Ultrastructural Studies of Chromatic Cells in Tristeza-Diseased Lime

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IN A REPORT on light microscope studies of tristeza, the name "chromatic cell" was applied to mature ray and phloem parenchyma cells and to meristematic cells that became darkly stained following infection with the tristeza causal agent (6). As mature phloem parenchyma cells became affected, the parietal layer of cytoplasm thickened and the large central vacuole reduced to several small ones. In some cases, the cytoplasm lost its characteristic structure and became translucent, but the translucent cytoplasm became colored with stains. Masses of darkly staining structures resembling elongated crystals or strands

were present in the structureless stained cytoplasm. These idioblasts (chromatic cells) remained alive for a long time as indicated by the facts that the nucleus was intact and the affected cells did not collapse, but in the degenerating phloem they were often necrotic. This phenomenon of chromatism of phloem parenchyma cells was the first observed pathological change that followed graft inoculation. In isolated locales of pitting type hosts (e.g., lime and sweet orange), this condition spread from phloem parenchyma cells to meristems, namely the ground meristem of very young stems and developing phloem and vascular cambium of older stems. Disruptive changes in structure and organization of other cells adjacent to chromatic meristem cells led to vein clearing and pitting.

Tanaka, Shikata, and Sasaki (8) and Shikata and Sasaki (6) described "necrotic" cells with masses of parallel tubules with diameters of 15–18 nm resembling tubular "P protein." They also reported accumulations of long, flexuous, threadlike particles of 10–12 nm with central cores. The cells in which they occurred were no doubt chromatic cells. Their observations were on Hassaku and Mexican lime plants inoculated from Hassaku dwarf disease. The threadlike particles were sometimes located around the masses of P protein, but were occasionally interspersed within them.

The purpose of this endeavor was to study cells as they became infected with the tristeza causal agent in newly forming tissue, using the light and electron microscopes. Emphasis was on the kind and nature of structures that appeared in the cells as they became affected.

Materials and Methods

Between 1966 and 1969, 10 batches of plant material were collected and sectioned. "Trunks" of small lime seedling were side grafted low with scions infected with a causal agent type that produces a moderate amount of pitting. Controls were grafted with healthy scions. Two to 3 weeks after grafting, the single stemmed lime seedlings were cut off 2 or 3 buds above the uppermost of the scions to force new growth. When new shoots from axillary buds were of variable lengths, leaf lateral veins or stem internodes of various stages of development of the new shoots were fixed in either glutaraldehyde, acrolein, or formaldehyde-glutaraldehyde fixative, postfixed in osmic acid, dehydrated in ethanol and propylene oxide, embedded in epon, and sectioned. The pieces selected were far too large for ultrathin sections, and survey sections about 1 micron thick for light microscopy were cut from the surface of the epon-embedded material. Low power photomicrographs were taken of the sections, and areas of cortex, phloem, and cambium with chromatic cells, and the various accompanying pathological disturbances were marked off on the micrographs. The epon blocks containing the tissue were mounted in a trimming device, and

all but the tissue marked on the photographs was trimmed away under a dissecting microscope. From these trimmed blocks, ultrathin sections and occasional monitor sections were made.

Particle sizes were determined from enlarged prints of electron micrographic plates with a compound microscope equipped with a 2X objective and a 12.5X ocular containing a micrometer. Four particles of apparent average size on each of several prints were measured.

Results

ANATOMY OF TISSUES STUDIED.—The parts of the tender young shoots studied were the major lateral veins of leaves and their traces within the stems. To simplify the presentation, only the stem sections will be described in detail, with occasional references to the veins. The vascular tissue of veins and young stems is similar in many respects. The principal tissue studied was the phloem, with considerable attention to the cambium, young xylem, and cortex.

The axial metaphloem and secondary phloem tissue consists of sieve tubes with their associated companion cells and the parenchyma cells. These 3 kinds of "cells" may be referred to as phloem elements. The radial tissue was composed of the interfascicular parenchyma cells. The functional portion of the stem phloem differed from that in the leaf veins by having larger-sized elements and more highly developed companion cells.

In most of the stems and veins studied, the metaphloem was fully developed and the cambium was actively contributing cells to the layer of developing secondary phloem. The meristematic cells in the developing phloem, like the chromatic cells, contained thick cytoplasm and small vacuoles; but they differed in having normal mitochondria, ribosomes, plastids, dictyosomes, and endoplasmic reticula, and none of the rods, tubes, and vesicles found in chromatic cells. Meristematic cells of the developing phloem were sometimes converted to chromatic cells.

Of course there was a layer of functional phloem, and the older sieve tubes of the primary phloem were undergoing degeneration and obliteration. Protophloem fibers were in various stages of formation.

SLIME BODIES.—Only 1 slime body was encountered, and it was in a differentiating secondary phloem sieve tube in the base of a tender, young, infected stem about 3 cm long. The slime body, which was believed to be a normal structure, was composed of tubules about 19–20 nm in diameter. Tubules in the small type of Cucurbita slime bodies are 24 nm (1). In mature sieve tubes, the dispersed slime was composed of nontubular filaments of small diameter.

PATHOLOGICAL ABERRATIONS.—The intensity of the disease varied with the stage of infection and the age of the tissue affected. The initial invasion of the causal agent caused shock symptoms. In the young

acropetal portion of stems, shock effects were very severe in the protophloem and the adjoining ground meristem, with the chromatic condition spreading to the ground meristem and to meristematic developing phloem. In these tissues, cells adjacent to chromatic cells hypertrophied or became necrotic and the tissue was severely pathological. In secondary phloem there was little wound reaction of noninvaded cells adjacent to chromatic cells.

CHROMATIC CELL FORMATION.—Chromatic cell formation involved changes within the cytoplasm of cells of varying stages of maturity. Some phloem elements of the developing phloem were still in the meristematic condition, and their cytoplasm nearly filled the cells. Other cells were mature ones, and pathological changes occurred in parietal layers of cytoplasm.

Three kinds of structures—tubules, filaments, and vesicles—appeared in early stages of chromatic cell formation. It was not established which one appeared first. In some sections one entity and not the other was present, but each electron micrograph is only a very small sample of an entire cell.

Tubules of a diameter of about 15–18 nm occurred in parallel bundles (Fig. 1,A,B). These tubules were often straight, but sometimes they were wavy or curved. The walls of the tubes were composed of spherical particles of about 25 A in diameter. The bundles of tubules were the most conspicuous entities in the cells.

Filaments, 10–12 nm in diameter, similar to those reported by Tanaka, Shikata, and Sasaki (8) were abundantly present in some collections (Fig. 1,B,C). They were randomly arranged in masses and somewhat twisted and curved. An electron transparent core was not visible in our preparations. The filaments were found most frequently in the chromatic cells formed from meristematic developing phloem cells.

Vesicles occurred in clumps of about 5–25 per section and were often enclosed by a membrane (Fig. 1,B). They varied from a few in any one section to the principal component present. The largest of the vesicles were 85–125 nm in diameter. It was not determined how these formed; but occasionally in newly infected cells, apparent vesicles appeared as lone individuals, which possibly by division gave rise to the clumps. Each vesicle was composed of an enclosing membrane with a few particles and filaments internally. Ribosomes were not recognized, and the vesicles probably are not mycoplasma. As the chromatic cells became necrotic, the vesicles like organelles tended to disappear, as did also the cellular organelles.

Discussion

Price (5) and Shikata and his coworkers (7, 8) were apparently not aware that the idioblasts described by them were chromatic cells; it is clear that they are as judged from their published electron micrographs and the work of Kita-

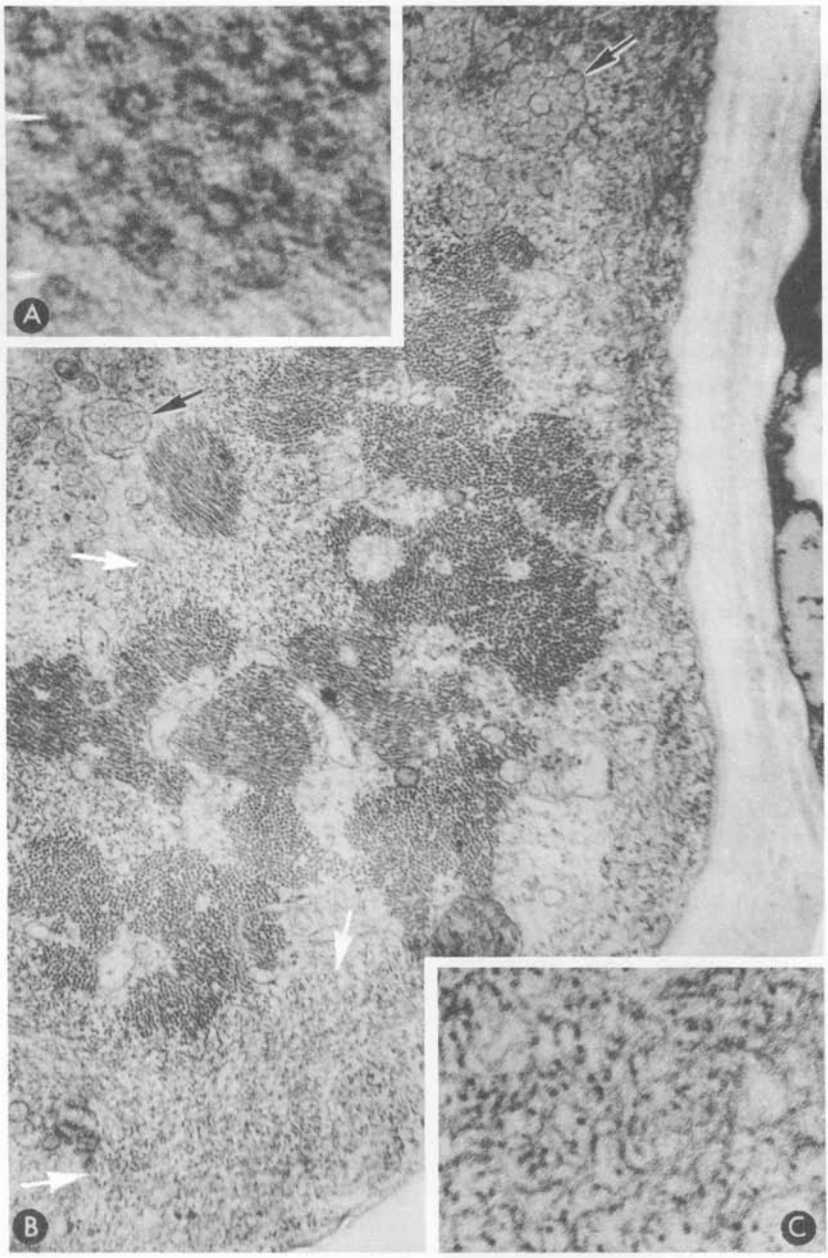

FIGURE 1. *A. Cross section of tubules showing subunits (X 360,000). B. Portion of a cell showing large dark masses of tubules, filaments (white arrows), and vesicles (black arrows) (X 26,000). C. Filaments (X 90,000).*

jima and Costa (4). There are 2 kinds of thready particles in chromatic cells. An attempt at classifying them is presented in Table 1, which is concerned with data in this report and that of 3 published articles.

There are discrepancies in the measurements of the diameters of the large, orderly arranged, parallel tubes. Judging from substructure, tubular nature, and arrangement of tubes, it may be concluded that all 4 papers report these structures. However, reported diameters fall into two ranges, 10–13 nm and 15–18 nm. Workers reporting the smaller measurements did not prefix their material with one of the aldehydes and the tubes may have shrunk. Two groups of workers thought the large tubes to be virus and others thought them to be P protein. The tubes and the masses they compose are almost identical to nonvirus bodies described for parenchyma of Nicotiana infected with tobacco mosaic virus (3), and they also resemble tubular "P protein" described by Cronshaw and Esau (1) as well as tubes of a slime body described herein.

The 10–12 nm thready, flexuous filaments described by workers in Japan (7) are like those described herein although we did not resolve a central core.

The vesicles resemble vesicles associated with the beet-yellows virus in cells of beet leaves (2).

TABLE 1. KINDS AND SIZES OF STRUCTURES OCCURRING IN CHROMATIC CELLS

Reference	Randomly arranged filaments	Parallel tubes	Vesicles
Price 1966	11–13 nm[a] 2–3 nm core "virus"		
Kitajima Costa 1968	6–8 nm. Occur around tubes	10–12 nm "crystaline" "virus"	
Tanaka Shikata A. Sasaki 1969	10–12 nm 2–3 nm core "virus"	15–18 nm P protein	
Schneider P. J. Sasaki	10–12 nm	15–18 nm	85–125 nm grouped in membranes

a. Tubes and filaments were not distinguished one from another.

Conclusions

Chromatic phloem parenchyma cells of tristeza diseased citrus contain 3 kinds of structures not found in phloem parenchyma cells of healthy plants. They are: 85–125 nm vesicles that occur in clumps, each of which is surrounded by a

membrane; parallel tubules that are 15–18 nm in diameter; and filaments that are 10–12 nm in diameter. The causal agent of tristeza remains unknown. The 10–12 nm filaments could be a causal virus, but further study is needed to determine whether the vesicles might be organisms.

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