Mode of Penetration of Sycamore Leaves by *Gloeosporium platani*

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ABSTRACT

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The chronology of the infection process of the sycamore anthracnose fungus on seedling sycamore leaves was investigated by using scanning and transmission electron microscopy. Under laboratory conditions, spore germination on the leaf surface exceeded 90% after 6 hr of incubation. Growth on the surface was vigorous, of short duration, and random. Almost without exception, termination of germ tube growth occurred within 6 hr after inoculation and was characterized by development of prominent appressoria firmly attached to the leaf surface. Direct penetration of leaf surfaces by infection pegs emanating from appressoria was observed frequently.

Sycamore (*Platanus occidentalis* L.), a fast growing, easily propagated tree, produces high yields of quality pulp and straight, well-formed logs for commercial veneer. This species grows faster and on more sites, with less intensive management, than most hardwood species (2). Since it is, however, a minor component in natural eastern hardwood stands, establishment of extensive plantations is required if the potential of this species is to be realized.

Of growing concern among forest pathologists is the lack of screening for resistance to the sycamore anthracnose fungus, *Gnomonia platani* Kleb. [= *Gloeosporium platani* (Mont.) Oud]. This fungus occurs throughout the range of sycamore (7) and has caused considerable damage even where monoculture was not being practiced (T. Filer, *personal communication*). Sycamore anthracnose occurred throughout the state of Illinois in 1952, developed into an epiphytotic in 1954 (1), and was severe again in 1961 (12). This disease resulted in 75% defoliation of sycamore trees in some areas of the Mississippi-Yazoo Delta during the spring of 1970 where over 33% of the sycamore trees 30.5 cm (12 in) dbh (diameter-at-breast-height) were infected (10). A disease survey of sycamore plantations in the South conducted in 1977 by State and Private Forestry, U.S. Forest Service, reported that 45% of the trees in plantations over 9 yr of age had top dieback and 100% showed various levels of defoliation (T. Filer, *personal communication).*

Investigations of environmental requirements for penetration and infection of sycamore by *G. platani* are presently being conducted. Studies of conidial germination, growth habit of *G. platani* on sycamore leaf surfaces, and mode of penetration and colonization are reported here.

MATERIALS AND METHODS

The sycamore anthracnose fungus was grown in petri plates on sycamore medium (40 g dried, ground sycamore leaves, 10 g agar, 950 ml distilled water) for 14 days at 32 C. Conidia were washed from agar plates with distilled water, separated from mycelial fragments by filtration through Whatman No. 1 filter paper under vacuum, and collected in a filter flask.

Sycamore seedlings were grown in hydrated peat pellets for 90 days at 32 C. Each plant (7–15 cm tall) was inoculated by atomizing 2 ml of sterile, distilled water containing 10,000 conidia per milliliter onto leaf surfaces and incubated for 24–48 hr at 22–23 C.

Tissue samples 1-mm square were cut from inoculated and uninoculated leaves, fixed in 3% glutaraldehyde at 4 C for 4 hr, and rinsed 4 hr in Millonig's phosphate buffer (4). The samples were postfixed in 2% osmium tetroxide for 4 hr at 4 C, dehydrated in a graded acetone series, and embedded in EPON-912. Thin sections were stained in saturated uranyl acetate for 20 min at 26 C followed by Reynolds' lead citrate for 1 min (13) and examined with a transmission electron microscope.

Additional seedlings were grown and inoculated as previously described. Tissue samples 10-mm square were cut from inoculated leaves after 3, 6, 12, 24, and 48 hr, fixed in 2% osmium tetroxide at 4 C for 24 hr, dehydrated in a graded ethyl alcohol series, dried in a critical-point drier, mounted on specimen studs, and coated with palladium-gold. Specimens were examined and micrographs were taken with a scanning electron microscope.

RESULTS

Observations 3 hr after inoculation. Ten percent of the *G. platani* conidia observed on the surface of sycamore leaves had germinated within 3 hr after inoculation. Germ tube growth was random; appressoria had not formed (Fig. 1A).

Observations 6 hr after inoculation. Conidial germination approached 100% 6 hr after inoculation. Three characteristic germ tube growth-types were observed: (i) single, short germ tubes (0.25 to $< 1 \times$ spore length) terminating in an appressorium (Fig. 1E, arrow), (ii) single, long germ tubes ($1 \times$ spore length) terminated by an appressorium (Fig. 1B), and (iii) multiple germ tubes from a single conidium (Fig. 1C). Germ tube growth was random and could not be associated with any particular topographical feature of the sycamore leaf surface. We saw no indication of differential growth rate or orientation of germ tubes between veinal and interveinal tissues, or attraction to stomatal openings. On the contrary, growth of germ tubes around or away from stomata occurred frequently (Fig. 1D), even in those instances where conidial germination occurred adjacent to stomata (Fig. 1E). Penetration through stomatal openings was not observed. Under conditions previously described, germ tube growth ceased within 6 hr after inoculation and a large, round, firmly anchored appressorium formed at the tip of almost every germ tube (Figs. 1E, 2A, 2B).

Observations 24 hr after inoculation. The initial step in the penetration process occurred as a bulging of the appressorium at the point of contact with the leaf surface (Fig. 3A, arrow). This was accompanied by development of a funnel-shaped, electron-dense structure (fundibulum) within the cytoplasm of the appressorium

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near the host-parasite interface (Fig. 3A). That the fundibulum is an extension of the appressorial cell wall is clearly evident in Fig. 3B. Intimate contact between appressorial contents and the host surface was accomplished via this structure.

Observations 48 hr after inoculation. Actual penetration of the host surface was observed by 48 hr after inoculation as growth between epidermal cells (intercellular) (Fig. 4A) or directly into an epidermal cell after a period of subcuticular growth (subcuticular/intracellular) (Fig. 4B). Enzymatic digestion of intercellular components appears to occur in advance of the hyphal

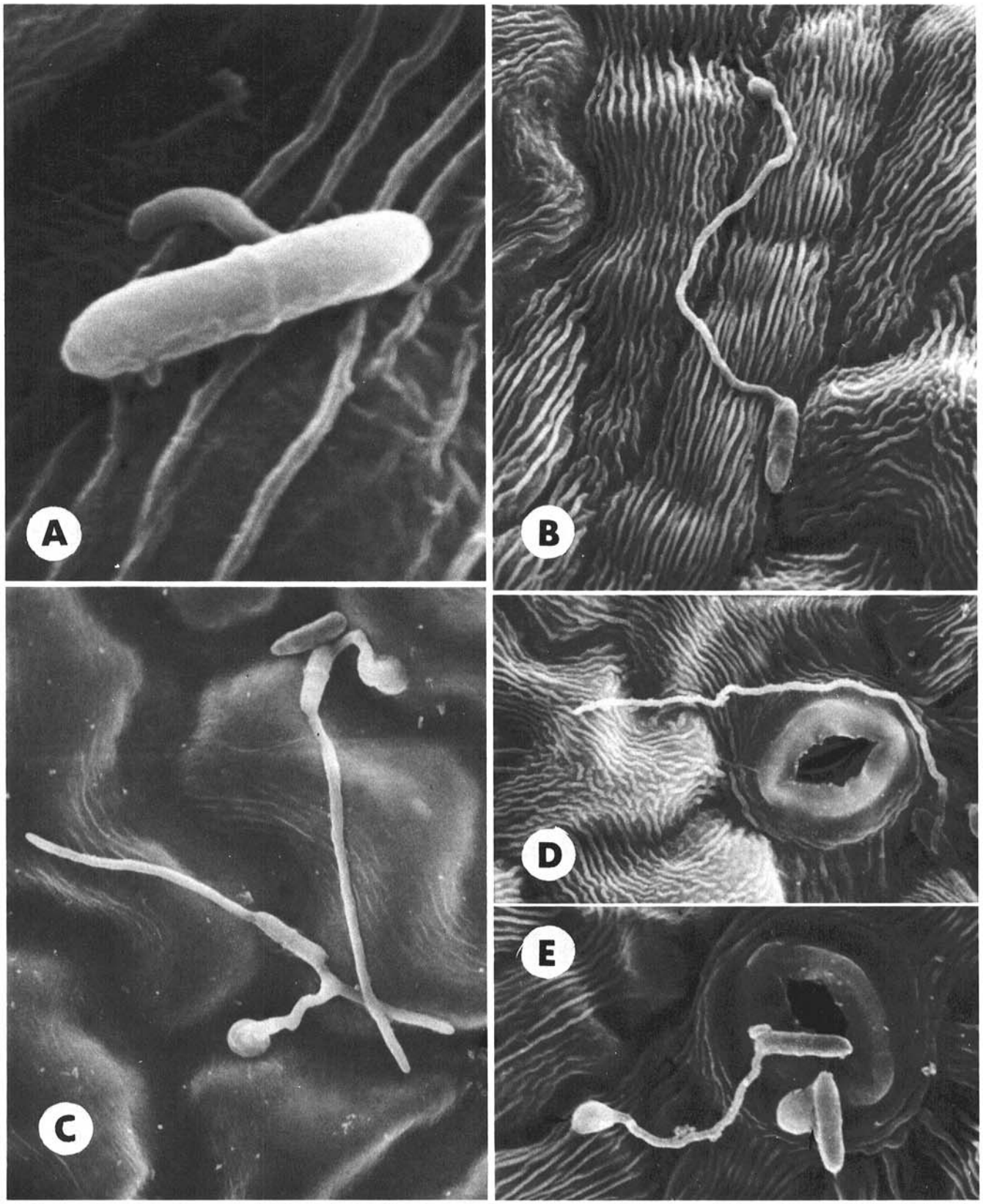

Fig. 1. Germ tube growth and appressorium formation by *Gloeosporium platani* on sycamore leaf surfaces; **A,** Germ tube growth 3 hr after inoculation (×6,440); **B,** Growth of germ tube after 6 hr with appressorium present (× 1,610); **C,** Multiple germ tubes from individual conidia (× 1,265); **D,** Growth of germ tube around and away from stomatal openings (× 1,075); and **E,** Conidial germination adjacent to and growth away from a stomate (× 1,466).

tip (Fig. 4A). Membrane disruption is suggested by membrane fragments in cells adjacent to the area of penetration (Fig. 4A, arrows). The subcuticular/intracellular method of penetration occurred initially as a movement of the appressorial contents via the fundibulum through a small pore (Fig. 4B, arrow) in the host cuticle. This was followed by lateral growth of the fungus between the cuticle and the epidermal cell wall and then into the cell interior through a pore somewhat larger in diameter than the cuticular pore. The appearance of the host cytoplasm indicated that necrosis of the host cell had occurred (Fig. 4B).

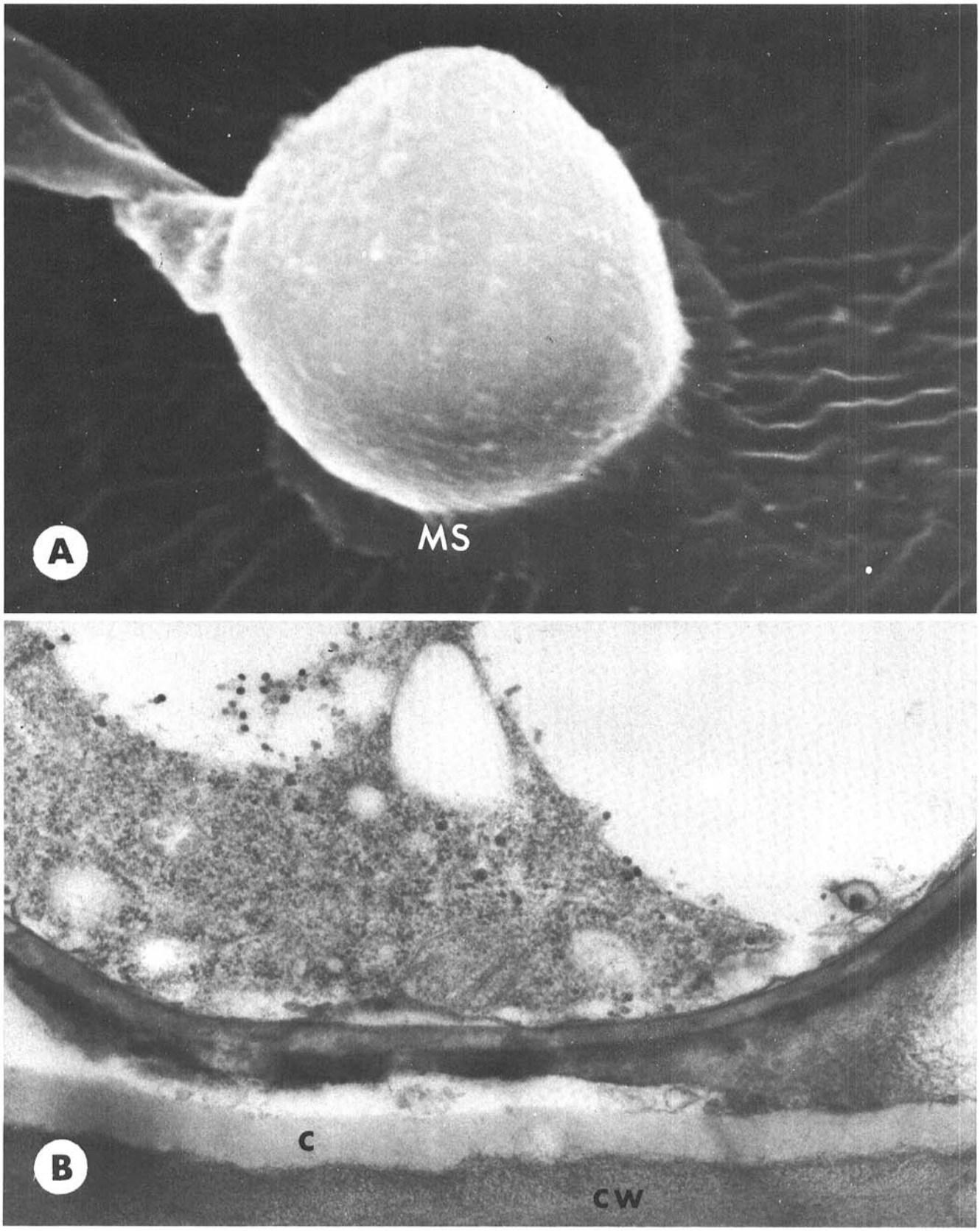

Fig. 2. Scanning and transmission electron micrographs of appressoria of *Gloeosporium platani* on sycamore leaves; **A,** Mucilaginous secretion (MS) at base of appressorium (× 15,640); and **B,** Cross-section of appressorium showing base of appressorium above cuticle (C) and cell wall (CW) (× 52,440).

DISCUSSION

The establishment of sycamore plantations in the southeastern USA over the past 4–6 yr has increased the potential for sycamore anthracnose epiphytotics. Planting stock has not been screened for resistance to the sycamore anthracnose fungus primarily because of economics of the species has not justified the long range research support required. Consequently, little is known about the biology of the host-parasite association. Information about conidial germination, subsequent penetration and colonization of the host, and the environmental factors affecting disease development is needed for a successful screening program. The data presented

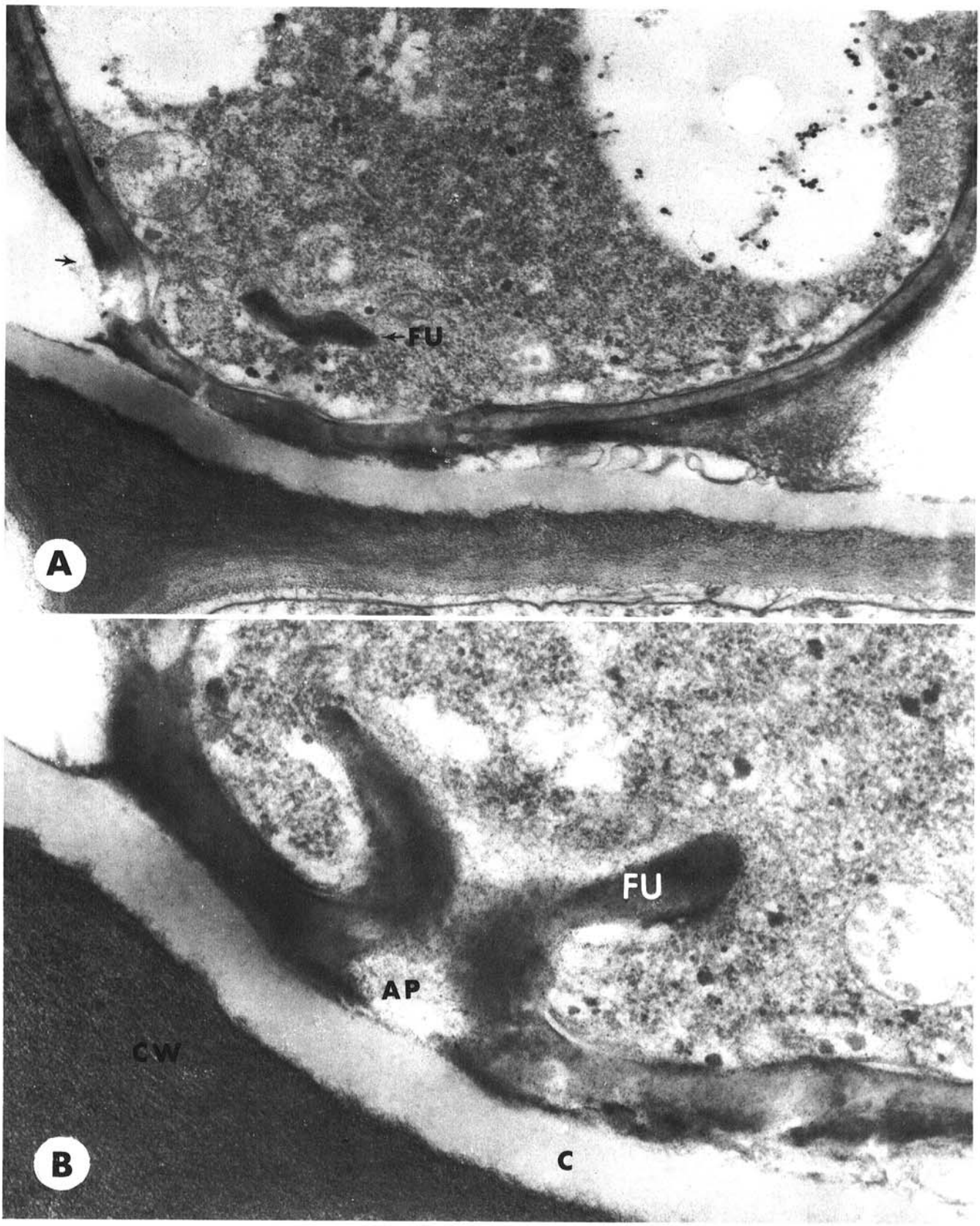

Fig. 3. Cross-sections of appressoria of *Gloeosporium platani* on sycamore leaves showing fundibular (FU) structure; **A,** Fundibulum (FU) at base of appressorium (× 39,330); and **B,** Fundibulum (FU) with appressorial pore (AP) above cuticule (C) and cell wall (CW) (× 74,260).

herein provide pictorial evidence of the ability of *G. platani* to germinate, penetrate, and colonize sycamore leaf tissue.

Schuldt (14) reported mean spore germination percentages of 3.5% – 16.1% in nonsupplemented sycamore anthracnose cultures and 29.7% – 73.3% germination when spores were incubated in 0.5% sodium oleate plus 2.0% dextrose. Our observations of approximately 100% germination within 6 hr after spores were atomized onto the leaf surface in nonsupplemented water droplets indicates that conidia of *G. platani* germinate profusely on sycamore leaf surfaces at room temperature and that if a germination stimulant is required, apparently it is obtained from the host.

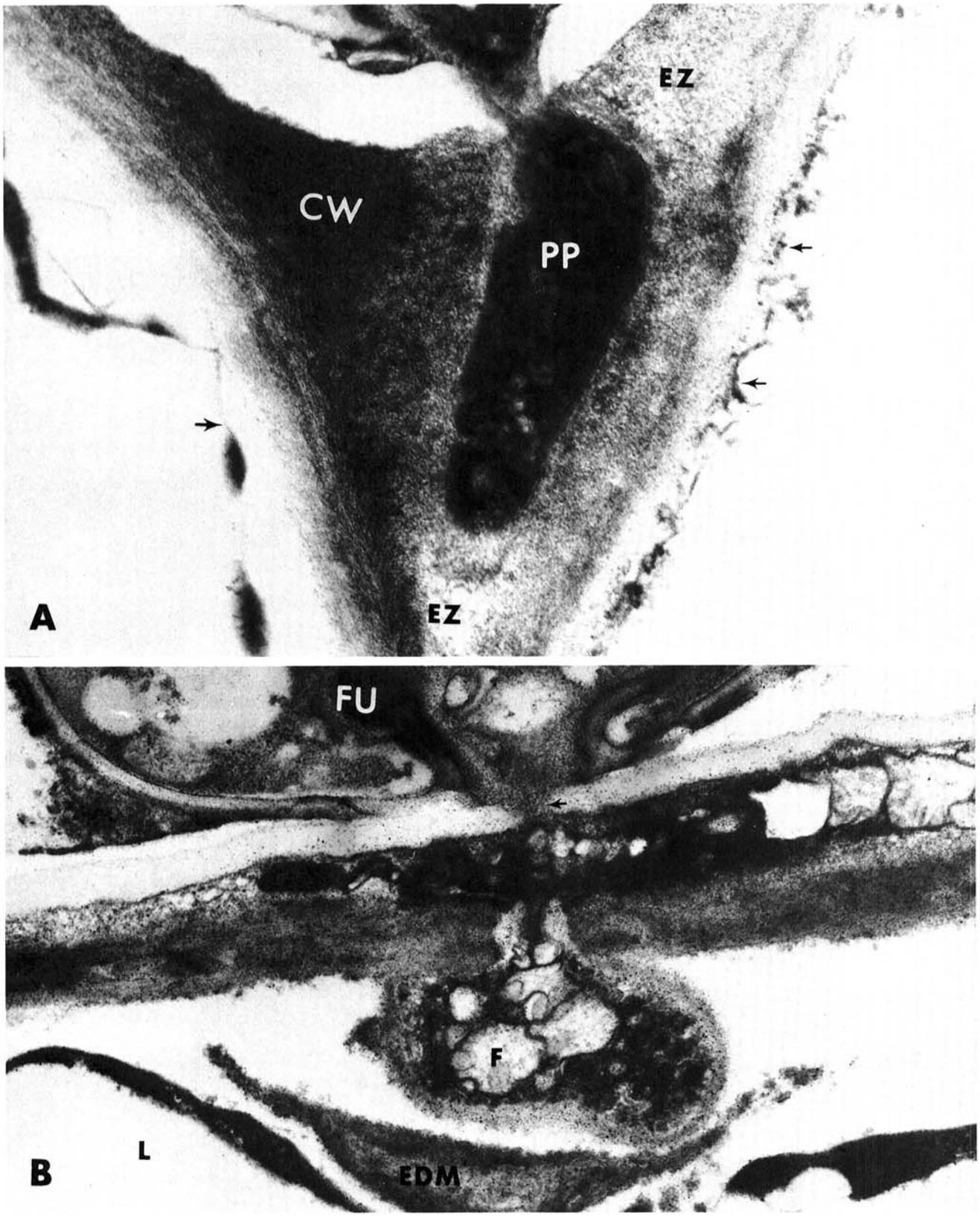

Fig. 4. Cross-section of appressorium of *Gloeosporium platani* on sycamore leaves showing penetration of host surface; **A,** Constriction of penetration peg (PP) and presumed enzymatic deterioration (EZ) in the host cell wall (CW) (× 66,930); and **B,** Subcuticular and intracellular penetration of the fungus (F). Electron dense material (EDM) in the cell lumen (× 40,020).

Germ tube length varied as markedly in these investigations (Figs. 1B, C, and E) as in those with *Colletotrichum lindemuthianum* on *Phaseolus vulgaris* (11). In some instances, growth was so slow or of such short duration that germ tubes were difficult to recognize (Fig. 1E, arrow). Germ tubes 5–6 times as long as the spore represented the other extreme (Figs. 1B and C). The variation observed in germ tube length of *G. platani* probably represents normal differences in vigor found in a population of spores. These observed differences could probably be eliminated or reduced by providing an exogenous energy source as was done by Mercer et al (11) in their investigations. They reported a suppression of appressorial development and a concomitant increase in germ tube elongation when nutrients such as orange extract were added to the inoculation medium.

The transition from hyphal extension to appressorial formation by *G. platani* occurred within 6 hr after inoculation. We did not investigate the factor(s) responsible for this quick transition, but stimuli known to be necessary for appressorium development in other host-parasite associations (contact with the host surface and chemical substances present in susceptible host tissue) (6, 9) may be involved in this disease. The rapid development of appressoria was similar to that reported for other anthracnose fungi (3, 5). The appressoria of *G. platani* appeared to be firmly anchored to the host surface (Fig. 2A). Although the composition of this anchoring material has been reported to be a mixture of host-fungus reaction products (15), micrographs of sections through appressoria of this fungus (Figs. 2B, 3A, and 3B) do not allow a similar conclusion to be made.

Mercer et al (11) described a cone-shaped appressorial pore for the bean anthracnose fungus. We found a similar structure (Figs. 3B and 4B) in *G. platani.* However, the fine-structure of the pore of *G. platani* differs considerably from the three-dimensional model of *C. lindemuthianum* drawn by Mercer et al.

The first evidence of *G. platani* breaking the outer protective layers of sycamore leaves occurred by 48 hr after inoculation (Fig. 4B). Penetration of the cuticle by the infection peg appears to be a combination of mechanical pressure and chemical softening. Presumably, enzymes are released which soften the cuticle and facilitate growth of the infection peg into the host. The production of a cutinase enzyme by a related species (*Colletotrichum piperatum* Ell. & Halst.) has been suggested (8). Direct penetration by mechanical pressure alone is discounted since there is no evidence of the cuticle being pushed inward or compressed. Subsequent fungal growth appears to be subcuticular similar to growth habits of other species of *Gloeosporium* and *Colletotrichum* (14). This may, in part, explain why *G. platani* can be routinely isolated from inoculated sycamore seedlings which are asymptomatic (authors' *personal observations*). Similar observations of apparent latent infections were reported for species of *Colletotrichum* and *Gloeosporium* on tropical fruits by Simmonds (15). He demonstrated that the presence of the fungus was not manifested in the form of characteristic symptoms until the fruit began to ripen. Conditions required for symptom development following infection by *G. platani* are being investigated.

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