Comparative Anatomy of Healthy and Exocortis Virus-Infected Citron Plants

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ABSTRACT

Specimens from healthy and exocortis virus-infected citron, *Citrus medica*, plants were sectioned and studied before and after fixation. The following anatomical characteristics of the disease in citron leaves were observed: (i) abnormal darkening of contents of parenchyma cells; (ii) hypertrophy and hyperplasia; (iii) granulation of cell contents; (iv) death of cells; and (v) cracking and sloughing of necrotic tissue. All these reactions occurred in the cortical tissue on the underside of the midrib of exocortis virus-infected leaves. Some hypertrophy and necrosis were observed in the phloem. Phytopathology 61:990-993.

Much research has been directed toward the development of methods of indexing for exocortis and other citrus viruses (1, 2, 3). Detection of infection by these viruses has depended mostly on foliar or other external symptoms on susceptible indicator citrus plants. Childs et al. (3) noticed certain anatomical abnormalities in exocortis-affected bark of *Poncirus trifoliata*, and described a staining technique which provides a specific color reaction in affected phloem ray cells of infected plants.

The purpose of the present investigation was to determine what anatomical abnormalities occur in tissue of citron infected with exocortis virus.

MATERIALS AND METHODS.—Arizona 861 and USDCS 60-13 'Etrog' citron plants grown in the greenhouse were used as test plants and as healthy controls. Leaf samples were collected from 3- to 12-month-old plants infected with a severe strain of exocortis virus. These leaves showed various degrees of browning and cracking on the lower surface of the midveins (Fig. 1-A).

Most of the anatomical work was done on leaf midveins. Healthy and exocortis virus-infected specimens of corresponding ages and degrees of maturity were sectioned and studied after being frozen or fixed in Formalin-acetic acid-alcohol solution and embedded in paraffin (7, 8). Midveins were used because the first visible symptoms of the disease usually were confined to the lower surface of the midveins, and midveins were more easily sectioned than stems. The tissue sections were stained with either hematoxylin-lacmoid (11) or 1% safranin (8) and mounted in Canada balsam.

RESULTS.—Sections from midveins of healthy leaves show an epidermal layer and several layers of cortical parenchyma cells containing chloroplasts (Fig. 1-B). Sections in Fig. 1-C and 2-A, B were cut from the midrib of a leaf infected with exocortis virus which had as its only external symptom a light yellow line on the lower surface of the midvein; Fig. 1-C and 2-A show that the diseased cortex contains a greater number of small parenchyma cells, mostly near the lower epidermis, than does healthy cortex. Parts of a few diseased cortical cells stained a very dark purple or deep red (Fig. 2-B). No cells of similar appearance were found in sections obtained from comparable healthy leaves. When viewed at high magnification, most of the darkly staining cells were found to contain a crystalline body (Fig. 1-D). Often the nucleolus in the nucleus of an adjacent cell was found to be larger than the nucleolus in comparable cells from healthy leaves.

The externally visible symptom of an advanced stage of the disease on leaves from which the section in Fig. 1-E was made appeared as a longitudinal yellowish-brown line on the lower side of the midveins. In this section, several cells in the cortical tissue stained a dark purple with hematoxylin-lacmoid. The cytoplasm of these darkly stained cells appeared abnormally dense, and some adjacent cells were hypertrophied. The affected portion of the cortex was near the phloem. Some cells in the phloem and in the cortex had collapsed. It appears that as a lesion in the midrib develops, cell division occurs around the affected part of the tissue, and cells in the cortex and in the phloem become necrotic. Sometimes a few cells in the inner layers of the infected cortex near the phloem contain vacuolated and crystal-like bodies that stained light purple (Fig. 1-F).

In advanced stages of the disease, the macroscopic symptoms are necrotic areas on the lower side of the midvein and a curling downward of the leaf as a whole; Fig. 2-C and D are photomicrographs of sections from

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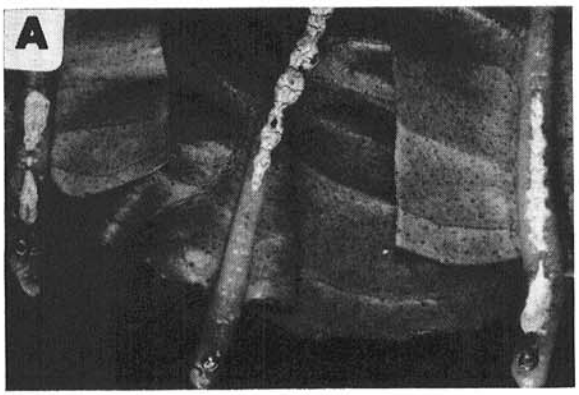
Fig. 1. **A)** Diseased leaves from Arizona 861 citron plant infected with a severe strain of exocortis virus showing browning and cracking on the lower surface of midveins. **B)** Cross section of part of the midvein of a healthy leaf with cortical cells of normal size and rich in chloroplasts. **C)** Cross section of part of the midvein of an exocortis-diseased leaf that had a light yellow line on its lower surface; cortex contains many small parenchyma cells, mostly near the epidermis, and a few darkly stained necrotic cells. **D)** Section through part of cortex of a midvein in C with darkly stained cell containing a crystalline body; nucleolus of the adjacent cell is hypertrophied. **E)** Longitudinal section in cortex of the midvein of an infected leaf with a brown line on its lower surface. Note hypertrophy and several darkly stained cells. **F)** Longitudinal section of cortex near the phloem of an affected leaf as in E. A few cells in the inner layers of the cortex near the phloem contained vacuolated and crystal-like bodies that stained light purple with hematoxylin-lacmoid. c = Cortex; cb = crystalline body; cm = cambium; d = cells stained dark purple or deep red; e = epidermis; h = hypertrophied cells; n = necrotic tissue; nu = enlarged nucleolus; p = phloem; and v = vacuolate bodies.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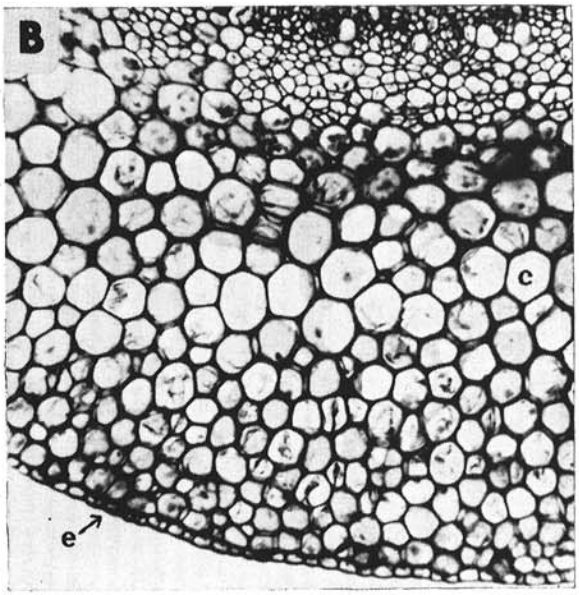
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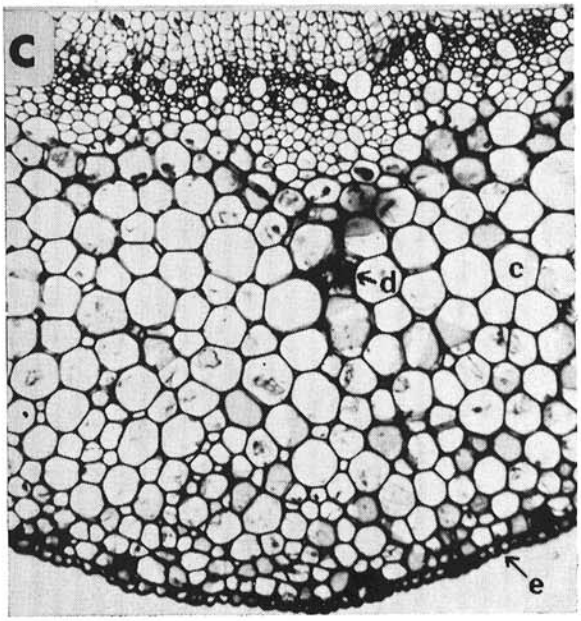
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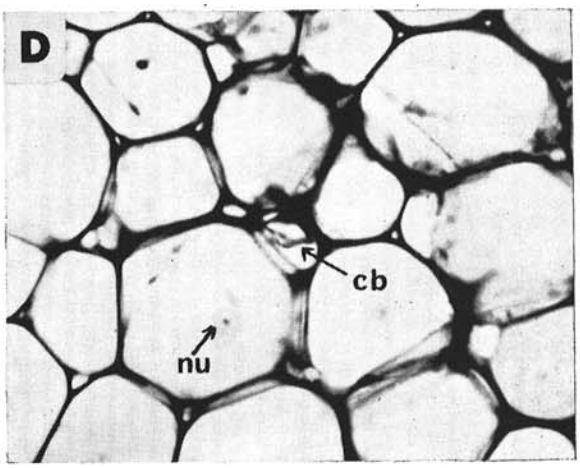
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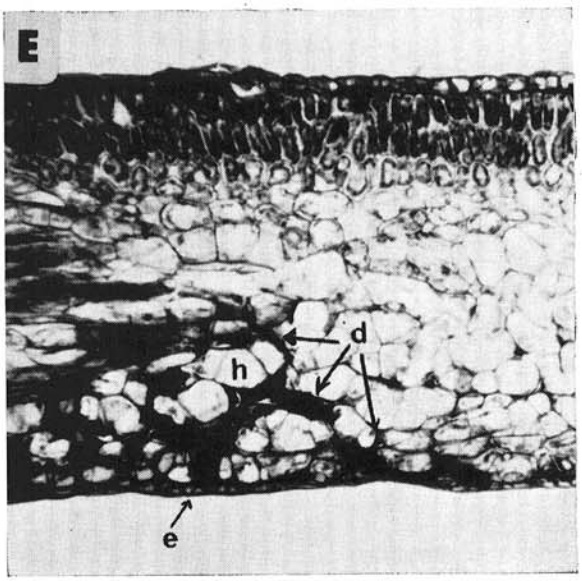
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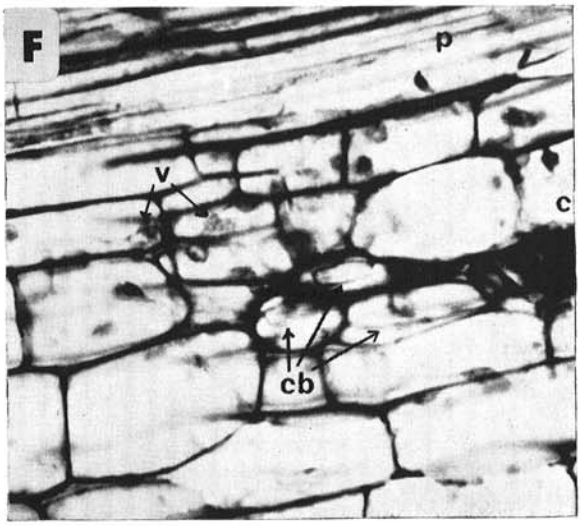
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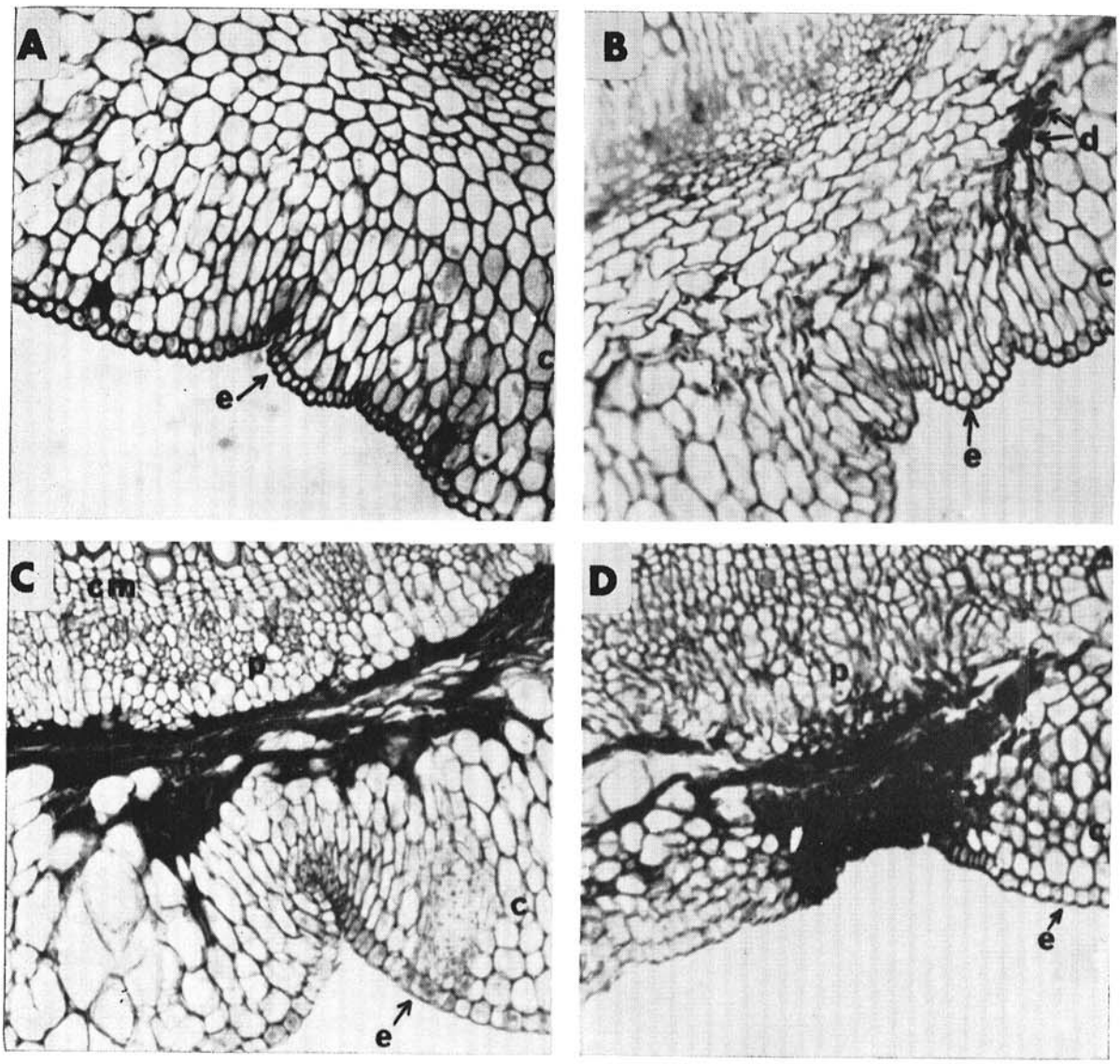

Fig. 2. A, B) Cross sections from part of the underside of the midvein of a diseased USDCS 60-13 citron leaf infected with a severe strain of exocortis virus, and showing a light yellow line on the lower surface of the midrib. Cells near the epidermis are small, and some stained deep red with safranin. Note involution and creasing in the affected area. **C)** Cross section from the underside of the midvein of an infected leaf in an advanced stage. The leaf was curled downward from the tip. Many cortical cells near the phloem were necrotic (stained deep red). Involution has progressed to form a deep crease. **D)** Cross section from the underside of the midvein of an infected leaf that had necrotic areas on the lower surface of the midvein. Much of the affected cortical tissue is necrotic and some cells are hypertrophied. Many of the phloem cells stained very deep red with safranin stain. Some necrotic and some functional sieve tubes were present.

a leaf in an advanced stage of the disease; Fig. 2-C shows progressing involution and creasing of the midvein as more and more parenchyma cells in the cortex become necrotic. Later, the cortex and epidermis rupture between the necrotic tissue and the lower surface of the midvein, and the parenchyma cells surrounding the necrotic area become meristematic. The new mass of parenchymatous tissue around the lesion eventually becomes necrotic, as in Fig. 2-D. A series of new meristematic layers may form beneath the necrotic area as it enlarges in the cortex and through the rays in the phloem. The contents of some parenchyma cells in the rays appear abnormally dense and stain dark purple, revealing a granulated cytoplasm. As the lesion enlarges, the dead mass of cells may fall away, leaving a pit or crack in the midrib.

Discussion.—The nature of the primary symptom, abnormal darkening of small cortical parenchyma cells of midveins, is important in understanding the histology of exocortis-diseased citron. The development of these darkly stained cells in exocortis virus-infected citron plants appears similar to the formation of the chromatic

cells described by Schneider (10) in citrus infected by tristeza virus, except that granulation of cytoplasm is absent in the chromatic cells. The presence of vacuolate or crystal-like bodies in darkly stained citron cells might be due to accumulation of exocortis virus per se, to normal products of host metabolism, or to virus-host interaction.

Necrosis in virus-diseased plants may begin in superficial cells or in deeper layers of tissue, may then spread, and may be followed by hypertrophy or hyperplasia (4) as in exocortis virus-infected citron. Similar anatomical changes were observed long ago by Gardner (6), who noted that necrosis in the stems and petioles of tomato plants infected with a streak-type virus was frequently followed by growth and division of cells surrounding the necrotic pockets.

Necrosis resulting from virus infection may spread inward from the epidermis into the cortex and phloem in bean stems inoculated with southern bean mosaic (9, 14), outward from subepidermal into epidermal cells in leaves of *Nicotiana glutinosa* infected by aucuba mosaic virus (12), or into the phloem and epidermis from the cortex as we have observed in citron leaves infected by exocortis virus. The site of primary necrosis in citron suggests that the parenchyma cells in the cortex are more sensitive than other cells to the effects of exocortis virus infection, and that the virus may be more abundant in the cortical tissue. The latter remains to be proven.

Tobacco mosaic virus can prevent normal expansion of young tobacco leaves by reducing activity of the marginal meristems (13). In young citron leaves infected by exocortis virus, pathological changes in secondary veins usually are less conspicuous than those in midveins. Our observations provide some indication of the cause of the epinasty and curling of infected citron leaves. Downward bending of infected, partially expanded leaves started at about the time midveinal browning or cracking first appeared. While tissues near the upper surface of the infected leaf apparently grew normally, extensive necrosis and reduced growth near the lower surface of the midvein forced the leaf to bend downward. Continuing but unequal growth in tissues near lesions caused cortical cracking. The rapid extension of necrosis in young tissues of Arizona 861 and USDCS 60-13 citron plants causes visible symptoms and makes these clones good exocortis indicators.

The cracking and sloughing of necrotic tissue of exocortis virus-infected citron plants from the living inner portion of the cortex resemble the cracking and bark shelling or scaling of exocortis-diseased trifoliate orange, in which the disease was first observed by Fawcett & Klotz (5), and the deterioration of phloem ray cells in citron resembles that described in *Poncirus trifoliata* by Childs et al. (3).

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