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Etiology

The Morphology and Disease Cycle of Ergot Caused by *Claviceps fusiformis* in Pearl Millet

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ABSTRACT

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Sclerotia of the pearl millet ergot pathogen, *Claviceps fusiformis*, germinated in moist sand in the laboratory, in potted soil in the screenhouse, and in the field. Germinating sclerotia produced one to 16 fleshy stipes each with a globular capitulum which contained pyriform perithecia. Asci contained in perithecia were long with an apical pore. Ascospores trapped on cellophane tape and greased microscope slides averaged 127.7 × 0.5 μm. Macroconidia contained in fresh honeydew were hyaline, unicellular, fusiform, and averaged 15.9 × 3.9 μm. Microconidia produced in chains from the tips of the germ tubes of macroconidia were globular, unicellular, hyaline, and averaged 5.9 × 2.5 μm. The primary disease cycle of ergot was shown to begin with airborne ascospores discharged from the germinating sclerotia and infecting pearl millet at flowering. Honeydew appeared 6–7 days after inoculation with ascospores and contained numerous macroconidia which play an important role in secondary spread of the disease. Conidia-to-conidia cycle took 4–6 days, and matured sclerotia were observed 20–25 days after inoculation.

Additional key words: *Pennisetum americanum*.

Ergot of pearl millet [*Pennisetum americanum* (L.) Leeke], caused by *Claviceps fusiformis* Loveless, has become an increasingly important disease with increased commercial cultivation of hybrid pearl millet in India (10,13,14). In addition to directly reducing grain yield the disease adversely affects grain quality by contaminating the grain with the toxic alkaloid-containing sclerotia of the pathogen (1–4,8). To develop reliable control measures, knowledge of biology and epidemiology of the disease is important. Although studies on sclerotial germination (5,9,11) and on conidial germination and infection (12) have provided some useful information on the biology of the pathogen, the relative roles of sclerotia and conidia in the epidemiology of the disease have not been clearly understood. During the past 4 yr at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Center we have studied several aspects of the biology and epidemiology of the disease which are reported in this paper.

MATERIALS AND METHODS

Sources of conidia and sclerotia. Sclerotia and conidia were collected from pearl millet inflorescences infected with *C. fusiformis* in the ICRISAT Center ergot nursery.

Morphology and anatomy of sclerotia. Matured sclerotia were examined for variation in shape, size, color, and compactness. To study the internal details, sclerotia of various morphology were washed with tap water and dried on blotter paper. Hand sections were made transversely and mounted in lactophenol-trypan blue on microscope slides.

Germination of sclerotia. During June 1978, an experiment was set up in petri dishes (9-cm diameter) at 25 C as well as in pots (25-cm diameter) in a screenhouse at 25–30 C. Sclerotia were placed in sterile sand in petri dishes and sterile red soil in pots. A total of 16 treatments including two storage periods (1 mo at 25–30 C and 12 mo at 15–30 C), two sizes (large and small), two depths (completely and partially buried), and two methods of placement (vertical and horizontal) with four replications were made in a completely randomized block design. In each replication, 25 sclerotia were used per petri dish or pot. Sand and soil were kept moist by watering. Observations on sclerotial germination were made at weekly intervals for 56 wk.

Germinating sclerotia were observed microscopically for various structures including stroma, perithecia, asci, and ascospores.

Trapping of ascospores. Strips of cellophane tape were fixed across the rim of a petri dish containing germinating sclerotia in moist sand such that the sticky side of the tape faced germinating sclerotia. After 3 days incubation at 25 C, the strips were removed, placed in cotton blue on microscope slides and observed for the presence of ascospores. Also, greased microscope slides and potato-dextrose agar (PDA) plates were exposed for 24 hr to the germinating sclerotia placed on moist blotter paper in a petri dish. The exposed PDA plates were incubated at 25 C for 7–10 days. The pathogenicity of the culture obtained was tested in the screenhouse.

Sclerotial germination and flowering time in pearl millet. During the rainy seasons of 1979 and 1980, pearl millet hybrids ICH-105 and BJ-104 were raised outdoors in pots with and without sclerotia. Observations were recorded on the time of initiation of sclerotial germination relative to the commencement of flowering in the hybrids.

Sclerotial germination and ergot development. *Experiment 1.* During July 1978, at ICRISAT Center farm, pearl millet hybrid ICH-105 was sown in two isolation plots (14 rows × 10 m) having no history of pearl millet culture. Rows were spaced at 75 cm and plants at 20 cm in a row. Each isolation plot was located at least 500 m from any other pearl millet crop. In one isolation plot, 1 wk after seedling emergence, 25 sclerotia (stored for 12 mo) were placed 1 cm deep at 1-m intervals in perforated plastic pipes (4 cm diameter × 6 cm long) in alternate rows. The other plot of the hybrid without sclerotia was maintained as the control.

Experiment 2. In July 1980, the two treatments (hybrid ICH-105 plots with and without sclerotia) were replicated three times in six isolation plots. Observations on sclerotial germination were made at weekly intervals beginning 30 days after seeding until appearance of ergot symptoms. Ergot incidence (percent inflorescences infected) was assessed three times in experiment 1 and four times in experiment 2 from the appearance of honeydew symptoms to crop maturity.

Inoculation with ascospores. In the screenhouse experiment, a set of six protogynous inflorescences of a male-sterile (ms) line, 5141-A, grown in pots, was exposed for 72 and 96 hr by positioning the inflorescences 8–10 cm above germinating sclerotia on the

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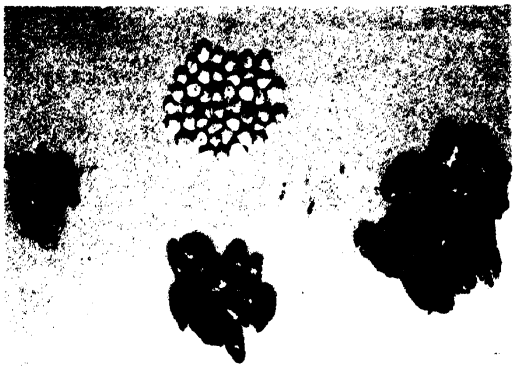

Fig. 1. Various sizes of sclerotia of *Claviceps fusiformis* compared to pearl millet grain (top).

Fig. 2. Germinating sclerotia of *Claviceps fusiformis* showing: several fleshy stipes each with a capitulum (top), and capitula with perithecial projections (bottom).

surface of the potted soil. To prevent contamination, double-layered muslin cloth was placed over inflorescences and tied underneath the brim of the pot. Another set of protogynous inflorescences of the same 5141-A plants was exposed to nongerminating sclerotia placed in the potted soil and serving as the control. High relative humidity was maintained by frequently spraying the inflorescences with water. Inoculated inflorescences were scored for ergot severity according to an ergot severity assessment key (14).

Morphology and germination of conidia. An aqueous conidial suspension obtained from fresh honeydew on infected inflorescences was incubated in cavity slides in moist chambers at 25 C and observed microscopically at 2-hr intervals for germination. Macro- and microconidia (100 each) were measured with an ocular-grid eyepiece and examined for shape and color.

Inoculation with conidia. Ten protogynous inflorescences of an ms line, 5141-A, grown in pots in the screenhouse and protected from pollen by covering with parchment bags, were spray inoculated with a honeydew conidial suspension ($\sim 10^6$ conidia per milliliter) obtained from infected inflorescences, and rebagged immediately after inoculation. Ten other inflorescences of the same line were water-sprayed and bagged to serve as the controls. High relative humidity (about 80%), provided by operating overhead mist sprays 3–4 times a day, 10 min each, and moderate temperature (20–25 C) were maintained in the screenhouse throughout the period of incubation and disease development. Ergot severity was assessed 10 days after inoculation by reference to a standard ergot severity assessment key (14).

RESULTS

Morphology and anatomy of sclerotia. Sclerotia varied in shape (elongated to round), size (3.6–6.1 × 1.3–1.8 mm), color (light brown to dark brown), and compactness (hard to brittle with cavities) compared to the normal grain of pearl millet (Fig. 1).

Transverse sections of sclerotia revealed a dark-brown, thick outer rind and a medulla composed of pseudoparenchymatous tissue traversed by plectenchymatous strands and usually with cavities. These cavities contained numerous microconidia and few macroconidia with low germinability (2–5%).

Germination of sclerotia. The earliest germination of sclerotia was observed about 4 wk after placement in petri dishes at 25 C or in pots in the screenhouse at 25–30 C. Some sclerotia germinated even at 56 wk after incubation at 25 C. In the screenhouse, however, no germination was observed later than 20 wk after placement in the pots. In repeated experiments, both in pots and petri dishes, the time from placement of the sclerotia to the initiation of germination was erratic. In general, more sclerotial germination was observed in a 12-mo-old lot (12–52%) than in a 1-mo-old lot (6–36%). Variations in sclerotial placement (horizontal and vertical), burial (partial and complete), and size, however, had no marked effect on germinability.

Sclerotia germinated by producing one to 16 fleshy, purplish stipes 6–26 mm long. Each stipe terminated in a globular capitulum which was light to dark-brown with perithecial projections (Fig. 2). Longitudinal sections through capitula revealed numerous pyriform perithecia embedded in somatic tissue in the peripheral region (Fig. 3A). Pyriform perithecia were seen with asci interspersed with paraphyses emerging through ostioles (Fig. 3B, a and b). The asci were long and hyaline with apical pores and narrow ends (Fig. 3B,c). Ascospores released from asci were long, hyaline, and nonseptate 103.2–176 × 0.4–0.5 μm (average of 100 spores 127.7 × 0.5 μm) (Fig. 3B,d).

Ascospores from germinating sclerotia were trapped on cellophane strips and greased-slides as revealed by microscopic examinations. All the PDA plates exposed to germinating sclerotia showed numerous ascospores. White mycelial growth appeared 4 days after incubation at 25 C and sporulation occurred 7 days after incubation. Initially, macroconidia were produced which on germination produced microconidia in chains. Microscopic examination confirmed these to be conidia of *C. fusiformis*. Positive pathogenicity tests further confirmed the identity of the

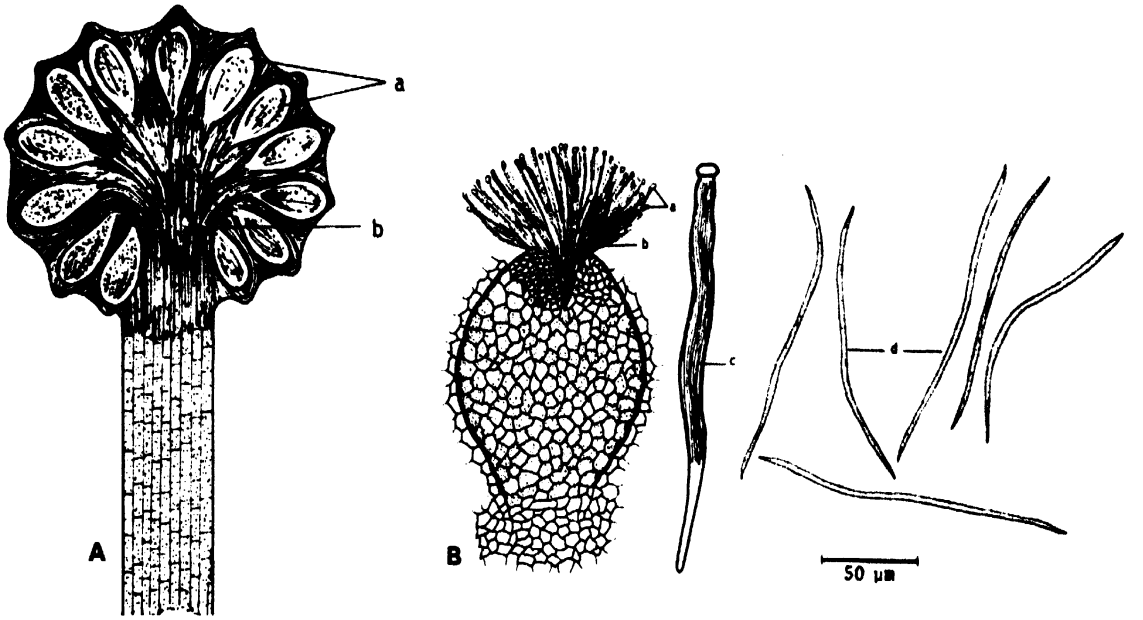

Fig. 3. Diagrammatic representation of the perfect state of *Claviceps fusiformis* showing: **A**, longitudinal sections of a stroma and peripheral embedding of perithecia in the somatic tissue: a = perithecia, b = somatic tissue; **B**, a matured perithecium releasing: a = asci and b = paraphyses; and c = an ascus containing eight ascospores; and d = ascospores.

TABLE 1. Percent ergot severity in pearl millet inflorescences after indicated exposure to sclerotia of *Claviceps fusiformis*

	Germinating sclerotia[a]		Nongerminating sclerotia[a]	
Inflorescence	72 hr	96 hr	72 hr	96 hr
1	30	40	0	0
2	10	40	0	0
3	5	10	0	0
4	5	20	0	0
5	20	10	0	0
6	5	60	0	0
Mean	12.5	30.0	0	0

[a] Same sets of germinating or nongerminating sclerotia in the pot soil were used for both exposures.

pathogen.

Morphology and germination of conidia. Macroconidia present in the fresh honeydew were hyaline, unicellular, and fusiform 12–26.4 × 2.4–6 µm (average of 100 conidia 15.9 × 3.9 µm) (Fig. 4, a). Microconidia were globular, unicellular, hyaline 2.4–10.8 × 1.2–4.8 µm (average of 100 conidia 5.9 × 2.5 µm) (Fig. 4,b). Conidial germination began 16 hr after incubation at 25 C. Each conidium germinated by producing one to three germ tubes from the ends and/or sides (Fig. 4,c). Germ tubes produced septa at the tips before producing macro- or microconidia. Microconidia were produced in chains from the tips of the germ tubes (Fig. 4,d).

Sclerotial germination and flowering time in pearl millet. Sclerotial germination in pots grown with pearl millet hybrids ICH-105 and BJ-104 occurred 40 days after seeding. This coincided with flowering in the hybrids. Many germinating sclerotia were also observed in isolation plots when ICH-105 began flowering.

Infection by ascospores. All the protogynous inflorescences exposed to germinating sclerotia for 72 or 96 hr developed ergot with severity ranging from 5 to 60% within 6–7 days. There was no ergot on inflorescences exposed to ungerminated sclerotia (Table 1).

In both field experiments, the earliest sclerotial germination (2%)

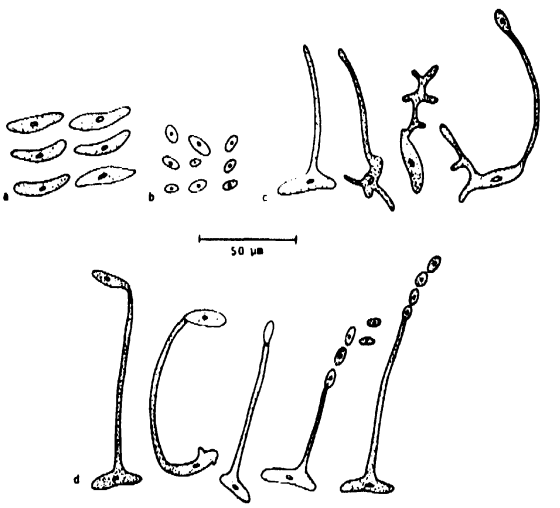

Fig. 4. Conidial types in *Claviceps fusiformis*: a = macroconidia, b = microconidia, c = germinating macroconidia with one to three germ tubes, and d = production of macro- and microconidia on the tips of the germ tubes.

was observed at 31 days after seedling emergence, when the hybrid was at the boot-leaf stage. By 38 days after emergence when the hybrid was at 50% flowering stage, about 5% sclerotia had germinated and ergot symptoms (honeydew) had been visible. Ergot incidence in ICH-105 in the two field experiments is presented in Fig. 5. In both experiments the first ergot infection (2–10% incidence) was observed 45 days after emergence in the plots with sclerotia and no symptoms appeared in the plots without sclerotia. In subsequent observations, however, ergot developed in the nonsclerotial plots also, but the incidence remained low compared with that of the sclerotial plots.

Infection resulting from conidia and sclerotial development. Honeydew appeared on all the inoculated inflorescences 4–6 days

after inoculation. The slimy, turbid droplets, which later turned pink, initially contained only macroconidia which on germination produced numerous microconidia within 2–3 days. The honeydew produced in infected florets dried up slowly and sclerotia became visible 8–10 days after inoculation. The ergot severity ranged from 80 to 90% on the inoculated inflorescences and there was no ergot in the control. Within 20–25 days after inoculation, fully developed sclerotia were seen in the florets (Fig. 6). The process of sclerotial development and maturation was more rapid than that of normal grain in pearl millet, which takes 30–40 days after pollination.

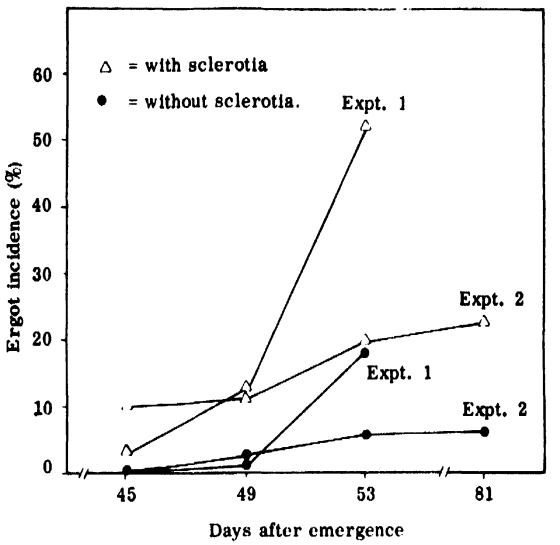

Fig. 5. Incidence of ergot in pearl millet hybrid ICH-105 grown in plots either infested with or without sclerotia of *Claviceps fusiformis* in two experiments during the rainy seasons of 1978 and 1980.

Fig. 6. Inflorescences of pearl millet infected with *Claviceps fusiformis*: the honeydew phase (left) and the sclerotial phase (right).

DISCUSSION

Our observations on morphology and germination of sclerotia and conidia were similar to those of Loveless (5) and Siddiqui and Khan (12), confirming that the causal fungus of ergot in pearl millet is *C. fusiformis* and not *C. microcephala* as reported earlier (11).

Under both field and farmers' grain-storage conditions sclerotia can survive for 8–10 mo with exposure to about three winter mo (December–February) when temperatures ranged from 10–15 C at night and 20–30 C during the day. This is supported by our results that sclerotia stored for 12 mo in the laboratory (15–30 C) germinated and produced ascospores that initiated infection. Prakash and Shetty (9) obtained maximum sclerotial germination (81%) when sclerotia were stored at 37 C for 8 wk, and storage at lower temperatures resulted in reduced germination. However, the storage period (8 wk) and the temperature (37 C) are not comparable to those used by farmers and hence a detailed investigation is needed to clearly understand the effect of storage temperatures and storage periods on sclerotial germination.

The results indicate that the primary disease cycle in pearl millet ergot begins with sclerotia left over in the field after harvest and/or sclerotia mixed with seed at the time of threshing and sown with the seed the next season. Following rain showers, these sclerotia germinate and release ascospores, which initiate infection in pearl millet that is flowering. The coincidence of sclerotial germination and flowering in the crop, both in pot and field experiments and the levels of initial infection in the hybrid crops in the isolation plots infested with sclerotia, and supportive results from the screenhouse experiments, clearly indicate the positive role of sclerotia in the epidemiology of the disease. Sundaram (13) suggested, without providing experimental evidence, that sclerotia get mixed with the soil along with the pearl millet seed and take about 30 to 40 days to germinate, thus coinciding with flowering, and that infection caused by airborne inoculum occurs. In dallisgrass ergot (caused by *C. paspali*), Luttrell (6) observed sclerotial germination in outdoor trays when dallisgrass had begun flowering in the field. A similar observation was recorded by Mantle and Shaw (7) for blackgrass ergot (caused by *C. purpurea*).

The secondary disease cycle within a crop is initiated with the appearance of honeydew. Macro- and microconidia present in the honeydew are disseminated by splashing rain, wind, insects (?), and physical contact with healthy inflorescences. The conidia-to-conidia cycle takes 4–6 days, depending upon weather conditions.

In areas where pearl millet is grown and there is no evidence of alternative hosts; sclerotia from the previous crop are the major source of primary inoculum. Use of sclerotia-free seed might help reduce primary inoculum. Other cultural practices, such as deep plowing, crop rotation, etc, may also reduce sclerotial germination.

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