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Foreword

Why Ergot, Why Brazil and Why Now

In June 1996, several of us had the opportunity to see sorghum ergot in Brazil and the damage that the disease can do We saw severe damage in seed production plots and witnessed the pain that seed producers had to go through to deal with this new problem. There was a wide spread scare in the sorghum community in the Americas because of the infamous reputation of the disease in causing damage in seed production fields As a silver lining, we also observed first hand, the excellent research program that the Brazilians had in place It became apparent that all of the sorghum researchers, seed producers and regulators of plant pathogens worldwide needed to come together to study and review this disease There could be no better venue for such a Global Conference than Sete Lagoas, Brazil Hence this meeting was proposed and with the support and assistance of the Director of Sorghum and Maize Research (CNPMS) of EMBRAPA, this meeting has been arranged. At the time the meeting was proposed, the disease was known only in a few countries but it has spread today to include much of the Americas and Australia. Obviously, we can no longer avoid the disease, we can only learn to live with it and manage it in future growing seasons It has been my pleasure to work with the research leaders on ergot at EMBRAPA in developing this conference, to receive cooperation from many of the global leaders on ergot to bring their knowledge to the conference and financial support from the American Seed Trade Association, as well as the support and encouragement from Texas A & M University and INTSORMIL in recognizing the importance of our collaboration on combating sorghum ergot

In this conference, we will learn from the experiences of countries where ergot had existed for a long time while examining the ergot situation in newly invaded countries We have several dedicated papers dealing with the biology of the pathogen, the epidemiology of the disease, and disease management strategies We will also chart the movement of the disease and plot ideas on how to collaboratively work to lessen the impact of sorghum ergot in the Americas

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Session I — Sorghum Ergot Around the World

Session President — John M. Yohe

Sorghum Ergot· an Overview

Peter G Mantle

Ergot disease of sorghum appears to be a 20th century phenomenon, formally recognized to the extent that some of the first samples, from Burma (1927), Kenya (1924), and Uganda (1926) were deposited in the herbarium of the former Commonwealth Mycological Institute at Kew, UK (Mantle 1968) Mycologically, the aspect of the pathogen with the greatest obvious taxonomic value, in the absence of any obvious sexual stage, was the sphacelial fructification established on infected ovaries and associated with honeydew exudate Thus, the connection with other ergot parasites was established by the description of *Sphacelia sorghi* in India (McRae 1917) The material was characteristic of the Indian sub-continent and the description was the taxonomic basis for recognizing sorghum ergot pathogens in both Asia and Africa for the next 70 years

In Africa the pathogen was recognized again in Tanganyika (1949), South Africa (1953), Nigeria (1955), Zambia (1965), Ethiopia (1973) and Mozambique (1984) but the disease became most prominent in northern Nigeria in the early 1960s associated with sorghum hybrid breeding (Futrell and Webster 1965, 1966) research when the particular natural susceptibility of male-sterile sorghums became quite clear However, the precise epidemiological source of the pathogen by which it maintained its potential for opportunistic disease was, and still remains, uncertain Indeed, in Africa the generally cryptic nature of the pathogen and its disease on sorghum, not producing prominent sclerotia as does *Claviceps purpurea*, even failed to catch the attention of A R Loveless at the University of Southern Rhodesia who otherwise described several new *Claviceps* species in East Africa, most notably *Claviceps fusiformis* (Loveless 1967), the characteristic pathogen of pearl millet It is, however, somewhat ironic that the common sorghum pathogen in Africa was shown experimentally to infect pearl millet (Frederickson and Mantle 1996), though this is probably not important as a functional alternate host

The author first became involved with a sorghum ergot pathogen in the mid 1960s concerning sclerotia of Nigerian origin thought to contain the alkaloid agroclavine Scepticism about identification of that alkaloid led instead to the isolation and characterization of a new naturally-occurring dihydrogenated ergot alkaloid, dihydroergosine, and the recognition of its reduced clavine alkaloid biosynthetic intermediates (festuclavine and dihydroelymoclavine) as minor components (Mantle and Waight 1968) Attention then focused on obtaining ergot alkaloid production in axenic culture in yields of 0 5 mg ml ^{1} (Mantle 1973) and the extension of the technique to production in stirred fermenters of up to 300 L capacity (Attwell 1981) Efficient axenic culture led to experimental demonstration of the biosynthetic interactions between the various alkaloids (Barrow et al 1974) and

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the flexibility of natural biosynthetic enzyme systems to accept an unnatural biosynthetic precursor to produce, for example, the hydroxy-prolyl analogue of dihydroergosine (Atwell and Mantle 1981) These secondary metabolite characteristics of the African sorghum ergot pathogen were thus recognized as a unique combination which, therefore, has in effect, diagnostic value for the fungus However, it also implies affinity with the very special *Claviceps gigantea* which parasitises maize exclusively in Mexico (Fuentes et al 1964) *C gigantea* also elaborates the dihydrogenated clavine alkaloids, festuclavine and dihydroelymoclavine, but takes the biosynthetic pathway no further into the larger cyclic tripeptide derivatives as does the African sorghum pathogen It is, however, hard to see whether there is any close phylogenetic relationship between the sorghum and maize pathogens that are separated geographically by long distances, and therefore the dihydrogenation step in the ergoline alkaloid biosynthetic pathway may have evolved independently in the two fungi It is notable that infection of maize ovaries by the African sorghum pathogen has been demonstrated experimentally by the author, although the parasitic mycelium does not become organized into a typical sclerotium, confirming findings of Futrell and Webster (1966)

Failure to differentiate a noticeable sclerotium in sorghum is a common feature of ergot disease in Africa Either the fungus barely fills the floral cavity with a sphacelium and then often becomes infested with saprophytic fungi, including *Cerebella spp* , or it may differentiate into only a small, roughly spherical, sclerotium 2-3 mm in diameter, located between abducted glumes Consequently, the disease was widely known as sugary disease because the honeydew stage was the most diagnostic symptom

In contrast, *S sorghi* in India produced an elongated sclerotium, protruding upwards 1 cm from the floret, though only constituting a really compact sclerotial body in its proximal portion Sufficient production of this tissue may give rise subsequently to the typical *Claviceps* stromata in the capitula of which the teleomorphic formation of ascospores occurs A taxonomically valid short description of *Claviceps sorghi* was made (Kulkarni et al 1976), from which it was widely assumed that it applied also to *S sorghi* of Africa No ergoline alkaloids were detected in sclerotia of *C sorghi* but an isolate was shown to produce agroclavine in small amounts (23 $\mu g\ ml^{-1}$) after 3 weeks of stationary liquid culture (Manzarpour 1985) Agroclavine was recognized by thin-layer chromatography and by electron impact mass spectrometry This further emphasized genotypic distinction between the African and Indian ergot pathogens of sorghum

Following fundamental studies on the mechanism of resistance of certain pearl millets to ergot disease that was being exploited at the ICRISAT Centre, Hyderabad (Willingale and Mantle 1985, Willingale et al 1986), attention was redirected towards sorghum, using an Indian isolate of *C sorghi* from Maharashtra State as a model for defining the path of infection (Frederickson and Mantle 1988) Concomitantly, the present author observed iterative germination of *S sorghi* (*C sorghi*) macrospores on agar media, an observation which led to the

description (Manzarpour 1985) of the ation phenomenon, expression of which is regulated by temperature At moderate temperatures, up to 27°C, virtually all spore germination was in the form of a short sterigma-like projection from the top of which a secondary spore formed, usually at the complete expense of the cytoplasm of the macrospore At temperatures above 27°C, typical hyphal germ tube germination occurred and became the exclusive mode of germination at 37°C This did not seem to be important to the parasitic biology of the pathogen until the pathology of the Indian pathogen *(C sorghi*) was compared directly with that of an African isolate in similar horticultural tunnel conditions in England in 1989 However, it was readily apparent that the honeydew stages were quite dramatically different *C sorghi* produced honeydew which was usually viscous and rich in sugars and in which no macrospore germination occurred, whereas the African pathogen exuded a very dilute honeydew which quickly became covered by a white crust (Frederickson et al 1991) This crust was shown to consist of a palisade of secondary spores borne on sterigmata which pierced the honeydew liquid surface (Frederickson et al 1989) Therefore the secondary sporulation of *C sorghi* was mainly a laboratory phenomenon Analogous profuse parasitic manifestation was apparently confined to the African pathogen Further study of secondary sporulation *in vivo* was performed at ICRISAT, Hyderabad (Bandyopadhyay et al 1990) showing that in high relative humidity under glasshouse conditions *C sorghi* expressed secondary sporulation on honeydew Under these experimental conditions it can be assumed that exuded honeydew was more dilute than is natural, in the absence of the desiccating influence of field conditions Therefore, secondary sporulation is a constitutive characteristic of *S sorghi*, whether as the expression of the anamorph of the Indian or of the African ergot pathogen of sorghum The nature of the relevant honeydews, that are partly phloem exudate and partly the parasite's biotransformed excreta, influences whether or not, and to what extent, secondary sporulation of submerged macrospores occurs The question remains why, when macrospores are spread across an agar surface in the laboratory, the normal mode of germination is iterative and is only replaced by hypha formation at high temperatures When macrospores are deposited on the surface of a stigmatic hair of sorghum, germination is consistently by hyphal formation and quickly leads to intercellular penetration and subsequently traverses through the stigmatic transmission tract Clearly, iterative germination on a stigmatic hair would be biologically futile and therefore macrospores respond to stigmatic influences (whether chemical and/or physical) to ensure penetration tube production irrespective of the temperature, which may fluctuate between wide diurnal limits In the absence of the direct influence of a potential host, as in the case of being submerged in exuded honeydew, iterative germination of macrospores is promoted in principle, if not in rate of response, by lowering temperatures which in the field will be associated with night conditions and the consequent diluted nature of freshly exuded honeydew under high relative humidity Whereas this may all make logical biological sense, it emphasizes some sophisticated mechanisms of

signal recognition in a unicellular fungal spore of two remarkable ergot pathogens of sorghum However, the biological success of the African pathogen is largely due to the versatile function of its macrospores and perhaps it is not surprising that they have special abilities Apparent reliance on asexual spores for parasitic establishment of the African pathogen is due to the rarity of stromatal formation from sclerotia, reported only in general terms by Mower et al (1973) but later used specifically by Frederickson et al (1991) as the basis for describing the african pathogen as *Claviceps africana* This species is distinct from *C sorghi* not only in mycological differences of the respective *Claviceps* teleomorphs, but also by the particular and consistent occurrence of dihydroergosine and related clavine alkaloids in sclerotia of *C africana*

A remarkable feature of *C africana* is its apparently spontaneous appearance in parts of Africa, particularly in male-sterile sorghums grown even in virgin localities for sorghum cultivation It should be emphasised that, although various *Sorghum* species can be, and are, hosts for *C africana,* no documentation of a plausible step-by-step sequence explaining the origin of epidemics of disease in seasonal crops has yet been made Assumptions are not adequate for explaining what is becoming globally an important disease All that was demonstrated experimentally is the windborne spread by secondary spores, registered on a spore trap, from an insect-proof primary focus of infection across a plot of male-sterile sorghums to give a widespread high incidence of disease (Frederickson et al 1993) Long distance dissemination of secondary spores has not yet been detected by spore trapping The spores can readily be recognized morphologically by their lemon shape but extensive scanning of spore trap slides would be very time-consuming Nevertheless, the dramatic spread of sorghum ergot throughout much of South America, after the causal agent was identified as *C africana* (Reis et al 1996), must be partly attributable to the windborne dissemination of propagules and the apparently susceptible sorghums grown extensively in South and Central America and in the United States Similarly, the pathogen established a new endemic focus in Queensland, Australia These rather sudden aspects of global spread had been foreshadowed by dissemination at some stage from Africa to Thailand (Boon-Long 1988, Frederickson et al 1991) and to Japan as determined by Mantle and Hassan (1994) who warned of the danger of spread into the Americas

The relatively insignificant size of sclerotia of *C africana* has resulted in the principal focus of disease significance being purely on loss of grain With an increase in disease incidence, the fact that the pathogen is an ergot fungus, some of whose close relatives produce metabolites which are toxic to animals, makes possible toxicity worth considering The effect of sclerotia from Nigeria on reproductive performance in mice revealed no effect even at very high intake (Mantle 1968) This contrasts with the marked effect on both blastocyst implantation and lactation by alkaloids of the pearl millet ergot pathogen *C fusiformis* It was concluded, therefore, that *C africana* sclerotia did not constitute a significant toxic hazard to farm animals or to humans This was in spite of the fact that dihydroer-

gosine possesses some of the pharmacological activity of its close relatives ergosine and ergotamine (Berde and Schild 1978) This view might be sustainable, in general, were it not for recent definition of a new dimension in *C purpurea* toxicity towards dairy cattle A so-called 'summer syndrome' in the Cape region of South Africa has been ascribed plausibly to small amounts of ergot alkaloids in a grass-seed based feed supplement used in summer when pasture is in short supply Decline in milk yield was attributed to reduced feed intake on account of the discomfort of hyperthermia induced by peripheral vasopressor effects of the ergot alkaloids (Schneider et al 1996) ascribed to sclerotia of *C purpurea* (Mantle 1996) The present author is also aware of an analogous syndrome associated with feeding ergotised sorghum to dairy cattle in South Africa and this aspect of possible toxicity will be explored further to establish whether there is a clear cause and effect It should also be noted that the ergot pathogen of Paspalum grass (*Claviceps paspali)* elaborates tremorgenic mycotoxins that are the cause of the neurological disorder Paspalum Staggers that can affect cattle grazing tall ergotised *Paspalum* spp forage Sclerotia should be examined for any indole-diterpenoid compounds which could have tremorgenic activity However, a recent experiment in Australia with beef cattle found no obvious adverse effect of feeding ergotised sorghum (M Ryley, personal communication)

There is no monopoly of sorghum ergot disease causation by pathogens with a *S sorghi* anamorph An interesting additional distinctive ergot pathogen was recognized in, and is apparently confined to, Japan (T Tsukiboshi, personal communication) which has a *Sphacelia segetum*-like (*C purpurea)* anamorph and grey spur-shaped sclerotia containing only a trace of some unusual ergot alkaloids (Ohmomo 1990) Asexual spores apparently do not undergo secondary sporulation in honeydew from parasitized male-sterile sorghum in the present author's experience, although the phenomenon was observed in spores washed from their honeydew and then spread on agar media (Hassan 1996) In lacking a mechanism for long distance dissemination, and therefore not necessarily a significant global threat to sorghum, the question arises as to whether the association between this fungus and sorghum is an ancient one limited by geography, or whether it has arisen recently and is indicating that an analogous association could develop elsewhere in the world

Pearl millet, in common with some other grasses, has a special stigmatic mechanism for excluding from neo-fertilized ovaries further ingress of pollen tubes or pathogen hyphae that traverse the pollen transmission tracts in the fused stylodia (Willingale and Mantle 1985) The separate stigmas of sorghum have no such mechanism and indeed can still appear quite fresh after ingress by ergot pathogens There is nothing in the floral biology of sorghum to assist the relatively slow pathogen-excluding histological changes that occur in the ovary following fertilization Several days may be required to differentiate tissues which constitute physical barriers to ergot penetration hyphae on their slower progress towards the ovule Ideally, self-pollination should occur before floret gaping, which not only exposes the ovary

through the wall of which ergot penetration can occur, but leaves one or both of the feathery stigmas protruding from the floret, trapped by the closing glumes Such a system was found to explain the mechanism of one particularly 'resistant' sorghum accession from East Africa (Frederickson et al 1994) Other accessions, which are free from ergot in their locality of origin, became susceptible when grown in a different latitude where their flowering behaviour was disturbed by the different daylength pattern Regrettably, it seems safe to conclude that there is no intrinsic exploitable natural resistance to ergot infection of *Sorghum* spp other than efficient pollination which arises from the particular floral biology In other words, all unfertilized sorghum ovaries are susceptible to ergot at the time of first floret gaping

In conclusion, the way in which the ovary parasite of sorghum, with versatile and extensive powers of dissemination, has been able to spread so fast globally, and to become so economically significant, is a reflection partly of the extension of monoculture agriculture However, it is more consequent on the artificial creation and wide use of male-sterile hosts in F_1 hybrid seed production which provides a window of opportunity for ergot pathogen spores to beat pollen grains for the controlling influence over the fate of unfertilized sorghum ovaries It is possible that disease control may be addressed agrochemically, at a recurring cost through the use of fungicides, but perhaps better strategies will focus on the male-sterility which is at the center of the present globally-exacerbated disease problem for sorghum

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Geographic Distribution and Spread of Sorghum Ergot: Causes and Implications

R Bandyopadhyay

Abstract

Sorghum ergot was observed for the first time in 1915 in India and remained restricted in Asia and Africa The distribution of the disease expanded outside these continents when it was found in Brazil in 1995, and in Australia in 1996 Since 1995, it has been reported in quick succession in 14 countries in South, Central and North America including the U S In Brazil and Australia the introduced pathogen was ***Claviceps africana*** *The pathogen was probably introduced with seed or as airborne secondary conidia, but other possibilities of introduction exist The disease spread rapidly in seed production fields due to conditions that favored production and spread of spores (primarily airborne secondary conidia) and predisposed flowers to infection Due to the vulnerability of male-sterile lines to the disease, its importance has increased internationally since male-sterile based hybrids are used in these countries The introduction of the disease will impinge on quarantine regulations seed production, commercial grain production, seed trade (including prices) and research prioritization*

Sorghum [*Sorghum bicolor* (L) Moench] is the fifth most important cereal crop and cultivated on about 45 million ha for food, feed, beverage and fodder Normally the crop is grown in drought-prone areas where few other crop choices are available In most parts of Asia and Africa, and some parts of Latin America and the Caribbean, sorghum is primarily grown for food under low input production systems by resource-poor farmers Yields average less than 1 t ha^{1} and vary substantially from year to year In contrast, sorghum cultivation in Australia, the United States, most South and Central American countries, and parts of South Africa is characterized by intensive, commercial production systems optimized to grow the crop almost completely as animal feed The most significant technological change since the 1960s was the development and use of F_1 hybrid seed (FAO and ICRISAT 1996) that lead to a dramatic improvement in productivity of the crop For example, India has experienced 55% coverage of the rainy season sorghum area under hybrids and a doubling of yield in the 30 years since hybrids were first introduced Sorghum cultivation in intensive, commercialized systems relies almost totally on F_1 hybrid seeds, and yields average 3–5 t ha^{1}

The sudden observation and rapid spread of ergot in Latin America beginning in Brazil in 1995, and in Australia in 1996 perplexed the sorghum industry around the world The global appearance of ergot made U S sorghum workers and quarantine officials feel rather vulnerable

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to the potential introduction of ergot However, the importance of sorghum ergot in hybrid seed production was highlighted in the mid-sixties in Nigeria (Futrell and Webster 1965) Indeed, the disease gained prominence only after the introduction of F_1 hybrids in India since A-lines are vulnerable to the disease This not only makes F_1 hybrid seed production difficult, but also creates problems in producing adequate quantities of A-line seed

Ergot is a disease of the ovary It reduces grain yield because the ovary of an infected flower is replaced by a fungal mass that secretes a sweet fluid, called honeydew Healthy grain are smeared with honeydew on which fungal saprophytes grow thereby reducing seed quality, making threshing difficult, reducing germination and seedling emergence, and predisposing seedlings to other diseases (McLaren 1993) Two teleomorphs of the ergot pathogen have been described These are *Claviceps sorghi* by Kulkarni et al (1976) and *C africana* by Frederickson et al (1991) The anamorph of both teleomorphs is *Sphacelia sorghi* (McRae 1917)

This paper provides a brief account of the distribution of the disease and the pathogens, the possible reasons for the sudden resurgence of the disease, and its implications

Distribution

The disease is normally not of concern in warm and dry areas, where sorghum is traditionally grown The problem becomes acute in cool temperature (even on male-fertile cultivars) and in male-sterile lines These two factors are primary contributors for lowering or nullifying pollen activity (McLaren 1992), adversely affecting efficient pollination and fertilization that protects flowers from infection (Futrell and Webster 1965, Musabyimana et al 1995)

The Afro-Asian era

Sorghum ergot was first observed in India in 1915 and described by McRae in 1917 That it was more widespread in India was confirmed in 1948, when Ramakrishnan recorded ergot in Madras State (Sangitrao et al 1999, for distribution in India) In 1927, ergot was reported in Burma These reports and samples represent the pathogen *C sorghi* In Africa, sorghum ergot was recognized first in the eastern region, having been sampled in Kenya in 1924 Table 1 summarizes the first reports of occurrence of the disease in different countries The disease was also observed in experimental hybrid seed production plots in Mali and Burkina Faso in West Africa (D S Murty, personal communication), but the identity of the pathogen is not known The fungus *Cerebella andropogonis* normally grows on the sugar exudates of *S sorghi* and is a good indicator of ergot in the field A few reports of ergot, such as those from Ghana, Sudan, and the Philippines, are based on the association of *C andropogonis* with the sphacelial stage of *S sorghi*

The pathogen in India and Myanmar was identified as *C sorghi* (Kulkarni el al 1976, Mantle 1968) All reports and samples from Africa, mentioned in Table 1, possibly represent the distinctive pathogen *C africana* (Frederickson et al 1991) In 1991, the ergot pathogen in Thailand was, contrary to expectations,

Table 1 Distribution of sorghum ergot in different countries until 1991[1]

Continent/Country	Reference	Remarks
Africa		
Angola	de Milliano et al 1991	
Botswana	Molefe 1975	193099[2] & 193661
Burundi	[3]	R Bandyopadhyay personal observation
Ethiopia	Woldekidan 1985	88616 & 225570
Ghana	[4]	*Cerebella andropogonis*[5] 63524
Kenya	McDonald 1924	*C andropogonis* 91149
Lesotho	de Milliano et al 1991	-
Malawi	de Milliano et al 1991	
Mozambique	de Carvalho 1958	
Nigeria	Wiltshire 1956	6280 & 99530
Rwanda	[3]	L K Mughogho, personal communication
Senegal	Jaubert 1953	-
South Africa	Dyer 1947	*C andropogonis*
Sudan	Anonymous 1932	*C andropogonis*
Swaziland	de Milliano et al 1991	
Tanzania	Mason 1926	*C andropogonis* 5541
Uganda	Small 1926	*C andropogonis* 14170
Zambia		Angus 1965
90016b		
Zimbabwe	Whiteside 1966	
Asia		
Burma	Rhind	1471 1472
India	McRae 1917	First report
Japan	Nishihara 1973	
Philippines	Langdon 1955	*C andropogonis*
Sri Lanka	Petch and Bisby 1950	*C andropogonis* 43654
Taiwan	Chen et al 1991	-
Thailand	Boon-long 1983	-
Yemen Arab Republic	Kamal and Agbari 1980	

[1]Based on CABI (1987)
[2]IMI numbers that refer to records in the herbarium of the CAB International Mycological Institute
[3]Observed in 1987
[4]Observed in 1956
[5]Association with *C andropogonis*

identified as *C africana*, not *C sorghi* (Frederickson et al 1991), and in Japan, one of the ergot species infecting sorghum was similarly identified (Mantle and Hassan 1994) The second *Claviceps*

species in Japan is yet to be identified (Bandyopadhyay et al 1996)

The America-Australian era

This period begins in 1995 when the disease appeared for the first time outside Asia and Africa causing a widespread epidemic and serious losses in hybrid seed production plots in Brazil (Bandyopadhyay et al 1996, Reis et al 1996) In another geographically distant continent, Australia, the disease appeared in 1996 on forage sorghums (Ryley et al 1996) Fortunately most of the commercial sorghums had flowered, thereby escaping infection *C africana* was identified as the causal fungus in Brazil (Reis et al 1996) and Australia (Ryley et al 1996) In Australia and the Americas alike, the disease spread very rapidly after initial appearance causing widespread concern

Following the appearance of ergot in Brazil in February, 1995, the disease was monitored carefully in South and Central Americas and the Caribbean given the high volume and frequency of seed exchange in the region for research and trade purposes By mid-1996, the disease had been recorded in Argentina, Bolivia, Paraguay, and Uruguay, by the end of 1996 in Colombia, Venezuela and Honduras, and during the first quarter of 1997 in Puerto Rico, Haiti, the Dominican Republic, Jamaica, and northern Mexico On 26 March 1997, the disease was observed in the U S on ratooned tillers in a sorghum field just North of the Rio Grande River near Progresso, TX The disease is most likely present in other countries of the Americas from where it has not been reported as yet The formal identity of the pathogen in these countries (except Brazil) is pending, but is most likely *C africana* Rapid dissemination of information on spread throughout the Americas was due to the e-mail network and the World Wide Web

Sources of Ergot Inoculum and Mode of Pathogen Spread

An understanding of sources of primary inoculum and mode of spread of the pathogen is required to postulate hypotheses for its introduction and spread in new areas The primary sources of inocula include sclerotia, infected plant debris, collateral hosts, and secondary conidia (Frederickson 1999) Ergot-infected spikelets exude sweet, sticky drops of fluid honeydew containing macroconidia and microconidia Rain splashes (Bandyopadhyay et al 1991) and possibly insects (Futrell and Webster 1966) spread conidia borne in the honeydew matrix These two modes of spread are more relevant for short distance spread than longer distance transport Wind dissemination of secondary conidia is the most significant mode of dispersal for both local and long-distance spread of the sorghum ergot pathogen (Frederickson et al 1989 and 1993) Secondary conidiation occurs when RH rises and temperature falls (Frederickson et al 1989 and 1993) These environmental conditions also predispose flowers to infection by reducing pollen activity and dispersal

Disease Introduction

The recent intensification in the geographic distribution of the disease is intriguing Why was the disease found in geographically distant Brazil and Australia within a year of each other after being

restricted in Asia and Africa for 80 years? What was the mode of entry of the pathogen into these two countries? Was the disease present in these countries earlier but not noticed? Is it related to the recent expansion in the collaborative seed exchange programs between seed companies and movement of genetic material of transnational seed companies, NGOs, farmers' groups, private farmers and scientists across national boundaries? The ergot pathogen in Brazil and Australia has been identified as *C africana*, the same as the one that is endemic in southern Africa and Thailand Besides sorghum, ergot was also reported for the first time in 1995 on pearl millet in Brazil and Australia The pathogens causing ergot on sorghum and pearl millet are different but cross-inoculation experiments have shown that the sorghum ergot pathogen can infect pearl millet in Australia (Ryley et al 1999) 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
- 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 A similar case may be true for Australia with the origin of the pathogen being a south-east Asian country This may sound science fictional, but explains why ergot appeared simultaneously on sorghum and pearl millet in Brazil Although Mantle and Hassan (1994) claimed that secondary conidia are short-lived, research is required to determine the longevity of secondary conidia
- Ergot could have been introduced to Brazil and Australia earlier than 1995 and was 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
- Someone walking through an infested field in a *C africana* endemic region could have had their apparel 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
- With increased air cargo shipments internationally, the pathogen may have moved in cargo holds from ergot endemic Africa to Brazil Such a theory has been propounded for introduction of Karnal bunt of wheat into the U S
- A naturally occurring ergot pathogen on grasses may have undergone a change in virulence to diversify its host range to include sorghum The possibility of such a change occurring simultaneously in Brazil and Australia is unlikely

Initially, infection in the introduced countries could have been in low proportion, but suitable weather helped develop an epidemic from a few small foci The pathogen has remarkable ability to rapidly spread from a small focus particularly when susceptible panicles are continuously available (Bandyopadhyay et al 1994 and 1994, Frederickson et al 1993) An understanding of the mode of introduction of the pathogen may help to devise methods to prevent its spread to new areas

Disease Spread

The introduction of the disease to Brazil and Australia, and its spread within these countries and across national boundaries offer good examples of the potential for long-distance spread It also exemplifies the rapidity with which epidemics can develop to cover large geographical areas In Brazil, the disease was first noticed during mid-February in seed production plots in Sao Paulo state Within a few weeks, the disease was reported from Ribeiro 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 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 within which the disease was noticed within a short time show the extraordinary capacity of the pathogen to develop into an epidemic rapidly in terms of both time and space

In Australia, the disease was first seen in Gatton on 26 April 1996 Within one week the disease was confirmed over an area of approximately 16,000 km^2, which, within a month, had expanded to 70,000 km^2 around the locality of the first sighting The disease was found throughout Queensland in 1996 and now occurs also in New South Wales (Ryley et al 1999) Disease spread to New South Wales was most likely due to secondary conidia with sclerotia playing an insignificant role, if any This is because seed movement from Queensland to New South Wales was allowed after sclerotia were removed by floatation in salt solution

In the Americas, it is most likely secondary conidia played a major role in the progressively north-eastward sightings of the disease from South America, to Latin America, to Central America and the Caribbean, to Mexico, and then to the US An examination of the weather parameters and air movement patterns over these countries up to a month before disease sightings can provide useful clues for the mode of spread Whether sclerotia also were responsible for the spread is undetermined due to unconfirmed reports of shipments of ergot-contaminated seed from Brazil to other South American countries and to the Bajio of Mexico (Bandyopadhyay et al 1996)

Environment and the Recent Epidemics

Environment has played a major role in fueling the ergot epidemics in Brazil and Australia after the introduction of the pathogen Two distinctly different environmental factors were perhaps responsi-

ble, wetness in Brazil and low temperature in Australia

Examination of Brazilian weather data for January and February 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 the first report of occurrence of the disease in Ribeiro Preto (15 February) was extraordinarily wet During this period, 446 mm rainfall occurred in 21 days From 1 to 15 February alone, 343 mm of rain fell in 14 days Minimum temperature during mid-January to February 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

In Australia, exceptionally cool and wet weather before and during flowering predisposed the sorghum crops to infection by inactivating pollen (Ryley et al 1999)

Implications of the Disease

There are several ramifications of the enhanced geographic scope of the disease in newer areas of the world where sorghum cultivation is dependent on hybrid seed

Plant Quarantine Regulations

One of the immediate issues pertains to the plant quarantine policy owing to the seedborne nature of the pathogen National policies for local and international quarantine regulations will require immediate attention Bandyopadhyay et al (1996) suggested that imported seed must be viewed as an important source of infection and thoroughly inspected for sclerotia, sphacelia, and honeydew before distribution is permitted However, experiences in the U S and Australia have shown that quarantine had limited impact in preventing disease introduction and spread due to the extraordinary capacity of ergot to spread rapidly by means other than seed Nevertheless, quarantine may delay disease spread into new areas by eliminating introduction of new disease foci through seed Research information can provide a valuable framework upon which to base quarantine regulations for reducing the chances of disease spread without unduly constraining seed trade and exchange

Seed Trade

The potential for introduction is intimately associated with the high frequency of sorghum seed exchange and trade

among countries of South and Central America, and to some extent, the United States The global nature of the hybrid sorghum industry, with seed production in one region and its distribution for planting at multiple distant locations, increases the chances of pathogen spread through seed The high vulnerability of the male-sterile parent, and possibly the enhanced survival of the pathogen in the cool, dry hybrid seed storage conditions further increase disease risk In 1996, there was a fear of banning seed imports from ergot infested countries to non-ergot countries (Bandyopadhyay et al 1996) due to the restricted distribution of the disease in the Americas With the pathogen's presence in the major sorghum-growing countries, the disease has become endemic across national boundaries by-passing the quarantine stage Therefore, the scenario of seed import bans can be now avoided, if appropriate seed certification and sanitation standards are maintained Nevertheless, restrictions on the movement of seed from infested to non-infested areas are likely to continue until shown ineffective

Seed Production

The hybrid seed industry will be most affected by the introduction of *C africana* requiring changes in several operational and policy aspects such as seed production, processing, quality control and handling practices, contractual arrangements with seed growers, pricing of seed, etc (Dolezal 1999, Ribas 1999) Traditional locations and sowing dates for seed production may require changes to avoid risk-prone areas and time, but cannot guarantee total control Repeated application of fungicides, reduction in the number of male-sterile rows to accommodate more pollen-donating restorers, rouging of infected plants, modification of seed processing facilities, additional quality control tests, and some loss in seed yield and quality despite these practices to control the disease would increase the price of the seed According to an estimate in Australia, an additional A$800 would be required to produce 1 t seed (M Ryley and others, unpublished)

Commercial Production

Grain production in newly ergot-prone areas that are characterized by cool temperature during the pre-flowering stages and wet conditions at flowering will be at an increasing risk due to the introduction of the disease Damage will be accentuated in hybrids that have inherently prolonged time interval between stigma emergence and fertilization necessitating withdrawal of productive hybrids In the absence of ergot, such a trait would have been of little consequence and productive hybrids would have continued to be produced Risk avoidance may necessitate alterations in normal sowing dates thereby reducing flexibility of sowing time and forcing growers to reevaluate the value of sorghum in their farming strategies Infected late tillers may continue to produce honeydew when the main crop is ready for harvest, thus, making combine harvesting difficult Honeydew-encrusted panicles complicate threshing, resulting in further grain losses Such threshed grain may have poor storability and marketability because honeydew lumps absorb moisture, become sticky, form clumps of grain, and the grain lots may become unsatisfactory

Genetic Vulnerability

Male-sterile genotypes are particularly vulnerable to ergot because they do not produce functional pollen Ergot susceptibility in relation to cytoplasmic male-sterility has been demonstrated in ergot of pearl millet caused by *C fusiformis* (Thakur et al 1989) Effective pollination reduces susceptibility so that male-fertile varieties rarely exhibit high ergot severities Cool, wet conditions before or during pollination reduce pollen fertility and pollen deposition, resulting in increased ergot infection in all genotypes (McLaren 1992) Valuable or scarce seed stocks of male-sterile lines are thus at risk of depletion if conditions allow extensive ergot infection at the production stage To avoid the loss of valuable male-sterile lines, seed should be multiplied at multiple locations preferably minimizing the ergot risk by manipulation of the planting date or through the use of fungicides Adequate stocks of diseasefree seed should be maintained

Potential Toxicity to Animals

The question of the potential toxicity of sorghum ergot sclerotia inevitably arises through analogy with *C purpurea* and *C fusiformis*, which synthesize such potent alkaloids as ergotamine and agroclavine However, the quantity and spectrum of alkaloids found in sorghum ergot sclerotia are not significant to cause toxicity in animals (Bandyopadhyay et al 1996, Mantle 1968) Therefore, it is highly unlikely that sclerotia contaminated sorghum grain has any implications for animal health For more detailed information, see Mantle (1999)

Research Priorities

An added dimension of the introduction of ergot to countries dependent on sorghum hybrids is the change in research priorities necessary to find solution to a new important problem that is specific for hybrid seed The criteria used to prioritize problems by different organizations and their mandates would determine the priority, resource allocation, and role of each organization Reliable assessments of the impact of the disease should consider aspects of sorghum research, production, processing, marketing, utilization and trade Unless new sources of funding are available, it would entail diversion of resources for research and development aspects from other important problems Due to the major implication of the disease for the seed sector, it is hoped that the seed industry would play a major role in ergot research It is encouraging to note that this is occurring in a few countries

Conclusions

Sorghum ergot has become an internationally important disease within a short span of two years since 1995 This is because adequate availability and profitability of F_1 hybrids seeds, one of the mainstays of intensive and productive sorghum agriculture in the developing and developed world alike, is at risk Commercial production of sorghum hybrids is also threatened, particularly in areas where sorghum experiences cool temperature at or before flowering It is not a new disease Nor is this the first time that it is causing concern to the sorghum seed industry having been a problem in India, Zimbabwe and South Africa The present worldwide attention that the disease is re-

ceiving stems from the rapid spread of the disease in the Americas and Australia where it can cause potential damage Sorghums in these geographical areas have never been exposed before to the ergot pathogens and have not gone through the natural selection processes The disease provides new challenges, opportunities and partnerships for the sorghum community

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Session II — Ergot Scenario in Brazil

Session President — E.M Reis (UFPF)

Sugary Disease of Sorghum in Brazil: an Overview

R Casela, A S Ferreira and N F J A Pinto

Abstract

*Since it was observed in Brazil in April 1975 sugary disease of sorghum (**Claviceps africana**) has been a serious threat to seed production The impact of the disease on sorghum seed business in Brazil resulted in an integrated effort involving private sector, University of Passo Fundo and Empresa Brasileira de Pesquisa Agropecuaria (Maize and Sorghum Research Center) to develop strategies to adequately manage this disease This paper summarizes the achievements from this effort*

Sugary disease of sorghum, caused by *Claviceps africana* Frederickson, Mantle & de Milliano, was reported for the first time in Brazil in April, 1995 simultaneously in Cravinhos (São Paulo) and Lavras (Minas Gerais), 400 km apart In a period of 30 days, several additional reports of the presence of the disease were made from different locations including the states of Minas Gerais, São Paulo, Mato Grosso do Sul, Goias and Parana The disease was later reported in the State of Rio Grande do Sul in a late plantings of sorghum Honeydew exudations were also observed in many different grasses close to the sorghum crop

The primary concerns of sorghum scientists in Brazil about the disease were its impact on hybrid seed production in the country and the presence of any substance that could be toxic to animals According to reports from private companies, the majority of seed production fields were at the grain filling stage when the disease was first observed Losses at that time were estimated at roughly US $400,000 and was primarily due to the effects of sugary exudation on seed quality In the second year, in an attempt to escape from more favorable environmental conditions for disease development, seed production fields were established earlier than usual This change resulted in poor nicking of flowering in the parental lines and poor pollen production Losses were higher and estimated to be around U$800,000

Research Achievements and Decisions Taken

Due to the impact of the disease on the sorghum production and seed business in Brazil, an proactive effort including the private seed sector, Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA), and the University of Passo Fundo (RS) was initiated in June 1995 to develop strategies for adequately managing this disease Part of the achievements resulting form these efforts will be reported in this paper, and in more details by others in these Proceedings

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Sources of Resistance

The greatest need of sources of resistance to sugary disease is in male-sterile lines Chances of identifying male-sterile resistant lines are very small considering the role of pollen in the disease (Bandyopadhyay 1992) Despite this low probability, all A lines of the sorghum breeding program of CNPMS/EMBRAPA were evaluated under natural infection in 1996 All lines evaluated were susceptible

Chemical Control

Information on chemical control of *C africana* on sorghum are very limited In South Africa, the fungicides benomyl, bitertanol, carbendazim + flusilazole, procymidone, propiconazole, tebuconazole, and triadimenol did not control *C africana* economically when sprayed once at flowering time (McLaren, 1994) Results obtained by scientists of CNPMS/EMBRAPA showed that fungicides Tebuconazole and Triadimenol were efficient in the control *C africana* although Triadimenol showed some phytotoxicity to sorghum seeds More recent results have indicated that the fungicide Propiconazole and the mixture Propiconazole + Difenoconazole are also efficient in the control of this pathogen (Pinto et al 1999)

Alternate Hosts

The host range of *C africana* under Brazilian conditions has been examined at CNPMS/EMBRAPA Sorghum was inoculated with honeydew from *Panicum maximum* (colonião), *Panicum* sp (coloniãozinho), *Brachiaria mutica* (bengo), *Brachiaria decunbens* (brachiaria), *Sorghum verticuliflorum*, *Cenchrus* sp , *Zea mays*, and *Pennisetum typhoides* (pearl millet) In all tests a non-inoculated control was included Test hosts were also inoculated with honeydew from sorghum Isolates from *P maximum*, *Panicum* sp , *B mutica* and *B decunbens* had low pathogenicity to sorghum and isolates from sorghum were nonpathogenic to *P maximum* *Panicum* sp , and *B decunbens* and of low pathogenicity to *B mutica* Isolates from sorghum were of intermediate pathogenicity to *S verticiliflorum* Infectivity of inoculum from *S verticiliflorum* plants was not tested on sorghum

Other Initiatives

As part of an integrated strategy several decisions were taken in the search for ways to minimize the problem, These include

- Several meetings involving farmers and the extension services were held to inform them about the presence of the disease in Brazil
- Training courses in seed production and seed quality control, to correctly identify the disease symptoms in the field and the presence of sclerotia in contaminated seeds
- A 0% tolerance of sclerotia was fixed as a standard in all processed seed for the State of Minas Gerais
- Registration of a fungicide for the control of *C africana* in sorghum, based on research conducted by CNPMS/EMBRAPA
- Monitoring of seed production fields and elimination of sclerotia during seed processing

- A national workshop on ergot was held to establish standards in seed quality control on a national basis

Final Comments

Sugary disease of sorghum has become a serious constraint in hybrid seed production in Brazil as well as in other parts of the world where the disease was recently observed The collaborative research program developed by Brazilian scientists, from the public and private sector, has been, mostly successful in finding ways to reduce the impact of the disease on the sorghum seed business despite the large area and explosive occurrence of the disease in the country We believe that the establishment of research strategies based on international cooperation, which is the main goal of this Conference, is fundamental for the control of this disease

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Strategies for the Control of Ergot in Sorghum Seed Production in Brazil

Paulo Motta Ribas

Abstract

A sudden outbreak of the sugary disease (ergot disease) of sorghum in Brazil in the fall of 1995, forced the public and private sectors to act rapidly to reduce or neutralize its effects on hybrid sorghum seed production A strategy was established to seek short, medium and long term research solutions combined with communication education and training of researchers, extensionists, seed production technicians, farmers and usersof forage and grain sorghum Government sectors were sensitized to participate in the joint effort The outcome of these efforts guaranteed the production and supply of seeds in the first two years of coexistence with the sugary disease

The private sector has played a strong role in Brazilian research, development and production of sorghum hybrid seed since its introduction to the country in the late sixties National and international companies established in the country, together with Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA), are responsible for the development and local production of almost 100% of sorghum seed sold in Brazil The volume of imported seed into Brazil is currently not significant Practically 100% of the sorghum seed planted in the country is hybrid The amount of open pollinated varieties commercialized is negligible The sorghum business in Brazil is small when compared to other grain crops, such as corn, soybeans, rice and dry beans In the last six years, however, interest in the crop and the utilization of its products as an animal feed has generated a growing demand for seed Table 1 shows the amounts of seed sold in Brazil from 1991 to 1996 and its value was estimated at nearly US $15 million in 1997, according to the national sorghum seed market The crop is likely to occupy the huge savanna (cerrado) areas of the country's West-Center and should be integrated into the Northeast semi-arid production system Its' excellent adaptation to water stress, prevalent in these regions, and the growing need for grains and forage for animal feed in agro-industrial projects being set up in the area, may be the decisive factors from which Brazil will become one of the greatest cultivated areas of sorghum in the South American continent

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Impact of the Outbreak of Sugary Disease on Hybrid Sorghum Seed Production in Brazil

The area dedicated to sorghum seed production in Brazil is concentrated in a region that spans from the North of São Paulo state (20°) to the Southwest of Goias state (17°) After the summer crops (corn and soybeans), sorghum is sown between January and March The crop is

Table 1 Sorghum seed market in Brazil Sales evolution from 1991 to 1997

Agricultural Year	Total Seed Sale (20 kg bags 44 lb.)	Change in sale from previous year(%)	Value (US $00)
1991/92	95 700	0 0	2 661
1992/93	152 500	59 3	4 497
1993/94	131 000	14 0	4 285
1994/95	152 100	16 1	6 042
1995/96	182 800	20 1	7 706
1996/97	223 800	22 4	10 037
1997/98	336,000	50.1	14.192

*Production estimate Source Pro Sorgo Group/APPS 1997

harvested between July and August Less than 30% of the area is cultivated without irrigation In the remaining irrigated areas, center pivot sprinkler systems are preferred

In the fall of 1995, around the 24-25 April, the seed industry acknowledged the outbreak of the sugary disease in Brazil, through simultaneous reports from Lavras, South of Minas Gerais, and Cravinhos, North of São Paulo These places are 400 km apart

Examinations carried out by the Plant Pathology Department of the Lavras Federal University, confirmed the presence of the fungus of the genus *Claviceps*, a fact later reconfirmed by EMBRAPA Within 30 days, the disease was reported in various regions in South-central Brazil, from the northern parts of Parana state in the South, to the West of Goias in the North and from Sete Lagoas, MG in the East, to Campo Grande, MS in the West By this time, seed production fields were in the final phase of flowering or beginning to fill grain However, the disease symptoms were observed in tillers or isolated plants that were not properly pollinated Later, however, heavy losses occurred in late-planted fields that flowered under low temperatures in the Triângulo Mineiro region, resulting in the total loss of seeds smeared with honeydew In general, the losses of the seed industry in 1995 did not jeopardize that year's seed supply and the financial loss did not surpass US $0 5 million

In 1996, with control measures recommended by research already in use by the industry, some indirect losses occurred Unsure of the efficiency and workability of the fungicide applications at flowering time, some companies decided to alter the sowing date, so that flowering could coincide with periods of high temperatures in February and March, resulting in less infection The results of this action were negative Although the fungicide application was efficient, unexpected wet conditions harmed pollination and general field performance This resulted in an estimated loss of US $1 million to the industry, indirectly caused by the sugary disease The reduction in seed production of these companies was counter-balanced by the competition, so the market was reasonably supplied again in 1996 With two years of experience behind us, we expect a normal market supply in 1997

The Reaction of the Private Sector

Once the first moments of incredulity, perplexity and almost panic had passed, private companies started to confront the new situation with the certainty that the disease was here to stay The first and obvious action was to relay all the information we had to the Centro Nacional de Pesquisa de Milho e Sorgo (CNPMS), of EMBRAPA, and to propose an immediate meeting to evaluate the situation and to establish a strategy to confront the problem Technicians from EMBRAPA and representatives of Pró Sorgo - a group of sorghum seed producing companies - met on 14 June 1995 in Uberaba, MG In the meeting, it was decided to integrate private and public action on ergot with shared efforts and resources, with the following objectives

- Guarantee the continuity of sorghum hybrid seed production in Brazil
- Inform, educate and train researchers, production technicians, extensionists, producers and sorghum consumers in general about ergot
- Inform and sensitize government sectors responsible for the regulation of seed production control in the country about ergot
- Manage the information propagated by the national media

It was also decided to invite Dr Erlei Melo Reis, from the University of Passo Fundo, Rio Grande do Sul, a recognized specialist of the genus *Claviceps* in winter cereals, to participate in the joint effort Dr Melo Reis accepted the invitation EMBRAPA, private companies and the University of Passo Fundo established strategies to tackle the problem, in a meeting in Ribeirão Preto-SP, in August 1995

Integrated actions

EMBRAPA and the University of Passo Fundo divided the scientific work The task of CNPMS was to immediately organize a breeding program aimed at varietal resistance The University of Passo Fundo took responsibility for the basic studies on the biology of the pathogen and epidemiology of the disease Private companies and EMBRAPA rapidly began testing fungicide seed treatment and spray application for controlling the disease as indicated by the international literature (McLaren 1994, Bandyopadhyay et al 1996)

Besides the scientific activity, the Pro-Sorgo Group and EMBRAPA initiated intense activity in other areas It was necessary to avoid the panic which is very common in Brazil every time an epidemic attacks some segment of the agricultural sector Past experiences such as the coffee rust and the African swine pest, and, particularly the official report of sorghum downy mildew in 1974, left no doubt about what to do about the sugary disease The immediate spread of correct information to the farmers and users of grain and forage sorghum was necessary Toxicological analyses of infested plant parts and silage samples were performed to confirm the safety of these feeds to animals Many technical meetings and releases to the press were made and continue to be made throughout the country Panic was contained Apparently, sorghum farmer's confidence was not shaken On the administrative and political levels, action was concentrated in the

State Seed Sub-commissions, technical and administrative committees comprising of government and private sector representatives, to establish quality standards and norms for the production of certified seed in the country The information about the disease and the debate about its consequences to sorghum seed production were introduced in the Sub-commissions meetings

In 1996, The Minas Gerais State Sorghum Sub-commission, (the most important sorghum seed producing state in the country) also joined the effort to control the sugary disease, and monitored all seed production fields in the state Also, registration of commercial fungicides for applications in sorghum seed production fields was extended Results began to appear from this defined strategy and actions co-financed by the Pro-Sorgo Group

Results

The identity of the Brazilian sorghum ergot pathogen was established as *Claviceps africana*, Frederickson, Mantle & de Milliano (Reis and Blum 1996), teleomorphic or sexual form of the imperfect form *Sphacelia sorghi* McRae This was possible due to collaborative work of Drs Melo Reis and Peter Mantle, Imperial College of Science, Technology and Medicine, UK It became especially clear to the Brazilians that risks of ergot toxicity were negligible Also the shape of sclerotia could easily be confused with atrophied sorghum grains (Frederickson et al 1991) Until then, we were looking for spur shaped structures (the meaning of ergot) It was not, therefore, appropriate to continue calling the disease "ergot", sugary disease was more appropriate

The seed industry needed quick answers to solve urgent problems Some seed lots were impregnated by the honeydew, posing problems with seed quality (McLaren 1993) The question was should another product be added to the conventional Captan treatment? Or should Captan be replaced to guarantee inactivation of conidia? Researchers at the University of Passo Fundo showed that conidia were inactivated by Captan alone, at the same concentration used by the industry

Next question what level of control would application of fungicides offer in the field and what kind of operational problems would it pose to the industry? The efficacy of fungicides was tested concurrently by EMBRAPA (Sete Lagoas-MG), Agroceres S/A (Capinopolis-MG) and Zeneca (Cravinhos-SP), in August and September, 1995 The results obtained were similar and indicated the triazole group of fungicides could be used by the seed industry (Ferreira et al 1996) Table 2 shows a summary of the results for 1995 As reported above, commercial products based on tebuconazole and propiconazole were recommended for use in sorghum, through an effort of the Sorghum Sub-commission of Minas Gerais

One last question remained how to apply the fungicides on a commercial scale? Practice brought the answer Between March and May 1996, seed companies that opted for land application had to mount relatively complicated operations to be able to spray all their fields at 5-7

Table 2 Chemical control of the sugary disease (Sphacelia sorghi McRae) in the field Tested fungicides, dosages, and selected products for chemical use

Tested Fungicides	Dosage (g or ml of a. i. ha^{1})
Tebuconazole*	375 0
	250 0
	125 0
	62.5
Propiconazole*	250 0
	125 0
	62.5
Triadimenol	250 0
	125 0
	62.5
Benomyl	500 0
	750.0
Carbendazin	500 0
Thiabendazole	100 0
Mancozeb	2400 0
Fentin Hydroxide	500 0
Metalaxyl + Mancozeb	960 0 + 216 0
Tiofanato Metílico	700.0

Recommended for commercial use at 250 g or 250 ml of a i ha^{1} total dose divided in 3 or 4 applications 300 to 400 l of spray volume ha^{1} Source EMBRAPA/Pró Sorgo Group 1996

day intervals during flowering using high spray volumes (300-400 l ha^{1}) Other companies chose aerial spraying, with much less operational complexity, but at a higher cost A third group used the center pivot irrigation system to apply the fungicide, with mixed results In spite of the indirect losses mentioned earlier, the industry managed to contain the disease, combining traditional management practices to improve 'nicking' between parents in the seed production field and fungicide applications

Fungicide Application

Experimental work indicated the need for 3 applications at 5 to 7 day intervals, from the beginning of anthesis to the complete flowering, covering a period of 20 days in the field The large volume of spray mixture required for land application using tractors (average of 350 l ha^{1}) made the operation more complex than normal, requiring the use of tank trucks for constant refilling of equipment The operational efficiency was considered low

The importance of uniform crops to limit the number of fungicide applications to a maximum of three became clear Aerial spraying, with low volume of mixture, was duplicated to guarantee the perfect wetting of the panicles, increasing the cost significantly Table 3 shows comparative costs of the aerial and land systems The use of the center pivot irrigation to deliver fungicides needs more analysis before it can be recommended

Sclerotia Elimination

Elimination of sclerotia in seed lots was another challenge Technicians were trained to monitor the sclerotia during seed processing Ample training for field and lab technicians, research and production managers and government inspectors was provided in February 1996 at the College of Agriculture-USP, with participation from EMBRAPA, the University of Passo Fundo and the Pro-Sorgo Group The harvest of 1996 was well monitored by companies and inspecting services for the presence of sclerotia

The total elimination of sclerotia was obtained during several normal steps in seed processing, with practically no new investment required in processing (Tables 4, 5 and 6) Combine harvesting recovered most of the sclerotia and the gravity table removed the remainder to a "zero" level The majority of sclerotia present in the field are discarded at the

Table 3 Comparative cost (US$ ha^{1}) of land-based and aerial application of fungicides for the control of sugary disease of sorghum

	Land Application*	Air Application**
I—Product		
Fungicide	42 45	169 80
Surfactant	3 02	12 08
Sub-Total I	45 47	181 88
II—Services		
Application	20 21	100 00
Water transport	5 90	
Labor	2 88	
Sub Total II	28 99	100 00
III—Grand Total	74 46	281 88
IV—Seed yield* **	80	80
V—Increase in cost for 20 kg bag	0.93	3.52

Fungicide 1 l ha^{1} Surfactant 2 l ha^{1}
* Fungicide 4 l ha^{1} Surfactant 8 l ha^{1}
**20 kg (44 lb) ha^{1} Source Pro Sorgo Group 1997

harvesting stage and fall or return to the soil, and this situation needs better evaluation, up to what point does sclerotia in the soil make these areas potentially unsuitable for seed production? Work conducted in 1996, however, indicated that the abundant production of sclerotia could be minimized by a combination of field management practices and also that they could be totally eliminated by adequate processing

Marketing of Sorghum Seed Without Sclerotia a Challenge and a Pledge of quality from the Brazilian Seed Industry

The Brazilian seed industry has been able, in general, to evolve with our country's agriculture, supplying it with quantity and quality seed, even to the point of reaching foreign markets Sorghum hy-

Table 4 Ergot infected sorghum plants found during inspection of hybrid seed production fields

Hybrid	Field	Area (ha)	Plants evaluated (No.)	Plants infected (No.)	% Infected
	1	15	1 000	89	8 9
	2	43	2 000	123	6 1
	3	60	3 000	8	0 3
A	4	30	4 000	16	0 4
	5	48	6 000	16	0 3
	6	30	3 000	23	0 8
	7	25	2 500	30	1 2
	1	58	2 000	3	0 2
B	2	12	3 000	85	2 8
	3	100	5 000	4	1 7
	1	35	4 500	78	1 7
C	2	106	7 000	148	2 0
	3	50	5 000	14	0 3
	4	70	8,000	306	3.8

Source Pro Sorgo Group 1996

Table 5 Evaluation of the presence of ergot sclerotia in various stages of sorghum seed processing

	% of Sclerotia*	
Phases	Sub Field 1	Sub Field 2
Pre Harvest	0 24	2 34
Post-Harvest	0 10	1 65
Post Cleaning	0 10	0 84
Post Air Column	0 04	0 56
Post Sizing	0 04	0 32
Post Gravity Table	0.00	0.00

* % of sclerotia in 500 g samples Source Pro Sorgo Group 1996

Table 6 Presence of ergot sclerotia in combined samples of hybrid sorghum seed lots

Hybrid	Seed lots Sampled (No.)	No of sclerotia present per lot
A	29	0
	4	1
B	38	0
C	7	0
D	11	0

Samples of 500 g lot^{1}
Lot of 8 metric tons Source Pro Sorgho Group 1996

brid seed production is a private-sector activity supervised by the government in Brazil These companies are also reaching out to export markets

The State Commissions are preparing themselves to adopt the "zero sclerotium" standard in their certified seed The Minas Gerais State Commission, leader in the sorghum seed production in the Country, has already adopted it By the end of the decade no Brazilian State will be producing sorghum seed that does not comply with this quality standard, essential in helping to control the spread of this disease

Conclusion

The results obtained by the Brazilian strategy for the disease control will be certainly debated and analyzed in the conference Our work is just beginning, and many questions concerning the seed industry are still without a conclusive answer From on-going research projects we expect, for example, a better definition of the epidemiological role of sclerotia, as well as better ways to manage sclerotia infested fields

The Brazilian private sector makes itself completely available to the national and international scientific community, here represented by eminent researchers, for the exchange of experiences that could lead to the control of this disease by sorghum producing Countries

Our short experience with the sugary disease-but our past experiences with other epidemics-authorize us to state that the first and strongest factor of success in any strategy to control an epidemic outbreak is to establish an union of efforts among all the sectors connected with the affected activity, devoid of all vanity or any other secondary interest

Secondly, that science from the North must be involved in all actions and decisions We don't solve a problem of this nature under the influence of emotions or utilizing attitudes that are against reason or ethics

The seed industry thanks the organizing committee for providing the opportunity to speak in an event of such quality with the hope that our modest contribution can be useful to reduce the "phantom" of sugary disease to a dimension that is realistic and manageable by all of us

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Session III — Country Reports

Session President — Gary Odvody

Introduction to the Country Reports Section at theGlobal Conference on Ergot of Sorghum

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The country and regional reports section of the global conference on ergot of sorghum represents both the new and the old concerning experience with these pathogens A few scientists will relate their many years of research and interaction with ergot, however, most will share about a pathogen that suddenly swept into their country or region within the last year or the last few months even as this conference was already being assembled A preponderance of the reports are from the Western hemisphere where in a majority of the countries there was first a sudden awareness of Claviceps africana, then expectation and finally occurrence across many sorghum production areas The reports will chronicle preparation and response to that ergot invasion, current distribution and impact of ergot, and other reactions to the occurrence of the pathogen There will be both a uniqueness and a similarity in each ergot experience that represents the interaction of the ergot pathogen with the regional environments and local sorghum production systems This chronology of ergot spread and regional response provides us with a current yet historically unique perspective enabling analysis of an ongoing global epidemic of a plant disease More importantly we have the opportunity to gain a collective insight into the biology and epidemiology of the ergot pathogens, especially *C Africana*, and to identify the research, management, and regulatory efforts in process Our collective knowledge, interaction, and collaboration on several issues involving sorghum ergots will be necessary to sustain the international and global nature of the sorghum seed industry

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Experiences with Ergot of Sorghum in Southern Africa

D C Nowell and N W McLaren

Abstract

*Ergot (**Claviceps africana**) has been present in the Southern African region for many years However, it was only in the mid-1980s that the disease increased in importance and started causing economic losses Although ergot can be of economic importance in commercial sorghum production, seed production fields are most affected by the disease The disease became more important in the region with increased hybrid seed production, and the introduction of foreign germplasm (primarily from the USA) Losses are linked to a number of different attributes with grain yield (seed set and fill) harvestability, plantability, germination and seedling vigor being the more important The attributes are of particular importance to the seed industry Considerable effort has to be expended to ensure a high quality seed reaches the farmers Controlling the disease is complex and includes managing planting date, irrigation, cleaning of the seed, fungicide application cold tolerance of germplasm pollen pressure and cultivar choice*

Ergot of sorghum in Southern Africa is caused by the fungus *Claviceps africana* Frederickson, Mantle & de Milliano (Frederickson et al 1991) and not by *Claviceps sorghi* Kulk et al as originally assumed (Mantle 1968, Sundaram 1980, Frederickson and Mantle 1988, Frederickson et al 1989, McLaren and Wehner 1990, Bandyopadhyay 1992, de Milliano 1992) Although the fungus was first listed in South Africa in 1953 by Doidge et al (1953), a specimen, found in KwaZulu-Natal, was deposited in the National Herbarium in Pretoria in 1936 by Sellschop (No 28658) but it is thought to have been present in South Africa prior to this The exact time of the first occurrence of the disease in other countries in the region are not known Thus, the disease has been present in the Southern African region for a considerable period

Ergot of sorghum was considered to be of limited economic importance and was associated only with specific cold and moist conditions By the late 1980s, the pathogen had increased in economic importance and was considered a serious economic threat to the production of hybrid seed (Bandyopadhyay 1992) This increase in economic importance was mirrored in the Southern African region Ergot only became a significant economic problem in this region when a late 1970s and early 1980s in hybrid seed production fields In South Africa, the first major ergot epidemic in seed production fields occurred during the 1985/1986 growing season Significant losses (quality and seed yield) were experienced, and have been present since then to a greater or lesser de-

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gree in most seasons This increase in incidence, and severity of ergot in the region coincided with increased hybrid seed production, and the increased direct use of inbreds from the U S in these hybrids It can be concluded that ergot is widespread and economically important in commercial and seed production fields, but primarily of economic importance in seed production

The causal organism will not be described as a detailed description of *C africana* can be found in Frederickson et al (1991) Similarly, Frederickson and Mantle (1988) and Frederickson et al (1989) have described the infection process and disease development

Distribution

This fungus is widely distributed in Southern Africa with Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Zambia and Zimbabwe having recorded the pathogen (Bandyopadhyay 1992, de Milliano 1992, Nowell unpublished) The severity of the disease varies considerably within, and between growing seasons, and over locations

The infection of sorghum, and subsequent development, of ergot is highly weather dependent (Sundaram 1980, Frederickson and Mantle 1988, Frederickson et al 1989, McLaren and Wehner 1990 and 1992, Bandyopadhyay 1992) Climatic conditions will be dealt with in depth in another paper during the conference (McLaren 1999) It has been found that overhead irrigation can significantly increase ergot severity

Problems/Losses

The major practical problem with ergot infection is the sugary exudate that forms on the head This substance is very sticky, and interferes with harvesting of the crop and processing, as well as planting, if destined for the seed market At the end of the season, sclerotia are formed in the infected glumes However, these sclerotia seldom have the typical black outer layer and seldom germinate to produce perithecia In a seed crop, the seed contaminated with ergot exudate tend to aggregate, particularly under conditions of high relative humidity, making harvesting, processing and planting extremely difficult, if not impossible This means that seed companies have to wash the seed once it has been harvested to remove the exudate from the seed This process is time consuming and the seed has to be artificially dried immediately after having been washed It is estimated that this process costs the industry (valued at about $2 million per annum), which is small on an international scale, an additional $200 000 per annum Similar losses are experienced in Zimbabwe but the value of the crop and losses are lower McLaren (1993) showed that ergot exudate could significantly affect both germination of the seed and plant vigor If this washing process is not completed correctly, the quality of the seed can be adversely affected due to increased moisture of the grain and the toxic effect of the exudate It has also been found that storage life of the seed can be reduced as a result of lower quality grain

Infection of the sorghum head also results in a decrease in seed set and hence a decrease in the grain yields Under ideal conditions for *C africana*, the effect on

yield can be large The yield loss ranges from 0–$900 ha^{1} (70%) in seed production fields

Mantle (1968) found that culture filtrates of a Nigerian strain of *Sphacelia sorghi* produced the alkaloids dihydroergosine, festuclavine, pyroclavine, dihydroelymoclavine and chanoclavine, which accounted for over 99% of the total alkaloid production These alkaloids are of relatively low toxicity Feeding of *C africana* sclerotia to mice failed to inhibit lactation or implantation Indications are that the alkaloids produced by this strain of *S sorghi* are the least toxic of all the alkaloid containing ergots

There are large variations in hybrid or inbred susceptibility to ergot in the field Resistance to ergot in sorghum does not increase in a linear manner with increased disease pressure However, specific lines/hybrids are consistently more resistant to the fungus than others lines/hybrids (McLaren 1992) This can be seen very clearly when a number of hybrids are produced in the same region and are planted at the same time Seed and quality losses for each inbred vary significantly

Solutions

In order to control the pathogen it is necessary to use integrated management practices In South Africa the following guidelines are used

i planting date ensure that the seed crop is planted so that the crop flowers when the mean maximum daily temperatures are above 28°C and mean night temperatures are above 13°C This will ensure that daily temperatures will limit the amount of infection and the night temperatures will not predispose the plants to infection (McLaren and Wehner 1990 and 1992, McLaren unpublished) This has found to be true in India as well (Anahosur and Patil 1992)

ii irrigation management — overhead irrigation can significantly decrease the ambient temperature and thus make conditions suitable for infection by *C africana* Thus irrigation should be undertaken in such a way as to not lengthen the dew period

iii cleaning of seed before harvest — seed produced under overhead irrigation can be washed shortly before harvest while still on the head should there be a very large amount of exudate present However, the wetting of the grain at this stage significantly increases the risks of reduced seed quality, vigor and viability

iv cleaning of seed during processing — infected seed crops have to be washed during the seed conditioning process Failing to do this could result in reduced germination, plantability and seedling vigor after planting Another factor that plays a role is the aggregation of exudate contaminated grain during storage and before planting when the relative humidity increases The whole packet of seed can become a solid, unusable, sticky mass of seed

vi slight seed contamination — slight contamination can cause a buildup of exudate in the planter hopper during planting Although planting may be perfect in the beginning, the

amount of seed planted becomes progressively less and will result in a progressively lower plant density (after 3 hours of planting the amount of seed planted is half of the desired plant density, and after 6 hours of planting the amount of seed planted is <10% of the desired plant density) This problem can only be eliminated through washing the seed during processing/conditioning

vi Fungicides — fungicides can be used to limit the amount of infection However, the cost effectiveness of such a practice is questionable (McLaren 1994) and will vary between each country

vii timing of pollen production and silk emergence — as the *S sorghi* conidia compete with the pollen for fertilization/colonization of the embryo, it is vital to have early, high and viable pollen pressure Failure to have this increases the risks of ergot greatly

viii ratio of males to females — linked to vii is the ratio of male rows to female rows as this has a large effect on the pollen pressure Normally, the Southern African region works on 4 or 6 rows of females to 2 rows of males In exceptional circumstances (when the male is a very strong pollen parent and cold tolerant) 8 rows of females to 2 males can be planted

ix cold tolerance — due to the predisposition of the plants to ergot infection by a cool period (<13°C night temperature) before flowering in some germplasm, it is important to plant the more cold tolerant parents in areas of higher ergot risk Cold tolerant/ergot resistant hybrids should be planted in areas of high risk for ergot

x seed treatments — the use of fungicide seed treatments are used on seed that had been infested in the fields to improve storage and seedling vigor However, they will have no effect on the incidence of ergot

Future

Primarily the research in Southern Africa will be focused on

i the identification of resistant/cold tolerant germplasm in the field This could be supplemented by the identification of molecular markers for resistance/cold tolerance

ii improved seed production practices

iii identifying the most suitable seed production regions

iv improving seed processing practices

v testing the newer generation fungicides for efficacy against C africana

vi testing fungicide seed treatments to improve germination and seedling vigor of infected seedlots

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Sorghum Ergot in India

C S Sangitrao, S Indira and R Bandyopadhyay

Abstract

Ergot is the most important disease constraint in sorghum seed production causing 10-80% yield losses The biology of the pathogen ***Claviceps sorghi*** *and ergot epidemiology have received considerable research attention in India The roles of sclerotia and collateral hosts in the disease cycle have been established Sclerotia could be separated from seed by flotation in 10% salt solution Delayed sowing, non-synchronization of seed parents, and poor crop management are some of the factors that favor the incidence, development, and spread of ergot in F_1 hybrid seed production fields Varieties hybrids, and their seed parents have variable degree of susceptibility Varieties are less susceptible than hybrids Male-steriles are more susceptible than maintainers and restorers In spite of self-pollination, varieties and B and R lines are also susceptible Recommended strategies for integrated disease management in India include early sowing, synchronization of male and female parents destruction of infected panicles and collateral hosts, fungicide application, removal of ergot sclerotia by flotation in salt solution and adherence to seed certification standards*

India is a major sorghum growing country in the world Sorghum is cultivated on 12 55 million ha in this country, representing 30% of the world's sorghum area The total annual production of 11 8 million t constitutes 11 6% of the total world production (FAO 1996) Sorghum is also the third major cereal in India after rice and wheat It is extensively grown for human food, animal feed, fodder and other industrial uses under rain-fed conditions in the dry regions with unpredictable rainfall

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Low-yielding traditional varieties in the major sorghum-growing areas have been gradually replaced by high-yielding hybrids since the release of the first hybrid CSH 1 in 1964 in India Hybrids are now extensively grown in almost 80% of the area in the major sorghum growing State of Maharashtra where productivity has increased from 587 kg ha^{1} (in 1970) to 1407 kg ha^{1} (in 1996-97) Doubling grain yields in the last 30 years in India has been possible due to the release of 15 hybrids for country-wide cultivation by the All India Coordinated Sorghum Improvement Project (AICSIP), Hyderabad, of the Indian Council of Agricultural Research (ICAR), New Delhi, and rapid adoption of hybrid technology In the last decade, more than 30 private seed companies have become active contributing significantly to this achievement Of the released hybrids, CSH 5, CSH 6, CSH 9,

and CSH 14 are highly popular in the rainy season

According to an estimate, more than 24 000 t of certified F_1 hybrid seed are produced in India which is sufficient to sow only 3 4 million ha in the rainy season out of the 7 million ha area under high-yielding varieties (B S Rana, personal communication) Presently there is a shortfall in supply, and hybrid seeds are in great demand

In Maharashtra State, where primarily hybrids are grown, and several seed companies are located, 10-80% losses in seed yield have been reported due to ergot Severe incidences of this disease and grain mold have forced the seed companies to shift seed production from Maharashtra to the States of Andhra Pradesh and Karnataka In spite of shifting the seed production to the normally hot and dry areas of Andhra Pradesh and Karnataka, the disease continues to be a threat to seed production particularly when cool and humid weather occur during anthesis For example, during 1994-96, seed production of the most popular hybrid CSH 9 was only 1 25 t ha^{-1}, compared with the expected 3 75 t ha^{-1} (B N Khule, Maharashtra State Seed Corporation, India, personal communication) Ergot has gained economic importance after the introduction of hybrids, and has made it difficult to produce F_1 hybrid seed and the female parent

We briefly review research done in India on the different aspects of sorghum ergot, and highlight the thrust areas to help formulate future strategies

Disease Distribution

McRae (1917) reported ergot for the first time in the world after he observed the sphacelial stage of the disease in Madras State, India, in 1915 Since then, the disease has been reported in other Indian States such as Maharashtra (Ajrekar 1926), Karnataka (Kulkarni 1942), Tamil Nadu (Nath and Padwick 1941, Thomas et al 1945, Ramakrishnan 1947) Andhra Pradesh (Ramakrishnan 1948, Sundaram 1967, 1969, Patil et al 1968), and Delhi and Haryana (AICSIP 1969-70) Ramakrishnan (1963) observed the sclerotial stage of the pathogen in Koilkuntla, Andhra Pradesh The disease is endemic in Maharashtra (Vidarbha and Ahmednagar regions), Karnataka, Andhra Pradesh, and Tamil Nadu AICSIP takes advantage of this endemic nature to test breeding lines in these States

The Pathogen

Sphacelial Stage

In India, McRae (1917) described the imperfect, sphacelial stage of the ergot pathogen of sorghum as *Sphacelia sorghi* Subsequently, the sphacelial stage was described with respect to conidiophores, conidia, and variations in the size and shape of conidia, (Ajrekar 1926, Ramakrishnan 1948, Sundaram et al 1970, Chinnadurai and Govindaswamy 1971, Khadke 1975, Kulkarni et al 1976, and Sangitrao 1982) Microconidia were almost of the same dimensions irrespective of the source, but the size of macroconidia varied (Sangitrao 1982) Secondary conidia are also known to occur in the greenhouse and in the field (Bandyopadhyay et al 1990)

Conidial Germination

Conidial germination was from both or either ends of the spore (Ajrekar 1926) Another report (AICSIP 1965-67) mentioned that, within 3 h, the macroconidia germinated by thin germ tubes ending in pyriform, hyaline secondary conidia that detached and in turn also germinated This was confirmed by Sangitrao (1982) Germination of both macroconidia and microconidia in 5% sucrose solution has been reported (Sangitrao, 1982) Germination of conidia from different hosts—*Sorghum bicolor, Pennisetum typhoides, Ischaemum pilosum, Dicanthium annulatum, Dicanthium caricosum* and *Sehima nervosum* was only 2 5% in the first 9 h, 50-70% in 12 h, and 95-98% in 48 h

The conidia of *S sorghi* remained viable on the heads for 1 year when maintained at room temperature (max 29°C and min 17°C) When smeared on the seed surface, 1-2% conidia germinated for up to 48 h, and lost viability completely in 72 h Conidia developed on modified Kirchoff's medium showed 80-100% germination (Sangitrao 1982)

Sclerotial Stage

The perfect stage of *S sorghi* in India has been reported as *Claviceps sorghi* Several workers reported variation in color, shape, size and mass of sclerotia (Ramakrishnan 1948, Tarr 1962, Patil et al 1968, Kulkarni et al 1976) But all these variations were due to the differences in genotypes, climatic conditions and the stage of development (Sangitrao 1982) Though there are two ovaries in twin-seeded cultivars as in Lav-Kush and IS 8070, only one ovary was infected (Sangitrao and Bade 1979)

Sclerotial Germination

Sclerotial germination was tried by Ramakrishnan (1948) by exposure to alternating temperatures of 5 and 20°C Instead of germination, sclerotia turned into a slimy mass (AICSIP 1965-67, Sundaram 1967) Rama Sastri (1973) mentioned that there is no possibility of existence of physiological races since sclerotia failed to germinate

Kulkarni et al (1976) for the first time successfully germinated sclerotia from a genotype IS 5353 in 35 days by keeping it in moist soil, near an open window in the laboratory, but sclerotia from CSH 1 did not germinate when kept for even up to 2 months

In his detailed studies using a "sclerotial germination assembly", Sangitrao (1982) reported that up to 55% sclerotia germinated at 27°C The sclerotia absorb water and the basal ends become swollen in 15-20 days of incubation, and subsequently develop a white tuft of mycelium in 5 days Pinhead-sized, pinkish red stroma, 6-15 mm long, developed from the base of the mycelial tuft A single sclerotium was seen to produce 1-7 stroma Sometimes, during the initial stages of sclerotial germination, a number of slimy pinkish pinnotes were seen on the surface of sclerotia, and contained numerous macroconidia and microconidia (Sangitrao 1982)

Another interesting feature observed while germinating the sclerotia on different substrates and positions is that they

germinated when placed horizontally on sterilized sand, while they did not germinate in the vertical position Fragments of sclerotia with intact basal ends germinated to produce stroma, while fragments from other parts did not germinate

Sclerotia were viable for 3 years at room temperature in the laboratory, and germinated when kept up to 60 cm deep in soil in the greenhouse Germination of sclerotia obtained from different genotypes ranged from 50-55% (in CK60 A and IS 84) to 25-35% (in 2077 A, CSH 1, and R 473) This suggests that the efficiency of germination is affected by host genotypes Fungicides such as Aureofungin®, Bavistin®, Ceresan®, thiram, and captan did not completely inhibit germination but reduced it to 2 3%

In a study on biological control, the fungus *Chaetomium globosum* was most effective in inhibiting sclerotial germination while *Rhizoctonia*, *Azotobacter*, *Fusarium*, and *Curvularia* had very little or no effect (Sangitrao 1982)

Internal Structures of Stroma on Germinated Sclerotium

Kulkarni et al (1976) observed ascospores in the stromata of germinated sclerotium in Dharwad, Karnataka Perithecia containing ascospores were embedded in the stroma and were flask-shaped, slightly protruding at the ostiolar region, and measured 132 8-232 4 x 66 4-124 5 µm Asci were hyaline, cylindrical with somewhat tapering ends with a hyaline cap at the apex, and measured 56-112 x 2 4-3 2 µm Ascospores were eight per ascus, filiform, measuring 40-85 x 0 4-0 8 µm The perfect stage of *S sorghi* in India was named as *Claviceps sorghi*

The perfect stage in Akola was also confirmed as *C sorghi* (Sangitrao 1982) corresponding to IMI herbarium number 262578 for the species originally deposited by Kulkarni et al (1976) However, the pinnote formation and stages of perithecial formation were reported for the first time by Sangitrao (1982)

Ascospore Germination

Ascospore germination was studied using 5% sucrose solution Initially they became swollen and broke into fragments before germination began Germination was 1-3% after 9 h, 5-7% after 12 h, 12-20% after 24 h, and 15-24% after 48 h Each fragment produced germ tubes (Sangitrao 1982)

Infectivity of Spores

The efficiency of infection was tested by using spores from different pathogen propagules Inocula used were 1) conidia from artificial cultures, fresh sphacelium, inner part of sclerotia, and pinnotes on germinating sclerotium, and 2) ascospores obtained from well developed stromata All these sources of inocula initiated 100% disease incidence within 6-8 days when applied artificially on the heads of male-sterile 2077 A and CK60 A under controlled conditions The conidial inocula produced 30-60% disease severity, compared with only 2-3% severity with ascospore inoculum (Sangitrao 1982)

Toxicity to Animals

Poisoning due to sorghum ergot resulting in injury to either humans or animals has not been reported in India Ramakrishnan (1948) assayed sclerotia of sorghum but failed to demonstrate the poisonous alkaloid ergotoxin Chinnadurai and Govindaswamy (1971) obtained alkaloids to the extent of 0 025% in honeydew and 0 08% in sclerotia of sorghum ergot, as ergotinine equivalent, and recommended the study of mammalian toxicity of sorghum ergot sclerotia They did not specifically extract and assay alkaloids but assayed powdered sclerotia The result probably reflects the general detection of indolic compounds by the reagent *p*-dimethylaminobenzaldehyde Sangitrao (1982) and Phadnaik et al (1994) reported that sorghum ergot sclerotia contained clavine derivatives, and that the total alkaloid content varied from 0 008 to 0 0032% (expressed in terms of ergometrine base) in the cultivar 2077 A

Phadnaik et al (1994) studied the toxicity on albino mice They reported that low alkaloid content explains the high LD_{50} value (1 875 g kg^{-1}) of sorghum ergot on experimental mice, compared with 100 mg kg^{-1} in rhesus monkeys 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 diarrhea, dyspnea, muscular spasm, hyperexcitability and gangrene In chronic toxicity studies, diarrhea 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

Flowering Behavior

Quinby (1958) described that the long stigmas in sorghum, which extend beyond the glumes after the flower closes, remain receptive to pollen and could be a route for infection by the pathogen House (1980) described that all the heads did not flower at the same time, the anthers dehisced when they were dry When caught in the rains, pollen dispersal was restricted because rain washed them away Similar observations were made for stigma receptivity by Chopde et al (1973)

Shekar et al (1981) showed that seed setting was poor upon pollination on the first day following anthesis, and was up to 83% on the second day in 2077 A and 296 A Temperature below 10°C also affected anther dehiscence in 296 B, resulting in no seed set in 296 A, the female parent of the popularly grown sorghum hybrid CSH 9 In a few cases low temperature has been found to restrict the release of pollen from anther sacs (Sangitrao 1982)

Puranik et al (1973) observed that the fertilized ovaries were susceptible to infection for 3 days The time of pollination and fertilization in sorghum is 2-4 h (Neergaard 1977), and the embryo has a dormant period of 6-22 h (Paulson 1969) without any cell division This suggests that infection can take place even after

fertilization, but this hypothesis needs confirmation

Pollination, Fertilization, and Ergot

When pollen and conidia were placed simultaneously on the stigmas, pollen germinated earlier than the conidia and the pollen tube reached the embryo faster than the fungal hyphae In pollinated and fertilized pistils, the fungus grew slowly or failed to grow, resulting in reduced ergot incidence Sangitrao (1982) and Bandyopadhyay et al (1992) observed that fertilization was effective in controlling ergot However, fungal hyphae were found in the fertilized ovaries, which needs to be confirmed

Host Range

There are numerous reports on possible collateral hosts of *C sorghi* The comprehensive list given by Bandyopadhyay (1992) indicates that alternate hosts of *C sorghi* include *Cenchrus setigerous*, *Ischaemum pilosum*, *Pennisetum orientale*, *Sorghum arundinacearum*, *S caffrorum*, *S halepense*, *S membranaceum*, *S nitens*, *S verticilliflorum*, and *Zea mays* Conflicting results have been reported for *C ciliaris* and *Pennisetum typhoides* (Sundaram et al 1970, Chinnadurai and Govindaswamy 1971, Sundaram 1974) Besides these, a few more hosts and non-hosts were recorded by Ramakrishnan (1947), Singh (1964), Reddy et al (1969), Sundaram (1970), Chinnadurai and Govindaswamy (1971), Mutkekar and Madane (1976), Patil and Madane (1979), and Sangitrao (1982) Sangitrao and Moghe (1995) reported that a triangular spore form of *S sorghi* collected from *Dicanthium caricosum* could infect sorghum in India

Epidemiology

Environmental Effects

Sugary disease is severe when cool climatic conditions prevail at the time of flowering (Tarr 1962) Environmental conditions favorable to the disease have been reported by several workers These include cool temperatures of 21-27°C (Ajrekar 1926, Chinnadurai et al 1970), 19±1°C and humid and cloudy weather (Sundaram 1970) and 17±2°C (Rama Sastri 1973) Sangitrao (1980) observed that temperature ranging from 13° to 19°C and relative humidity of 76-94% favored disease spread under artificial inoculation Similar studies have been conducted by Sundaram (1970)

In five years of detailed epidemiological studies, Sangitrao (1982) revealed that more than 75% relative humidity (RH) and 15-19°C minimum temperature were favorable for disease development The incubation period ranged from 5 to 15 days, with an optimum of 7 days

Sclerotial formation is favored by dry weather Bandyopadhyay et al (1990) reported that 14-28°C and >90% RH for 12-16 h, and 28-35°C and <80% RH for 12 h were favorable for conidial and sclerotial production, respectively

In India, weather conditions in 38th to 4th meteorological weeks were favorable for 80-100% of disease incidence, and 35-70% disease severity Sclerotial formation was directly proportional to the severity of the sphacelial stage Cool tem-

peratures, high humidities, rain splashes, and constantly changing wind direction are congenial for the spread of the disease (Sangitrao 1980)

Dispersal of Conidia

Such insects as thrips, *Orius* species, beetles, midge, head bugs, and hymenopterous insects failed to transmit the pathogen when released in cages containing susceptible panicles suggesting that insects do not play any role in the spread of the disease The groups of insects that normally visit flowering panicles and panicles with developing grains are different (Bandyopadhyay et al 1991) Sangitrao (1982) observed that ovaries with midge maggots were later utilized by the ergot fungus and developed honeydew for further spread Bandyopadhyay et al (1991) observed that secondary conidia were produced when honeydew dropped on wet soil These secondary conidia can act as the source of primary inoculum

Disease Cycle

Based on field observations, Singh (1964) and Sangitrao (1982) hypothesized that conidia and ascospores could infect collateral hosts that flower before sorghum, thus providing primary inoculum The ascospores from sclerotia can infect sorghum or collateral hosts in the following season Macroconidia are produced and protected in the sclerotial locules (Sangitrao 1982), and in due course sclerotia disintegrate and release macroconidia These macroconidia can germinate on moist soil surface, produce secondary conidia which can infect the collateral hosts, thus completing the disease cycle

Disease Survey and Yield Loss Estimation

During 1978-81, a survey of the Vidarbha region and western Maharashtra for sorghum ergot (Sangitrao 1982) concluded that certain factors accounted for the incidence and spread of the disease These were

- Delayed sowing
- Late rains
- Non-synchronization of male and female parents
- Presence of grass weeds harboring primary inoculum
- Improper crop management, e g , use of poor quality seed resulting in low male parent population, uneven growth in the field, inadequate control of pests and weeds, and improper application of fertilizer leading to failure of staggering or proper nicking for pollination
- Non-removal of diseased panicles and tillers in rejected or harvested plots
- Disposal of infected panicles close to the fields

Sangitrao (1982) conducted a replicated trial in the field to ascertain the yield losses due to sorghum ergot Sixteen sorghum cultivars including two male-sterile lines (CK60 A and 2077 A) were evaluated under an infector row system Reduction in yield was correlated with disease incidence and severity of honeydew and sclerotial stages There were significant differences among the cultivars with respect to incidence and severity of honeydew and sclerotial stages Loss in yield

had a linear relationship with honeydew incidence A logistic equation explained the yield loss when plotted against honeydew severity, sclerotial incidence, and sclerotial severity (Fig 1)

Seed Production Programs

The major areas of seed production programs in India are mainly concentrated in Andhra Pradesh and Karnataka In these States, seed production plots are sown during October/November such that warm and dry weather normally prevails at the time of flowering However, the crop succumbed to ergot in recent years due to delayed sowing and erratic rains during flowering Both these situations led to parental lines flowering in cool, and humid climate, and resulted in poor pollen production of the restorer lines affecting seed set in male-sterile lines of various hybrids including the most popular CSH 9

The practices followed to manage the disease in seed production plots are

- Proper staggering with 4th and 7th day of sowing of the male parent for synchronization
- Spraying 2% urea to make up growth of male or female parent for synchronization
- Removal of a few alternate leaves to retard the growth
- Spraying gibberellic acid (20 ppm) growth hormone to induce quick growth of the male parent
- Fungicidal sprays

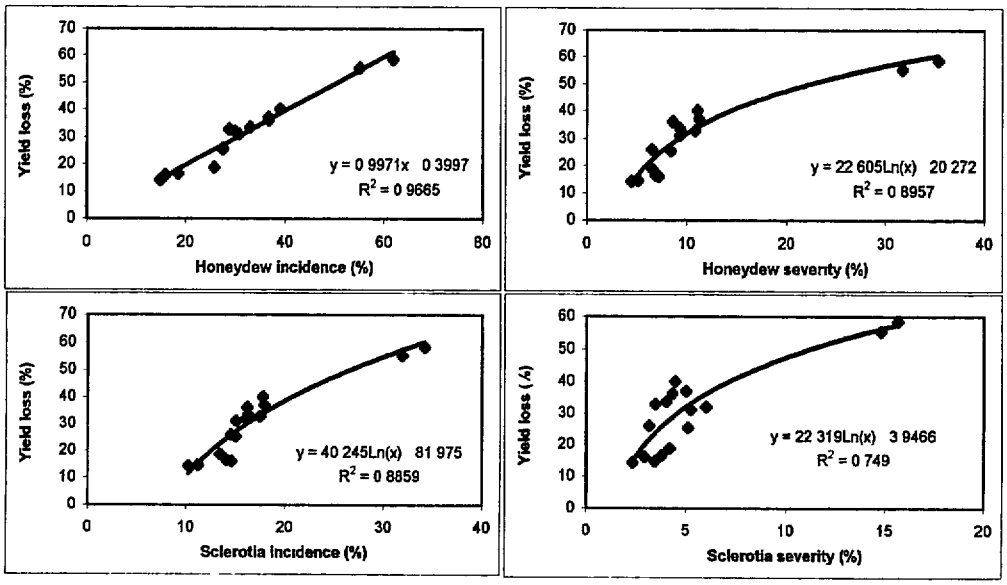

Figure 1 **Effect of honeydew incidence and severity and sclerotial incidence and severity on yield loss in the sorghum male-sterile 2077A**

Disease Control Strategies

Cultural Methods

Effect of Sowing Dates

At Navasari (Gujarat), sorghum sown later than normal on 20 July showed less infection in all the cultivars (Desai et al 1979) A similar report by Sangitrao et al (1979) showed disease escape on the crop sown on 15 July at Akola (Maharashtra) Anahosur and Patil (1982) emphasized early sowing to escape the disease, but this was not always possible in Dharwad (Karnataka) where cool and humid weather prevailed at flowering suggesting that early sowing is location specific

Effect of Fertilizer

The incidence of sugary disease increased significantly with more nitrogen and less potash Phosphorous did not influence disease incidence, but appeared to stimulate honeydew production

Physical Methods

Separation of sorghum sclerotia using 30% salt solution was found to render the grain safe for sowing and human consumption (More et al 1977) This recommendation was adopted by the Government of Maharashtra Later, Sangitrao (1982) reported that even 10% salt solution can also separate 100% sclerotia

Several methods have been evaluated to remove ergot sclerotia from large quantities of seed (Kalmegh et al 1994) Among these, 10% salt solution removed 100% sclerotia The separation efficiency was more than 90% with the gravity separator, colorimetric sortex, and belt draper (Table 1)

Table 1 Efficiency of separation of *Claviceps sorghi* sclerotia from sorghum seed by different methods[1]

Method	Separation efficiency (%)
Hand winnower	86
Machine winnower	84
Grading sieve 7/64	88
Grading sieve 8/64	89
Grading sieve 9/64	89
Gravity separator	91
Colorimetric sortex	94
Belt draper	98
Salt solution (10%)	100

[1]Source Sangitrao (1982) and Kalmegh (1994)

Reduction of Inoculum and Seed Certification Standards

The male-sterile lines CK60 A, 2077 A, 2219 A, and 296 A are female parents of CSH 1, CSH 5, CSH 6 and CSH 9, respectively, and are highly susceptible to the honeydew and sclerotial stages

Ad hoc seed certification standards were introduced in 1976, following ergot incidence during 1967-75 in parts of western Maharashtra and Vidarbha (Eastern Maharashtra) A Government circular declared the disease as objectionable and seed producers were instructed to rogue infested heads showing sclerotial formation The permissible limits were 1 2500 heads for certified seed plots, and 1 5000 for foundation seed plots Four field inspections were recommended to record infected heads At the last field inspection (preharvest), the seed plot would be liable for rejection if the heads with ergot sclerotia were more than the prescribed limit

Using these seed certification standard guides (Anonymous 1977), seed produced in a few seed processing plants were rejected during 1976-79 in Maharashtra However, the suitability of this certification is often questioned, due to the presence of other, more efficient sources of primary inoculum

Pollen-Based Management

Chinnadurai et al (1970) reported that pollination of susceptible varieties with fertile pollen reduced spore germination, and hence host infection by the ergot pathogen Sangitrao (1982) and Sangitrao et al (1992) observed the effect of pollination at different times, before and after inoculation at 100% anthesis, on disease severity of the honeydew stage The results indicated that pollination 8 h before inoculation reduced the disease severity to 5-8% in five male-sterile lines 36 A, CK60 A, 296 A, 2077 A, and 2219 A, compared with 49-56% severity in the inoculated non-pollinated control

Biological Control

Reddy et al (1965) reported the presence of *Cerebella volkensii* at the sphacelial stage When conidia of *C volkensii* and *S sorghi* were sprayed together, the sphacelial stage of the pathogen appeared initially without any trace of growth of *C volkensii* However, after 3 weeks, there were numerous stromata of *C volkensii* associated with sphacelia of *S sorghi* suggesting that the sphacelia is important for the growth of *C volkensii* stromata Subsequently, Sangitrao (1982) reported that the application of a culture of *Cerebella* at different stages of flowering had no significant effect on incidence of the sphacelial stage However, it reduced the disease severity The *Cerebella* colonies appearing on the sphacelium were more in number in the treated heads than in untreated heads affecting the sclerotial development The application of *C volkensii* at 50% and at complete flowering had only 7 9 and 4 7% sclerotial severity, against 14 3% in the untreated control

Chemical Control

Ergot control by fungicides in seed production plots of sorghum is the most effective preventive measure Fungicidal control of *C sorghi* has been extensively investigated in India (Nagarajan and Saraswathi 1971, Gangadharan et al 1976, Sundaram 1976, Anahosur 1979, and Sangitrao 1982) Two or three sprays of 0 2% Ziram®, thiophanate methyl, Vitavax® and captafol at 2-week intervals starting at the panicle emergence stage increased yield from 1 to 4 t ha^{1} Two sprays of Bavistin® 0 1%, one at the time of 50% flowering and the second after a fortnight was also effective However, Nagarajan and Saraswathi (1971) obtained little field control of *C sorghi* with systemic fungicides Benlate®, Vitavax 75W®, and Tecto 60® This is true in field situations when disease pressure is high, but disease control by fungicides is usually better than unsprayed situations if inoculum pressure is low

Host-Plant Resistance

Not much work has been reported on resistance in sorghum to ergot disease Ajrekar (1926) tested numerous varieties at Pune and Dharwad, under natural conditions and found that none was immune

Resistance to ergot on sorghum has been extensively reported However, due to lack of proper screening techniques and unreplicated trials, many lines previously reported resistant have proved to be susceptible in subsequent trials across locations A list of sorghum lines reported to be resistant in India has been published (Bandyopadhyay et al 1996)

Bandyopadhyay and Reddy (1991) tested male-sterile and male-normal sorghum and inoculated at various times after pollen shed They concluded that those panicles that were more efficient self pollinators were more effective in escaping the disease There is a need to develop reliable and rapid methods for identifying these efficient self-pollinators

In India, AICSIP has a network of 16 locations and several cooperating test centers in eight States where sorghum research is conducted Among these, ergot naturally appears in Akola, Dharwad, Coimbatore, Surat, and Palem where breeding lines are evaluated for ergot reaction Table 2 shows that most of the elite varieties, hybrids, and their seed parents have variable degree of disease susceptibility In general, the varieties (average score 2 40 on a 1-5 scale where 1 = no disease, and 5 = maximum disease) were less susceptible than the hybrids (average score 2 91) The seed parents had a higher degree of susceptibility (average score 3 47) than their maintainer (average score 2 87) and restorer parents (average score 2 69) It is evident that in spite of self pollination, varieties, and B and R lines also have moderate susceptibility to disease

Integrated Disease Management

Integrated disease management includes cultural and chemical methods,

Table 2 Ergot severity[1] in high-yielding sorghum varieties, hybrids, and their seed parents in field screening at five locations[2] during 1992-96

Cultivar	1992/93	1993/94	1994/95	1995/96	1996/97	Mean
Varieties						
SPV 462	3 1	1 2	2 9	2 3	2 4	2 58
SPV 881	2 8	1 4	2 3	3 0		2 37
CSV 13	2 8	1 3	2 8	2 8	2 8	2 50
CSV 15	3 2	1 2	2 6	2 2	2 2	2 28
Mean				-		2 43
Hybrids						
CSH 5	3 4	1 0	3 2	3 0	3 2	2 76
CSH 9	3 5	2 2	4 2	2 2	3 2	3 06
CSH 14	3 2	2 2	3 3	2 7	3 1	2 90
Mean		-			-	2 91
Parents						
296 A	-	3 9	4 2	3 2	3 3	3 65
296 B	2 8	3 1	3 2	3 9	3 1	3 22
RS 29	2 7	2 0	2 7	3 2	2 8	2 68
AKR 150	3 0	2 5	2 8	2 7	2 5	2 70
AKMS 14A	-	3 4	4 0	2 3	3 4	3 30
AKMS 14B		2 9	3 1	1 8	2 3	2 52
Mean			-			3.01

[1]Mean of five locations Disease severity scored on a 1 5 scale where 1 = no disease and 5 = maximum disease
[2]The locations were Dharwad Coimbatore Akola, Surat, and Palem

biocontrol, and genetic resistance The following practices are recommended in India

- Early sowing to escape disease
- Synchronization of male and female parents to ensure availability of ample viable pollen for effective fertilization of A lines
- Rouging of early infected panicles to reduce the sources of inoculum for secondary spread
- Destruction of collateral hosts by mechanical removal and weedicide sprays
- Preventive sprays of fungicides are necessary
- Mechanical removal of ergot sclerotia by flotation of seeds in 10% salt solution

Research Needs

Though the biology of the pathogen has been well studied, considerable gaps remain in our understanding of the epidemiology of the disease and the practicality of ergot management strategies under field conditions Some of the specific areas that need attention are

Biology

- Studies on taxonomic features of a large number of isolates of the pathogen to determine if variation exists at the species level

Epidemiology

- Understand the sources of primary inoculum and their relative relevance to initiate the disease in the field
- The extent of pathogen spread by secondary conidia needs a critical evaluation Such studies should include the role of environmental factors in the production, spread, and survival of secondary conidia
- Better understanding of the effect of environmental conditions, particularly low temperature, on infection and flowering biology and their interactions
- A disease forecasting model based on environment and host parameters is required to predict the occurrence of disease in seed production plots This will enable judicious and need-based use of fungicides

Host-Plant Resistance

- Exploit the use of rapid self pollination under low temperature as a means of escape resistance in male-fertile cultivars There is a need to develop appropriate methodologies to assess rapid self pollination
- Intensify search for resistance in male-steriles

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The Ergot Disease of Sorghum in Africa

S Z Mukuru

Abstract

Sorghum is an important staple cereal crop in most countries of Africa The crop is primarily cultivated by small scale resource poor farmers Most of these farmers grow their local landraces A total of over 20 million hectares are under sorghum cultivation in Africa Nigeria and Sudan are the major sorghum producers in Africa but sorghum is widely cultivated and a key food staple in large parts of the continent

Sorghum in Africa is attacked by a variety of diseases including ergot but incidence and severity of anyone of the sorghum diseases depends to a large extent on climatic condition Ergot caused by *Sphacelia sorghi* has been observed on sorghum in many countries of Africa but it is generally of low economic importance The disease affects individual florets in a panicle causing poor development or preventing grain development This paper reviews the geographic distribution and importance of ergot in Africa and discusses strategies for its control

The Ergot Disease Symptoms and Cycle

The disease affects individual florets in a panicle causing grain yield reductions because the infected florets do not produce grain The disease is recognized by the presence of sticky pinkish to brownish liquid drops that exude from infected ovaries The drops, which contain masses of conidia of the causal fungus, are sweet to taste and attract numerous insect which feed on them and spread the conidia to other florets A saprophytic fungus *Cerebella volkensii* (syn *C sorghi vulgaris)* may overgrow the honey dew and convert it into a sticky matted black mass (Mughogho 1988, Bandyopadhyay 1992, Sundaram 1980)

Primary infection in the field is probably established by conidia from collateral hosts and infected panicle debris in soil The spread of the disease in the field is by conidia disseminated from panicle to panicle by insects, wind and rain splashes (Tarr 1962, Bandyopadhyay 1992)

Geographical Distribution and Importance of the Disease

Ergot, also commonly known as honey dew or sugary disease has been observed on sorghum in several countries of Africa where the crop flowers under cool wet climatic conditions Ergot was observed and reported on sorghum in Uganda and Kenya in 1926, Tanzania in 1949, Republic of South Africa in 1953 and Nigeria in 1956 (Tarr 1962) Ergot has since then been observed in Sudan, Rwanda, Burundi, Malawi, Zambia, Mozambique, Zimbabwe, Lesotho, and Botswana (de Milliano 1992, Mughogho 1988) In 1987 ergot epiphytotics occurred in Lesotho and Malawi (de Milliano 1992) Mengistu and Gebrekidan (1980) reported that er-

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got is relatively new in Ethiopia It was recognized in 1972 in experimental plots at Alemaya Ergot was also reported to be bothersome at Arsi Negele, Ethiopia

Incidence and severity of ergot on sorghum depends mostly on weather conditions at the time of flowering If days are cool (minimum of 19°C±1°C), highly humid and cloudy during the anthesis period, the spread of the disease is rapid and damage can be severe (Sundaram 1980) The disease is more common on sorghum in medium and high altitude areas of eastern Africa In Rwanda and Ethiopia ergot is regarded as a priority disease for research and these countries have had a research program on ergot In Southern Africa, ergot is important in Zimbabwe and Botswana especially on tillers of late planted sorghums The disease is of low economic importance in West Africa

Ergot is, however, important in exotic breeding material at research stations Male steriles or plants with unfertilized florets are highly susceptible We have frequently observed a high incidence of ergot in male steriles and hybrids and other exotic breeding material at Alupe, Western Kenya

Strategies for Controlling the Disease

Several strategies for the control of sorghum ergot have been proposed These include sowing of cultivars resistant to ergot, cultural practices and use of fungicides to control the disease Sowing of resistant cultivars is regarded as the most practical, economical and effective method to control ergot (Bandyopadhyay 1992)

Resistance to Ergot

In Ethiopia and Rwanda where ergot disease is regarded as a priority disease for research, a collaborative research program between the national programs of these countries and with SAFGRAD/ICRISAT in Nairobi and ICRISAT Center in India began in 1987 The objectives of the collaborative research was to develop a resistance screening technique and identify sorghum germplasm resistant to ergot

Screening Technique for Ergot Resistance

An effective and simple field technique was developed and used by the national programs of Rwanda and Ethiopia (ICRISAT Annual Report 1989) The technique is as follows

- All spikelets that underwent anthesis prior to inoculation are removed from the panicles
- The remaining spikelets are sprayed with a conidial suspension (1×10^6 conidia ML^{-1}) prepared from fresh honeydew
- Inoculated panicles are covered with paper bags to enhance humidity
- Bags are removed 2 days after inoculation, panicles are reinoculated and rebagged
- Bags are finally removed 7-10 days after first inoculation
- Five panicles of each genotype are evaluated 24-25 days after first inoculation by counting infected spikelets in 100 spikelets born on primary branches Ergot severity is recorded as the percentage of ergot-infected florets

Sources of Resistance to Ergot

The national programs of Ethiopia and Rwanda in collaboration with ICRISAT carried out a major ergot resistance screening activity in 1987 and 1988 at "hot spot" locations in both countries

In Rwanda, 65 local varieties were screened at Rubona and Karama Six lines IS 25535, IS 25549, IS 25550, IS 25553, IS 26669 and Ikinyaruka were identified to be resistant In 1988, an additional 245 genotypes collected from Ethiopia, Rwanda, Yemen, Lesotho and Burundi were screened at Karama and Rubona and 34 lines that had up to 10% ergot infected florets were selected for advanced screening (Table 1) (Musabyimana 1988)

In Ethiopia, 248 genotypes with intermediate and highland adaptation were screened for ergot resistance at Arsi Negele (Bandyopadhyay 1980) Twenty seven accessions with less than 5% infected florets were selected The selected lines were screened at Arsi Negele for three years and ergot resistance confirmed in six lines (ETS 1446, ETS 2448, ETS 2465, ETS 3155, ETS 4457 and ETS 2113)

Research on ergot in both these countries has since been abandoned due to shortage of funds Because of this, several lines that were initially identified as having good levels of ergot resistance have not been confirmed and utilized in breeding

Other Control Strategies

Other ergot control strategies other than resistance reported in the literature

Table 1 Origin and ergot reaction of selected[1] genotypes screened for ergot resistance in 1988 at Rubona, Rwanda

Sorghum genotype	Origin	% infected florets	IS N°	Origin	% infected florets
IS 25543	Rwanda	10		Yemen	
IS 25561	Rwanda	4	28994 YAR-1	Yemen	3
IS 25565	Rwanda	4	29011 YAR 8	Yemen	5
IS 25584	Rwanda	5	29012 YAR-19	Yemen	3
IS 25588	Rwanda	5	29013 YAR-20	Yemen	0
			29089 YAR-101		10
IS 25590	Rwanda	10	29381 PHM 25	Lesotho	10
IS 25591	Rwanda	3	29382 PHM 26	Lesotho	1
IS 25593	Rwanda	3	29404 PHM 48	Lesotho	1
			29405 PMH 49		0
ET 2427 BG 15	Ethiopia	5	29407 PHM 51	Lesotho	0
ET 2787 BG 75	Ethiopia	10	29420 PHM 64	Lesotho	5
ET 2531 BG 120	Ethiopia	5	29438 PHM 83	Lesotho	3
ET 2421 BG 9	Ethiopia	0	29446 PHM 91	Lesotho	7
ET 2467 BG 55	Ethiopia	10			
ET 2413 BG 1	Ethiopia	5	29421 PHM 65	Lesotho	0
			29421 PHM 95	Lesotho	3
			25485 PB 21	Burundi	7
ET 2440 BG 28	Ethiopia	6	25489 PB 27	Burundi	10
ET 2423 BG 19	Ethiopia				
ET 2443 BG 13	Ethiopia				

[1]The selected genotypes had up to 10% ergot infected florets Source Musabyimana (1988)

include the following (Tarr 1962, Sundaram 1980)

- Identify suitable sowing dates in relation to climatic conditions to avoid ergot attack
- Destruction of wild grasses especially perennials, which are collateral hosts
- Rouging of ergot infected plant to prevent spread in the field
- Sowing of uninfected seeds
- Crop rotation to avoid sowing sorghum in fields which had an infected crop in the previous season
- Using fungicides to control the disease

Conclusion

Ergot on sorghum has been observed in several countries in Africa but is only of minor importance except in Ethiopia and Rwanda However, most national research programs in Africa have initiated research to develop sorghum hybrids and it is expected that hybrids will become popular with farmers in future

Ergot is a serious limiting factor in production of hybrid seeds, particularly if fertilization in male steriles is delayed due to lack of viable pollen caused by non synchronous flowering of male steriles and restorers (Bandyopadhyay 1992) Ergot is therefore likely to become increasingly important as demand for hybrid seed increases

It is essential that effective ergot control methods should be developed and disseminated to farmers and seed companies to ensure that high quality hybrid seeds free of ergot are produced

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Current and Future Research on Sorghum Ergot in Australia

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Abstract

Research on sorghum ergot in Australia is being conducted in 3 major areas-toxicity, fungicide efficacy and climate risk analysis The effects of sclerotia of ***Claviceps africana*** *(containing 0 1% dihydroergosine) on grazing cattle and pigs and chickens in intensive situations, have been studied Cattle grazing on a standing sorghum crop with 0 3% sclerotia were not adversely affected, whereas pigs fed 5% sclerotia displayed reduced growth, and some chickens fed the same sclerotia concentration displayed diarrhoea and/or died In a fungicide efficacy trial, triadimenol at 62 and 125 mL a i ha^{1} and tebuconazole at 250 mL a i ha^{1} provided very high levels of protection A climatic risk analysis indicated that there was a higher probability of ergot infection at the beginning and at the end of the Australian sorghum growing season (November-April) than in the middle of the season The following research needs have been identified in Australia - toxicity studies on lotfed beef, development of fungicide spraying protocols, importance and control of seedborne inoculum, taxonomy and genetic relationships of* ***Claviceps*** *on sorghum, and novel genes for resistance Australia has the expertise and facilities to conduct much of this research, but there is considerable scope for fully collaborative international research*

Current Research

Toxicity Studies

It is generally considered that *C africana* is unlikely to be a major threat to animal health, based on the work of Peter Mantle and his colleagues Mantle and Waight (1968), and Frederickson et al (1991) demonstrated a relatively low toxicity of its major alkaloid component, dihydroergosine (DHES) to mice and rats compared to alkaloids of *Claviceps purpurea* (Fr) Tul and *Claviceps fusiformis* Loveless In Queensland only a single batch of sclerotia-contaminated sorghum grain has been obtained in sufficient quantity for toxicity studies Sclerotia from this crop had a morphology identical to *C africana* and had an average alkaloid concentration of approximately 0 1% The primary alkaloid detected was chromatographically identical to DHES

A herd of 23 cattle (14 heifers and 9 steers) was grazed for 28 days on this grain sorghum crop to assess the acute toxicity of sclerotia of *C africana* The crop was sampled before and after grazing for nutrient analysis, and the sclerotia concentration in the average diet was estimated at 0 3% by weight No clinical

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signs associated with ergot alkaloid poisoning were observed during the trial The animals grazed and exercised normally Consumption of feed and nutrients was estimated as being above maintenance, although insufficient for maximum growth, and the animals maintained liveweight

Several tons of contaminated grain were then harvested from the remaining area of the same sorghum crop and a sclerotia-rich fraction was separated from the grain by flotation in a solution of 10% $NaCl_2$, then air dried The concentration of sclerotia in a sub-sample of this fraction was determined by visual separation and weighing It was then incorporated into pig and meat chicken diets, which were otherwise similar in nutrient contents

Pigs averaging 20 kg liveweight were fed the equivalent of up to 5% sclerotia for 28 days in a growth trial No significant clinical abnormalities were observed Slight impairment of performance (reductions in feed intake and poor feed conversion) was observed over the first 7 days with diets containing more than 0 6% sclerotia, and over the 28 day trial period growth was reduced by 30% in pigs receiving 5% ergot sclerotia

Meat chickens were fed the equivalent of up to 5% sclerotia from 3 to 23 days of age Diarrhoea was observed in up to 31% of the birds fed 5% sclerotia, and mortality at this concentration was 21% In a few cases, apparent gasping for breath preceded death Growth of surviving birds was reduced by about 8% compared to controls Diarrhoea was also significant in birds fed 2 5% sclerotia, but reduction in growth overall compared to controls was slight Diarrhoea is among the effects noted in cocks dosed with ergotamine and related alkaloids, and gasping for breath has been reported in rodents given ergosine and related alkaloids However, these effects have not been reported to occur with dihydrogenated derivatives (Loew et al 1978) In fact, the general effects of dihydrogenated alkaloids tend towards behavioural depression Although no clinical signs associated with effects on the vascular system were observed, it is noted that the dihydrogenated ergot alkaloids tend to lower body temperature in rabbits, in contrast to the hyperthermia caused by their respective parent compounds (Loew et al 1978) In any case, these broiler chickens were housed in temperature-controlled sheds

Total alkaloid intake of pigs fed the 5% ergot was estimated to be approximately 50 mg day^{1}, or about 2 5 mg per liveweight kg day^{1} during the 28 day trial The chickens fed 5% ergot would have ingested a similar amount on a liveweight basis These intakes of DHES appear low to account for the depressed performance observed Manev, Pericic and Muck-Seler (1989) found that single doses of 10 mg liveweight kg^{1} (intraperitoneal) inhibited fighting between mice, but we did not observe any behavioural changes in our animals

Agalactica is one serious effect of some alkaloids that our trials have not yet addressed, but Frederickson (in Bandyopadhyay et al 1996) found that diets of 50% ergot sclerotia (15 mg alkaloid day^{1}, presumably about 500 mg live weight kg^{1}) had no effect on lactation in mice

Although our results support previous work showing that *C africana* has a low toxicity to livestock compared to other *Claviceps* species, they do not support the proposition that it is harmless The depressed performances of pigs and chicken fed with diets containing 5% sclerotia may be due to the presence of low concentrations of alkaloids other than DHES in the sclerotia It might even be due to non-alkaloid components of the ergot Modern commercial breeds of pigs and chickens are genetically selected for maximum growth under ideal nutritional conditions, and may have a different order of susceptibility to certain alkaloids than laboratory animals Investigations are underway to clarify some of these issues As an interim measure, Queensland has placed a limit of 0 3% for sclerotia of *C africana* in stockfeeds (as compared to 0 02% for other *Claviceps* species)

Fungicides

The relative efficacies of 10 fungicide x concentration combinations for the control of *Claviceps africana* on an A-line were determined in a trial conducted near Gatton, southern Queensland (Table 1) The A-line was planted in mid February, 1997 and watered by furrow irrigation when necessary Plot size was 3 x 7 m rows, 70 cm apart, with only the center row being sprayed and rated, and there were 4 replicates Fungicides were applied at 200 L water ha^{1} using a handheld boom, with one hollow cone Teejet® TX4 nozzle above the row and two TX4 nozzles on droppers on either side of the row directed towards the panicles Pressure at 450 kPa was supplied by propane gas The first spray was applied approximately 5 days before first stigmas were visible, and a further 6 applications were made at 4 day intervals except for the last spray which was applied 6 days after the previous spray The adjuvant Nufarm® Spraymate Bond, a sticker/extender consisting of 450 g L^{1} synthetic latex and 100 g L^{1} surfactant was added to the fungicide solutions at 0 1% In each plot, % infected panicles was determined and the % spikelets infected on each infected panicle was assessed on 2 occasions

Table 1 Efficacies of fungicides for control of *Claviceps africana*

Fungicide and active rate (mL or g ha 1	% Infected panicles		% Infected spikelets	
	Rating 1[1]	Rating 2[1]	Rating 1[1]	Rating 2[2]
Triadimenol (125)	0	0	0	0
Tebuconazole (250)	1 2	2 3	0 1	
Triadimenol (62)	4 6	3 1	0 2	
Mancozeb (1500)	18 6	58 9	2 6	10
Fluazinam (500)	23 3	52 3	1 5	-5
Benomyl (1000)	34 7	58 9	0 9	10
Fluazinam (250)	46 7	89 4	5 6	70
Procymidone (250)	57 8	96 9	5 0	10 70
Untreated	58 0	96 5	7 4	10 80
Triademorph (300)	60 4	88 1	7 0	10 70
Procymidone (125)	69.4	97.9	8.3	20 70
LSD (P=0.05)	18.0	14.3	3.4	n.a.

[1]Mean rating for each treatment
[2]Range of ratings for each treatment

Triadimenol (Bayfidan®) at 125 mL a i ha^{1} was the best treatment, followed by tebuconazole (Folicur®) at 250 mL a i ha^{1} and triadimenol at 62 mL a i ha^{1} The efficacies of these fungicides have been reported previously (G Odvody, unpublished data), and are now confirmed under Australian conditions The results of another trial conducted in southern Queensland suggests that triadimenol may have eradicant properties The protectants mancozeb (Mancozeb®, Penncozeb®) and benomyl (Benlate®) provided reasonable control in the A-line and may prove to be particularly useful in commercial seed production blocks to reduce costs The threat of strains of *C africana* resistant to the DMI fungicides developing after repeated applications of fungicides such as triadimenol and tebuconazole must be averted by the use of other fungicides in alternating blocks

Climatic Analysis

In response to considerable grower and industry concern after the outbreak of ergot in southern Queensland, a climatic risk analysis was conducted to assess the likely timing and frequencies of further outbreaks of the disease on grain sorghum across the main growing areas in Australia (Meinke and Ryley 1997) The following set of rules, based on published research results and other information, was used to interrogate long term weather data (1960-1990) for 10 localities

1 Minimum daily temperature <14°C for at least 3 consecutive days, 14-21 days before anthesis This rule was based on the results of McLaren and Wehner (1990) who established a highly significant positive correlation between disease incidence and minimum daily temperatures <14°C at microsporogenesis, approximately 21 days prior to anthesis

2 Maximum daily temperatures <27°C for at least 2 consecutive days at anthesis combined with at least 5 mm of rain during that period A significant relationship between maximum daily temperatures <27°C and ergot incidence was established by McLaren and Wehner (1992) The rainfall requirement assumes overcast weather and a necessity for free moisture on stigmas for infection by conidia

3 A minimum of 380 thermal units (accumulated daily average temperature) and a maximum of 510 thermal units separating periods 1 and 2 (Hammer and Muchow, 1994)

4 Anthesis occurring between 15 October and 1 May, except for Kununurra, Western Australia (1 June-15 September)

A similar analysis was conducted for hybrid seed production, using only rules 2 and 4, and assumed <100% pollination Multiple events can occur in each season and are accounted for in the analysis To analyse the frequency distribution and timing of events the flowering window was split into 10-day intervals and the number of events within each 10-day interval was accumulated over the 30 year period

For grain sorghum production a bimodal distribution was apparent at most locations, showing a high probability of infection events occurring at the beginning

and at the end of the growing season in Australia Based on this analysis, the risk of ergot infection when the vast majority of grain sorghum is flowering in Australia (January-March) is low In October, 1996 ergot was found on grain sorghum ratoon crops in central Queensland, confirming this prediction, however ratoon cropping is not a common practice in Australia Sorghum crops (particularly tillers) flowering late in the season when temperatures are lower are at a high risk from infection, as experienced in the 1995-96 and 1996-97 seasons A general north-south, as well as a west-east, gradient was apparent, whereby the number of events late in the season increased for locations further south and further east The temporal and geographic risk patterns for hybrid seed production (and on forage sorghum) followed the same trend, but the numbers of events were greater than for grain production The analysis predicted the likelihood of ergot infection at Narromine, central New South Wales (a seed production area) in mid season - *C africana* was first reported in that area in February, 1997 There is an extremely low probability of sorghum ergot at Kununurra, where winter hybrid seed production and parent seed production is conducted

Future Research

Research Agencies and Funding

Agricultural research in Australia is conducted by state departments of agriculture, [such as the Queensland Department of Primary Industries (QDPI)], Universities, CSIRO (Commonwealth Scientific and Industrial Research Organisation), Cooperative Research Centres (CRCs), and private companies (including seed companies)

As in other countries, governments are turning towards agriculture industries to pay for research undertaken in their interest Consequently, while government maintains much of the research infrastructure, most of the operational funding for agricultural research is sourced from industry-funded agencies such as Grains Research and Development Corporation (GRDC) and Rural Industry Research and Development Corporation (RIRDC) Growers pay a production levy into these agencies which is matched on a $ for $ by the Commonwealth government Allocation of funds for research is on a competitive basis CSIRO is a Commonwealth-funded research organisation, whose agricultural research budget is derived mainly from the external agencies such as GRDC Universities have a duel role of teaching and research, with funding for the latter coming from external sources and from within the University system The Cooperative Research Centre concept was designed by the Commonwealth Government to enable researchers with a common interest from different organizations (both public and private sector) to conduct research Funding for CRCs is on a competitive basis, and there are inputs of staff and facilities from the contributing organizations Funding for research within these Centres is derived from the CRCs and from external funding agencies The Cooperative Research Centre for Tropical Plant Pathology (CRCTPP) at the University of Queensland, is a partnership between the University of Queensland, QDPI, CSIRO, Bureau of Sugar Experimental Stations and the Queensland University of Technology

The CRCTPP has a major interest in molecular biology, including molecular-based pathogen identification, genetic engineering for disease resistance, and the genetics of host-parasite interactions

Research Needs

In Australia we have identified a number of areas of research on sorghum ergot which we believe are essential for the development of effective management strategies for the commercial grain industry and for the seed industry There is considerable expertise in Australia to conduct critical studies in most of these areas Undoubtedly, some generic work can be conducted elsewhere, and in addition there is considerable scope for collaborative research with other researchers overseas However, some areas of research, particularly those involving taxonomy, fungicide efficacy for registration purposes and epidemiology will need to be conducted in Australia

Toxicity Studies

Studies on the toxicity of sclerotia of *C africana* to grazing cattle, and to pigs and poultry in intensive situations are being conducted in DPI in response to considerable grower concern by a team of scientists led by B J Blaney The team includes J S Kopinski, R A McKenzie, P F Mannion, P R Martin, K G Reichmann and R Maryam, ably supported by a number of colleagues

Protocols for the extraction of alkaloids have been set up, and the alkaloid profiles of collections of sorghum ergot sclerotia from throughout Queensland are being compared to assess their variability

Lotfed beef is a very valuable export for Australia, and considerable interest in the potential effects of sclerotia-contaminated grain on lotfed cattle has been generated Large scale trials will be conducted in the near future to study these effects To our knowledge, similar field studies have not been conducted elsewhere, in the past the effects of ergot alkaloids have been determined on laboratory-bred animals

Fungicides

Although trials conducted overseas, particularly in Brazil, and more recently in Australia have identified effective fungicides for application in seed production blocks and in nurseries at flowering, there is a critical need for further research in a number of areas Firstly, the fungicides found to be most effective (triadimenol, tebuconazole and propiconazole) belong in the dimethylation inhibitor group (DMI), and there is a risk that the repeated applications necessary in seed production blocks and nurseries could lead to the development of fungicide-resistant isolates Effective fungicides from other groups must be identified to enable a fungicide rotation strategy to be developed In particular, fungicides with eradicant activity or residual activity would be particularly useful Our trials indicated that the protectant fungicide mancozeb may have a role to play in such a strategy We do not have the resources in Australia to conduct large-scale fungicide screening trials and will have to rely on overseas workers to identify effective fungicides However, efficacy trials will still have to be conducted on candidate fungicides for registration purposes Appropriate application techniques, especially for aerial spraying

will also need to be developed Australia has a small role to play in this area, and we believe that a process for the exchange of information on fungicides will greatly assist in the development of effective fungicide protocols

Effective fungicides are needed for the control of sclerotia and conidia in honeydew in infested seed lots, but before this is done the relative importance of these sources of inoculum in initiating epidemics needs to be established In addition, the roles that alternative hosts, volunteer sorghum and fallen infected heads which survive from one season to the next, play in the survival of sorghum ergot must be studied We are planning to conduct research into some of these areas, in particular the survival of conidia in seed lots and their control with fungicides, and the importance of sclerotia in the biology of the pathogen

Climatic Analysis

Many of the parameter values used in the analysis outlined previously in this paper are not very well defined and it is therefore paramount to closely monitor the critical environmental and host factors associated with ergot epidemics (Meinke and Ryley 1997) Such monitoring will provide the necessary benchmark data to enable a better quantification of the disease risk A more thorough analysis could be conducted using dynamic simulation models (eg Hammer and Muchow 1994) combined with the rule-based system outlined in this study This would integrate our physiological understanding of sorghum growth and development, and the interactions between disease outbreaks and environmental factors Meinke and Hammer (1995) used a similar approach to quantify possible yield losses due to rain at harvest for the Australian peanut industry Further, a simulation approach would allow the integration of recent advances in long-range weather forecasting (Stone et al 1996) to develop a probabilistic forecasting system with a minimum lead-time of 3 months (Meinke et al 1996, Meinke and Hammer 1997) This would give the industry and producers an indication of likely production risks either prior to, or shortly after, sowing a crop, thus allowing strategic adjustments to their operations GRDC and CRCTPP have provided short-term funding to Drs Holger Meinke and Malcolm Ryley to develop such models

Taxonomy and Genetic Relationships within Claviceps Species on Sorghum

As pointed out in a previous paper in this Conference (Sorghum Ergot in Australia) some collections in Australia differed in critical morphological characteristics to *C africana* It is possible that there is more than 1 species of *Claviceps* on sorghum in Australia, or that there is considerable morphological and/or genetic variation in *C africana* Studies on the morphology and development of the anamorphs and teleomorphs of a range of collections from throughout Australia could be supplemented by comparing their alkaloid profiles, and by conducting RFLP analyses A knowledge of the genetic variation within the *Claviceps* species on sorghum and their relationship to virulence is essential if novel sources of resistance are to be effective RFLP analysis of populations over time may also assist in determining the role that sexual reproduction plays in the life cycle

of species There is also scope to study the genetic relationships between populations in Australia and in nearby Southeast Asian countries The CRCTPP has the expertise and resources to conduct such studies

Host Plant Resistance to C africana

According to published literature, there are no sources of effective resistance in *Sorghum bicolor* However, there is considerable variation for pollen production, particularly under cool conditions, in species Breeders can therefore have a significant impact on the disease in commercial lines

The problem of susceptibility of A-lines remains Novel genes for resistance offer some hope Antifungal proteins (AFPs) which inhibit germination and/or growth of a pathogen have been identified by a number of research centres, including the CRCTPP Researchers at this centre have a number of AFPs derived from Australian native flora which have activity against ubiquitous pathogens such as *Sclerotinia sclerotiorum* (Lib) de Bary and *Sclerotium rolfsii* Sacc We have obtained funding from GRDC to conduct a preliminary screening of these AFPs for activity towards isolates of *C africana* If one or more of these AFPs is shown to be effective considerable research will still be needed before resistant genotypes are developed The AFPs must be tested for their effects on pollen germination and pollen tube growth, genes which encode the AFPs must be identified and cloned, the genes must be expressed in transgenic plants, and there will be a need to pyramid different genes to ensure that the resistance is durable The costs of development and assessment of such genotypes is considerable and may require the sharing of resources and technology However, issues of patents and intellectual property are likely to impede this exchange

International Collaboration

There is considerable scope for a fully collaborative international effort to conduct research on many areas of the biology and management of sorghum ergot The success of such an approach depends on the willingness of major research organisations in each country to cooperate, and on the free exchange of results and sorghum germplasm Specific research in each country should be concentrated in areas where there is the expertise and facilities However, aspects of intellectual property, patents and plant variety rights may impact on the effectiveness of international collaboration

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Sorghum Ergot in the United States — Public Sector Response

G N Odvody and T Isakeit

Abstract

*Spread of sorghum ergot (**Claviceps africana**) to the United States was confirmed with its late March 1997 observation on sorghum regrowth of abandoned commercial grain sorghum fields in the Rio Grande Valley (RGV) of Texas adjacent to the border with Mexico The pathogen was subsequently observed on grain sorghum regrowth and on johnsongrass (**Sorghum halepense**) proximal to these fields and it continued to increase on these hosts during persistent cooler and wetter than normal conditions By mid-May ergot was observed on the current grain sorghum crop (trace to 10% incidence, minimal severity) and had reached the northern limits of the RGV which is separated from the next major sorghum growing Coastal Bend by 80 km of rangeland Ergot is anticipated to eventually spread throughout Texas and threaten the 114 000 ha (quarter million acres) sorghum hybrid seed production industry on the High Plains of Texas Spread throughout all sorghum growing regions of the United States is also anticipated but with minimal impact on commercial grain sorghum production except for possible cool temperature pollen sterility in northern production areas This increased ergot susceptibility could similarly affect winter nurseries in Southern regions and increase ergot incidence on feral sorghum hosts including johnsongrass Chemical control of ergot on seed and in hybrid production fields is being pursued with extensive collaboration between public researchers, governmental regulatory agencies, commodity groups and private companies Collaboration is both national and international in scope*

In 1996 grain sorghum [*Sorghum bicolor* (L) Moench] production was a $2 2 billion dollar (U S) industry in the United States (National Grain Sorghum Producers Association, NGSPA, Abernathy, TX) Most of the 20 4 metric tons (4 5 billion lbs) of grain produced was consumed domestically and 30% went to the export market with Mexico as the largest importer The main U S production areas are several states in the middle of the country in a region called the Great Plains (Figure 1) More importantly, sorghum ergot, *Claviceps africana* Frederickson, Mantle, & de Milliano (Frederickson et al 1991), threatens the 114, 000 hectares (quarter million acres) devoted to hybrid seed production on the High Plains of Texas (Figure 1) This production represents about 35% of the hybrid seed produced in the world and 90% of that produced in the United States Sorghum ergot represents a serious threat to the entire sorghum industry because of the extreme vulnerability of male-steriles to *C africana* Commercial grain sorghum production itself may have little direct damage from ergot due to its high self-fertility but all producers will be affected if control of ergot forces higher seed production

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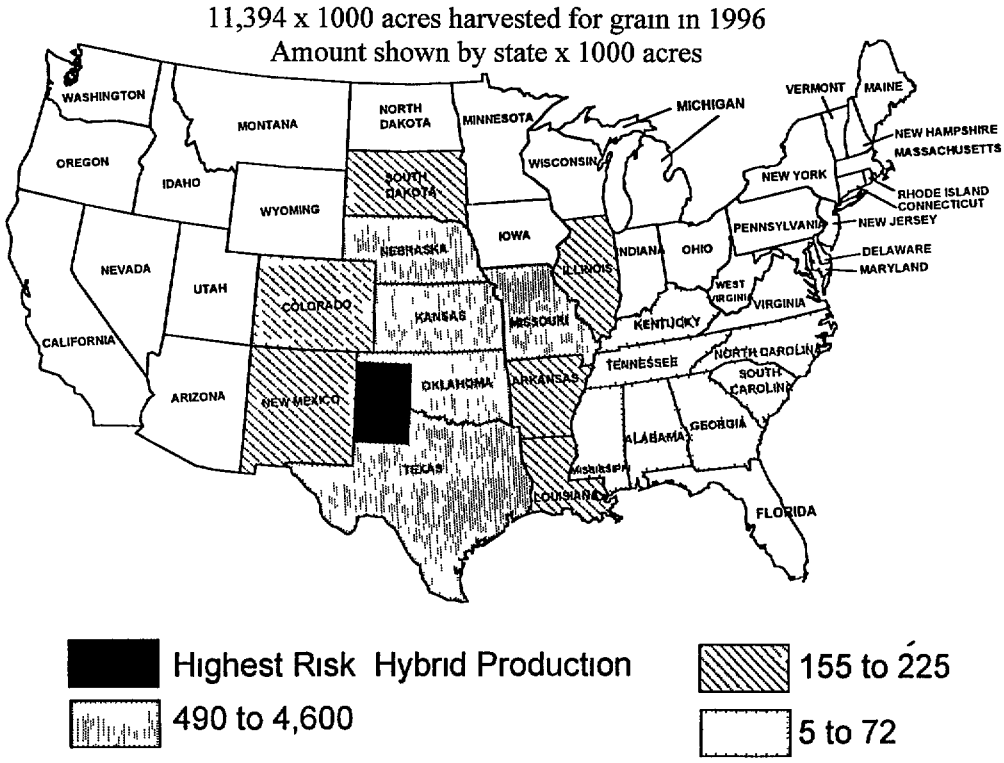

Figure 1 **Sorghum production areas in the United States potentially vulnerable to sorghum ergot**

costs that must be passed on to the consumer There is the possibility that ergot could also reduce the availability and quality of seed

Concern about the eventual introduction or spread of ergot to the United States began with the initial reports of ergot in Brazil in 1995 (Ferreira and Casela 1995, Reis et al 1996) In 1996 with continued spread across the Western Hemisphere and elsewhere, global research collaboration was sought by U S scientists with scientists having both an extensive and developing experience with ergot in various countries around the world The hope was that participation in development of ergot controls in other countries would prevent or slow the spread of ergot in the Western Hemisphere and provide for rapidly implementable controls once the pathogen spread to new geographic areas including the U S Issues concerning need for and efficacy of seed import quarantines and other public policy concerning ergot were not fully resolved before the advent of *Claviceps africana* at the Southern border of Texas with Mexico in late March 1997 The rapid spread of ergot to the U S was anticipated since its initial observation in Mexico in late February 1997 The mode of introduction of *C africana* into the U S is unknown but the pathogen probably spread across the border as aerial conidia

Initial Observation and Spread

The first report of ergot on sorghum in the United States was on ratooned and volunteer sorghum (plants established from F_2 seed that remained in the field from the harvested crop) in a field adjacent to the Rio Grande River near Progresso, Texas on March 26, 1997 The ergot was subsequently identified on similar sorghum in abandoned fields and in johnsongrass [*Sorghum halepense* (L) Pers] at several sites in the Rio Grande Valley (RGV) near the southernmost tip of the state Full identification of the ergot pathogen has not been completed but it is almost surely *C africana* because this is the only species spreading across the Western Hemisphere on sorghum and its characteristics are consistent with this species

From those initial observations the ergot has continued to increase on later blooming (May) sorghum tillers at the same locations By mid-May ergot began to be observed in several commercial grain sorghum fields just completing bloom in the RGV Incidence ranged from a trace to 2 to 10 % of the heads and severity was generally at one to a few florets infected per head but a few self-fertile sorghum heads had 35 to 50% of the florets infected The cooler than normal temperatures and continued high rainfall and high humidity during flowering and pollination are thought to have contributed to the higher than expected amount of ergot seen in the self-fertile commercial sorghum crop of the RGV There are apparent differences in susceptibility to ergot among commercially-available grain sorghum hybrids that is only now becoming apparent in field observations in South Texas and Northern Mexico where many of the same hybrids are grown Reasons for higher vulnerability to ergot in these hybrids is unknown but probably involves some factors related to incomplete fertility or delayed fertilization under the wet, rainy conditions The diversity of planting dates in the RGV and elsewhere across South Texas also assures that the ergot infection in the primary crop will provide inoculum for spread to later-blooming heads and fields if conditions remain favorable for infection

Incidence of ergot in johnsongrass also increased from initial early April observations but the disease appeared to occur primarily in subpopulations that may have had some infertility that contributed to a higher incidence of ergot Often only a small rhizomatous clump of related tillers of johnsongrass would have a high incidence of ergot and other nearby johnsongrass at the same maturity would have almost no visible ergot infection These observations are consistent with reports that some johnsongrass populations are essentially sterile To date, ergot in johnsongrass has only been observed near its occurrence on commercial grain sorghum

By the end of May 1997 the northern limit of sorghum ergot in the U S and Texas was in commercial sorghum production fields at the northern edge of the RGV near Raymondville Natural spread of the pathogen by aerial inoculum would require crossing an 80 km region of the King Ranch to the North before contacting sorghum production fields again near Riviera, Texas

Potential for Ergot by Climate and Geographical Location in the United States

A greater knowledge of the biology and epidemiology of *C africana* is needed, especially concerning aerial spread and seed dissemination of inoculum, and survival of the pathogen on seed, and in soil and on alternate hosts in nature As in most other affected countries there are questions about how the variable environments and climates across diverse sorghum production areas will affect incidence and severity of *C africana* on both cultivated and feral sorghum and other hosts The impact of sorghum ergot in the U S will probably be determined by the differential susceptibility that exists in the types of sorghum being grown and by environments present during time-critical phases of their floral development and during bloom when infection actually occurs Commercial grain sorghum hybrids across most of the U S will likely sustain little damage from ergot through their self-fertility that provides an escape type of resistance However, pollen sterility associated with cool temperatures in some sorghum production regions in the Northern Plains or winter production in Southern regions could increase the risk of ergot in any sorghum crop (McLaren and Wehner 1992) Forage sorghums represent an increased ergot risk but sterile forage hybrids are likely to sustain the greatest damage from ergot because they have no pollen source and tillering provides a succession of sorghum flowers for rapid cycling of the disease While seed production is not of importance in these latter forages the high incidence of ergot would produce tremendous amounts of honeydew that could interfere with harvest and, under continued wet conditions, it supports growth of saprophytic fungi

Endemic Nature of Sorghum Ergot in the United States

Once ergot is established in the U S , it will probably become endemic in feral johnsongrass which is present throughout most of the Great Plains sorghum producing states but its highest populations occur in Texas and other states across the Southern U S The potential role that johnsongrass may have in the survival and spread of *C africana* is further increased by the probability of cool temperature sterility increasing ergot incidence on this host in the more Southern regions of the U S , especially if a killing frost is delayed or does not occur The differential susceptibility that may exist in johnsongrass populations even under warmer environments could also be an important factor affecting the endemic nature of ergot in the United States The initial observation of ergot on surviving ratooned and volunteer sorghum despite killing frosts in extreme South Texas indicate an even greater risk of ergot increase and survival in winter months when frosts are marginal in their intensity or they do not occur Early season growth of johnsongrass at Southern locations may also increase the likelihood of initial inoculum and pathogen increase prior to the commercial crop reaching the bloom stage Shattercane, a feral weedy grain sorghum (*S bicolor*) more common in the Northern sorghum growing regions of Kansas and Nebraska, needs to be investigated for possible contribution to long term survival and establishment of *C africana* in this region

Concern about Spread of *C africana* by other than Natural Means

Ergot may naturally spread rapidly by aerial conidia but there may also be inadvertent man-derived dissemination across more vast regional areas through various other means These may include planting ergot-contaminated seed in non-ergot areas, or the transport of ergot-contaminated seed, other crop materials, equipment, or wearing apparel to or through non-ergot areas These and other potential sources of introduction require consideration to determine their actual risk and the feasibility of making any appropriate response through sanitation, exclusion or other methods

Ergot-contaminated seed from commercial grain sorghum production might potentially introduce inoculum of *C africana* during its transport to and through areas where ergot has not yet been observed In March 1997, near Tampico, Mexico, ergot was common on plants established along the roadsides from harvested seed, which had fallen from grain transport equipment or trucks An additional concern is for the possibility of pathogen introduction through honeydew or sclerotia which may remain in harvest equipment that progressively moves into non-ergot regions to the North as the crop matures for harvest In the latter case, introduction of ergot might not be readily apparent until the next cropping season

The popularity of sudan grass or sorghum × sudan grass hybrids (both sterile and self-fertile) planted for forage or hay across the Great Plains and Southern states further increases the risk of ergot spread both by introduction into new areas and by their generally higher susceptibility than commercial grain sorghum hybrids These sorghums usually have a higher incidence of ergot that is related to their incomplete fertility which can vary highly between different hybrids and varieties The sterile sorghum-sudan hybrids are extremely susceptible to ergot because they have no intended pollen source and they are constantly producing tillers of susceptible inflorescences Timely harvest of forages before heading would minimize this risk Sclerotial production requires a extended time period (Bandyopadhyay et al 1996) so only forage remaining in the field long after maturity would appear to be a sclerotial risk Under wet conditions saprophytic fungal growth and colonization of sphacelia by Cerebella reduce the production of sclerotia but raise questions about toxicity beyond that of ergot-derived tissues themselves The survival of conidia in dried honeydew on forage sorghum heads and other plant tissues would probably be maximized if cutting, drying and baling of the forage occurs soon after infection has occurred but prior to saprophytic colonization Bales from such fields at least theoretically might pose the greatest risk of introduction into new areas Johnsongrass as a primary component or contaminant in hay bales may also function in the same regard

Industry Response and Domestic and International Research

Collaborative research and other cooperative initiatives between various U S seed industry organizations, regulatory agencies, and public institutions from the U S and other countries will enhance the ability of participant countries in deter-

mining mutually beneficial control approaches for sorghum ergot Examples include formal collaboration like Texas A&M with INIFAP of Mexico and EMBRAPA/CNPMS of Brazil, and INTSORMIL projects with ergot research and control objectives However, informal collaborative linkages among public and private sorghum researchers across the sorghum producing states have been established to provide for rapid communication and information exchange regarding the most current status of ergot distribution and the results of research Several state universities and organizations including the National Grain Sorghum Producers Association, State sorghum boards, the American and Texas Seed Trade Associations, the Texas Department of Agriculture, and various components of the USDA have also been at the forefront of ergot awareness and education efforts that have both national and international implications This communication effort is also reflected in a similar global exchange of ergot information and research data concerning sorghum ergot Computer access to e-mail and postings on World Wide Web sites has greatly enhanced the effectiveness of the communication process because of its ability to contact a wide audience rapidly and economically The ability to share digital images as well as text in this manner has greatly aided education efforts concerning sorghum ergot which was largely unrecognized by sorghum workers in the Western Hemisphere prior to 1995

An ad hoc committee of the American Seed Trade Association is gathering all relevant data about the biology, epidemiology, and control of *C africana* to determine U S industry strategies to prevent or reduce spread of ergot to non-ergot areas and provide for appropriate controls in current and potential areas of distribution The committee will address various domestic and international issues concerning seed distribution policies and regulations and the production, processing, quality and treatment of seed from ergot areas, The committee will also promote research and other efforts to (1) develop fungicide controls for ergot in hybrid production fields and for use as seed treatments, (2) identify and develop host plant resistance, (3) identify and recommend feasible cultural and other alternative controls, (4) identify and develop recommendations for the special needs associated with maintenance of private and public sorghum research programs and seasonal nurseries in ergot regions, and (5) promote industry education about ergot

Of these goals, education of the industry, the producer, and the general public concerning sorghum ergot is probably the most important initial objective Communicating knowledge about ergot symptomatology, biology, epidemiology, relative economic threat, toxicity, and other relevant ergot issues will be a continuous process undertaken by various public and private sorghum workers across the U S

A primary immediate benefit will be the ability to more accurately and rapidly determine the current distribution and spread of ergot throughout the U S Sterile sorghum × sudangrass hybrids and other highly susceptible sorghums are being used as indicator or trap crops across large areas of the U S sorghum growing region to facilitate detection and documentation of the spread of ergot through the region Information about ergot from

informed, knowledgeable sorghum workers will provide for a more timely response by the industry, regulatory agencies and public researchers and reduce time-consuming diagnostic errors Education will also promote a more stable, reasoned response to the threat of ergot by minimizing the prospect of either an over reaction to the threat of ergot or an equally unwise dismissal of its potential economic impact on sorghum

Chemical Control of Ergot in Hybrid Seed Production Fields

One of the more immediate concerns of the U S sorghum industry is the development of chemical control methodology that will provide for near 100% control of ergot in hybrid seed production fields Chemical controls are being pursued based on the methodologies successfully utilized in Brazil (Ferreira et al 1996) where triazole fungicides were applied to sorghum heads at strategic intervals beginning at initiation of bloom The U S industry will largely depend on aerial application of the fungicides The efficacy of aerial applications versus the more accurate ground equipment applications are currently being evaluated on hybrid production fields in Northern Mexico and South Texas

A crisis exemption has been granted by the EPA (Environmental Protection Agency) to allow application of Tilt (propiconazole) to control sorghum ergot in hybrid production fields in the United States The exemption allows Tilt to be applied at 125 g a i ha^{1} (4 oz ac^{1} of Tilt 3 6E, 0 113 lb a i ac^{1}) in each of three applications for a total of 725 g a i ha^{1} Currently there is much experimentation by commercial companies to determine the most appropriate time of initial fungicide application and the best balance of water content and pressure to maximize fungicide coverage and penetration of the head for aerial application Other triazole fungicides are also being evaluated for efficacy in control of ergot Fungicides with other modes of action are also being evaluated so that fungicide application schemes might be devised to reduce the risk of selecting fungicide tolerant strains of *C africana* The majority of the hybrid seed production areas are planted during May on the High Plains of Texas Fungicide control results from small research plots in the RGV and from commercial hybrid production fields in the RGV and international locations including Northern Mexico are hoped to provide the information base for an effective aerial application chemical control methodology for the High Plains production region if needed

Chemical Seed Treatments

Although not empirically proven, ergot contaminated seed planted in another region has the potential to introduce *C africana* either from honeydew-encrusted seed or as sclerotia Encrusted seed could provide aerial inoculum for susceptible hosts if it remains on a moist soil surface and sclerotia may either later germinate to produce ascospore inoculum or they may have viable conidia surviving in sclerotial cavities (Bandyopadhyay et al 1996) Standard seed treatment with the contact fungicides captan and thiram prevent germination of macroconidia in encrusted honeydew on seeds (G Peterson unpublished information) Most commercial seed companies already use captan above levels known to completely inhibit conidial germination in honeydew on seeds and

the Texas Department of Agriculture has issued guidelines for seed treatment with captan or thiram to reduce the risk of planting seed produced in ergot areas These contact fungicides may do little or nothing against the sclerotial stage of *C africana* where triazoles might be more effective since they are reported to be more active against those of other *Claviceps* spp (Bandyopadhyay et al 1996)

Host Plant Resistance and Cultural Practices to Control Ergot

Initial effort in development of host plant resistance is being conducted by establishment of nurseries to evaluate previously reported sources of host plant resistance Many of these nurseries are located in ergot areas at various global locations with several participating international and U S scientists exchanging germplasm All current male-sterile sorghums appear to be fully susceptible to ergot and it will take considerable time and possibly some novel breeding approaches to develop adapted, agronomically acceptable ergot-resistant sources Because chemical controls are expensive and may not be 100% effective it will be necessary to incorporate all feasible cultural and other ergot control or prevention methods into hybrid production schemes Any cultural practice which will facilitate rapid and maximum pollination of the female parent will directly reduce the risk of ergot and complement the effectiveness of applied fungicides Although ergot is not yet present in the primary hybrid production region many commercial U S companies are already implementing some of these practices including multiple planting dates of the male pollinator and a smaller ratio of female to male rows

Conclusions

The emergence of *C africana* as a global disease problem on sorghum has created considerable concern about its present and future impact on hybrid seed production and the entire sorghum industry That concern and the continuing spread of sorghum ergot, especially in the Western Hemisphere, has generated an increasing amount of research into virtually every aspect of the biology of *C africana* that can be potentially exploited to provide for control of sorghum ergot In the same manner that the global nature of the sorghum industry and the international seed trade probably contributed to the spread of *C africana* it is also this same factor that will encourage scientists from around the world to collaborate in their development of integrated and sustainable controls for sorghum ergot

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United States Private Sector Response

W E Dolezal

Abstract

Ergot has been positively identified in the U S and poses a threat to sorghum production Private seed companies in cooperation with governmental agencies seed trade organizations federal and university plant pathologists have developed a voluntary action plan for reducing the risk of accidental seed transmission of ***Claviceps africana*** *into disease free areas The view expressed in this paper represent those of Pioneer Hi-Bred International Inc , and not those of the entire seed industry Details of the plan for ergot management in commercial, parent seed and research nurseries are presented*

Ergot has long been recognized as a serious disease of sorghum [*Sorghum bicolor* (L) Moench] The disease is caused by fungi within the genus *Claviceps*, most commonly two species *C sorghi* and *C africana* The recent introduction and the rapid spread of the pathogen in the Americas poses new challenges for the US sorghum producers Discovery of *C africana* infected sorghum plants near Weslaco, TX Texas confirmed the pathogen's spread into the U S Most of the U S sorghum seed producers believe that *C africana* will be disseminated, via wind, to the major sorghum seed production region known as the Texas High Plains Seed producers recognize that there is a risk factor in transporting the pathogen via seed movement A voluntary ergot management policy was developed through the cooperative efforts of personnel from sorghum seed companies, seed trade associations, agricultural professional societies, federal and state regulatory agencies and university plant pathologists The views expressed in this presentation reflect only the views of Pioneer Hi-Bred International, Inc (PHI) These views are not intended to represent those of entire U S sorghum seed producers PHI is a member of the American Seed Trade Association (ASTA) and has collaborated with that association, and other private sector agencies, in developing the ergot management action plan presented in this paper Compliance with this action plan is strictly voluntary

The accidental introduction and rapid spread of *C africana* in the Americans and Australia has brought the potential for major economic losses to several businesses in the agricultural community There is possibly one benefit from this disease It has brought together many of the world's leading agricultural scientists to assist in a worldwide problem, as demonstrated by this conference It has also help foster a new era of cooperative research between governmental agencies, between countries and between public and private institutions Pioneer Hi-Bred International, Inc conducts sorghum research at 18 worldwide locations, working with many public institutions to help

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develop our products This disease has caused a shift in research priorities within our company Resources are being reallocated to meet this new threat Part of these resource reallocation involves PHI personnel being reassigned new ergot research goals, while another involves providing increased financial support to public research programs which are focused on ergot Budgetary constraints over the past few years have reduced staffing at many public institutions to low numbers Many national and local disease surveys have been discontinued due to staffing shortages This has resulted in limited or no pathogen monitoring database PHI and other private seed companies have cooperated in sharing ergot disease data with the public sector Special ergot websites, like the one established by the USDA-ARS-TARS, have been very helpful in monitoring the spread of the fungus

Today's telecommunication systems, like electronic mail, allow for instant updating of developing disease situations There needs to be more effort to establish a worldwide database for pathogen monitoring, especially for the newly emerging diseases Public institutions and private companies need to share their disease monitoring data to help develop research priorities and update phytosanitary laws

A successful private company, like PHI, wants to follow a good agricultural stewardship policy This includes practicing a sound phytosanitary policy It is a good moral policy to follow, but it also makes good business sense Good business relationships are built on trust, a trust between the customer and the company The goal is to have the customer purchase his seed from us year after year There is also a trust built between the company and phytosanitary regulatory officials The accidental introduction of an exotic disease into an agricultural region can cause severe economic damage to the farmer and his community The linkage of that accidental disease introduction with a private seed company can have a severe economic impact to the business Financial losses to the company may occur through litigation cost, civil penalties and loss of business reputation and the loss of future seed sales A company must often make a choice between a legally correct versus a biologically correct phytosanitary decision It may be legal to import seed with a known exotic pathogen, however, the pathogen may not be on the current phytosanitary restricted listing It would be a poor decision based on biological standards, but it is a legally correct one under current law PHI and other companies impose higher internal quality standards than what are legally required PHI imposes internal quarantines on seed movement from various regions These restrictions are necessary to reduce the risk of accidental pathogen introduction PHI has long maintained this restriction on seed movement from Africa to the Americas because of the risk of ergot

All researchers, both in the public and private sector should review the biological risk factor in moving seed from one

continent to another Legal restrictions may prevent the movement, but when legally permissible, the risk for pathogen introduction need to be determined There can probably never be a total elimination of risk, it can be minimized by implementing a high seed quality policy (use of fungicides, insecticides, etc)

Ergot Management Quality Plant adopted by PHI, and other private seed companies, for reducing the risk or transporting *C africana* with sorghum seed shipments in 1997, is as follows

Commercial & Parent Seed Production Fields

- Appropriate fungicides will be applied to fields during pollination
- Fields will be regularly inspected for the presence of ergot
- Segregate seed lots pending seed health testing
- Seed should be cleaned and treated in the country of origin
- Seed from infected areas should not be shipped into known disease free production areas
- Zero sclerotia tolerance in seed shipped into disease free areas

Research Nurseries

- Proactive fungicide applications during pollination
- Fields regularly inspected for the presence of ergot
- Panicles inspected during harvest Infected panicles are to be destroyed
- All panicles dried and threshed in the country of origin
- Nursery seed treated with appropriate fungicide in the country of origin
- Seed known to be infected should not be shipped into known disease free production areas (Zero tolerance of seed with honeydew & viable conidia)

Worldwide standards need to be established for determining seed quality for seed production in areas where ergot is known to occur Seed from ergot infected regions should be allowed to be shipped to other ergot infected regions, but this seed must meet the grain quality standards established for each country This is not a quarantine issue but it is a seed quality issue Can we determine what is a realistic, and physically obtainable, quality standard for sclerotia and honeydew seed using industry standard seed cleaning equipment (gravity tables & aspirators) commonly used at most sorghum production facilities? What is the point of diminishing return in cleaning seed lots for sclerotia? What are the risk factors of sclerotia contamination versus economic cost of seed production? Other questions to be resolved involve use of seed treatment fungicides Companies often face the dilemma of a mandated seed treatment fungicide in the country of production that is not approved for use in another county where the sorghum seed is sold Can regulations be amended for these circumstances?

Ergot will continue to challenge sorghum researcher and seed producers The finding from research presented at this conference should help the entire seed industry in the years to come Pioneer Hi-Bred International, Inc is pleased to be an active participant in this conference and will continue collaborative research efforts with the public sector

Sorghum Ergot in Argentina

L M Giorda, M J Martinez, M Nassetta, and S Palacios

Abstract

In 1996, sorghum ergot was observed for the first time in Argentine during March/April and identified as ***Claviceps africana*** *The outbreaks of ergot disease in Argentina were mainly associated with cool weather conditions during the growing seasons of 1996 and 1997 The average minimum and maximum temperatures were 14 1 and 27 9°C prior to and during anthesis and were within favorable limits for disease development Flowering by the end of January resulted in a low risk of favorable disease conditions in both years Consequently, early sowing allowed to escape from the disease Different experiments carried out at Manfredi Experiment Station, Cordoba indicated that* ***Astyllus atromaculatus*** *Blanchard transmitted* ***C africana*** *locally*

Grain sorghum in Argentina is cultivated throughout the subtropical and temperate regions between latitudes 22-40° South and longitude 56-66° West It is grown primarily as a feed crop on approximately 800,000 ha in dryland areas (Giorda 1997) and is usually cultivated in a rotational system with either soybeans or peanuts Sorghum production is concentrated in the states of Cordoba, Santa Fe, Entre Rios, and La Pampa

During March and April 1996, ergot was observed for the first time in Argentina in commercial fields of grain and forage sorghum F_1 hybrid seed production and identified as *Claviceps africana* (Giorda et al 1996) In 1997, the disease appeared earlier which affected some late-planted seed production plots and farmers' fields where honeydew was present and resulted in problems of harvesting the crop

Ergot Alkaloids

Sclerotia and honeydew from infected sorghum male sterile lines were collected in 1997 and used to extract ergot alkaloids The extracts were separated by thin layer chromatography (TLC) on silica gel The alkaloids were visualized by dimethylaminobenzaldehyde spray as blue-purple spots (Fig 1) and seen as blue fluorescent spots under long-wavelength UV light (Giorda 1997, Mantle and Waight 1968, Mantle 1968, Stahr 1991) Ergometrine was used as the standard of ergot alkaloids

Sclerotia from 1996 and 1997 showed similar TLC patterns with an average Rf value of 0 61 for the major alkaloid component The alkaloid spectrum in sclerotia collected from diverse sources and years was uniform but their concentrations were different

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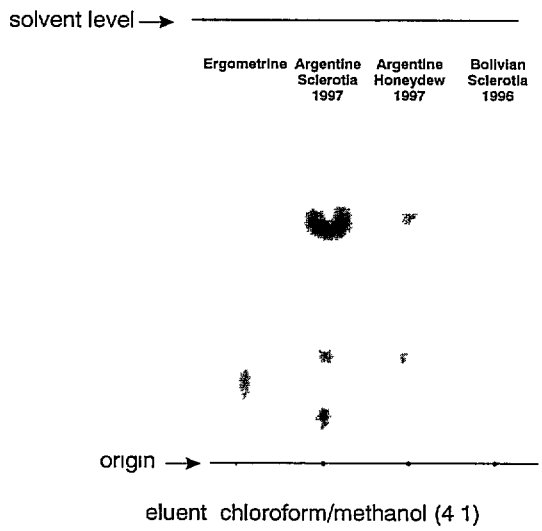

Figure 1 **Thin layer chromatography on silica gel visualizing ergot alkaloids from sclerotia and honeydew of *Sphacelia sorghi* collected from Argentine infected sorghum fields Ergometrine was used as the ergot alkaloids standard**

Epidemiology

The outbreak of ergot disease in 1997 was mainly associated with cool weather and a mean relative humidity (RH) of 75 7% The average minimum and maximum temperatures were 14 1 and 27 9°C These temperatures and humidity levels following panicle emergence or about 10 days prior to onset of anthesis and during anthesis, were sufficient for infection and development of the disease in male sterile sorghum lines (Table 1)

Table 1 **Average minimum and maximum temperature and relative humidity at Manfredi Experimental Station, Córdoba, Argentina during the 1997 growing season***

	Temperature °C		RH
	Minimum	Maximum	Mean
January	18 4	30 7	83 7
February	14 1	27 9	75 7
March	14 7	26 8	70 9
April	11.9	27.4	63.0

* planting date 11 Nov 1996 main flowering period 25 Jan 1997 anthesis period of late flowering male sterile lines 15 25 Feb 1997 and disease occurrence 4 Apr 1997

The first symptoms of ergot were observed on 4 Apr 1997 on late flowering female lines at the Manfredi Experiment Station (Cordoba nursery located at 31° 49'12" South latitude and 63° 46'00"longitude and 292 m above sea level) At the same time, the disease was also observed on a F_1 hybrid seed production field near Pergamino, Buenos Aires state, about 500 km away from Manfredi, with similar minimum and maximum temperatures (14 5 and 27 6°C) and higher humidity prior and during flowering (Table 2) The prevailing wind directions were from the north and northeast with some air movements from the south and southeast

Table 2 **Average minimum and maximum temperature at Pergamino Experimental Station, Buenos Aires, Argentina during the 1997 growing season**

	Temperature °C	
	Minimum	Maximum
February	14 5	27 6
March*	15.1	29.4

* Average from the first 15 days of the month

Minimum and maximum temperature of about 14 and 28°C combined with RH levels of 70-90% are conducive to conidial production (Bandyopadhyay et al 1996) and hence pathogen spread These environmental conditions are similar to those that occurred in Argentine in 1996 and 1997 in various sorghum locations from mid-February and March

The average mean RH was 85 4% and 87 9% during February and March 1996, respectively, corresponding RH in 1997 were 75 7% and 70 9%, respectively (Tables 1, 3) The dry weather after infection during the 1997 season suppressed the development of different saprophytes which were very common during the 1996 season when the spread of *C africana* occurred more rapidly and intensively

Table 3 **Average minimum and maximum temperature and relative humidity at Manfredi Experimental Station, Córdoba, Argentina during the 1996 growing season***

	Temperature °C		RH
	Minimum	Maximum	Mean
January	16 3	28 5	83 7
February	15 4	26 6	85 4
March	13 8	27 4	87 9
April	11.2	22.7	91.4

* planting date 30 nov 1995 main flowering period 6 Feb through 6 Mar 1996 anthesis period of late flowering male sterile lines 6 15 Mar 1997 and disease occurrence 25 Mar and 15 Apr 1996

McLaren (Bandyopadhyay et al 1996) in South Africa used the maximum daily temperature of 28°C (temperature above which disease incidence is minimal) and 12°C minimum temperature (temperature below which maximum predisposition occurs) to identify high and low risk flowering periods Using McLaren's methods and Argentina's environmental data of 1996 and 1997, we determined low and high-risk periods for the country (Figures 2 and 3) However, the average minimum temperature used in our analysis was slightly higher (14 and 28°C, 15 and 27°C for 1996 and 1997, respectively) than those mentioned by McLaren (Bandyopadhyay et al 1996)

Flowering before the end of January resulted in low risk of ergot favorable disease conditions in both years Consequently early sowing favored escape from the disease whereas later sowing, that resulted in flowering during the cooler part of the season (by the end of February), promoted ergot incidence

In other experiments conducted at the Manfredi Experiment Station, we found that the latent period varied between 4 to 8 days depending on environmental conditions Honeydew exudation initiated two days after the sphacelium was observed

Local Spread Vectors

Infected spikelets exude sticky drops of fluid honeydew containing macroconidia and microconidia Several authors suggested that conidia are spread also by insects that feed on honeydew while others (Bandyopadhyay et al 1996) suggested that insects may not play a significant role in ergot spread

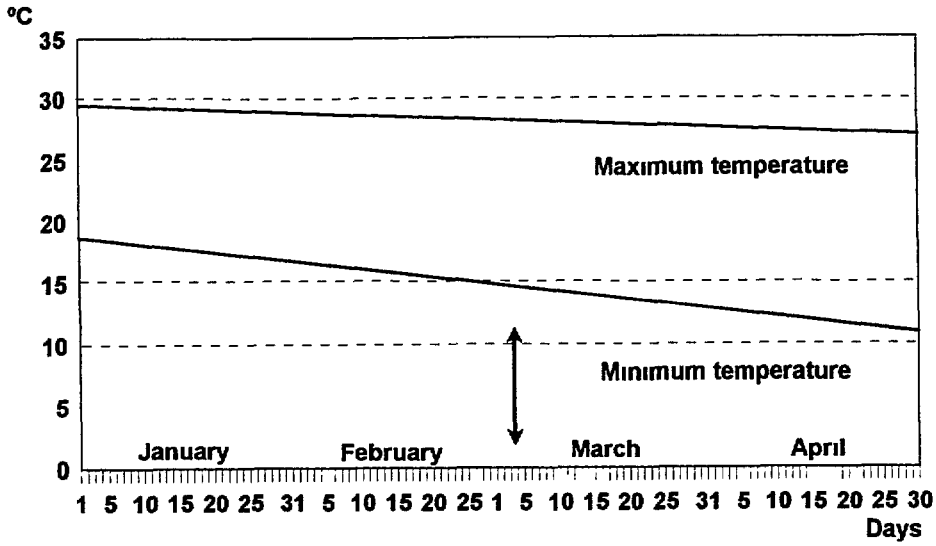

Figure 2 Maximum and minimum temperatures at Manfredi Exp Sta , Cordoba, Argentina associated with ergot disease development First symptoms and signs appeared on late flowering female lines in 03/04/97

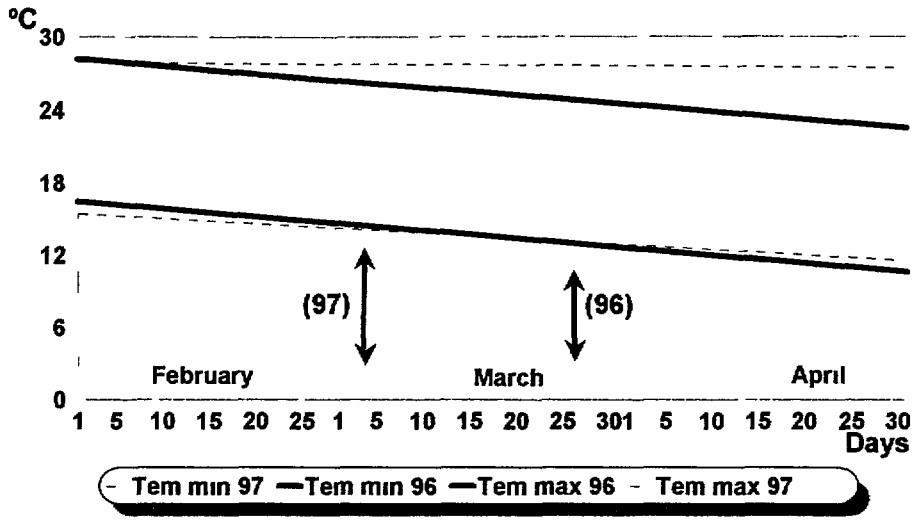

Figure 3 Daily minimum and maximum temperatures at Manfredi Exp Sta , Artentina 1996 and 1997 Arrows indicate outbreak of ergot disease

Experiments carried out at Manfredi Experiment Station, Cordoba, indicated that *Astylus atromaculatus* Blanchard transmitted *C africana* Fifteen grain sorghum panicles of female lines were covered with pollination bags 8 days before

flowering, and were infested one day before anthesis or at the onset of anthesis with two honeydew-contaminated insects per panicle An additional 15 panicles were inoculated with a honeydew suspension Control panicles were protected from contact with insects by bagging them with thick pollination bags 8 days before flowering until the dough stage of growth The trial was repeated three times and performed during March and April Visible ergot symptoms were observed 8 to 9 days after vector inoculation while fewer days (4 to 5 days) were needed for disease development when panicles were inoculated with honeydew suspension No infection occurred on the protected control panicles *Astylus atromaculatus* normally "feeds" on pollen and it is conceivable that the insect became infested with conidia and consequently served as vectors

Management Strategies

Considerable research information is available on different aspects of the biology of the pathogen However more work is needed on the disease epidemiology and variability of the pathogen to develop an integrated ergot management strategy Based on the scarce data available from the two years of ergot incidence in Argentina, the following management practices are suggested

- Early planting dates (October to beginning of November) to escape the disease
- Chemical control in hybrid seed production fields Systemic fungicides mixed with contact fungicides and applied 3 times at 5-7 days intervals, beginning 10 days before flowering (thiabendazole, propiconazole and terbuconazole might be effective as preventive but not as curative treatments)
- Pollen-based management
- Use seed free from sclerotia (within tolerance levels) and disinfected with fungicides
- Develop a forecasting system based on temperature and relative humidity conditions
- Identify host-plant resistance

Acknowledgment

We would like to thanks Ing Agr C Barberis for providing the Pergamino, Buenos Aires data, to Geol E F Lovera for providing the meteorological data from Manfredi Experiment Station, Cordoba and to D Alverani for his collaboration on the field and laboratory trials

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Incidence of Ergot in Honduras

J Moran and F Gomez

Abstract

Ergot is a very aggressive disease with a capacity for wide dispersal compared to other sorghum diseases In 1996 it moved from Brazil to Bolivia, Argentina Colombia and Venezuela Also in 1996, ergot was identified in Honduras, specifically in the valleys of Zamorano, Jamastran, Comayagua, and Talanga in yield trials and in a commercial field

Most of the sorghum in Honduras, mainly maicillos criollos, is grown in the southern region where the yields are very poor (854 kg ha^{1}) due to unfavorable weather conditions such as low precipitation and high temperatures The northeast region of the country is second in importance with a mean yield of 2,826 kg ha^{1} since the farmers in this region (mainly Olancho) have adopted improved growing and harvesting technologies including more uniform high yielding hybrids compared to the maicillos criollos Also weather conditions in this region are more favorable for growth and development of sorghum There are limited commercial hybrid production areas in the eastern part of the country, such as the Jamastran valley, and recently an increase in sorghum production throughout the north coast was observed

Basically, sorghum is grown in many regions with a wide range of weather conditions These diverse environments provide ideal conditions for pathogen development and therefore, yield loss In Honduras, the most common sorghum pathogens *are Colletotrichum graminicola, Peronosclerospora sorghi, Cercospora fascimaculans, Gloeocercospora sorghi, Puccinica purpurea* and *Exserohilum turcicum which can cause yield losses* of up to 80% Ergot (*Claviceps africana*) was reported for the first time in 1996

Spread of Ergot in Honduras

Ergot was observed in Zamorano (Fig 1) in a 1996 commercial sorghum hybrid strip test The trial was established on August 31 and consisted of 29 commercial hybrids from six different private companies from the USA, Mexico, Guatemala and Brazil Seeds from all hybrids were treated prior to planting with PrometÆ Each plot consisted of four rows, 125 m long, and 80 cm in width Different maturities were observed, with anthesis ranging from 63 to 79 days after planting (dap) Honeydew was observed in some panicles of the hybrid ICI 730 produced by ZENECA

In the following weeks, white crusts of honeydew were observed on the leaves of ICI 730 which produced secondary inoculum that infected other hybrids and ergot

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Figure 1 Geographical locations where ergot was confirmed in Honduras

symptoms developed on nearby hybrids Of the contaminated hybrids, ICI 737 showed the greatest amount of honeydew although it was 25 meters away from ICI 730 However, we were unable to determine if the presence of ergot in ICI 737 was the result of inoculum from ICI 730 Differences in ergot appearance between the hybrids could be caused by variation in blooming as ICI 730 bloomed 63 dap and ICI 737 five days thereafter Honeydew was only observed on the north and south borders of the research plot and not observed in the middle

Honeydew exudation was also observed in ICI 730 in the PCCMCA trial in a nearby field This trial was arranged as a randomized complete block design with 30 entries and four replications The honeydew was observed in almost all panicles and is attributable to the small size of the plot (16 m^2) As in the strip test, ICI 737 exhibited honeydew symptoms after ICI 730 With time, the exudation was colonized by secondary organisms and assumed a blackish appearance

Climatic conditions during anthesis in Zamorano were

- Accumulated precipitation 60 1 mm
- Mean maximum temperature 25 9°C
- Mean minimum temperature 16 4°C

- Mean relative humidity at 7 00 95%
- Mean relative humidity at 13 00 84 1%

The PCCMCA trial was also established at two other locations in Honduras (Fig 1) Comayagua, at Centro de Entrenamiento y Desarrollo Agrícola (CEDA), and Jamastran, at Las Acacias Experiment Station Comayagua is a valley located 80 km northwest from Tegucigalpa at 580 meters above sea level (masl) At this location, the trial was established on September 19 and was identical to the Zamorano trial Time to anthesis ranged from 63 to 70 dap Up to 100% of the plants of ICI 730 were infected Other hybrids sowed near, showed some exudation, however, the same hybrids failed to produce any honeydew if located far from ICI 730 plots in other replications Also in two replications, ICI 737 had 15% disease incidence With time, white crusting was observed on leaves, similar to plants in Zamorano and the panicles eventually were colonized by secondary organisms Low ergot incidence was reported in Jamastran valley, which is, located 70 km east of Zamorano at 600 masl Ergot incidence in ICI 737 was as low as 5%, but, other hybrids were ergot-free

A research group from INTSORMIL who visited the country in early December confirmed ergot Drs Gary Peterson, Darrel Rosenow, William Rooney and Mr Delroy Collins identified the disease in the three locations previously mentioned At the same time Dr Phil Arneson, a pathologist from Cornell University, identified ergot in four different weeds but it is unknown if the causal agent was *C africana*

Seed samples from the PCCMCA trial were sent to Dr Richard Frederiksen at TAMU, to determine if seed was the conduit for ergot introduction All the seed were treated with recommended fungicides before sowing Since seed treatment kills conidia of *C africana* on seed surface and no sclerotia were found in the seed lots, it was not possible to conclude that contaminated seed was the cause for introduction of ergot to Honduras (R A Frederiksen, personal communication)

The PCCMCA trial was distributed throughout Central America and Mexico two locations in Mexico, three in Guatemala, one in El Salvador, two in Nicaragua, one in Costa Rica, two in Panama, and one in the Dominican Republic When we found out that ICI's hybrid might be a possible source of ergot, we contacted the other regional locations to find out if ergot had shown up Fortunately, none reported the appearance of ergot in their trials

Recently Mr Jaime Paredes, a sorghum researcher from El Salvador, diagnosed ergot in that country A seed increase nursery of sorghum lines had up to 80% incidence of ergot This nursery was located only 100 meters from the field where the PCCMCA trial was planted in 1996 In October 1996, ergot was reported on a commercial hybrid in the Talanga valley, located 50 km northeast of Tegucigalpa (Fig 1) That hybrid was the 887 V2, produced by Cristiani Burkard S A , a seed company from Guatemala This same hybrid was used as a check in the strip test established at Zamorano earlier that year However, honeydew was not observed in the panicles at Zamorano In commercial fields, ergot incidence was

minimal Honeydew was observed on plants at the margins of the field where pollen availability was minimal

Future directions

A germplasm grant was approved by the Sorghum Germplasm Committee for evaluation of sorghum lines for ergot resistance We will cooperate with Dr William Rooney from TAMU Lines will be evaluated for genetic variability to *C africana*

The main objectives of these research efforts include

1 Evaluate resistance to *C africana* from different sorghum genotypes that will be used directly in breeding programs or as genetic sources

2 Characterize major resistance mechanisms for use in development of integrated control of ergot

3 Evaluation sites of ergot to the exotic sorghum germplasm will be at two or more locations in Honduras to maximize screening opportunities

Ergot, Honeydew or Sugary Disease of Sorghum in Venezuela

Gino Malaguti and Ninoska Pons

Abstract

*Sugary disease of Sorghum [**Sorghum bicolor** (L) Moench] appeared suddenly in Venezuela by June 1996 F_1 hybrid seed and commercial grain sorghum production fields as well as the common weeds **S halepense S arundinaceum S verticilliflorum** and **Panicum maximum**, were infected The diseased panicles exhibited abundant, sticky, sugary exudation which flowed down as droplets falling on leaves and soil before drying to form creamy whitish crusts Microscopic examination of cross sections of infected ovaries revealed the presence of stroma, conidiophores and conidia with characteristics referable to the species **Sphacelia sorghi** On the basis of the analysis of sugar composition of dried drops of honeydew this anamorphic state was related to **Claviceps africana** by P G Mantle The sphacelial structures were colonized by the fungus **Cerebella andropogonis** Ascogenous forms were not observed It can be assumed that the primary inoculum comes from wild hosts or volunteer sorghum plants growing along fields or roadsides and from infected debris, containing conidia which are easily transported by the wind or animals Until resistant lines are available, protective fungicide treatments, integrated with suitable agronomic practices are being employed*

Sugary disease or honeydew of sorghum [*Sorghum bicolor* (L) Moench] has been known since 1917 in many regions of India and various countries of Africa (Bandyopadhyay et al 1990,1996) In 1995, it was reported in Brazil where an epidemic covered far and wide in a few months Later it was found in Argentina, Colombia, Paraguay (Bandyopadhyay et al 1996) and, in 1996, in Venezuela

In Venezuela, the disease appeared suddenly in male sterile lines of sorghum in the F_1 hybrid seed production fields, in the states of Carabobo (San Joaquin and Guacara), Aragua (Magdaleno, Cagua and Villa de Cura) and Anzoategui (El Tigre) It was also observed, but with a less incidence, in extensive hybrid plantations for commercial grain production in Guarico state (Altagracia de Orituco, Chaguaramas and other localities) In Venezuela, about 350,000 ha of grain sorghum is annually sown with hybrid seed, which are mostly produced in the country

The first visible and distinctive manifestation of the disease is the abundant secretion of a honey-like, sticky exudation that flows down from the flowers infected by the fungus *Sphacelia sorghi* McRae, mentioned as the anamorph or imperfect

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stage of species of *Claviceps* (Ascomycete)

The fungus penetrates the flowers through the emerging stigma and style, replaces the ovary with its own mass thereby preventing grain formation The damage is extremely severe in fields for hybrid seed production if the male sterile flowers remain nonpollinated for a long time The fertilizing pollen, from the restorer lines, is sometimes delayed, because of poor synchronization or adverse environmental conditions, thus allowing the fungus to penetrate through the stigma, and colonize the ovary The ovule, once fertilized, is mostly resistant to the infection (Bandyopadhyay et al 1996)

The role of pollination in reducing infection was reaffirmed in an experimental field for seed production, where in the same male sterile plants, the side of the panicles exposed to wind carrying pollen, had more normal grains than in the opposite side of the panicle, where more infected ovaries were found (M Riccelli, personal communication)

In the commercial F_1 hybrid used for grain production, the flowers produce normal pollen leading to rapid fertilization Therefore, infection is restricted to a few flowers It has been observed that the sugary sticky honeydew exudation causes difficulty during the harvesting operations

Ergot of sorghum is favored by abundant and prolonged rains which keep relative humidity higher than 90% and temperature, especially at night, relatively low, between 18 and 28°C In Brazil, rainfall during 1995 was twice than normal, in Venezuela (Maracay, Aragua state) during 1996, it was 1421 mm, which was higher than the annual rainfall average during the past 12 years (1078 mm) at the same locality These wet conditions are regarded to be favorable to epiphytotic outbreaks of the disease (Bandyopadhyay et al 1996)

Symptoms

The first symptom and sign of the disease is the sticky sugary exudation This is so abundant that it comes from the ovaries of infected flowers as translucent drops, which flow down along the panicle, falling on leaves of the plant or on the soil (Figures 1 and 2) It seems that the pathogen *S sorghi* secretes inhibitory enzymes which prevent the conversion of plant sugars into starch as have been shown for *Claviceps purpurea* (Campbell 1959) These sugary drops, at first colorless, later turn opaque and brown, and when dry, produce a whitish powdery cracked crust on panicles, leaves and soil (Figure 3)

The diseased panicles may be easily recognized by their whitish dry-sugary crust, which contains a great amount of conidia Later and especially under high humidity, the infected panicles turn to a blackish color and the deformed ovaries enlarge and crack and are later colonized by parasitic or saprophytic fungi

The described symptoms, induced by *Sphacelia sorghi,* were also observed in the wild sorghums (*Sorghum halepense* (L) Pers , *S arundinaceum* (Desv) Stapf, *S verticilliflorum* (Steud) Stapf, and *Panicum maximum* Jacq These weeds are very common in Venezuela fields

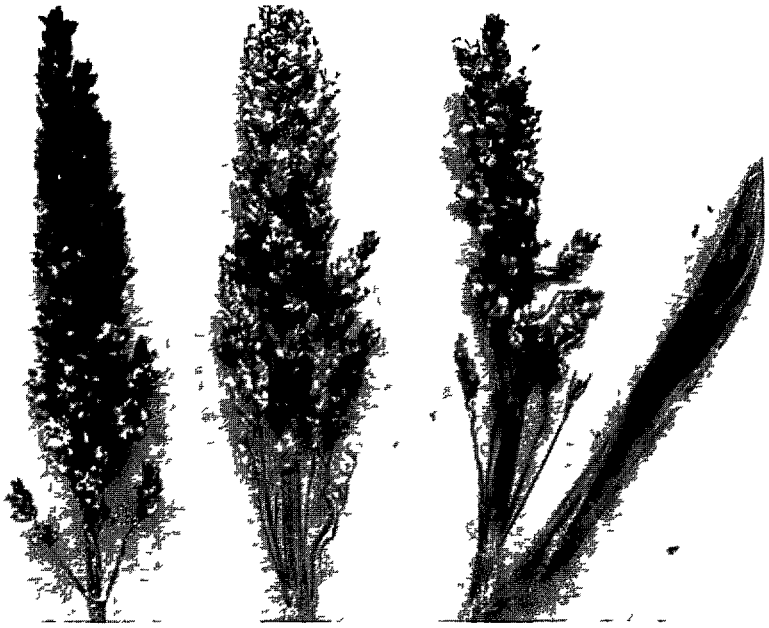

Figure 1 Symptoms induced by *Sphacelia sorghi* in sorghum panicles

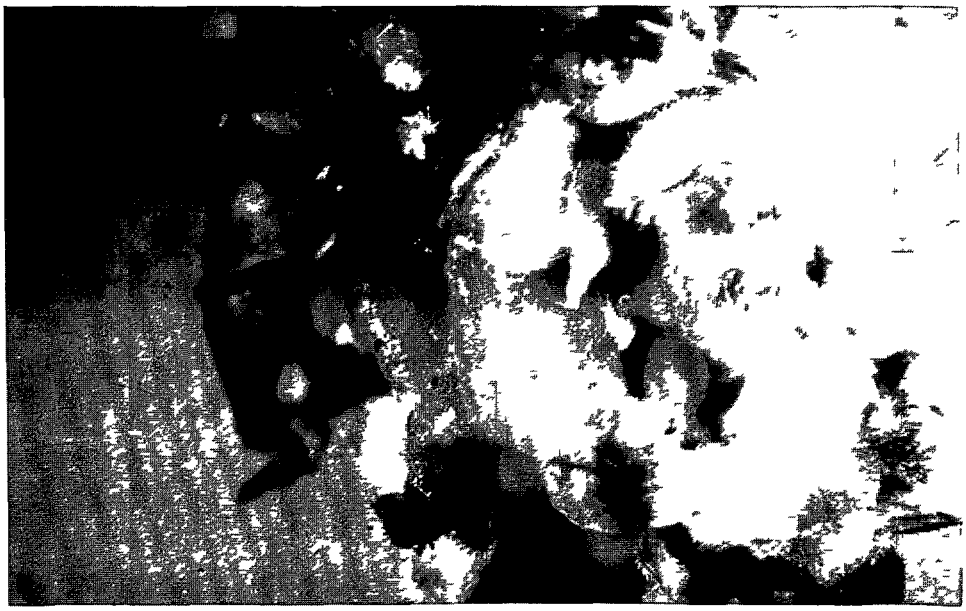

Figure 2 Droplets of the sugary exudation in sorghum panicle

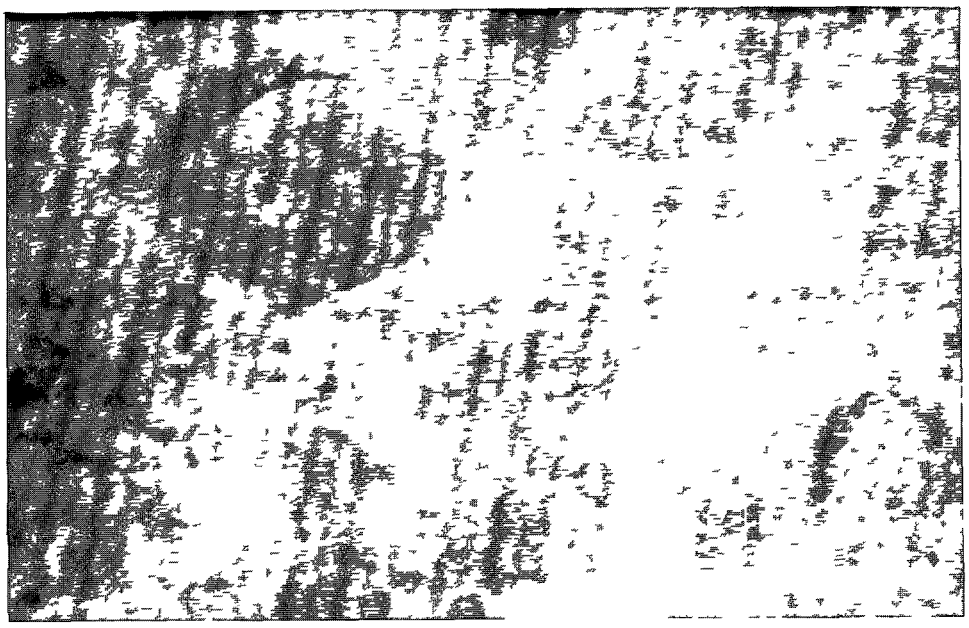

Figure 3 Droplets of the sugary exudation and powdery crust of dried drops on foliar surface

The sugary exudation and the sphacelial tissue filling the ovary were observed in the above mentioned host plants Typical sclerotia, curved as a little horn (= ergot in French), from which the perfect stage of the fungus would arise, were never found Thus, the ascogenous forms were not observed in Venezuela

Pathogen

Microscopic examination of cross sections of infected ovaries revealed the presence of fungal structures whose characteristics are similar to the ones described by various authors for the fungus *S sorghi* (Bandyopadhyay et al 1990, 1996, Fredericksen et al 1991)

Infected ovaries are almost entirely filled with a stromatic, not very compact, convoluted mass formed by angular hyaline cells which, together, exhibit a white, creamy color, with short conidiophores in dense palisades, bearing phialidic conidiogenous cells in which conidia are produced (Figures 4 and 5) Conidia are very abundant, slimy, oblong, elliptical, slightly constricted at the middle, hyaline, guttulate, 15-19 (m long and 4-5 (m wide at the base and at the apex (Figure 6)

The sugary exudate and the sphacelial stroma are colonized by *Cerebella andropogonis* Ces which seems to inhibit sclerotium development and therefore the sexual stage formation *C andropogonis* is easily recognized for its blackish brown colored conidiophores in dense palisades of velvet-like appearance, forming characteristic convolutions like those on the surface at the brain mass (hence the name *Cerebella*) (Figures 7 and 8) (Langdon 1955, Tarr 1962)

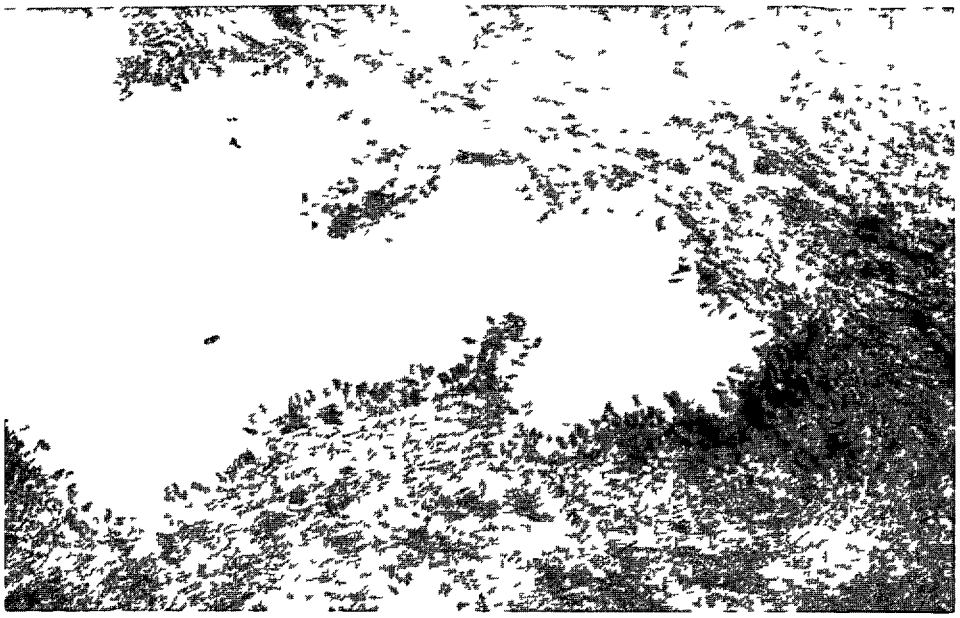

Figure 4 **Cross section of an ovary infected by *Sphacelia sorghi*, showing stroma with conidiophores in palisade**

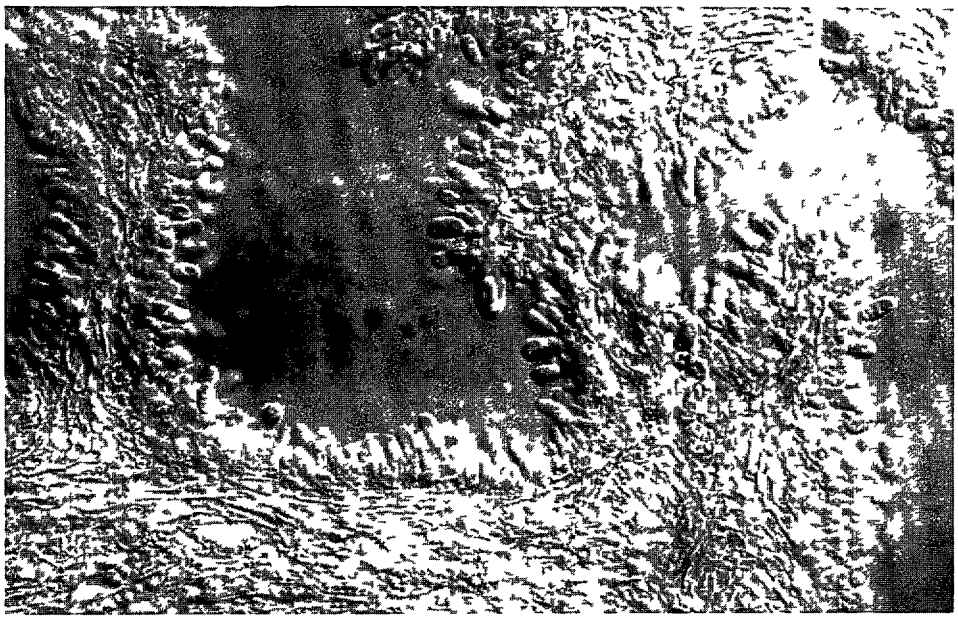

Figure 5 ***Sphacelia sorghi.*** **Stroma with conidiophores, conidiogenous cells and conidia**

Figure 6 Conidia of *Sphacelia sorghi*

Samples of inflorescences of cultivated and wild sorghum sugary exudations, were sent to Professor P G Mantle, Imperial College, University of London, who confirmed the presence of sphacelial fructifications and typical sugars of the sexual stage of *Claviceps africana* Frederiksen, Mantle and de Milliano, and the lack of well developed sclerotia The absence of sclerotia reduces the risk of toxicity of false grains

Disease Introduction

The possible explanations for the presence of the disease in Venezuela, are as follows

a Introduction of the fungus with commercial grain sorghum imported for animal consumption, or with experimental samples from Africa, Asia or South America

b Conidial dispersion by strong air movements from adjacent countries such as Brazil and Colombia, as it happens with rust uredospores

c *C africana* might have been present in Venezuela for some years in host plants, and remained unnoticed, until the particular environmental conditions of 1996 favored inoculum pressure thereby producing the epiphytotic

Primary Infection

In Venezuela, it can be assumed that in the absence of ascospores, primary infection originates from conidia from wild hosts or volunteer sorghum plants grow-

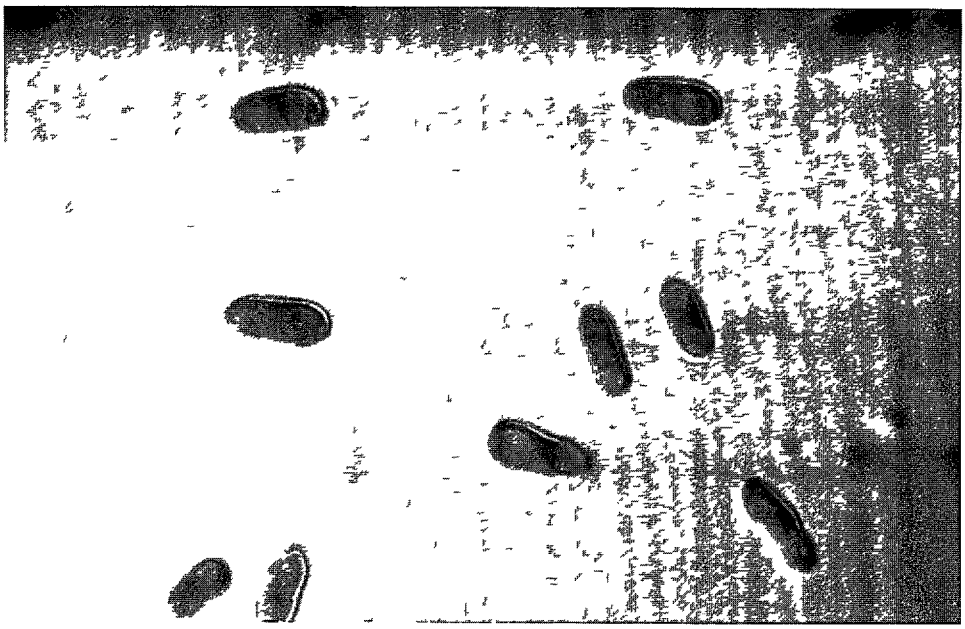

Figure 7 ***Cerebella andropogonis*** **growing on the** ***S sorghi*** **honeydew**

Figure 8 ***Cerebella andropogonis*** **on inflorescence of** ***Panicum maximum*** **infected with** ***S sorghi***

ing along field borders or roadsides Conidia may also come from the sugary powdery crust on panicles or infested debris left in the field after harvest Conidia may remain viable for several months and then spread by wind, insects, birds, animals and farm implements (Bandyopadhyay et al 1996)

Seeds from diseased fields may carry the sugary exudate along with the fungus mycelium and conidia leading to reduced germination, and increased predisposition to seedling diseases (McLaren 1993)

Considerations about Control

The use of resistant hybrids is the first option to control ergot However, resistant male sterile lines are not available In most countries, work is directed towards the selection of these lines, in places where environmental conditions favorable to the disease are naturally present Germplasm can also be tested by inoculating the panicles by spraying an aqueous sugary suspension prior to pollination (Futrell & Webster 1966)

As long as resistant material is not available, other control options may be considered for managing the disease The precautions can be summarized as follows

Hybrid seed production

- Sowing in appropriate periods avoiding high humidity and low temperatures during flowering period
- Moving seed production to drier areas with low relative humidity
- Eliminating all wild hosts, especially *P maximum* and wild sorghum plants present in the field or in nearby areas
- Changing the male female proportion to increase the male lines to enhance the pollen quantity and thereby obtaining a higher fertilization and a lower infection
- Obtaining a better synchronization of the male and female flowering, with the respective study of the exact cycle and line development
- Using healthy parental seeds to reduce primary inoculum
- Reducing tillers by increasing plant population in the female lines and eliminating, if possible, the suckers before flowering
- A very important measure is to protect the fields with an appropriate fungicide, with three applications (every 5 to 6 days) starting just before anthesis Spray droplets must be evenly distributed over the whole panicles

Grain Production

In grain sorghum fields, the disease is normally less severe than in seed production due to self-pollination Protective fungicides are not recommended due to its high cost, but an economic study on cost-benefit ratio is required Defensive strategies are more pertinent These are

- Eliminating all possible wild hosts of the pathogen from the field and neighboring areas before sowing
- Eliminating volunteer sorghum plants, especially if infected
- Assuring that the seeds are healthy, coming from pathogen free areas An appropriate seed treatment fun-

gicide should be applied if any doubt exist

According to Brazilian investigations carried out in 1995 and 1996 (Odvody 1996), the "Triazole" family can satisfactorily control the disease Commercial products such a Tilt® (Ciba-Geigy), Folicur® and Baytan® (Bayer) are all used in concentrations around 0 5-1 L ha

Baytan was more effective, but it is toxic at the indicated level Seed treatment can eliminate the conidia that can be present in the sugary exudation dried on the grains

Final Consideration

Ergot, honeydew or sugary disease of sorghum, is a disease that can disseminate quickly by means of wind, animals and seeds, reaching long distances and spreading in vast areas It perpetuates in debris, grains and a variety of host plants The control measures listed above must be studied and assessed for each circumstance since some are difficult to follow, such as the elimination of wild sorghum plants As the disease is present in most seed producing areas of Venezuela, care should be taken in selecting fields for seed multiplication

As happens with most diseases, an evolution will take place in which we find ways that will allow us to coexist with this disease Diseases such as downy mildew and maize mosaic of corn, the bacterial blight of sesame and the white leaf of rice are examples of this evolution and adaptation

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Cereal Ergots in Uruguay

Maya S Piñeiro

Ergot alkaloids produced by *Claviceps* on forage grasses (tall fescue, ryegrass) and cereal grains are widespread in Uruguay Local climatic conditions are ideal for sclerotia development and germination, dissemination of ascospores, and infection The weather and agronomic practices make sclerotia as important propagules The presence of ergot in cereals is quite common Most of it is attributed to admixture of grains with ryegrass Annual ryegrass is a common weed, which invades cultivated lands in Uruguay In these wild grasses, infection by *Claviceps purpurea*, *Pithomyces chartarum*, *Claviceps spp*, etc is very common and the fungi are widely distributed in our fields Annual ryegrass seeds infected with fungal endophytes or *C purpurea* ergot sclerotia very often become components of feed Other species such as *C paspali* and *Ustilago paraguaiensis* also affect cattle, producing nervous disorders

Contamination with ergot occurs in all cereal grains, bran and other cereal by-products and feed Feed components often include mixtures of third grade screenings, which have weed grass seeds and ergot sclerotia Low-grade categories of cereals, such as barley, wheat, rye, may contain ergot bodies from the cereal itself or from the weed grasses making the ration unsuitable for animal feed

In response to increasing ergot infection resulting in large animal health and economic losses, the Uruguayan authorities, in 1986, issued regulations establishing limits These set a maximum value of 0 03% (0 03 g of ergot sclerotia per 100 g of animal feed) or 450 ng g^{1} of total alkaloids for all species, with the exception of feed for sows, rabbits and chinchillas during lactation and pregnancy, where there is a zero tolerance These regulatory limits take into account a daily dose of 59 µg kg^{1} of alkaloids as the maximum permissible intake without having toxic effects in dairy cows This is a very strict standard, comparable to Canada's 0 05% for their highest grade rye, and 0 01% for export wheat

Even when applying commercial grading systems and the 1986 regulations, precautions had to be taken as toxic levels passed into the grains because alkaloid values higher than those permitted could not be detected by routine grain quality laboratories Determination of ergot sclerotial tissue was not possible once mixtures were milled Up to 1993, ergot was detected mainly by microscopic counts of sclerotia or ergot bodies and sporadically by colorimetric assays, pigment content and biological trials

Human toxicosis has not been detected after the enforcement of the ergot regulations, grain sanitary limits and trade control, but animal cases are endemic Since

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1992, ergot toxicity syndromes and field outbreaks have been on the rise in cattle, sheep, swine, horses and poultry These local animal toxic signs include poor weight gains, abortions and reproductive problems, agalactia, staggers in heifers, summer hyperthermia, heat stress, peripheral necrosis and gangrene, nervous disorders, convulsions and other clinical manifestations of vasopressor action of ergot alkaloids All members of the family *Clavicipitaceae* are capable of producing ergot alkaloids and should be suspect in these toxic cases In addition endophytic fungi can also be found in pasture grasses and can be toxic to animals

Cereal grains are very susceptible to ergot infection During the last decade, improvement in agricultural practices, milling techniques and grading have eliminated the potential risk of widespread human outbreaks of ergotism However the chronic effects of ingesting low levels of ergot alkaloids over long periods and the animal cases detected locally led us to investigate the actual levels of ergot alkaloids present in our commonly consumed cereals and feeds In Uruguay, ryegrass was considered the major source of ergot infection although there was no conclusive alkaloid data At Laboratorio Tecnologico del Uruguay (LATU) we have been monitoring animal case studies for ergot alkaloid levels in the feed and components used, as well as in all suspected toxic cereals since 1993

Our first objective was to compare, develop or adapt and standardize analytical methods for chromatographic identification and quantification of four major ergot alkaloids The principal aim, however, was to evaluate the levels and incidence of these alkaloids in samples of our main cereals and animal feeds

In 1993-1995, a pilot study monitoring mycotoxin contamination in food and feeds, including ergot alkaloids was conducted by LATU with FAO's financial assistance (Piñeiro et al 1996) Ergot alkaloids were detected in 26% of the feed samples at levels above the legal allowance The major pharmacologically active ergot alkaloids analyzed were ergotamine, ergocristine, ergocornine, and alpha-ergokryptine Since then, we have routinely determined these ergot alkaloids in our laboratory (Piñeiro et al 1995) Analyses were by liquid chromatography using a 5-µm Hypersil C18 column (100 x 4 6 mm), with an isocratic Hewlett Packard 1050 system with fluorescence detector (Scott and Lawrence 1980, Ware et al 1986)

Sugary disease of sorghum has not been overtly observed in Uruguayan fields although cases have been suspected The results presented here on ergot alkaloid levels and incidence refer to sorghum and mixed feed, where sorghum was a large part of the feed components used

As combined toxicities of alkaloids may be a more insidious detriment to both livestock and human health than the individual compounds, the results of our studies have been expressed as total ergot alkaloids Our data presented pertains to samples originating from feed involved in toxic cases or from suspected contaminated grains

Out of 177 total samples analyzed since 1993 (Table 1), 109 samples had de-

Table 1 Level of total ergot alkaloids in 177 cereal-based products in Uruguay

Product	No samples	<6	5 150	151 450	451 700	701 1000	1001 2000	2001 3000	>3000
Sorghum & mixed feed	105	42	11	22	15	5	8	0	2
Bran & wheat based feed	41	16	9	9	1	1	2	1	2
Oats	1	1	0	0	0	0	0	0	0
Wheat & Flour	17	5	2	2	3	1	4	0	0
Rice & by products	9	3	0	4	1	1	0	0	0
Corn	2	1	0	1	0	0	0	0	0
Barley	2	0	0	0	0	1	1	0	0
Total positive samples >ng g^{1}						109			
Total positive samples >450 ng g^{1}						49			
Total samples						177			

tectable values (detection limit 6 ng g^{1}) for total ergot alkaloids Ergotamine was the major alkaloid present in all cases Of all samples, 49 surpassed the legal limit of 450 ng g^{1} Levels varied from <ng g^{1} to >3000 ng g^{1} for total alkaloids (Table 2) The percentage of total positive samples above the detection limit was 61 9% High levels of alkaloids, with values above 1001 ng g^{1} to >3000 ng g^{1}, were found in sorghum and mixed feed, bran and by products and wheat Such high alkaloid content was detected in 5 6% sorghum and mixed feed samples

Of the samples exceeding regulations, sorghum and mixed feed represented the largest part (16 9%), followed by wheat and flour (4 5%), bran and wheat based feed (3 9%), for a total of 27 7% samples over legal allowances

Table 2 Percent samples of various cereal products (total 177) with alkaloid levels below or above the tolerance level (450 ng g^{1}) in Uruguay

Product	<450 ng g^{1}	>450 ng g^{1}
Sorghum & mixed feed	42 0	16 9
Bran & wheat based feed	19 2	3 9
Oats	0 6	0 0
Wheat & flour	5 1	4 5
Rice & by products	3 9	1 1
Corn	1 1	0 0
Barley	0.0	1.1
Sub Totals	71.9	27.5

Total elimination of ergot poses difficulties However, good preventive measures based on mycological identification, alkaloid detection and quantification, pasture, silage and feed handling can reduce toxic levels, improving sanitary and economic aspects considerably

As general recommendations, reduction in ergot contamination is possible by clearing wild grasses near cultivated fields, sowing cereal varieties less susceptible to ergot, rotation with non-hosts, post harvest removal of sclerotia, and deep plowing From the industry point of view, segregation, density, sieves, separators, flotation, milling, etc can remove over 80% of ergot

The data presented here should raise awareness of the possibility of toxicity by the consumption of ergot contaminated cereals on a daily basis by humans as well as animals It would assist in evaluating health risks from intake of ergot alkaloids and setting and enforcing appropriate standards

Acknowledgement

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Sorghum Ergot in Mexico

J H Torres-Montalvo and N Montes-Garcia

Abstract

Grain sorghum is one of the three most important crops in Mexico and its production is now threatened by ergot, which was first observed in February, 1997 Initial ergot infestation covered parts of the states of Tamaulipas, Veracruz and San Luis Potosi As of May, 1997, ergot was present in northern Tamaulipas, and the states of Sinaloa, Nayarit, Jalisco, and Colima The seed industry is attempting to reduce the impact of ergot in seed production fields by applying triazole fungicides More information is needed about chemicals, rates of application, and phytotoxicity INIFAP is conducting research in different areas to control the disease Monitoring is being done to evaluate the spread and the severity of ergot Chemical control and host plant resistance are priority research areas Regulatory measures have been applied to prevent the dissemination of ***Claviceps africana***

Grain sorghum [*Sorghum bicolor* (L) Moench] has increased in economic importance in Mexico during the last four decades Its adoption by farmers has been so successful that it was considered the second green revolution (DeWalt and Barkin 1985) This success was due to its high yield, wide adaptation to the different ecological conditions in Mexico, drought tolerance, and to the demand of the grain by the feed industry Another important factor was the availability of hybrids that are used in 95% of the production area

Today, grain sorghum is the third most important crop in the country sown in 1 4 million ha More than 4 2 million tons of grain is produced per year (FAO 1996)

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The main production areas are northern and central Mexico in the states of Tamaulipas, Sinaloa, Guanajuato, Michoacan and Jalisco (Fig 1), where 87% of the total grain is harvested (SAGAR 1996) The highest yields in the world have been achieved in the state of Guanajuato (19 t ha ^{1})

Mexico is the fifth largest producer of grain sorghum in the world following the US, India, China, and Nigeria In spite of this large production, Mexico imports 3 5 million t of sorghum grain annually which is equivalent to 45% of the total grain required by the industry This makes Mexico the largest sorghum grain importer in the world

Many diverse factors limit grain yield including drought, soil salinity, pests and diseases It was estimated that diseases are responsible for 10-15 % of crop losses in Mexico (Narro et al. 1992) Ergot (caused by *Claviceps africana*), a potentially devastating disease of sorghum,

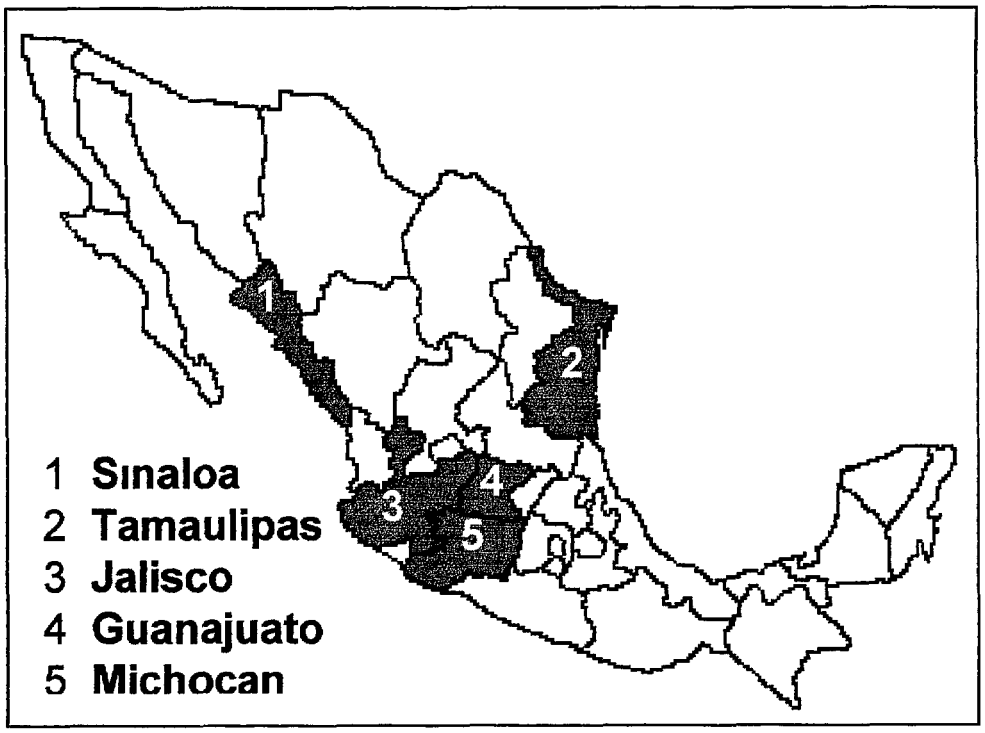

Figure 1 Main grain sorghum producing areas in Mexico

was first observed in Mexico in February 1997 Our paper presents the evolutionary status of ergot in Mexico

Initial Spread of the Disease

During the first week of February 1997, the ergot epidemic spread to and within Mexico Ergot was initially observed in several sorghum fields in the states of Tamaulipas, San Luis Potosi, and Veracruz (Fig 2) In Tamaulipas, the main sorghum producing state, infested plants were reported in the central and southern part of the state In central Tamaulipas, ergot was observed in ratoon crops and volunteer plants that flowered at the end of January In southern Tamaulipas and the neighboring states of Veracruz and San Luis Potosi, ergot was found in commercial fields, seed production fields, and nurseries with A-lines, R-lines, grain hybrids, and forage sorghums Moist conditions persisted after the flowering stage and saprophytes such as *Cerebella* sp were observed Ergot was also reported infecting johnson grass [*Sorghum halepense* (L) Pers] throughout the area and several plants of pearl millet [*Pennisetum glaucum* (L) R Br] under high inoculum pressure in a winter grow-out nursery in southern Tamaulipas established by the Texas Department of Agriculture (TDA)

Figure 2 Spread of sorghum ergot in Mexico

The highest incidence of infection was reported in the sorghum male-sterile lines and the forage sorghums Some commercial hybrids with various levels of male-sterility, believed to be caused by the sensitivity of the plant to temperatures below 12°C during pollen formation also had a high incidence of ergot Lack of pollen most likely contributed to colonization of the ovary by spores of *C africana* Microsporogenesis is the developmental stage that is most susceptible to adverse environmental conditions (Manjarrez-Sandoval et al 1989)

Initially, ergot failed to move further north because host plants in this area suffered freeze damage during the month of January (Fig 3) The causal agent was identified as *Claviceps africana,* based on sclerotia morphology, amount of secondary conidiation (Frederickson et al 1989, Frederickson et al 1991), and the fact that this species was the causal agent in other countries of the Western Hemisphere (Reis *et al* 1996) Studies on alkaloid content are being conducted to confirm this identification

Continued Dissemination of Ergot

There are two sorghum growing seasons per year in northern Tamaulipas, during which 90-95% of the state's grain is produced During the main season, sorghum is sown throughout the month of February and during the first 8 days of March (Herrera and Betancourt 1981), however, the farmers usually sow sorghum from January to late March,

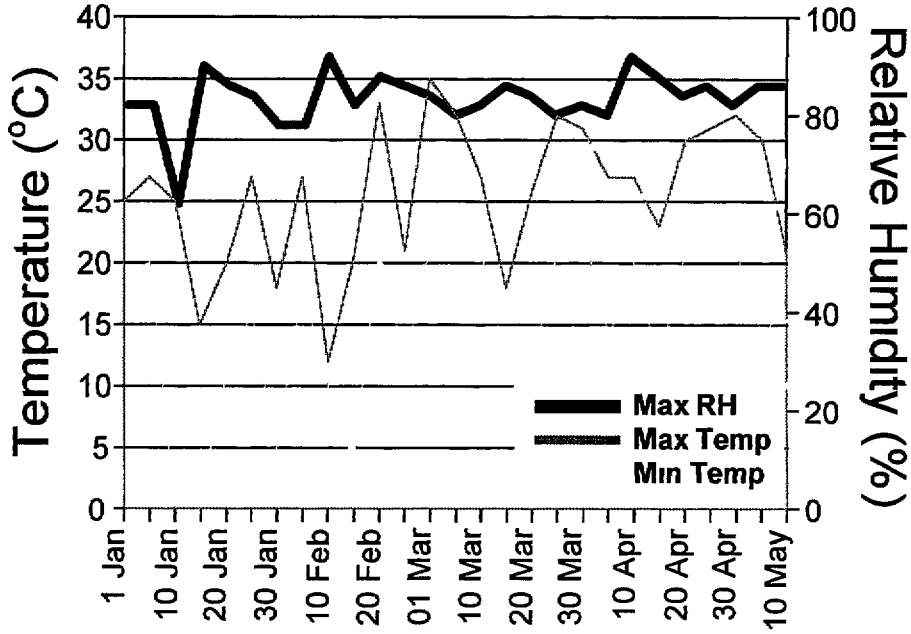

Figure 3 **Maximum and minimum temperatures (°C), and maximum relative humidity (%) in northern Tamaulipas, 1997, Rio Bravo, Mexico**

depending on availability of the soil moisture

After the first appearance of ergot, it was expected that incidence would be less in northern Tamaulipas during the main growing season since sorghum usually flowers during April-May This is based on historic climatic data, which suggests that temperature and relative humidity at this time interval are not conducive for development of ergot However, the 1997 growing season was unique Two factors have contributed to the continued spread of ergot 1) the environmental conditions were favorable for development of the disease as cool temperatures and high relative humidity have been present most of the season (Fig 3) including temperatures below 12°C that caused pollen sterility in some commercial fields and 2) multiple planting dates have resulted in florets blooming over extended periods of time which contributes to availability of plant host to the fungal spores Some farmers had sown during the first week of January and others were still sowing in May The first observations suggests differences in susceptibility to ergot among different hybrids but more research is needed before definite conclusions can be made Up to 80% incidence and 40% severity were recorded in some commercial hybrids, particularly those that flowered in late March and early April

Besides the above listed factors, johnson grass is an endemic weed in the area where estimates of more than 40 000 ha are infested in northern Tamaulipas

(Rosales 1994) This dramatically increases the risk of ergot incidence in sorghum fields, because johnson grass could act as an alternate host when sorghum florets are not available

Ergot was observed in the Pacific region in the states of Sinaloa, Nayarit, Jalisco, and Colima (Fig 2), and was of high incidence in production fields and research nurseries that flowered in April and May As on May, 1997, ergot has not been reported in the Bajio, which is the second largest sorghum production area in Mexico and comprises the states of Guanajuato and Michoacan The first sorghum fields will not initiate flowering until late June There is a high risk that ergot will be present in the Bajio because environmental conditions would be nearly ideal for ergot development

Impact of Ergot on Seed Production

Besides grain sorghum, northern Tamaulipas is also the main sorghum seed production area in Mexico Most hybrid seed was produced in this region in the 1980's (Table 1), however, due to increased costs, seed production has decreased considerably In 1995 less than 1% of the sorghum seed was produced in Northern Tamaulipas [National Service of Inspection and Certification of Seeds (SNICS) 1996] The remaining seed was imported and primarily from the United States Eight seed companies and one farmer organization produced 22 different hybrids on more than 2 000 hectares in 1997 (SNICS 1997) Advantages for seed production in this region are favorable environmental conditions for production of good quality seed, highly mechanized farms, acceptable yields, a short split between flowering for both parents, constant winds that favor pollination, adequate infrastructure for seed processing, availability of labor for rogueing the fields, availability of irrigation, and a large local market (Williams-Alanis and Torres-Montalvo 1988)

After the first report of ergot in Mexico, the seed industry was shocked at the implications that this disease carried for seed trade and the fact that ergot primarily affects the male sterile-lines in the production of F_1 hybrids, particularly if there is poor pollination by the restorer lines (Bandyopadhyay et al 1996)

Table 1 Sorghum seed produced in Mexico, 1988-95

Year	Area (ha)	Seed Needed[1] (t)	Seed Produced (t)	Surplus or Deficit[2]	Yield (t ha^{-1})
1988	17160	42790	73816	113 0	2 6
1989	9247	37312	17323	117 0	1 9
1990	10716	42146	21016	41 0	2 0
1991	5834	33206	9809	63 0	1 7
1992	5543	32072	9980	31 0	1 8
1993	946	21404	1333	47 0	1 4
1994	308	20652	309	6 5	1 0
1995	2213	35031	3754	0 9	1 7

Source National Service of Inspection and Certification of Seeds (SNICS)
[1]Hybrid seed needed for commercial use the following year
[2]For the growing season the following year

During a disease survey in early May 1997, ergot was not observed in the seed production fields blooming although inoculum was present because infected plants were found in nearby commercial fields that had flowered in April

Seed companies are using chemical control to prevent ergot by applying triazole fungicides based on the success in Brazil (Ferreira et al 1996) Adequate equipment for ground application of chemicals is not available and, therefore, aerial applications are being used Propiconazole (Tilt®) or tebuconazole (Folicur®) have been sprayed 2-3 times by the seed companies Rates varied from 250 to 500 ml of chemical (250 g a i L^{-1}) The cost of three sprays is estimated at US \$625 ha^{-1}, including the cost of the chemical and aerial applications Most applications were done during blooming Seed producers are concerned with possible phytotoxic effects of fungicides on the parental lines when applied during the flowering stage sprayed twice before the plants began to bloom The first application was at the 50% flag leaf stage, and the second 5-6 days later The efficiency of aerial applications to control the possible presence of ergot and phytotoxicity are being evaluated

Research

When ergot was first reported in Mexico, the National Research Institute for Forestry, Agriculture and Livestock (INIFAP) immediately initiated a comprehensive program to control the disease The first priority was to determine the extent of the disease A research team, primarily from the Rio Bravo Experimental Station, conducted regional and national monitoring to evaluate incidence and severity of ergot

Studies on preventive chemical control are being conducted using both systemic and contact fungicides Time of application, chemicals, rates, and methods of application are some of the variables under study Chemical seed treatments are also being conducted Management practices such as planting dates, temperature effects on fertility and viability of sorghum pollen, and identification of alternate hosts are being evaluated

Host plant resistance is the most economical and logical control strategy if sources of resistance are available All parental lines and hybrids of INIFAP are currently being evaluated Resistance, when identified, needs to be incorporated into elite sorghum germplasm Other research projects include studies on toxicology, biology of the pathogen, and DNA marker assisted selection

Scientists from INIFAP and Texas A&M University conducted a meeting in April 1997, to plan and implement collaborate research in areas of mutual interest Chemical control and monitoring the spread of ergot in Mexico and Texas were of the greatest concerns

Regulation

Sanidad Vegetal, the phytosanitary agency in Mexico imposed an emergency plan to control the spread of ergot to other regions of the country The objective of this plan was to diagnose, prevent, control and eradicate sorghum ergot (SAGAR, Diario Oficial de la Federacion, 1997) These measures included incineration of

fields with more than 50% incidence, limited the sale of infected grain to areas where sorghum is not produced, and disinfecting of equipment used in infested fields with 5% NaCl

After the announcement of this plan, other regulations have been made public The measures can be divided into three areas management of infected grain, inspection and management of seed production fields and storage facilities, and prohibit importation of infected grain and/or seed These measures were reviewed by the responsible regulatory agency which also cooperates with scientists and the sorghum industry to obtain information to regulate the movement of contaminated grain/seed and to approve use of the effective fungicides needed to control ergot

Education

Sorghum producers, extension specialists, and the sorghum industry will need additional research to provide answers on ergot and effective methods for control They need to be informed of problems and methods to reduce the impact of the malady Several seminars, meetings and conferences were organized by different agencies to provide information about ergot to producers, the private sector, and institutions involved in the sorghum industry

Information is vitally needed in Spanish The bulletin "Ergot — A global disease threat to sorghum" produced by the Sorghum Improvement Conference of North America (SICNA) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), was translated into Spanish and is now in press INIFAP is also producing technical articles

Acknowledgments

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Session IV — Ergot Biology

Session President — R.A. Frederiksen

DNA Comparisons and Alkaloid Toxicity of the Ergot Fungi — A Contribution to Ergot Phylogeny

Sylvie Paloutová

Abstract

The RAPD patterns of ***Claviceps purpurea*** *revealed great variability even among the isolates from the same plant and head Two groups were found, based on the relatedness of their RAPD patterns as well as on the presence or absence of an EcoRI restriction site in the 5 8S rDNA One of them (EcoRI+) predominated on the cereals and grasses from open habitats whereas the other (EcoRI-) preferred glycophytic hosts Unlike* ***C purpurea****, the RAPD patterns of* ***C fusiformis, C paspali*** *and* ***C gigantea*** *did not show intra--species variability The DNA sequence comparison of ITS spacers and the 5 8S rDNA region from 12* ***Claviceps*** *species revealed the existence of two distinct groups Group 1—****C viridis, C africana, C gigantea, Claviceps*** *sp ex* ***Sorghum, Claviceps*** *sp ex* ***Panicum maximum, C phalaridis*** *and* ***C citrina*** *and Group 2—****C purpurea, C sulcata, C fusiformis, C grohii*** *and* ***C paspali***

Both Claviceps sp , ***C citrina, C phalaridis*** *and* ***C paspali*** *shared a deletion in the ITS1 region,* ***C phalaridis*** *and* ***C citrina*** *exhibited another deletion in the ITS2 region, about 30 bp long The missing regions were also found in the outgroup species* ***Fusarium sambucinum*** *suggesting their older phylogenetic origin*

The alkaloids in sclerotia and shaken cultures of ***C gigantea*** *were analyzed The* ***C gigantea*** *pathway leads from dihydro-chanoclavine through festuclavine and dihydrolysergol to dihydrolysergic acid The occurrence of dihydroergosine, the only dihydrogenated peptide alkaloid known from the phylogenetically related species* ***C africana*** *indicates that in* ***C africana*** *a similar pathway could be operating*

The Position of *Claviceps* among Hypocreales

The evolution and to some extent the taxonomy of the parasitic fungi belonging to the genus *Claviceps* are influenced by their host plants—grasses, rushes and sedges The genus *Claviceps* belongs in the family Clavicipitaceae The family is placed in the order Hypocreales, but in the fifties, doubts concerning the morphology of the conidiogenous stroma led some mycologists to transfer it into Xylariales (Luttrell 1951) Another mycologists erected the order Clavicipitales (Gaumann 1952) Recently, molecular taxonomy was used to solve the relationships among different members of Hypocreales and the original placement of monophyletic Clavicipitaceae into this order was confirmed The molecular phylogenies

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also show that the genus *Claviceps* was the first group derived from the common ancestor line, then *Epichloe/Neotyphodium* followed by *Atkinsonella* and last clade contains species of *Balansia* and *Myriogenospora* (Spatafora and Blackwell 1993, Rehner and Samuels 1995, Glenn et al 1996) These results contradict the formerly held hypothesis about *Balansia* being the most primitive clavicipitoid group

The Intraspecific Variation of *C purpurea*

The undoubtedly most widespread ergot is the type species *C purpurea* that colonizes all pooid and arundinoid grasses and also occurs on chloridoids and rarely on panicoids This wide host spectrum led many mycologists to search for varieties or races based on host preferences Indeed, several groupings were described during this century, but when compared they differ, e g , Stager's (cf Campbell 1957) European groups from the groups observed in Japan by Tanda (1979) Campbell (1957) cross-inoculated the *C purpurea* isolates from 37 grasses and found that when quite aggressive methods of artificial inoculation were used, no host preferences occurred

The search for varieties continues using the methods of DNA fingerprinting in our laboratory and in the group of Prof Tudzynski in Germany Results were the same in both laboratories (Jungehulsing 1995) There are at least two groups that share 1-2 major RAPD bands and we found that they also differ in the presence of EcoRI conservative restriction site in the 5 8S rDNA EcoRI+ group (70 % of isolates) was found on cereals and the grasses from open habitats (Figure 1) EcoR- group consisted of 30% of isolates and seems to prefer the glycophytic hosts, like *Molinia, Agrostis, Poa annua, Calamagrostis*, but was also found on *Festuca rubra* and *Dactylis* sp In this group, there are two basic RAPD patterns with the primer 257

After confirming the stability of fingerprint patterns during the asexual propagation of the isolates, experiments were car-

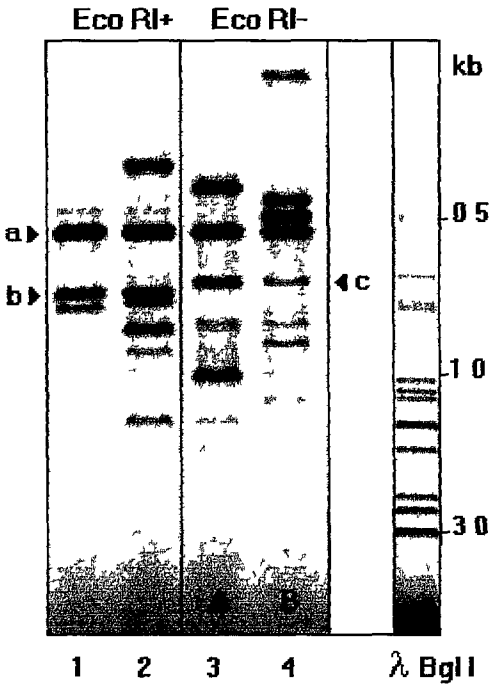

Figure 1 RAPD banding patterns of *C purpurea* subgroups with the primer 257

1 isolate from *Lolium* sp England 2 isolate from *Festuca ovina* Czech Rep 3 isolate from *Poa annua* Czech Rep 4 isolate from *Agrostis* sp Belgium
a *C purpurea* species specific band
b Eco RI+ group specific band
c Eco RI group-specific band
A type of pattern found in 16 Eco RI isolates
B type of pattern found in 2 EcoRI isolates

ried out to look at mutation and reisolation, variability through comparing the isolates from different florets on the same flower head, different heads from the same plant, different plants at the same locality and from different species of this locality Pattern differences were found even among isolates from the same head (Figure 2) that confirm multiple infection steps

Jungehulsing, from Tudzynski's group, found that the chromosome number in *C purpurea* isolates is variable (3 - 6) and that their size ranges from 1 8 to 7 Mb She confirmed that the sexual crossing (by double infection) of parents with equal chromosome number but unequal chromosome size was possible, but each isolate from the progeny had different karyotype due to translocations during meiosis Translocations during the sexual cycle were the major source of RAPD variability Chromosomal polymorphisms were observed among the isolates from the same place, year and host species, probably due to chromosomal rearrangements

To find out if the RAPD polymorphism variability of *C purpurea* was reflected also in other genome parts, the rDNA regions containing ITS spacers and 5 8 rDNA from the strain *C purpurea* P695/S

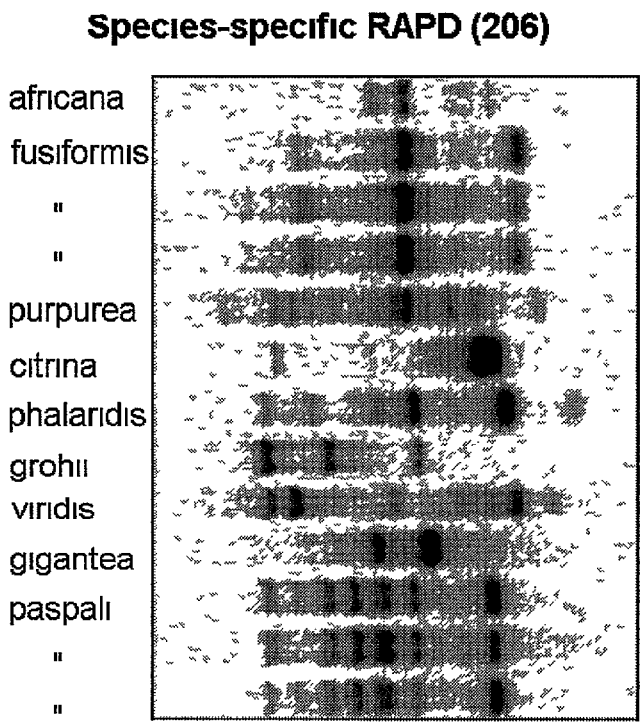

Figure 2 The RAPD variability of the field isolates from Lolium

(Schumann et al 1982), isolated probably from European cereals, and from *C purpurea,* isolated in our laboratory from sclerotia on *Phalaris* sp collected in Australia, were sequenced These sequences were compared to two American *C purpurea* sequences isolate GAM 12885 collected on *Dactylis glomerata* near Athens, Georgia, USA (Glenn and Bacon 1996, sequence U57669 in GenBank) and isolate 109 from *Festuca arundinacea,* Lexington, Kentucky (Schardl et al 1991)

The European and Australian isolate differed only in a single base out of 556 localized in ITS2 The American isolates GAM 12885 and 109 differed from the European P695/S each in the 5 and 6 positions, respectively They varied in the ITS1 spacer, but none were common whereas the difference between them was in 10 positions of ITS1 GAM 12885 also lacked the conservative EcoRI restriction site located in the 5 8S rDNA sequence which documents that EcoRI+ and EcoRI- group exists also in America

The Variability of other *Claviceps* species

DNA fingerprinting of nine *Claviceps* species was performed by RAPD technique with primer 206 (TCAA-

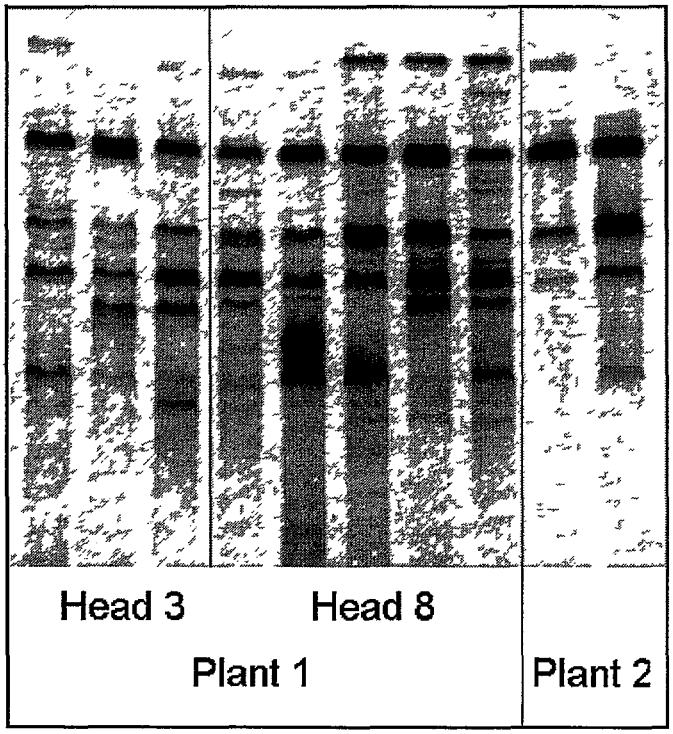

Figure 3 Species-specific RAPD with the primer 206

CAATGTCGGCCTCCGT) (Figure 3) Unlike *C purpurea*, different isolates of *C paspali* and *C fusiformis* exhibited very low RAPD pattern variability The patterns of three Mexican *C gigantea* isolates were identical and also *C africana* isolates from Bolivia and Australia did not differ It may be argued that *C africana* is spreading too rapidly to develop genome differences among distant isolates The patterns with the primer 206 were markedly species-specific and this could enable the identification of sphacelial stages once the teleomorph RAPD was determined The RAPD pattern was used as the first criterion for excluding the possibility that the newly discovered ergot from *D spicata* (*C citrina*) is *C purpurea* (Paloutova et al 1998)

The *Claviceps* Phylogeny

Langdon (1952, 1954) presented the first outline of Claviceps evolution Its main course is connected with the evolution of grasses and the global climatic changes, so that brief outline of grass evolution will be given here

Grass Evolution

Brown and Smith (1972) and Jones (1991) summarize the evolution of grasses Grasses probably appeared during the Jurassic period (Mesozoic), in the wet tropics The subfamilies Phareae, Centothecae and Arundinoidae (C3) are presumed to be the most primitive Descendants of early arundinoids are the subfamilies Chloridoidae and Panicoideae that developed C4 photosynthetic pathways and thus acquired competitive advantage in warmer regions

Chloridoideae, according to the geographical distribution, acquired C4 photosynthesis and expanded before the breakup of the Pangea (Middle Cretaceous), the greatest number of species being concentrated in South Africa

Part of the Panicoideae (the tribe Paniceae) acquired C4 photosynthesis shortly before South America was separated from the rest of Gondwana (Late Cretaceous) In isolated South America, Paniceae grasses became dominant C3 photosynthesis was retained in apparently more primitive shade loving species (*Oplismenus, Icnanthus*, and partially *Panicum*) whereas C4 species colonized open habitats of tropical and subtropical zones (some species of *Panicum, Paspalum, Cenchrus, Brachiaria, Pennisetum*)

In southern Asia, the Andropogoneae (*Sorghum, Zea, Saccharum, Bothriochloa, Imperata*) developed from some C4 ancestor and rapidly occupied savanna habitats and dry open woodlands It is assumed that this occurred some 25-30 million years ago (mya) Andropogonoid species form 30-40% of all grass species in India and Africa The transfer of Andropogoneae to America occurred probably via southern Europe, before their separation in Tertiary and they reached Australia via island chains

Pooidae subfamily retained C3 photosynthesis, as C4 does not present any advantage in a cooler climate It expanded mainly into the Northern Hemisphere The Southern Hemisphere does not support its climatic needs except in New Zealand, southern South America and Australia

The grass phylogenies derived from plastid as well as nuclear DNA sequences show two main groups In the PACC group (Panicoids, Arundinoids Chloridoids and Centothecae), chloridoids and arundinoids are sister groups to the ancestor of panicoids, which correlates well with the theory based on the geographical distribution of grass subfamilies The group BOP contains Bambusa-Oryza and Pooids (Mathews and Sharrock 1996)

Ergot Distribution

The global distribution of *Claviceps* species is influenced by the presence of the grass hosts and also by the human factor With the exception of Brazil and West India at the end of the 19th century (Moller 1901, Hennings 1899), the information concerning the occurrence of tropical forest ergots is very scarce As far as I know, no primitive ergot with an undifferentiated sclerotium has been found in an African tropical forest However, this could be also explained by the periodical changes of climate in Africa that led to extinction of original rain forests The best information about geographical and host distribution is available for the ergots that colonize cereals and pasture grasses (Langdon 1952)

The distribution of ergot species throughout the world has several interesting features First, there is a striking difference in the number of ergot species colonizing the main subfamilies of Poaceae The colonization of chloridoid, arundinoid and pooid hosts is somewhat obscured by the vast occurrence of *C purpurea*, but in fact ergot occurrence outside panicoids is as rare as its' occurrence on cyperaceous hosts The only chloridoid-specialized species known so far are *C cynodontis* (Cynodon), *C yanagawaensis* (Zoysia), and *C cinerea* (Hilaria) and we added a new species, *C citrina*, found in Central Mexico on *Distichlis spicata* which was described in our laboratory (Paloutova et al 1998) Also the occurrence of *C purpurea* on chloridoids was documented (Campbell 1957, Gray et al 1990)

The pooid ergot is represented by *C purpurea* with Laurasian distribution and parasitic/endophytic *C phalaridis* endemic to Australia Both species colonize arundinoid species like *Danthonia* and have a wide host spectrum, although *C purpurea* is considered as an advanced species and *C phalaridis* a more primitive one *C litoralis* occurs on northern Japanese islands, and Sakhalin, on *Elymus* and *Hordeum* (Kawatani 1944) Some mycologists, however, doubt its' species status and consider it a variety of *C purpurea C grohii*, *C cyperi* and *C nigricans* that colonize sedges of the North temperate regions bear some morphologic and metabolic similarity to *C purpurea* (Mower and Hancock 1975)

In the genus *Claviceps*, the panicoid species prevail The most primitive species, *C orthocladae* which is found on Centothecae and *C flavella* and *C diadema* (undifferentiated sclerotium encompassing the flower parts, germination directly on the host) which are found on panicoids, occur only in the South America tropics, whereas none have been found in wet tropical and subtropical forests of Africa and South Asia None of the South America ergots were found in other regions except *for C paspali* that was

spread by human influence They evolved as a separate group In contrast, several species are found both in Africa and India and some also in Australia, so that the origin of these species is obscured by a relatively recent exchange No colonization of arundinoid grasses was observed in panicoid ergots

Claviceps Phylogenetic Tree

Following the study of ergot distribution and grass evolution, the species for phylogenetic analysis based on the comparison of internal transcribed spacers ITS1, 2 and 5 8S rDNA were collected Four species (Table 1) were obtained from the collections, while the remaining eight were isolated in our laboratory

The aligned DNA sequences were analyzed by the neighbor-joining method contained in Puzzle (Strimmer and von Haeseler 1996) and MEGA packages (Kumar et al 1994) *Fusarium sambucinum* sequence X65477 from GenBank (O'Donnell 1992) was used as an outgroup species The tree (Figure 3) shows two highly supported distinct groups

1 C viridis, C africana C gigantea, C phalaridis, Claviceps sp ex Sorghum and *Panicum* and *C citrina*

2 *C purpurea, C sulcata, C fusiformis, C grohii* and *C paspali*

The more basal species *C phalaridis, Claviceps sp ex Sorghum* and *Panicum, C citrina* and also *C paspali* from the second group shared a marked deletion in the ITS1 region, *C phalaridis* and *C citrina* exhibited another deletion in the ITS2 region, about 30 bp long These deletions were found not only in the *Fusarium* outgroup but to some extent also in another two sequences of nectriaceous fungi that we tested as suitable outgroup species and could be interpreted as a primitive character

A schematic restriction map of the sequences and the position of deletions are shown on Figure 4 As the tree was made only from one quarter of known *Claviceps* species, the relationships are

Table 1 Characterization of ergot species used in phylogeny tree

Species	Host	Origin	Alkaloids
C purpurea	Pooidae Arundinoidae	Europe America Africa, Australia	clavines ergopeptides
C fusiformis	*Pennisetum Cenchrus*	Africa	clavines
C sulcata	*Brachiaria*	Africa	+
C grohii	*Carex*	Canada	+
C paspali	*Paspalum*	South America	clavines lysergamides
C viridis	*Oplismenus*	India, Japan	
C africana	*Sorghum*	Africa	dihydroergosine
C gigantea	*Zea*	Mexico	dihydroclavines dihydrolysergamide
C phalaridis	Pooidae Arundinoidae	Australia	-
Claviceps sp	*Sorghum*	Japan	unknown
Claviceps sp	*Panicum maximum*	Paraguay	unknown
C citrina	*Distichlis*	Mexico	-

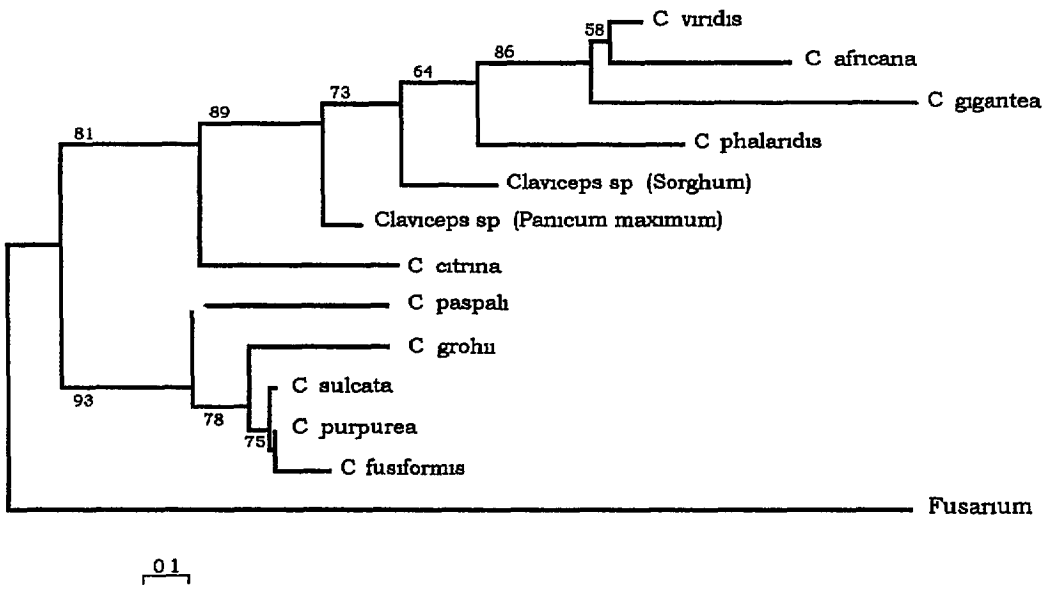

Figure 4 Neighbor joining tree for 12 *Claviceps species*
The gamma distances were computed according to Jukes and Cantor algorithm parameter a=0 32 with pairwise deletion option contained in the MEGA software NJ tree was constructed with 500x bootstrap The branch support BCL values (higher than 50%) are shown on the respective branches

far from absolute However, they confirm the existence of two main groups and the close relationship of South American species to the ergot ancestors The *Claviceps* tree corresponds to some extent to the scheme of grass evolution published by Mathews and Sharrock (1996) The first group corresponds to the grasses of PACC clade *C phalaridis* probably developed on arundinoids and survived in Australia because its pooid hosts were not colonized by *C purpurea* The grouping of Mexican andropogonoid parasite *C gigantea* with two *Sorghum* parasites from Africa confirms that the andropogonoids took their ergots with them during their radiation from South Asia

The second branch is quite puzzling Advanced cold resistant species distributed on the Northern Hemisphere and found on pooids and/or sedges are united with African savanna species strictly bound to panicoid hosts What is even more striking is the similarity of DNA sequences in this group—at least 97% among the most distant *C purpurea* and *C grohii* that speak for the relatively recent evolution. Rehner and Samuels (1995) observed 95 6% identity in 960 bp fragment of 28S rDNA of *C purpurea* and *C fusiformis*

The first ergot species probably appeared on the predecessors of PACC grasses in the warm and humid climate of

the South America region of former Gondwana This hypothesis is supported not only by the geographic distribution and molecular phylogeny of contemporary primitive *Claviceps* species but also by the lack of primitive tropical forest ergot species in Africa

Main spreading of ergot proceeded together with the expansion of panicoid species The change in world climate on the Cretaceous-Tertiary boundary led to reduction of tropical forests and increase in open grassy habitats The ergot speciation then continued independently on all continents of the former Gondwana The ergots of the first group probably originate from this event The exchange among African and Indian species was enabled at the same time as the spreading of andropogonoid grasses in the Oligocene

The contemporary ergots of the second group occurring in the colder climatic regions and semi-arid Africa probably evolved relatively recently to fill the ecological niches available which required the adaptation to survive low temperatures and/or dryness Where their ancestors came from is unclear and more species from these regions should be analyzed Tentatively we speculate about a Laurasian origin and relatively recent transfer to Africa

Alkaloids of Different *Claviceps* species

Until now, the qualitative analysis of alkaloid content has been made in only seven species of the 43 described, these being *C purpurea* (peptide alkaloids of the ergotamine or ergotoxine group), *C fusiformis* (agro-, elymo- and chanoclavine), *C paspali* (clavines and lysergic acid amides), *C gigantea* (dihydroclavines) (for review see Flieger et al 1997) The peptide alkaloid dihydroergosine is the main component in *C africana* and traces of agroclavine were found in *C sorghi (Mantle* 1968) Tanaka and Sugawa (1952) detected peptide alkaloids, ergometrine and agroclavine in sclerotia of *C imperatae* Porter et al (1974) detected ergometrine-related alkaloids in ergotized *Cynodon dactylon*, but the ergot species was not identified Taber and Vining (1960) found alkaloids in submerged cultures of *C maximensis* and an ergot from *Zizania* sp

In his descriptions of new Japanese ergot species (Tanda 1981, 1991) only assayed alkaloids in sclerotia using blue coloration with vanUrk's reagent and their toxicity to mice Some positive colorimetric reaction was found only in *C bothriochloae* (Tanda 1991) In *C yanagawaensis*, however, the mice toxicity was observed despite the absence of measurable alkaloids (Tanda 1981), suggesting the presence of other toxic secondary metabolites

An indirect proof of alkaloid production by *C cinerea* was the occurrence of abortions in cattle grazing *Hilaria mutica*, colonized by this ergot in North Mexico (Zenteno-Zevada 1958)

In our laboratory we study the alkaloid content of different *Claviceps* species as well as the intraspecific variation in alkaloids of *C purpurea* The type of alkaloids produced could also help in the elucidation of relatedness amongst species

C gigantea

We analyzed the alkaloids produced in sclerotia as well as shaken cultures of *C gigantea* Dihydroderivatives of the compound known from the usual clavine-lysergic acid pathway were found, suggesting that *C gigantea* alkaloid biosynthesis leads from dihydrochanoclavine through festuclavine and dihydrolysergol to dihydrolysergic acid The precursor of this pathway is not known It is interesting that dihydroergosine, the only dihydrogenated peptide alkaloid known, is produced by the related species *C africana* (Mantle 1968, Frederickson et al 1991) This indicates that in *C africana*, a similar pathway is operating

Other Claviceps species

Our preliminary analyses show that alkaloids are present in the shaken cultures of *C grohii* and *C sulcata* Walker (1957, 1970) found no alkaloids in *C phalaridis* sclerotia This interesting ergot species is noted by its endophytism on pooid and arundinoid grasses in Australia We analyzed the sclerotia and cultures in our laboratory on HPLC enabling the detection of alkaloids on the ppm level and the preliminary results confirmed their absence as well as the presence of group of unknown metabolites We obtained, after the analysis, sclerotia of a new species of Mexican ergot *C citrina* from *D spicata*

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Host × Pathogen × Environment Interaction in Sorghum Ergot Disease

N W McLaren

Abstract

Weather is a primary determinant of ergot incidence and severity Low pre-flowering temperatures predispose florets to infection by reducing pollen viability Reduced pollen viability extends the susceptible period for infection which is from flower opening and early anthesis to pollination and fertilization Genotypes differ in the extent of predisposition and hybrids with cold tolerance and an ability to produce viable pollen despite reduced temperatures have been identified Similarly, weather during early anthesis affects ergot severity Optimum disease occurs at 19°C and high humidity with little or no infection at temperatures exceeding 28°C Secondary sporulation and sclerotium development are also affected by prevailing weather conditions The relationship between weather and disease can be used to modify production practices to reduce the risk of encountering ergot favorable conditions Planning of flowering dates based on long- or medium-term weather data for a specific locality has proved successful in reducing the risk of ergot in South Africa Selection of germplasm under ergot-favorable conditions has yielded more resistant lines and hybrids which, when grown commercially, reduce the risk of the disease

Numerous examples of discontinuities between optimum conditions for growth of a plant and the growth and pathogenic development of disease causal organisms have been described Optimizing growing conditions for the host plant by means of management practices can result in concomitant sub-optimal conditions for pathogen development and visa versa Implementation of control strategies based on such discontinuities, however, requires a thorough knowledge of both host and pathogen responses to their respective environments Variation in host tolerance levels, in particular, requires quantification so as to determine the crossover points at which host or pathogen acquire the respective advantage of prevailing environmental conditions

Host predisposition is defined as the tendency of nongenetic factors, acting prior to infection, to affect a plant's susceptibility to disease (Yarwood 1959) Predisposition of male-normal sorghum [*Sorghum bicolor* (L) Moench] to infection by *Claviceps africana* Frederickson, Mantle & de Milliano has been a major area for research at the Agricultural Research Council's - Grain Crops Institute in South Africa (McLaren 1992, McLaren and Wehner 1992, McLaren 1997) Avoiding predisposing environmental conditions and identifying hybrids and

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lines tolerant to predisposing factors has formed the basis of ergot research and control Similarly, consideration of environmental factors which favor pathogen growth, infection and dispersal have led to changes in production practices so as to reduce the risk of conditions favorable for the pathogen during the critical stage of plant development In this paper, emphasis will be on these two aspects of the host × pathogen × environment interaction

Disease Favorable Environment

Ergot is a disease of unfertilized ovaries The critical period for infection of sorghum by the ergot pathogen is from flower opening and the onset of anthesis until effective pollination and fertilization make pistils escape or resist further infection (Futrell and Webster 1965, Sangitrao 1982, Musabyimana et al 1995) Infection occurs primarily through the stigma, and infection hyphae essentially follow the same path as the pollen tube (Frederickson and Mantle 1988) Infection or fertilization depends on environmental conditions, which determine whether infection hyphae or pollen tubes obtain the competitive advantage for the ovary Under optimum daily temperatures for host development (>28°C) and night temperatures >17°C (depending on host adaptation) pollen germinates within 30 min and fertilization occurs within 2 to 12 h (Stephens and Quinby 1934, Artchwager and McGuire 1949) In contrast, at similar temperatures conidia require 8 to 12 hours for germination on the stigma and 36-48 hours to reach the ovary (Sangitrao 1982, Frederickson and Mantle 1988, Frederickson 1990) At cooler temperatures pollen release and growth become retarded Conversely, pathogen activity increases In vitro studies have indicated the optimum temperature for germination and growth of the pathogen to be 19-20°C (Bandyopadhyay et al 1996, Frederickson 1990, McLaren, unpublished (Figure 1)) No or limited growth was recorded above 30°C

Cool (19±1°C), wet, cloudy weather during early anthesis favors rapid development and spread of the disease (Sundaram 1971) Kulkarni (1942) reported most severe infections within the range of 21 to 31°C when humidity was high Most damage occurred in fields, which flowered during the cooler periods Frederickson (1994) found that ergot severities were significantly higher at 20°C and 25°C than at 30°C At 30°C, ergot severities of 0 9% were recorded, as opposed to 26% at 20°C and 14% at 25°C The latent period for infection was also significantly shorter at lower temperatures

Cloudiness during anthesis promotes disease development (Anahosur and Patil 1982), probably due to delayed anther dehiscence and pollen deposition and activity under these conditions (Quinby 1958) Futrell and Webster (1966) reported that near 100% RH for 24 h during early flowering was optimal for infection of a male-sterile line Sangitrao and Bade (1979) believed that when humidity is high, rainfall is not essential for ergot development Anahosur and Patil (1982) concluded that temperatures of 19 to 21°C with 67-84% RH following panicle emergence, were sufficient for infection in male-sterile sorghum

The above-mentioned conditions are similar to those defined by McLaren and

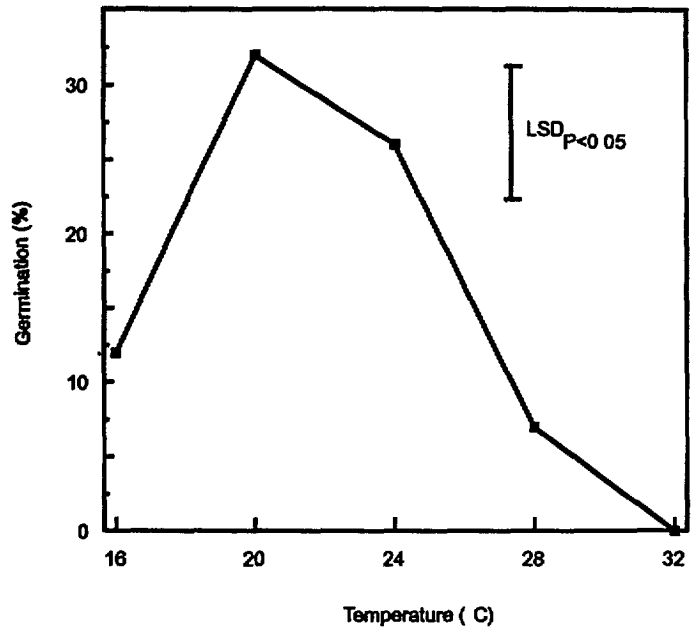

Figure 1 **Effect of temperature on germination of *Claviceps africana* macroconidia on water agar after 12 h**

Wehner (1990), who determined the optimum temperature for disease development at 19 5°C and the upper maximum limit at 28°C above which disease severity was negligible (Figure 2) They, too, found an inverse relationship with humidity, maximum disease tending to occur at high humidity In their studies, however, they recorded a strongly negative intercorrelation between daily maximum temperature and rainfall (r = 0 71), and sunshine hours (r = 0 86), and suggested that maximum temperature was a suitable indicator of disease favorable conditions under South African conditions

Predisposition

Futrell and Webster (1965) suggested that any factor that prolongs the period from flower opening until fertilization could increase the risk of 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 that night temperatures of 13°C or less during meiosis could induce male sterility in sorghum Brooking (1976) estimated the critical stage for cold induced sterility to be 2 to 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 In-

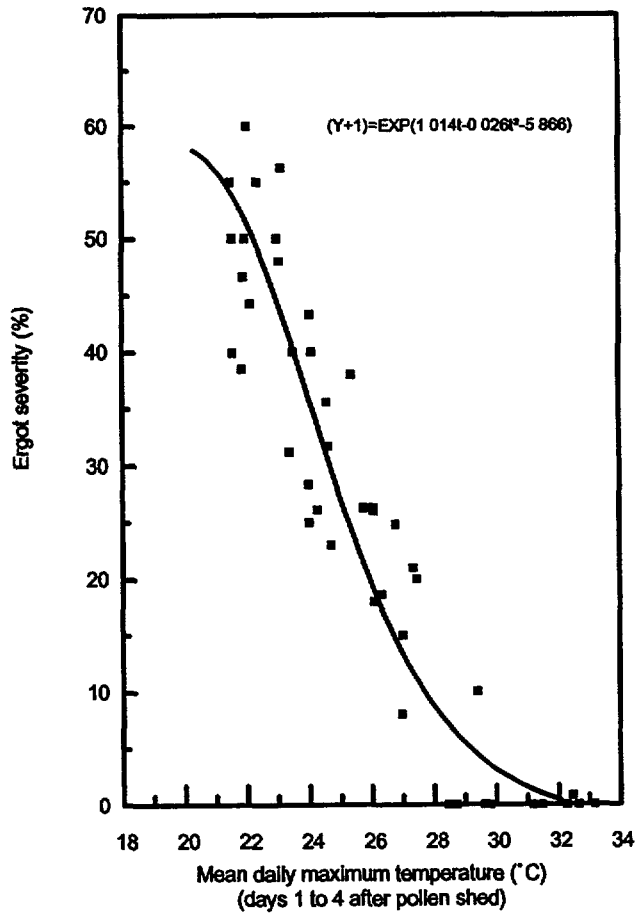

Figure 2 **Relationship between ergot severity and mean daily minimum temperature at Potchefstroom 1986/87, 1987/88, 1988/89 and Kempton Park 1988/89 in plots not subjected to preflowering cold stress (>16°C) (McLaren and Wehner 1990)**

florescences showing low temperature-induced sterility developed anthers that were exserted normally at anthesis 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, pollen from plants not subjected to cold stress was densely cytoplasmic and packed with starch grains

McLaren and Wehner (1992) demonstrated the relationship between cold-induced sterility and ergot susceptibility In field trials with male-normal sorghum hybrids, night temperatures <12°C at 3 to 4 weeks prior to flowering increased susceptibility to ergot to the equivalent of that of a male-sterile line (Figure 3) Seed set in noninoculated heads under pollination bags was also reduced, suggesting that increased susceptibility was the result of low temperature-induced sterility Er-

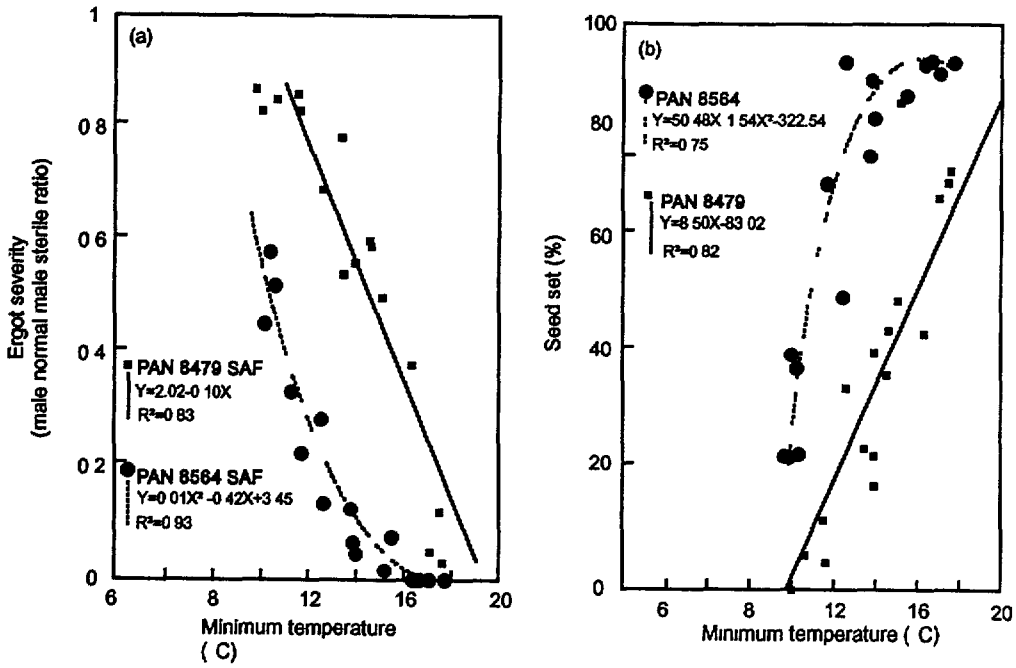

Figure 3 **Relationship between mean minimum temperature (23-27 days pre-flowering) and ergot severity in two sorghum hybrids expressed as a ratio of ergot development in a male sterile line (a) and seed set (b)**

got incidence and seed set were inversely correlated (r = -0 92) Hybrids differed in their ability to tolerate preflowering cold stress, with PAN8564 maintaining seed set and ergot resistance until 12°C In contrast, a progressive increase in sterility and concomitant increase in ergot was recorded in PAN8479 This result is similar to cold-induced sterility recorded by Brooking (1979), who suggested that the linear response of some genotypes to temperature reduction indicate that sterility is a quantitative response and not a qualitative one occurring below some critical temperature

Molefe (1975) reported ergot in Botswana in panicles that flowered when humidity was high, minimum temperature was 14°C, and maximum temperature was 27°C The low minimum temperature may have been a predisposition factor favoring disease development Similarly, Sangitrao and Bade (1979) found mini-

mum daily temperatures of 13 to 18°C and 76 to 84% RH most favorable for ergot development

Studies carried out by McLaren (unpublished) using experimental hybrids showed that those with cold tolerance in relation to the production of viable pollen (determined by an iodine starch test) were able to escape disease despite high ergot potentials (Figure 4) Conversely, those with low cold tolerance and hence low pollen viability (P22/96, PAN8494) were more predisposed to infection and yielded high disease severities despite low ergot potentials Pepper and Prine (1972) shaded sorghum with black plastic fabric (25% light) and found two critical periods that adversely affected grain yield components One of these was from the pre-

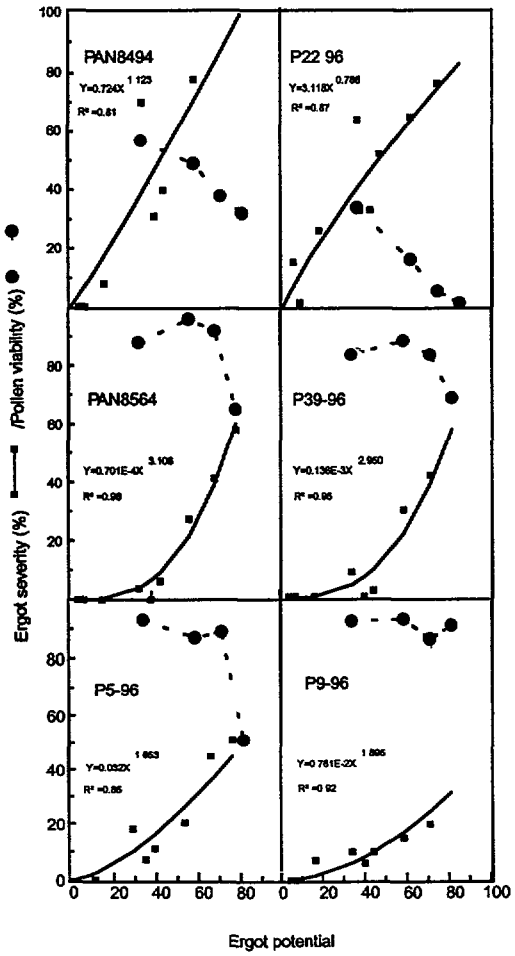

Figure 4 **Relationship between ergot potential and observed ergot severity and concomitant changes in pollen viability in experimental sorghum hybrids**

boot vegetative stage when the last leaf was just visible above the whorl, to full panicle expansion or 50% anthesis (approximately 2 weeks preceding anthesis) Shading during these times resulted in reduced numbers of seeds per panicle Low radiation-induced sterility may, therefore, be an additional factor to be considered in ergot epidemiology and may explain significant increases in ergot severity during prolonged cloudy weather

Inoculum Production

Bandyopadhyay et al (1990) showed that temperature and relative humidity affected honeydew formation and spore production, but not sphacelia development Temperatures from 14-28°C combined with RH above 90% for 12-16 h day^{1} favored conidial production and hence pathogen spread In contrast, at 28-35°C and RH <90% for 22 h day^{1} sphacelia developed into sclerotia and honeydew and spore production were suppressed Manzarpour (1985) found that most conidia germinated to form microconidia at 24-30°C, whereas at higher temperatures they germinated by forming a hyphal germ tube

Frederickson et al (1993) demonstrated how rapid, epidemic development of *C africana* could result from secondary sporulation of the pathogen They considered secondary sporulation to be the primary epidemiological agent within their experimental area Secondary sporulation is most profuse at 28/23°C, followed by 24/14°C and least at 35/28°C (Bandyopadhyay et al 1990) Frederickson et al (1989, 1993) studied diurnal patterns of secondary conidia concentrations and found the greatest occurrence at nightfall, coinciding with a sharp rise in RH and fall in temperature Secondary sporulation did not occur following several hot days in succession

The effect of temperature on germination of sclerotia has received limited attention due to difficulties obtaining sufficiently high germination frequencies Frederickson (1990) obtained 5% germination in *C africana* after 16 weeks of incubation in sterile sand at temperatures ranging from 4 to 28°C and subsequently, 10% after 4 weeks at diurnal oscillations of 10 to 28°C Mantle (1968) incubated sclerotia of a Nigerian isolate at 24, 27 and 30°C, achieving initial germination after 4 weeks at 27°C

Germination of sclerotia of *C sorghi* was achieved after 5 weeks incubation on moist sand at 24°C (Frederickson et al 1991) Sangitrao (1982) found that sclerotia of *C sorghi* germinated at 5 to 55°C, with 20 to 30°C being the optimum range

Environmental effects may be secondary by way of their effects on saprophytes that affect sclerotial development Sclerotia develop poorly in a rainy season due to the growth of *Cerebella* sp which suppresses their development, whereas dry weather after infection allowed formation of less contaminated, mature sclerotia (Futrell and Webster 1966) Sangitrao et al (1979) found that molds on sclerotia under wet conditions result in the elongated outer portion of the sclerotia becoming thin and papery and ultimately weathering They also found that the portion of the sclerotia protected by glumes does not weather and suggested that this

plays an important role in the perpetuation of the pathogen

Implications of the Host × Pathogen × Environment Interaction in Disease Control

The purpose of disease control is to prevent disease damage from exceeding the level at which profit or required yield is diminished A number of management strategies have been employed to exploit the host × pathogen × environment relationships to control ergot and reduce the risk of yield losses

Sowing Dates

In India, early sowing which ensures that sorghum flowers during the warmer and drier part of the growing season reduces the risk of ergot (Singh 1964, Desai et al 1979, Sangitrao et al 1979, Anahosur and Patil 1982) Early flowering crops are also less likely to be subjected to low temperatures during microsporogenesis and thus escape being predisposed to infection McLaren (1996) used the criteria maximum daily temperature of 28°C (temperature above which disease incidence is minimal) and 12°C minimum temperature (temperature below which total sterility or maximum predisposition occurs) to identify high- and low-risk flowering periods, based on long- and medium-term weather data for various production areas in South Africa (Figure 5) Also indicated in Figure 5 are predicted ergot severities based on weather variables during flowering (McLaren and Flett in press) using methodology indicated below (Equation 1) Generally, flowering before the end of January in South Africa results in low risk of ergot-favorable disease conditions

Predicting Disease Severity

McLaren and Flett (in press) developed a model to predict ergot severity (sensu McLaren, 1992) based on weather variables as follows

$$Y=(aX_1\ )\ +EXP(bX_2_+cX_2\ +d)\ +\ (e*X_3)\ \text{(Eq 1)}$$

where Y=expected mean ergot severity

X_1=mean minimum temperature (°C), 23-27 days before flowering

X_2 =mean daily maximum temperature (°C), 1-5 days after flowering

X_3=mean daily maximum humidity (°C), 1-5 days after flowering

and a, b, c, d and e are regression coefficients

The model was calibrated to predict ergot potential i e , expected ergot severity in a sorghum nursery with a broad genetic base subjected to artificial inoculation (inoculum sprayed at weekly intervals with a spore suspension in water adjusted to ca 10^4 spores ml^{-1}) Application of the model during the 1996/97 season yielded a high "index of agreement" (Wilcox 1982) (d = 0 846, Table 1) These results indicate that ergot severity can be accurately predicted based on maximum and minimum temperature and maximum relative humidity during critical phases of host development The model will, however, need to be recalibrated for specific germplasm, applications and risk analysis

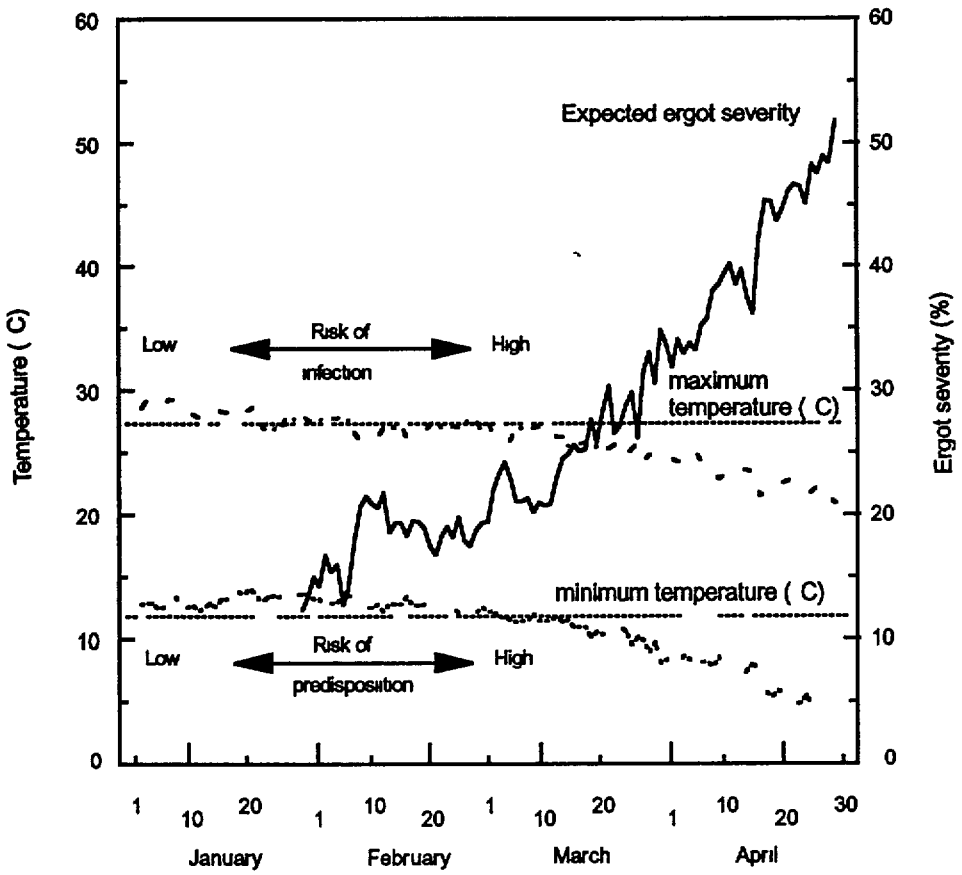

Figure 5 Example of the use of long-term maximum and minimum temperatures for the estimation of high and low risk ergot periods (Bethlehem, South Africa) Expected ergot severity is calculated according to Equation 1 (see text)

Table 1 Observed and predicted ergot severities associated with flowering dates at two localities in South Africa during 1997 illustrating the accuracy of ergot prediction based on weather variables

Flowering date	Locality	Maximum temperature (C)	Minimum temperature (C)	Maximum RH (%)	Observed ergot severity (%)	Predicted ergot severity (%)
17 February	Potchefstroom	32 4	17 6	76.2	7 3	7 7
18 February	Bethlehem	30 2	15 8	93 6	16 5	24 9
25 February	Bethlehem	23 0	16 0	96 0	31 1	36 4
4 March	Potchefstroom	25 2	16 8	91 8	34 3	29 7
10 March	Potchefstroom	23 8	16 2	87 2	40 1	31 6
14 March	Bethlehem	21 3	13 6	97 0	58 6	45 6
17 March	Potchefstroom	23 8	17 5	86 2	29 3	27 0
25 March	Bethlehem	19.3	12.7	96.0	61.3	48.5
Index of agreement (Wilcox 1982					d=0.846	

of various flowering dates or seed production localities If applied to natural epidemics, an inoculum algorithm will need to be developed The model is currently being used effectively in the evaluation of lines and cultivars for ergot resistance (McLaren 1992, Figure 4), but no doubt could be adapted and applied elsewhere, notably to determine the need for fungicide applications

Germplasm Evaluation for Resistance

Sundaram (1980) suggested that in order to compare ergot severities in breeding lines, particularly those differing in maturities, 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 5 days after pollen shed significantly affect ergot incidence (McLaren and Wehner 1990, 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 (1997) demonstrated variation in production of viable pollen in sorghum lines subsequent to pre-flowering cold stress Whereas most lines yielded <40% viable pollen with a pre-flowering temperature <14°C, some selections yielded in excess of 70% viable pollen Similar responses are reflected in Figure 4

McLaren (1992) used regression analyses to quantify resistance of sorghum genotypes to ergot Sowing nurseries at two locations over a range of sowing dates created temperature gradients At flowering individual heads were marked with the date of anthesis and artificial inoculation Ten heads of each genotype 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 associated with each inoculation date for each genotype 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 More recently, the above model (Equation 1) which determines ergot potential based on weather variables has been applied

Non-linear regression analysis, using the model $Y=ax^b$ (where Y=observed disease incidence, x=disease potential, and a and b are regression coefficients) was used to determine the relationship between ergot potential associated with different inoculation dates and observed disease incidence within genotypes Regression 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 (Figure 4) Genotypes in the latter group differed

with respect to resistance breakdown points Rearrangement of the regression model also enabled the subsequent rate of resistance breakdown at any point to be calculated An advantage of this method was that ergot severity in sorghum genotypes that flowered at different times could be statistically compared

Conclusions

Weather during early anthesis and during meiosis prior to flowering are the primary determinants of ergot severity The response of sorghum to weather differs according to tolerance of pre-flowering cold stress and flowering behavior during anthesis These factors have become major selection criteria in the development of hybrids and varieties with ergot resistance in South Africa However, detailed studies of mechanisms of escape resistance in the host × pathogen × environment relationship are still required Brooking (1976) stated that pre-flowering low temperatures do not affect female sterility Personal observations in fields in South Africa suggest that studies on cold-induced female sterility or reduced male x female compatibility under cold stress are warranted Similarly, studies on a wide range of germplasm are required to determine the limits of predisposition Currently, 12°C is the lowest tolerance limit recorded to date (McLaren and Wehner 1992) The possibility of selecting for lower temperature tolerance needs investigation

Other environmental factors, which may significantly affect ergot incidence and severity, have been largely ignored Chinnadurai (1971) found that the incidence of ergot of sorghum in India was significantly increased with increased nitrogen applications Potassium decreased disease incidence Phosphorus did not affect disease incidence but stimulated honeydew secretion Chinnadurai (1972) found that trace elements affected growth and sporulation of *C sorghi* Lack of manganese caused the most reduction in mycelial growth, iron was essential for sporulation and molybdenum was inhibitory to both growth and sporulation Similar results have been recorded with ergot caused by *C purpurea* and *C fusiformis* Herbicides have also been observed to promote infection of rye grass by *C purpurea* (Macek and Milevoj 1978) Thus, although many aspects of the host × pathogen × environment interaction have been studied and exploited in disease control, further studies of the relationships may yield improved disease control strategies which will reduce the risk of, and losses due to ergot

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The Lifecycle, Spread and Survival of the Sorghum Ergot Pathogens

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Abstract

Knowledge of the biology of a pathogen as described in its lifecycle enables the effective targeting of control measures The lifecycles of the three sorghum ergot pathogens are compared to the lifecycle of ***Claviceps purpurea*** *and to one another The rapid build-up of disease in the field is attributed to the polycyclic nature of the asexual stage Windborne secondary conidia of* ***Claviceps africana*** *formed under high relative humidity in the field, are the chief means of spread of ergot in Zimbabwe Experiments show that the secondary conidia can travel at least up to 300 m and that even a small initial concentration of 10 secondary conidia per cubic meter of air is enough to start an epidemic Later in the epidemic concentrations may reach as high as 350 per cubic meter In unprotected A-lines in Zimbabwe all 38 rows of a 30 meter plot became infected after 1 cycle of honeydew production Although severities declined rapidly from source, only 2 more cycles were required to eliminate obvious disease gradients so that more uniform final severities of 50-70% were obtained Overall apparent disease rates (r) reached 0 2 per unit per day in A-lines varying between 0 14 and 0 29 in individual A-lines and reaching as high as 0 58 in single plots of some A-lines These values compare well to those obtained in epidemics of the potato late blight and wheat stem rust pathogens At the end of the crop season with the cessation of rains and the maturity of the crop, conidial production ceases The pathogens have to survive between crops in some form Perennation of the sorghum ergot pathogens as conidia on seed and crop debris or in sphacelia or sclerotia may be possible The perfect stages of the 3 pathogens are contrasted and it is suggested that the teleomorph of* ***C africana*** *does not play a major role in survival or dissemination Alternate hosts such as other* ***Sorghum*** *species are probably much more important in perennation, survival and spread than are any other grasses Reports of alternate hosts in the literature are confusing and contradictory*

Three distinct species of *Claviceps* have been described as pathogens of sorghum 1) *Claviceps sorghi* Kulkarni, Seshadri and Hegde (1976) as found in India and other parts of Asia, 2) *Claviceps africana* Frederickson, Mantle and de Milliano (1991), the pathogen of Africa, Australia, the Americas and Thailand and Japan, 3) *Claviceps species* also of Japan, the formal description to be published by Tsukiboshi (personal communication) The life-cycle(s) of the three pathogens is the culmination of their strategies for spread and survival and is important for considerations on disease control

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Claviceps species are important ovary pathogens of cereal crops and grasses Therefore one turn of the lifecycle depends upon the success of host infection at a quite specific crop stage i e the ovary at flowering, and of subsequent perennation in the absence of the host between cropping seasons The lifecycle of *C purpurea*, found in any text book of plant pathology, is the most familiar, and the best investigated and substantiated of all the ergot pathogens Because of this, the lifecycle tends to be rather over emphasized as a yardstick for sorghum ergots Whilst providing a useful model for the lifecycle of sorghum ergot pathogens, it should only be used as a analogy because of important differences in pathology The lifecycle of *C purpurea* is as follows *C purpurea*, a pathogen of rye, wheat and barley in temperate climes, overwinters as sclerotia in seed or on the ground In the spring, sclerotia germinate producing numerous stomata with capitulae Each perithecium bears many asci containing the ascospores, constituting the primary inoculum Infection of host stigmas by windborne ascospores results in the invasion of the ovary by fungal hyphae and development of the fungal sphacelium About a week later honeydew, containing millions of infectious conidia, is produced Dissemination of honeydew to uninfected flowers by insects and rain enables further disease spread By the time honeydew secretion ceases, the purple/black, hard, elongate sclerotia are very obvious on the infected spike Sclerotia are dislodged at around harvest and fall to the ground or become mixed with seed, remaining dormant over winter, and so the cycle is completed

For the sorghum ergot pathogens, the best starting point is with the portion of the lifecycle that is most well defined and studied, and well proven i e the imperfect stage The most observant of us would, in the field, first notice *C africana* infections as sphacelia emerging from between host glumes at about 6 days post-infection More usually, for all 3 of the sorghum ergot pathogens, we see the honeydew at about 7 days post-infection, dripping from infected florets This early honeydew is usually initially thin, transparent and devoid of spores, becoming progressively more sticky, colored honey-yellow to brown or pinkish and opaque with conidia Alternatively honeydew may remain of thin viscosity and droplets of honeydew may become superficially white at the surface, even where honeydew drips onto soil This feature, secondary conidiation, is characteristic of natural *C africana* infections It may occur less readily with *C sorghi* but never with *C species* When honeydew has this characteristic the oblong to oval macroconidia at the surface have germinated to produce secondary conidia Smeared on a slide, or viewed with Cryo-SEM, the characteristic pear-shaped secondary spores, borne on long sterigma-like processes, can be recognized This phenomenon would not be expected by analogy with *C purpurea*

In one season epiphytotics of disease may quickly occur under favorable weather conditions, when conidia are transmitted to uninfected flowers and later maturing tillers i e this part of the cycle of infection, asexual spore production, infection, is polycylic *C purpurea* conidia are disseminated by rainsplash

and insects (Ingold 1971, Moreno et al 1970), leading to limited disease development (epidemics are very unusual) Conidia of *C sorghi* are also disseminated by rainsplash for limited distances (Bandyopadhyay et al 1991) but there is no evidence of insect transmission Instead, the secondary conidia introduce a new, and highly significant, dimension to the lifecycle secondary conidia become airborne and are dispersed by wind The presence of secondary conidia in air and their relationship with infection has been investigated for the pathogen, *C africana*, under natural conditions in Zimbabwe during several crop seasons (Frederickson et al 1989, 1993) Their presence in air shows a diurnal pattern, their concentration gradually increasing throughout the day to reach a peak around nightfall, concurrent with the rise in relative humidity This may reduce desiccation of the thin-walled, hyaline spores Initial low incidences and severities of ergot follow low concentrations of 10/m^3/hr secondary conidia in air, and disease build-up to high severity follows higher concentrations of up to 350/m^3/hr (Frederickson et al 1989, 1993) Frederickson et al (1993) detected secondary conidia at 15 m from source, Frederickson (unpublished) trapped secondary conidia at 30 m from source and secondary conidia can probably travel much further, by inference from the presence of infections up to 300 m away in adjacent fields (Frederickson et al 1993) Secondary conidiation probably accounts for the rapidity with which even small initial infections give rise to large scale epiphytotics in *C africana* and to outbreaks in areas previously unplanted to sorghum

To put the potential for spread of *C africana* in perspective, in one experiment with *C africana* in Zimbabwe (Frederickson et al 1993) a central plot of sorghum was inoculated and began to provide inoculum (secondary conidia) to surrounding A-line plots at 11 days post-inoculation Nine days later (day 20) the first outbreaks were visible in surrounding plots By day 26, still within 1 cycle, infection was fairly well distributed over the entire experimental area Represented graphically, the overall rate of disease spread, measured as apparent infection rate (r) (Van der Plank 1963) reached 0 2 per unit per day Individual values for different A-lines ranged from 0 14 to 0 29, with values as high as 0 48 and 0 58 in individual plots These rates compare favorably to those measured in epidemics of the two quite devastating pathogens, potato late blight (*Phytopthora infestans*), and wheat stem rust (*Puccinia graminis tritici*) For potato blight r, 0 42, in a highly susceptible cultivar and 0 11 in a less susceptible cultivar The wheat stem rust pathogen achieved r, 0 41 (Van der Plank 1963) *Claviceps africana* is clearly in the same league

Towards the end of the crop season, the dormant tissue is sclerotial, rather than sphacelial Spore production ceases and a more resilient, solid body is found in the floral cavity *C sorghi* and *C species* sclerotia are usually quite visible as elongate structures protruding from infected florets However the true sclerotia of *C africana* are almost entirely enclosed within the host glumes because parasitic bodies of this species are smaller and globose in shape Anything visible will almost certainly represent the sphacelial

part of the structure Sclerotia are held quite firmly in the floral cavity and are not likely to be dislodged easily Pathogens will thus only remain in the field at harvest in the form of sphacelia or sclerotia (containing conidia) in discarded, infected panicles, honeydew on discarded panicles and other trash, and honeydew on the soil surface Threshing may cause the mixing of sphacelia and sclerotia with seed, harvested seed may be coated with honeydew

Therefore the number of options for perennation may be manifold However most are as yet unproven as generating viable primary inoculum in the new season The most usual assumption by analogy to *C purpurea* is that ascosporic infection must play the major role Ascosporic infection by *C purpurea* is common in nature and has been used repeatedly by researchers to initiate infections in wheat experiments (Mantle & Shaw 1976) Germination of sclerotia and ascosporic infection, occur readily, simply by burying sclerotia from the previous season in pots of soil between plants Tsukiboshi (personal communication) also reports the 50% germination of *C species* sclerotia after 1 month incubation in moist sand However sclerotia of *C sorghi* and *C africana* are not so compliant After many unsuccessful attempts, Frederickson et al (1991) finally achieved germination of *C africana* sclerotia in vitro After 1 month, 10% of sclerotia, previously maintained at winter room temperatures (20-25°C) at Matopos, Zimbabwe, germinated when incubated in pots at the ambient outdoor temperatures of the growing season (10-28°C) After 6 weeks, 80% had germinated to produce stromata with capitulae, but only a few of these matured to produce asci *C sorghi* sclerotial germination occurs only slightly more readily but with more consistency Both Sangitrao (1982) and Frederickson et al (1991) obtained germination of sclerotia in the temperature range of 20-30°C after 5 weeks in moist sand Ascosporic infection by *C africana* in Africa is probably extremely rare in nature, in fact there is no evidence for the natural occurrence of the sexual stage at all The role of airborne dispersal by ascospores has been assumed by the secondary conidia at another stage in the lifecycle altogether and secondary conidia may also be involved in perennation

In Africa, many researchers have routinely used *C africana* conidia from panicles, sphacelia and sclerotia, stored at "winter" room temperatures, as inoculum in infection experiments 9-10 months later (Futrell & Webster 1966, Mower et al 1973, Frederickson et al 1991, 1993) Initial severities following from the repeated spraying of such inoculum onto panicles are very low, usually only a few sphacelia result However a small initial source is sufficient to start an epiphytotic, as shown earlier In nature viable conidia remaining after winter would have to reach flowering panicles from ground level, whether as honeydew on soil, trash or seeds, or associated with sphacelia or sclerotia Germination of macroconidia to form airborne secondary conidia may permit this and merits investigation, as does the survival of conidia on all these vehicles

One final possibility for perennation is the involvement of alternate hosts The al-

ternate host could be involved at two possible stages Primary inoculum may infect an earlier- maturing host and the resultant sphacelial honeydew could infect later-maturing sorghum This is the usual situation for *C purpurea in England* Blackgrass (*Alopecurus myosuroides*) is infected by *C purpurea* ascospores in advance of wheat anthesis (Mantle & Shaw 1976) and the honeydew subsequently infects wheat Secondly, honeydew from sorghum could infect the alternate host, which could then act as a reservoir of infection The continual flowering of *Sorghum halapense* in Australia (Ryley, personal communication) would seem to permit the latter Most tests for alternate hosts have at best been host range tests, involving the inoculation of putative alternate host with honeydew from sorghum or vice versa to prove susceptibility The literature presents a confusing array of examples, for many species have been investigated with conflicting results For example *Panicum maximum* was positive as an alternate host of *C africana* in Thailand (Boon-long 1992) but not of *C africana* in Zimbabwe (Frederickson 1990) The only consistent involvement appears to be with wild sorghum species as alternate hosts, and possibly pearl millet (*Pennisetum glaucum*) Caution is needed for many ergot species naturally infect wild grasses (Loveless 1964) and so honeydew on a grass alone is not evidence for infection by the sorghum ergot pathogens, even if adjacent to the sorghum field Conidial characteristics must be measured (Loveless 1964) and Koch's postulates attempted

The lifecycle of an organism represents a summary of its biological strategy for spread and survival Clearly if we hope to exploit our knowledge of ergot pathogen biology to effect control, there are still many questions to be addressed, and gaps in our knowledge of the lifecycle to close, before we can do so

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Putative Control of Ergot Disease Epidemics in Hybrid Sorghum Production through the Inhibition of Secondary Sporulation by *Claviceps africana*.

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Abstract

A strategy is proposed for limiting epidemics of ergot disease in the production of F_1 hybrid sorghum seed Certain sweet sorghums inhibit the secondary sporulation which occurs on exuded ergot honeydew by supplying the parasite with excess sucrose which is transformed to unique free oligosaccharides with spore-germination inhibiting properties Therefore the strategy seeks to incorporate a sweet character into A lines for hybrid seed production to restrict secondary disease spread

The African ergot pathogen of sorghum, *Claviceps africana* and its anamorph *Sphacelia sorghi*, can cause significant economic losses in the production of F_1 hybrid seed Recent spread of the pathogen in the Americas and Australia has focused attention on control measures

Assuming that secondary sporulation of *Sphacelia sorghi* macrospores *in vivo* is the most important factor in creating epidemics of ergot disease in sorghum (Frederickson et al 1989), it is necessary to consider two particular biological features

The first is probably common to all ergot fungi, and concerns the way in which each pathogen utilizes the sucrose supplied by the host plant in phloem translocate to the diseased ovaries There seems to be a strong nutritional preference for the glucose moiety of sucrose, as opposed to the fructose If fructose is as growth inhibitory in all ergot fungi as it is in the best studied example, *Claviceps purpurea*, it is readily understood how the pathogen has developed a means of glucose release which at the same time avoids creating increased concentration of free fructose Hence, the fructofuranosidase of *C purpurea* provides an ideal way of making glucose available for respiration and as a source of biosynthetic carbon skeletons It does so by forming a series of oligosaccharides, first by transfer of a fructose residue to a sucrose molecule and then by addition of further fructose units and glucose is thereby obtained as a free monosaccharide These oligosaccharides might temporarily inhibit germination of spores while they are embedded in honeydew Indeed any such inhibition might only be advantageous to the fungus since there is no point in germination occurring until conidia in much diluted honeydew are transferred to other uninfected flowers by rainsplash or head to head contact in hu-

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mid conditions Therefore, no special properties have been ascribed to the oligosaccharides, they just apparently provide a convenient way of maintaining a low molar concentration of unwanted fructose

The second biological feature is the differential parasitic behavior expressed by the sphacelial stages of the sorghum ergot pathogens of India (*S sorghi*) and Africa (*C africana*) Their *S sorghi* anamorphs are mycologically so similar that it is impractical to differentiate them by microscopic examination of the spore-laden honeydew Nevertheless, they are genotypically distinct in so far as, for example, when allowed to develop parasitically on the same male-sterile sorghum host, the honeydew from *C africana* produced abundant secondary sporulation whereas none was evident on the honeydew from *C sorghi* (Frederickson et al 1991) Further parasitic development of the mycelia allows organization of morphologically distinctive sclerotia and the accumulation of dihydrogenated ergot alkaloids (notably dihydroergosine) only in *C africana* Subsequently, there may be the elaboration of the distinctly different teleomorphs, though this may be extremely difficult to achieve with *C africana* in experimental conditions and there is uncertainty concerning the extent to which this occurs naturally Consequently, to make a rather simple combination of these two features was the most prudent (Frederickson et al 1991) in attempting to explain why secondary sporulation was so much more evident *in vivo* in infections of sorghum by *C africana* However, it did allow the initially plausible deduction that the extent of expression of secondary sporulation *in vivo* might change (reduce) if a sweet stem sorghum were the host in which the pathogen had access to more abundant sucrose This, therefore, is the origin of our first-declared strategy to utilize a sweet sorghum character in the A-line of F_1 hybrid sorghum seed production (Hassan et al 1995) The most that could initially have been expected in terms of mechanism was that this honeydew would contain a higher than usual concentration of untransformed sucrose which, by an osmotic effect, might suppress secondary sporulation

The first step in testing the feasibility of this strategy was to explore responses of sweet sorghum accessions under field conditions in South Africa In two successive years (1994-1995), this was achieved, though with great difficulty due to the widespread prevailing droughts in Southern Africa Several sweet sorghums were selected which consistently failed to support overt secondary sporulation on the honeydew exuded from infected florets, particularly during warm dry conditions

As an indication of what is meant by sweetness in sorghum in the flowering stage, analysis in April 1997 of juice squeezed from the last internode of accessions grown in South Africa frequently contained at least 10% sugar, mainly in the form of sucrose How this related to the concentration of phloem translocates available to the pathogen is unclear, but the analysis at least indicates liberal availability of sugar

Concomitantly, the sugars in honeydew exuded from infected grain sorghums and from sweet sorghums were studied in greater depth than had been previously attempted (Frederickson 1990) It gradually became clear that the oligosaccharides arising from *C africana's* parasitism of sorghum were not identical to those produced in an analogous way by *C purpurea* parasitising rye or wheat, the latter having been elucidated to considerable depth of enzymology (Dickerson 1972) Nor did the sorghum ergot honeydew oligosaccharides readily match the complex saccharides described by Mower et al (1973) for the same organism (Frederickson et al 1991) obtained from Nigeria It was therefore decided to explore *C africana* honeydew from sorghum without any preconceived expectations except that whatever occurs must be derived enzymologically from the sucrose supplied to the pathogen by the host in leaked phloem translocate Preparative descending paper chromatography was the primary separation technique adopted since glucose, fructose and sucrose can readily be resolved, and increasing polymerization of saccharides progressively retards migration rates on paper in the propan-1-ol ethyl acetate water (7 1 2) solvent system employed Subsequently, preparative high voltage paper electrophoresis was advantageous in further resolving mixtures which co-chromatographed in paper partition chromatography methodology

Acid hydrolysis, in conditions that efficiently cleave the glucose-fructose bond in sucrose, showed that the most abundant honeydew oligosaccharide, chromatographing typically as a trisaccharide, yielded only fructose Similarly, a disaccharide also appeared to be composed only of fructose Fast atom bombardment mass spectrometry of peracetylated and permethylated trisaccharide gave data consistent with a constitution of three hexoses The deduced tri-fructose, occurring naturally through the ergot fungus's enzymology, was likely to be constructed by the 2-1 or 2-6 linkages that are the options characteristic of the inulin or levan series of oligofructosides, respectively The inulin series are typically derived by partial hydrolysis of storage carbohydrate polymers (of the order of ~ 50 fructose residues) of some Compositae plants (e g Jerusalem Artichoke [*Helianthus* sp], chicory [*Cichorium* sp] and *Dahlia tuberosa*) Levan oligosaccharides may similarly be obtained by partial hydrolysis of fructosans obtained from some grasses or arising from the fermentation utilization of sucrose by certain bacteria (e g *Serratia* sp) Since mass spectrometry could not distinguish between 2-1 and 2-6 linkages between fructose units, and also that obtaining purified honeydew sugars in >20 mg amounts was feasible, a nuclear magnetic resonance spectroscopy approach was adopted Subsequently, literature showed that in recent years helpful ^{13}C data on small, mainly inulo-, oligosaccharides was published Consequently, our attention was also directed to obtaining our own model inulobiose and inulotriose purified by the same methodology as used for ergot honeydew constituents, which were subjected to the same NMR instrumentation for data acquisition and analysis

A striking feature of the principal trisaccharide (tri-fructose) revealed by

NMR spectroscopy was its homogeneity, after isolation only by descending paper chromatography, indicated by 9 strong ^{13}C resonances This was in contrast both to the extreme complexity of resonances seen in inulotriose obtained by partial acid hydrolysis of inulin and purified similarly and also to the relative complexity even after further high voltage electrophoresis It was quite clear that the ergot sugar was not of the inulin series Correlations of ^{13}C and ^{1}H NMR data have led to a firm conclusion that the *C africana* trifructose is levantriose, which previously was known only as a laboratory compound and was never found as a free natural product Further, the levantriose in thin films of potato dextrose agar was found to inhibit germination of macrospores, applied to the surface, completely at 2% w/v and almost completely at 1% Such concentrations are readily achieved in the honeydew exuded from infected florets of certain sweet sorghums

There is, therefore, the unexpected scenario that the African sorghum ergot pathogen predominantly transforms the hosts' sucrose to a potentially toxic unusual fructoside which on grain sorghum only accumulates in rather low amounts in honeydew and therefore permits the profuse secondary sporulation that is so characteristic of honeydew exudate and that poses such a potent factor in epidemic development The reason why honeydew from infected grain sorghum is so dilute with respect to sugars is that the metabolic demands of the large sphacelium, which *C africana* produces, nearly exhausts the plant's phloem translocate The vigor in producing a large parasitic sphacelium is notably evident in the sorghum infections seen in the recent epidemics in South America, and particularly in a Bolivian isolate used to infect male sterile grain sorghum in London in 1996

Analysis of the honeydew exuded from certain sweet stem sorghums infected with *C africana* in experimental agricultural field conditions, but not supporting secondary sporulation, showed that rather high concentrations of levantriose accumulate and are maintained even in humid conditions

Logically it follows that the failure to support secondary sporulation was consequent on transformation of the sucrose-richer phloem translocate in these particular sweet sorghums to the biologically-active levantriose This sugar is not widely fungitoxic since it did not inhibit germination of conidia of two plant pathogenic Penicillia (*P digitatum* and *P expansum*) even at a concentration of 2%

An exploitable biochemical mechanism is therefore available in certain sweet sorghums, and the use of a sweet character in the male-sterile A-line in F_1 hybrid seed production is proposed as a feasible strategy to suppress ergot disease epidemic development

The strategy relies on the persistent expression of the particular, and rather unusual, enzymology in C africana So far our experience with the pathogen on its spread northwards through South America shows a consistent pattern of typical oligosaccharides in honeydew, even to the extent that the composition has in effect taxonomic value in disease diagnosis and was invoked as a complementary

character when the pathogen was recently first encountered in Brazil (Reis et al 1996)

The first step in this experimental construction of agronomically suitable A lines, expressing a sweet character during 1-2 weeks after flowering when suppression of secondary sporulation on honeydew is epidemiologically most important, is to demonstrate that male sterile forms of our selected sweet sorghums will behave similarly to these fertile forms with respect to suppression of secondary sporulation Construction of male steriles is currently in progress in South Africa and, provided that they come up to expectations, the challenge will be to introduce appropriate sweet character expression into sorghums that are agronomically suitable to act as A lines in F_1 hybrid seed production systems It will still be desirable to achieve efficient pollination as rapidly as possible from the B line Nevertheless, primary foci of disease due to ergot may arise in some florets of the A line but these foci should not then function as sources of further windborne inoculum causing epidemic development It may of course be prudent to destroy B line plants immediately after pollen shedding in case they develop their own foci for disease spread, though this may be of minor importance if the B line is efficiently self-pollinated

Ergot disease causes most seed losses in male sterile sorghums and therefore persistence of the sweet character expression in the F_1 hybrid need not be an objective, especially if restored fertility has been efficient The sweet character in the A line could therefore be recessive to a dominant pure grain sorghum genome of the B line If fertility is not efficiently restored persistent expression of the sweet character may be desirable

Amplification of the biochemical mechanisms tentatively perceived for *C africana* in producing oligosaccharides which can inhibit secondary sporulation is currently in progress in London, with strong indication already that the disaccharide is levanbiose, again recognized for the first time that this small fructoside occurs as a free natural product It will be important to define at least two more of the minor oligosaccharides and to show whether or not they are biologically active concerning secondary sporulation In any case it is already clear that *C africana* has rather unique mechanisms for sucrose utilization which are especially efficient in making virtually all the glucose component available for nutrition and consigning the fructose into fructosans, that for the purpose of F_1 hybrid seed production can be exploited with long-term cost benefit both to the seed producers and to the farmer in the semi-arid tropics

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Session V — Ergot Management Strategies

Session President — Ranajit Bandyopadhyay

Host Plant Resistance: Sorghum Ergot

J A Dahlberg

Abstract

*Sorghum [**Sorghum bicolor** (L) Moench] is the fifth most important cereal crop in the world In 1995, sorghum ergot caused by **Claviceps africana** was first reported in the Americas Since then it has moved to cover the hemisphere Fungicides have become the short-term solution for control, but treatment is expensive Host-plant resistance offers a possible solution to limiting ergot and reducing the costs of seed production Because of its unique life history with sorghum, sterile plants and any plant that has problems with sterility is vulnerable to the disease Disease management strategies include three basic approaches, 1) escape in space and/or time 2) accommodation to the pathogen (tolerance), and 3) confrontation (use of physical or chemical defenses, including both antibiosis and nonpreference resistance) Each has limitations based on our limited knowledge of the sorghum-ergot relationship*

Sorghum [*Sorghum bicolor* (L) Moench] ergot, identified as *Claviceps africana*, was first reported in the Americas in mid-1995 in Brazil (Reis et al 1996) Since that time, 33% of the countries in the Americas and the Caribbean have had confirmed reports of ergot The disease has moved approximately 8000 km (5000 miles) in two years

In the spring of 1997, ergot was reported in Puerto Rico, Jamaica, the Dominican Republic, and Mexico including several winter nursery sites utilized by the U S Sorghum Industry The economic impact of this disease was felt immediately Several research programs have lost a full breeding cycle due to a primarily self-imposed ban on growing the material in the summer in the United States Breeder's seed that is imported into the U S is being treated with selected fungicides as a precaution Breeding programs will have additional costs in fungicide treatment to minimize ergot in the field and reduce the chances of seed contamination

Crisis exemptions are being sought and granted to spray the systemic propiconazole Tilt® on nurseries and production fields this summer These emergency exemptions are defined by the Animal and Plant Health Inspection Service (APHIS) of the United States Government as, "Emergency Exemptions The authority given to the Environmental Protection Agency (EPA) to exempt a State or Federal agency from the requirements of FIFRA, if emergency conditions exist There are four types of emergency exemptions which may be used based on the following circumstances (1) Crisis An exemption

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requested if the time available from discovery or prediction of a pest outbreak is insufficient for a pesticide to be registered for the particular use (2) Specific An exemption requested if a pest outbreak has or is about to occur, and no pesticide is registered and readily available or no other appropriate method of control is available to eradicate or control the pest (3) Quarantine An exemption requested if significant economic or health problems will occur without the use of the pesticide (4) Public Health An exemption to control a pest that will cause a significant risk to human health" (APHIS 1995)

Cost estimates have run from approximately $30 00 per acre for treatment using Tilt with an overall projected cost of $2 00-3 00 for every bag of hybrid seed produced Ergot has also been reported in Australia, and they are projecting increased costs of $20 00 Australian dollars for each 25 kg bag of seed with an increased cost of $4 0 million Australian dollars annually to the industry Brazil is also experimenting with several different applications of systemic fungicides such as Tilt, triadimenol (Bayfidan®), and tebuconazole (Folicur®) for use in control of ergot in hybrid seed production fields

Because of ergot's sudden appearance in the America's, short term strategies in this region have been limited to research on fungicidal treatments However, given the overall cost, both financially and environmentally, of using chemicals as the main control strategy, resistant cultivars seem to be a practical and economical form of control for the future This will involve host-plant resistance and perhaps the use of specialized biotechnology to achieve this goal

Host-Plant Resistance

Host-plant resistance has been recognized by farmers for several thousand years Early farmers used deliberate human selections as a means of selecting specific heads or panicles for planting in the next generation Selections were based on larger heads, larger seed, more seed, better seed, and more than likely, disease free panicles This type of selection pressure created populations that became an array of deliberately chosen components It may still be rich in variation because cultivators of traditional agriculture have an appreciation for mixtures, but the mixtures will conform to whatever an individual selector chooses The total potential range of variation will be fragmented into landrace populations or primitive cultivars (Harlan 1975) Individual plant characteristics and the physical and chemical structure of these plant populations would, therefore, directly and indirectly affect the evolution and ecology of plant pathogens and the ability of the plant population to deal with them (Fritz and Simms 1992)

This co-evolution of plants with their respective pathogens was eloquently stated by Flor (1955, 1956) in what has become known as the gene-for-gene hypothesis Simply stated, genes in a host plant that control susceptibility or resistance have a corresponding gene in the pathogen that determines avirulence or virulence This interaction determines whether or not the disease will be

expressed in the host (Fehr 1987) Several authors and books have discussed the gene-for-gene hypothesis and its effects on breeding for plant resistance and the mechanisms involved (Bagga and Boone 1968, Ellingboe 1981, Fritig and Legrand 1993, Fritz and Simms 1992, Jacobs and Parlevliet 1993, Johnson and Jellis 1992, Martin et al 1993, Michelmore 1995, Toxopeus 1956) From these discussions, three basic strategies for disease management in host-plant resistance have developed, 1) escape in space and/or time, 2) accommodation to the pathogen (tolerance), and 3) confrontation (use of physical or chemical defenses, including both antibiosis and nonpreference resistance) (Kennedy and Barbour 1992)

These disease management strategies have the potential for the development of plants that are better adapted to handling outbreaks of ergot in the future However, in order to understand how these strategies might be used and developed, it is essential that one understands some of the basic morphology involved in susceptibility of sorghum to ergot and ergot's own life history

The Sorghum Plant and Ergot

Sorghum is a robust and genetically variable plant Most sorghums are photoperiod sensitive and days to flowering range from 36 to 199 days It has strong stems and ranges in height from less than 1 m to greater than 4 m Stems are juicy or dry, sweet or bitter Leaves are arranged alternately and have prominent midveins and parallel lateral veins Long, overlapping leaf sheaths are attached at the nodes

The sorghum head is a panicle consisting of a central axis with whorls of main branches, each with secondary and sometimes tertiary branching The length of the rachis branches permits a variety of panicle shapes and sizes The branches carry the racemes of the spikelet (Figure 1) The panicle emerges from the flag leaf sheath, or boot

As sorghum flowers undergo anthesis, spikelets form at the apices of the rachis branches Spikelets come in pairs on the racemes with one being sessile and fertile and the other being pedicelled and sterile Each sessile spikelet contains one fertile and one sterile floret Anthesis occurs early in the morning and proceeds from the apex to the bottom of the panicle over a six-to-nine day period, depending on the environment, size of the panicle, and the variety Sorghum flowers open as a result of the lodicules swelling and forcing the glumes apart (Doggett 1988) The stigmas emerge and the papillae fluff out Stigmas generally extend beyond the glumes which close after one to four hours (Stephens and Quinby 1934) Stigmas may be receptive to pollen one to two days pre-flowering and may be receptive for up to a week after flowering Jaster (1985) reported that under certain environmental conditions, stigmas may be viable up to 12 days after 100% anthesis

Seeds begin to appear shortly after flowering Physiological maturity, recognized by the development of the black-layer, occurs between 25-55 days after flowering Seed is normally harvested 10-20 days after black-layer formation when seed moisture content is

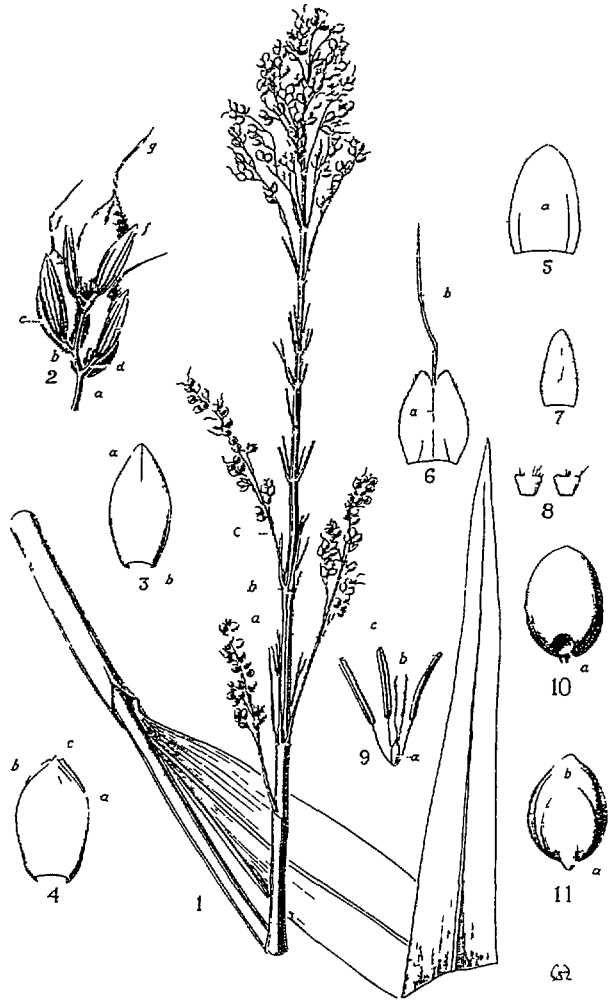

Figure 1 **The inflorescence and spikelets of sorghum (1) parts of the panicle a, internode of rhachis, b, node with branches, c, branch with several racemes (2) Raceme a, node, b, internode, c, sessile spikelet, d, pedicel, e, pedicelled spikelet, f, terminal pedicelled spikelets, g, awns (3) Upper glume a, keel, b, incurved margin (4) Lower glume a, keel, b, keel-wing, c, minute tooth terminating keel, (5) Lower lemma a, nerves (6) Upper lemma a, nerves, b, awn (7) Palea (8) Lodicules (9) Flower a, ovary, b, stigmas, c, anthers (10) Grain a, hilum (11) Grain a, embryo mark, b, lateral lines (Snowden 1936)**

roughly 15% Seeds are made up of primarily three major components the endosperm, embryo, and pericarp Associated with the seeds of many sorghum cultivars is a specialized layer called the testa, composed of phenolic compounds, which separates the endosperm from the pericarp

Because the life and disease cycle of ergot will and has been discussed extensively in this conference, discussion

on ergot will be limited to its life cycle and its potential interactions with sorghum The life cycle of *Claviceps africana* is outlined in Figure 2 It is critical that in its life cycle non-pollinated stigmas are present for colonization by either conidia or ascospores of the pathogen The primary site of infection is the stigma, although the conidia may also infect through the style and the ovary wall Once germinated conidia reach the vascular bundles of the rachilla, rapid colonization of the ovary takes place (Bandyopadhyay et al 1996) The focus on plant-host resistance must therefore revolve around the pathogen's interaction with the pollinating mechanisms involved in the sorghum plant

Potential Strategies for Disease Management in Sorghum

Three basic strategies for disease management in host-plant resistance for sorghum are available, 1) escape in space and/or time, 2) accommodation to the

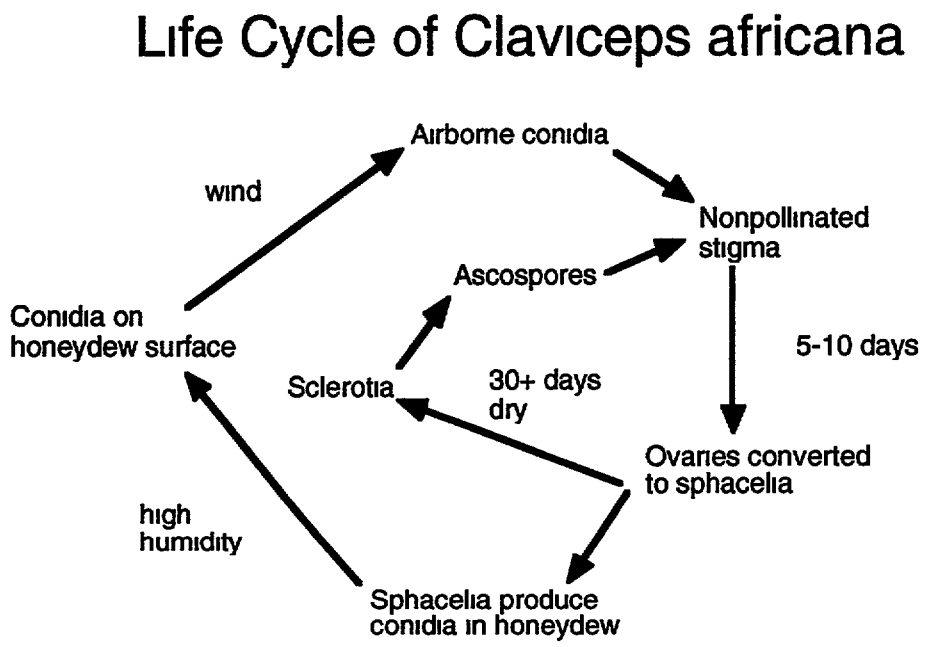

Figure 2 Life cycle of sorghum ergot (*Claviceps africana*) (after Odvody)

pathogen (tolerance), and 3) confrontation (use of physical or chemical defenses, including both antibiosis and nonpreference resistance) In order for these systems to be utilized, several programs must be in place to support these strategies The first step in the development of these tools are proper screening and evaluation techniques for ergot Bandyopadhyay et al (1996), in an excellent review on ergot, outline the research that has taken place in the development of inoculation methods, screening techniques, evaluation procedures, and finally sources of resistance, considering that, lines with significant and stable levels of resistance were not available (Bandyopadhyay et al 1996)

To fully implement these options, disease management must consider that in sorghum we are dealing with two different developmental programs in our breeding strategies, 1) development of maintainer and restorer (B/R) lines and 2) development of male-sterile lines In B/R lines, germplasm accessions that show some promise of resistance have been found within many of the collections from around the world (for sources of resistance, see Bandyopadhyay et al 1996) and this would be expected if one accepts the gene-for-gene hypothesis

Landraces or traditional cultivars presented populations in which co-evolution of the ergot pathogen could evolve and both resistant and virulence genes could co-exist Male-sterile lines, however, pose a different problem A major assumption within the gene-for-gene hypothesis is that plant populations exist in nature in which interaction between host plants and pathogens can exist Disease expression is modifiable through the interaction of genes from both the plant population and the pathogen Both individual plants and population and pathogens survive Male-sterile populations of sorghum are an artifact of our agricultural scheme and are not viable forms in nature, consequently, genes for resistance and virulence within a male-sterile population would not have evolved Therefore, genes for resistance in our germplasm sources may be difficult to elucidate This does not preclude, however, the use of escape mechanisms that might assist in limiting outbreaks of ergot in the future and the potential of biotechnology, specifically, transformation as possible tools within our disease management strategy

Escape in Space and/or Time

In both B/R lines and male-steriles, manipulation of flowering dates (maturity) to allow flowering to occur during optimal pollination and fertility times is one method that can be incorporated into breeding programs These genes could produce cultivars and hybrids that flower at times when weather conditions are unfavorable to ergot development Germplasm is currently available to begin this process Flowering in sorghum germplasm ranges from 36 to 199 days This would also require a thorough understanding of weather conditions throughout the proposed developmental stages of sorghum

Male-steriles have been bred for prolonged periods of stigma receptivity

and this has been important in the hybrid seed industry when pollen parents flower later than females (Frankel and Galum 1977) De Vries (1971) also reported that greater periods of stigma receptivity produced greater seed set Jaster (1985) found significant differences in stigma receptivity among selected cytoplasmic genetic male steriles Greater periods of stigma receptivity could also possibly lead to greater susceptibility to ergot and we might have to begin developmental programs to produce female lines with shorter periods of stigma receptivity This is one area of research that requires further funding and evaluation Again, germplasm exists to meet this challenge

Accommodation to the Pathogen (Tolerance)

This strategy may be the most difficult to implement, especially on large scale production and hybrid seed production areas Unfortunately, even small numbers of infected panicles may be enough to completely contaminate production seed given the mechanical harvesting that is used in such operations Harvesters could act as efficient spreaders of honeydew onto harvested seed In order for tolerance to be an effective tool in our disease strategy arsenal, finding accessions that would allow for infection and production of sphacelia in sterile florets in B/R lines, but would then limit the production of honeydew would have to be found within our germplasm collections

Confrontation (Use of Physical or Chemical Defenses, Including both Antibiosis and Nonpreference Resistance)

Post-fertilization, ultrastructural changes in the gynoecium following pollination, prevent ergot hyphae from infecting the ovary along the same pathways (Bandyopadhyay et al 1996) In B/R lines, several strategies can be employed to enhance sorghum's ability to confront ergot Mechanisms that enhance pollen shed, pollen fertility and fertilization can be used McLaren and Wehner (1992) found the preflowering cold stress was highly correlated to ergot incidence Bandyopadhyay and Reddy (1991), using different B-lines arrived at basically the same conclusions Both studies relate to fertility issues interacting with environmental conditions Lansac et al (1994) looked at pollen viability and germination and found that sorghum pollen was similar to other Gramineae species in that it was desiccation sensitive and lost viability during drying Further research is needed to evaluate the potential of this mechanism as a viable tool in our disease management strategy Dr Keith Schertz (personal communication) indicated that work was done in the 1930s on pollen shed by Quinby and Stephens (USA) and Rangoswamy (India), however, little research has been carried out since then

Due to the inverse relationship of ergot severity with pollination, screening for resistance to both *C sorghi* and *C africana* in male-steriles has not shown much promise Most research in this area has concentrated on A_1, A_2, A_3, and A_4 cytoplasmic male-steriles sources and

further evaluation of other sources is needed One promising area of research might be in stigmatic secretions that may influence spore germination Chinnadurai et al (1970) found varietal differences in stigmatic secretion of such products as malic acid, succinic acid, arginine, aspartic acid, tartaric acid, and tyrosine which might be related to spore germination in ergot Similar results have been found in pearl millet (Kannaiyan et al 1973) Further research efforts need to be carried out to validate these findings

Due to the inherent difficulties in finding resistance in male-steriles, biotechnology or more specifically, transformation may provide a window of opportunity in the development of resistant female lines Anti-fungal proteins expressed in the stigma, style, and ovary might provide a level of resistance in females such that we may be able to move away from chemical control in the field Transformation is possible in sorghum and testing of anti-fungal proteins and tissue-specific gene expression will need to be carried out

Conclusions

Ergot is a serious and costly disease to sorghum production, especially in hybrid seed production Estimated costs for chemical control are high and will increase production costs for farmers and the seed industry However, host-plant resistance may help control this disease and eventually reduce costs of production Germplasm sources exist that may either escape or resist the disease in B/R lines Extensive research and collaboration will be needed to further elucidate mechanisms that will be important in male-sterile lines and the use of biotechnology offers some promise, albeit in the future Development of world-wide screening nurseries, inoculation techniques, and evaluation of germplasm resources are required in order to build the knowledge base that will allow us to utilize host-plant resistance This conference and this disease provides us with that opportunity to lay the foundation for this world-wide effort to combat ergot for the future

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Chemical Control of Sugary Disease of Sorghum (*Claviceps africana*)

N F J de Almeida Pinto, A da Silva Ferreira, and C R Casela

Abstract

Sugary disease, caused by ***Claviceps africana****, represents a major threat to hybrid seed production in Brazil Since the disease was observed in 1995, a collaborative research program involving private and public sector has been developed for control of the disease This paper reports results of recent trials developed in the EMBRAPA Maize and Sorghum Research Center Experiment Station, in the evaluation of fungicides and doses for the control of sugary disease in seed production fields*

Sugary disease of sorghum, caused by *Claviceps africana*, has been considered a major threat to hybrid seed production in Brazil The disease was observed in Brazil in 1995 and by mid 1996, Brazilian scientists, through a collaborative research program involving public and private sector, identified two active ingredients that were highly effective in the control of the disease

Very few reports are found in the literature regarding the control of *C africana* by fungicides No fungicidal activity towards sugary disease was reported for the residual action of benomyl, bitertanol, carbendazim plus flusilazole, procymidone, propiconazole, tebuconazole, and triadimenol On the other hand, Dithane M-45, Difolatan, Cuman, Carbendazim plus Tridemorph, Carbendazim plus Thiram, and Thyophanate methyl proved to be effective in reducing disease severity and significantly increased seed production In vitro studies indicated that Benomyl, Captan, Ziram, Thiram, Vitavax, and Miltox inhibited growth and sporulation of *Sphacelia sorghi* In this paper we present some of the recent results of fungicide trials performed at the Embrapa Experiment Station to evaluate the impact of fungicides in the control of sugary disease

Fungicide trials were divided into three experiments In the first trial fungicide treatments consisted of four sprays, at three-day intervals using a backpack sprayer, starting at the beginning of anthesis In all treatments sorghum panicles were sprayed so that they were completely covered by the fungicide Disease evaluations were based on the percentage of infected panicle according to a 1 to 5 scale, where 1 = no disease and 5 = above 50% of disease Tebuconazole was very effective in controlling sugary disease (Table 1) Some toxicity to sorghum seeds was observed as a result of application of Triadimenol, despite its high efficiency in the control of the disease (Table 2)

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Table 1 **Effect of fungicides in the control of sugary disease (*Claviceps africana*) in a seed production field of the cultivar BR304, Embrapa Corn and Sorghum Research Center, Lagoas, MG, Brazil, 1997**

Treatment	Dose (ml a i ha^{-1})	DP[a] (%)	DP[b] (%)	DP[c] (%)	DS[d] (%)	DS[e] (%)	DS[f] (%)	NE1[g]	NE2[h]	NE3[i]
Propiconazole	75 0	0 0	4 4	77 3	0 0	1 0	3 2	1	2	2
Propiconazole	125 0	0 0	0 0	24 6	0 0	0 0	1 2	1	1	2
Propiconazole	50 0+	0 0	7 5	82 9	0 0	1 0	3 2	1	2	2
Difenoconazole	50 0									
Propiconazole	75 0+	0 0	12 6	73 3	0 0	2 0	4 0	1	2	2
Difenoconazole	75 0									
Tebuconazole	60 0	0 0	2 3	41 1	0 0	0 5	1 2	1	1	2
Testemunha		58 9	100 0	100 0	3 0	37 5	65 0	2	4	5

[a]=diseased panicles three days after last spraying [b]=diseased panicle 14 days after last spraying [c]=diseased panicle final rating
[d]=disease severity three days after last spraying [e]=disease severity 14 days after last spraying [f]=disease severity final rating
[g]=disease grade three days after last spraying [h]=disease grade 14 days after last spraying [i]=disease grade final rat ing

Table 2 **Effect of fungicides in the control of sugary disease (*Claviceps africana*) in a seed production field of the cultivar BR304, Embrapa Corn and Sorghum Research Center, Lagoas, MG, Brazil, 1996**

Treatment	Dose (g or ml a.i ha^{-1})	DP1[a] (%)	DP2[b] (%)	DS1[c] (%)	DS2[d] (%)	NE1[e]	NE2[f]
Tebuconazole	375	19 8	38 4	2 6	5 0	2	2
Benomyl	750	49 2	86 3	5 0	15 0	2	3
Mancozeb	2400	79 4	100 0	20 7	50 0	3	4
Triadimenol	250	0 0	0 0	0 0	0 0	1	1
Fentin hydroxide	500			Phytotoxic to panicles and leaves			
Metalaxyl + Mancozeb	960+216	91 4	100 0	21 3	66 7	3	5
Tiofanato Metilico	700	88 9	100 0	21 7	50 0	3	4
Testemunha		87 2	100 0	58 3	93 3	5	5
Tebuconazole	375	57 4	90 1	12 3	15 0	3	3
Tebuconazole	375	30 9	42 5	4 0	5 3	2	2

[a]=diseased panicles three days after last spraying [b]=diseased panicle 14 days after last spraying [c]=disease severity three days after last spraying [d]=disease severity 14 days after last spraying [e]=disease grade three days after last spraying [f]=disease grade 14 days af ter last spraying

In the second trial, plots were sprayed in a three-spray program at 5-day intervals The highest doses of Tebuconazole were the most effective in controlling sugary disease, resulting in increased seed weight and percentage of germination, and a reduced number of diseased spikelets and number of sclerotia per 100g of seed (Table 3) The third trial included a 7-day interval spraying program for the highest dose of the combination Propiconazole + Difeconazole The fungicides Propiconazole, Propiconazole +

Table 3 Effect of fungicides in the control of sugary disease (*Claviceps africana*) in a seed production field of the cultivar BR304, Embrapa Corn and Sorghum Research Center, Lagoas, MG, Brazil, 1997

Treatment	Dose (kg or l a i ha[1])	Disease spikelets (%)	Seed weight (g 1000[1])	Germination (%)	Vigor (%)	Seed weight panicle[1] (g)	No sclerotia per 100 g of seed
Tebuconazole	0 2500	4 33	32 15 a	76 67 a	64 33 abc	11 29 a	2 7
Tebuconazole	0 1250	8 00	30 31 a	78 33 a	63 00 abc	10 55 a	4 3
Tebuconazole	0 0625	12 00	26 63 b	73 00 ab	62 67 abc	8 23 ab	17 7
Propiconazole	0 2500	4 00	26 87 b	76 67 a	67 33 ab	8 13 ab	3 3
Propiconazole	0 1250	10 60	25 87 b	73 67 ab	69 67 a	10 57 a	8 0
Propiconazole	0 0625	12 30	25 62 b	70 00 bc	59 67 abc	9 55 a	28 0
Triadimenol	0 2500	0 63	25 46 b	68 33 bc	54 33 c	10 84 a	0 3
Triadimenol	0 1250	0 30	26 94 b	69 67 bc	58 67 bc	8 78 a	0 0
Triadimenol	0 0625	1 00	26 59 b	65 67 c	56 67 bc	9 97 a	0 7
Testemunha	-	57 00	19 37 c	68 33 bc	60 00 abc	5 48 b	54 0

1=average values followed by the same letter are indicative of no significant differences at 5% probability (Tukey)

Difeconazole, and Tebuconazole were effective in controlling the disease (Table 1)

In all the trials, plots artificially or naturally inoculated before fungicide spraying, fungicides did not give complete control of disease, indicating the non-curative effect of the fungicides Residual fungicide activity was not enough to protect the crop throughout the flowering period

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Integrated Management of Sugary Disease of Sorghum — Practical Aspects

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Abstract

This paper discusses the strategies involved in the control of sugary disease of sorghum caused by ***Claviceps africana*** *The knowledge of the biological cycle of the pathogen is of fundamental importance to establish integrated disease control strategies The strategies aim to reduce the inoculum at its source Main inoculum sources are seed inoculum, crop residues, sclerotia, and secondary hosts Disease may be managed through seeding time, pollen management, breeding to increase sugar content in honeydew, and chemical protection of the infection court*

Sugary disease is known also as ergot of sorghum Nevertheless the common name that should prevail is sugary disease because long, black, hard sclerotia like spurs are not produced Diagnosis would be facilitated if sugary disease would be used for the disease caused by *Claviceps africana* Frederickson, Mantle & de Milliano and ergot for *C sorghi* McRae Two distinct pathogens and diseases of sorghum are involved

Sugary disease causes damage mainly in seed production fields It is known as the male sterility disease since any factor that negatively interferes with fertilization predisposes the female flower to infection Infection does not take place in those florets where fertilization occurs Hence disease is more important in seed producing fields than in commercial ones

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Damage may be direct or indirect Direct when ovary infection takes place, and seed or kernels are not formed Indirect damage results from the sugary secretion or sphacelium on seeds which may reduce seedling emergence in the field by stimulating attack by soil fungi or from toxins present in the sphacelium Nevertheless this has not been confirmed in Brazil yet Direct losses in seed production in the 1996 growing season in Brazil were estimated as in excess of one million dollars No damage has been determined for commercial fields yet

Integrated Disease Management of Plant Diseases

In integrated disease management (IDM), all available control strategies within a unified program are used to keep the harmful organism population (pathogens) below the economic damage threshold (EDT) in such a way as to minimize negative side effects on the environment (Anonymous, 1969) To achieve this goal a detailed understanding to the pathogen life cycle is required

Biological cycle of ***Sphacelia sorghi***

*McRae (teleomorph **Claviceps africana** Frederikson, Mantle & de Milliano)*

The target of control measures of any disease should be the causal agent Hence, a detailed knowledge of the biological cycle of the etiologic agent is fundamental Control strategies aim at interrupting one or more phases of the life cycle in order to impede continuous disease development The life cycle of *S sorghi* is based on information from literature mainly from Africa and India

Main Phases of the Life Cycle to be Targeted by IDM are

Inoculum sources — The reduction of inoculum at its source is the principle on which strategies are based

1) Seeds as source of inoculum The literature reports that two types of inoculum may be present with the seeds

a) Conidia in the honeydew contaminate seeds during harvesting Similar to wheat and rye ergot, macroconidia on the seed surface should not play any role in the pathogen life cycle (Wiese 1977) The biological function of conidia is to infect ovaries of unfertilized flowers, this is the only known infection site for ergot diseases Hence, no control should be directed at this inoculum Since inoculum as conidia is in the wrong infection court (seed surface) and unable to cause infection Additionally, laboratory experiments have shown that a seed treatment with captan (75% WP) at concentrations >40 $\mu g\ ml^{1}$ inhibited spore germination (Reis & Blum, unpublished data) It was also shown that viability of the inoculum as conidia on the seed surface lasts for only one month (Reis & Blum, unpublished data) McLaren (1993) reported that seed washing in seed processing plants might improve seedling emergence in the field by lowering sugar and toxic substance concentrations

b) Sclerotia within seeds The presence of sclerotia in sugary disease is abundant Sclerotia of *C africana* are not soft, long, hard and black like those of the true ergot diseases such as *C purpurea* in wheat or rye (Wiese 1977) Thus it is suggested to name them pseudosclerotia or sclerotia-like bodies The amount of sclerotia produced in infected panicles is very high Thus a large number is found among threshed seeds Little is known about the biological function of these resting structures in the continuation of the parasite's life cycle Germination of *C africana* sclerotia in the laboratory has been reported once by Frederickson et al (1991) No reports of their germination under field conditions have been found in available literature Germination, formation and release of ascospores, the true structures for air-borne dispersal of inoculum, needs investigation Work carried-out in Brazil by commercial sorghum seed producing companies has demonstrated that the mechanism of seed cleaning and classification allows the complete removal of sclerotia from infested seed lots Even if they could easily germinate, they would be eliminated from seeds by seed processing equipment

2) Sorghum residues as source of inoculum Futrell & Webster (1966) reported that *S sorghi* may survive in the

dry honeydew in the anamorphic form (conidia) in infected panicles, which remain in the soil after harvesting Secondary conidia can be released from this inoculum source and carried by wind to infect ovaries of sorghum flowers in the next season Under field conditions and monoculture, the required survival period would be about 6-7 months However, research is required to quantify the time required to complete decomposition of sorghum residues as well as inoculum viability under southern Brazilian conditions In Passo Fundo, Rio Grande do Sul State, sclerotia are naturally broken down, nearly 98% of their weight, in a 11 month period The experiment is still in progress and no germination has been detected in any sclerotia kept at the soil surface to simulate no-till farming and at a depth 10 cm to simulate conventional tillage Experiments on decomposition of naturally infected panicles and macroconidia viability are being carried-out Currently, one may infer that a single season's crop rotation with a non-susceptible plant species may reduce inoculum in infected panicles remaining on the soil, both as sclerotia or macroconidia A summer rotation would imply that sorghum could be cultivated again after an 18 month period The potential control of sugary disease through crop rotation however is unclear taking in to consideration more important sources of inoculum such as secondary hosts

Seeding Time of Sorghum in the Americas and Relation to the Pathogen Survival in its Parasitic Phase

Table 1 shows the seeding and flowering time of sorghum throughout the Americas Secondary conidia produced on sorghum panicles of cultivated plants would ensure inoculum for the whole continent Every month should there be sorghum at flowering prone for infection everywhere Thus pathogen does not need other alternative mechanism for survival in the Americas, even sclerotia

Secondary hosts as source of inoculum Secondary hosts can be defined as alternative non-profitable susceptible species, such as weeds and wild plants

Table 1 Seeding and flowering time of sorghum in the Americas

	Seeding time		Flowering time	
Country	1st Season	2nd Season	1st Season	2nd Season
Brazil	October/December	February/March	January/February	April/May
Mexico	November/December		March/April	
	April		June/July	
	January/March		July/August	
Colombia/ Venezuela	March/April	September/ October	May/June	November/ December
USA	March/May		June/August	
Bolivia	May/June		July/August	
Argentina	October/November		December/February	
Honduras	August/September	May	October/November	October

Futrell & Webster (1966) and Bandyopadhyay (1992) reported the following secondary hosts of S sorghi in Africa and India *Cenchrus ciliaris*, *C setigerus*, *Ischaemium pilosum*, *Panicum maximum*, *Pennisetum typhoides*, *P orientalis* and *Sorghum halepense* Although not being a secondary host, Futrell & Webster (1966) include maize as a susceptible species However, field observations in Brazil both in experimental areas and on farms, failed to reveal any visible symptoms on maize plants growing in close proximity to infected sorghum plants One may speculate that in Brazil maize is not naturally infected and thus it is not a susceptible species or inoculum source for sorghum

In Brazil, a number of species have been reported as showing signs of sugary disease Conidia of *Sphacelia*-like fungi were measured and inoculated on to a male sterile sorghum line Results are presented in Table 2 The only infective inoculum to *S bicolor* came from *S halepense* in which conidia shape and size were similar to *S sorghi* The remaining isolates appear to be different and possibly are undescribed *Sphacelia* species, when compared with the descriptions of Loveless (1964)

These results suggest that only the species *bicolor* and *halepense* and probably *sudanense* and *verticiliflorum* in the genus *Sorghum* should be considered host of the sorghum sugary disease fungus Among them is Johnson grass an important species in Brazil The incidence of this weed in South America is particularly high in Argentina while less so in Brazil

Technically, the control of secondary hosts would be recommended to avoid an alternative source of inoculum, but this is not feasible taking into consideration the large areas infested by these species,

Table 2 Measurement of *Sphacelia* spp macrononidia

	Macroconidia			
	Length		Width	Mean
Host plant	µm			
Sorghum bicolor	10 0 22 5	x	5 0 10 0	14 8 x 7 4
Brachiaria brizantha	10 0 20 0	x	4 5 7 5	15 2 x 6 1
Cenchrus sp	15 0 30 0	x	3 7 7 5	22 3 x 5 4
Lolium multiflorum	7 5 13 8	x	3 0 7 5	9 1 x 4 5
Panicum maximum	13 7 - 27 5	x	5 5 - 10 5	20 6 x 7 6
Paspalum dilatatum	8 7 - 22 5	x	5 0 - 8 0	15 4 x 6 9
Paspalum guenoarum	10 0 - 23 7	x	3 7 7 5	16 2 x 5 2
Pennisetum americanim (own inoculum)	10 0 18 7	x	4 5 8 7	14 8 x 6 8
*Pennisetum americanum**	10 5 21 5	x	5 0 10 0	15 9 x 7 2
*Sorghum halepense**	10 0 28 7	x	5 0 10 0	17 2 x 7 6
Frederickson Mantle & deMilliano (1991)	9 0 17 0	X	5 0 8 0	

* Sorghum inoculum

especially Johnson grass Besides that, there is a need to confirm the production of secondary conidia in these plants as they are on sorghum according to Frederikson et al (1991) Control of this disease will be impossible by the reduction of the inoculum from these sources If secondary conidia are abundantly produced and easily transported through wind at very large distances, they would be sufficient to ensure inoculum for a large geographic area such as a country or a continent (Table 2)

The host range reported in the literature has not been confirmed for Brazil The isolated pathogen has been restricted to the genus *Sorghum*, and may be a new strain or a new species when compared with the literature More investigation is needed to prove this hypothesis From now on, *Sorghum bicolor, S halepense, S sudanense* and *S verticiliflorum* should be considered as the only host of *C africana* in Brazil Other plant species should be inoculated under natural conditions to test Koch's postulates

Pollen Management

As sugary disease is the result of infection of unfertilized ovaries and flower predisposition is directly related to poor pollination, the management of factors involved in pollination may lead to damage reduction Recommended measures are

- selection of sorghum lines with high pollen production
- optimization of the ratio of female-male lines
- selection of parents adapted to the environment in such a way that pollen quantity and viability is not affected
- management of seeding time to synchronize the flowering of male and female lines
- nitrogen or irrigation may be used in the later flowering line to anticipate flowering
- pollination may be also improved by promoting air turbulence by plane or atomizer
- knowledge of the climate of the region where seed is produced so that sorghum flowers within the temperature range of 20 to 25°C and with blowing wind
- improvement of lines for a higher amount of pollen production, higher viability, larger period of pollen production should be pursued with reduced susceptibility of these features to environmental conditions

Under field conditions in Brazil commercial hybrids exhibit differences in disease severity This may be attributed to better adaptation or to the more efficient pollination that occurs in more resistant lines compared with more susceptible lines

Genetic Improvement

A high sugar content of the honeydew may suppress germination of macroconidia and as a consequence reduce the production of secondary conidia, the main infecting structures of the pathogen This strategy may be achieved by incorporation of the sweet character present in sweet sorghum into the genome of male sterile lines

According to Hassan et al (1995), breeding for resistant cultivars that may be done through the introduction of this trait could reduce the rate of spread of the disease through a seed production (male sterile) field

Control by Chemical Protection of Infection Courts

While resistance is not available, fungicides may be used to reduce losses in seed production fields

McLaren (1994) and Ferreira et al (1996) demonstrated the potential of triazole fungicides in the control of sugary disease In Brazil three applications are recommended as follows (a) 250 ml/ha of tebuconazole (Folicur 200 EC), at the beginning of flowering when sorghum plants are 80% with emerged panicles, (b) 500 ml/ha, 5-6 days after the first spray, and (c) 250 ml/ha 5-6 days after the second application The use of ground equipment is recommended using a three nozzle spray boom for each sorghum row Two spraying jets are directed to the panicle sides and one to its top Volumes of about 300-400 l ha^{1} have been used for the technical control of the disease in seed production fields No recommendation is available yet for disease control in commercial fields

Final Remarks

The number of secondary conidia produced both in seed production fields and in commercial ones and their efficient transport by wind to long distances may explain the recent occurrence and rapid spread of this disease in the Americas and Australia This may be aided by the continuous cropping of sorghum during all seasons on the continent (Table 1) This ensures the presence of inoculum at all times and all places The presence of Johnson grass may add to this as an important source of inoculum in South America These suppositions require investigation At present the only feasible means of controlling the disease in seed fields is by fungicide application

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Session VI - International Collaboration in Ergot Research — Global Network

Session President — Robert E. Schaffert

Role of ICRISAT in Sorghum Ergot Research

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Abstract

ICRISAT is currently preparing to operationalize its Medium-Term Plan (MTP) for 1998-2000 One of the key components of the MTP is to further intensify participatory approaches in research involving networking and partnerships with National Agriculture Research Systems (NARS), Advanced Research Institutes, the private sector and Nongovernmental Organizations (NGO) to capture complementarities and comparative advantages Due to funding constraints, ICRISAT has decided to continue sorghum ergot research only with noncore, complementary grants ICRISAT has experience in ergot research in the Center and through collaborative projects in eastern Africa ICRISAT can help in the joint development of a suitable network on sorghum ergot and contribute to it by information exchange, assisting in seeking donor funding for collaborative research, and developing International Public Goods such as information on epidemiology to design disease management strategies, risk assessment modeling and identifying ergot-resistant sorghums We recommend a collaborative approach with shared responsibilities and devoid of undue overlapping efforts among interested partners for achieving the common goal of controlling the disease

Ninety percent of the world's sorghum production area is located in developing countries, where it is a staple for millions of the very poorest people, who lack economic access to more expensive cereal grains (FAO/ICRISAT 1996) Because sorghum is a crop of only the poorest minority, national average figures often do not reflect the large dietary intake by these disadvantaged groups Over the past two decades, sorghum areas in Asia and Latin America have declined, although grain yields are increasing to compensate Even these yield increases may be underestimations due to the increasing areas sown for fodder use, which are accounted for in area estimates but not grain in flows The importance of sorghum as a feed and forage source in the mixed crop-livestock systems characteristic of the Semi-Arid Tropics (SAT) is gaining momentum The grain of sorghum is emerging as a significant livestock feed in India, and is the major use of the crop in Latin America and the developed world Feed usage of sorghum not only improves farm incomes and diversifies the enterprise, but also helps make milk and meat products more affordable, thus improving the nutrition of the poor

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) was established in 1972 as the first international research center under the auspices of the Consultative Group on International Agricultural Research

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(CGIAR) The mandate of ICRISAT is as follows

- Serve as a world center for the improvement of grain yield and quality of sorghum, millets, chickpea, pigeonpea, and groundnut and act as a world repository for the genetic resources of these crops
- Develop improved farming systems that will help to increase and stabilize agricultural production through more effective use of natural and human resources in the seasonally dry semi-arid tropics
- Identify constraints to agricultural development in the semi-arid tropics and evaluate means of alleviating them through technological and institutional changes
- Assist in the development and transfer of technology to the farmer through cooperation with national and regional research programs, and by sponsoring workshops and conferences, operating training programs, and assisting extension activities

ICRISAT's mission statement reads as follows through international research and related activities, and in partnership with national agricultural research systems (NARS), to contribute to sustainable improvements in the productivity of agriculture in the SAT (plus other countries in which ICRISAT's mandate crops have relevance) in ways that enhance nutrition and well being, especially of low-income people

ICRISAT's expertise in science-based agriculture development, together with its international, apolitical, non-profit, and humanitarian nature, uniquely position it to help the peoples of the SAT to work together to achieve effective practical solutions ICRISAT's mission is carried out through strategic partnerships with a wide range of national and local organizations, in which each partner contributes according to its special expertise and mandate ICRISAT, as a global center of scientific excellence, contributes broadly applicable international public goods (IPGs), while its regional, national, and local partners translate these goods into the final products that help improve the lives of the rural poor in their areas of responsibility

Sorghum Diseases in the Medium-Term Plans (MTP) of ICRISAT

The MTP constitutes a statement of the strategic directions and priorities of the institute In the past decade, MTPs for 1989-93 and 1994-97 have been operationalized During the first of these MTPs, sorghum disease research encompassed grain mold, anthracnose, leaf blight, foliar diseases, downy mildew, long smut and ergot Due to funding constraints, research on downy mildew, long smut and ergot was dropped from the core research agenda during the 1994-97 MTP period

The MTP for the period 1998-2000 is currently pending approval from the Technical Advisory Committee of the CGIAR (ICRISAT 1997) This MTP is not inconsistent with the priorities, objectives, goals, and values of the past It may not be surprising that the proposed 1998-2000 agenda is evolutionary, rather than revolutionary, since strategic research in close partnership with stakeholders requires a long-term

approach, and emerges from a wide and ongoing consensus-building process Nevertheless, strong currents of change underlie this evolution, particularly in response to strong donor interests, new scientific developments, and the increasing strength of a number of NARS partners Despite the importance of many research opportunities, funding levels constrain the extent to which they can be pursued ICRISAT determines which aspects it can afford to address by dividing the entire research agenda into a number of specific distinctive topics, and ranking them in prioritized order It then pursues as many of the highest priority topics as funding will permit

To achieve objectivity in the process of ranking ICRISAT's priorities, specific criteria were established against which the merits of the suggested topics were judged These included equity, efficiency, internationality, sustainability, new science opportunities, relevance to NARS priorities and future trends, which could change basic assumptions Based on these criteria and considering the emerging importance of sorghum ergot, ICRISAT has decided to reinstate ergot research but it can only be conducted using external (non-core) funding Grain mold, anthracnose and foliar diseases of sorghum remain on the core agreed agenda Research on these diseases will be conducted at ICRISAT locations in India, Mali and Kenya in collaboration with NARS and Advanced Research Institutes (ARIs)

Sorghum Ergot Research at ICRISAT

During the mid-seventies, ergot research began with resistance screening activities in India This activity was discontinued after two years since resources were needed for research on other more important diseases at that time Ergot research was initiated again in 1986 at the request of the NARS in Ethiopia and Rwanda where the disease was considered important During 1986-90, our research objectives were

- study the biology of the pathogen and epidemiology of the disease
- determine the role of pollination and fertilization in disease development
- develop methods to screen for ergot resistance
- identify sources of locally-adapted, ergot-resistant germplasm

Some of the highlights of our results are as follows

Biology of the Pathogen

The variation in symptomatology of the disease as affected by environmental conditions was described (Bandyopadhyay et al 1990) The occurrence of secondary conidia in the Indian isolate of the pathogen and the unique process of secondary conidiogenesis was reported (Bandyopadhyay et al 1990) The epidemiological implications of secondary conidia are considerable and the production of easily detachable secondary conidia outside the surface of sticky honeydew matrix suggests that the fungus can be disseminated by wind

Epidemiology

Knowledge of sources of inoculum that initiate ergot infection in the field, and of the mode and extent of spread of the disease is required to devise disease management strategies Three graminaceous weeds (*Cenchrus ciliaris*, *Panicum maximum*, and *Urochlora panicoides*) were identified as sources of primary inoculum in the field Large-scale field experimentation showed that wind-driven, water splash-dispersed macroconidia disseminate the disease Secondary foci appeared to be randomly established in down-wind areas There were 2-3 infection cycles, each of 6-8 days duration, in a flowering season The spatial pattern of infected spikelets changed from a focal to a random pattern as disease progressed

Field experiments also indicated that the pathogen can be dispersed by wind Wind dispersal is primarily due to secondary conidia produced on the surface of honeydew It is important to consider that secondary conidia are produced only at high (>90%) relative humidity, and cool temperature (15-20°C), both conditions are also favorable for infection after spore deposition Thus, the pathogen has ecologically adapted itself to produce a type of conidia (secondary) that has greater possibilities of dissemination (by wind) only when conditions for infection are optimal

Although honeydew from infected plants usually drips and falls on soil, the epidemiological significance of soilborne conidia was not known It was observed that honeydew that dripped on to dry field soil remained light brown, whereas honeydew that fell on wet soil developed a white fluffy growth Scanning electron microscopy observations showed that honeydew that fell on dry soil contained only nongerminated microconidia embedded in their original viscous matrix In wet soil, the fluid matrix was not evident and microconidia germinated to produce a crop of secondary conidia raised above the soil surface Properly designed field experiments with caged plants (to avoid insects) showed that secondary conidia from the soil surface were wind disseminated and infected the caged plants Thus, soilborne secondary conidia may be an important inoculum source in the field

Pollination Effects

It is generally believed that pollinated flowers resist infection and are not colonized by the fungus Ergot development by inoculating flowers at different time intervals before or after pollination was studied Pollination- and infection-related events were monitored by fluorescence microscopy The fertilization event was related to reduction in ergot because the fungus failed to grow or grew slowly in pistils inoculated after fertilization Similarly, pollen tube growth was retarded in flowers inoculated before pollination However, a few flowers produced grains with the fungus growing at the basal end It was hypothesized that such grain probably resulted from pistils in which infection hyphae and pollen tubes were observed growing simultaneously, each succeeding in their respective function — colonization and fertilization Occurrence

of ergot-infected grain is a phenomenon not reported before Information on pollination effects was vital for the development of a screening technique and identification of resistance

Identification of Ergot Resistance in Hybrid Parents

Identification of ergot-resistant seed parents is a priority for the development of ergot-resistant hybrids Variation was observed in seed set (20-93%) in hybrid parents and hybrids in seasons when night temperatures were low (9-11°C) Low night temperatures are known to adversely affect self-pollination and, therefore, reduce seed set Reduction in the efficiency of self-pollination predisposes plants to ergot infection Based on these observations, it was hypothesized that self-pollination efficient lines at low temperature are more likely to resist ergot than lines that are not self-pollination efficient Evaluation of seed parents at low (10°C night, 25°C day) and normal (15°C night, 25°C day) temperatures in controlled environment growth chambers proved the hypothesis and assisted in the identification of ergot-resistant seed parents

Collaborative Research on Sorghum Ergot in Eastern Africa

In eastern Africa there was a sorghum research network with an Advisory Committee to determine research priorities This committee considered ergot a priority disease and recommended that scientists in Ethiopia and Rwanda, where ergot is a serious problem, should collaborate with ICRISAT on research on the disease in the region ICRISAT, therefore, restructured its ergot research by conducting all its activities on host resistance aspects through two collaborative projects in Ethiopia and Rwanda The objectives of the collaborative projects were to develop a resistance screening technique and to identify ergot resistance Training was an integral part of the projects ICRISAT and national program scientists jointly planned and conducted all the experiments on ergot Together, scientists maintained inoculum, inoculated and evaluated panicles, analyzed results and planned further experiments Each year, from 1987 to 1989, an ICRISAT scientist worked at ergot 'hot spots' in Ethiopia and Rwanda together with the national program counterparts A plant pathologist from each country spent 3-7 months at ICRISAT working on ergot

Through this collaborative research, simple and effective artificial inoculation methods were developed and the importance of inoculation demonstrated in resistance screening A field screening technique was developed that has been suitably used by both national programs to identify locally adapted, ergot-resistant sorghum lines (Tegegne et al 1994, Musabyimana et al 1995) Ethiopian and Rwandan scientists have exchanged ergot-resistant lines and shared these with scientists in southern Africa, Kenya, and India where ergot is also an important problem

The impact of the collaborative projects has been the development of expertise and materials that are of immediate benefit to the national

programs because the research was conducted at national research stations using plant materials and physical facilities available to the national programs The Ethiopian and Rwandan national programs now conduct ergot research independently

Dimensions of ICRISAT's Role

Comparative Advantages and Partnerships

Within its agreed agenda, ICRISAT will only engage in research that generates benefits, such as IPGs, widely across its mandate region Internationality was a specific criterion considered during prioritization of research topics, and local or sub-regional relevance was a reason that the priority for ergot research was lowered Outside the agreed agenda, ICRISAT will continue to engage in projects focused on national priorities using bilaterally targeted local funds These national projects often provide the CGIAR with opportunities to identify and deliver the benefits of IPGs (e g , germplasm) which would otherwise not have been possible

The emerging capacity of some National Agricultural Research Institutes (NARIs), networks, regional organizations, private sector entities, and other alternative suppliers of research goods, makes it possible for ICRISAT to refocus its own limited resources towards other opportunities For ergot research, several research entities have strength that can be captured imaginatively through collaborative research To further advance the devolution of parts of its research agenda, ICRISAT will pursue a deliberate strategy encouraging the emergence of alternative suppliers This strategy is embodied by the development of enabling technologies that will increase the attractiveness of investments by other groups in ergot research By developing prototypes that can be finalized for release by NARS and ultimately generate a profit for the private sector, ICRISAT can help strengthen national and development capacities, and phase out its own role in final product development Profits from technologies can be reinvested in research, leading to sustainable research and development by alternative supplies ICRISAT, NARIs, ARIs, and the private sector have always maintained close working relationships Clearly each partner has comparative advantages that result in the whole being far more effective than its individual parts As different partners mature and evolve, their roles may need to change so that complementarity is maintained

During the MTP period, ICRISAT will seek opportunities to outsource parts of its research agenda where this can provide clear increases in efficiency while maintaining a satisfactory standard of quality However, an operational definition of outsourcing in relation to the research agenda needs clarification ICRISAT sees little comparative advantage in engaging in a strict subcontracting mode of research, other institutions could handle the mechanics of such transactions equally well Instead, ICRISAT sees value in extending the breadth and depth of its research work through cost-effective joint efforts with NARS, ARIs, and other organizations ICRISAT's commitment to greater

inclusiveness blends nicely with this outsourcing strategy, since closer partnerships in research provide a framework in which an increasing share of the joint agenda can be outsourced to partners, simultaneously accomplishing important training and technology dissemination functions as well Mechanisms for these outsourcing modes include visiting scientist positions, studentships, networking, joint proposal development and marketing For example, the SADC/SMIP/ICRISAT project provided facilities for the Ph D research of D E Frederickson that provided several key IPGs on ergot that have relevance worldwide (Frederickson 1990)

Developing Human Resources of NARS Partners

The NARS fora have given visibility and support to a fundamental shift that has been underway over recent years in the modality through which ICRISAT (and other CGIAR Centers) provide assistance in the development of national scientific resources This shift involves a transition from a conventional training-course mode, towards full research partnerships formed around joint priorities Through these partnerships NARS colleagues gain experience in all stages of an active research program, including priority setting, project planning and proposal development, fund seeking, project execution, review and impact assessment Advanced skill sets are needed to carry out these partnerships, including scientific creativity, flexibility, innovativeness, and the ability to communicate the value and relevance of research The development of these skills promises to substantially strengthen NARS capacity to provide the full spectrum of outputs required to achieve sustainable agricultural development in their countries These partnerships are now organically embedded in ICRISAT's research agenda

Information Exchange

The availability of information is widely recognized as being a key factor in the achievement of sustainable self-sufficiency in developing countries Recent enhancements in information technology have generated opportunities for the exchange of information on a scale that was previously impractical ICRISAT is dedicated to making information relating to its mandated areas of interest as widely available as possible

Following the outbreak of ergot epidemics in Brazil and Australia, ICRISAT, and the Sorghum and Millet Improvement Conference of North America (SICNA) collaborated in making information on the disease available rapidly A review article on ergot was commissioned from scientists with expertise on the disease in South Africa, Zimbabwe, India and ICRISAT for publication in the International Sorghum and Millets Newsletter (ISMN) An extensive review article was published (Bandyopadhyay et al 1996) on an urgent basis to make it available at the International Sorghum and Millet Improvement Conference, Lubbock A selected bibliography on sorghum ergot was also published in the ISMN A color flier with pertinent information on the disease was inserted in the ISMN with the

review The flier has been distributed to sorghum growers, seed industry, researchers and policy makers It was also put on the home page (www cgiar org/ ICRISAT) of ICRISAT in the World Wide Web for easy accessibility It is now being translated into Spanish and Portuguese The flier was produced by ICRISAT with funding from National Grain Sorghum Producers (Abernathy, TX) and SICNA, and is an example of the importance of collaboration in achieving shared goals

Continued communication of results through the ISMN, other publications, and through meetings and conferences such as this one in Brazil will facilitate the dissemination of the most recent information The current and future capability of communication through e-mail and the Web (e g , www ars-grin gov/ars/SoAtlantic/Mayaguez/sorghumhtml) will vastly increase the global ability to communicate and exchange information on ergot These communication modes have been phenomenally successful just within the past year

Networks

Networks are essential for interlinking ICRISAT, NARS, and ARIs They provide cost-efficient mechanisms for joint research, technology and information exchange, and strengthening NARS capacities They build collaboration, capitalizing on the complementary strengths of institutions around the world Enabled by regionally targeted donor support, ICRISAT continues to foster the development of the following networks

- CLAN (Cereals and Legumes Asia Network)
- WCASRN/ROCARS (West and Central African Sorghum Research Network)
- WCAMRN/ROCAFREMI (West and Central African Millet Research Network)
- SMIP (Sorghum and Millets Improvement Program) in southern Africa
- CLAIS (Commission Latinoamericana de Investigadores en Sorgo)

Networks are ideal for building bridges among countries, regions and institutions This attribute contributes strongly to ICRISAT's target of Inclusiveness during the MTP period An emerging trend is the increased participation of such "non-traditional" NARS such as NGOs, private sector organizations, and farmer cooperatives that are becoming important agents of technology delivery over time Another trend is the increased participation in network partnerships of ARIs that can contribute the basic research components needed to solve recalcitrant problems

ICRISAT's ultimate objective is to help networks become fully NARS-driven, and self-sustaining In its catalytic and convening roles, ICRISAT continues to seek increased NARS-to-NARS technology and information exchange, helping achieve regional self-sufficiency For example, CLAIS can seek to increase Brazil's role in the exchange of ergot management technology in Latin America By reducing the dependence of

NARS on ICRISAT for direct support and finished technologies, ICRISAT will be able to more fully focus on its global and strategic responsibilities as its continuing contribution to network objectives

Conclusions

With past experience on collaborative research on sorghum ergot and information exchange, ICRISAT is well placed to foster and participate in collaborative research in a global, regional or bilateral framework ICRISAT can collaborate with national partners and ARIs in seeking regional and bilateral funds for collaborative projects in which NARS have greater research involvement than ICRISAT We can assist in exploring possible interest from non-traditional donor sources, such as developing-world governments, the private sector, philanthropists, charitable organizations, foundation support and others ICRISAT with its excellent environmental control and field facilities and germplasm can participate in research to deliver such IPGs as information on epidemiology, risk-assessment modeling, and screening for cold-tolerance and ergot resistance

In such projects, ICRISAT would cultivate the use of visiting scientist, personnel exchanges, secondments, and joint appointments with ARIs to enhance complementarities and enable strong outputs In the current scenario of constrained funding opportunities, a collaborative approach with shared responsibilities is more likely to succeed than individual overlapping efforts

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The Role of the International Sorghum and Millet Collaborative Research Program on Management of *Claviceps africana* in Sorghum

Richard A Frederiksen and John M Yohe

INTSORMIL has a team of researchers from the U S and developing countries with interest in collaborative research on diseases of importance to sorghum with an emphasis on those constraints that are most important Consequently, INTSORMIL has a mandate to address the problems associated with ergot However, there are far too many constraints worldwide to address all of them simultaneously, with equal vigor The network of sorghum workers in Africa, Asia, and Latin America gives INTSORMIL an opportunity to outline existing information and focus these limited resources in the most efficient manner as well as to derive resources from other agencies and donors for additional support While it may appear that a delay in response to ergot has occurred, the facts dictate differently INTSORMIL scientists were informed of ergot being present in Brazil shortly after it was noted there Discussions among interested parties were initiated and some commercial firms made decisions as to how to handle seed stocks from nurseries in Brazil, and later movement of seed from other Latin American countries where ergot had been reported INTSORMIL and ICRISAT scientists visited the nurseries in Brazil in 1996 and proposed collaborative programs of research in areas of host reaction to disease These initiatives were thought to be preemptive Additionally, this global conference on sorghum ergot was proposed by INTSORMIL scientists and has been jointly sponsored by INTSORMIL This conference was needed because at the time there were many questions being asked regarding ergot and the implications that this disease will have on the industry where sorghum is grown under intensive production through the use of hybrids Currently, there is a cooperative initiative with Mexico to monitor the spread of *Claviceps africana* as it moves northward Presently, ergot is approximately 50 km north of the Mexican border This represents the extent of the distribution of the pathogen before crossing the Wild Horse Desert of Southern Texas This is a region of nearly 100 km of range land with limited crop production separating the Rio Grande Valley from the upper coastal plains of Texas Appearance of ergot in this region would indicate that the pathogen has the ability to move over greater distances and would present even a greater threat to sorghum production In fact the pathogen continues to move and as of October 7, 1997, was found in Nebraska

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INTSORMIL researchers now have more scientists interested in the management of ergot For example, feeding trials involving animals in Mexico and Texas are planned, as are studies on the presence of secondary metabolites following ergot honeydew encrusting on sorghum grain These studies, while critical in determining the importance of ergot, are not essential for control INTSORMIL, ICRISAT, and International scientists can bring the necessary individuals together to work on control Disease management through host resistance, chemical controls, and other strategies are all important Control of ergot in seed production fields using fungicides will hopefully only be a temporary arrangement Eventually, ergot, like most other diseases of sorghum, will be controlled through a management system that relies less on pesticides and more on natural host protection INTSORMIL can provide the scientific expertise to make this possible

The Role of USDA in Research on Sorghum Ergot

J A Dahlberg

The United States Department of Agriculture (USDA)* is made up of 17 agencies, each with various responsibilities and duties as they relate to American Agriculture The two major agencies that will deal with ergot are the Animal and Plant Health Inspection Service (APHIS) and the Agricultural Research Service (ARS) APHIS's role in American Agriculture is to provide leadership in securing the health and care of animals and plants and improving productivity and competitiveness in agriculture, thereby contributing to the national economy and the public health of our citizens To carry out this mission, APHIS guards U S borders against foreign agricultural pests and diseases, searches for and monitors agricultural diseases and pests, takes emergency action if a foreign pest or disease threatens the U S , facilitates agricultural exports through scientifically based sanitary and phytosanitary standards, and ensures the safety of genetically engineered plants and other products of agricultural biotechnology On March 10, 1997 a New Pest Advisory Group (NPAG) was formed to discuss issues and make recommendation concerning ergot From this meeting, APHIS recommended that no regulation should be introduced that would affect the movement of sorghum into the country This decision was based primarily on the fact of ergot's rapid spread in the Americas and the fact that ergot was becoming established in the continental U S as a result of natural spread by airborne spores APHIS continues to monitor the spread of the disease and will continue to be updated on progress made in the control of the disease via the NPAG and various other organizations working on ergot

The ARS will play a major role in the National effort to gather information and develop research on ergot The ARS is the in-house research arm of the USDA and its' mission is to "provide access to agricultural information and develop new knowledge and technology needed to solve technical agricultural problems of broad scope and high national priority, to ensure adequate availability of high-quality, safe food and other agricultural products, to meet the nutritional needs of the American consumer, to sustain a viable and competitive food and agricultural economy, to enhance quality of life and economic opportunity for rural citizens and society as a whole, and to maintain a quality environment and natural resource base " The ARS currently plays a strong role concerning research activities on ergot by maintaining and updating a World Wide WebHomepage dealing with ergot (http //www ars-grin gov/ars/ SoAtlantic/ Mayaguez/sorghumnews html), releasing research dollars ($100,000 this past year) for study of the disease, and

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evaluating reported resistant sources of exotic germplasm at our Tropical Agricultural Research Station, located in Mayaguez, Puerto Rico Two major research facilities are gearing up to do further research on ergot, our research facility at Fort Detrick, Maryland, USA, and our site in Mayaguez These two sites have already collaborated on evaluating fungicide treatments of honeydew infected seed and have published their findings on the Web site Most seed companies and State and Local regulatory agencies to ensure the safe movement of research seed into the United States have approved these recommendations for use Our Fort Detrick facility offers state-of-the-art lab facilities for importation of exotic diseases and pests for study and evaluation, and current plans of action include, 1) development of molecular methods for species identification, 2) direct morphological comparison of the different species under the same environmental conditions, and 3) determination of environmental factors that influence the formation and viability of sclerotia Our Mayaguez facility provides excellent environmental conditions for screening of exotic accessions and the development of improved sources of germplasm for disease resistance Plans of action include, 1) evaluation of reported sources of resistance, 2) crossing of these accessions to A3 cytoplasmic sources to evaluate them in a male-sterile background, 3) evaluate other sources of cytoplasmic sterility, 4) enhancement of these sources of resistance into improved genetic backgrounds, and 5) develop markers for study of genetic variability of ergot found within the Americas Through collaborative efforts and contacts, such as this conference, the USDA looks forward to working with the worldwide sorghum scientific community to strengthen our focus on research areas in which we can excel to provide additional research and information in our effort to safeguard sorghum from ergot in the future

*Much of the general information on the agencies within the USDA was taken from their respective Homepages on the World Wide Web(http //www usda gov)

Sorghum Ergot in Australia

Malcolm Ryley and Bob Henzell

The Australian Grain Sorghum Industry

Grain sorghum is a major component of farming systems in north east Australia due to the dominance of summer rainfall, its vital contribution to management of many soilborne diseases, and in providing stubble cover for erosion control Between 400,000 ha and 600,000 ha are grown annually, with production varying from approximately 500,000 t to 1 5 million t, depending mainly on seasonal conditions Australia is among the top 10 producers in the World, but well behind the United States, India, Mexico and China Approximately 70% of the crop is grown in central and southern Queensland, with the remainder in northern New South Wales (Figure 1) The grain is used exclusively for stockfeed, particularly poultry, beef cattle, pigs and dairy cattle and none is used for direct human consumption Stockfeed usage in Australia increased from 340,000 t in 1985-86 to 1 1 million t in 1995-96, with a consequential decrease in the volume exported

In Australia, planting times are influenced mainly by the availability of adequate soil moisture for germination and also by considerations of frosting and potential damage from sorghum midge (*Stenodiplosis sorghicola* [Coquillett]) late in the growing season The optimum planting period in central Queensland is late December to mid February, while on the Darling Downs in southern Queensland, October is the preferred time Further south in New South Wales the optimum planting time is in late October and November Consequently, most sorghum crops are flowering during hot weather (maximum daily temperatures >28°C) when the risk from ergot should be very low However, some late-planted crops may still be flowering in April, when temperatures are considerably lower than in mid-season Ratoon cropping, a practice in which seed is harvested from the previous season's regrowth, is conducted by some growers in central Queensland but it is considered to be an opportunistic practice in response to good rainfall in spring

The Australian Seed Industry, Parent Seed Production and Breeders' Nurseries

Four seed companies (Pacific Seeds/Advanta, Pioneer Hi-bred, Grainco and Hylan Seeds) produce 5,000-6,000 t of planting hybrid seed annually, with about 60% being commercial grain seed Most is produced during the summer months (planted between October and late November) under irrigation in the Macquarie Valley of central New South Wales and in the Murrumbidgee Irrigation Area in southern New South Wales, and a small quantity is produced

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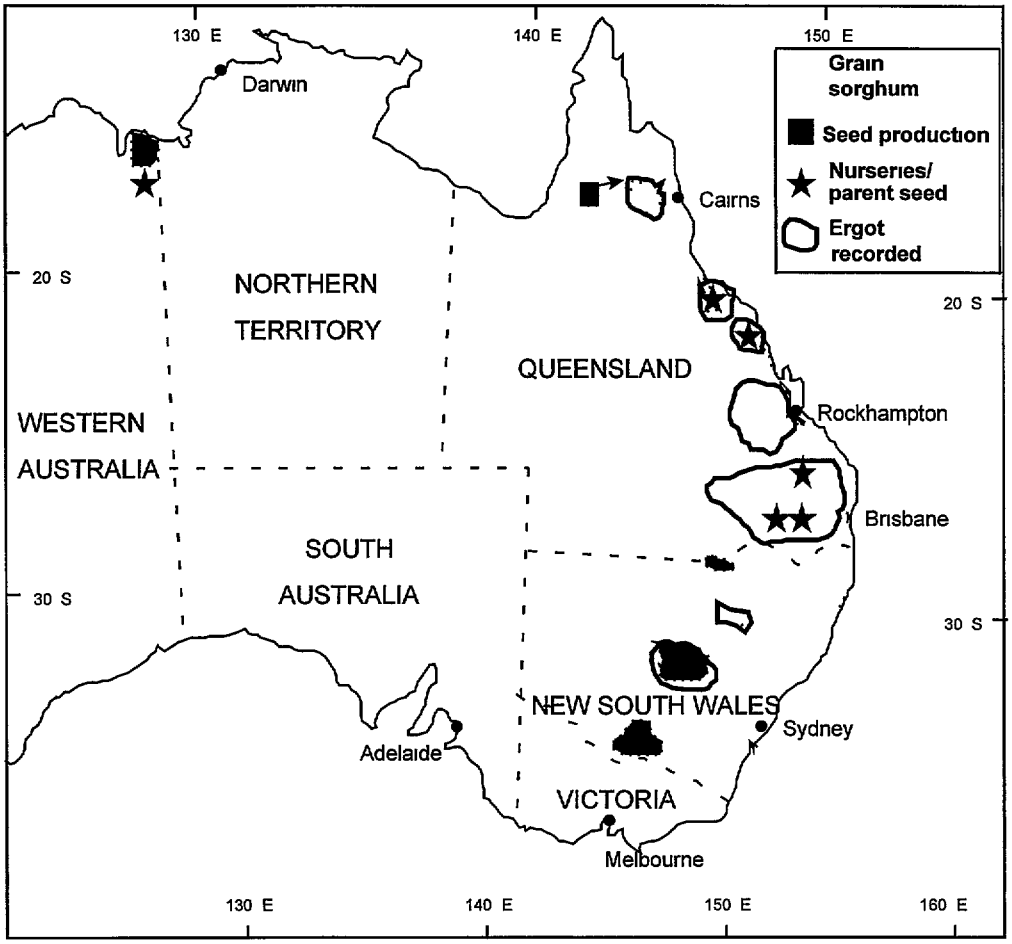

Figure 1 **Commercial sorghum production areas, seed production areas, breeders' nurseries and parent seed production blocks, and distribution of *Claviceps africana* in Australia, 1996 - 1997**

near the New South Wales-Queensland border Some winter production is conducted in irrigation areas on the Ord River, Western Australia, the Burdekin River on the northern Queensland coast, and at Lakeland Downs in northern Queensland, but mainly for short daylength cultivars and in emergency situations when there has been a shortfall in summer production (Figure 1)

Breeders' nurseries and parent seed production blocks are often conducted at the same site Pacific Seeds/Advanta plants two nurseries at Gatton in southern Queensland, the first planted in early September (flowering in December) and

the other in late January (flowering in late March-April) Some basic seed is produced at the Ord River in winter Pioneer Hi-bred conducts 2 summer nurseries at Wyreema, near Toowoomba in southern Queensland which generally is cooler and has lower humidity during the summer and early autumn months than Gatton One is planted in mid October and the other in late November A winter nursery and grow-out blocks for hybrids and inbreds are planted in the Burdekin area in early April Grainco conducts parent seed production/ nurseries in summer at Cambooya and in winter at Bowen on the northern Queensland coast Hylan conducts their parent seed production in the Burnett district, approximately 120 km north of Toowoomba The Queensland Department of Primary Industries (DPI) breeding program has summer nurseries at Hermitage (planted October), Gatton (planted late November), Biloela planted September and January) Research Stations and a winter nursery at Ayr Research Station in northern Queensland, planted in early April

The Outbreak of Sorghum Ergot

Sorghum ergot was first identified in Australia on April 26, 1996 on a specimen which had been presented to the Plant Pathology group at the DPI, Toowoomba (Ryley et al 1996) The ergot sample had been collected from a research farm at Gatton Quarantine authorities in DPI and in the Australian Quarantine Inspection Service (AQIS) were immediately notified, and a strategy for further short-term action was developed The site was visited the following day and infection levels of 100% in A-lines, but only about 5% in the R-lines were found in both grain and forage sorghum blocks A white waxy coating covered entire panicles and white deposits of dried honeydew were evident on the plant parts and on the ground below the affected panicles Honeydew, ranging from translucent through opaque to honey-yellow and finally white, exuded from spikelets The honeydew contained 3 types of hyaline, thin-walled, aseptate conidia Macro-conidia were oblong, rounded at both ends, often constricted in the median region, 10-20 µm x 5-8 µm, secondary conidia were narrowly obovoid-clavate, 11-17 µm x 5 5-8 0 µm, with a short apiculus at the base, and microconidia were spherical and 2-3 µm in diameter Oval-spherical sclerotia, 3 5-5 0 µm x 2 0-2 5 µm, which were protruding between the glumes of many spikelets were collected at the same site 14 days later A tentative identification of *Claviceps africana* Frederikson, Mantle and de Milliano (Frederikson et al 1991) was confirmed by Dr P Mantle, Imperial College of Science, Technology and Medicine, London

Two days after the first report, ergot had been found in other nurseries and in forage sorghum crops within a 10 km radius Quarantine notifications which prohibited the movement of produce and also restricted some farming activities were placed on 3 properties (including 2 research stations) Within 1 week, ergot had been found over an area of approximately 16,000 km^2 during surveys of forage and grain sorghum crops in southern Queensland Most records were on forage sorghum, which tends to flower unevenly often resulting

in poor pollination, and on late-flowering tillers in grain sorghum crops Quarantine restrictions on the 3 properties were lifted when it was realised that the disease was not restricted to a small geographic area A DPI sorghum ergot task force consisting of quarantine, plant pathology and management personnel was set up to coordinate surveys, to provide information to growers, seed companies, advisers and the general public, and to liaise with authorities in other Australian states

Current Distribution of Sorghum Ergot

One month after the intial sighting of sorghum ergot in Australia, the disease had been found on forage sorghum [*Sorghum bicolor* (L) Moench, *Sorghum sudanense* (Piper) Staph and *S bicolor* × *S sudanense*], on late flowering grain sorghum tillers, and on johnson grass *Sorghum halepense* (L) Pers over an area of nearly 70,000 km^2 in southern and central Queensland, and on forage sorghum on the Atherton Tableland, north Queensland approximately 1,400 km north of Gatton Within another 2 months it had been found on forage sorghum or in nurseries and parent seed blocks at several localities near the coast between the southern and northern outbreaks In early August the disease was identified at Lakeland Downs, some 160km north of the Atherton Tablelands, which is now the northernmost known limit of ergot in Australia Between October, 1996 and May, 1997, ergot has been reported at many localities within the known area of distribution In February, 1997 the disease was recorded in forage sorghum crossing blocks in the Namoi Valley of northern New South Wales, and in hybrid seed production blocks in the Macquarie Valley of central New South Wales, about 600 km southwest of the previously known southern limit of the disease In early 1997 the pathogen was reported on *Sorghum almum* Parodi, a common weed in central Queensland Secondary conidia have been implicated in the spread of sorghum ergot in Africa (Frederickson et al 1989, 1993) and Brazil (Bandyopadhyay et al 1996) and they undoubtedly contributed to the spread of the pathogen in southern Queensland Weather conditions in southern Queensland before and immediately after the first detection of *C africana* were ideal for infection, disease development and dispersal of secondary conidia At Gatton between May 1 and May 6, 1996 there were 6 consecutive days with rainfall above 20 mm, maximum daily temperatures below 25°C and strong easterly to south-easterly winds The outbreaks in New South Wales may also have been the result of infection by secondary conidia However there is no conclusive evidence that sorghum ergot spread rapidly from a single focus (Gatton) or possibly 2 foci (Gatton and northern Queensland) in Australia in mid 1996 It is possible that sorghum ergot had been present in Australia for a number of years at very low levels due to drought conditions

Identity of the Causal Agent

The morphology of the conidia and/or sclerotia of most of the isolates collected from grain sorghum, forage sorghums and *S halepense* were identical to the original isolate from Gatton Cross inoculation studies using macroconidia

Table 1 Morphology of conidia in collections from northern Queensland, May, 1996

Characteristic	*Claviceps africana*		*Claviceps* species	
	Range[1]	Means[2]	Range[1]	Means[2]
macroconidia				
length (µm)	10 20	12 6 14 8	9-19	12 1 14 1
width (µm)	5 8	7 0 7 1	4 5 5	4 6 5 0
secondary conidia				
length (µm)	11 17	13 8 15 4	9 14	10 5 12 0
width (µm)	6 8	6.3 7.1	4 5.5	4.7-4 9

[1]Range of dimensions of conidia from 50 collections
[2]Range of means of 50 collections

from *S bicolor* and *S halepense* also confirmed that the latter was a host of *C africana* However some collections from forage sorghums differed from *C africana* A careful examination of macroconidia and secondary conidia (produced in vitro) from individual spikelets in the same panicles in a collection from north Queensland (May, 1996) revealed that most spikelets were infected by *C africana*, but on the remainder the conidium morphology did not resemble that species The macroconidia of this taxon were similar in length to those of *C africana*, but were consistently narrower, while the secondary conidia were shorter and narrower than *C africana* (Table 1) According to Frederickson et al (1991) macroconidia of *Claviceps sorghi* are narrower (4-6 µm) than those of *C africana* (5-8 µm) as are macroconidia of the north Queensland isolates These and other differences (such as conidium shape and sterigma dimensions) suggest that the species may be *C sorghi*

Two distinct types of sclerotia were found in inflorescences of forage sorghum at a locality on the Darling Downs (west of Toowoomba) in June, 1996 The first type, typical of those described for *C africana* (Frederickson et al 1991) were oval-spherical, 4 0-5 5 mm long and 2 0-4 0 mm wide with a rough surface The second type were cylindrical, with longitudinal grooves in the smooth basal portion, 5 0-8 0 mm long and 1 8-2 5 mm wide Although the sclerotia were similar in some respects to those described for *C sorghi* (Frederickson et al 1991, Bandyopadhyay et al 1996) they should not be assigned to that species at present, pending further study

Considerable variation in the morphology (particularly dimensions) of both conidia and sclerotia have been reported (Bandyopadhyay et al 1996, present report) which may be a reflection of the influences of environmental factors and the hosts There is an urgent need to reexamine the taxonomy of *Claviceps* taxa which infect *Sorghum* species This reassessment must be conducted using as many traditional characteristics as possible, as well as new molecular techniques such as RFLPs and RAPDs Insights into the genetic relationships between isolates from different countries and within countries and into the role of sexual reproduction in the life cycle can also be obtained from such molecular studies

Losses in Commercial Grain Crops and Forage Sorghums

In the 1996 and 1997 seasons losses in grain sorghum crops have been extremely low In 1996 most commercial grain crops in southern and central Queensland had flowered before the disease was discovered and it was only late flowering tillers which were infected, with infection levels <5% The low temperatures at this time of year (particularly night temperatures) and the uneven flowering of the tillers, hence poor pollination and fertilisation, were ideal for ergot infection Some harvesting difficulties due to honeydew were experienced in southern Queensland in late flowering crops, but direct yield losses were negligible In central Queensland, ergot was observed in ratoon crops in November, 1996 with infection in the order of 5-10%, in tillers from November onwards, and on primary heads with <5% infection in April, 1997 In southern Queensland losses were negligible in 1996 and to the present time there have been no reports of ergot in commercial grain crops in the region Of greater concern than the direct yield losses are the uncertainties regarding potential alkaloid poisoning of stock in the lotfeed industries, and the implications for export However, Australian research indicates that ingestion of grain contaminated with 1% sclerotia does not severely affect livestock, pigs or poultry Although some growers have been concerned with grazing livestock on ergot-infected forage sorghum and with using the hay for silage there have been no reports of adverse effects on livestock in either situation

Despite the small losses experienced in the commercial grain industry the potential for serious losses remains It is apparent that there is abundant inoculum present for most of the year to initiate an epidemic In 1996 ergot was recorded from April-July and again from October onwards in central and southern Queensland Some hosts, particularly forage sorghums, *S almum* and *S halepense* appear to be particularly susceptible and can be considered to be very important sources of inoculum when commercial grain crops are flowering The roles of other sources of inoculum, such as ascospores from germinating sclerotia, conidia in honeydew on fallen inflorescences and conidia in locules of immature sclerotia are unknown Given environmental conditions which lead to poor pollen production, poor pollination and fertilisation, followed by a period of relatively cool weather an epidemic on grain sorghum in one or more of the growing areas in Australia is possible

Losses in Breeders' Nurseries and Seed Production Blocks

The greatest impact of sorghum ergot in Australia has been on the seed industry To the present time ergot has only been recorded in seed production blocks near Narromine, central New South Wales in February, 1997, following a short period of cool, wet weather Immediately after this period approximately 1% of the total seed production area was sprayed once or 3 times by helicopter with Folicur® (43% a i tebuconazole) Infection levels in these seed production blocks were negligible, and after harvest no sclerotia could be detected in the seed sample

Losses in nurseries and parent seed production blocks have been far more severe At the site of the initial recording in April, 1996, infection levels of 100% were experienced in A-lines and approximately 5% in R-lines across all nurseries As a consequence, no AxB or hybrid crosses were harvested Losses of approximately 30% were experienced in an experimental hybrid seed production block on the northern Queensland coast in August-September, 1996 An unexpected outbreak was recorded in A-lines in breeders' nurseries at Gatton in December, 1996 Examination of weather data indicated that approximately 8 and 17 days before symptoms were noticed there were 2 days with maximum temperatures <27°C, minimum temperatures <15°C and rainfall of >5 mm Between these two periods the maximum daily temperatures were >30°C It is apparent that temperatures following infection have little influence on disease development However losses were negligible in single rows of A-lines but were higher in multiple rows Attempts to manage the infection with aerial applications (by both helicopter and plane) of Folicur at 0 5 L ha^{1} proved ineffective A second nursery at Gatton, with lines flowering between late March and early May, a higher risk period due to low temperatures than in December-January, was not sprayed with Folicur and had negligible infection

Nurseries are also conducted by seed companies on the eastern Darling Downs, which is generally cooler and less humid than the Gatton district due to its elevation In one nursery where breeding lines flowered in January, 1997, ergot incidence was approximately 5% in grain sorghum lines and 50% in forage sorghum lines despite having been sprayed twice a week for 4 weeks with Folicur at 0 5L/ha At this same site spraying lines which flowered a month later with this fungicide on a 4 day cycle resulted in approximately 85% control In another nursery, lines which flowered during an overcast, wet and cool period in late January-early February and which were not sprayed were heavily infected (50% in A-lines and <5% in R-lines) The September 1996-planted nursery at Biloela was the only DPI nursery infected with ergot in 1996-97 High levels of infection occurred in A-lines which flowered in late November, when the risk from ergot should have been low due to high temperatures expected at that time

Consequences and Control in Commercial Grain Crops

Consequences Apart from the potential direct yield losses associated with ergot, there are other consequences of sorghum ergot which have, and will, impact on the Australian sorghum industry Seed costs will undoubtedly increase due to the increased costs associated with management of ergot in seed production blocks The Seed Industry estimates that given the worst-case scenario the cost of a 25 kg bag of seed could increase by AUS $20 (AUS $ 0 77 = 1 US $) The outbreak in Australia has export trade implications, particularly to countries which demand a nil tolerance to ergot sclerotia A 0 3% tolerance to ergot sclerotia has been set for the domestic stockfeed market, and the impact of this limit on the availability and price of lotfeed sorghum grain is

unknown Before the 1996-97 season confidence by some growers in grain sorghum was lowered due to uncertainty in the potential damage which ergot was likely to cause, but that confidence had been partly restored towards the end of the season in which losses have been negligible The presence of ergot has also reduced the flexibility of planting times in an attempt to avoid the high risk period at the end of the season (crops flowering in April-early May)

Control The following recommendations have been made to growers to minimise losses from ergot - (i) planting times should be selected to ensure that crops flower during the low risk period (January-March), (ii) crops should be well managed to ensure even flowering, and (iii) if necessary kill late-flowering tillers with herbicides to minimise potential harvesting problems To assist growers in the selection of planting times a climatic risk analysis has been conducted for selected localities in Australia A set of climatic parameters, based on overseas research results, was used to interrogate long-term weather data, resulting in a prediction of the number of infection events which had occurred over the past 30 years The analysis indicated that crops flowering early (before December) or late (after March) in the season were at greatest risk The climatic parameters used in this model are being modified to more closely reflect the field situation For example, experience in Queensland has shown that infection can occur without rain, prolonged heavy dew at night is apparently sufficient for infection Other models have the potential for use as a warning system for fungicide application in seed production blocks

Consequences and Control in Seed Production and Nurseries

Consequences Although direct yield losses from ergot in seed production blocks have been moderate to date, the potential for significant losses remains A crop failure due to ergot could have serious consequences not only for the supply of adequate planting seed for the Australian plantings, but also for the seed company which has the failure, due to a loss in market share The effect will also flow on to domestic production levels Serious losses in nurseries and parent seed blocks can result in loss of important breeding material and impact on the development and release of new cultivars Seed companies have faced extra production costs such as labour for handling contaminated seed lots, costs associated with interstate quarantine requirements, purchase of specialised spraying equipment and fungicides There has also been a loss in flexibility in planting times and locations, and in the future new production areas may have to be sourced

Control At present, ergot can be considered to be endemic in Queensland and most of New South Wales, and only southern New South Wales and the Ord River Irrigation Area can be classed as being ergot-free As mentioned before, ergot has been found in the seed production area in central New South Wales, but not in southern New South Wales Selection of planting times and localities which are likely to result in the smallest losses can be made with the aid

of a climatic model, but even 1 or 2 days of unseasonly cool wet weather can result in significant losses Measures designed to minimise the risks of introducing ergot into ergot-free areas, such as grading, fungicide seed dressings such as thiram, washing of honeydew-contaminated seed, are being practised Improved pollen management through alterations in male female ratios has also been recognised by seed companies However, fungicides applied during flowering will remain the major management tool Permits have been granted for emergency use of tebuconazole, triadimenol and procymidone in nurseries and seed production blocks, and the first 2 fungicides are currently being used routinely However, there still needs to be considerable research on aspects of application including nozzle type, water rates and the relative efficacy of aerial and ground application Fungicides with eradicant activity need to be identified to enable applications after conditions suitable for infection have occurred, as do fungicides with residual activity to reduce the numbers of applications

The Future

Sorghum ergot has had little direct impact on grain sorghum production in Australia, but the seed industry has been seriously affected by the disease The potential for epidemics both in seed production and commercial grain production exists, and although low risk times and localities can be identified, only short periods of unseasonal weather are needed for ergot epidemics It is impossible to predict the long-term impact of ergot in Australia, but it is apparent that losses will vary from year to year

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Global Conference on Ergot of Sorghum

Recommendations Report

Participants of the **Global Conference on Ergot of Sorghum** met in plenary sessions and in work groups to make general and specific recommendations for collaboration and networking with the overall goal of developing sustainable control strategies for sorghum ergots

I General Recommendations

Establish an International Steering Committee to serve and assist in coordinating sorghum ergot research activities and communication within the international community In response to this recommendation the following International Steering Committee Members, as shown in the table below, were selected

Responsibilities of the International Steering Committee

- Maintain and expand the **World Wide Web site** for dissemination of information on ergot of sorghum including links to other pertinent web sites and other sources of ergot information accessible by computer or other means
- Develop a mechanism for **posting** of the most recent sorghum ergot control or management information on the ergot web site
- Develop an e-mail directory of individuals specifying sorghum ergot expertise or interest and post the directory on the ergot web site and disseminate through other appropriate means An initial listing should include attendees of this conference
- Develop international working groups to target specific sorghum ergot research projects
- Maintain and promote collaboration and integration of sorghum ergot research between the public and private sectors of the sorghum research and production industry
- Promote funding for integrated research and development for the sustainable control of ergot in sorghum

Name	Year of Expiration	Organization	Country
Bandyopadhyay Ranajit	2002	ICRISAT	India
Casela, Carlos Roberto	2002	Embrapa Milho e Sorgo	Brazil
Dahlberg Jeff	2001	USDA	USA
Mantle Peter George	2000	Imperial College of Science	UK
Maunder Bruce	2000	NGSP	USA
McLaren Neal W	2001	Grain Crops Institute	S Africa
Odvody Gary (Chairman)	2002	Texas A&M	USA
Ribas Paulo	2000	Agroceres	Brazil
Ryley Malcolm J	2001	QLD Dept of Primary Ind	Australia

II Recommendations for Research Strategies in the Area of Genetic Resources

- Evaluate sources of resistance (reported in the literature) by utilizing sorghum germplasm available within national and international collections (Jeff Dahlberg, USDA and Ranajit Bandyopadhyay, ICRISAT were elected to coordinate this effort)
- Develop and distribute a standard methodology for evaluation of genetic resources
- Develop and distribute information about standard inoculation techniques
- Evaluate the role of different sorghum cytoplasms and fertility on infection
- Evaluate alternate sources of cytoplasmic male sterility for resistance to ergot
- Develop standardized screening techniques for pollen fertility, pollen shed, fertilization, and seed set
- Evaluate tillering ability in germplasm collections of both B, A, and R lines
- Ten sites were identified as possible locations for initial screening of accessions reported by ICRISAT and others as ergot-resistant sources

Argentina	India
Australia	Mexico
Brazil	Puerto Rico
Ethiopia	South Africa
Honduras	United States

III Recommendations for Control and Management Strategies

Phytosanitary Issues

In areas where sorghum ergot exists Sclerotia should not be used as a phytosanitary restriction measure because sclerotia and sclerotium-like material pose little risk for initiating disease compared to external airborne inoculum

Sclerotia, sclerotium-like material, and honeydew encrusted seed pose the same qualitative risk concerning epidemiology of sorghum ergot caused by *Claviceps africana* because sclerotial germination is low and there is no evidence for sexuality from isolates in the Americas

Seed intended for planting should be treated with an appropriate fungicide (e g captan or thiram) that completely eliminates inoculum of the ergot pathogen on the seed surface

Host Range of *Claviceps africana*

Sorghum bicolor, S bicolor subspecies bicolor S halepense, and other sorghum species are recognized as hosts for *C africana* Other (reported) secondary hosts will be recognized only after Koch's postulates have been demonstrated under natural conditions *Zea mays* is not a host of sorghum ergot under natural conditions

Chemical Control

Development of improved chemical control methodology is needed, especially for ergot control in hybrid seed production

Risk of strains of *C africana* tolerant to triazole fungicides should be assumed and there must be a proactive effort to identify alternate fungicides with a different mode of action

Exercise restraint in the indiscriminate use of fungicides to minimize the risk of developing fungicide-resistant strains

Alternate Control Strategies

Alternate control strategies need to be developed to both minimize the need for chemical control and maximize its effectiveness

Biological Research

Research is needed on several aspects of the biology of the *Claviceps* spp attacking sorghum (see section IV) As new scientific knowledge is gained it should be incorporated into current and projected ergot control and management strategies

Epidemiology

Encourage further development of disease prediction models for ergot with regional calibration and interactive capabilities to aid in ergot management decisions including fungicide application

Monitoring of sorghum ergot

A mechanism is needed to provide for the rapid sharing of information concerning the spread, distribution and severity of ergot across different global locations The ergot web page may be the primary means to accomplish this goal

Pollen Management

The critical importance of pollination/fertilization events as the primary ergot escape mechanism for sorghum indicate a need for more research in this area These should include studies on host pathogen environment interactions affecting sorghum pollination/fertilization and further development and refinement of pollen management strategies Knowledge in these areas may also provide a basis for the development of sustainable host plant resistance

IV Recommendations for the Biology of Sorghum Ergot

- Continue research on sclerotia or sclerotia-like bodies and determine functions of these bodies in the disease and life cycle of sorghum ergot
- Determine the dispersal of asexual inoculum, vis-a-vis rates, spatial, temporal and any other aspects affecting dissemination of the pathogen
- Expand, apply, and validate disease model in other geographic areas
- Use multidisciplinary approaches to toxicological studies in feeding trials of ergot contaminated sorghum
- Determine the role(s) of saprophytic fungi in toxins, synergisms, biocontrol and other interactions of ergot with other microorganisms

- Determine the host range of the sorghum ergot pathogens and influence of different hosts on the production of toxic alkaloids

V Recommendations for Molecular Approaches to the Study of Sorghum Ergot

- Evaluate the suggestion of using fertility/incompatibility genetic mechanisms as genes for transforming sorghum for resistance to ergot
- Utilize molecular level approaches for evaluation of pathogen population biology
- Increase the use of fingerprinting technologies in determining relations among ergot collections for species and other taxonomic relations
- Determine the potential of transforming sorghum to express antifungal proteins at infection sites

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