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Ozone Damage Detection in Cantaloupe Plants*

Ozone-damaged plants were distinguishable from nondamaged plants by reflectance measurements in the 1.35- to 2.5-μm near-infrared water absorption waveband.

Abstract: *Ozone causes up to 90 percent of air pollution injury to vegetation in the United States; excess ozone affects plant growth and development and can cause undetected decrease in yields. Laboratory and field reflectance measurements showed that ozone-damaged cantaloupe (Cucumis melo L.) leaves had lower water contents and higher reflectance than did nondamaged leaves. Cantaloupe plants which were lightly, severely, and very severely ozone-damaged were distinguishable from nondamaged plants by reflectance measurements in the 1.35- to 2.5-μm near-infrared water absorption waveband. Ozone-damaged leaf areas were detected photographically 16 h before the damage was visible. Sensors are available for use with aircraft and spacecraft that possibly could be used routinely to detect ozone-damaged crops.*

Introduction

Ozone (O_3), which may reach a concentration of 70 parts per hundred million (pphm) in the Los Angeles basin, is probably the most important air pollutant affecting plant growth, development, and reproduction in the United States (Walker and Barlow, 1974). Ozone causes as much as 90 percent of pollution injury to vegetation (Marx, 1975), some of which is invisible and can cause yield decreases. Ozone can also harm people, fabrics, and rubber (Craker and Manning, 1972).

Ozone is formed by a photochemically-induced reaction between the hydrocarbons and nitrogen oxides of automobile exhaust (Craker and Manning, 1973): nitrogen oxides produce atomic oxygen (0) that combines with O_2 in the air to form O_3. Some O_3 may descend to the Earth's surface from the stratosphere, or it can be formed from electrical storms and electrical discharges (Heggestad and Heck, 1971).

Literature published before 1971 on plant responses to air pollutants, including O_3, was intensively reviewed by Heggestad and Heck (1971). Usually O_3 causes small necrotic spots to develop on the upper (adaxial) surface of fully-expanded leaves of herbaceous plants (Heggestad and Heck, 1971), and injures their palisade cells first (Evans and Ting, 1974; Heggestad and Heck, 1971; Howell and Kremer, 1972; Thomson *et al.*, 1966). On grasses without palisade cells, O_3 injury develops in the leaf mesophyll and on both upper and lower (abaxial) leaf surfaces (Heggestad and Heck, 1971).

We studied effects of O_3 damage on the

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reflectance and photographic responses of cantaloupe plant leaves and canopies to determine the best wavelengths to detect O_3 damage and to determine if O_3 damage could be detected before lesions became visible.

Materials and Methods

We conducted preliminary O_3 studies on eight 3-week-old vegetable plants—cucumber (*Cucumis sativus* L., cv Ashley); cantaloupe (*Cucumis melo* L. var. *cantalupensis* Naud., cv Perlita); cowpea (*Vigna sinensis* Savi, cv unknown); sweet pepper (*Capsicum annum* L., cv Rio 66); squash (*Cucurbita pepo* L., cv Early Prolific Straightneck); lima bean (*Phaseolus limensis* Macf., cv Jackson Wonder); pinto bean (*Phaseolus vulgaris* L., cv Pinto); and watermelon (*Citrullus vulgaris* Schard., cv Charleston Gray)—exposed to 16 pphm of ozone for 2 h in a 0.17 m^3 plexiglass chamber (Craker and Manning, 1972, 1973). About 24 h later, the cucumber's, cantaloupe's, and cowpea's foliage showed more O_3 damage than did those of the other five crops. Therefore, we selected cantaloupe plants for further O_3 studies. The plants for the preliminary study were grown in a different greenhouse than those below.

We planted five seeds in each of 50 0.2-liter plastic pots, containing a sandy clay loam mixed with a 10-25-5 fertilizer to give an N rate equivalent of 67.2 kg/ha. The experiment was conducted in a greenhouse and the pots were subirrigated. About three weeks after plant emergence, 25 pots were placed in each of two plexiglass chambers for two simultaneous treatments—one aerated (control) and the other O_3-treated. An O_3 meter (MAST† 724-2) connected to the chamber was used to measure the O_3 concentration generated by an O_3 apparatus (Craker and Manning, 1972, 1973). For 3 h, O_3-treated plants were exposed to 18-pphm O_3 and the control plants were aerated. Flow rates to the O_3 and aerated chambers were 4.3 and 3.8 liters per minute, respectively. Light intensity and temperature inside both chambers were about 4,600 ft-c and 30°C, respectively. Humidity was not controlled.

Primary leaves of control and O_3-treated plants, 16 h after O_3 treatments, were used for reflectance measurements. Five leaves (each from a different plant) were collected from the control plants; and 15 leaves (each from a different plant) were collected from ozone-treated plants: five with light, five with severe, and five with very severe visible damage (Plate 1). Levels of O_3-induced, leaf-flecking symptoms were selected arbitrarily: lightly-damaged leaves had small sporadic grayish-green spots, severely-damaged leaves had larger grayish-green central and marginal leaf surface-damaged areas with loose upper epidermal layers; and very severely-damaged leaves had very large, dark grayish-green, surface-damaged areas with missing upper epidermal layers. These degrees of injury were present at 16, 24, or 38 h after treatment.

At the time we made spectral measurements, we photographed a representative leaf for each treatment. However, the film was ruined during processing (this was known 26 h later). We rephotographed other leaves after they had been exposed to some sunlight. Nevertheless, Plate 1 typifies the control and ozone-damaged leaves that were used for spectral measurements.

Immediately after we collected each leaf, we wrapped it in Glad wrap (plastic wrap) to minimize dehydration and transferred it to the laboratory for measurements. Leaf reflectance, thickness, green weight, and area measurements and tissue cross section samplings were completed for all leaves within 6 h.

Total diffuse reflectance of upper (adaxial) surfaces of single leaves over the 0.5- to 2.5-μm waveband was measured with a Beckman Model DK-2A spectrophotometer, equipped with a reflectance attachment. To measure the reflectance of O_3-treated leaves, the spectrophotometer's light beam was impinged only on O_3-damaged areas. Data were corrected for decay of the barium sulfate standard to give absolute radiometric data (Allen and Richardson, 1971). Leaf thickness was measured by using a linear displacement transducer and digital voltmeter (Heilman *et al.*, 1968). Leaf areas were measured with a planimeter. Water content was determined on an oven dry-weight basis by drying leaves at 68°C for 72 h and cooling them in a desiccator before final weighing.

For the reflectance measurements, we used seven wavelengths from the 41 wavelengths measured over the 0.5- to 2.5-μm waveband—0.55 μm (green reflectance peak), 0.65 μm (chlorophyll-absorption band), 0.85 μm (near-infrared plateau), 1.45 μm (water absorption band), 1.65 μm (reflectance peak after water-absorption band at 1.45 μm), 1.95 μm (water absorption band), and 2.20 μm (re-

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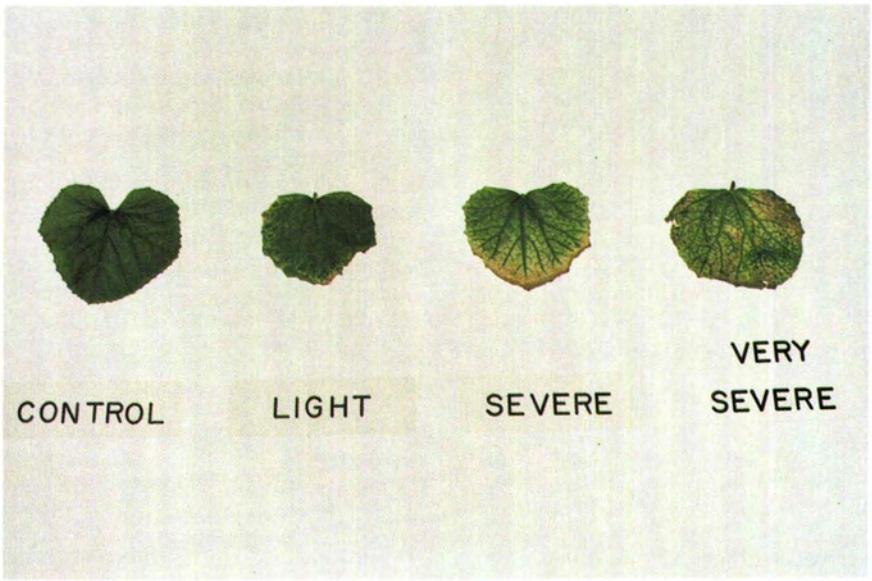

PLATE 1. Photograph showing typical control and lightly, severely, and very severely ozone-damaged cantaloupe leaves.

flectance peak after water absorption band at 1.95 μm). We analyzed reflectance data for each of these wavelengths for variance and used Duncan's multiple range test to test differences among treatment means, $p=0.01$ (Steel and Torric, 1960).

Tissue pieces from near the center of leaves were fixed in formalin-acetic acid-alcohol, dehydrated with tertiary butanol, embedded in paraffin, stained with safranin-fast green, and transversally microtomed at 12-μm thickness (Jensen, 1962). Photomicrographs were obtained with a Zeiss Standard Universal Photomicroscope. This was done to relate internal leaf structure with reflectance.

In order to support laboratory results, control and O_3-treated cantaloupe plants contained in flats were taken from the greenhouse to the field where their canopies were measured spectroradiometrically. Reflectances of control and ozone-treated plant canopies were measured over the 0.5- to 2.4-μm waveband 1 hr after treatment with a ground-based Exotech Model 20 Spectroradiometer (Leamer *et al.*, 1973). Its sensor had a 15-degree field-of-view (0.08m^2) at 1.2 m above the plant canopies.

In order to determine whether O_3 leaf damage could be detected before it was visible, we simultaneously compared control and O_3-treated plants visually and photographically. We photographed the plants hourly in a photographic laboratory with a Polaroid Land Camera Model 108 using Pola-Color film-type 108, and with a Hasselblad camera, using 70-mm Kodak Aerochrome infrared film 2443 (infrared color) and a yellow Hasselblad filter. The only light source was provided by 75 Watt incandescent Grow Light bulbs (Kyung-hung Trading Company, Box 635, Central Seoul, Korea), whose primary spectral output was blue light.

RESULTS AND DISCUSSION

LEAF STRUCTURE

Internal leaf structures for the control and for lightly, severely, and very severely O_3-damaged leaves are shown in Figure 1, A, B, C, and D, respectively. Leaf structure had collapsed from dehydration for severely (C) and very severely (D) O_3-damaged leaves as compared with the control (A) and lightly O_3-damaged leaves (B). Leaf water contents ranged from 82.6% for very severely damaged to 90.3% for control leaves.

REFLECTANCE SPECTRA

Laboratory reflectance spectra over the 0.5- to 2.5μm waveband are shown in Figure 2 for the control and for the lightly, severely, and very severely O_3-damaged cantaloupe leaves.

Mean light reflectances at the 0.55 and 0.65-μm wavelengths in the visible region (0.5 to 0.75 μm) among the control, and

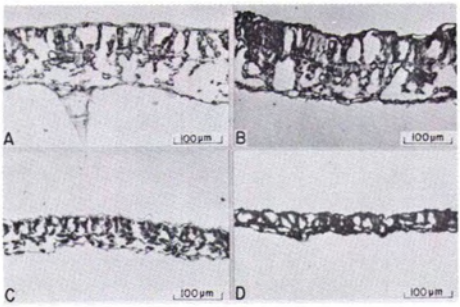

FIG. 1. Transections of control (A) and lightly (B), severely (C), and very severely (D) ozone-damaged leaves.

lightly and severely O_3-damaged leaves were not different statistically, but the mean reflectance of very severely O_3-damaged leaves was significantly greater ($p = 0.01$) than that for the other treatments.

Mean reflectances among the treatments were not different statistically at the 850-μm wavelength in the near-infrared region (0.75 to 1.35 μm).

The reflectances for the leaves of all treatments were different statistically ($p = 0.01$) for the 1.45-, 1.65-, 1.95-, and 2.2-μm wavelengths in the near-infrared water absorption region (1.35 to 2.5 μm). As severity of O_3 damage increased, leaf reflectance increased because of dehydration as caused by stress and senescence (Gausman, 1974; Hoffer and Johannsen, 1969; Schubert, 1972; Sinclair, 1968; and J. R. Thomas, unpublished data, Weslaco, Texas). The 1.65- and 2.2-μm wavelengths with atmospheric windows could be useful to detect O_3-damaged plants. There are sensors available for use in aircraft and spacecraft in this region.

Field reflectance measurements over the 0.5- to 2.4-μm waveband for control and O_3-treated cantaloupe plant canopies (Figure 3) supported the laboratory results. The reflectance of O_3-treated plants was the same as that for the control plants in the visible region, but it was higher than that for the control plants in the near-infrared (0.75 to 1.35 μm) and near-infrared water absorption (1.35 to 2.5 μm) region.

PHOTOGRAPHIC DETECTION

We compared hourly visual and Polaroid photographic results to determine if O_3 leaf damage could be detected before it was seen. We detected O_3 leaf damage photographically as light brownish-colored areas (Cardenas *et al.*, 1969-70; Cardenas *et al.*, 1972; Leamer *et al.*, 1978), 16 h before we could see it (38 h after treatment).

Infrared photos as compared with the Polaroid photos did not show O_3 damage. Apparently, the predominantly blue light source was responsible for our success in detecting O_3 damage with Polaroid photography. However, possibly more work with different films, filters, and light sources will give even earlier detection of O_3 leaf damage than we have obtained.

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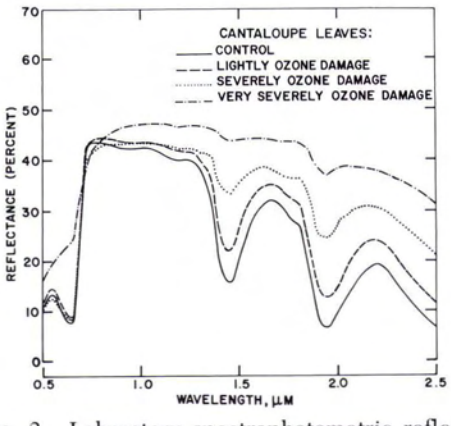

FIG. 2. Laboratory spectrophotometric reflectance spectra over the 0.5- to 2.5-μm waveband for control and lightly, severely, and very severely ozone-damaged cantaloupe leaves.

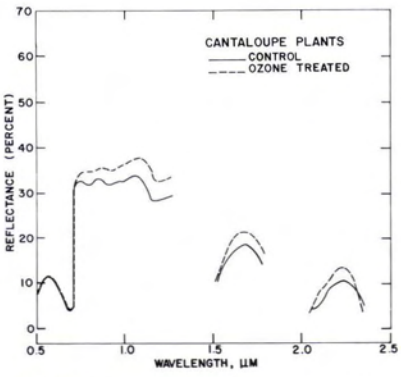

FIG. 3. Field spectroradiometric reflectance spectra over the 0.5- to 2.5-μm waveband for control and O_3-treated cantaloupe plants.

References

Allen, W. A., and A. J. Richardson. 1971. Calibration of a laboratory spectrophotometer for specular light by means of stacked glass plates. *Rev. Sci. Instrum.* 42:1813-1817.

Cardenas, R., H. W. Gausman, W. A. Allen, and Marcia Schupp. 1969-1970. The influence of ammonia-induced cellular discoloration within cotton leaves (*Gossypium hirsutum* L.) on light reflectance, transmittance, and absorptance. *Remote Sens. Environ.* 1:199-202.

Cardenas, R., H. W. Gausman, and C. E. Thomas. 1972. Photographic previsual detection of watermelon mosaic virus in cucumber. *J. Rio Grande Valley Hort. Soc.* 26:73-75.

Craker, L. E., and W. J. Manning. 1972. Demonstrating air pollution with plants. *Misc. Bull.* Suburban Experiment Station, Univ. of Mass., Waltham. 17 p.

———— 1973. Demonstrating ozone injury on plants. *J. Agronomic Education.* 2:23-24.

Evans, L. S., and I. P. Ting. 1974. Ozone sensitivity of leaves: relationship to leaf water content, gas transfer resistance, and anatomical characteristics. *Amer. J. Bot.* 61:592-597.

Gausman, H. W. 1974. Leaf reflectance of near-infrared. *Photogramm. Eng.* 40:183-191.

Heggestad, H. E., and W. W. Heck. 1971. Nature, extent, and variation of plant response to air pollutants. *Advan Agron.* 23:111-145.

Heilman, M. D., C. L. Gonzalez, W. A. Swanson, and W. J. Rippert. 1968. Adaptation of a linear transducer for measuring leaf thickness. *Agron. J,* 60:578-579.

Hoffer, R. M., and C. J. Johannsen. 1969. Ecological potentials in spectral signature analysis. P. 1-16 *in* P. Johnson, ed. *Remote Sensing in Ecology.* Univ. Georgia Press, Athens. 244 p.

Howell, R. K., and E. F. Kremer. 1972. Ozone injury to soybean cotyledonary leaves. *J. Environ. Quality* 1:94-97.

Jensen, W. A. 1962. *Botanical Histochemistry.* W. H. Freeman & Co., San Francisco. 408 p.

Leamer, R. W., J. R. Noriega, and C. L. Wiegand. 1978. Seasonal changes in reflectance of two wheat cultivars. *Agron. J.* (In Press).

Leamer, R. W., V. I. Myers, and L. F. Silva. 1973. A spectroradiometer for field use. *Rev. Sci. Instrum.* 44:611-614.

Manzer, F. E., and G. R. Cooper. 1967. Aerial photographic methods of potato disease detection. *Maine Agric. Exp. Sta. Bull.* 646.

Marx, J. L. 1975. Air pollution: effects on plants. *Science* 187(4178):731-733.

Schubert, J. S., N. H. MacLeod, and R. K. Howell. 1972. Changes in optical properties of stressed and diseased leaves. *Amer. Soc. Agron. Abst.* p. 167.

Sinclair, T. R. 1968. Pathway of solar radiation through leaves. M. S. Thesis, Purdue University Library, Lafayette, Indiana. 179 p.

Steel, R. G. D., and J. H. Torrie. 1960. *Principles and Procedures of Statistics.* McGraw-Hill, New York. 481 p.

Thomson, W. W., W. M. Dugger, Jr., and R. L. Palmer. 1966. Effects of ozone on fine structure of the palisade parenchyma cells of bean leaves. *Can. J. Bot.* 44:1677-1682.

Walker, J. T., and J. C. Barlow. 1974. Response of indicator plants to ozone levels in Georgia. *Phytopathology* 64:1122-1127.
