Cell Wall Changes in Juice Vesicles Associated with "Section Drying" in Stored Late-harvested Grapefruit

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Abstract. Normal and collapsed juice vesicles were removed from stored late-harvested grapefruit segments (Citrus paradisi Macf. cv. Marsh) and the cell wall anatomy of epidermal and internal parenchyma was compared with light, scanning electron, and transmission electron microscopy. Normal juice vesicles were turgid and elongate, and epidermal cells and internal parenchyma were intact. Collapsed juice vesicles appeared flattened, and internal parenchyma were compressed. Cell wall thickening occurred in internal parenchyma and single or clustered epidermal cells of collapsed vesicles. Cell walls of the same cells in normal vesicles were thin. Epidermal and internal parenchyma cell walls of collapsed vesicles were 10 to 50 to 10 to 20 times the thickness, respectively, of corresponding normal cell walls. Lignin was detected in thickened cell walls of epidermal and internal parenchyma of collapsed vesicles. The results suggest that cell wall synthesis in vesicles is a symptom of section drying in grapefruit.

Materials and Methods

Transmission electron microscopy. Grapefruit were stored for 4 months at 16 to 21°C. Flavedo, albedo, and segment membrane were removed, and individual normal and collapsed juice vesicles were carefully dissected from the segments of at least 10 fruit. Normal vesicles were obtained from turgid or SD sections. Whole vesicles were immersed in 1% OsO₄ and 0.3 m sucrose in 0.2 m K₂PO₄ buffer, pH 7.4. We have found OsO₄ prefix-
Fig. 2. Scanning electron micrographs of epidermal and internal parenchyma (juice cells) of grapefruit juice vesicles. (A) Epidermal cells from normal vesicles. (B) Juice cells from normal vesicles. (C) Epidermal cells from collapsed vesicles. (D) Juice cells from collapsed vesicles.

Table 1. Histochemical reaction of epidermal and internal parenchyma within normal and collapsed juice vesicles of stored late-harvested grapefruit. + = positive staining reaction, – = negative staining reaction.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Normal vesicles</th>
<th>Collapsed vesicles</th>
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<tr>
<td></td>
<td>Epidermal cells</td>
<td>Internal parenchyma</td>
</tr>
<tr>
<td>Aniline blue</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phloroglucinol</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Toluidine blue</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ruthenium red</td>
<td>+</td>
<td>+</td>
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Embedding. Successful embedment of this delicate tissue in resin. After 30 min, the vesicles were washed in buffer and osmoticum, then fixed in buffered 3% glutaraldehyde. In some cases, it was necessary to vacuum-infiltrate fixatives into the tissues for 3 to 4 hr. The vesicles were then post-fixed in 2% OsO₄, 0.3 M sucrose in 0.2 M K₂PO₄, for 1 hr at room temperature. The vesicles were washed with distilled water and dehydrated in ethanol. After placing in 30% L. R. White resin (Polysciences, Warrington, Pa.) in absolute ethanol, the vesicles were carefully cut in half with a razor blade. Infiltration was extended over 5 days in vacuo (–60 kPa). The tissue embedded in resin was polymerized overnight at 60°C. Thick sections (1 μm) through normal and collapsed vesicles were made and stained with 0.1% aqueous toluidine blue for light microscopy. Thin sections (0.1 μm) of epidermal and internal parenchyma of the two vesicle types were made and stained with 4% aqueous uranyl acetate for 15 min and poststained with 0.5% lead citrate (13) for 5 min. The sections were then viewed on a Philips 201 transmission electron microscope.

Cryomicrotomy. Fresh tissue was quickly frozen with liquid CO₂. Thick sections (50 to 100 μm) were made on a Harris model WRC cryostat (Harris Manufacturing, North Billerica, Mass.). Sections were stained for at least 30 min with 0.1% toluidine blue (lignin), saturated phloroglucinol in 20% HCl (lignin), 0.02% aqueous ruthenium red (pectin), or 0.005% aniline blue in 50% ethanol (callose) (9, 12). Staining reaction was determined by viewing with light microscopy.

Scanning electron microscopy. Whole normal or collapsed vesicles were fixed in 3% glutaraldehyde in 0.2 M K₂PO₄ for 3 to 4 hr. They were washed in buffer and cut in half with a razor blade. The cut vesicles were then postfixed in buffered 2% OsO₄ for 2 hr at room temperature. After dehydration in ethanol, the tissue was critical-point-dried. In some cases, for successful
Fig. 3. Transmission electron micrographs of epidermal and internal parenchyma (juice cells) of grapefruit juice vesicles. (A) Epidermal cells from normal vesicles; adaxial surface of epidermal cell wall. (B) Juice cells from normal vesicles. (C) Epidermal cells from collapsed vesicles. (D) Juice cells from collapsed vesicles. c = Cuticle, p = primary wall, sw = secondary wall.

dehydration, the tissue remained in ethanol at 4°C for 1 to 3 days. Sections were made with a razor blade and viewed with a Hitachi S530 scanning electron microscope. Alternatively, after dehydration, the vesicles were quickly frozen in 100% ethanol after fixation at liquid N₂ temperatures, fractured, and viewed with SEM.

Results

The juice vesicle of Citrus consists of an epidermal layer covered with epicuticular wax and cuticle and progressively larger interior juice cells or internal parenchyma (6). The internal parenchyma is highly vacuolate and the cell walls are thin. The internal structures of vesicles were very fragile after 4 months of storage, while the epicuticular material remained firmly attached to the epidermal cell layer. Therefore, permeation of fixation and embedding solutions was incomplete. Because of the impermeable layer of epicuticular material, fixation and embedding solutions were carefully vacuum-infiltrated into the vesicles. As a result, most of the fine architecture of the cytoplasm was lost. However, the cell wall structure appeared to remain intact, as judged by transmission electron microscopy.

Internal parenchyma of affected vesicles had collapsed, which gave the vesicle a flattened appearance. In contrast, normal vesicles were elongate and turgid, and the internal parenchyma cells were large and intact (Fig. 1A and B). Examination of normal vesicles with light microscopy indicated that the internal parenchyma cell walls were very thin. Epidermal cells of normal vesicles appeared more regular in shape and were covered with a thin layer of cuticular material. The internal parenchyma within collapsed vesicles appeared compressed and the cell walls were
thickened. Further observation indicated that epidermal cells with thickened cell walls occurred singly or appeared in clusters throughout the epidermal layer of the collapsed vesicle.

Because fixatives and resin used for tissue embedment may inhibit histochemical reaction, sections from fresh tissue were made with a cryostat, stained, and observed with light microscopy. The cell walls of the interior parenchyma or epidermal cells from normal vesicles did not contain lignin (Table 1). However, lignin was present in walls of collapsed internal parenchyma and in cells with wall thickening in the epidermal layer. Internal parenchyma and epidermal cells from normal vesicles stained positively for the presence of pectin. Stain intensity was similar in the cell walls of both cell types of collapsed vesicles. The absence of a staining reaction with aniline blue indicated that callose was not present in either the internal parenchyma or epidermal cell wall of both vesicle types.

Scanning electron micrographs of cryofractured and hand-cut vesicles revealed thickened cell walls of isolated epidermal and internal parenchyma cells of collapsed vesicles. Cell walls did not thicken in the two types of cells in normal vesicles (Fig. 2 A-D). Thickened cell walls were also confirmed in transmission micrographs of epidermal cells and internal parenchyma of collapsed vesicles. No cell wall thickening was observed in the same cell types from normal juice vesicles (Fig. 3 A-D).

Random measurements taken from 10 separate tissue blocks indicated that the cell walls of internal parenchyma from normal vesicles were ≈200 nm thick. Epidermal cell walls were slightly thicker than cell walls from internal parenchyma (250 to 300 nm), with the cell wall facing the cuticle slightly thicker than the others. Cells in juxtaposition with thickened epidermal cells of collapsed vesicles were of the same thickness as epidermal cells from normal vesicles. In areas where cell wall thickening was observed, epidermal cell walls varied between 2 to 4 μm in thickness. Further, collapsed internal parenchyma cell walls varied between 2 to 10 μm in thickness (Figs. 2 and 3).

Discussion

Vesicle collapse in grapefruit is a disorder that is manifested in the cell wall of internal parenchyma as an accumulation of cell wall materials. Epidermal cells, single or in clusters, also accumulate cell wall materials. Seemingly normal epidermal cells can be found in juxtaposition to the thickened ones. All collapsed vesicles examined, whether from stylar-end, stem-end, or core area, exhibited some degree of cell wall thickening in the internal parenchyma and epidermal layer. The presence of cell wall thickening in disordered vesicles of late-harvest fruit suggests that cell wall synthesis has occurred in these tissues. Deposition of lignin in both cell types indicates that the cells have been traumatized, possibly by increased dehydration or other stress, and have shifted to some degree to secondary metabolism (10). The lack of callose histochemical reaction indicates that cell wall thickening is not the result of injury. We were unable to accurately determine differences in pectin amounts between the two vesicle types even though a positive staining reaction was attained in both tissues. Turrell and Bartholomew (15), in contrast, did not detect pectin in lignified disordered vesicles. This apparent discrepancy may be explained by the removal of soluble pectin from the affected tissue since they allowed tissue to stand for 16 to 18 hr before reaction was recorded. The increase in H₂O-soluble pectin and concomitant decrease in NaOH-soluble pectin in disordered tissue supports this premise (5). Wall thickening of the epidermal cells from collapsed vesicles was strikingly similar to helical or annular secondary wall thickening characteristic of xylem, which suggests that secondary wall formation had occurred. Although uncommon, under cultured conditions, vascular elements with helical wall thickenings were found in lemon juice vesicle stalk cells (11).

SD is a late-season physiological disorder in which vesicles within the segment either appear collapsed or granulated. Granulated vesicles are often larger than normal ones. Their contents have been referred to as gelled, with increased pectin amounts but similar water contents (3-5, 8). Because water can be tightly bound to the pectic fraction, extractable juice from section-dried fruit is greatly reduced. In grapefruit, the segment of SD fruit is composed predominantly of collapsed vesicles. In over-mature tangerines, tangelos, and 'Valencia' oranges, granulated vesicles almost exclusively make up the dried section (2-4). Both types of vesicles can occur in each fruit, however. In addition, collapsed vesicles can be found in the same position of the dried grapefruit segment that granulated vesicles are often found. This suggests a similarity between the two vesicle symptoms of SD.

Bartholomew (3) described the granulated vesicle of 'Valencia' orange as one that initially began to increase in size in the early stage, but ultimately became collapsed in the final stage of the disorder. The increase in size was attributed to the increase in pectic substances that were thought to form a hydrated gel within the vesicle (3-5, 8, 13, 14). Whether the gel occurs extra- or intracellularly has not been established. Increased cell wall thickness and lignin production has been reported for internal parenchyma of late-stage granulated vesicles (3, 15). Thus, granulation in other Citrus fruit and vesicle collapse in grapefruit appear to have common cell wall features that suggest, but do not prove, the two may be related. Our preliminary observations with so-called early stage granulated grapefruit vesicles have indicated that less wall thickening occurs and lignin is absent, which suggests that the collapsed vesicle may be a more senescent stage of the granulated vesicle. Another type of collapse may exist in which vesicles do not enlarge, but the contained cells lose their cellular material and shrink (3). These vesicles are predominantly found at the blossom-end or the core of the fruit section.

Plasmolysis prevented comparison of cytoplasmic components in normal and collapsed vesicles. If cytoplasmic characteristics are to be analyzed, techniques for fixation and embedment must be developed that will not disrupt cytoplasmic components. Plasmolysis is of concern and efforts should directed towards more effective fixation and embedment.

Literature Cited


Permeability of Polymer Film Wraps for Citrus Fruit Fumigated with Hydrogen Cyanide to Control California Red Scale

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Abstract. Permeability to the postharvest fumigant hydrogen cyanide (HCN) varied markedly among 13 plastic film-wrapping materials. Permeability was determined by comparing California red scale (Aonidiella aurantii [Maskell]) (CRS) surviving fumigation on film-wrapped and nonwrapped, insect-infested, fruit. HCN transmission rates for several films also were determined by a permeation cell technique. Some films partially restricted passage of the fumigant to the fruit and CRS survival was high, while permeability of other films differed little from unrestricted exposure on nonwrapped fruit and CRS survival was low. For films with low permeability to HCN, increasing the HCN concentration or the length of fumigation time are possible methods of increasing the amount of HCN that penetrates to the fruit for control of quarantined insects. The permeability of film wrapping materials to fumigants should be a prime consideration when selecting films for wrapping citrus fruit in quarantine situations.

Fumigation treatments are often required for commodities that are shipped from producing areas where insect pests are established to markets in areas free of the target insects (Albrigo et al., 1981; Monro, 1969). Some films may restrict transmission of fumigants used to control insects that infest the fruit (Albrigo and Ismail, 1983; Albrigo et al., 1981; Houck and Mackey, 1989), which would limit the use of such films for fruit shipped to export markets. Therefore, film wraps are not now allowed on citrus during quarantine fumigation (Albrigo and Ismail, 1983; Albrigo et al., 1981; Animal and Plant Health Inspection Service, 1976).

Recent tests with ethylene dibromide (EDB) and methyl bromide (MB) have shown that films vary in their transmission of these fumigants (Houck and Mackey, 1989). These studies showed that some films severely restricted transmission of EDB while most films readily transmitted MB. HCN is often used on citrus as a postharvest quarantine fumigant for control of surface insects such as thrips (Franklinella species), various scale species, and Fuller rose beetle (Pantomorus serinus [Boheman]). The purpose of this study was to determine transmission characteristics of several commonly available films to HCN using CRS as a biological indicator for penetration of HCN.

Materials and Methods

Films. Identification and characteristics of the films tested are summarized in Table 1. Recently extruded polymeric films supplied by the manufacturers in small rolls were used for test purposes. Some of the films also were tested in previous EDB and MB experiments (Houck and Mackey, 1989).

Fruit and insects. Two initial HCN fumigation tests were conducted with ‘Reed’ white seedless grapefruit (Citrus paradisi Macfadyen) from an orchard infested with various stages of California red scale (CRS) [Aonidiella aurantii (Maskell)]. The grapefruit were hand-picked from a block of trees heavily infested with CRS in a test plot at the Univ. of California Lindcove Field Station. Many CRS were dead or parasitized by the internal parasitoid, Comperiella bifasciata Howard (Hymenoptera: Encyrtidae), which often was, in turn, parasitized by its hyperparasitoid Marietta carnesi Howard (Hymenoptera: Aphelinidae). Grapefruits were used not washed or waxed, washed (using FMC Cleaner 39), or washed and waxed (FMC Sta-Fresh 223 wax).

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