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THE ANATOMY OF THE AVOCADO PEDICEL AND THE LOCALIZATION OF DIPLODIA MYCELIUM

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ABSTRACT

Avocado fruit is often attacked during softening by *Diplodia natalensis*, which causes stem-end rot. The pedicel anatomy and the localization of the fungal mycelium in various tissues were studied in artificially inoculated pedicels. Mycelium was found to be present in most tissues of the pedicel. Vessel elements of the pedicel seem to serve as the main route of fungal penetration. In the vessels the mycelium advances mainly via perforation plates and to some extent through pits. The intrusion into fibers, sclereids, and parenchyma cells is similarly through pits. The anatomy of the pedicel and that of the stem were compared.

Introduction

Avocado fruit is often attacked shortly after harvest by the fungus *Diplodia natalensis* Pole-Evans, which causes stem-end rot. It was observed by SCHIFFMANN-NADEL (1968) that fruit harvested with short pedicels was severely rotted at the softening stage, whereas in fruit with long pedicels, the amount of rot was low. CUMMINGS and SCHROEDER (1942), in their study of avocado fruit anatomy, have briefly mentioned the pedicel, with reference to its compact vascular cylinder.

It was assumed that detailed anatomical knowledge of the avocado pedicel would contribute to a better understanding of the relation between the pedicel tissues and the route of the fungus. Therefore, the anatomy of the avocado pedicel was studied, and the localization of the fungus in the various tissues was traced.

Material and methods

Avocado (*Persea americana* Mill. var. Fuerte) fruits with pedicels ranging from 10 to 20 mm in length were picked twice during the picking season, that is, in February and in April. The fruits were artificially inoculated at the cut surface of the pedicel with a conidial spore suspension of the fungus. At various intervals after inoculation, the pedicels were collected and fixed in formalin-acetic acid. Transverse and longitudinal sections (20–40 μ thick) were prepared with the sliding microtome. The following reagents and stains were used for histochemical identification of tissues, cell walls, and inclusions: zinc-chlor-iodide for cellulose (SASS 1940); phloroglucinol-HCl for lignin (FOSTER 1949); sudan IV for cuticle, fats (JOHANSEN 1940); $FeCl_3$ for tannins (JENSEN 1962); IKI for starches (SASS 1940); and the triple stain, safranin-fast green-orange G (JOHANSEN 1940), as a general differential stain. Maceration of the pedicel was carried out using JEFFREY's method (FOSTER 1949); epidermis separation was carried out with KOH. Cotton blue (RAWLINS 1933) was used to stain fungal hyphae in the pedicel tissues.

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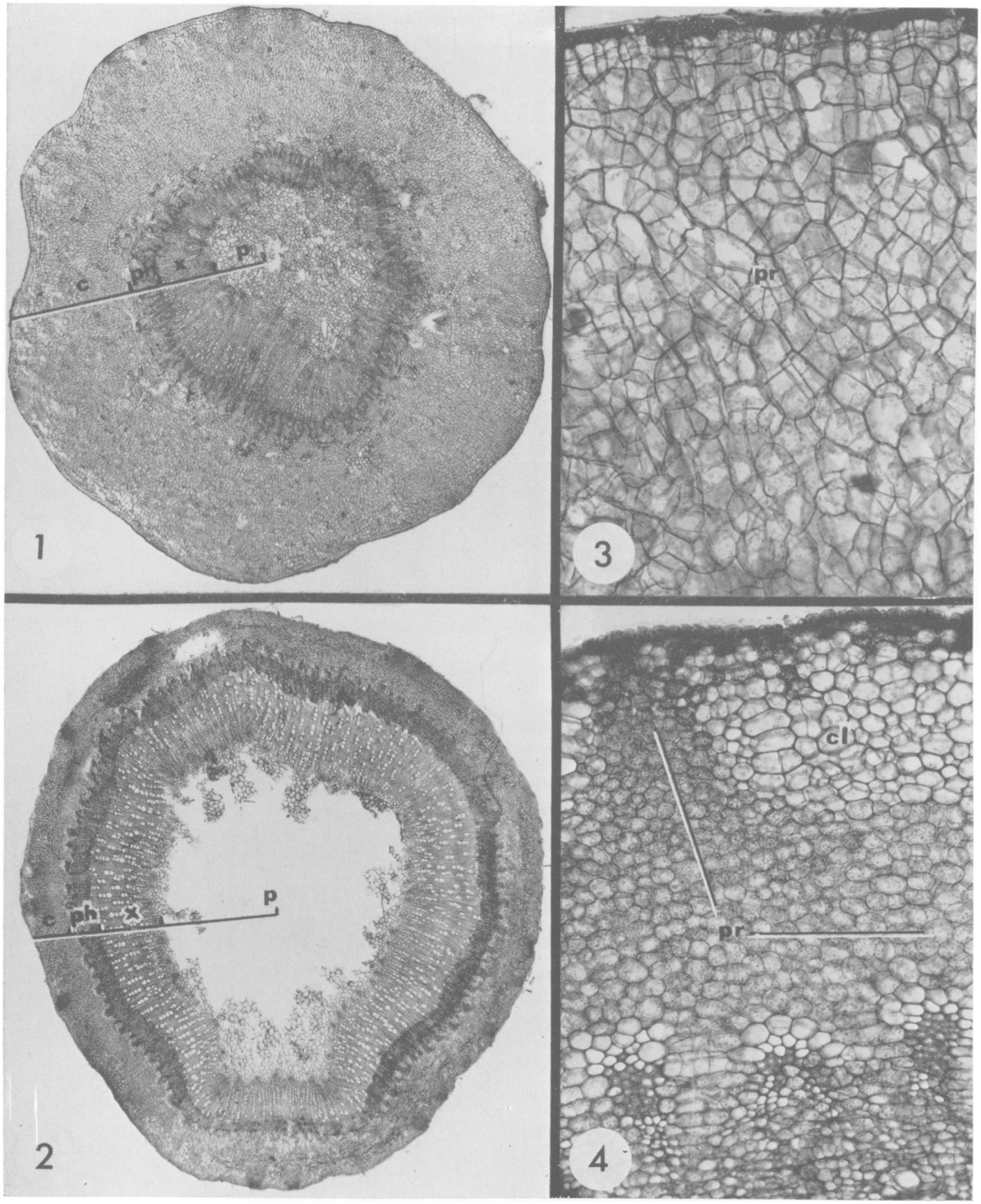
Observations and discussion

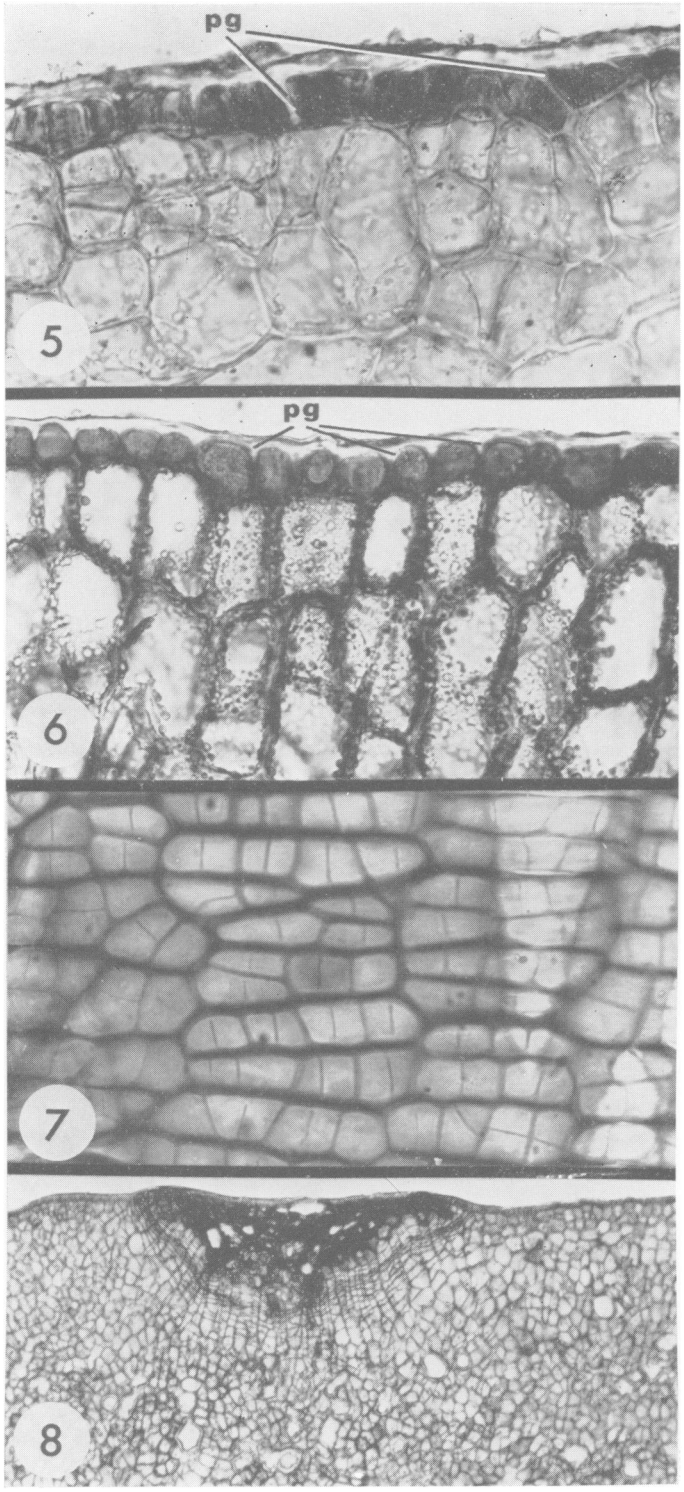
The avocado fruit is borne on a pedicel about 20 mm long and 6–10 mm in diameter. Comparative examination of cross sections of the pedicel and the stem, similar in age and diameter and collected from the same branch, revealed pronounced differences in cortex/stele ratio, pith diameter, and cortex structure (figs. 1, 2). In the pedicel the radial ratio of cortex/stele is approximately 1/1 compared with 1/9 in the stem. The pith of the pedicel is quite narrow and is composed of compact parenchymatous tissue (fig. 1), whereas the pith of the stem has almost twice the diameter of that of the pedicel and the tissue is mostly disintegrated (fig. 2). These differences in cortex/stele ratio and in pith size might possibly be related to the different forces acting upon the pedicel and the stem. It may be assumed that the pedicel is subjected relatively more to tensile forces caused by fruit weight, while the stem is subjected mainly to bending forces. It is noteworthy, however, that the sequence of the tissues is similar in both cases. A detailed description of the pedicel tissues follows.

EPIDERMIS.—The epidermis is composed of a single layer of cells of unequal size covered by a thick cuticle. In cross sections of the pedicel, radial peglike thickenings of the epidermal walls could be observed at intervals of from two to seven cells (fig. 5). In longitudinal section, such radial wall thickenings appear in each cell (fig. 6). From a surface view the epidermis appears as horizontally elongated groups of cells, one cell wide and from two to seven cells long, surrounded by heavy walls (fig. 7). This cell arrangement appears to result from subdivision of epidermal cells with the newly formed cells aligned at right angles to the pedicel axis; the divisions and cell enlargement keep pace with the radial thickening of the pedicel.

Thick-walled, unicellular hairs, 150–350 μ long, are scattered sparsely over the pedicel, becoming more abundant toward the sepals, where they may number up to 100–200 per square millimeter.



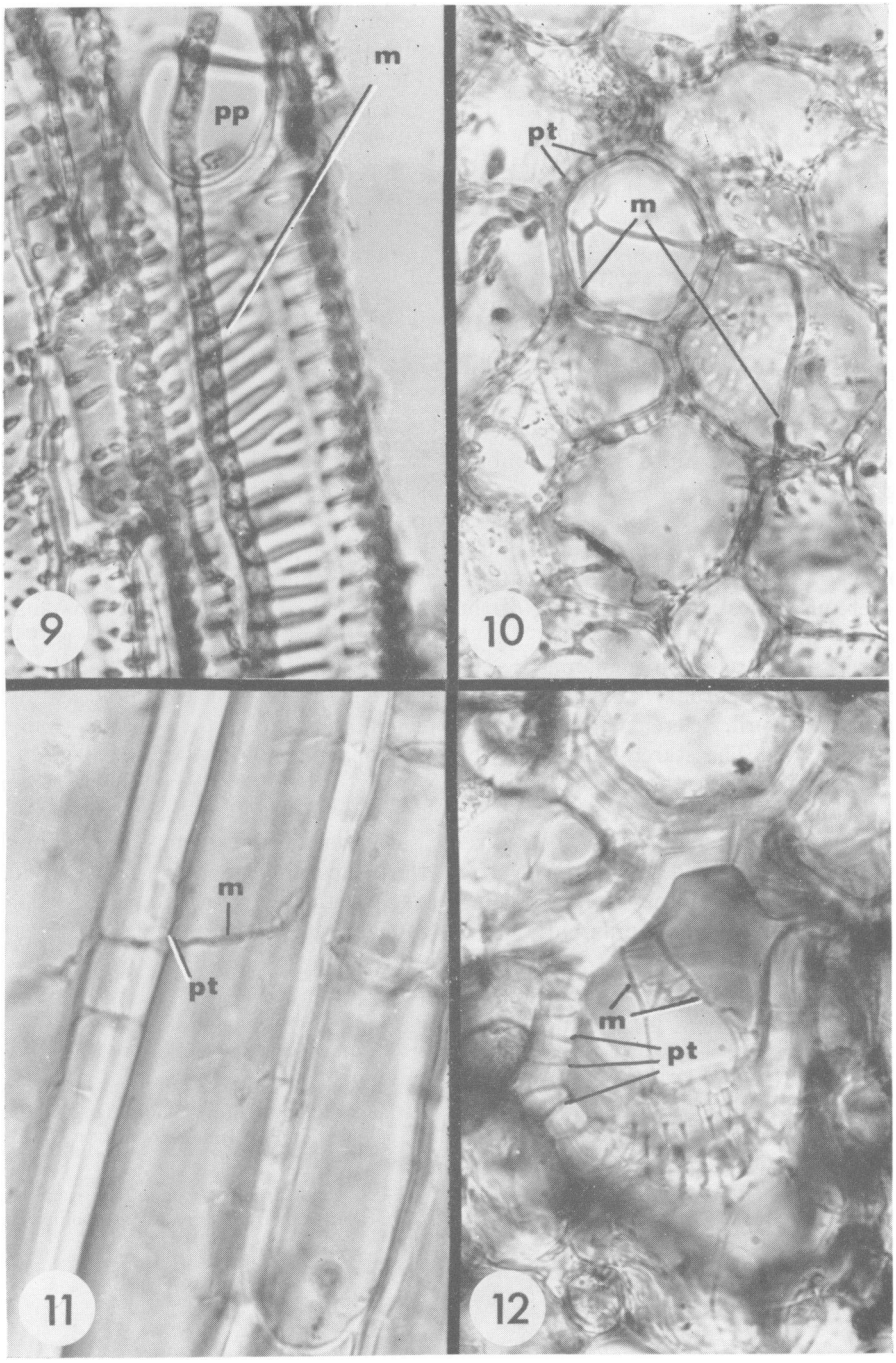
FIGS. 1–4.—Figs. 1–2, Transections of avocado pedicel and stem, respectively, showing differences in cortex/stele ratio and in pith diameter. Fig. 1, ×10; fig. 2, ×12. Fig. 3, Part of fig. 1, magnified, showing homogeneous cortex parenchyma, ×120. Fig. 4, Part of fig. 2, magnified, showing cortex parenchyma and collenchyma, ×120. Cortex, *c;* phloem, *ph;* xylem, *x;* pith, *p;* collenchyma, *cl;* parenchyma, *pr;* stained with safranin-fast green-orange G.



FIGS. 5–8.—Figs. 5–7, Pedicel epidermis. Fig. 5, Transection of pedicel showing epidermis with spaced radial wall thickening, ×450. Fig. 6, Longitudinal section of pedicel, showing radial wall thickening in each cell, ×450. Fig. 7, Surface view of separated epidermis showing horizontal groups of cells, ×450. Fig. 8, Transection of pedicel showing a lenticel, the epidermis still present, ×60. Peglike thickening, *pg;* stained with safranin-fast green-orange G except fig. 7, stained with safranin only.

Stomata are paracytic and very scarce; the subsidiary cells are located sidewise and underneath the guard cells, thus causing elevation of the latter above a large substomatal cavity. The epidermal cells are generally rich in tannin. Lenticels of various sizes can be observed while the epidermis is still persistent (fig. 8). The onset of periderm formation, however, was observed only in pedicels of fruit picked late in the season.

CORTEX.—The thick cortex of the pedicel is strikingly homogeneous, consisting of large isodiametric parenchyma cells (fig. 3). The stem cortex on the



FIGS. 9–12.—The occurrence of mycelium in pedicel tissues. Mycelium, *m;* perforation plate, *pp;* pit, *pt;* longitudinal and transections, stained with cotton blue. Fig. 9, Mycelium in vessel member, ×650. Fig. 10, Mycelium in pith parenchyma cells, ×950. Fig. 11, Mycelium in fibers, ×650. Fig. 12, Mycelium in sclereids, ×650.

other hand, is composed of both collenchyma and parenchyma tissues (fig. 4). Among the parenchyma cells of the pedicel, solitary sclereids or groups of sclereids, mostly of the brachysclereid type, were observed. The sclereids were at different stages of wall thickening, from very thin to almost complete elimination of cell lumen. Starch, fats, and tannins were generally found in the parenchyma cells. In the pedicels of fruit picked in February, starches were predominant and very little tannin was present; in April, at the end of the season, a marked rise in tannin and a depletion in starch were noticed.

STELE.—The phloem occupies about one-seventh and the xylem about one-fifth of the total pedicel radius, which is similar to the ratio in the stem (figs. 1, 2). Primary phloem fibers are arranged in fascicles; some viable septate fibers were observed. The phloem parenchyma is high in fats and, later in the season, in tannin as well.

In macerated material, the xylem was found to consist of tracheary elements (tracheae, tracheids) as well as fibers (140–800 μ long) and parenchyma cells. The tracheae consist of tailed vessel members 90–190 μ long, 25–60 μ wide. The vessel members are variously thickened (netted, with simple or bordered pits or mixed), and have oblique scalariform to simple perforation plates. The xylem parenchyma contains starch in greater quantities at the beginning of the season than at the end.

The pith parenchyma cells are isodiametric; the cell wall is cellulosic in February and has become noticeably lignified in April. Sclereids are also found in the pith parenchyma. The starch content in the pith parenchyma varies during the season, similarly to that in the cortex.

Presence of an abscission layer between the pedicel and the fruit could be observed several days after picking, while the fruit was still firm. Upon softening of the fruit, this layer became more differentiated.

THE LOCATION OF THE FUNGUS.—Anatomical examination of longitudinal and cross sections of the pedicel made at several intervals after inoculation with spores of *Diplodia natalensis* showed the following results. Four days after inoculation, mycelium was observed mainly in vessel elements (fig. 9), 3–4 mm from the cut surface; there was also some mycelium in the parenchyma cells of the cortex and pith (fig. 10), close to the cut surface. After 7 days the mycelium was more profuse in the same tissues and had penetrated more deeply into the pedicel. Later, the mycelium advanced and became more abundant; several hyphae could frequently be observed in a single vessel element. After two weeks, the mycelium was found throughout the pedicel, including the torus. At the late stage of fungal penetration, the mycelium could be observed in sclerenchyma elements, fibers (fig. 11) and sclereids (fig. 12), as well as in the phloem elements, but not in epidermal cells. The mycelium was inter- as well as intracellular.

The advance of the mycelium through the vessel members occurred mainly via the perforation plates, and to a much lesser extent through pits. The intrusion into the fibers and sclereids was through their pits.

The penetration of the mycelium from the pedicel into the fruit seems to be mainly via vessel elements. This mode of penetration was also reported for citrus fruit (SCHIFFMANN-NADEL 1947). The rate of progress of mycelium in the pedicel and its significance in relation to the appearance of stem-end rot in avocado fruit are discussed in more detail in another paper (SCHIFFMANN-NADEL, COHEN, and ARZEE, "Rate of advance of *Diplodia* mycelium in avocado pedicel," in preparation).

In view of differences observed in the anatomy of the pedicel and the stem, further studies are indicated to show the distribution and mode of advance of the fungal mycelium in inoculated stems as well.

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