Anatomical Changes Associated With the Development of Gold Fleck and Fruit Pox Symptoms on Tomato Fruit

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ABSTRACT

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Anatomical changes associated with three developmental stages of gold fleck-pox syndrome, a physiological fruit disorder of some tomato cultivars, are described. In green fruits, where dark green flecks are visible, the subepidermis and/or the outermost cortex are transformed into a typical spongy mesophyll tissue containing many chloroplasts. When the fruits ripen to the pink stage, the flecks change from dark green to golden yellow; this is accompanied by disintegration of the chloroplasts and secretion of cell wall material into the cell cavities and the intercellular spaces. At the red-ripe stage, external symptoms remain either as gold-colored flecks or develop into necrotic lesions (poxlike symptoms). Proliferation of the cells immediately surrounding these lesions, rupture of the epidermis, and protrusion of underlying cells are observed at this stage.

Additional key words: physiological disorder, *Lycopersicon esculentum*, Mill.

Tomato (*Lycopersicon esculentum*, Mill.) fruit pox has been recognized as a serious disorder for several years and is thought not to involve a biotic pathogen (1, 5). It is characterized by small lesions on the fruit that are usually brown, rough, and corky, and may be either slightly raised or slightly sunken. This disorder can seriously reduce the market value of tomato fruits, and furnish points of entry for pathogens. Fruit pox symptoms are easily distinguished from other fruit necroses such as bacterial speck, or bacterial spot (3, 6). Two types of lesions have been described as pox and as flecks. In 1973, Crill et al. (2) concluded that there were two distinct diseases and named them fruit pox and gold fleck. They were able to distinguish between the two diseases on immature fruits and reported that susceptibility was controlled by different genes.

During the 1974-1976 seasons, we observed both gold fleck and fruit pox on some newly-introduced cultivars in Calfironia. The sporadic occurrence indicated that environmental as well as genetic factors are involved. The objective of this study was to observe anatomical changes related to symptom development. Since we have concluded that both gold fleck and fruit pox symptoms can be manifestations of the same disorder, we will give preference to the earlier name and use 'fruit pox complex' to refer to the gold fleck/fruit pox syndrome. The terms 'fruit pox' and 'gold fleck' will refer to the distinctive symptoms.

MATERIALS AND METHODS

Occurrence and symptom development.—During the seasons 1974, 1975, and 1976, fruits of several fresh market tomato cultivars grown in cultivar evaluation plots in various California locations (Merced, Davis, Stockton, and Westly) were examined at harvest for the incidence and severity of gold fleck and fruit pox. Fruits exhibiting gold fleck symptoms were harvested and held at 20 C to observe postharvest changes.

Anatomical studies.—Tomatoes (cultivar Calmart) used in these studies were grown at Davis, California. Fruit showing various stages of development of gold fleck were harvested, and immediately sectioned for histological observations. Fresh, 100-μm-thick sections of mature-green and dark-pink fruit were made using a Reichert sliding microtome. These sections were infiltrated with water and examined without staining. Epidermal strips were obtained from the fruit about halfway between the stem end and the equatorial diameter by submerging them briefly in boiling water. Epoxy-embedded, 2.5-μm-thick sections were obtained using a Sorvall JB4 microtome. Then, 3-5 mm^3 blocks of the outer fruit wall were fixed in 3% glutaraldhyde in 0.1 M phosphate buffer at pH 7 for 4-6 hr. Following several buffer rinses, some of the samples were postfixed in osmium tetroxide. The samples were dehydrated in a graded ethanol series and embedded in Spurr's low-viscosity epoxy medium.

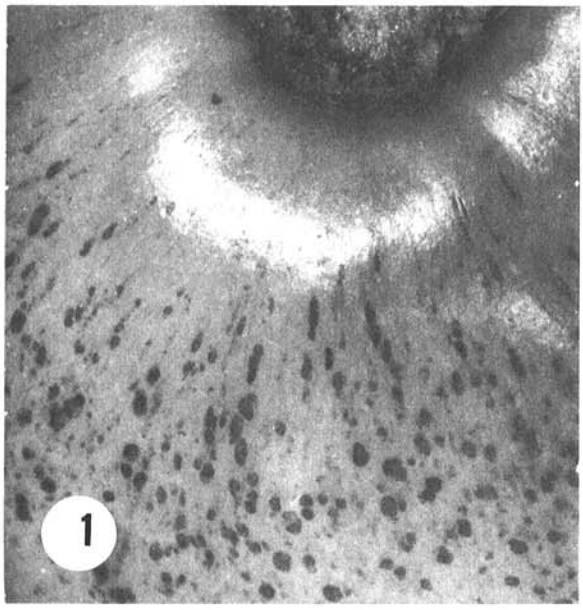
RESULTS AND DISCUSSION

Visual symptoms.—The gold fleck-pox complex developed in three stages:

Stage 1.—On immature- and mature-green fruits, round, elongated or irregular dark green specks or spots, about 0.1 to 3 mm wide, were distributed over the entire fruit surface, especially the proximal half near the stem scar (Fig. 1).

Stage 2.—On partially ripe fruits, the dark green spots became golden yellow. This change occurred whether the fruits ripened while on the plant or during storage at 20 C.

Stage 3.—On red-ripe fruits, the golden yellow specks either remained unchanged or developed into necrotic tan colored lesions (similar to fruit pox symptoms). The gold fleck and pox symptoms both were observed on the same fruit (Fig. 2). Although the necrotic lesions developed on fruits of the plant, none of those showing gold fleck at harvest developed necrotic lesions during subsequent storage at 20 C.



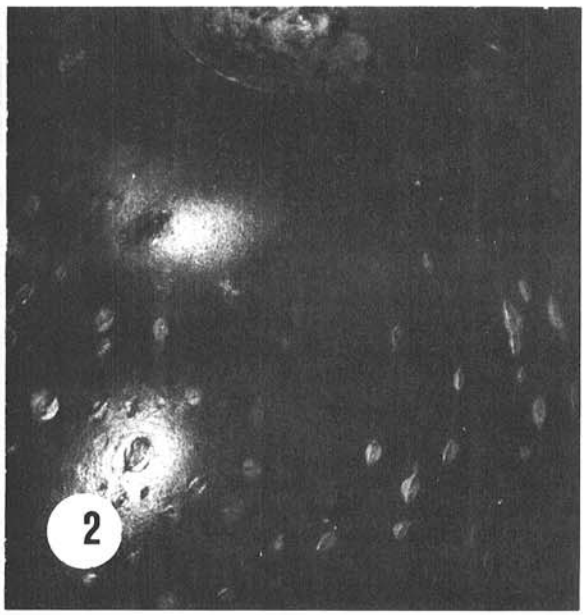


Fig. 1-2. 1) Portion of a mature-green tomato fruit affected by gold fleck in stage 1, where dark green specks are distributed over the entire fruit surface, especially the proximal half near the stem scar. (× 2). **2)** Portion of a vine-ripened tomato fruit showing pox lesions (stage 3) (× 2).

These symptoms are similar to those described by Crill et al. (2) for gold fleck. We never observed the initial symptoms of fruit pox (white lesions on green fruits) described by Crill et al. (2).

Occurrence during the 1974-1976 seasons.—Gold fleck was observed on fruits of several fresh market tomato cultivars and advanced breeding lines; i.e., Castlemart, Castle X29, Castle X1001, Florida MH-1, Calmart, and Jackpot. However, the incidence and severity of gold fleck on these cultivars varied greatly from year to year and from one production area to another in the same season. In 1975, about 20% of the fruits from the above-mentioned cultivars grown in Merced showed gold fleck, but no such symptoms were observed on the same cultivars grown in Davis. In 1976, both Castlemart and Calmart grown in Davis showed gold fleck on approximately 30% of the fruits. Based on examination of 100 plants in each of three fields of Castlemart in Merced in July, 1976, we found large variation in gold fleck incidence from one field to another; the incidence of gold fleck varied among plants within a given field, and almost all fruits on some plants exhibited the symptoms but no fruits were affected on other plants. Obviously several environmental factors influence the expression of gold fleck. In contrast, Crill et al. (2) concluded that various seasonal and environmental conditions did not influence the incidence of the gold fleck/fruit pox syndrome. They also reported that cultivar Florida MH-1 is free from fruit pox and gold fleck, but we observed gold fleck on that cultivar grown in Westly, Calfironia, in the fall of 1974.

Histology of symptom development.—Normally the outer fruit wall of mature-green tomatoes has small, pale-green chloroplasts in both the subepidermal and cortical cells. In affected fruits, the dark green specks (Stage 1), contained more chloroplasts. Individual plastids were larger, and brighter green. Cells with such modified characteristics were found immediately below the epidermis and, less frequently, deeper in the cortex.

The golden yellow spots on partially-ripe tomatoes still contained chlorophyll, but carotenoids appeared to be more prominent. The typical red fluorescence of chlorophyll observed with ultraviolet light was much less than in tissue containing green flecks. Plastids appeared more diffuse, and opaque material interfered with observation of the cells.

The epidermal cells associated with a dark green speck appeared distended with altered cell walls (Fig. 4) as compared with healthy cells (Fig. 3). In pink and red tomatoes (Fig. 6) this trend was further enhanced. The cell walls are very thin, and the three-dimensional appearance of the healthy cells (Fig. 3 and 5) is lost. When oil Red-O staining was used for cutin, the cuticle in normal tissue stained orange, whereas the cuticle in affected tissue remained almost colorless. This indicates that the cuticle of affected tissue is not only thinner, but also differs chemically from that of normal tissue.

On dark-pink and red fruit, some of the gold flecks developed into necrotic lesions similar to the symptoms of fruit pox described by Ivanoff and Young (5) and Butler et al. (1). Anatomically, the epidermis was ruptured (Fig. 7) and an interdigitating lattice of tan colored cell walls emerged. The walls did not stain positive for cutin.

We believe that the necrotic pox-like lesions observed on red-ripe Calmart fruits are a more advanced stage of gold fleck symptoms and not the separate disorder reported by Crill et al. (2). This is supported by (i) when examining whole tomatoes with a dissecting microscope, we never saw incipient white lesions on immature-green fruits, nor the ruptured lesions on mature-green fruits which were described by Crill et al. (2) as initial stages of fruit pox, and (ii) on dark-pink fruits, we observed nonruptured golden-yellow flecks with tan, ruptured lesions at their center, whereas the yellow peripheral areas appeared identical to other nonruptured gold flecks.

Microscopy of epoxy-embedded tissue.—Examination of thin epoxy sections revealed more clearly the differences between normal and gold fleck-affected tomato fruit wall (pericarp). Figure 8 illustrates the organization of the epidermis, subepidermis and several layers of the outer cortex from a mature-green fruit. Figure 9 is a section through a dark green spot on a mature-green fruit. This tissue resembles the spongy mesophyll tissue of a leaf because it contains large air spaces and is composed of the thin-walled, polymorphic

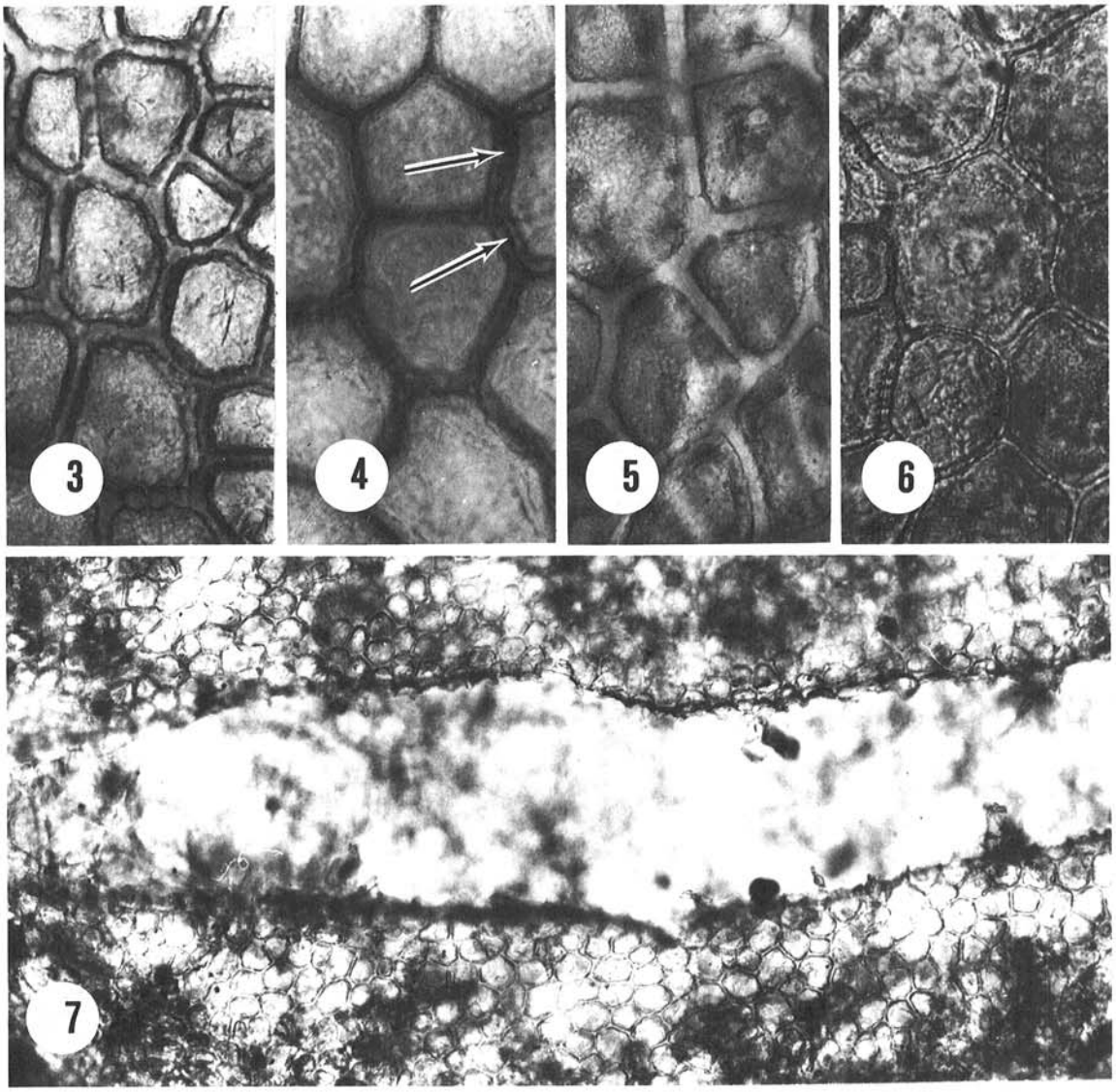


Fig. 3-7. (3-6) Surface views of fresh epidermal strips from tomatoes. The cells are from about midway between the stem end and the equator of the fruits (× 450). **3)** Epidermal cells from a healthy mature-green tomato. Note the three-dimensional effect of the massively walled cells. **4)** Epidermal cells from a dark green region of a mature-green tomato. The cells are distended and the walls appear weakened. The middle lamella is visible at arrows. **5)** Epidermal cells of a healthy ripe tomato. **6)** Epidermal cells from an advanced gold-fleck region in a red tomato. The walls are thin and the cell contents granular. The three-dimensional surface effect is lost. **7)** Surface view of a reptured pox lesion from a ripe tomato. The material within the lesion consists of dead cells with thick, tan-colored walls (× 120).

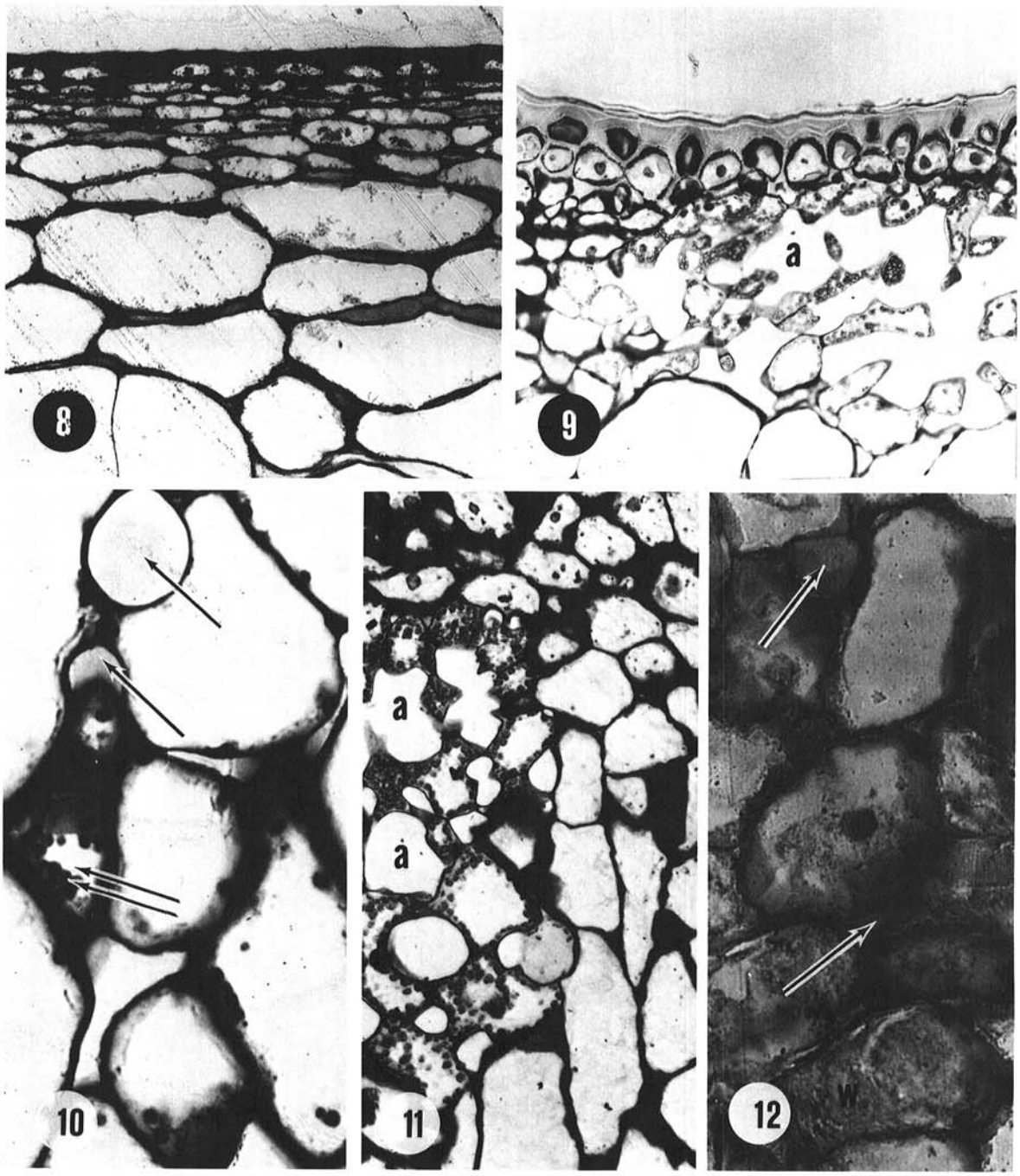


Fig. 8-12. 8) Radial epoxy section from a mature-green tomato through a normal outer fruit wall (pericarp). Plastids are inconsipicuous in the epidermis, the subepidermis, and in the cortex (× 150). **9)** Radial epoxy section through a mature green tomato fruit affected by gold fleck. Note the spongy mesophyll appearance of the pericarp; a = airspace (× 190). **10)** Cells from a tangential epoxy section through a slightly immature-green tomato. Airspaces (arrows) and chloroplasts (double arrows) are evident. The cell containing many chloroplasts is thick walled, as opposed to the thin-walled cells of more advanced gold fleck regions (Fig. 9, 11) (× 480). **11)** An advanced stage from a gold fleck spongy mesophyll region. In this section all the plastids contained starch (× 300). **12)** Cells from advanced gold fleck lesion on a dark-pink fruit. Several cells appear covered with wall material (W) whereas other cells have secreted an amorphus, wall-like material into the intracellular spaces (arrows) and into the cell cavities (× 480).

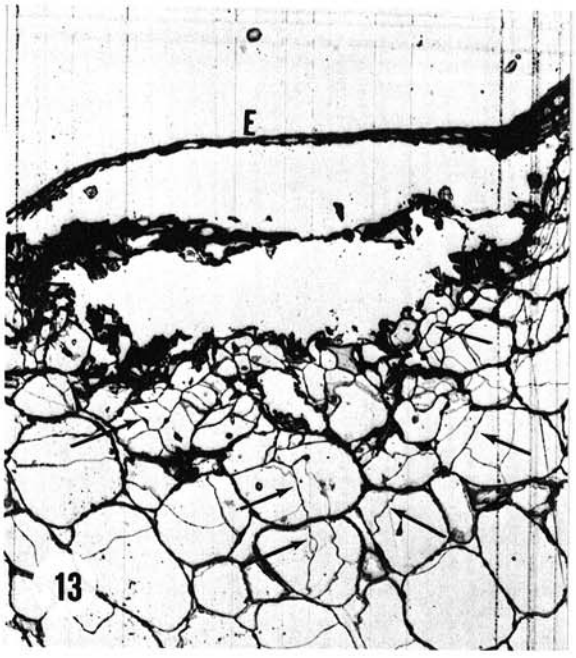


Fig. 13. Radial epoxy section through a ruptured gold fleck lesion (pox stage). In this section the epidermis (E) is present and loosely attached. The black material beneath the epidermis is similar to the tan colored walls seen in fresh pox lesions. These shattered upon cutting. The lesion is surrounded by a large number of newly divided cells; the walls of some of these are indicated by arrows (× 70).

cells with numerous chloroplasts. Such "mesophyll" lesions may contain as few as three cells to as many as 50 or more cells. In Fig. 9, the transformed cells lie directly under the epidermis. Here and at the boundary between transformed and normal tissue, some pressure seems to be exerted and some cells appear to have collapsed.

Figure 10 illustrates two ways in which cortical cells could be transformed into mesophyll-like cells. At the arrows, air spaces have developed between a cortical cell and its neighbors. Only a few plastids are present but the cell wall appears thinner. At the double arrows, a cortical parenchyma cell appears to have elaborated many plastids but still is encased in a thick cell wall.

An advanced mesophyll-like stage from a light-pink tomato is shown in Fig. 11. The subepidermal cells (at the top) and the normal cortical cells (at right) contain a fine precipitate; the cells have thick, swollen walls. The airspaces in the gold fleck-affected mesophyll appear empty, but the cells are glutted with plastids. In this particular section all plastids contained starch grains (as observed by IKI staining). A further symptom development in pink tomatoes is the secretion of an amorphous cell wall-like material into the cell cavity and the spaces between cells (Fig. 12). This material was difficult to stain in epoxy sections. Cell nuclei were still prominent at this stage.

A radial section through the most advanced symptom (tan colored, ruptured lesion) is shown in Fig. 13. The lesion was a rounded structure consisting of thick-walled dead cells surrounded by a newly differentiated disorganized callus. It appeared that rupture of the epidermis and formation of pox-like areas were directly related to this hyperplastic growth, because when these did not occur the gold fleck did not rupture and remained confined beneath the epidermis. Hyperplasia is common in diseased tomatoes and has been described by Gardner (4) for mosaic fruit and by Spurr (7) for tomatoes with blossom-end rot.

The first two stages of gold-fleck do not detract appreciably from the visual quality of fresh tomatoes, but the fruit pox stage can result in economically important losses. Fruit pox is unsightly and can cause cullage at the packinghouse or serve as entry points for decay organisms. Additional work is needed to identify the environmental factors which may contribute to the incidence of the gold fleck-pox complex. The possible involvement of a virus merits investigation. New cultivars and advanced breeding lines should be evaluated for susceptibility to this physiological disorder. Plant breeders should also identify genetic sources of susceptibility and avoid using them in the development of new cultivars.

The anatomical changes associated with gold fleck can be used as an interesting model to study developmental morphology. The developmental stages described in this study are similar to those in a true leaf; green mesophyll formation, aging accompanied by destruction of chlorophyll but preservation of the yellow carotenoids, and finally death of the cells. We have not studied the very early stages of gold fleck and one question is whether the mesophyll-like cells arise from division of the nondifferentiated cells in the carpel or whether they dedifferentiate later from normal fruit wall cells. Our observations, as shown in Fig. 8, suggest that the latter could be the case.

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