A Laboratory Guide to the Identification of *Claviceps purpurea and Claviceps africana* in Grass and Sorghum Seed Samples

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Introduction and overview of the genus *Claviceps*

The genus *Claviceps* includes very specialized fungi which parasitize only the flowers of specific grasses, no other part of the plant is infected. During infection, the ovary is replaced by a specialized fungal structure called a sphacelium (plural = sphacelia) that in time becomes another structure called a sclerotium (plural = sclerotia). The sclerotium is a hard, compact mass of fungal tissue with a thin outer layer (rind). Sclerotia of most *Claviceps* species are one to four times larger than the host seed. Grasses with small seeds, e.g. *Agrostis* will yield much smaller sclerotia than larger seeded grasses e.g. *Lolium*. Sclerotia may be white, tan, or black, depending on the species of *Claviceps*. The disease caused by *Claviceps* and the sclerotia that develop have the common name ergot. Ergot is a general term that applies to all species of *Claviceps.*

During development of ergot, spores called conidia (singular=conidium) are produced and mix with a sugary liquid produced from plant sap to form honeydew. During the early stages of infection, honeydew can accumulate and drip from infected flowers in large drops. Honeydew is attractive to flies and other insects which feed on the syrup and disseminate the conidia. The honeydew resulting from ergot infection should not be confused with the honeydew from insects such as aphids. When the honeydew of *C. africana* is exposed to high humidity, the spores can produce another type of conidia (secondary conidia) at the honeydew surface which can be disseminated long distances by wind. Secondary conidia are not produced by *C. purpurea*. Many cycles of infection followed by transfer of conidia can occur as long as there are unpollinated/unfertilized flowers available.

Eventually the supply of susceptible flowers is depleted, or the crop is harvested, and *Claviceps* species somehow have to survive a considerable period of time in the absence of the host. The sclerotia provide one possible means of perennation for *Claviceps* pathogens. Once favorable environmental conditions reappear at the start of the next crop season, sclerotia may germinate to produce stalked structures (stromata) with knob-like apices (capitula). The cavities, or perithecia, in each capitulum contain sac-like asci. The asci contain the ascospores, which are formed by a sexual process. The ascospores of some, but not all, *Claviceps* species are very important for providing the new (initial) inoculum in a season.

The sclerotia of many *Claviceps* species contain alkaloids which can cause toxicity problems if consumed by animals or humans. However, some of the alkaloids present in *C. purpurea* and some other *Claviceps* species have been long recognized for pharmaceutical uses, including drugs helpful in childbirth, and in treatment of migraine headaches or psychiatric disorders. Alkaloids from *C. purpurea* are among the most important natural products used by the pharmaceutical industry. Prior to the industrial cultivation of *C. purpurea* in pure culture, ergot was grown as a crop on rye (*Secale cereale*) under field conditions for use in manufacture of important medicinal drugs.

Most *Claviceps* species are restricted to only one or several grass genera. The exception is the common ergot caused by *C. purpurea*, which has a host range exceeding 200 species of grasses. *Claviceps purpurea* is distributed worldwide in temperate climatic zones. Other species of *Claviceps*, such as *C. africana,* and *C*. *sorghi*, which attack sorghum, prefer a warm or subtropical climate.

In this manual, the fundamental differences among sphacelia, sclerotia and conidia of *C. purpurea* and *C. africana* are detailed. Additional information is provided on the life cycle, host range, distribution, and control of the two ergot species with emphasis on their identification in the field and in harvested seed. Where appropriate, the sclerotial differences between

C. africana and *C. sorghi* are described, although *C. sorghi* does not occur in the Americas and is now rarely observed in its limited area of occurrence in India.

Life cycle

Claviceps purpurea (Fr.:Fr.) Tul.

The sclerotium of *C. purpurea* is comprised of a compact mass of fungal tissue encased in a darkly pigmented rind. It is in this state that the fungus survives through the winter in temperate regions. In fact, a period of one-two months of cold (0-10 C) temperatures are required for germination of the sclerotia. The sclerotia are well adapted to temperate regions such as the northern United States. In warmer regions, such as the southeastern U.S., sclerotia are colonized by other fungi and do not survive well.

In the spring, at or prior to flowering of grasses, the sclerotia germinate (Fig. 1), producing stalked fruiting bodies in which tiny, threadlike ascospores are produced. Rainfall, or high soil moisture, is required for production and release of ascospores. The spores are ejected into the air and are carried by air currents to grass flowers. The ovary is the only part of the grass plant susceptible to infection. *C. purpurea* does not affect forage or turf quality.

In most grasses the period of susceptibility is very brief, including only the period from the beginning of flowering until fertilization. Fertilized ovaries are resistant to infection. Environmental conditions, such as cool temperatures which delay pollination and subsequent fertilization, increase the period of susceptibility. Male sterile lines are especially susceptible to ergot because of the intrinsic lack of pollen. *C. purpurea* attacks many grasses but does not naturally infect species of *Sorghum* or *Zea*.

Within a week after infection, conidia are produced in abundance. A mixture of sap and conidia can accumulate in large drops at the site of infection (Fig. 1). This is commonly referred to as the honeydew stage. Secondary spread can occur by movement of conidia to healthy flowers by rain-splashing, direct contact of infected and non-infected heads, or by insects such as flies or moths that are attracted to the sugary honeydew.

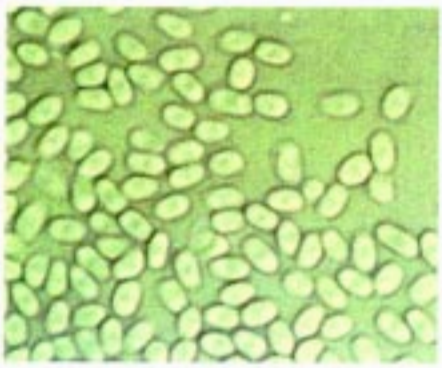
Within about two weeks after infection, sclerotia begin to appear (Fig. 1). Maturity of the sclerotia coincides with maturity of the infected grasses. As the grasses mature, the life cycle of *C. purpurea* is completed when sclerotia fall to the ground, where they survive the winter.

Claviceps africana Frederickson, Mantle, & de Milliano

In *C. africana* the sclerotia are not separate structures from sphacelia but are so closely physically associated that it is better to regard them only as different tissues. Sclerotial tissues form from inside, and to the base of, sphacelial tissues under dry conditions, 20-40 days after infection. Sphacelia may contain variable amounts of sclerotial tissue depending upon the extent of differentiation. Therefore, what is commonly known as the sclerotium of *C. africana* is actually the remains of the white to cream sphacelium on top, with the true orange-brown tissues of the sclerotium towards the base. With this definition, sclerotia of *C. africana* are rounded in shape and at 4-6 x 2-3 mm are similar in size to a sorghum seed. They barely protrude from the sorghum flowers. In contrast, the sclerotia of *C. sorghi*, the pathogen found only in India, are elongate, cylindrical, straight, or curved structures, protruding 3-14 mm from flowers. The role of sclerotia of *C. africana* in survival of the species is not well established since germinated sclerotia have not been seen in nature. However, *C. africana* can survive in the conidial state on feral sorghum and alternate hosts, such as *Sorghum halepense*, which can flower throughout the year in some parts of the United States and Mexico.

Honeydew on *Poa pratensis*

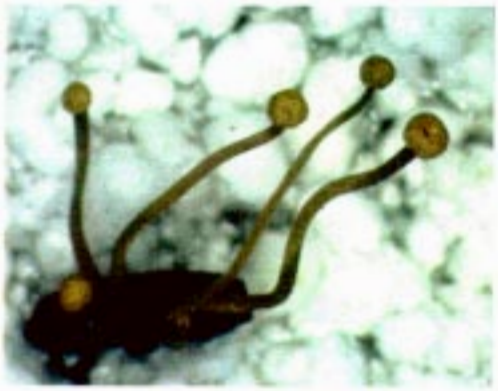
Reinfection



Conidia

Sclerotia on *Festuca* species

Ascospores



Germinating sclerotium

Figure 1. General life cycle of *Claviceps purpurea*.

The flower (including ovary, style, and stigma) is the only organ of the plant susceptible to infection. Susceptibility is limited to the period between beginning of flowering and fertilization. Resistance to infection develops after fertilization of the ovary. Male-sterile sorghums used in hybrid seed production are especially susceptible to infection because they require an external pollen source for fertilization. The high fertility of most hybrids and other sorghums normally protects them from ergot but all sorghums can develop high levels of ergot when pollen viability is reduced by cool temperatures that occur prior to and at flowering. Heavy rainfall at flowering can also increase the potential for ergot by interfering with the normal pollination/fertilization process.

Cool to moderate temperatures (14-28 C) with wet, cloudy conditions, favor rapid disease development and spread. Warmer temperatures (>28C) restrict ergot severity. Within 7-8 days after infection, honeydew containing macroconidia can accumulate in large drops at the site of infection (Fig 2.). Disease spread can occur through transfer of honeydew by various means but the primary method of pathogen dispersal is windborne secondary conidia. Under conditions of high (greater than 90%) humidity a conidium can produce an elongated tube on the surface of honeydew which extends into the air above the conidium and on which a secondary conidium is produced. These secondary conidia, visible as a white powdery growth on the surface on honeydew, (Fig. 2) disperse through air currents, providing rapid dissemnation of *C. africana* over a very wide geographical area.

The name *Claviceps africana* follows the description of the sexual stage of the pathogen in Zimbabwe, Africa, in 1991. Before this time it was assumed that all sorghum ergot pathogens worldwide were the same species and so it was designated as *Sphacelia sorghi*, the name originally applied to the asexual stage of *C. sorghi*. Any reference in the literature to *S. sorghi* in Africa or the Americas can safely be assumed to refer to *C. africana.*

Host range and distribution

Claviceps purpurea

The best known species of *Claviceps* is *C. purpurea*, often referred to as common ergot. It is a common pathogen of cereal grains and grasses grown for seed although rarely of economic concern. Over 200 grass species, almost all genera in the subfamily Pooidea are susceptible. This group includes many of the important cool season grass genera such as *Agrostis, Avena, Dactylis, Festuca, Hordeum, Lolium, Poa, Secale, Triticum.* However, *Sorghum* species are not hosts for *C. purpurea*.

C. purpurea is widely distributed and common in temperate regions. *C. purpurea* is known to occur in Africa, Asia, Australasia and Oceania, Europe, and the Americas. In the U.S., *C. purpurea* is common in the northern two-thirds of the country. The sclerotia of *C. purpurea* do not survive well in warmer climates like the southeastern U.S., where they are more easily colonized by other fungi.

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Claviceps africana

Reports of ergot in Africa from as early as 1924, believed to be *C. sorghi*, were identified as *C. africana* in 1991. During the 1990's *C. africana* spread to sorghum production regions worldwide at a remarkable rate. By 1997, *C. africana* was recognized in southern Asia, Africa, Australia, and the Americas.

The principal hosts for *C. africana* include *Sorghum bicolor* (L.) Moench, *Sorghum halepense* (L.) Pers., and other *Sorghum* species. *Pennisetum glaucum* L.R. Br. was infected

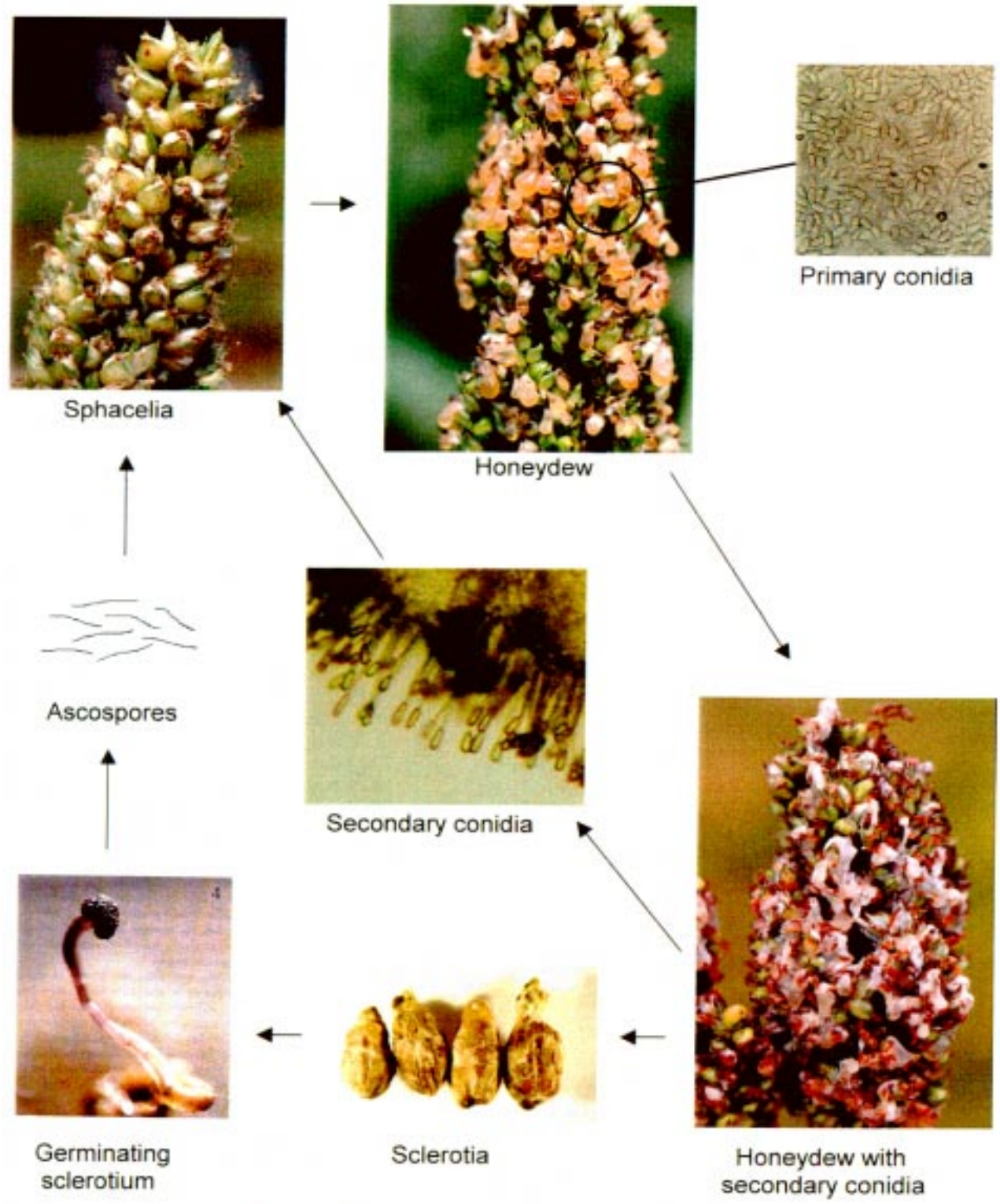


Figure 2. General life cycle of *Claviceps africana* on Sorghum

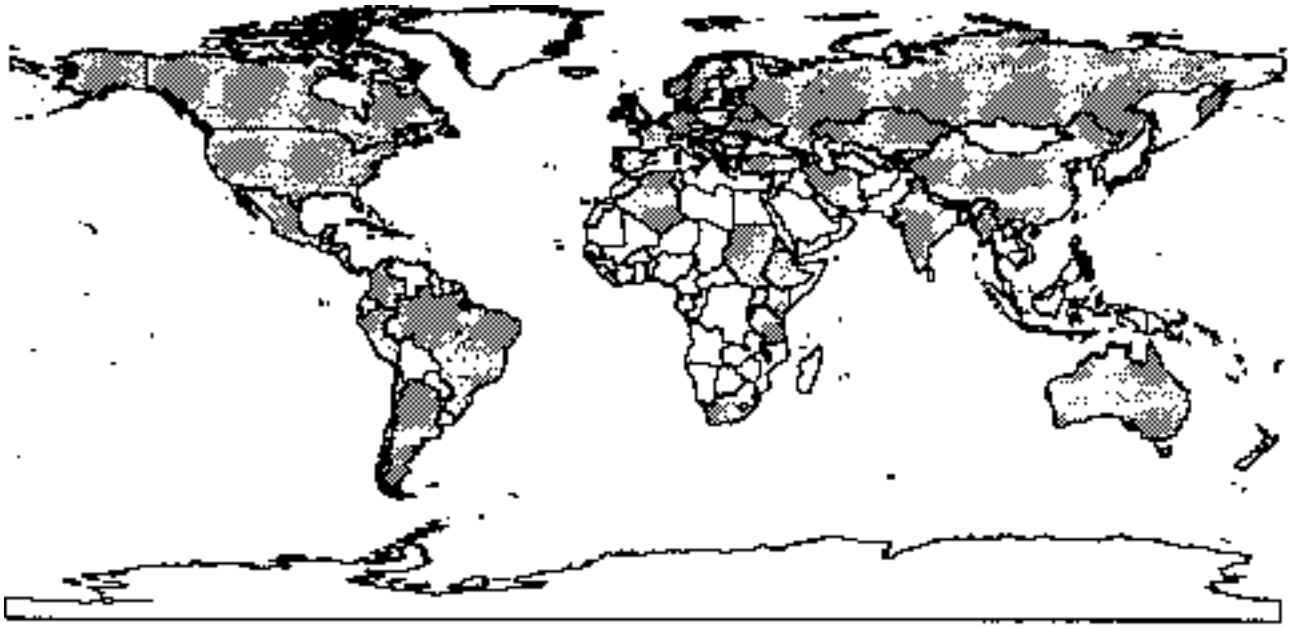


Figure 3. Distribution of *Clavicops purpurea*

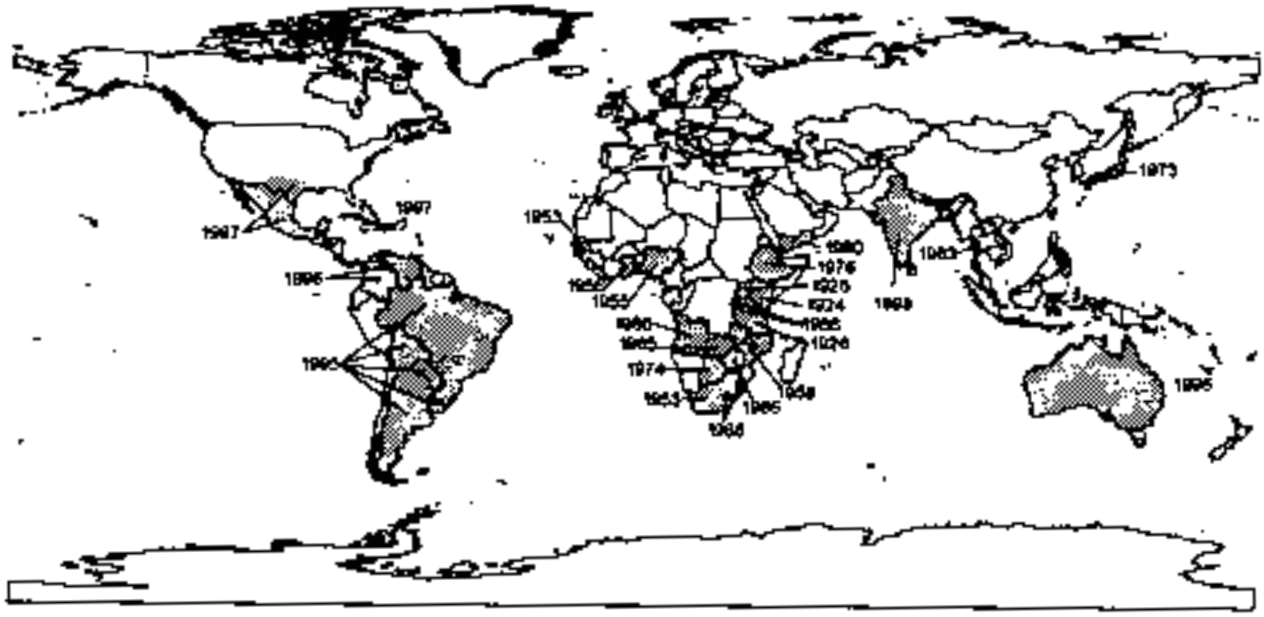


Figure 4. Distribution of *Claviceps africana*

by *C. africana* under greenhouse conditions but it is not a host under field conditions. Except for *P. glaucum*, only *Sorghum* species were infected under field or controlled environment conditions.

Economic impact

Claviceps purpurea

Ergot is common on grasses worldwide, although rarely of economic importance. The tolerance for ergot in cereal grains ranges from 0.3% in rye to 0.1% in wheat and barley. Higher levels impact grain quality. In production of cool season forage and turf grasses for seed, ergot is of little concern, except for susceptible cultivars of *Poa pratensis*. In ergot infested fields of *P. pratensis* the sticky honeydew can aggregate seed and plant debris, making harvest difficult. Additional seed loss occurs during cleaning to remove sclerotia.

Claviceps africana

Ergot caused by *C. africana* can be a serious disease of the female seed parent, (male-sterile) in hybrid sorghum seed production fields. Losses to 80% in India and 25% in Zimbabwe have been reported. In addition to direct seed loss from flower infection, sticky honeydew covering infected panicles can make seed harvest difficult to impossible.

Sorghum ergot can greatly reduce the quality of grain from infected fields and the presence of ergot in seed of *Sorghum bicolor* can impact international seed markets. However, sphacelia and sclerotia can be removed by standard seed cleaning operations and captan seed treatments on hybrid sorghum seed for planting gives complete control of honeydew on the surface of seed, sphacelia, or sclerotia.

Although the impact of C. africana can be significant, the impact of C. sorghi is negligible because this pathogen is restricted to India and is now rarely or never observed in that region.

Control

Claviceps purpurea

Disease resistance is a primary means of control for *C. purpurea*, especially in cereal grain crops such as *Secale cereale*. In seed production of *Poa pratensis*, fungicides, applied at flowering, may also be used for ergot control. Sclerotia of *C. purpurea* can also be removed from seed during seed cleaning.

Claviceps africana

A seed treatment protocol used by ICRISAT includes fumigation with methyl bromide (32 g m-3) for 4 hours, followed by seed treatment with a mixture of Benlate and Thiram. Evaluation of fungicides by USDA-ARS and Texas A&M University indicated that Thiram and Captan were effective in preventing conidial germination and conidial production.

Field control measures for ergot are warranted primarily in the male-sterile seed parents in hybrid sorghum seed production fields. These control practices include: 1) planting disease free seed or fungicide treated seed, 2) timing of planting to optimize rapid pollination and fertilization, and 3) the use of fungicidal sprays during bloom. The removal and destruction of infected plants and infested plant residues from the previous crop and alternate hosts will likely provide limited benefit across the many areas of Mexico and South Texas where *C.*

africana is endemic. The pathogen has the ability to increase and spread rapidly across great distances from nondetectable, small sources of surviving inoculum. In these endemic areas, sorghum ergot is likely to occur every season on inaccessible wild and other sorghum hosts even in the absence of any cultivated sorghum crop. Development of greater host plant resistance in sorghum will play an important role in ergot control, especially for male-sterile sorghums.

Claviceps africana in the Americas

The first report of *Claviceps africana* on sorghum in the United States was by Dr. Tom Isakeit (Texas A&M University) who observed it on ratooned sorghum near Progresso, Texas on March 26, 1997. The ergot was subsequently identified on *Sorghum halepense* in the same South Texas region of the Rio Grande Valley. The host status of grass species other than Sorghum is difficult to establish and none are likely to be natural hosts for *C. africana*.

The spread of *C. africana* into the U. S. was anticipated since its initial observation in the Western hemisphere in Brazil in 1995 and subsequent spread across South America in 1996. The mode of introduction of *C. africana* into South America is unknown but the pathogen has the potential for dissemination by nontreated sorghum seed contaminated by sclerotia or encrusted by dried honeydew that contain viable macroconidia. The pathogen may also have been introduced as dried honeydew on clothing, shoes, machinery or other means. The macroconidia have the ability to germinate and produce secondary aerial conidia in less than 24 hr. The rapid spread of *C. africana* over large geographic areas is attributed to aerial conidia but it is unlikely to be capable of intercontinental dissemination although its distance limitations are unknown.

By mid-February 1997 ergot had spread to Western hemisphere regions outside of South America including Honduras, the Dominican Republic, Jamaica, Puerto Rico, and Mexico. The sorghum crops most severely affected by ergot in these countries were those with male-sterility or other fertility problems which maximize susceptibility through lengthening the time a sorghum flower ovary remains unfertilized. Commercial grain sorghum hybrids had negligible incidence of ergot during normal growing seasons because they are highly self-fertile and their rapid pollination prevents infection by *C. africana*. Male-sterile (female parent) sorghums in hybrid seed production fields are generally the sorghums most heavily damaged by ergot because they require an external pollen source for fertilization. The variable fertility of forage sorghums was reflected in the incidence of ergot observed. Pollen sterility induced by cool temperatures, usually late in the season, increased incidence of ergot in forages and in the normally-resistant commercial grain sorghum hybrids.

Now that *C. africana* is established in the U. S., it will probably become endemic on *Sorghum halepense,* which may develop an increased incidence of the disease late in the year when cool temperatures are more likely to induce pollen sterility. The host status of grass species other than Sorghum is difficult to establish and none are likely to be natural hosts for *C. africana.* The impact of sorghum ergot in the U. S. will probably be determined by the differential susceptibility that exists in the types of sorghum being grown and by environments present during time-critical phases of their floral development and during the time when infection occurs.

Commercial grain sorghum hybrids will likely sustain little damage from ergot through their self-fertility that provides an escape type of resistance. However, pollen sterility associ

ated with cool temperatures in some sorghum production regions in the Northern plains or winter production in Southern regions could increase the risk of ergot in any sorghum crop. Forage sorghums represent an increased ergot risk but sterile forage hybrids are likely to sustain the greatest damage from ergot because they have no pollen source and tillering provides a succession of sorghum flowers for rapid cycling of the disease. While seed production is not of importance in these latter forages, the high incidence of ergot would produce tremendous amounts of honeydew that could interfere with harvest and, under continued wet conditions, support the growth of saprophytic fungi.

Toxicity of the saprophytic fungi is of some concern but previous research indicates that sclerotia and sphacelia of *C. africana* are of negligible toxicity. Male-sterile sorghums in hybrid seed production fields have the greatest risk for damage by ergot but triazole fungicides applied to heads of blooming sorghum have provided adequate control in Brazil and other production regions. Commercial seed companies are anticipating the need for fungicidal protection of their fields but many are also reducing ergot risk through pollen management techniques. These practices will maximize the timely production and distribution of pollen from the male-pollinator parent so it will be present for rapid fertilization when the male-sterile seed parent begins to bloom.

Public and private sorghum researchers throughout sorghum growing regions of the U. S., and others in state agencies, commodity and other groups, and various divisions of the USDA are collaborating in research and survey efforts to detect the spread of sorghum ergot across the U. S. Collaborative research efforts extend beyond U. S. borders to a global network of scientists attempting to develop integrated control methodologies for *C. africana* because it also spread to Australia in 1996 and it continues to have negative impact in areas of Africa where it is endemic.

Some of the most extensive collaborative research efforts on sorghum ergot are those currently established between scientists in the U.S. and those of INIFAP in Mexico. Their research has focused on several aspects of the biology of *C. africana* including yearly occurrence, spread, survival, and economic impact of sorghum ergot in the two neighboring countries.

Detection of *Claviceps* in harvested seed and seed samples

Sclerotia of *Claviceps* species are best identified and differentiated under magnification. At the ICRISAT Asia Center the Plant Quarantine Unit, seed lots are examined for *C. africana* under 10X magnification for the presence of sclerotia and their presence is noted in the purity report. At the Oregon State University Seed Laboratory ISTA rules are followed for purity testing of grass seed. If ergot is found during the purity exam it is recorded as ergot (*C. purpurea*) under inert matter in the purity report.

An examination of seed at 10X or greater magnification is required to detect the presence of sclerotia, sclerotial fragments, and other fungal bodies of *C. purpurea* and *C. africana*.

Flotation test and limitations

The flotation test, where seed is vigorously stirred in 10% sodium chloride solution, is often used as a definitive test for ergot sclerotia. The floating component is assumed to consist entirely of sclerotia but, in practice, *C. africana* fungal bodies will only float if they contain sufficient amount of sclerotial tissue to render them buoyant. Otherwise they, and

sphacelia, will sink. However, seed that is immature, small, damaged, incompletely filled, or with attached glumes may be easily retrieved with flotation procedures and mistakenly identified as sclerotia or other structures of *Claviceps,* especially when they are black from colonization by saprophytic fungi. Other floating plant debris like glumes and other inflorescence material may also be colonized by saprophytes and have an appearance of ergot bodies. Therefore, it is imperative that all products of the flotation test be examined closely under magnification at 10X or above to confirm identity. Additional tissue manipulation including scraping, dissecting, and teasing apart of structures will probably be necessary before final identity can be determined.

Characteristics of sclerotia and conidia

Claviceps purpurea (Fr.:Fr.) Tul.

The sclerotia of *C. purpurea* are elongated, generally 1-4 times longer than the host seed. The sclerotia are typically cylindrical with rounded ends, straight to curved and tapered at the distal end. They appear black to purplish black in color on the outside, grayish-white on the inside with a lighter colored sphacelial cap. The rind is hard but will break if pressed firmly with forceps. The surface may appear longitudinally wrinkled and rugose near the sphacelial cap. Dried conidia may be present on the exterior of the sclerotium and appear grayish in color. Conidia are released if placed in a drop of water.

In seed samples, sclerotia generally occur unbroken, but may be present in half or thirds and rarely smaller.

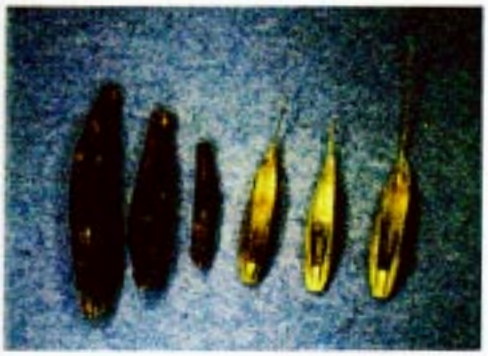
Conidia are single celled, hyaline, 4-6 x 2-3 μm in size. They are oval to elongate and may be slightly depressed at the center. A vacoule or guttule may be present at one or both ends.

Claviceps africana Frederickson, Mantle and De Milliano

The sclerotium of *C. africana* develops from within, and to the base of the sphacelium. Even when the sclerotial tissues are fully developed, the sphacelial tissues usually remain in place on top. Therefore what is commonly called the sclerotium actually consists of sphacelial and sclerotial tissues. With this definition, sclerotia are oval to spherical, tapering at the distal end, 4-6 x 2-3 mm. The white to cream tissues at the top are sphacelial and the true sclerotial tissues towards the base are white internally, with a thin, orange brown to red-brown rind. The rind will often be covered by a thin, white layer of sphacelial tissue.

The typical sphacelial fructification of *C. africana* produces two types of primary conidia: macroconidia and microconidia. Macroconidia are 9-17 x 5-8 μm, single-celled, hyaline, oval to oblong and slightly depressed at the center. A vacoule is generally present at each end. Microconidia are 2-3 μm in diameter, spherical, single celled, and hyaline.

Since sclerotial tissues are associated with sphacelial tissues, macroconidia and microconidia may also be found on the surface of sclerotial tissues (Fig. 6). These conidia are also present in dried honeydew that either dripped onto healthy seed or was deposited onto it during harvest operations (Fig. 6) .



Sclerotia of *Claviceps purpurea* with *Lolium multiflorum* Lam.

Sclerotia of *Claviceps purpurea* with *Poa pratensis* L.

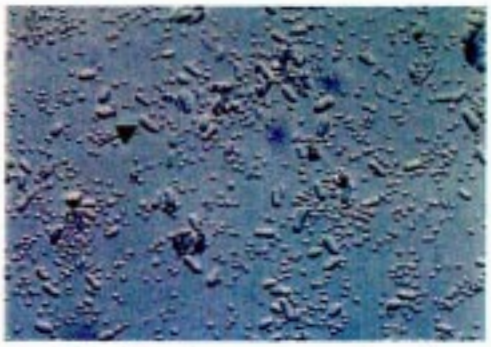
Conidia of *Claviceps purpurea*

Figure 5. Characteristics of sclerotia and conidia of *Claviceps purpurea*.

Sorghum seed coated with variable amounts of dried honeydew and sphacelia of *C. africana*

Seed screenings with sphacelia/sclerotia of *C. africana*

Sphacelia/sclerotia of *C. africana* (top) and healthy sorghum seed (bottom)



Conidia of *Claviceps africana* from the surface of sphacelium in harvested seed (Arrow indicates macroconidium)

Figure 6. Characteristics of sclerotia and conidia of *Claviceps africana*

Comparison of *Claviceps africana* and *Claviceps sorghi*

In *Claviceps sorghi*, sclerotia are cylindrical, 3-14 x 1-2.5 mm, elongate, straight or curved, narrowing at the distal end. They may have two longitudinal grooves on each side with a pattern of diagonal striations and a large sphacelial portion present at the distal end. The color of the distal tissues is initially cream to buff, becoming grey to light brown; proximal tissues internally white, encased in a reddish-brown rind. Lemma and palea often attached at base of sclerotium.

Other seed contaminants

Smuts

Claviceps can be differentiated from the smuts based on features of the sclerotium. Scle-rotia are hard with a solid, usually white to buff colored interior. The smuts yield a black powdery mass when ruptured. Spores of the smut fungi can be confirmed by microscopic examination.

Figure 7. Comparison of sclerotia of *Claviceps africana* and *C. sorghi*.

Cerebella species

Cerebella is a black-colored saprophytic mold that extensively colonizes the honeydew and sphacelia produced by *Claviceps* species. The fungus is black throughout with a deeply invaginated surface and spherical shape that resembles a brain, hence the name *Cerebella*. The *Claviceps* sphacelia beneath these growths are ramified by hyphae of *Cerebella* and also appear black. Because infection by *Cerebella* is most common under periods of prolonged high moisture it often occurs with additional black discoloration from extensive colonization of honeydew by other saprophytic fungi. The latter also give a black discoloration to seed, inflorescence and other plant tissue where honeydew has been exuded.

The presence of *Cerebella* should be considered only as a sign or indicator of possible ergot that must be confirmed by identification of actual fungal bodies or other structures of *Claviceps* spp. Seed may be discolored by grain mold and grain weathering fungi so discoloration alone is probably not a sign of *Claviceps* unless it is associated with remnant honeydew containing conidia of *Claviceps*. Then the seed sample should also be searched more rigorously for ergot fungal bodies.

Cerebella on *Lolium multiflorum*

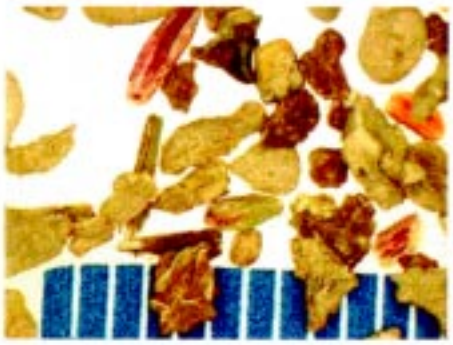
Cerebella on *Sorghum*

Figure 8. Characteristics of *Cerebella*.

Other debris

Various debris, including stem pieces, other seeds, rodent and insect droppings, and insect parts, some of which may resemble *Claviceps* sclerotia, can occur in seed samples. Rodent and insect droppings in particular may appear similar to sclerotia in size and shape. The droppings are softer than sclerotia and break when pressed with forceps and are uniform in color throughout. Debris is differentiated under magnification of at least 10X.

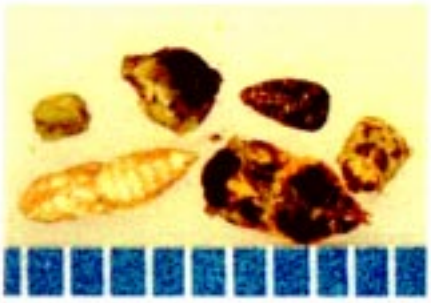
Sclerotium with stem pieces
(arrow points to sclerotium)



Soil and seeds

Rodent droppings

Insect droppings



Insect parts

Seeds

Figure 9. Characteristics of other contaminants.

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