Histopathology of Sweet Potato Root Infection by *Streptomyces ipomoea*

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ABSTRACT

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Fibrous feeder roots and fleshy storage roots of sweet potato inoculated with the soil rot pathogen, *Streptomyces ipomoea*, were examined using light and scanning and transmission electron microscopy. Hyphal growth on the surface of fibrous roots was extensive, and the pathogen penetrated the root surface by either direct penetration of the periclinal wall by short lateral branches from the surface hyphae or by growing into fissures at the junctures between epidermal cells. Development on the surface of unwounded storage roots was minimal, and the hyphae did not penetrate the root. Postpenetration development within fibrous roots and wounded storage roots was similar. Hyphae ramified through tissue intracellularly and intercellularly. Localized degradation of host cell walls was observed in areas where hyphae either grew along the inner surface of the walls or penetrated the walls. Hyphae apparently penetrated the walls either by simply growing through extensively degraded sites or by growth of lateral branches through the walls. *S. ipomoea* did not produce specialized structures analogous to fungal penetration structures. Spore chains were observed within host tissue at late stages of infection.

Soil rot or pox is an economically important disease of sweet potato, *Ipomoea batatas* (L.) Lam., that causes substantial yield reductions as well as disfiguring lesions on the fleshy storage roots, which render the storage roots produced unmarketable (7). It is caused by an actinomycete, *Streptomyces ipomoea* (Person & Martin) Waksman & Henrici. A similar but distinct pathogen, *S. scabies* (Thaxt.) Waksman & Henrici, causes common scab on several root and tuber crops. Although several other minor actinomycetous plant pathogens are known, *S. ipomoea* and *S. scabies* are the most economically significant and widely distributed. Scant attention has been given to the mechanisms of pathogenesis by actinomycetous plant pathogens. Although they are prokaryotes, they are often compared to fungi because of their filamentous growth habit. However, it is not known whether or not they have morphological adaptations analogous to the appressoria, infection cushions, haustoria, etc., produced by plant pathogenic fungi (2). Previous histopathological studies of *S. scabies* infection of potato have been limited to light microscopy. It has been shown that hyphae of the pathogen are present within potato root and tuber cells, but because the hyphal dimensions approach the limits of resolution of light microscopy, finer details of the infection process were not observed (4–6). More recently, scanning electron microscopy (SEM) has been used to study the colonization of the surface of lenticels and stomates through which *S. scabies* enters potato tubers (1). However, there has been no report of how plant pathogenic actinomycetes enter roots that lack such natural openings or how they penetrate the host cell walls once within the tissue. This work was undertaken to study the morphological aspects of pathogenesis of a plant pathogenic actinomycete, *S. ipomoea*.

MATERIALS AND METHODS

Isolates 78-49 and 81-45 of *S. ipomoea* were grown on growth agar (3) in petri dishes incubated at 24–28 C for 7–14 days. Inoculum was prepared either by cutting plugs of agar with a flamed cork borer from the margin of colonies or by making a suspension of aerial mycelia and spores (AMS) by flooding the plates with sterile distilled water and gently scraping the surface with a flamed scalpel.

The soil rot-susceptible sweet potato cultivars Centennial or Jewel were used in all studies. To study the development of the pathogen on the surface of fleshy storage roots (SR), whole SR were washed in tap water and placed in plastic boxes with moistened paper towels. A drop of AMS was transferred to the surface of the periderm with a Pasteur pipette, and the SR were incubated at room temperature. To reduce the potential of secondary contamination by other actinomycetes, bacteria, and fungi, all other inoculations were conducted with tissues that had first been surface sterilized in 0.525% sodium hypochlorite for 10 min. Development of *S. ipomoea* within SR tissue was studied using slices taken from peeled, surface-sterilized SR, which were inoculated by placing agar plugs bearing mycelia on the freshly cut surface of the slice and then incubated at room temperature in sterile petri dish moist chambers. Development on and within fibrous feeder roots (FR) was studied using adventitious FR that developed from surface-sterilized vine nodes preincubated on water agar in petri dishes. The FR were inoculated on the water agar by placing either a mycelium-bearing agar plug or drop of AMS on the tip of the roots, which were then incubated at room temperature.

FR were prepared for light microscopy by immersing 1-cm lengths of infected FR in 10% KOH and autoclaving them for 3 min at 15 psi. The roots were then rinsed three times with tap water and once with 1% HCl. The FR were transferred directly from the HCl to 0.05% trypan blue in lactoglycerol (87.5 ml of lactic acid, 6.3 ml of glycerol, and 6.2 ml of water) and allowed to stain at room temperature overnight. The FR were rinsed and mounted on slides in lactoglycerol.

Specimens were prepared for scanning electron microscopy (SEM) by excising 1- to 2-mm-thick sections of tissue from the infected roots with a razor blade. The samples were fixed in Formalin-acetic acid at 4 C for at least 24 hr then rinsed twice in 0.1 M sodium phosphate buffer (pH 7.0) and once in distilled water for 15 min. Samples were dehydrated in an acetone series. The samples were critical-point dried using acetone as the transitional solvent and mounted on stubs using double-stick tape. They were then coated with gold/palladium at 10 mA and 85 millitorr in a Hummer I sputter coater (Anatech Ltd., Alexandria, VA) for 3 min (giving a coating of about 300 A). Samples were examined on a Hitachi S-500 scanning electron microscope (Hitachi Instruments, Inc., Santa Clara, CA) at 25 kV.

Tissue was prepared for transmission electron microscopy (TEM) by cutting infected SR into 1-mm cubes, which were fixed

overnight at 4 C in 2.5% glutaraldehyde:2.5% acrolein in 0.05 M sodium cacodylate buffer (pH 7.2). Samples were postfixed in buffered 1% osmium tetroxide for 2 hr and dehydrated in an ethyl alcohol series at room temperature. Uranyl acetate (2%) was added to the 80% ethyl alcohol during dehydration. The tissue was embedded in L. R. White medium-grade resin (SPI Supplies, West Chester, PA) at 60 C for 24 hr. Sections were poststained with lead citrate and uranyl acetate and examined in a JEOL-JEM 100CX transmission electron microscope (JEOL, U.S.A., Inc., Peabody, MS) at 80-100 kV.

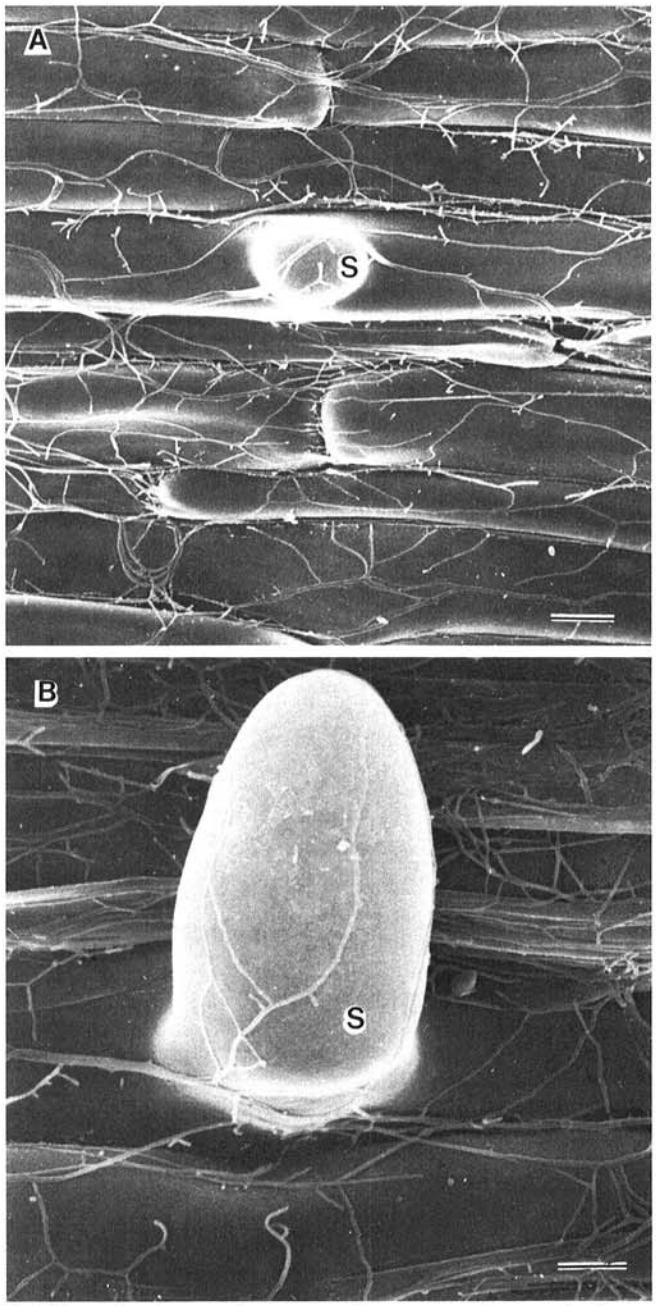

Fig. 1. Scanning electron micrographs of *Streptomyces ipomoea* hyphae on the surface of sweet potato fibrous roots (FR). **A,** Hyphae of *S. ipomoea* that grew on the surface of FR before penetration. S = root hair. (Scale bar = 10 μm) **B,** Hyphae of *S. ipomoea* on the surface of FR and a root hair (S). (Scale bar = 5 μm)

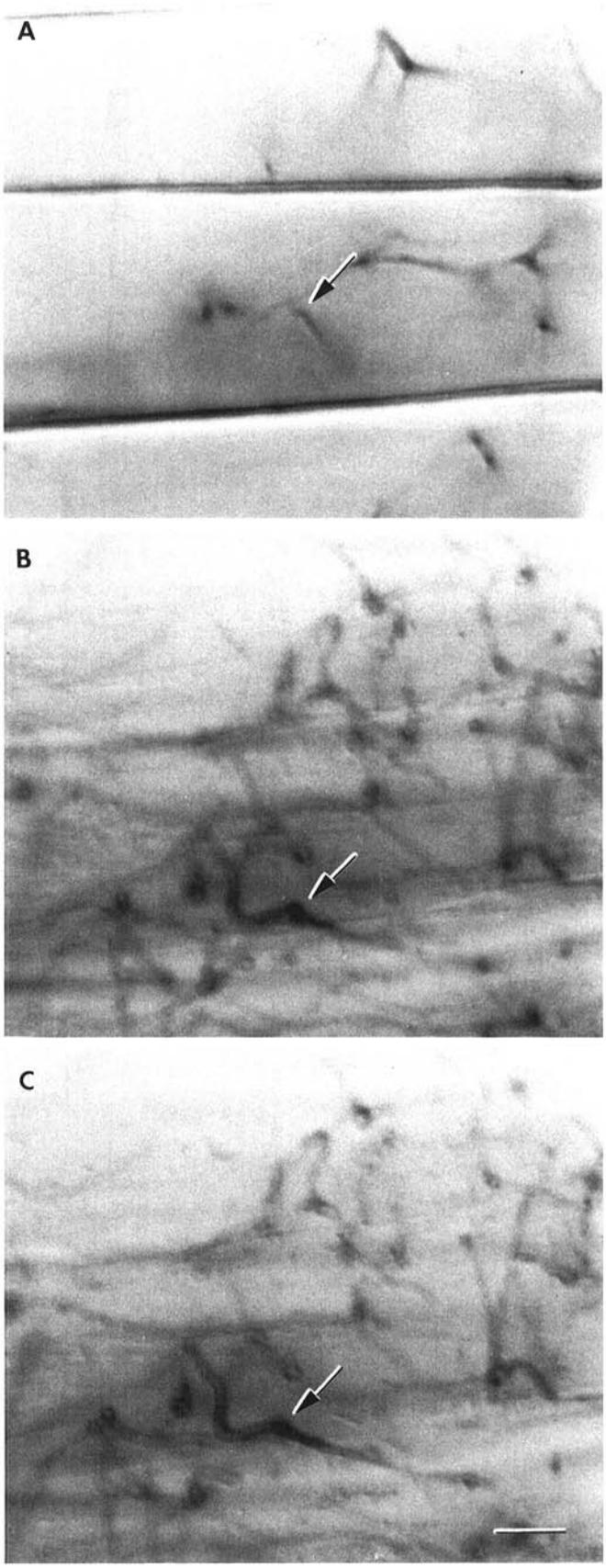

Fig. 2. Scanning electron micrographs of *Streptomyces ipomoea* on the surface of sweet potato fibrous roots (FR). **A,** Presumed penetration (arrow) of *S. ipomoea* by growth of hypha into the juncture between two epidermal cells. (Scale bar = 5 μm) **B,** Direct penetration (arrow) of *S. ipomoea* by growth of a lateral branch from a hypha through the periclinal wall of an epidermal cell. (Scale bar = 1 μm) **C,** Presumed multiple penetration of the periclinal wall of an FR epidermal cell by lateral hyphal branches (arrow) from a single surface hypha of *S. ipomoea*. (Scale bar = 0.5 μm)

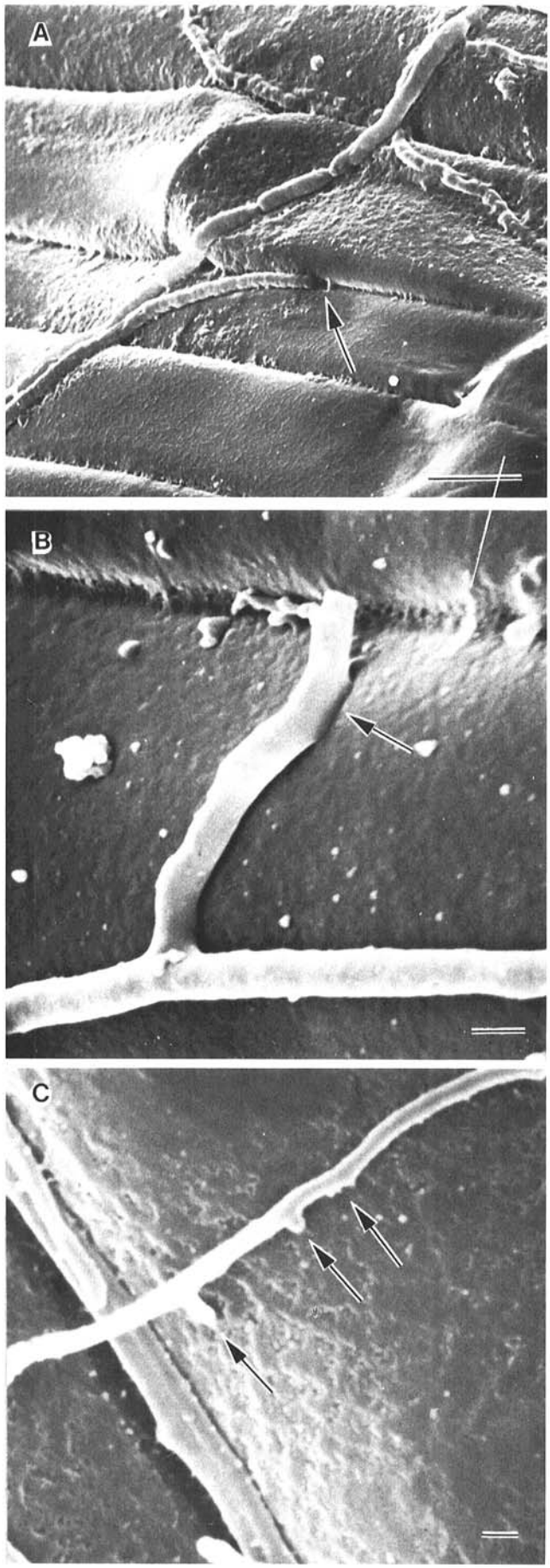

Fig. 3. Nomarski interference contrast micrographs taken in different focal planes of the same field of view of the surface of a whole mount of a sweet potato fibrous root infected with *Streptomyces ipomoea*. **A,** Focused on the lumen of the epidermal cell and hyphae within the cell. **B,** Focused on the periclinal wall of the epidermal cell. **C,** Focused on hyphae on the root surface. Arrows indicate one apparent point of penetration in each of the focal planes. (Scale bar = 5 μm)

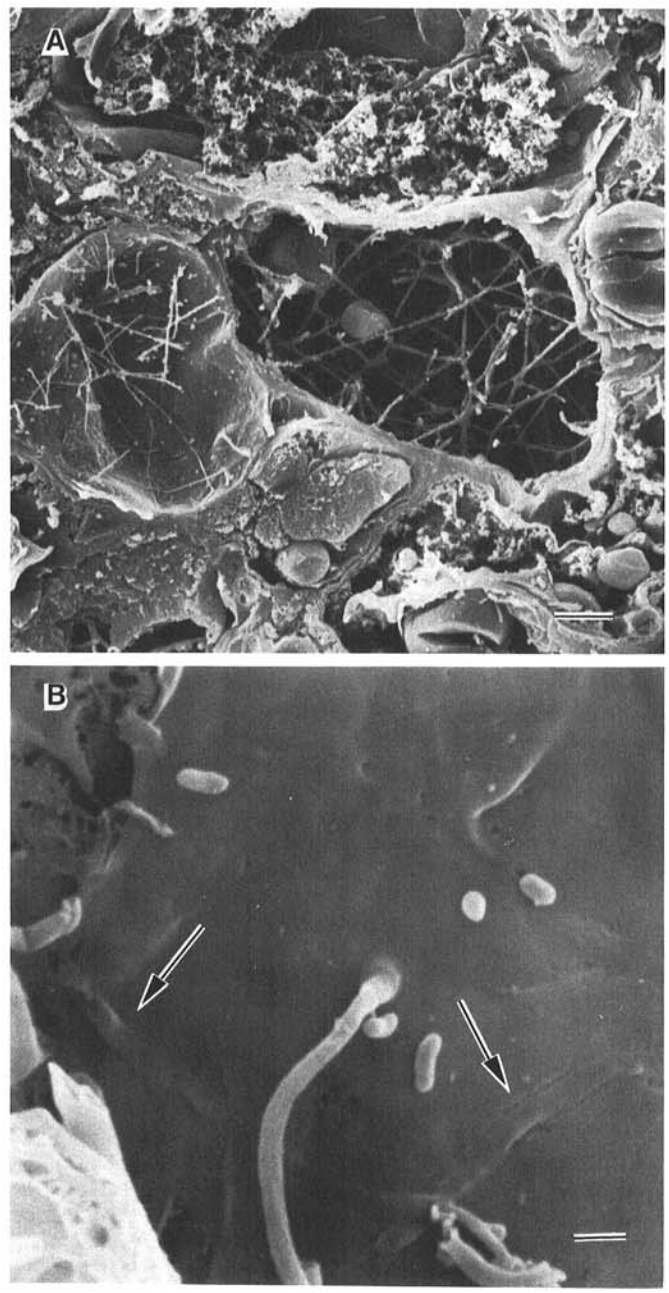

Fig. 4. Scanning electron micrographs of sweet potato storage roots infected with *Streptomyces ipomoea*. **A,** Hyphae of *S. ipomoea* within parenchyma cells. (Scale bar = 10 μm) **B,** Hyphae within a parenchyma cell. One hypha has grown through the cell wall while other hyphae (arrows) appear to be growing within the wall and/or middle lamella. (Scale bar = 1 μm)

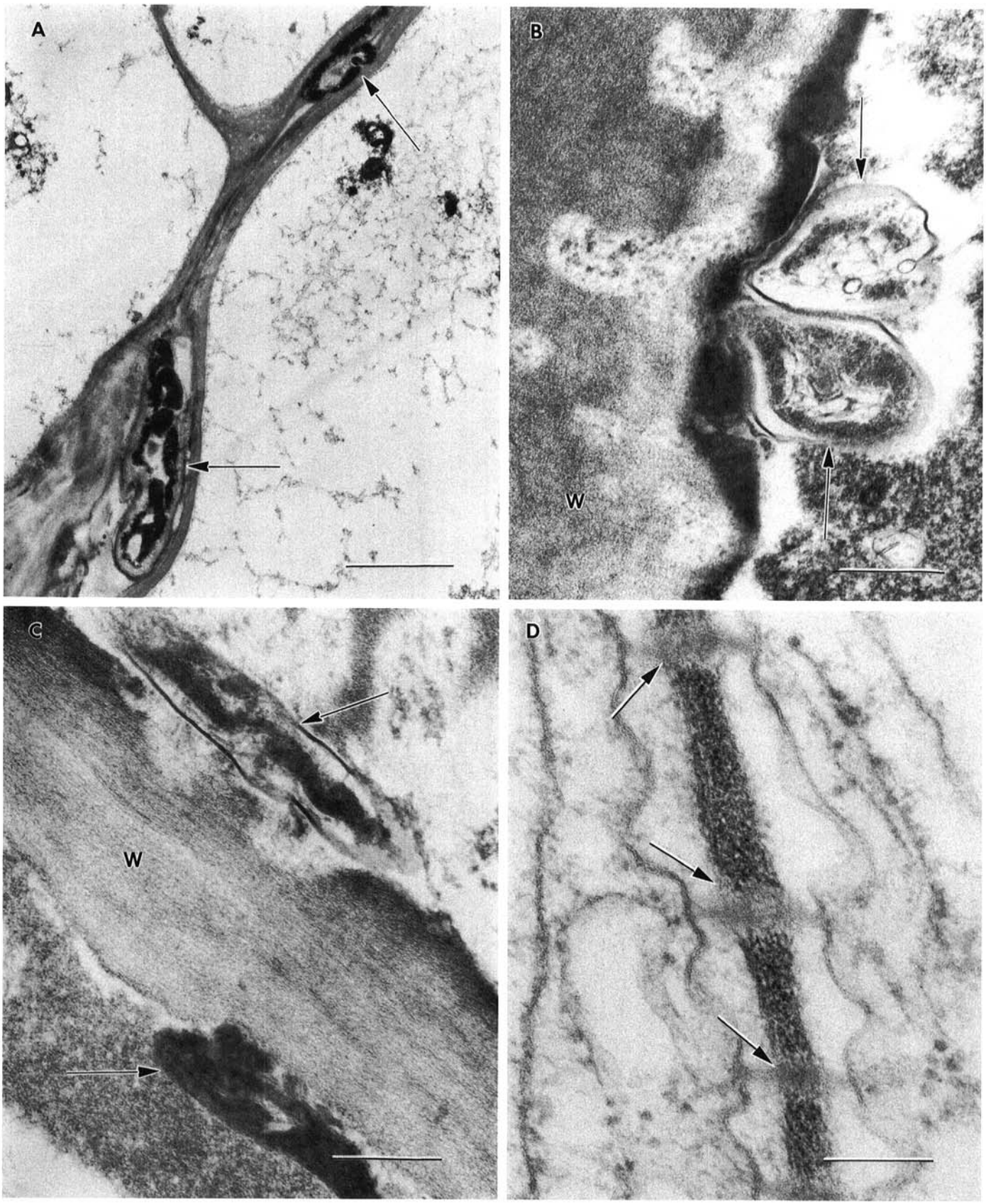

Fig. 5. Transmission electron micrographs of sweet potato storage root parenchyma. **A, B,** and **C** are from tissue infected with *Streptomyces ipomoea* and **D** from healthy tissue. **A,** Hyphae (arrows) of *S. ipomoea* within the middle lamella between two parenchyma cells. (Scale bar = 1 μm) **B** and **C,** Less densely stained channels are evident within the host cell wall (W) adjacent to hyphae (arrows) of *S. ipomoea*. (Scale bar = 1 μm) **D,** Healthy tissue with plasmodesmata (arrows) within the cell walls. (Scale bar = 1 μm)

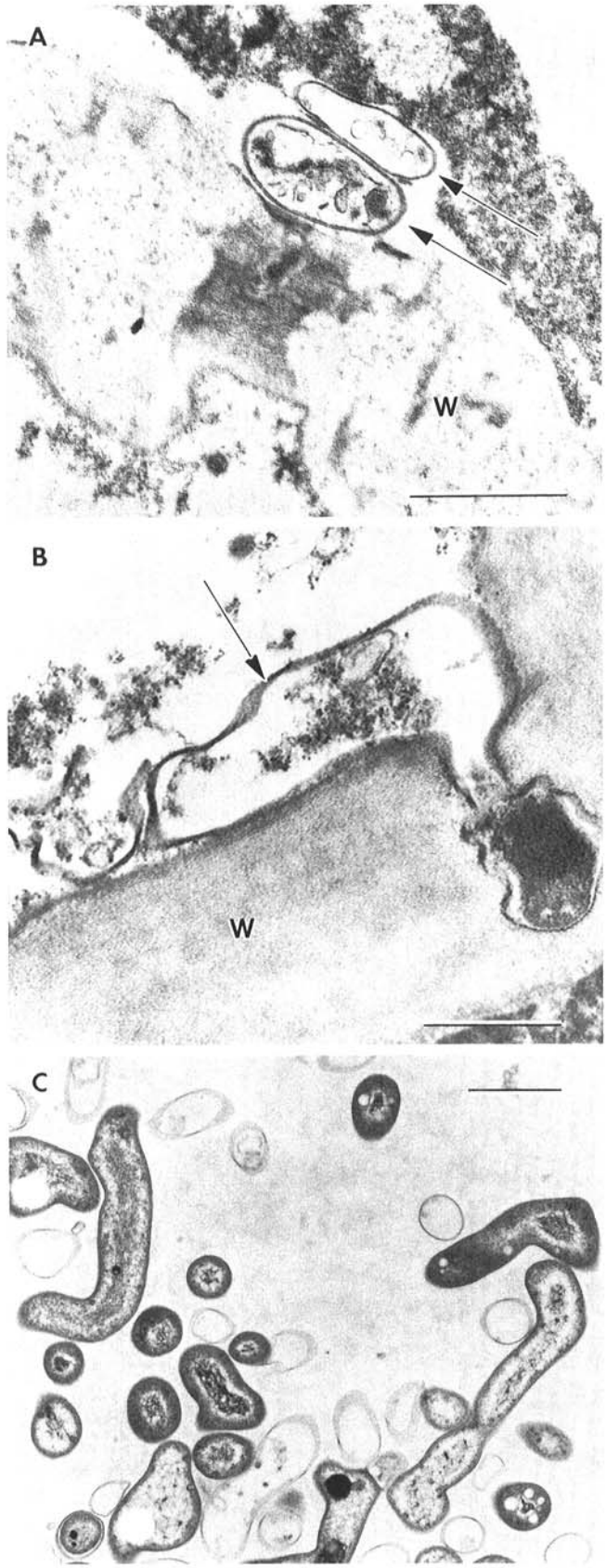

Fig. 6. Transmission electron micrographs of *Streptomyces ipomoea* in infected sweet potato storage root parenchyma (**A** and **B**) and pure culture (**C**). **A,** Severely affected tissue with swelling of the host cell wall (W). Note the laminar appearance of the host wall. A channel next to hyphae (arrows) of *S. ipomoea* is more densely stained and less swollen than the surrounding wall. (Scale bar = 1 μm) **B,** Hypha (arrow) of *S. ipomoea* with a lateral branch extended into the host cell wall (W). (Scale bar = 1 μm) **C,** Substrate hyphae of *S. ipomoea* within agar in pure culture. (Scale bar = 1 μm)

RESULTS

Hyphae of *S. ipomoea* elongated on the surface of unwounded SR periderm without branching, penetrating, or inducing lesions. By contrast, colonization of the surface of FR was extensive. Hyphae grew for considerable distances, often parallel to the longitudinal axis of the root, and formed many branches (Figs. 1A and 2). Hyphae also grew over the surface of secondary roots and root hairs (Fig. 1B). Some hyphae appeared by SEM to extend into cracks or fissures at the junctures between epidermal cells (Fig. 3A). More frequently, however, short lateral branches were observed by SEM, which appeared to extend from the side of hyphae in contact with the root surface directly into the periclinal wall of the epidermal cell (Fig. 3B). In some instances, several branches appeared to extend into a single host cell from a single hypha (Fig. 3C). Lateral branches from hyphae on the root surface, viewed by light microscopy, had extended through the periclinal

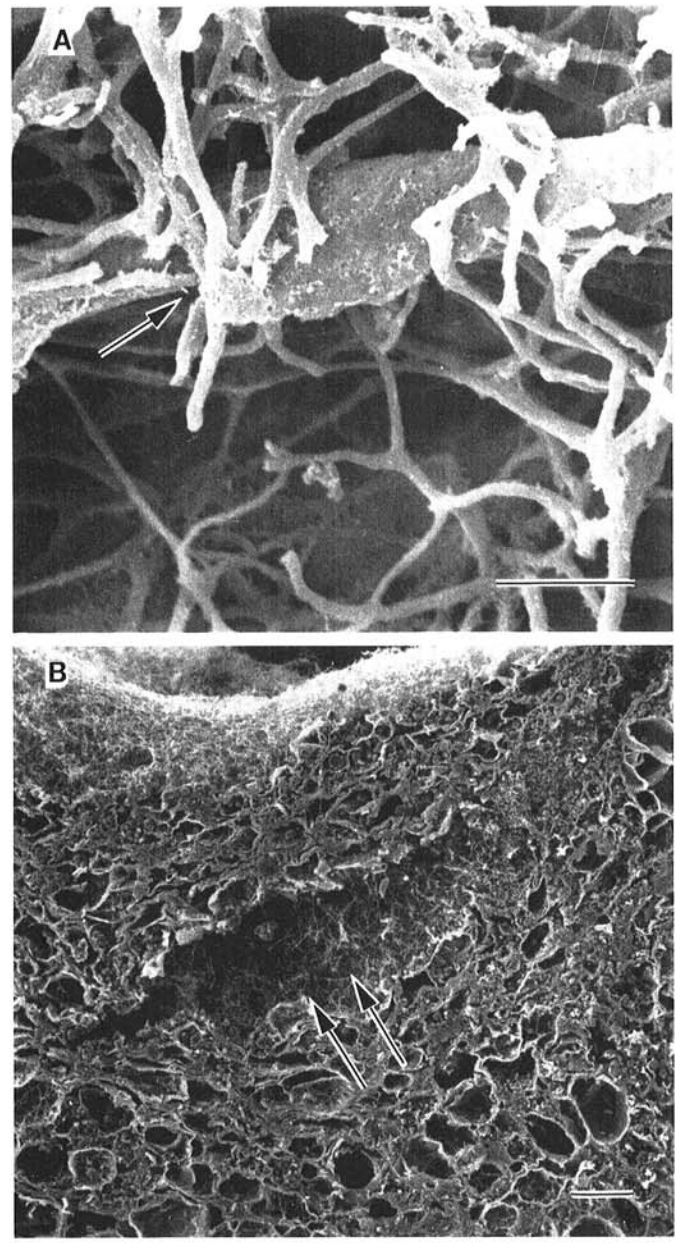

Fig. 7. Scanning electron micrographs of *Streptomyces ipomoea* within infected sweet potato storage roots (SR). **A,** Hyphae of *S. ipomoea* that have penetrated (arrow) an SR parenchyma cell wall. The wall appeared to have been torn and had many small holes and may have been partially degraded. (Scale bar = 5 μm) **B,** A schizogenous cavity (arrows) within an *S. ipomoea*-infected SR. (Scale bar = 100 μm)

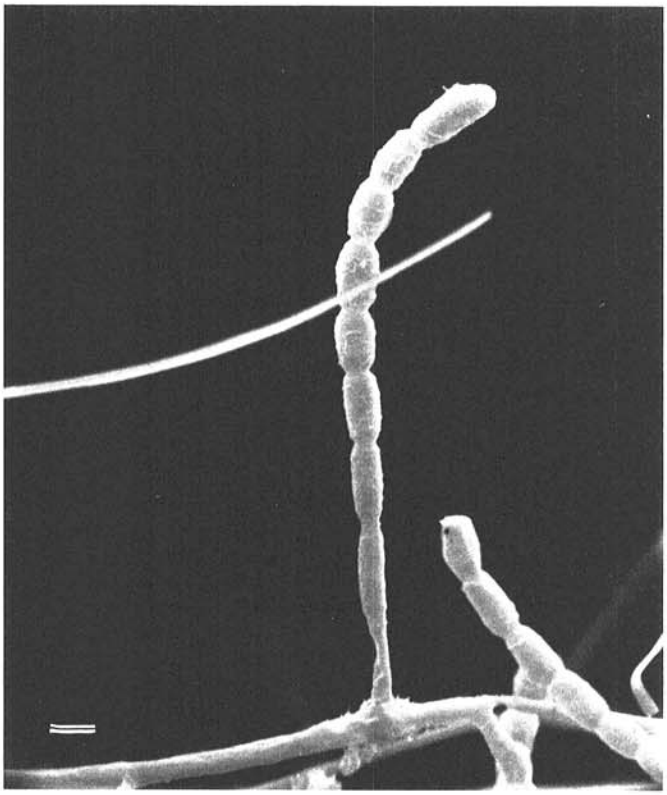

Fig. 8. Scanning electron micrograph of a sporogenous hypha and spore chain of *S. ipomoea* within a schizogenous cavity in an infected sweet potato storage root. (Scale bar = 1 μm)

wall and produced ramifying hyphae within the epidermal cell (Fig. 2).

No major differences were observed between postpenetration development of *S. ipomoea* in SR and FR. Hyphae colonized infected tissue extensively and grew both intercellularly and intracellularly. They were observed frequently on or near the inner surface of the host cell wall, and many host cells contained numerous hyphae (Fig. 4A). Two patterns were observed by which the hyphae appeared to have penetrated the host cell walls. In one, hyphae entered the wall at an oblique angle, extended for short distances within the wall or middle lamella, and eventually emerged from the wall (Fig. 4B). Occasionally, hyphae extended within the middle lamella (Fig. 5A). In most cases, channels through the host wall appeared less densely stained near hyphae (Fig. 5B and C). The channels were similar in appearance to plasmodesmata in healthy tissue (Fig. 5D) but less densely stained and slightly larger in diameter. Frequently, a hyphal branch extended from the substrate hypha directly into such channels in the host cell wall (Fig. 6B). Host cell walls in tissue heavily colonized by *S. ipomoea* hyphae were swollen and less densely stained and had a laminar appearance (Fig. 6A). However, channels adjacent to hyphae in such tissue were less swollen and more densely stained than the surrounding wall. Figure 6C shows the appearance of hyphae grown in culture, for comparison.

Cell walls were collapsed in older regions of lesions, and groups of hyphae crossed such walls without evident change (by SEM) in appearance or direction (Fig. 7A). Large schizogenous cavities developed within infected SR tissue and often contained numerous hyphae of the pathogen (Fig. 7B). A few short spore chains were observed within older infected tissue (Fig. 8). Host cells affected by the pathogen had granular cytoplasm and were frequently collapsed, and membranes lacked structural integrity. Hyphae of *S. ipomoea* were observed within all types of FR and SR tissues, including vascular elements. All such infected tissue, with the exception of secondary xylem wall thickenings, eventually collapsed and lost structural integrity.

DISCUSSION

Despite the superficial similarity between the filamentous growth habit of *S. ipomoea* and plant pathogenic fungi, distinct differences were evident in the way in which they penetrated plant cell walls. The actinomycete apparently does not produce appressoria or infection cushions analogous to those of most fungi (2). Hyphae of *S. ipomoea* grew within host cells, often adjacent to the cell wall, without penetrating the host wall. Penetration of host cell walls apparently occurred as a result of growth of lateral branches arising from the hyphae at certain sites where the hypha was in contact with the host wall. There appeared to be some cell wall dissolution at these sites, suggesting that enzymatic cell wall degradation may be an important if not essential part of wall penetration by *S. ipomoea*. Because channels in host walls were sometimes similar in appearance to host plasmodesmata, perhaps hyphae penetrate through plasmodesmata following enlargement of the plasmodesmatal channel by enzymatic degradation of some of the surrounding cell wall.

Spore chains of *S. ipomoea* were observed in infected tissue only infrequently and late in the infection process. Their open loop morphology was similar to that originally described for *S. ipomoea* (8) and typically associated with *Streptomyces* spp. This suggests that the 'false sporangia' previously observed on artificial media may be an aberration induced by a component of the culture environment (3).

The observation that *S. ipomoea* is not restricted to the cortex of the roots as are many other root-rotting pathogens may partially explain the pathogen's ability to drastically reduce growth and yield of the plant. Destruction of the cortex might reduce selective absorption of water and nutrients, but destruction of the vascular system might also reduce translocation to other parts of the plant.

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