EPIDEMIOLOGICAL ASPECTS OF *Claviceps africana,*

CAUSAL AGENT OF SORGHUM ERGOT

A Dissertation

by

NOE MONTES GARCIA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2004

Major Subject: Plant Pathology

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ABSTRACT

Epidemiological Aspects of *Claviceps africana,* Causal Agent

of Sorghum Ergot. (December 2004)

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Sorghum ergot, caused by *Claviceps africana* Frederickson, Mantle & de Milliano, is a disease that affects non-fertilized ovaries in sorghum male-sterile plants and infects hybrids if there is pollen sterility at flowering time. Sphacelia containing macroconidia could play a role in the survival of the pathogen. This study developed risk assessment models and evaluated environmental conditions affecting viability of macroconidia and transition from sphacelial to sclerotial tissues. Effect of weather on ergot severity was evaluated under natural conditions (in monthly planting dates) in nine sorghum genotypes at College Station, Weslaco, Rio Bravo, and Celaya. Panicles were inoculated daily beginning at flower initiation with a suspension of 1.6×10^6 *C. africana* conidia ml^{-1}. Weather triad values were used to identify weather parameters correlated with the disease. Ergot severity was statistically greater in A-lines than hybrids because of the possible interference of pollen on some dates. Celaya had the greatest amount of ergot in hybrids. A-line ATx2752 had the lowest average ergot severity throughout years, locations and planting dates, as did the hybrid NC+8R18. Maximum and minimum temperature had a negative correlation with ergot at Rio Bravo, College

Station and Weslaco, while at Celaya it was positive. The highest correlation was 7 to 9 days before initiation of flowering, suggesting that cooler temperatures during this period could cause male sterility. A-lines showed the same relationships between ergot and maximum and minimum temperatures after initiation of flowering. Minimum relative humidity had a positive correlation with ergot after initiation of flowering in both sorghum plant types. Sphacelia stored under cool temperatures (-3°C to 7°C) maintained conidial viability, and newly-formed sphacelia located on the sphacelia surface had the highest conidial viability. However, they show a greater viability reduction through time compared with conidia from older sphacelia, showing that conidial maturity can play a role in the survival of the conidia. Sphacelia on plants grown at 10°C, 20°C and 30°C with low relative humidity did not had any sclerotial development up to 4 weeks after formation of sphacelia. However, higher temperatures promoted an increase in the sphacelia dry weight during that time.

DEDICATION

This dissertation is dedicated to my wife, Maria de Jesus, my daughters, Perla Rubi, Lourdes Adriana and Nicole Dominique, and to my sons, Noe Jr. and Alexander (†), for their love, support and comprehension during all this new and final odyssey of my personal education. To my father Carlos and my mother Elvira, my brothers Miguel Angel and Carlos Jr., and my sisters Griselda and Sandra Luz for their support and love since my early years. Also, to my uncles Jose Guadalupe, Jose Trinidad, Manuel, Cristina, Margarita, Elvia, and to the entire family of relatives that have been always so supportive of me and my loved ones. !!Thanks and god, bless you!!

To the memory of my grandmother
Luciana

and of my grandfather
Justo

and of my aunt
Maria Elena

and of my son
Alexander

!!You will live forever in our hearts!!

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CHAPTER I

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is cultivated worldwide on more than 45 million hectares and used for feed, food and industrial purposes. It is the fifth most important cereal crop in the world. The most significant event that promoted the increase in the production of this cereal was the development and commercialization of hybrid seed production during the late 1950's.

Since 1997, sorghum seed production fields and commercial grain fields in North America have been exposed to a sorghum disease called "ergot". Ergot is a fungal disease of cereals and grasses that is caused by species in the genus *Claviceps*. According to Agrios (1), ergot of cereals (caused by *C. purpurea*) is one of the oldest recorded plant diseases on earth, because of poisoning of people and animals from ergot-contaminated food. Nevertheless, sorghum ergot, caused by *Claviceps africana,* Frederickson, Mantle, & de Milliano, is a disease that affects only non-fertilized ovaries in hybrid seed production, where is particularly severe on male sterile plants (A-lines), especially when pollen can not reach stigmas of the A-line, can not be released from anthers of the pollinator, and when there is pollen sterility of the pollinator due to some environmental factors. Economic losses are associated with poor seed quality and reduced yield (6). Another problem associated with this disease is the contamination

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This dissertation follows the style of Plant Disease.

of seed with pathogen structures (sphacelia and sclerotia), resulting in a quarantine problem for many seed companies established in the around the world. Sorghum seed importations to Mexico and Central America have been targeted by government inspectors because of honeydew on the seed surface, sphacelia and seed debris have been misidentified as sclerotia[*]. Contaminated seed shipments that contain sphacelia or sclerotial structures can be important because macroconidia located on sphacelia could play a role in the survival and spread of the pathogen. Exposed macroconidia in sorghum debris or in seed that fall to the ground during planting can germinate in a conducive environment, producing windborne secondary conidia that can infect a susceptible host.

Ergot can also be a problem on sorghum hybrids if there is pollen sterility at flowering or if pollen can't be released from anthers due to rain during the flowering period. Control of ergot is difficult because of the lack of physiological resistance in commercial hybrids and male-sterile lines used in sorghum hybrid seed production (6). Some sorghum lines with claimed ergot resistance show susceptibility when they are evaluated under different environmental conditions. The following are hypotheses of this study:

Hypotheses

- Environmental factors (maximum and minimum temperature, and minimum relative humidity) will increase sorghum ergot, and there will be severity differences among hybrids and male-sterile lines.

[*] Sphacelia do not pose the same toxicity concerns as sclerotia.

- Conidia associated with the sphacelium can survive at low temperatures and low relative humidity for several months, and retain greater viability if they are on the inside of the sphacelium.
- Sphacelial tissues require dry-cool environmental conditions to develop into sclerotial tissue.

Objectives

- Determine the relationship between the host, pathogen and environment to develop a sorghum ergot risk assessment model.
- Determine the effect of temperature and age of the sphacelium on survival of *C. africana* conidia located outside and inside the sphacelium.
- Identify environmental factors leading to the process of transition from sphacelium to sclerotium.

CHAPTER II

REVIEW OF LITERATURE

Pathogen

Economic importance

Losses due to ergot in seed production fields can be high. In India, losses up to 80% have been reported in seed production fields whereas in Zimbabwe the annual losses are between 12 and 25% and sometimes up to 100% (6). In 1997, nearly 45% of the hybrid seed production fields in the Texas Panhandle had ergot with varying degrees of severity (85). Losses from import rejection can occur. For example, in 1999, the Nicaraguan Inspection and Certification Department intercepted seeds with honeydew and sphacelial tissues mixed with seed in a shipment from the USA. This shipment was quarantined, resulting in losses of millions of dollars to seed companies.

Usually, this disease is not important in hybrid grain sorghum fields. Losses of seed quality can be an issue, because of honeydew contamination of healthy sorghum grain, increasing colonization by saprophytic fungi. McLaren (47) found such seed had reduced germination. In addition, honeydew stickiness can interfere with harvest.

Causal agent

Sorghum ergot is caused by the ascomycete *Claviceps africana* Frederickson, Mantle & de Milliano. There are two other species of the genus *Claviceps* that are pathogenic to sorghum*:* *Claviceps sorghi* Kulkarni, Seshardi & Hedge and *Claviceps sorghicola* Tsukiboshi, Shimanuki & Uematsu*.* These species are in the family *Clavicipitaceae*, which are perithecial ascomycetes (pyrenomycetes) (3). Frederickson et al. (26) observed that the main differences between *C. sorghi* and *C. africana* are: (1) the sclerotia shape of *C. africana* is oval to spherical, while it is long, cylindrical, curved or straight shape with *C. sorghi*; (2) *C. sorghi* has red-brown stroma*,* compared with blue-purple stroma of *C. africana*; (3) production of ergoline alkaloids (mainly dihydroergosine) in sclerotia of *C. africana*, with no production of alkaloid in *C. sorghi;* (4) honeydew sugars (mainly sucrose) are present in greater proportion (30-70% w/v) in *C. sorghi*, compared with 2-16% (w/v) in *C. africana*, and (5) faster formation of the sphacelial tissue before honeydew formation with *C. africana*, whereas *C. sorghi* exudes honeydew first from a more slowly-colonized ovary. In Japan, *C. sorghicola* was described based on smaller macroconidia, no microconidia or secondary conidia, and different alkaloids in sclerotia (80).

Geographical distribution

Ergot has been observed in the majority of sorghum production areas in the world. In Asia, sorghum ergot caused by *Claviceps sorghi*, was observed for the first time in India around 1915 (50). In Africa, the disease was first observed in Kenya in 1924, and

later, in other countries of Africa (19). It was in Africa where Frederickson et al (26) identified a different species, *C. africana*. In 1988, ergot was observed in Thailand (10), and in 1991 in Taiwan (13), although the pathogen was not identified to species. In Japan, another sorghum ergot species, *Claviceps sorghicola,* was discovered (80).

Since early 1995, sorghum ergot caused by *C. africana* was observed for the first time outside Africa and Asia, in Brazil (71), Australia (72) and in North America (2,41).

Pathogen genetic diversity

Genetic diversity among isolates of *C. africana* has been analyzed by random amplified microsatellite (RAM) and amplified fragment length polymorphism (AFLP) techniques (76,77,65). Tooley et al. (77) grouped isolates from Australia, India and Japan into one cluster, and isolates from U.S., Mexico and Africa into another. This supports results obtained by Pazoutová et al. (65), who found American isolates were similar to South Africa isolates. In another study, Tooley et al. (76) using AFLP analysis, found 95 to 100% similarities among isolates from United States, Puerto Rico and South Africa, suggesting Africa as the origin. Also, they observed a number of polymorphisms in the United States group, indicating that the pathogen population contains multiple genotypes.

Disease occurrence in Mexico and Texas

According to Frederickson et al. (27) spread by windborne secondary conidia is a factor in long distance dispersal. The initial ergot observation in Mexico was on

sorghum plants that were planted out of the 1996 normal fall planting dates in San Fernando, Tamaulipas that flowered on January-February period in 1997. Later, the disease was observed in normal planting dates in Tamaulipas south in the region known as "huasteca" (2). Since the introduction of ergot to Mexico, the disease incidence has been observed from 5 to 20% in commercial fields and up to 100% in seed production fields. During the following months, ergot was observed in Veracruz, Nuevo León, Nayarit, Sinaloa, Sonora and Jalisco, and from August 1997, in Michoacán, Guanajuato, Querétaro, Coahuila, Morelos, Puebla and Chiapas. Sorghum ergot has been active mainly in its principal hosts (johnsongrass and forage sorghum) across all the sorghum production areas. During early 1998, ergot was observed in the Pacific area (Sonora, Sinaloa and Nayarit), following storms caused by "El niño" and cold weather from February to May. In September ergot was observed around the "Bajio" area and the high plains of Mexico (Puebla and Morelos). By the end of 1998, ergot was in more than 20 Mexican states.

During September 1998, high amounts of rainfall throughout south Texas supported ratooning of plants, which were exposed to low temperatures from October to December. The extended flowering period associated with a cool and wet environment caused a fast development and spread of the pathogen on forage sorghum, grain sorghum along roads and johnsongrass plants (63). During 1999 ergot was observed in several sorghum winter nurseries located on the west coast of Mexico and Puerto Rico, and south Texas. During November-December, ergot was observed in the Bajio area and Tamaulipas. In early 2000, ergot had been observed in Puerto Vallarta, Mexico, with

disease incidence up to 35% and severity up to 100% in commercial hybrids. The epidemic in 2000 showed that *C. africana* is well established in the major sorghum production areas of Mexico and the United States, and has shown its capability to overwinter and survive hot and dry weather conditions.

Pathogen biology

C. africana infects only unfertilized ovaries, throughout the stigma or the ovary cell wall (25). Conidia germinate on the stigma, producing 1 to 4 hyphae. After penetration of the stigma, mycelia colonize the stigma rachis, growing downward to the ovary through internal tissues near the ovule and reaching the rachilla vascular tissue. Then, colonization proceeds upwards, leading to full colonization of the ovary. Mycelia grow intracellularly between the ovary cell wall and the epidermal tissue until the ovary is converted into a soft and whitish fungal mass, the sphacelium[1]. This structure increases its size and gives the ovary a swollen appearance.

The sphacelial interior consists of irregular channels and pores that are covered by simple conidiophores bearing conidia. Conidia (macroconidia and microconidia) are liberated to the exterior via the sucrose sap from the injured floret. *C. africana* macroconidia measure 9-17 x 5-8 µm and are hyaline, unicellular, oblong and with a constriction at the center. Usually there is a vacuole at each end. Microconidia measure 2-3 µm in diameter, and are circular, unicellular and hyaline. Germination of

[1] Sphacelium is derived from a Greek word, which means gangrene. "Sphacelium" is defined as the structure forming conidia in *Claviceps* from which the sclerotium develops (Dictionary of the fungi, 9th. Ed.).

macroconidium occurs only under a high relative humidity, leading to production of pyriform secondary conidia.

Honeydew droplets sometimes inhibit the macroconidium germination due to the high osmotic potential caused by high sugar concentration or to some other inhibitory compounds (37). The types of sugars that occur in the honeydews of some ergot fungi are hexoses, alditols, fructofuranosylglucoses and fructofuranosylalditols (57). Additional findings suggest the fungus can form sugars during parasitism. When moisture is absorbed from rain, dew or relative humidity, the osmotic potential of honeydew is reduced allowing macroconidia on the honeydew surface to start germination (6), producing a conidiophore that bears secondary conidia exterior to the honeydew. Secondary conidia are transported by wind and infect a host under high relative humidity (24). Ergot spread by windborne secondary conidia in conjunction with a sphacelial conidia source, have significant economical and epidemiological implications for sorghum hybrid seed production (27). Additionally, Prom and Lopez (67) demonstrated that adult corn earworm moths (*Helicoverpa zea*) can internally carry viable spores for several days and can act as a primary dispersal agent for *C. africana* spores. Moths can migrate from ergot endemic areas to disease free areas.

Upon development of the sphacelium, certain alterations in host physiology are observed, and host-parasite interactions may be important in triggering the morphogenesis of the sphacelium to the sclerotium (58). The sphacelial tissue transforms into sclerotial tissue under low temperature and dry environments. The sclerotium is a

mass of whitish fungal structure surrounded by a brown-reddish rind that can produce ascomata (29).

Symptoms

The disease can be easily identified by observing honeydew exudates from the sphacelium. Honeydew is a thin or viscous, sweet fluid that contains fungal conidia and sugars. Newly-formed honeydew droplets are transparent in color and can turn opaque. Droplets fall onto leaves and soil. Another sign of sorghum ergot, rarely seen with *C. africana*, is the sclerotium.

There is a required dormancy period for sclerotia of *Claviceps* species before they can germinate, which can be up to 9 months. Sclerotia of different species may require different germination conditions. Sclerotia of *Claviceps sulcata* germinated on filter paper after they had been exposed to lab conditions for six months. To date, only Frederickson et al. (26) demonstrated germination of the sclerotia of *C. africana*. The sclerotia germinated after one year under dry storage conditions at 20-25 °C, and incubation under moist conditions for four weeks. In India, Bandyopadhyay et al. (6) found that sclerotia of *C. sorghi* grown at 27 °C on the soil surface had more than 50% germination, while *C. sorghicola* sclerotia have been germinated under wet sand with diffuse light at 25°C. Sclerotia of *C. africana* from Mexico and Texas did not germinate (64).

Survival

Many pathogenic ascomycetes that produce resting structures generate ascospores following carpogenic germination. There are differences among the maturity of such structures and their capability to survive. Within a crop, *C. africana* produces sphacelia, or perhaps also sclerotia. At harvest, sphacelia differ in age or have different degrees of sclerotial tissue development. Survival of the pathogen may be affected by the level of fungal development or by environmental conditions. Cox and Scherm (15) found that the highly-mature *Monilinia vaccinii-corymbosi* pseudosclerotia that contained fully melanized entostromata had the highest level of survival, whereas the immature stromata had the lowest survival. Also, Coley-Smith et al. (14) observed that *Sclerotium cepivorum* sclerotia buried in the field for two years survived better than immature, nonmelanized sclerotia, or newly formed melanized sclerotia.

Bhuiyan et al. (8) showed that *C. africana* macroconidia present in sorghum panicles that were held above soil surface survived for more than eight months over winter, suggesting that local survival can provide inoculum for future epidemics in Australia. Storage of sphacelia at high temperature (>32°C) resulted in a rapid decrease in viability of *C. africana* macroonidia with no spores viable after two weeks of storage. Conidia germinated after 17 weeks storage at 20°C. The effect of cool temperatures (6°C) were evaluated by Odvody et al. (62), who observed that conidia of sorghum ergot maintained viability at its maximum up to 12 weeks, and then decreased 50% at 22 weeks of storage. In other study, Prom et al. (68) showed that conidia located on

sphacelia that were held above soil surface for a year survived and infect sorghum florets on male-sterile line

Host

History and taxonomy

A sorghum collection was first described by Linnaeus in 1753 under the name *Holcus*. However, in 1794 Moench re-classified this collection as the separate genus *Sorghum.* The genus *Sorghum* belongs to the tribe Andropogoneae, and is divided into three species: *Sorghum halepense* (L.) Pers., which is a native perennial of southern Eurasia east to India; *S. propinquum* (K.) Hitch., which is a native perennial of Sri Lanka and southern India, and from Burma eastward to the islands of south-eastern Asia, and *S. bicolor* (L.) Moench which includes all the domesticated taxa, a widely-distributed and ecologically variable African complex, and stabilized weedy derivatives derived from inter-breeding between domesticated sorghums and their closest wild relatives (39).

Importance of sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal in the world. In developing countries of the semi-arid regions, sorghum is one of the most important cereals that make the difference between food security and famine (40). Sorghum is more prevalent than other crops in semi-arid areas due to its drought tolerance. Sorghum grain has been used in malts, beers, fermented foods, starch production, animal compound feed, and more importantly, as flour for human consumption. Plant parts, such as leaves and stalks, have been used for house

construction, animal forage, ethanol and sugars. Sorghum grain yield can be up to 15 MT ha^{-1} in areas such as Guanajuato, Mexico, where environmental conditions are excellent for the development of this crop (Jesus Narro, personal communication).

Sorghum production

Sorghum is planted worldwide in about 43.2 million ha (average from 1997 to 2003) of which 7.6% and 4.4% are planted in the United States and Mexico, respectively (FAO, 2004). In the United States and Mexico the grain yields of 3.176 and 3.859 MT ha^{-1}, respectively, are more than double compared with the 1.358 MT ha^{-1} worldwide production. This is reflected by a greater production in US and Mexico, despite the larger proportion planted to sorghum in Africa (57%). According to FAO, during 2003, 59.5 million MT of sorghum were produced worldwide, of which 38% was produced in Africa, mainly for human consumption, whereas the United States and Mexico produced 17.5% and 10.8%, respectively, mainly for livestock feed to produce meat. In the last four years, there has been a reduction (18.5%) in the average yield of sorghum in the US compared with the yield obtained from 1997 to 1999, which was 4.3 MT ha^{-1}. Meanwhile, in Mexico grain yield increased 3.4% during the same period.

Sorghum plant

Sorghum is a robust and genetically variable member of the grass family, which looks very similar to maize and sugar cane (39). Sorghum has a strong stem and ranges in height from less than 1 m to 4 m. Stems can be juicy or dry, sweet or bitter. Leaves

are arranged alternatively and have prominent midveins and parallel lateral veins. Long overlapping sheaths are attached at the nodes.

Panicle development

Physiological aspects play a significant role on the understanding of sorghum ergot, especially those ones related to the flowering pattern which has been described in detail by Stephens and Quinby (75) and House (39). Development of the sorghum panicle starts from 40 to 45 days after planting (depending on the sorghum genotype). There is a small growing point on top of the apical tissue formed basically of younger leaves. This small structure forms the primary rachis, secondary and tertiary rachis. The individual florets start to differentiate individually (22). Structures as lemma and palea appear, followed by the differentiation of the stigmas and anthers. At this stage, the panicle measures between 1 and 1.5 cm and can be seen with the eye. Pollen formation starts inside the anthers (microsporogenesis). From this point, the panicle elongates until it reaches the flowering stage.

The young inflorescence or panicle is forced up through the sheath (boot) of the flag leaf. The peduncle may be well exerted above the flag leaf or sometimes the lower portion may remain inside the boot, surrounded by the sheath. Panicles may be short or long, compact to very lax and open, and oval, conical, long and slender, or pyramidal. Panicles have a central rachis that give rise to secondary and tertiary branches that hold racemes of spikelets, which normally occur in pairs. Each pair consists of a sessile bisexual spikelet and a pedicelled spikelet, which may be male or sterile. Sessile

spikelets are normally fertile, oval, and elliptic with two glumes. The lower glume partially envelops the upper glume. The pedicelled spikelet is sterile and consists only of glumes and/or lemmas. Frequently the sessile spikelet is fertile and bears three stamens, which produce functional pollen. Very rarely pedicelled spikelets may contain a rudimentary ovary that could produce smaller seeds that those of the sessile spikelets (59).

Flowering development

In general, most flowering occurs during the night and early in the morning (75). They comment that this may be due because anthers and pollen are easily affected by high temperature during the day. These authors also noticed that during cloudy and cold nights, the flowering process was delayed. The normal period in which a sorghum panicle completes the flowering period is seven days, but when plants are exposed to cold temperatures (near winter time) the flowering period can be up to 15 days (59,39). Usually, the time at which flowers open is between 02:00 and 08:00 hours, but sometimes can be delayed up to 10:00 hours due to environmental conditions. Sorghum has basipetal flowering, which occurs as follows: glumes normally open during day time from top to bottom, anthers and stigma emerge, anthers release pollen, followed by the closing of the glumes. Usually, anthesis of the pedicelled spikelet occurs a few days after sessile spikelets. The flowering process vary significantly according to the sorghum genotype used and to the climatic conditions, therefore it is highly important to know

how the flowering pattern can be affected by the environmental conditions and also in which manner they are contributing to infection by *C. africana*.

Potential hosts

According to Reed et al., (70), the potential non-crop hosts for *C. africana* are shattercane (*S. bicolor* subsp. *drummondi*) and johnsongrass [*S. halepense* subsp. *halepense* (L.) Pers]. These species could serve as over-wintering or alternative hosts of *C. africana* outside of the normal growing season. In their study, they found that non-sorghum accessions were not susceptible to the pathogen. However, Futrell and Webster (32) observed that conidia from *Sphacelia sorghi* collected on sorghum panicles, infected *Z. mays,* and ergot conidia collected from common guinea grass (*Panicum maximum*) infected sorghum and pearl millet plants. Another study of the host range of *C. africana* in Africa was conducted by Frederickson and Mantle (28), who observed that pearl millet can be an alternative host for *C. africana* under high disease pressure, especially in lines that showed the highest ergot incidence with *C. fusiformis.*

Plant-pathogen interaction

Stigmas are the only sites for entrance of the pathogen. Nevertheless, fertilized flowers are resistant to infection (6). In general, the stigma is pollinated by pollen grain as soon as it is exposed, then the pollen grain germinates in 30 minutes and fertilization occurs in the following 2-12 hours (75). Alternatively, conidia of *C. africana* require at least 12 hours to germinate on the stigma, and 36-48 hours to reach the base of the

ovary. However, environmental conditions may affect this interaction, e.g. ovaries were not fertilized 48 hours after pollen arrival (5). Komolong et al. (44) observed that *C. africana* conidia germinated within 1 day after inoculation (a.i.), the pathogen colonized the ovary at 1.3 days a.i., and 50% of the ovary was transformed into sphacelial tissue between the 5th and the 6th day a.i. They also observed changes in the chitin cell wall content of the fungus and callose deposition in the sorghum tissue as the pathogen continued infection.

Environment

Effect on pollen sterility

Environmental factors influence sorghum ergot by their direct effects on the pathogen and through their effects on pollen production in hybrids and restorer lines, and the pollination and fertilization processes in seed production fields. *C. africana* readily infects male-sterile plants in the absence of viable pollen, but self-fertile plants may become more susceptible because of sterility induced by certain environmental conditions. Downes and Marshall (21) demonstrated that night temperatures of 13°C or lower during meiosis can induce sorghum sterility in some genotypes. Brooking (11) observed that once the fertilization has occurred, there is no risk of having sterility due to cold temperatures. Also, he found that the period of cold temperature sensitivity of a sorghum panicle extends from the emergence of the flag leaf ligule to the time when the flag leaf is 20 cm in length.

Temperatures of 10 to 12°C are required for several days to induce complete sterility of the sorghum panicle. If there is a short interruption of such temperatures, only some portions of the panicle may be affected. The relationship between the sterility induced by cold temperatures and ergot susceptibility was demonstrated by McLaren and Wehner (48), who observed that some sorghum genotypes grown at 12°C for 3-4 weeks before flowering had the same flowering characteristics of male-sterile plants, and therefore were susceptible to ergot. In recent studies, Montes et al., (54) observed a significant effect between the minimum temperatures below 13°C and ergot incidence in sorghum hybrids, especially if these temperatures were present between 9 to 11 days before blooming (boot stage).

Effect on infection process

Any factor that increases the period between floret opening and fertilization will promote ergot infection (31). The ideal weather conditions for sorghum ergot development are temperatures around 19°C, high relative humidity, and cloudy conditions during the flowering period (46,84). Ergot is reduced with increasing temperature and it does not occur at maximum temperatures exceeding 28 to 30°C (46). Gupta et al. (36) observed that *C. microcephala* (cause of pearl millet ergot) was not observed at air temperatures exceeding 32 °C[*].

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[*] Odvody notes: We have observed natural infections of *C. africana* in Texas occurring on male-sterile lines during time periods when daytime highs exceed 38-39°C, and nighttime minimums were probably no lower than 24°C.

The optimum relative humidity for disease development is closer to 100% during the infection process (32). Workneh and Rush (84) observed that prevalence of ergot was related to the average relative humidity and precipitation. These same authors suggested that minimum temperature is less of a factor than maximum temperature in development of sorghum ergot in the Texas panhandle seed production area. High humidity can delay anther dehiscence and release of pollen (69). Temperatures between 14 to 28°C, combined with high relative humidity (>90%), are required for secondary conidiation and for conidial dispersion, but they are not required for sphacelium-sclerotium differentiation (5). The effect of temperature on secondary conidia germination were observed by Bhuiyan et al. (7), who showed that secondary conidia germinated up to 37°C, although the optimum was 20°C.

Plant disease management

Since farmers in ancient times began cultivating plants, people have been concerned with crop losses caused by pathogens. The decisions of when, where and what to plant and the development of production technologies through generations of trial and error have resulted in the successful farming that suppresses plant pathogen development. The discovery of the causes of plant diseases and the understanding of the interactions of pathogen and host has enabled us to develop measures for plant disease control.

Sorghum ergot is a disease that can produce several infection cycles during a growing season especially if the flowering pattern is not uniform. Yield losses can be

minimized by management approaches such as use of resistant cultivars, chemical control, and cultural practices. In seed production fields, pollen management has been very effective. Such management involves increasing the number of rows of the pollinator, establishing several planting dates of the pollinator and use of pollinator lines that produce high quantities of pollen.

Genetic resistance

In the past few years, attempts have been made to identify resistant sources. Nevertheless, no true genetic resistance to sorghum ergot has been found. Resistant cultivars at one location may be susceptible at another, e.g. sorghum germplasm from Uganda (IS8525J and IS8525D) that expressed high levels of resistance to ergot in Puerto Rico (16), but were susceptible in Mexico and the US (Prom, unpublished data). McLaren (47), evaluating sorghum lines in South Africa, didn't observe any correlation between the levels of sorghum ergot at one location compared with ergot levels at other locations. He classified sorghum lines as: (1) those linearly related to disease potential, (2) those highly susceptible even at low disease potentials, and (3) those with various degrees of resistance despite increasing disease potentials.

Moran (56) noted that differences in ergot vulnerability among sorghum genotypes within lines and hybrids, are most likely due to differences in floral structure, pollen viability and plant characteristics, all of them influenced by the interaction with the environment. Even though lines showed some resistance or vulnerability in their progenies, it is important that this information be utilized carefully, since drastic shifts in

the levels of ergot are expected depending on the environmental conditions present during the flowering period. Some of these differences can be explained by the work of Komolong et al (44), who observed that the rate of ovary colonization differed among male-sterile lines that also differed in ergot susceptibility, suggesting that there is a possible histological basis for partial resistance in male-sterile sorghum lines. New sources of resistance that have been observed in sorghum accessions such as IS14131 and IS14257 will promote further research on this aspect (70).

Chemical and biological control

Use of fungicides to control flower infection (12,35,61,73,78) has been effective with ground application (53), but this is not a suitable technique due to economics, application difficulties, and residual toxicity of some fungicides. The use of biological control to manage sorghum ergot is experimental. Bhuiyan et al (9) observed that undiluted cultures of *Trichoderma spp*, and two isolates of *Penicillium citrinum* completely inhibited the germination of *C. africana* conidias *in vitro* and reduced the severity of the disease *in vivo*. Similarly, *Pseudomonas aeruginosa* and *Burkholderia cepacia* inhibited conidial growth *in vitro*. However, *in vivo*, these bacterial isolates failed to suppress conidial germination of *C. africana*.

Crop management

Crop management is the most reliable and economic practice that a farmer or seed company can do to manage ergot (6). This entails ensuring: (1) a good nicking

(flowering at the same time) between A and R lines in seed production fields; (2) increasing the ratio of R to A lines to make more pollen available at shorter distances from the male-sterile lines; and (3) using hybrids with high pollen production. Another possible cultural approach is avoidance, which minimizes exposure of flowering grain sorghum to periods of time when environmental conditions are conducive for disease development.

Risk assessment models

Models can be used to develop a management program that identifies the pathogen's weakest point in its life cycle. The risk assessment model can tell growers when is the best time to control the pathogen or when the pathogen will not be favored by certain environmental conditions. There have been several studies to develop sorghum ergot risk assessment models which utilize weather variables. For example, Meinke and Ryley (51) conducted a climatic risk analysis in Australia and found that disease risk appears to vary strongly with season type, location, and the timing of anthesis. The analysis also indicated that either early or late plantings subjected grain sorghum to a greater risk of ergot. Additionally, the number of potential ergot events in any month differed strongly between years, season type and location (82).

Environmental factors such as temperature and relative humidity, have been used to obtain disease prediction models by McLaren and Flett (49). They used weather variables to quantify ergot incidence in sorghum experimental lines and found that daily average minimum temperature observed 23 to 27 days before flowering, daily average

maximum temperature 1 to 5 days after flowering, and daily average of maximum relative humidity from 1 to 5 days after flowering were the weather factors that had a relationship with ergot incidence. In other studies, Montes et al. (54) and Montes-Belmont et al. (55) observed that daily cool minimum temperature and maximum temperature present before flowering, and daily minimum relative humidity present around flowering were the weather factors that had a high correlation with ergot incidence on sorghum hybrids. If there is natural inoculum present, the response will be very similar to inoculations, due to the main effect that environmental conditions have on the disease.

Workneh and Rush (85) reported that rain appeared to be a major factor influencing sorghum ergot development in the Texas Panhandle, and the use of risk forecasting models will be a useful tool for growers and seed production companies. Weather variables have been used to predict presence of mycotoxins in wheat infected by *Fusarium graminearum* the causal agent of fusarium head blight. Both rainfall and temperature were important weather variables in the disease expression, especially if they occurred around heading (34,38).

Seed treatment

The problem of honeydew on the surface of sorghum seeds had been addressed by Dahlberg et al. (17), who found that contact fungicides captan (Captan 400®) and thiram (42-S Thiram®) were effective in inhibiting conidiophore and secondary conidia formation without drastically reducing the viability of the sorghum seed (1-4%).

Frederickson and Odvody (30) observed that conidia viability of newly intact sphacelia treated with captan (Captan 400®) was significantly reduced (63%) and cores from treated sphacelia didn't show a major reduction compared with the control. They suggested that this could be due to the slight penetration ability of captan within the sphacelia or desiccation of the sphacelia.

Toxicity

Toxicity associated with consumption of sclerotia or ergot bodies is not a recent problem. Historically, there is evidence to indicate that ergot poisoning (caused by *C. purpurea*) have plagued man and animals for centuries. Fortunately, as a human toxicosis, diseases caused by *Claviceps* species are no longer prevalent due to improvements in preventing contaminated grain products from entering the food supply. However, there is always a poisoning risk to farm animal, and the severity of the problem will depends on factors such as animal species, age, general health status of the animal, period of exposure, ergot species and amount consumed (66). The toxicity and concentration of the alkaloid of ergot-contaminated grains depend on the strain and the maturity stage of the fungus, as well as the host, and growing and geographical conditions (66).

Exposure to a variety of ergot alkaloids can be manifested in a wide range of symptoms such as gangrene and convulsions. Bailey et al., (4) studied the effects of *C. africana* sphacelia and sclerotia added to feed on poultry (broilers). The total alkaloid content (11.3 ppm to 235 ppm) didn't cause any significant mortality or obvious

symptoms of ergot toxicity. However, they found effects such as an increase in liver weights after three weeks of feeding, reduction of weight gain in the fourth week, and the reduction of feed efficiency. They mentioned that there is slight toxicity of ergot to poultry and suggest that concentrations less than 1 or 2% are not likely to result in significant mortality or obvious symptoms of toxicity.

Another effect of ergot alkaloids is that they act like dopamine to depresses blood prolactin in the sow. This hormone is an essential factor in the process of preparing the mammary glands to produce milk after farrowing. This symptom is analogous to that reviewed for *C. africana*-infected sorghum fed to piglets and cows containing the alkaloid dihydroergosine (6).

Organisms associated with sorghum ergot

Three of the most common genera associated with *Claviceps*-infected sorghum are *Fusarium sp, Cladosporium sp.* and *Epicoccum* (32). *Epicoccum andropogonis* (Ces.) Schol-Schwarz or *Cerebella andropogonis* Cesati is a reliable indication of previous infection with a species of *Claviceps* (45). It is worth mentioning therefore that the conspicuous, black, convulted mycelium of *Cerebella andropogonis* on sorghum or grass inflorescences has proved a most useful field indicator of ergot-infected plants. *Alternaria spp.* has also been observed on *Claviceps*-infected sorghum. *Epicoccum spp*. are reported as mycoparasites of *C. africana* (6), while *Alternaria* is one of the most common plant pathogens that cause opportunistic diseases in many crops. Although little is known concerning the toxicity of *Epicoccum*, this fungus co-exists as a hyperparasite

of the sphacelia and has been found on almost all *Claviceps*-infected sorghum. In particular, the co-occurrence of the *Claviceps, Fusarium , Epicoccum and Alternaria* toxins in cereal grains should be considered in any risk assessment for human and animal health and nutrition.

CHAPTER III

RELATIONSHIP BETWEEN ERGOT AND WEATHER FACTORS

Introduction

Models that simulate the development of plant diseases can aid in crop management. Critical skills for disease management are early diagnosis, knowledge of the behavior of the pathogen in a specific geographic area, and the ability to forecast disease development. Generally, a disease model is developed to estimate the probability or risk of an undesirable event occurring at a given location and time (20). The reason for developing models is because growers need decision systems for plant disease management. Usually, models involve the interaction among factors described by Vanderplank (81) in the disease triangle such as pathogen, environment and host. One of the factors involved are the environmental conditions of a specific geographical area that can affect ergot incidence and severity.

The objectives of this study were: (1) to develop a risk assessment model for each location (Rio Bravo and Celaya, Mexico, and College Station and Weslaco, Texas) and develop a general prediction model to advise growers, seed companies and scientists about the ergot risk, and (2) to determine the differences in ergot susceptibility among sorghum hybrids and three widely-utilized male-sterile lines exposed to variable environments at multiple locations.

Materials and methods

Evaluation sites

The experiments were planted at College Station (central), and Weslaco (south), Texas, USA; and Rio Bravo (north), and Celaya (central), Mexico under irrigated conditions during 2002 and 2003. These locations were chosen because they are located in some of the largest sorghum grain production areas. They are subsequently denoted as CS, WE, RB and CE, respectively (Table 1). Planting at CS and WE was done using a John Deere two-row planter, while planting at RB and CE was done using a semi-mechanical planter. At all locations, population density used was around 125 thousand plants ha^{-1} . Weeds were controled by hand hooded sprayer with Roundup® (Glyphosate) at a 25 ml ha^{-1} per 12 L. of water. Sorghum midge (*Contarinia sorghicola*) was controlled as needed with Asana XL (70 ml ha^{-1}).

Table 1. Geographical and ecological characteristics of testing locations sown during 2002 and 2003.

Location	Latitude	Longitude	Mean anual Precipitation (mm)	Mean anual Temperature (^{o}C)	Mean length of warm season (days)
Weslaco	26° 10'N	98° 01'W	510	23.3	320
College Station	30° 25'N	96° 40'W	1000	20.1	280
Rio Bravo	25° 58'N	98° 00'W	500	23.1	320
Celaya	20° 34'N	100°50'W	640	17.7	220

Genotypes

Sorghum hybrids AP 2233 (Syngenta®), KS 310 (Sorghum Partners®), NC+7W97 (NC+ seeds®), GARST 5664 (Syngenta®), ATx399 x Tx430 (Texas A&M University), NC+8R18 (NC+ seeds®) and A-lines ATx635, ATx2752 and ATx623 (all Texas A&M University) were planted every month at each location. Hybrids were chosen based on previous ergot reaction studies conducted during the fall in Weslaco, Texas (42), and similarities in flowering pattern.

Planting date

The number and planting dates* at each location depended upon weather conditions. At RB, there were 10 planting dates, while at CS and WE there were six planting dates. At CEL there were five planting dates.

Experimental design

Plants were grown at each location in a complete randomized block design with four replications. The experimental unit was one row of 5 m length, with a row spacing of 0.81 to 0.91 m (32” to 36”). In each row, five panicles of similar maturity were selected and tagged (total genotype sample= 20) at bloom initiation.

* On the figures, Julian day is the planting date.

Pathogen inoculum and inoculation

Inoculation was conducted using local *C. africana* isolates at each location. At the CS, RB and CE locations, inoculum was increased under greenhouse conditions and applied every other morning between 8:00 a.m. and 10:00 a.m. Targeted panicles were marked and inoculated using a hand atomizer until runoff with a suspension of 1.6 x 10^6 conidia ml^{-1} from flower initiation until full bloom of each plant. At WE, inoculum was obtained from field-infected panicles and the spore suspension was stored at 7-10°C between inoculations, which were performed every two days.

Data collected

The period from initial and final bloom dates for each inoculated panicle was recorded (the period ranged from 4 to 13 days depending on weather conditions and type of plant. i.e. A-lines showed a wider period than hybrids), as well as the ergot severity (percentage of infected florets observed in each inoculated plant) measured at milk stage (10-12 days after 50% flowering). Weather data was obtained from weather stations located near each experimental site.

Statistical analysis

Disease severity was transformed using arcsine of the square root of ergot severity to satisfy assumptions of normality. Data were analyzed taking into consideration the chronological variation (dates) nested in location by year (loc x year), instead of the spatial variation due to replications (blocks) in each planting date. Because

male-sterile sorghum lines are highly susceptible to *C. africana* in comparison with hybrids, data were analyzed in two different groups (A-lines and hybrids). Bartlett's (χ^2) test of homogeneity was performed to determine homogeneity of variances among environments. If the variances were homogeneous, data were combined. Analysis of variance was conducted for each location considering genotypes and locations as fixed effects, and years and dates as random effects. PROC GLM (SAS Institute, Cary, NC) procedure was used to estimate total variance, means of genotypes, dates and years. Genotype and planting date means were compared using Tukey's ($P< 0.01$) at each year and location.

The most important weather variables that contributed to ergot expression were identified by use of stepwise regression (PROC STEPWISE; SAS Institute, Cary, NC). Weather triad values, consisting of the mean of three consecutive days, were correlated with ergot severity. To obtain each triad value, a 30-day period before flowering for each panicle was used. This period was divided into 10 stages, each consisting of three days, which were named X1 to X10 (table 2). Also, a period up to nine days after flowering was considered in the analysis and divided into three stages (named X11 to X13). Correlation analyses (PROC CORR; SAS Institute, Cary, NC) were performed to identify the weather parameter stages that were correlated with disease. Final models derived from multiple regression (PROC REG; SAS Institute, Cary, NC) analysis were chosen based on the highest coefficient of determination and the lowest mean square error value. Actual data was analyzed and put into surface response regression models capable of predicting ergot severity.

Table 2. Stages in which the sorghum plant reproductive period was divided.

STAGE	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
Days before bloom	28 to 30	25 to 27	22 to 24	19 to 21	16 to 18	13 to 15	10 to 12	7 to 9	4 to 6	1 to 3			
Days after bloom											1 to 3	4 to 6	7 to 9

Results

Pathogen development

a) General analysis

Since at WE, the inoculum and inoculation method used were different compared to the other locations, the combined ANOVA only included data from RB, CS and CEL locations. The combined analysis for ergot severity on hybrids (table 3) and A-lines (table 4) showed a highly significant effect ($P< .0001$) of location, year, date(loc*year) and genotypes, and the interactions among them.

Table 3. Combined ANOVA for ergot severity observed in sorghum hybrids.

Variation factor	df	Sum of squares	Mean square	F value	Prob > F
Location	2	5.1227	2.5614	100.08	< .0001
Year	1	5.3989	5.3989	210.95	< .0001
Loc x Year	2	0.9225	0.4613	18.02	< .0001
Date (loc*year)	32	204.7889	6.3996	250.05	< .0001
Genotype	5	2.9916	0.5983	23.38	< .0001
Gen x Loc	10	1.7959	0.1796	7.02	< .0001
Gen x Year	5	0.7184	0.1437	5.61	< .0001
Gen x Loc x Year	10	2.5237	0.2524	9.86	< .0001
Error	3650	93.4159	0.0256		
Total	3717	325.8339			

Table 4. Combined ANOVA for ergot severity observed in A-lines.

Variation factor	df	Sum of squares	Mean square	F value	Prob > F
Location	2	3.4725	1.7362	31.80	< .0001
Year	1	4.6042	4.6042	84.32	< .0001
Loc x Year	2	2.7190	1.3595	24.90	< .0001
Date (loc*year)	31	181.2520	5.8468	107.08	< .0001
Genotype	2	1.5517	0.7758	14.21	< .0001
Gen x Loc	4	8.9114	2.2278	40.80	< .0001
Gen x Year	2	1.1623	0.5811	10.64	< .0001
Gen x Loc x Year	4	0.4980	0.1245	2.28	< .0586
Error	1701	92.8826	0.0546		
Total	1749	312.1165			

In general, sorghum ergot severity was statistically greater (P<.01) in 2002 in both hybrids and A-lines, compared with 2003 (table 5). The CEL location had the greatest amount of ergot on hybrids (12.47%), followed by CS and RB, which was

9.79% and 7.2% respectively. Locations as CEL and CS had greater (P<.01) ergot severity in A-lines compared with RB. In general, A-lines had the greatest ergot severity. According to the average severity presented across years, locations, and dates, male-sterile line ATx2752 had the lowest ergot (22%), while ATx635 and ATx623, had 33.01% and 31.15%, respectively. Sorghum hybrid AP2233 was the most susceptible to ergot followed by GARST 5664, ATx399 x RTx430, and NC+7W97. The hybrid NC+8R18 showed the least amount of ergot (5.10%), which was 38% of the severity of the most susceptible one (table 5).

Table 5. Effect of year, location and genotype on mean ergot severity in sorghum hybrids and A-lines.

	Hybrids	A-lines
Year	*	
2002	12.81 a	34.58 a
2003	4.89 b	22.29 b
Location		
College Station	9.79 b	32.29 a
Rio Bravo	7.19 b	26.35 b
Celaya	12.47 a	28.11 a
Genotype		
AP2233	13.42 a	
GARST 5664	9.89 b	
ATx399 x RTx430	9.48 bc	
NC+7W97	8.69 cd	
KS 310	7.30 d	
NC+8R18	5.10 e	
ATx635		33.01 a
ATx623		31.15 b
ATx2752		22.29 c

*= Treatments with the same letter in each category are statistically similar according to Tukey, P< 0.01

b) Location analysis

After transforming the original data, the ANOVA for hybrids showed a highly significant ($P< .01$) difference among genotypes and dates at all locations (table 6). The ANOVA for A-lines showed a highly significant effect of genotype, date and the interaction (genotype x date) in all the locations (table 7) with the exception of CEL, where date and interaction were not significant.

Table 6. Mean squares and test of significance of factors generated in the ergot severity ANOVA for hybrids evaluated at several locations.

Variation Factor	Rio Bravo		College Station		Weslaco		Celaya	
	2002	**2003**	**2002**	**2003**	**2002**	**2003**	**2002**	**2003**
Genotype	0.15**	0.41**	1.18**	0.16**	--	.001ns	0.21**	0.02*
Date	14.4**	3.76**	7.24**	0.45**	--	0.01**	1.42**	0.11**
Gen x Date	0.23**	0.21**	0.58**	0.13**	--	.001ns	0.18**	0.01ns
Error	0.021	0.013	0.0135	0.013		0.0007	0.0191	0.0093

Note: **=highly significant according to Tukey's P<0.01; *= significant according to Tukey's P<0.05; ns=not significant.

Table 7. Mean squares and test of significance of factors generated in the ergot severity ANOVA for A-lines evaluated at several locations.

Variation Factor	Rio Bravo		College Station		Weslaco		Celaya	
	2002	**2003**	**2002**	**2003**	**2002**	**2003**	**2002**	**2003**
Genotype	4.08**	1.12**	0.70**	2.79**	--	1.46**	1.75**	0.32**
Date	7.50**	6.71**	0.35**	5.14**	--	2.15**	0.12ns	1.19**
Gen x Date	0.45**	0.62**	0.48**	0.77**	--	0.62**	0.05ns	0.12**
Error	0.045	0.038	0.033	0.037		0.023	0.035	0.036

Note: **=highly significant according to Tukey's P<0.01; ns=not significant.

The differences in hybrid response are shown in figure 1. At RB and CS disease had similar trends, ranging from almost-zero infection during the spring and summer, to almost 50% ($P < .01$) infection (table 8) during the fall. Meanwhile, the CEL location showed an inverse relationship, where the highest ergot level was during the summer and the lowest during the fall may be due to complete sterility including the ovaries and/or poor pathogen development. At WE, infection levels were not significantly different throughout the year.

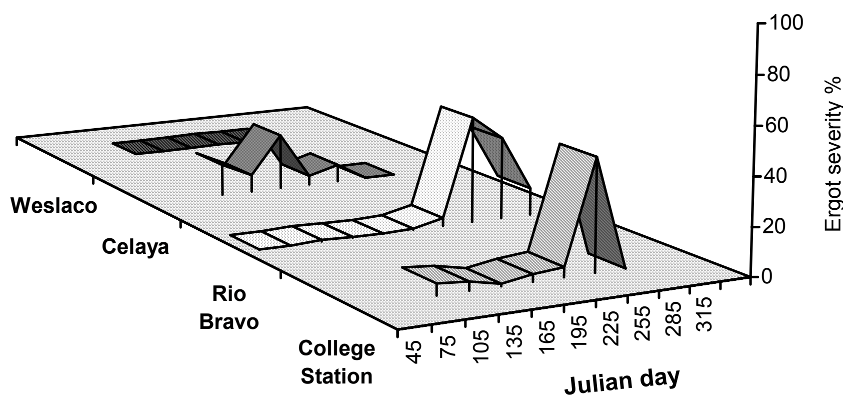

Fig. 1. Average (2002-2003) ergot development on sorghum hybrids observed at four locations.

Table 8. Mean ergot severity values observed in sorghum plants at four locations and several planting dates.

	Rio Bravo				College Station				Weslaco				Celaya			
Date	**Hybrid**		**A-line**		**Hybrid**		**A-line**		**Hybrid**		**A-line**		**Hybrids**		**A-line**	
1	0.08	d	2.3	f		*										
2	0.04	d	13.3	d					0	b	1.2	c				
3	0.64	d	5.7		4.7	b	44.6	a	0	b	8.5	b	13.5	b	40.8	ab
4	0.18	d	1.1	f	3.6	bc	35.2	a	0.1	b	7.2	b	7.3	bc	26.2	bc
5	0	d	4.9	ef	0.9	c	21.3	b	0.3	a	1.5	c	23.8	a	45.4	a
6	0.43	d	35.0	c	2.9	bc	40.2	a	0	b	0	c	3.9	c	13.4	c
7	3.30	d	61.8	a	4.1	bc	24.2	b	0	b	32.5	a	7.4	bc	7.4	c
8	44.6	a	62.4	a	46.4	a	21.3	b								
9	34.6	b	44.9	b												
10	11.4	c	10.4	d												

*= Treatments with the same letter in each location-plant type are statistically similar according to Tukey, $P < 0.01$

With A-lines, ergot was almost identical at CS and CEL, with 95 to 98% more infection than RB and WE during the spring (fig. 2). The reverse was seen at RB, where the highest infection (66% more than CS) was during the fall.

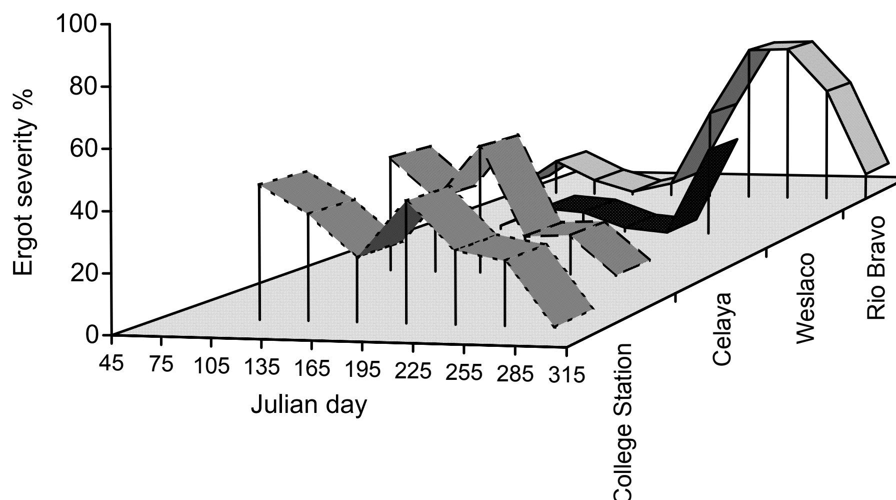

Fig. 2. Average (2002-2003) ergot development on A-lines at four locations.

b.1) Rio Bravo

In RB, ergot severity during planting dates in the first five months was very low in both years. No ergot was seen in May and sorghum hybrids and females planted during June had little ergot development. The greatest severity was observed with August and September planting dates, where ergot exceeded 70% severity. Sorghum planted in October had lower ergot in both years (fig. 3).

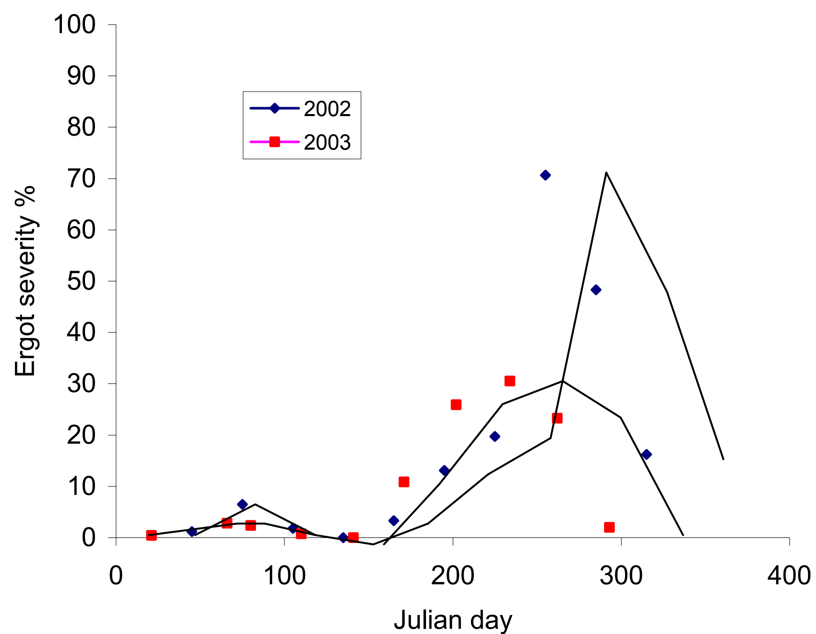

Fig. 3. Effect of planting date on the combined ergot severity of sorghum hybrids and A-lines observed in two years at Rio Bravo, Tamaulipas, Mexico.

Also, in RB, A-lines showed greater susceptibility to ergot than hybrids during the whole year. This susceptibility was greater during June and July planting dates, with 99% and 95% more ergot, respectively (fig. 4). Ergot infection declined from August to October planting dates, with ergot values ranging from 29% to 10%, respectively. A-lines ATx635 was the most susceptible (*P*< .01) at all planting dates with the exception of August and September (255 and 285 Julian days, respectively) dates, when ATx2752 was the most susceptible (fig. 5).

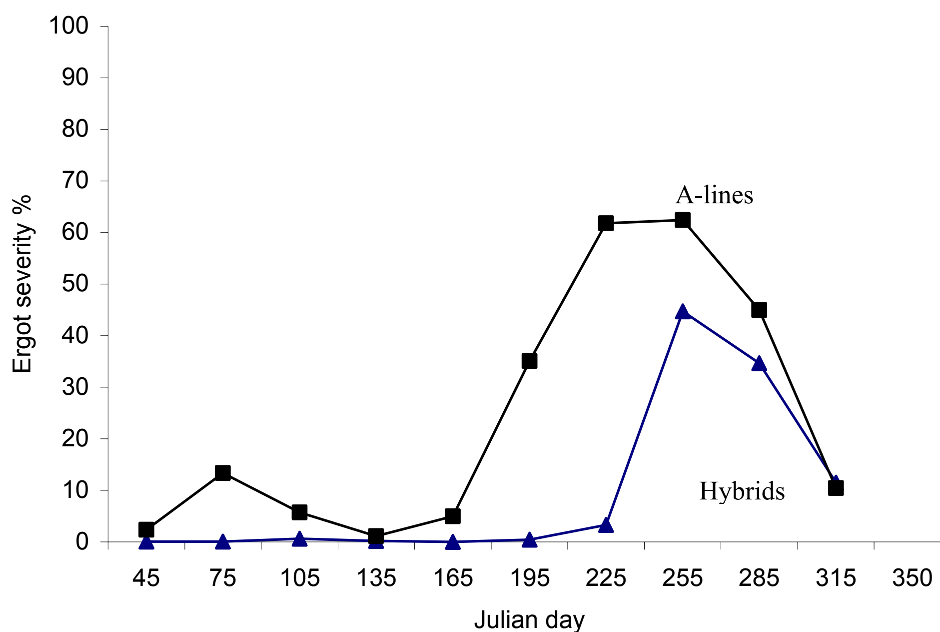

Fig. 4. Effect of planting date on subsequent sorghum ergot severity observed in hybrids and A-lines at Rio Bravo, Tamaulipas, Mexico.

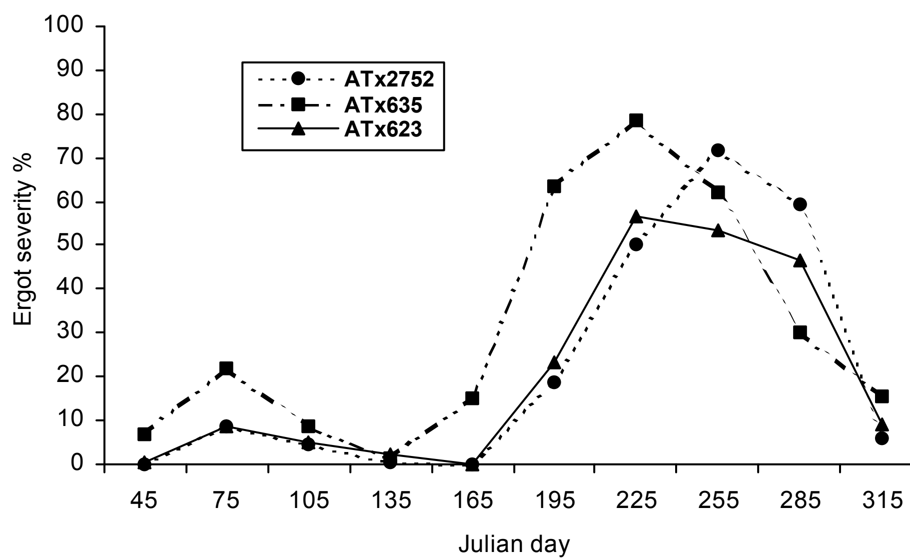

Fig. 5. Differences in ergot development among A-lines planted throughout the year at Rio Bravo, Tamaulipas, Mexico.

Since ergot severity increased in the fall season planting dates, sorghum hybrids showed a highly significant difference among them (fig. 6). Sorghum hybrid KS310 was the most susceptible across dates except in the last two planting dates, when AP2233 was more susceptible to ergot.

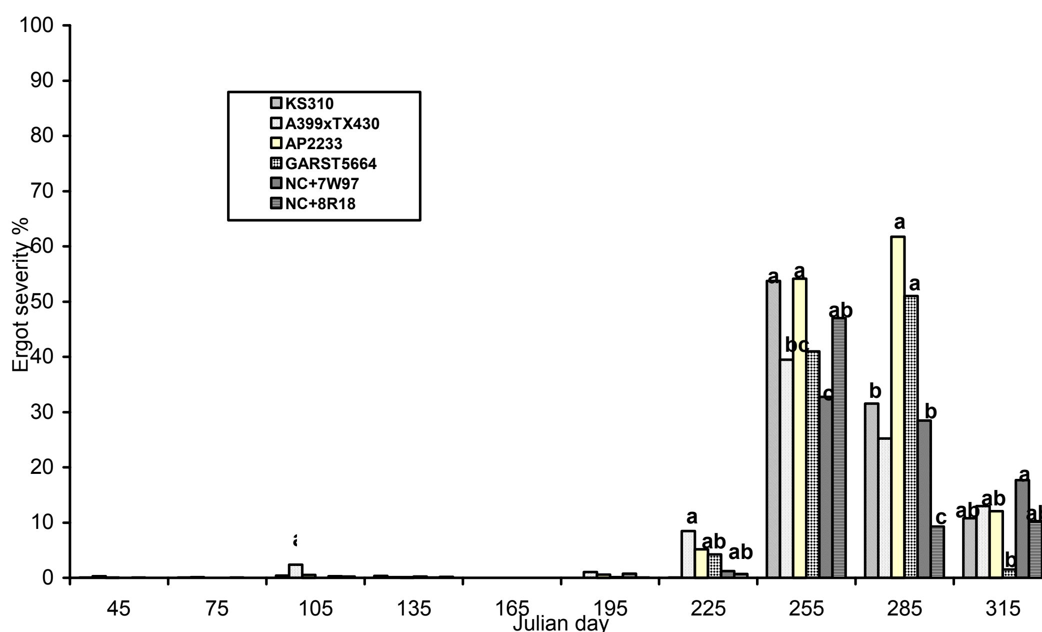

Fig. 6. Mean differences in ergot development among sorghum hybrids observed throughout the year at Rio Bravo, Tamaulipas, Mexico.

(hybrids with similar letters in each planting date are statistically similar according to Tukey's P<.01).

b.2) College Station

Results were a little different at CS in both years, where ergot was present across all planting dates. Nevertheless, ergot was lowest (1%) in sorghum planted during June (fig. 7). Planting during early spring (April) and the fall (September) promoted ergot development up to 28% and 38%, respectively. This is quite different from the results at RB, where ergot development during the spring was almost negligible.

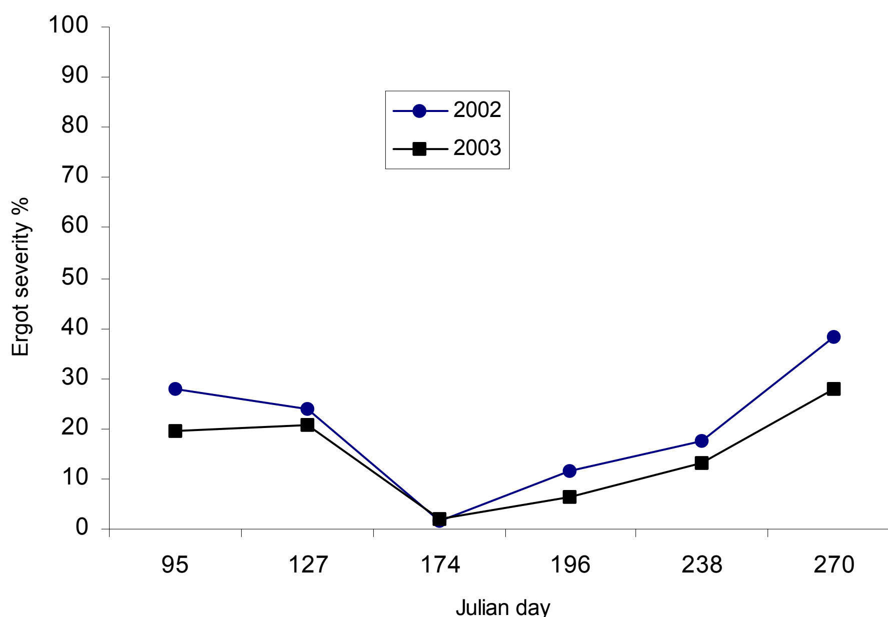

Fig. 7. Effect of planting date on the subsequent combined ergot severity of sorghum hybrids and A-lines observed in two years at College Station, Texas, USA.

There was a highly significant difference ($P < 0.01$) among hybrids and A-lines at this location, where the A-lines showed between 84% and 93% more ergot than hybrids (fig. 8) across planting dates. However, hybrids showed twice as much ergot than A-lines at the 270 Julian day planting date. This could be due to frost that killed florets prior to ergot infection.

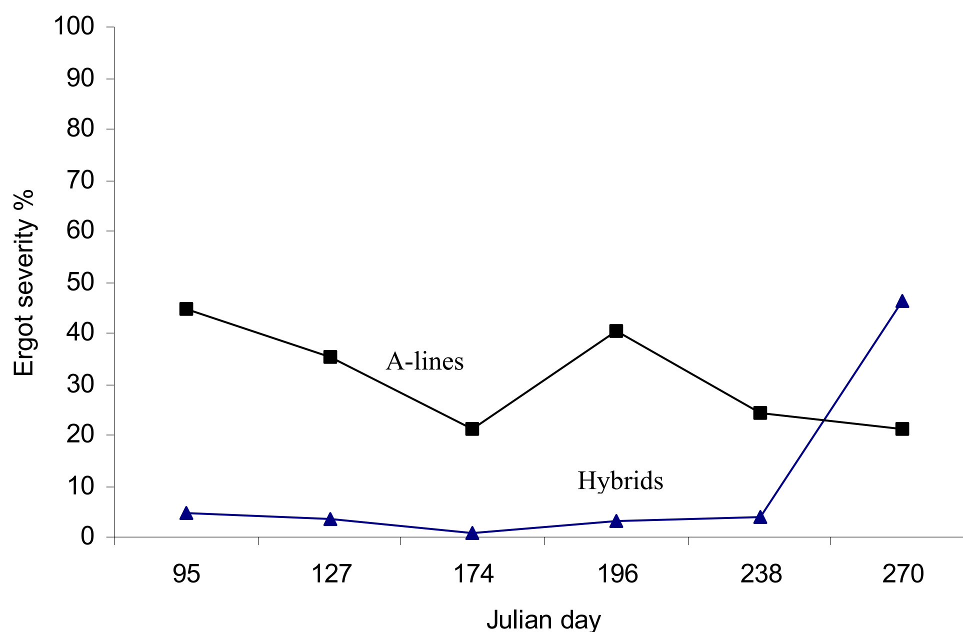

Fig. 8. Effect of planting date on the subsequent sorghum ergot severity observed in hybrids and A-lines at College Station, Texas, USA.

The high values of ergot at CS were attributed to the high susceptibility of sorghum A-line ATx623, which had significantly (*P*< 0.01) more ergot severity than the other A-lines (fig. 9). The difference in severity between this line and ATx635 ranged from 16 to 40%, and from 25 to 92% with ATx2752.

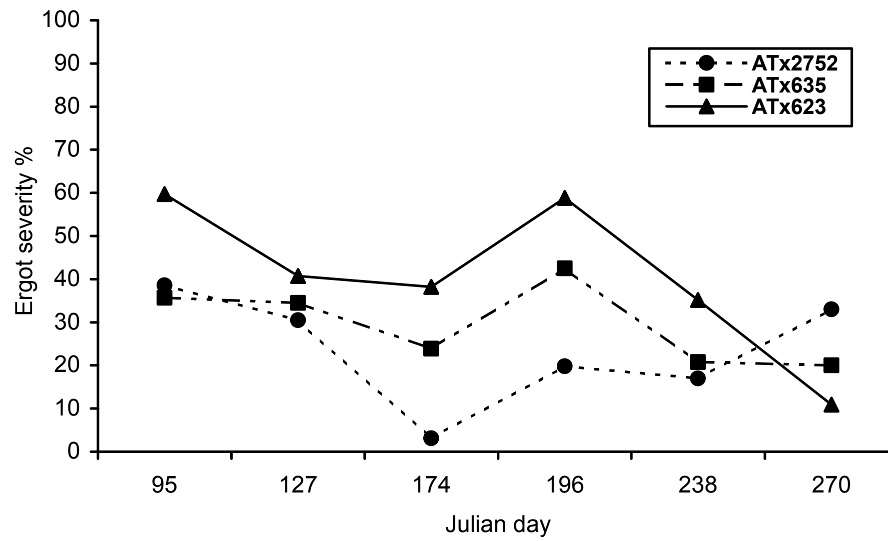

Fig. 9. Differences in ergot development among A-lines planted throughout the year at College Station, Texas, USA.

Sorghum hybrids KS-310 and NC+8R18 had the least amount of ergot across planting dates (fig. 10) at CS. This difference among hybrids was more noticeable on the last planting date, when ergot severity values reached 70%.

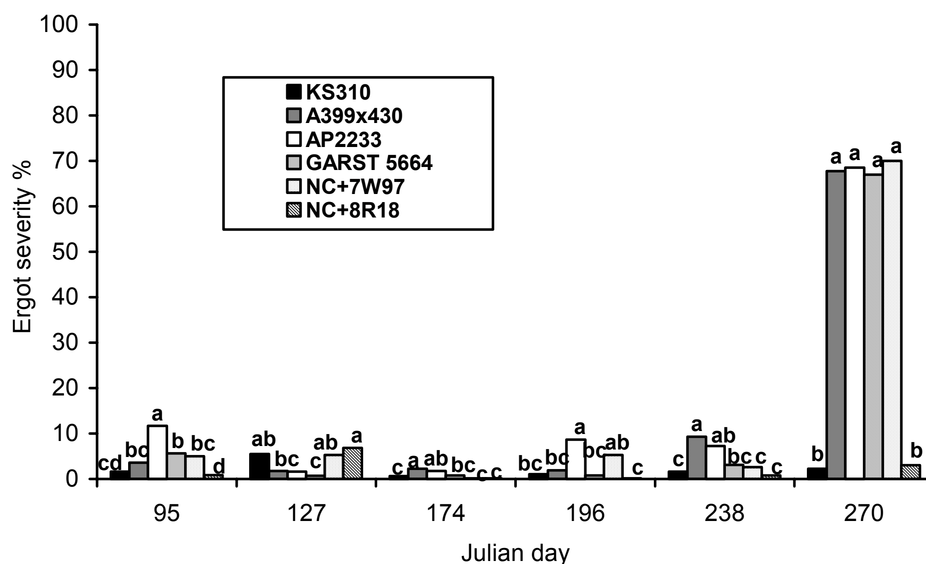

Fig. 10. Effect of planting date on subsequent sorghum ergot development in hybrids at College Station, Texas, USA.
(hybrids with similar letters in each planting date are statistically similar according to Tukey's P<.01).

b.3) Weslaco

At the WE location, ergot severity was much lower compared to the other locations. A-lines planted around May-June had some disease development (7-8%), while those planted in the middle of September had the highest ergot severity (fig. 11). The highest ergot level of sorghum hybrids was lower than 1% (fig. 12). This may be related to inoculum viability or differences in inoculation methods. Sorghum hybrid KS 310 showed the highest level of susceptibility, which was not significantly different from the other hybrids planted in July. A-lines planted around May-June, had some disease development (7-8%), while A-lines planted in the middle of September had the highest

ergot severity (fig. 12). A-line ATx635, was highly susceptible especially in the 253 Julian-day planting date (fig. 13), when it was 7 times more susceptibility than ATx2752. These results are similar to the ones obtained at RB, where ATx635 showed the highest ergot susceptibility.

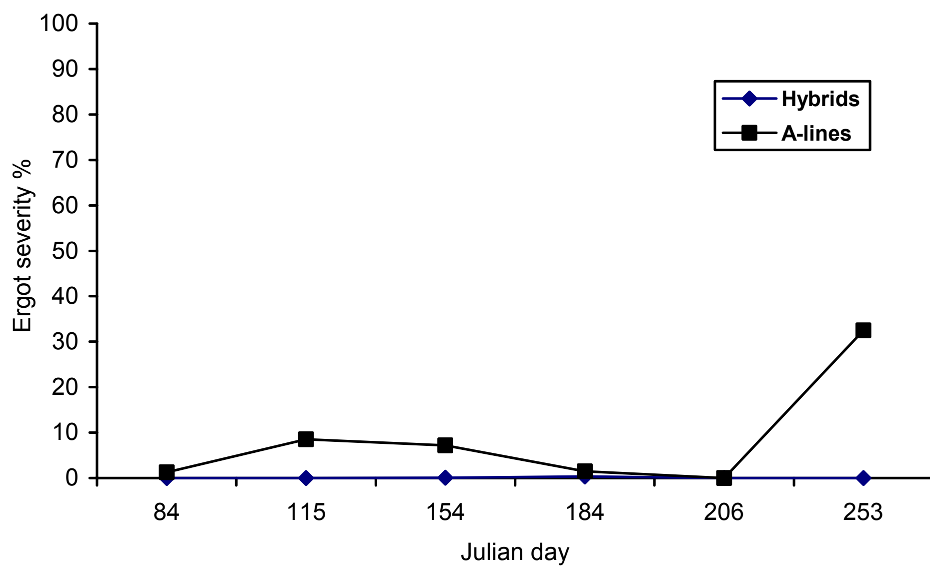

Fig. 11. Effect of planting date on subsequent sorghum ergot severity observed in hybrids and A-lines at Weslaco, Texas, USA.

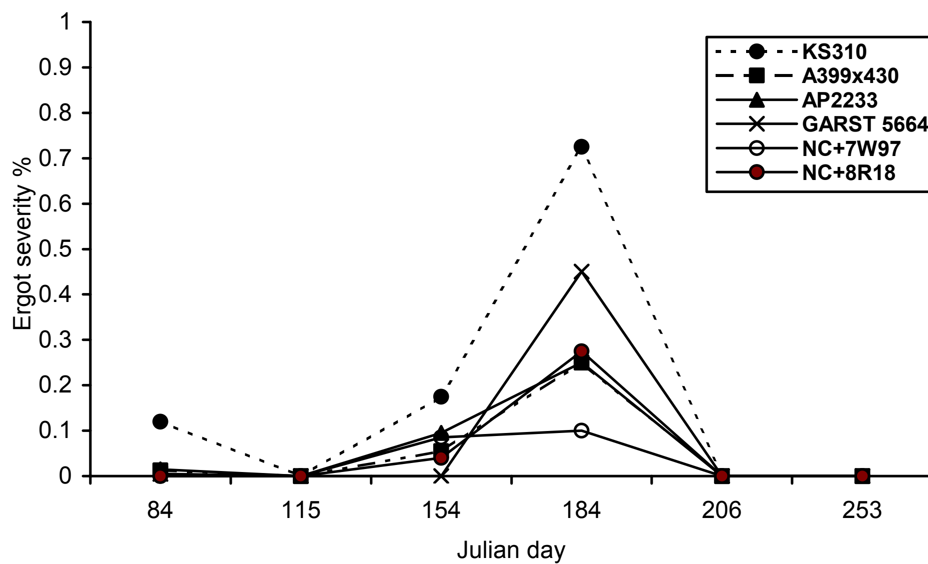

Fig. 12. Differences in ergot development among hybrids planted throughout the year at Weslaco, Texas, USA.

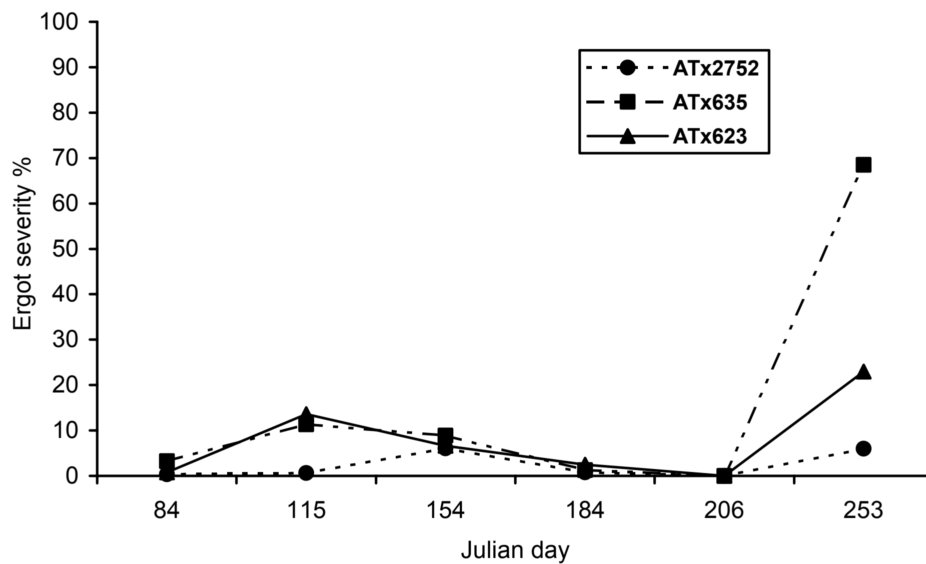

Fig. 13. Differences in ergot development among A-lines planted throughout the year at Weslaco, Texas, USA.

b.4) Celaya

At CEL, ergot development during 2002 was greater than that observed the following year (fig. 14). Ergot was observed throughout the spring and summer plantings with a reduction trend as the year continued. Fall plantings (starting from August) were affected by frost that coincided with the reproductive stage of plants. A-lines showed 47% to71% more ergot infection than hybrids (fig. 15).

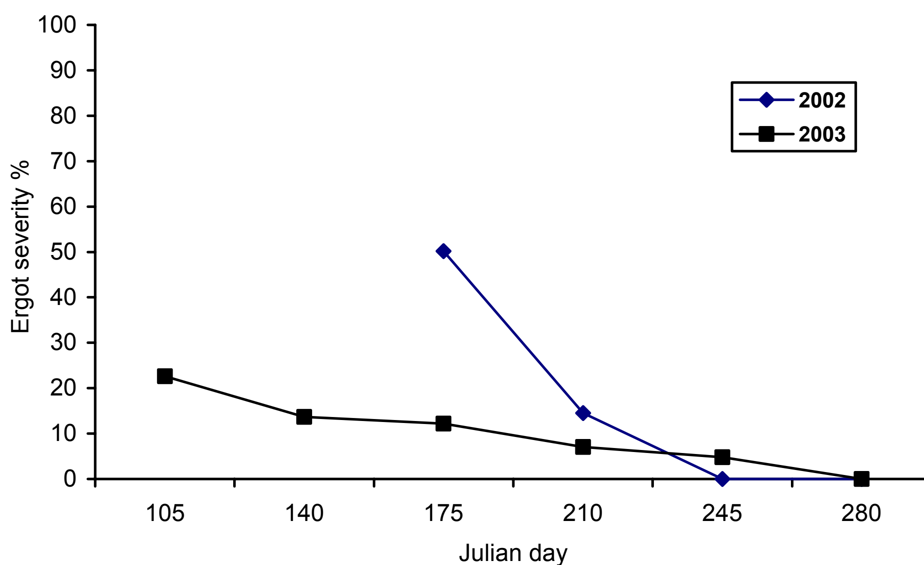

Fig. 14. Effect of planting date on the subsequent combined ergot severity of sorghum hybrids and A-lines observed in two years at Celaya, Guanajuato, Mexico.

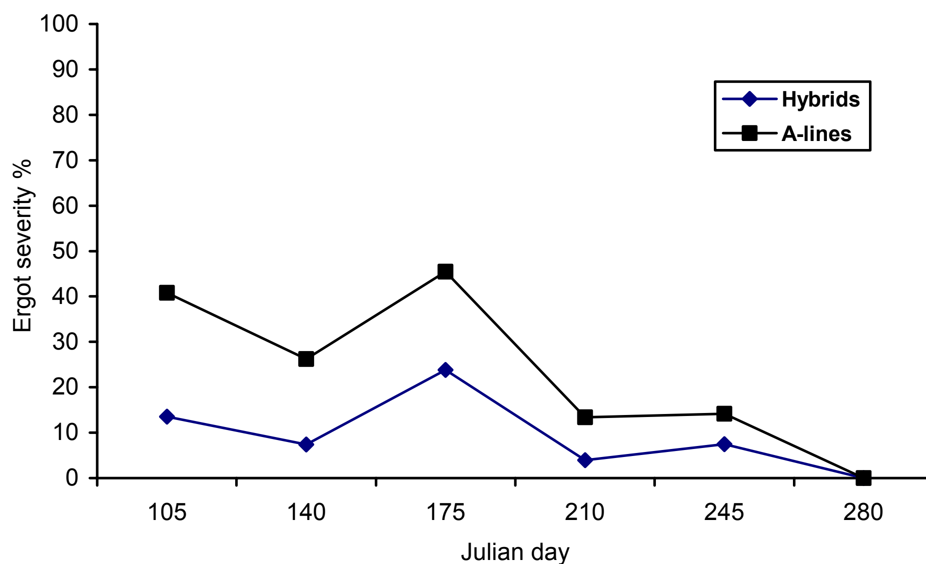

Fig. 15. Effect of planting date on the subsequent sorghum ergot severity observed in hybrids and A-lines at Celaya, Guanajuato, Mexico.

Significant differences among hybrids were observed only at 105 and 175 Julian day planting dates, when there were two and three times more ergot than other planting dates, respectively (fig. 16). Hybrids planted in the middle of April showed more susceptibility than those planted at the end of May. At 105 Julian day, NC+7W97 was the most susceptible, while at 175 Julian day, it showed less susceptibility. In all planting dates, NC+8R18 had the least amount of ergot.

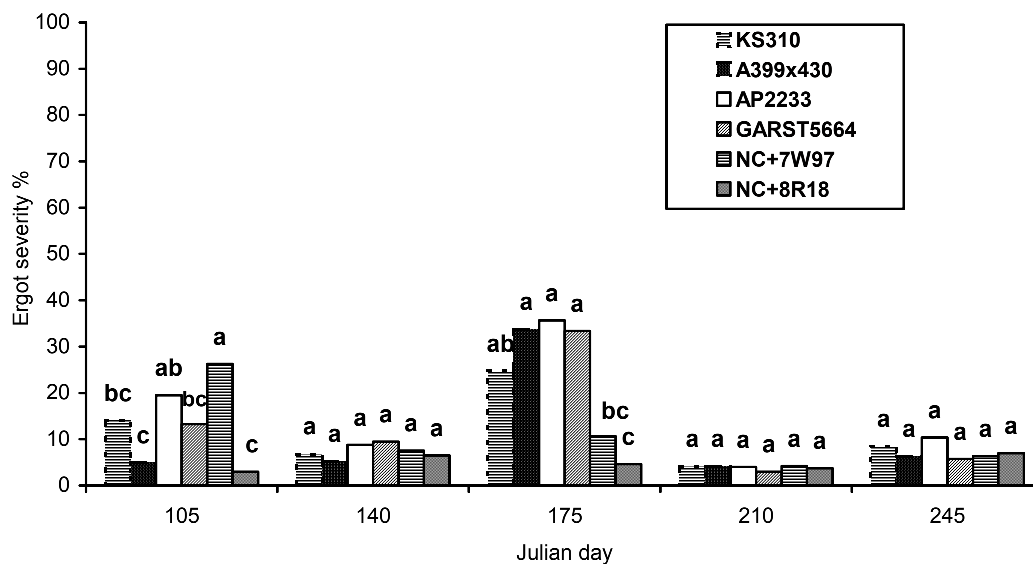

Fig. 16. Differences in ergot development among hybrids planted throughout the year at Celaya, Guanajuato, Mexico.
(hybrids with similar letters in each planting date are statistically similar according to Tukey's P<.01).

Within A-lines, there were significant differences in susceptibility with all planting dates (fig. 17). A-line ATx623 showed the highest susceptibility to ergot, with severity ranging from 37% to 89% compared with ATx2752, and from 53% to 88% compared with ATx635 across all planting dates.

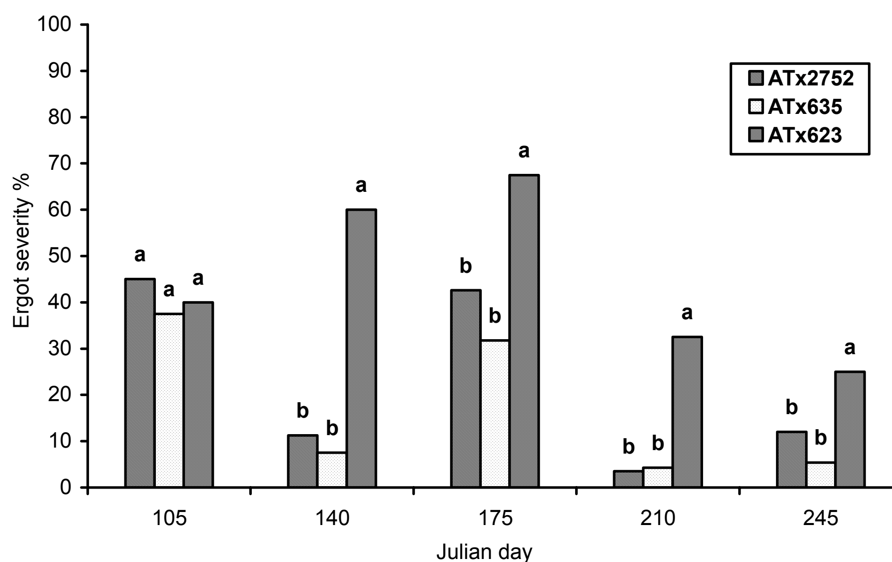

Fig. 17. Differences in ergot development among A-lines planted at Celaya, Guanajuato, Mexico.
(A-lines with similar letters in each planting date are statistically similar according to Tukey's P<.01).

Environment

Environmental conditions had a great impact on ergot development in all locations. Since previous studies conducted by McLaren and Flett (49), Montes et al. (54) and Montes-Belmont et al. (55) suggested that weather factors such as maximum temperature (TMAX), minimum temperature (TMIN), and relative humidity (RH) are the most important determinants of ergot susceptibility in sorghum plants, I selected these factors. In this study I included TMAX, TMIN and minimum relative humidity (RHMIN) data recorded during 2002 and 2003 years. Since RB and WE had similar weather conditions throughout the year with minimal variations among them, I

considered the weather data as one location (RB-WE) in the weather analysis. CS and RB-WE locations had the same TMAX trend throughout the season, with differences of up to 5°C occurring in the winter months (fig.18).

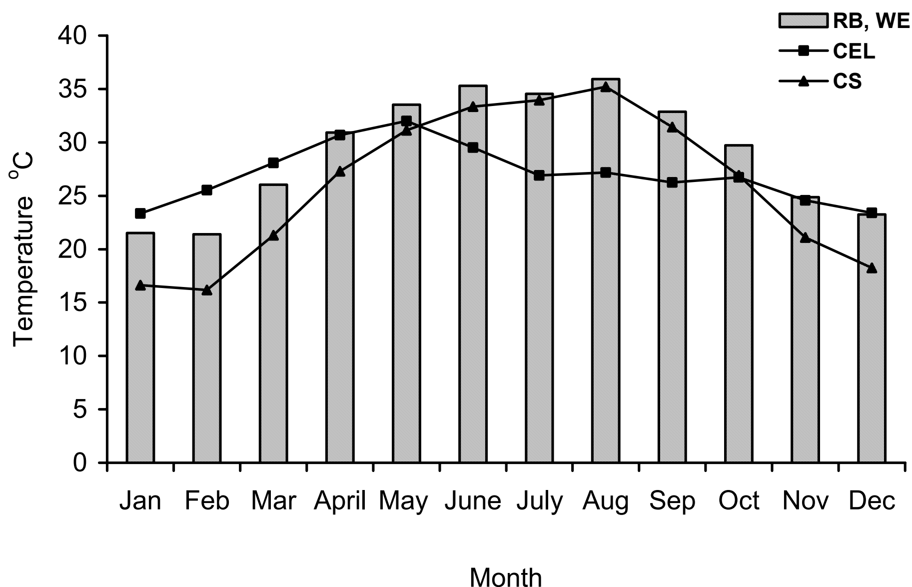

Fig. 18. Average monthly maximum temperature recorded at four locations during 2002-2003 period.

These locations showed higher temperatures (up to 8°C) during the entire summer compared to CEL, which in May had TMAX values declining to approximately 20°C during the winter months. TMIN values were different at all locations. RB-WE locations had the highest TMIN values, which reached almost 25°C during July-August. CS had the same TMIN trend as RB and WE but with a reduction of 2 to 3°C every

month compared with these locations. CEL had the lowest TMIN values that were just above the 13°C limit for pollen sterility (21) only during May to July (fig. 19). These cool temperatures can predispose sorghum plants to infection (48,52). Cooler temperatures, in conjunction with frost during November-March, affected growth of plants at CS and CEL locations, where sorghum florets were killed before *C. africana* infection.

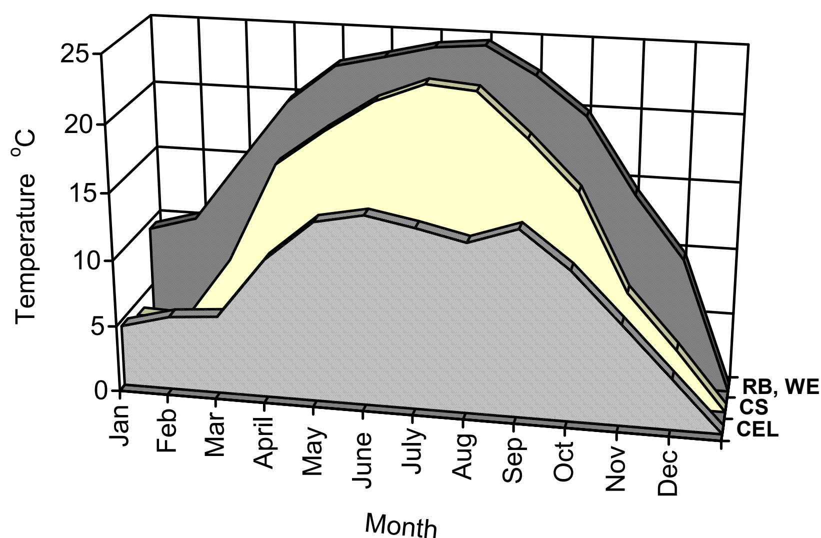

Fig. 19. Average monthly minimum temperature recorded at four locations during 2002-2003 period.

Maximum temperature

Pearson's correlation coefficients were obtained to determine the period in which the weather factors had a greater influence on ergot severity. Correlation coefficients showed differences among years, locations and sorghum plant types. With hybrids, there was a negative relationship between ergot and TMAX, with a highly significant correlation coefficient of –0.71at RB location at X8 stage (7 to 9 days before flowering; table 9). Ergot severity also was negatively related to TMAX values recorded at RB, CS and CEL in post-flowering stages.

Table 9. Pearson's correlation coefficients between ergot severity and maximum temperature.

Stage	Hybrids						A-lines					
	RB		CS		CEL		RB		CS		CEL	
X_1	-0.55	**	-0.04	ns	0.09	ns	-0.23	**	-0.12	ns	0.09	ns
X_2	-0.54	**	-0.04	ns	0.04	ns	-0.26	**	-0.07	ns	0.13	ns
X_3	-0.55	**	-0.09	ns	0.02	ns	-0.32	**	0.04	ns	0.04	ns
X_4	-0.57	**	-0.09	ns	-0.03	ns	-0.36	**	0.00	ns	0.04	ns
X_5	-0.58	**	-0.14	*	-0.07	ns	-0.46	**	-0.01	ns	-0.39	**
X_6	-0.60	**	-0.19	**	-0.11	ns	-0.56	**	0.03	ns	-0.31	**
X_7	-0.70	**	-0.13	*	-0.10	ns	-0.59	**	-0.02	ns	0.12	ns
X_8	-0.71	**	-0.19	*	-0.15	*	-0.59	**	0.16	ns	-0.16	ns
X_9	-0.66	**	-0.29	**	-0.24	**	-0.62	**	0.07	ns	0.13	ns
X_{10}	-0.66	**	-0.40	**	-0.20	**	-0.68	**	-0.01	ns	-0.15	ns
X_{11}	-0.65	**	-0.48	**	-0.26	**	-0.71	**	-0.09	ns	0.17	ns
X_{12}	-0.68	**	-0.51	**	-0.14	ns	-0.65	**	-0.16	ns	0.19	ns
X_{13}	-0.64	**	-0.50	**	-0.29	**	-0.63	**	-0.09	ns	0.32	**

Note: Correlation coefficients in each location were obtained using 5200 data points in RB, 3120 data points in CS and 2600 in CEL. **= Statistically significant at *P*< 0.01; *= Statistically significant at *P*< 0.05; ns=not significant.

Similar results were observed with A-lines. They had a highly significant correlation of –0.71 between ergot and TMAX after flowering at RB. The combined analysis of this group at CS showed no significant relationship between ergot and TMAX at any stage, however, during 2003 (appendix), a highly significant relationship was observed during post-flowering stages. At CEL, a highly significant and positive correlation was seen with TMAX and ergot at 7 to 9 days after initiation of flowering. This may be due to the low values of TMAX that are present throughout the year (fig. 18).

Polynomial regression analysis was used to obtain an ergot development model under TMAX. According to the observed ergot data on hybrids and TMAX conditions recorded at RB during the period from 7 to 9 days before initiation of flowering, ergot will not have any impact if TMAX value exceeds 33°C during this stage. However, cooler temperatures will predispose the sorghum florets to further ergot infection during flowering (fig. 20).

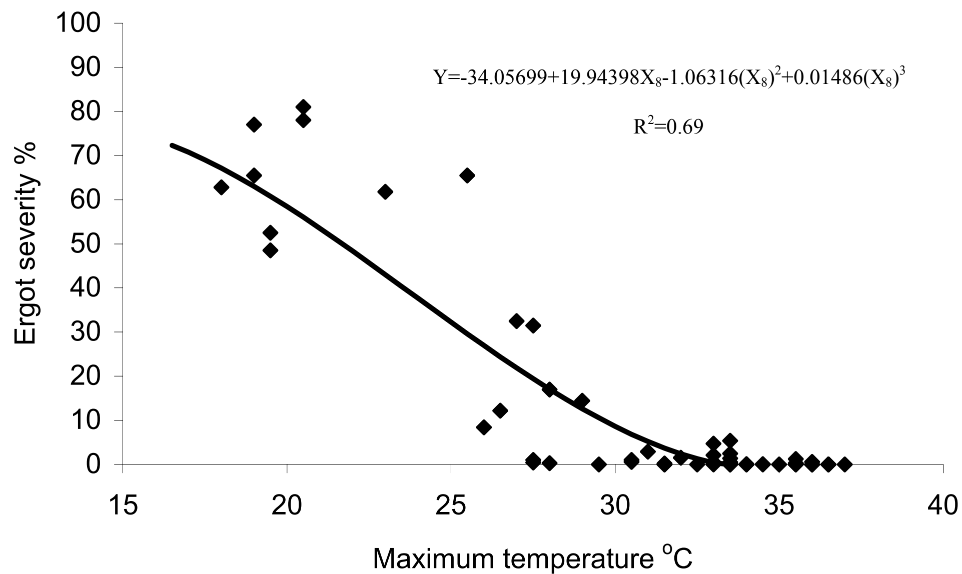

Fig. 20. Effect of maximum temperature recorded 7 to 9 days before bloom initiation on ergot severity in sorghum hybrids at Rio Bravo. (Each dot represents the mean of 20 plants. Regression line was obtained from 400 data points).

With A-lines, the relationship of TMAX 4 to 6 days after initiation of flowering to ergot development (fig. 21), showed that the ideal weather conditions for maximum ergot expression are temperatures around 23°C, however, ergot can develop up to 38°C.

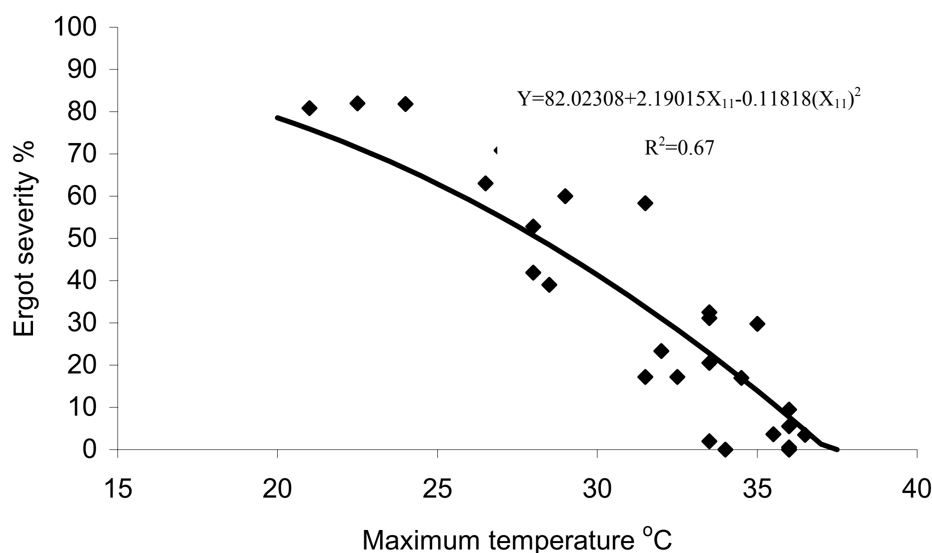

Fig. 21. Effect of maximum temperature recorded 4 to 6 days after bloom initiation on ergot severity observed in sorghum A-lines evaluated at Rio Bravo.
Note: Each dot represents the mean of 20 plants. Regression line was obtained from 400 data points.

Minimum temperature

There was a highly significant correlation between ergot and TMIN during the pre-flowering stages of sorghum hybrids (table 10). A negative relationship was found at RB and CS locations, especially in the X7 and X8 stages. However, at CEL, a positive relationship was observed. Results were similar with the A-line plants at this location, where ergot increased as warmer conditions appeared. The inverse was observed at RB and WE, which showed a high negative correlation 1 to 3 days after flowering.

Table 10. Pearson's correlation coefficients between ergot severity and minimum temperature.

Stage	Hybrids						A-lines					
	RB		CS		CEL		RB		CS		CEL	
X_1	-0.54	**	-0.08	ns	0.01	ns	-0.29	**	-0.29	**	0.23	*
X_2	-0.48	**	-0.02	ns	0.01	ns	-0.34	**	-0.17	ns	0.12	ns
X_3	-0.48	**	-0.05	ns	-0.03	ns	-0.18	**	-0.12	ns	0.14	ns
X_4	-0.53	**	-0.07	ns	0.04	ns	-0.33	**	-0.08	ns	0.12	ns
X_5	-0.58	**	-0.22	**	0.09	ns	-0.47	**	-0.14	ns	0.26	*
X_6	-0.63	**	-0.33	**	0.09	ns	-0.46	**	-0.16	ns	0.42	**
X_7	-0.69	**	-0.32	**	0.18	*	-0.54	**	-0.11	ns	0.20	ns
X_8	-0.69	**	-0.21	**	0.18	*	-0.57	**	-0.16	ns	0.36	**
X_9	-0.59	**	-0.18	**	0.21	**	-0.56	**	-0.07	ns	0.12	ns
X_{10}	-0.68	**	-0.28	**	0.15	*	-0.59	**	-0.09	ns	0.05	ns
X_{11}	-0.65	**	-0.40	**	0.07	ns	-0.61	**	-0.02	ns	0.12	ns
X_{12}	-0.64	**	-0.47	**	-0.08	ns	-0.59	**	-0.10	ns	0.19	ns
X_{13}	-0.68	**	-0.42	**	0.11	ns	-0.57	**	-0.09	ns	0.31	**

Note: Correlation coefficients in each location were obtained using 5200 data points in RB, 3120 data points in CS and 2600 in CEL. **= Statistically significant at $P< 0.01$; *= Statistically significant at $P< 0.05$; ns=not significant.

TMIN during X8 stage had a significant effect on ergot in sorghum hybrids (fig. 22). Cooler temperatures around 10°C increased ergot development. TMIN above 22.5^{o}C during X8 stage did not have any effect on ergot development during flowering time.

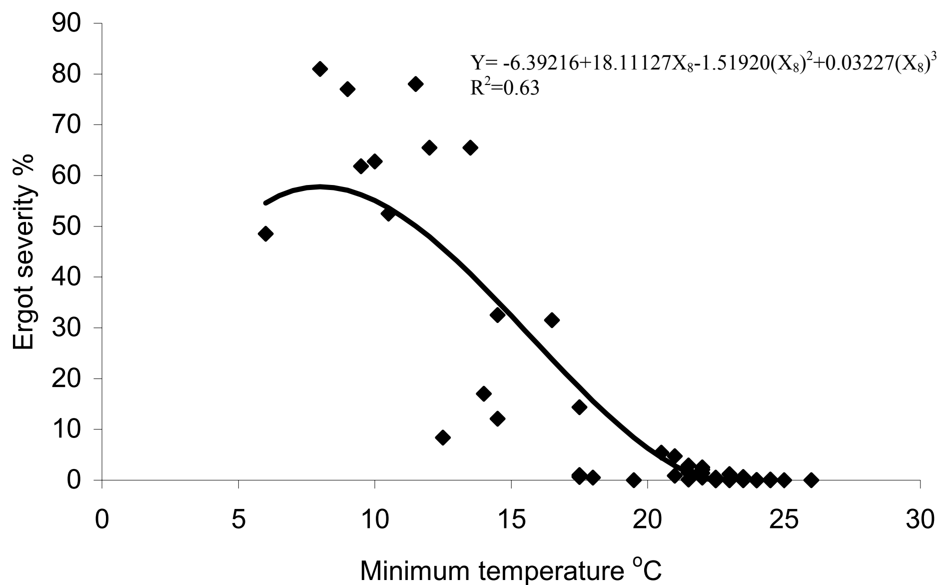

Fig. 22. Effect of minimum temperature recorded 7 to 9 days before bloom initiation on ergot severity observed in sorghum hybrids evaluated at Rio Bravo.
Note: Each dot represents the mean of 20 plants. Regression line was obtained from 400 data points.

A-line plants also showed a highly negative relationship between ergot and TMIN recorded at 1 to 3 days after initiation of flowering. The relationship of TMIN to ergot is shown in figure 23, which shows higher disease at lower TMIN (below 20°C). Also, the polynomial model shows that ergot is diminished with TMIN greater than 25°C during this stage.

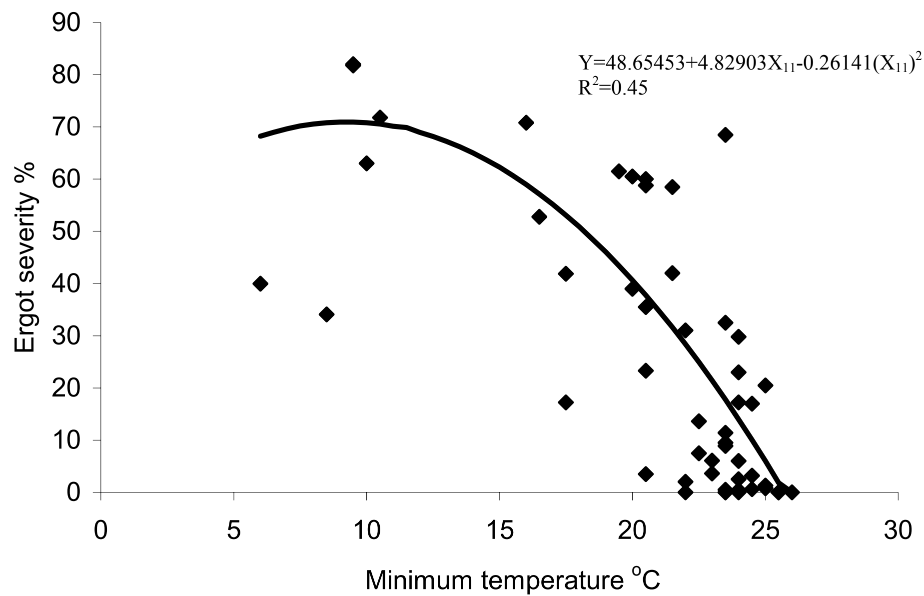

Fig. 23. Effect of minimum temperature recorded 1 to 3 days after bloom initiation on ergot severity observed in A-lines evaluated at Rio Bravo. (Each dot represents the mean of 20 plants. Regression line was obtained from 400 data points).

An inverse relationship was observed at CEL, where TMIN showed a positive relationship to ergot on A-lines. This location had a short range of TMIN (9.5 to 15.5°C). A linear regression analysis of the data showed that ergot is minimal with cooler temperatures (near 9°C) and increases with warmer TMIN (fig. 24).

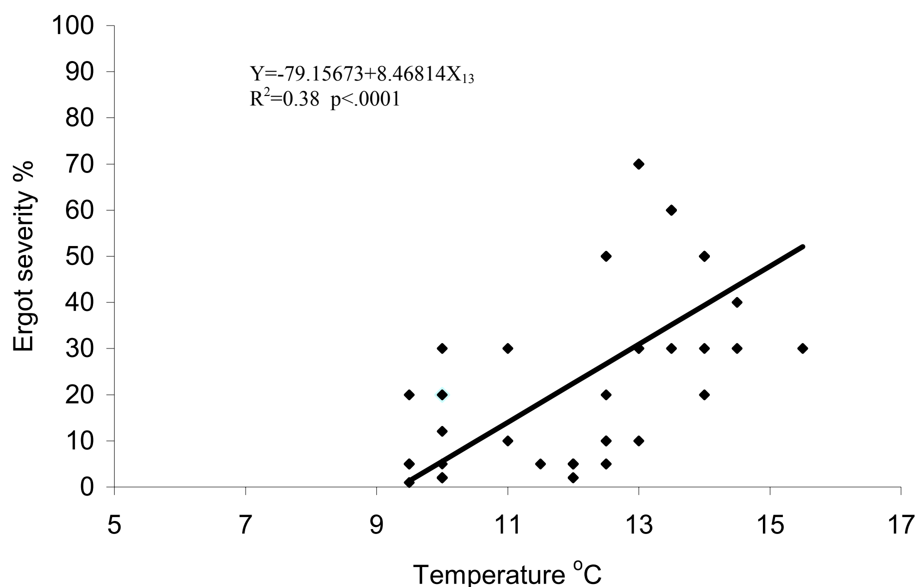

Fig. 24. Effect of minimum temperature recorded 7 to 9 days after bloom initiation on ergot severity (r=0.62, P<.0001) observed in sorghum A-lines evaluated at Celaya. (Each dot represents the mean of 20 plants. Regression line was obtained from 200 data points).

Relative humidity

One of the most important weather factors to have an influence on disease is moisture and relative humidity. With sorghum ergot, high relative humidity promotes conidia germination, host infection and pathogen reproduction. RB, WE, and CS locations have high (approx. 100%) maximum relative humidities (HRMAX) during the whole year, with daily variations in RHMIN, while CEL has an HRMAX between 50 to 80%. Correlation coefficients (combination of 2002 and 2003 years) at RB and CS between HRMIN and ergot were very low but significant (table 11). However, RB showed a high correlation (r= 0.56) at X11 stage (appendix) during 2003. This

correlation indicates that HRMIN could play a key role on the infection process during the flowering period of the hybrids. At RB, A-lines showed a highly significant correlation between ergot and HRMIN during the flowering period. At CEL, a low significant negative relationship was observed.

Table 11. Pearson's correlation coefficients between ergot severity and minimum relative humidity.

Stage	Hybrids						A-lines					
	RB		CS		CEL		RB		CS		CEL	
X_1	0.34	**	-0.06	ns	-0.18	*	0.04	ns	-0.25	**	0.09	ns
X_2	0.23	**	-0.01	ns	-0.18	*	0.13	ns	-0.19	*	-0.16	ns
X_3	0.22	**	0.02	ns	-0.28	**	0.22	**	-0.33	**	-0.08	ns
X_4	0.11	*	-0.04	ns	-0.13	ns	0.01	ns	-0.22	*	-0.21	ns
X_5	0.18	**	-0.12	*	-0.11	ns	0.04	ns	-0.25	**	0.02	ns
X_6	0.14	**	-0.15	**	-0.13	ns	0.08	ns	-0.28	**	0.05	ns
X_7	0.39	**	-0.22	**	-0.04	ns	0.22	**	-0.23	**	-0.13	ns
X_8	0.26	**	0.04	ns	0.13	ns	0.26	**	-0.39	**	-0.07	ns
X_9	0.10	*	0.22	**	0.08	ns	0.36	**	-0.18	*	-0.26	*
X_{10}	0.03	ns	0.25	**	0.06	ns	0.38	**	-0.18	*	-0.19	ns
X_{11}	0.22	**	0.29	**	-0.15	*	0.55	**	0.04	ns	-0.30	**
X_{12}	0.11	*	0.32	**	-0.11	ns	0.49	**	0.11	ns	-0.16	ns
X_{13}	0.17	**	0.31	**	-0.17	*	0.52	**	0.01	ns	-0.01	ns

Note: Correlation coefficients in each location were obtained using 5200 data points in RB, 3120 data points in CS and 2600 in CEL. **= Statistically significant at $P< 0.01$; *= Statistically significant at $P< 0.05$; ns=not significant.

The effect of HRMIN on ergot in hybrids is shown in figure 25. The polynomial regression model suggest that values around 40% HRMIN are needed to support ergot infection. At RB, the range of HRMIN data was from 17 to 79% (62% difference).

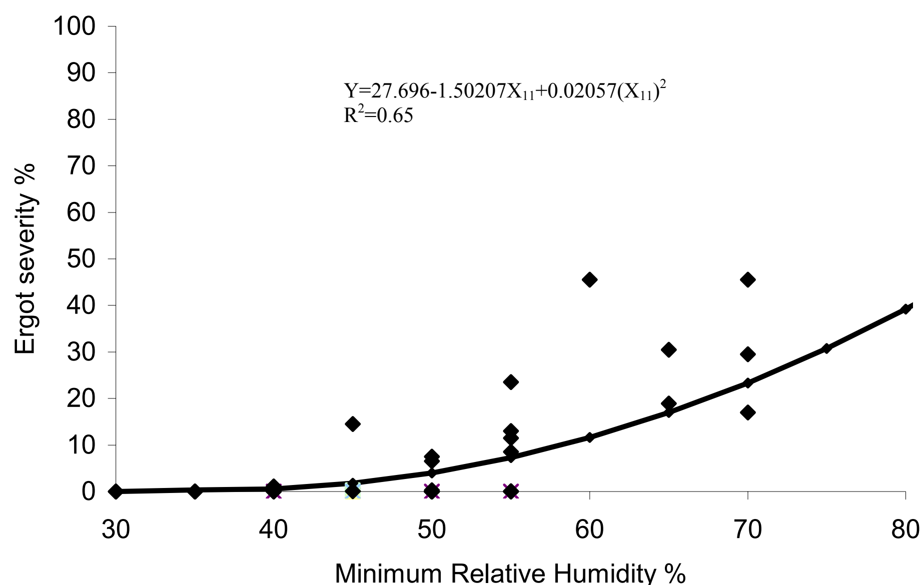

Fig. 25. Effect of RHMIN recorded 1 to 3 days after bloom initiation on ergot severity observed in sorghum hybrids at Rio Bravo during 2003.
Note: Each dot represents the mean of 20 plants. Regression line was obtained from 200 data points.

Surface response regression models

Weather data that had a relationship to ergot at each location were subjected to a stepwise procedure and then into a two-factor surface response regression model. The resulting prediction models were statistically significant for both types of sorghum plants. Since the effects of weather factors on ergot development were different at each location, several models were obtained for each one of them. According to the two-factor surface regression model that accounted for 46% of the variation of the data, sorghum ergot on A-lines at CS was influenced by TMIN and HRMIN after bloom

initiation, especially when HRMIN was above 30% in conjunction with cool TMIN values (fig. 26) from 10°C to 22°C with its optimum at 12-13°C.

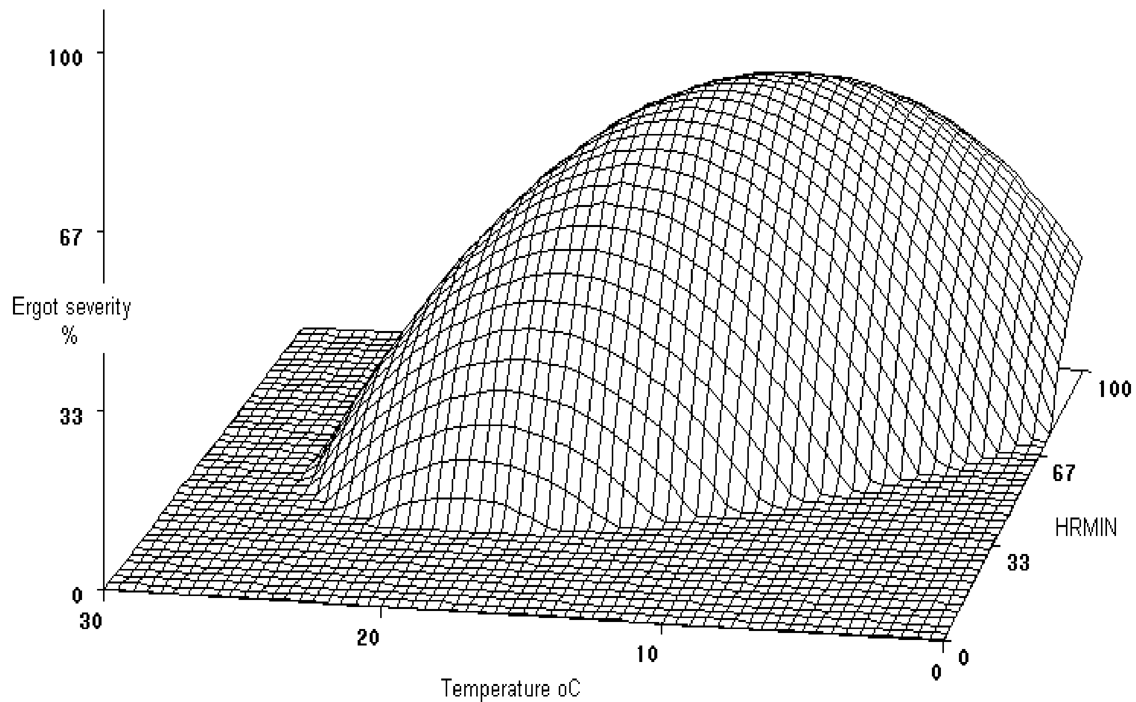

Fig. 26. Predicted effect of HRMIN and TMIN after bloom initiation on ergot in A-lines at College Station.

$Y=-341.500773+21.169045(TMIN)+7.179676(HRMIN)-0.445575(TMIN)^{2}-0.137607(TMIN*HRMIN)-0.035415(HRMIN)^{2}$

In CEL, TMAX also influences ergot, especially those temperatures present during the flowering period that in combination with HRMIN supported an ideal environment for the disease. The predicted model obtained showed that TMAX of 25 to 32°C and HRMIN from 30 to 75% were conducive for ergot development (fig. 27). The maximum ergot expression was with TMAX around 30°C and HRMIN around 50%. This two-factor regression model accounted for 45% of the total variation of the data.

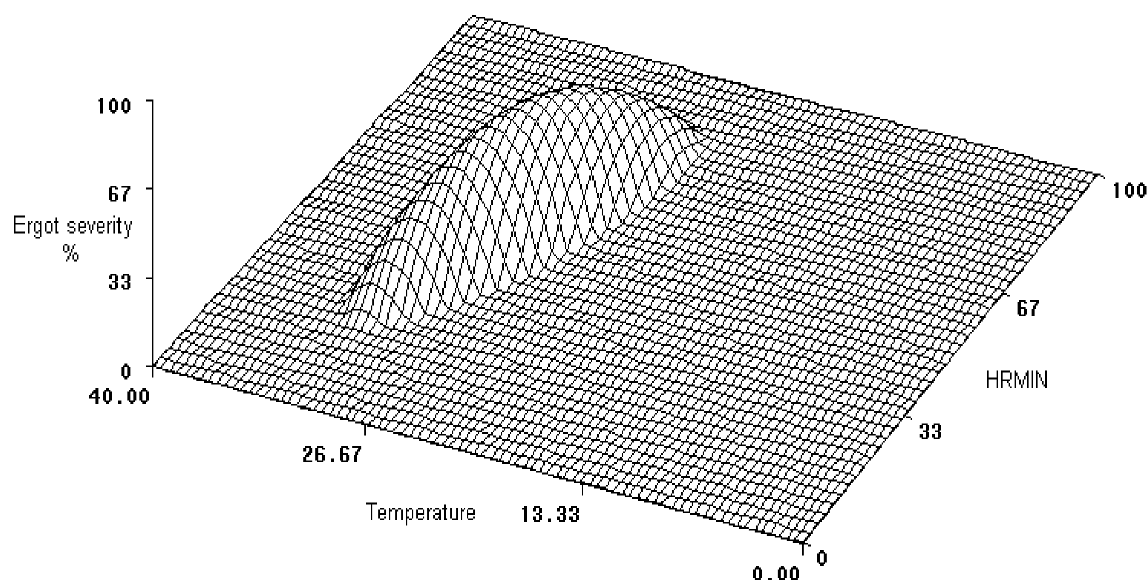

Fig. 27. Predicted effect of HRMIN and TMAX present after bloom initiation on ergot in A-lines at Celaya, Mexico.

$Y=-3523.482386+200.153558(TMAX)+30.632924(HRMIN)-2.907150(TMAX)^2-0.755054(TMAX*HRMIN)-0.092247(HRMIN)^2$

A-lines were also affected by TMAX at RB, where they promoted ergot development with increasing HRMIN during the flowering period. The surface regression model accounted for 60% of the total variation and showed a highly statistical significance (fig. 28). The predicted ergot infection at this location was very high, especially when high values of HRMIN and TMAX are combined. Maximum ergot is around 30°C with HRMIN above 65%.

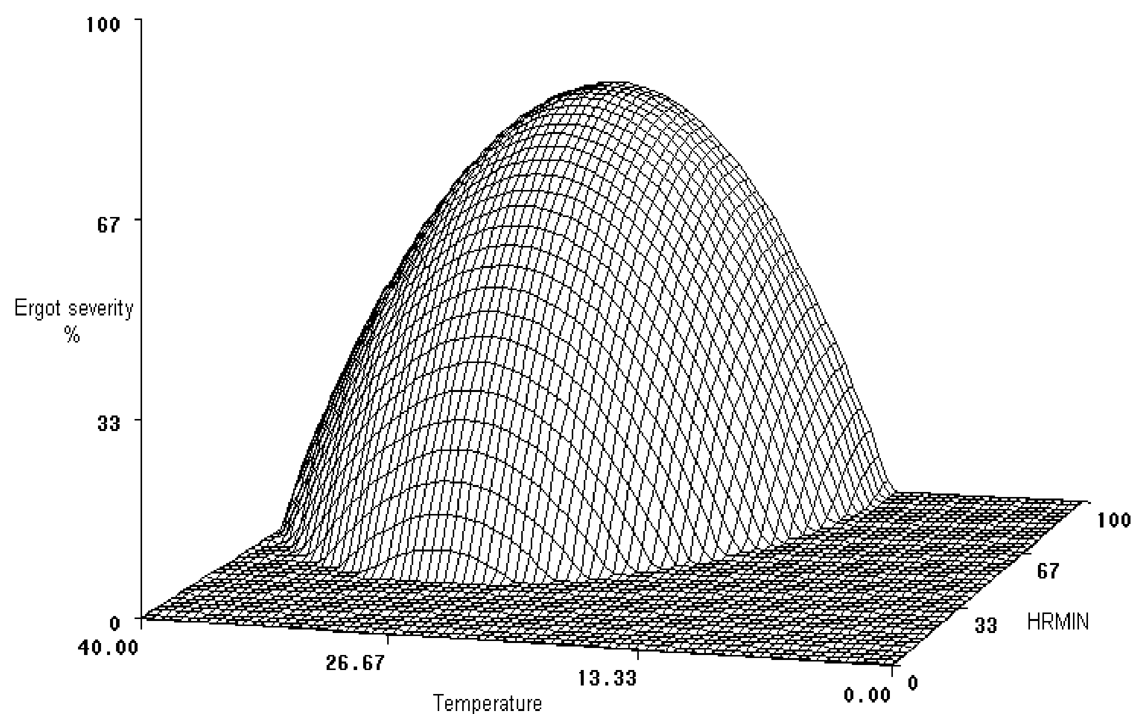

Fig. 28. Predicted effect of HRMIN and TMAX present after bloom initiation on ergot in A-lines at Rio Bravo, Mexico.

$Y=-325.815702+16.727277(TMAX)+3.687857(HRMIN)-0.288226(TMAX)^2-0.014591(TMAX*HRMIN)-0.017411(HRMIN)^2$

The ergot prediction model for sorghum hybrids at CS was also affected by the combination of HRMIN and TMIN, especially temperatures during 7 to 9 days before initiation of flowering, and relative humidity during flowering. According to the model, ergot will develop under cool and humid conditions. The model accounted for 50% of the variation (fig. 29).

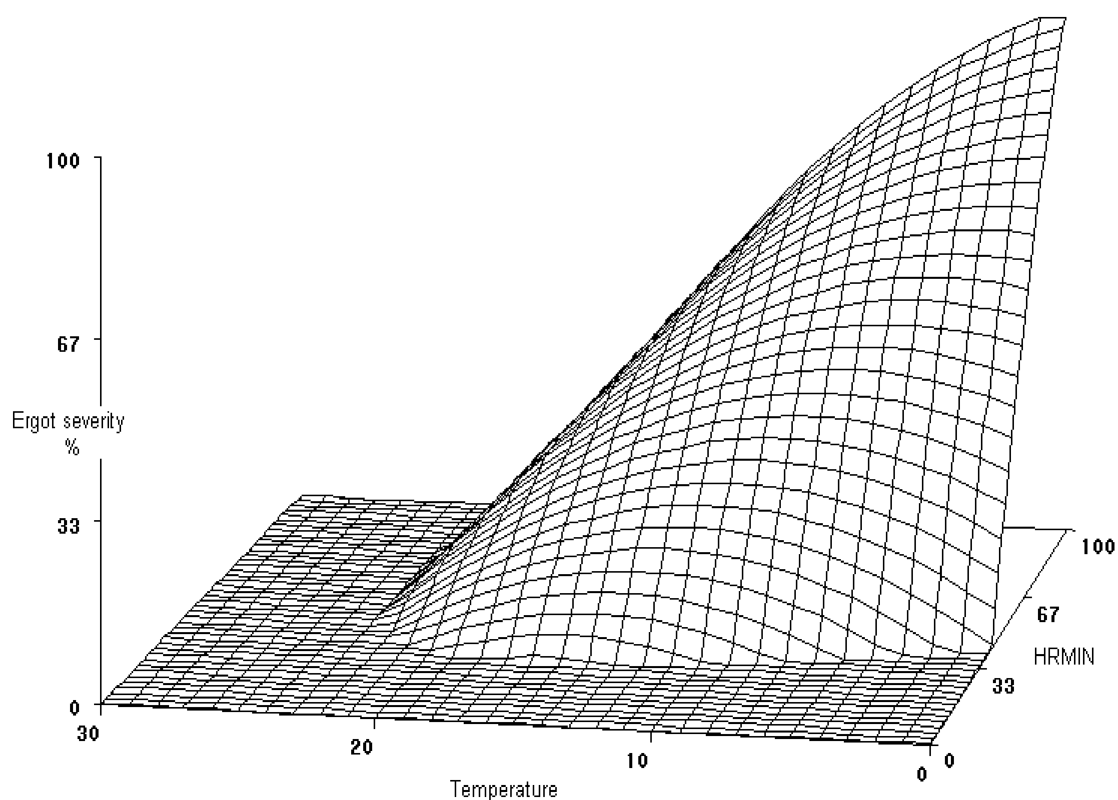

Fig. 29. Predicted effect of HRMIN (after bloom) and TMIN (before bloom) on ergot in sorghum hybrids at College Station, Texas.

$Y=-117.949338+7.387763(TMIN)+3.226328(HRMIN)-0.156154(TMIN)^2-0.091867(TMIN*HRMIN)-0.010254(HRMIN)^2$

The inverse relationship occurred in CEL, where the predicted ergot severity in hybrids is affected by warmer temperatures (7-9 days before bloom initiation) in combination with HRMIN (after bloom initiation) values exceeding 30% during the flowering period is shown in figure 30.

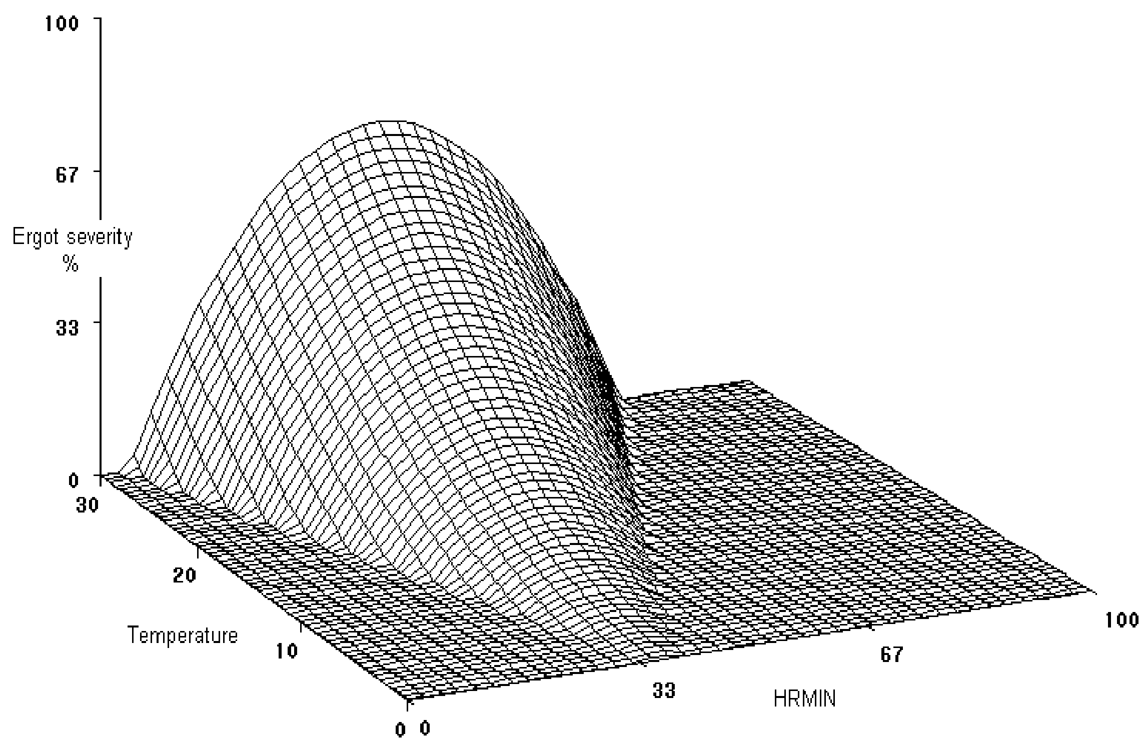

Fig. 30. Predicted effect of HRMIN (after bloom) and TMIN (before bloom) on sorghum hybrids at Celaya, Mexico.

$Y=-62.324576-0.550622(TMIN)+3.491509(HRMIN)+0.070196(TMIN)^2-+0.018804(TMIN*HRMIN)-0.048370(HRMIN)^2$

Discussion

The main objective of this study was to understand the relationship between ergot and weather, and compare these relationships among locations. The predicted ergot potentials obtained appear accurate for future use at each location due to the high R^2, nevertheless, a validation of them is needed. The results and the models derived from the present study assume the presence of viable and abundant *C. africana* inoculum.

The results show great variability in susceptibility to sorghum ergot among genotypes that are evaluated at a single planting date, and also the variation in susceptibility of a single genotype evaluated at different planting dates within a year, locations and years. Therefore, sorghum ergot resistance studies should be done using all these components to assure that the disease resistance is genetic and not a type of avoidance mechanism. These results corroborate McLaren (48), who found that ergot reaction of a specific line is quantified by comparing observed ergot severities associated with different flowering dates with the ergot potential of those dates. The data showed that none of the nine hybrid and A-line genotypes had genetic resistance. However, NC+8R18 showed the least amount of ergot over planting dates, years and locations. This confirms the observations of Bandyopadhyay et al (5), who found that variations in ergot incidence and severity in sorghum lines is related to tolerance of lines to ergot favorable conditions, such as pre-flowering cold stress and the ability to escape infection by ensuring rapid and effective pollination.

At all locations, ergot caused by *C. africana* would be influenced by weather conditions. These results are similar to those obtained by Gupta et al (36), who observed

that rainfall, sunshine hours, relative humidity and air temperature accounted for almost 100% of the variation in ergot. In this study, high temperature and low relative humidity inhibit ergot. In this study, as in others (46,49,55,54), favorable weather conditions prior to and after bloom, promoted sorghum infection by *C. africana*.

Ergot development is very sensitive to a moist environment. These results suggest that high humidity provided by rain or morning and afternoon drizzle promotes infection. Male-sterile plants showed that the ideal weather conditions for maximum sorghum ergot expression are temperatures around 23°C. However, this study suggests that ergot can develop up to 38°C, while temperatures above inhibited the infection by *C. africana*. This may be due to the inhibition of germ tube growth above this temperature, as shown for *C. microcephala* (36). These results differ from those of McLaren and Wehner (46), who observed that the ideal temperature for sorghum ergot development is around 19°C, and that ergot is reduced with increases in temperature, until it is stopped at temperatures exceeding 28-30°C. These high temperatures can be associated with reduced relative humidity.

The high correlation between ergot severity, TMIN, TMAX and HRMIN in this study, as well as results from the studies of McLaren and Wehner (46), McLaren and Flett (49) and Montes et al (54) reaffirms the close relationship between weather variables and ergot severity in sorghum. In general, weather factors that had a high correlation with ergot severity, showed the highest correlation coefficients around flowering time. Nevertheless, with hybrids, TMAX ($r= -0.71$, $P<.0001$) and TMIN ($r=-0.69$, $P<.0001$) values at RB before initiation of flowering had a direct effect on ergot,

especially those values present 7 to 9 days before initiation of flowering. This may reflect predisposition of the hybrids before flowering for a later infection. Ergot severity also showed a good relationship with TMAX values recorded at RB, CS and CEL locations in post-flowering stages. The observed negative relationship suggests that cool temperatures promoted fungal invasion and reproduction. CEL location had the lowest TMIN values, which were just above the 13°C limit for pollen sterility (21) only during the period of May to July. This represents a high risk for ergot, because low night temperatures prior to flowering (11,48,52), or during pollen mother cell meiosis (21) and in the period between flag leaf ligule emergence and flag leaf sheath elongation (11) result in male-sterility to sorghum heads. Thus, male-sterility caused by low temperatures becomes a significant factor in the epidemiology of the disease. At 7 to 9 days before initiation of flowering, sorghum hybrids showed the highest correlation between ergot severity and TMIN. Additionally, A-lines showed the greatest correlation with TMAX ($r=-0.71$, $P<.0001$), TMIN ($r=-0.61$, $P<.0001$) and HRMIN ($r= 0.55$, $P<.0001$) coefficients after initiation of flowering. The differences in the response to weather conditions could be due to the genetic background of the A-lines, to their flowering pattern, and interference with infection because of pollination. These results confirm that A-lines are the most susceptible to ergot. Differences among these genotypes rely on the time that they have being exposed during flowering time to weather conditions that favor ergot development. The A-line ATx2752, shows the least amount of ergot through years, locations and planting dates. This is perhaps due to its

short flowering period that reduces the length of time ovaries are receptive, or because it blooms at the same time as some of the hybrids, allowing fertilization.

At CEL, a positive relationship with ergot was observed with TMIN on hybrids. This was also observed on the male-sterile plants at this location, where ergot increased with warmer conditions. The results observed at CEL are due to the low TMIN conditions that are present during the growing season, which can cause pollen sterility and cause ergot development, especially as the growing season advances from summer to fall. However, colder conditions will produce complete sterility and/or inhibit pathogen development.

Moisture is a key factor in the infection process of fungal pathogens. The results showed that HRMIN values from 30% present during the flowering period were sufficient to promote infection. Nevertheless, at CEL a low significant negative relationship was observed between ergot and HRMIN. The possible explanation for this discrepancy is that this data uses the HRMIN average triad value, and conidia germination and infection can be activated within a few hours under low moisture or with 23-24% HRMIN (54), and not with the high relative humidity values that Futrell and Webster (32), and McLaren and Wehner (48) observed. However, McLaren and Wehner (46) found the same period, 1 to 4 days after pollen shed, to have the most significant relationship between ergot and HRMIN.

It is clear that the hybrid seed production system is at high risk for ergot because it can develop on A-lines at all locations during the normal growing season. Ergot infection in CS and CEL locations during the fall season did not occur due to frost that

reduced flower development and cool temperatures that inhibited the pathogen. Situations like this and that at RB, where there are no commercial sorghum fields planted after September, result in no new infections during the winter, so new infections in the next growing season could arise from overwintering of inoculum or secondary conidia coming from infected hosts elsewhere.

At WE location ergot severity was drastically lower compared to the other locations. These results may be attributable to the *C. africana* inoculum and the inoculation method that were used. Inoculum used at this location came from infected panicles preserved at room temperature and inoculum was not newly prepared between inoculations, while in the other locations a freshly-prepared spore suspension was used.

Models developed in this study consider the effect of weather factors during the infection process as well as plant predisposition (on hybrids) on ergot severity. Also, they assume high amounts of viable inoculum are present during the flowering period. The predicted ergot potentials can be used as a tool in sorghum hybrid seed production to: 1) distinguish the seed production location risk, and 2) apply fungicides more efficiently, or 3) identify the possible crop windows were grain sorghum could be established and cultivated. The recommendation to farmers will be to plant sorghum hybrids in periods in which plants can avoid exposure to low minimum temperatures before flowering. For seed production fields, the recommendation is to apply control measures (chemical control and crop management) to reduce ergot. The analysis of historical weather data and application of these models can give a better idea of the possible *C. africana* impact in sorghum hybrids and male-sterile lines.

CHAPTER IV

EFFECT OF STORAGE TEMPERATURE AND SPHACELIUM AGE ON CONIDIA SURVIVAL

Introduction

In the sorghum hybrid seed production industry, the main goal is to obtain and maintain sorghum seed with high viability. Viability is highly influenced by storage conditions. The most critical conditions for seed in storage are low seed moisture and low temperature. In addition to these factors, seeds need to be free from inert material such as plant debris that could contain pathogens and insects. The USDA-ARS National Seed Storage Laboratory stores seeds at low seed moisture content (5-8 %) in a sealed, moisture-free container at low or subzero temperature, which inhibit insects and fungal pathogens (60).

Pathogens that are carried in seed lots may be either internally seed-borne or present on the seed surface, in plant debris, or infected weed seeds. These pathogens can survive long periods of storage along with dried seeds in a dormant stage, and usually do not resume activity until seeds germinate (33,34). Bhuiyan et al. (8) demonstrated that macroconidia of *C. africana* can survive on honeydew-coated seed for more than 12 months at 4°C (42-100% RH), suggesting that international seed exchange was a possible route for the accidental introduction of this pathogen to Australia (43). Since ergot was detected during 1997 in the Texas seed production area, its possible that

pathogen structures could be spread with shipments exported overseas, possibly to sorghum producing areas that are free of the pathogen. Ellis (23) reports that temperature has a dramatic effect on seed longevity and concluded that each 5°C reduction in seed temperature doubles the life of seeds. However, low temperatures can maintain pathogen viability.

The objectives of this study were: (1) to determine the effect of storage temperature on the viability of *C. africana* macroconidia located on the surface and within the sphacelium, and (2) observe the effect of sphacelium age on the macroconidial survival.

Materials and methods

Sorghum A-line ATx623 was planted in the greenhouse during 2001, 2002 and 2003 at College Station, Texas. Flowering panicles were tagged and inoculated by hand atomizer until runoff with a suspension of 1.6×10^6 *C. africana* conidia ml^{-1}. Several panicles were selected according to their sphacelial development 7 to 10 days after inoculation. Sphacelia were collected at several stages depending on their maturity. Sphacelial structures were grouped into four maturity classes based on sphacelium development:

Class 1: newly-formed sphacelia showing slightly transparent honeydew ooze.

Class 2: One-week-old sphacelia showing a lot of transparent honeydew ooze.

Class 3: Two-week-old sphacelia with dark-brown dried honeydew.

Class 4: Three-week-old sphacelia showing hardness on the sphacelia surface and honeydew crust.

Sphacelia, attached to the panicle rachis were placed in petri dishes containing silica gel (as a dessicant). Dishes were sealed with parafilm. The incubation temperatures of sphacelia were: fluctuating sub-freezing (-3°C or less), 7°C, 14°C and 21°C.

Dishes were arranged in a factorial experiment with 16 treatments out of the combination of sphacelia maturity (four levels) and temperature (four levels); each plot was replicated four times in a randomized complete design. The model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

Where μ is the overall mean conidia germination, α_i is the effect of the *i*th level of temperature, β_j is the effect of the *j*th level of sphacelia age, and $\alpha\beta_{ij}$ is the interaction effect of the *i*th level of temperature with the *j*th level of sphacelia age.

Conidia survival was measured as the proportion of germinated macroconidia showing conidiophore formation with secondary conidia at their tips and was measured almost every month by sampling macroconidia located on the sphacelium surface and within the sphacelium interior.

A random sample of 20 sphacelia was taken from each one of the treatments, and after removing plant tissue, was placed into vials containing 20 ml distilled water. Vials were stirred for one minute and a portion of the suspension (1 ml) was placed onto

water-agar plates. Four replications per treatment were made and incubated overnight at room temperature (21°C). The germination observed in this sample was named “germination on the sphacelium surface”. After rinsing the remaining sphacelia with a jet of water for 30 seconds, they were macerated using a mortar, suspended in 10 ml distilled water, stirred for 30 seconds and placed onto water-agar plates. The germination percentage obtained here was named “within the sphacelium”. Original data was transformed using the arcosine of the square root of each value to comply with normality distribution assumptions. To determine significant differences between means, Tukey's mean separation was used at $P<.01$.

Results

There was a highly significant effect of the main factor temperature across the six month study on germination of *Claviceps africana* conidia located on the sphacelium surface, while sphacelium age had a highly significant effect up to the fifth month, same as the interaction between these two factors (table 12). Almost same results where obtained in the ANOVA table for germination of conidia located within the sphacelium, that was affected by both factors up to the fifth month (table 13).

Table 12. Observed mean squares and test of significance of main factors on germination of *C. africana* conidia located on the sphacelium surface.

		Month											
		1		2		3		4		5		6	
Source	**df**	**MS**		**MS**		**MS**		**MS**		**MS**		**MS**	
Temp (A)	3	0.444	**	0.794	**	2.202	**	0.664	**	0.604	**	0.923	**
Age (B)	3	0.968	**	0.719	**	0.308	**	0.069	**	0.054	*	0.055	ns
A x B	9	0.140	**	0.089	**	0.097	**	0.147	**	0.063	**	0.029	ns
Error	176	0.055		0.032		0.032		0.032		0.001		0.026	

**= Highly significant effect at $P<0.01$; *= significant effect at $P<0.05$, and ns= not significant.

Table 13. Observed mean squares and test of significance of main factors on germination of *C. africana* conidia located within the sphacelium.

		Month											
		1		2		3		4		5		6	
Source	**df**	**MS**		**MS**		**MS**		**MS**		**MS**		**MS**	
Temp (A)	3	0.614	**	0.544	**	1.199	**	0.550	**	0.431	**	1.009	**
Age (B)	3	0.648	**	0.377	**	0.078	**	0.058	**	0.033	**	0.004	ns
A x B	9	0.090	**	0.162	**	0.062	**	0.108	**	0.053	**	0.032	**
Error	176	0.035		0.033		0.021		0.003		0.0013		0.026	

**= Highly significant effect at $P<0.01$; *= significant effect at $P<0.05$, and ns= not significant.

Warmer storage temperatures (21°C) significantly reduced germination across the 6-month period in conidia located on the sphacelium surface. The reduction ranged from 42% to 100% compared with frozen temperatures, and from 26% to 100% compared with cool temperatures (7°C). At the end of the 6-month study, frozen temperatures show the highest significant conidia germination, with a reduction of 59% in that period. Conidia on younger sphacelium showed significantly more germination (47-65%) compared with older sphacelium conidia in the first 3-month period. However, this situation was reversed in the second 3-month period where newly-formed sphacelium gave the lowest conidia germination. At the end of the 6-month trial, all sphacelial ages gave statistically the same conidia germination on the sphacelium surface (table 14). Identical situations were observed in conidia located within the sphacelium, where the warmer treatment reduced conidia viability from 46 to 100% compared with frozen temperatures, and newly formed sphacelia showed 42 to 73% more conidia germination than older sphacelia in the first 3-month period. After that, conidia germination was statistically similar in both sphacelial ages (table 15).

Table 14. Effect of main factors on the average *C. africana* conidia germination located on the sphacelium surface.

Factor	Month 1		2		3		4		5		6	
Storage Temperature (°C)		*										
0	40.50	a	32.19	a	31.96	a	20.06	a	18.94	a	16.53	a
7	31.75	a	28.94	a	23.29	b	19.75	a	18.06	a	9.31	b
14	31.39	a	26.16	a	10.79	c	12.06	b	9.19	b	2.56	c
21	23.35	b	7.16	b	2.98	d	0.13	c	0.25	c	0	c
Sphacelium age (days)												
0	47.87	a	37.84	a	22.50	a	7.63	c	6.06	b	5.13	a
7	30.75	b	22.94	b	17.89	b	17.75	a	12.44	a	11.22	a
14	28.35	bc	20.75	b	16.81	b	15.44	ab	14.31	a	7.19	a
21	20.02	c	12.91	c	11.81	b	11.19	b	13.63	a	4.88	a

*= Treatments with the same letter in each category are statistically similar according to Tukey, $P < 0.01$

Table 15. Effect of main factors on the average *C. africana* conidia germination (%) located within the sphacelium.

Factor	Month 1		2		3		4		5		6	
Storage Temperature (°C)		*										
0	24.52	a	25.19	a	17.02	a	13.06	c	15.19	a	11.03	a
7	28.31	a	18.53	a	17.02	a	26.00	a	12.75	b	12.59	a
14	30.06	a	18.94	a	10.91	b	17.06	b	3.00	c	2.03	b
21	13.21	b	6.09	b	1.52	c	2.19	d	0.5	d	0	c
Sphacelium age (days)												
0	36.19	a	20.06	a	13.33	a	11.56	c	11.25	a	5.97	a
7	25.44	b	23.63	a	12.75	ab	17.88	a	8.75	ab	6.53	a
14	21.58	b	19.56	a	12.67	ab	16.19	b	5.56	c	6.63	a
21	12.89	c	5.50	b	7.73	b	12.69	bc	5.88	b	6.63	a

*= Treatments with the same letter in each category are statistically similar according to Tukey, $P < 0.01$

Comparing the average germination across years and dates in both conidia locations, conidia from the sphacelium surface had more germination at all levels of storage temperature and sphacelium age (table 16) than interior conidia. Conidia from within the sphacelium and the sphacelium surface stored at warmer conditions showed a significant reduction of 50% and 75%, respectively compared with frozen temperatures in the same location. Also, conidia from younger sphacelium showed 53 to 75% more germination than conidia from older sphacelium in both conidia locations.

Table 16. Effect of storage temperature and sphacelium age on germination of *C. africana* conidia located on the surface and within the sphacelial tissue.

Factor	Sphacelium surface	Within sphacelium
Storage temperature (°C)	*	
0	29.48 a	25.89 a
7	23.29 b	20.37 b
14	17.10 c	18.43 b
21	7.81 d	12.99 c
Sphacelium age (days)		
0	19.75 a	18.62 a
7	18.77 ab	16.79 ab
14	15.45 b	14.74 b
21	4.92 c	8.71 c

*= Treatments with the same letter in each category are statistically similar according to Tukey, $P < 0.01$

The combined analysis showed significant differences among storage temperatures at each sphacelium age (fig. 31). At all sphacelia ages, conidial germination decreased as temperature increased from 0 to 21°C (fig. 32; r= -0.75 at p< .0009). This suggests that temperatures at locations with summer-fall planting dates could promote survival of conidia outside and inside the sphacelia, creating a viable source of inoculum for the next crop season.

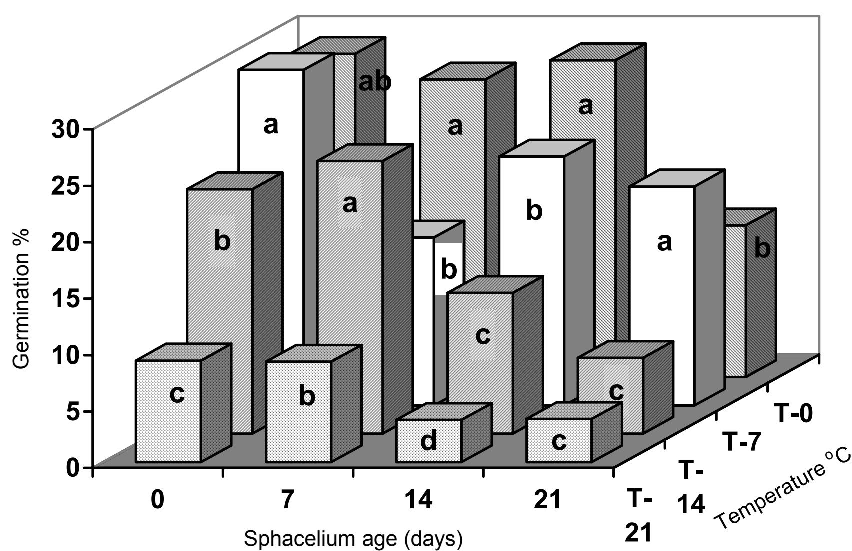

Fig. 31. Effect of the interaction between sphacelium age and storage temperature on germination of *C. africana* conidia.

Note: Temperature treatments with the same letter in each sphacelial age, are statistically similar according to Tukey's P<0.01

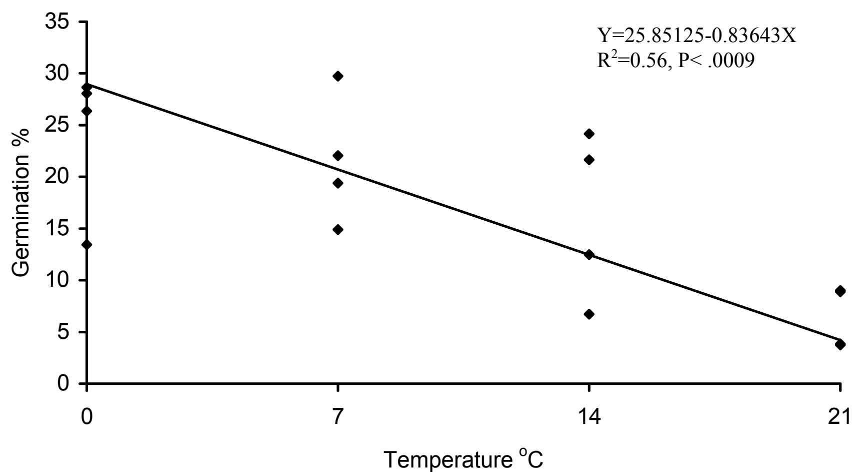

Fig. 32. Regression model showing the relationship between storage temperature and *C. africana* conidia germination.

The effect of sphacelial age on conidia germination was better described using exponential decay regression models, which show that conidia from the surface of newly formed sphacelium had statistically more germination (table 14) than the other sphacelia ages during the first three months (fig. 33), and then decreased after this time. During the 6-month period, newly-formed sphacelia had half the conidial germination of the older sphacelia for every unit increase of time. The models explained up to 77% of the variation in the sampled population.

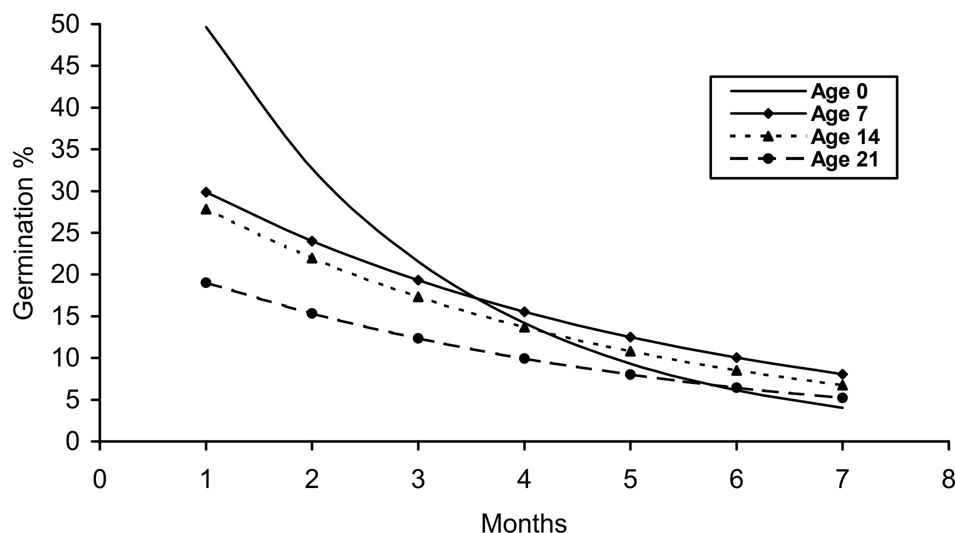

Fig. 33. Effect of sphacelium age on germination of *C. africana* conidia located on the sphacelial surface. Exponential decay regression models obtained with 272 data points for Age 0 (R^2= 0.77), $Y=75.4040e^{-0.4179X}$; Age 7 (R^2= 0.56), $Y=37.1572e^{-0.2180X}$; Age 14 (R^2= 0.66), $Y=35.2801e^{-0.2367X}$ and Age 21 (R^2= 0.46), $Y=23.5959e^{-0.2158X}$.

Viability of conidia located within newly-formed sphacelia was higher during the first two months. Older sphacelia had lower viability of conidia up to the fourth month (fig. 34). Conidia on newly-formed sphacelia decline in viability three times faster than the oldest sphacelia. Models accounted up to 69% of the total variation in each sphacelia age populations.

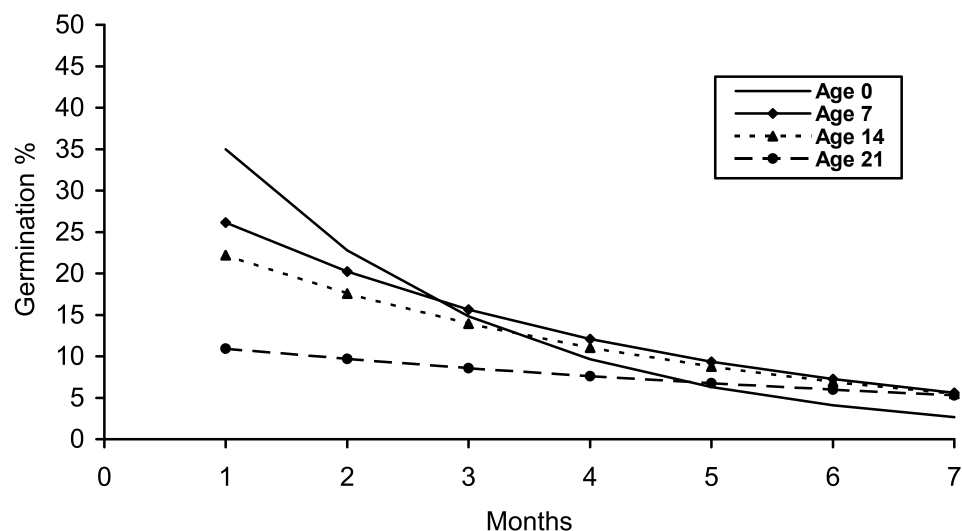

Fig. 34. Effect of sphacelium age on germination of *C. africana* conidia located within the sphacelial tissue. Exponential decay regression models obtained with 272 data points for Age 0 (R^2= 0.69), $Y=75.4040e^{-0.4179X}$; Age 7 (R^2= 0.58), $Y=37.1572e^{-0.2180X}$; Age 14 (R^2= 0.58), $Y=35.2801e^{-0.2367X}$ and Age 21 (R^2= 0.53), $Y=23.5959e^{-0.2158X}$.

Exponential decay regression models for cooler storage temperatures showed significantly highest viability values through the 6-month period with 42% to 99% higher than the warmest temperature (fig. 35). Models accounted for up to 81% the total variation of the population. Conidial germination rate declined 5 times faster at the warmest storage temperature as compared with the coolest. Conidial viability at the 21°C treatment was nil at the fifth month, while conidia viability was more than 10% at cooler storage after the sixth month. Similar trends were observed with models of the conidia from within the sphacelia (fig. 36). However, the viability was lower at all the storage temperatures. Models accounted for up to 72% of the total variation of the data. The

warmest storage temperature showed 4.5 times more reduction in the conidial germination as compared with the coolest, and twice compared with the 14°C.

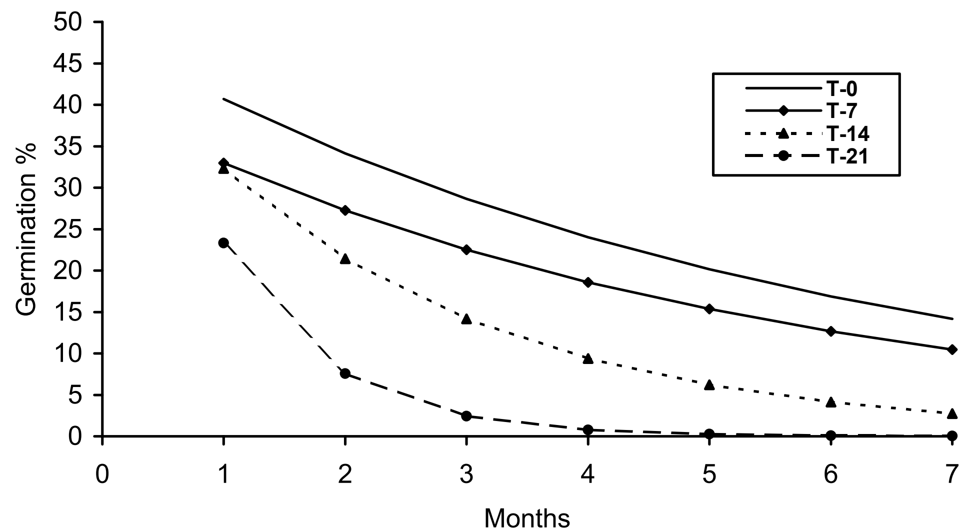

Fig. 35. Effect of storage temperature on germination of *C. africana* conidia located on the sphacelium surface. Exponential decay regression models obtained with 272 data points for T-0 (R^2= 0.81), $Y=48.5155e^{-0.1759X}$; T-7 (R^2= 0.66), $Y=39.9308e^{-0.1911X}$; T-14 (R^2= 0.58), $Y=48.8018e^{-0.4120X}$ and T-21 (R^2= 0.54), $Y=71.9075e^{-1.1263X}$.

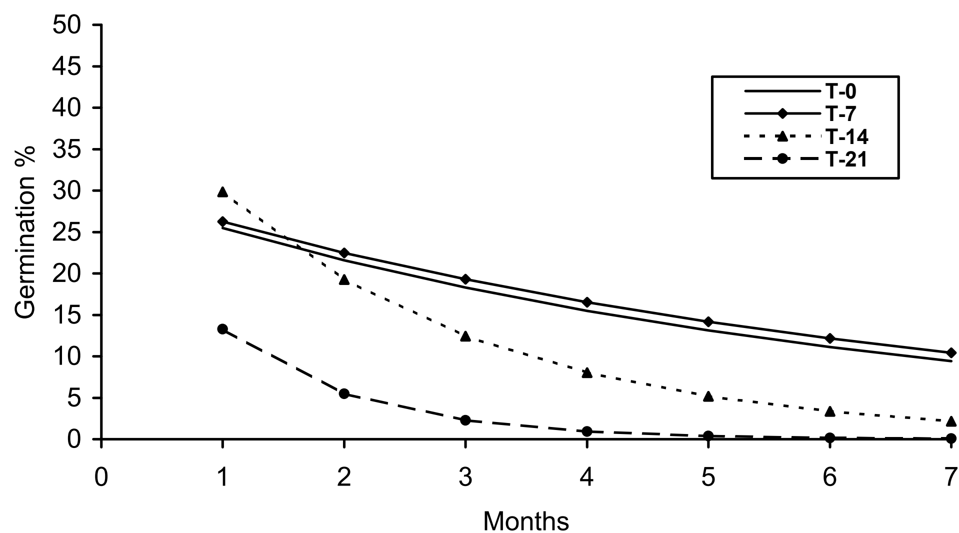

Fig. 36. Effect of storage temperature on germination of *C. africana* conidia located within the sphacelium. Exponential decay regression models obtained with 272 data points for T-0 (R^2= 0.72), $Y=30.0636e^{-0.1656X}$; T-7 (R^2= 0.71), $Y=30.6050e^{-0.1539X}$; T-14 (R^2= 0.58), $Y=46.2400e^{-0.4374X}$ and T-21 (R^2= 0.38), $Y=32.1480e^{-0.8842X}$.

Discussion

This study shows that survival of conidia of older sphacelia, which are most common during harvest of commercial or seed production fields, is very sensitive to the warmer temperatures that would be present during the summer in spring-planted sorghum production areas. Where cooler temperatures prevail following a crop (e.g. with summer or fall-planted crops), conidia may survive longer, perhaps contributing to local survival of inoculum for the next crop season.

Conidia located on the sphacelium surface had greater germination than the conidia located inside the sphacelium. This may be due to the developmental maturity of the conidia located on the outside. Warmer storage temperatures (21°C) significantly reduced conidia viability compared with freezing or cool temperatures (<21°C). Dry and cool temperatures are required to preserve conidia viability, and newly-formed sphacelia have the highest conidial viability especially if conidia are located on the sphacelium surface. Averaged over all sphacelia ages, conidial viability decreased as temperature increased from 0 to 21°C ($r= -0.75$ at $P< .0009$). Similar results obtained by Odvody et al. (62) showed that *C. africana* macroconidia maintained viability stored at 6°C, whereas Bhuiyan et al. (8) showed that storage of sphacelia at high temperature (>32°C) resulted in a rapid decrease in viability of *C. africana* macroconidia, with no spores viable after two weeks of storage.

Conidia from the surface of newly-formed sphacelia had statistically more germination than the other sphacelial ages during the first three months. During the 6-month period, newly-formed sphacelia had a reduction in conidial viability of twice the value of the older sphacelia for every increase unit of time.

Viability of conidia at the highest storage temperature decreased 5 times as much as the coolest storage temperature. Conidia viability at the 21°C treatment declined to zero at the fifth month, while conidial viability at cooler temperatures were more than 10% at the sixth month. Similar trends were observed with the conidia from within the sphacelia. Conidial viability at the highest storage temperature was 4.5 times reduced as compared with the coolest, and was half of the 14°C treatment. These results are similar

to those of Bhuiyan et al. (7), who found that *C. africana* conidia showed little germination after 17 weeks storage at 20°C. Also, conidia survived for more than eight months stored outside over the winter months. These results support those of Prom et al (68), whom showed that conidia could be viable up to 12 months under field conditions in Texas. Cool temperatures (6°C) evaluated by Odvody et al. (62), maintained conidia viability from fresh sphacelia at its maximum up to 12 weeks, and then decreased 50% at 22 weeks of storage.

CHAPTER V

EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON SCLEROTIA FORMATION

Introduction

Upon development of the sphacelium, certain alterations in host physiology are observed, and these host-parasite relationship may be important in triggering the morphogenesis of the sphacelium and sclerotium (58). The sphacelium is transformed into another structure that will provide protection to the fungus during the over wintering period. The sphacelial tissue transforms into sclerotial tissue at low temperature and dry environments (Odvody, personal communication). Three stages in sclerotia formation and development were distinguished by Townsend and Willets (79): 1) initiation, the appearance of small distinct initials formed by the interwoven hyphae, 2) development and increase in size, 3) maturation, characterized by surface delimitation, internal consolidation, pigmentation, and often associated with droplets excretion. Active vegetative mycelial production always precedes the initiation of sclerotia, and nutrients are absorbed by the fungus from the substrate (83).

Sclerotial tissue is a mass of whitish fungal mycelium surrounded by a protective brown-reddish rind. The rind is composed of thickened, pigmented, parenchyma-like cells that usually develop on the outer surface of sclerotia (83). A characteristic feature of sclerotial rind formation is a change of color from white to buff to dark brown or

black. The color develops by the accumulation of melanin (83). Sclerotia are hard, rugose and orange-brown colored structures that can survive the winter conditions and produce ascomata (29), and remain viable for several years. Sclerotial tissue development is very important because it ensures survival and has the potential to introduce new pathogen genotypes via sexual reproduction.

Materials and methods

This experiment was done at Texas A&M University (College Station, Texas) during the spring-summer of 2003 under controlled environmental conditions. Plants of A-line ATx623 were grown under greenhouse conditions and inoculated at flowering time with a suspension containing 1.6×10^6 *C. africana* conidia ml^{-1}. Infected plants showing sphacelium formation were transported to growth chambers and kept under constant temperature/relative humidity regimes. Treatments consisting of temperature regimes of 10, 20 and 30°C combined with low relative humidity (<30%) were applied to 10 plants per growth chamber. Plants were sampled every week to observe sclerotia development. A dissecting microscope was used to observe sclerotial tissue differentiation, rind formation or changes in the sphacelial surface. Dry weight as a measure of sphacelia growth was taken and analyzed using PROC GLM (SAS Institute, Cary, NC.). Dry weight was obtained by oven-drying the sphacelia ≈ 100°C until the dry weight was constant (5-10 min). Separation of means was performed using Tukey's at $P<0.01$ level of significance.

Results

No sclerotial tissue or rind formation, nor even changes in the structural shape of sphacelia were detected during the four-week study. Also, no secondary conidia production was detected at this level of relative humidity. The Analysis of variance showed that temperature and sampling date had a highly significant effect on the dry weight of sphacelia. Also, the interaction between these factors was significant (table 17). In general, lower temperatures resulted in the lowest sphacelia dry weight (8.22 mg), followed by the 20°C treatment with 11.38 mg.

Table 17. ANOVA for sphacelia dry weight (mg) obtained from male-sterile line ATx623.

Source	df	SS	MS	F value	***P*** **> F**
Sampling date (A)	3	378.78	126.26	262.99	< .0001
Temperature (B)	2	378.22	189.11	393.90	< .0001
A x B	6	43.73	7.28	15.18	< .0001
Error	36	17.28	0.48		

At all sampling dates, higher temperatures promoted significantly more accumulation of dry matter in the sphacelia (fig. 37). This could play a role on the inoculum amount available for the following season. Up to three weeks after sphacelial formation, dry weight increased, and then it decreased thereafter.

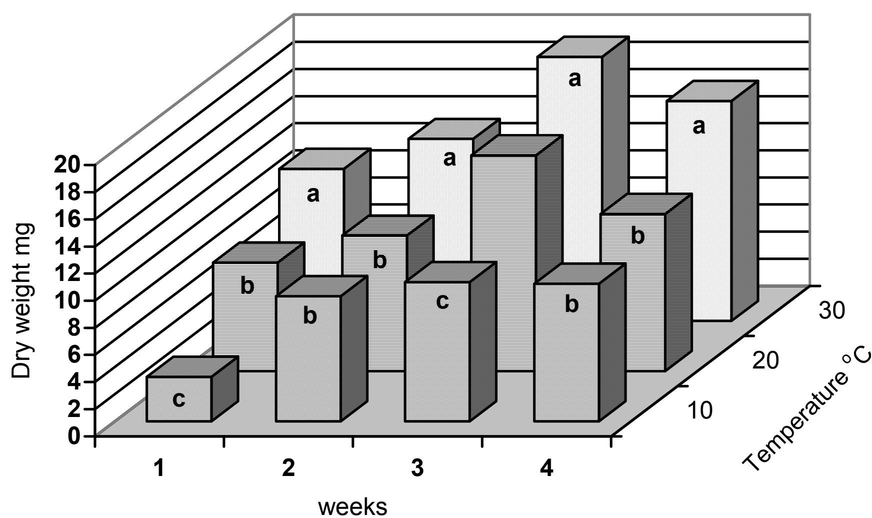

Fig. 37. Effect of temperature on sphacelium dry weight of A-line ATx623.

Discussion

Weather conditions that trigger the sclerotial development have been described as dry and cool (Odvody, personal communication). However the conditions in this study did not change the sphacelial tissue. There was no production of secondary conidia at 30% relative humidity. In general, sphacelia incubated at cooler temperatures had the lowest sphacelia dry weight under dry conditions. These results are supported by the observations of Bandyopadhyay et al. (5), who found that sphacelium-sclerotium differentiation was not triggered by the combination of temperatures between 14 to 28°C

and high relative humidity, which are required for secondary conidiation. According to Mower and Hancock (58), when the sclerotium of *C. purpurea* is developing, certain alterations in the host physiology are observed, and they may be important in the host-parasite relationship in triggering the morphogenesis of the sphacelium into sclerotium. Factors such as day/night light regimes, CO^2 concentration, sphacelia maturity, and the temperature regime and changes during the day may have an impact on sclerotial tissue differentiation and need to be investigated.

CHAPTER VI

CONCLUSIONS

A-lines had greater ergot susceptibility than hybrids. Nevertheless, differences between A-lines suggests, that breeding for resistance is a possible control option.

Sorghum hybrids are at great risk for ergot at CEL, due to the low minimum temperatures that are present throughout the year that can cause pollen sterility. A-lines are highly susceptible to *C. africana* due to the conducive environment during the summer and fall seasons.

Maximum and minimum temperature had a negative correlation with ergot at 7 to 9 days before initiation of flowering, suggesting that cooler temperatures during this period could cause pollen sterility in sorghum hybrids, predisposing plants to ergot infection. Also, male-sterile lines showed more susceptibility to the disease under cooler temperatures after initiation of flowering.

Moisture provided by minimum relative humidity had a positive correlation with ergot, especially if moisture was present after initiation of flowering in both sorghum plant types.

Models developed in this study identified environmental factors involved during the infection process and affecting predisposition of hybrids. The predicted ergot potentials can be used as a tool in sorghum hybrid seed production to distinguish seed production risk areas, and apply fungicides more efficiently, or in grain sorghum fields to identify the possible crop windows where sorghum could avoid ergot.

Cool-dry conditions did not had any effect on sclerotial tissue development up to 4 weeks after formation of sphacelia. Further studies on sphacelia-sclerotia transition need to be done with emphasis on weather conditions present after sorghum harvest time and with fluctuation in the temperature regimes (day/night).

Because of the viability of conidia associated with sphacelia, the presence of such fungal tissue in seed could pose a risk of spreading the pathogen into new areas.

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APPENDIX

Table A1. Pearson's Correlation coefficients between ergot severity and maximum temperature observed at Rio Bravo.

Stage	Hybrids						A-lines					
	2002		2003		Combined		2002		2003		Combined	
X_1	-0.54	**	-0.15	*	-0.55	**	-0.40	**	0.15	ns	-0.23	**
X_2	-0.53	**	-0.22	**	-0.54	**	-0.39	**	0.03	ns	-0.26	**
X_3	-0.58	**	-0.19	**	-0.55	**	-0.48	**	0.05	ns	-0.32	**
X_4	-0.57	**	-0.37	**	-0.57	**	-0.44	**	-0.16	ns	-0.36	**
X_5	-0.60	**	-0.29	**	-0.58	**	-0.52	**	-0.33	**	-0.46	**
X_6	-0.65	**	-0.26	**	-0.60	**	-0.67	**	-0.36	**	-0.56	**
X_7	-0.70	**	-0.34	**	-0.70	**	-0.62	**	-0.50	**	-0.59	**
X_8	-0.71	**	-0.40	**	-0.71	**	-0.65	**	-0.48	**	-0.59	**
X_9	-0.70	**	-0.43	**	-0.66	**	-0.70	**	-0.45	**	-0.62	**
X_{10}	-0.70	**	-0.44	**	-0.66	**	-0.74	**	-0.55	**	-0.68	**
X_{11}	-0.70	**	-0.55	**	-0.65	**	-0.75	**	-0.63	**	-0.71	**
X_{12}	-0.72	**	-0.54	**	-0.68	**	-0.73	**	-0.49	**	-0.65	**
X_{13}	-0.68	**	-0.54	**	-0.64	**	-0.72	**	-0.48	**	-0.63	**

Note: Correlation coefficients in each location were obtained using 1440 ergot data points per year. **= Statistically significant at P< 0.01; *= Statistically significant at P< 0.05; ns=not significant.

Table A2. Pearson's Correlation coefficients between ergot severity and maximum temperature observed at College Station, Texas.

Stage	Hybrids						A-lines					
	2002		2003		Combined		2002		2003		Combined	
X_1	-0.05	ns	-0.22	**	-0.04	ns	-0.10	ns	-0.17	ns	-0.12	ns
X_2	-0.03	ns	-0.22	**	-0.04	ns	0.03	ns	-0.15	ns	-0.07	ns
X_3	-0.07	ns	-0.35	**	-0.09	ns	0.13	ns	-0.06	ns	0.04	ns
X_4	-0.12	ns	-0.21	**	-0.09	ns	0.17	ns	-0.22	ns	0.00	ns
X_5	-0.12	ns	-0.35	**	-0.14	*	0.12	ns	-0.25	ns	-0.01	ns
X_6	-0.20	**	-0.30	**	-0.19	**	0.17	ns	-0.23	ns	0.03	ns
X_7	-0.18	*	-0.21	**	-0.13	*	0.14	ns	-0.33	*	-0.02	ns
X_8	-0.29	**	-0.26	**	-0.19	*	0.41	**	-0.32	*	0.16	ns
X_9	-0.47	**	-0.35	**	-0.29	**	0.24	*	-0.25	ns	0.07	ns
X_{10}	-0.55	**	-0.29	**	-0.40	**	012	ns	-0.30	*	-0.01	ns
X_{11}	-0.67	**	-0.29	**	-0.48	**	0.01	ns	-0.26	ns	-0.09	ns
X_{12}	-0.74	**	-0.40	**	-0.51	**	0.01	ns	-0.45	**	-0.16	ns
X_{13}	-0.72	**	-0.39	**	-0.50	**	0.10	ns	-0.38	**	-0.09	ns

Note: Correlation coefficients in each location were obtained using 720 ergot data points per year. **= Statistically significant at P< 0.01; *= Statistically significant at P< 0.05; ns=not significant.

Table A3. Pearson's Correlation coefficients between ergot severity and maximum temperature observed at Celaya, Guanajuato, Mexico.

Stage	Hybrids						A-lines					
	2002		2003		Combined		2002		2003		Combined	
X_1	0.58	**	0.26	**	0.09	ns	0.65	**	0.18	ns	0.09	ns
X_2	0.51	**	0.32	**	0.04	ns	0.22	ns	0.42	**	0.13	ns
X_3	0.61	**	0.24	**	0.02	ns	-0.34	ns	0.39	**	0.04	ns
X_4	0.43	**	0.32	**	-0.03	ns	-0.69	**	0.48	**	0.04	ns
X_5	0.22	ns	0.32	**	-0.07	ns	-0.62	**	-0.22	ns	-0.39	**
X_6	0.11	ns	0.25	**	-0.11	ns	-0.51	**	-0.14	ns	-0.31	**
X_7	-0.02	ns	0.22	**	-0.10	ns	-0.30	ns	0.31	**	0.12	ns
X_8	0.03	**	0.17	ns	-0.15	*	-0.35	ns	0.17	ns	-0.16	ns
X_9	-0.36	*	0.15	ns	-0.24	**	-0.24	ns	0.36	**	0.13	ns
X_{10}	-0.32	**	0.22	**	-0.20	**	-0.08	ns	-0.01	ns	-0.15	ns
X_{11}	-0.39	**	0.36	**	-0.26	**	0.60	**	0.33	**	0.17	ns
X_{12}	-0.14	ns	0.22	**	-0.14	ns	0.62	**	0.37	**	0.19	ns
X_{13}	-0.46	**	0.28	**	-0.29	**	0.56	**	0.42	**	0.32	**

Note: Correlation coefficients in each location were obtained using 720 ergot data points per year. **= Statistically significant at $P< 0.01$; *= Statistically significant at $P< 0.05$; ns=not significant.

Table A4. Pearson's Correlation coefficients between ergot severity and minimum temperature observed at Rio Bravo.

Stage	Hybrids						A-lines					
	2002		2003		Combined		2002		2003		Combined	
X_1	-0.66	**	-0.03	*	-0.54	**	-0.46	**	0.12	ns	-0.29	**
X_2	-0.62	**	0.04	**	-0.48	**	-0.49	**	0.07	ns	-0.34	**
X_3	-0.67	**	0.03	**	-0.48	**	-0.31	**	0.16	ns	-0.18	**
X_4	-0.63	**	-0.15	**	-0.53	**	-0.43	**	-0.05	ns	-0.33	**
X_5	-0.68	**	-0.18	**	-0.58	**	-0.57	**	-0.23	*	-0.47	**
X_6	-0.72	**	-0.23	**	-0.63	**	-0.58	**	-0.20	*	-0.46	**
X_7	-0.76	**	-0.40	**	-0.69	**	-0.62	**	-0.38	**	-0.54	**
X_8	-0.78	**	-0.45	**	-0.69	**	-0.68	**	-0.38	**	-0.57	**
X_9	-0.66	**	-0.39	**	-0.59	**	-0.64	**	-0.40	**	-0.56	**
X_{10}	-0.75	**	-0.50	**	-0.68	**	-0.63	**	-0.50	**	-0.59	**
X_{11}	-0.72	**	-0.58	**	-0.65	**	-0.66	**	-0.51	**	-0.61	**
X_{12}	-0.71	**	-0.51	**	-0.64	**	-0.67	**	-0.43	**	-0.59	**
X_{13}	-0.78	**	-0.56	**	-0.68	**	-0.68	**	-0.37	**	-0.57	**

Note: Correlation coefficients in each location were obtained using 1440 ergot data points per year.
**= Statistically significant at P< 0.01; *= Statistically significant at P< 0.05; ns=not significant.

Table A5. Pearson's Correlation coefficients between ergot severity and minimum temperature observed at College Station, Texas.

Stage	Hybrids						A-lines					
	2002		2003		Combined		2002		2003		Combined	
X_1	-0.10	ns	-0.27	**	-0.08	ns	-0.27	**	-0.31	*	-0.29	**
X_2	0.00	ns	-0.12	ns	-0.02	ns	-0.12	ns	-0.21	ns	-0.17	ns
X_3	-0.03	ns	-0.16	ns	-0.05	ns	-0.08	ns	-0.18	ns	-0.12	ns
X_4	-0.03	ns	-0.24	**	-0.07	ns	0.07	ns	-0.26	ns	-0.08	ns
X_5	-0.24	**	-0.34	**	-0.22	**	0.03	ns	-0.36	**	-0.14	ns
X_6	-0.41	**	-0.39	**	-0.33	**	-0.04	ns	-0.28	*	-0.16	ns
X_7	-0.42	**	-0.39	**	-0.32	**	0.07	ns	-0.39	**	-0.11	ns
X_8	-0.26	**	-0.32	**	-0.21	**	-0.01	ns	-0.30	**	-0.16	ns
X_9	-0.26	**	-0.40	**	-0.18	**	0.17	ns	-0.35	**	-0.07	ns
X_{10}	-0.49	**	-0.37	**	-0.28	**	0.14	ns	-0.37	**	-0.09	ns
X_{11}	-0.59	**	-0.18	*	-0.40	**	0.23	**	-0.39	**	-0.02	ns
X_{12}	-0.68	**	-0.25	**	-0.47	**	0.09	ns	-0.38	**	-0.10	ns
X_{13}	-0.66	**	-0.31	**	-0.42	**	0.14	ns	-0.50	**	-0.09	ns

Note: Correlation coefficients in each location were obtained using 720 ergot data points per year.
**= Statistically significant at P< 0.01; *= Statistically significant at P< 0.05; ns=not significant.

Table A6. Pearson's correlation coefficients between ergot severity and minimum relative humidity observed at Rio Bravo.

Stage	Hybrids						A-lines					
	2002		2003		Combined		2002		2003		Combined	
X_1	0.52	**	0.19	*	0.34	**	0.24	*	-0.11	ns	0.04	ns
X_2	0.29	**	0.29	**	0.23	**	0.28	**	0.04	ns	0.13	ns
X_3	0.27	**	0.28	**	0.22	**	0.38	**	0.07	ns	0.22	**
X_4	0.10	ns	0.41	**	0.11	*	0.02	ns	0.05	ns	0.01	ns
X_5	0.20	**	0.31	**	0.18	**	-0.06	ns	0.22	*	0.04	ns
X_6	0.30	**	-0.01	ns	0.14	**	-0.06	ns	0.37	**	0.08	ns
X_7	0.63	**	-0.06	ns	0.39	**	0.18	ns	0.28	**	0.22	**
X_8	0.45	**	-0.03	ns	0.26	**	0.23	**	0.38	**	0.26	**
X_9	0.18	**	0.07	ns	0.10	*	0.39	**	0.37	**	0.36	**
X_{10}	0.03	ns	0.14	*	0.03	ns	0.39	**	0.40	**	0.38	**
X_{11}	0.11	ns	0.56	**	0.22	**	0.55	**	0.54	**	0.55	**
X_{12}	0.13	*	0.09	ns	0.11	*	0.51	**	0.42	**	0.49	**
X_{13}	0.27	**	-0.06	ns	0.17	**	0.39	**	0.71	**	0.52	**

Note: Correlation coefficients in each location were obtained using 1440 ergot data points per year.
**= Statistically significant at P< 0.01; *= Statistically significant at P< 0.05; ns=not significant.

Table A7. Pearson's correlation coefficients between ergot severity and minimum relative humidity observed at College Station.

Stage	Hybrids						A-lines					
	2002		2003		Combined		2002		2003		Combined	
X_1	-0.01	ns	-0.12	ns	-0.06	ns	-0.24	*	-0.17	ns	-0.25	**
X_2	0.05	ns	0.17	ns	-0.01	ns	-0.23	ns	-0.22	ns	-0.19	*
X_3	0.05	ns	0.27	**	0.02	ns	-0.33	**	-0.44	**	-0.33	**
X_4	0.04	ns	-0.03	ns	-0.04	ns	-0.31	**	-0.10	ns	-0.22	*
X_5	-0.13	ns	0.01	ns	-0.12	*	-0.31	**	-0.11	ns	-0.25	**
X_6	-0.19	*	0.07	ns	-0.15	**	-0.37	**	-0.04	ns	-0.28	**
X_7	-0.29	**	0.01	ns	-0.22	**	-0.30	**	0.01	ns	-0.23	**
X_8	0.09	**	0.05	ns	0.04	ns	-0.55	**	-0.01	ns	-0.39	**
X_9	0.35	**	0.20	ns	0.22	**	-0.27	*	-0.01	ns	-0.18	*
X_{10}	0.34	**	0.14	*	0.25	**	-0.15	ns	-0.21	ns	-0.18	*
X_{11}	0.44	**	0.16	ns	0.29	**	0.09	ns	0.05	ns	0.04	ns
X_{12}	0.45	**	0.23	**	0.32	**	0.05	ns	0.24	ns	0.11	ns
X_{13}	0.45	**	0.15	ns	0.31	**	-0.01	ns	0.13	ns	0.01	ns

Note: Correlation coefficients in each location were obtained using 1440 ergot data points per year.
**= Statistically significant at P< 0.01; *= Statistically significant at P< 0.05; ns=not significant.

Table A8. Pearson's correlation coefficients between ergot severity and minimum relative humidity observed at Celaya.

Stage	Hybrids						A-lines					
	2002		2003		Combined		2002		2003		Combined	
X_1	-0.32	*	-0.39	**	-0.18	*	0.74	**	-0.10	ns	0.09	ns
X_2	-0.21	ns	-0.33	**	-0.18	*	0.46	*	-0.41	**	-0.16	ns
X_3	-0.39	**	-0.41	**	-0.28	**	0.62	**	-0.41	**	-0.08	ns
X_4	-0.07	ns	-0.37	**	-0.13	ns	0.26	ns	-0.42	**	-0.21	ns
X_5	-0.02	ns	-0.30	**	-0.11	ns	0.39	ns	-0.13	ns	0.02	ns
X_6	0.29	*	-0.40	**	-0.13	ns	0.01	ns	0.17	ns	0.05	ns
X_7	0.44	**	-0.26	**	-0.04	ns	0.36	ns	-0.11	ns	-0.13	ns
X_8	0.55	**	-0.29	**	0.13	ns	0.49	*	0.01	ns	-0.07	ns
X_9	0.56	**	-0.17	ns	0.08	ns	-0.20	ns	-0.26	*	-0.26	*
X_{10}	0.46	**	-0.09	ns	0.06	ns	-0.55	**	-0.16	ns	-0.19	ns
X_{11}	-0.10	ns	-0.35	**	-0.15	*	-0.85	**	-0.18	ns	-0.30	**
X_{12}	-0.17	ns	-0.24	**	-0.11	ns	-0.81	**	-0.09	ns	-0.16	ns
X_{13}	-0.42	**	-0.18	**	-0.17	*	-0.65	**	0.31	**	-0.01	ns

Note: Correlation coefficients in each location were obtained using 1440 ergot data points per year.
**= Statistically significant at P< 0.01; *= Statistically significant at P< 0.05; ns=not significant.

Table A9. Average germination means of *C. africana* conidia located on the sphacelium surface.

Month	Storage Temperature (°C)	Germination %	*	Sphacelium age	Germination %	*
1	0	40.50	a	0	47.88	a
	7	31.75	ab	7	30.75	b
	14	31.39	ab	14	28.35	bc
	21	23.35	b	21	20.02	c
2	0	32.19	a	0	37.84	a
	7	28.94	a	7	22.94	b
	14	26.16	a	14	20.75	bc
	21	7.16	b	21	12.91	c
3	0	31.96	a	0	22.50	a
	7	23.29	b	7	17.89	ab
	14	10.79	c	14	16.81	ab
	21	2.98	d	21	11.81	b
4	0	20.06	a	0	7.63	c
	7	19.75	a	7	17.75	a
	14	12.06	b	14	15.44	a
	21	0.13	c	21	11.19	b
5	0	18.94	a	0	6.06	b
	7	18.06	a	7	12.44	a
	14	9.19	b	14	14.31	a
	21	0.25	c	21	13.63	a
6	0	16.53	a	0	5.13	b
	7	9.31	b	7	11.22	a
	14	2.56	c	14	7.19	ab
	21	0	c	21	4.87	b

*= Treatments with the same letter in each month are statistically similar according to Tukey, $P < 0.01$

Table A10. Average germination means of *C. africana* conidia located within the sphacelium.

Month	Storage Temperature (°C)	Germination %	*	Sphacelium age	Germination %	*
1	0	24.52	a	0	36.19	a
	7	28.31	a	7	25.44	b
	14	30.06	a	14	21.58	b
	21	13.21	b	21	12.89	c
2	0	25.19	a	0	20.06	a
	7	18.53	a	7	23.63	a
	14	18.94	a	14	19.56	a
	21	6.09	b	21	5.50	b
3	0	17.02	a	0	13.33	a
	7	17.02	a	7	12.75	a
	14	10.92	b	14	12.67	a
	21	1.52	c	21	7.73	b
4	0	13.06	a	0	11.56	c
	7	26.00	b	7	17.88	a
	14	17.06	b	14	16.19	ab
	21	2.19	c	21	12.69	bc
5	0	15.19	a	0	11.25	a
	7	12.75	b	7	8.75	b
	14	3.00	c	14	5.56	c
	21	0.50	d	21	5.88	c
6	0	11.03	a	0	5.96	a
	7	12.59	a	7	6.53	a
	14	2.03	b	14	6.63	a
	21	0	b	21	6.53	a

*= Treatments with the same letter in each month are statistically similar according to Tukey, $P < 0.01$

VITA

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