Stroma Development, Honeydew Formation, and Conidial Production in *Claviceps sorghi*

R. Bandyopadhyay, L. K. Mughogho, S. K. Manohar, and M. V. Satyanarayana

Plant pathologist and principal plant pathologist, Cereals Program; research associate, Electron Microscope Unit; and research associate, Cereals Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P.O., Andhra Pradesh 502 324, India.

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ABSTRACT

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The first sign of ergot (*Claviceps sorghi*) disease in sorghum was the appearance of superficial mycelial growth on the proximal end of the ovary 3 days after inoculation with conidial suspension. The ovary was converted into a fungal stroma 2 days later, followed by honeydew exudation from the stroma. Honeydew contained three types of conidia—macroconidia, secondary conidia, and microconidia. Macroconidia were elliptical in shape and were the first to be released in the honeydew. Under humid conditions some macroconidia on the surface of the honeydew germinated by germ tubes that enmeshed to form a hyphal mat; others germinated by erect conidiophores on which apical, pyriform secondary conidia were formed outside the honeydew surface. Small, obovate microconidia were later found in the honeydew. All three conidial forms germinated on and penetrated the stigma. Stromata developed at 14–35 C. Honeydew and conidial production occurred at 14–28 C and RH above 90% for 12–16 hr day^{-1}. Sclerotia developed at 28–35 C and RH below 90% for 2 hr day^{-1}. Above 90%, RH, stromata, and honeydew were colonized by saprophytic fungi and sclerotia were not formed.

Additional keywords: ergot, *Sorghum bicolor, Sphacelia sorghi*, sugary disease.

Ergot of sorghum, *Sorghum bicolor* (L.) Moench, caused by *Claviceps sorghi* Kulkarni, Seshadri & Hegde, is widely distributed in Africa and Asia (7). Severe incidence of this disease has been reported in Botswana (17), India (2), Nigeria (12), and South Africa (4). Ergot causes serious losses in sorghum grain yield and quality, particularly in hybrid seed production in India (21).

The path of infection of the ergot fungus in sorghum florets has recently been described (9). Conidia of the pathogen germinate by germ tubes that penetrate the stigma and grow down the style and colonize the ovary. The ovary is converted into a fungal mass (stroma) from which honeydew is exuded. Later, under suitable environmental conditions, the stroma is transformed into a hard sclerotium. The teleomorph of *C. sorghi* is formed when the sclerotium germinates to produce a stalked globose stroma containing perithecia and ascospores (14). Because sclerotia do not readily germinate in nature, the role of ascospores in ergot epidemiology is unknown. Also little known are details of the development of the anamorph of *C. sorghi*, called *Sphacelia sorghi* McRae, which is the prevalent form of the pathogen. Futrell and Webster (13) suggested conidia from alternate grass hosts and infected panicle debris in soil were sources of primary inoculum.

The honeydew contains macroconidia (14,22) and microspores (16) or microconidia. Recently Frederickson et al (10) reported that an isolate of *S. sorghi* from Zimbabwe produced secondary conidia that originated from macroconidia. They also showed that secondary conidia could be disseminated by wind. In this paper we describe the development of the fungal stroma in infected ovaries, and the formation of macroconidia, secondary conidia, and microconidia at different temperatures and relative humidity.

MATERIALS AND METHODS

Stroma development and spore production. Three sorghum genotypes (male-sterile line 296A, the improved cultivar ICSV 1, and a landrace cultivar IS 24450) were grown in a Vertisol in 30.5-cm-diameter plastic pots. Panicles were spray-inoculated until runoff with 10^6 conidia ml^{-1} suspension of *C. sorghi* in water (prepared from fresh honeydew) when stigmas of the top 25% spikelets of 296A had emerged. Spikelets of five primary branches of ICSV 1 and IS 24450 were hand emasculated before flowering, and were inoculated similarly when stigmas had emerged. After inoculation, plants (24 plants, four to a pot for each genotype) were kept for 24 hr in a chamber with humidifiers

TABLE 1. Signs and symptoms of sorghum ergot (*Claviceps sorghi*) during the early stages of stroma development and conidial production

Hours after inoculation	Signs and symptoms on florets
24	No change in pistil.
48	Stigma lost turgor and became limp. Stigma feathers ash colored. Grey to light brown discoloration visible in stylar area adjoining the stigma.
72	Stigma collapsed and drying. The transmitting tissue in style light brownish and visible through the translucent stylar wall tissue. Basal part of ovary whitish green in some pistils, but in others a white fungal stroma visible on the lower third of the ovary.
96	Stigma dried but still attached to discolored style. Nearly half to two-thirds of the ovary from the base upwards converted into a stroma. Longitudinal section of stroma showed the basal part of ovary wall and ovule replaced by involulating mycelium that formed the stroma. Stroma visible above the glumes.
120	Ovary almost completely colonized and converted into a stroma except for part of the ovule cap. Stigma dried but ovule cap and style still attached to the stroma. Honeydew containing macroconidia began to exude from the stroma.
144	Whole ovaries in most florets converted into stromata. Abundant honeydew containing macroconidia and secondary conidia dripped from florets.
168	Honeydew contained macroconidia, secondary conidia, and microconidia.

to provide high humidity (above 90% RH). After incubation, two pots of each genotype were kept for symptom development and conidial production in a greenhouse, and in two growth chambers (Conviron Model CG 1011, Controlled Environments Ltd., Winnipeg, Canada) labeled A and B. Day and night temperatures and the duration of RH above 90% were 24 and 14 C and 12 hr day^{-1} in growth chamber A, 35 and 28 C and 2 hr day^{-1} in growth chamber B, and 28 and 23 C and 16 hr day^{-1} in the greenhouse. The growth chambers were lighted with fluorescent lamps (4.5×10^3 lx) to provide a 12-hr photoperiod.

Ten pistils of inoculated spikelets were removed from the glumes and examined for ergot symptoms on a daily basis up to 14 days after inoculation, and thereafter at 1- to 3-day intervals until sclerotia were formed. Infected spikelets that oozed droplets of honeydew were marked on the glumes with ink to identify the day when honeydew oozed. This helped to determine the age

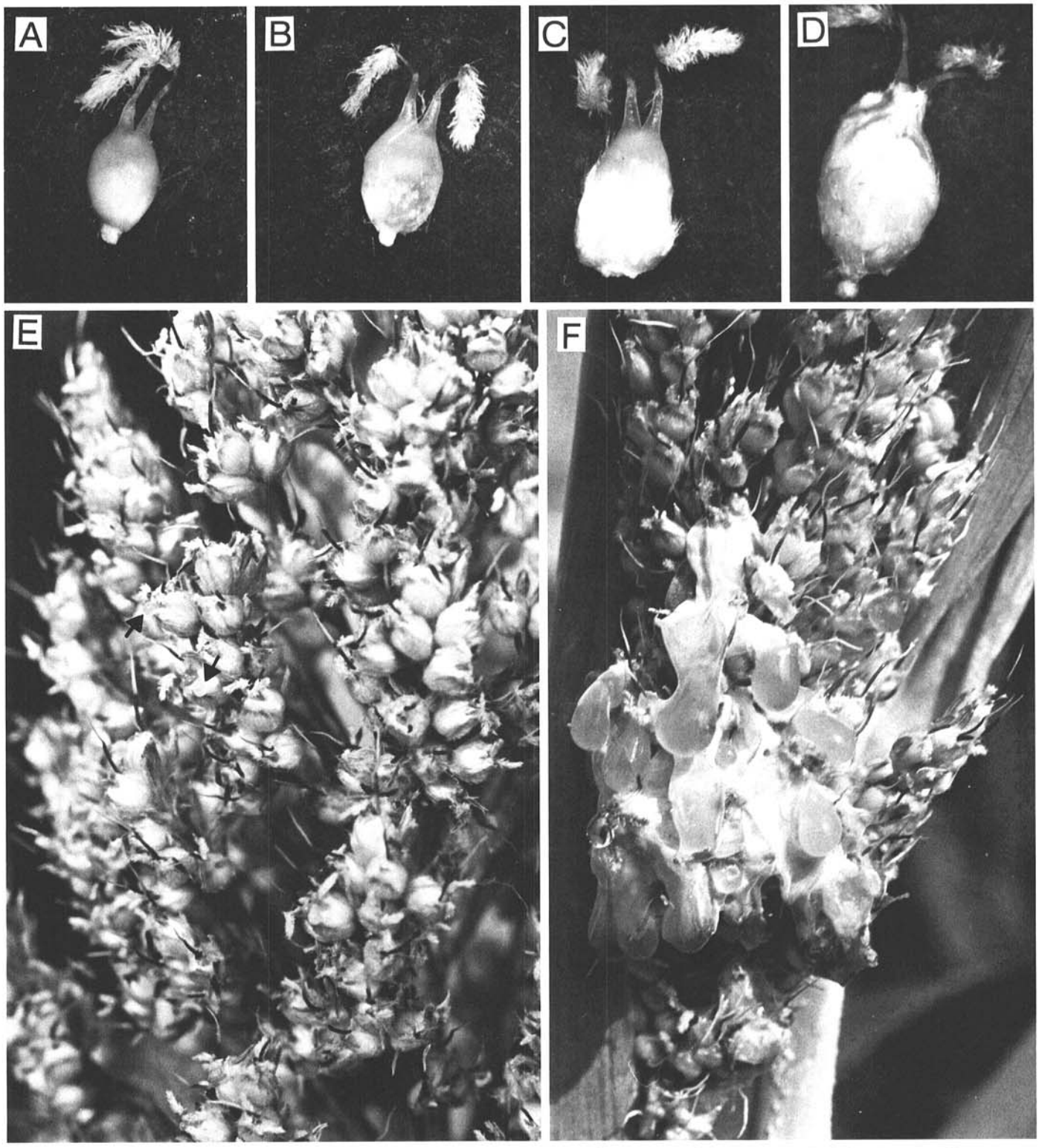

Fig. 1. Early signs of sorghum ergot caused by *Claviceps sorghi*. **A–D,** Acropetal development of fungal stroma in infected ovary. Glumes were removed from the spikelets to show the white stroma on the ovary. **E,** White stromata visible above the glumes of spikelets of severely infected panicle 6 days after inoculation. Small honeydew drops (arrows) are visible in a few spikelets. **F,** Large, brown honeydew with a fungal mat at its proximal end. The powdery appearance on the mat is due to secondary conidia.

of the honeydew when observed on subsequent days. Honeydew was sampled at 0.5, 1, 1.5, 2, 4, 7, and 24 hr after first appearance, and then at 24- to 48-hr intervals up to 14 days to determine the types and amount of conidia produced. Drops of honeydew formed during the first 4 hr were too small to collect for determining conidial concentration. Instead, individual drops were collected with an uncalibrated capillary tube, smeared on to microscope slides, and examined under the light microscope to record the occurrence of conidia. Later, when sufficient honeydew was oozing out of the stroma, 1 ml was collected at 7 and 24 hr after honeydew formation, diluted with water, and the concentration of conidia measured with a hemacytometer. At least 100 conidia of each type were measured to determine size.

The formation of secondary conidia from macroconidia was examined with a scanning electron microscope (SEM) and a light microscope. Intact infected spikelets with 2- to 3-day-old honeydew, whitish in appearance, were excised from panicles and directly attached to stubs, sputter-coated with gold (18), and observed under a JEOL 35CF scanning electron microscope. To study the formation of macroconidia and microconidia, sporulating fungal stromata were fixed for 72 hr in 3% glutaraldehyde and dehydrated for 24 hr each in ethylene glycol monomethylether, ethanol, propanol, and butanol series (8). Tissues were then embedded in methacrylate Historesin (LKB-Produkter AB, S-16126, Bromma, Sweden), sections 2 μm thin prepared, affixed to glass slides, stained with toluidine blue (19), and observed under a light microscope.

Conidial germination and penetration of stigma. Infectivity of the three conidial forms was determined on the sorghum male-sterile genotype 296A, and on the emasculated spikelets of the ergot-susceptible, male-fertile genotype IS 24450 in the greenhouse. Freshly emerged stigmas of these genotypes were sprayed with a mixed suspension of macroconidia, secondary conidia, and microconidia prepared from 4- to 5-day-old honeydew. Pistils were sampled 8, 24, and 72 hr after inoculation and observed for conidial germination and penetration of germ tubes under SEM and fluoroscence microscope. For SEM, pistils were fixed for 3–4 hr in 2% osmium tetroxide, dehydrated for 10 min each in 30, 50, 70, 90, and 100% acetone series, critical point dried in liquid CO_2, sputter-coated with gold (18), and observed in a JEOL 35CF SEM. For observation in fluorescence microscopy, pistils were softened in 2 N NaOH for 2 hr, washed overnight in running tap water, stained, and mounted in aniline blue, and observed by using epifluorescence optics (5).

Fig. 2. A well-developed sclerotium from an infected spikelet that was not colonized by saprophytes.

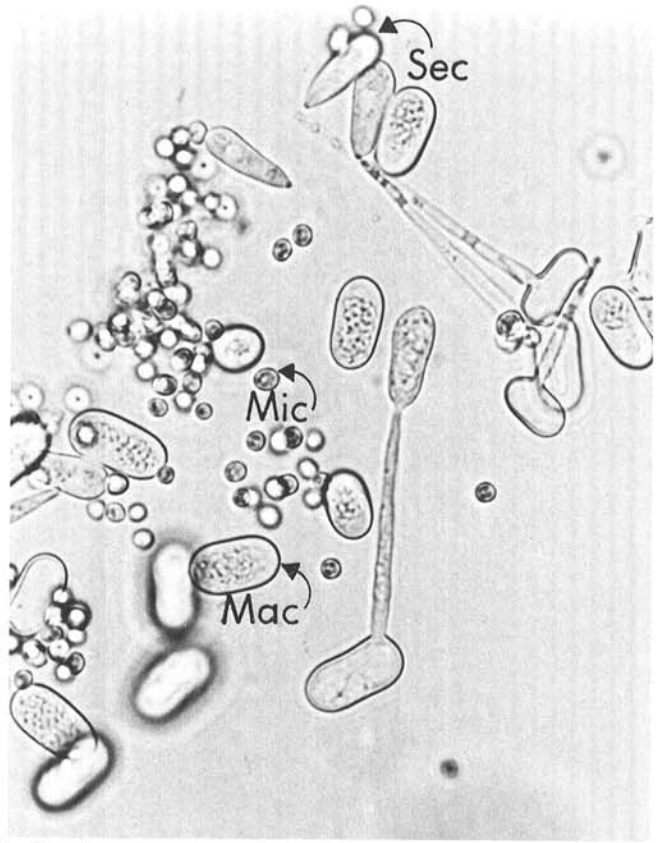

Fig. 3. Micrograph of diluted honeydew showing three types of conidia (macroconidia, Mac; secondary conidia, Sec; and microconidia, Mic) produced by *Claviceps sorghi* in infected sorghum spikelets (×2,640). Note the hilum in secondary conidia and difference in sizes and shapes of the three conidial types.

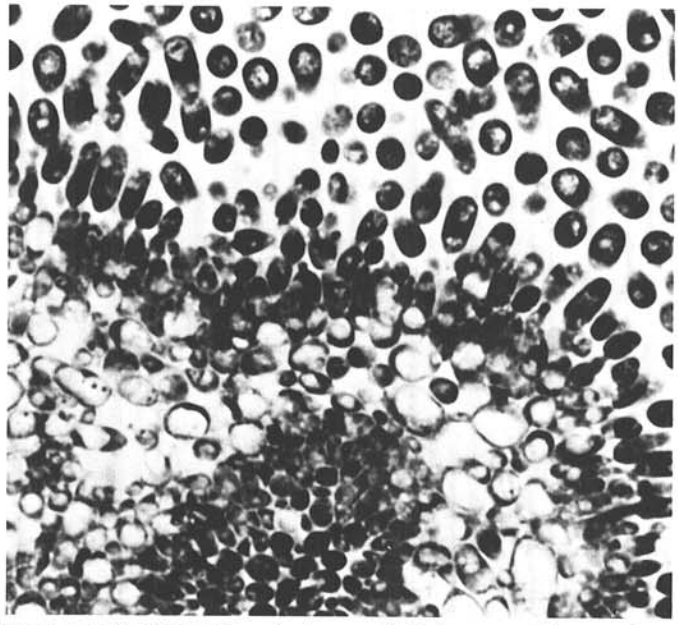

Fig. 4. Longitudinal section of a stroma of *Claviceps sorghi* in a sorghum ovary showing macroconidia originating from conidiophores on the surface of the stroma and in locules inside the stroma (×2,490).

RESULTS

Stroma development and honeydew formation. Table 1 gives information on the early stages of stroma development and honeydew production. The first sign of infection was the appearance of superficial, white mycelial strands at the proximal end of the ovary 3–4 days after inoculation. The mycelial strands grew acropetally through the ovary wall and transformed it into a white fungal stroma (Fig. 1A–D).

Honeydew formed 5–6 days after inoculation when at least three-fourths or more of the ovary was converted into a stroma that was visible above the glumes (Fig. 1E). Honeydew oozed from stromata as tiny, transparent, shiny, and sticky drops that increased in size and dripped from spikelets 30–48 hr after formation. Honeydew production was profuse in the greenhouse and growth chamber A, but scanty in growth chamber B.

In the greenhouse and growth chamber A, the honeydew surface became firm on which a white, sparse, and powdery growth appeared 24 hr after the formation of the honeydew. The whole surface of the honeydew became white in 48 hr (Fig. 1F). The honeydew turned pinkish in color 3–7 days after formation. On examination, such honeydew was found to contain, in addition to conidia of *C. sorghi*, spores of *Fusarium* spp., *Cladosporium* spp., and budding yeast cells. Later, the honeydew had a black crust on the surface due to growth of fungal saprophytes. Stromata also turned pink and dirty brown to black in color 10–14 days after inoculation. Later, such stromata shrank in size, became fibrous, and failed to develop into sclerotia.

In contrast, in growth chamber B, the honeydew lost moisture and became thick in consistency, and no whitish growth or fungal contaminants were seen on it. Stromata gradually hardened, and 21–30 days after inoculation, well-developed sclerotia were formed

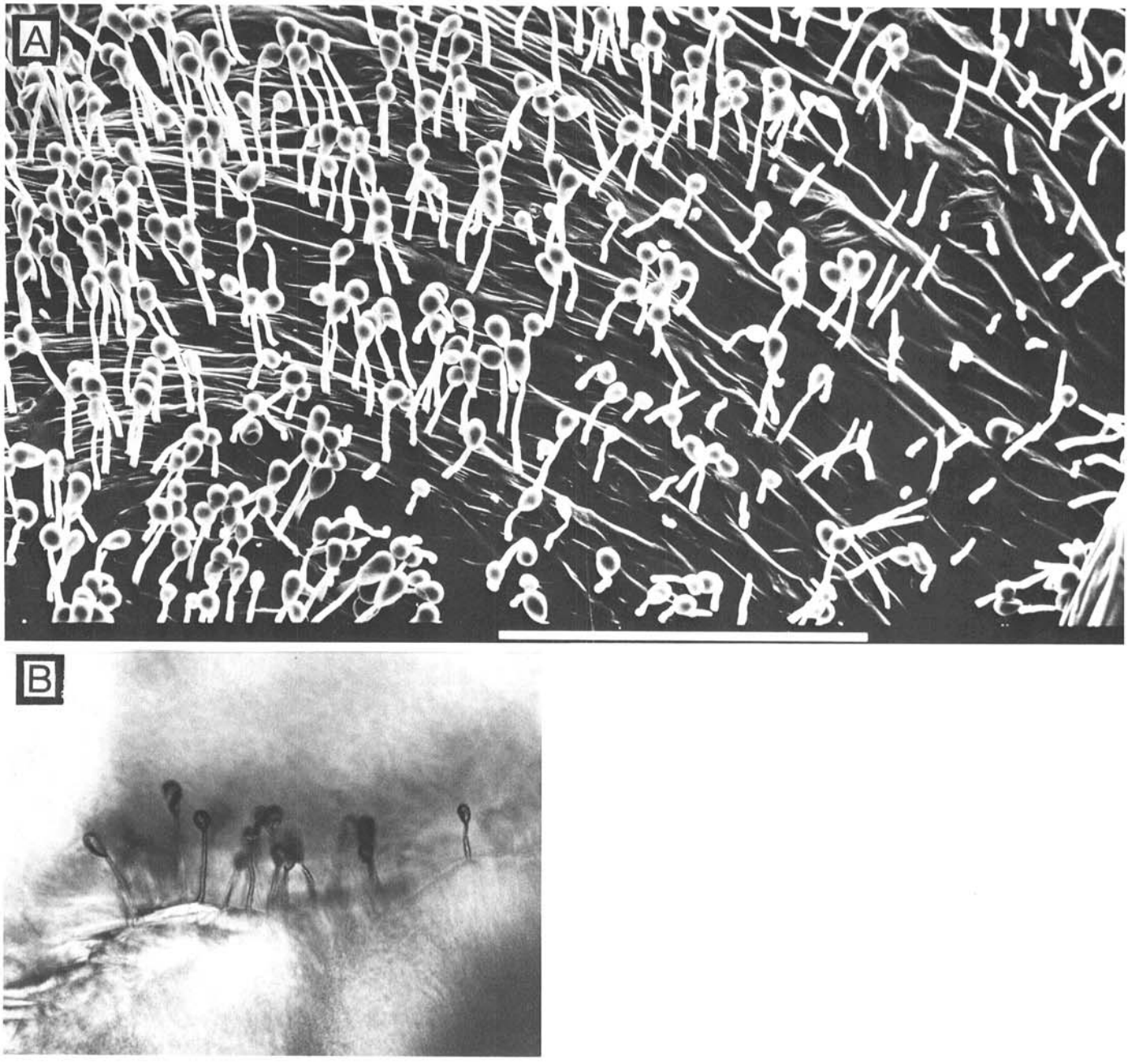

Fig. 5. In vivo production of secondary conidia by *Claviceps sorghi* on the surface of honeydew exuded from infected sorghum spikelets. **A,** Scanning electron micrograph showing secondary conidia borne outside the honeydew at the apex of simple conidiophores that originated from macroconidia (not shown) embedded in the honeydew (see Fig. 6B). Also seen are conidiophores that have emerged or are emerging from the honeydew and have grown to different lengths before producing secondary conidia. Scale bar = 100 μm. **B,** Intact conidiophores bearing secondary conidia on honeydew surface as observed under light microscopy (×1,285).

(Fig. 2). These sclerotia had reddish-flecked broad bases concealed by the glumes, grey to white cylindrical middle, and tapered ends on which stigmas and styles were sometimes still attached.

Spore production. Honeydew production by stromata preceded conidia production. Three types of conidia were produced: macroconidia, microconidia, and secondary conidia (Fig. 3). Macroconidia were produced and released in the honeydew from conidiophores in and on the stroma within 1 hr of honeydew formation (Fig. 4). The number of macroconidia in the honeydew was approximately 16.7×10^6 ml^{-1} 7 hr after its formation and increased by nearly 4 times to 6.4×10^7 ml^{-1} 17 hr later. Macroconidia were hyaline, unicellular, elliptical to oblong with round ends, and measured 5.1–7.7 × 7.7–23.0 μm (mean of 100 macroconidia: 7.3 × 13.2 μm) in the greenhouse, and 5.1–7.7 × 6.4–17.9 μm (mean of 100 macroconidia: 6.9 × 10.9 μm) in growth chamber B. Size of macroconidia in growth chamber A appeared similar to those in the greenhouse and, therefore, not measured.

The white powdery growth on the honeydew surface (Fig. 1F) mentioned earlier was due to the production of secondary conidia (Fig. 5A and B) from macroconidia. The sequence of events leading to the formation of secondary conidia was as follows. The cytoplasm of a few macroconidia on the honeydew surface became thinly granulated and such macroconidia germinated from the apical ends by producing short and thick germ tubes with rounded ends. This occurred as early as 6 hr after the honeydew had oozed from the stroma, and secondary conidia were formed within the next 16 hr. During this period more macroconidia germinated and the germ tubes became longer, with or without branching, and intertwined to form a thin but firm hyphal mat (Fig. 6A) over the surface of the still fluid honeydew containing ungerminated macroconidia. As the flow of honeydew from the stroma increased, the shape of the honeydew mass lengthened, but the enclosing hyphal mat remained firm and intact.

Some macroconidia enmeshed in the hyphal mat germinated by acuminate germ tubes that were thin, unbranched, and 20–52

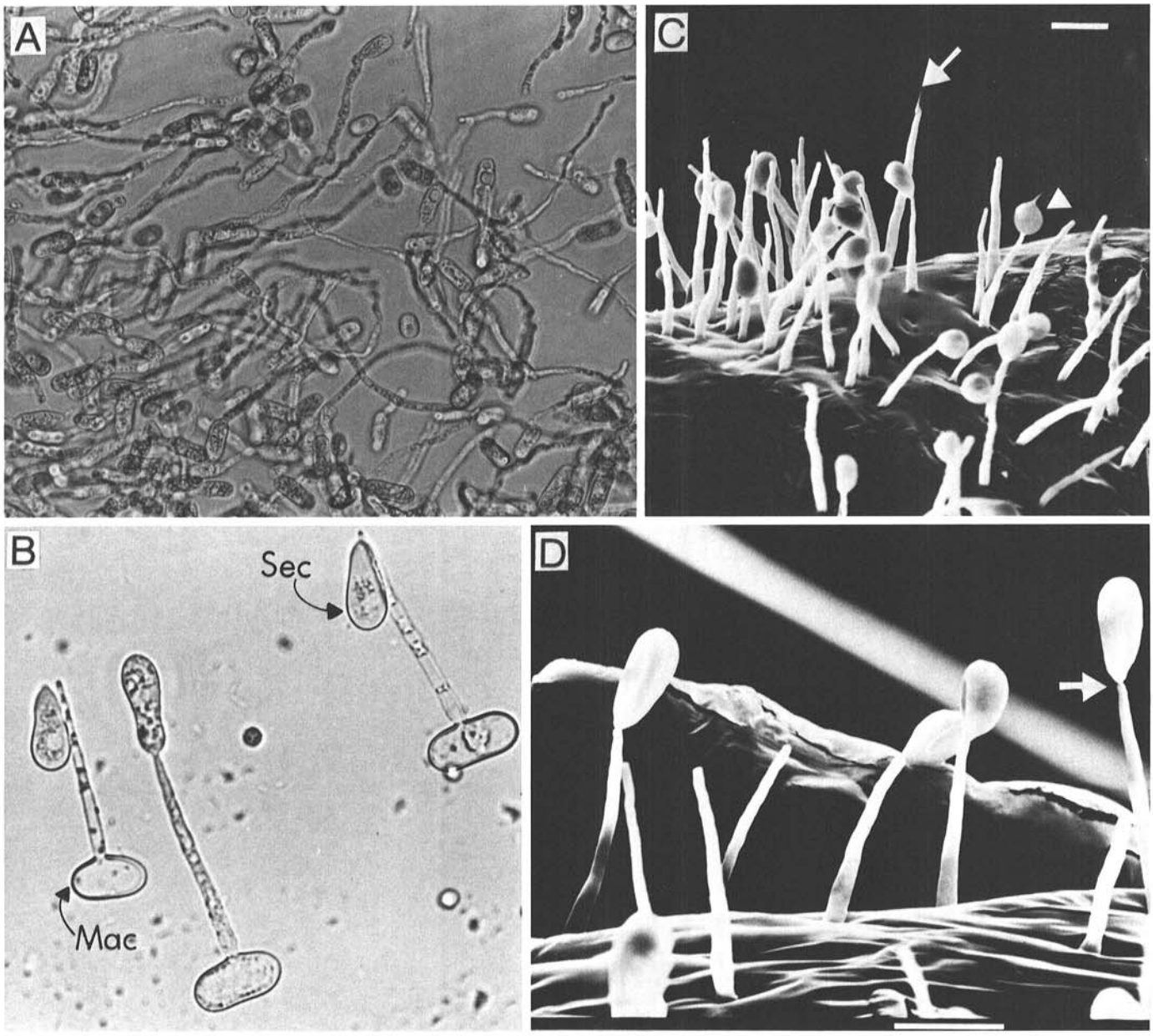

Fig. 6. Events related to formation of secondary conidia of *Claviceps sorghi* on the surface of honeydew in infected sorghum spikelets. **A,** Macroconidia lining the honeydew surface germinated from apical ends and produced germ tubes that enmeshed to form a fungal mat. The mat was crushed on a slide before it was photographed (×1,210). **B,** Several macroconidia in the mat produced aerial conidiophores on which secondary conidia were borne apically. Also note the difference in shapes of macroconidia (Mac) and secondary conidia (Sec) (×2,500). **C,** Scanning electron micrograph of the honeydew surface showing conidiophores with a pointed structure at the apical end (arrow) that formed a bulb (arrowhead) and finally differentiated into secondary conidia. Scale bar = 10 μm. **D,** Close-up scanning electron micrograph of mature secondary conidia on the honeydew surface. Note the delicate hilar attachment (arrow) between the secondary conidium and conidiophore. Scale bar = 10 μm.

μm long (Fig. 6B). Other macroconidia produced two germ tubes, or a single germ tube which branched later. The erect germ tubes or conidiophores pierced through the hyphal mat and their acuminate ends expanded into a bulb (Fig. 6C) which differentiated into pyriform, hyaline secondary conidia measuring 5.2–7.7 × 7.7–20.5 μm (mean of 100 secondary conidia: 7.2 × 12.1 m) (Fig. 6B). The cytoplasm of the macroconidia passed through conidiophores into secondary conidia during their formation (Fig. 6B). A mature secondary conidium was readily detached from the conidiophore by a constriction mechanism at its proximal end (Fig. 6D), which left a scar that distinguished it from a macroconidium (Fig. 3).

Honeydew also contained hyaline, round to obovate microconidia (Fig. 3) that measured 2.6–5.1 × 2.6–6.7 μm (mean of 100 microconidia: 3 × 4 μm). They were observed 2 days after

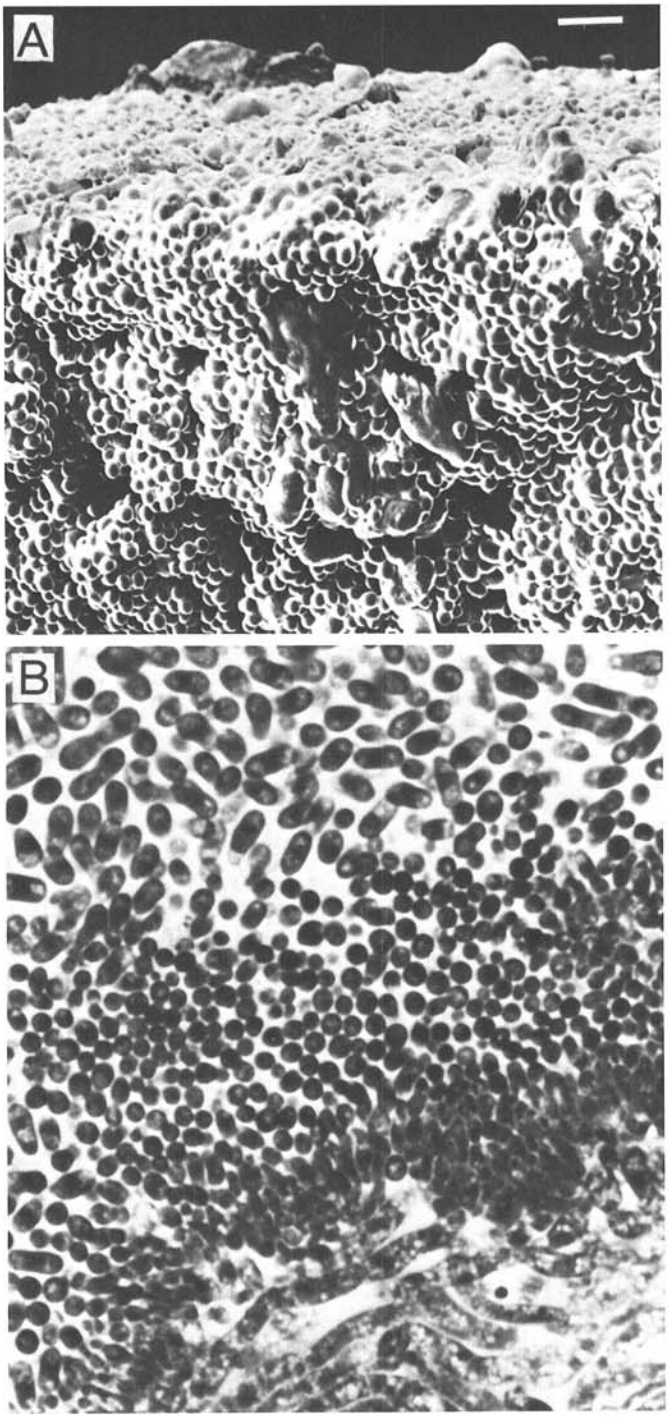

Fig. 7. Microconidia of *Claviceps sorghi* in infected sorghum spikelets. **A,** Many small microconidia and a few macroconidia (surrounded by microconidia) in the fragile crust that formed around developing sclerotia of the sorghum genotype IS 24450. The scanning electron micrograph is a cross-sectional view of the crust. Scale bar = 10 μm. **B,** Longitudinal section of fungal stroma showing round to obovate microconidia originating from conidiophores on the fungal stroma. Also seen are macroconidia above the layer of microconidia (×2,490).

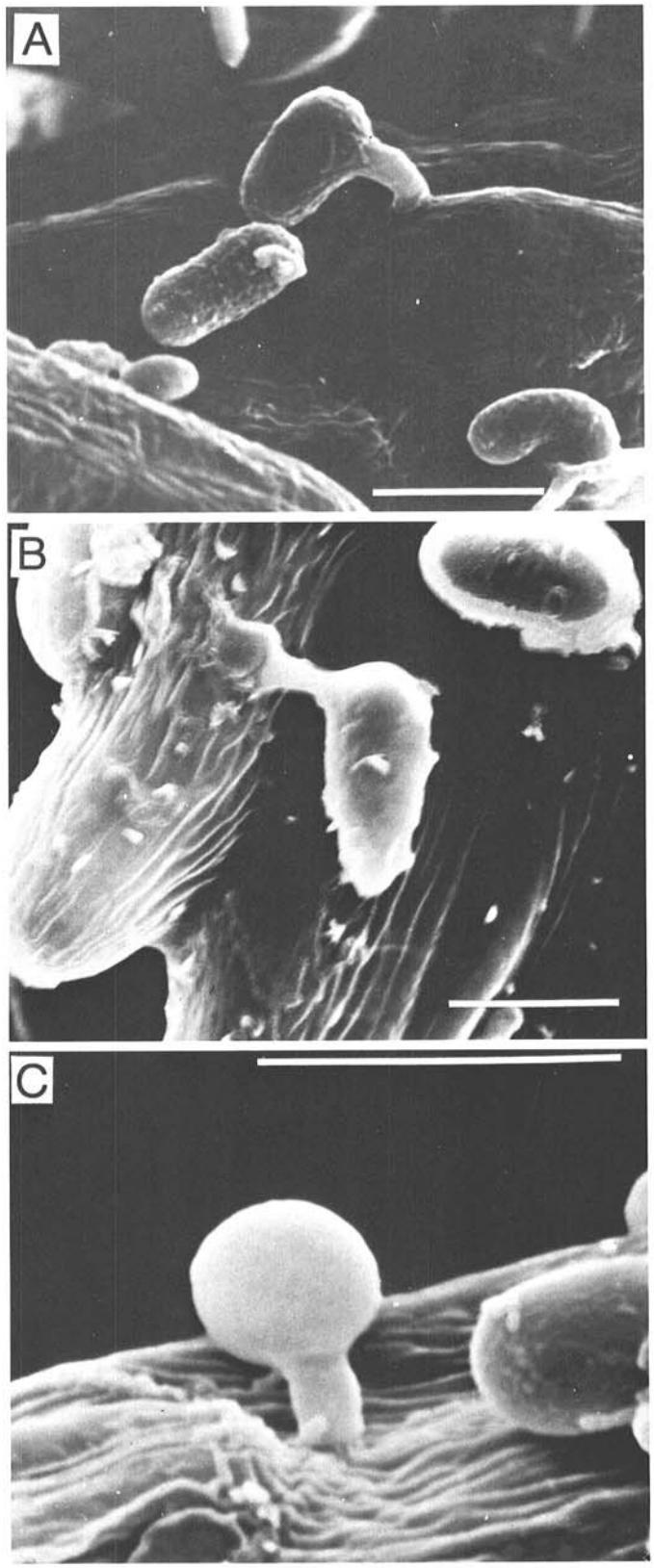

Fig. 8. Scanning electron micrographs showing germination on, and penetration of stigmatic papillae by **A,** *Claviceps sorghi* macroconidia, **B,** secondary conidia, and **C,** microconidia 72 hr after inoculation of the stigma of a male-sterile sorghum genotype 296A. Scale bar = 10 μm.

honeydew formation, and their numbers increased as the honeydew aged. A dense matrix of microconidia was found in a white, fragile crust around the developing sclerotia in the sorghum genotype IS 24450 (Fig. 7A) in growth chamber B. The origin of microconidia was unclear. They appeared to have originated either from conidiophores in the stroma (Fig. 7B), or by budding of macroconidia with which they were closely associated (Fig. 7A).

Conidial germination and penetration of stigma. Macroconidia germinated on the stigma by producing one to four germ tubes, one of which penetrated between cells of the stigmatic papillae (Fig. 8A) within 24 hr after inoculation. Secondary conidia germinated usually by one germ tube that also penetrated the stigmatic papillae (Fig. 8B). Microconidia germinated and penetrated the stigmatic papillae directly by a thick germ tube (Fig. 8C).

DISCUSSION

Like ergots of other cereals (15), sorghum ergot appears to be a grain replacement disease because a fungal stroma grows in place of the grain after successful infection of the ovary (9, Table 1). Our studies confirm the report of Sundaram (20) that the earliest manifestation of infection is evident as superficial fungal growth on the ovary at least 3 days after inoculation, and can be seen if glumes that enclose the infected ovary are opened. The pathogen colonized the ovary for another 2 days before honeydew formed and oozed from infected spikelets. This is the most common external sign of the disease (12,17,22).

Our observations showed that temperature and RH affected honeydew formation and spore production, but not stromata development. Temperatures from 14 to 28 C combined with RH above 90% for 12–16 hr day^{-1} were highly conducive to conidial production, and hence pathogen spread, but not for the differentiation of stromata into sclerotia. In contrast, at 28–35 C and RH below 90% for 22 hr day^{-1} stromata developed into sclerotia, but honeydew and spore production were suppressed. Molefe (17) also reported honeydew production at 20 and 25 C but not at 40 C. At this latter temperature no indication was given as to whether the ovaries had been infected. There is need for further detailed studies under controlled conditions on the effects of temperature and relative humidity on sorghum ergot disease development.

Our studies confirm previous reports that the growth on the honeydew of fungal saprophytes, such as species of *Fusarium*, *Cerebella*, and *Cladosporium* (13), and yeast cells (3) arrest sclerotial growth. Colonization by saprophytes may be one of the reasons why sclerotia do not readily germinate in nature.

C. sorghi has tremendous potential for reproduction and spread as shown by the production of three types of conidia, all capable of germination and penetration of the stigma. The occurrence of macroconidia (14,22), microconidia (16), and secondary conidia (10), and the formation macroconidia from conidiophores in the stroma (9), have previously been reported. In addition to confirming these reports, we provide information on how secondary conidia and microconidia are produced in vivo. The method by which microconidia are formed from conidiophores in the stroma, and from macroconidia in the honeydew of *C. sorghi* appears to be similar to that in *C. gigantea* Fuentes, Isla, Ullstrup & Rodriquez in maize (11) and *C. fusiformis* Loveless in pearl millet (23). The formation of secondary conidia from macroconidia in in vitro tests has also been reported in unidentified ergot fungi from the grasses *Cynodon dactylon* (L.) Pers., *Pennisetum hohenackeri* Hochst. ex Stued (24), and *Cenchrus ciliaris* L. (1).

Secondary conidiogenesis in *C. sorghi* is unique because of the rapidity and the manner in which it occurs in the honeydew. Macroconidia inside the honeydew do not usually germinate, probably because of the high osmotic potential caused by the high sugar concentration in the honeydew matrix (6). However, being hygroscopic (20), the honeydew surface may absorb water from the atmosphere, which would lower the osmotic potential. As a result, under humid conditions, macroconidia on the honeydew surface would germinate as shown in this study. Two methods of germination were observed. In the first method, macroconidia germinated by long germ tubes that enmeshed to form a firm hyphal mat on the honeydew surface. The second method of germination involved the extension of germ tubes outside the honeydew. These germ tubes were functionally aerial conidiophores bearing apical secondary conidia that were easily detached and are dessiminated by wind (10). The wind dispersal mechanism increases the potential for the rapid spread of the pathogen, which is otherwise believed to spread by rainsplash and by insects that feed on the honeydew (13).

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