Spectral Reflectance and Transmittance of Corn Leaves Infected with Helminthosporium maydis

G. R. Safir, G. H. Svits, and A. H. Ellingboe

First and third authors, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48823; second author, Infrared and Optics Laboratory, Willow Run Laboratory, University of Michigan, Ann Arbor 48104.

Michigan Agricultural Experiment Station Journal Paper No. 5683.

Accepted for publication 1 May 1972.

ABSTRACT

Corn leaves with Texas male sterile cytoplasm have greater reflectance and transmittance after inoculation with *Helminthosporium maydis* than do healthy leaves. The differences were the most pronounced in the chlorophyll (0.5-0.7 μ) and water (1.45 and 1.95 μ) absorption regions of the spectrum. The first measurable, reproducible increase in reflectance of diseased over healthy tissue was detected when disease lesions became visible to the eye, about 40 hr after inoculation. The earliest changes were confined to the chlorophyll absorption regions of the spectrum. On normal cytoplasm corn, the increases in reflectance and transmittance of diseased over healthy tissue are smaller in magnitude and show up later than on T cytoplasm corn.

Phytopathology 62:1210-1213.

Additional key words: southern corn leaf blight.

The need for methods which would enable rapid detection of disease and evaluation of disease severity over large land areas has become increasingly important. The outbreak of southern corn leaf blight (SCLB) in 1970 illustrated the rapidity with which an epidemic can develop and the need for rapid assessment of disease severity.

Remote sensing has been used to detect physiologically stressed and diseased crops (7, 8, 10, 13, 14). In 1970, remote sensing from high-altitude planes enabled the detection and classification of five levels of infection of SCLB (2). The different levels of SCLB severity were detected in some experiments as different color tones on infrared-sensitive film (3). The different tones arise from differences in the total reflectivity of the crop canopy in the visible and near-infrared which were correlated with blight severity.

The spectral reflectance and transmittance of corn leaves, as measured in the laboratory, are affected by many factors. These include chlorophyll content, water content, leaf thickness, mesophyll structure, and the angle of illumination (4, 5, 6, 8, 9, 10, 11, 14). According to Allen et al. (1), the effective absorption spectra of the corn leaf between the light wavelengths of 0.5 and 2.5 μ appear to be described by the absorption coefficients of chlorophyll and pure liquid water.

The contribution of the diseased leaves to the canopy reflectance and transmittance in the visible and the near-infrared is the subject of this paper.

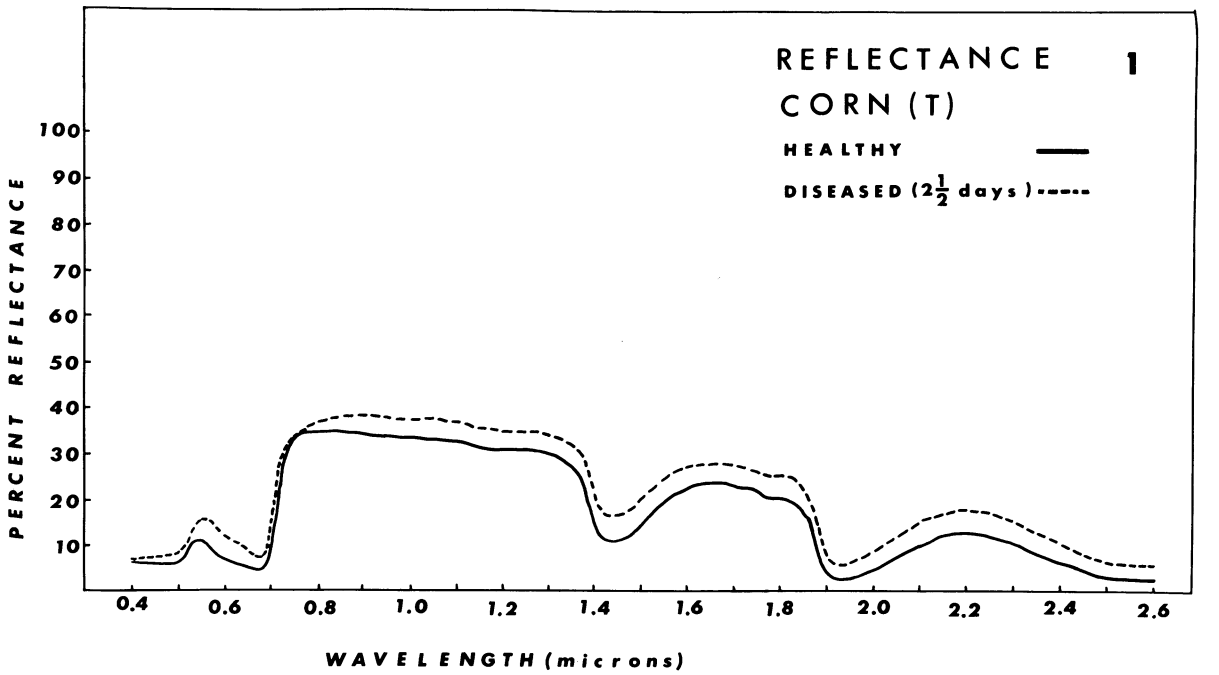
MATERIALS AND METHODS.—*Growth of corn plants.*—Corn plants of the hybrids W64A X OH B with Normal "N" and W64A X OH B with Texas Male Sterile "T" cytoplasm were grown in the greenhouse in 5-inch clay pots in a steam-sterilized 1:1:1 soil-sand-peat mixture. Each pot which contained 3 N and 3 T cytoplasm corn plants was fertilized twice weekly with ca. 200 ml of Rapid Grow 23-19-17 solution (Rapid Grow Corp., Dansville, N.Y.). The solution contained four teaspoons of fertilizer/gallon of water.

Culture of the fungus.—*Helminthosporium maydis* (race T) was grown on migration-complete medium (12) plus 1% yeast for 1 to 2 weeks at 22 C. The fungus mycelium, including agar, was ground with 100 ml sterile distilled water in a Waring Blendor for 30 sec. Five ml of the mixture was pipetted onto sterile filter paper in each 15-cm-diam petri dish. The fungus sporulated on the filter paper in 1 to 3 weeks. Spores of *H. maydis* were scraped from the surface of the filter paper and suspended in 100 ml sterile distilled water which contained 1 drop of Tween 80 (polyoxyethylene sorbitan monooleate). The spore suspension was sprayed onto 4- to 5-week-old corn plants. Inoculated corn was then incubated in a humid chamber for ca. 30 hr at 22 C. Lesions were macroscopically visible at 36-42 hr after inoculation.

Measurements of leaf reflectance and transmittance.—Leaf hemispherical reflectance and hemispherical transmittance of inoculated and noninoculated corn plants were measured with a Beckman DK 2A ratio-recording spectrophotometer. Measurements were made over the spectral range 0.4 to 2.6 μ at an incident angle of 5 degrees from normal. Reflectance was compared with a $BaSO_4$ standard.

Spectral measurements were taken from the fifth or sixth leaf from the soil line on tissue halfway between the stalk and the leaf apex. Reflectance measurements were taken from intact leaves on the plants. Transmittance measurements were taken from the same leaves immediately after detachment to reduce the possibility of significant water loss.

The spectra of leaf samples from 30 inoculated and 30 noninoculated corn plants of both "N" and "T" cytoplasm were taken in order to establish whether any new absorption bands existed which could be attributed to the presence of *H. maydis*. The spectral differences between diseased and healthy tissue were attributable to the fractional areas, esti-



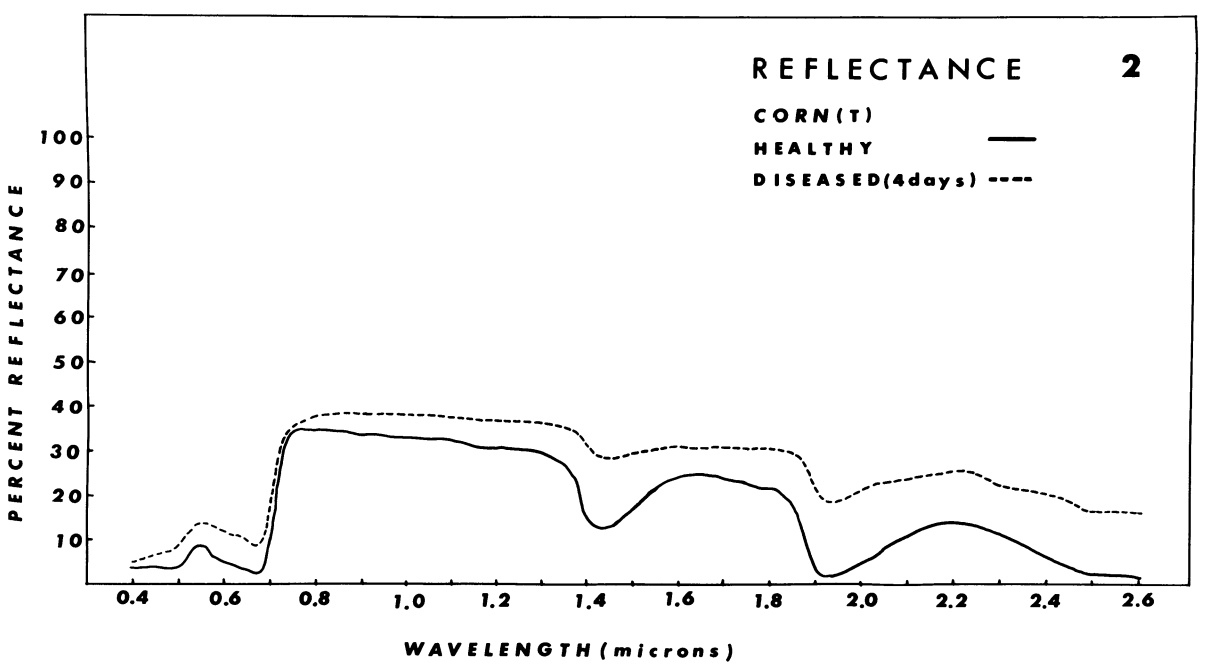


Fig. 1-2. 1) Spectral reflectance of a typical "T" cytoplasm corn leaf at 2.5 days after inoculation with *Helminthosporium maydis* as compared with a healthy leaf. 2) Spectral reflectance of a typical T cytoplasm corn leaf at 4 days after inoculation with *H. maydis* as compared with a healthy leaf.

mated by eye, of green, chlorotic, and necrotic tissue exposed to the spectrophotometer port. The data reported here were taken from leaves inoculated to such an extent that the individual lesions coalesced, and thus the lesions were not measured in terms of size and number. These spectral data represent the most severe tissue damage at various times after inoculation with the SCLB fungus. Only diseased portions of the tissue were exposed to the spectrophotometer port.

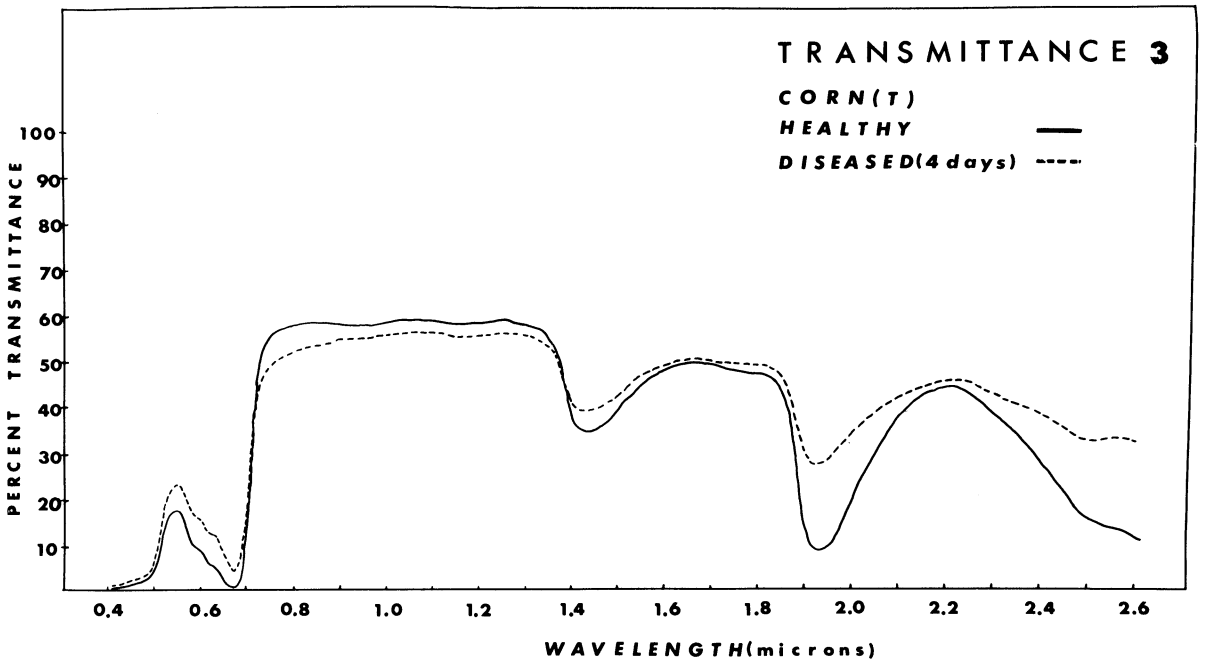


Fig. 3. Spectral transmittance of a typical "T" cytoplasm corn leaf 4 days after inoculation with *Helminthosporium maydis* as compared with a healthy leaf.

RESULTS.—At 1.5 days after inoculation, diseased T cytoplasm corn leaf tissue had a slightly higher reflectance in the chlorophyll absorption regions of the spectrum than did healthy tissue. No such differences occurred in the water absorption regions at that time after inoculation.

T cytoplasm corn 2.5 days after inoculation with *H. maydis* (race T) had higher leaf reflectance in most of the spectral region of 0.4- to 2.6-μ wavelength than did healthy corn (Fig. 1). These differences were more pronounced in the chlorophyll (0.5 to 0.7 μ) and water absorption (1.45 μ and 1.95 μ) regions of the spectrum. Greater reflectance in these regions indicates a decreased concentration of chlorophyll and water, respectively, in the leaf tissues (9, 10, 14). At the wavelength 0.675 μ, the maximum reflectance of healthy and diseased tissues was 4.5% and 6.5%, respectively. The maximum reflectance of healthy and diseased tissues was 12.5% and 16%, respectively, at 1.45 μ, and 2.5% and 5.5%, respectively, at 1.95 μ.

At 2.5 days after inoculation of resistant N cytoplasm corn, there were no detectable spectral differences between healthy and inoculated leaf tissue. The reflectance spectrum of healthy N cytoplasm corn was similar to that of healthy T cytoplasm corn.

At 4 days after inoculation of T cytoplasm corn (Fig. 2), the increase in reflectance of diseased tissue over healthy tissue was greater than at 2.5 days after inoculation. The maximum reflectance of diseased tissues at 0.675 μ, 1.45 μ, and 1.95 μ was 8.5%, 28.5%, and 19.5%, respectively.

At 4 days after inoculation of N cytoplasm corn, the diseased leaves had a higher reflectance than healthy leaves in the chlorophyll absorption region. These differences were smaller than those occurring on T cytoplasm corn at 4 days after inoculation. No differences occurred in the water absorption regions at this time after inoculation.

The transmittance of T cytoplasm corn at 4 days after inoculation was higher than that of healthy tissue, and the increases were more pronounced in the chlorophyll and water absorption bands (Fig. 3). The maximum transmittance of healthy tissue at 0.675 μ, 1.45 μ, and 1.95 μ was 2%, 33.5%, and 10%, respectively. For diseased tissue at 0.675 μ, 1.45 μ, and 1.95 μ, the maximum transmittance was 4.5%, 39%, and 27%, respectively.

The transmittance of N cytoplasm corn at 4 days after inoculation was slightly higher than that of healthy tissue in the chlorophyll absorption region. These differences were considerably less than those reported here for T cytoplasm corn, and were not present in the water absorption bands. The transmittance of healthy N and healthy T cytoplasm corn was similar.

DISCUSSION.—We have shown that T cytoplasm corn leaf tissue, when infected with *H. maydis* (race T), has a higher reflectance and transmittance than does healthy tissue. These differences are more pronounced in the chlorophyll (0.5-0.7 μ) and water absorption (1.45 μ and 1.95 μ) regions of the spectrum, and are probably caused in part by a loss of chlorophyll and water in diseased tissue. Increases in

reflectance of diseased over healthy tissue were first detectable when disease lesions became macroscopically visible to the eye. These differences, however, were confined to the chlorophyll absorption region of the spectrum. With normal cytoplasm corn, the differences in reflectance and transmittance between healthy and diseased tissue were smaller and showed up later than on T cytoplasm corn. The fact that an increase in visible reflectance of diseased corn tissue preceeded any detectable changes in infrared reflectance is in contrast to many generalized statements, which lead readers to believe that disease causes a previsual change in infrared reflectivity.

Data from infrared photography and other airborne sensors have lead to some important hypotheses. One of these is that the predominant factor in the distinction between diseased and healthy plants may be differences in foliage density and leaf area (13). These differences may arise from supressed growth or loss of leaves (10). Also, changes in leaf orientation, such as those which may arise from water deficiency and leaf wilting, can be extremely important (10). In both cases, the total infrared reflectance of the foliage area viewed would be reduced as would the visible reflectance, but to a lesser degree than would the infrared (10). The spectral characteristics of a blighted canopy as interpreted from aerial color infrared photography (3) suggested a decreased reflectance in the near infrared region of the spectrum. However, our data support the hypothesis that blighted leaf tissue per se has an increased infrared reflectance over that of healthy tissue. Soils generally have a lower infrared reflectance than a plant canopy (10), and it is possible that the soil itself may have contributed to the reduced reflectance of a blighted field as mentioned above (3). Further indications of this possibility arise from the fact that blighted corn leaves can exhibit changes in geometry due to wilting, death of tissue, and decreased growth in extreme cases. We are currently investigating these aspects of the remote detection of SCLB.

LITERATURE CITED

1. ALLEN, W. A., H. W. GAUSMAN, A. J. RICHARDSON, & J. R. THOMAS. 1969. Interaction of isotropic light with a compact plant leaf. J. Optical Soc. Amer. 59:1376-1379.
2. BAUER, M. E., P. H. SWAIN, R. P. MROCZYNSKI, P. E. ANUTA, & R. B. MAC DONALD. 1971. Detection of southern corn leaf blight by remote sensing techniques. Seventh Int. Symp. on Remote Sensing of Environment, p. 693-699. Willow Run Laboratories, Univ. Michigan, Ann Arbor.
3. COFFMAN, B. 1971. Corn blight from 11 miles up, p. 31-33. Farm J.
4. GATES, D. M., H. H. KEEGAN, J. C. SCHLETER, & V. R. WEIDNER. 1965. Spectral properties of plants. Appl. Optics 4:11-20.
5. GAUSMAN, H. W., W. A. ALLEN, V. I. MYERS, & R. CARDENAS. 1969. Reflectance and internal structure of cotton leaves (Gossypium hirsutum L.). Agron. J. 61:374-376.
6. GAUSMANN, H. W., W. A. ALLEN, V. I. MYERS, R. CARDENAS, & R. W. LEAMER. 1970. Reflectance of single leaves and field plots of cycocel-treated cotton (Gossypium hirsutum L.) in relation to leaf structure. Remote Sensing of Environment 1:103-107.
7. KEEGAN, H. J., J. C. SCHLETER, W. A. HALL, & G. M. HAUS. 1956. Spectrophotometric and colorimetric study of diseased and rust resisting cereal crops. Nat. Bur. Standards, Rep. 4591.
8. KNIPLING, E. B. 1967. Physical and physiological basis for differences in reflectance of healthy and diseased plants. Workshop Infrared Color Photogr. Plant Sci. Proc., Florida Dep. Agr., Winter Haven.
9. KNIPLING, E. B. 1969. Leaf reflectance and image formation on color infrared film, p. 17-29. *In* P. L. Johnson [ed.]. Remote sensing in ecology. Univ. Georgia Press, Athens.
10. KNIPLING, E. B. 1970. Physical and physiological basis for the reflectance of visible and near infrared radiation from vegetation. Remote Sensing of Environment 1:155-159.
11. RABIDEAU, G. S., C. S. FRENCH, & A. S. HOLD. 1946. The absorption and reflection spectra of leaves, chloroplast suspensions, and chloroplast fragments as measured in an Ulbricht sphere. Amer. J. Bot. 33:769-777.
12. SNIDER, P. J., & J. R. RAPER. 1958. Nuclear migration in the Basidiomycete Schizophyllum commune. Amer. J. Bot. 45:538-546.
13. THOMAS, J. R., C. L. WEIGAND, & V. I. MYERS. 1967. Reflectance of cotton leaves and its relation to yield. Agron. J. 59:551-554.
14. WOOLEY, J. T. 1971. Leaf reflectance and transmittance. Plant Physiol. 47:656-662.