscopy, 0.5 µm cross-sections of plastic-embedded leaf tissue were dried onto glass slides, stained with toluidine blue, cover-slipped and photographed. The inset in Figure IC is a whole mount which was

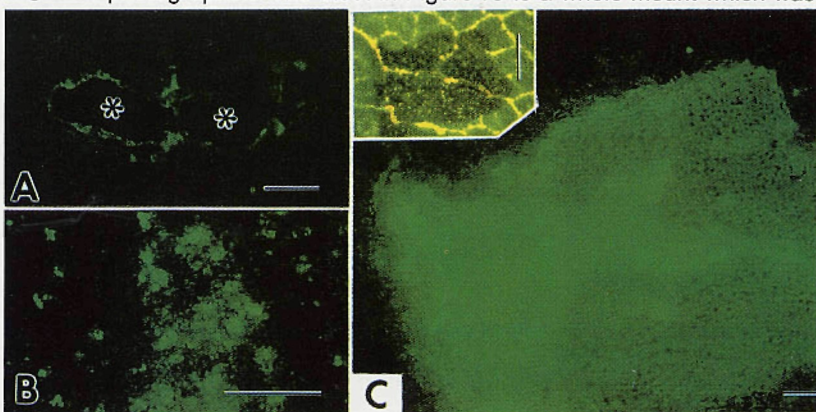

Figure 1. Endogenous fluorescence in dead plant cells. A. Fluorescence appears within minutes around the edges of a hole (asterisks) in a velvetleaf plant leaf made by poking the leaf with forceps. B. Spotty and patchy fluorescence from dead cells near the leaf margin in a senescing velvetleaf plant. C. Endogenous fluorescence in a leaf from a velvetleaf plant following exposure to a drop of herbicide. Inset. Lower magnification light micrograph of the damaged area illustrated in C. A, IOx, bar 1 mm; B and C, 58x, bars = 250 µm; Inset, 17x, bar = 500 µm.

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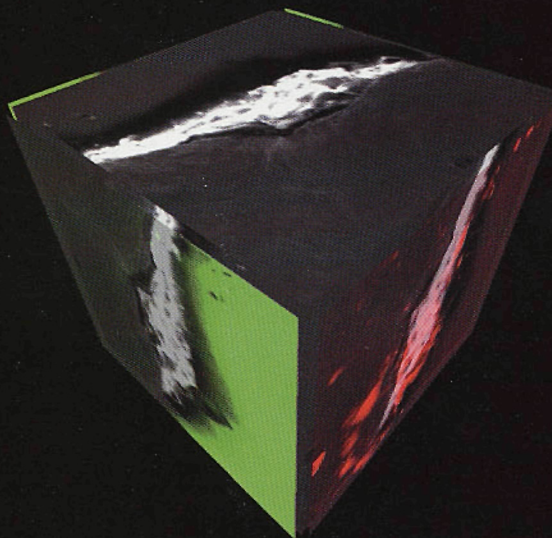

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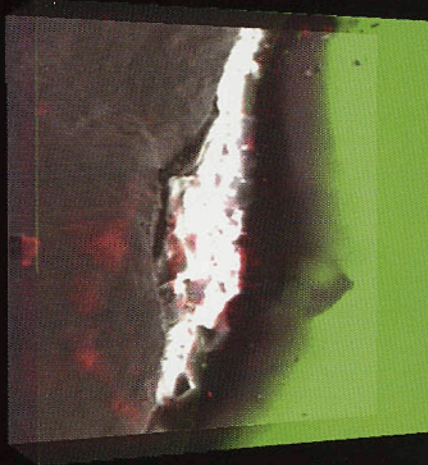

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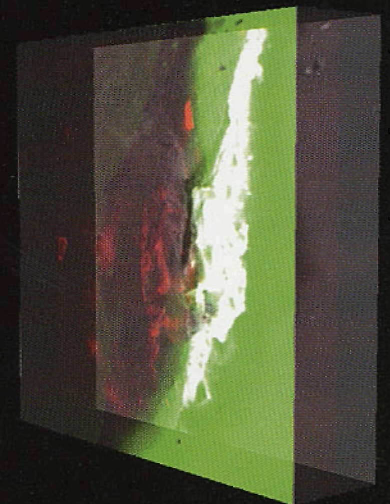

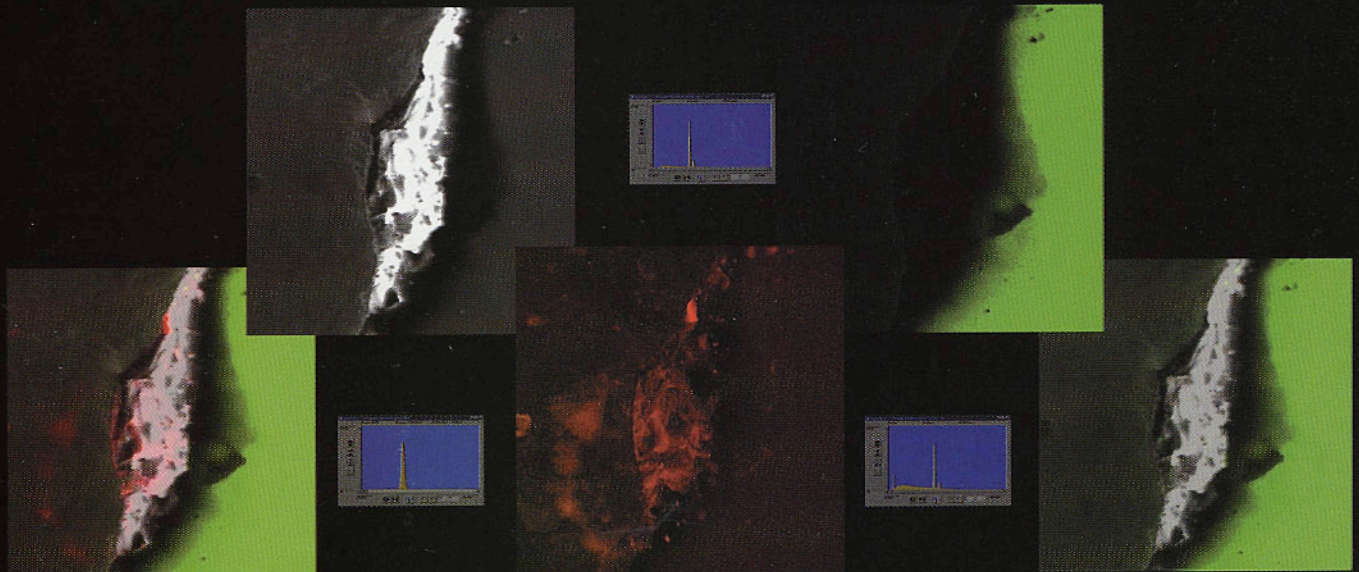

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photographed with a Zeiss research dissecting microscope and bright field illumination. The micrographs were taken with Kodak Elite II 400 ASA color slide film and the color prints were prepared from color slides with a Canon 200 color photocopier. ■

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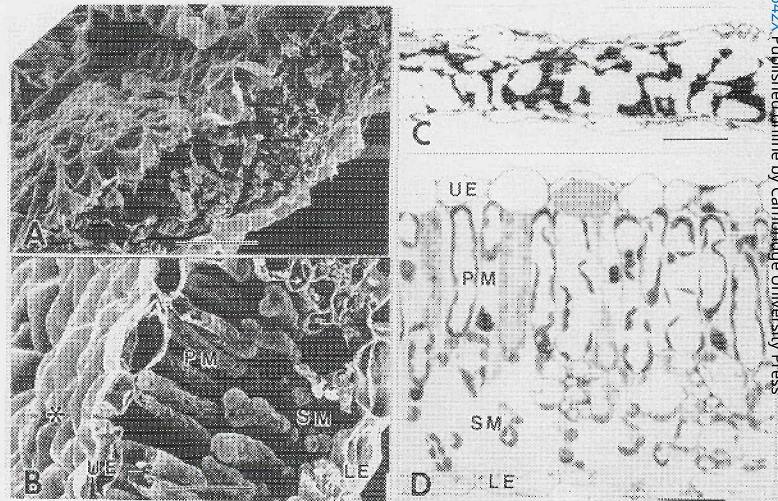

Figure 2. A, B. Scanning electron micrographs of (A) a herbicide-treated leaf showing collapse of the upper epidermis and extensive cytolysis and pcynosis in the pallisade and spongy mesophyll cell layers and (B) a control leaf with cobblestone-like upper surface (asterisk) and intact cell layers. C, D. Light micrographs of cross-sectioned (C) herbicide-treated leaf showing collapse of the upper epidermis and extensive cytolysis and pycnosis in the pallisade and spongy mesophyll cell layers and (D) control leaf for comparison. UE - upper epidermis, PM - pallisade mesophyll, SM -spongy mesophyll, LE - lower epidermis. A and B, 360x, bars = 50 µm; C and D, 300x, bars = 50 µm.

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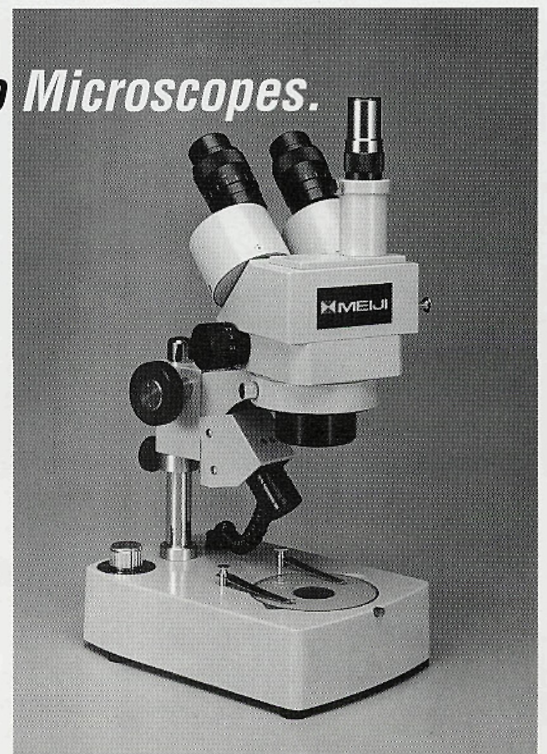

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