Histopathology of the Interaction of Sorghum bicolor and Sphacelotheca reiliana

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ABSTRACT

Sphacelotheca reiliana, the causal organism of sorghum head smut, develops only in actively growing host meristematic tissue. Three types of mycelium are found in a developing sorus. A thin parasitizing mycelium was intercellular with haustoria. A large, reproductive mycelium produced teliospores from the centers of the intervascular regions. The peridium consisted of a partially segmented, nonreproductive mycelium surrounded by a thin layer of host cells.

The distribution of mycelium in the apical meristem determined the type of sorus produced. This mycelium was carried by the elongating cells to areas in the developing inflorescence at the initiation of the host reproductive cycle. Widespread colonization of the host apical meristem at the beginning of elongation and floral differentiation resulted in the formation of a smooth sorus. When the mycelium was limited to the basal regions of the apical meristem before elongation and differentiation, the floral primordia were not colonized, and the result was a sterile or vegetative (phyllodied) head. If the mycelium was carried into the lowest regions of floral primordia, a small sorus was produced at the base of a sterile inflorescence. Widespread but sparse mycelium at the time of elongation resulted in a sorus in the shape of a partially differentiated inflorescence.

Reproductive and peridial cells of the fungus in a developing sorus were mainly uninucleate. Phytopathology 60:828-832.

Head smut of sorghum, caused by *Sphacelotheca reiliana* (Kühn) Clint., has been reported from all parts of the world where sorghum is grown. Although there is considerable literature about specific disease characteristics, the histopathology of the host-parasite relationship is poorly described.

Infected *Sorghum bicolor* (L.) Moench plants exhibit only slight symptoms until appearance of the inflorescence. Once the inflorescence appears, the disease is manifested in several ways. The panicle may be partially or entirely replaced by the smut sorus. When partially replaced, that portion of the panicle free of the sorus is sterile. Another common reaction is the complete absence of a sorus with a sterile and sometimes phyllodied panicle (Fig. 1-A).

Sphacelotheca reiliana is a dimorphic fungus. The overwintering stage is the thick-walled teliospore. On artificial media, teliospores germinate by producing an epibasidium or promycelium on which haploid sporidia are formed. During germination, the presumably diploid nucleus undergoes meiosis to produce the haploid sporidia of two mating types. Sporidia reproduce on artificial media by budding, and clones arising from buds may be maintained. The sporidial stage is monokaryotic, and nonparasitic whereas the parasitic stage is reported to be dikaryotic (3).

Fusion of compatible haploid sporidia occurs in the soil, and subsequent host penetration by dikaryotic hyphae has been reported (6, 7). However, experimental evidence supporting this phenomenon is lacking. Presumably, this hypothesis of penetration mode was derived from observed germination behavior of the organism on culture media. Al-Sohaily (1) observed that teliospores germinating in the soil produce hyphae without sporidia. Clinton (2) reported that penetration by *S. sorghi* may be either by sporidial germ tubes or hyphae.

In 1914, Potter (8) reported on the mycelial distribution in nodes and lateral buds of infected plants. He noted that the fungus invaded and developed in the meristematic tissues, and proposed that the lateral buds become infected when the culm differentiates the nodes.

The present investigation was initiated to determine the infection mode and the developmental processes of the host-pathogen complex.

MATERIALS AND METHODS.—*Histopathology.*—Inoculum was maintained as two monosporidial lines of *S. reiliana* on potato-dextrose agar (PDA). The parasitic stage was produced by mating the two haploid, monosporidial lines. The monosporidial lines were separately transferred to individual 250-ml Erlenmeyer flasks, each containing 50 ml of sterile potato-dextrose broth. The flasks were placed on a reciprocal shaker for 3 to 5 days at room temp. At inoculation time, equal volumes of the broth suspensions of each monosporidial line were mixed.

Two susceptible sorghum varieties, Combine Shallu (SA 394) and SA 7078, were inoculated at two different times. The first inoculation was made on 1-week-old seedlings. Seeds were germinated in soil in flats in the dark at 30 C to elongate the mesocotyl and force the growing point above the soil line. The seedlings were then transferred to the greenhouse and hypodermically inoculated in the nodal area below the shoot apex (Fig. 1-B). All plants in the study were grown at 24 to 35 C and watered every 4 days with Hoagland's solution. At 3-day intervals following inoculation, two seedlings of each variety were removed, and a portion of each seedling including the shoot apex was placed in Formalin-acetic acid (FAA).

In the second treatment, 3- to 4-week-old plants were hypodermically inoculated in the growing point with the sporidial suspension. The main shoots were then allowed to head. Infected panicles were removed and secondary shoots allowed to develop. At various stages

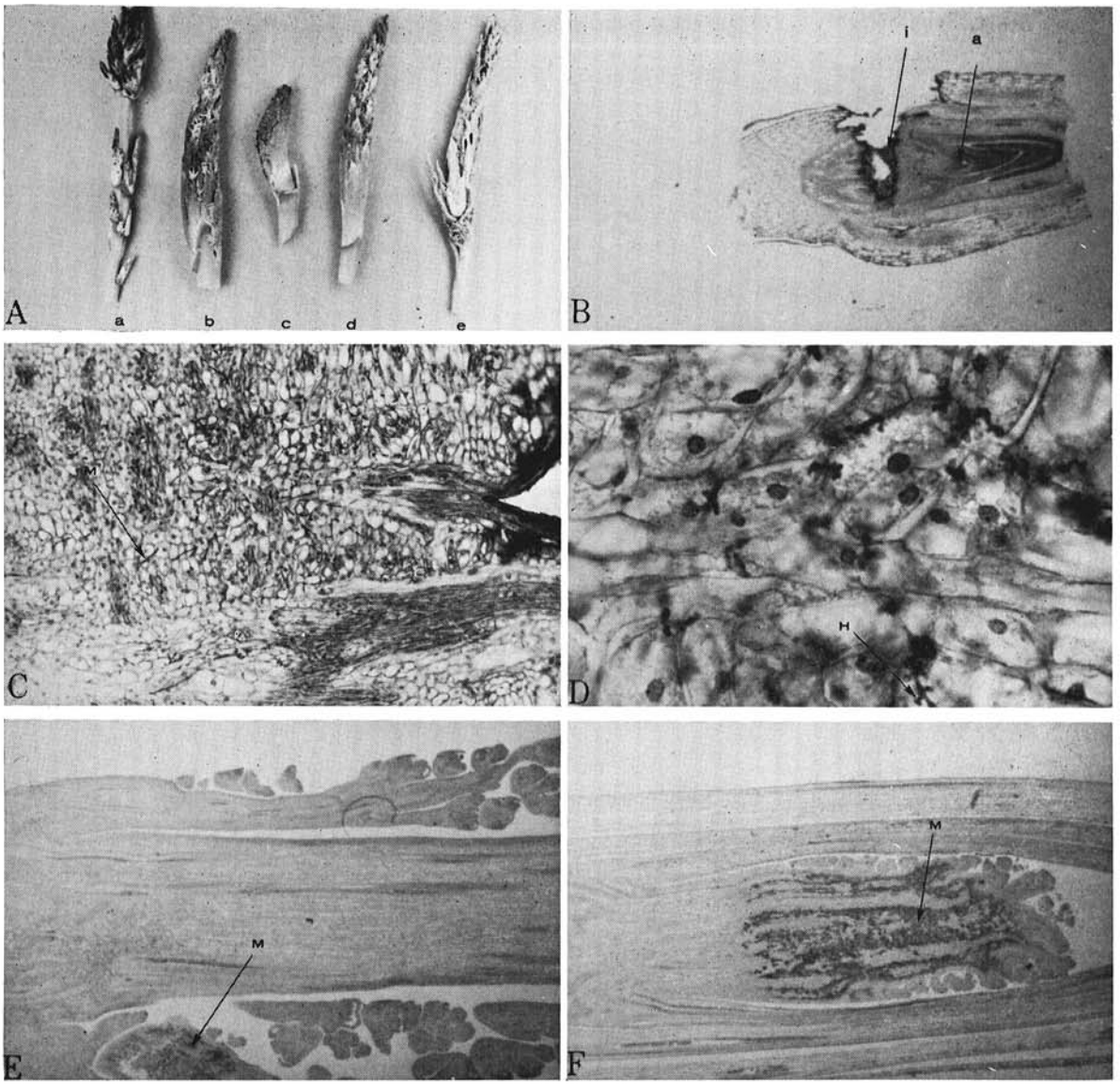

Fig. 1. Gross and microscopic aspects of sorghum infected with *Sphacelotheca reiliana*. **A)** Infected "head" type; (a) panicle branches differentiated; (b,d,e) sori in partially differentiated heads; (c) a "smooth sorus". **B)** Longitudinal section through coleoptile of 10-day-old seedling. Inoculation point (i) and shoot apex (a). (×23) **C)** Longitudinal section of Combine Shallu 3 days after inoculation. Intracellular mycelium (m). (×400) **D)** Combine Shallu 6 days after inoculation. Intercellular mycelium with haustoria (h). (×1600) **E)** Longitudinal section of inflorescence colonized by *S. reiliana* (m) in lower panicle branch only. (×40) **F)** Longitudinal section through an immature sorus in shape of partially differentiated inflorescence. (See Fig. 1-B.) Mycelium (m). (×40)

of development, secondary shoots were removed and fixed in FAA. Tissues were dehydrated in a t-butanol series, embedded in paraffin, and 8-10 μ sections cut on a rotary microtome. Twenty-five to 50 sections of each shoot apex were stained with Thionin and Orange-G or Flemming's triple stain (9).

Penetration.—Teliospores of the pathogen were mixed with sterile sand in flats. Seeds of susceptible varieties were planted 2.5 mm in depth in the flats. The seedlings were removed after 6 days, washed, and fixed in FAA. One and one-half-cm sections of the seedlings, including a portion of the mesocotyl and the shoot apex, were placed in lactophenol containing 0.1% acid fuchsin and autoclaved at 122 C for 15 min. The sections were then mounted in lactophenol and examined microscopically.

Nuclear study.—Fresh developing sori were squashed and smeared on glass slides. The nuclei were stained using Giatgong's modification of the Giemsa technique (5).

RESULTS.—*Histopathology.*—In seedlings inoculated at 1 week of age, intracellular mycelium had developed 3 days after inoculation. Mycelia were observed in both meristematic and parenchymous tissues of the nodal area some distance from the point of inoculation (Fig.

1-C). This scattered distribution was probably due to the inoculation method in which the sporidia were forcefully injected into the tissue and dispersed throughout the tissues. Mycelium had not invaded the meristem of the shoot apex or vascular bundles.

Six days after inoculation, the mycelium was intercellular, and haustoria had developed (Fig. 1-D). The amount of mycelium observed 27 days after inoculation appeared to be about the same as that observed after 3 days. Mycelial development was confined to the nodal region of inoculation in all specimens, and was not observed in the meristem of the shoot apex.

Thirty-six tillers from plants inoculated at 3 to 4 weeks of age were examined to determine extent of panicle colonization. Inactive tiller buds contained only traces of mycelium in the meristematic tissues, whereas some of the growing tiller apexes were completely colonized at a relatively early stage of development. Tissues between the vascular bundles of the panicle were heavily colonized, and the periphery of the young panicle was smooth and continuous, showing no characteristic differentiation into floral structures. Some panicles were fully colonized except at the apex of the elongated panicle. In these cases, the panicle apex was free of the mycelium and similar to differentiating panicle branches on noninfected plants. Mycelium was commonly localized in an area of the panicle, usually near its base (Fig. 1-E), involving only one or a few panicle branches with the remainder of the panicle free of the pathogen. All panicles or panicle branches of infected plants which lacked sori were sterile.

Complete or partial colonization of a panicle in which floral differentiation had begun was common. The periphery of the panicle exhibited varying degrees of differentiation into floral branches, but usually did not proceed beyond the elongation of panicle branch initials when the interior was either fully or partially colonized by the pathogen (Fig. 1-F).

Panicle proliferation was uniformly produced on plants in a greenhouse test planted in December. Several weeks of cold weather occurred 2 weeks after inoculation, and greenhouse temperatures reached 0 C on several nights. When approximately 50% of the plants had headed, each plant had a sterile or proliferation reaction. None of the diseased plants produced sori. Main shoots of the remaining plants were collected and examined prior to heading. These panicles differentiated in a seemingly normal manner, and were free of mycelium. However, mycelium was observed to a limited extent in the node at the base of the panicles.

Several plants inoculated at 4 weeks of age were maintained in the greenhouse for 9 months. More than 50 tillers were produced on each plant. After producing predominantly smutted heads and then predominantly proliferated heads, the plants began producing healthy heads.

In young sori, the mycelium is of three types: peridial, reproductive, and vegetative. The fungal tissue of the peridium consisted of hyphae 2-4 μ in diam which failed to stain uniformly with either staining method used. These cells underlie a thin layer of host tissue, and do not participate in fungal reproduction (Fig. 2-A).

Peridial cells were isolated and grown in shake culture. Susceptible sorghum seedlings were inoculated at 3 weeks of age with these peridial cells. In contrast to sporidial inoculations which resulted in 100% infection of all main shoots and tillers, the peridial culture infected less than 50% of the plants. Plants infected with peridial hyphae produced only small or no sori on the main shoot, and many of the subsequent tillers were healthy.

Reproductive mycelium was observed in the centers of the intervascular areas, running parallel to the vascular bundles. Hyphae were 3-5 μ in diam, and lacked haustoria. There was no evidence of host cells in the areas occupied by these masses of hyphae (Fig. 2-B).

Branching from the intervascular mycelium at the peripheries of the reproductive "mats" were vegetative hyphae, 1-2 μ in diam (Fig. 2-C). These hyphae were intercellular with haustoria. They grew from the reproductive mycelium toward the vascular bundles.

Cells of the older reproductive mycelium produced teliospores by rounding up into large spore balls composed of masses of teliospores. Spore formation and maturation proceeded from the sorus apex downward, and from the centers of the intervascular areas toward the vascular bundles.

Numerous aggregates of hyaline spores were observed in mature sori. These spores produced germ tubes when left in distilled water for several days. The pathogenic potential of these cells was not investigated.

Penetration.—Only 10 germinated teliospores were found among the many thousands observed on almost 100 sorghum seedlings. In all cases, the germinated teliospores produced hyphae several times the length of the epibasidium (promycelium) produced in culture (Fig. 2-D). There was no evidence of sporidia, and the hyphae appeared to penetrate the epidermis directly.

Nuclear study.—The technique employed to study the nuclear condition of the parasite within the host was unsatisfactory. The smear of the young sorus released masses of indistinct fungal hyphae. Because of the size of the mycelium observed with this technique, it was believed that only the reproductive and peridial cells were dislodged from the sorus.

The stained mycelial cells were predominantly uninucleate. The nuclei of binucleate cells were slightly smaller than the single nuclei.

Discussion.—The sporidial stage of *S. reiliana* may be produced only rarely in nature, at least in soil, as found by Al-Sohaily (1). The hyphal mode of host penetration would reduce the potential genetic recombination that could occur following mating of sporidia in the soil. Since there has been no report of any other method of fungal genetic recombination within the host, this smut organism might be expected to be relatively stable in its pathogenic reaction. This is the case since, until recently (4), there have been many opportunities in Texas for observing changes in pathogenicity.

Observations of the plants inoculated at 1 week of age and of tillers of plants inoculated at 4 weeks indicated that the precise infection site within the growing point influenced the type of host reaction. The seem-

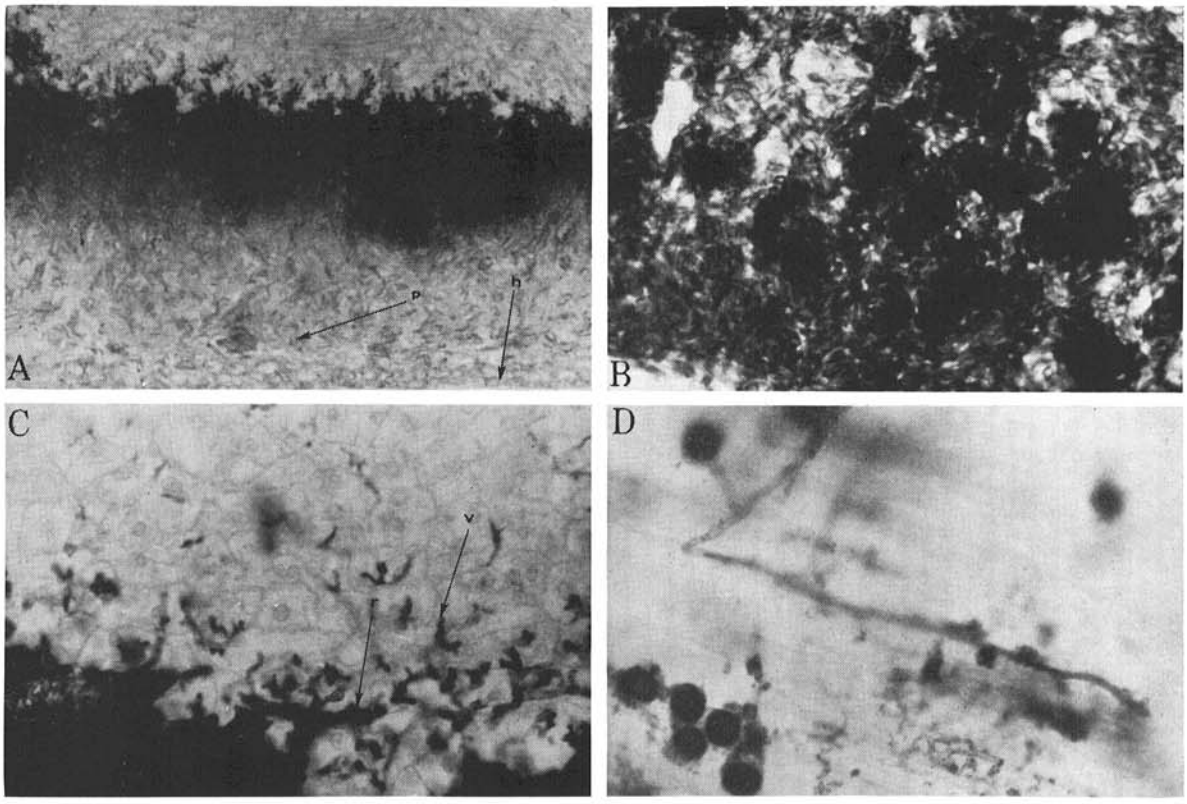

Fig. 2. **A)** Mycelium of *Sphacelotheca reiliana* in the peridium (p) underlying the epidermis (h). (×400). **B)** Aggregation of reproductive mycelium immediately prior to teliospore formation. (×1600) **C)** Intercellular vegetative mycelium (v) arising from reproductive mycelium (r). (×1600) **D)** Teliospore germination of *S. reiliana* on Combine Shallu seedling 6 days after planting. (×1600)

ingly quiescent nature of the organism in tissues other than active floral meristems supports this view. Mycelium failed to grow from subapical tissues into the apical meristem even after 3 weeks. Apparently, initial invasion of the apical meristem is necessary for colonization and sorus development within the primary shoot. Invasion and establishment of the fungus in the subapical meristems results in sori on tillers originating from the colonized tissues.

A widespread colonization of apical meristem prior to initiation of the host reproductive cycle results in a conical sorus with a smooth, continuous membrane. The mycelium in the shoot apex becomes distributed throughout the elongated floral primordium in a passive manner. Pathogen growth stimulation in the floral primordia is simultaneous with the host floral initiation. Complete colonization of the apical meristem at the time of elongation results in the arrest of further development of the floral meristems. The result is a short, smooth-walled sorus.

A widespread colonization of apical meristem which arrested floral differentiation at a more advanced stage results in a sorus resembling a partially differentiated inflorescence. It is probable that this "leafy" sorus reaction results from a more sparse colonization of the apex compared to that which occurs when a smooth sorus reaction is observed.

Mycelium confined to the lower or lateral portions of the shoot apex causes the formation of a local sorus with the remainder of the panicle sorus-free, but sterile. Colonization of the entire base of the shoot apex produces a sorus enveloping the lower portion of the panicle with the upper portion blasted. Mycelium localized at only one side of the apex results in a sorus on only one or more panicle branches.

When fungal colonization is below the apical meristem tissues which elongate to form the panicle, the inflorescence will be sterile and sorus-free.

The parasite is latent in all tissues other than active floral meristems. It invades and colonizes the meristem of the inflorescence only after floral initiation. Lateral buds become infected at the time of their formation. These observations are in agreement with Potter (8) and Clinton (2). This passive-type infection would seem to invalidate the term "systemic", which is frequently used to describe the infection process. The latency of the pathogen was further demonstrated by the observations on 9-month-old greenhouse plants that produced smutted, blasted, and healthy panicles. This reaction should not be expected if the parasite freely invaded tissues other than active floral meristems.

Following floral initiation, mycelium in the apical shoot bud becomes active and grows throughout the floral primordium as the cells divide and elongate.

Colonization at the centers of the intervascular areas becomes extremely dense, and the host tissue in these areas apparently disintegrates. The vegetative mycelium changes physiologically and cytologically to reproductive mycelium. Vegetative or parasitic mycelium emerges from this reproductive mycelium to invade previously uncolonized host tissue.

The fungal cells beneath the host epidermis function as the peridium of the sorus. These cells failed to stain uniformly as did other growth phases of the organism. This staining reaction was at first interpreted as an indication of nonliving tissue. Isolation and subsequent host inoculation with these cells, however, indicated they are living. Because the majority of the sorus cells are uninucleate, it is believed that the peridial cells have undergone karyogamy. The possible role of this diploidization in the observed reduction in pathogenicity in the peridial cells is purely speculative at this time.

Hyaline spores observed in sori have been described as "sterile" cells (3). These spores were viable, and produced germ tubes when placed in distilled water. They are probably teliospores which lack spore walls.

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