

Histopathology of Scar Skin Disease of Apple

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ABSTRACT

Scar skin disease of apple, a fraet-transmissible disease of unknown etiology, produces characteristic external and internal fruit symptoms. Early symptoms consist of raised, red, pimple-like areas. Red coloration in these areas precedes the appearance of brown necrotic scar tissue, which later replaces the red pigmented tissue in epidermal and subepidermal layers of cells. At certain points on the fruit surface, the tissue appears raised due to hyperplastic activity beneath the epidermis. This results in formation of "pockets" of hyperplastic and, later, necrotic cells underlain by

phellogen and periderm. Pressure from these hyperplastic tissues and the underlying periderm causes fracturing of the cuticle. Pocket formation occurs only at scattered points in the affected tissues, while the remaining scar tissue consists primarily of necrotic epidermal cells. The subepidermal layers underlying the necrotic epidermis also become necrotic as the fruit matures. Beneath the necrotic epidermal and subepidermal areas, several layers of periderm form, resulting in the rupture of the cuticle.

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Scar skin, a graft-transmissible disease of apples long considered to be caused by a virus, but still of unknown etiology, was first noticed by Millikan in 1955 as an unusual symptom on Red Delicious fruit in northern Missouri orchards. The symptoms consisted of a corky periderm on the fruit skin similar to that caused by spray damage (10). Symptoms commenced with the formation of light, water-soaked blemishes which radiated from the calyx end. Later, scar tissue developed in the epidermis. This was followed by the development of patches of scar tissue on the sides of the fruit (9). Histological work has been lacking, not only with scar skin, but with most virus and virus-like diseases of pome fruits. The few histological studies which have been conducted of such diseases of pome fruit trees have dealt mostly with the histopathology of stem-pitting problems (4, 5).

Scar skin disease produces distinct fruit symptoms, and offers an opportunity to study the interrelationship between development of external fruit symptoms and the internal histological changes responsible for them.

MATERIALS AND METHODS.—Representative fruits exhibiting scar skin symptoms were collected each week from bud-inoculated 8-year-old Red Delicious apple trees grafted on East Malling VII rootstocks. Control fruits were obtained from noninoculated Red Delicious trees in the same row.

Microscopic observations of apple tissue were made on either fresh-frozen tissue or paraffin-infiltrated tissue. Fresh-frozen tissue embedded in Ames O.T.C. embedding compound was sectioned in a cryotome at 10-20 μ m. The sections were placed on microscope slides and observed immediately. Paraffin-infiltrated tissue was fixed in either FAA followed by a tertiary butyl alcohol dehydration series (7), or in a 10% solution of acrolein according to the method of Feder and O'Brien (2). Dehydrated tissues were placed in a (1:1, v/v) mixture of tertiary-butyl alcohol and paraffin oil, Tissuemat (61 C MP), and sectioned at 10 μ m. Sections were stained in a 0.5% solution of safranin O in 50% alcohol, counterstained with 0.5% fast green in (1:1, v/v) clove oil and absolute ethyl alcohol (7,8) and mounted in Permount.

RESULTS.—*External symptoms.*—The first symptoms on scar skin-affected Delicious apples were observed on June 27 when fruit was 2-3 cm in diameter as small, reddish-brown areas, 0.5-1 mm in diameter,

radiating from the calyx end. Examination with a stereoscopic microscope revealed raised, pimple-like, red-pigmented structures which were more abundant along the ridges of the fruit. During the following week, the symptoms became more intense as a result of coalescence of the small pimples into reddish blotches 5 mm or more in diameter. Nearly all fruits on scar skin-affected trees exhibited similar symptoms at this time. Three weeks after the first appearance of symptoms, when fruits were about 3-4 cm in diameter, small areas of brownish, rough, scar-like tissue appeared within the now enlarged red blotches (Fig. 1). About 30% of the surface was covered with the red coloration at this point. The rough, scar areas were more distinct, appearing as necrotic islands surrounded by red-pigmented areas. Six weeks after the appearance of symptoms, small fissures developed in the brown, scar-like areas. Areas of scar material increased in size and covered most of the red areas, often forming large, brown, shield-like zones. Small fissures increased in depth and width and eventually developed into cracks 2-3 mm in depth and 2-3 mm wide (Fig. 2). As the cracks enlarged, various secondary microorganisms became established in them. By the middle of September, fruits were discolored and distorted in shape, reduced in size, and covered with large cracks and extensive zones of shield-like scar tissue.

Histological observations.—The first histological symptoms in fresh-frozen, cryotome-sectioned tissue appeared as zones of reddish-pigmented tissue corresponding to the macroscopically visible red areas on the fruit surface. The cell shapes and sizes in the epidermal, subepidermal, and parenchyma tissues of the reddish-pigmented zones were exactly like those of the respective nonpigmented control areas. The reddish coloration was localized in the epidermal and subepidermal cell layers. At first the epidermal layer, and later two or three subjacent cell layers, became necrotic. The transition from normal to red-colored to brown to necrotic tissue areas was gradual. In some areas surface necrosis was light and involved only the epidermis, but in others it was more extensive and resulted in scar tissue formation, while at the same time necrosis advanced into the subepidermal layers.

By the third week of symptom development, two basic types of symptoms could be observed in fixed tissue.

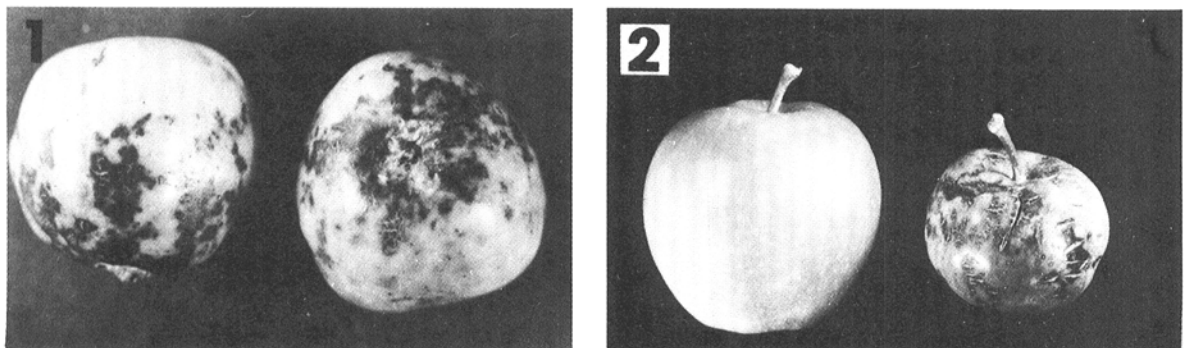
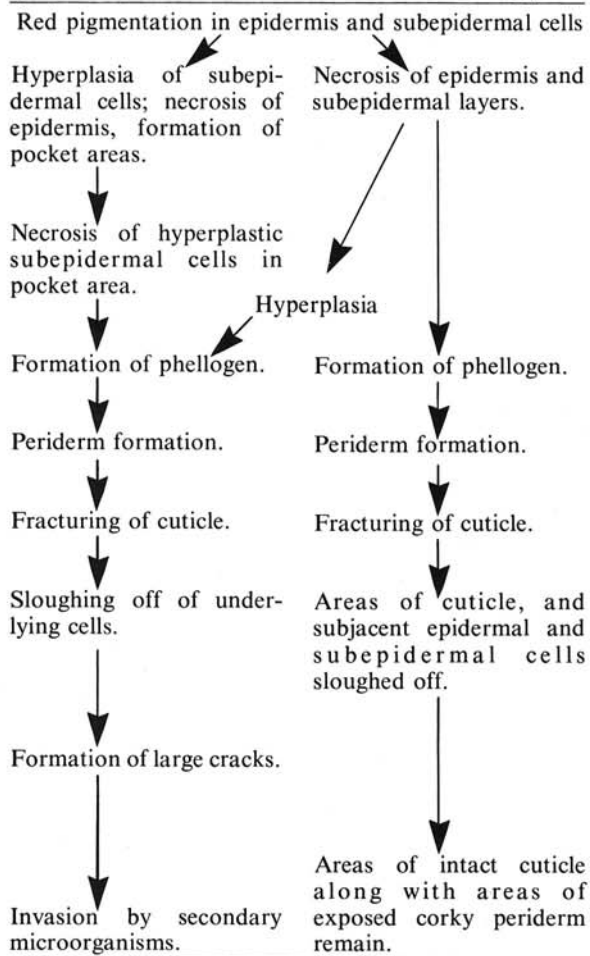


Fig. 1-2. External symptoms of scar skin-infected Red Delicious apples. 1) Small pimples and fissures on young infected fruit. 2) Healthy, mature fruit (left) and infected mature fruit (right) with distorted shape, smaller size, and deep cracks.

These consisted of small protuberances and of thin layers of necrotic epidermal and subepidermal cells. The two symptom types followed the sequential steps diagrammed in Scheme 1 below.

Scheme 1. Sequence of events in the two types of symptoms produced in the scar skin disease of apple.



Within the red-pigmented areas, small protuberances of the cuticle developed. Beneath the raised cuticle, fan-shaped clusters of epidermal cells pushed upward (Fig. 3, 4). Some epidermal cells in the slightly raised area were partially separated from adjacent epidermal cells and were somewhat distorted (Fig. 4, 5). Directly subjacent to these epidermal cells, a zone of hyperplastic activity developed. It appeared to originate in the first cell layer below the epidermis and occupied the area between the epidermis and the subepidermal cells (Fig. 5, 6). Cells within the hyperplastic zone appeared disorganized (Fig. 6) and were arranged in lens-shaped pockets (Fig. 7). Within the protuberances certain epidermal cells began to become necrotic (Fig. 5, 6, & 7). Epidermal cells later collapsed and this was soon followed by necrosis of underlying hyperplastic cells. The increased cell division within the pocket areas resulted in the formation of protuberances. Cells within the pocket became more

deeply stained with safranin, suggesting suberization of cells and possible lignin deposition. At approximately the same time at which cells became necrotic, a layer of phellogen developed subjacent to the zone of hyperplastic, necrotic cells (Fig. 7, 8). The phellogen continued to develop until it produced a complete periderm (Fig. 9). Associated with the periderm layer was a zone of lightly stained cells (Fig. 8, 9, 10) which circumscribed the pocket area. The periderm walled off the pocket from surrounding tissues, and contributed further to outward expansion of the pocket tissues. Cells outside of, but immediately next to, the phellogen appeared relatively normal in that they did not retain as much safranin as the necrotic cells that lay close to the center of the pocket area. These cells, however, retained the safranin slightly more than did remote healthy cells. During phellogen development the cuticle remained intact, but as successive layers of phellem were produced and internal pressure increased, fissuring of the cuticle occurred (Fig. 10). Fissuring appeared at first as small cracks on the surface of the cuticle over the center of pocket areas. The cracks then deepened until the epidermal layer was reached, at which point the cuticle sometimes tended to peel back from the fissure, exposing underlying cells. The fissures increased in size by the sloughing off of the cells lining the walls of the fissure until large open cracks formed (Fig. 11). The phellogen and phellem layers continued developing underneath the pocket areas and, when finally complete, the phellem contained from four to six layers of cells. The cracks, which enlarged due to sloughing off of cells, also increased in depth and reached the phellem at various points. Portions of the phellem and adjacent cells surrounding the crack area became compressed into an area of disorganized, crushed cells (Fig. 12). Various secondary fungi appeared in exposed areas. Periderm development in areas of secondary invasion appeared extensive, but whether or not the periderm excluded these organisms was not determined.

In red-pigmented cells which did not develop directly into raised pimple areas, necrosis of epidermis occurred (Fig. 13). At first, only the epidermal layer of such red-pigmented areas became necrotic, but later the underlying subepidermal layers also became necrotic. At certain points, zones of hyperplastic cells formed pocket-like protuberances in the same manner as described previously for the raised pimple areas in younger fruit. Actually, these pockets could not be distinguished from those described earlier. The areas which did not develop the hyperplastic response developed instead a layer of phellogen which ran parallel to the fruit surface and subjacent to the necrotic epidermal and subepidermal layers (Fig. 14). The amount of periderm produced appeared to depend on the age of the symptoms. Areas which had just become necrotic showed no meristematic activity or only a phellogen layer, while tissue with more advanced symptoms (older necrotic zones) had a phellem of from one to six layers (Fig. 14 & 15). In all these stages, the cuticle remained intact, even though a thick periderm existed below the necrotic epidermis. The developing periderm was not restricted to the areas beneath the heavily necrotic portion of the epidermis and subepidermis, but extended outward, underneath an area

of normal epidermal cells and, finally, disappeared. The phellem varied in thickness from one cell at the periphery of necrosis to four or six cells at the center of necrosis. The thickness of the developing periderm decreased as the

epidermal layer under which it spread became more normal looking. In all areas of phellogen development some safranin was always retained by the cells directly above the periderm, although in some cases the stain

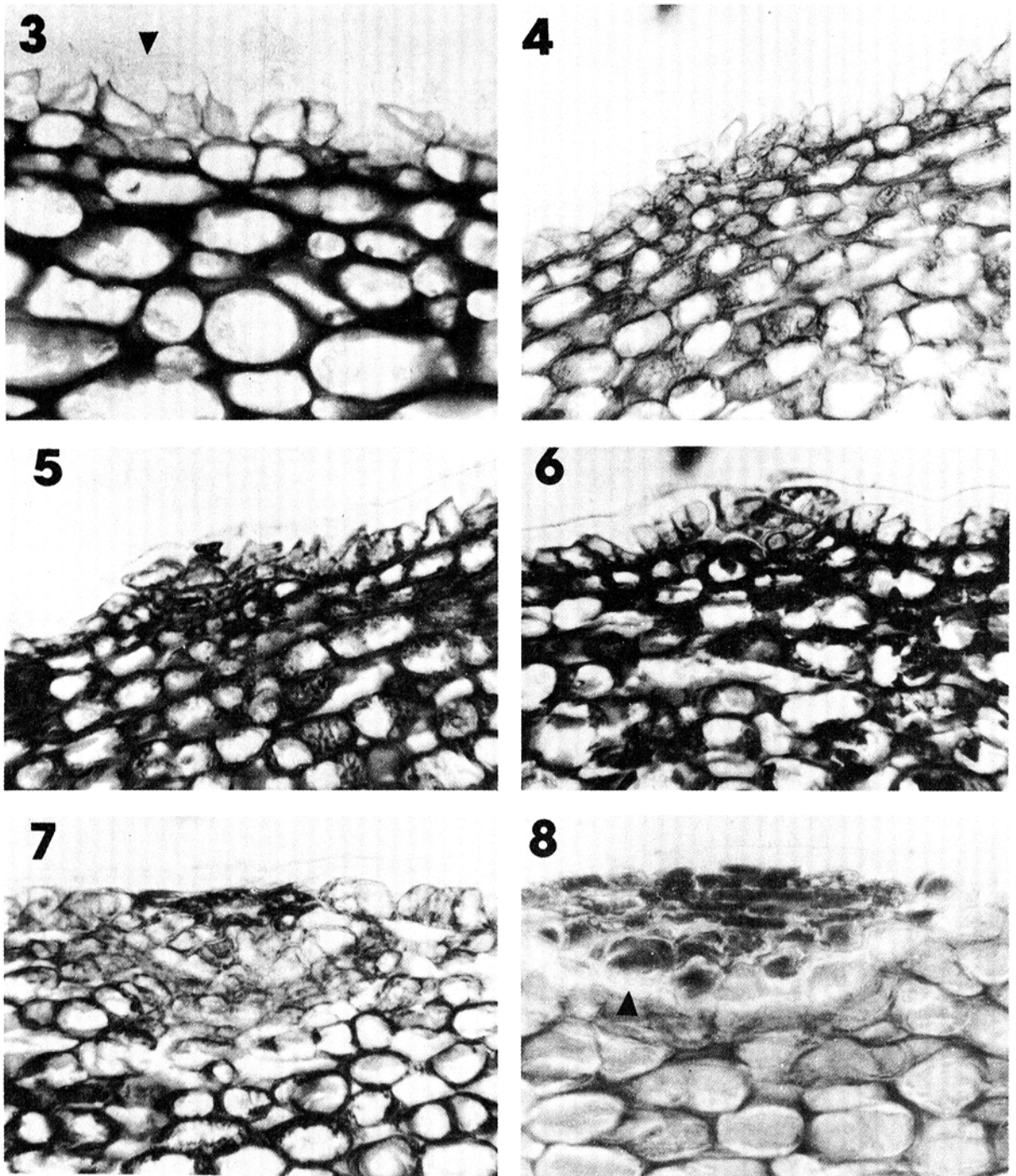


Fig. 3-8. Internal symptoms of scar skin-infected Red Delicious apples. **3)** Radial section of healthy fruit showing normal cuticle (arrow), epidermis and subepidermal cells. **4-8)** Sequence of histopathological symptoms. **4)** Hyperplasia of subepidermal cells. **5)** Increased hyperplasia and appearance of necrotic epidermal cells. **6)** Necrosis and collapse of epidermal cells. **7)** Necrosis of epidermal and subepidermal cells, appearance of phellogen and formation of "pocket." **8)** Zone of lightly stained cells (arrow) underlying "pocket."

seemed to be present intercellularly rather than within the cells. When four to six layers of phellem had been produced, breaking of the cuticle occurred, apparently due to pressure from the developing periderm. As the phellem increased in thickness, small fissures occurred at scattered points on the cuticle. The fissures increased in size due to sloughing off of cells and, possibly, due to expansion of the underlying periderm. Islands of unbroken cuticle remained on the fruit surface surrounded by areas of exposed phellem cells (Fig. 16). Extensive areas of corky periderm developed underneath the intact cuticle and the exposed phellem areas.

DISCUSSION.—The appearance of red pigments, presumably anthocyanins, in epidermal and subepidermal cell layers of scar skin-infected apples preceding hyperplasia or epidermal necrosis is a common occurrence in many plant diseases resulting from localized infections by fungi and bacteria. Reddening has also been observed in several virus and mycoplasma-caused disorders; e.g. in the virus-caused red node disease of bean in which reddish concentric rings form on the pods (6), and in the mycoplasma-caused X-disease of

cherry in which the entire foliage takes on a reddish hue (11).

Hyperplasia similar to that observed in scar skin of apple was noted in tobacco mosaic virus (TMV)-infected tomato fruits by Gardner (3). Gardner suggested that hyperplasia is probably a response to epidermal necrosis in tomato. In scar skin, however, hyperplasia appears not to be directly associated with necrosis. Even in older pockets the hyperplastic (meristematic) tissue is deeper in the fruit, and is separate from the necrotic tissue, suggesting that hyperplasia is caused by some continuous stimulus supplied by the infecting agent, rather than by the necrotic tissue. It is true, however, that in older tissue, in which the epidermis has become necrotic, it is more difficult to separate hyperplasia from necrosis. In the case of hyperplasia following necrosis of the epidermis, hyperplasia may well be a response to necrosis.

Necrosis is a commonly observed plant response to systemic viral infection. In numerous virus-host combinations, necrotic areas appear on the stem, leaves, or fruit of the host following infection (6). The appearance of necrosis may be linked to increases in

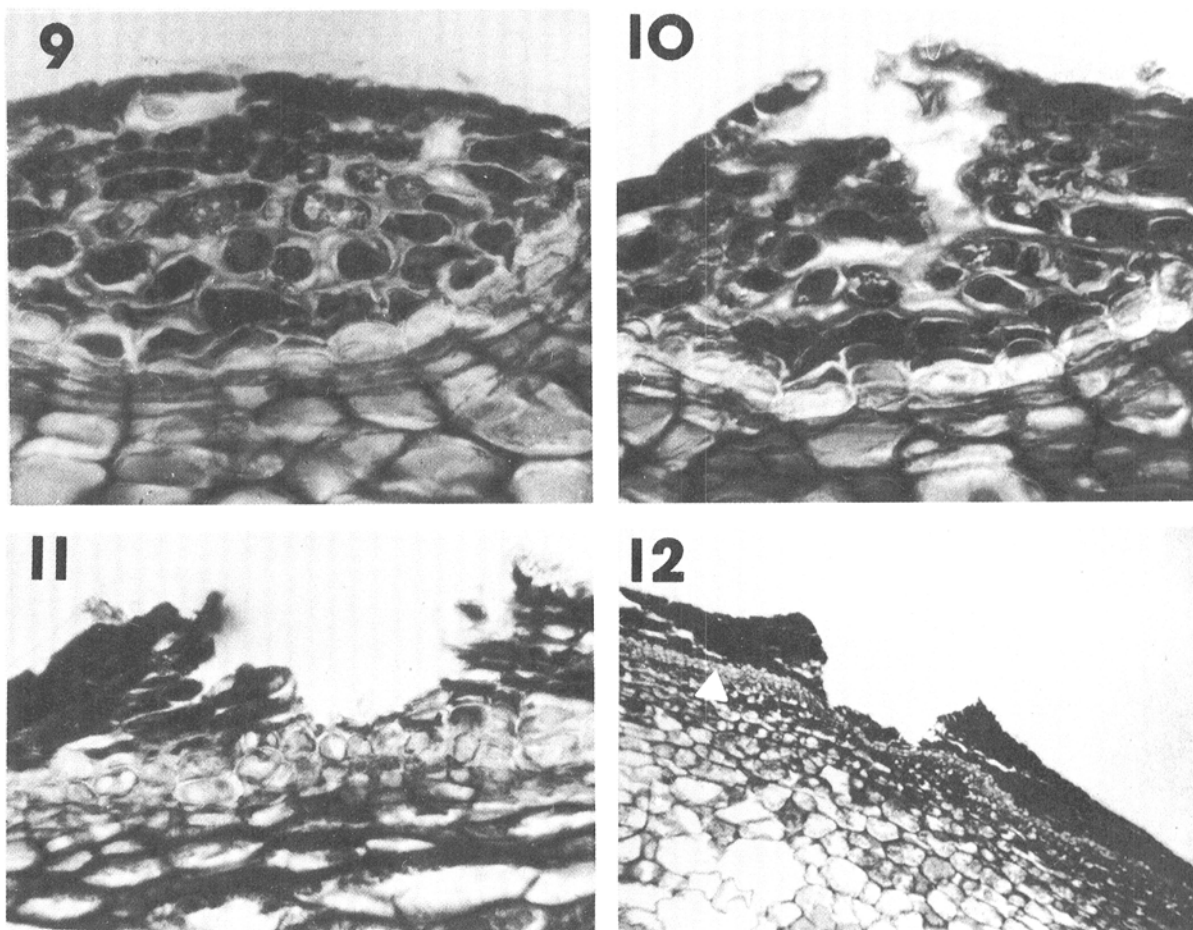


Fig. 9-12. Sequence of events in the formation of the "pocket" and of cracks on the surface of scar skin-infected Red Delicious apples. 9) The "pocket" area, underlain by periderm and with the cuticle intact, is raised above surrounding tissue. 10) Cuticle and epidermis rupture and cells slough off. 11) Enlarged crack of "pocket" exposes phellem cells. 12) Old "pocket" area with crack and underlying band of lightly stained cells (arrow) which appear to be the developing periderm.

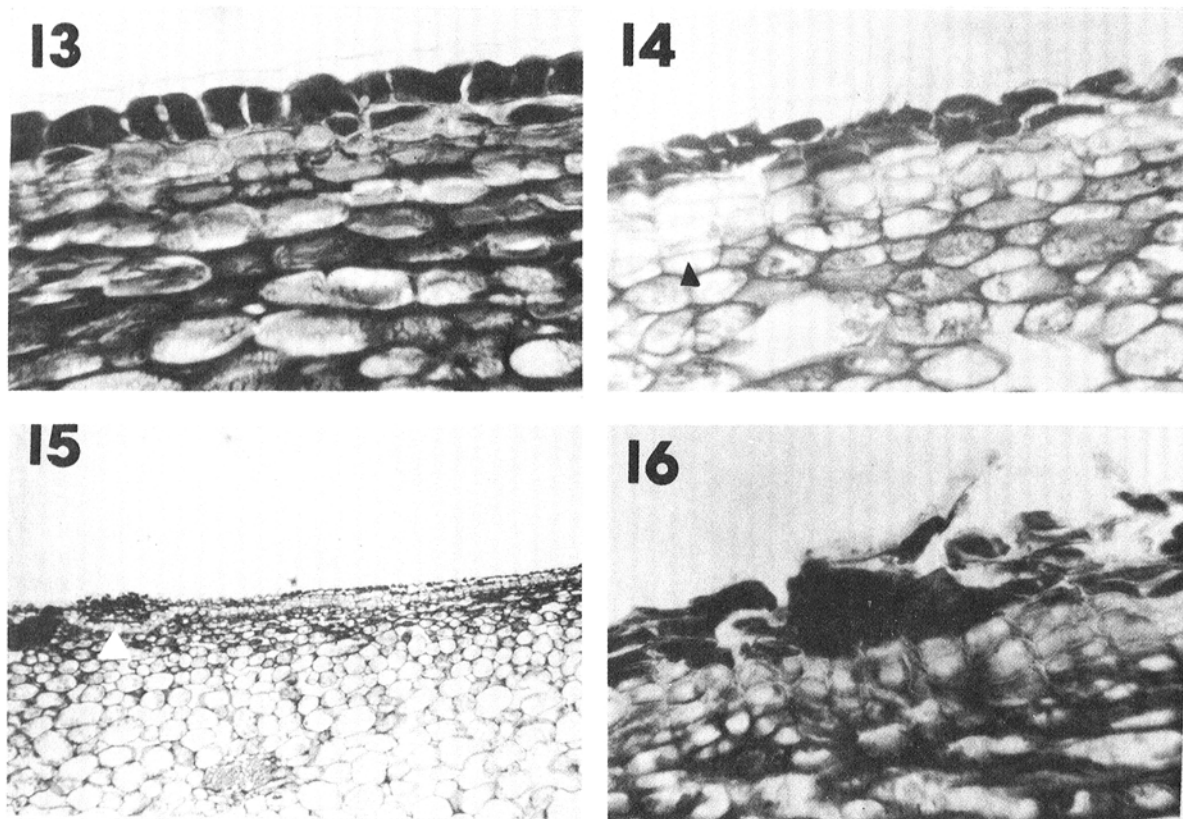


Fig. 13-16. Sequence of events in the necrosis of epidermis and subepidermal layers without formation of "pockets." **13)** Necrosis of epidermis and formation of periderm layer while cuticle remains intact. **14)** Necrotic epidermis and subepidermal layers underlain by two to three layers of phellem (arrow). **15)** A pocket area on the left with periderm (arrow) subjacent to it and continuing outward under necrotic epidermal and subepidermal cells. **16)** Old scar skin-affected fruit showing exposed phellem cells and area of intact cuticle.

polyphenoloxidase and peroxidase activity. Quinones, the product of polyphenoloxidase action on phenolic compounds, may promote the breakdown of protoplasmic structure with the observable result being cell death (1). It is possible, therefore, that the brown coloration and necrosis, which follows the red pigmentation in scar skin, could be due to increased levels of phenolics and, subsequently, quinones in the area affected by the disorder.

Periderm development, which occurred after hyperplasia and/or necrosis were initiated, is also typical of many other plant diseases as well as of other forms of mechanical damage (14). Cork formation has been reported in psorosis in citrus (15), and russetting of the fruit was observed in certain virus diseases of apple (12). Thus, periderm formation in scar skin disease of apple appears to be part of the generalized response of apple fruits to cell injury and epidermal cell death.

The hyperplastic response on parts of the fruit brings about a swelling effect and an uplifting of the cuticle. The cuticle, however, remains intact even though subjacent epidermal cells are necrotic and suberized. Retention of safranin stain by the cells in the pocket indicates cell suberization and possible lignin deposition (6). A somewhat similar arrangement of leathery necrotic

epidermis under an intact cuticle has been noticed in the buckskin disease of cherry (13). In the scar skin disease of apple, the cuticle appears to be quite flexible, with fracturing of the cuticle occurring only after extensive periderm formation.

In scar skin, pocket formation and necrosis of epidermal and subepidermal cells appear not to be associated with any other fruit tissue. No association was observed between pocket formation and vascular, particularly phloem, tissues. Thus, scar skin symptoms appear to be restricted to epidermal and adjacent subepidermal cells.

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