Cytology and Histology

Histological Development of *Sphacelotheca reiliana* on *Zea mays*

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ABSTRACT

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Shoot apices of corn seedlings infected by *Sphacelotheca reiliana* are colonized in the first 6 wk after planting. This results in sorus development in mature plants in place of ears and tassels. A young sorus has a peridium consisting of a dense layer of hyphae beneath a two- to three-cell layer of host tissue. Beneath the peridium, hyphae grow abundantly between and within columns of highly vascularized host tissue. As the sorus matures the hyphae form aggregates of intercalary teliospores within a gelatinous matrix. Newly formed teliospores are covered with small rounded processes which continue to elongate as the spore enlarges, and a second group of processes begin to develop as the spore reaches maturity. Partitioning hyphae grow between the teliospore aggregates throughout their development. As the sorus emerges, the peridium deliquesces, exposing a mass of teliospores enmeshed in vascular strands of the host.

Additional key words: head smut.

Head smut is a systemic disease caused in corn, *Zea mays* L., by *Sphacelotheca reiliana* (Kühn) Clint. [*Sporisorium reiliana* (Kühn) Langdon and Fullerton]. Plants are infected by soilborne teliospores during emergence or as seedlings. The infection process and events leading to sorus development have not been investigated. Mycelial distribution and sorus development of *S. reiliana* on *Sorghum bicolor* (8,11) and *Sorghum vulgare* var. *sudanense* (3) and of *Sphacelotheca sorghi* on broom corn (*Andropogon sorghum* var. *technicus* [1]) and *Sorghum leiocladium* (2,5) have been partly described. Few of these studies have utilized modern cytological techniques that provide increased resolution and clarity. Often description supplemented with hand drawings is the only documentation given.

The purpose of this investigation was to describe sorus development of *Sphacelotheca reiliana* on *Z. mays*.

MATERIALS AND METHODS

A susceptible corn hybrid, P 3978, was planted in a "greenhouse-mix" soil that had been infested with teliospores of *S. reiliana*. Plants were grown at 22 ±5 C for 6–12 wk, and shoot apices and intact sori were dissected at various stages of development.

Light microscopy. Material for sectioning was cut into 3 × 3 mm pieces and fixed in cold 2.5% glutaraldehyde in 0.05 M phosphate buffer for 24 hr, washed in the same buffer, and dehydrated in an acetone series. Tissue pieces were embedded in Spurr's low

viscosity resin (9). Sections 0.5–1.0 μm thick were cut with glass knives on an LKB ultramicrotome, fixed to glass slides, and stained with toluidine blue.

Shoot apices were dissected from 24 6-wk-old corn seedlings. Twelve of these seedlings had chlorotic spots on the leaves indicating infection by *S. reiliana* (7), and 12 had no chlorotic spots. The apices were hand sectioned, cleared in lactic acid at 50 C for 48 hr, and stained with toluidine blue in lactophenol.

Scanning electron microscopy. Tissue pieces were fixed as above, postfixed in osmium tetroxide for 1 hr, transferred to 1.0% thiocarbohydrazide (4) in buffer for 1 hr, returned to osmium for 30 min, washed in buffer, dehydrated in an acetone series, dried in a critical-point dryer, mounted on specimen stubs, and coated with
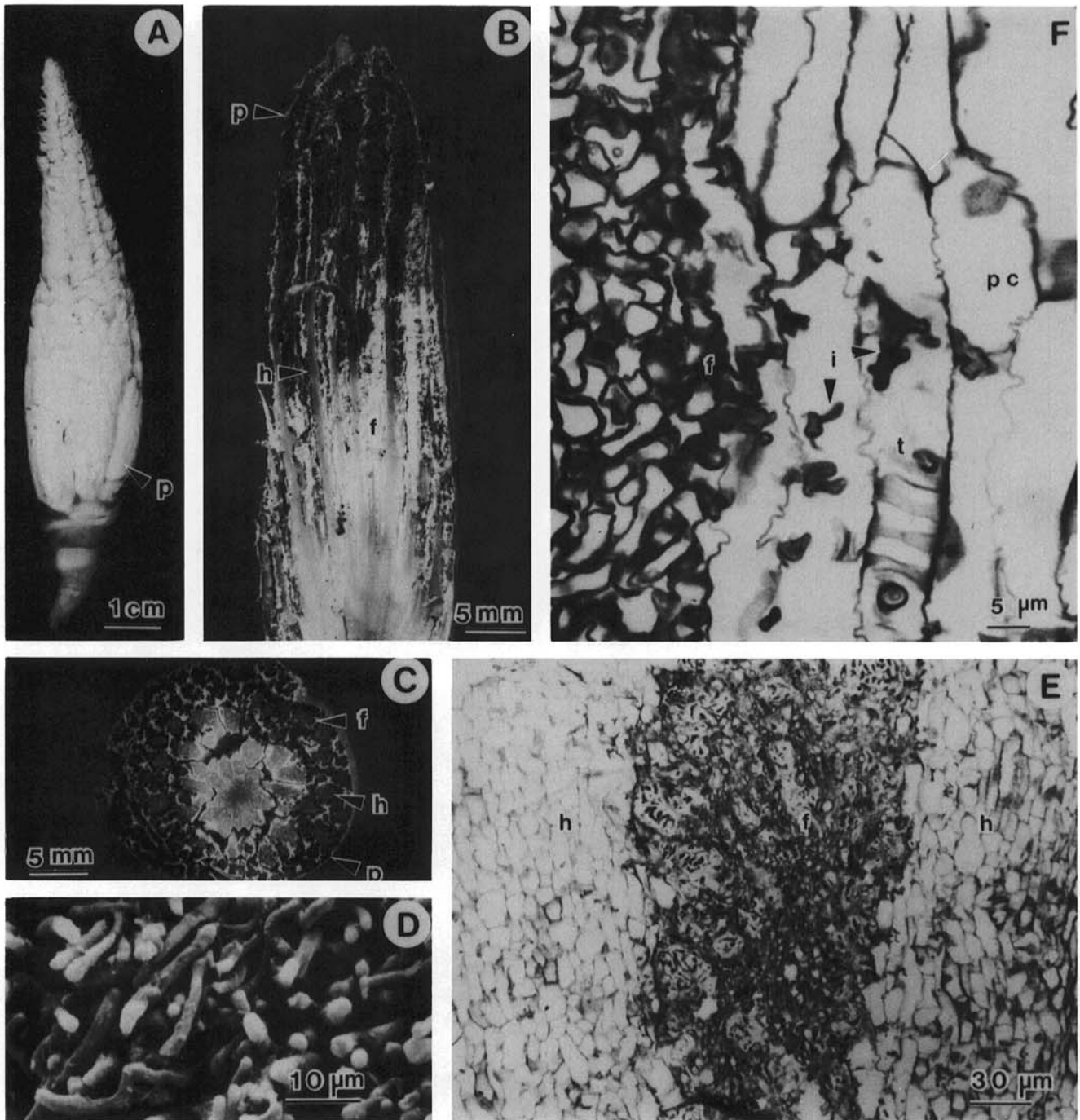

Fig. 1. Surface and sectioned views and light and scanning electron micrographs of young sori of *Sphacelotheca reiliana* on *Zea mays*. **A,** Surface view of an immature sorus with intact peridium. **B and C,** Longitudinal and cross sections, respectively, of a sorus showing peridial layer, host tissues, and mycelium. **D,** Scanning electron micrograph of immature hyphae between host tissue. **E,** Light micrograph showing juxtaposition of host and mycelium. **F,** Light micrograph of border between host and mycelium showing intracellular and intercellular hyphae (i) in tracheary element (t) and parenchyma cells (pc). Legend: p = peridium, h = host tissue, and f = mycelium.
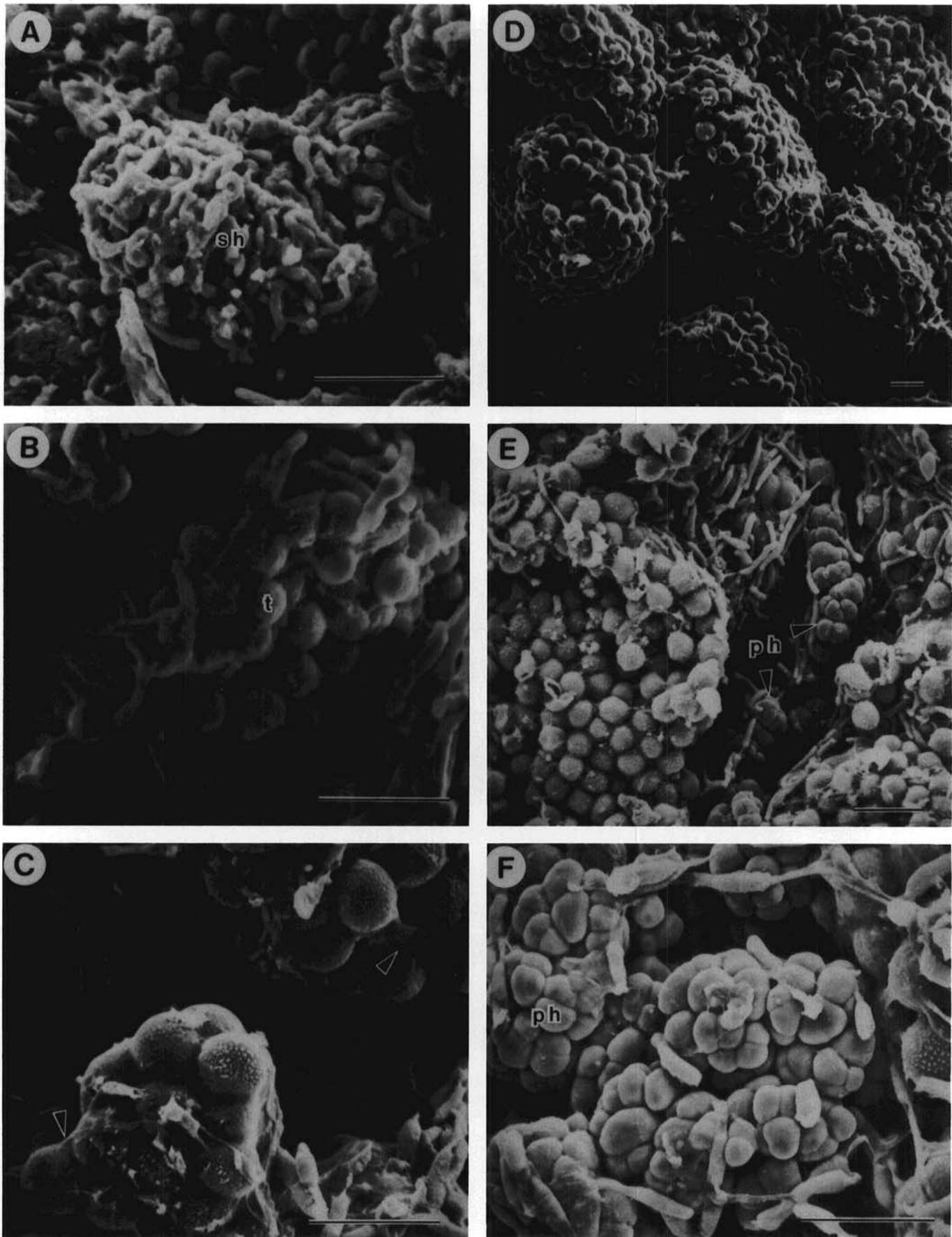
Fig. 2. Scanning electron micrographs of sporogenous hyphae, teliospores, and partitioning hyphae. **A,** Massed sporogenous hyphae (sh). **B,** Newly formed teliospores (t) intertwined with sporogenous hyphae. **C,** Newly formed teliospores in a gelatinous matrix (arrow). **D,** Enlarged spore balls. **E,** Partitioning hyphae (ph) growing between masses of teliospores. **F,** Close-up of partitioning hyphae. Scale bars = 20 μm.

carbon. Specimens were viewed with a Phillips scanning electron microscope.

RESULTS

Immature sori have an intact peridium (Fig. 1A), and consist of elongating columns of host vascular and parenchymal tissue with hyphae 2.0–3.2 μm in diameter growing concurrently between them (Fig. 1B to E). Hyphae slightly smaller in diameter (1.0 μm) are found in and among the outer layer of host cells in parenchymal and vascular elements (Fig. 1F). As the sorus matures, these hyphae

Fig. 3. Light micrographs of cross sections of sporogenous hyphae and teliospore masses. **A,** Sporogenous hyphal masses (sh). **B,** Newly formed teliospores (t) intermixed with sporogenous hyphae and an adjacent sporogenous hyphal mass not yet forming teliospores. **C,** Partitioning hyphae (ph) growing between masses of teliospores (t).

between the vascular columns intertwine and form globose to subglobose sporogenous hyphal masses 25–50 μm × 20–28 μm (Figs. 2A and 3A). Each hyphal mass eventually forms a ball of intercalary spores (Figs. 2B and 3B) which appeared to be encased in a gelatinous matrix (Fig. 2C). The individual spores increase in size causing the mass to enlarge to 75–100 μm in diameter (Figs. 2D and 3C). These spore balls are separated by the growth of nonsporogenous hyphae which are termed partitioning hyphae (Figs. 2E to F, and 3C).

When first formed, spores are 2–4 μm in diameter with a dense uniform distribution of small rounded processes (Fig. 4A). As the spores enlarge to 10–11 μm, the processes become better defined possibly due to the dissolution of the gelatinous matrix (Fig. 4B). Mature spores attain a maximum diameter of 12–15 μm and develop a second batch of processes. The larger processes achieve a maximum length of 0.5 μm, while the smaller group develops to 0.2 μm (Fig. 4C). Spore formation proceeds from the sorus apex downward and from the center of the intervascular area toward the vascular bundles and the peridium.

The peridium enclosing the young sorus consists of an outer two- to three-cell layer of host tissue, sometimes having trichomes on the outer surface, and an inner layer of fungal hyphae 3.2–4.0 μm in diameter (Fig. 5A to C). As the sorus matures, the peridium deliquesces (Fig. 5D) and is totally absent at the time of sorus emergence. Peridial hyphae do not participate in spore development, and a sharp boundary can be observed where partitioning hyphae separate peridial from sporogenous hyphae (Fig. 6).

Mycelium of *S. reiliana* grows throughout the shoot apices of 6-wk-old corn seedlings with chlorotic flecks on leaves (Fig. 7A). The distribution of hyphae in these tissues varies from total colonization to an occasional hypha. No hyphae are found in the shoot apices of seedlings without chlorotic spots on their leaves.

Nodes above and below the site of sorus development also contain a few hyphae (Fig. 7B) but internodal parenchyma tissue is free from hyphae.

Sorus development precludes normal development of ears and or tassels. Although variation in sorus development has been observed (10), basic sorus anatomy is similar whether it is replacing a male or female inflorescence.

DISCUSSION

The results of my observations of sorus development and spore ontogeny are similar to those of Clinton (1), Wilson and Frederiksen (11), Fullerton (2) and Langdon and Fullerton (5) who studied other systemic smut diseases. These workers suggested several names for the various types of hyphae in a sorus. Peridial, reproductive, and vegetative hyphae are the terms suggested by Wilson and Frederiksen (11) for hyphae that form the peridium, the spore balls and those within host cells, respectively. Fullerton (2) and Langdon and Fullerton (5) used the terms "sporogenous hyphae" for hyphae that form spores, "partitioning hyphae" for those that grow between spore balls, and "nonsporogenous" or "intercellular hyphae" for all remaining hyphae in the sorus. These terms have been used in this paper where the author judged them to be appropriate. In earlier studies, some authors used the term "haustoria" to describe the intercellular hyphae of some smut fungi (11). In more recent investigations (2), however, these hyphae have not been termed haustoria based on the lesser degree of branching compared to fungi traditionally considered to form haustoria.

Ledin (6) proposed a tunica-corpus theory of development of the vegetative shoot apex of *Z. mays*. Using this as a model, it can be speculated that colonization of the subtunica layer or the rib meristem of the shoot apex results in distribution of hyphae up to leaf and axillary buds (ear primordia) during the first several weeks of plant growth. Midribs and blades of leaves of infected seedlings often contain hyphae that cause chlorotic spots 1–2 mm in diameter. These have been described by Matyac and Kommedahl (7). Occasionally, sori develop along the midrib of these leaves, confirming earlier observations (10).
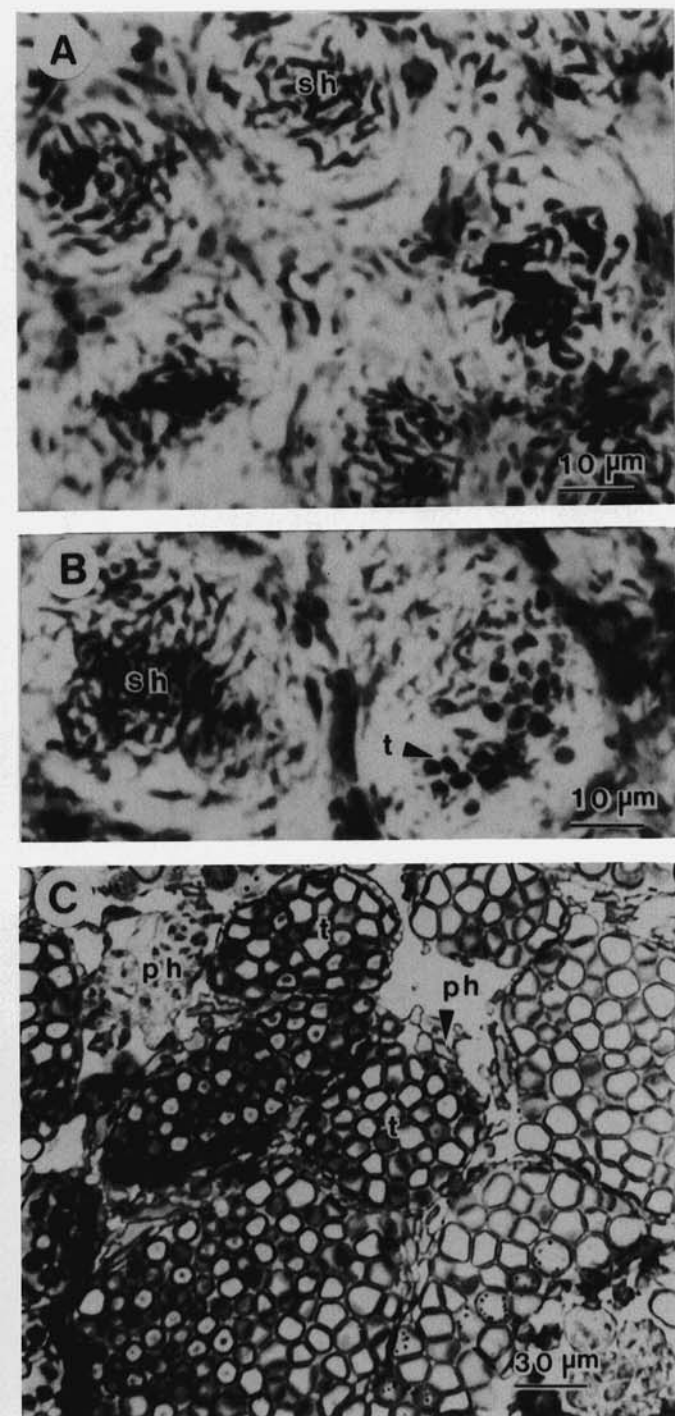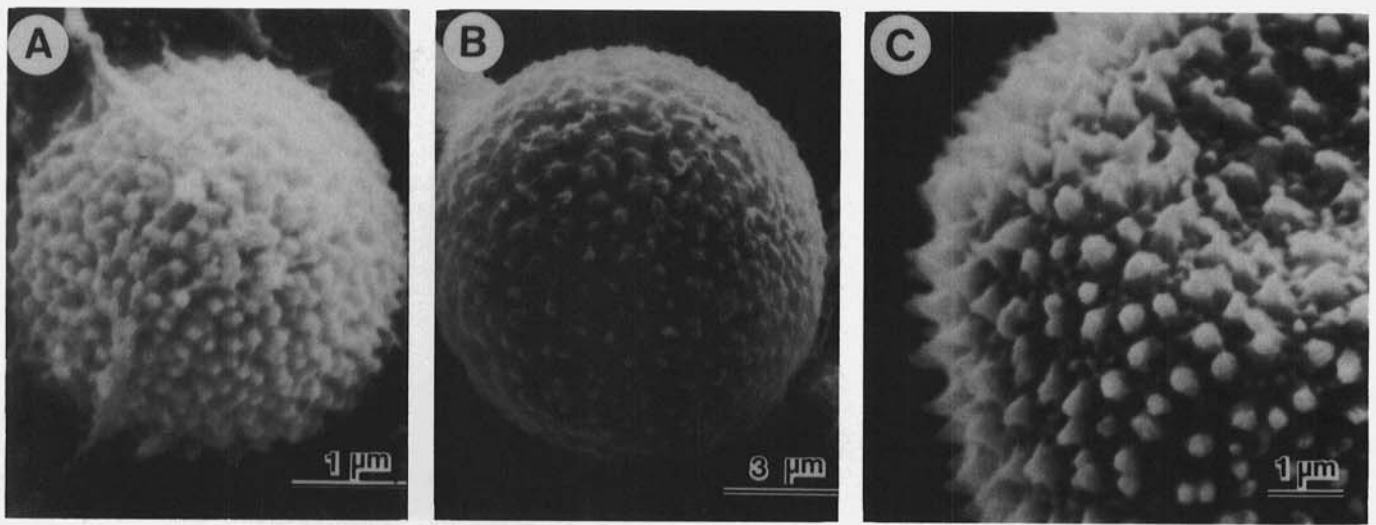
Fig 4. Scanning electron micrographs of teliospore ontogeny. **A,** Newly formed teliospore densely covered with rounded processes. **B,** Partially mature teliospore with well defined and spaced processes. **C,** Mature teliospore with short rounded processes and longer pointed processes.
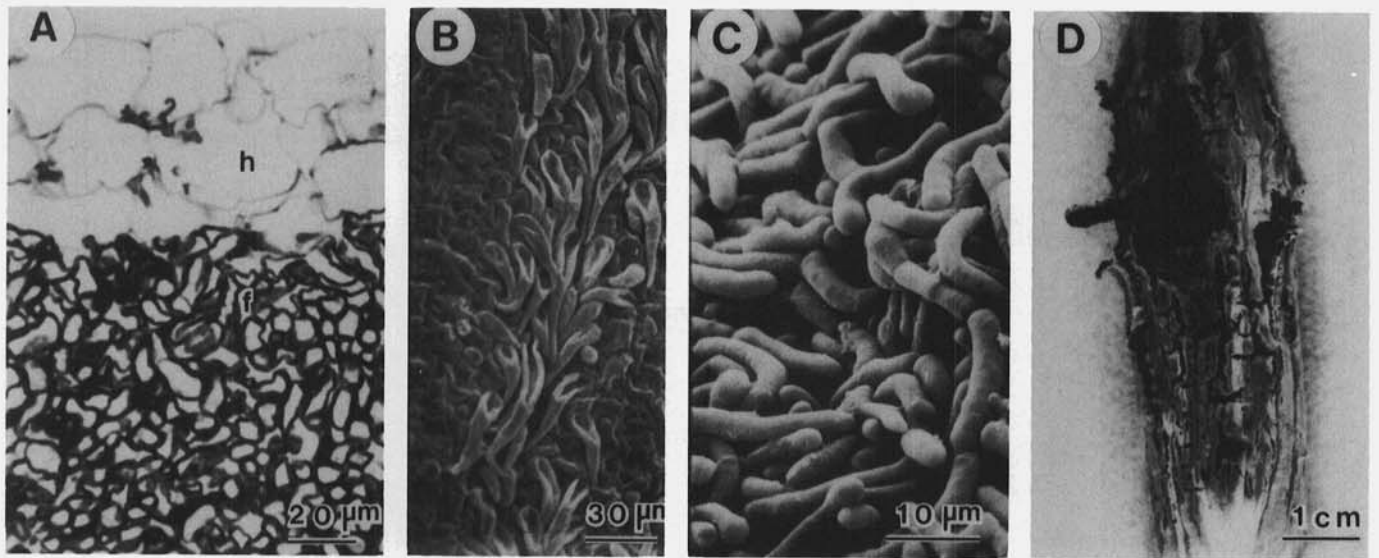

Fig. 5. Surface view and light and scanning electron micrograph of host and fungal contribution to sorus peridium. **A,** Light micrograph of cross section of peridium with an outer host cell layer (h) and an inner fungal layer (f). **B,** Scanning electron micrograph of trichomes on the outer surface of the peridium. **C,** Scanning electron micrograph of peridial hyphae. **D,** Surface view of a preemergent sorus with a deliquescing peridium.
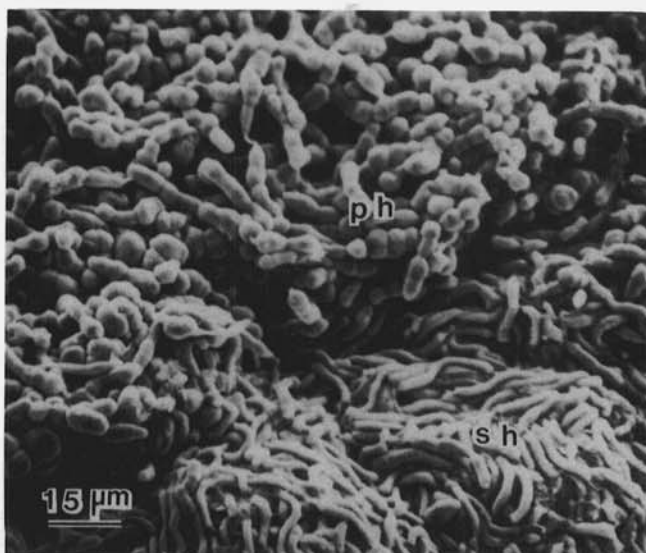
Fig. 6. Scanning electron micrograph of the interface between masses of sporogenous hyphae (sh) and partitioning hyphae (ph).
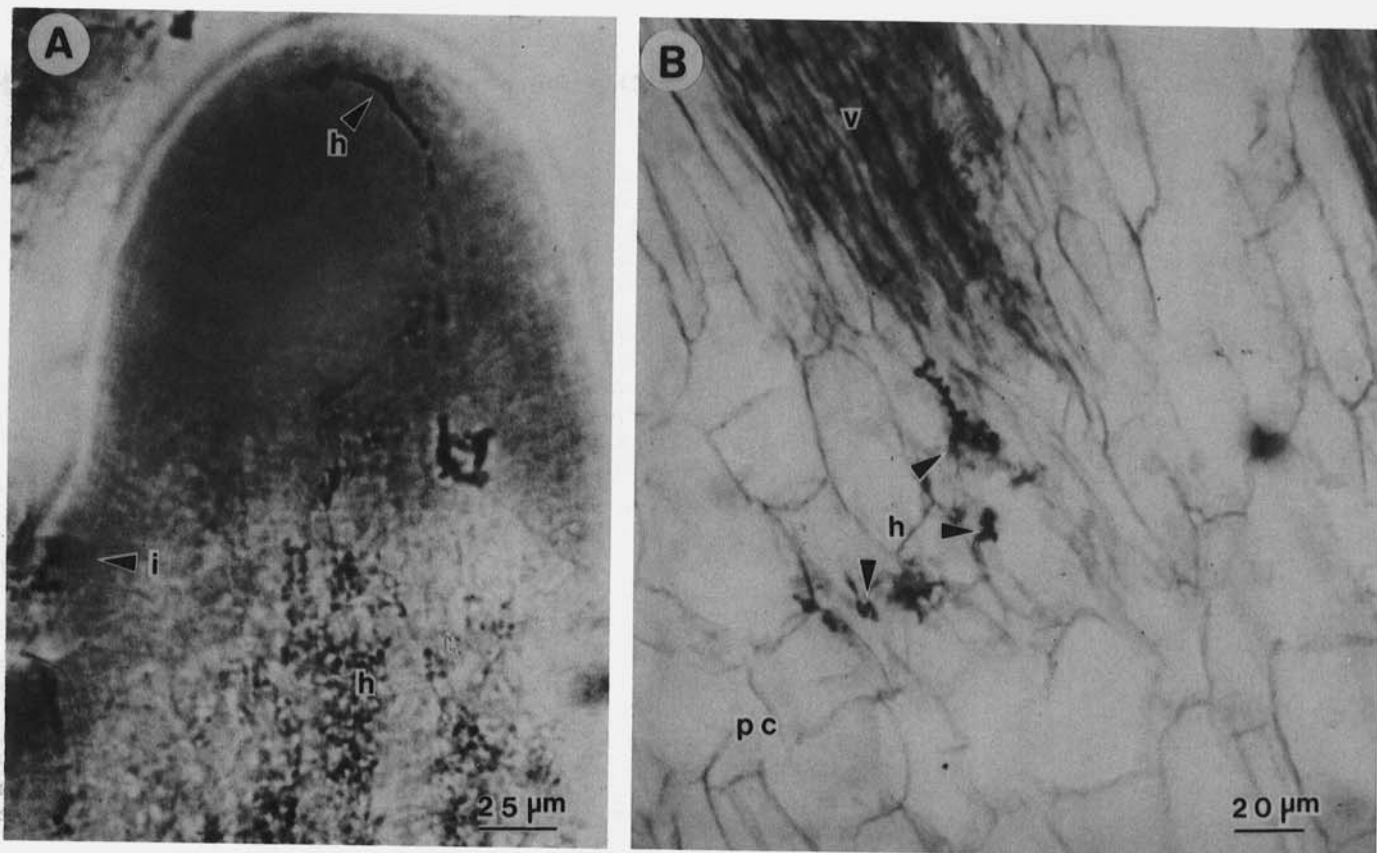
Fig. 7. Light micrograph of hyphae of *Sphacelotheca reiliana* in a vegetative shoot apex and node of *Zea mays*. **A,** Shoot apex heavily colonized with hyphae (h) and the site of an immature axillary node (i). **B,** Node of *Z. mays* containing hyphae (h) in parenchyma cells (pc) adjacent to vascular tissue (v).

The presence of hyphae in the axillary buds results in the development of a sorus in place of a normal ear. Colonization of the shoot apex at the time it changes to a reproductive apex (beginning of tassel formation), results in sorus development in place of a tassel.

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