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A scanning electron microscopic study of the infection of water oak (*Quercus nigra*) by *Taphrina caerulescens*

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Abstract: The fungal pathogen *Taphrina caerulescens* was isolated from leaves of water oak (*Quercus nigra*) exhibiting symptoms of oak leaf blister. Healthy leaves were inoculated with a suspension of cells from pure culture in order to examine the infection process. Scanning electron microscopy was used to monitor budding of *T. caerulescens* cells, formation of germ tubes, and indirect penetration of leaf tissue through stomata, which occurred within 48 h post-inoculation. Direct penetration was not observed.

Key Words: conidia, indirect penetration, infection process, oak leaf blister

Taphrina caerulescens (Mont. & Desm.) Tul. is a plant pathogenic fungus that causes oak leaf blister disease. Infected leaves develop raised, irregular lesions in early spring, with leaf tissue necrosing by midsummer. Oak leaf blister results in defoliation and sometimes death of various oak species in the southern USA (Mix 1949, Horst 1978).

Taphrina caerulescens is an ascomycete with both parasitic and saprophytic phases that have varying morphologies (Sinclair et al 1987). In the parasitic phase, the pathogen infects leaves at the time they emerge from buds. Infection stimulates hypertrophy and hyperplasia in the host to produce a blisterlike overgrowth, and a layer of asci emerges on the leaf surface. Blastospores, often referred to as conidia, bud directly from ascospores while they are still within the ascus. These blastospores are discharged forcibly and can reinfect the host or give rise to the saprophytic phase, in which the fungus grows in a yeastlike form. Some of these somatic cells overwinter on twigs or among bud scales and can cause new infections in the spring (Fitzpatrick 1934, Mix 1935).

The infection process in oak leaf blister disease has not been characterized fully. Martin (1925) artificially inoculated bur oak (*Quercus macrocarpa* Michx.) with cultured cells of *T. caerulescens* and used light microscopy to visualize cotton blue stained leaves at approximately 3 wk post inoculation. She observed hyphal strands entering host stomata. Our research reports the use of scanning electron microscopy (SEM) to visualize infection structure development by *T. caerulescens* and documents the sequence of events involved in the transition from saprophytic to parasitic growth in this species.

Pathogen isolation.—Leaf tissue of water oak (*Quercus nigra* L.) bearing asci of *T. caerulescens* was fastened to petri dish lids over potato dextrose (Martin 1925). Spores were discharged onto the agar within 24 h and isolated colonies (blastospores originating from single asci) that formed were transferred to fresh media and incubated at 4 C. Cultures grew as yeast-like cells that exhibited frequent budding. No mycelial growth was evident. Characteristics of the isolates were consistent with previous reports (Martin 1925, Mix 1924, 1949). Cell width (N = 20) from 2-wk-old cultures was 2.68 ± 0.73 μm. Colony diam at this time was 9–13 mm. Colonies were opaque and pale pink in color, turning a darker shade of pink with age. They were circular with entire margins and viscid in consistency, having a smooth, glistening appearance.

Inoculation.—Cultured cells from a single 2-wk-old isolate (approximately 1.35×10^9 cells/mL) were suspended in 0.01% Tween 80 and atomized onto the lower surfaces of leaves newly emerged from buds on 2-mo-old greenhouse grown water oak seedlings (Martin 1925, Mix 1924). Inoculated seedlings were covered with plastic bags and maintained in a growth chamber (22 C). For microscopic examination samples were taken at 24 and 48 h post inoculation. The experiment was repeated three times.

Sample preparation.—Inoculated leaf pieces were fixed overnight at 4 C in a 1:1 mixture of 5% glutaraldehyde and 100 mM potassium phosphate buffer, pH 6.8. The tissue then was rinsed in 50 mM buffer and post fixed in a 1:1 mixture of 2% OsO_4 and 100 mM buffer for 2 h at 4 C (Mims 1981). Following thorough rinsing in distilled water, specimens were dehydrated in a graded ethanol series to 100% ethanol. Leaf pieces were critical point dried with carbon dioxide as the transition fluid, mounted on specimen stubs, and sputter coated with gold-palladium. Samples were examined with a Hitachi S-405A scanning electron microscope operating at 15 KV.

Observations.—SEM revealed that many *T. caerulescens* conidia on the host leaf surface exhibited budding within the first 24 h following inoculation (FIG.

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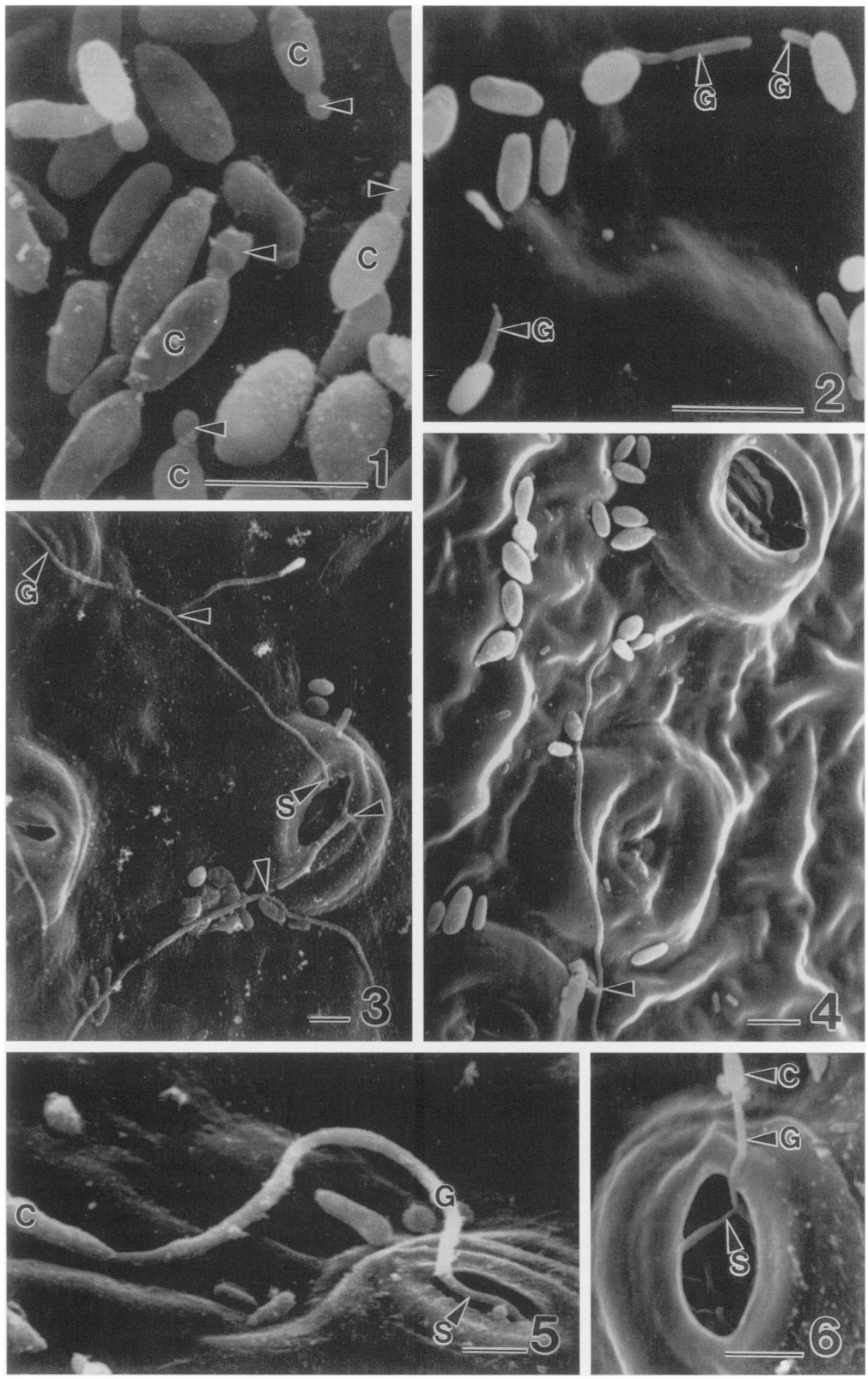

FIGS. 1–6. Scanning electron micrographs of water oak leaf surfaces inoculated with *T. caerulescens*. 1. At 24 h post inoculation. Budding conidia (C); buds indicated at arrowheads. 2–6. At 48 h post inoculation. 2. Early stages in germ tube (G) formation. 3, 4. Branched germ tubes (at arrowheads) and stomatal penetration (S). Germ tube (G) that grew over a closed stoma is present in upper left of 3. 5, 6. Conidia (C), short, unbranched germ tubes (G), and stomatal penetration (S). Bars = 5 μm.

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1). A bud formed at one end of the conidium, and a constriction was prominent at the point of delimitation between the parent cell and the newly formed spore.

Budding was observed infrequently in the 48 h post inoculation samples. At this time period, approximately 20% of conidia present had formed germ tubes (FIGS. 2–6). Germ tubes emerged from the apical end of each conidium (FIG. 2), and had a long, thin morphology that was very distinct from the shape and size of immature buds. Germ tubes frequently grew to extensive lengths. Longer germ tubes were usually branched (FIGS. 3, 4), and their growth appeared random rather than directional. Germ tube tips were observed to extend over guard cells and into stomatal openings (FIG. 3). Occasionally these hyphal strands grew over closed stomata (FIG. 3). Tilting of samples revealed that germ tubes were appressed closely to the host leaf surface, following contours of the epidermal layer (FIG. 4). Conidia with short germ tubes that appeared to exhibit more directional growth habits also were present (FIGS. 5, 6). No appressoria or attempts at direct penetration were observed.

Inoculated oak seedlings maintained in a growth chamber (22 C) after samples were taken began to show typical oak leaf blister symptoms in approximately 4 wk, indicating that the penetration events observed microscopically were successful in initiating infection.

SEM proved to be successful in the characterization of host leaf penetration by *T. caerulescens*. Results of this investigation indicate that conidia of this pathogen are capable of initiating infection within 48 h of contacting a susceptible host leaf surface. Spore germination events and indirect penetration of host stomatal openings by *T. caerulescens* were documented microscopically for the first time. These observations support the camera lucida drawings of indirect host penetration by *T. deformans* (Berk.) Tul. published by Martin (1925).

Fitzpatrick (1934) and Mix (1935) questioned Martin's (1925) results. They observed direct penetration of the intact host cuticle (Fitzpatrick 1934) and epidermis (Mix 1935) by *T. deformans* on upper and lower peach leaf surfaces. Using the techniques of leaf clearing and serial sectioning, they found that germ tubes emerged from one end of the spore, attached firmly to the epidermis, and penetrated the cuticle to form intercellular mycelium within the epidermal layer. Such penetration events would not be seen readily with SEM; it is likely that conidia would block the view of short germ tubes. On the other hand, indirect penetration would be difficult to detect using the techniques of Fitzpatrick (1934) and Mix (1935). Clearing or sectioning easily could displace the longer, branching germ tubes observed in this study. Additionally, Mix (1935) inoculated emerging leaves before stomatal openings had fully differentiated.

Although not observed in this investigation, it is probable that *T. caerulescens* is capable of directly penetrating an intact host leaf in the absence of a natural opening. This speculation is supported by observations of concave, ascus bearing depressions on both upper and lower leaf surfaces of water oak (Birdwell 1996), with stomata present only on the lower surface. Hyphae of *T. caerulescens* are subcuticular and intercellular in the epidermis (Camp and Whittingham 1974) and not known to traverse the mesophyll like those of *T. deformans* (Fitzpatrick 1934, Mix 1935, Syrop 1975). Thus, asci would appear only on the upper leaf surface if that epidermal layer had been penetrated directly.

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