Systemic Infection of Sorghum by *Acremonium strictum* and Its Transmission Through Seed

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ABSTRACT

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The fungus *Acremonium strictum* was isolated from stems of wilted sorghum plants collected from Pantnagar, Uttar Pradesh, India, and its pathogenicity was established in the greenhouse. Of the several inoculation methods examined, drenching soil in pots containing 10-day-old plants of the susceptible cultivar IS 18442 with a conidial suspension of the pathogen resulted in more than 70% disease incidence. Disease symptoms in the greenhouse were similar to those in the field. Grains from diseased plants were small and shrunken with reduced weight, germination, and seedling vigor. *A. strictum* was isolated from all parts of infected plants, from roots to grains, indicating the systemic colonization of the host. Infected seeds produced diseased plants when sown in autoclaved soil.

Additional key words: inoculation technique, *Sorghum bicolor*

In July 1983 at Pantnagar, Uttar Pradesh State, India, we observed many sorghum (*Sorghum bicolor* (L.) Moench) plants with wilt symptoms typical of those caused by *Acremonium strictum* W. Gams (15). The disease was most prevalent in the sorghum line of Indian origin, IS 18442, which had been sown in every sixth row as a disease spreader in a 2.5-ha leaf disease screening nursery.

The disease was first noticed 40 days after emergence as isolated, distinctly pale yellow, stunted plants. Single diseased plants or two or three adjacent diseased plants were interspersed between healthy plants in the same row. Initially, the lower leaves turned pale yellow and developed reddish brown streaks that later enlarged and coalesced. Such leaves dried within a few days. Gradually, the symptoms progressed acropetally and the apical leaves turned yellow at first, became necrotic, and then dried without plant lodging. Severely infected plants did not produce panicles. When longitudinally split, stalks of such plants showed internal discoloration; the pith was disintegrated and creamy pink and contained slightly fluffy intertwining fungal mycelia visible to the naked eye. The cortex tissues were deep reddish

brown. Close examination revealed necrotic xylem tissue crossing the nodes. In some cases, the apical meristems were rotted. Internode length near the apical meristem was considerably reduced. Externally, the stalks looked green until the apical leaves began to turn yellow and dry.

A. strictum has a worldwide distribution in soil and the atmosphere (4), but as an incitant of disease in sorghum, it has been reported only from Argentina (7), Honduras (18), and the United States (15). A *Cephalosporium* sp. reported as the cause of sorghum wilt in Egypt (5) was probably *A. strictum*, and the disease probably occurs in more sorghum-growing countries than so far reported (8). Recently, we saw similar disease symptoms on a few isolated sorghum plants in several African countries, Lesotho, Zimbabwe, Malawi, Tanzania, and Mali (L. K. Mughogho and R. Bandyopadhyay, *personal observations*), where it does not appear to be a serious disease on varieties currently grown by farmers. In Honduras, however, the disease is important on local land races (18). In maize, *A. strictum* (= *Cephalosporium acremonium* Corda) causes wilting of mature plants, infects seedlings (3), and internally colonizes grain (11,13). This paper reports the identification of *A. strictum* as the cause of a wilt of sorghum in India, inoculation methods for establishment of infection, its systemic colonization of the host plant, and transmission through seed.

MATERIALS AND METHODS

Isolation and identification of causal agent. Stems of wilted sorghum plants collected from the field at Pantnagar were split open, and small pieces of fibrous, discolored internal tissues were plated on potato-dextrose agar (PDA) after surface sterilization in a 0.15% mercuric chloride solution for 1 min followed by three washings in sterile distilled water. The plates were incubated at 30 C under diurnal fluorescent light with a 12-hr photoperiod. Slide cultures (17) of the fungus that grew out of the plated tissues were made to preserve the fruiting structures for identification. The cultures were sent to the Commonwealth Mycological Institute (CMI), Kew, Surrey, UK, and the Centraalbureau Voor Schimmelcultures (CBS), Baarn, Netherlands, to confirm the identity of the pathogen.

Pathogenicity test and recovery of pathogen from different plant parts. Pathogenicity was tested on cultivar IS 18442 grown in the greenhouse from disease-free, surface-sterilized seeds in 30.5-cm-diameter plastic pots filled with a Vertisol autoclaved at 121 C for 2 hr. Three plants were grown in each pot. Inoculum was multiplied on sand-oatmeal medium for 30 days at 30 C. Pots were inoculated when the plants were 30 days old by removing the top 5-cm layer of soil in the pot, jabbing a scalpel into the soil to cause slight injury to roots (15), spreading 150 g of the inoculum over the loosened soil in each pot, and covering the inoculum with the soil removed earlier. Thirty pots (90 plants) were inoculated with sand-oatmeal inoculum, and another 20 received autoclaved soil for use as checks. The pots were arranged in a randomized block design and watered frequently to keep the soil moist. The plants were observed for disease symptoms each day until maturity.

At grain maturity (black-layer formation growth stage), different plant parts, from roots to grain, from inoculated and uninoculated plants were surface-sterilized and plated on PDA (pH 5.5) amended with dicrysticin-S (streptomycin sulfate and procaine penicillin G). Fungi growing from these
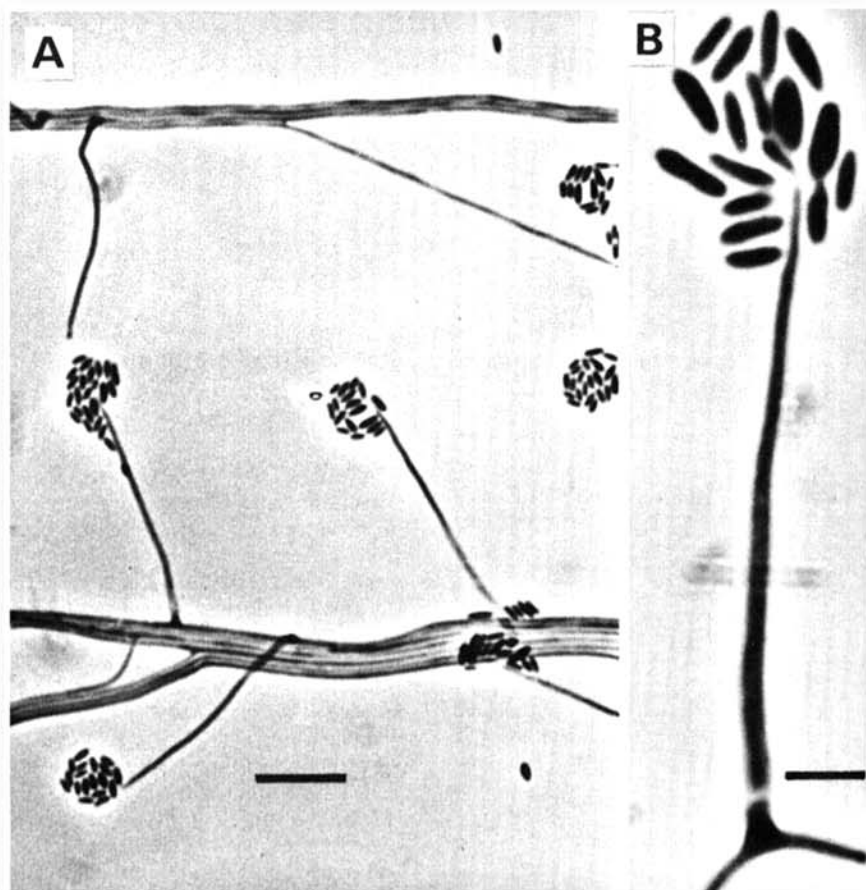
Fig. 1. *Acremonium strictum.* **(A)** Erect conidiophores arising from strands of fasciculated hyphae. Scale bar = 20 μm. **(B)** Simple conidiophore with a bunch of cylindrical conidia at the tip. Scale bar = 4 μm.
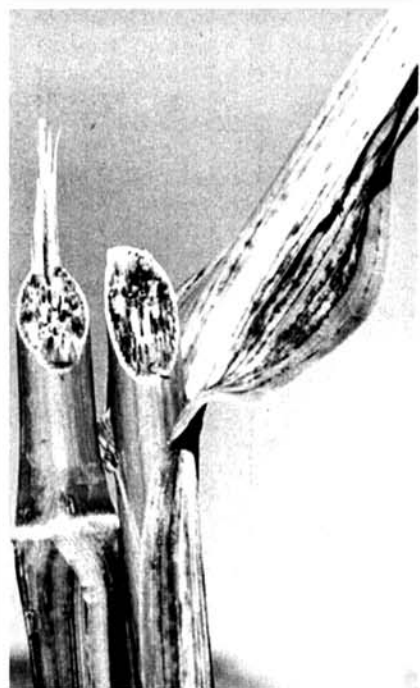

Fig. 2. Leaf and stem of a greenhouse-grown plant infected by *Acremonium strictum.* Symptoms include necrotic streaks on leaf veins, interveinal desiccation of leaf and leaf sheath, faint brown stripes on internode (rind) surface, and necrotic xylem tissue in tangential section of internode.
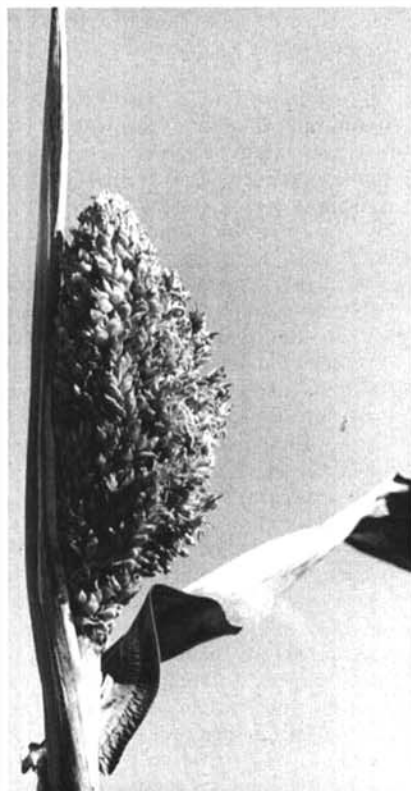

Fig. 3. Sorghum panicle of plant systemically infected by *Acremonium strictum* showing sterile florets.

pieces were transferred to fresh PDA for identification.

Pieces of necrotic xylem tissue from different plant parts were sampled at grain maturity and prepared for fluorescence microscopy by fixing in 3% glutaraldehyde, dehydrated, embedded, sectioned at 3 μm, and affixed to glass slides (6). Sections were stained with 0.01% Cellufluor (Polyscience, Inc., Warrington, PA) for 1 min, rinsed in running tap water for 10 min, air-dried, and mounted in low-fluorescent immersion oil. Sections were examined under an Olympus Vanox microscope fitted with an epifluorescent incident light attachment (model AH-RFL-LB) consisting of HBO 200W high-pressure mercury lamp, an UG-1 exciter filter, and a DM-400+L-420 dichoric mirror-cum-absorption filter.

Seed transmission of pathogen. Grain from plants in the pathogenicity test was harvested at a moisture content of 15% from wilted and healthy plants grown in inoculated and check pots. After storage at room temperature (25 ± 2 C) for 12 days followed by surface sterilization with 0.15% mercuric chloride for 1 min, the seed was sown in pots filled with a sterilized Alfisol. Twenty pots (60 plants) were sown with seed from healthy plants, and 20 pots were sown with seed from wilted plants. The resulting plants were observed for disease symptoms from emergence to grain maturity. Isolations of the pathogen were made from different plant parts as described.

Inoculation tests. Inoculum of the pathogen was multiplied on autoclaved sorghum grain incubated at 30 C for 15 days. Test plants were grown as in the pathogenicity experiment, except the soil used was an Alfisol. The following inoculation methods were used. 1) Soil inoculation: Autoclaved soil was thoroughly mixed with inoculum in a 125:1 (w/w) ratio and used to fill 30.5-cm pots. Each pot received 5 kg of inoculated soil (10.2-cm layer) at the bottom, 4 kg of autoclaved soil (6.3-cm layer) on which seeds were placed, then 3 kg of autoclaved soil (5.1-cm layer) and was watered regularly until the plants reached maturity. 2) Soil drenching: Soil in pots containing seedlings at the four-leaf growth stage was drenched with a 40-ml conidial suspension (10^6 ml^{-1}). 3) Soil drenching with root injury: The soil was inoculated as in 2, then jabbed with a scalpel around the plants to slightly injure the roots. 4) Leaf inoculation: The youngest fully opened leaf at the four-leaf growth stage was rubbed with cotton wool soaked in inoculum suspension. 5) Inoculation of injured leaves: Leaves were inoculated as in 4, but Carborundum powder was sprinkled on the leaves before inoculation to cause injury during inoculation. 6) Inoculation of leaf sheath: Inoculum was placed between the stalks and leaf sheaths of the fourth leaf of 19-

day-old plants. 7) Uninoculated check with roots injured as in 3. 8) Uninoculated check with leaf injury as in 5. 9) Uninoculated check without root or leaf injury. Pots were arranged in a randomized block design with three replicates and nine pots per treatment. There were thus nine treatments each with 27 pots, i.e., 81 plants. Disease incidence was recorded at 7- to 14-day intervals up to the soft dough growth stage.

RESULTS

Isolation and identity of causal agent. *A. strictum* was the predominant fungus isolated from all wilted plants collected in the field. This identification was confirmed by both the CMI and CBS. Visible fungal growth as slimy dots or sparse hyphae appeared 48 hr after plating infected leaf pieces, and sporulation occurred 24 hr later. At 30 C, 1-wk-old colonies were 3–4 cm in diameter, creamy pink, and mostly slimy at the center but occasionally fluffy with conical tufts of hyphae. Many erect conidiophores with conidial heads were observed arising from raised columns of hyphae. Hyphae were thin-walled, septate, 1–1.5 μm in diameter, and frequently joined together to form fascicles (Fig. 1A). Often, two fasciculated hyphal strands were connected by a single hypha or two or three fused hyphae. Conidiophores were simple, erect, hyaline, septate at base, 32.5–60 ×1–1.8 μm (mean 46 × 1.7 μm), and distinct at the peripheries of the colonies (Fig. 1B). Conidia were produced in a slimy head at the tip of the conidiophores, were unicellular, hyaline, cylindrical, 2–5 × 1–1.5 μm (mean 3.6 × 1.2 μm), and germinated with a single germ tube. Numerous conidia were produced at the center of the colony in a slimy mass. Sclerotia and chlamydospores were not found in culture.

Pathogenicity test and isolation of pathogen from different plant parts. Ten days after inoculation, a few veins on the sheaths showed faint brownish specks that quickly elongated along the veins and within 24 to 36 hr spread to leaf veins, where necrosis occurred randomly on the lamina. The veinal necrosis elongated to become large reddish brown streaks within a few days (Fig. 2). Leaves with two or more necrotic veins turned yellow and finally dried. The area between the necrotic veins on the leaf sheaths became light to deep brown and finally dried. In the later stages, faint reddish stripes also occurred on the rind surface. This pattern of symptom expression progressed upward, leaf by leaf, to the flag leaf.

When the stems were split longitudinally or cut transversely, xylem tissues were distinctly seen as reddish brown necrotic strands (Fig. 2) from the base of the plant to the peduncle and main rachis. Necrotic streaks were also noticed on the surfaces of some primary and secondary branches of the rachis and the rachilla.

All inoculated plants produced panicles; however, some plants desiccated immediately after flowering and did not produce grains, whereas others produced small shrunken grains of variable size depending on the stage of grain development when the plant completely wilted (Fig. 3). Germination and test weights of grains from infected plants and vigor of seedlings raised from such grains were significantly less than with grains from healthy plants (Table 1).

A. strictum was seen in the tracheids of xylem tissue under fluorescence microscope and was isolated from roots, stalks, leaf laminae, midribs, leaf sheaths, peduncles, main raches, rachis branches, rachillae, glumes, and grains of inoculated plants in the greenhouse (Fig. 4A,B). It was absent from all parts of healthy plants. *Fusarium moniliforme* Sheld. was also isolated from a small number of grains.

Seed transmission of pathogen. Plants grown from seeds of infected plants showed leaf veinal necrosis 37 days after emergence when the boot leaf was in the whorl. At that stage, 12% of the plants showed symptoms, and at maturity, 20% of the plants were wilted. At maturity, plants were uprooted and cut transversely at several points to observe xylem necrosis. Plants with necrotic streaks on their leaves also had xylem necrosis. *A. strictum* was reisolated from plants with disease symptoms. All check plants were disease-free.

Inoculation methods. Information on the progress of disease in the different inoculation treatments is presented in Figure 5. The most effective inoculation method was drenching soil around plants with a conidial suspension of the pathogen; the additional injury to roots gave a higher incidence of infected plants (87%) than in plants without root injury (72%). Disease symptoms appeared earlier and progressed more rapidly in plants with injured roots. These two inoculation methods caused complete plant desiccation in 62% of the plants at the soft dough growth stage (Fig. 5, inset). Disease symptoms appeared 27 days after emergence in plants sown in inoculated soil. Thereafter, the disease progressed slowly, and 40% of the plants showed wilt symptoms at the soft dough growth stage. Leaf sheath inoculation produced disease symptoms within 8 days, but only 18% infected plants were recorded at the soft dough growth stage. Leaf inoculation with or without injury was the least effective, causing veinal necrosis in only 1% of the plants, and symptoms did not progress to other plant parts. Disease symptoms were not observed in uninoculated check plants.

DISCUSSION

The results of this investigation show that *A. strictum* was responsible for the wilt of sorghum plants at Pantnagar. This is the first report of this disease on sorghum in India. The occurrence of this pathogen in sorghum seed has been
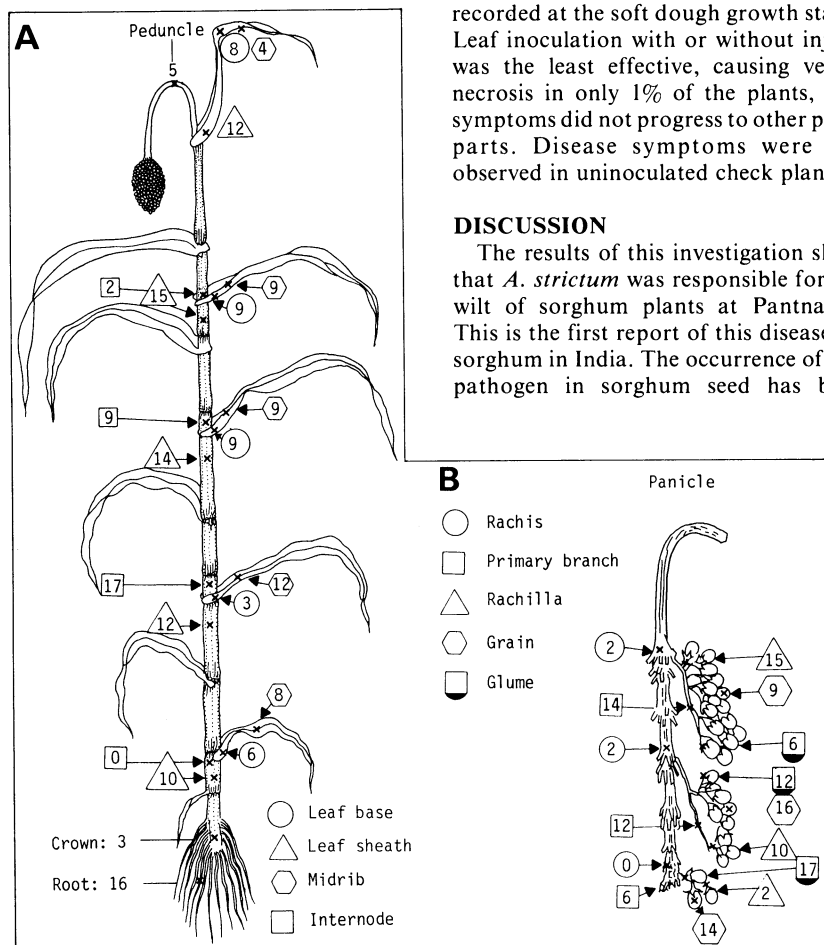

Fig. 4. Parts of **(A)** sorghum plant and **(B)** panicle from which *Acremonium strictum* was isolated. Numbers refer to the plant pieces from which *A. strictum* was isolated out of 20 pieces plated on potato-dextrose agar.
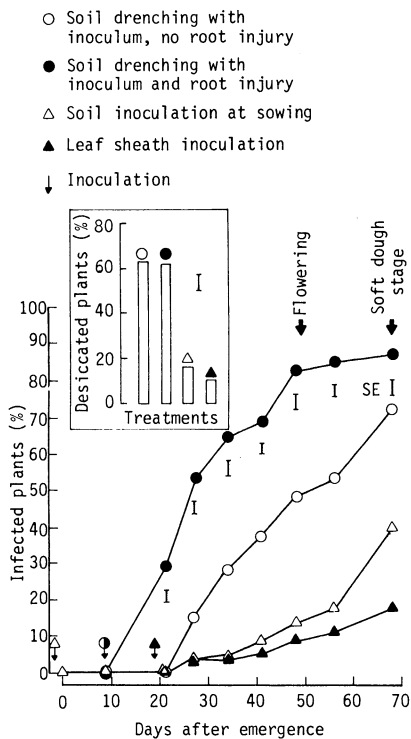
○ Soil drenching with
  inoculum, no root injury
● Soil drenching with
  inoculum and root injury
△ Soil inoculation at sowing
▲ Leaf sheath inoculation
↓ Inoculation

Fig. 5. Progress of Acremonium wilt incidence over time and percentage of plants desiccated at soft dough growth stage (inset) in inoculation treatments that produced symptoms away from inoculation/infestation sites.

reported (12), but its systemic colonization of the plant and disease transmission through seed has not. The nature of symptom expression, occurrence of the pathogen in several aerial plant parts separated by considerable time and space from the inoculated roots, and the presence of the pathogen in the entire plant system suggest that *A. strictum* systemically colonized the sorghum plants. The possibility of airborne inoculum infecting aerial plant parts was negligible in our experiments, because these were not directly exposed to

inoculum and no sporulation occurred on infected plant parts.

Systemic colonization of plants by vascular wilt fungi mainly occurs because spores are transported in the transpiration stream (9). The extent of systemic movement of *A. strictum* in sorghum was such that it migrated from roots to grains in the panicle through the stem and from a mother plant to its progenies through seeds. There are three possible routes of entry into the grains from a mother plant: the pathogen may colonize the spikelets from an infected boot leaf while the panicle is enclosed in it, infected glumes may provide inoculum for grain infection after panicle emergence, and the pathogen may enter the grain through the hilar region from infected vascular tissues of the mother plant, as has been demonstrated in fusarial wilts (1,14). When infected grains were plated on PDA, the initial fungal growth occurred at the hilar end, suggesting that the hilar tissues were colonized. Internal colonization of hilar tissues has been demonstrated in chickpea wilt (10). Our studies on seed transmission of *A. strictum* satisfy the requirements for proof of seed transmission (2); however, the actual potential of seed transmission of the disease under field conditions remains to be studied.

The requirements of wounds for successful penetration of host plants by different wilt fungi are variable (16). Although root injury was not essential for entry of *A. strictum*, our data confirmed a previous report (15) that the lag period between inoculation and symptom expression is shorter in root-injured plants, showing their increased susceptibility. In the field, root injury may be caused by cultural operations (e.g., thinning or weeding), by natural plant senescence of the cortex, and by insects, nematodes, and fungi. Direct entry of the pathogen through intact leaves or stem is unlikely unless the

pathogen has direct access to the vascular tissues, e.g., when the leaves are clipped (15). This would require prior damage by such agents as leaf-feeding and boring insects. The role and relevance of edaphic and environmental factors in Acremonium wilt incidence deserve attention.

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Table 1. Grain weight, germination, length, and dry weight of roots and shoots of 8-day-old sorghum seedlings raised from *Acremonium*-infected and healthy grains

Treatment	100-Grain weight (g)	Germination (%)	Shoot Length (mm)	Shoot Weight (mg)	Root Length (mm)	Root Weight (mg)
Infected seed	1.2	59 (50.0)[a]	18.7	9.6	15.0	25.3
Healthy seed	4.4	88 (69.8)	38.4	32.6	24.0	61.4
SE	±0.09	±1.99 (±1.31)	±3.55	±1.97	±1.06	±8.37

[a]Figures in the parentheses are arc sine-transformed values.