Feature Articles

Ergot - a Global Threat to Sorghum

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Introduction

Productivity of sorghum has increased significantly since the early 1970s in Asia. In 1970, the average sorghum yield in Asia was 747 kg ha^{-1}; it had increased to 1214 kg ha^{-1} by 1994 (FAO 1996). One of the significant contributors to the increase in productivity was the cultivation of highly productive F_1 hybrid seeds by Indian farmers. In Africa, South America, and Latin America commercial sorghum hybrids are increasingly being used. Farmers in USA and Australia achieve substantial yields from sorghum, and they rely almost totally on hybrid seeds.

Ergot is a serious disease of sorghum that affects the production of F_1 hybrid seeds particularly in male-sterile lines if seed set is delayed due to the lack of viable pollen caused by nonsynchronous flowering of male steriles and restorers. In western Maharashtra and Vidarbha, India, 10-80% losses due to the disease have been reported in seed production plots (C S Sangitrao, personal communication). Similar yield loss is not uncommon in southern Africa. Ergot can also cause widespread damage to male-fertile cultivars in farmers' fields when favourable environmental conditions occur at flowering. In 1986, an epidemic on a newly introduced hybrid, CSH 11, caused serious losses in Karnataka, India. As a result, the otherwise productive hybrid was withdrawn from cultivation (R Bandyopadhyay, unpublished). Ergot is a disease of the ovary; it reduces grain yield because infected flowers do not produce grains. The disease lowers grain/seed yield and quality, makes threshing difficult, reduces germination and seedling emergence, and predisposes seedlings to other diseases (McLaren 1993). Until 1995, the disease was limited to Asia and Africa in distribution.

The sorghum industry around the world was shocked by the sudden observation and rapid spread of ergot in Latin America beginning in Brazil in 1995. A similar epidemic broke out in Australia in April 1996. The global appearance of ergot has made US sorghum workers and quarantine officials feel rather vulnerable to the potential introduction of ergot. That vulnerability was underscored with news that ergot-contaminated seed from Brazil may have been shipped to other South American countries and to the Bajio of Mexico. If established in Mexico, US scientists wondered how quickly it might spread across the border into South Texas where the pathogen would have direct access to the Great Plains states in central USA where most of the country's sorghum is grown.

The purpose of this review is to provide a current status report on sorghum ergot for the benefit of those who are interested to learn more about the disease. A bibliography of the disease replaces the usual Selected References section in this issue of ISMN.

Widening geographical influence

Sorghum ergot was first observed in India in 1915 and was described by McRae in 1917. That it was more widespread in India was confirmed in 1948, when Ramakrishnan recorded ergot in Andhra Pradesh State. In 1927, ergot was sampled in Burma (IMI accession 14172). All reports and samples represent the pathogen *Claviceps sorghi.* In 1991, ergot was also reported from Taiwan (Chen et al. 1991).

In Africa, sorghum ergot was recognized first in the eastern region, having been sampled in Kenya in 1924 (IMI accession 93464), Uganda in 1926 (IMI accession 14170), and Tanzania in 1949 (Wallace and Wallace 1949). In 1953, ergot was reported in South Africa (Doidge et al. 1953), in 1955 in Nigeria (IMI accession 62801), Zambia in 1965 (Angus 1965), Botswana in 1974 (Molefe 1975), Ethiopia in 1976 (IMI accession 225570), and Mozambique in 1984 (Plumb-Dhindsa and Mondjane 1984). In 1986, ergot was confirmed in Zimbabwe, Swaziland, and other southern African countries (de Milliano et al. 1991). All reports and samples from Africa represent the distinctive pathogen *Claviceps africana* (Frederickson et al. 1991).

The worldwide distribution of *C. africana* is currently expanding rapidly. In 1991, the ergot pathogen in Thailand was, contrary to expectations, identified as *C. africana,* not *C. sorghi* (Frederickson et al. 1991), and in Japan, one of the ergot species parasitizing sorghum was similarly identified (Mantle and Hassan 1994). The same

pathogen caused a widespread ergot epidemic in Brazil in 1995 (Reis et al. 1996) and is possibly now in Argentina, Bolivia, Colombia, and Paraguay. This year extensive *C. africana* infections have been confirmed throughout Queensland, Australia. Ergot presents a very serious threat to sorghum production in Central and North America, although it has yet to be recorded in USA.

Quarantine and the seed trade

The sources of recent *C. africana* infections in Thailand and Japan, and the Americas and Australia in particular, have not been determined, although seed traded intercontinentally for commercial or research purposes is obviously implied. Once arrived in a country, however, the disease most probably relies on airborne dispersal, that is likely to be a more important means of dissemination between adjacent countries or states on the same continent. Therefore, quarantine must play the leading role in continued exclusion of the pathogen. The initial source of *C. africana* inoculum is unclear even in Africa; sclerotia, sphacelia, and honeydew are all likely possibilities. Although *C. africana* sclerotia germinate with difficulty (Frederickson et al. 1991, Bandyopadhyay 1992), only a small source of inoculum is needed under favorable conditions to initiate an epiphytotic in male-sterile sorghum (Frederickson et al. 1989 and 1993). Imported seed must therefore be viewed as an important source of infection and thoroughly inspected for sclerotia, sphacelia, and honeydew before distribution is permitted. For continued exclusion of the disease, e.g., from the USA, seeds with the slightest contamination must be rejected.

Reports of ergot in Latin America and the probable quarantine and other trade restrictions instituted to prevent or slow the spread of ergot to other Western Hemisphere countries including USA will have an immediate, negative impact on the US sorghum industry. The global nature of the US sorghum industry ensures that ergot in such other countries as Brazil and Argentina will have an immediate economic effect; there will be a loss or limitation of winter nursery facilities and sorghum markets in Latin America, due to the banning of seed imports from ergot-affected countries into non-ergot countries. With every additional Western Hemisphere country affected by ergot there is both an increased negative impact on the US sorghum industry, and an increased potential for ergot introduction into USA, either through contaminated seed, or through natural spread across the southern border with Mexico. There will be similar effects in such other countries where ergot has not yet been observed.

If established in USA, ergot could have two especially devastating effects on the sorghum industry; through direct damage to hybrid seed production fields, and through the loss of international and domestic markets. Most hybrid seed production in USA is done in the high plains of the panhandle region of Texas where vast areas of highly vulnerable male-sterile sorghums could be heavily damaged, and provide for rapid spread of the disease. The hybrid seed produced in ergot-infested fields would not only be of lower quality, but there would be less of it; it would require additional sanitation procedures, and it would be restricted in both US and international commerce. The presence of ergot in USA may also cause the loss of some international markets for sorghum, even where production may be from fields or areas of USA not yet affected by ergot.

Toxicity to animals

One of the key issues of ergot on cereals is the stigma associated with the toxicity of alkaloids produced in sclerotia. Mantle (1968a) and Mantle and Waight (1968) first reported that *C. africana* sclerotia contain unique clavine alkaloids, chiefly dihydroergosine. These researchers, together with Mower (1973), Frederickson (1990), and Frederickson et al. (1991) have found that sclerotial alkaloid content varies between 0.02 and 0.98% w/w, 88% of which is dihydroergosine. Other clavine alkaloid intermediates in the biosynthetic pathway to dihydroergosine were confirmed by TLC, HPLC, and mass spectrum analyses as pyroclavine, festuclavine, chanoclavine, and dihydroelymoclavine (Mantle 1968, Frederickson 1990, Frederickson et al. 1991, Mantle and Hassan 1994, Reis et al. 1996). Ramakrishnan (1948) and Mantle (1968a) noted the total absence of alkaloids in sclerotia of *C. sorghi,* the Asian pathogen. The account by Chinnadurai and Govindaswamy (1971a) of 0,025 and 0.08% w/w alkaloid in *C. sorghi* sclerotia is probably erroneous, since they did not specifically extract and assay alkaloid, but assayed powdered sclerotia. The result probably reflects the general detection of indolic compounds by the reagent p-dimethylaminobenzaldehyde. Analysis for dihydroergosine and the other minor alkaloids in sclerotia can thus distinguish between *C. africana* and *C. sorghi,* The question of the potential toxicity of sorghum ergot sclerotia inevitably arises through analogy with *C. purpurea* and *C. fusiformis,* that synthesize such potent alkaloids as ergotamine and agroclavine (Youngken 1947, Shone et al. 1959, Loveless 1967). In contrast to the agalactia exerted by agroclavine on pregnant and lactating mice and sows (Shone et al. 1959, Mantle 1968b, Mantle 1969), diets of up to 50% sclerotia of *C. africana* (15 mg alkaloid day^{-1}) following insemination or before parturition, had no effect on blastocyst implantation, litter size, pup growth, or

lactation in mice (Mantle 1968a and b, 1969 and 1990). Therefore it is highly unlikely that sclerotia-contaminated sorghum grain has any implications for animal health.

Phadnik et al. (1994) reported that sorghum ergot sclerotia contained clavine derivatives and the total alkaloid content varied from 0.008 to 0.032% (expressed in terms of ergometrine base). The low alkaloid content explains the high LD_{50} value (1.875 g kg^{-1}) of sorghum ergot in experimental mice, compared to 100 mg kg^{-1} in rhesus monkey for pearl millet ergot. Mortality in mice occurred even at a single oral dose of 0.75 g kg^{-1} body weight. Common symptoms of toxicity were diarrhoea, dyspnea, muscular spasm, hyperexcitability, and gangrene. In chronic toxicity studies, diarrhoea was observed on the second day after administering one-sixth of the LD_{50}. Significant reduction was observed in body weight and relative organ weight of lungs, liver, heart, spleen, and kidney. Shrinkage at the tail tip, gangrene, and sloughing of tail also occurred. Histopathological changes in the affected part of the tail include occlusion of the blood vessels due to the presence of red thrombi and necrosis. Further critical studies on toxicity to livestock are required to determine if ergot sclerotia are hazardous to animals.

There are indications that sclerotia of *Claviceps* species found in Japan contain the alkaloid palielavine (T Tsukiboshi, personal communication). There is no information on its potential toxicity.

Symptoms

Ergot only attacks unfertilized ovaries. Few or all flowers in an inflorescence may be infected. The most obvious external sign of the disease is the exudation, from the infected flowers, of honeydew, a thin-to-viscous, sweet, sticky fluid that gives the name 'sugary' or 'honeydew' disease to the malady.

The ovary is infected much before the initiation of honeydew exudation. In fact, the earliest symptoms of infection can be seen on the ovary if flowers are dissected 3-4 days after infection. The infected ovary appears dull green and smaller (*C. sorghi*) or larger (*C. africana*) than the healthy, fertilized ovary which is dark green and round. Superficial, white mycelial growth initially appears at the basal end of the ovary and extends upward as the pathogen colonizes ovary tissues both internally and externally. Finally, the complete ovary is converted into a white, fungal mass, or sphacelium, that is visible between the glumes. Then, honeydew exudation begins.

Newly formed honeydew droplets are colorless and transparent, and become progressively opaque. Honey-

dew can be uniformly yellow-brown to pink, or superficially matt white. Continued production of honeydew causes droplets to lengthen, smearing seeds and leaves, and falling to the ground. When infection is severe, affected panicles can be recognized from a distance. They may be white with fresh honeydew, or black if the honeydew is saprophytically colonized.

During wet or humid periods at relative humidities above 90%, the ergot fungus produces secondary conidia on the surface of the honeydew which appear as a white scum or powdery growth. This white growth on the honeydew appears wherever it is present including the panicle, leaves, and soil. If moist conditions persist, infected flowers may be highlighted by several saprophytes (one of these is *Cerebella* sp) that grow on the honeydew as a large, black, globose convoluted mass concealing the sphacelium. If conditions are warm and dry after the honeydew is formed, it desiccates, forming a brittle, hard, white crust on panicles and leaf surfaces. Under warm dry conditions, sphacelia gradually harden to form solid dense sclerotia. But, in moist conditions, the sphacelia shrivel and become fibrous, and fail to develop into sclerotia.

Ergot is not the only cause of honeydew exudation in sorghum. Insects, such as aphids, also secrete sticky honeydew that is often interspersed with the white molts of the insects. Leaves can also exude honeydew at temperatures below 20°C due to a physiological disorder called 'leaf sugary disease'. Honeydew from insects and leaf sugary disease does not contain spores of the ergot fungus.

Claviceps africana

According to Frederickson (1990) and Frederickson et al. (1991), when ovarian tissues have been fully colonized after 6-8 days, individual infections of *C. africana* become visible as bulky, soft, white, oval to spherical fungal sphacelia, protruding between the glumes. Honeydew exudation commences a day later, as a small drop of transparent liquid devoid of conidia at the tip of the sphacelium. Over successive days, the honeydew droplet enlarges, appearing superficially matt white, but non-viscous, and transparent below. The surface layer contains germinating macroconidia with aerially supported secondary conidia (Frederickson et al. 1989). Honeydew droplets may spill in a cascade over panicles rendering them maeroscopically white in appearance, a very distinctive symptom. Following rain, small patches of white honeydew may collect on the ground at the base of an infected plant. Under dry conditions, honeydew from infected florets forms viscous, orange-brown droplets. After several weeks, the soft, sphacelial tissues are replaced

by hard, compact, sclerotia, 4-6 x 2-3 mm, still largely enclosed by the glumes. A small sphacelial cap remains infected. Dried honeydew may encrust panicles.

Claviceps sorghi

Frederickson and Mantle (1988) and Frederickson (1990) reported that honeydew is the first sign of infection, visible after about 8-10 days. However Bandyopadhyay et al. (1990) noted a visible stroma after 5-6 days, concurrent with transparent honeydew. Honeydew superficially white due to secondary conidiation can occur when relative humidity is high (90%), but honeydew is more usually brown and viscous or pink to light honey in color under field conditions in India (Kulkarni et al. 1976, Mughogho 1986) because normally humidity remains low (<80%) when honeydew exudation occurs in nature. The sphacelia are cylindrical, curved or straight, bilaterally-grooved, visible 1-2 days after honeydew exudation begins (Frederickson et al. 1991). At maturity, the protruding sphacelial portion of the parasitic biomass is discolored, whilst the proximal part, largely within the glumes, contains the true sclerotium, composed of compact, white plectenchymatous tissue under a thin, red-brown cortex (Sangitrao and Bade 1979b; Frederickson et al. 1991). The shape and size of mature sclerotia depend on host genotype, environment, and nutritional fac-
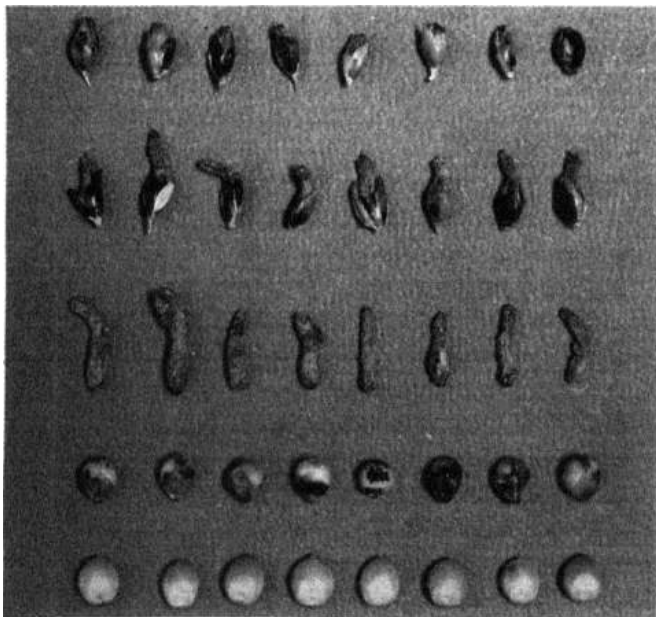

Figure 1. Differences in the size of Claviceps sorghi sclerotia, and grain discoloration because of honeydew. Top to bottom: small sclerotia concealed inside glumes; long sclerotia with glumes; long sclerotia removed from the glumes; discolored seed; healthy seed. Sclerotia were collected from the same field in Akola, Maharashtra State, India.

tors (Fig. 1). They measure 3-14 x 1-2.5 mm (Frederickson et al. 1991), 9-20 x 1.5-2 mm, and 4-7 x 1.5-2 mm (Kulkarni et al. 1976), 10-25 x 4-6 mm (Ramakrishnan 1948).

Claviceps species

Tsukiboshi and Frederickson (unpublished) describe sphacelia as elongate structures, straight or curved, the protruding part conical in shape. Sclerotia are purple-black, 14 x 2 mm, capped by a very small sphacelial remnant. Orange-yellow honeydew is exuded from infected florets, upon which secondary conidiation never occurs.

Causal organisms

Current evidence suggests that sorghum is uniquely host to three different ergot pathogens: Claviceps sorghi (Kulkarni et al. 1976), Claviceps africana (Frederickson et al. 1991) and a Claviceps species (possibly C. panicoidea). Claviceps sorghi is found only in Asia, whereas C. africana, the typical sorghum pathogen of Africa (Frederickson 1990, Frederickson et al. 1989 and 1991), is also known in Thailand and Japan (Frederickson et al. 1991, Mantle and Hassan 1994), and most recently in Brazil (Reis et al. 1996) and Australia (M Ryley, personal communication). The third pathogen, Claviceps species, appears to be present currently only in Japan (T Tsukiboshi, personal communication). The anamorphs of C. africana and C. sorghi are both known as Sphacelia sorghi M[c]Rae, Claviceps africana and C. sorghi have been compared and contrasted in a number of features, including phytopathology, teleomorphs, alkaloids, and honeydew sugar composition by Frederickson et al. (1991).

Anamorph of *C. africana* (S. sorghi)

Frederickson et al. (1991) describe the sphacelial stage as a highly convoluted, white parasitic body (5-8 mm long) bearing in discrete pockets the hyaline, mononucleate, oblong to oval macroconidia of 9-17 x 5-8 μm, slightly constricted at the center and with 2 polar vacuoles, and spherical microconidia 2-3 μm in diameter. Pear-shaped secondary conidia borne on sterigma-like processes are 8-14 x 4-6.5 μm with a distinct, protruding hilum.

Anamorph of C. *sorghi* (S. sorghi)

Bandyopadhyay et al. (1990) describe hyaline, unicellular, elliptic to oblong macroconidia with round ends of

13.2 x 7.3 µm. Pyriform, hyaline, secondary conidia are 12.1 x 7.2 µm. Hyaline, round to obovate microconidia are 3 x 4 µm. The original description of Kulkarni et al. (1976) describes macroconidia of 12-19 x 5.8 µm, whereas Frederickson et al. (1991) give measurements as 8-19 x 4-6 µm.

Anamorph of *Claviceps* species

Conidia are small, 5-11.3 x 2.5-5 µm; no secondary conidia are produced (personal observation, Frederickson and Tsukiboshi).

Teleomorph of *C. africana*

Frederickson (1990) and Frederickson et al. (1991) describe the stromatal origins as pale, globose proliferations of the sclerotium. Fully-extended stipes (8-15 x 0.3-0.6 mm) are pigmented purple, adjacent to the capitulum. Capitula (0.5-1.3 mm) are sub-globose, and intensely purple. Perithecia are 86-135 x 123-226 µm; mature asci in situ, 140 x 3.2-4.2 µm; 8 ascospores of up to 45 x 0.8-1.2 µm. Mower et al. (1973) depicted, but did not describe, stromata of *C. africana* from Nigeria.

Teleomorph of *C. sorghi*

Kulkarni et al. (1976) gave an incomplete description of the teleomorphic stage: 2-3 stromata, stipe, and capitula of unspecified sizes. Perithecia, 132.8-232.4 x 66.4-124.5 mm. Asci (56-112 x 2.4-3.2 µm), cylindrical with tapering ends and a hyaline apical cap; 8 ascospores, filiform, 40-85 x 0.4-0.8 µm. The description of the teleomorphic stage provided by Sangitrao (1982) is almost similar to that of Kulkarni et al. (1976) except for a few minor details. Frederickson et al. (1991) describe the stipes as 6-8 x 0.5 mm, burnished-bronze/ deep terracotta in color. Capitula 0.7 mm diameter, buff-colored but with darker, papillate perithecial ostioles, the stipe insertion point is surrounded by a white frill. Perithecia measure 130-250 x 60-125 µm. The white frills were not described by Kulkarni et al. (1976) and Sangitrao (1982).

Teleomorph of *Claviceps* species

According to Tsukiboshi (personal communication) the stromata resemble those of *C. sorghi,* with a buff to terracotta stipe and terracotta-colored capitulum, but lacking a frill at the capitulum base. Ascospores are 92.5-205 µm long, and larger than those of *C. sorghi.*

Biology of the pathogens

Path of infection

The path of infection by *C. africana* (Frederickson 1990) and *C. sorghi* (Frederickson and Mantle 1988) have been studied in great detail. The latter study has been corroborated by ICRISAT (1989) and Bandyopadhyay (1992).

Ergot is a tissue-specific disease; only pistils are infected. The patterns and events during the course of infection for *C. africana* and *C. sorghi* are similar. The stigma is the principal site of infection, although conidia can germinate (Frederickson 1990) and infect (R Bandyopadhyay, unpublished) through the style and ovary wall. The primary route of ovary colonization is as follows. Conidia germinate on the stigma producing 1-4 germ tubes. After penetrating the stigmatic papillae, the infection hyphae grow intercellularly through the stigmatic rachis, progress through the transmitting cells of the style up to the ovary, then down through the ovary in the inner ovary wall tissues adjacent to the ovule, and finally reach the vascular bundles within the rachilla. Only upon contact with these vascular bundles docs the fungus rapidly colonize the ovary acropetally. Bundles of hyphal strands grow intercellularly along the inner layer of the ovary wall, integuments, and epidermis. The fungus also colonizes the ovary tissues from the focus of the initial infection tract. Ovule tissues are colonized by the invasive hyphae from the ovary. Finally, the fungus replaces the ovary with its own deeply involuted soft mass, called sphacelia. Initially, sphacelia secrete a clear liquid which becomes more opaque (honeydew) upon production and release of macroconidia originating from short conidiophores on the surface of sphacelia. The sphacelia possess several internal locules. Conidiophores lining the locule also produce macroconidia that stay trapped within the locule. The sphacelia later harden and are converted into sclerotia. Throughout the course of invasion, the fungus retains contact with the vascular bundles in the rachilla without destroying it, and thus allows continued nutrient transport to support the sphacelia and honeydew production.

Time course of infection and colonization events

A summary comparison of time-course of infection events from reports by Sangitrao (1982), Frederickson and Mantle (1988), ICRISAT (1989), Frederickson (1990), and Bandyopadhyay (1992) is given in Table 1. The first two reports deal with isolates of *C. sorghi* collected from the same location (Akola, India), but differ in environmental conditions used during the experiments.

Table 1. Colonization of different parts of the sorghum ovary by *Claviceps africana* and C. *sorghi* on a time scale.

Event	*Claviceps africana*[1]	*Claviceps sorghi* Report A[2]	Report B[3]	Report C[4]
Germination of conidia	15-24 h	16 h	12 h	12 h
Infection hyphae at the stylar end of ovary	-	3 d	2 d	2-3 d
Infection hyphae at the basal end of ovary	4 d	5 d	3 d	5 d
Complete ovary colonized	6-8 d	8-10 d	5-8 d	6-7 d

Source: 1. Frederickson (1990). Study conducted at 10-30°C, and 65-100% RH; 2. Frederickson and Mantle (1988). Study conducted at 10-30°C, and 65-100% RH; 3. ICRISAT (1989). Study conducted at 14-28°C, and 45-90% RH; and 4. Sangitrao (1982). Study conducted at 17-33°C, and 30-60% RH.

In the study by Frederickson (1990) on *C. africana,* conidia germinated on stigmas within 15-24 h, and the ovary wall had been substantially colonized, with hyphae visible even in the peripheral ovule tissues, at the base of the ovary and on the lower ovary surface, by day 4. At 6-8 days the macroscopic sphacelium was visible.

The study by Frederickson and Mantle (1988) on *C. sorghi* occurs within the constraints of diurnal temperature and relative humidity (RH) oscillations of 10-30°C and 65-100% RH and can be compared directly to the study of *C. africana* (Frederickson 1990), which occurred concurrently, Macroconidia germinated on stigmas within 16 h of deposition. Hyphae travelled to the top of the ovary in 3 days. Predominantly abaxial colonization of the inner ovary wall proceeded, and by day 5, hyphae were visible at the ovary base. At day 6, ovary base colonization was so extensive that hyphae emerged onto the ovary surface, permitting acropetal, surface colonization in the next 2-3 days. Also on day 6, hyphae had penetrated the chalazal region of the ovule. Subsequent colonization was very rapid so that at 8-10 days, the whole ovary had been colonized, sphacelial fructifications were evident, and honeydew exudation commenced.

Influence of environment

In vitro germination of conidia

Claviceps africana. In vitro on potato dextrose agar (PDA) or water agar (WA), macroconidia began to germinate after 12 h at 14-35°C; the optimum temperature was 19°C (Frederickson 1990, McLaren and Wehner 1990), and conidia failed to germinate at 37°C. Germination was always iterative, i.e., producing a secondary conidium. However, the sterigma-like process bearing the secondary conidium became extended with increasing temperatures to a maximum of five times the conidial length at 30°C (Frederickson 1990). The proximity of the macroconidia to pollen stimulated germination by germ tube formation. Macroconidia were unable to germinate once the sucrose content of the media reached 10% w/v.

Claviceps sorghi. Frederickson (1990) observed that macroconidia required 16 h to germinate in vitro at all temperatures. Conidia germinated from 14°C to 37°C, optimally at 35°C. Germination percentage did not vary significantly over the 18-30°C range but was always iterative. At 35°C, germination was in the form of two, sometimes three, germ tubes. Macroconidia were able to germinate even with 34% w/v sucrose in the media.

Germination of macroconidia was studied on water agar with or without amendment with a suspension of stigma macerate at 15, 20, 25, 30, and 35°C (R Bandyopadhyay, unpublished). Macroconidia germinated in two modes, iteratively from lateral sides by producing thin, slender, sporogenous germ tubes terminating in secondary conidia, and noniteratively by producing thick germ tubes from both ends. Macroconidia almost always germinated noniteratively in association with stigma macerate, and iteratively in the absence of stigma macerate. When in contact with stigma macerate agar, 96-100% macroconidia germinated noniteratively at 15-30°C, but only 30% at 35°C after 48 h. Germ tubes were 10 times smaller (40 μm) at 15°C than at 20°C. On water agar, iterative germination was 47% at 15°C, 94% at 20°C, 6% at 25°C, and 3% at 30°C; no germination was observed at 35°C. Iterative germination is responsible for the production of secondary conidia, whereas noniterative germination is a prerequisite for infection. These data suggest that infection can occur within a wide tern-

perature range, but 20±2°C is most favorable for secondary sporulation.

Claviceps species. At the optimum temperature of 27.5°C, conidia of *Claviceps* species begin to germinate in vitro after only 10 h producing four germ tubes. At 20-25°C germination is iterative (T Tsukiboshi, personal communication).

In vivo germination of conidia

Germination of macroconidia of *C. africana* on stigmas to form penetrative germ tubes started 15 h after inoculation (Frederickson 1990) under diurnal temperature oscillations of 12-28°C. Iterative germination of macroconidia was never seen on stigmas. Frederickson and Mantle (1988), noted microconidia of C. *sorghi* germinating by one to several germ tube(s) within 16 h at 12-28°C and Bandyopadhyay et al. (1990) observed the same thing after 24 h at 14-28°C following inoculation onto the stigma.

Sphacelia and sclerotia formation, and sporulation in vivo

Temperature plays a major role in the rate of colonization of ovary tissues by C. *sorghi* (R Bandyopadhyay, unpublished). At constant temperature, sphacelia appeared at the basal end of the ovary 5 days after inoculation (DAI) at 25°C, 6 DAI at 20°C, and 8 DAI at 15°C. Honeydew exudation began 6-7 DAI at 25°C, 7-8 DAI at 20°C, and 13-14 DAI at 15°C. Therefore, the rate of colonization was slower, and more obviously, sporulation was delayed at lower temperatures. However, constant temperatures do not occur in nature. With diurnal variations in temperature, differences in colonization rates were less discrete in growth chamber studies. With day/night temperatures of 35/28°C (RH 90% for 2 h d^{-1}) and 28/23°C (RH 90% for 16 h d^{-1}), sphacelia were visible at the hilar end of the ovary 3 DAI compared with 5 DAI at 24/14°C (relative humidity 90% for 12 h d^{-1}). Honeydew exudation began 5 DAI at 28/23°C, and 6 DAI at 35/28°C and 24/14°C. Production of honeydew and formation of secondary conidia were most profuse at 28/23°C, followed by 24/14°C, and least at 35/28°C (Bandyopadhyay et al. 1990). Conditions favorable for the production of secondary conidia are least conducive for the production of microconidia, and vice-versa. For example, the proportion of microconidia in the honeydew was 17.5% at 35/28°C compared with 4% at 28/23°C.

Sangitrao and Bade (1979a) reported that low temperature and RH increase the incubation period (inoculation to honeydew production). Sclerotial formation was favored by dry weather. Bandyopadhyay et al. (1990) found that temperatures of 14-28°C and RH above 90% for 12-16 h favored conidial, but not sclerotial, production. Higher temperatures of 28-35°C and <80% RH for 12 h were conducive to sclerotial formation (R Bandyopadhyay, unpublished).

Formation of secondary conidia

Increased RH, leading to continued exudation of 'thin' honeydew stimulates iterative germination of macroconidia at the honeydew surface, and results in the formation of secondary conidia in both *C. africana* and C. *sorghi.* Secondary conidiogenesis is unique because of the rapidity and the manner in which it occurs in the honeydew (Frederickson et al. 1989, Bandyopadhyay et al. 1990). Macroconidia inside the 'thick' honeydew do not usually germinate because of the high osmotic potential caused by the high sugar concentration in the honeydew matrix. However, being hygroscopic, the honeydew surface might absorb water from the atmosphere. This would lower its osmotic potential and make the honeydew 'thin'. As a result, under humid conditions, macroconidia on the honeydew surface germinate by two methods. In the first method, macroconidia germinate by long, thick, branched or unbranched germ tubes that enmesh to form a firm hyphal mat which provides a stable surface on the otherwise flowing, inner fluid of the honeydew. The second method of germination is iterative and involves the extension of germ tubes outside the honeydew surface. These germ tubes are functionally conidiophores terminating in apical secondary conidia that are easily detachable and disseminated by wind.

In spore-trapping experiments, a diurnal pattern of secondary conidia concentration was evident, with the greatest occurrence at nightfall, coinciding with the sharp rise in RH and fall in temperature (Frederickson et al. 1989 and 1993). Secondary conidiation did not occur when there were several hot, dry days in succession.

Germination of sclerotia

Claviceps africana

Most attempts by Frederickson (1990) to germinate sclerotia proved futile, with only 5% germination after 16 weeks of incubation in sterile sand at a range of temperatures from 4° to 28°C. However, Frederickson et al. (1991) subsequently reported that 10% of sclerotia from Matopos, Zimbabwe, maintained for 1 year in dry storage at 20-25°C, germinated after 4 weeks of burial 3 cm deep in moist soil, and diurnal temperature oscillations of 10-28°C. Globose, creamy-white initial germination

structures were first seen. By 6 weeks, 80% of the sclerotia had germinated, with differentiating stipes and capitula. Few stromata survived to maturity. Mower et al. (1973) reported stromata of a Nigerian pathogen but did not describe the conditions for inducing germination. Mantle (1968a) incubated scierotia of a Nigerian pathogen at 24°, 27°, and 30°C, achieving initial germination after 4 weeks at 27°C, which was subsequently aborted.

Claviceps sorghi

In Bangalore, India, Kulkarni et al. (1976) induced germination by incubation of small, hard, scierotia in soil in petri dishes. Initial germination structures were observed after 35 d in 19 of 24 scierotia. Full differentiation to stromata was seen after 45 d. Details of environmental conditions were not given. Frederickson et al. (1991) reported germination of elongate scierotia to give stromata after 5 weeks of incubation on moist sand at 24°C. In Akola, India, Sangitrao (1982) reported stromata differentiated in 50-55 d in up to 55% scierotia.

Sangitrao (1982) also conducted detailed studies using a sclerotial germination assembly that gave consistent germination of scierotia at 27°C. Among the different substrates tested, scierotia germinated best when placed horizontally flat on moist sterilized sand. Scierotia placed vertically with either end buried did not germinate. Fragments of scierotia with intact basal ends germinated to produce stroma. Fragments from other parts did not germinate. Sclerotia were viable after storage for 3 years at room temperature in the laboratory. Although field-exposed scierotia failed to germinate, scierotia buried up to 60 cm deep in soil in the greenhouse germinated readily in the germination assembly. Scierotia germinated at 5-50°C, 20-30°C being the optimal range. Host genotypes had significant effects on germination. While 50-55% scierotia germinated from CK60A and IS 84, germination was about 25-35% for scierotia obtained from such genotypes as 2077A, CSH 1. and R 473. Fungicides Aureofungin®, Bavistin®, Ceresan®, thiram, and Captan® did not inhibit germination completely, but reduced it to some extent.

Claviceps species

In contrast to C. *africana* and C. *sorghi,* scierotia of *Claviceps* species germinate very easily on moist sand under scattered light at 25°C. Germination is approximately 50% after 1 month. Germination has also been observed in the field (T Tsukiboshi, personal communication).

Pollen-pathogen-environment interaction

Flowering behavior

Since the ergot pathogens infect and colonize only the gynaecia, knowledge of the inflorescence is essential to understanding the disease. Flowering behavior of sorghum has been described in detail (Stephens and Quinby 1934, Quinby 1958, House 1985). The sorghum panicle is a raceme. Anthesis in spikelets occurs basipetally. During anthesis, the glumes normally open in the morning, the stigma and anthers emerge, anthers shed pollen to pollinate the stigma, and later the glumes close. However, the flowering process is known to vary significantly in different environments and genotypes. For example, in some genotypes stigmas emerge from glumes and remain exposed at least 72 h before anthesis, while in others a cleistogamous condition occurs. The time of flower opening during the day, duration of flower opening, viability of pollen, and the duration and extent of stigma receptivity, all depend on host and environmental conditions. For example, almost all spikelets flower during the night and pollen loses viability after 5 h when sorghum flowers at 26-39°C and 25-60% RH (Stephens and Quinby 1934), but at 10-30°C and 30-95% RH, anthesis occurs only after sunrise, and pollen remains viable for longer periods (Sanchez and Smeltzer 1965). Host factors also determine pollen production, stigma receptivity, stigma size, and other floral characters.

Infection and pollination on a time scale

The spikelet parts most critical for infection and colonization are stigma and anthers. Normally, a stigma is pollinated soon after it is exposed, pollen germinates within 30 min, and fertilization occurs within another 2-12 h (Stephens and Quinby 1934, Artschwager and McGuire 1949). On the other hand, conidia require 8-12 h for germination on the stigma, and 36-48 h to reach the base of the ovary (see Table 1). However, the times for these events vary from floret to floret and are strongly influenced by the environment. For example, fluorescence microscopy studies showed that not all pistils were fertilized even 48 h after pollen shed and successful pollination (R Bandyopadhyay and N W McLaren, unpublished observations).

Relationship between pollination, fertilization, and ergot infection

Several studies have shown that effective pollination and fertilization make pistils escape or resist ergot (Futrell and Webster 1965, Sangitrao 1982, Musabyimana et al. 1995). In an experiment using a fluorescence microscope,

the progress of pollen tube growth and fungal growth was traced at 24-h intervals in pistils of spikelets inoculated 4, 3, 2, and 1 d before or after anthesis, and also at anthesis (Bandyopadhyay et al. 1992). The percentage of spikelets with pollen on the stigma (pollinated), and pollen tubes in ovules (fertilized) were calculated from the microscopy data. The percentage of infected spikelets was calculated from the records of the number of inoculated and infected spikelets in the field. When pollen and conidia were placed concurrently on the stigma, pollen germinated earlier than conidia, and the pollen tube reached the embryo sac faster than the colonizing infection hypha. In pollinated and fertilized pistils, the fungus grew slowly, or failed to grow, thereby reducing ergot severity. In a few cases, infection hyphae and pollen tube grew together up to the ovule, and, as a result, both sphacelia and grain developed together in some spikelets. Data showed that spikelets inoculated 1-4 d after anthesis were all pollinated, but 7.8% were not fertilized and thus remained susceptible, as confirmed by data showing 4-10% ergot severity in the field. Ergot severity increased to 52-95% in spikelets inoculated 1-4 d before anthesis, since the efficiency of fertilization was low. Among these spikelets, 91-100% were pollinated, but only 11-14% were fertilized. Therefore, efficient pollination did not ensure high fertilization rates. The data also show that fertilization was effective in controlling ergot, but that it did not prevent the pathogen from colonizing a few pistils.

Effect of environment on pollination, infection, and their interaction

Futrell and Webster (1965) suggested that any factor that prolongs the period from flower opening until fertilization, promotes ergot infection. In controlled pollination frequency trials they found a strong correlation (r=0.87) between the percentage of unpollinated florets and ergot infection.

Downes and Marshall (1971) demonstrated in greenhouse experiments that night temperatures of 13°C or less during meiosis can induce male sterility in sorghum. Brooking (1976) estimated the critical stage for this to be 2-3 weeks prior to anthesis. The greatest temperature sensitivity was during the late archesporial cell—pollen mother cell development period, up to the leptotene stage of meiosis. Once meiosis progressed beyond leptotene, sterility was not induced. Development from microspore release through to fertilization was particularly insensitive to prolonged night temperature treatment. Inflorescences showing low temperature-induced sterility, developed anthers that were exserted normally at an-

thesis, but were only partially dehiscent. Pollen grains in these anthers at anthesis were mainly vacuolate, two-celled grains, equivalent to control pollen just prior to the onset of the maturation phase. In contrast, most of the control pollen was densely cytoplasmic and packed with starch grains.

The relationship between cold-induced sterility and ergot susceptibility was demonstrated by McLaren and Wehner (1992). In field trials with three male-normal sorghum genotypes, night temperatures <12°C, 3-4 weeks prior to flowering increased susceptibility to ergot to the equivalent of that of male-sterile genotypes. Seed set in noninoculated heads under pollination bags was also reduced, suggesting that increased susceptibility was the result of low temperature-induced sterility. Ergot incidence and seed set were inversely correlated (r=-0.92). Genotypes differed in their ability to tolerate pre-flowering cold stress, and in two of the three genotypes a progressive increase in sterility and concomitant increase in ergot was recorded from 16°C and below. This result is similar to cold induced sterility recorded by Brooking (1979), who suggested that the linear response of some genotypes to temperature reduction indicates that sterility is a quantitative response and not a qualitative one occurring below a critical temperature. Greenhouse and growth-chamber trials by McLaren and Wehner (1992) confirmed that cold stress applied 7-8 weeks after sowing reduced pollen viability, and that this was the primary reason for increased susceptibility to ergot.

Watkins and Littlefield (1976) studied ergot infection of wheat caused by *C. purpurea* and found an increase in ergot at low temperatures. They suggested that anthers may be sensitive to cool temperatures so that pollination is delayed and the ovaries remain susceptible for a longer period. Suneson (1937) reported the absence of functional anthers at flowering in wheat plants chilled from -3 to 2.5°C 1-5 weeks before they emerge from the flag leaf sheath. This could be the reason why pollination does not occur and ovaries remain susceptible to ergot for a longer period of time (Watkins and Littlefield 1976).

Brooking (1979) recorded some form of partial sterility in the hybrid CK60 x 606 at 25-20°C, as only 76% of the pollen grains were starched. Similarly, Thakur and Williams (1980) reported incomplete fertility restoration in F_1 hybrids of pearl millet as a factor reducing effective pollen availability and hence increasing ergot susceptibility in some hybrids. Pollen viability studies by McLaren (unpublished) suggest that this may be a major reason for differences in ergot escape resistance in sorghum lines. Only 5 of 58 lines evaluated showed 100% pollen viability despite minimum temperatures of <19°C during the period 3-4 weeks prior to anthesis. These
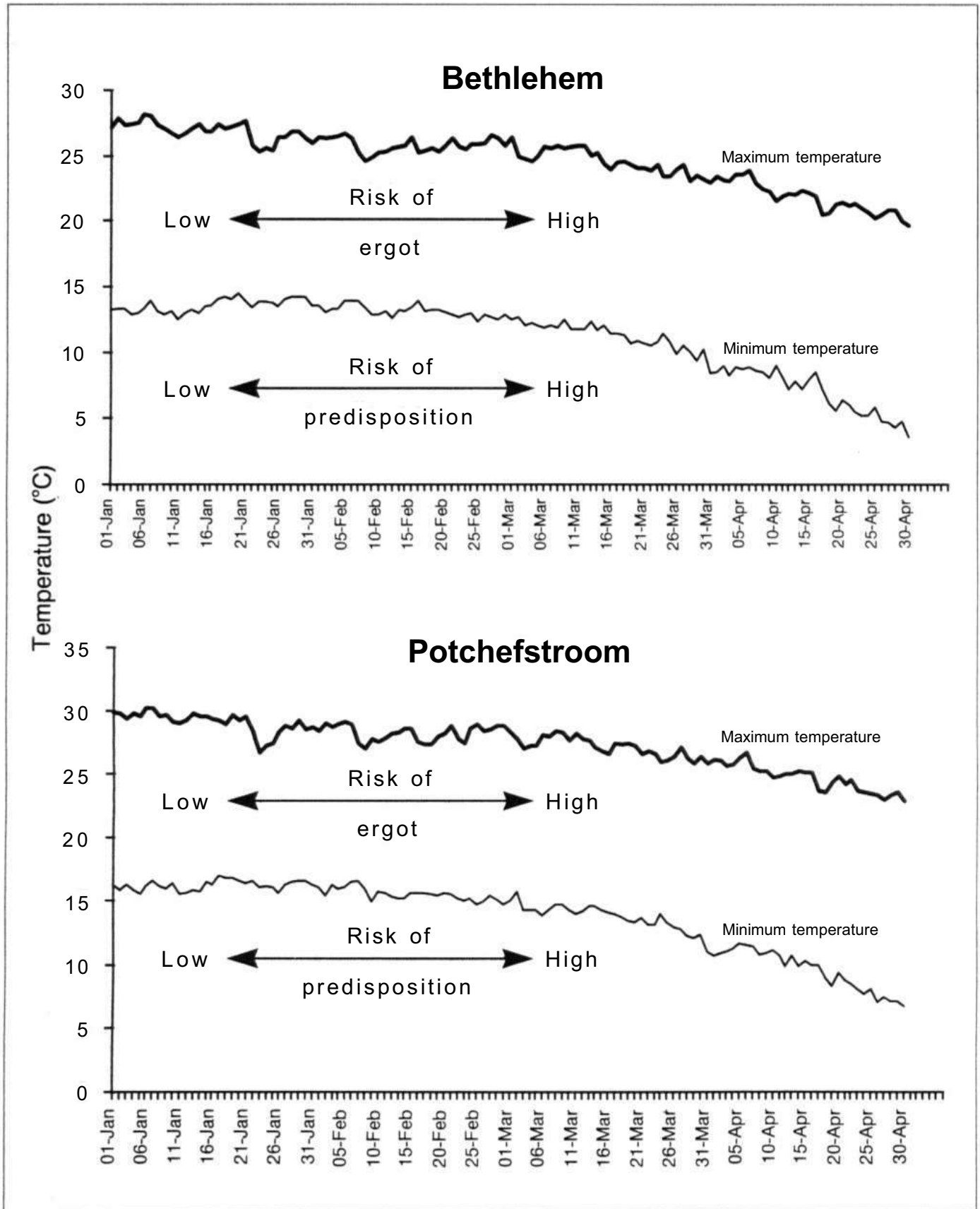
Figure 2. High and low ergot risk periods based on long-term daily maximum and minimum temperatures at two locations (Bethlehem and Fotchefstroom) in South Africa.

Local spread

Claviceps africana

Insect vectors, including moths and flies, have been linked with the local transmission of *Claviceps purpurea* and C. *paspali* (Langdon and Champs 1954, Moreno et al. 1970, Ingold 1971, Beerwinkle et al. 1993). *Claviceps paspali* is also transmitted by wind and rain (Noble 1936). The perfect stage of *C. purpurea* is formed readily and ascospores are known to act as infectious agents (Youngken 1947, Mantle and Shaw 1976). By analogy, the spread of sorghum ergot by wind-driven rain, mechanical contact and windborne ascospores has been assumed (Tarr 1962, Futrell and Webster 1966, Sundaram 1976), but without any real evidence. Instead Frederickson et al. (1989 and 1993) provide strong evidence for the role of windborne secondary conidia of *C. africana* in transmitting disease over moderate distances under natural environmental conditions in Zimbabwe. Secondary conidia are formed readily under natural conditions in southern Africa. In one experiment, an inoculated row of male-sterile sorghums exhibited prolific secondary conidiation of honeydew. Disease subsequently spread to panicles across several meters, a distance precluding physical contact, in one cycle of infection. Of 15 panicles protected from insects by fine-mesh netting, 14 became infected and germinating secondary conidia were observed on their stigmas. Using a spore-trap, secondary conidia were captured close to the panicles. In a second experiment, a disease source was created at the center of a 23.5 x 33 m area. The secondary conidiation of the source was apparently responsible for initial disease outbreaks up to 15 m away. Secondary conidia were trapped in air at this time. After one more cycle of honeydew production, when secondary conidia were even more abundant in the air, nearly all the panicles had become infected. Panicles protected from contact with large, flying insects became infected as did those in the first experiment. Secondary conidia were thus judged at least partially responsible for secondary spread. That rain occurred after the establishment of the initial outbreaks suggests that later spread may have been partially due to the distribution of honeydew by rain splash, although airborne secondary conidia were present at the same time. Frederickson captured macroconidia from the air during rain at panicle height and 0.75 m from infected panicles in a later study (unpublished).

Claviceps sorghi

In disease spread experiments similar to those of Frederickson et al. (1989 and 1993), Bandyopadhyay et al. (1991) attributed disease spread to water splash from sprinkler irrigation in rain-free situations. Their data show that in a male-fertile genotype, the proportion of infected plants in the total flowering plants d^{-1} increased from 40% 7 DAF to 100% 20 DAF. The number of infected spikelets plant^{-1} increased from 40 to 180 in a similar time-frame (Bandyopadhyay et al. 1994a) indicating an increase in inoculum from secondary foci. There were 2-3 infection cycles, each of 6-8 d duration in a flowering season. From a point source, the disease spread over a 33 x 33 m field resulting in more than 25% plant infection. Ergot spread and focus formation was also mapped (Bandyopadhyay et al. 1994b). The spatial pattern of infected spikelets changed from a focal to a random pattern as the disease progressed through the distribution of macroconidia. Dispersal gradients obtained by fitting calculated dispersal data with power model, decreased over time. The Lloyd Index of patchiness for distribution of diseased spikelets also decreased as the disease progressed. The secondary foci appeared to be randomly established in the down-wind areas of the fields.

Long distance spread

The introduction of the disease in Brazil and Australia, and its spread within these countries offer good examples of the potential for long-distance spread. It also exemplifies the rapidity with which epidemics can spread to cover large geographical areas.

In Brazil, the disease was first noticed during mid-February in seed production plots in Sao Paulo state. Within one week, the disease was reported from Ribeirao Preto, Capinopolis, Lavras, Sete Lagoas, Pirapora, Paracatu, and Parana towards the north, and Xapeco (Santa Catarina state), and Pelotas (Rio Grande state) towards the south. The locations in Sao Paulo and Minas Gerais states where the disease was found surrounded an area approximately 800 000 km^2. The distance from Paracatu in the north to Pelotas the farthest point of infection in the south is nearly 2000 km. The magnitude of the area and distance covered by the pathogen within the short period of 1 week show the extraordinary capacity of the pathogen to spread rapidly in terms of both time and space.

Examination of weather data for Jan and Feb 1995 (provided by Dr O Brunini, Instituto Agronomico de Campinas, Campinas, Sao Paulo) showed that nearly twice the normal rainfall occurred in February in central and southern Brazil. The 25-day period before first report of occurrence of the disease in Ribeirao Preto (15 Feb) was extraordinarily wet. During this period, 446 mm rainfall occurred on 21 days. From 1-15 Feb alone, 343 mm of rain fell in 14 days. The predominant wind

directions at mid-day were north, northwest, and east. After 15 Feb, the predominant wind directions were south and southeast. Minimum temperature during mid-Jan to Feb was between 19-21°C and, therefore, not limiting for pollen production. However, the exceptionally wet conditions severely affected anther emergence, anther dehiscence, and pollen deposition. The inefficient pollination of male-sterile lines predisposed their stigmas to infection. The wet conditions and cloudy weather also provided an ideal environment for the continual production of abundant secondary conidia. Therefore, all the constituents for an epidemic were available at the same time—a susceptible host with an extended susceptible period, that was exposed to abundant inoculum of a newly introduced pathogen during favorable weather. A shorter incubation period, extended susceptible period, longer favorable period for infection and sporulation, and rapid transport of airborne secondary conidia aided several cycles of infection in the same area, and long-distance spread to newer areas. A reconstruction of *probable* events that led to the epidemic is as follows:

- Small foci of infection developed somewhere in south-central Brazil during 21-23 Jan.
- Secondary conidia produced in these foci were lifted in the cloud layer by convective currents (common during this part of the year) during 24-26 Jan.
- Traveling north with the clouds, secondary conidia deposited in the Ribeirao Preto area and further north with rain on flowering sorghum during the remaining days of Jan and early Feb.
- Initially in the newly introduced areas, few plants became infected and, by 7-12 Feb, produced numerous secondary conidia.
- Local and long-distance transport of these conidia helped to spread the extensive epidemics.

In Australia, the pathogen was first seen near Brisbane on 26 Apr 1996. Within 3 weeks, the disease became endemic in 60 000 km^2 of southern Queensland, and in northern Queensland in a much smaller area approximately 1500 km from the southern outbreak. The disease is now found throughout Queensland. Exceptionally cool and wet weather before and during flowering predisposed the sorghum crops to infection (M Ryley, personal communication).

The ergot pathogen in Brazil and Australia has been identified as *Claviceps africana,* the same as the one that is endemic in southern Africa and Thailand. Besides sorghum, ergot was also noticed for the first time in 1995 on pearl millet in Brazil. The pathogens causing ergot on sorghum and pearl millet are different. No one may ever know how the sorghum ergot pathogen was introduced to Brazil and Australia, despite strict quarantine. We can only hypothesize now:

- Sorghum seeds contaminated with ergot sclerotia from a *C. africana*-endemic region may have been illegally carried and sown in Brazil and Australia.
- Anyone walking through an infested field in a *C. africara*-endemic region, could have had their clothes smeared with honeydew, and/or brought back some soil (with macroconidia) on footwear. The same person perhaps walked into a sorghum field in Brazil wearing the contaminated clothes and/or shoes, thereby introducing the pathogen. Macroconidia can germinate on soil to produce secondary conidia which, being airborne, can infect sorghum.
- Due to an unusual intercontinental air current pattern from southern Africa to South America, secondary conidia could have been lifted from Africa to the cloud layer, crossed the Atlantic, and subsequently deposited in southern Brazil. This may sound science fictional, but explains why ergot appeared simultaneously on the two cereals in Brazil.
- Ergot could have been introduced into Brazil and Australia earlier than in 1995 and been present in low, undetectable frequencies. The exceptionally favorable conditions in 1995 (in Brazil) and 1996 (in Australia) could have helped the development of easily noticeable epidemics.
- A naturally occurring ergot pathogen on grasses may have undergone a change in virulence to diversify its host range to include sorghum. Initially, such an infection could have been in low proportion, but suitable weather helped development of an epidemic from a few small foci.

An understanding of the mode of introduction of the pathogen may help to devise methods to prevent its spread to new areas.

Survival of conidia in sphacelia/sclerotia

Sangitrao (1982) found that up to 16% sclerotia stored for 3 years were able to germinate by producing well-differentiated sexual structures and ascospores, and macroconidia in pionnotes. Macroconidia and microconidia can also survive in conidia in the dried honeydew on infected panicles that fall to the ground and remain viable for 7 months (Futrell and Webster 1966). Frederickson et al. (1991 and 1993) routinely used 9-12-month-old, dry-stored sphacelia of *C. africana* to generate inoculum for infection experiments. The initial resultant infections were few (usually only up to 10 sphacelia), but the honeydew had its normal infectivity restored following one passage through the host. Conidia from sphacelia stored

at 4°C or at room temperature, had equally low infectivity. Mower (1973) also used dried honeydew, stored at 5°C for 9 months, to infect sorghum.

Host range

There have been numerous reports on possible collateral hosts of *C. africana* and *C. sorghi.* The comprehensive list of Bandyopadhyay (1992) indicates that alternate hosts of *C. sorghi* include *Cenchrus setigerous, Ischaemum pilosum, Pennisetum orientate, Sorghum arundinacearum, S. caffrorum, S. halepense, S. membranaceum, S. nitens, S. verticilliflorum,* and *Zea mays,* whilst conflicting results have been reported for *Cenchrus ciliaris* and *Pennisetum typhoides* (Chinnadurai and Govindaswamy 1971b, Sundaram et al. 1970, Sundaram 1974). Recorded alternate hosts of *C. africana* are few to date. Futrell and Webster (1966) reported infection of *Zea mays* in Nigeria following artificial inoculation with *C. africana* conidia, but no infection of P. *typhoides.* However, conidia from *Panicum maximum* infected sorghum. This latter result is in accordance with Boon-Long (1992) but in disagreement with Frederickson, who could not infect sorghum with conidia from *P. maximum* in Zimbabwe. However, infection of several *Pennisetum americanum* varieties was successful. Conidia of ergot from *Cynodon dactylon, Urochloae brachyura, Brachiaria bryzantha, Digitaria tenata, Chloris guyana, Sporobolus pyrimidalis, Hyparrhenia* sp, and *Andropogon* sp, collected in South Africa, Zambia, and Zimbabwe did not infect male-sterile sorghum following artificial inoculation (Frederickson 1990). These results are not surprising because, according to Loveless (1964), the differences in size and shape of honeydew conidia on these grasses suggest that the pathogens are not *C. africana.* However, Sangitrao and Moghe (1995) reports that a triangular spore form of *S. sorghi* collected from *Dicanthium caricosum* could infect sorghum in India. Boon-Long (1992) reported infection of sorghum in Thailand with conidia from *P. maximum, Dicanthum annulatum, Brachiaria mutica, Sorghum sudanensis, S. almum,* and *S. halepense.* Only honeydew from a wild sorghum species in Zimbabwe, probably *S. versicolor,* has induced disease on cultivated *Sorghum bicolor* following inoculation.

In Japan, *Claviceps panicoidearum* from *Miscanthus* species can infect sorghum. Moreover, the teleomorph and anamorph show such a striking resemblance to *Claviceps* species (not *C. africana)* on sorghum that they may be the same organism (Tsukiboshi and Frederickson, unpublished).

Disease cycle

Primary infection in the field is probably established by conidia from collateral hosts and infected plant debris. Ascospores are also likely to serve as sources of primary inoculum. Based on field observations Singh (1964), Futrell and Webster (1966), and Sangitrao (1982) hypothesized that ascospores and conidia could infect collateral hosts that flower prior to sorghum, and that the conidia in the honeydew of collateral hosts provide fresh inoculum to initiate primary infection in sorghum. Infected spikelets exude millions of conidia in the honeydew which are spread by wind, rain, and possibly insects. In dry weather, well-differentiated sclerotia are produced and honeydew forms a hard white crust after the completion of the sporulation phase. In the following season, ascospores are produced from scierotia; conidia are produced from dried honeydew (Futrell and Webster 1966) or as pionnotes on germinating scierotia (Sangitrao 1982). Scierotia have internal locules in which macroconidia are produced and protected from the elements. With time, scierotia disintegrate and release macroconidia. These macroconidia can germinate on the surface of moist soil to produce secondary conidia. Secondary conidia are easily disseminated by wind and can initiate infection. However, the locule-macroconidia-secondary conidia hypothesis is still untested. The sexual and asexual spores subsequently infect collateral hosts, or may infect sorghum to complete the disease cycle.

Disease control options

Plant quarantine

Until recently, the disease has been successfully kept out of Australia. Australian quarantine laws require that all sorghum seeds imported for research or cultivation must be grown for one generation in a quarantine greenhouse, and be inspected by a qualified plant pathologist. The cordon of quarantine was, however, breached by an epidemic of the disease in Apr 1996. The strict quarantine regulations of the USA regarding sorghum ergot need to be strongly enforced to avoid entry of the pathogen into that country.

A standard protocol for the detection of *C. africana* in seeds does not exist and needs prompt development. Heavily contaminated seed lots can be recognized by an experienced eye following dry seed inspection, with dry white or orange-brown honeydew being evident on seeds or on sphacelia or scierotia. Small, spherical to conical sphacelia or scierotia, often with glumes still attached, can be seen among the more spherical seeds. Errors may have arisen in the past through quarantine officials failing

to recognize the small *C. africana* sclerotia when expecting the elongate sclerotia, typical of *C. sorghi,* as shown popularly in the literature. Following dry seed inspection a washing test may assist further. Seeds containing sclerotia, sphacelia or honeydew when ground and then agitated in very little water should release the distinct primary and secondary conidia of *C. africana* for verification under a microscope. The effectiveness of using a saline soak to float-out sclerotia of *C. africana* has not been demonstrated. Also, a method based on the detection of the sclerotial alkaloids of *C. africana* following extraction can be devised.

At ICRISAT Asia Center, quarantine regulations are followed rigorously to eliminate the possibility of exporting ergot-contaminated seeds. The protocol followed by ICRISAT is a simple one. The seed crop is normally grown during the hot and dry postrainy season with irrigation to avoid the disease. Seed lots are then physically cleaned to remove glumes, soil and other particulate matters. The Plant Quarantine Unit subsequently examines the seed lots under 10 x magnification for the presence of sclerotia. The seed lot is rejected if sclerotium is found. Then the lots are fumigated with methyl bromide (32 g m^{-3}) for 4 h. Finally, the seed is treated with a mixture of Benlate® and thiram (both are toxic to ergot conidia at very low doses) before despatch. However, the US quarantine regulations stipulate that seeds should not be treated with any chemicals. The chances of introduction of the pathogen through seed are minimized if the quarantine regulations are followed rigorously.

Host-plant resistance

Inoculation methods

Artificial inoculation methods must take into consideration the importance of flowering biology in disease development. It is inappropriate to inoculate spikelets after anthesis because fertilization interferes with infection. Evaluating flowers inoculated after pollination measures disease escape, rather than resistance. Essentially an inoculation method involves at least one artificial inoculation of nonpollinated spikelets and bagging of panicles (Musabyimana et al. 1995). Trimming of spikelets to remove all pollinated spikelets from a panicle before inoculation ensures that only nonpollinated spikelets are inoculated. Alternatively, inoculation of panicles on the first day of anthesis (Tegegne et al. 1994) or with approximately only 10% pollen shed (McLaren 1992) will achieve nearly the same objective without expending the time and effort required for trimming. Frederickson et al. (1994) selected panicles that had not flowered, inspected them daily in the morning, marked freshly anthesizing

florets, and inoculated them with a spore suspension. Only the marked florets were subsequently used to measure disease. After inoculation, bagging enhances ergot severity by maintaining the high humidity that favors infection. Bagging also ensures that each panicle is tested without interference from external pollen, that could interfere with disease development. Furthermore, bagging facilitates production of selfed seeds, thus allowing direct selection of ergot-resistant plants.

Screening techniques

Hot spots. A hot spot is a location where environmental conditions are favorable and natural sources of inoculum are available for severe occurrence of the disease in most years. For ergot, such locations should have low night temperature and high humidity or wetness during preflowering and flowering periods during the growing season. The availability of overhead sprinkler irrigation to maintain high humidity for infection and pathogen spread is desirable, but not an absolute necessity. Hot spots have been used to effectively screen sorghum for ergot resistance at Arsi Negele in Ethiopia (Tegegne et al. 1994), Rubona in Rwanda (Musabyimana et al. 1995), Henderson and Panmure in Zimbabwe (Frederickson et al. 1994), and Bethlehem in South Africa (McLaren 1992). In India, sowing dates are adjusted such that preflowering and flowering periods occur when environmental conditions favor infection and disease spread.

Use of temperature gradients. The identification of resistance to ergot under artificially induced epidemics does not necessarily ensure that resistance will remain effective under natural epidemic conditions (Thakur et al. 1989). This is because ergot incidence in sorghum is extremely sensitive to changes in pre- and postflowering climatic conditions (McLaren and Wehner 1990 and 1992, Frederickson 1993). Desai et al. (1979) and Sangitrao et al (1979) showed a relationship between sowing date and ergot-favorable conditions during sorghum flowering. Early sowing tended to favor escape from the disease, whereas later sowing, that resulted in flowering during the cooler part of the season, promoted ergot incidence. Thus, in screening trials it is essential that the stability of resistance over a range of climatic conditions be quantified.

McLaren (1992) illustrated the need for temperature gradients in ergot resistance screening trials. Of 70 lines that failed to develop the disease at Potchefstroom in the warmer North West Province of South Africa, only three remained resistant when screened at Bethlehem, a cooler locality in the Free State. This was attributed to the

cooler conditions affecting the flowering pattern and pollination efficacy of sorghum genotypes. The extent of resistance breakdown also differed significantly between genotypes.

Preflowering cold stress reduces pollen viability in sorghum, thus reducing pollination efficacy and predisposing sorghum to infection by the ergot pathogen (McLaren and Wehner 1992). Genotypes differed in both cold sensitivity and the threshold temperature required to induce sterility.

McLaren and Wehner (1992) and McLaren (1992) created temperature gradients during the pre- and post-flowering periods by evaluating sorghum nurseries using a series of sowing dates at two localities. Individual heads of each genotype were marked with the date of onset of anthesis and artificial inoculation. This enabled subsequent graphic plots of ergot severity, seed set under pollination bags, and climatic conditions to be compared and gave indications of resistance and pollination efficacy under changing environmental conditions. Studies of this nature can also reveal significant disease-weather interactions that would otherwise not be recorded in single-sowing evaluation plots.

Self-pollination efficiency. The susceptible period for infection by the ergot pathogen is from stigma emergence to fertilization (Futrell and Webster 1965). Any factor that reduces pollination efficiency will therefore, increase susceptibility to infection.

The major factor contributing to reduced pollination efficiency is preflowering cold stress (McLaren and Wehner 1992), and it is therefore late-sown sorghum that is generally most severely infected. Preflowering minimum temperatures below 12°C reduced self-pollination in a tolerant hybrid (PAN 8564) to 21 %, as indicated by seed set under pollination bags. In contrast, seed set at higher temperatures was 80%. A concomitant increase in ergot incidence was recorded with decreased self-pollination efficiency. Pollen viability was reduced by 35-37% in susceptible hybrids, but only by 16% in a resistant hybrid in the greenhouse under a 25/10°C regime. Thus, differences in tolerance to preflowering cold stress is an important criterion in ergot escape resistance. Brooking (1979) found similar differences in genotype tolerance to cold induced sterility. In a sensitive hybrid, he found pollen sterility to be a linear response to night temperature with both 14° and 11°C lowering pollen fertility and thus, seed set. Molefe (1975) found ergot to be more severe in Botswana in fields associated with minimum temperatures of 14°C than in those that flowered during warmer conditions.

McLaren (unpublished) found differences in the rate of flower opening and self-pollination efficiency of sorghum lines. In a greenhouse trial male-sterile lines were inoculated with the ergot pathogen at various times after anthesis. Susceptibility to ergot lasted from 4 to 8 days, depending on genotype. Those panicles that were more efficient self-pollinators were more effective in escaping disease, as indicated in Fig. 3. A key aspect in efficient self-pollination is the rapidity with which fertilization occurs after pollination (Bandyopadhyay and Reddy 1991). There is a need to develop reliable and rapid methods of identifying efficient self-pollinators.

Jaster (1985) found a reduction in sorghum grain set with delayed anthesis of 3, 6, 9, and 12 days on male-sterile lines. Genotypes differed in the extent of these reductions. He also found that A-lines did not all start and finish flowering in the same length of time. The range from first to 100% anthesis averaged 4.2-6.4 days. Thus, even in the absence of ergot, inefficient or delayed self-pollination may induce permanent sterility. Whether this may promote ergot incidence needs more intensive study. Frederickson and Mantle (1988) found that if inoculation is delayed for 4 days after the florets 'gape', their Stigmas will cease to be a suitable substrate for the pathogen. In contrast, McLaren (unpublished) found that male-sterile lines remain susceptible to infection in the greenhouse for up to 16 days (Fig. 3). Bandyopadhyay (unpublished) also found that male-sterile lines could be infected by inoculating flowers up to 12 days after stigma emergence,

Chinnadurai et al. (1970a) reported that pollination of susceptible varieties with fertile pollen reduced spore germination, and hence host infection by the ergot pathogen. They attributed this to pollination affecting the nature of stigmatic exudates and subsequent effects on spore germination. Thus, rapid self-pollination may act as an inhibitor of pathogen development and play a role in reducing disease severity.

Frederickson et al. (1994) found that Rwandan and Ethiopian sorghums, resistant to ergot in their natural environments tended to be photoperiod-sensitive when grown in Zimbabwe, resulting in prolonged vegetative growth and ergot susceptibility. They suggested that the resistance expressed in their natural environments resulted from efficient self-pollination, that allowed insufficient time for colonization of the ovary by the slower growing pathogen. Their photoperiod-sensitivity in Zimbabwe could have affected this balance in these accessions. One accession, IS 25485 however, remained disease-free. Examination of the florets of this genotype showed that cleistogamous pollination had occurred in a large percentage of the florets 1 day before the flowers
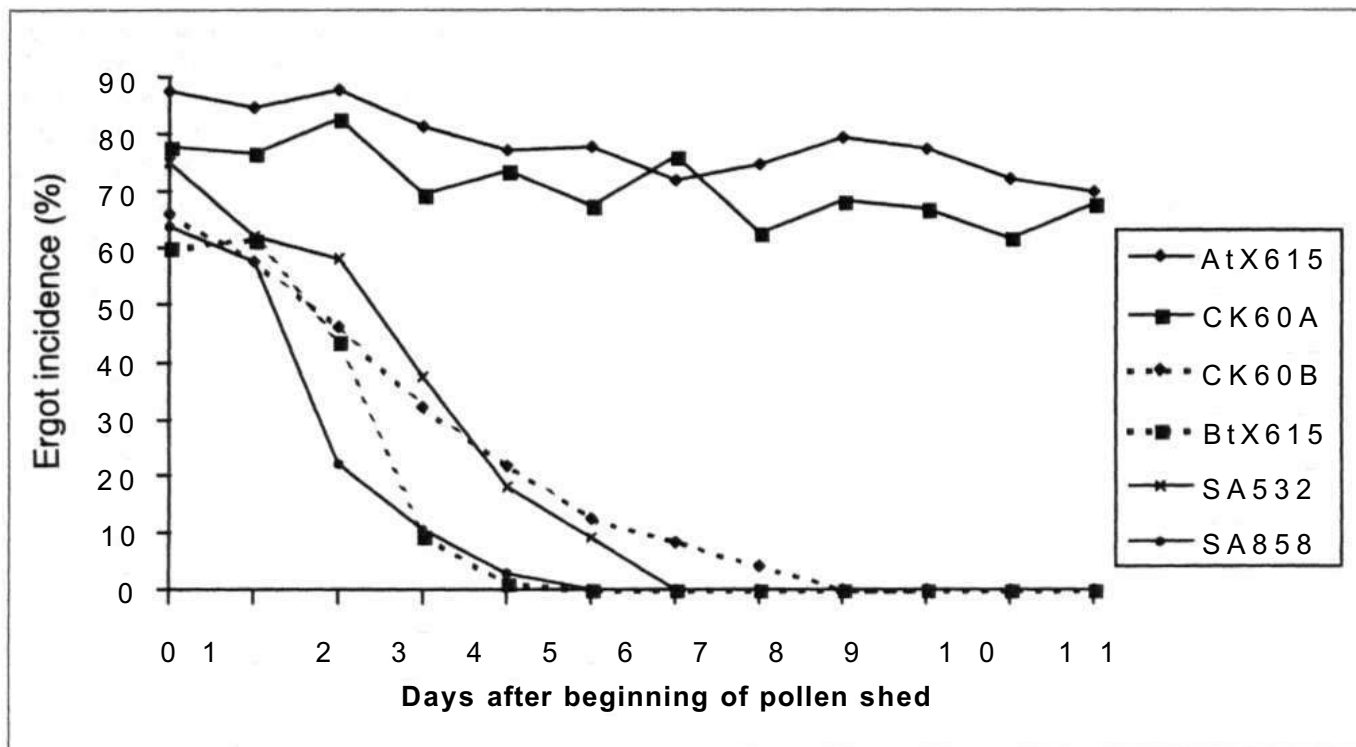
Figure 3. Ergot incidence in male-sterile and male-normal sorghum lines inoculated at various times after pollen shed.

gaped. By the time of floret gaping and artificial inoculation with the ergot pathogen, pollination had occurred, and disease escape was complete.

Evaluation procedures

Disease evaluation. Different procedures have been followed by various researchers to measure ergot in disease-evaluation trials. Disease has been measured qualitatively by visually estimating the proportion of panicle area infected (Anahosur et al. 1990), or the percentage of flowers infected (McLaren et al. 1992, Musabyimana et al. 1995), or disease expressed on a 1-5, or 1-9 scale. Frederickson et al. (1994) quantitatively measured disease incidence by counting the proportion of diseased panicles, and disease severity by counting the proportion of inoculated flowers that became infected. Tegegne et al. (1994) and Musabyimana et al. (1995) sampled one primary branch from each node of the rachis, counted the number infected flowers in the composite sample, and determined the proportion of infected flowers in the panicle. The visual, qualitative method underestimates disease in a panicle because some infected flowers are not visible on the single plane of the panicle or because sphacelia and sclerotia may be concealed in the glumes. However, since it is rapid and simple, this method can be used to reject susceptible lines in large screening trials. Quantitative evaluation is tedious and time-consuming, but is appropriate in trials requiring accurate data. Tegegne et al. (1994) suggested that both quantitative and qualitative methods can be used in resistance screening trials. The qualitative method can be used in the field to make a first rapid evaluation of large number of entries. After rejecting panicles with susceptible scores; the ratings of other panicles can be confirmed by quantitative evaluation. Tegegne et al. (1994) and Musabyimana et al. (1995) used both methods in trials to identify sources of resistance.

Sangitrao (1982) measured sphacelial and sclerotial phases of the disease separately by rating individual panicles qualititavely on a 0-5 scale, where 0 = no ergot, 1 = 1-20 (or 0.05 to 1%) infected spikelets in a panicle, 2 = 21-50 (or 1.05 to 2.5%) infected spikelets, 3 = 51-100 (or 2.55 to 5%) infected spikelets, 4 = 101-500 (or 5.05 to 25%) infected spikelets, and 5 = 500 (or 25%) infected spikelets. The rating scale was pictorially depicted using a standard area diagram. He evaluated at least 50 panicles plot[-1], and expressed ergot severity as the percentage disease index (PDI), calculated by dividing the product of the total rating of all panicles and 100, with the product of the total number of observed plants and the maximum rating (5).

Seed set Frederickson (1993) found that seed set in inoculated R-lines was higher at 25°C than at 20°C or 30°C. She suggested that pollination and seed set have the competitive advantage over ergot at 25°C. Reduced seed set at 30°C may have been heat induced, although at. this temperature disease development is known to be restricted (Frederickson 1993, McLaren and Wehner 1990).

McLaren and Wehner (1992) found seed set under pollination bags to be correlated with preflowering cold stress. The induced sterility was highly correlated (r=0.92) with ergot incidence. Genotypes differed in their seed set x cold stress interactions, with concomitant differences in ergot severity. Bandyopadhyay and Reddy (1991) came to a similar conclusion based on concurrent, but similar studies with a different set of maintainer lines from those of McLaren and Wehner (1992).

In pearl millet, Thakur et al. (1983) found that ergot infection in hybrids was positively correlated with grain yield. The increase in grain yield was attributed to the effects of rapid pollination resulting in seed set which would otherwise be lost to ergot.

Care needs to be taken when using seed set as an evaluation criterion. Frederickson et al. (1994) found that Rwandan and Ethiopian lines resistant to ergot in their native environments showed photoperiod-sensitivity when evaluated in Zimbabwe. This was accompanied by a reduction in seed set in most accessions. Despite sterility, some accessions failed to become infected. Frederikson suggested that gynoecial formation was abnormal in the different environment, or that in this environment gynoecia were unreceptive to both pollen and the pathogen. For this reason, undue emphasis on seed set as an indication of predisposition to ergot may skew the results of ergot evaluation trials.

Regression methods. Sundaram (1980) suggested that in order to compare ergot severities in breeding lines, particularly those differing in seasonal requirements, sowing dates must be adjusted so that plants will flower, and can be inoculated at the same time. In most semi-arid regions climatic cycles are unpredictable and synchronous flowering in desired climatic conditions is virtually impossible. Furthermore, temperature variations of relatively small magnitude before flowering and during the first 4 days after pollen shed significantly affect ergot incidence (McLaren and Wehner 1990 and 1992). This, together with natural variation in flowering dates both within and across sorghum genotypes, has resulted in inaccurate comparison of ergot incidences. The extent to which the differences in disease incidences reflect the host genotype, or the result of differences in climate associated with differing flowering dates is questioned.

McLaren (1992) used regression analyses to quantify the resistance of sorghum genotypes to ergot. An advantage of this method was that ergot severity in sorghum genotypes that flowered at different times could be statistically compared. Temperature gradients were created by sowing nurseries at two locations over a range of sowing dates. At flowering, individual heads were marked with the date of anthesis and artificial inoculation. Ten heads genotype^{-1} were inoculated on 3 to 4 different dates at each location. Visual estimations of percentage infected florets head^{-1} were made and these values were used to calculate the mean ergot severity genotype^{-1} associated with each inoculation date. An index of ergot-favorable conditions was determined, as the mean disease incidence over all genotypes associated with a specific flowering date. This was termed the disease potential, or expected disease severity, associated with a specific flowering date.

Non-linear regression analysis, using the model $Y=ax^b$ was used to determine the relationship between ergot potential associated with different inoculation dates and observed disease incidence within genotypes. Lines could be classified into three categories; those linearly related to disease potential, those highly susceptible (even at low disease potentials), and those with various degrees of resistance despite increasing disease potentials (Fig. 4). Genotypes in the latter group differed with respect to resistance breakdown points. The latter was defined as the disease potential required to induce 5 % or 1 % disease severity (termed 5% or 1% breakdown point) and could be calculated by substitution into the regression model. Re-arrangement of the regression model also enabled the subsequent rate of resistance breakdown at any point to be calculated. These criteria proved to be useful for quantifying resistance as they are fixed values, independent of fluctuations in flowering dates and variations in climatic conditions during flowering and infection. A disadvantage, however, is that large, diverse populations are required to allow the calculation of ergot potentials for different flowering dates. McLaren (in preparation) has developed a method of estimating disease potential based on climatic variables prior to, and during flowering. This will enable the breakdown points of smaller nurseries to be determined without large diverse populations being required for disease potential determinations.

Sources of resistance

Bandyopadhyay (1992) stated that the lack of confirmed sources of resistance to sorghum ergot underscores the urgent need to identify ergot resistance sources. Many
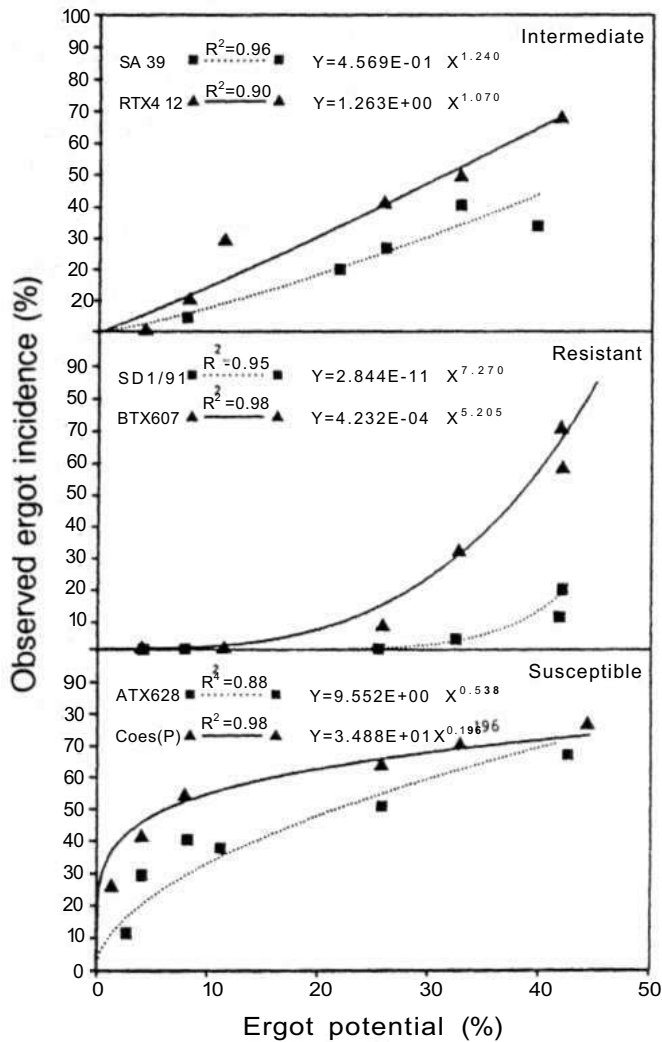
Figure 4. Illustration of three relationships between ergot incidence in sorghum lines and ergot potential using the regression model $Y = ax^b$.

reports of resistance to ergot have been made on sorghum (see Table 2). However, as pointed out by Tegegne et al. (1994) most reports are based on the results of unreplicated trials and many lines previously reported resistant have proved to be susceptible in subsequent trials and at other localities (Frederickson et al. 1994). McLaren and Wehner (1992) and McLaren (1992) showed that, given the correct preflowering predisposition and climatic conditions during early flowering, all genotypes are probably susceptible to ergot. For example, the sorghum lines found resistant to ergot in Ethiopia were susceptible in Rwanda, and vice-versa (R Bandyopadhyay unpublished). Since G x E interaction is critical for disease development, it is necessary to qualify resistance in particular lines with respect to the limits of environmental conditions in which it is operable. As a first step, it is

necessary to retest the lines reported as resistant and quantify their resistance using the method described by McLaren (1992).

Genetics and mechanisms of resistance/susceptibility

Even early reports on sorghum ergot outbreaks recognized that cytoplasmic male-sterile or A-line sorghum cultivars were highly susceptible to ergot, whereas the B-lines, R-lines, and other male-fertile varieties had low susceptibilities (Futrell and Webster 1965 and 1966, de Milliano et al. 1991). As with other ergot pathogens and their hosts, the primary reason for susceptibility is the delay or failure of pollination, so that even male-fertile varieties can become susceptible during or following weather conditions that reduce pollen fertility, or retard and reduce pollination (Puranik and Mathre 1971, Done 1973, Watkins and Littlefield 1976, Brooking 1979, Thakur and Williams 1980, Wood and Coley-Smith 1980, Thakur et al. 1983, Thakur et al. 1989, McLaren and Wehner 1990, McLaren 1992b). Normally, following pollination of the stigma, the post-fertilization, ultrastructural changes in the gynoecium prevent the subsequent invasion of ergot hyphae by the same pathway. In sorghum, infection by *C. africana* was close to zero 48 h post-pollination (Musabyimana et al. 1995). The extreme stigma constriction phenomenon in pearl millet gynoecia after the passage of pollen tubes down stylodia, similarly excludes *C. fusiformis* (Willingale and Mantle 1985 and 1986). Frederickson et al. (1994) found that rapid, even cleistogamous, pollination was responsible for the resistance of Ethiopian and Rwandan sorghums tested in Zimbabwe *C. africana* under natural and artificial disease pressure. Because of this inverse relationship with pollination, extensive screening of sorghum for resistance to *C. sorghi* and *C. africana* has failed to provide a resistant male-sterile sorghum (Ajrekar 1926, Chandrasekaran et al. 1985, Rajkule et al. 1985a and 1985b, Bandyopadhyay 1992, McLaren 1992a, Frederickson et al. 1994, Tegegne et al. 1994, Musabyimana et al. 1995).

Screening for resistance is complicated by the differing sensitivities of genotypes to environmental conditions (see Effect on environment on pollination, infection, and their interactions). Field studies by McLaren and Wehner (1990 and 1992) and McLaren (1992b) with *C. africana* in South Africa clearly show that ergot disease incidence increases if maximum temperatures are below 12°C at flowering, with optimum disease intensity at a 19.5°C maximum. There may also be interactions with hours of sunshine and rainfall. Minimum temperatures below 12°C at 3-4 weeks prior to flowering and in the 4 days

Table 2. Sorghum lines reported to be resistant to sorghum ergot.

Genotypes	Reference
SPV 126 and SPV 232	Rajkule et al. 1983
IS 2205, 4358, 5125, 5687, 5689, 7960; Ms 7999, 8030, 8268, 8289; As 6142, SOR 766; and SOR 831	Lakshmanan et al. 1988
SPV 617 (SPV 671 is susceptible)	Chandrasekaran et al. 1985
SPV 671, 677, 683, 686, 698, 351; 695, 696, and 697; SPH 329, 332, 341, 342, and 346; and MSH 56	Rajkule et al. 1985b
IS 625, 2867, 3413, 8101, 8545, 8614, 14332, 14380, and 14387	Rajkule et al. 1985a
SPV 59B, 220, 224, 260, 35, 300, 138, 233, 289, 295, and 296	Kukadia et al. 1982
SB 1085, 2413, 2415, 5501, and 1079; IS 2217, 2328, 3443, 3547', 8283, 14332, and 18758; M 35610; CSV 4	Anahosur and Lakshman 1986
IS 25480, 25527, 25530, 25531, 25533, 25537, 25551, 25554, 25555, 25570, 25576, and 25583	Musabyimana et al. 1995
ETS 1446, 2448, 2465, 3135, 4457, and 4927	Tegegne et al. 1994
SA 1304, SD1/91, SA 1619, SA197; RTAM 428, and 29 others	McLaren 1992
Tunis grain, G 4, and Sumac	Singh 1964
ETS 3252, 3251-1, 4145, 4457, 4927, 2448, 3125, 3912, and 1446; IS 25542, IS 25576, IS 25555, IS 25485, 83/8/1/1, 83/54/4/2-2, 83/54/1/2, 83/42/1/1, 12192, and TURA	Frederickson et al. 1994
IS 2495, 5337, 6449, 6705, 6891, 7584, 8051, 8070, 8609, 8930, 8936, and 9091	(C S Sangitrao, personal communication)
K.3, *S. nodulosunu* IS 1122, IS 5285	Chinnadurai et al. 1970b
J-604, M 35-1, and M 47-3	Khadke et al. 1978
IS 2444, 4530, 5285, 3248, 8970, and 7239	Sundaram 1970
CO 25, CO 23, TNS 23, TNS 28, TNS 35, TNS 24, TNS 30	Lakshmanan and Mohan 1989
IS 14332, IS 3443, and IS 3547	Anahosur et al. 1990
MS 7960, 8030 and others; IS 2205, 5125, 4358, 4006, 4300 and others; SOR 831, 766 and others; AS 6142 and others	Lakshmanan et al. 1987

1. Also resistant to sorghum downy mildew, ehareoal rot, grain mold, rust, zonale leaf spot, and anthracnose.

after pollen-shed, induce pollen sterility, resulting in increased ergot infection. Screening methods which therefore fail to consider the differing temperature sensitivities of genotypes lead to inaccurate ergot disease incidence comparisons. Environmental sensitivity also means that cultivars must be locally adapted to ensure the stability of their resistance. Comparing 'sugary disease breakdown point'(SDBP), the disease level required to produce 1% ergot severity in genotypes, and breakdown rate (rate at which disease reaches 1%), circumvents this problem

(McLaren 1992), allowing accurate comparison of disease in genotypes with different flowering dates grown at different localities, and in different seasons. Given the critical interactions outlined, it would seem logical to search for R-lines with increased cold tolerance in microsporogenesis and in pollen germination, and for A-lines exhibiting a fundamental physiological gynoecial response to limit infection. Apart from one study by Frederickson et al. (1994) in which the accession IS 25570 with poor seed set and low *C. africana* severity exhibited a necrotic reaction at the mid-point of its styles, studies to date reiterate that resistance is in reality 'escape' through pollination.

Breeding for resistance

Bandyopadhyay (1992) suggested that resistant cultivars are probably the most practical and economical method of ergot control, but also pointed out that lines with significant and stable levels of resistance were not available. This is consistent with a report by Willingale et al. (1986) who stated that, despite *Claviceps* sp occurring on many graminaceous crops, there is no consistent evidence of physiological sources of resistance in any of the crops that ensures protection against the particular *Claviceps* spp that parasitizes that crop plant.

Because there is no physiological resistance to ergot, attention should be given to indirect or escape resistance. This has proved particularly useful in breeding for ergot control in pearl millet. Willingale et al. (1986) found that ergot-resistant lines possess very short periods of protogyny and could relate ergot severity to the length of protogyny and rate of self-pollination. In a few cases, millets were protandrous with a concomitant increase in pollination rate and escape resistance. Although not so extensively studied, similar variations in sorghum flowering patterns exist, and floral characteristics that promote pollination should be selected. IS 25485 from Rwanda is such a line. As described by Frederickson et al. (1994), this line is characterized by cleistogamous pollination and efficient self-pollination before gaping, such that insufficient time for colonization of the ovary by the pathogen is allowed. As a result, this line remained resistant over a range of localities, from Rwanda to Zimbabwe.

McLaren and Wehner (1992) studied cold tolerance in sorghum hybrids. The hybrid PAN 8564, that was more cold-tolerant than other hybrids, was less susceptible to preflowering cold-induced sterility, and hence more efficient at self-pollination, seed set, and ergot escape. Brooking (1979) in New Zealand found the line 606 to be particularly resistant to preflowering cold stress with normal pollen in cold-stress regimes of 25/5°C. Data sug-

gested that selection for tolerance to cold-induced sterility can contribute to ergot escape resistance.

McLaren (unpublished) found significant differences in the rate of flower opening, self-pollination, and length of the ergot susceptible period (Fig. 3). Flowering and the concomitant ergot susceptible period in SA 858 lasted for 5 days as opposed to 8 days in CK 60 B. This results in a risk period reduction of 37% in SA 858 compared with CK 60 B. Thus, selection for rapid reduction of the ergot-susceptible period can contribute to integrated ergot risk reduction.

Chinnadurai et al. (1970b) found that the varietal reactions of sorghum were related to the nature of stigmatic secretions. Susceptible cultivars secreted large amounts of substances which stimulated spore germination, including malic acid, succinic acid, arginine, and aspartic acid; whereas resistant cultivars secreted tartaric acid and tyrosine that were inhibitory to spore germination. Kannaiyan et al. (1973) found a similar mechanism in pearl millet resistant to *Claviceps fusiformis,* with tryptophane as the spore germination inhibitor. Thus, identification of inhibitor substances (notably certain amino acids) and selection of genotypes with high levels of inhibitory substances can contribute towards resistance to, or escape from, ergot.

Hassan et al. (unpublished) found that secondary sporulation was less in the exudates of high-sugar (sweet) sorghums was restricted than in those of grain sorghums. No apparent differences in the severity of infection in sweet and grain sorghums were recorded. However, reduction in secondary sporulation, and the resultant reduction in inoculum levels could have an epidemiological significance and contribute to integrated ergot control.

McLaren (unpublished) found significant differences in male:female compatibility of sorghum. A poorly compatible relationship between pollen and stigmatic tissues prolongs the period from flower opening to fertilization and hence the length of the ergot-susceptible period. Conversely, good compatibility will promote ergot escape resistance. Jaster (1985) found that pollen x stigma compatibility in ATx 623 was the best he tested based on the percentage of pollen tubes in the ovary (32%) 60 min after pollination, with ATx 399 (17%) and ATx 3197 (23%) were the least comparable.

Thus, it is evident that although physiological resistance to ergot is lacking, or yet to be identified, many minor floral characteristics could, if selected for, contribute to ergot escape resistance. The breeding strategy to incorporate ergot resistance in sorghum should rely on traits that allow rapid pollination and fertilization in environments favorable for disease development.

Very little has been published on the genetics of ergot resistance in any graminaceous crop except pearl millet. Thakur et al. (1989b) showed that ergot resistance in pearl millet is controlled by polygenic recessive genes implying that to breed ergot-resistant hybrids, resistance should be incorporated into both male-sterile and pollen parents (Rai and Thakur 1995). The greatest need is to identify ergot-resistant male-sterile lines, although the chances of identifying, or breeding ergot-resistant male-steriles appear slim in view of the role of pollen in disease development. However, ergot-resistant male-sterile lines of pearl millet have been developed (Thakur et al. 1993) suggesting that it might be possible to develop ergot-resistant male-stcriles in sorghum too. Ergot-resistant maintained, restorers, and hybrids of pearl millet have also been bred (Thakur et al. 1993). Futrell and Webster (1965) reported that in screening trials, sorghum lines with cytoplasmic sterility were more susceptible to ergot than sterile lines due to chromosomal aberrations: McLaren (unpublished) in preliminary trials compared isogenic lines of 'Martin' with A_1, A_2, A_3, and A_4 cytoplasm, and found that the A_4 cytoplasm tended to be less susceptible to ergot. Although of no immediate economic value, these results suggest that cytoplasm may be a factor in ergot susceptibility, and that evaluation of alternate cytoplasms in relation to ergot susceptibility is warranted.

Chalal et al. (1981) screened pearl millet germplasm under epiphytotic conditions and found a total lack of major gene resistance to *C. Jusiformis*, They pursued a strategy involving recurrent selection to concentrate the minor genes controlling polygenic resistance for intra-population improvement. Inbred lines with less than 5% ergot severity were selected and intermated to produce two diallel populations. After 3-4 cycles of recurrent selection the proportion of plants with 0-5% severity increased, indicating that an appreciable level of resistance can be successfully generated. Similar studies have yet to be conducted on sorghum. Good progress has been made in breeding for ergot resistance in pearl millet and there is much to learn from the well-documented extensive research on pearl millet ergot conducted at ICRISAT (Thakur and King 1988, Thakur et al. 1993) during the 1980s and 1990s.

Cultural methods

Adjustments in sowing date and location

In Zimbabwe, farmers claim that sowing early in November (when permitted by the season), results in ergot infecting only tillers, perhaps partially because microsporogenesis occurs well before minimum tempera-tures fall to below 12°C, or A-line flowering coincides with a mid-season dry spell. Since severe ergot epiphytotics, resulting in nearly total seed loss, occur every 5 years on sorghum A-lines in Zimbabwe, sowing adjustments clearly give only limited control. This is re-emphasized in studies by Frederickson (unpublished). In India, sowing in early July in preference to early August also resulted in reduced *C. sorghi* infections (Sangitrao et al. 1979, Anahosur and Patil 1982) because plants flower at temperatures favorable to rapid pollination and fertilization.

Although most of the seed companies in India are located in Maharashtra, F_1 hybrid seeds are rarely multiplied there because of the risk of ergot. Most of the sorghum seed is produced in Andhra Pradesh under irrigation in the dry season areas (e.g., Telangana) where environmental conditions are not favorable to ergot attack. In Zimbabwe, seed production plots in the Muzarabani area usually escape ergot infection. However, ergot is able to infect male-sterile sorghums virtually wherever and whenever they are grown in southern Africa provided that environmental conditions are favorable, be it on research stations or in commercial fields (W A J de Milliano, personal communication). Neither production in a dry area, nor irrigated production in the dry season guarantees escape from ergot.

Reduction of inoculum

Farmers who produce hybrids in Zimbabwe remove infected panicles from the field at harvest, plow residues into soil, and practice crop rotation (Swift and Hurrell, personal communication). These precautions would appear to be reasonable, but are without obvious effect, perhaps due to the ability of the pathogen to spread rapidly by secondary conidia from a small focus of infection (Frederickson et al. 1993). Even though germination of *C. africana* sclerotia, and thus ascosporic infection, is probably a rare event in nature, sclerotia would have very low infectivity at only a few centimeters of soil depth (Anonymous 1972). Nevertheless, sowing seeds free from sclerotia will eliminate the possibility of introducing the disease into new areas. Sphacelial conidia of C. *africana* can live for 9 months in panicles maintained at the field surface between crops (Frederickson, unpublished). Macroconidia on such residues may possibly germinate to produce airborne secondary conidia for the initiation of new-season infections, and plowing would again reduce initial inoculum sources. Although the ascosporic stages of *C. sorghi* and *C. africana* have not been confirmed in nature, removal of contaminating sphacelia and sclerotia from seed batches is advised. In

India, seeds are soaked in water and sclerotia floated-off in 5% salt solution, followed by seed drying (Bandyophadhyay 1992). The same practice has been attempted in South Africa, with only slight loss in seed viability (McLaren, unpublished). However, all these practices will remain arbitrary as control measures, until the source(s) of initial inoculum is determined.

Pollen-based management

Poor 'nicking', or nonsynchrony of flowering in male-sterile lines and restorers, and lack of viable pollen during stigma emergence are the major reasons for the increased susceptibility of male-sterile lines in seed production plots. Efficient pollination reduces the window of infection, i.e., the time between stigma emergence in male-sterile lines and pollen shed by restorers, and is the most effective strategy to significantly reduce ergot damage. In Zimbabwe, farmers routinely stagger the sowing of the pollen-donating R-lines, sowing the first R-line row 7-10 days before the second row and the A-lines, to ensure a more regular supply of pollen to the A-line (Hurrell and Swift, personal communication). This gives mixed success in reducing *C. africana* ergot infections, because of the more critical effect of the environment around the time of flowering (McLaren and Wehner 1990, McLaren 1992). Frederickson and Obilana (1993) evaluated pollinator:female ratios in field trials. Seed set was more variable between rows with a 6:4 (A:R line ratio than with a 4:4 layout. Highest seed set was found in rows adjacent to the pollen source. Furthermore, disease increase was more rapid in 6:4 ratio plots than in the 4:4 layout. Similar results were recorded by Thakur et al. (1983), who found a significant decrease in ergot of pearl millet caused by *C. fusiformis* with pollen donor:female ratios of 1:2 compared with 1:4, and 1:8.

Control with fungicides

It is uneconomical for small-scale farmers but acceptable for seed producers to use fungicides to control ergot. Appropriate fungicides sprayed at stigma emergence stage and at 5-7 day intervals has been shown to reduce the disease. The control is good if inoculum pressure is low, and in the absence of rains; but disease control is not absolute at high inoculum pressure. Fungicides do not have curative action, and are most effective as preventive measures. Therefore, sprays should begin before the onset of the disease in the field, and continue until flowering is complete, they have little effect after the onset of the disease.

Fungicide control of *C. sorghi* has been extensively investigated (Nagarajan and Saraswathi 1971, Gang-

adharan et al. 1976, Sundaram 1976, Anahosur 1979, Lakshmanan et al. 1986), less so for *C. africana* (McLaren 1994). In India, 2 or 3 sprays of 0.2% Ziram®, thiophanate methyl, and Captafol® at 2-week intervals starting at the boot stage increased yields from 1 to 4 t ha^{-1}. Other fungicides including thiram, Ziram®, zineb, and Dithane-M45® were also effective. Nagarajan and Saraswathi (1971) obtained little field control of *C. sorghi* with the systemic fungicides Benlate®, Plantvax 75W®, Vitavax 75W®, and Tecto 60®. This is true in field situations when disease pressure is high, but control is usually good if inoculum pressure is low. McLaren (1994) controlled *C. africana* in South Africa using one of several systemic fungicides (benomyl, carbcndazim/flusilazol, propiconazole, triadimenol, terbucanazole and CG169374) but bitertanol at 150 or 300 g a.i. ha^{1} and procymidone at 250 g a.i. ha^{-1} gave significant disease reductions. However, McLaren calculated that yield gains were not sufficient to justify economic use of fungicides. In Zimbabwe, Frederickson and Leuschner (unpublished) found that benomyl at 0.2% a.i. reduced ergot disease significantly in A-lines if sprayed once at heading, and calculated that control was economically feasible. The use of fungicides would be invaluable for the production of hybrid seed even though control may not be absolute. Fungicides normally used do not have male gameticidal effect, and thus seed set should not be affected. Much research on fungicidal ergot control is currently in progress in Brazil and Australia. Fungicide use by seed producers in Brazil reduced losses due to the disease in 1996.

Dressing of seed with thiram (1:250) was recommended by Sundaram (1976) to prevent sclerotial germination. Mantle and Hassan (1994) recommend seed treatment as a routine precaution to prevent the entry of seedborne *C. africana* into the Americas, but did not suggest a specific fungicide. Except for Sangitrao (1982) who found that fungicidal seed treatment reduced, but did not prevent, sclerotial germination, no other information is available on the toxicity of fungicides to sclerotia. Neither is it known if fungicides can penetrate sclerotia to kill conidia embedded in internal locules. *Claviceps purpurea* sclerotial germination is limited following seed treatment with triadimenol, a chemical that may have a potential use against *C. sorghi* and *C. africana* sclerotia. In Brazil, triadelmenol applied as a spray has been found effective in reducing disease in the field.

Adjustments in seed processing plants

Removal of sclerotia from seed harvested from affected fields during seed processing may be a useful strategy.

During the seed-cleaning process, a gravity table aided by an air-stream could effectively separate sclerotia that are lighter in weight than normal seeds. However, further research on this technique is required. The use of salt water solution to float out sclerotia is not practical in seed-processing plants because such a treatment would increase the seed moisture content at a stage when the seed needs to be dried.

Integrated ergot management strategies

Although ergot is of greater consequence in hybrid seed production fields, damage in commercial grain crops is also often significant in pollen-limiting environments. The approaches to ergot management in hybrid seed production and grain production require different emphasis on the various components. Pollen-based management, ergot escape, chemical control, and removal of sclerotia in seed-processing plants are of overriding importance in the production and processing of hybrid seeds. Host-plant resistance is most likely to succeed economically in grain production plots. In both production situations, the use of methods that reduce inoculum in the field are relevant in reducing damage. Further research is essential to test combinations of different components of ergot control to develop integrated ergot management strategies to suit the socioeconomic and technological needs of each location and end user.

Research needs

Considerable research information is available on the infection process, role of pollination and fertilization on disease development, and techniques to screen for resistance. Future work should build upon past research in developing integrated ergot management (IEM) practices. Thus, the future thrust of research should be directed toward strategic research on pathogen biology, disease epidemiology, and on components of IEM practices.

Biology

- Identify the species of *Claviceps* prevalent on sorghum in different parts of the world and determine relationships among these species, if any.
- Re-examine the taxonomy in *Claviceps* spp that attack sorghum using large samples of the pathogen population from different areas.
- Develop simple and rapid detection methods for sclerotia/ sphacelia in seed for use by quarantine officials to prevent entry of *Claviceps* spp into disease-free countries via contaminated seed, and by seed quality

laboratories to certify pathogen-free seed. Since *C. africana* has elaborate sclerotial alkaloids, direct chemical analysis for alkaloids may be appropriate. Alternatively an immunological technique could be developed as was done recently for C. *purpurea* (Shelby and Kelley 1992). This latter method may also be appropriate for sphacelia. Effective ways to establish the identity of *Claviceps* spp in seed lots need to be developed.

Epidemiology

- Determine sources of initial inoculum, especially with respect to preventing entry of the pathogen into disease-free countries. Possible sources include sclerotia (ascosporic infection and conidia) and sphacelia (macroconidia on internal fructifications, secondary conidia).
- Find the alternate hosts of the pathogen and determine their role in the epidemiology of the disease.
- Determine the role of sclerotia as a source of primary inoculum. Further explore the possibility of sclerotial germination by sexual means under natural conditions. Also, study the prospect of the following mechanism for the survival and initial spread of the pathogen: thick-walled sclerotia over seasons protecting macroconidia contained within their locules from edaphic elements; the sclerotial wall slowly degrades with the onset of rains thereby releasing macroconidia on the soil surface; macroconidia germinate on moist soil to produce secondary conidia; secondary conidia become windborne and initiate primary infection in the field. Also, study the longevity of macroconidia in soil, and the possibility of the process described above occurring in the absence of macroconidia protected within the locules of sclerotia.
- Focus investigations on the relationship between temperature and longevity and infectivity of conidia, and the conditions under which primary conidia on field residues produce secondary conidia.
- Conduct studies to find the environmental factors leading to secondary conidiation and the release of secondary conidia into air; germination and longevity.
- Determine the length of survival of sclerotia in different environments and quantify the extent of sclerotia seed contamination that is necessary to spread the pathogen.
- Ascertain if seed contaminated with conidia serve as a source of primary inoculum in the field (it is generally believed that seedborne conidia are not sources of primary inoculum).

- Determine the effect of different environmental conditions on flowering biology, infection, and their interactions to better understand factors influencing disease incidence.
- Develop a statistical model to predict the occurrence of disease using data on weather (temperature, humidity, rainfall, solar radiation, etc.), host (pollen production and viability, stigma receptivity, nicking, etc.) and disease incidence. Such a model could help to forecast the occurrence of the disease and to better schedule and optimize fungicidal spray application.

Components of management practices

- Develop a spray schedule of effective fungicides with details of timing of application, requirement of stickers, frequency and dose of application, volume of spray water required, and economics for the practical control of the disease. Also, suitably modify spray equipment to avoid damage to plants.
- Determine the effect of seed treatment with fungicide on the viability of sclerotia and the conidia contained in seed, and on conidia contaminating the seed surface. An economical and effective method of decontaminating infected seed lots would have immediate application.
- Ascertain the efficacy of removal of alternate hosts from the field on disease severity in the field.
- Identify genotypes that support less secondary conidiation and thereby reduce secondary spread of the pathogen. Most sorghum genotypes seem to permit secondary conidiation on honeydew but Mantle (personal communication) has found some sweet sorghum genotypes with high sugar content that reduce secondary conidiation.
- Identify resistant sources possibly with novel mechanism of physiological resistance. Expand research to identify and develop male-sterile lines with ergot resistance.
- Develop productive hybrids and their seed parents with the following characteristics:
  - restorers with the ability to release abundant fertile pollen at low temperature and high humidity,
  - male-sterile lines that are tolerant to low temperature, i.e., with receptive stigmas that have the capacity to be rapidly fertilized with scant pollen at low temperature, and
  - the resultant F_1 hybrids combining the above-mentioned attributes of restorers and male-steriles.
- Determine the genetics of resistance of factors associated with ergot resistance and rapid fertilization (seed set) at low temperature.

- Explore the possibility of using gravity tables, air streams, and other methods for the removal of sclerotia from seed lots in seed-processing plants. Develop ways to rapidly clean seed processing equipment and to handle infected seed lots without contaminating healthy seed lots.
- Search for warm and dry periods during the year and/or areas to avoid ergot in hybrid seed production fields.

International collaboration

Until now, research on sorghum ergot has been carried out in Asia (India and Japan) and Africa (Botswana, Ethiopia, Nigeria, Rwanda, South Africa, and Zimbabwe). Research interest and expertise exist in South Africa, at the University of Zimbabwe, at the Imperial College (University of London, UK), and at ICRISAT Asia Center, Patancheru, India. The Imperial College has had significant strategic participation in sorghum ergot research in Zimbabwe. However, sorghum ergot research has been de-emphasized in some of these institutions during the last 4 years since it was not considered to be of a high priority.

Enhanced geographic distribution and the resurgence in importance of the disease in countries with near-total dependence on hybrid seed have escalated the demand for research on the disease. There is a need for renewed emphasis on sorghum ergot research with the participation of the public institutions and the seed industry in Australia, Brazil, and USA, and for active involvement of researchers with earlier experience in ergot-related work, A collaborative approach with distribution of responsibility is more likely to succeed than individual overlapping efforts, given the current scenario of constrained funding opportunities.

Quarantine procedures designed to prevent the introduction of pathogens are sometimes limited in their effectiveness, as observed with the recent outbreak of ergot in Australia. An excellent adjunct to quarantine procedures is collaborative research to control and study ergot with scientists and sorghum workers in those regions where the pathogen is already established. Such research will simultaneously reduce the risk of ergot introduction and provide controls that can be rapidly implemented if ergot is eventually introduced.

The three main areas of collaborative interest are fungicidal control, host-plant resistance, and ecology of ergot sclerotia. Fungicidal control will enable production of ergot-free seed, or at least with minimal numbers of ergot sclerotia. Knowledge about the contribution of sclerotia to ergot survival and spread, especially through seed, may be exploited to detect and either remove or destroy

ergot sclerotia in contaminated seed lots. These types of ergot control are hopefully needed only as interim procedures during attempts to identify host-plant resistance. The latter will be, at the very least, a time-consuming process to both confirm host-plant resistance to ergot, and then incorporate that resistance into agronomically useful germplasm adapted to regions where it is needed.

As sorghum workers collaborate to control ergot and produce ergot-free seed within and across geographical regions the risk of ergot spread to new regions will be reduced.

The development of highly effective fungicidal control in other regions would provide a control method that could be immediately employed in USA, or elsewhere, to reduce the impact of ergot when it is first observed. Fungicidal control may function in the short term for hybrid seed production but will be expensive, and may possibly be overwhelmed by high inoculum pressure and optimal conditions for ergot development. The loss of pollen viability in self-fertile sorghums under cool temperatures is also of concern in USA because much sorghum in the Northern Great Plains States of Kansas, Nebraska, and South Dakota flowers and matures during increasingly cooler temperatures. Fungicide application would not be an option in such fields. This is another indication of the importance of host-plant resistance that maintains resistance to ergot simply by producing viable pollen even in cool temperatures.

The identification of host-plant resistance requires a mobilization of global germplasm resources to assemble the best candidates for resistance screening. There needs to be a screening of all sources previously identified as having some level of ergot resistance, although there may be generally few (or no?) good sources of physiological resistance to ergot. Resistance to ergot could be greatly influenced by the environment, and it is imperative that sources be screened or that resistance be confirmed under multiple environments where ergot is present. In the absence of physiological resistance to ergot, additive factors that may be associated with reduced incidence and severity of ergot need to be identified. Such traits or resistance mechanisms in sorghum germplasm will then need to be incorporated singly or additively into sorghum A-lines or R-lines as appropriate. Because this is a time-consuming process, global cooperation and contribution are needed to address such global threats as ergot today.

Collaborative research on ergot at multiple global locations with a shared evaluation of methodology and germplasm across those locations should provide integrated controls for ergot that are best suited for each region. Perhaps, there is even the possibility of utilizing the ergot hyperparasite, *Cerebella,* as a biocontrol agent within an integrated control program for ergot. Continued communication of results through the International Sorghum and Millets Newsletter (ISMN), other publications, and through meetings and conferences will facilitate the dissemination of the most recent information and genetic resources. The current and future capability of communication through e-mail and the World Wide Web will vastly increase the global ability to communicate and exchange scientific information about ergot. The use of e-mail to globally exchange information on ergot has been phenomenally successful just within the past several months.

Acknowledgment

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