Infrared Fluorescence of Corn Leaves Infected by Helminthosporium maydis

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ABSTRACT

Corn leaves (Texas male-sterile cytoplasm) were inoculated with *Helminthosporium maydis* (Race T) and infrared (IR) fluorescences of lesions, adjacent areas, and healthy tissues were measured. The IR fluorescences of lesion areas were lower in all cases than were the surrounding tissues. The IR fluorescences of tissues surrounding lesions were similar to those of healthy tissues on noninoculated leaves.

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Additional key words: remote sensing, disease detection, southern corn leaf blight.

The detection of southern corn leaf blight in early stages of infection by remote sensing techniques could provide a means for tracing the seasonal progress of the disease, provide a timely and economical means for taking remedial action, and provide a data base for estimating loss of yield due to the disease.

In order to detect blighted fields by remote sensing, a radiative property of blighted fields must be found which is sufficiently unique and characteristic of the disease that the detection of this property can be definitive. Mayer (6) has shown that in ivy leaves only the green (chlorophyll-containing) portions of the leaves fluoresce in the near-infrared region of the spectrum. In addition, Best (1) has shown that necrotic areas of tobacco leaves infected with

tomato spotted wilt virus or with tobacco mosaic virus fluoresce in the visible spectrum when exposed to ultraviolet light.

In the work reported here, the infrared fluorescence properties of healthy corn leaves and corn leaves infected with the southern corn leaf blight fungus, *Helminthosporium maydis* (race T), Nisik. and Miyake, were explored.

MATERIALS AND METHODS.—Laboratory tests of infrared fluorescence on healthy and infected corn (*Zea mays* L.) plants were made using a filtered Xenon photographic flash lamp for the excitation source. The reason for choosing the short duration excitation method was to simulate the effect of an airborne scanning ultraviolet laser which would be used as the source in a suitable remote sensing system. The flash duration of the Xenon lamp is about 100 μs. The flash duration due to the dwell time of a scanner would be significantly less. A bench scanner was built to test the time dependence of infrared fluorescence for flashes as short as 50 μs on leaves which were dark adapted for 1-2 h. The leaves were dark-adapted to induce a physiological condition similar to that which would occur at night because remote sensing fluorescence sensors would most likely be utilized at night. The results indicated that the magnitude of the infrared fluorescence of dark-adapted corn leaves was independent of flash duration from 1.0 ms to 50 μs. It was assumed that this time-independence would hold for a flash duration of 5 μs which would be typical of scanner dwell times. Therefore, the fluorescence photographs using a flash duration of 100 μs should provide a valid measure of the fluorescence under field conditions.

A standard studio 250-J photographic flash lamp was enclosed in a metallic container with a window fitted with a Corning glass filter 9782. Although the spectral intensity of the source was not measured, the expected spectral intensity was derived from published flash lamp data (5) and the spectral transmittance of the 9782 filter (2). The purpose of the filter was to remove all spectral components with wavelengths longer than 650 nm which will record on high-speed infrared film (2). The camera with high-speed infrared film and either the Wratten 70, 89B, or 87 filters was used to measure the fluorescences (approximately 650-920 nm) of corn leaves during 100-μs exposures to the flash lamp. The relative sensitivities of the high speed infrared film with these filters (3) are shown in Fig. 1.

The spectral radiance of the infrared fluorescence of corn leaves was not accurately measured for these experiments because the usual recording spectrophotometers require excitation times of the order of seconds and minutes and the resulting spectra may not be the same as for short-duration excitation flashes. The spectral radiance of fluorescence of chlorophyll in vivo under steady state excitation can be expected to have a major sharp peak, the main band at 685 nm between 660 nm and 700 nm and a lower broad satellite band peaking at about 740 nm with a long "tail" extending to 800 nm (8). One can see from Fig. 1 that, with the use of the Wratten 87 filter, the radiation in the "tail" of the spectrum will be recorded. The Wratten 89B permits the recording of most of the satellite band. The Wratten 70 will permit the recording of some significant fractions of the main band along with the longer wavelength satellite band.

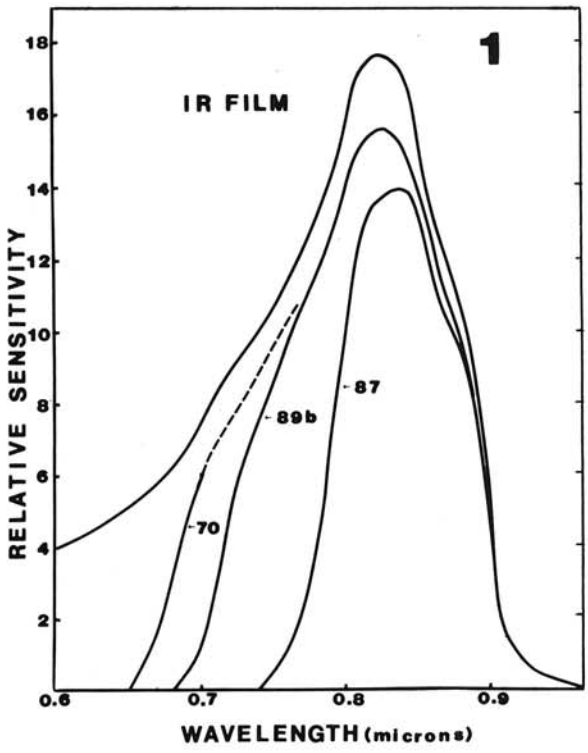

Fig. 1. Relative sensitivity of high speed infrared film with filters. Kodak technical publication data were used to derive these sensitivity curves.

Corn plants of the hybrid W64A × OHB with Texas male-sterile cytoplasm were grown in clay pots in the greenhouse for 30 days in steam sterilized soil as described previously (9). One half of the plants were then spray-inoculated with spores of *H. maydis* (race T) suspended in distilled water (9). Both inoculated and noninoculated plants were then incubated in a humid chamber for 20 h. Nine inoculated and 18 noninoculated corn leaves were used for each of three test periods at 1, 2, and 5 days after inoculation. A leaf of a control plant was placed to either side (top and bottom in figures) of a leaf of an inoculated plant for photography. After the plants were dark-adapted for 1-2 h, the infrared fluorescence flash exposures were made. The fluorescent radiance in the spectral band defined by the Wratten 70 and film sensitivity was more than a factor of 10 greater than that defined by the Wratten 87 and film sensitivity as was to be expected. However, in all cases, there was no evidence for spectral differences other than that.

RESULTS AND DISCUSSION.—At one day after inoculation, densitometer readings on the negatives showed that there was no significant difference between the average radiance of the healthy and inoculated leaves.

The slightly chlorotic lesions are not visible to the unaided eye by reflection or panchromatic photography at this stage of development, but they can be detected by the eye if they are viewed by transmitted light.

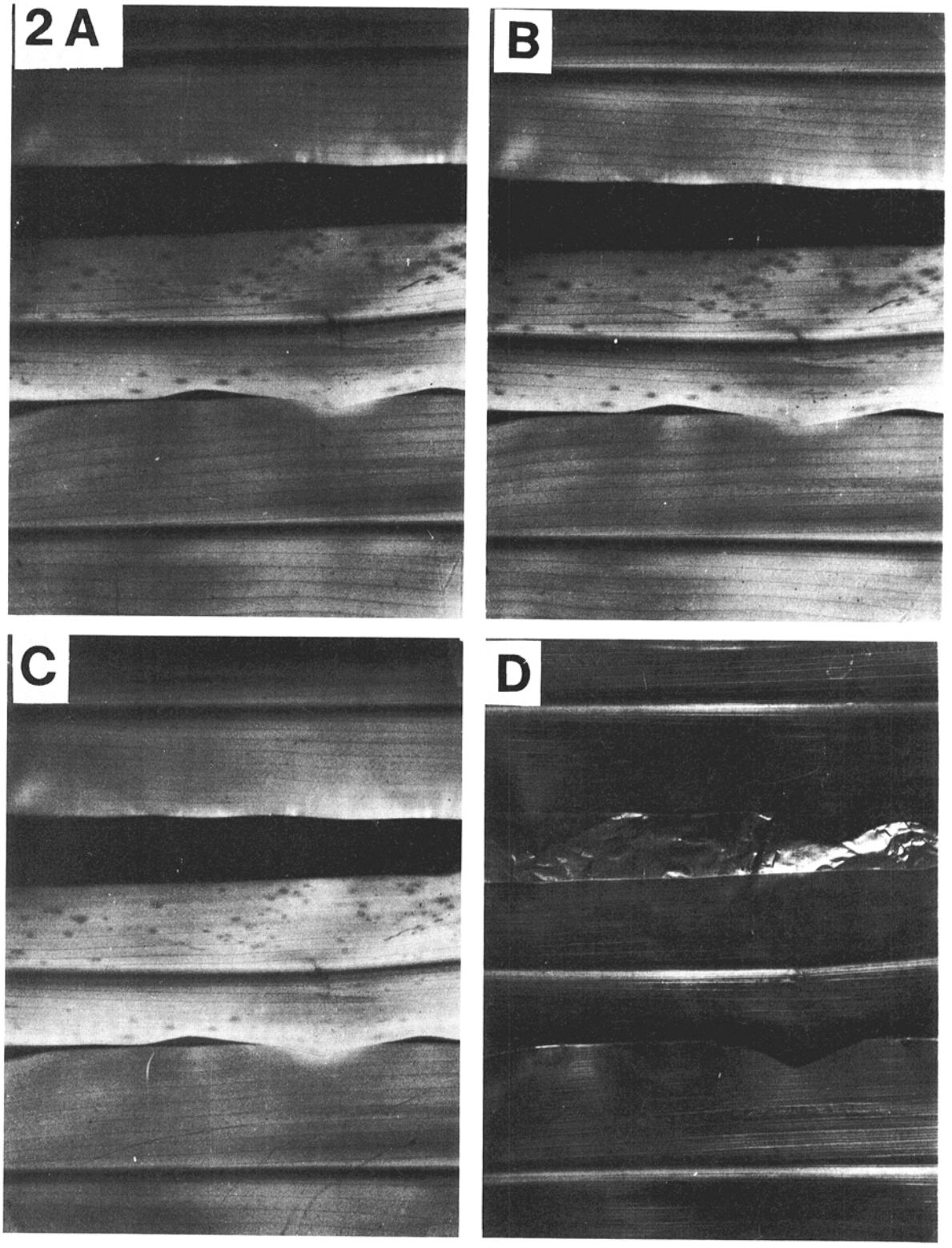

Fig. 2-(A to D). A typical sample 2 days after inoculation is shown by fluorescence. The fairly high gamma of the film makes small radiance differences easier to see by eye. The Wratten filters 87, 89B, and 70 were used for **A**, **B**, and **C**, respectively. Unfiltered panchromatic film was used for **D** to obtain a photograph by reflected light.

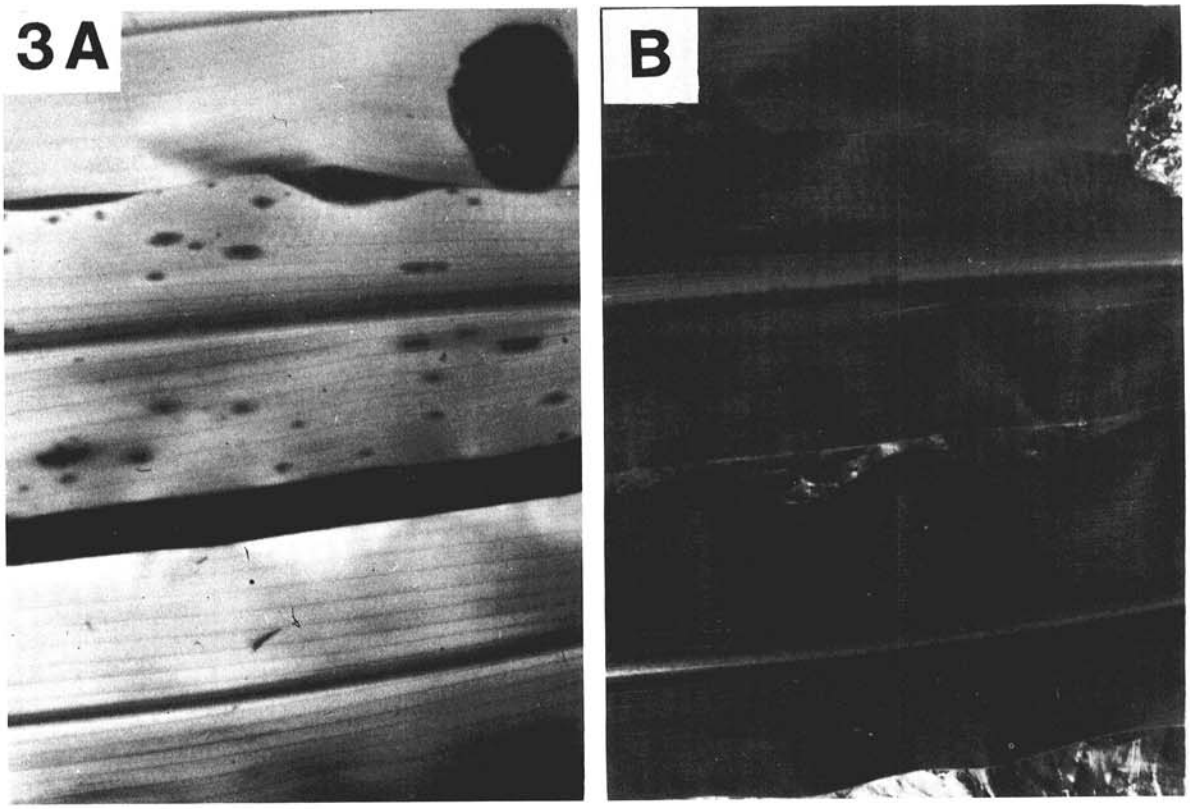

Fig. 3-(A , B). A typical sample is shown 5 days after inoculation. The fluorescent photograph, **A**, was made with the Wratten 70 filter. The reflected light photograph, **B**, was made with unfiltered panchromatic film.

A typical sample at 2 days after inoculation is shown by fluorescence photography in Fig. 2-A, B, C using filters 87, 89B, and 70 respectively. The lesions (chlorotic) are still difficult to see by reflected light as shown by panchromatic photography (Fig. 2-D), although the number and degree of development of the lesions have increased. The radiance in the lesion area is approximately 10% below that of the neighboring tissue.

In some instances, blighted leaves showed a slightly greater radiance (as much as 5%) than the unblighted leaves. Since the variation of radiance (as much as a factor of two) along each leaf was so much greater than the small average rise in radiance of some blighted leaves, the rise in the average could be attributed to minor spatial variations in irradiance of the excitation radiation. The corn leaves did not lie perfectly flat, therefore, the natural undulations caused the variation which can be expected from leaf orientation toward and away from the excitation source.

As infection progressed, the number and size of observable lesions increased, but in all cases, the lesion areas were less fluorescent than were the surrounding tissues. Apparently the toxin (4, 7) produced by the fungus did not affect the fluorescence of tissues surrounding the lesions since the surrounding tissues had fluorescences similar to those of healthy tissues on noninoculated plants.

Figure 3 shows a typical sample 5 days after inoculation. The developing chlorotic and necrotic lesions are just visible to the naked eye but do not show well in panchromatic photography (Fig. 3-B). The complete quenching of fluorescence in the lesion area is shown in 3-A using the Wratten 70 filter. The large round dark spot in the upper right of Fig. 3-A is a crumpled piece of aluminum foil to serve as an indicator of any reflected radiation reaching the infrared film. The aluminum foil should appear dark in fluorescence photography except where reflection of fluorescent radiation from the leaves occurs.

The results of these experiments show that the influence of an infection of *Helminthosporium maydis* on the fluorescence of 1-mo-old Texas male-sterile corn is insignificant except in the lesion area where the fluorescence is decreased. The degree of fluorescence in nonlesion areas is determined primarily by the orientation of the leaf to the excitation source. The influence of an infection of *Helminthosporium maydis* on the infrared fluorescence of corn may be useful for measuring the lesion areas on infected leaves; however, fluorescence characteristics would probably not serve as a practical signature for detection of southern corn leaf blight by remote sensing.

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