Spectrofluorescence of Extracts from Virus-Infected Tissues

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THE URGENT NEED for quick and reliable means of diagnosing citrus virus diseases has caused virologists to investigate new methods. Dyes and spectrophotometric methods have been used, but the results have not been wholly satisfactory. Recently, tissues of sweet orange affected by greening were shown to be fluorescent. This paper reports the results of preliminary investigation of virus-infected citrus tissues with a spectrofluorimeter.

Materials and Methods

The following materials were examined: 1) apical leaves from both healthy and exocortis-affected 2-year-old Etrog citron 60-13 (*Citrus medica* L.) plants; 2) mature leaves from both healthy and exocortis-affected 2-year-old Etrog citron 60-13 plants; 3) young and mature leaves from both healthy and crinkly-leaf psorosis-affected Tarocco and Moro sweet orange [*C. sinensis* (L.) Osb.], sour orange (*C. aurantium* L.), and Avana mandarin (*C. reticulata* Blanco) trees; and 4) fruits from both healthy and impietratura-affected Moro sweet orange trees. Some leaves and fruits of the Moro sweet orange trees affected by impietratura also showed symptoms of psorosis B.

In each trial, a variable quantity of leaves, ranging in weight from 20 to 25 g was homogenized by means of ultrasounds with ten times as many grams of distilled water. The homogenate was centrifuged at 10,000 rpm for 5 min, and the clear, almost colorless extract obtained was filtered and diluted 1/10. In preliminary trials, the final dilution of 1/100 was found to be the most suitable dilution for observations at the spectrofluorimeter.

The extract of the fruit (10 healthy and 10 unhealthy) was obtained from the inner part of the fruit only, and great care was taken to prevent the essential oils of the skin from mixing with it. The extract so obtained was treated as above. In this case the liquid was slightly pink.

Finally, we placed 3 ml of each of the above extracts in quartz cells (1 cm thick) and examined their fluorescence spectrum by means of the Zeiss PMQ II spectrophotometer and the device for fluorimetry ZFM 4. As exciting radiation we used the Hg line at 365 mμ (= 3650 A).

Results

It should be stressed that the scale according to which the intensity of fluorescence is measured is arbitrary and different for each diagram. In our investigation, citrus tissue extracts showed fluorescence values in the interval between 300 and 700 mμ wavelength, as follows. 1) Extracts from apical leaves from Etrog citron (Fig. 1), both the healthy and the exocortis-affected, showed two maxima of fluorescence in the ranges of 366 and 460 mμ. However, at 366 mμ the extract of healthy leaves showed substantially higher fluorescence than that of the virus infected one. At 460 mμ exactly the opposite occurred. 2) Extracts from mature leaves of Etrog citron, both healthy and the exocortis-affected leaves, behaved in the same fashion relative to each other as did the apical leaf extracts. 3) Extracts from healthy and crinkly-leaf-affected

young and mature leaves of Tarocco and Moro sweet orange, sour orange, and Avana mandarin, all behaved, relative to each other, in the same fashion as did the Etrog citron leaves. However, at 460 mμ, the leaves of virus-infected trees failed to show fluorescence substantially higher than that of the healthy leaves (Fig. 2). 4) Extracts from fruits of Moro sweet orange (Fig. 3), and the extracts of healthy and impietra-

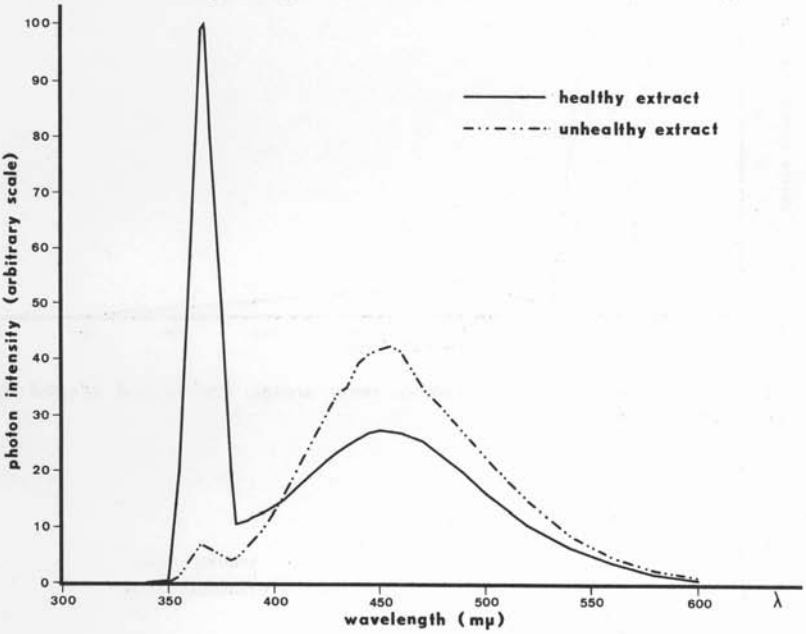

FIGURE 1. *Extracts of apical leaves of Etrog citron 60-13, healthy and affected by exocortis.*

tura-affected fruits behaved, relative to each other, in the same fashion as did the extracts of sweet orange, sour orange, and mandarin leaves.

Discussion and Conclusions

The difference in the behavior of the extracts from healthy trees compared to those from infected ones is possibly due to differences in chemical composition. It is very likely that in the healthy extracts there is a substance, or a group of substances similar to each other, which shows a maximum of fluorescence at 366 mμ. One the other hand, the extracts of infected tissues would have only a very small quantity of such a substance. Similarly, extracts of the infected tissues, particularly those from Etrog citron, may contain a substance, present only in very small quan-

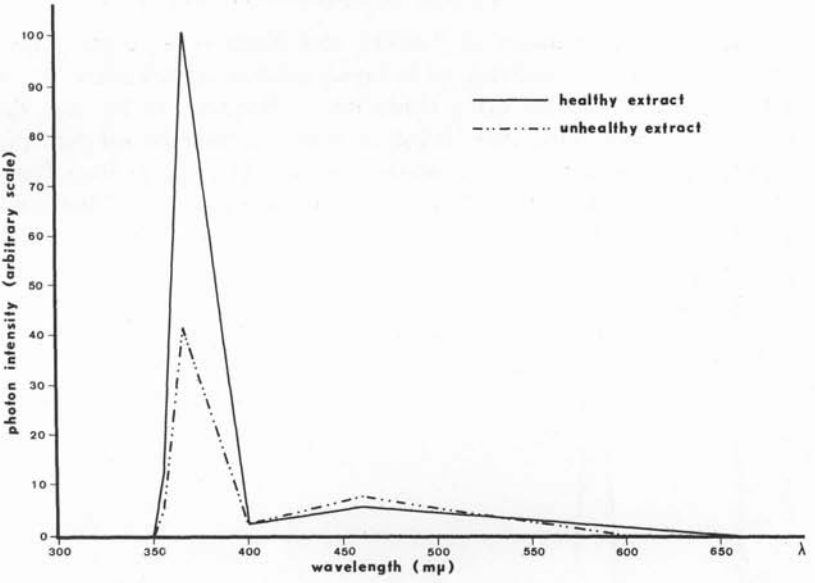

FIGURE 2. *Foliar extracts of Tarocco sweet orange, healthy and affected by crinkly-leaf psorosis.*

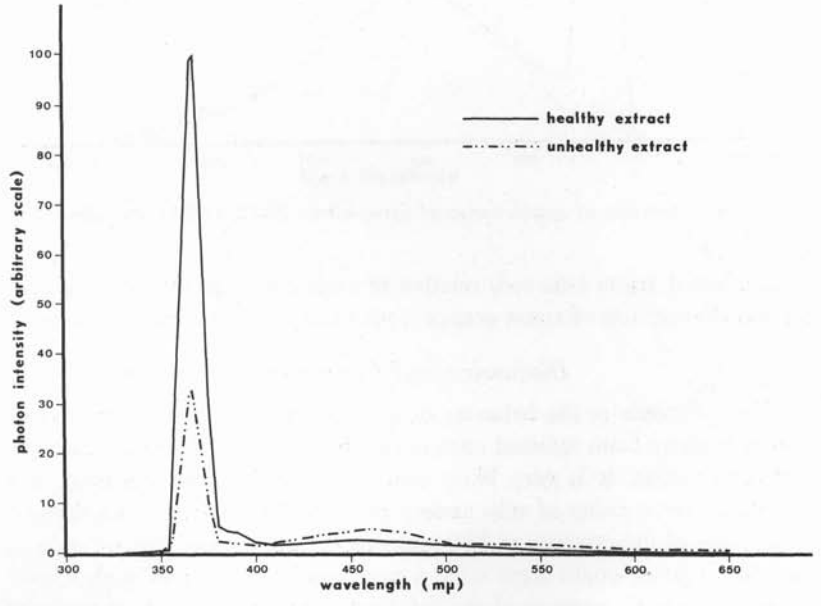

FIGURE 3. *Extracts of fruits of Moro sweet orange, healthy and affected by impietratura.*

tity in the healthy extracts, and having a maximum of fluorescence at 460 mμ. We have not investigated the nature of these substances, nor have we ascertained whether the different composition of the extracts of infected tissues, as compared to the healthy ones, may be ascribed to the reaction of the tree to the virus or directly to the virus bodies.

Literature Cited

1. SCHWARZ, R. E. 1965. A fluorescent substance present in tissues of greening-affected sweet orange. S. African J. Agr. Sci. 8: 1177-1180.