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J. Amer. Soc. Hort. Sci. 112(5):812–817. 1987.

Morphological Characteristics of Tannin Cells in Japanese Persimmon Fruit

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Additional index words. *Diospyros kaki*, astringency, cell wall structure

Abstract. **Tannin cells from fruit of Japanese persimmon (*Diospyros kaki* L.) cvs. Fuyu [pollination-constant and nonastringent (PCNA)], Chokenji [pollination-variant and nonastringent (PVNA)], Hiratanenashi [pollination-variant and astringent (PVA)], and Kuramitsu [pollination-constant and astringent (PCA)], were observed by florescence microscope (FM) and scanning electron microscope (SEM) on 4 July, when all cultivars were very astringent, and on 5 Sept., when 'Fuyu' and 'Chokenji' fruit had completely lost their astringency. 'Hiratanenashi' and 'Kuramitsu' fruit, however, were still quite astringent on the latter date. FM observations on 4 July indicated that tannin cells of all fruit possessed some discontinuous portions in the cell walls. SEM observations of fractured surfaces of fruit flesh verified the existence of pores in the tannin cell walls. Moreover, coagulated internal contents of tannin cells (caused by the fixatives) had protruded through the pores. On 5 Sept., however, the pores in tannin cell walls of 'Fuyu' and 'Chokenji' were not present, and the surface of coagulated internal contents had become smooth. 'Hiratanenashi' and 'Kuramitsu' fruit on 5 Sept. showed little change in the structure from that observed on 4 July. Pore occlusion occurred in 'Hiratanenashi' fruit that were treated on the tree with ethanol fumes to remove astringency, which indicates that loss of astringency induces structural changes in tannin cell walls.**

Japanese persimmons are classified into the following four cultivar types depending on the effect of pollination on flesh color and whether the fruit lose astringency on the tree: 1) PCNA, 2) PVNA, 3) PCA, and 4) PVA (5, 8). Types 1 and 2 lose astringency naturally during fruit growth and are edible at maturity. Types 3 and 4 do not lose astringency on the tree. Astringent fruit need to be treated with ethanol after harvest to remove astringency. To determine the basic reaction of ethanol in astringency removal, many workers have focused their attention on the chemical properties of tannins (4, 6, 7, 10, 11).

Tannins are known to be stored in large specialized cells in Japanese persimmon fruit. However, studies of the morphology of tannin cells have been lacking, except for those of Howard (2) and Tokugawa and Yuasa (17). The morphology of tannin cells is not well understood and changes in them associated with deastringency are unclear.

In the present study, FM and SEM observations were made to clarify the morphology of tannin cells in Japanese persimmon fruit and to determine if morphological changes are related to the process of deastringency.

Materials and Methods

Five fruit of 'Fuyu' (PCNA), 'Chokenji' (PVNA), 'Hiratanenashi' (PVA), and 'Kuramitsu' (PCA) were collected on 4 July and 5 Sept. 1983, from mature trees at Kyoto Univ,. Kyoto, Japan. The trees had bloomed in mid-May, and the fruit matured late in October. Specimen blocks were removed from the equatorial portion of fruit and immediately fixed with 4% paraformaldehyde and 5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2). Following dehydration in EtOH series, the blocks were embedded in plastic resin, Acrytron E (Mitsubisi Rayon) (13), composed mainly of glycol methacrylate. A molding cup tray and plastic block holder (DuPont Instruments) were used for embedding. At least two embedded blocks per cultivar were sectioned 2–4 μm in thickness on an AO rotary microtome, model 820 (American Optical) equipped with a thin-section adapter and a glass knife. The sections were observed by epifluorescent illumination using an Olympus BHS-RFK fluorescence microscope after staining with 0.1% Kayaphor NL (Nihonkayaku), a fluorochrome similar to Calcofluor White (3).

Received for publication 10 Apr. 1986. We gratefully acknowledge the kind advice of A. Sugiura, Laboratory of Pomology, Kyoto Univ., Kyoto, Japan, and K. Uriu, Dept. of Pomology, Univ. of California, Davis. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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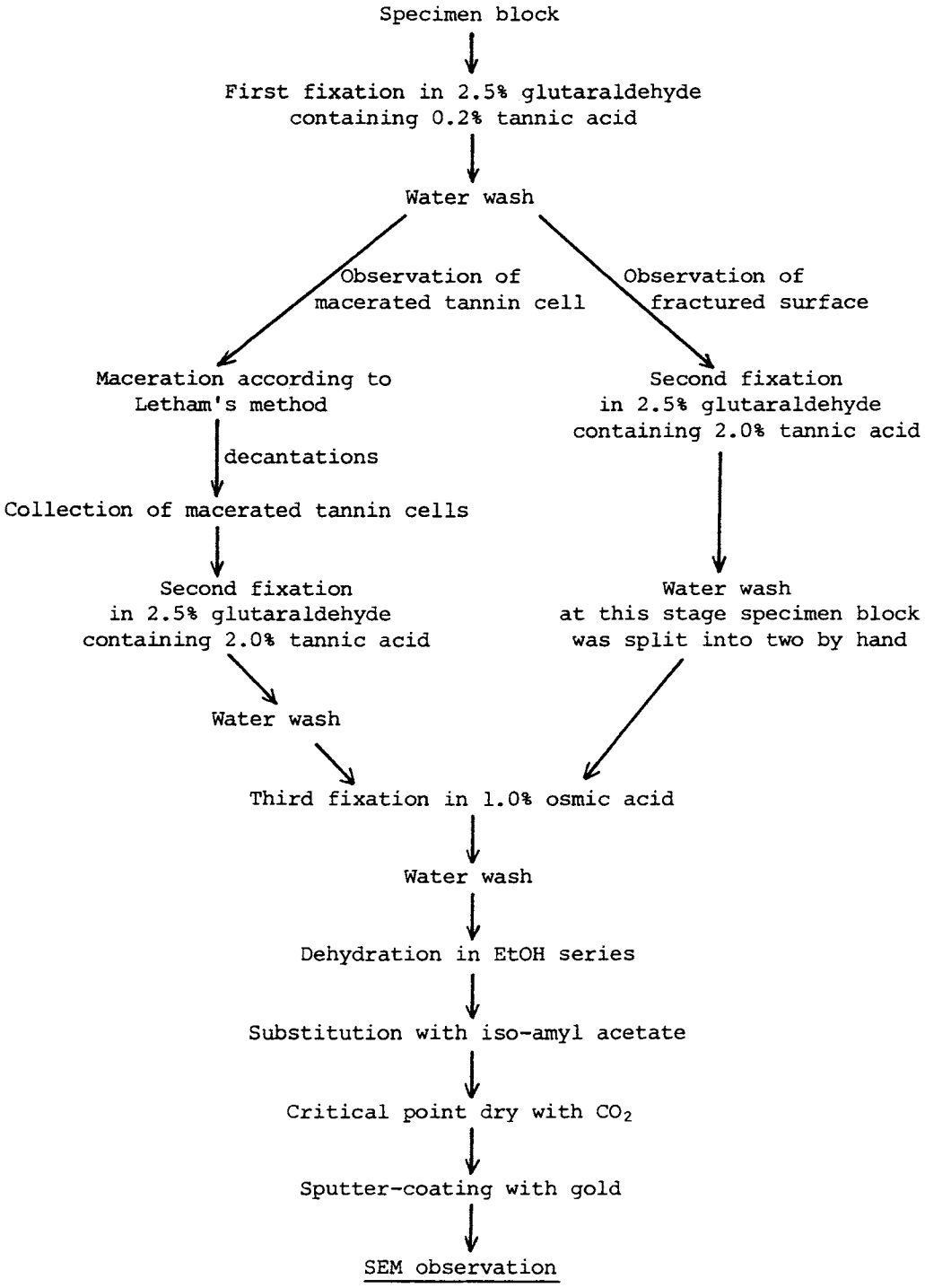
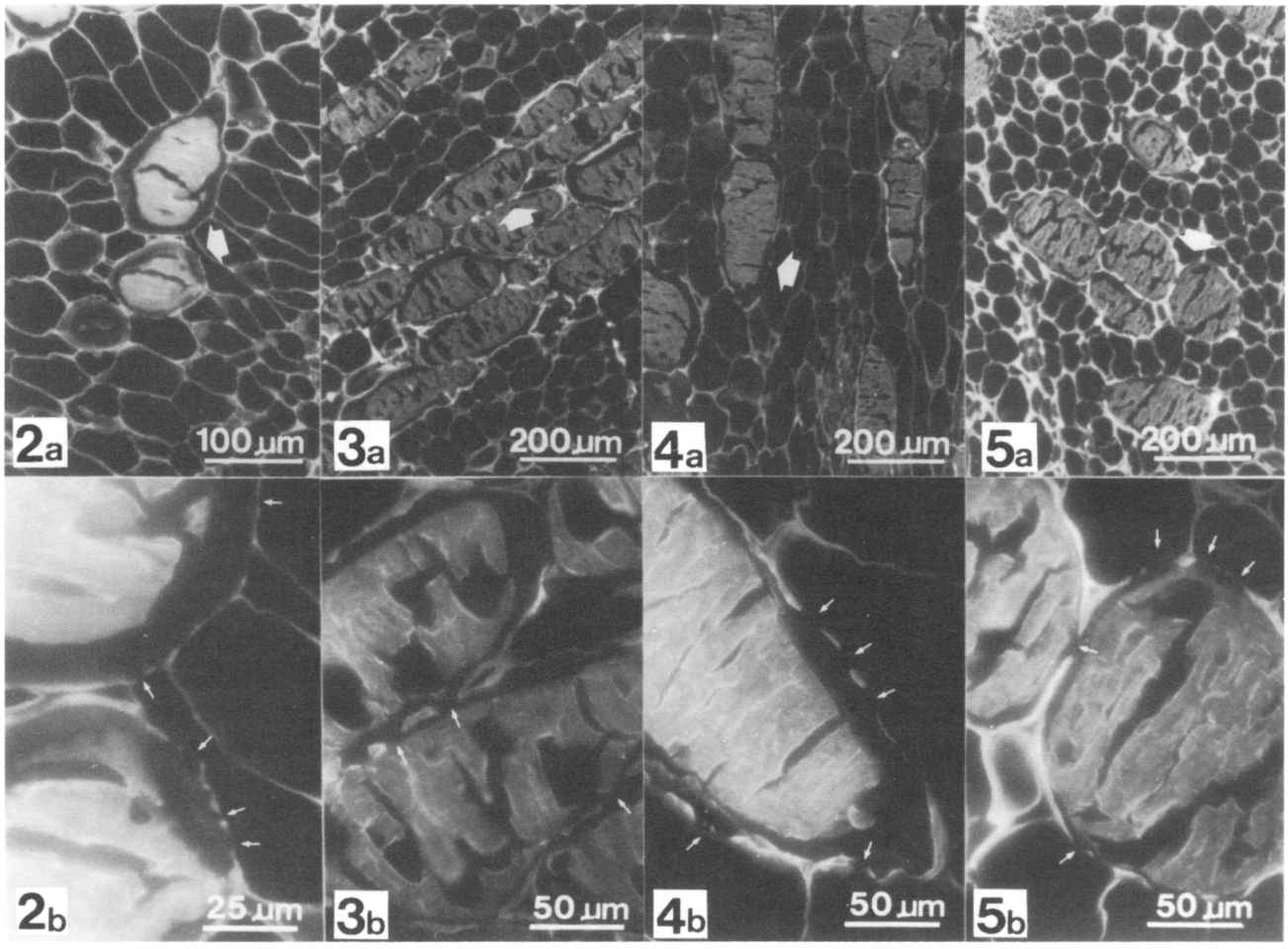


Fig. 1. Procedure for SEM observation of fractured surface of flesh tissue and macerated tannin cells in Japanese persimmon fruit.

Specimen blocks also were taken from the equatorial portion of the fruit and fixed immediately. The procedure for preparing the specimens is shown in Fig. 1. After the first water wash and the second fixation step, the hardened block was split into two by hand to expose the fractured surfaces. In so doing, tannins were coagulated by the fixative and the coagulated internal contents remained within the tannin cells when the specimen was split. Thus, the tannin cells were readily distinguishable from other cells on the fractured surfaces. These surfaces of each cultivar derived from at least two blocks were observed with a Hitachi HHS-2X SEM operated at 15 kV.

Fifty 'Hiratanenashi' fruit on the tree were enclosed on 19 July 1984 in polyethylene bags containing ≈5 ml of 5% ethanol to remove astringency (14). Five days later, when the fruit had completely lost their astringency, the bags were removed and five fruit were picked at 4-day intervals until 9 Aug. Five untreated fruit were picked on 24 July and 9 Aug. Specimen blocks were removed from the fruit and fixed with 2.5% glutaraldehyde containing 0.2% tannic acid. The blocks were washed with water and macerated at 45°C for 5 hr by oscillating at 90 times/min in a 0.05 M EDTA solution adjusted to pH 10.0 (9). Tannin cells were separated from parenchymatous cells by decanting several times. They were prepared for SEM observation according to the procedure shown in Fig. 1. The SEM was op-



Figs. 2–5. Fluorescence micrographs of flesh of (left to right) 'Fuyu', 'Chokenji', 'Hiratanenashi', and 'Kuramitsu' Japanese persimmon fruit on 4 July. Flesh was stained with Kayaphor NL, a fluorochrome similar to Calcofluor White. Lower micrographs (**b**) are high magnifications of regions of upper micrographs (**a**) indicated by arrows. Arrows in lower micrographs indicate discontinuous portions in the tannin cell walls. Note higher magnification in Fig. 2.

erated as described, and all observations were made on 20 to 30 tannin cells.

Results and Discussion

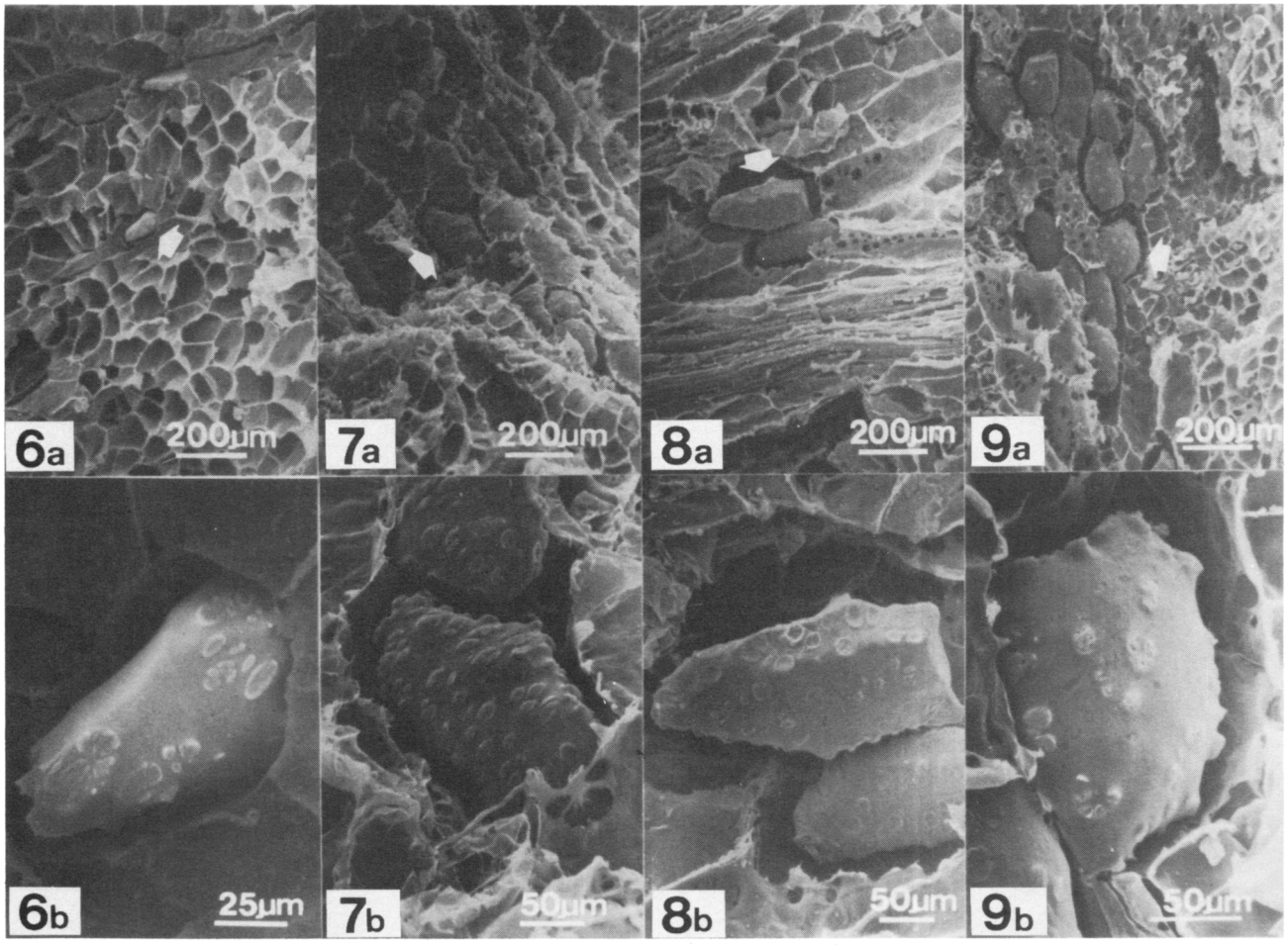
All cultivars were very astringent on 4 July; 'Fuyu' and 'Chokenji' fruit lost their astringency by 5 Sept., when the browning reaction of the flesh appeared. 'Hiratanenashi' and 'Kuramitsu' fruit, however, were still quite astringent on the latter date.

FM observations on 4 July revealed that tannin cells of all cultivars had some discontinuous areas in their cell walls (Figs. 2–5). These areas were formed not only between adjacent tannin cells but also between adjacent parenchymatous cells. The openings were much smaller in 'Fuyu' than in the other cultivars. Furthermore, coagulated internal contents of the cells in all cultivars had very rough surfaces with many protrusions (Figs. 6–9). In this respect, portrusions in 'Fuyu' also were smaller than in the other cultivars.

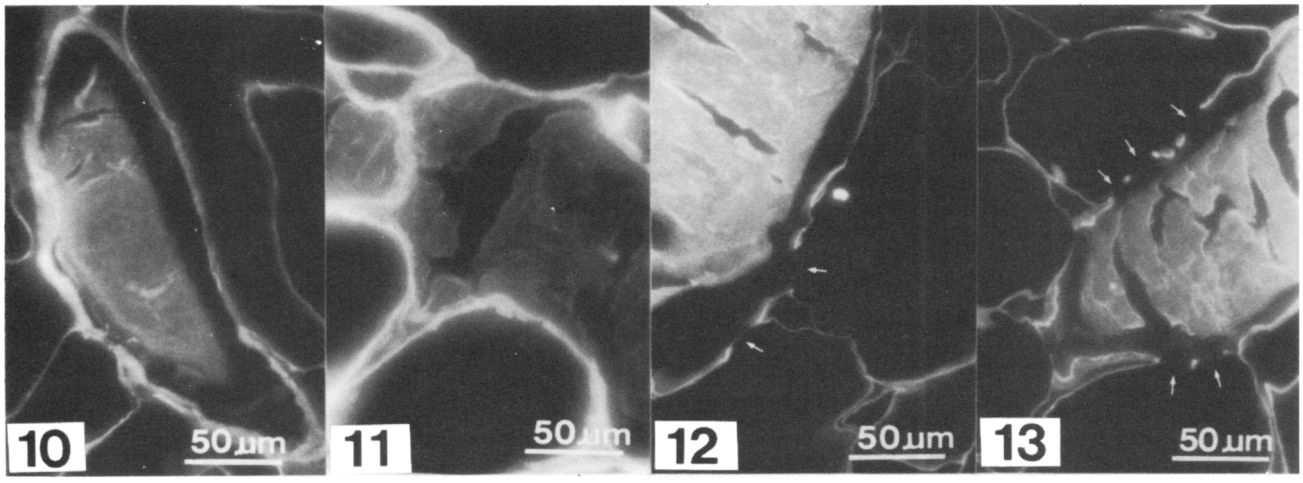
With progressing deastringency, these tannin cell features changed. On 5 Sept., the openings in the tannin cell walls were not present in 'Fuyu' and 'Chokenji' (Figs. 10 and 11), whereas in 'Hiratanenashi' and 'Kuramitsu' they continued to exist (Figs. 12 and 13). Also, the surfaces of the coagulated cell contents had lost their protrusions and had become smooth in 'Fuyu' and 'Chokenji' (Figs. 14 and 15). In 'Hiratanenashi' and 'Kuramitsu', however, there were many protrusions (Figs. 16 and 17).

In addition to the FM observations, we also verified the existence of pores in the tannin cell walls by SEM observations (Fig. 18). The pores appeared to be positioned opposite the protrusions of the coagulated cell contents. Moreover, some micrographs showed that two coagulated cell contents were connected by the protrusions. (Fig. 19). This connection would suggest that the protrusions through the pores of the cell walls are the means by which tannin cells communicate with each other and with neighboring parenchymatous cells.

It is well known that there are areas in plant cell walls, called pits, which are comprised only of primary wall and concentrated plasmodesmata (1). Thus, pore regions observed in persimmon tannin cell walls are probably modified pit fields. Existence of pore-like perforations in tannin cell walls of Japanese persimmon fruits was reported by Howard (2). Our observations confirm this and further show that the protrusions of the coagulated cell contents are located at corresponding pore sites. When the contents of tannin cells are coagulated by fixatives, the vacuoles shrink with a high condensation force pulling the plasma membranes inward. Therefore, the surfaces of coagulated cell contents are considered to be the plasma membranes. If so, our

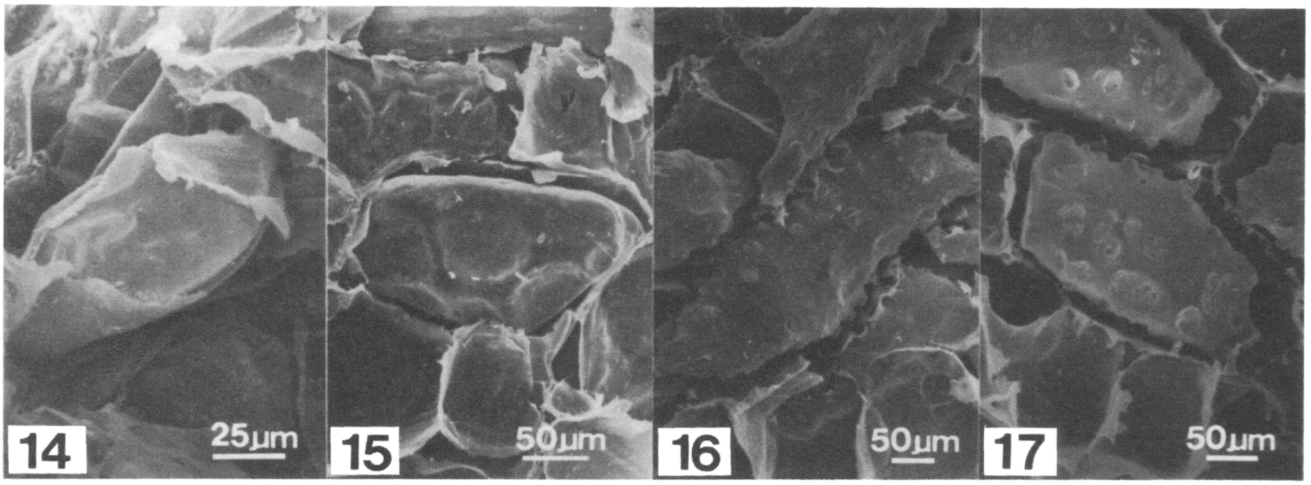


Figs. 6–9. SEM micrographs of fractured flesh surfaces of (left to right) 'Fuyu', 'Chokenji', 'Hiratanenashi', and 'Kuramitsu' Japanese persimmon fruit on 4 July. Lower micrographs (**b**) are high magnifications of regions of upper micrographs (**a**) indicated by arrows. Lower micrographs show surfaces of coagulated tannin cell contents. Note higher magnification in Fig. 6b.

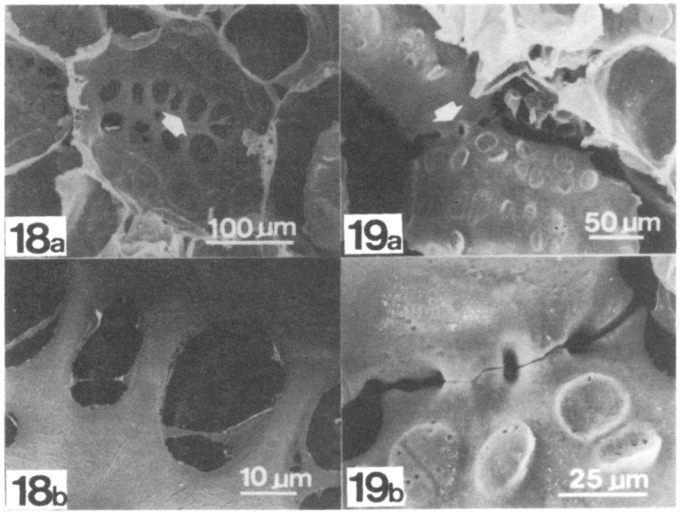


Figs. 10–13. Fluorescence micrographs of tannin cells in flesh of (left to right) 'Fuyu', 'Chokenji', 'Hiratanenashi', and 'Kuramitsu' Japanese persimmon fruit on 5 Sept. These samples were stained with Kayaphor NL. Arrows in micrographs indicate discontinuous or very thin portions in the cell walls. 'Fuyu' and 'Chokenji' had no discontinuities in their cell walls.

observations would indicate that there is membrane-to-membrane contact among tannin cells by extension of the plasma membrane through the pores in their cell walls. It may also imply that there are cytoplasmic connections traversing these areas of contact. Scott et al. (12) demonstrated that the oil-sac idioblast of the mesocarp of avocado fruit had pores in the suberized layers of the cell walls marking the path of plasmodesmata, and that they were related to the accumulation of oil



Figs. 14–17. SEM micrographs of coagulated contents of tannin cells in flesh of (left to right) 'Fuyu', 'Chokenji', 'Hiratanenashi', and 'Kuramitsu' Japanese persimmon fruit on 5 Sept. Tannins were coagulated naturally in 'Fuyu' and 'Chokenji' before fixation and had no protrusions on their surfaces. Note higher magnification in Fig. 14.



Figs. 18–19. SEM micrographs of tannin cells in flesh of 'Kuramitsu' fruit on 4 July. Lower micrographs (**b**) are higher magnifications of regions of upper micrographs (**a**) indicated by arrows. Fig. 18, cell wall showing pores; Fig. 19, view of tannin cells showing continuity of internal contents between cells.

in the idioblast. Tannin cells in 'Fuyu' fruit, which are smaller than in the other non-PCNA cultivars at any fruit growth stage (18), had smaller pores and protrusions on 4 July, which would suggest that these structures of tannin cells are related to tannin accumulation. To demonstrate this, further investigation using transmission electron microscopy will be necessary.

The other interesting feature is that tannin cell morphology changes as deastringency progresses. PCNA- and PVNA-type fruit lost their astringency differently. In the former, a major factor is dilution due to fruit enlargement. Tannin coagulation is a minor factor and occurs in the later stages of fruit growth (18). On the other hand, the latter loses its astringency due to coagulation of tannins induced by seed-produced ethanol and acetaldehyde in the middle stages of fruit growth (15, 16). Tannin coagulation in PCNA fruit leading to complete loss of astringency is never brought about by ethanol and/or acetaldehyde (15). Whatever the mechanism of the loss of astringency might be, the morphological changes in tannin cell walls seem to be associated with loss of astringency. This association was verified by applying ethanol to remove astringency in 'Hiratanenashi' fruits on the tree. Although tannin cells from untreated fruit still had many pores in their walls on 24 July and 9 Aug. (Figs. 20 and 21), cells in treated fruit gradually occluded the pores, with total occlusion on 9 Aug. (Figs. 22–24). Thus, tannin coagulation is responsible for the morphological changes in tannin cell walls.

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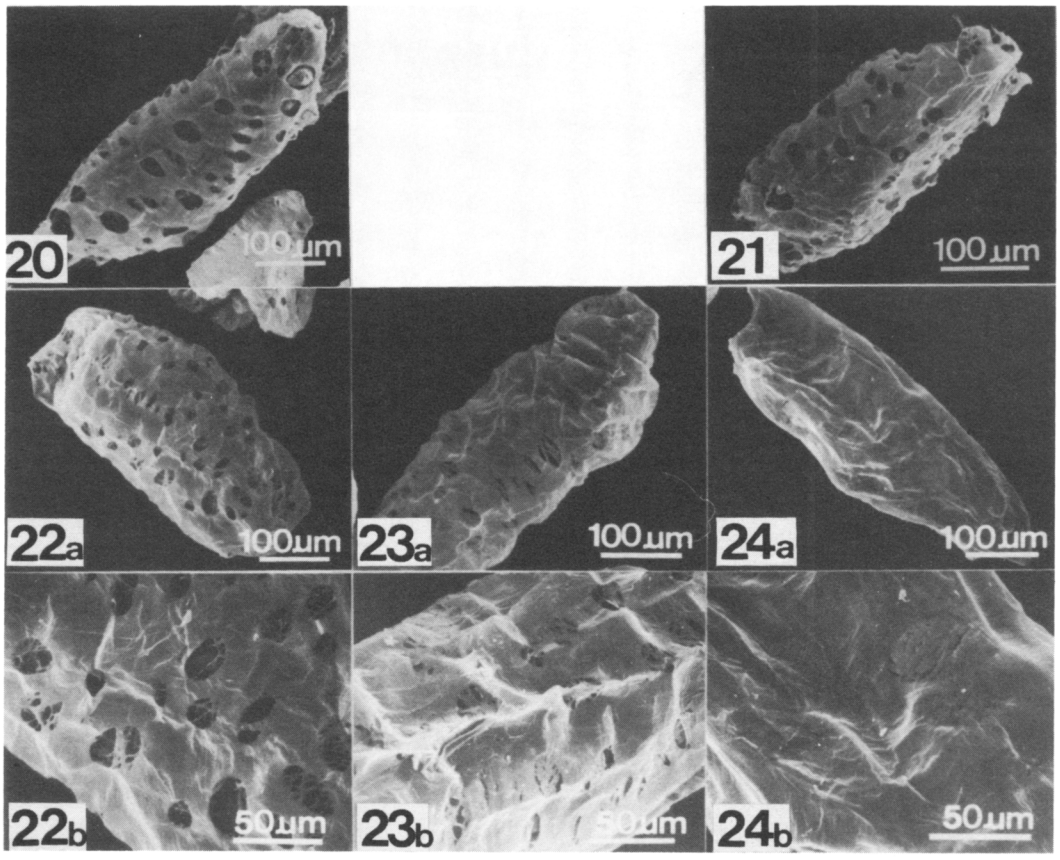

Figs. 20–24. SEM micrographs of macerated tannin cells from untreated and ethanol-treated fruit of 'Hiratanenashi'. Lower micrographs (**b**) are high magnifications of middle micrographs (**a**). Figs. 20 and 21, untreated: Figs. 22–24, ethanol-treated. Figs. 20 and 22, 24 July: Fig. 23, 1 Aug.: Figs. 21 and 24, 9 Aug.

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