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Retention of Chlorophyll in Lesions Produced by Tobacco Ringspot Virus

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Retention of Chlorophyll In Lesions Produced By Tobacco Ringspot Virus[1]

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Abstract. On intact and detached cotyledons of Caserta squash development of lesions produced by the tobacco ringspot virus was observed macroscopically and microscopically. Chlorophyll was destroyed in all portions of infected cotyledons except at the periphery of lesions and at the basal cells of trichomes in the center of lesions. Lesions were smaller on detached, infected cotyledons. Chlorophyll was retained in intact plastids clumped in a reticulate pattern throughout lesions on detached leaves. Chloroplasts in lesions were still deeply pigmented 40 days after cotyledons had been detached, whereas all other areas of the cotyledons were devoid of chlorophyll 1 week after the cotyledons were detached. Bacteria were observed in chlorotic cells in developing lesions and may be involved in cell disruption in portions of lesions.

Certain virus infections of plants result in chlorosis of leaves. The exact mechanism of virus interference with chlorophyll production has not been determined. Cook (1930) and Sheffield (1936) reported that chlorophyll was not broken down by viruses and that chlorosis was produced by the inhibition of plastid formation. The fact that chlorosis develops to a greater extent on young leaves than on older ones, frequently is cited as evidence for this view. A number of viruses, however, cause chlorosis in old, fully developed leaves (Smith, 1935; Peterson and McKinney, 1938). The latter authors have shown that the concentration of chlorophyllase in virus-infected plants was highest in chlorotic leaves and lowest in green leaves. It thus appears that chlorophyll is destroyed in certain cases. In the course of investigations involving viruses of soybeans, peculiarities in the development of chlorosis in lesions produced by the tobacco ringspot virus were observed. An investigation of these peculiarities was the purpose of the research reported herein.

MATERIALS AND METHODS

An isolate of tobacco ringspot virus (TRSV) isolated originally from muskmelon, was used for all inoculations. The virus produced typical concentric necrotic rings on infected tobacco (*Nicotiana tabacum* L.) plants and necrotic local lesions on pri-

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mary leaves of zinnia (*Zinnia elegans* Jacq.) and Cowpeas (*Vigna sinensis* Early Ramshorn (Torner) Savi).

Development of TRSV-incited lesions was studied on cotyledons of squash (*Cucurbita pepo* L. Caserta). Plants were grown in 4-inch pots filled with fertile soil. Cotyledons were inoculated when the first true leaf was about ¾ in. in diameter. Inoculum consisted of diluted juice from TRSV-infected Turkish tobacco leaves that showed concentric necrotic rings. One volume of crude juice was diluted with 9 volumes of 0.01 M phosphate buffer of pH 7 and fine Carborundum (600 mesh) was added immediately before inoculation. The upper surface of each cotyledon was rubbed with a cheesecloth pad saturated with inoculum.

Symptom development on detached cotyledons in sterile Petri dishes containing about 2 mm of sterile, distilled water was observed.

Results

Lesion development began with the appearance of small, light-green areas 2-4 mm in diameter. During the next 24-48 hours, the lesions enlarged to 6-8 mm and became chlorotic. The first indication of a ring pattern was observed with the development of a dark-green ring, 1-2 mm wide, around the chlorotic area. Small groups of necrotic cells then began to appear in the chlorotic area in a circular pattern with a 2-4 mm diameter. Each necrotic area enlarged until a ring of necrotic tissue was formed around the chlorotic center of the lesion. The centers of some of the lesions occasionally were not completely chlorotic at this stage and were very light yellow-green. The necrotic ring expanded to the margin of the dark-green ring of cells. The tissue at the center of the lesion collapsed and turned necrotic 10-12 days after inoculation. The lesions then consisted of a greyish-green center, 2-4 mm in diameter, surrounded by a light-brown 2-4 mm ring. These areas were surrounded by a 1-2 mm ring of dark-green tissue that had not yet collapsed. Beyond this ring was a halo of chlorotic tissue 2-3 mm wide which gradually enlarged, leaving the entire leaf chlorotic except at the lesions.

The persistence of the dark-green rings around the necrotic centers of the lesions was of considerable interest. Cells in this area were examined under the microscope at intervals as the lesions developed. Cell activity was greatly reduced at the earliest stage at which lesions could be detected. Cytoplasmic streaming had nearly ceased, and chloroplasts began to clump in large masses against cell walls. By the time this clumping was completed, the dark-green ring was easily visible.

A similar examination of tissue at the center of the lesions

showed that chloroplasts gradually disintegrated. Disintegration started at many locations in this area, but only a few cells were involved at each location. The affected cells were pale-yellow, and structural changes in the plastids were frequently observed. The chloroplasts finally collapsed in a mass of pale-yellow or colorless material. Chlorotic cells were most evident about 2 mm from the centers of the lesions. These cells later collapsed and formed the rings of necrotic tissue. Motile bacteria were observed in cells showing chlorosis. Bacteria were most numerous in the most chlorotic cells and probably aided tissue break down. As chlorosis progressed in the central areas of the lesions, only the basal cells of trichomes retained their initial green color. Chloroplasts in these cells remained intact until the supporting cells collapsed and died. After the centers of the lesions had become necrotic, these basal cells were easily discernible because of their darker color. Bacteria were not observed in the green basal cells of trichomes or in tissue forming the rings of dark-green cells. However, as many as 12 bacterial cells were observed in nearby chlorotic subepidermal cells. The bacteria ranged from motile rods that averaged 1.7 x 2.5 microns to coocoid forms only 0.5 micron.

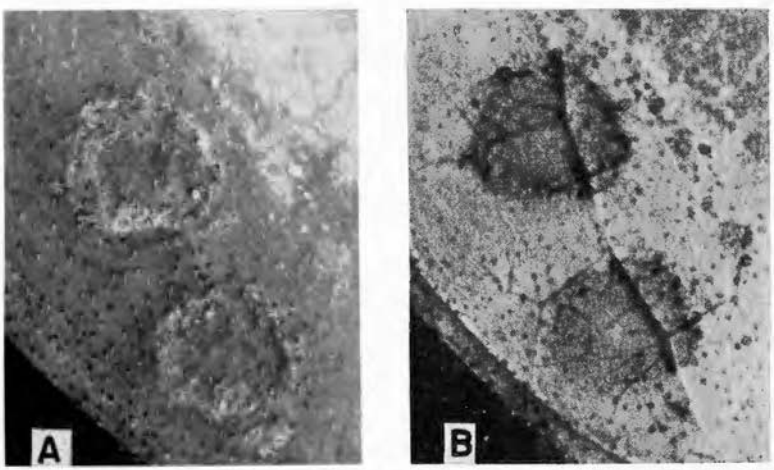

Figure 1. A, lesions produced by the tobacco ringspot virus on Caserta squash cotyledons viewed with incident light. The area of cell disruption is light colored and extends around the periphery of the lesion in a band. B, the same lesions photographed in transmitted light. The dark color of the lesions is caused by presence of chlorophyll. Basal cells of some trichomes also contain chlorophyll and appear as dark dots near the lesions. (X 80)

Detached, infected cotyledons with incipient lesions were kept moist in Petri dishes, and lesion development was observed. Lesions were smaller, averaging 5 mm in diameter. Necrotic areas did not develop because of the presence of sufficient

moisture; however, there was disruption of the cells in this tissue, especially in the epidermal and subepidermal layers (Fig. 1A). This was best observed with incident light. The pattern of cellular disruption was circular and analogous to the ring of necrotic tissue on lesions of attached cotyledons. Presence of chlorophyll in the lesions was best observed in transmitted light (Fig. 1B). The green color of the lesions was most intense in portions of a narrow band at the periphery. Basal cells of trichomes near the lesions were the only green cells outside the lesions on the entire cotyledons. Noninoculated cotyledons developed no lesions and had lost all green color 1 week after they were detached and placed in dishes. Chloroplasts were lysed, and cells were invaded by bacteria and fungi. Very few fungi were observed in deteriorating cotyledons with lesions.

Lesions on detached cotyledons retained their chlorophyll 40 days after they were placed in dishes. At this time they were examined and discarded. The chlorophyll-bearing plastids were still intact and heavily pigmented. Chloroplasts were clumped in elongate masses, and gave the lesions a reticulate appearance (Fig. 2). The chloroplasts were extremely small, averaging 3 microns but sometimes being as small as 1 micron. The pigmented plastids were much more numerous near the upper surface of the cotyledons.

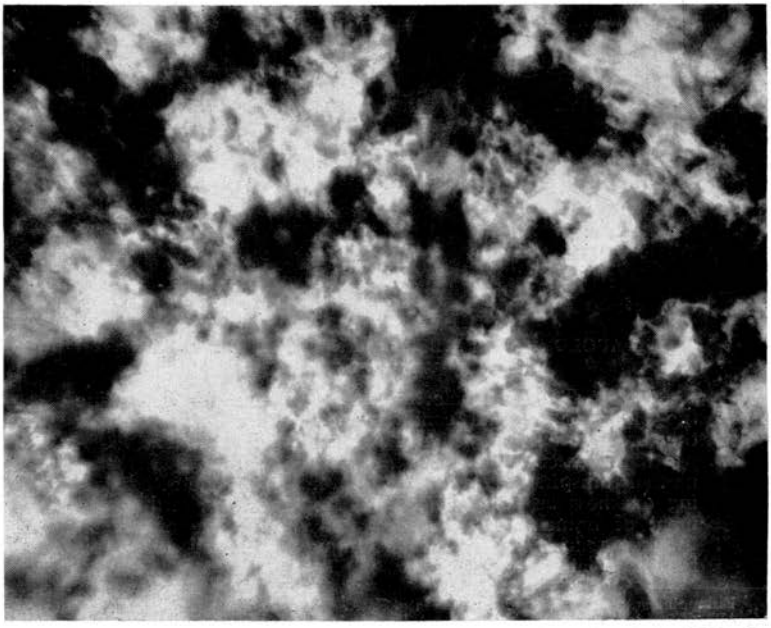

Figure 2. Reticulate pattern of clumped chloroplasts in a lesion produced by the [illegible] on Cucurbita squash cotyledons. (X200)

Discussion

The retention of chlorophyll in virus-produced lesions is of interest in better understanding the effects of viruses on the host plant. The reason that chlorophyll is not broken down in certain areas of lesions remains obscure. Possibly the chlorophyll is somehow fixed by the virus so that it is resistant to the action of chlorophyllase. Inclusion bodies have been reported in trichomes of virus infected plants on numerous occasions (Littau and Black, 1952; Thaler, 1956; Milicic and Plavsic, 1956). Retention of chlorophyll in basal cells of trichomes of infected plants might be due to a high concentration of virus in these cells that inhibits bacterial destruction of chlorophyll.

Disruption of cells in the periphery of lesions on detached cotyledons in which bacteria are active suggests the possibility that damage in this area might be due more to bacteria and bacteria-produced enzymes than to the virus.

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Measurement of Xylary Fluid Movement in Elm by the Thermoelectric Method[1]

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Abstract. The thermoelectric method was used to determine the effect of external conditions and diurnal variation on the direction of xylary fluid movement in elm branches. Upward movement was detected under conditions favoring normal transpirational rates. No movement was detected during darkness or rainy conditions. Possible downward movement of branch fluid was indicated during late afternoon. Further refinement of the technique is needed for clarifying the latter observation.

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