Histopathology of Sweet Potato Roots Infected with *Monilochaetes infuscans*

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Portions of a thesis submitted in partial fulfillment of M.S. degree requirements, North Carolina State University. This research was funded in part by USDA Cooperative Agreement 12-14-7001-821.

Journal Series Paper 6460 of the North Carolina Agricultural Research Service, Raleigh.

The use of trade names in this publication does not imply either endorsement by the N. C. Agricultural Research Service of the products named, or criticism of similar ones not mentioned.

Accepted for publication 23 July 1980.

ABSTRACT

Lawrence, G. W., Moyer, J. W., and Van Dyke, C. G. 1981. Histopathology of sweet potato roots infected with *Monilochaetes infuscans*. Phytopathology 71:312–315.

Infection of sweet potato roots by *Monilochaetes infuscans* occurred by direct penetration of cell walls. Conidia germinated within 6 hr, producing one or two germ tubes. Each germ tube terminated in an appressorium from which an infection peg penetrated the cell wall on the root surface. The fungus colonized the root periderm by growing intracellularly and forming appressorialike structures and infection pegs to facilitate cell-to-cell growth. Colonization was essentially limited to the periderm with occasional hyphae in the first layer of cortex cells. No appressoria were observed in the cortex. Infection and colonization of underground stem tissue was similar to that in the root periderm.

The scurf disease of sweet potato (caused by *Ipomoea batatas* (L.) Lam) was first described in 1890 and the causal agent was named *Monilochaetes infuscans* Ell. and Halst. ex Harter (3). Sweet potato is the only known natural host; however, other *Ipomoea* spp. became infected under artifical conditions (6). Signs of the disease first appear on roots and underground stems as small, brown-to-black spots consisting of mycelial mats and conidiophores that produce chains of conidia. Colonization is confined to the periderm; however, the pathogen spreads until spots occur on all root and underground stem surfaces. Eventually these spots coalesce to give infected portions of the root surface a dark brown to black appearance (Fig. 1). Losses are primarily cosmetic in fresh market roots; however, shrinkage may be enhanced during storage due to moisture loss through the ruptured periderm. The pathogen may survive on debris left in the field and infect transplants (3,11,12) or overwinter on seed roots and then infect sprouts. All sweet potato cultivars currently grown in the United States are susceptible to infection by *M. infuscans*.

This disease has remained a potential problem wherever sweet potatoes are grown, although controls are available. Little is known about the process of pathogenesis; only one report describes infection and colonization (11). Poole (11) suggested that infection occurs through broken cell walls in the root periderm; however, this would not account for stem infection nor was the mechanism of colonization explained. That report also describes intracellular structures in infected periderm cells that resemble sclerotia; no further studies have been conducted to confirm the role of these structures in survival.

The objective of this study was to elucidate infection and colonization of sweet potato by *M. infuscans*. A portion of this research has been previously presented (8).

MATERIALS AND METHODS

An isolate of *M. infuscans* was obtained from naturally infected cultivar Jewel sweet potato storage roots collected in Columbus County, North Carolina. The organism was isolated and maintained in pure culture as previously described (6,7). Observations in these studies were made on inoculated Jewel roots

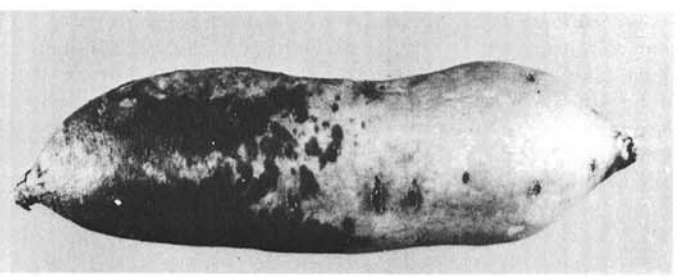

Fig. 1. A storage root of sweet potato cultivar Jewel infected with *Monilochaetes infuscans*. Colonization has occurred from the proximal end; this pattern of development is indicative of spread from infected transplants.

0031-949X/81/03031204/$03.00/0

and stems.

Light microscopy. Stem cuttings (~5 cm long) were rooted in a beaker of tap water. All roots were inoculated at the point of emergence from the stem with a drop of an aqueous conidial suspension (9.5×10^5 conidia per ml) and incubated in a moist chamber at 23 C. Infection was monitored by collecting 4-mm-long root sections from the site of inoculation at 3-hr intervals for 50 hr beginning 6 hr after inoculation. The tissue was killed and fixed in formalin-acetic acid-ethanol (10:5:50; v/v) (FAA) followed by dehydration in a graded series of ethanol and tertiary butyl alcohol (5,7). Samples were infiltrated with paraffin and embedded in Paraplast ([melting point 56–57 C] Sherwood Medical Industries, St. Louis, MO 63103). Embedded samples were chilled in an ice water bath before serial sections (12 μm) were cut on a rotary microtome. Serial sections were mounted on glass slides with chrome alum gelatin adhesive (10) and stained with Triarch quadruple stain (Triarch, Inc., Ryson, WI 54971). Observations were made with a Leitz Ortholux microscope and photographed with a Leitz Orthomat 35-mm camera and Kodak Plus-X Pan film. Structure sizes are reported as a range obtained from a minimum of 50 measurements. Germination and germ tube elongation were observed in vitro by placing conidia in distilled water on glass slides and observing them at the same intervals as those on inoculated roots.

Scanning electron microscopy. Rootlets were inoculated as described for light microscopy. Rootlet sections were fixed in FAA (5) or glutaraldehyde and dehydrated in an ethanol-Freon 113 series. Dehydrated samples were critical-point dried and affixed on scanning mounts with DAG (graphite in isopropanol, Ted Pella Co., Tustin, CA 92680). Mounted samples were coated with gold and observed on an Etec Autoscan scanning electron microscope (Etec, Hayward, CA 94545).

RESULTS

Germination of conidia and development of appressoriumlike structures were similar when conidia were incubated in distilled water or on the surface of fine roots (Figs. 2 and 3). Germ tubes, 1.5–3.1 μm in diameter, began to emerge from conidia approximately 6 hr after inoculation. Occasionally two germ tubes were produced from opposite ends of the same conidium. A bulbous swelling formed at the distal end of the germ tube approximately 11 hr after incubation began (Figs. 2A and 3A). The mature structure was dark brown to black with a septum delimiting it from the germ tube, measured 4.6–7.7 μm × 4.5–7.7 μm, and resembled an appressorium. Occasionally germ tubes would branch, resulting in more than one appressoriumlike structure per germ tube. These structures appeared to be partially attached to the cell surface by a mucilaginous material (Fig. 3B) and penetration was by a thin infection peg (Fig. 2B). These observations support the designation of these structures as appresssoria. Penetration always occurred by this method. Following penetration of the cell wall, the hyphae expanded from 0.8–1.5 μm to 2.3–4.8 μm in diameter and grew intracellularly (Fig. 2C). The intracellular hyphae were septate and occasionally branched (Figs. 2E and 3E–G). When the intracellular hyphae encountered an opposing cell wall the tip became swollen (Fig. 2C,D) and formed another structure similar to the external appressoria formed on the root surface (Figs. 2E,F and 3C–G). These structures were thick walled, appeared to be delimiting from the intracellular hyphae by a septum (Fig.

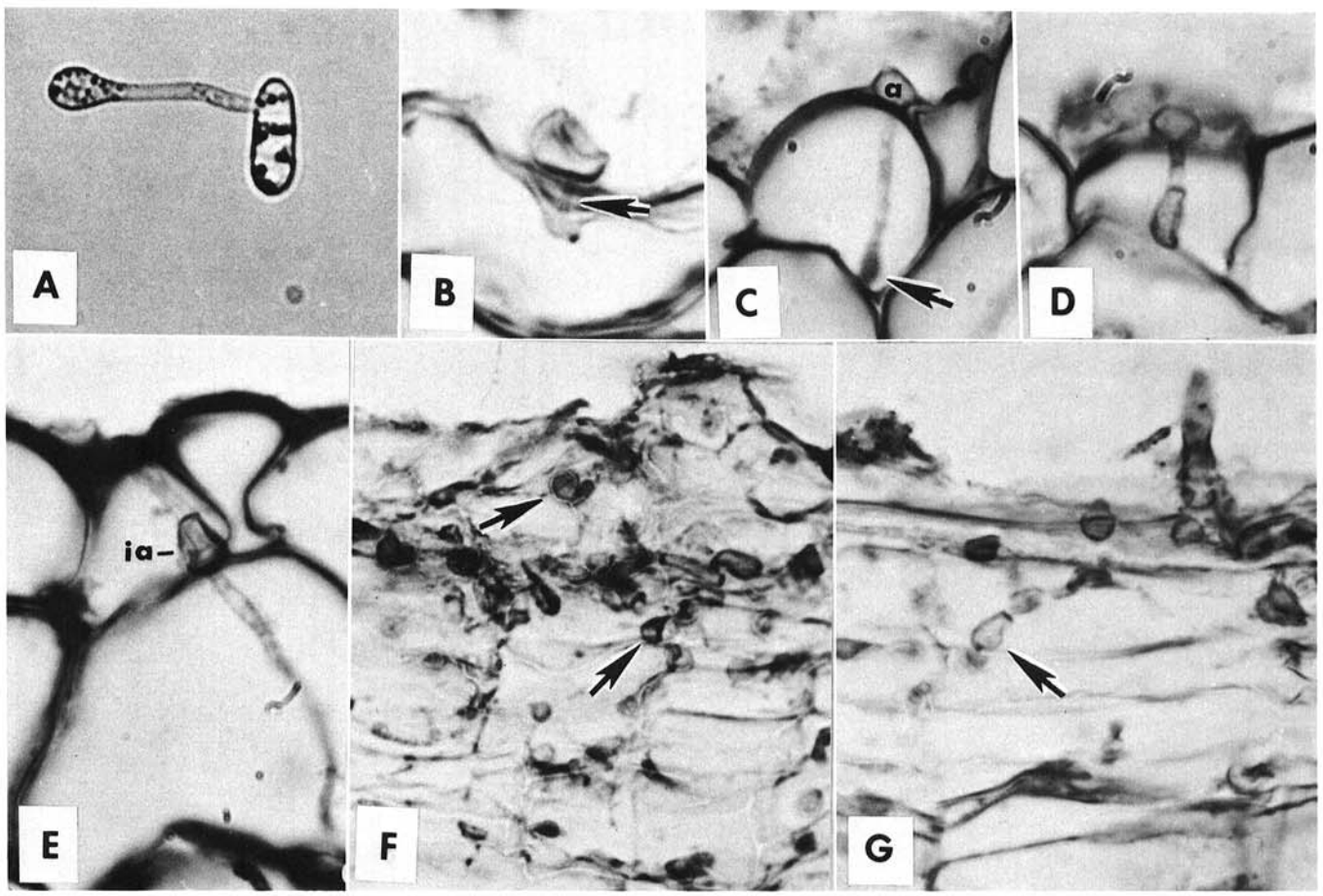

Fig. 2. Light micrographs of conidial germination, surface and intracellular appressorial formation, and colonization of sweet potato roots and underground stems by *Monilochaetes infuscans*. **A,** Conidium, germ tube, and appressorium formed in distilled water (× 560). **B,** Transverse section of an appressorium and a penetration peg (arrow) passing through the cell wall. Note the apparent host wall thickening in the region of penetration (×1,600). **C,** Appressorium (a) on cell surface, intracellular hypha with swollen tip near opposing cell wall (arrow) (×1,600). **D,** Surface appressorium, intracellular hypha with developing intracellular appressorium (×1,600). **E,** Intracellular appressorium (ia) fully developed, and hypha extending into lower adjacent cell (×1,600). Colonization of **F,** root periderm (×400) and **G,** stem tissue (×1,000). Dark bodies are intracellular appressoria (arrows).

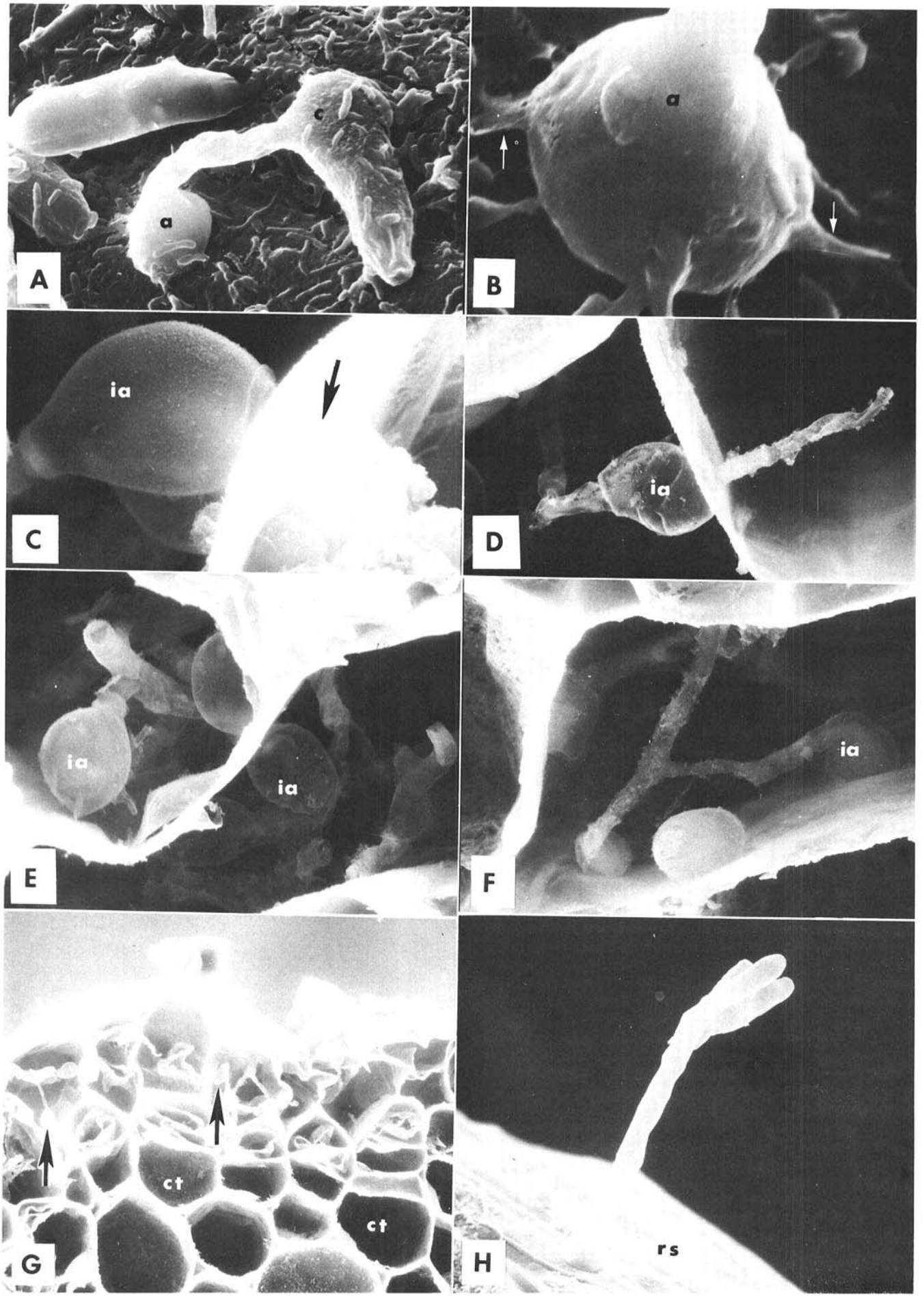
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Fig. 3. Scanning electron micrographs of penetration, colonization, and sporulation of *Monilochaetes infuscans* in sweet potato root tissue. **A,** Germinated conidium (c) with elongated germ tube and appressorium (a) on the root surface (note bacteria) (×4,600). **B,** Appressorium (a) and mucilaginous material on the root surface (arrows) (×15,000). **C,** (×8,000) and **D,** (×3,900) Intracellular appressorium (ia) during penetration of cell wall (note host wall bulge at arrow). **E,** (×5,000) and **F,** (×3,000). Colonization of root periderm showing many intracellular appressoria (ia) and branched hyphae. **G,** Colonization, limited to the root periderm, not in cortex (ct) showing intracellular hyphae and appressoria (arrows) (×1,000). **H,** Three conidia accumulated at the tip of a conidiophore projecting from the root surface (rs) (×1,700).

2D,E), and are similar in size (2.31–5.39 μm × 4.62–7.70 μm) to the external appressoria. We have designated these swollen structures as intracellular appressoria. Swellings in the cell wall opposite the appressoria (Figs. 2B and 3C) often were observed, but not always (Figs. 2E and 3D). Light micrographs of cross sections suggest that this may be papilla formation by the host (Fig. 2B). In cross sections, a thin penetration hypha (peg) passing through the cell wall was observed similar to those that initiate infection on the root surface.

Extensive colonization of the periderm was evident in both light and SEM micrographs (Figs. 2F and 3E–G). The hyphae were branched in many host cells, which resulted in several intracellular appressoria per cell (Fig. 3E,F). There was no evidence of colonization from the periderm beyond the first layer of cortex cells (Fig. 3G). Hyphal growth was limited, and no intracellular appressoria were observed in the cortex cells.

Conidiophores with bulbous basal cell were produced on the root surface approximately 1 wk after inoculation (Fig. 3H). The small, light brown spots that are characteristic of the scurf disease were visible within 2 wk after inoculation. These spots continued to enlarge and coalesce until all roots and underground stems became infected (Fig. 1). Surface appressoria, intracellular hyphae, and intracellular appressoria were observed in cross sections of infected stem tissue (Fig. 2G). Initial penetration occurred directly through intact cell walls in both root and stem tissue.

DISCUSSION

The isolate of *M. infuscans* collected from sweet potato storage roots and used in this investigation did not differ culturally or morphologically from those used by other research workers (4,11,12). However, a previous study (11) described infection as occurring through broken cell walls on the surface of the periderm and the presence of intracellular sclerotia. The structures we observed on the root surface are characteristic of appressoria as previously defined (2): they are produced from germ tubes (Figs. 2A and 3A), appear to be attached to the cell surface (Fig. 3B), and facilitate entry into the host (Fig. 2B–D). Although the fungus may penetrate the root surface through broken cells under other conditions, penetration in our study occurred only via appressoria. The intracellular structures described here are morphologically and functionally similar to those structures produced on the root surface. However, these structures facilitate cell-to-cell movement of intracellular hyphae rather than entry and are delimited from the hyphae by a septum. Similar structures produced by other fungi have been referred to as endotrophic appressoria (9). We provide evidence for direct penetration of cells via appressoria and that the intracellular sclerotia referred to in a previous study (11) were perhaps the intracellular appressoria.

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