

Sporodochium Development and Conidium Production in *Cephalosporium gramineum*

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ABSTRACT

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Cephalosporium gramineum was induced to grow in and on moist, naturally-infested wheat straw (*Triticum aestivum* L.) in the field and laboratory. Its colonization of straw, sporulation, and sporodochium development were monitored with light and electron microscopes. In immature wheat plants, the fungus was confined to xylem vessels. In straw it developed extravascular, asporogenic hyphae that ramified all subepidermal tissues. Stomata were principal sites of hyphal emergence onto the straw surface where hyphal branching intensified and sporulating phialides differentiated at branch tips. Sporulation was independent of the organization of phialides into simple clusters or into sporodochia. Sporodochia developed above stomata and at sites within effuse, superficial mycelium. They originated from groups of short, branching hyphae (some branched penicillately) that proliferated centrifugally into hymenia. Sporodochia were convex initially but flattened with age.

Their expanse of tightly compressed phialides supported masses of phialospores atop a thin, superficial stroma. Mucus covered the fungus, much of the substrate surface, and suspended masses of phialospores. The fungus became completely superficial as mycelium within the straw eventually lysed. Sporodochia developed within 4-8 wk and were most abundant on straw, especially leaf sheath tissue, kept moist and cool (12 C in the laboratory and on straw either upon or within 5 cm of the soil surface in the field). They developed slowly or not at all on air-dry straw, on straw at 21 C, or at 10-cm depths in soil. Phialides in sporodochia were typically elongate (1-2 × 5-25 μm) whereas on mycelium in vitro they were shorter, some being reduced to sessile collars on hyphae or on individual conidiogenous cells. Blastogenous-like sporulation, which was prevalent within the xylem of immature plants and in vitro, was not apparent on straw.

Cephalosporium gramineum Nis. & Ika. is an important pathogen of wheat, other small grains, and numerous grasses in the USA and Europe. It is the only true vascular fungal parasite of the Gramineae. In Michigan and certain other areas, the fungus persists as a sporodochium-producing saprophyte (*Hymenula cerealis* El. & Ev.) on the residues of infected hosts (1, 2). In this stage, numerous conidia are produced which infest soil and infect successive cereal or grass crops.

Morphological descriptions of the *Cephalosporium* genus and of *C. gramineum* and its sporodochial stage are available (1, 4, 6, 10). However, the ontogeny of sporodochia has not been described. This study expands earlier observations (12, 14) and employs scanning electron microscopy to monitor the transition of the fungus from a vascular pathogen to a superficial, sporodochium-forming saprophyte. The mechanism of its migration from xylem vessels to the surface of wheat straw, the developmental morphology of sporodochia and the morphology of sporulation within sporodochia, within xylem elements of host plants, and within cultures of the fungus in vitro were of principal interest.

MATERIALS AND METHODS

A Michigan isolate of *Cephalosporium gramineum*

was maintained in vitro on Difco potato-dextrose agar (PDA) for direct microscopic observation and for use as inoculum for infection of wheat seedlings (12). The cultures, grown from conidia transferred onto PDA in 10 × 150-mm plastic petri plates, were incubated at 21 C for 7-10 days on a laboratory bench and agar blocks (approximately 1 mm³) bearing visible fungal growth were removed. The blocks were prepared for light- and transmission electron microscopy as previously described for leaf segments (12).

Sporodochia were available for direct observation on old, field-collected wheat straw (cultivar Genesee). Since the ontogeny of sporodochia was of interest, new straw known to be infested with *C. gramineum* was identified from tags placed earlier on immature plants with leaf stripe symptoms (11, 12). The straw was cut into 5- to 6-cm lengths and groups of 10 straw segments were incubated in field and laboratory environments to induce sporodochium formation.

For incubation in the field (the same field in which the straw was collected), groups of straw segments were placed in 10 × 10-cm nylon bags (1.0 × 1.5-mm mesh). The bags were placed on the soil surface or at 3- or 10-cm depths in soil. Field incubations began in autumn (August) and were terminated at intervals ranging to 130 days (December). This period spanned the seasonal optimum (October) for sporodochium formation in Michigan (13).

In the laboratory, similar groups of straw segments were incubated on different substrates for up to 90 days. The substrates, all in plastic petri plates, were 2% water agar, PDA, and steam-sterilized and nonsterilized wheat field soil. The soil was maintained between 20 and 30% moisture (dry weight basis) and all substrates were prepared in duplicate for continuous incubation on a laboratory bench at 21 C or in the dark at 12 C.

Straw segments were removed from incubation approximately at 1-wk intervals. Any segments in which microorganisms other than *C. gramineum* were prominent were discarded. Those dominated by *C. gramineum* were sectioned into 1-cm lengths and transferred to 3% buffered glutaraldehyde (9). Subsequent handling for scanning electron microscopy was as previously described (9). To observe the progress of *C. gramineum* within straw, representative segments were freeze-fractured after critical-point drying and prior to mounting (7).

OBSERVATIONS

Appearance of sporodochia on wheat straw.—Sporodochia of *C. gramineum* developed on straw remaining in Michigan wheat fields from harvest (July) through early winter (December). They were especially abundant on straw from fields in which wheat, other small grains, or grasses were grown for two or more consecutive years. Sporodochia appeared in autumn (October) on culm and leaf sheath tissues held moist near the soil surface beneath clumps of straw. Their distribution on culms was random (Fig. 1) or oriented in patterns indicative of the fungus' earlier vascular parasitic habit (Fig. 2, 8).

Naturally-occurring sporodochia were tan to deep brown, up to 1 mm in diameter, superficial, and easily dislodged when mechanically disturbed. They were raised, yellow-brown, and glistening with mucus-bound masses of conidia when moist (Fig. 1, 2, 16). When dry, they were flattened, crusty, and deep brown-black (Fig. 8, 14, 15).

Colonization of infested straw.—*Cephalosporium gramineum* made extravascular mycelial growth in all straw segments incubated in moist, aerated environments (Fig. 3, 4). Growth was minimal or absent in air-dry straw or in straw buried 10 cm deep in field soil. In the laboratory, incubation at 21 C promoted extensive mycelial growth but sporodochium differentiation was sparse or absent. Incubation at 12 C slowed mycelial growth and promoted the differentiation of numerous sporodochia.

In all environments, growth of mycelium within straw was sterile, nondirectional, and inter- and intracellular (Fig. 3, 4). The fungus indiscriminately expanded through all subepidermal straw tissues in contrast to its confinement within xylem in living plants. Extravascular growth was evident after 2 wk in the field and within 3 days in the laboratory at 21 C.

Sporodochium initiation.—Mycelium within straw eventually reached the epidermis, but exit onto the straw surface was not direct. Stomata and the severed ends of straw segments were the initial and major avenues to the exterior (Fig. 5, 6, 7).

Spore production and the development of sporodochia

were initiated only after hyphae emerged from the straw. External hyphae branched penicillately (Fig. 10) or monodirectionally (Fig. 13) and proliferated into sporodochia. Soon thereafter, the short, branching hyphae were clustered and nondirectional (Fig. 10, 11). Phialides readily differentiated at hyphal tips and began spore production before a hymenium was clearly organized (Fig. 6, 10, 11). Phialide differentiation began on the central hyphae in clusters and hymenia expanded laterally. Sporulation continued throughout the organization and maturation of sporodochia. In the field, sporodochia of average size (approximately 0.7 mm in diameter when moist) appeared within 4-8 wk, often immediately above stomata (Fig. 8) where they developed from a minimum of superficial mycelium (Fig. 6, 13). At 12 C in the laboratory, superficial mycelium was more extensive than on field-incubated straw and sporodochia originated at random sites within it (Fig. 10, 11, 12) in 2-4 wk.

Sporodochia initially were convex with a mounded stroma (Fig. 12, 16) but they flattened with age (Fig. 15). Mucus accumulated and covered the periphery of sporodochia and much of the substrate surface (Fig. 9, 14). It also suspended masses of phialospores above sporodochia. Much mucus and most of the conidia were lost during preparation of specimens for microscopy. Where mucus remained, it appeared in crusty layers (Fig. 9, 14, 15).

Conidium production.—*Cephalosporium gramineum* generated large numbers of spores in vitro, in host plants (12), and on straw. In the field, conidia infested straw and soil remote from sporodochia (Fig. 19). With all substrates, except internal straw tissues (Fig. 3, 4), phialides readily differentiated at hyphal apices in unorganized mycelium (Fig. 6, 10, 11). Their organization into sporodochia occurred only on straw and only in cool, moist environments.

All phialospores from sporodochia developed singly through an apical pore flanged by an extension of the generative cell wall (Fig. 17, 18). The extension formed a collar that supported each developing spore until it was set off by a septal constriction. In vitro, a similar collar supported spores developed from phialides set laterally on hyphae (Fig. 20), from the ends of individual conidiogenous cells (Fig. 21), and from lateral, sessile sites on hyphae (Fig. 22). Blastospore-like sporulation (Fig. 21) occurred in vitro and within parasitized wheat plants (12) but was not evident on straw. Sporodochial phialides (Fig. 17, 18) ranged to 25 μm in length, whereas phialides on hyphae in vitro (Fig. 20) typically were 5-15 μm long.

DISCUSSION

This study microscopically monitored the transition of *C. gramineum* from a vascular pathogen to a nondiscriminating saprophyte in wheat straw. The results indicate a need to investigate the anatomical and/or biochemical factors that confine the fungus to xylem in living plants. Because growth within and sporulation upon straw apparently does not occur at depths greater than 8 cm in soil, an investigation of light and/or aeration as limiting factors also is warranted. Furthermore, it is

unclear why sporulation did not occur within straw but was profuse on its exterior in all environments that permitted mycelial growth.

The mucus produced by *C. gramineum* (Fig. 9, 14) has been described previously as a mucopolysaccharide (2, 4,

11), implicated in pathogenesis (11), and observed in infected plants (12). Its most significant role may be in augmenting the fungus' saprophytic survival. It may protect spores and hyphae from desiccation and from radiation injury (5). Another supportive role could be in

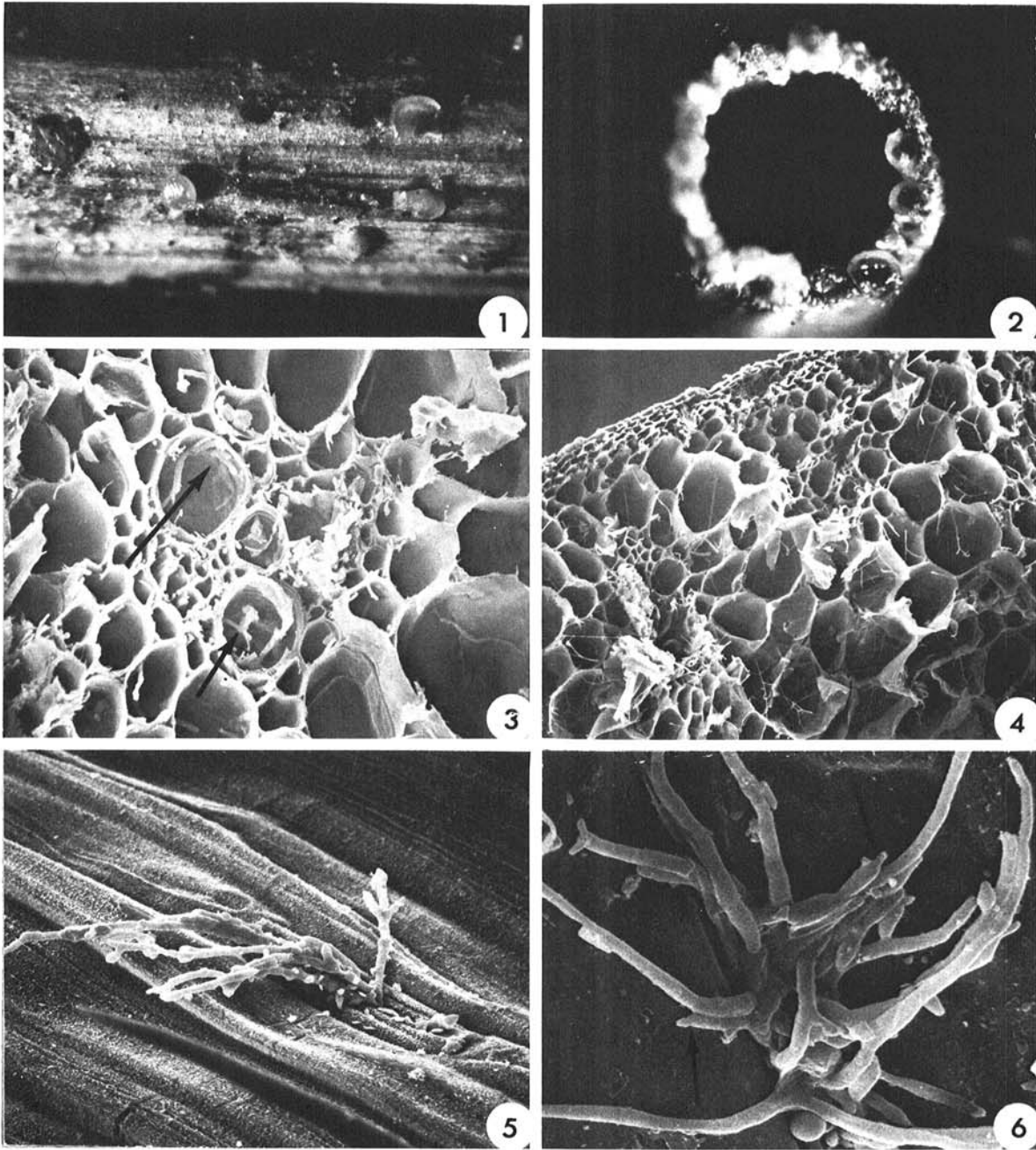


Fig. 1-6. (1-2). General view of raised, moist sporodochia of *Cephalosporium gramineum* formed on naturally infected wheat straw. 1) Lateral view of culm ($\times 12$). 2) End view of culm ($\times 12$). (3-4) Wheat culms fractured transversely to reveal infestation with *Cephalosporium gramineum*. 3) Fungus (arrows) in xylem elements after 1 wk of incubation on field soil ($\times 500$). 4) Fungus colonizing subepidermal tissues after 4 wk of incubation on field soil ($\times 200$). (5-6) *Cephalosporium gramineum* exiting wheat straw through stomata. 5) Nonsporulating hyphae exiting stomate on leaf blade ($\times 800$). 6) Sporulation initiated (arrows) on hyphae outside stomate on culm ($\times 1,500$).

substrate dominance (Fig. 9, 14). Its abundance might explain the paucity of contaminant organisms in our soil-incubated straw segments (2, 3, 8, 11).

Cephalosporium gramineum readily sporulated on host residues apart from sporodochium formation. This form of inoculum production may explain why the fungus

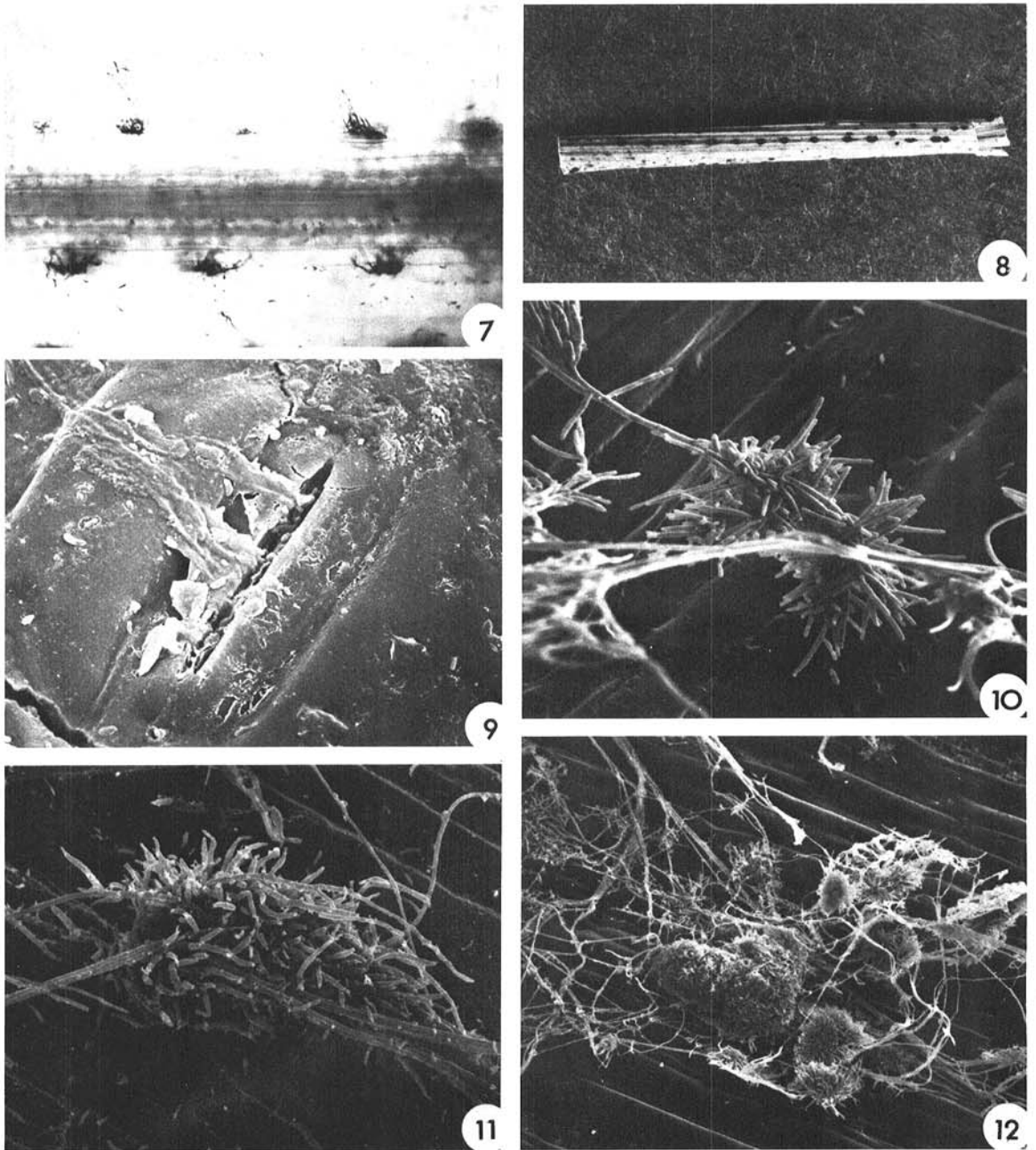


Fig. 7-12. 7) Stomata on wheat culm occluded by sporulating hyphae of *Cephalosporium gramineum*. Note spores dispersed on culm surface ($\times 170$). 8) Segment of a field-incubated wheat culm with dry sporodochia of *Cephalosporium gramineum* surmounting an infested xylem element ($\times 1.3$). 9) Mucus of *Cephalosporium gramineum* discharged through a stomate on an infested wheat culm ($\times 1,900$). (10-12) Stages of sporodochium development by *Cephalosporium gramineum* on wheat straw. Note ongoing sporulation at hyphal tips and spores dispersed on straw. 10) Incipient sporodochia (center) originating from penicillately-branched hyphae (upper-left) ($\times 950$). 11) An early, expanding hymenium ($\times 800$). 12) Sporodochia in various stages of organization at sites within superficial mycelium ($\times 200$).

is a persistent wheat pathogen in portions of Montana and western Europe where sporodochia rarely occur. Sporodochium development apparently is dependent on environment and is not requisite for successful saprophytism or parasitism.

This study further revealed a capacity for sporulation in vitro from structures resembling degenerative phialides. The fungus, which is known to sporulate blastogenously (6, 12) and from elongate phialides (Fig. 17, 18, 20), also may develop phialospore-like conidia

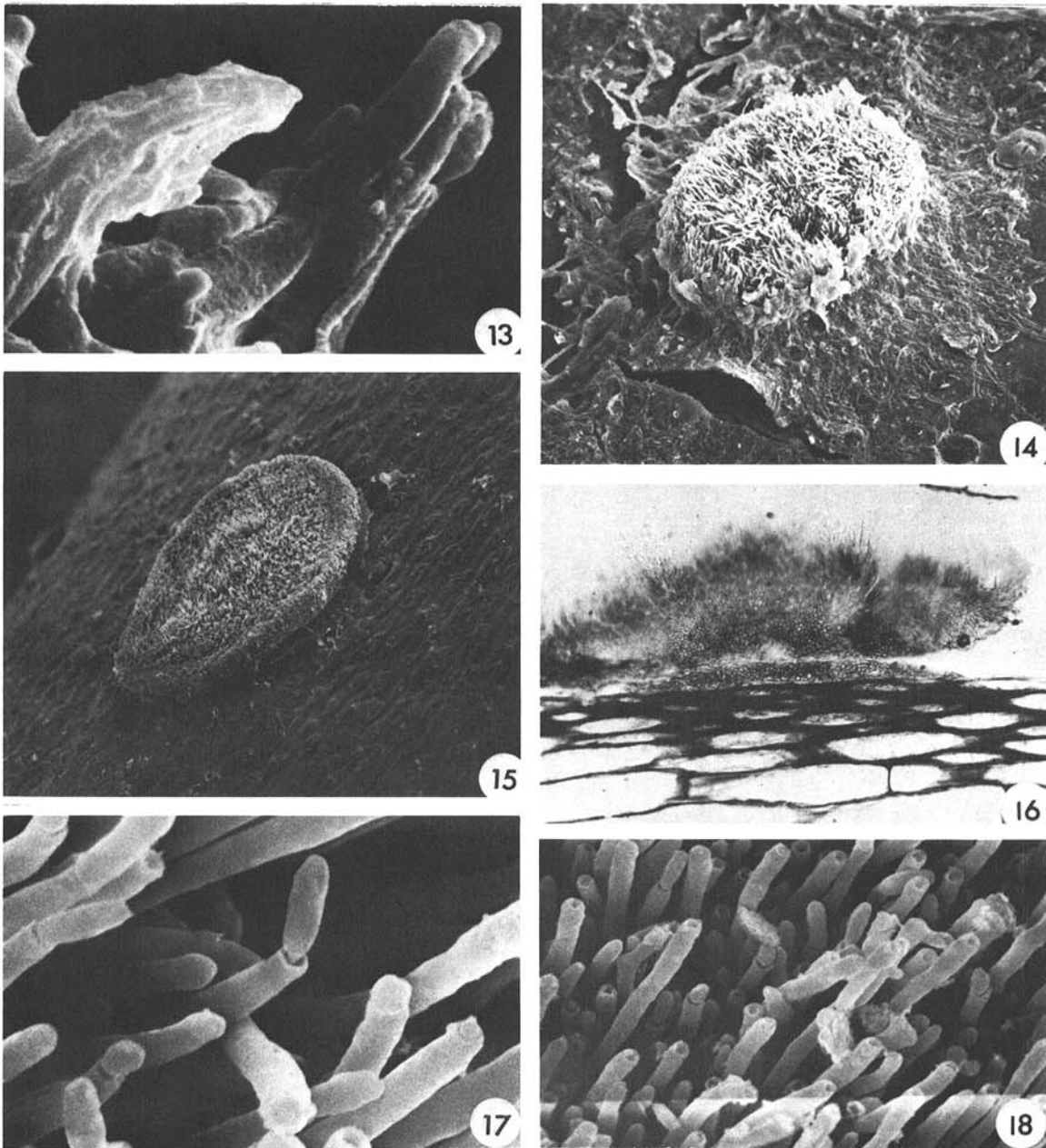


Fig. 13-18. 13) A juxtaposed, nonpenicillate cluster of hyphae of *Cephalosporium gramineum* on wheat straw prior to proliferation into a sporodochium ($\times 8,000$). 14) Young, expanding sporodochium of *Cephalosporium gramineum* and its surrounding substrate encrusted by mucus ($\times 450$). (15-16) Mature sporodochia of *Cephalosporium gramineum* on wheat straw. 15) Fully expanded, dry, flattened sporodochium with spores and mucus removed ($\times 150$). 16) Transverse section through sporodochium showing thin, superficial stroma and depletion of subepidermal hyphae ($\times 200$). (17-18) Clusters of phialides comprising the hymenial surface of sporodochia of *Cephalosporium gramineum*. 17) Liberation of a mature phialospore ($\times 9,000$). 18) Various stages of phialospore development ($\times 4,500$).

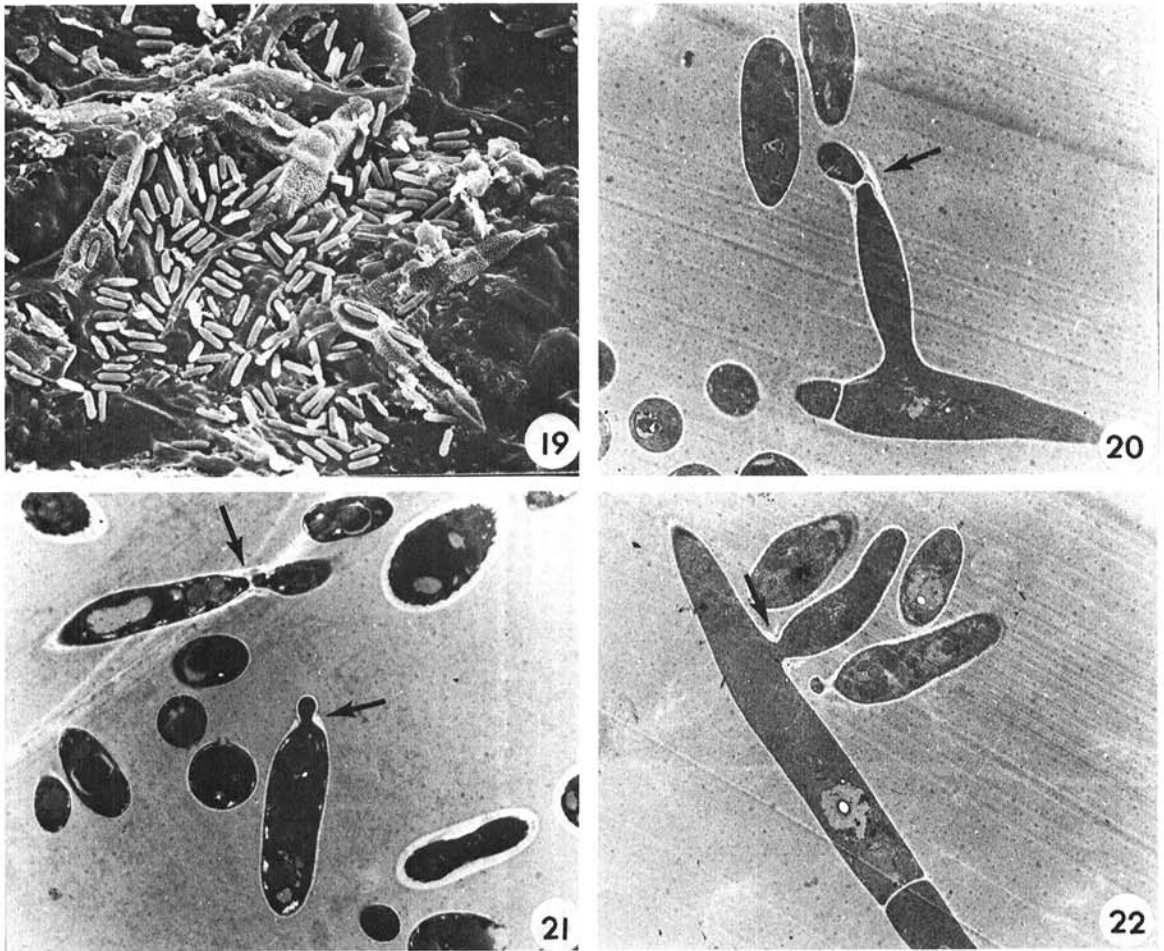


Fig. 19-22. 19) Phialospores of *Cephalosporium gramineum* in soil ($\times 1,800$). (20-22) Spore production by *Cephalosporium gramineum* in vitro viewed in thin section. 20) Phialide with prominent collar (arrow) at base of developing phialospore ($\times 5,500$). 21) Blastosporic (lower arrow) and phialide-like sporulation (upper arrow) from conidia ($\times 5,500$). 22) Phialide-like sporulation from sessile, lateral site on a hyphal cell ($\times 5,500$).

through heretofore undescribed, sessile, collared pores on hyphae (Fig. 22) and on individual cells (Fig. 21). These structures may represent a rare transition between blastogenous and phialogenous sporulating mechanisms.

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