Cytology and Histology

Histological Evidence that *Fusarium lateritium* is an Exopathogen on Sweetpotato with Chlorotic Leaf Distortion

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ABSTRACT

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Apical meristems, axillary buds, leaves at different stages of development, and nodes from vines of sweetpotato (*Ipomoea batatas*) with symptoms or in remission from chlorotic leaf distortion (caused by *Fusarium lateritium*) were examined by light and scanning electron microscopy. Apical meristems and axillary buds from healthy plants and plants affected with chlorotic leaf distortion were covered with numerous pearl glands and a mucilagelike material. On plants affected with chlorotic leaf distortion, hyphae were observed on and within this mucilage on the apical meristem, leaf primordia, and young, folded leaves. A dense layer of hyphae was observed between halves of developing, folded leaves and on the adaxial surface of recently unfolded leaves. On older, expanded leaves, mucilagelike material was observed only rarely around the base of pearl glands, and hyphae were present only as scattered clumps with broken ends. No fungal penetration structures were observed on the surface of the plant, and no fungal structures were observed inside any part of the shoot system. It is concluded that the *F. lateritium*/sweetpotato chlorotic leaf distortion system represents an exopathogen-suscept relationship. Implications of this relationship are discussed.


Chlorotic leaf distortion is a disorder of sweetpotato (*Ipomoea batatas* (L.) Lam.), incited by the fungus *Fusarium lateritium* Nees:Fr. (1). Many species of *Fusarium* cause plant diseases, but they are generally vascular wilts, cortical rots, or other necroses

(10). Chlorotic leaf distortion is distinctively different from most other diseases caused by species of *Fusarium*. Symptoms are systemic, and *F. lateritium* has been isolated from many NaOCl-treated parts of affected shoots, including shoot apical meristems, but not from roots. Symptoms develop only during extended periods of sunny weather on plants that have been growing vigorously. The first evidence of chlorotic leaf distortion is the

appearance of white hyphae that are visible macroscopically at the margins of young, folded leaves and on the surface of the youngest unfolded leaves. As leaves expand and mature, the hyphae are evident only as isolated clumps near the margins of the leaves. During weather conducive to chlorotic leaf distortion, an intense general chlorosis develops on young leaves (which also may become distorted), but as the leaves expand, they recover from chlorosis. When nonconducive (cloudy) weather occurs, all leaves on plants recover from chlorosis, and if cloudy weather persists, hyphae are no longer evident on newly unfolding leaves (1). Plants free of chlorotic leaf distortion and *F. lateritium* can

be produced by regenerating plants from meristem-tip culture if meristems are excised when plants are free of symptoms (1).

A histological study was conducted to determine if the physical relationship between *F. lateritium* and sweetpotato shoots could help explain the unusual nature of symptom development. This paper provides evidence that *F. lateritium* develops extensively on and in an uncharacterized mucilagelike material secreted on the surface of healthy and chlorotic leaf distortion-affected sweetpotato apical meristems but that it does not enter sweetpotato shoots affected with chlorotic leaf distortion. A preliminary report has been published (2).
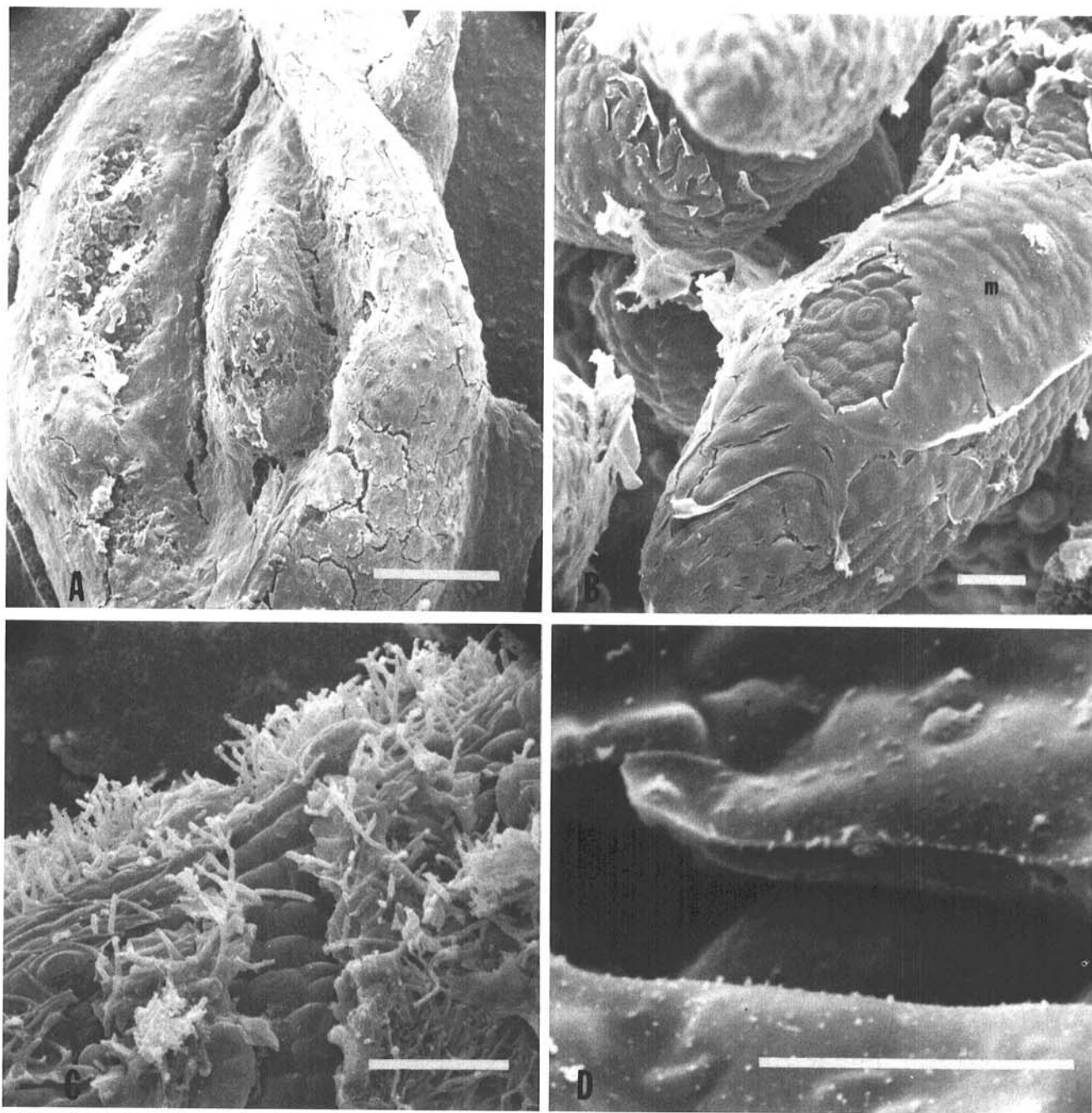
Fig. 1. Scanning electron micrographs of shoot tips from sweetpotato plants with or without chlorotic leaf distortion. **A,** Plant affected with chlorotic leaf distortion—most of the apical dome, leaf primordia, and immature, folded leaves are covered with a mucilagelike material (bar = 500 μm). **B,** Tips of leaf primordia from a shoot tip of a sweetpotato mericlone free of chlorotic leaf distortion. A layer of mucilagelike material (m) partially covers the leaf primordia (bar = 50 μm). **C,** Leaf primordium of a plant affected with chlorotic leaf distortion. Part of the mucilagelike material has broken apart, revealing the epidermis. Hyphae are visible within and on the mucilagelike material (bar = 50 μm). **D,** Surface of a leaf primordium from the apical meristem of a sweetpotato free of chlorotic leaf distortion. The mucilagelike material was cracked, and the epidermis can be seen through the crack (bar = 10 μm).

MATERIALS AND METHODS

Samples. Leaves and vine terminals (10–30 cm) were collected from sweetpotato plants grown in the greenhouse or the field

at Baton Rouge, LA, during the growing seasons of 1989–1991. All plants were originally derived from meristem-tip culture (mericlones) and found to be apparently free of chlorotic leaf distortion (1). Plants affected with chlorotic leaf distortion were maintained in the greenhouse from prior pathogenicity tests (1) and transplanted to the field each year from May to June. Samples were collected before development of chlorotic leaf distortion, when chlorotic leaf distortion symptoms were severe, and when plants were in partial or total remission from chlorotic leaf distortion. The oldest leaf that had not unfolded was arbitrarily designated as leaf 0, older leaves that had unfolded were assigned positive numbers progressing from the 0 leaf and younger, still folded leaves were assigned negative numbers from the 0 leaf. Genotypes examined included the cultivars Beauregard, Hernandez, Jewel, and Porto Rico and the clone NC-845.

Isolation. Terminal 20-cm segments from vines of Jewel were collected from the greenhouse on 7 November 1989 and on 12 August 1991, when chlorotic leaf distortion symptoms had been prominent for several weeks, and on 18 January and 19 February 1990, when plants were in remission. Nodes and shoot tips were excised, surface-disinfested in 0.525% NaOCl for 5 min, rinsed in sterile distilled water, and placed on Komada's medium (8) in petri dishes 9 cm in diameter. They were incubated for 1 wk at 28 C, and the number of explants from which salmon to pink colonies with white aerial hyphae and a feltlike texture formed was recorded as positive for isolation of *F. lateritium* (1).

Light microscopy. Specimens were prepared for light microscopy by cutting small pieces from shoot tips, leaves, and nodes from vines. Tissue was fixed at 4 C in 2.5% glutaraldehyde:1.0% acrolein in 0.05 M sodium cacodylate buffer (pH 7.2). Samples

TABLE 1. Frequency of isolation of *Fusarium lateritium* from different parts of chlorotic leaf distortion-affected Jewel sweetpotato vines collected from the greenhouse during periods of chlorotic leaf distortion symptom development and remission

Part of shoot[x]	Number of isolations per 10 attempted[y]	
	Symptomatic	Remission
Apical meristem	3	0
Leaf primordia	6	0
−1 leaf	9	6
0 node, cross section	9[z]	0
0 axillary bud	ND[z]	4
+1 leaf piece	8	1
+2 leaf piece	4	1
+2 node, cross section	4	1
+2 axillary bud	10	5
+4 node, cross section	6	3
+4 axillary bud	10	4
+6 node, cross section	6	3
+6 axillary bud	10	4

[x] The oldest leaf that had not unfolded was defined as the 0 leaf. Older leaves were assigned + numbers and younger leaves − numbers progressing from the 0 leaf.

[y] Symptomatic shoots were collected on 11 Nov 1989 from plants in the greenhouse that had shown chlorotic leaf distortion symptoms for several weeks. Shoots from plants in remission from chlorotic leaf distortion were collected from the same group of plants in the greenhouse on 18 Jan. 1990.

[z] Axillary buds were left on the node cross sections and not plated separately.
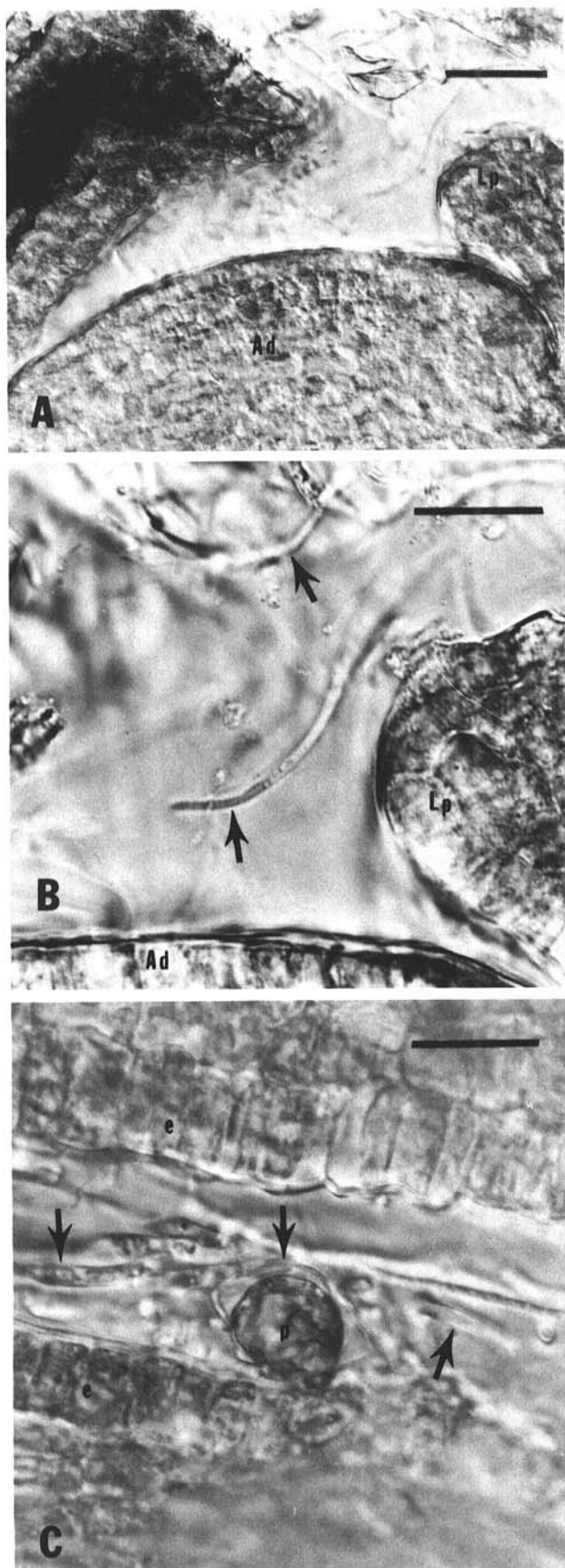
◀ **Fig. 2.** Light micrographs of sections through the shoot tip region of a sweetpotato vine with chlorotic leaf distortion. **A,** Longitudinal section through an apical meristem showing apical dome (Ad) and leaf primordium (Lp) (bar = 50 μm). **B,** Higher magnification of **A** showing hyphae (arrows) in space between leaf primordium (Lp) and apical dome (Ad) (bar = 25 μm). **C,** Cross section through a young leaf that had not yet unfolded. Hyphae (arrows) are present between the adaxial epidermal layers (e) of the opposing halves of the leaf and around a pearl gland (p) (bar = 25 μm).

were postfixed in buffered 1% osmium tetroxide for 2 h and dehydrated in an ethyl alcohol series at room temperature. Tissue was embedded in L. R. White medium-grade resin (SPI Supplies, West Chester, PA) at 60 C for 24 h. Sections were cut about 0.5 µm thick with a Sorvall/Porter-Blum Ultramicrotome MT-2

and poststained with Toluidine blue. Freshly collected tissue was also frozen in 1% gum arabic on the stage of a sliding microtome (AO 880, American Optical, Buffalo, NY) with a CO_2-freezing attachment and sectioned at about 25 µm.

The epidermal layer was peeled from areas on unfolded leaves
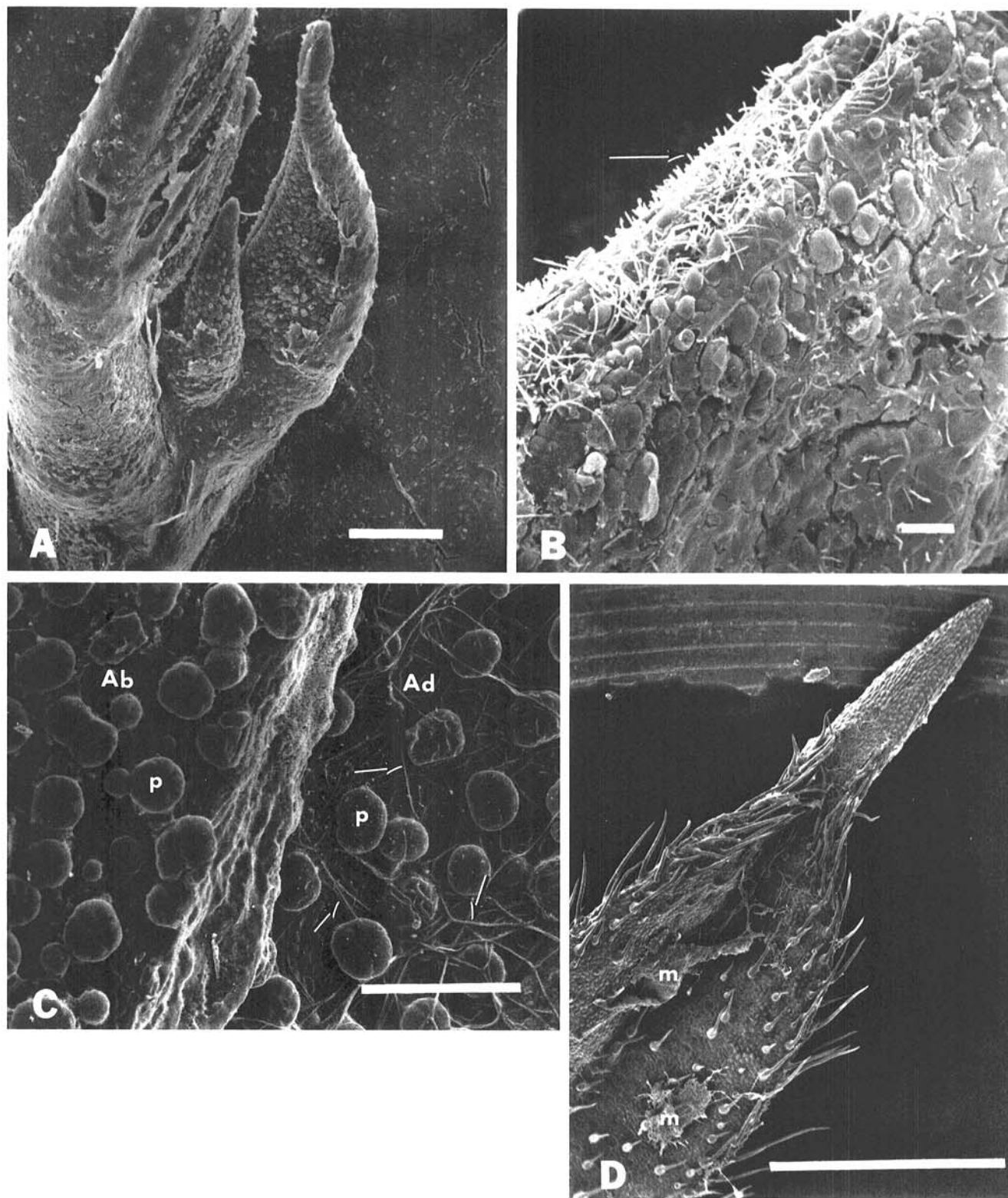
Fig. 3. Scanning electron micrographs of parts of sweetpotato shoots with chlorotic leaf distortion. **A,** Shoot tip showing distribution of pearl glands and mucilagelike material (bar = 500 µm). **B,** A young, folded leaf (−1). Phialides (arrow) protrude from between the halves of the leaf (bar = 50 µm). **C,** A young, folded leaf (−1) from a sweetpotato vine with chlorotic leaf distortion. A portion of one half of the leaf was removed so that the abaxial surface (Ab) of one half of the leaf can be seen on the left and the adaxial surface (Ad) of the other half on the right. Hyphae are numerous (arrows) on the adaxial surface, and pearl glands (p) are numerous on both surfaces (bar = 100 µm). **D,** The tip of a young unfolded leaf (+1) from a sweetpotato vine that had partially recovered from symptoms of chlorotic leaf distortion. Mucilagelike material (m) is present near the tip and hyphae are present on and within this material (bar = 1 mm).

and immediately mounted in 0.1% Tween-20 in distilled water on a microscope slide. Sectioned tissue and epidermal peels were examined using bright field and Nomarski interference contrast with a Zeiss Universal light microscope.

Scanning electron microscopy (SEM). Shoot tips (<5 mm long), vine nodes with axillary buds, and pieces of unfolded leaves (about 2–3 × 2–3 mm) were examined by SEM. Tissue was fixed for 1 h at 4 C in the glutaraldehyde-acrolein fix as above, then rinsed for 15 min twice in 0.01 M sodium cacodylate buffer (pH 7.2) and once in distilled water. Samples were dehydrated in either an ethanol or an acetone series and critical-point dried using either ethanol or acetone as the intermediate solvent and CO_2 as the transitional fluid. Samples were mounted on stubs and coated with gold/palladium at 10 mA and 85 millitorr in a Hummer I sputter coater (Anatech Ltd., Alexandria, VA) for 2 min (giving a coating of about 200 Å). Samples were examined on a Hitachi S-500 (Hitachi Instruments, Santa Clara, CA) or a Cambridge S-260 (Cambridge Instruments, Deerfield, IL) scanning electron microscope.

RESULTS

Isolations. Isolation frequencies were similar in both attempts from symptomatic plants and in both attempts from plants in remission. One attempt for each is presented in Table 1. *F. lateritium* was isolated from all parts of the surface-disinfested vine terminal of plants showing chlorotic leaf distortion symptoms (Table 1). Frequency of isolation was lower from apical meristems and leaf primordia but was almost 100% from folded leaves and axillary buds. Isolation frequency was relatively low from plants that were in remission from chlorotic leaf distortion, and the frequency was greatest from axillary buds on these plants (Table 1). No other organisms were isolated on Komada's medium.

Microscopic observations. Using SEM, a mucilagelike layer was observed that almost completely covered the apical dome and youngest leaf primordia of shoot tips from plants affected with chlorotic leaf distortion (Fig. 1A). Hyphae of similar diameter to those of *F. lateritium* were observed on and within this layer (Fig. 1C). Bacteria were rarely observed within the mucilagelike
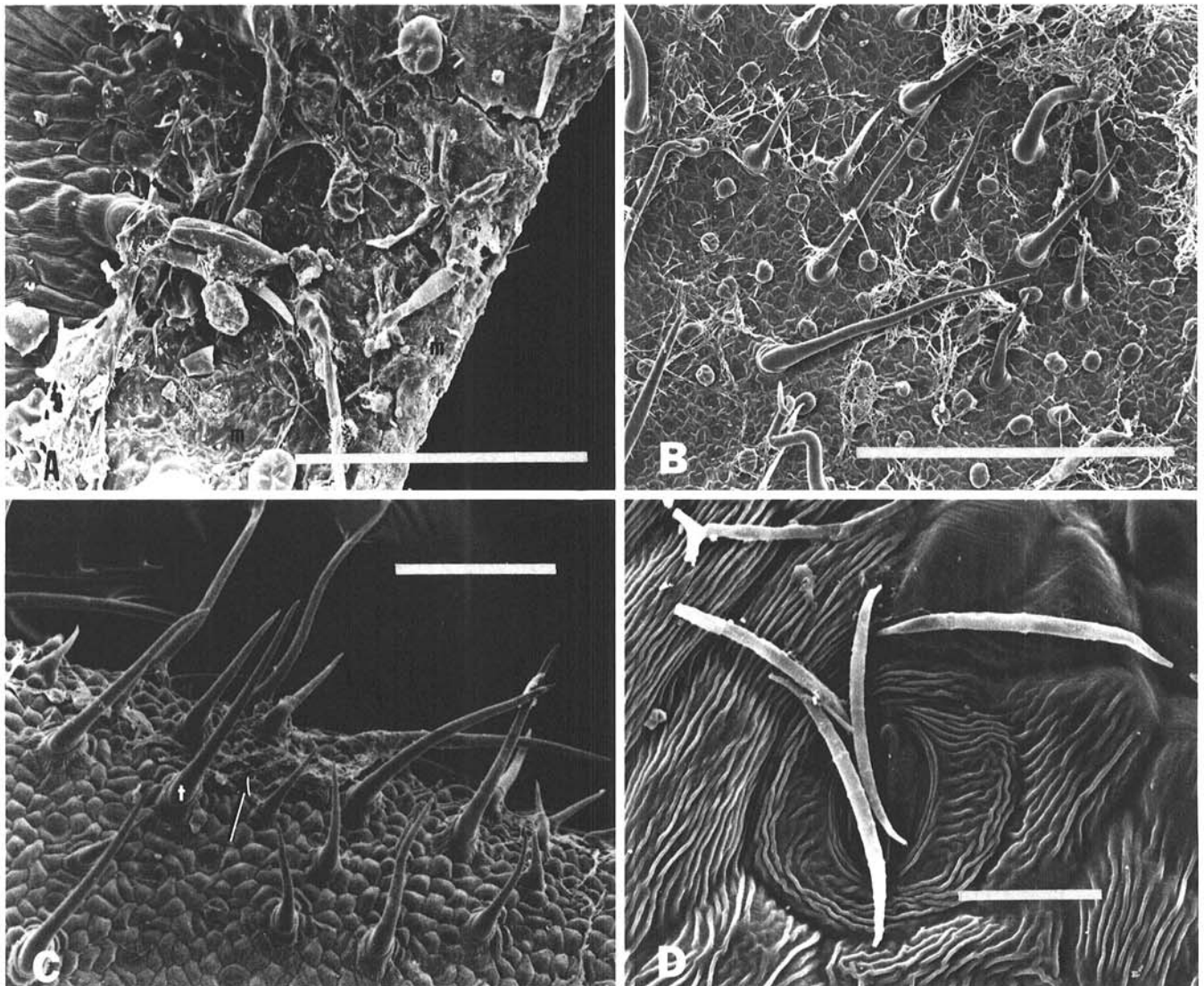
Fig. 4. Scanning electron micrographs of leaves of sweetpotato with chlorotic leaf distortion. **A,** The margin of a young, unfolded leaf (+1) from a sweetpotato vine that had partially recovered from symptoms of chlorotic leaf distortion. Mucilagelike material is present near the margin but not on the rest of the lamina (bar = 200 μm). **B,** A young, unfolded leaf (+1) from a sweetpotato vine with severe symptoms of chlorotic leaf distortion. A nearly continuous layer of hyphae is present on the adaxial surface of the leaf (bar = 500 μm). **C,** An unfolded leaf (+1) from a sweetpotato plant (cv. Porto Rico) that had chlorotic leaf distortion symptoms. Hyphae (arrows) are present around the bases of trichomes (t) near the margin of the leaf (bar = 200 μm). **D,** Macroconidia typical of *Fusarium lateritium* on the surface of an unfolded leaf (+1) from a sweetpotato vine with chlorotic leaf distortion (bar = 20 μm).

material on the shoot tips of field-grown plants. Shoot tips from healthy mericlones free of chlorotic leaf distortion also had a mucilagelike layer covering the same portions of the shoot tip but hyphae were not observed (Fig. 1B and D). Several attempts to obtain sections through the apical-meristem region were compromised by the apparent lack of proper infiltration of embedding resin into tissue underlying the mucilagelike layer. However, in some resin-embedded sections and in sections from fresh tissue, fungal hyphae were observed within and outside the mucilagelike layer and in the spaces between leaf primordia and the apical dome but were not observed within the plant (Figs. 1C and 2A and B). The mucilagelike layer was present on some of the youngest folded leaves, was broken and covered only a portion of some of the intermediate folded leaves, and was sparse on the oldest folded leaves. Pearl glands were numerous and well developed on the leaf primordia and were close to, but not actually on, the apical dome (Fig. 3A).

On young leaves that had not unfolded or expanded (−1 to −2), hyphae were visible with the unaided eye or at low magnification along the margin of the opposite halves of these leaves. With SEM, margins often were observed to have numerous phialides protruding from between the halves of the leaf (Fig. 3B). Hyphae were abundant on the adaxial surface of these leaves and between the halves of these leaves but were not as numerous on the abaxial surface (Figs. 2C and 3C). Pearl glands were numerous on both leaf surfaces, and mucilagelike material was also present on both surfaces, especially the abaxial surface during periods of severe symptom development (Fig. 3C).

On young unfolded leaves (+1), some mucilage was present near the leaf tip and along the margins of the leaf (Figs. 3D and 4A). During periods of chlorotic leaf distortion symptom expression, the adaxial surface was nearly covered with a layer of hyphae (Fig. 4B). On the cultivar Porto Rico, clumps of hyphae were also observed near the bases of trichomes, which were more numerous at the margins of the leaves. However, mucilagelike material was not as evident at margins of leaves on cv. Porto Rico as on other genotypes (Fig. 4C). Macroconidia of a size and shape typical for *F. lateritium* were observed on the leaf surface during these periods (Fig. 4D). Pearl glands were more widely scattered than on younger, folded leaves. In cross sections or epidermal peels from such leaves, hyphae were observed frequently on the leaf surface, but no hyphae were observed inside the leaf (Fig. 5A and B). No appressoria or other penetration structures were observed. During periods of chlorotic leaf distortion remission, hyphae were in separated clumps, often near or around pearl glands, and the ends of the hyphae often appeared broken (Figs. 5A and 6A and C).

On older, unfolded leaves (+2), clumps of hyphae were more widely scattered and were more common near the margin of the leaf. During periods of chlorotic leaf distortion remission, the hyphae at the edges of the clumps appeared broken (Fig. 6C). During periods of symptom expression, tips of the hyphae appeared intact, phialides were often present, and conidia were often observed on the leaf surface (Fig. 6B and D). No fungal structures were observed inside the tissue of leaf sections or epidermal peels.

Axillary buds were covered with a mucilagelike layer (Fig. 7A) and appeared very similar to the apical meristems. Hyphae were observed on and within the mucilagelike layer (Fig. 7B) but were not observed on the surface of the adjoining stem. Examination of cross sections through stem nodes did not reveal the presence of any fungal structures within the nodes. Hyphae were observed in and on a mucilagelike secretion near the base of a few of the pearl glands present on the stem.

DISCUSSION

Chlorotic leaf distortion appears to result from a unique relationship between *F. lateritium* and the sweetpotato plant. The fungus induces a severe chlorosis without entering the plant tissue. Symptoms develop systemically probably because the fungus grows on the surface of or very near the apical meristem and

thus grows along with the developing shoot.

Portions of the shoot most extensively colonized by the fungus were also completely or partially covered with a layer of an unidentified mucilagelike material. This material is probably produced by the sweetpotato plant as similar material was observed on healthy mericlones in the apparent absence of the fungus. Although mucilage hairs have been listed as occurring on members of the Convolvulaceae (7), there is a lack of literature on the pearl glands on sweetpotato. Generally, plant pearl glands are considered to be secretory structures (5). On other plants, glands of various types secrete substances including enzymes, polysaccharide slimes, sugars, salts, and secondary metabolites, such as waxes, terpenoid resins, and oils (9). It is possible that the mucilagelike material on sweetpotato is secreted by the pearl glands. This is supported by two observations: 1) pearl glands were most densely distributed on the young portions of the shoot that were also covered with the mucilagelike material, with the pearl glands forming a nearly continuous layer on the surface of leaf primordia and young, folded leaves; and 2) on older portions of the shoot, mucilagelike material was seldom observed
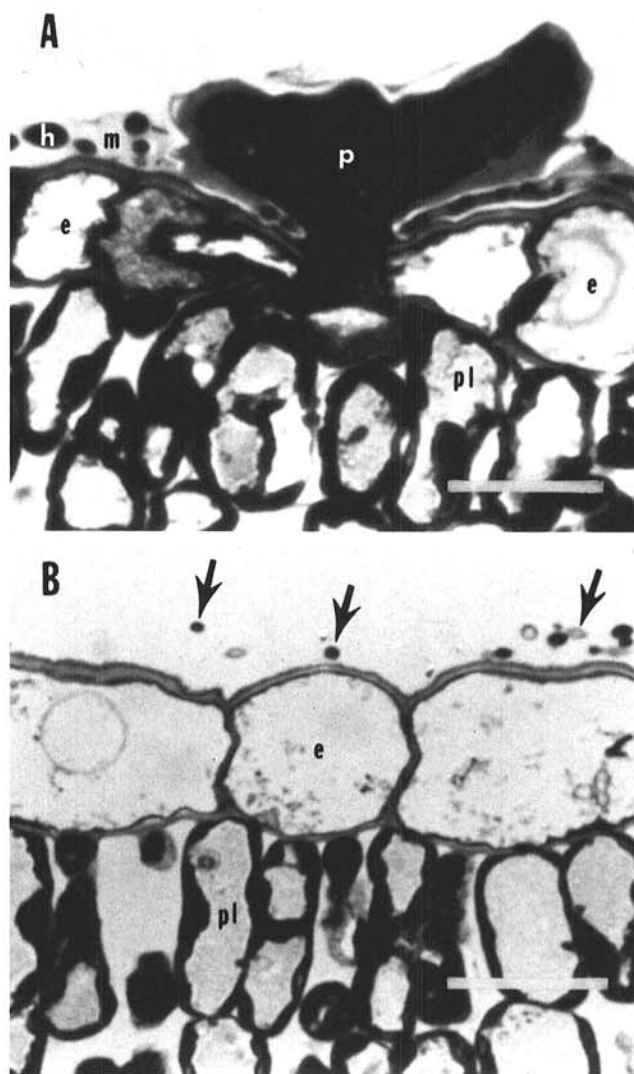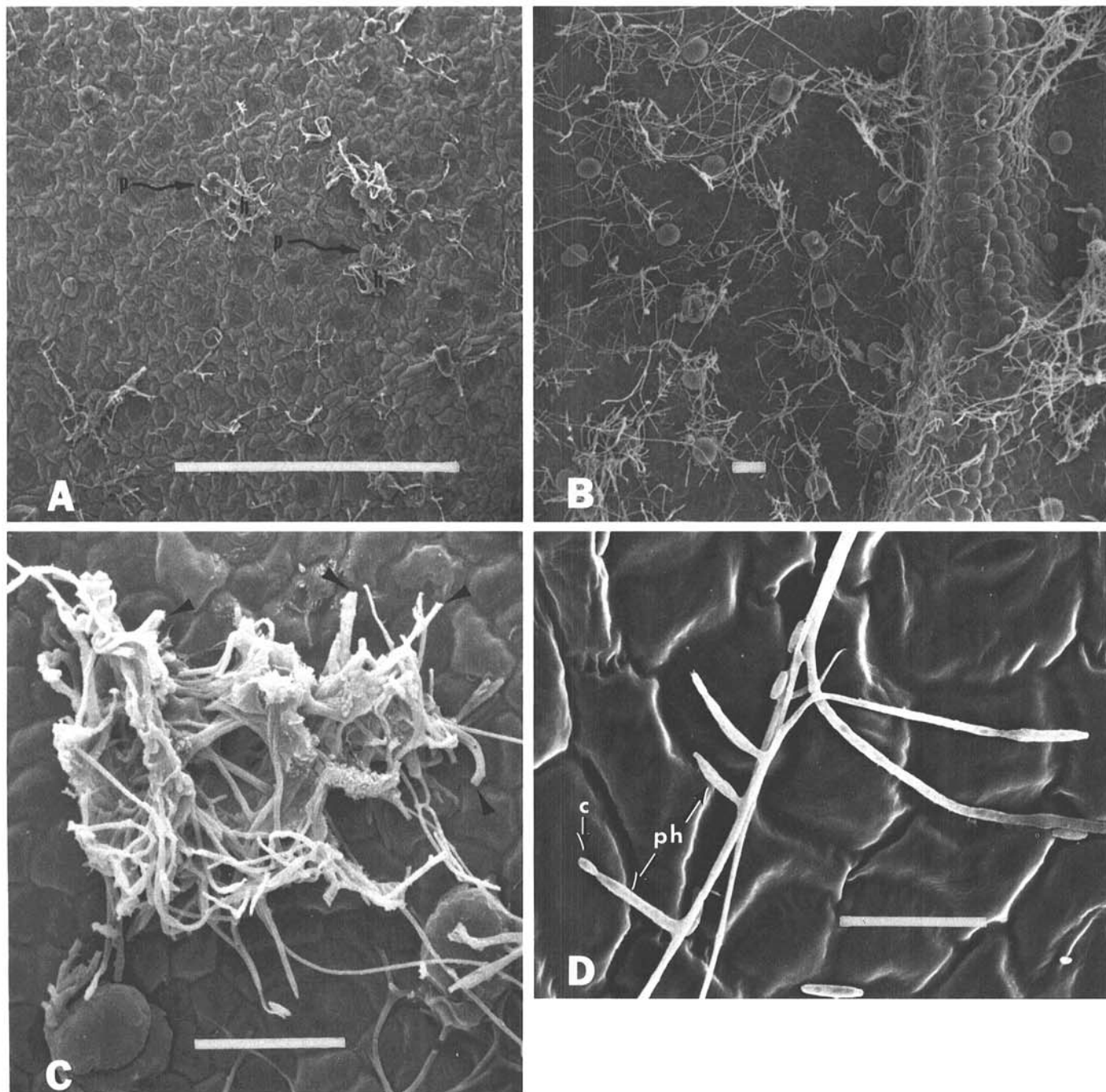
Fig. 5. Light micrographs of cross sections of unfolded leaves (+2) from a sweetpotato vine that had recovered from chlorotic leaf distortion. Hyphae are present on the surface of the leaves but were not observed within the leaves. **A,** A pearl gland (p) is surrounded by mucilagelike material (m), within which are hyphae (h) (e = epidermal cell, pl = palisade mesophyll cell) (bar = 20 μm). **B,** Hyphae (arrows) are present on the surface of the leaf, but no mucilagelike material is evident (e = epidermal cell, pl = palisade mesophyll cell) (bar = 20 μm).

and was primarily located around the base of the widely scattered pearl glands.

Many functions have been suggested for mucilage secretions in plants including reduction of transpiration and protection against radiation or herbivory (7). The roles of pearl glands and the mucilagelike material on sweetpotato are unknown. However, it appears that the mucilagelike material may provide a unique niche for *F. lateritium*. It may afford the fungus protection from various environmental factors, such as desiccation and radiation. It may also account for the fact that the fungus was readily isolated from surface-disinfested tissue even though all available histological evidence indicates that the fungus does not enter the plant. This may have practical implications, as treating plants with fungicides that are toxic to *F. lateritium* in vitro has had little effect on chlorotic leaf distortion development (Clark, *unpublished data*). It may be that the mucilagelike material also prevents direct contact between the fungicides and the fungus. A mucilage produced by the fungus *Colletotrichum graminicola* is thought to protect spores of this fungus from phenolic compounds leaching from within the host plant (11). *F. lateritium* probably is also protected while it is located between halves of the folded immature sweetpotato leaves and possibly, to a lesser extent, at the margins of leaves on cultivars with numerous trichomes.

Fig. 6. Scanning electron micrographs of unfolded leaves of sweetpotato. **A,** A young leaf (+1) from a sweetpotato vine that had recovered from chlorotic leaf distortion. Hyphae (h) were present in clumps, often near the base of pearl glands (p) (bar = 500 μm). **B,** An unfolded leaf (+2) from a sweetpotato vine with chlorotic leaf distortion. Hyphae appeared intact at the tips and phialides were present (bar = 50 μm). **C,** A clump of hyphae on the surface of an unfolded leaf (+2) from a sweetpotato vine that had recovered from chlorotic leaf distortion. Some ends of hyphae (arrows) appear broken (bar = 50 μm). **D,** The surface of an expanded leaf (+2) with chlorotic leaf distortion symptoms. Several phialides (ph) are present with developing conidia (c) (bar = 20 μm).

Observations of chlorotic leaf distortion development over several years have indicated that severe symptoms develop only after periods of sunny weather (1), but the role of sunlight in this stimulation is unknown. Number of pearl glands or amount of mucilagelike material on sweetpotato has not yet been quantified. However, it appeared in this study that more mucilagelike material was present and covered a greater portion of the shoot on plants that had developed severe chlorotic leaf distortion compared to plants in remission. In *Althea rosea*, more mucilage cells were found on plants grown in sunny sites (6). Thus, intense sunlight may stimulate increased production of pearl glands and/or the mucilagelike material, which may in turn afford the fungus greater protection for development on the shoot surface.

That *F. lateritium* can induce a severe chlorosis on sweetpotato leaves without entering the plant warrants further study. It is

possible that the fungus produces a toxin, which can diffuse into the leaf and cause chlorosis. It has been suggested that some fungi are exopathogens (15), inducing toxic effects in their suscepts without entering the suscept, or telepathogens (14), inducing effects without direct contact with the suscept or as a prelude to entering the host. Albinism in citrus and cotton seedlings has been attributed to toxins produced by *Alternaria tenuis* (6,13) or *Aspergillus flavus* (4). These fungi grow on the seed coats but do not enter the seedlings. Brown spot on Emperor mandarin citrus is induced by *Alternaria citri* without entering the leaf by elaboration of a toxin (12). Chlorotic leaf distortion differs from these diseases, however, in that it involves an extended exopathogenic relationship. While there is ample evidence that the relationship between *F. lateritium* and the sweet potato vine system is predominantly exopathogenic, it is not possible to prove that the fungus never enters the plant. It is conceivable that some environmental conditions may favor penetration of some part of the extensive vine system of the sweet potato by the fungus.

Some toxins produced by plant pathogenic fungi are known to be light activated (3). Thus, an alternative hypothesis for the role of sunlight in chlorotic leaf distortion development would be that light may be required to activate a toxin.

Chlorotic leaf distortion is also unique because of the recovery phenomenon. Whole plants recover during periods of nonconducive weather, but individual leaves also recover as they expand and mature even during conducive weather (1). Recovery of individual leaves might be explained by the observation that the fungus is epiphytic on the plant and appears to stop growing as leaves expand, mature, and no longer have a mucilagelike covering. It may be that the fungus is inactive on older leaves and thus no longer able to sustain production of the putative chlorosis-inducing substance.

Fig. 7. Scanning electron micrographs of axillary buds from nodes (+6) of sweetpotato vines with chlorotic leaf distortion. **A,** Numerous pearl glands (arrows) are present and a mucilagelike material is evident between and covering pearl glands in some areas (bar = 500 μm). **B,** Leaf tips in an axillary bud showing mucilagelike material (m) and hyphae (arrows) on and within the material (bar = 50 μm).

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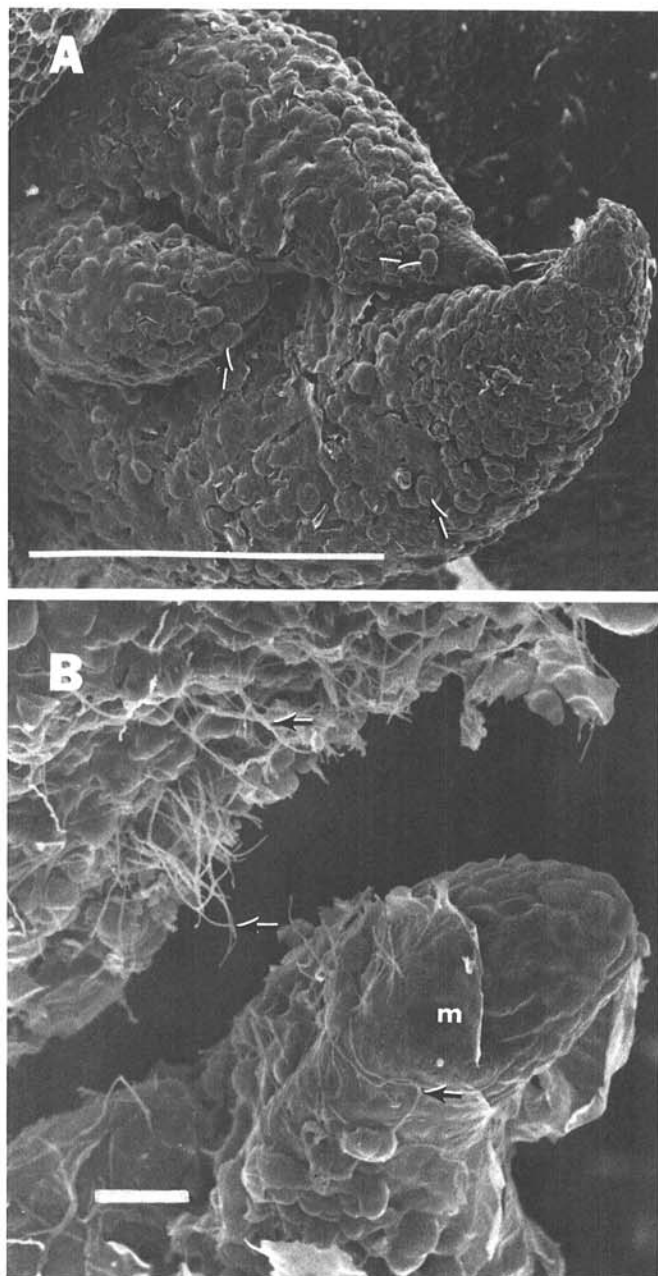15. Woltz, S. S. 1978. Nonparasitic plant pathogens. Annu. Rev. Phytopathol. 16:403-430.