Claviceps africana sp. nov.; the distinctive ergot pathogen of sorghum in Africa

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Stromata arising from ergot sclerotia from sorghum in Zimbabwe were different in colour and texture from those of *Claviceps sorghi* from sorghum in India, compounding other differences in the dynamics of the early stages of parasitism, the sugar composition of honeydew, the quantitative expression of secondary conidiation and the morphology of sclerotia. The distinctive sexual stage forms the basis of describing the African material as a new *Claviceps* species which also uniquely elaborates a group of dihydrogenated ergot alkaloids that are biosynthetic intermediates leading to the principal product dihydroergosine.

Whereas *Claviceps fusiformis* Loveless was first described as parasitizing pearl millet (*Pennisetum typhoides* Staph & Hubbard) in southern Africa (Loveless, 1967) but typifies also the pearl millet pathogen in India, it has recently become evident that there is no such uniformity amongst African and all Asian ergot pathogens of sorghum, the other small grain crop important in the semi-arid tropics.

Ergot disease of sorghum, otherwise known as sugary disease, was first reported as a conidial fructification from India (McRae, 1917) and first observed in Africa (Kenya) in 1924 (IMI 93464). Following the interest in F1 hybrid tropical cereal production in the late 1960s, ergot was recognized also in Nigeria as a problem in experimental male-sterile sorghums (Futrell & Webster, 1965). Mower *et al.* (1973) observed the teleomorph of the Nigerian pathogen as typical of *Claviceps* but did not describe it. The only mycological descriptions of African sorghum pathogens represent the anamorph and assume analogy with Indian type material of *Sphacelia sorghi* McRae (Loveless, 1964; Mantle, 1968). It has further been assumed that the brief, though acceptable, description of the ascosporogenous stage of an Indian ergot pathogen of sorghum as *Claviceps sorghi* (Kulkarni *et al.*, 1976) applies equally to the ergot pathogens of Africa and Asia; the pathogen does not appear to be present in sorghum grown in the Americas. However, recent comparative studies on Indian and African sorghum ergot pathogens (Frederickson & Mantle, 1988; Frederickson *et al.*, 1989; Frederickson, 1990) have recognized considerable differences between the organisms during parasitism, justifying protracted attempts to generate the teleomorphic fructification from ergot sclerotia collected on sorghum in Zimbabwe, where the fungus is widely endemic. A few such resultant stromata, which appeared to be adequately differentiated for ultrastructural study at Imperial College, provided the classical evidence for the present description of the African pathogen as a new *Claviceps* species, sufficiently distinct from *C. sorghi* by conventional criteria. The distinction is complemented by comparisons of some other biological and biochemical characteristics.

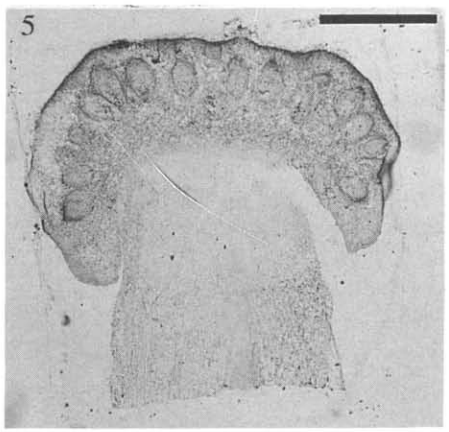
MATERIALS AND METHODS

Germination of sclerotia

Approximately 100 sclerotia (Fig. 1) collected from ripe sorghum at Henderson Research Station, Harare, Zimbabwe during Mar. 1987 were incubated in moist sand in the dark at a temperature cycling daily in the range 19–28 °C. Within 4 wk, a few sclerotia showed signs of stromatal initiation. Three months later one sclerotium had produced clavicipitaceous stromata (Fig. 3), histology of which showed that the capitulae only contained immature perithecia. However, the material provided preliminary evidence of novel morphological characteristics.

Sclerotia collected from ripe sorghum at Matopos, Zimbabwe during Mar. 1988 and stored dry at room temperature (approx. 20–25°) were, in Mar. 1989, enclosed in fine mesh nylon with red Matopos clay soil and buried just below the surface of similar soil in a 20 cm diam. plastic pot. The pot, maintained outside the laboratory, was watered daily. After 4 wk in soil, 10% of the sclerotia had developed one or two globose structures erupting through the cortex. By 6 wk, 80% of sclerotia had germinated, the most precocious having stromata differentiating into the characteristic clavicipitaceous stipe and capitulum (Fig. 4). Despite transfer to fresh, moist

Fig. 1. Sclerotia of *C. africana* (left) and *C. sorghi* (right) from sorghum. The left hand moiety of *C. sorghi* sclerotia consists largely of sphacelial tissue. **Fig. 2.** Germinated sclerotium of *C. sorghi* with a clavicipitaceous stroma arising from the proximal sclerotial portion. **Fig. 3.** Germinated sclerotium of *C. africana* with clavicipitaceous stromata, preserved in formoacetic-alcohol which has removed stipe pigmentation. **Fig. 4.** Fresh *C. africana* stroma showing characteristic pigmentation.
Fig. 5. Spurr resin-embedded longitudinal section of a *C. africana* stroma showing the distribution of perithecia in the capitulum (bar; 0·3 mm). **Fig. 6.** Detail of a perithecium (bar; 52 μm) of the stroma illustrated in Fig. 4. **Figs 7–10.** Transmission electron micrographs of sections through perithecial tissues of a *C. africana* stroma (Fig. 4) showing oblique (Fig. 7) and longitudinal (Figs 8–10) views of asci. Figs 9–10 show an ascospore septum in detail. Bars; 2 μm (Figs 7–9), 0·5 μm (Fig. 10).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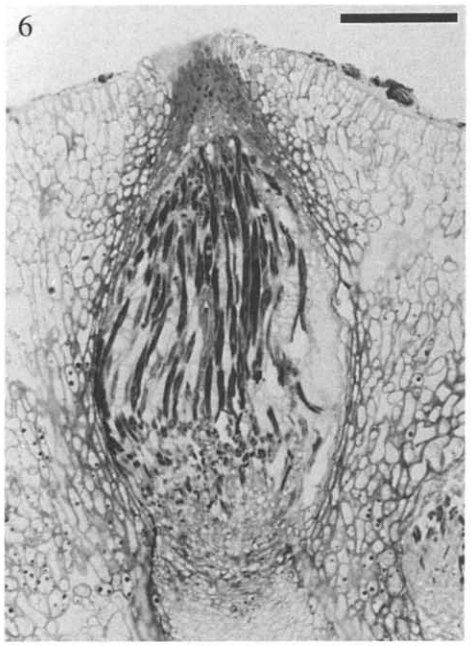
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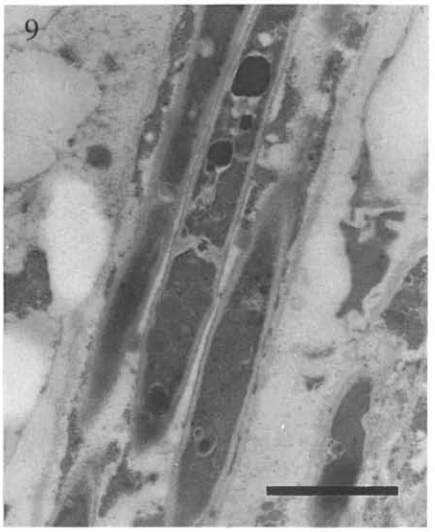
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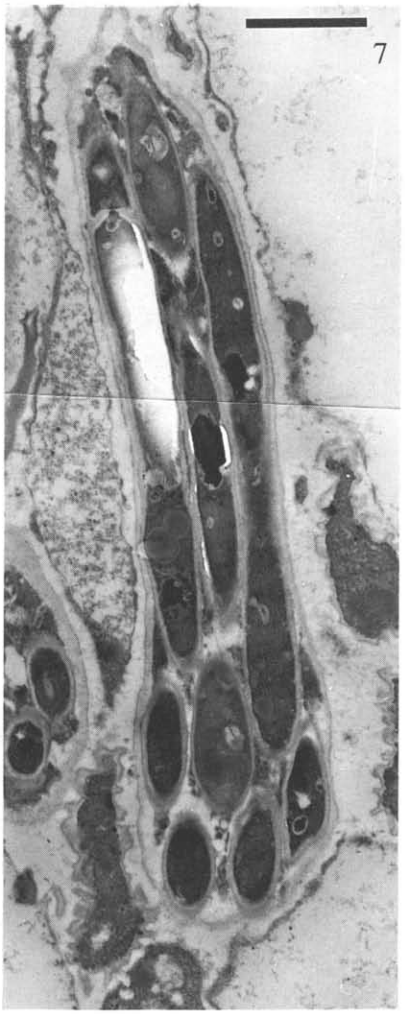
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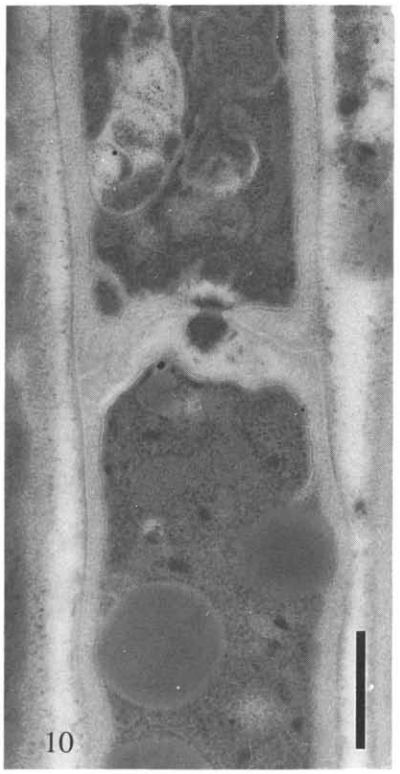
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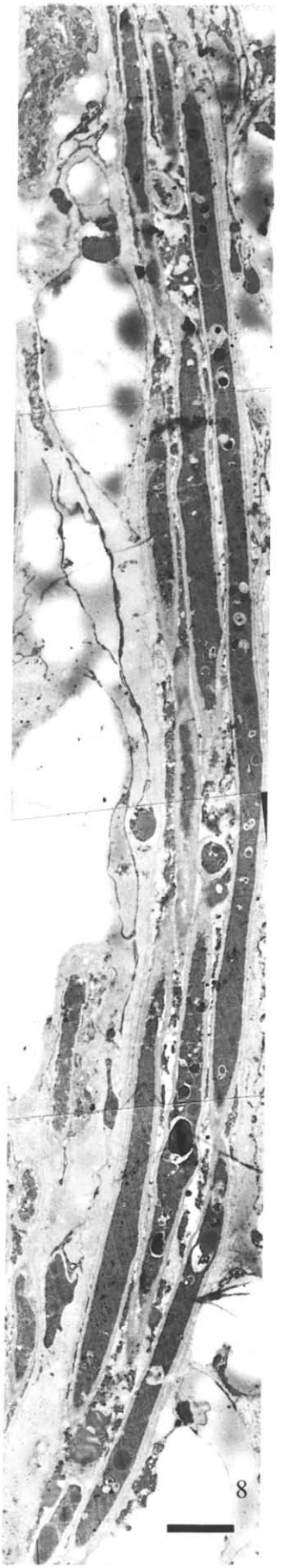
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soil, most of these stromata subsequently deteriorated in the laboratory, leaving only two sclerotia with fully extended stromata. These were the subject of detailed histology.

Sclerotia of *C. sorghi* from sorghum, of the same 1983 collection in Akola, Maharashtra State, India used for infection studies (Frederickson & Mantle, 1988), were incubated on moist sand in the laboratory at 24° and produced stromata within 5 wk.

Experimental pathology and histology

Comparative experimental phytopathology of sorghum ergot fungi was studied on male-sterile sorghum grown in horticultural tunnels as previously described (Frederickson & Mantle, 1988). Histology techniques for light and transmission electron microscopy were as previously described (Frederickson & Mantle, 1988).

Analytical

Ergot alkaloids were extracted from sclerotia, quantified and resolved chromatographically as previously described (Mantle, 1968). Analysis was further refined by reverse-phase HPLC using a methanol:ammonium hydroxide mobile phase (Frederickson, 1990).

Honeydew sugars were analysed qualitatively by descending chromatography on Whatman No. 1 paper in propan-1-ol:ethyl acetate:water (7:1:2). Sugars were quantified by microlitre-scale modification of the Fehlings method for total reducing sugars before and after hydrolysis, and for glucose specifically by the glucose oxidase assay (Frederickson, 1990). Fructose concentration was calculated as the difference between glucose and the total reducing sugars before hydrolysis.

RESULTS AND DISCUSSION

Observation of the teleomorph of the sorghum ergot fungus from two locations in Zimbabwe, and studies of its histology, has confirmed the organism as a *Claviceps* (Figs 3–8, 11–12). The indirect evidence from the original description of *C. sorghi* (Kulkarni *et al.*, 1976), and the direct evidence of the *C. sorghi* ascosporogenous stage derived from Indian sclerotia in the laboratory in London (Fig. 2; Table 1), do not fit the African pathogen. One striking difference is in stromatal colour, involving red-brown pigments in *C. sorghi* (Fig. 2) and blue/purple pigments in the African organism (Fig. 4). Another difference is in the superficial stromatal texture, the prominent white hyphae at the stipe base and around the capitulum edge in *C. sorghi* contrasting with the glabrous characters of the African species. Stromatal differences are complemented by differences in ascus and ascospore dimensions (Table 1), though such parameters do not appear to have critical differential value in *Claviceps* speciation.

Prior to recent observation of the teleomorph, the distinctiveness of African sorghum ergot pathogens had been apparent from comparisons of the sclerotia collected in India and Africa and deposited in the IMI herbarium, but was reinforced when pathotypes from India and Zimbabwe were grown in adjacent horticultural tunnels in the U.K. The dynamics of early ovary parasitism show that the African pathogen becomes more quickly established, first visible by the sphacelial mass forcing the glumes apart before honeydew exudes, whereas Indian *C. sorghi* leaks honeydew first from a less profusely, and more slowly, colonized ovary. Consequently, the sugar composition of exuded honeydew was quite different (Table 1), the bulky African pathogen removing much more sucrose from the plant sap exudate than the slimmer *C. sorghi* parasitic biomass (Frederickson, 1990). The mode of differentiation of sclerotial tissue, as parasitism proceeds, is unique to each organism. *C. sorghi* forms a proximal plectenchyma below an extended sphacelial fructification (Fig. 1), within which elongation is evident from the thin red core, representing cortical tissue enclosing some of the first differentiating sclerotial medulla. By contrast the African pathogen largely differentiates by transformation of the hyphae of the nearly spherical sphacelium, with little change in dimensions.

African sorghum ergot appears to be unique in elaborating a dihydrogenated cyclic tripeptide ergot alkaloid, dihydroergosine, first characterized in Nigerian material (Mantle, 1968) but more recently found as the principal ergot alkaloid in sclerotia from Botswana, Zimbabwe and South Africa. In contrast, Indian *C. sorghi* sclerotia, both native and those grown on sorghum experimentally in the U.K., contain no ergoline alkaloids. However, traces of agroclavine have been detected in cultured mycelia of *C. sorghi* and in herbarium material from Burma (IMI 14171; Mantle, 1968) implying an absence of the dihydroergoline pathway which occurs in the African material.

The stromatal features of the African pathogen are so similar to those quoted for *C. fusiformis* (Loveless, 1967) that alone they would hardly justify separation as a distinct species. Yet the macroconidia (Loveless, 1964), and many other characteristics of these ergot fungi, are quite distinct. Thus the close morphological similarity of the sphacelial conidial states of *C. sorghi* and the African pathogen should not detract from the distinctive ascosporogenous fructifications of these two groups of ergot fungi. Similarly, although the phenomenon of secondary conidiation in African pathogens, contributing an important aerobiological dimension to their phytopathology (Frederickson *et al.*, 1989), was absent in the Indian pathogen in U.K. comparative studies, the differences in expression may only reflect the widely different sugar compositions of the honeydews. Profuse secondary conidiation *in vivo* seems to be associated with dilute honeydew but in laboratory spore germination tests *C. sorghi* expresses the same phenomenon. Secondary conidiation has also been observed in *C. cynodontis* in Africa (Frederickson *et al.*, 1989).

Ultrastructural details of the Zimbabwean stromata, such as ascospore septation and the implied rearrangement of an initially linear sequence of juvenile spores in the ascus by sliding into a parallel orientation of filiform propagules, are not peculiar to this species but serve to illustrate these fundamental *Claviceps* features (Figs 7–12).

Although it is not yet possible to define favourable experimental conditions for sclerotial germination to produce a functional teleomorph, the demonstration of well differ-

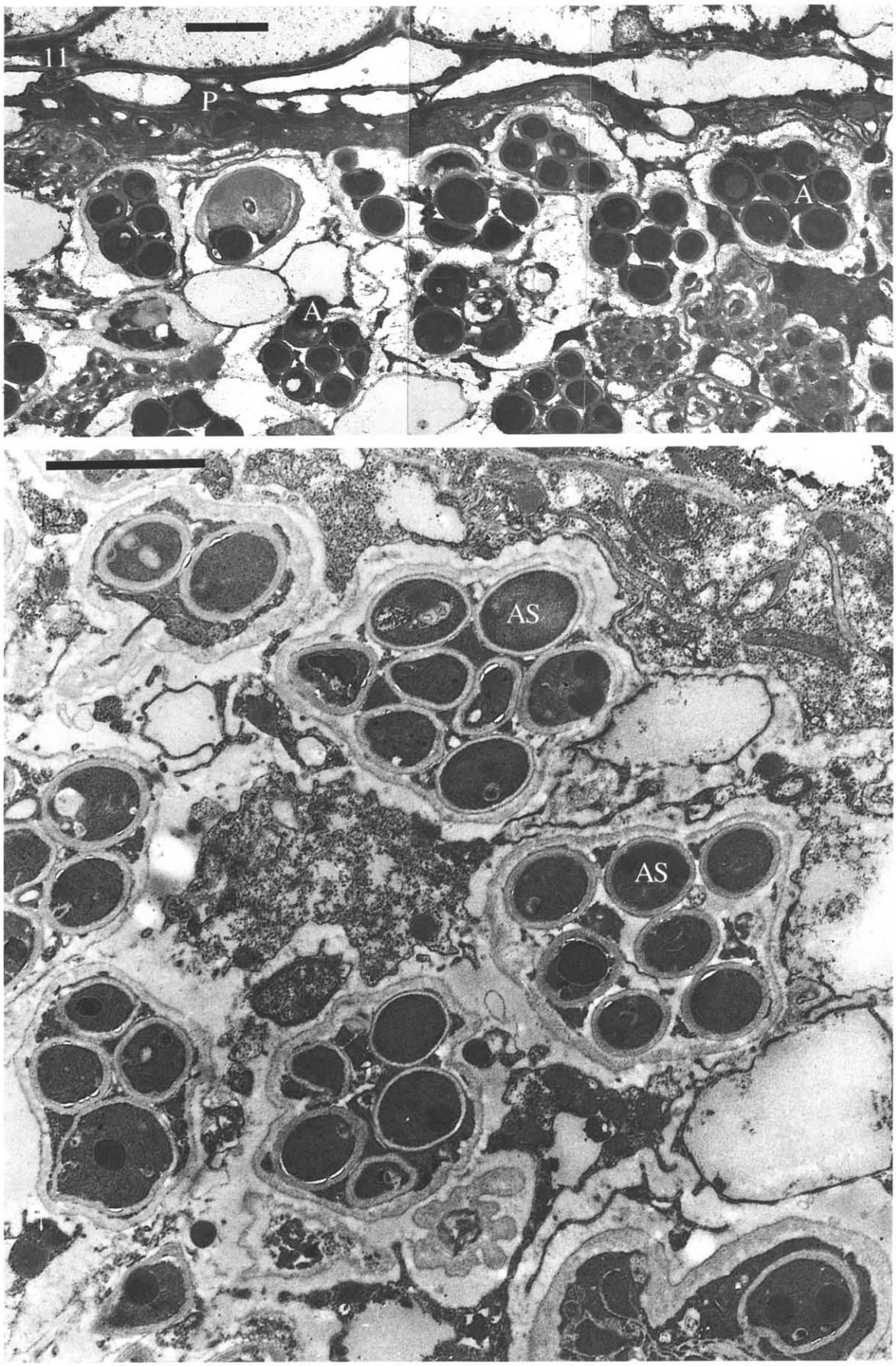

Fig. 11. Transverse section through perithecium (Fig. 6) showing asci containing ascospores, up to 8 according to the level of section (P, perithecial wall; A, ascus). **Fig. 12.** Detail of two asci in transverse section showing arrangement of ascospores (AS, ascospore). Bars; 2 μm.

Table 1. Distinguishing *Claviceps africana* of Africa from the analogous *Claviceps sorghi* of India

Claviceps africana	*Claviceps sorghi**
Sclerotia Oval or spherical parasitic structures bearing a small distal sphacelial cap, overall 4–6 × 2–3 mm. White plectenchymatous medulla bounded by a thin red-brown cortex, appearing patchily flecked red where covered by adherent sphacelial fructification. Flower parts persistent on sclerotia.	**Sclerotia** Long, cylindrical, curved or straight (3–14 × 1–2·5 mm), sometimes with two longitudinal grooves, one on each side, and associated pattern of diagonal striations from glume adherence during phases of parasitic extension. Cream to buff during late development on the host, becoming grey or light brown after maturity and darkening with age. Tissue protruding beyond adherent floral parts generally composed of loosely woven sphacelial hyphae. Proximal part enclosed within glumes may have differentiated to form a sclerotium composed of white compact plectenchymatic tissue, under a thin red-brown cortex. Sclerotial tissue narrowing to a red core in the distal sphacelial tissue.
Stromata Initially pale, translucent tissue proliferates on the sclerotium at one or two places from which stromata, occasionally up to 5 or 6, arise. Notably glabrous stipes (translucent) and capitula (opaque), becoming pigmented purple in the distal part of the stipe and more intensely so in the capitulum. Stipes 8–15 × 0·3–0·6 mm; capitula sub-globose, 0·5–1·3 mm; perithecia, 86–135 × 123–226 µm; most mature ascus *in situ*, 140 × 3·2–4·2 µm; ascospores, usually up to 45 × 0·8–1·2 µm. Perithecial paraphyses absent at maturity.	**Stromata** 2–3 per parasitic body, arising only from the true sclerotial portion (approximately the proximal one third). Appears initially as a lanose mound of white tissue erupting through the cortex. Stipes 6–8 × 0·5 mm, white close to the point of attachment to the sclerotium, otherwise burnished bronze or deep terracotta in colour, darker near insertion into the capitulum where it is surrounded by a white frill. Capitula 0·7 mm diam., buff but with darker, papillate perithecial ostioles; perithecia 130–250 × 60–125 µm; asci, 56–114 × 2·4–3·2 µm. Ascospores 8 per ascus, filiform, hyaline, 40–97 × 0·4–0·8 µm.
Asexual fructification (*Sphacelia sorghi* McRae) Macroconidia: hyaline, mononucleate, oblong to oval, slightly constricted at the centre with a vacuole at each end, 9–17 × 5–8 µm. Microconidia: hyaline, mononucleate, spherical, 2–3 µm diam.	**Asexual fructification** (*Sphacelia sorghi* McRae) Macroconidia: hyaline, mononucleate, oblong to oval, slightly constricted at the centre with a vacuole at each end, 8–19 × 4–6 µm. Microconidia: hyaline, mononucleate, mostly spherical, 2·5 µm diameter.
Phytopathology In the U.K., first disease symptom (6 d after inoculation) is white parasitic biomass, forcing the host glumes apart. The next day, the first honeydew exudes, soon becoming opaque as conidia are swept from the sphacelial fructifications. Superficial whiteness of honeydew surface becomes the dominant symptom; the whole inflorescence may become prominently white. The phenomenon is the result of germination of macroconidia at the honeydew surface to give an aerial layer of secondary conidia. Secondary conidia: pear-shaped, bearing a scar at the narrow end where attached to the sterigma-like process, 8–14 × 4–6·5 µm. Subsequent sclerotial growth of the pathogen is by differentiation within the sphacelial biomass, without excessive elongation or lateral extension, to give a roughly spherical true sclerotium.	**Phytopathology** In direct comparison with the African pathogen in the U.K., first disease symptom (8–10 d after inoculation) is transparent honeydew exuding from florets, associated with a sphacelium totally enclosed within the floral cavity. Subsequently the honeydew becomes opaque with conidia, parasitic biomass lengthening to protrude between the glumes.
Biochemistry Ergoline alkaloids in sclerotia: uniquely mainly dihydroergosine, together with biosynthetic intermediates festuclavine, dihydroelymoclavine, and chanoclavine. Total alkaloid usually 0·2–0·5%; readily assayed from 1 g sclerotia. Honeydew sugars: total sugar (mainly sucrose) 2–16% (w/v) including fructose (up to 6%) and glucose (trace).	**Biochemistry** Ergoline alkaloids in sclerotia: none. Honeydew sugars: total sugar (mainly sucrose) typically 30–70% (w/v) including fructose (up to 30%) and glucose (trace).

* Including data from Kulkarni *et al.* (1976).

entiated germination of one year old sclerotia has theoretical implications for disease aetiology. However, the natural role of sclerotia of the African pathogen in initiating ergot disease remains obscure.

An ergot disease pathogen seriously affecting F1 hybrid sorghum production in Thailand (Boon-Long, 1988) and subsequently studied experimentally during parasitism of male-sterile sorghum in the U.K., produced disease symptoms typical of an African sorghum ergot pathogen, including the profuse secondary conidiation which had also been recognized as a gross symptom in Thailand. The sclerotia were small and compact, containing dihydroergosine and its familiar associated alkaloids, typical also of an African pathotype. The contrast between the Thai ergot fungus and that collected from the adjacent Burma in 1927, implies that the current pathogen in Thailand may have been introduced from Africa and would be included within *C. africana*.

Claviceps africana Frederickson, Mantle & De Milliano, sp. nov.

Sclerotia (Fig. 1) ovalia ad sphaerica, 4–6 mm longa, 2–3 mm lata; pars pyramidalis apicalis (status *Sphaceliae*) ultra floris partes protrudens; pars dura basalis (sclerotium ipse) cum cortice rubro-brunneo in quo fragmenta adhaerentia *Sphaceliae* adsunt, et cum medulla plectenchymatis albi. *Stromata* (Figs 3–4) 1–9, ex 1 aut 2 locis in superficie sclerotii orientia. *Stipites* 8–15 mm longi, 0·3–0·6 mm lati, glabri, primum albi translucidi postea purpurei, praesertim in parte distale. *Capitula* subglobosa, 0·5–1·3 mm, primum opaca et

pallido-bubalina postea atro-purpurea, maturitate papillata et insertionem stipitis involventia. *Perithecia* (Fig. 6) ovato-pyriformia, 123–226 µm longa, 86–135 µm lata. *Asci* cylindrici, in perithecio ad 140 µm longi, 3·2–4·2 µm lati. *Ascosporae* filiformes, hyalinae, septatae, ad 45 µm longae, 0·8–1·2 µm latae. *Conidia*: macroconidia (*Sphacelia sorghi* McRae) hyalina, 1-nucleata, 9–17 µm × 5–8 µm, oblonga ad ovalia, in parte media paulum constricta; microconidia 2–3 µm diam., sphaerica.

In flosculis *Sorghi bicoloris* (L.) Moench., Matopos, Zimbabwe, D.E. Frederickson, Mar. 1988. Holotypus: IMI 343772.

Sclerotia (Fig. 1) oval to spherical, 4–6 mm long, 2–3 mm wide; the pyramidal apical sphacelial portion protruding beyond the floral parts; the hard basal portion (the true sclerotium) appearing flecked red by fragments of adherent sphacelial tissue overlaying a red-brown cortex. Medulla consists of white plectenchyma. *Stromata* (Figs 3–4) 1–9, arising from one or two points on the sclerotial surface. *Stipes* 8–15 mm long, 0·3–0·6 mm wide, glabrous, initially translucent whitish becoming purple, especially in the distal portion. *Capitula* sub-globose, 0·5–1·3 mm, initially opaque and light buff, becoming dark purple, papillate with maturity and enveloping the stipe insertion. *Perithecia* (Fig. 6) ovate-pyriform, 123–226 µm long, 86–135 µm wide. *Asci* cylindrical, up to 140 µm long, 3·2–4·2 µm wide within the perithecium. *Ascospores* filiform, hyaline, septate, up to 45 µm long. 0·8–1·2 µm wide. *Conidia* (*Sphacelia sorghi* McRae) hyaline, mononucleate, 9–17 µm × 5–8 µm, oblong to oval and slightly constricted at the centre (macroconidia); 2–3 µm diam, spherical (microconidia).

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