Peanut Science (1982) 9, 82-86

Cylindrocladium crotalariae-induced Periderm Formation in Taproot and Fibrous Roots of *Arachis hypogaea*[1]

Nancy E. Harris and M. K. Beute[2]*

ABSTRACT

Nine greenhouse grown *Arachis hypogaea* lines susceptible or resistant to Cylindrocladium black rot were examined histologically for periderm formation. No differences were observed in the basic formation of original or additional taproot periderms or in the suberization of periderm in susceptible and resistant peanut lines. However, extensive periderm were observed more frequently in resistant lines. Additional phellogens occurred in all parts of the taproot that contained living parenchyma. First order branch roots also produced successive periderms.

Key Words: *Arachis hypogaea*, root, periderm, disease resistance.

Periderm is a protective dermal tissue derived from the phellogen, a secondary meristem, that replaces the epidermis during secondary growth. This tissue also develops as a result of injury (1). Once initiated, a phellem of radial files of suberized prismatic cells is formed which is considered, theoretically, to be structurally and chemically resistant to most pathogen attack (5).

Peanut *(Arachis hypogaea* L.) taproots have phellogens at 10 days post-germination and a few layers of phellem are present. Branch roots are initiated in the pericycle after the cortex and epidermis are sloughed (6). These grow through the periderm leaving gaps in it.

Johnson and Beute (4) reported that formation of the original periderm on Florigiant peanut hypocotyles appeared to limit invasion of the Cylindrocladium black rot (CBR) fungus into the stele if conditions for disease development were unfavorable. If conditions were favorable, the fungus aggregated on the periderm with necrosis spreading into the vascular tissue. Occasionally, an additional periderm formed in advance of the necrosis and appeared to limit further invasion by the fungus. A possible relationship between the rapid invasion of fibrous roots and the absence of an intact periderm in these roots were suggested. The objective of this study was to describe periderm formation in taproot and fibrous roots of CBR-susceptible and resistant plants.

Materials and Methods

General Procedures:

The isolate of *Cylindrocladium crotalariae* used in this work was obtained from a peanut plant in eastern North Carolina and maintained on acidified potato dextrose agar (PDA). Microsclerotia (ms) were produced by growing the fungus on PDA in darkness for 3 to 5 weeks, after which cultures were comminuted in a Waring blender (Waring Products, New Hartford, Conn.) for 2 minutes and passed through nested sieves with 246 and 140 μm openings (60 and 100 mesh, respectively). Microsclerotia were rinsed to remove myclial fragments and concentrated in a beaker of water. Inoculum densities of 1, 2 and 4 ms per mm^2 taproot surface were selected as described by Harris and Beute (2). Individual ms of uniform size were placed on the roots. Points of inoculation were marked with colored pins placed adjacent to the roots.

Plant Material and Culture. - Peanut lines included commercial multiline Florigiant (CBR-susceptible), commercial NC 2 (CBR-susceptible), and single-plant selection seed of the following CBR-resistant lines: NC 3033, Argentine, NC 3033 X Florigiant, Florigiant X NC 3033, Argentine X Florigiant, Florigiant X Argentine, and NC 3033 X NC 2. All single-plant selection seed was of the F_5 generation or greater. Seeds were dusted lightly with a 30-30% botran-captan protectant (Upjohn Company, Kalamazoo, MI) and germinated in wet vermiculite. Approximately 5 days after germination, seedlings were removed, dipped in a solution of Rhizobium inoculum ("Nitragin", Nitragin Company, Inc., Milwaukee, WI), and transplanted 2 per box, into hinged polyethylene boxes (25 X 16.5 X 4 cm, Tri-State Plastics, Henderson, KY) containing washed vermiculite previously amended with one teaspoon per 1512 cm^3 of osmocote (19-6-12, Sierra Chemical Company, 1001 Yosemite Dr., Milpitas, CA). Two 2.5 cm diameter holes were drilled at one end of each box to accommodate the emerged epicotyls and two 0.5 cm diameter holes at the opposite end for watering. Boxes were wrapped in aluminum foil and tilted in flats lined with plastic for subirrigation. The taproot and a considerable proportion of the fibrous root system grew along the surface of the vermiculite in the tilted box, permitting observation. Plants were allowed to grow for 10 days before inoculation. Temperatures within the boxes averaged 25 C.

Histological Technique.- Taproot and fibrous root samples wre killed and fixed in formalin-2-propanol-propionic acid (3), infiltrated and embedded with Paraplast (Brunswick Company, St. Louis, MO), sectioned on a rotary microtome at 12 μm and stained with Triarch's quadruple stain (George H. Conant, Triarch, Inc., P. O. Box 98, Ripon, WI). Histochemical tests included Sudan IV for suberin and phloroglucinol and orcinol for wound gum (3). In all histological tests, 1 cm tissue samples were taken at the point of inoculation and sectioned completely. Ribbons were taken uniformly through the block for mounting, giving approximately the same number of sections for each treatment.

Formation of additional periderms was evaluated in the susceptible multiline Florigiant as compared to the most resistant available germplasm, NC 3033. After 5 weeks, a 1 cm segment of taproot at the point of inoculation was taken for histological processing. A small piece of taproot tissue adjacent to the 1 cm sample was plated on acidified PDA to verify the presence of *C. crotalariae*. A comparison of host responses for the 9 germplasms consisted of a 3 or 5 week long incubation with 6 or 10 plants per inoculum density per line, respectively. The potential for periderm formations was evaluated in primary and secondary branch roots of Florigiant and NC 3033. Ten days after transplanting, several primary or secondary branch roots on each plant were inoculated with 2 ms placed 3 cm from the point of emergence from the taproot. Each treatment was replicated 4 times. Fibrous root samples were taken 3 weeks after inoculation and fixed for histological observation.

Results and Discussions

All peanut lines tested showed the development of an original periderm (op) on the taproot consisting of a 1-2 layered phellogen (pg), several layers of Sudan IV positive phellem (pm) and 1-2 layers of phelloderm (pd) cells (Fig. 1A). The number of cork cell layers typically exceeded that of phelloderm. In all experiments the Sudan IV test for suberin revealed neither qualitative nor quantitative differences among the 9 lines. The taproot cortex (c) was typically collapsed and sloughed by the end of the 3 or 5 week incubation periods, leaving the periderm as the protective dermal tissue (Fig. 1A). Natural breaks which oc-

[1]Paper No. 8468 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, N. C.

Use of trade names in this publication does not imply endorsement by the N. C. Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.

[2]Former graduate student (now Assistant Professor of Biology, Elon College, Elon, N. C.) and Professor of Plant Pathology, North Carolina State University, Raleigh 27650, respectively.

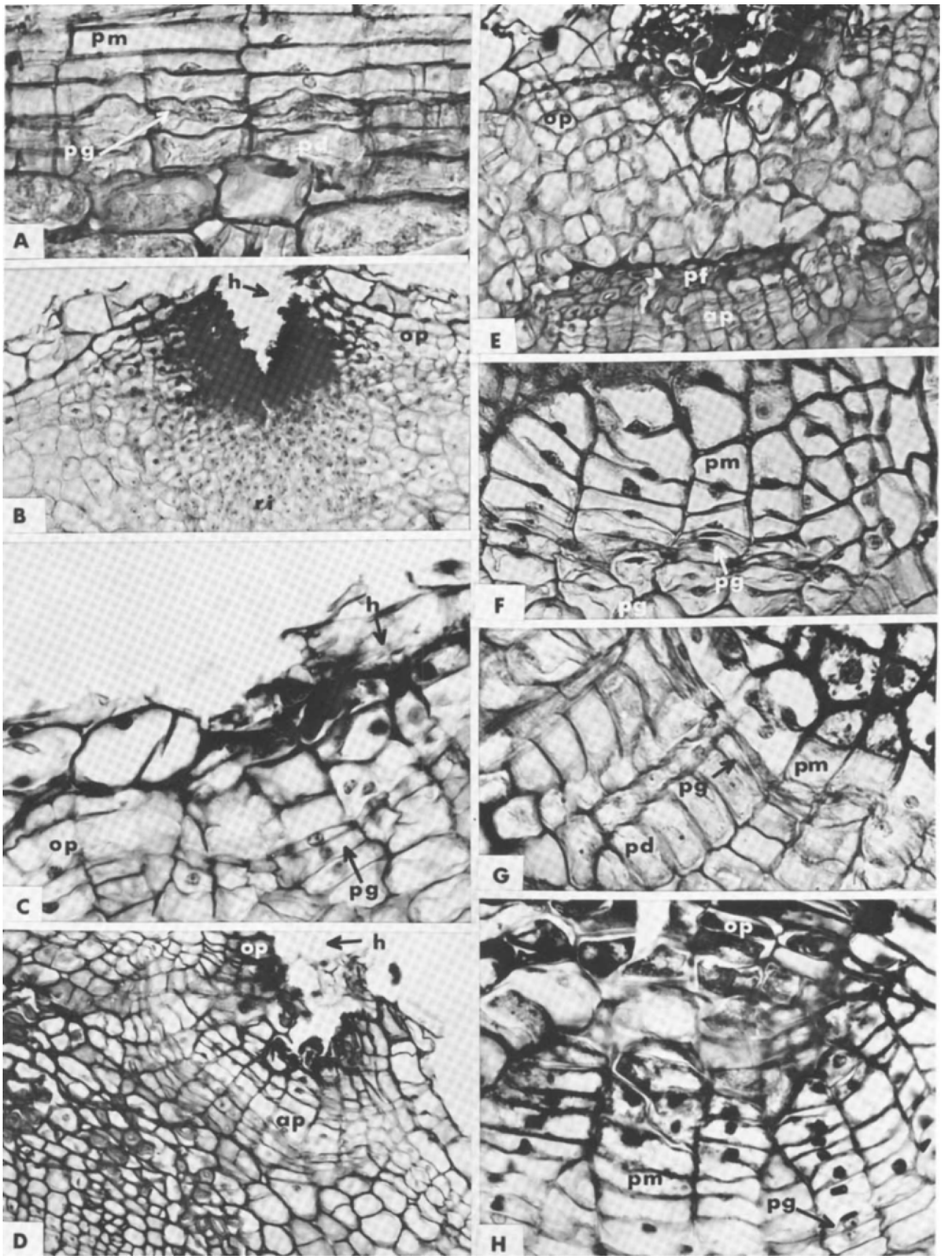

Fig. 1. Normal peanut taproot periderm and taproot periderm induced by *Cylindrocladium crotalariae:* (A) composition of original taproot periderm (X250); (B) disruption of original periderm by emerging fibrous root initial (X100); (C) cell divisions in phelloderm or pericyclic cells of taproot forming additional phellogen (X250); (D) additional periderm in pericyclic cells of taproot in response to necrosis of original periderm (X100); (E) phellogen and periderm formation beneath primary phloem fibers (X100); (F) cpmposition of additional periderm (X250); (G) formation of many layers of phelloderm in an additional periderm (X250); (H) formation of many layers of phellem in an additional periderm of a resistant line (X250); original periderm (op), additional periderm (ap), phellogen (pg), phelloderm (pd), phellem (pm), phloem fibers (pf), hyphae (h), root initial (ri).

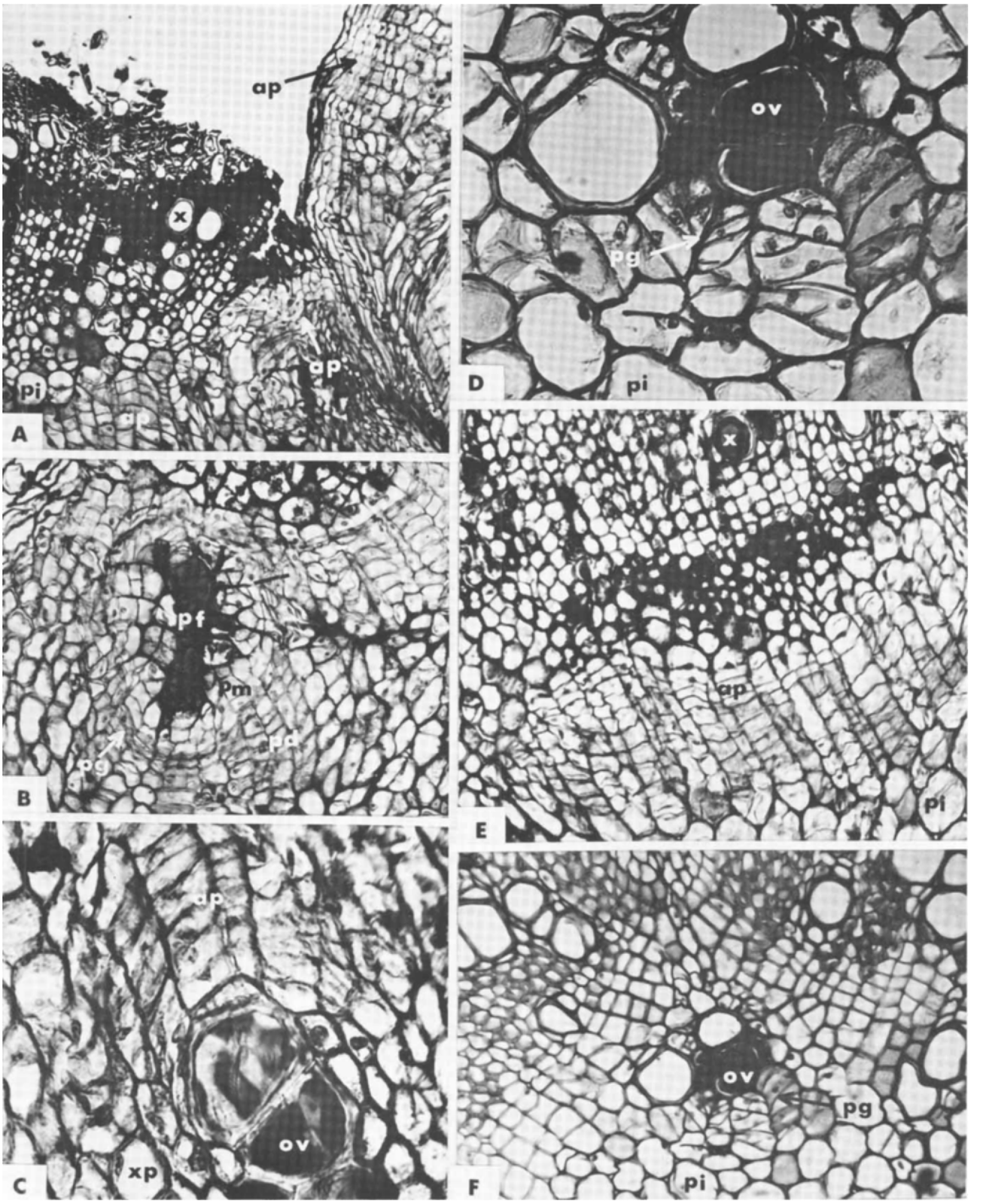

Fig. 2. Periderm formation in peanut taproots induced by *Cylindrocladium crotalariae:* (A) lateral spread of necrosis in taproot with formation of additional periderm on margins (X63); (B) additional periderm formation around a necrotic tissue projection (X100); (C) additional periderm formation adjacent to necrotic xylem parenchyma and occluded vessels (X250); (D) cell divisions in pith parenchyma adjacent to necrotic xylem parenchyma and occlued vessels (X250); (E) extensive additional periderm in pith (X100); (F) necrotic cells and phellogen initiation in otherwise healthy taproot tissue (X200); additional periderm (ap), phellogen (pg), pith (pi), occluded vessel (ov), phloem fibers (pf), xylem (x), phellem (pm), phelloderm (pd), xylem parenchyma (xp).

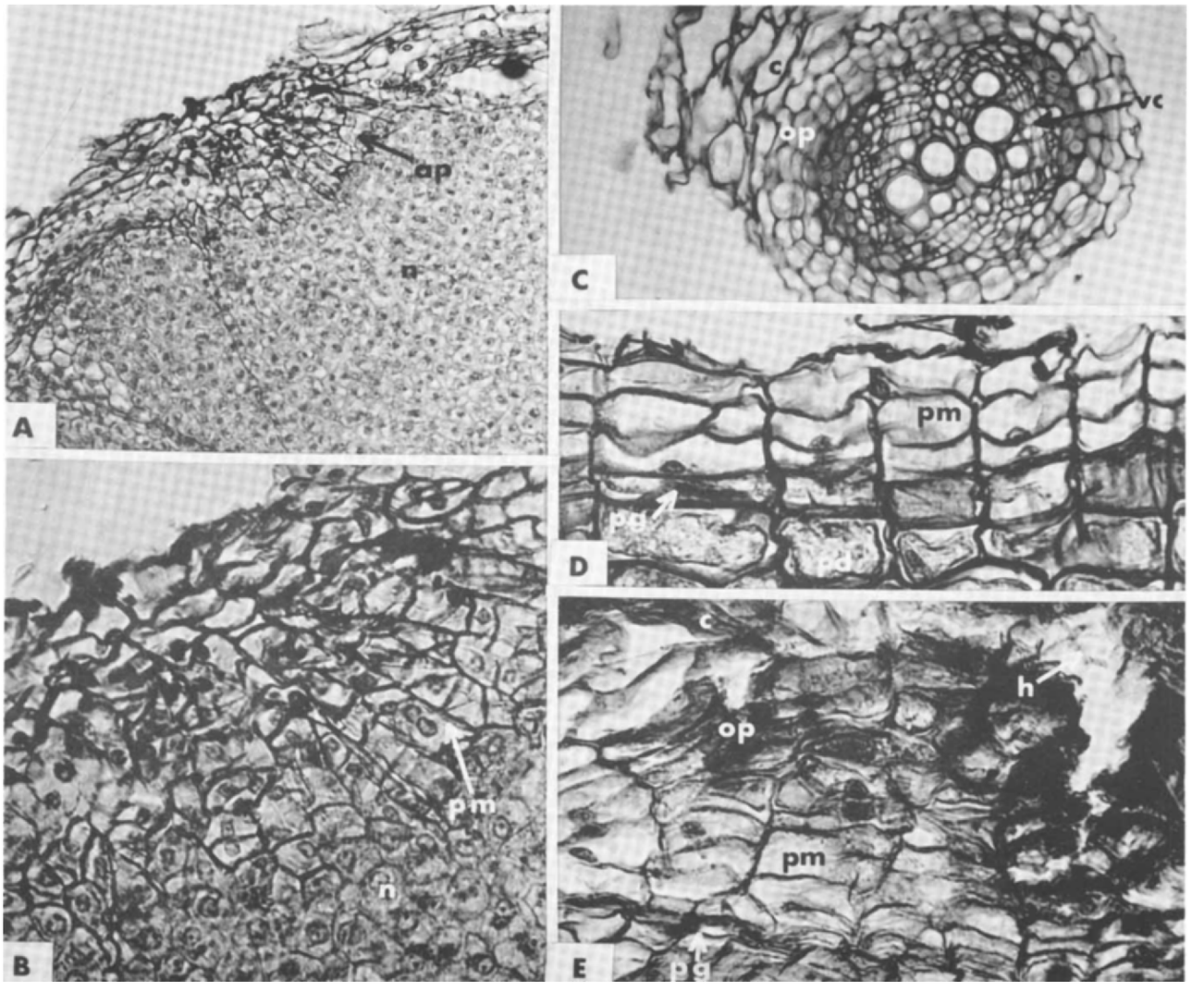

Fig. 3. Periderm formation in taproot nodules and fibrous roots of peanut infected with *Cylindrocladium crotalariae:* (A) additional periderm walling off a nodular lesion (X63); (B) radial files of phellem in additional periderm of nodule lesion (X100); (C) primary branch root showing secondary growth (X63); (D) composition of original periderm of a primary branch root (X250); original periderm (op), additional periderm (ap), phellogen (pg), phelloderm (pd), phellem (pm), cortex (c), nodule (n), hyphae (h), vascular cambian (vc).

curred in the original periderm cylinder as the result of growth of emerging branch roots provided entrance points for *C. crotalariae* into the stele (Fig. 1B).

As reported by Johnston and Beute (9), a strong tissue reaction to some toxic substance(s) occurs in advance of the fungal hyphae (h). Sensitive cells become prenecrotic, may or may not collapse, and eventually become necrotic as evidenced by abnormal staining and cytology. In all lines tested as the original periderm layers became prenecrotic, cell divisions were observed in cells below the lesion. The initiation of first additional phellogens usually involved either phelloderm cells or pericyclic parenchyma cells immediately beneath the original periderm (Fig. 1C and 1D). Frequently, however, the first cells to dedifferentiate and become meristematic were primary or secondary phloem parenchyma cells adjacent to the primary phloem fibers (pf) (Fig. 1E). Additional periderms (ap) consisted of phellogen, suberized phellem and phelloderm layers (Fig. 1F and 1G); however, the number of layers of phellem and phelloderm varied from one to many. More recently initiated phellogens had fewer associated cork and phelloderm layers than did older phellogens (Fig. 1H). Also, extensive periderm barriers were observed more frequently in resistant than in susceptible lines (2). This suggests that either the barrier itself was more effective in resistant lines than in susceptible ones in retarding fungal invasion and was, thus, able to develop to a greater extent, or that some other mechanism in resistant lines retarded fungal invasion allowing the periderm to expand secondarily (2).

As the prenecrotic reaction spread beyond the additional periderm, lesions extended both inward and laterally, with phellogens developing along the margins in healthy parenchyma cells (Fig. 2A). Subsequent additional phellogens formed in secondary phloem parenchma cells, xylem parenchyma (xp), and in pith parenchyma (pi). As the lesion expanded three dimensionally in finger-like fashion in the taproot, small pockets of prenecrotic or necrotic tissue were walled off with additional periderm. Regardless of the orientation of the le-

sion relative to the basic taproot anatomy, phellem was always laid down towards the necrotic tissue and phelloderm laid down away from the lesion (Fig. 2B). This strict relationship of cell division product to immediate cellular environment regardless of orientation of phellogen to the whole root appeared to hold true for periderm production in all peanut taproots observed. Xylem parenchma cells were as capable of resuming meristematic activity as phellem parenchyma and when lesions expanded into the stele, phellogens frequently were initiated in parenchyma adjacent to occluded vessel elements (Fig. 2C). As necrosis approached the center of the root, phellogen frequently developed within the pith parenchyma in resistant entries resulting in a periderm within the pith (Fig. 2D and 2E). Whole sectors or quadrants of the taproot were occasionally sloughed as the result of extensive lesion development and periderm formation.

It is suspected that the toxic substance(s) may move for some distance in the vascular system as evidenced by the appearance of isolated groups of necrotic vascular parenchyma cells adjacent to occluded vessels occurring above and below the lesion site in otherwise healthy tissue. Strands of pith phellogen (in resistant lines) were frequently associated with these isolated necrotic cells, suggesting that the simulus for dedifferentiation arose from the adjacent necrotic tissue (Fig. 2F).

Invasion and destruction of nodules was frequently observed. In many cases a phellogen was initiated presumably either in nodular cortical tissue or in the meristematic zone retained in the nodule (1). This gave rise to a suberized periderm barrier which sometimes effectively walled off the nodular lesion (Fig. 3A and 3B). All lines produced nodular phellogens in response to *C. crotalariae*.

Although they are believed not to have a periderm (4), peanut branch roots of the first order behaved similarly to the taproot. Many fibrous roots showed indications of initiation of secondary growth including initiation of a vascular cambium with production of secondary xylem and phloem, initiation of a phellogen with production of suberized phellem and phelloderm, and sloughing of the cortex (Fig. 3C and 3D). Both susceptible Florigiant and resistant NC 3033 primary branch roots were capable of limited normal periderm production. No additional phellogens were observed beyond the primary phloem fibers during the 3 week incubation period (Fig. 3E). Branch roots of the second order never formed a periderm. These roots were in a primary state of growth with an intact epidermis and cortex. All peanut branch roots of the second division or greater are presumably incapable of secondary growth under normal conditions.

Peanut taproots of both resistant and susceptible germplasm have a great potential for periderm formation when infected by *C. crotalariae*. Although periderm formation appears histologically similar in all lines tested, qualitative and quantitative differences are suspected to exist which contribute to CBR resistance. Susceptible Florigiant generally sustains more breachments of the original taproot periderm and effectively confines fewer of its lesions by additional periderm formation than the other line tested (2). Secondary or additional phellogens may form in all parts of the peanut taproot where potential meristematic responding cells exist. Susceptible and resistant peanut branch roots of the first order are capable of limited secondary growth including the initiation of an original and additional phellogens. The potential for periderm formation in peanut root is considered to be an important feature of the plant's natural defense system against tissue injury.

Literature Cited

1. Esau, K. 1977. Anatomy of seed plants. John Wiley and Sons, Inc., New York, pp. 183-197.
2. Harris, N. E. and M. K. Beute. 1982. Histological responses of peanut germplasm resistant and susceptible to *Cylindrocladium crotalariae* in relationship to inoculum density. Phytopathology 72:1250-1256.
3. Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill Book Company, New York City, NY. 523 pp.
4. Johnston, S. A. and M. K. Beute. 1975. Histopathology of Cylindrocladium black rot of peanut. Phytopathology 65:649-653.
5. Wood, R. K. S. 1967. Physiological plant pathology. Blackwell Scientific Publications. Oxford, England. pp. 130-131, 474.
6. Yarbrough, J. A. 1949. *Arachis hypogaea*. The seedling, its cotyledons, hypocotyl and roots. Am. J. Bot. 36:758-772.

Accepted November 3, 1982