Histopathology of *Peronospora tabacina* in Systemically Infected Burley Tobacco

R. D. Milholland, J. Papadopoulou, and M. Daykin

Professor, graduate research assistant, and research technician, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27650.

Journal Series Paper 6415 of the North Carolina Agricultural Research Service, Raleigh.

The assistance of Harvey W. Spurr, Jr. and F. A. Todd who provided burley tobacco stems systemically infected with *Peronospora tabacina* is greatly appreciated.

Use of trade names implies neither endorsement of the products by the North Carolina Research Service nor criticism of similar ones not mentioned.

Accepted for publication 29 July 1980.

ABSTRACT

Milholland, R. D., Papadopoulou, J., and Daykin, M. 1981. Histopathology of *Peronospora tabacina* in systemically infected burley tobacco. Phytopathology 71:73-76.

Field-grown burley tobacco plants (KY-14) systemically infected with *Peronospora tabacina* were examined histologically. The fungus was confined primarily to the vascular tissue in the stem, but caused severe necrosis of all tissues in diseased roots. The fungus progressed through the tissues intercellularly causing the host cell walls to collapse and eventually become necrotic. Intracellular hyphae were observed in xylem vessels and parenchyma cells. Single and branched haustoria also were observed. The fungus caused vascular plugging, hyperplasia of cambium and phloem, xylem disintegration, and the formation of vascular cavities. Infected stem and leaf tissues both contained mature oospores.

Additional key words: blue mold, downy mildew.

Blue mold of tobacco (*Nicotiana tabacum* L.) is caused by the fungus *Peronospora tabacina* Adams. The disease first was reported from Australia where it occurs on cultivated and wild *Nicotiana* species (1). In the United States, the blue mold fungus is confined primarily to leaves, whereas in Australia and Europe, mycelium frequently spreads from the leaves into the stem and may invade the plant systemically (3).

In 1979, systemic infection by the blue mold fungus was observed in tobacco fields in North America and it resulted in a $250 million loss to growers (5). According to Spurr and Todd (5), burley tobacco losses in North Carolina averaged 40% and many fields were completely destroyed, while flue-cured tobacco losses were about 4%. Oospores were abundant in leaves and systemically infected stems of burley tobacco.

The research reported herein was undertaken to study the histopathology of burley tobacco stem and root tissues systemically infected with *Peronospora tobacina*.

0031-949X/81/01007304/$03.00/0

MATERIALS AND METHODS

Burley tobacco plants (KY-14) known to be systemically infected with *Peronospora tabacina* were collected in August 1979 from a field in Yancey County, North Carolina. Stems and roots were cut into 10-mm sections and fixed in formalin-propionic-propanol (FPP) for 1 mo. Sections were dehydrated during 1 wk in an isopropyl series then infiltrated and embedded in Paraplast (Sherwood Medical Industries, St. Louis, MO 63100) (2). The specimens were softened for 48 hr in a solution of 90 ml of 1% sodium lauryl sulfate (the household detergent, Dreft) and 10 ml of glycerol and 12-μm sections were cut with a rotary microtome. Sections were mounted on slides with Haupt's adhesive and stained with a modified Conant's quadruple stain (2).

Stems and leaves of burley tobacco also were collected in December 1979 in five counties (Allegheny, Buncombe, Haywood, Mitchell, and Yancey) from fields in which plants previously had shown blue mold symptoms. The tissues were fixed in FPP and prepared for examination as described previously.

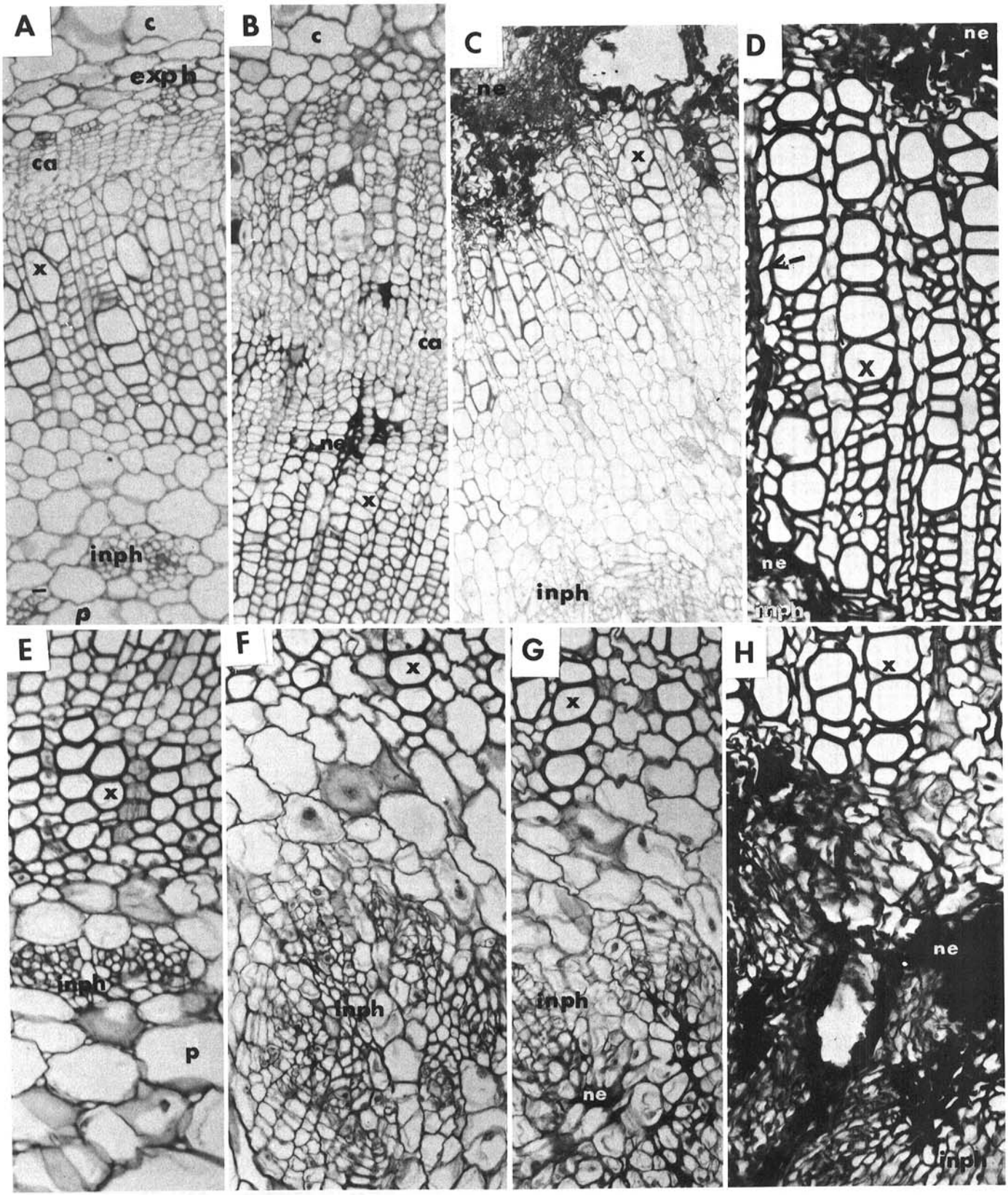

Fig. 1. Transverse sections of burley tobacco stems: sections B,C,D and F,G,H are infected with *Peronospora tabacina.* **A,** Healthy stem showing cortex (c), external phloem (exph), cambium (ca), xylem (x), internal phloem (inph), and pith (p) (×131). **B,** Early stage of necrosis (dark areas labeled ne) in external phloem, cambium, and xylem parenchyma regions (×131). **C,** Advanced stage of necrosis in the outer regions of vascular tissue showing large cavities (×131). **D,** Intercellular growth of hyphae from external region to internal phloem through xylem parenchyma (arrow) (×210). **E,** Healthy section showing xylem (x), normal size of internal phloem (inph), and pith (p) (×210). **F,** Hyperplasia of internal phloem (×210). **G,** Hyperplasia with partial necrosis of internal phloem (×210). **H,** Advanced stage of necrosis and cavity formation in the internal phloem (×210).

RESULTS AND DISCUSSION

According to Mandryk (4), the blue mold fungus enters the tobacco stem through the vascular system of infected leaf mid-veins and petioles. In our studies, the vascular cambium and external phloem regions appeared to be the initial site of infection, once the fungus gained entrance into the stem (Figs. 1A,B). The fungus grew intercellularly and spread throughout the cambium, external phloem, and into the xylem, resulting in severe necrosis. After the fungus became well established, large cavities formed between the xylem and external phloem (Fig. 1C). The fungus was not observed in the cortex of the stem at this stage of disease development. Spurr and Todd (5) report that systemically infected plants are severely stunted at this stage of disease development.

Mandryk (4) reports that necrosis is confined to the periphery of the secondary xylem, the cambium, and the inner part of the external phloem at flowering. As the xylem ages, it forms a barrier to the spread of the mycelium from external to internal phloem and the pith. In the tissues we examined, the fungus progressed intercellularly through the xylem parenchyma and into the internal phloem (Fig. 1D). Hyperplasia was observed in the cambium and internal phloem regions resulting in severe distortion of the tissues (Figs. 1E,F). Hyperplasia of the internal phloem was followed by cell necrosis (Fig. 1G), eventually resulting in severe necrosis and disintegration of the phloem and xylem (Fig. 1H). Large cavities also formed in this area.

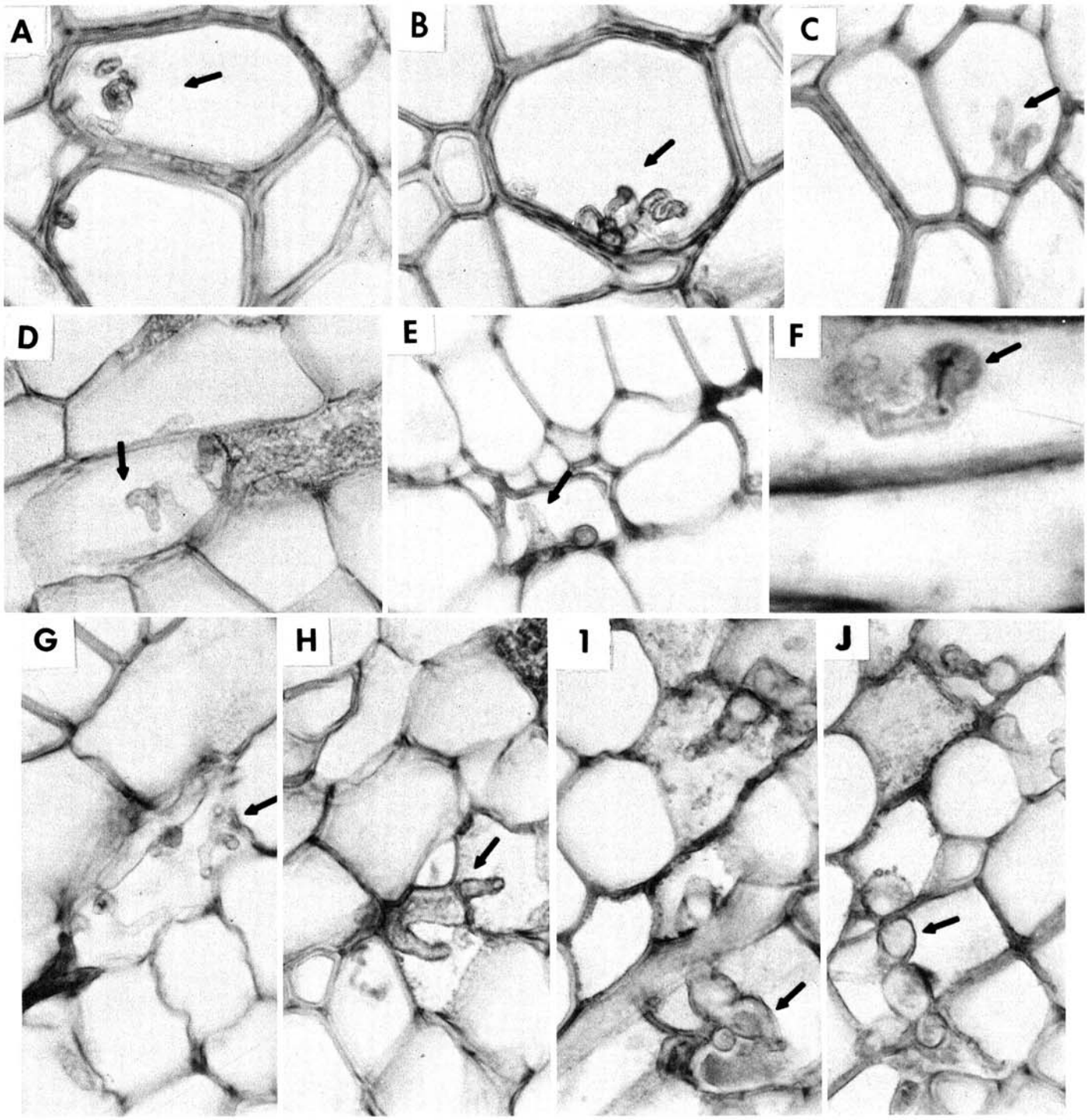

Fig. 2. Transverse sections through a burley tobacco stem infected with *Peronospora tabacina*. **A,B,** Intracellular hyphae in xylem vessel elements (×840). **C, D,** Branched haustoria in xylem parenchyma cells (×840). **E,F,** Nonbranched haustoria in parenchyma cells (×840 and ×2,100). **G,** Intracellular hyphae in a necrotic cell (×840). **H,** Thick hyphal strands growing intercellularly (×840). **I,J,** Enlarged, thickwalled fungal structures in xylem tissue (arrow) (×840).

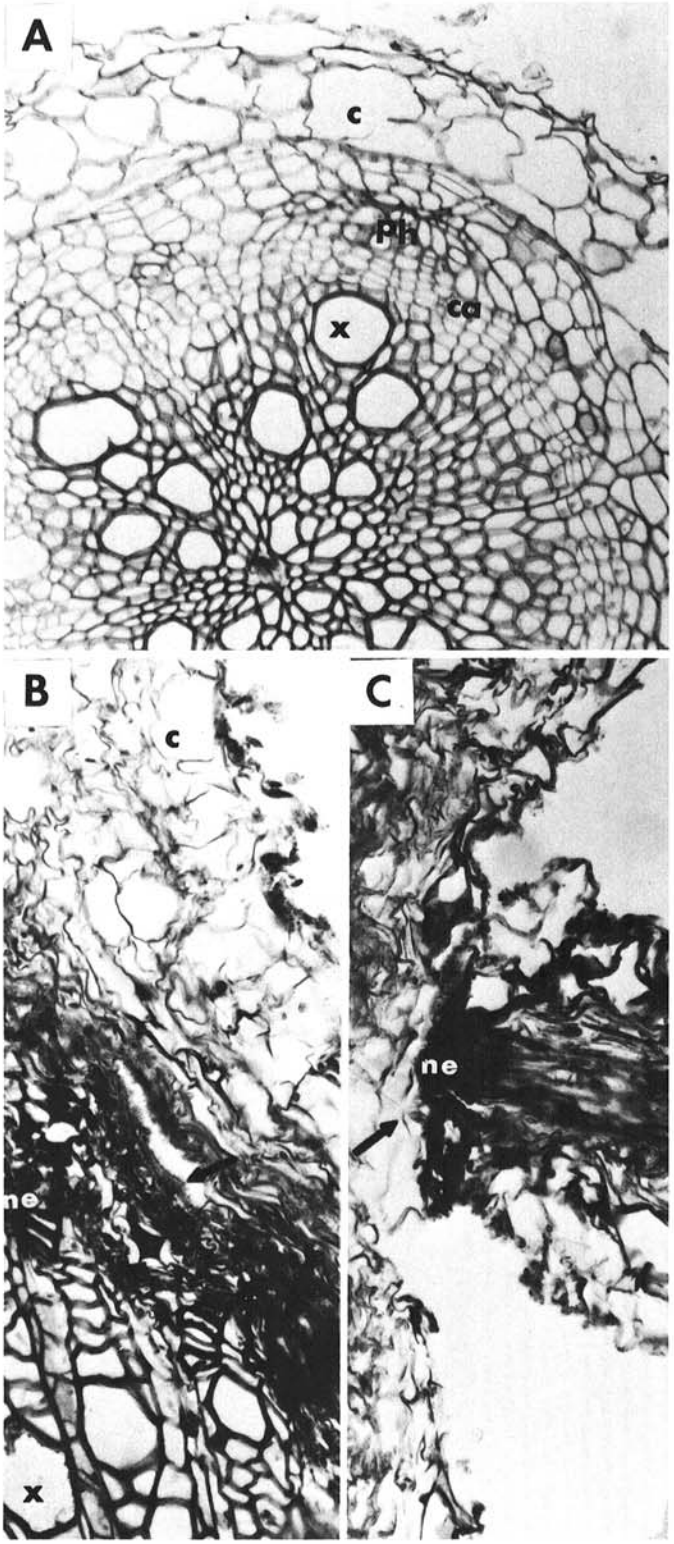

Fig. 3. Systemic infection of burley tobacco root caused by *Peronospora tabacina*. **A,** Healthy root showing cortex (c), phloem (ph), cambium (ca), and xylem (x) ×230. **B,** Diseased root with necrotic cortex, cambium, phloem, and xylem ×230. **C,** Lateral root infection ×230.

Although the fungus spreads intercellularly throughout the vascular tissue, intracellular hyphae were observed in some xylem vessels and parenchyma cells (Figs. 2A,B). Haustorialike structures with varying morphology were observed throughout the infected tissue. Both single and branched type haustoria were observed (Figs. 2C,D,E). In several instances, haustoria were closely associated with the host nucleus (Fig. 2F). In addition to the normal-appearing hyphae (Figs. 2G,H), enlarged thick-walled

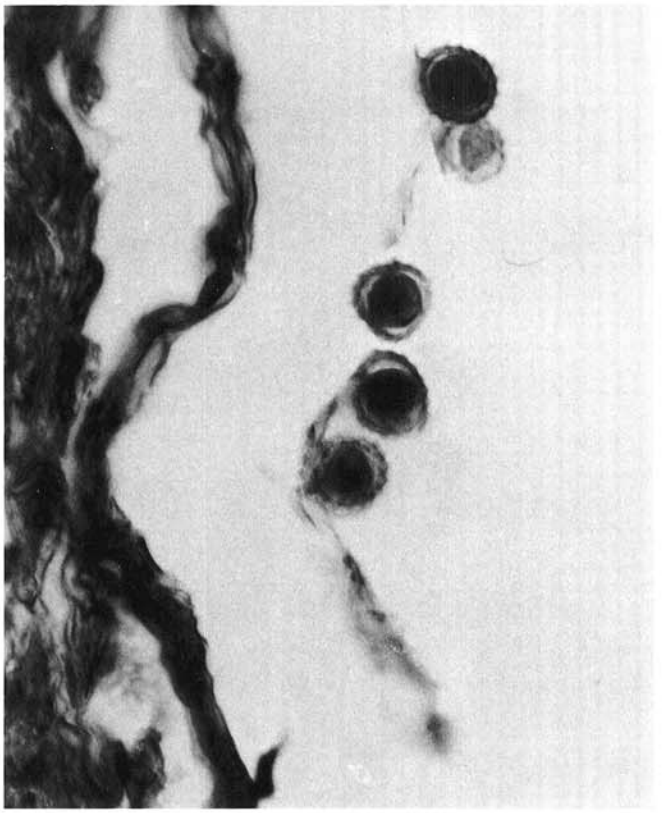

Fig. 4. Oospores of *Peronospora tabacina* on burley tobacco leaf × 567.

fungal structures measuring 13–15 μm in diameter were observed in the xylem (Figs. 2I,J). The importance of these structures and their relation to oospore development is unknown. Oospores were not observed at this stage of disease development.

All tissues in diseased roots were affected. The cortex, phloem, cambium, and xylem were necrotic and completely disorganized (Fig. 3A,B,C,).

Stem samples collected in December were necrotic and *Alternaria* hyphae had invaded the cortex which made it impossible to follow the progress of the blue mold fungus at this stage of development. Although oospores were not found in infected stem tissue collected in August, they were observed in both stem and leaf tissues sampled in December (Fig. 4). Mature oospores observed in stem sections usually were associated with severely necrotic tissue located in the outer cortex and epidermal region, and measured 18–21 μm in diameter with ridges in the outer wall.

The results of our studies indicated that burley tobacco stems systemically infected with *P. tabacina* are characterized by: intercellular and intracellular hyphae; branched and unbranched haustoria; the formation of enlarged, thick-walled fungal structures in the xylem; hyperplastic distortion of the cambium, phloem, and xylem; and the formation of cavities between the xylem and phloem. Vascular plugging, although not extensive, also was observed. Oospores developed after August in infected stems. The number of oospores produced in stem tissue was lower than that in infected leaves.

LITERATURE CITED

1. COBB, N. A. 1891. Notes on the diseases of plants. Agric. Gaz. N.S.W. 2:617-619.
2. JOHANSEN, D. A. 1940. Plant Microtechnique. McGraw-Hill, New York. 523 pp.
3. LUCAS, G. B. 1975. Diseases of Tobacco. Biological Consulting Associates, Raleigh, NC. 621 pp.
4. MANDRYK, M. 1960. Host-pathogen relationship in tobacco plants with stems infected by *Peronospora tabacina*. Aust. J. Agric. Res. 11:16-26.
5. SPURR, H. W., Jr., and F. A. TODD. 1980. Observations of the extensive 1979 tobacco blue mold epidemic. Phytopathology 70:(In press).