Histopathology of Marasmius Blight of American Beachgrass

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ABSTRACT

Hyphae of a Basidiomycete were found in internal tissues of diseased plants of American beachgrass, and parenchymatous tissues were apparently destroyed by the invading fungus. In addition to mycelium, the xylem vessel elements contained a gum or tanninlike substance. The mycelium of isolates from the basidiocarps of the *Marasmius* sp., associated with diseased plants, appeared to be the same as that of the fungus isolated from diseased tissues. The distribution of mycelium in cross- and longitudinal sections of beachgrass plants, grown and inoculated under sterile conditions, was similar to that of naturally infected plants.

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Along the North Carolina coast in recent years, a disease has become prevalent in American beachgrass (*Ammophila breviligulata* Fernald), the principal plant species used to stabilize coastal sand dunes along the Atlantic coast from Nova Scotia to North Carolina and along the shores of the Great Lakes (9). Circular areas of dead beachgrass have developed along dunes on the Outer Banks of North Carolina that range in size from 2 to 20 ft in diam (5). A *Marasmius* sp. was repeatedly isolated from the roots and basal stems of diseased plants, and sporophores of the fungus often developed at the base of diseased plants (9).

Marasmius spp. have been associated with the death of grasses for many years and some were reported to be pathogens (8). Stained sections of diseased wheat tissue indicated that the mycelium of the attached basidiocarps of *M. tritici* developed in basal stems while the host was still alive (10). Young (10) also showed that hyphal strands at the base of stipes were continuous with the mycelium within host tissues. The death of vegetation in some "fairy rings" has been attributed to *M. oreades* Fr. (1, 2, 7). Histological studies of three grass species infected by *M. oreades* Fr. indicated that the fungus could penetrate living tissue (3). The *Marasmius* sp. associated with diseased American beachgrass has been shown to be pathogenic to this species of dune grass (9).

The purpose of this study was to determine the distribution of hyphae of *Marasmius* sp. within the tissues of naturally infected and inoculated beachgrass plants.

MATERIALS AND METHODS.—Diseased and healthy beachgrass plants were collected from a 4-year-old beachgrass planting on Ocracoke Island, North Carolina. Isolations were made from diseased stems and roots that had been surface sterilized in 0.525% sodium hypochlorite for 10 min. Sections 0.4 to 0.6 cm were cut from healthy and diseased surface-sterilized roots and stems. Half of each section was fixed in Formalin: acetic acid: ethyl alcohol solution (FAA) and the other half was plated on Difco potato-dextrose agar (PDA) to determine whether the *Marasmius* sp. was present. The fixed sections from roots and stems, whose companion sections yielded *Marasmius* sp. in culture, were dehydrated with tertiary butyl alcohol and embedded in tissuemat (6). Root tissue was sectioned at 10 μ and stem tissue at 15 μ. The sections were stained with safranin:fast green (4).

Diseased and healthy beachgrass seedlings from laboratory pathogenicity tests were examined histologically by the same techniques except that root and stem tissues were sectioned at 8 and 12 μ, respectively. Seedlings were grown aseptically and inoculated in jars and tubes containing sand and Hoagland's solution at 24 C under white fluorescent lights at about 150 ft-c (9).

RESULTS.—A fungus with clamp connections at septa was isolated on PDA from sections of diseased plants. Isolates from *Marasmius* sp. basidiocarps that grew on dying plants were morphologically identical to the fungus isolated from diseased tissues. The fungus grew rapidly (1 cm/day) on PDA and formed a characteristic thin, white colony. Microscopically the mycelium was the same width with clamp connections at most septa.

Histological examination of healthy and naturally infected tissues showed large amounts of mycelium in diseased tissues (Fig. 1-A, B). Hyphae in diseased roots and stems had clamp connections (Fig. 1-C). Although many clamp connections were observed on the hyphae in plant tissues, not all of the hyphae in the tissues of naturally infected plants had clamp connections at all septa. A layer of mycelium covered the root surfaces similar to the mantles formed by mycorrhizal fungi. Hyphae were found in all stem

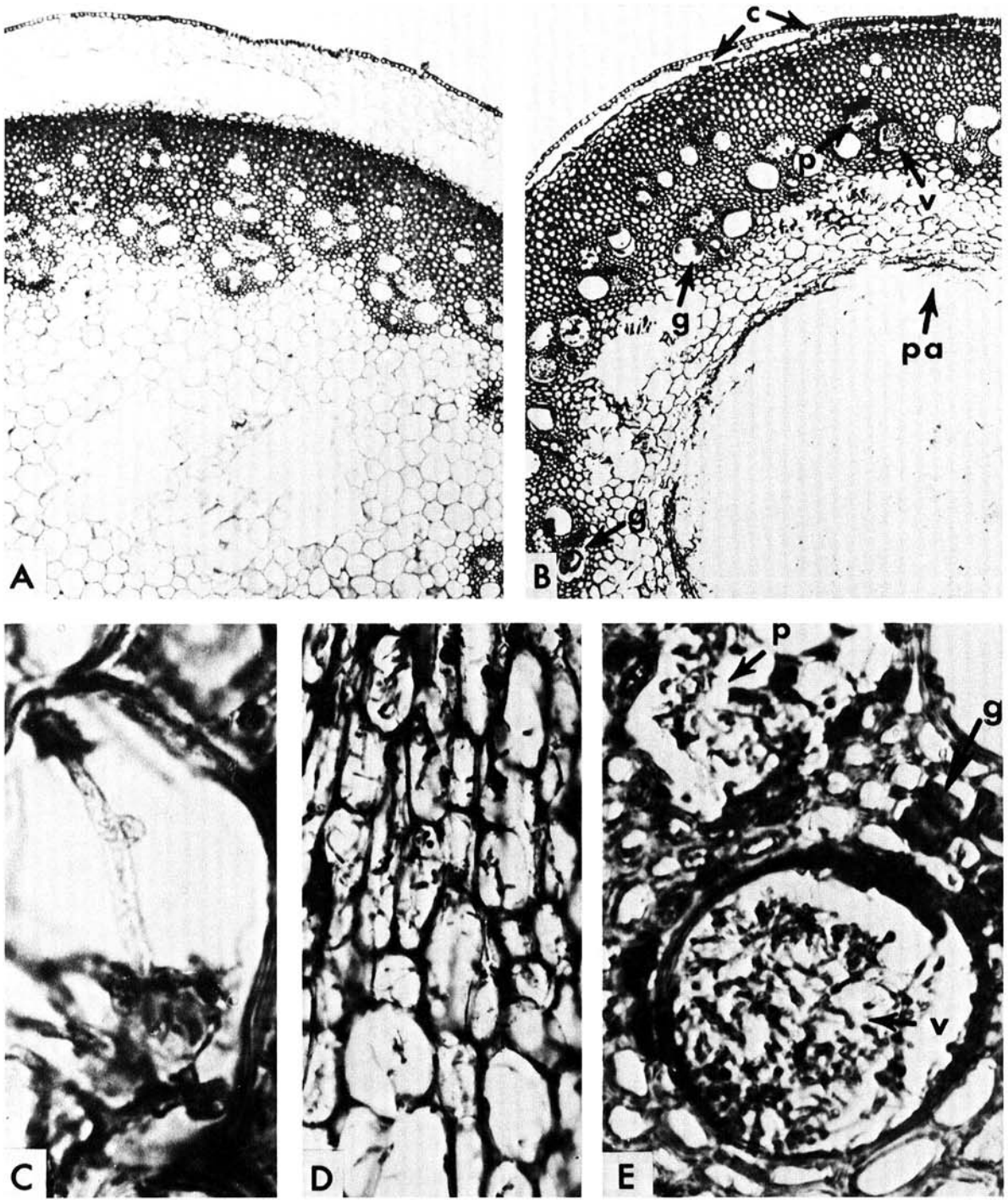

Fig. 1. Histology of healthy and naturally infected beachgrass plants collected from dunes. **A)** Cross section of a portion of a healthy stem (ca. ×30). **B)** Cross section of a portion of a diseased stem with disrupted parenchyma cells (pa) and phloem (p) and with a xylem vessel (v) element partially blocked by masses of hyphae. Many of the xylem vessel elements were partially filled with a gum or tanninlike material (g). Much of the cortical tissue (c) was absent or replaced by a layer of mycelium beneath the epidermis (ca. ×40). **C)** Clamp connection on a hypha of *Marasmius* sp. within a parenchyma cell of a leaf sheath (ca. ×1,500). **D)** Portion of a longitudinal section from a diseased stem with inter- and intracellular hyphae located throughout the parenchyma cells near the axil of a leaf sheath (ca. ×350). **E)** Mycelium in a xylem vessel (v) element and in the phloem (p) of a diseased beachgrass stem. Xylem vessel elements are also often partially filled with a gum or tanninlike substance (g) (ca. ×350).

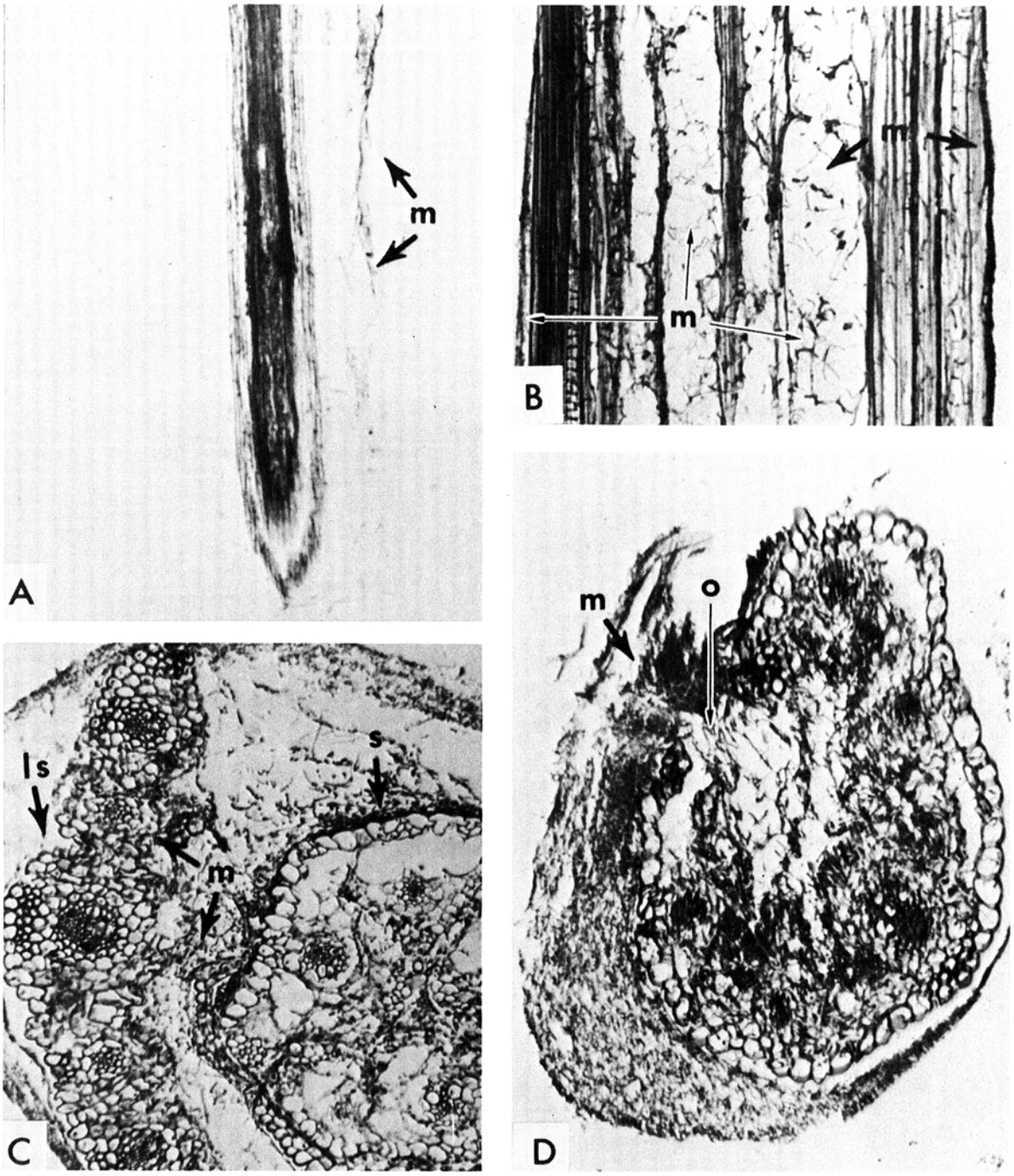

Fig. 2. Histology of aseptically grown beachgrass seedlings inoculated with an isolate of *Marasmius* sp. obtained from naturally diseased beachgrass plants. **A)** Longitudinal section of a diseased root with mycelium (m) on or near the epidermis (ca. × 15). **B)** Portion of a longitudinal section of a diseased stem with a layer of mycelium (m) on the epidermis and hyphae within the stem (ca. × 350). **C)** Portion of a cross section of a diseased stem with mycelium (m) between the leaf sheath (ls) and stem (s) (ca. × 35). **D)** Cross section of a diseased stem without a leaf sheath. A mass of mycelium (m) is present on the epidermis. Mycelium apparently entered the stem through an opening in the epidermis (o) (ca. × 35).

tissues (Fig. 1-B), including the xylem vessel elements (Fig. 1-E). Many vascular bundles in diseased roots and stems were filled with a gum or tanninlike substance, particularly the xylem vessel elements, tracheids, and lacunae (Fig. 1-B). The gum or tanninlike material was most prevalent adjacent to the cell walls. Many other cells were filled with this material which stained a bright red. In infected stems, the phloem and parenchymatous tissues appeared to be disrupted (Fig. 1-B, E). Much of the cortical tissue was absent or replaced by masses of mycelium beneath the epidermis (Fig. 1-B). Remaining cortical tissue contained numerous hyphae, and mycelium was abundant in the axil of leaf sheaths. Hyphae occurred both inter- and intracellularly (Fig. 1-D). Hyphae were not found in phloem and xylem tissues of the roots of naturally infected plants, but were found in other root tissues.

Inoculated seedlings grown under sterile conditions had masses of mycelium on the surface and inside of the roots (Fig. 2-A) and stems (Fig. 2-B). Distribution of hyphae in and on inoculated plants was approximately the same as for naturally infected plants except that there was more mycelium on the epidermis of the inoculated plants (Fig. 2-C, D). There was more mycelium in the cavities left by disrupted cells in inoculated tissues than in naturally infected tissues. The mycelium appeared to aggregate between the leaf sheath and stem (Fig. 2-C) and entered the stem through epidermal openings (Fig. 2-D). Clamp connections were abundant on hyphae in inoculated root and stem sections.

DISCUSSION.—The presence of clamp connections on mycelium in naturally infected plants indicated that the pathogen was a basidiomycete. Mycelium in the sectioned tissue was similar to that isolated from adjacent root and stem sections and to cultures obtained from the basidiocarps of the *Marasmius* sp. associated with diseased plants.

The phloem and the parenchymatous tissues of the stem were apparently killed by the fungus. The cortical cells of the roots and stems were invaded and the cell contents destroyed. Stem xylem vessel elements were invaded and appeared to be partially blocked by mycelium and a gum or tanninlike material. This material was possibly formed by the host in response to fungal invasion or by the fungus as it grew in host tissue or a combination of both. The disruption of phloem cells and large amounts of mycelium and a gum or tanninlike material in the xylem vessels of diseased plants suggests that a defective vascular transport system may result in decline and eventual death of the plant.

It is not known whether all mycelium observed in naturally infected plants was from the same fungus. Absence of clamp connections on some hyphae in naturally infected tissue may be due to cutting sections across hyphae between septa.

The abundance of mycelium on the surface of seedlings grown and inoculated under aseptic conditions, and the extent of mycelial growth on the plant above the soil line was attributed to high humidity within the jars and tubes in which the seedlings were grown.

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