EVALUATION AND HERITABILITY OF ERGOT RESISTANCE DERIVED

FROM SORGHUM GERMPLASM IS8525

A Thesis

by

RAFAEL MATEO MONCADA

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

MASTER OF SCIENCE

December 2003

Major Subject: Plant Breeding

EVALUATION AND HERITABILITY OF ERGOT RESISTANCE DERIVED

FROM SORGHUM GERMPLASM IS8525

A Thesis

by

RAFAEL MATEO MONCADA

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

MASTER OF SCIENCE

Approved as to style and content by:

_____   _____
William L. Rooney   Gary Odvody
(Chair of Committee)   (Member)

_____   _____
Byron Burson   Mark Hussey
(Member)   (Head of Department)

December 2003

Major Subject: Plant Breeding

ABSTRACT

Evaluation and Heritability of Ergot Resistance Derived from Sorghum Germplasm

IS8525. (December 2003)

Rafael Arturo Mateo Moncada, B.S., Escuela Agricola Panamericana, Honduras

Chair of Advisory Committee: Dr. William Rooney

Sorghum (*Sorghum bicolor* [L.] Moench) is fifth among the major cereal crops in the world in terms of production area and total production. Grain sorghum can be successfully produced in a wide range of environments, its productivity is severely limited by pathogens, insects and abiotic stresses. One of these pathogens is *Claviceps africana* Frederickson Mantle & de Milliano, commonly known as ergot. As is the case with many sorghum diseases, the best long term approach to control ergot may be the use of genetic resistance. There is limited information about resistance to *C. africana* in sorghum, and the reported resistance in most lines is fertility-based. Dahlberg (1999) first reported the line IS8525 to have the most tolerance to ergot of any of the accessions screened in Puerto Rico. The specific objectives of this research are: (1) to confirm the presence of *C. africana* resistance in IS8525 germplasm, (2) to determine if the resistance in IS8525 is pollen mediated or ovule based, and (3) to determine if the resistance in IS8525 is heritable and stable across environments. Ergot vulnerability ratings were determined for two recombinant inbred line populations, IS8525D and IS8525J, in four locations during 2001. Also, ergot vulnerability ratings were evaluated in four test-cross populations (using as testers A3Tx623 and A3Tx623) in two locations.

Evaluations of the original parents indicate that ergot tolerance in IS8525D parent was consistently better than that in IS8525J parent. As expected, neither parent provided complete resistance. The IS8525J recombinant inbred line population showed significantly more ergot susceptibility than the IS8525D recombinant inbred line population and this trend was consistent across environments. Variation for ergot vulnerability among recombinant inbred lines for both populations was detected, but the amount of variability was environment dependent. In the testcross hybrids, all four populations were susceptible to ergot, primarily due to male sterility in the hybrids, confirming that the tolerance shown in IS8525 germplasm is mostly pollen mediated. However, a greater level of tolerance in the IS8525 hybrid checks confirmed the reports of tolerance by Dahlberg et al. (1998) and Reed et al. (2002).

DEDICATION

This thesis is dedicated to our Lord Jesus, for reminding me that I should be grateful for all the things I have accomplished in life.

To my mother Juanita, I am just the result of every sacrifice you have made for me. Thank you so much for all your love and patience!!

To my sister Marcela and my dad Rafael.

ACKNOWLEDGMENTS

I would like to thank Dr. William Rooney for his support, time, and patience throughout these past years. Thanks Dr. Rooney for giving me the opportunity to become an Aggie, for sharing your knowledge in plant breeding and for your friendship.

I am very grateful to the members of my committee, Dr. Gary Odvody, Dr. Byron Burson and Dr. Louis Prom, for all their help and advice conducting my research. Special thanks to Dr. Lloyd Rooney for all his support since the first time I came here. Without your help it would not have been possible for me to get this degree. Thank you very much!

To my friends at the sorghum breeding lab, Karen Prihoda, Cindy King, Delroy Collins, Nelson, Stephen, and the newest addition, Amanda, for your invaluable help and friendship. To the "old" sorghum breeding crew, Jorge Moran, Cleve Franks, Adalberto Sanchez, Pushpak Metha, David Wooten and Selahattin Aydin, I will never forget the summer of 2000! To the "new" sorghum crew, Gabriel Krishnamoorthy, Les Kulhman, Hector Ramirez, Ryan Bading and the newest additions, Leo and Joaquin, just a couple of more summers to go guys!

I would like to thank the technicians at the Weslaco, Corpus Christi and Isabela Experiment Stations for their help in carrying out my experiments.

Very special thanks to my former roommate and "good friend" Gabriela Perez for always being there when I needed you. Also thanks to my friends, Nolvia Moran, Odete Mendes, Aline Teixeira, Laura Silva, Belem Magaña, Monica de la Torre, Ana Leal, Pablo Echeverria y Javier Bueso. To all the persons mentioned above and every other person that helped me out in one way or another, my sincere gratitude.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Sorghum (*Sorghum bicolor* [L.] Moench) is fifth among the major cereal crops in the world in terms of production area and total production (FAO, 2001). The crop is typically grown in tropical and subtropical regions of the world that are subject to drought and heat stress. The ability of sorghum to withstand these stresses and consistently produce grain remains one of its strongest traits.

While grain sorghum can be successfully produced in a wide range of environments, its productivity is severely limited by pathogens, insects and abiotic stresses. One of these pathogens is *Claviceps africana* Frederickson Mantle & de Milliano*,* which is commonly referred to "ergot" or sugary disease, and its recent appearance poses a new "threat" to profitable sorghum production (Bandyopadhyay et al., 1998).

Claviceps sorghi was first described in India in 1915. A similar disease was observed in Africa in 1924, thought to be *C. sorghi*, and became economically important in the 1960s, concurring with the development of hybrid seed production (Bandyopadhyay et al., 1998). Until recently, it was assumed that the pathogen present in Africa was the same causal organism as the one in India, but Frederickson et al. (1991) documented that

---

This thesis follows the style of Crop Science.

sorghum ergot in Africa was caused by a distinctly different species designated as *Claviceps africana* Frederickson Mantle & de Milliano.

In 1995, the African pathogen was observed in Brazil (Reis et al., 1996). In the following year, the disease was reported in Australia (Ryley et al., 1996). In both countries, the causal pathogen was *Claviceps africana* (Reis et al., 1996; Ryley et al., 1996). By the middle of 1996, the disease had spread from Brazil to Argentina, Bolivia, Paraguay, and Uruguay and it was in Colombia, Venezuela, Honduras, and El Salvador by the end of the year. In 1997, the disease continued to move north and it was reported in the Caribbean, Northern Mexico and soon after in the U.S. By the end of 1997, *C. africana* was reported in all sorghum growing regions in the western hemisphere. (Bandyopadhyay et al., 1998).

Previous screenings of sorghum germplasm have not identified any lines with complete resistance to ergot (Moran, 2000). Therefore, when normally-fertile sorghum encounters cool wet weather prior to and during anthesis, *C. africana* can infect all types of sorghum. However, because of the nature of ergot infection, male sterile lines are susceptible to significant infection by the disease. Since hybrid sorghum seed production relies exclusively on cytoplasmic male sterile lines, the sorghum seed industry is interested in any mechanism that will reduce the susceptibility of sorghum to ergot.

Ergot can be devastating in hybrid seed production fields because male-sterile seed parents are highly susceptible to the pathogen. Infection reduces yield by preventing seed and grain development. The organism specifically infects male sterile

lines because it only infects unfertilized ovaries. Once the ovary is fertilized, the developing zygote shows complete resistance to the pathogens' attempts at infection. When the ovary is infected, fungal hyphae develop into spore fungal masses. In addition to the reduction in seed yield, losses in seed quality occur because honeydew from infected florets contaminates surrounding grains. This contamination makes harvest, cleaning and the distribution of the seed difficult or impossible.

Three different approaches are being investigated to reduce the damage caused by this organism. These strategies are: (1) agronomic management of pollen, (2) chemical control, and (3) genetic resistance to *C. africana* (Bandyopadhyay et al., 1998). Currently, the most effective method is pollen management, because as mentioned previously, fertilization makes the developing embryo impervious to infection by the pathogen. Therefore, ensuring large quantities of viable pollen during anthesis reduces infection. While certain fungicides can control ergot, effective application of the chemicals to the plant at anthesis is almost impossible or economically inefficient in seed production systems. Because of this and the relatively high cost of fungicides, producers have only attempted chemical control under extreme conditions.

As is the case with many sorghum diseases, the best long-term approach to control ergot may be the use of genetic resistance. If feasible, it would be the most economically feasible control method for commercial grain production. However, there is limited information about resistance to *C. africana* in sorghum, and the reported resistance in most lines is fertility-based.

"Resistance" to *C. africana* in self-fertile sorghum is the ability to pollinate the plant and fertilize the egg cell and polar nuclei before infection occurs. In essence, this is an escape rather than resistance and is strongly linked to a post-fertilization mechanism. Therefore, most research in sorghum has dealt with identifying plants with traits that support escape resistance (Bandyopadhyay et al., 1998). However, the search for genes either in elite or exotic germplasm must be performed in order to determine sources of physiological resistance that will help researchers to develop more effective ergot control strategies.

In initial efforts to identify resistance to ergot, the line IS8525, from Ethiopia, was reported (Dahlberg et al., 1999) to have the most tolerance of any of the accessions screened. Not only did IS8525 show reduced infection in the line per se evaluation, but it also showed reduced infection in testcross male-sterile hybrids.

The purpose objective of this research was to determine if IS8525 has potential for enhancing ergot tolerance in sorghum. The specific objectives are: (1) to confirm the presence of *C. africana* resistance in IS8525 germplasm, (2) to determine if the resistance in IS8525 is pollen mediated or ovule based, and (3) to determine if the resistance in IS8525 is heritable and stable across environments.

CHAPTER II

LITERATURE REVIEW

The Host

Origin, Distribution and Adaptation

Sorghum (*Sorghum bicolor* [L.] Moench) originated in north-eastern Africa, where the greatest variability in wild and cultivated species is found. It was probably domesticated in Ethiopia by selection from wild sorghum between 5,000 and 7,000 years ago. From this center of origin, it was distributed along trade and shipping routes throughout Africa, and through the Middle East to India at least 3,000 years ago. Sorghum was first taken to the Americas through the slave trade from West Africa. It was reintroduced in late 19th century for commercial cultivation and is now widely found in the drier areas of Africa, Asia, Australia, North, Central and South America (ICRISAT, 2003).

Because sorghum evolved in arid areas of Africa, it is adapted to regions of the world where water availability and soil fertility are marginal. It has a number of morphological and physiological characteristics that contribute to its adaptation to dry conditions, including an extensive root system, waxy bloom on the leaves that reduces water loss, and the ability to stop growth during periods of drought and resume growth when conditions become favorable. It is also tolerant to waterlogging and can be grown

in high rainfall areas. It is, however, primarily a crop of hot, semi-arid tropical environments with 400 – 600 mm annual rainfall that are too dry to grow maize (*Zea mays* L.). It is also widely grown in temperate regions and at altitudes of up to 2300 m in the tropics.

Sorghum can be successfully grown on a wide range of soil types. It is well suited to heavy vertisols commonly found in the tropics, where its tolerance to waterlogging is often required, but is equally suited to light sandy soils. It tolerates a range of soil pH from 5.0 – 8.5 and is more tolerant to salinity than maize. It is adapted to poor soils and can produce grain on soils where many other crops fail (ICRISAT, 2003).

Importance of Sorghum

Sorghum has been traditionally classified into four groups: grain sorghums, grass sorghums, sweet sorghums and broom corns (ICRISAT, 2003). Grain sorghums are grown for their grain which can be used as a feed or food grain. Sometimes, the entire grain sorghum plant is made into silage. The grain is higher in protein and lower in fat content than corn also, yellow endosperm sorghums contain carotene while white corn does not. Forage sorghums are grown for green feed and hay but can also be weeds for instance, Sudan grass (*Sorghum vulgare* var. *sudanense* Hitchc.) an annual grown for feed and hay. Sweet sorghums have sweet juicy stems and are used to make sorghum syrup and ethanol. Sweet sorghums can also be used in animal feed or silage. Broom

corn is grown for the branches of the seed cluster, which are used to make brooms (http://www.cyberspaceag.com/sorghum history.html).

In the Western Hemisphere, sorghum is used for livestock feed, either as feed grain or as raw material for compound feeds, while it has been used for food for human consumption in South Asia, Africa and Central America. Sorghum grain is used as an ingredient in malts (Nigeria), ready to cook breakfast food (South Africa), flour and beers (Southern Africa), weaning foods (Botswana), tortillas (Central America), and noodles (Southeast Asia) (Rooney et al., 1980; Murty and Kumar, 1995). Leaves and stalk can be used as materials for building houses and fences, and fibers are utilized by the broom industry (Moran, 2000).

Sorghum Production

Sorghum is grown for grain production in a wide range of environments. Under optimal field conditions, grain yield can be as high as 15 MT ha^{-1}, and a good yield is usually between 7 and 9 MT ha^{-1} when rainfall is not a limiting factor. Under average conditions, sorghum yield can vary between 3 and 4 MT ha^{-1}, and decrease to 0.3 to 1 MT ha^{-1} under drought conditions (House, 1982).

Under optimal conditions, sorghum has a high yield potential comparable to other cereals such as rice (*Oryza sativa*), maize (*Zea mays L.*), or wheat (*Triticum aestivum*). However, sorghum is usually grown in environments where both biotic and abiotic stresses are common. Sorghum is attacked at all stages of development by a wide

variety of pests that compromise yield, ranging from fungi that infect the seed in the soil, to birds that feed on the grain (Moran, 2000).

In 2002, the total annual sorghum production was 54.5 million MT from approximately 42.5 million ha, making sorghum the fifth most important cereal in the whole world. Sorghum represented 5 % of the total harvested area and 2.7 % of the total cereal production around the world. The most important producers were the United States with an annual production of 9.3 million MT of grain from 2.9 million ha; India with 7 million MT from 9.5 million ha; Nigeria with 7.7 million MT from 7.0 million ha; Mexico with 5.8 million MT from 1.7 million ha; Sudan with 2.8 million MT from 4.8 million ha; and China with 2.7 million MT from 0.7 million ha (FAO 2001). In recent years, cereal grain production in general and sorghum production in particular have been decreasing. The total area of sorghum harvested decreased to 3.7% while all cereals experienced a reduction of 2.21%. Total production of cereals decreased by 3.67, while sorghum decreased to 8.37 % from the year 2001 (FAO 2002).

The Pathogen

Ergot (*Claviceps africana* Frederickson Mantle & de Milliano)

Sorghum ergot, commonly known as "sugary disease", is caused by the pathogen *Claviceps africana* Frederickson Mantle & de Milliano. Ergot results from the colonization of individual ovaries, replacing the infected ovary with fungal tissue, preventing seed development in the affected florets (Fredericksen and Odvody, 2000).

After the infection occurs, the fungal tissue produces a sugary liquid, known as "honeydew", which drips from the infected floret onto the plant tissue and the ground below. Honeydew causes significant problems in harvesting, reducing seed and grain quality, which are then colonized by fungal saprophytes (Bandyopadhyay et al., 1998). Infected seed have lower germination and seedling emergence and may be predisposed to other diseases (McLaren, 1992).

Because fertilization of ovaries prevents their colonization, the lack of pollen in the male-sterile A-lines used as females in F_1 hybrid production makes these lines particularly susceptible (Fredericksen and Odvody, 2000). However, when weather conditions are favorable for infection or when self pollination is delayed or inhibited, all types of sorghum germplasm, fertile or male sterile, inbred lines or hybrids, are susceptible to *C. africana* (Moran, 2000).

Ergot Distribution

Sorghum ergot, caused by *Claviceps sorghi*, was first observed in India in 1915 (McRae, 1917). Later the disease was recognized in Kenya in 1924. It is now widely distributed in eastern, western, and South Africa (de Milliano et., al. 1991). The pathogen became an economic problem in South Africa in the 1960's, when hybrid seed production began. Ergot in Africa was believed to be caused by *C.sorghi,* until 1991 when Frederickson, Mantle and de Milliano identified *C. africana* as the causal pathogen. The African organism differs from *C. sorghi* in stromatal color, superficial stromatal texture, differences in ascus and ascospore dimensions; and more importantly,

the dynamics of early ovary parasitism. The sphacelial mass formed by the African organism forces the glumes apart before the honeydew exudes, while *C. sorghi* exudes honeydew first from a less profusely and more slowly, colonized ovary (Frederickson et al., 1991).

In 1988, sorghum ergot was identified in Thailand (Boon-Long, 1992). Posterior analysis determined that the pathogen produced disease symptoms similar to those of *C. africana* (Frederickson et al., 1991). One of the two *Claviceps* species reported in Japan was also identified as *C. africana* (Bandyopadhyay et al., 1998).

In 1995, sorghum ergot was first observed in the Western Hemisphere, specifically in Brazil (Reis et al., 1996). During the following year, the disease was reported in Australia (Ryley et al., 1996). In both countries, the causal pathogen was *C. africana* (Reis et al., 1996; Ryley et al., 1996). By mid 1996, the disease spread from Brazil to Argentina, Bolivia, Paraguay, and Uruguay and by the end of the year, it had spread to Colombia, Venezuela, Honduras, and El Salvador. In early 1997, the disease was reported in the Caribbean, specifically in Puerto Rico, Haiti, Dominican Republic, and Jamaica. At the same time, *C. africana* was also reported in northern Mexico, and a month later it was observed in South Texas, U.S.A. By October 1997, the disease had spread throughout Texas, and was also recorded in Georgia, Kansas, Nebraska and Mississippi (Bandyopadhyay et al., 1998). The potential of airborne secondary conidia in spreading the disease was demonstrated by Frederickson et al. (1993). This is a key factor in the possibility of an ergot epidemic in sorghum. Sorghum ergot is a disease of

increasing importance to world sorghum production because occurs in Africa, Asia, Australia, North, Central and South America.

Symptoms

Ergot only attacks unfertilized ovaries. A few or all ovaries within florets on a panicle are individually infected, specifically in male sterile lines or hybrids with fertility restoration problems. There are two obvious symptoms of infection in the field. The first and most obvious is the production of sugary fluid (honeydew) from infected florets. The second is the presence of fungal sphacelia or sclerotia between the lemma and palea of infected florets (Bandyopadhyay et al., 1998; Moran, 1998). Honeydew is thin or viscous, sweet and very sticky. With time, honeydew can become uniformly yellow-brown in color. It may remain as intact droplets, or drip onto uninfected florets, seeds, leaves, and the ground. Honeydew contains the infectious conidia (primarily macroconidia) and it is the germination of conidia on the droplet surface that produces secondary conidia which has a white coloration. The fungal sphacelia or sclerotia of *C. africana* are not very noticeable prior to production of honeydew. However, the sphacelium, may or may not develop in place of seed even before honeydew is produced (Fredericksen and Odvody, 2000). At maturity, it is difficult to differentiate sphacelia /sclerotium from healthy seed coated with honeydew and saprophytic fungi, and it is even more difficult when the fungus *Cerebella* is present. *Cerebella* spp. is a frequent saprophyte of sorghum ergot, colonizing the sugary honeydew under humid conditions.

Causal Organisms

The genus *Claviceps* includes very specialized fungi which parasitize only the ovaries of specific grasses, no other part of the plant is infected. *Claviceps* are ascomycetes that belong to the family Clavicipitaceae. There are about 43 known species (Pazoutova and Parbery, 1999) which are capable of infecting approximately 600 monocotyledonous species (Temberge, 1999).

Ergot is a general term that applies to all species of *Claviceps*. The pathogens that cause sorghum ergot are different from the pathogens that cause ergot in other cereals. Sorghum ergot is caused by three *Claviceps* species: *C. sorghi* first described and endemic to India, *C. africana* endemic to Africa, and *C. sorghicola* endemic to Japan.

The pathogen life cycles and symptoms caused by these three *Claviceps* spp. are similar but not identical. The primary differences among the three species are: the role of secondary conidia in pathogen dispersal and epidemic development, the importance of sclerotia to survival, the presence of a sexual reproductive stage in nature and the synthesis of potentially toxic alkaloids (Stack, 2000).

Claviceps africana is the teleomorph or imperfect stage of the anamorph *Sphacelia sorghi.* The african pathogen was characterized as having stromatal color with blue-purple pigments with glabrous characters. This species also is unique in the production of an ergot alkaloid known as dihydroergosine, which is a dihydrogenated cyclic tripeptide (Frederickson et al., 1991).

Claviceps africana is the only sorghum ergot pathogen that has been found in the U. S. In order to determine if the pathogen in America and Australia came from the same region, Pazoutova et al., (2000) evaluated the relatedness of ergot strains from the U. S., Bolivia, Australia, Africa and India. The RAPD banding pattern grouped the isolates from the U.S. and Bolivia in one group, and the isolates from Australia and India in another group. Thus, it is believed that isolates from North and South America came from the same clone; whereas, isolates from Australia came from a different clone, related to the Indian isolates.

Genetic variation exists between U. S. populations of *C. africana* and populations from others countries. Variation among U. S. populations is being investigated. The potential for genetic variation leading to altered virulence or host range is uncertain.

Life Cycle

Ergot infection is organ specific. *Claviceps* spp. have a similar life cycle but the timing of infection events differs. The ergot pathogen infects unfertilized flowers in a manner that mimics pollination.

The stigma is the principal site of infection, although conidia can germinate and infect through the style and ovary wall. The conidia land on the stigma and within 16 to 24 hours they germinate. The hyphae grow down the style, onto the inner ovary walls, and the vascular bundles within the rachilla (Bandyopadhyay et al., 1998, Stack, 2000). Once the rachilla has been colonized, the hyphae establish a specific and persisting host-

parasite frontier (Tenberge, 1999). The pathogen starts to grow in the ovary and the outer ovary wall tissues and ovule until converts the ovary into a mass of fungal tissues called a sphacelium. At this time, from the sphacelia that has developed instead of seed, the most obvious ergot symptom (honeydew) appears, approximately six days after infection. (Bandyopadhyay et al., 1998, Stack, 2000). The sphacelium of *C. africana* produces at least two spore types; macro and micro-conidia. Both are located below the honeydew surface, and have the capability to germinate as well. The honeydew contains a very high population of macroconidia. As the honeydew flows down the panicle, the macroconidia can directly infect unfertilized florets. Because of the high sugar concentration, the honeydew attracts insects, and they served as vectors of the pathogen by carrying honeydew containing macroconidia to flowers of other sorghum plants. The macroconidia also germinate by producing secondary conidia. If the relative humidity is high and temperature decreases, secondary conidia are produced on the honeydew surface. A white film on the surface of the honeydew indicates the production of secondary conidia from macroconidia (Bandyopadhyay et al., 1998, Stack, 2000).

The secondary conidia production and release follow a diurnal pattern, with peak conidia release at sunset. Secondary conidia can be dispersed by wind. It is believed that conidia are capable of long range dispersal (region to region) via wind and/or insects. The production and dispersal of secondary conidia are considered to be the primary reasons for the rapid spread of sorghum ergot in Australia within one season and throughout South, Central and North America within two years. If deposited on a receptive stigma of an unfertilized sorghum floret, the secondary conidia are also

capable of repeated germination. If the macroconidia is deposited on the surface other than an unfertilized floret, it can germinate by producing another conidia for aerial dispersal. These secondary conidia are capable of undergoing at least three germination cycles (Bandyopadhyay et al., 1998).

Once the formation of the sclerotia begins, the production of honeydew and conidia decreases and eventually ceases. In *C. africana* the sclerotia are not separate structures from the sphacelia but are so closely associated that is better to regard them only as different tissues. Sclerotial tissues from inside, and to the base of, sphacelial tissues under dry conditions, 20-40 days after infection. What is commonly known as sclerotium of *C. africana* is rounded in shape and 4 to 6 x 2 to 3 mm in size, which is similar in size to a sorghum seed (Frederickson, 1991, Montes et al., 2000). Sclerotia in *Claviceps* species serve for sexual reproduction and as resting structure to survive during unfavorable conditions (Tenberge, 1999) However, the role of sclerotia of *C. africana* in survival is not well established because germinated sclerotia have not been observed in nature (Frederickson, 1999). For these reasons, sorghum pathologists assume that the primary inoculum for a new season probably comes from secondary conidia at another stage in the disease cycle of the pathogen (Frederickson, 1999). However, *C. africana* can survive in the conidial state or feral sorghum and alternate hosts, such as *Sorghum halapense* (L.) Pers., volunteer sorghum as well as surviving ratooned sorghum which can flower throughout the year in some parts of the U. S. (Bandyopadhyay et al., 1998).

Epidemiology

Cool to moderate temperatures (14-28 ºC) with wet, cloudy conditions during floret opening and from the onset of anthesis to fertilization favors the rapid disease development and spread (Bandyopadhyay et al., 1998, Stack, 2000). Warmer temperatures (> 28 ºC) restrict ergot severity. Near 100% relative humidity for 24 hours during anthesis is optimal for infection (Futrell and Webster, 1966). When humidity is high, wetness or rainfall is not essential for ergot development. Cloudiness during anthesis aids disease development, probably because of delayed anther dehiscence and pollen deposition and activity (Quinby, 1958). In 1992, McLaren and Wehner (1992) determined that male-fertile sorghum germplasm was as susceptible as male-sterile germplasm if they were exposed to night temperatures less than 12 ºC during microsporgenesis. The increased susceptibility is due to the lack of viable pollen because of cooler temperatures. Therefore, the probability of ergot infection increases when sorghum is exposed to low temperatures during microsporogenesis which reduces pollen viability (Moran 2000).

Once established, the development and spread of the disease is influenced by temperature and humidity as the disease progresses. Conidial production and pathogen spread occur at temperatures ranging from 14 ºC to 28 ºC, and high relative humidity at some time during the day. Under these conditions conidial spread is favored, but differentiation of sphacelia into sclerotia does not occur. However, higher temperatures of 25 ºC to 28 ºC and lower relative humidity (< 90%) induce the differentiation of sphacelia into sclerotia. Under these conditions, the pathogen is less unlikely to spread

because of reduced conidial production and less than optimal infection conditions (Bandyopadhyay et al., 1998). Another important issue is the combined effect of weather and saprophytic fungi. The environment affects ergot development indirectly by influencing the growth of saprophytes on developing sclerotia. Sclerotia develop poorly in the rainy season because the growth of *Cerebella* sp. and other mold fungi suppress their development; whereas, dry weather after infection allows the formation of less-contaminated, mature sclerotia (Futrell 1966).

Conidia and pollen germinate and grow to the ovary through stylar tissue. Both compete to reach the ovary. In this competition, weather conditions prior to and during anthesis will influence the severity of the disease. Under normal sorghum normal growing conditions, with average day and night temperatures of 28 ºC and 17 ºC, respectively, the pollen tube germinates within 30 minutes of landing on the stigma, and fertilization of the ovule occurs within 2 to 12 hours after pollination (Stephens and Quinby, 1934). At the same temperature, a secondary conidia of *C. africana*, requires 8 to 12 hours to germinate, and 36 to 48 hours to grow onto the ovary (McLaren 1999). Therefore, under normal weather conditions, the pollen tube has a significant competitive advantage of at least 24 hours over the infecting tube of *C. africana*. If temperatures are lower, the rate of pollen production, germination and growth usually decreases, while the ergot infection rate remains the same (Bandyopadhyay et al., 1998; Moran, 2000).

The environment affects ergot development indirectly by influencing the growth of saprophytes on developing sclerotia and old sphacelia tissue. Sclerotia develop

poorly in the rainy season due to the growth of *Cerebella* sp. and other mold fungi that suppress their development; whereas, dry weather after infection allows the formation of less-contaminated, mature sclerotia.

Conidia Dispersion

Pathologists have described several mechanisms of dispersion for ergot and the genus *Claviceps* in general. However, they have concluded that the favored method of transmission is species specific.

Wind dissemination of secondary conidia is the most important mode of dispersal for local and long distance spread of *C. africana* (Frederickson et al., 1993) and may explain the rapid, long-distance disease spread in Australia, South, Central and North America. Concentration of secondary conidia in the air shows a diurnal pattern, with greatest occurrence at nightfall, coincident with the sharp rise in relative humidity and fall in temperature. Secondary conidia are also produced on honeydew that drips and falls onto wet soil. Such soilborne secondary conidia can infect plants in the field and may also act as primary inoculum for disease initiation in the field (Bandyopadhyay et al., 1998).

Another mechanism of dispersion is insect transmission. Insects such as thrips, beetles, midge flies and head bugs can carry conidia attached to their bodies. Langdon and Champs (1954) documented insect transmission in the spread of *Claviceps purpurea* conidia. However, insects may not play a significant role in spreading *C. africana*

(Bandyopadhyay et al., 1998). Water splash transmission is also possible, but it has been reported in field trials only on *C. sorghi* (Bandyopadhyay et al., 1998).

Other mechanisms of transmission by head to head contact. During combine harvesting, machinery maybe contaminated and serve as mechanism of dispersion to surrounding fields of sorghum. Also during harvesting, sphacelia, sclerotia and honeydew coated seed may be picked up with grain, contaminating the seed lot. Any of these sources may provide the primary inoculum directly or through an alternate sorghum host, to start the infection process in the next cycle (Moran, 2000).

Disease Spread

Pathogen dissemination remains poorly understood; however, efforts have been made to understand how the disease is spread to essentially all sorghum growing regions of the world in only two years. Bandyopadhyay (1999) mentioned several possible mechanisms. Among them are: (1) the introduction of sorghum seed contaminated with ergot sclerotia from an endemic region, (2) spread of secondary conidia through an intercontinental air current, (3) ergot could have been present in these areas prior the appearance of the epidemics, but favorable weather conditions for pathogen development were present, (4) mechanical contamination, researchers apparel was contaminated with honeydew in Africa, (5) the pathogen may have moved in cargo folds from ergot endemic regions to Brazil or Australia, and (6) the pathogen that affects other grasses might have gone through a mutation and turn into a virulent strain that infects sorghum.

Economic Impact

Private and public sorghum breeding programs rely on male-sterile lines to produce F_1 hybrids. The production of F_1 hybrid seed is limited by factors that affect the efficiency of the cytoplasmic male sterility system such as low pollen production, low pollen viability, and the poor synchronization between R-lines and A-lines. These factors individually or in combination may make male-sterile lines extremely susceptible to ergot. However, when weather conditions are favorable for the *C. africana* infection or when pollination is delayed or inhibited, all sorghum germplasm, fertile or male sterile, inbred lines or hybrids, are susceptible (Moran, 2000).

Claviceps africana infects unfertilized sorghum flowers preventing seed development. Consequently, ergot directly reduces yield by preventing seed production. The impact of sorghum seed yield is directly correlated with disease incidence and severity in the field. In India, losses of 10 to 80% have been reported in hybrid seed production fields. Similarly in Zimbabwe, annual losses of 12 to 25 % and occasionally total losses have been reported (Bandyopadhyay et al., 1998). *Claviceps africana* can also cause an indirect reduction in yield and quality. Ergot-infected panicles frequently contain seeds covered with honeydew. The honeydew is extremely viscous and can interfere with harvesting operations by gumming-up the combine, lengthening the harvest process. Also, the honeydew makes the seed clump together and difficult to handle.

Sorghum ergot can also severely affect grain quality. Alkaloid production by *Claviceps* species is well known as quality problem in some grains such as wheat and

rye (*Secale cereale* subsp. *Cereale*). Researchers have indicated that in the U. S. the sorghum ergot pathogen, *C. africana*, does not produce significant amounts of toxic alkaloids in the honeydew. However, researchers in Australia have indicated toxicity (feed refusal and pulmonary dysfunction) to swine and poultry fed sorghum with very high levels of ergot (Bandyopadhyay et al., 1998, Stack, 2000). Sorghum ergot can also increase the incidence and severity of grain molds such as *Cerebella* spp., *Curvularia* spp., *Fusarium* spp., *Alternaria* spp., and *Cladosporium* spp. All these pathogens can affect sorghum grain quality by direct discoloration of the grain or by the production of toxic compounds. Also, the honeydew produced exuded to the panicles contains not only the macro and micro conidia of *C. africana* but also high concentrations of sugars. This sugar serves as readily utilizable energy source for many species of bacteria as *Bacillus* spp. and *Pseudomonas* spp. It is possible that the honeydew coating on grain and seed surfaces can provide a protective habitat for not only *C. africana* macroconidia but also other sorghum pathogens and grain mold species (Stack 2000).

Control Mechanisms

Different components of the sorghum industry such as hybrid seed production, grain production, and forage use different strategies to manage sorghum ergot. Successful management will require cooperation among production areas and distribution channels for both seed and grain (Stack, 2000). Control strategies are limited due to recent arrival of the disease. However, three different approaches are being studied to reduce the damage caused by ergot in hybrid seed production. These

strategies are: agronomic pollen management, chemical control and genetic resistance (Moran, 2000).

Pollen Management

Pollen management is the easiest, fastest and most effective method to manage this disease. Ensuring that large quantities of viable pollen are available during flowering prevents the pathogen from infecting the ovule. This also requires coordination of male and female flowering between A and R lines (Moran, 2000).

Producers have increased the ratio of R to A-line rows to reduce the distance that pollen must travel to fertilize the ovary. The closer the rows are to one another the less chance that any ovaries will remain unfertilized. However, this strategy is negatively affected by cool wet weather at flowering because pollen movement is greatly reduced. Therefore, cultural management will not solve all ergot infection problems (Bandyopadhyay et al., 1998).

Chemical Control

Beside pollen management, chemical fungicides are the only other means of controlling ergot. There are a number of triazoles fungicides that will reduce infection rates. However, chemical treatments are more effective as preventive measures when the disease pressure is low and the frequency of rainfall also is low (Bandyopadhyay et al., 1998). Chemicals also are expensive, difficult to apply to the target areas, and once the disease has developed in the field, chemical control is not effective.

Genetic Resistance

Because of the relatively short time that this organism has been a global problem, little research has addressed resistance to *C. africana*. So far, there is no consistent evidence that physiological resistance to *Claviceps* occurs in any crop species (Willingale et al, 1986).

Because of the limitations of both pollen management and chemical control, the best long-term solution to ergot may be the development of germplasm with genetic resistance. However, genetic resistance and/or tolerance must be identified and the heritability of the trait must be established.

Previous Research on Genetic Resistance to Ergot

There is relatively little research underway that addresses ergot resistance in sorghum (Global Sorghum ergot conference, 1997). Moran et al. (2000) evaluated twelve pairs of sorghum A and B-lines, twelve R lines and twelve hybrids. These were selected because they represented a wide range of maturity types and different pedigrees. They also were commercially important parental lines. Moran concluded that ergot occurred in all genotypes at four locations. In Rwanda, six resistant lines were identified (Mukuru, 1999). Musabyimana et al., (1995) identified 12 ergot-resistant lines, with disease severity below 10%. Dahlberg et al., (1999) evaluated 100 accessions from the USDA germplasm collection and found that IS8525 was the most promising line. It not

only had the lowest ergot ranking among all lines tested in Isabela, Puerto Rico, but it also showed some potential in a male sterile testcross hybrid.

Reed et al., (2002) evaluated 18 genetically diverse sorghum lines, including cultivated landraces and wild accessions, as well as in potential alternate hosts, including *S. halepense* for resistance to *C. africana.* They concluded that only *Sorghum* spp. was susceptible to ergot; however, within the sorghum germplasm pool, two wild accessions IS14131 and IS14257, were resistant to ergot. Both of these accessions were characterized in male sterile (A3 cytoplasm) genetic background to evaluate the physiological basis for their resistance. Based on the low levels of infection in male-sterile hybrids produced using IS14131 and IS14257, particularly under field conditions, they concluded that resistance in these accessions appeared to be physiological and not pollen mediated resistance.

Based on Dahlberg's (1999) and Reed's (2002) findings, IS8525, IS14131 and IS14257 are the only known sources with physiological resistance to ergot in male sterile genetic backgrounds. These results indicate that this form of resistance may be useful for controlling ergot in commercial seed production fields.

CHAPTER III

EVALUATION OF ERGOT RESISTANCE DERIVED FROM SORGHUM GERMPLASM IS8525

Introduction

The production of sorghum hybrid seed is especially susceptible to ergot (*Claviceps africana*) because of the use of male-sterile A-lines as a seed parent. The recent global spread of ergot has forced hybrid sorghum seed producers and researchers to search for a solution to this problem. To date, cultural control through pollen management has been the primary mechanism used to minimize the effects of ergot in hybrid seed production. This is accomplished by increasing the area planted with pollinator rows, but this reduces hybrid seed yield and economic returns (Moran, 2000).

However, cultural control does not eliminate the problem. As an alternative, chemical fungicides are effective in controlling ergot, but the high cost and inefficient delivery mechanisms limit their utility. If resistance in sorghum can be identified, the best long-term solution for this problem is genetic resistance. While there are several reports of genetic tolerance to sorghum ergot (Dalhberg et al., 1998), in most cases this resistance appears to be a pollen-mediated disease escape. While this mechanism is useful in fertile hybrids, it cannot be used in male sterile parents (Frederickson et al., 1994; Bandyopadhyay et al., 1998). For use in hybrid seed production, ergot resistance

must be functional in a male-sterile background, stable across environments and heritable if it is to be used in sorghum breeding programs.

Dahlberg et al. (1998) first reported non-pollen-based ergot resistance in sorghum germplasm IS8525. Intermediate resistance levels were also expressed in male-sterile hybrids of IS8525 inoculated with the pathogen. Reed (2002) evaluating hybrids in different cytoplasms with several different source of potential resistance identified IS8525 as the most tolerant to ergot of any sources evaluated.

Genotype by pathogen by environment interactions plays an important role in determining ergot severity in a genotype at a given location. Days after anthesis, temperature, and relative humidity are correlated with the increase of ergot severity (McLaren and Wehner, 1990; McLaren, 1992; McLaren and Flett, 1998). Thus, we must consider variable climatic conditions and quantify genetic resistance to ergot across environments to accurately make a conclusion about the resistance to ergot for a given genotype (Reed, 2002).

IS8525 has shown some levels of ergot resistance not related to efficient fertilization. It is now necessary to evaluate this resistance in segregating progenies (both fertile and sterile) across environments to determine if the tolerance is heritable and stable across environments. The objectives of this study were to (1) to confirm the presence of ergot resistance in IS8525 germplasm and (2) determine if the resistance in IS8525 germplasm is stable across diverse environments and (3) estimate the heritability of ergot resistance in these populations.

Materials and Methods

Population Development

Seed of IS8525 was obtained from Dr. Jeff Dahlberg, formerly the USDA-ARS sorghum curator located in Mayaguez, Puerto Rico. Upon growing this line in Texas, it was obvious that two distinct types were present. One type was characterized as juicy midrib (herein designated as IS8525J) and the other had a dry midrib (subsequently designated as IS8525D). In addition to midrib, the two lines differed in plant height and panicle shape. However, IS8525D and IS8525J are both caudatum sorghum, photoperiod insensitive, and have purple/red color plants with purple colored glumes and long to awnless lemmas. Their seed have a red pericarp, white endosperm and a pigmented testa layer.

Both types of IS8525 were crossed as the pollinator with BTx643 using plastic bag sterilization to obtain F_1 hybrid seed. Panicles of both F_1 hybrids were bagged and self-pollinated to create two F_2 populations. Each F_2 population was grown in Puerto Rico, where F_2 plants were randomly selected and pollinated to advance to the $F_{2:3}$. Plants from each $F_{2:3}$ derived lines were randomly selected and self-pollinated to advance to the F_4 generation. This process was repeated until a set of $F_{2:5}$ recombinant inbred line (RIL) was developed from each population. From the population (BTx643/IS8525J) a total of 34 $F_{2:5}$ lines were developed (designated as the IS8525J population), and from the population of (BTx643/IS8525D) a total of 49 lines were derived (designated as the IS8525D population).

Field Evaluation

Both populations were evaluated in four environments: College Station, Texas (CS01D, CS01J) and Corpus Christi, Texas (CC01D, CC01J) during the summer of 2001, and Weslaco, Texas (WE01D, WE01J) and Isabela, Puerto Rico (PR01D, PR01J). At each location, each test was arranged in a randomized complete block design with two replications. Each plot consisted of one 5 m row with intra-row spacing of 0.76 m. Sterile checks ATX623 and A3TX623, and original parents BTX643, IS8525J and IS8525D were planted as controls. Environmental conditions at the four locations varied widely. In CS, the plantings were made in late March and ergot ratings were made in July, which is typical of a normal production season. In CC, the plantings were made in early March and ergot ratings were made in June, which is typical of a normal production season. In WE, plantings were made in August and ergot ratings were made in November, which is an off-season evaluation in cooler temperatures. The PR location planting was conducted in August and evaluations were in November, when weather patterns are warm and humid.

Claviceps africana **Inoculum and Inoculation**

A conidial suspension of C. *africana* was prepared by washing fresh honeydew from infected sorghum panicles in water. The resultant suspension was filtered through two layers of cheesecloth and diluted in water to contain approximately 1×10^6 conidia per milliliter. The panicles of plants growing in the plots were tagged and inoculated with the *C. africana* suspension when the panicles were from 10 to 25% flowered. Each

plot was inoculated on two separate days. In each plot, five panicles were inoculated on the first day, and five additional panicles were inoculated on the second day. Twenty-eight days after inoculation, the ten inoculated and tagged plants were harvested and rated for ergot severity and incidence.

Disease Evaluation

Ergot incidence was measured as the percentage of tagged panicles that had at least one floret infected with ergot. Ergot severity was measured as the percentage of florets per panicle that were infected with ergot. Ergot severity was measured using a scale from zero to five, as described by Moran (2000) where 0 indicates 0% of florets infected; 1 indicates 1 to 5% of the florets infected; 2 indicates 6 to 10% of the florets infected; 3 indicates 11 to 25% of florets infected; 4 indicates 26 to 50% of florets infected; and 5 indicates that more than 50% of the florets were infected.

Data Transformation

Both ergot severity and incidence ratings are important variables to consider when characterizing the level of ergot tolerance in sorghum germplasm. However, analysis of two separate but equally important variables can lead to opposite conclusions. Combining two variables makes analysis and results easier to interpret. According to procedures described by Moran (2000), ergot severity and incidence data were used to create a single data point called ergot vulnerability.

Ergot severity and incidence data were combined into an ergot vulnerability rating using factor analysis. Factor analysis is a type of multivariate analysis used to combine two data points into a single point for analysis (Johnson and Wicherm, 1998). The purpose of the analysis is to describe the covariance relationship among the variables in terms of random quantities called factors. Factors for each observation were obtained through the principal component analysis included in the factor reduction procedure in SPSS[®.] The factor reduction procedure was run considering observations for all environments. Prior to running the factor analysis procedure, incidence ratings were divided by 20, in order to set incidence in the same scale (0-5) as severity. Because negative factors were obtained, it was necessary to add 1.4 to each of the factors to obtain values greater than zero. Negative values were obtained due to little variation on the two data factors to create the particular vulnerability single point.

Statistical Analyses

Individual environment analyses were performed for ergot vulnerability, ergot severity and ergot incidence for the two populations (IS8525D and IS8525J). Both populations were analyzed following a randomized complete block design (RCBD) assuming recombinant inbred lines as random effect components (Table 1). Mean comparisons within environments were performed using the least significance differences (LSD) procedure, with a probability level of 0.05, using the appropriate mean square depending upon the component under analysis.

To test the validity of combining data from individual environments, Bartlett's test for heterogeneity of error variances was performed (Little and Hills, 1978; Steel and Torrie, 1980). Results indicated that the error variances across environments were heterogeneous. However, transformation of data failed to normalize variances and because the data from each environment was no obvious problems with the data from each environment the data were combined and analysis was conducted without transformation. In the combined analyses, ergot vulnerability, ergot severity and ergot incidence were analyzed as dependent variables and lines and environments were considered random effects. When significant differences were detected, mean comparisons across environments were performed using the least significance differences (LSD) procedure, with a probability level of 0.05, using the appropriate mean square depending upon the component under analysis (Table 2).

Originally, the random factor day (for two different days of inoculation) was included in the ANOVA for individual environments and combined analysis. However, statistical differences between days were not detected at $P < 0.05$. Thus, this component (days of inoculation) was not included in the final analysis. All individual environment analyses as well as the combined analyses were generated using the GLM procedure included in SPSS[®.]

Table 1. Expected mean squares and degrees of freedom for the individual analysis of variance on ergot vulnerability, ergot severity, and ergot incidence for both populations.

Source	df †	Mean Squares	Expected Mean Squares‡
Replications	r-1	MS_R	$\sigma^2_e + g'\sigma^2_R$
Genotypes	g-1	MS_G	$\sigma^2_e + r'\sigma^2_G$
Error	(r-1)(g-1)	MS_e	σ^2_e
Total	rg-1		

† varied depending upon the number of missing observations at each environment.

‡ g' and r' denote means for genotypes and replications, respectively.

Table 2. Expected mean squares and degrees of freedom for the combined analysis of variance on ergot vulnerability, ergot severity, and ergot incidence for both populations.

Source	df †	Mean Squares	Expected Mean Squares‡
Environment	e-1	MS_E	$\sigma^2_e + g'\sigma^2_{R(E)} + r'\sigma^2_{GE} + r'g'\sigma^2_E$
Replications (Environment)	e(r-1)	$MS_{R(E)}$	$\sigma^2_e + g'\sigma^2_{R(E)}$
Genotypes	g-1	MS_G	$\sigma^2_e + r'\sigma^2_{GE} + r'e'\sigma^2_G$
Genotype x Environment	(e-1)(g-1)	MS_{GE}	$\sigma^2_e + r'\sigma^2_{GE}$
Error	e(g-1)(r-1)	MS_e	σ^2_e
Total	egr-1		

† varied depending upon the number of missing observations at each environment.

‡ g' and r' and e' denote means for genotypes and replications and environments, respectively.

Heritability Estimates

Broad sense heritability estimates for ergot vulnerability in the two recombinant inbred lines populations was calculated using the GLM procedure included in SPSS[®.] Heritability estimates are the ratio between the genotypic and the phenotypic variances of the population evaluated. Heritability (H^2) for ergot vulnerability for each environment was estimated using the following formula:

$$H^2 = \frac{\sigma^2_G}{\sigma^2_{G} + (\sigma^2_e / r')}$$

Where σ^2_G is the genotypic variance; σ^2_e is the error variance; and r' is the mean of replications.

Heritability (H^2) estimates for ergot vulnerability in the combined analyses were calculated using the following formula:

$$H^2 = \frac{\sigma^2_G}{\sigma^2_{G} + (\sigma^2_{GE} / r') + (\sigma^2_e / r'e')}$$

Where σ^2_G is the genotypic variance; σ^2_{GE} is the genotype by environment interaction variance, σ^2_e is the error variance; r' is the mean of replications and e' is the mean of environments.

Results and Discussion

Relationship between Ergot Vulnerability and Severity and Incidence

There was a strong and significant correlation among ergot vulnerability, ergot severity and ergot incidence. Pearson's correlation coefficient between severity and incidence across all locations and both populations was 0.689, which was significant at the 0.001 level. Pearson's correlation coefficient between severity and vulnerability was 0.918, while incidence and vulnerability was 0.920. Both were significant at the 0.001 level (Table 3). The variable ergot vulnerability, generated by the factor analysis, explained more of variability than either ergot incidence and ergot severity. Thus, the creation of a single vulnerability rating by combining severity and incidence was useful and this variable was used to perform all statistical analyses by individual environments as well as the combined analysis across environments for both populations.

The importance of creating a single data point variable can be seen later in the combined analysis of the populations across environments. For example, PR01 had the second highest ergot vulnerability ratings, but a relatively low ergot severity value. In this case if only ergot severity or incidence ratings were considered, the conclusion may have been different. The vulnerability rating accounts for variation in both severity and incidence. Researchers can still use the severity and incidence data to make the correct choices based on whether incidence or severity is more important for this situation.

Table 3. Pearson's correlation coefficients among ergot vulnerability, ergot severity and ergot incidence.

		Severity	Incidence	Vulnerability
Severity	Pearson Correlation	1	.689 †	.918 †
	Sig. (2-tailed)	.	.000	.000
	N	1134	1134	1134
Incidence	Pearson Correlation	.689 †	1	.920 †
	Sig. (2-tailed)	.000	.	.000
	N	1134	1136	1134
Vulnerability	Pearson Correlation	.918 †	.920 †	1
	Sig. (2-tailed)	.000	.000	.
	N	1134	1134	1134

† Correlation is significant at the 0.01 level (2-tailed).

Populations

Ergot was observed in both populations in all four environments. Significant differences (P < 0.05) in ergot vulnerability were detected between both populations in three of the environments (Table 4). The IS8525D population had significantly lower vulnerability ratings than the IS8525J population at all environments except at PR01, where differences (P < 0.05) were not detected (Table 5).

For the combined analysis, significant differences (P < 0.05) were detected between both populations. Significant differences were detected among environments for ergot vulnerability (Table 4). This was expected because environments have a significant effect on ergot development.

Table 4. Calculated mean squares for the combined analysis of variance on ergot vulnerability, ergot severity and ergot incidence for IS8525J and IS8525D sorghum recombinant inbred line populations at four environments.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
ENV	3	215.67**	657.54**	803.68**
REP(ENV)	4	0.33	0.15	3.64
POPULATIONS	1	34.19**	92.61	110.86
ENV * POPULATION	3	3.63**	13.55**	38.85**
ERROR	1110	0.31	0.73	1.97

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 5. Ergot vulnerability† ratings for IS8525J and IS8525D sorghum recombinant inbred line populations at four environments and the combined results for both populations across environments and environments across the two populations.

Population	Environment				Combined‡
	CS01	CC01	PR01	WE01	
IS8525D	1.07^b	0.19^c	1.55^a	2.31^d	1.28^a
IS8525J	1.69^a	0.75^b	1.54^a	2.66^c	1.66^b
Combined§	1.38^A	0.47^B	1.54^C	2.48^D	

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

‡ Populations followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

§ Environment means followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

For ergot severity, significant differences (P < 0.05) were detected between the populations in CS01 and WE01 (Tables 4 and 6). For the combined analysis, significant differences (P < 0.05) were detected between both populations. Significant differences were detected among environments for ergot severity (Table 4). For ergot incidence, significant differences (P < 0.05) were detected between the populations in CS01 and WE01 (Tables 4 and 7). For the combined analysis, significant differences were detected between both populations. Significant differences were detected among environments for ergot incidence (Table 4).

When comparing populations, the IS8525J population was the most vulnerable with an average vulnerability rating of 1.66, compared to 1.28 for the IS8525D population (Table 5). It was apparent from the data analysis and observation that greater levels of tolerance were present in the IS8525D population. While some male sterility was observed in the IS8525J population, this was also observed in IS8525D population.

Table 6. Ergot severity† ratings for IS8525J and IS8525D sorghum recombinant inbred line populations at four environments and the combined results for both populations across environments and environments across the two populations.

Population	Environment				Combined‡
	CS01	CC01	PR01	WE01	
IS8525D RILs	0.87^b	0.15^a	1.01^b	3.23^c	1.31^a
IS8525J RILs	1.90^c	0.61^a	0.97^b	4.26^d	1.93^b
Combined§	1.38^B	0.38^A	0.99^C	3.74^D	

† Ergot severity ratings were made on a scale of 0 to 5 where 0 = 0%, 1 = 1-5%, 2= 6-10%, 3 = 11-25%, 4 = 26-50%, and 5 = 51-100%.

‡ Populations followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

§ Environment means followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

Table 7. Ergot incidence† ratings for IS8525J and IS8525D sorghum recombinant inbred line populations at four environments and the combined results for both populations across environments and environments across the two populations.

Population	Environment				
	CS01	CC01	PR01	WE01	Combined‡
IS8525D RILs	62.40^b	11.20^a	96.40^c	98.60^c	67.15^a
IS8525J RILs	84.20^d	43.40^a	96.80^c	100.00^b	81.10^b
Combined§	73.30^B	27.30^A	96.60^C	99.30^C	

† Ergot incidence ratings are the percentage of panicles with at least one infection point on the panicle.

‡ Populations followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

§ Environment means followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

Population by Environment Interaction

For ergot vulnerability there was a significant population by environment interaction at the 0.05 level (Table 4), but the data showed no major shifts between the two populations, with the exception of the IS8525D population in PR01 that had a slightly higher ergot vulnerability rating that the IS8525J population (Table 5). There was a significant population by environment interaction at the 0.05 level for ergot severity (Table 4), but the data showed no major shifts between the two populations, with the exception of the IS8525D population in PR01 that had a slightly higher ergot severity rating that the IS8525J population (Table 6). For ergot incidence there was a significant population by environment interaction at the 0.05 level (Table 4), but the data showed no major shifts between the two populations, IS8525D population incidence ratings were always lower than the ones of IS8525J population (Table 7).

Environments

Significant differences (P < 0.05) in ergot vulnerability were detected among environments (Table 5). The same patterns among environments were observed for ergot severity and ergot incidence. Significant differences (P < 0.05) in ergot severity were detected among environments (Table 6). Significant differences among environments were detected for ergot incidence ratings (Table 7).

The high ergot vulnerability ratings at WE01 were expected as temperatures during the fall are considerably lower than in the normal spring growing season. According to McLaren and Whener (1992) and Bandyopadhyay (1998), low

temperatures reduce pollen production viability, making some genotypes more vulnerable to ergot. CC01 had the lowest ergot vulnerability ratings due to the high temperatures and humidity encountered during the evaluations of the populations in the summer season. The ergot vulnerability ratings in CS01 and PR01 were somewhat unexpected. While they are not as high as the ergot vulnerability ratings in WE01, both environments had ergot vulnerability ratings significantly different (P < 0.05) from those at CC01. Both populations were evaluated during the summer at these two locations as well, where the weather is also humid with high temperatures. The high infection rate in College Station was also reported by Moran (2000). These results indicate that infection can occur in warm temperatures.

Heritability Estimates

Broad-sense heritability (H^2) estimates for ergot vulnerability at individual environments and combine analysis varied widely. The combined H^2 estimates were higher for the IS8525D recombinant inbred line population (Table 8). Heritabilities in single environments were relatively low, and dropped even more in combined analysis due to the significant interaction variation and differential response of recombinant inbred lines. The combined analysis results in this study indicate that while ergot resistance is heritable, resistance is not stable across environments. Ergot resistance is not maintained under cooler conditions such as WE01. Also, the resistance to ergot is variable in environments with warm temperatures such as CS02. While these are broad-sense heritability estimates, since the evaluation was completed on F2:5 lines, most of

the genetic variation remaining would be due to additive main effects or additive by additive epistatic interactions. Therefore, it could be expected that narrow-sense heritability estimates would not differ much from these currently available.

Table 8. Ergot vulnerability† variance components coefficients and broad sense heritability (H^2) estimates for IS8525J and IS8525D recombinant inbred line sorghum populations by individual and combined environments.

| Environment | Population | Variance component coefficient | | | | |
		σ^2_G	σ^2_E	σ^2_{GE}	σ^2_e	H^2
WE01	IS8525J	0.04	.	.	0.06	0.56
CC01	IS8525J	0.39	.	.	0.35	0.68
PR01	IS8525J	0.00	.	.	0.05	0.00
CS01	IS8525J	0.09	.	.	0.28	0.39
Combined	IS8525J	0.01	0.60	0.12	0.21	0.13
WE01	IS8525D	0.09	.	.	0.09	0.67
CC01	IS8525D	0.00	.	.	2.95	0.00
PR01	IS8525D	0.00	.	.	0.06	0.00
CS01	IS8525D	0.18	.	.	0.24	0.60
Combined	IS8525D	0.01	0.77	0.09	0.14	0.21

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

IS8525 Juicy Population Results

Weslaco (WE01)

The highest value of ergot vulnerability for the IS8525J population was at WE01 with an average ergot vulnerability rating of 2.66, and significant variation around the mean (Fig. 1; Table 9). Since the IS8525J population was highly vulnerable in WE01, the distribution curve was skewed to the right and many genotypes had high ergot vulnerability ratings. Differences ($P < 0.05$) were detected among ergot vulnerability ratings of the IS8525J population and the original parents BTX643 and IS8525J (Table 10). No significant differences were detected ($P < 0.05$) among ergot vulnerability ratings of the IS8525J population and the sterile checks ATX623 and A3TX623 (Table 10).

For ergot severity, the highest means for the IS8525J population were observed in WE01 with an average ergot severity rating of 4.26. Significant differences in severity were detected among recombinant inbred lines of the IS8525J population (Table 9). Differences ($P < 0.05$) were detected among ergot severity ratings of the IS8525J population and the original parents BTX643 and IS8525J (Table 11). No significant differences were detected among ergot severity ratings of the IS8525J population and the sterile checks ATX623 and A3TX623 (Table 11).

For incidence, the highest value for the IS8525J population was observed in WE01 with an average ergot incidence of 100.0 %. No significant differences in incidence were detected among recombinant inbred lines of the IS8525J population (Table 9) and none were detected among ergot incidence ratings of the IS8525J population and the original parents BTX643 and IS8525J (Table 12). No significant differences were detected ($P < 0.05$) among ergot incidence of the IS8525J population and the sterile checks ATX623 and A3TX623 (Table 12).

Ergot severity incidence and ergot vulnerability seem to be the most appropriate variables to identify ergot resistant genotypes in an environment with cool temperatures such as WE01. These two variables allowed the detection of significant differences among recombinant inbred lines of the IS8525J population, parents and checks in an environment where the disease was highly expressed due to the natural weather conditions. Due to the high pressure of the disease, incidence will not explain much of the performance of genotypes, especially in environments where ergot can be seen in every single plant of a plot.
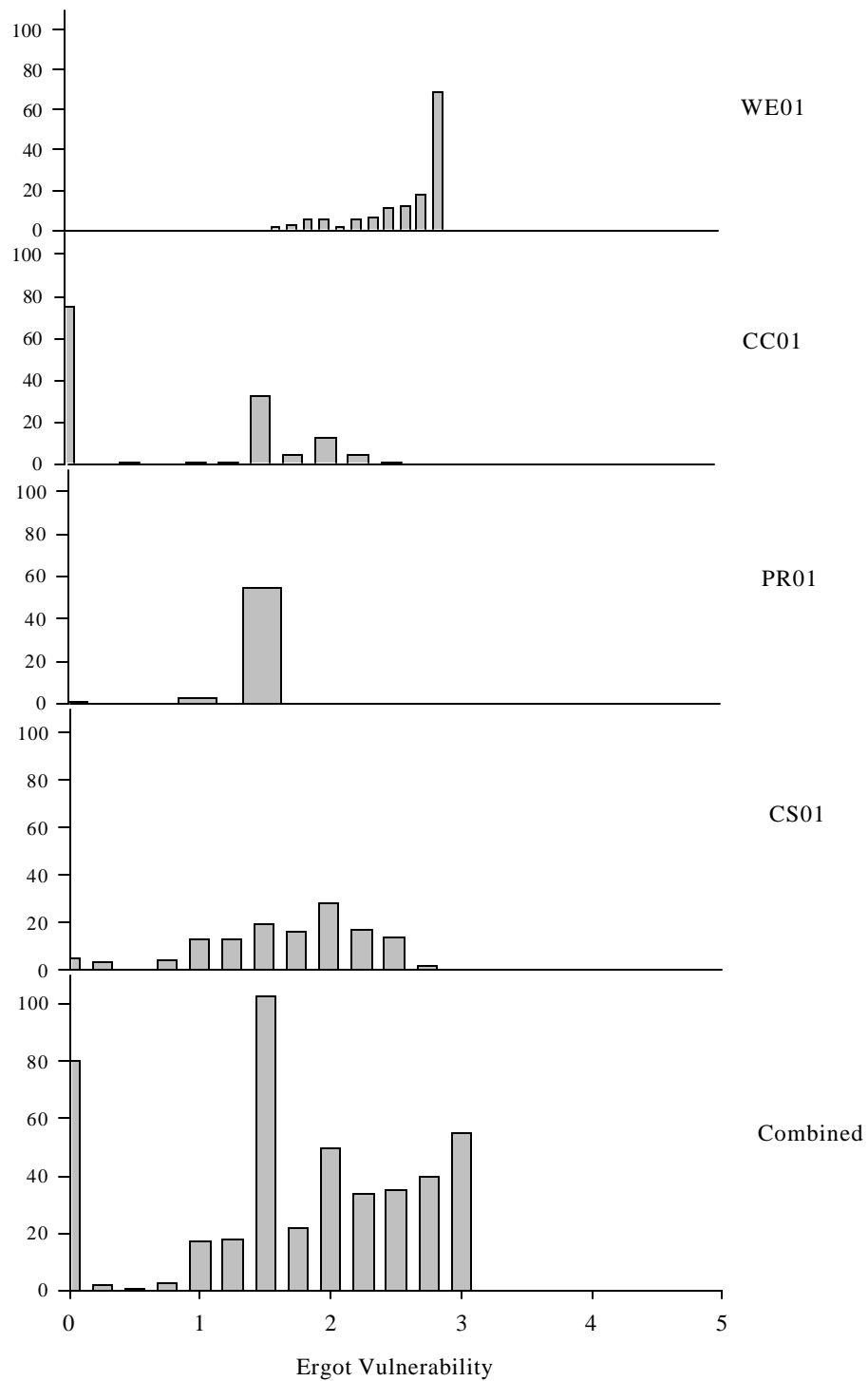
Figure 1. Distribution of ergot vulnerability ratings for the recombinant inbred line population from the cross of B1/IS8525J.

Table 9. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the recombinant inbred lines derived from the BTx643/IS8525J cross at WE01.

| Source | df | Mean Squares† | | |
		Vulnerability	Severity	Incidence
RILs	33	0.22**	2.06**	0.00
REP	1	0.00	0.02	0.00
Error	100	0.06	0.56	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 10. Ergot vulnerability† combined ratings for IS8525J and IS8525D sorghum recombinant inbred line populations, original parents and sterile checks across environments, and environments across IS8525J and IS8525D recombinant inbred line populations, original parents and sterile checks.

Populations, parents and checks.	Environment				Combined‡
	CS01	CC01	PR01	WE01	
IS8525D RILs	1.07ec[(B)]	0.19a[(C)]	1.55b[(A)]	2.31b [(D)]	1.28b
IS8525J RILs	1.69ad[(A)]	0.75b[(B)]	1.54b[(A)]	2.66a[(C)]	1.66a
ATX623	1.71ab[(B)]	0.40a[(A)]	2.15a[(B)]	2.70a[(C)]	1.74a
A3TX623	1.93a[(B)]	0.19a[(A)]	2.24a[(B)]	2.72a[(C)]	1.77a
BTX643	1.36bde[(B)]	0.24a[(A)]	1.42b[(B)]	2.26bd[(C)]	1.32b
IS8525J	1.82ab[(B)]	0.00a[(A)]	1.59b[(B)]	1.92d[(B)]	1.33b
IS8525D	0.75c[(A)]	0.00a[(A)]	1.53b[(A)]	1.08c[(A)]	0.84c
Combined§	1.48[B]	0.26[A]	1.72[C]	2.23[D]	

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

‡ Genotypes followed by the same small letter are not significantly different from each other at the 0.05 level (LSD).

§ Environment means followed by the same capital letter are not significantly different from each other at the 0.05 level (LSD).

Table 11.   Ergot severity† combined ratings for IS8525J and IS8525D sorghum recombinant inbred line populations, original parents and sterile checks across environments, and environments across IS8525J and IS8525D recombinant inbred line populations, original parents and sterile checks.

Population, parents and checks.	Environment				Combined‡
	CS01	CC01	PR01	WE01	
IS8525D RILs	0.87dc$^{(B)}$	0.15a$^{(A)}$	1.01b$^{(B)}$	3.23e$^{(C)}$	1.31b
IS8525J RILs	1.90e$^{(C)}$	0.61b$^{(A)}$	0.97b$^{(B)}$	4.26a$^{(D)}$	1.93c
ATX623	2.13ae$^{(B)}$	0.25a$^{(A)}$	2.70a$^{(B)}$	4.38a$^{(C)}$	2.36a
A3TX623	2.47a$^{(B)}$	0.20a$^{(A)}$	2.98a$^{(B)}$	4.44a$^{(C)}$	2.52a
BTX643	1.21bc$^{(B)}$	0.25a$^{(A)}$	0.89b$^{(AB)}$	3.11be$^{(C)}$	1.36b
IS8525J	2.10ae$^{(B)}$	0.00a$^{(A)}$	1.00b$^{(C)}$	2.20bc$^{(B)}$	1.32b
IS8525D	0.47c$^{(B)}$	0.00a$^{(A)}$	0.85b$^{(B)}$	1.65c$^{(B)}$	0.74b
Combined§	1.59^{B}	0.21^{A}	1.48^{B}	3.32^{C}	

† Ergot severity ratings were made on a scale of 0 to 5 where 0 = 0%, 1 = 1-5%, 2= 6-10%, 3 = 11-25%, 4 = 26-50%, and 5 = 51-100%.

‡ Genotypes followed by the same small letter are not significantly different from each other at the 0.05 level (LSD).

§ Environment means followed by the same capital letter are not significantly different from each other at the 0.05 level (LSD).

Table 12. Ergot incidence† combined ratings for IS8525J and IS8525D sorghum recombinant inbred line populations, original parents and sterile checks across environments, and environments across IS8525J and IS8525D recombinant inbred line populations, original parents and sterile checks.

	Environment				
Population, parents and checks.	CS01	CC01	PR01	WE01	Combined‡
IS8525D RILs	62.4bc[(B)]	11.2a[(A)]	96.4a[(C)]	98.6a[(C)]	67.1c
IS8525J RILs	84.2a[(D)]	43.4b[(A)]	96.8a[(C)]	100.0a[(B)]	81.1d
ATX623	85.9a[(B)]	25.0ab[(A)]	100.0a[(B)]	100.0a[(B)]	60.2ad
A3TX623	89.0a[(B)]	10.0a[(A)]	100.0a[(B)]	100.0a[(B)]	74.7ad
BTX643	76.2ac[(B)]	12.4a[(A)]	89.4a[(BC)]	97.8a[(C)]	68.9ac
IS8525J	90.0a[(B)]	0.00a[(A)]	100.0a[(B)]	95.0a[(B)]	71.2dc
IS8525D	47.4b[(B)]	0.00a[(A)]	99.4a[(B)]	42.4b[(B)]	47.3b
Combined§	66.4[B]	14.5[A]	97.4[C]	90.5[C]	

† Ergot incidence ratings are the percent of panicles with at least one infection point on the panicle.

‡ Genotypes followed by the same small letter are not significantly different from each other at the 0.05 level (LSD).

§ Environment means followed by the same capital letter are not significantly different from each other at the 0.05 level (LSD).

Corpus Christi (CC01)

Ergot vulnerability ratings in the IS8525J population averaged 0.75 in CC01 and bimodal variation was detected across the juicy populations (Fig. 1). While variation was reduced, significant differences in ergot vulnerability were detected among recombinant inbred lines of the IS8525J population (Table 13). Differences ($P < 0.05$) were detected among ergot vulnerability ratings of the IS8525J population and the original parents BTX643 and IS8525J, but no differences were detected ($P < 0.05$) among ergot vulnerability ratings of the IS8525J population and the sterile checks ATX623 and A3TX623 (Table 10).

For ergot severity, the lowest average values for the IS8525J population were observed in CC01, but significant differences in severity were still detected among the recombinant inbred lines (Table 13). Differences ($P < 0.05$) were detected among ergot severity ratings of the IS8525J population and the original parents BTX643 and IS8525J, and the sterile checks ATX623 and A3TX623 (Table 11).

For the variable incidence, the lowest value for the IS8525J population was observed in CC01 with an average ergot incidence of 43.4 %. Significant differences in incidence were detected among recombinant inbred lines of the IS8525J population (Table 13). Differences ($P < 0.05$) were detected among ergot incidence ratings of the IS8525J population and the original parents BTX643 and IS8525J, but no differences were detected ($P < 0.05$) for ergot incidence between the IS8525J population mean and the sterile check ATX623. However, significant differences were detected between the IS8525J population the sterile check A3TX623 (Table 12).

The increased susceptibility of the IS8525J population relative to the sterile checks was unexpected and its implications are unclear. It is obvious that some of the increased vulnerability is due to sterility that is present in the population. However, the sterile checks are 100% sterile, so there cannot be any greater sterility in the population. Since environmental conditions have a significant effect on ergot vulnerability, it is possible that the sterile checks are later in maturity than the population. At this location, the population was earlier than the checks and ergot infection was more favorable earlier in the season. However, this cannot be the complete explanation as analysis including date of inoculation did not detect significant differences among days of inoculation.

In CC01, ergot incidence, ergot vulnerability and ergot severity were equally important to identify ergot resistant genotypes. The three variables allowed detecting significant differences within recombinant inbred lines of the IS8525J population, parents and checks in an environment where the weather played an important role in ergot expression.   .

Table 13. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the recombinant inbred lines derived from the BTx643/IS8525J cross at CC01.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
RILs	33	1.87**	2.00**	13.99**
REP	1	4.20**	4.69**	27.27**
Error	97	0.34	0.23	3.21

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Puerto Rico (PR01)

In PR01 the general ergot vulnerability mean for IS8525J population was 1.54 (Table 10) and very little variation from the mean was evident (Fig. 1). Statistical analysis did not detect any variation among RILs (Table 14). In addition, no differences (P < 0.05) were detected among ergot vulnerability ratings of the IS8525J population and the original parents BTX643 and IS8525J, but differences were detected (P < 0.05) among ergot vulnerability ratings of the IS8525J population and the sterile checks ATX623 and A3TX623 (Table 10).

For ergot severity, the IS8525J population had a mean of 0.97. No significant differences in severity were detected among recombinant inbred lines of the IS8525J population (Table 14). No differences (P < 0.05) were detected among ergot severity ratings of the IS8525J population and the original parents BTX643 and IS8525J, but differences were detected among ergot severity ratings of the IS8525J population and the sterile checks ATX623 and A3TX623 (Table 11).

For the variable incidence, the IS8525J population had an average ergot incidence of 96.8% and differences in incidence were not detected among recombinant inbred lines of the IS8525J population (Table 14). No differences (P < 0.05) were detected for ergot incidence ratings between the IS8525J population and the original parents BTX643 and IS8525J or between the IS8525J population and the sterile checks ATX623 and A3TX623 (Table 12).

Table 14. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the recombinant inbred lines derived from the BTx643/IS8525J cross at PR01.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
RILs	32	0.00	0.02	0.45
REP	1	0.01	0.01	0.09
Error	25	0.06	0.02	0.64

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

College Station (CS01)

In CS01 the general ergot vulnerability mean for IS8525J population was 1.69 (Table 10), and the distribution around this mean was very close to normal (Fig. 1). There was also more variation in ergot vulnerability ratings compared to any other environment (Fig. 1).

Significant differences in vulnerability were detected among the recombinant inbred lines in CS01 (Table 15). No differences ($P < 0.05$) were detected for ergot vulnerability ratings between the IS8525J RILs population and the original parents BTX643 and IS8525J (Table 10). No significant differences were detected ($P < 0.05$) among ergot vulnerability ratings of the IS8525J RILs population and the sterile checks ATX623 and A3TX623 (Table 10).

For ergot severity, the IS8525J RILs population averaged 1.90 and significant differences in severity were detected among recombinant inbred lines of the IS8525J population (Table 15). Significant differences ($P < 0.05$) were detected for ergot severity ratings between IS8525J population mean and the original parent BTX643, but no differences were detected between the IS8525J population mean and the IS8525J parent (Table 11). Differences were detected ($P < 0.05$) for ergot severity between the IS8525J population and the sterile checks ATX623 and A3TX623 (Table 11).

For ergot incidence, the IS8525J RILs had an average ergot incidence of 84.2 %. No differences in incidence were detected among recombinant inbred lines of the IS8525J population (Table 15) nor were they detected between the population mean or any of the checks (Table 12).

Table 15. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the recombinant inbred lines derived from the BTx643/IS8525J cross at CS01.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
RILs	33	0.67**	2.60**	2.02
REP	1	0.00	0.189	0.35
Error	99	0.29	0.75	1.43

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Combined Analysis

Significant differences (P < 0.05) were detected among environments. In addition a significant genotype by environment interaction was detected (Table 16). As mentioned before, ergot resistance is not stable across environments indicating that selection for resistant lines is difficult. Performance of the IS8525J lines differed across environments as indicated by the significant genotype by environment interaction, indicating that no extrapolation of data from one environment to another can be done. Close evaluation of the performance of individual lines indicates that many lines were extremely variable in their response across environments (Table 17).

The level of disease observed in the IS8525J population in the overall population analysis across environments was similar to the trend of the IS8525J population in the individual analysis. Out of the four environments, the ergot vulnerability ratings for the IS8525J population were higher in WE01, followed by CS01, PR01 and CC01 (Table 10). The combined ergot vulnerability distribution of the IS8525J population was skewed to the right (Fig. 1). Significant differences were detected among the combined ergot vulnerability mean of the IS8525J population and the combined ergot vulnerability mean of the original parents BTX643 and IS8525J, but no differences were detected among the combined ergot vulnerability mean of the IS8525J population and the combined ergot vulnerability mean of the sterile checks ATX623 and A3TX623 (Table 10). For ergot severity, the highest ergot severity was in WE01 followed by CS01, PR01 and CC01 (Table 11). Significant differences were detected among the combined ergot severity mean of the IS8525J population and the combined ergot severity mean of the

original parents BTX643 and IS8525J (Table 11). In addition, significant differences were detected among the combined ergot severity mean of the IS8525J population and the combined ergot severity mean of the sterile checks ATX623 and A3TX623 (Table 11).

Table 16. Calculated mean squares for the combined analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the recombinant inbred lines derived from the BTx643/IS8525J cross at four environments.

| Source | df | Mean Squares† | | |
		Vulnerability	Severity	Incidence
Environment	3	80.72**	328.22**	205.62**
Rep(Environment)	4	1.05**	1.23**	6.93**
RIL	33	0.67	1.17	3.77
Env * RIL	98	0.62**	1.45**	3.76**
Error	321	0.21	0.48	1.47

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 17. Ergot vulnerability† combined ratings for recombinant inbred elite lines derived from the BTx643/IS8525J cross at four environments.

Line (entry #)	CS01	CC01	PR01	WE01	Combined
			Environment		
33	0.98[2] ‡	0.00[1]	1.59[9]	2.25[4]	1.20[1] §
9	1.03[3]	0.00[1]	1.59[9]	2.23[3]	1.21[2]
8	0.80[1]	0.00[1]	1.61[10]	2.79[20]	1.30[3]
BTX643	1.36[8]	0.24[3]	1.42[3]	2.26[5]	1.32[4]
IS8525J	1.82[21]	0.00[1]	1.59[9]	1.92[1]	1.33[5]
15	1.31[6]	0.00[1]	1.59[9]	2.72[18]	1.40[6]
17	2.19[28]	0.00[1]	0.79[1]	2.71[17]	1.42[7]
18	1.70[16]	0.00[1]	1.43[4]	2.63[11]	1.44[8]
21	1.64[15]	0.79[5]	1.62[11]	2.20[2]	1.56[9]
ATX623	1.71[19]	0.40[4]	2.15[34]	2.70[16]	1.74[23]
A3TX623	1.93[23]	0.19[2]	2.24[35]	2.72[18]	1.77[25]

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

‡ The superscript number indicates the line ranking in that particular environment.

§ The superscript number indicates the line ranking across environments.

The highest ergot incidence was observed in WE01 followed by PR01, CS01, and CC01 (Table 12). Significant differences were detected between the combined ergot incidence mean of the IS8525J population and the combined ergot incidence mean of the original parent BTX643, but no differences were detected between the combined mean of the IS8525J population and the IS8525J parent (Table 12). In addition, no significant differences were detected among the combined ergot incidence mean of the IS8525J population and the combined ergot severity mean of the sterile checks ATX623 and A3TX623 (Table 12).

IS8525 Dry Population Results

Weslaco (WE01)

The average ergot vulnerability rating for the IS8525D population was 2.31 and there was significant variation around the mean (Fig. 2). Since vulnerability was high, the distribution of the RIL was skewed to the right due to high ergot vulnerability ratings. Significant differences in vulnerability were detected among recombinant inbred lines of the IS8525D population (Table 18). No difference in vulnerability rating $(P < 0.05)$ was detected between IS8525D population and BTx643, but a significant differences was detected between the IS8525D population and IS8525D (Table 10). No significant differences were detected $(P < 0.05)$ among ergot vulnerability ratings of the IS8525D population and the sterile checks ATX623 and A3TX623 (Table 10).

For severity, the average for the IS8525D population was 3.23 and significant differences in severity were detected among recombinant inbred lines of the IS8525D population (Table 18). No significant differences in ergot severity was detected between the IS8525D population and BTx643, but significant differences (P < 0.05) were detected among ergot severity ratings of the IS8525D population and the other original parent IS8525D (Table 11). No differences were detected (P < 0.05) among ergot severity ratings of the IS8525D population and the sterile checks ATX623 and A3TX623 (Table 11).

Ergot incidence for the RILs in the IS8525D population averaged 98% and significant differences were detected among the RILs (Table 18). While differences (P < 0.05) were not detected for ergot incidence ratings between the IS8525D population and BTx643, but differences (P < 0.05) were detected between the IS8525D population and the parent IS8525D (Table 12). No significant differences were detected (P < 0.05) among ergot incidence of the IS8525D population and the sterile checks ATX623 and A3TX623 (Table 12).

Ergot pressure was high in WE01 and this was reflected in all three measurements. Each measurement detected significant differences within the IS8525D recombinant inbred lines.
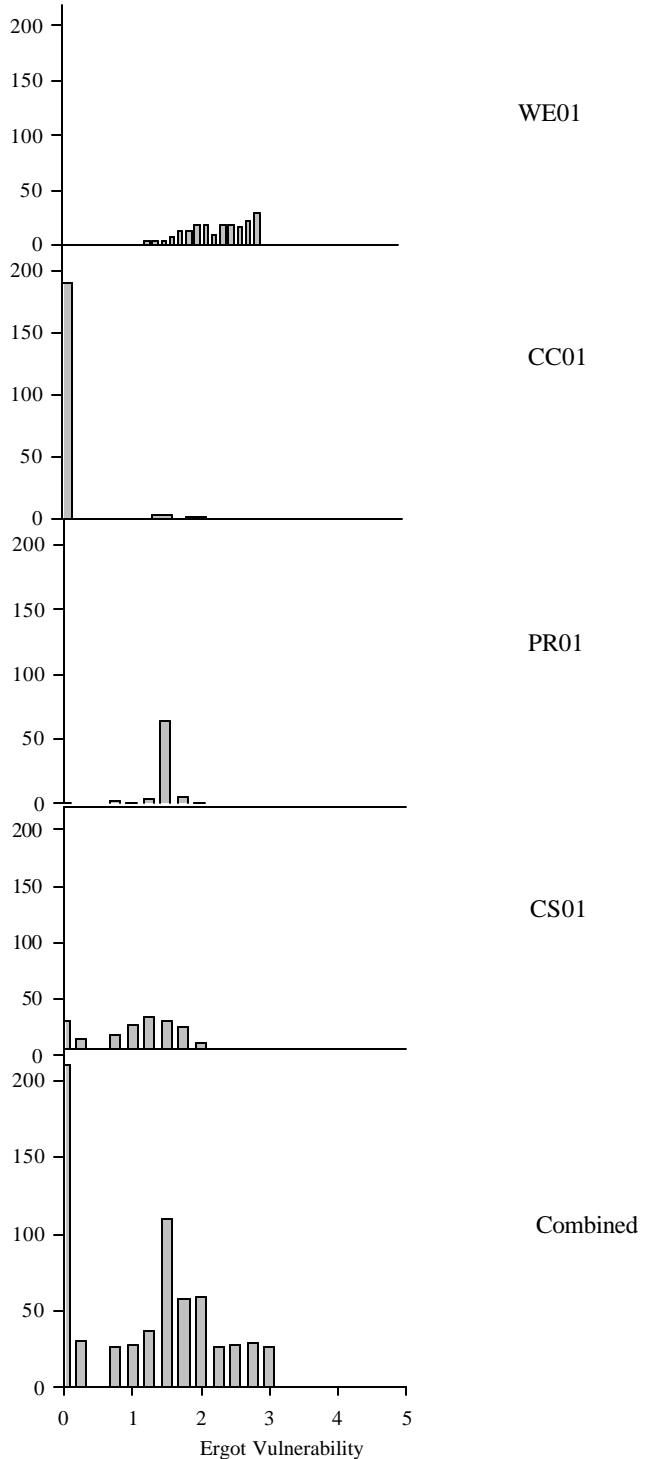
Figure 2. Distribution of ergot vulnerability ratings for the recombinant inbred
line population from the cross of B1/IS8525D

Table 18. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the recombinant inbred lines derived from the BTx643/IS8525D cross at WE01.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
RILs	48	0.47**	4.03**	0.37**
REP	1	0.02	0.00	0.07
Error	144	0.09	0.69	0.06

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Corpus Christi (CC01)

The IS8525D population mean in CC01 was 0.19. Unlike WE01, ergot vulnerability ratings in CC01 across the dry population were not as variable (Fig. 2). Significant differences in vulnerability were detected among recombinant inbred lines of the IS8525D population (Table 19). No differences ($P < 0.05$) in ergot vulnerability were detected between the IS8525D population mean and either parent (BTx643 and IS8525D) or either sterile checks (ATx623 and A3Tx623) (Table 10).

For ergot severity, the IS8525D population mean was 0.15 and significant differences in severity were detected among the recombinant inbred lines (Table 19). Significant differences for ergot severity were not detected between the IS8525D population mean and either the parents (BTX643 and IS8525D) or the sterile checks (ATx623 and A3Tx623) (Table 11).

For ergot incidence, the IS8525D population mean was 11.2% and significant differences in severity were detected among the recombinant inbred lines (Table 19). Significant differences for ergot incidence were not detected between the IS8525D population mean and either the parents (BTX643 and IS8525D) or the sterile checks (ATx623 and A3Tx623) (Table 12).

Table 19. Mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the recombinant inbred lines derived from the BTx643/IS8525D cross at CC01.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
RILs	48	0.75**	0.50**	6.52**
REP	1	0.75**	1.00**	4.59**
Error	146	0.14	0.11	1.16

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

In CC01, ergot vulnerability, ergot severity and ergot incidence were equally important to identify ergot resistant genotypes. The three variables allowed to detect significant differences among the recombinant inbred lines of the IS8525D population, and differences among IS8525D population, parents and checks in an environment where the weather played an important role in ergot expression.

Puerto Rico (PR01)

The general ergot vulnerability mean for the IS8525D population was 1.55 (Table 10) and the distribution of lines for ergot vulnerability was tightly focused with variation from the mean (Fig. 2). The lack of variation resulted in no significant differences for ergot vulnerability being detected among the recombinant inbred lines (Table 20). In addition, no significant differences ($P < 0.05$) were detected for ergot vulnerability between the IS8525D population and either parent (Table 10). Significant differences were detected for ergot vulnerability ratings between the IS8525D population mean and the sterile checks ATx623 and A3Tx623 (Table 10).

For ergot severity, the IS8525D population mean was 1.01 and no significant differences were detected among the recombinant inbred lines (Table 20). No significant differences ($P < 0.05$) were detected for ergot severity between the IS8525D population mean and either parent (Table 11). The severity mean of the IS8525D population was significantly lower that either sterile check (ATx623 and A3Tx623) (Table 11).

Table 20. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the recombinant inbred lines derived from the BTx643/IS8525D cross at PR01.

| Source | df | Mean Squares† | | |
		Vulnerability	Severity	Incidence
RILs	44	0.05	0.08	0.35
Rep	1	0.04	0.22	0.31
Error	31	0.07	0.07	0.31

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

For ergot incidence, the IS8525D population mean was 96.4%. Significant differences in incidence were not detected among recombinant inbred lines of the IS8525D population (Table 20). No significant differences (P < 0.05) were detected for ergot incidence between the IS8525D population mean and either parents or either sterile check (ATx623 and A3Tx623) (Table 12).

College Station (CS01)

The ergot vulnerability mean for IS8525D population was 1.07 (Table 10) and the distribution of the RILs was near normal. The range of variation in ergot vulnerability ratings was greater in College Station than it was in any other environment (Fig. 2). Significant differences in vulnerability were detected among recombinant inbred lines of the IS8525D population (Table 21). No significant differences (P < 0.05) were detected for ergot vulnerability between IS8525D population mean and either parent (Table 10). Significant differences were detected among ergot vulnerability ratings of the IS8525D population and the sterile checks ATX623 and A3TX623 (Table 10).

For ergot severity, the IS8525D population mean was 0.87. Significant differences in severity were detected among recombinant inbred lines of the IS8525D population (Table 21). No significant differences were detected for ergot severity between the IS8525D population mean and either parent (Table 11). As expected, the IS8525D population mean was significantly higher than the sterile checks (ATX623 and A3TX623) (Table 11).

Table 21. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the recombinant inbred lines derived from the BTx643/IS8525D cross at CS01.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
RILs	48	0.95**	1.16**	6.85**
REP	1	1.73**	0.84	16.15**
Error	145	0.24	0.26	1.91

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

For ergot incidence, the IS8525D population mean was 62.4% and significant differences were detected among recombinant inbred (Table 21). No significant differences (P < 0.05) were detected for ergot incidence between the IS8525D population mean and either parents (BTx643 and IS8525D) (Table 12). The incidence of IS8525D population mean was significantly lower than either sterile check (ATx623 and A3Tx623) (Table 12).

Combined Analysis

Differences (P < 0.05) for all three dependent variables were detected among environments. In addition a significant genotype by environment interaction was detected (Table 22). As mentioned before, ergot resistance is not stable across environments indicating that selection for resistant lines is difficult. Performance of the IS8525D lines differed across environments as indicated by the significant genotype by environment interaction, indicating that no extrapolation of data from one environment to another can be done. Close evaluation of the performance of individual lines indicates that many lines were extremely variable in their response across environments. (Table 23). The level of disease observed in IS8525D population in the overall population analysis across environments was similar to the trend of the IS8525D individual population analysis. The ergot vulnerability ratings for the IS8525D population were highest in WE01, followed by PR01, CS01 and CC01 (Table 10).

The combined ergot vulnerability distribution of the IS8525D population was near normal (Fig. 2). In the combined analysis, the IS8525D population mean was not different from BTx643, but the IS8525D population mean was lower than that of the IS8525D mean (Table 10). As expected, the ergot vulnerability mean of the IS8525D population was significantly lower than the vulnerability mean of the sterile checks (Table 10).

The results for ergot severity were similar to those of ergot vulnerability (Table 11). However, significant differences for ergot severity were not detected between the IS8525D population mean and the ergot severity mean of either parent (Table 11). As expected, the ergot severity rating for the IS8525D population was less than either sterile check (Table 11).

For ergot incidence, no significant differences were detected between the IS8525D population mean and the BTx643 parent (Table 12). The IS8525D population mean was significantly lower than the ergot tolerant parent, IS8525D (Table 12). As expected, the IS8525D population mean was significantly lower than the ergot incidence mean of the sterile checks ATX623 and A3TX623 (Table 12).

Table 22. Calculated mean squares for the combined analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the recombinant inbred lines derived from the BTx643/IS8525D cross at four environments.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
ENV	3	148.68**	338.66**	706.40**
REP(ENV)	4	0.63**	0.52	5.21**
RILs	48	0.59	1.48	3.18
ENV * RILs	140	0.47**	1.22**	3.22**
ERROR	466	0.15	0.34	1.02

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 23.   Ergot vulnerability† combined ratings for recombinant inbred elite lines derived from the BTx643/IS8525D cross at four environments.

	Environments				
Line	CS01	CC01	PR01	WE01	Combined
IS8525D	0.75[14] ‡	0.00[1]	1.53[8]	1.08[1]	0.84[1] §
43	0.32[4]	0.00[1]	1.56[9]	1.64[4]	0.88[2]
13	0.64[11]	0.00[1]	0.79[1]	2.23[20]	0.92[3]
8	0.24[2]	0.00[1]	1.43[7]	2.18[16]	0.96[4]
52	1.18[25]	0.00[1]	1.30[4]	1.54[2]	1.00[5]
49	1.08[20]	0.00[1]	1.59[15]	1.56[3]	1.06[6]
38	0.95[18]	0.00[1]	1.56[9]	1.81[5]	1.08[7]
19	0.24[2]	0.00[1]	1.56[9]	2.53[34]	1.08[7]
BTX643	1.36[34]	0.24[3]	1.42[6]	2.26[33]	1.32[33]
ATX623	1.71[46]	0.40[4]	2.15[21]	2.70[44]	1.74[47]
A3TX623	1.93[48]	0.19[2]	2.24[22]	2.72[45]	1.77[48]

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

‡ The superscript number indicates the line ranking in that particular environment.

§ The superscript number indicates the line ranking across environments.

Conclusions

To assess the value of each environment, it is important to consider the reaction of the parents in terms of ergot susceptibility. In each environment and for each method of assessing ergot susceptibility, the IS8525D parent consistently had lower ergot susceptibility ratings. Therefore, it does appear that IS8525D does show some level of tolerance to ergot. However, IS8525J does show susceptibility to ergot. Therefore, complete resistance to ergot is not available from this germplasm.

However, IS8525J showed significantly more ergot susceptibility than the IS8525D parent. This trend was consistent across environments and also was clearly evident in the populations derived from both IS8525J and IS8525D. The IS8525J population was significantly more vulnerable than the IS8525D population. One of the reasons why the IS8525J population was more vulnerable is because the population was segregating for male fertility. Some lines in the population were still producing male sterile plants and this increased the susceptibility of these lines. The exact cause of this sterility is not known as both parents are known to be maintainers of sterility in the A1 CMS system. Since both of these lines possess normal cytoplasm, the male sterility can not be caused by this system.

Variation for ergot vulnerability among recombinant inbred lines for both IS8525J and IS8525D populations was detected. However, for certain environments, variation was limited and not statistically detectable. This indicates that variation is

limited for any potential selection program and it will be defined by the environment in which evaluation can be conducted. Further compounding of this problem is the presence of a significant genotype by environment interaction. Close evaluation of the performance of individual lines indicates that many lines were extremely variable in their response across environments. There were several genotypes that were consistently poor in their performance which was not unexpected, but there also were several genotypes with a relatively stable and more tolerant response across environment. These results provide evidence that if the trait is heritable, selection could produce a line with greater ergot tolerance that is stable.

For individual environments, heritability estimates ranged from 0.00 to 0.68, indicating that genetic variability for this trait is highly dependent on the environment allowing its expression. Because these lines are F2:5, most of the variation among the lines will be due to additive gene action; therefore, the heritability estimates that are provided represent heritable variation and there is an expectation that progress could be made from breeding.

The results obtained from the different dependent variable indicate the importance of evaluating ergot susceptibility in different ways. Simply evaluating ergot incidence would reveal that little progress could be made, but ergot severity indicates a different response should be expected. Combining the two variables is probably the most appropriate single point measurement. However, it is important to always consider the relationship of this created variable to the two dependent variables that can be systematically measured.

CHAPTER IV

HERITABILITY ESTIMATES FOR ERGOT RESISTANCE IN TESTCROSS HYBRIDS OF RECOMBINANT INBRED LINES FROM SORGHUM GERMPLASM IS8525

Introduction

To improve a population with respect to a trait of interest, sufficient genetic variation must be present and a selection method that discriminates among individuals that differ in genotypic value must be used. To measure the genetic variability in a population, trait heritability estimates are necessary. Heritability is defined as the proportion of the observed variation in a progeny that is inherited (Poehlman, 1995). Also, heritability is defined as the proportion of observable field variation that is from genetic factors (Nyquist, 1991). Heritability estimates determine the value of a breeding population and often they can be used to define the appropriate breeding technique to be used to improve the trait of interest.

Ergot is a potentially dangerous disease of sorghum seed production. The most feasible and economical method to control ergot is genetic resistance. Due to the nature of the disease, sufficient quantities of viable pollen can prevent ergot infection. However, pollen mediated disease escape mechanism can be easily confused with true

physiological resistance. While pollen mediate disease escape mechanism is useful in fertile hybrids, it cannot be used in hybrid seed production, due to the use of male sterile parents. Ergot resistance must also be functional in a male-sterile background to be considered effective in a plant breeding program. Dahlberg et al. (1998) first confirmed an increase level of tolerance in male sterile testcross hybrids of IS8525. Thus, we want to evaluate IS8525J and IS8525D recombinant inbred line populations in a fertile and in a sterile background and confirm Dahlberg's results.

In the previous chapter the heritability of line per se resistance from IS8525J and IS8525D was evaluated using recombinant inbred line populations. However, ergot tolerance and its heritability are even more important as the level of tolerance and its heritability in hybrid combination. The objective of this chapter is to determine the heritability of ergot tolerance in the IS8525J and IS8525D RIL populations in testcross hybrids.

The objectives of this chapter are (1) to estimate heritability of ergot resistance in 4 testcross populations derived from the IS8525J and IS8525D RILs, (2) to compare heritability estimates between populations and (3) to determine if the resistance in IS8525 is heritable and stable across diverse environments.

Materials and Methods

Testcross Hybrid Development

From the recombinant inbred lines (RILs) used in Chapter III, four testcross hybrid populations were developed. Testcross hybrid populations were created in a crossing block at the Texas A & M University Experimental Station Agricultural Research Farm near College Station, Texas. Each RIL of the IS8525J and IS8525D populations was used as a pollinator onto ATX623 and A3TX623, creating four different testcross populations: ATX623 x IS8525D RIL (A1TCD), ATX623 x IS8525J RIL (A1TCJ), A3TX623 x IS8525D RIL (A3TCD), and A3TX623 x IS8525J RIL (A3TCJ).

These hybrids were made to determine the level of ergot vulnerability shown in male sterile and male fertile backgrounds. Since none of these pollinator lines possessed fertility restoration genes when crosses with an A3 cytoplasm type were made, the resultant F1 hybrids were sterile. However, some of these pollinator lines can restore fertility when crossed with A1 type of cytoplasm is made, producing a fertile hybrid. Not all RILs were hybridized because some of the RILs were still segregating for fertility and those crosses were impossible to make (Table 24).

Table 24. Number of hybrids derived from the crosses between the testers ATX623, A3TX623 and IS8525D and IS8525J RILs populations evaluated at two locations.

	Testcross			
LOCATION	A1TCJ	A3TCJ	A1TCD	A3TCD
WE01	22	26	41	39
CS02	28	28	40	38

Field Evaluation

The four testcross populations were planted in three different environments: Weslaco (WE01), Corpus Christi (CC02) and College Station (CS02), Texas. However, due to the lack of rainfall in Corpus Christi during summer 2002, several observation plots were lost making evaluation impossible. Corpus Christi results are not included in this thesis.

All trials were arranged in a randomized complete block design with two replications. Each plot consisted of one 5m row with intra-row spacing of 0.76m. In all trials, original parents ATX623 and A3TX623 were included as checks. Public hybrids ATX623 and A3TX623 crossed to BTX643, IS8525D, IS8525J, RTX430, RTX432 and RTX436 were also included as checks.

Claviceps africana Inoculum and Inoculation

The inoculum preparation and inoculation was completed using procedures described in Chapter III.

Disease Evaluation

The degree of ergot severity and incidence were noted for all tagged panicles four weeks after inoculation using the same procedures described in Chapter III.

Data Transformation

Data for ergot severity and incidence were combined into a single variable, designated ergot vulnerability using the same procedures described in Chapter III. As in the line per se adjustments, negative values were obtained and it was necessary to add 2.31 to each factor to avoid this problem. All other procedures remained the same.

Statistical Analyses

Individual environment analyses were performed for ergot vulnerability, ergot severity and ergot incidence for the four populations (A1TCD, A3TCD, A1TCJ and A3TCJ). The four populations were analyzed following a randomized complete block design (RCBD) assuming genotypes as random effect components (Table 25). Mean comparisons within environments were performed using the least significant differences (LSD) procedure, with a probability level of 0.05, using the appropriate mean square depending upon the component under analysis.

In order to combine data from individual environments, a Bartlett's test for heterogeneity of error variances was performed (Little and Hills, 1978; Steel and Torrie, 1980). Results indicated that the error variances across environments were heterogeneous and transformation of data failed to normalize variances. Since the data from each environment was good, the data were combined and analyzed without transformation.

In the combined analyses, ergot vulnerability, severity and incidence were analyzed as dependent variables and lines and environments were considered random effects. When significant differences were detected, mean comparisons across environments were performed using the least significant differences (LSD) procedure, with a probability level of 0.05, using the appropriate mean square depending upon the component under analysis (Table 26).

Originally, the random factor day (for two different days of inoculation) was included in the ANOVA for individual environments and combined analysis. However, statistical differences between days were not detected at 0.05 significance level. Thus, this component (days of inoculation) was not included in the final analysis. All individual environment analyses as well as the combined analyses were generated using GLM procedure included in SPSS[®.]

Table 25. Expected mean squares and degrees of freedom for the individual analysis of variance on ergot vulnerability, ergot severity and ergot incidence for four testcross populations.

Source	df †	Mean Squares	Expected Mean Squares‡
Replications	r-1	MS_R	$\sigma^2_e + g'\sigma^2_R$
Genotypes	g-1	MS_G	$\sigma^2_e + r'\sigma^2_G$
Error	(r-1)(g-1)	MS_e	σ^2_e
Total	rg-1		

† varied depending upon the number of missing observations at each environment.

‡ g' and r' denote means for genotypes and replications, respectively.

Table 26. Expected mean squares and degrees of freedom for the combined analysis of variance on ergot vulnerability, ergot severity and ergot incidence for four testcross populations.

Source	df †	Mean Squares	Expected Mean Squares‡
Environment	e-1	MS_E	$\sigma^2_e + g'\sigma^2_{R(E)} + r'\sigma^2_{GE} + r'g'\sigma^2_E$
Replications (Environment)	e(r-1)	$MS_{R(E)}$	$\sigma^2_e + g'\sigma^2_{R(E)}$
Genotypes	g-1	MS_G	$\sigma^2_e + r'\sigma^2_{GE} + r'e'\sigma^2_G$
Genotype x Environment	(e-1)(g-1)	MS_{GE}	$\sigma^2_e + r'\sigma^2_{GE}$
Error	e(g-1)(r-1)	MS_e	σ^2_e
Total	egr-1		

† varied depending upon the number of missing observations at each environment.

‡ g' and r' and e' denote means for genotypes and replications and environments, respectively.

Heritability Estimates

Broad sense heritability estimates for ergot vulnerability in the four testcross populations was calculated using the GLM procedure included in SPSS$^{®.}$ Heritability estimates are the ratio between the genotypic and the phenotypic variances of the population evaluated. Heritability (H^2) for ergot vulnerability for each environment was estimated using the following formula:

$$H^2 = \frac{\sigma^2_G}{\sigma^2_{G} + (\sigma^2_e / r')}$$

Where σ^2_G is the genotypic variance; σ^2_e is the error variance; and r' is the mean of replications.

Heritability (H) estimates for ergot vulnerability in the combined analyses were calculated using the following formula:

$$H^2 = \frac{\sigma^2_G}{\sigma^2_{G} + (\sigma^2_{GE} / r') + (\sigma^2_e / r'e')}$$

Where σ^2_G is the genotypic variance; σ^2_{GE} is the genotype by environment interaction variance, σ^2_e is the error variance; r' is the mean of replications and e' is the mean of environments.

Correlations

Combined ergot vulnerability mean ratings from the testcross populations were correlated with the combined ergot vulnerability mean from the respective IS8525J and IS8525D parent from the recombinant inbred line populations to see their relationship in ergot vulnerability.

Results and Discussion

Populations

Ergot was observed in all four populations at all four environments. Significant differences ($P < 0.05$) in ergot vulnerability were detected among the four populations (Table 27). In the combined analysis, the A1TCJ testcross population had significantly lower ergot vulnerability than any of the other testcross populations (Table 28). This was not consistent however, as the A1TCD population had lower ergot vulnerability scores in WE01 (Table 28).

Ergot severity followed the same pattern as ergot vulnerability. The A1TCJ testcross population had lower ergot severity ratings than A3TCJ, A1TCD and A3TCD testcross populations (Table 29). However, the A1TCD population performed better than A1TCJ in WE01 (Table 29). For ergot incidence, no significant differences ($P < 0.05$) were detected among the four populations (Table 27 and 30).

For the four populations in this study, the differences in ergot vulnerability are generally associated with comparisons between male-fertile and male-sterile test cross populations. In A1 cytoplasm, some of the testcross hybrids were restored to fertility. The presence of male fertility resulted in lower (P < 0.05) ergot vulnerability values than in the A3TCJ and A3TCD testcross hybrids, which were completely male sterile (Table 28). These results are consistent with previous ergot resistance studies. Futrell and Webster (1965) reported that ergot susceptibility is greatly influenced by male-fertility characteristics. Since ergot only attacks unfertilized ovaries, male-fertile genotypes are typically more resistant to ergot than male sterile genotypes because of pollen mediated escape. Also, this fully confirms the intermediate level of resistance reported by Dahlberg et al. (1998) and Reed (2002), when IS8525 was evaluated in a male sterile background.

Table 27. Calculated mean squares for the combined analysis of variance on ergot vulnerability, ergot severity and ergot incidence for A1TCJ, A3TCJ, A1TCD and A3TCD testcross sorghum populations at two environments.

| Source | df | Mean Squares† | | |
		Vulnerability	Severity	Incidence
ENV	1	100.19**	195.34**	0.00
REP(ENV)	2	0.00	0.05	0.00
POPULATIONS	3	8.59	16.73	0.00
ENV * POPULATION	3	5.62**	10.92**	0.00
ERROR	1013	0.27	0.62	

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 28. Ergot vulnerability† ratings for A1TCJ, A3TCJ, A1TCD and A3TCD testcross populations at two environments and the combined results for the four populations across environments and environments across the four populations.

Population	Environment		
	WE01	CS02	Combined‡
A1TCJ	2.68b(A)	1.56b(B)	2.12c
A3TCJ	2.80a(A)	2.22ac(B)	2.51b
A1TCD	2.56c(A)	2.08a(B)	2.32a
A3TCD	2.75ab(A)	2.34c(B)	2.54b
Combined§	2.69A	2.05B	

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

‡ Populations followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

§ Environment means followed by the same letter are not significantly different from each other at the 0.05 level (LSD)

Table 29.   Ergot severity† ratings for A1TCJ, A3TCJ, A1TCD and A3TCD testcross sorghum populations at two environments and the combined results for the four populations across environments and environments across the four populations.

	Environment		
Population	WE01	CS02	Combined‡
A1TCJ	4.82b	3.23b	4.02c
A3TCJ	4.99a	4.17ac	4.58a
A1TCD	4.66c	3.97a	4.31b
A3TCD	4.92ab	4.35c	4.63a
Combined§	4.84A	3.93B	

† Ergot severity ratings were made on a scale of 0 to 5 where 0 = 0%, 1 = 1-5%, 2= 6-10%, 3 = 11-25%, 4 = 26-50%, and 5 = 51-100%.

‡ Populations followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

§ Environment means followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

Table 30. Ergot incidence† ratings for A1TCJ, A3TCJ, A1TCD and A3TCD testcross sorghum populations at two environments and the combined results for both populations across environments and environments across the two populations.

	Environment		
Population	WE01	CS02	Combined‡
A1TCJ	100.00a	100.00a	100.00a
A3TCJ	100.00a	100.00a	100.00a
A1TCD	100.00a	100.00a	100.00a
A3TCD	100.00a	100.00a	100.00a
Combined§	100.00A	100.00A	

† Ergot incidence ratings are the percentage of panicles with at least one infection point on the panicle.

‡ Populations followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

§ Environment means followed by the same letter are not significantly different from each other at the 0.05 level (LSD).

Population by Environment Interaction

For ergot vulnerability there was a significant population by environment interaction at the 0.05 level (Table 27). This interaction was primarily due to a single shift in performance between A1TCD and A1TCJ populations in WE01, which was mentioned in the previous section (Table 28). There were no other shifts that were of significantly importance. An identical trend for population x environment interaction was observed for ergot severity (Table 27). Since ergot incidence was 100% in all environments, there was no population x environment interactions detected for ergot incidence (Table 27 and 30).

Environments

Significant differences (P < 0.05) were detected among environments for ergot vulnerability and ergot severity across environments (Table 27). This observation was expected as environments have a significant effect on ergot development. Because ergot incidence was high in all environments there were no significant differences (P < 0.05) among environments (Table 27 and 30).

The environment WE01 had much higher ergot vulnerability ratings than CS02 (Table 28). Because this was a fall season environment, temperatures at anthesis were cool and with consistent rainfall, the environment was very favorable for the development of the pathogen. In CS02, low to intermediate ergot vulnerability ratings were observed.

Heritability Estimates

Broad-sense Heritability (H^2) estimates for ergot vulnerability at individual environments and combined analysis varied widely. The combined H^2 estimates were higher for the fertile hybrids than for the sterile hybrids (Table 31). Heritabilities in single environments were relatively high, but decreased substantially in combined analysis due to the significant interaction variation and differential response of testcross hybrids. Heritabilities in A1 cytoplasm were generally higher than those in A3 cytoplasm, presumably because of the additional tolerance provided by male fertility. This likely increased the range of variation and consequently the heritability of the trait. In A3 cytoplasm, heritability estimates were low indicating that selection for tolerance in male sterile lines will be difficult. The combined analysis results in this study indicate that ergot resistance is heritable and can be transmitted to male-sterile and male-fertile progeny. This resistance is not stable across environments. We can see that ergot resistance is not maintained under cooler conditions such as WE01. Also, the resistance to ergot is variable in environments with warm temperatures such as CS02.

Correlation Between Inbred and Hybrid Performance

The combined Pearson's correlation coefficient between recombinant inbred lines populations and test crosses populations for ergot vulnerability was 0.096 (P< 0.05) (Table 32). While this correlation is significant, it is of little practical value. The low correlation indicates that if selection is to be practiced to improve ergot tolerance in the male sterile lines, it will be necessary to evaluate the ergot tolerance in a sterile testcross

because line per se evaluation is not predictable. This correlation suggests that a susceptible IS8525 recombinant inbred line will produce a susceptible hybrid when test crossed with ATX623 and A3TX623. In addition, different relationships between recombinant inbred lines and testcrosses may change with the use of a different tester.

Variation Within A1TCJ Population

 Weslaco 2001 (WE01)

  Ergot vulnerability ratings within the A1TCJ testcross population were strongly skewed to highly susceptible. Only a few genotypes had ratings less than 5 (Fig. 3). However, differences in vulnerability were detected among individuals of the A1TCJ testcross population (Table 33). In addition, transgressive segregation was observed. There were testcrosses with both higher and lower ergot vulnerability ratings than the hybrids of either parent (IS8525J or ATX623) (data not shown). Also, significant differences were detected between A1TCJ and checks for ergot vulnerability (Table 34).

Table 31. Ergot vulnerability† variance components coefficients and broad sense

heritability (H^2) estimates for A1TCJ, A3TCJ, A1TCD and A3TCD testcross sorghum

populations by individual and combined environment at WE01 and CS02.

| Environment | Population | Variance component coefficients | | | | |
		σ^2_G	σ^2_E	σ^2_{GE}	σ^2_e	H^2
WE01	A1TCJ	0.09	.	.	0.06	0.72
CS02	A1TCJ	0.19	.	.	0.33	0.53
Combined	A1TCJ	0.12	0.59	0.02	0.22	0.75
WE01	A3TCJ	0.00	.	.	0.00	0.00
CS02	A3TCJ	0.07	.	.	0.34	0.27
Combined	A3TCJ	0.01	0.16	0.03	0.16	0.04
WE01	A1TCD	0.08	.	.	0.11	0.60
CS02	A1TCD	0.27	.	.	023	0.69
Combined	A1TCD	0.03	0.11	0.15	0.17	0.33
WE01	A3TCD	0.11	.	.	0.01	0.96
CS02	A3TCD	0.07	.	.	0.23	0.36
Combined	A3TCD	0.01	0.07	0.06	0.12	0.21

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot

severity and incidence ratings measured in each environment.

Table 32. Ergot vulnerability Pearson's correlation coefficients between IS8525 recombinant inbred lines and four testcross population.

		Ergot vulnerability RILs	Ergot vulnerability testcrosses
Ergot vulnerability RILs	Pearson Correlation	1	.096†
	Sig. (2-tailed)	.	.003
	N	1122	984
Ergot vulnerability testcrosses	Pearson Correlation	.096†	1
	Sig. (2-tailed)	.003	.
	N	984	1023

† Correlation is significant at the 0.01 level (2-tailed).

For ergot severity, the highest value for the A1TCJ testcross population was also observed in WE01 with an average ergot severity rating of 4.82. The population showed transgressive segregation and significant differences in severity among individuals of the A1TCJ testcross population (Table 33). There were testcrosses with both higher and lower ergot vulnerability ratings than the hybrids of either parent (IS8525J or ATX623) (data not shown). Also, significant differences were detected among A1TCJ and checks for ergot severity (Table 35). For the variable incidence, every hybrid had ergot incidences of 100.00 %. Because no variation was detected, there were no differences in ergot incidence (Table 33 and 36).
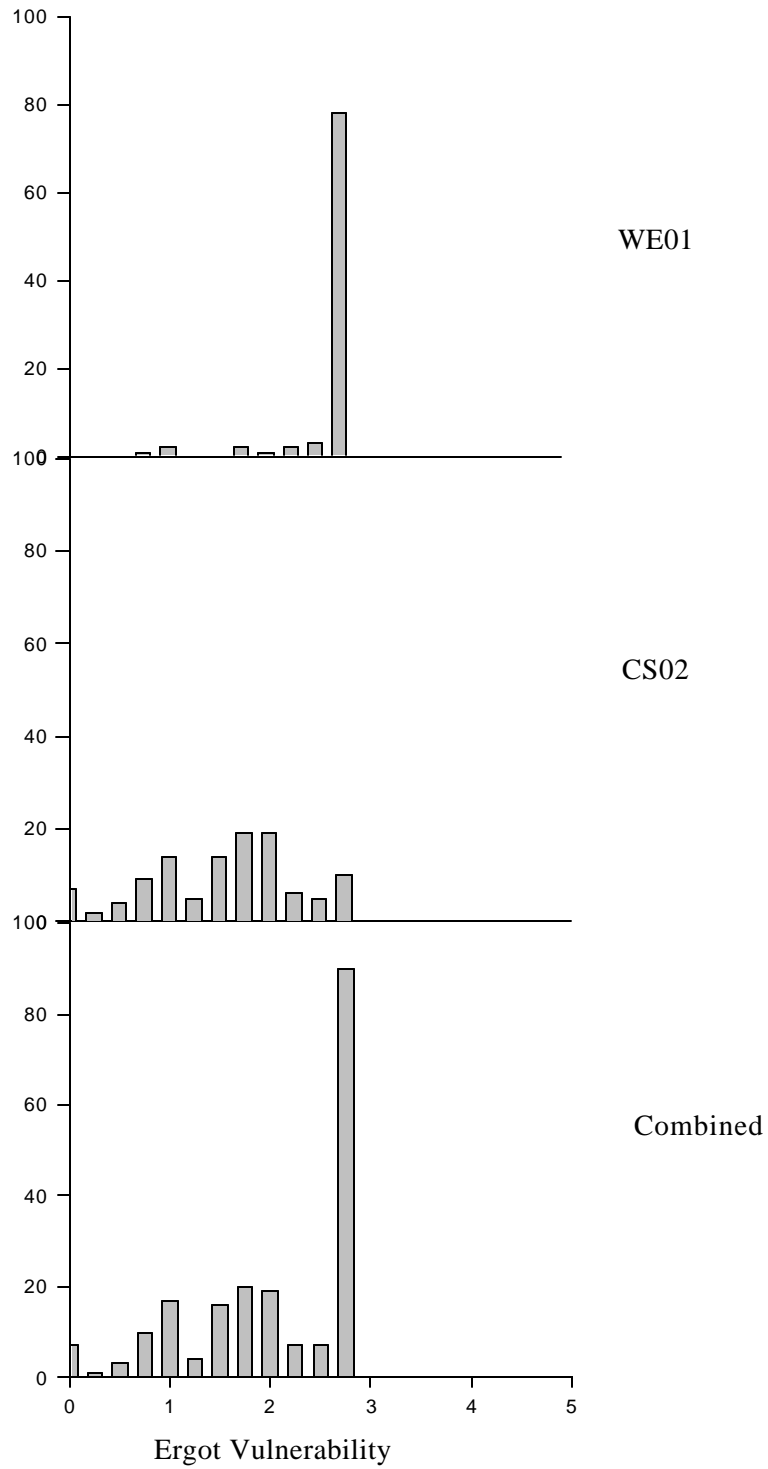
Figure 3: Distribution of ergot vulnerability ratings for the test cross population derived from the cross of ATX623/IS8525J recombinant inbred lines (A1TCJ).

Table 33. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the ATX623/IS8525J recombinant inbred lines cross (A1TCJ) at WE01.

| Source | df | Mean Squares† | | |
		Vulnerability	Severity	Incidence
Hybrids	21	0.40**	0.82**	0.00
REP	1	0.01	0.02	0.00
Error	64	0.06	0.14	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 34. Ergot vulnerability† combined ratings for A1TCJ, A3TCJ, A1TCD and A3TCD testcross sorghum populations and checks across environments, and environments across A1TCJ, A3TCJ, A1TCD and A3TCD populations and checks.

	Environment		
Population	WE01	CS02	Combined
A1TCJ	2.68	1.56	2.12
A3TCJ	2.80	2.22	2.51
A1TCD	2.56	2.08	2.32
A3TCD	2.75	2.34	2.55
ATX623	2.80	1.88	2.34
A3TX623	2.80	2.35	2.58
ATX623 x BTX643	2.75	1.85	2.30
ATX623 x IS8525D	2.58	1.56	2.07
ATX623 x IS8525J	2.57	1.75	2.16
ATX623 x RTX430	2.11	1.48	1.80
ATX623 x RTX432	1.80	0.77	1.28
ATX623 x RTX436	2.24	0.78	1.51
A3TX623 x BTX643	2.80	2.18	2.49
A3TX623 x IS8525D	2.80	1.75	2.27
A3TX623 x IS8525J	2.80	2.73	2.77
A3TX623 x RTX430	2.47	2.38	2.42
A3TX623 x RTX432	1.52	1.77	1.64
A3TX623 x RTX436	2.31	2.75	2.53
Combined	2.51A	1.90B	

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

§ Environment means followed by the same capital letter are not significantly different from each other at the 0.05 level (LSD).

Table 35. Ergot severity† combined ratings for A1TCJ, A3TCJ, A1TCD and A3TCD testcross sorghum populations and checks across environments, and environments across A1TCJ, A3TCJ, A1TCD and A3TCD populations and checks.

	Environment		
Population	WE01	CS02	Combined
A1TCJ	4.82	3.23	4.02
A3TCJ	4.99	4.17	4.58
A1TCD	4.66	3.97	4.32
A3TCD	4.92	4.35	4.63
ATX623	5.00	3.68	4.34
A3TX623	5.00	4.36	4.68
ATX623 x BTX643	4.93	3.65	4.29
ATX623 x IS8525D	4.69	3.22	3.95
ATX623 x IS8525J	4.67	3.50	4.08
ATX623 x RTX430	4.01	3.11	3.56
ATX623 x RTX432	3.57	2.10	2.83
ATX623 x RTX436	4.20	2.11	3.15
A3TX623 x BTX643	5.00	4.11	4.55
A3TX623 x IS8525D	5.00	3.50	4.25
A3TX623 x IS8525J	3.57	4.90	4.95
A3TX623 x RTX430	4.52	4.40	4.46
A3TX623 x RTX432	3.17	3.52	3.35
A3TX623 x RTX436	4.30	4.92	4.61
Combined	4.58A	3.71B	

† Ergot severity ratings were made on a scale of 0 to 5 where 0 = 0%, 1 = 1-5%, 2= 6-10%, 3 = 11-25%, 4 = 26-50%, and 5 = 51-100%.

§ Environment means followed by the same capital letter are not significantly different from each other at the 0.05 level (LSD).

Table 36. Ergot incidence† combined ratings for A1TCJ, A3TCJ, A1TCD and A3TCD testcross sorghum populations and checks across environments, and environments across A1TCJ, A3TCJ, A1TCD and A3TCD populations and checks.

	Environment		
Population	WE01	CS02	Combined
A1TCJ	100.00	100.00	100.00
A3TCJ	100.00	100.00	100.00
A1TCD	100.00	100.00	100.00
A3TCD	100.00	100.00	100.00
ATX623	100.00	100.00	100.00
A3TX623	100.00	100.00	100.00
ATX623 x BTX643	100.00	100.00	100.00
ATX623 x IS8525D	100.00	100.00	100.00
ATX623 x IS8525J	100.00	100.00	100.00
ATX623 x RTX430	100.00	100.00	100.00
ATX623 x RTX432	100.00	100.00	100.00
ATX623 x RTX436	100.00	100.00	100.00
A3TX623 x BTX643	100.00	100.00	100.00
A3TX623 x IS8525D	100.00	100.00	100.00
A3TX623 x IS8525J	100.00	100.00	100.00
A3TX623 x RTX430	100.00	100.00	100.00
A3TX623 x RTX432	100.00	100.00	100.00
A3TX623 x RTX436	100.00	100.00	100.00
Combined	**100.00A**	**100.00A**	

† Ergot incidence ratings are the percent of panicles with at least one infection point on the panicle.

§ Environment means followed by the same capital letter are not significantly different from each other at the 0.05 level (LSD).

College Station 2002 (CS02)

The average ergot vulnerability rating for A1TCJ testcross population was 1.56 and the population was widely and normally distributed around the mean (Fig. 3). Differences in vulnerability were detected among individual testcross hybrids of the A1TCJ population (Table 37). Transgressive segregation in both directions was observed in the testcross hybrids. Also, significant differences were detected between A1TCJ and checks for ergot vulnerability (Table 34).

For the variable severity, the A1TCJ testcross population had an average ergot severity rating of 3.23 and significant differences in severity were detected among individuals of the A1TCJ testcross population (Table 37). Transgressive segregation for ergot severity was observed. Also, significant differences were detected between A1TCJ and checks for ergot severity (Table 35). No variation was detected for ergot incidence.

Combined Analysis

Ergot was observed in both environments. Ergot incidences in both locations were consistently 100% (Table 36). Significant differences for ergot vulnerability and severity ($P < 0.05$) were detected between environments. In addition a significant genotype by environment interaction was detected for ergot vulnerability (Table 38). As mentioned earlier ergot resistance was not stable across environments indicating that the performance of the A1TCJ hybrids differed across environments. Close evaluation of the performance of individual hybrids indicates that many hybrids were extremely variable in their response across environments (Table 39)

Table 37. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the ATX623/IS8525J recombinant inbred lines cross (A1TCJ) at CS02.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
Hybrid	27	1.09**	2.23**	0.00
Rep	1	2.25**	4.58**	0.00
Error	83	0.33	0.68	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 38. Calculated mean squares for the combined analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the ATX623/IS8525J recombinant inbred lines cross (A1TCJ) at two environments.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
ENV	1	52.23**	106.30**	0.00
REP(ENV)	2	1.13**	2.30**	0.00
A1TCJ	27	1.15**	2.35**	0.00
ENV * A1TCJ	21	0.31**	0.64	0.00
ERROR	147	0.22	0.44	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 39. Ergot vulnerability† combined ratings (with their relative rank in parentheses) for the testcross hybrids derived from the ATx623/IS8525J recombinant inbred lines cross at two environments.

	Environment		
Testcross Hybrid from Entry	WE01	CS02	Combined
25	$1.58^{(1)}$	$0.35^{(1)}$	$0.96^{(1)}$
26	$1.78^{(2)}$	$0.56^{(2)}$	$1.17^{(2)}$
18	$2.52^{(4)}$	$1.09^{(5)}$	$1.8^{(3)}$
21	$2.48^{(3)}$	$1.35^{(9)}$	$1.92^{(4)}$
13	$2.8^{(7)}$	$1.63^{(6)}$	$2.01^{(5)}$
17	$2.8^{(7)}$	$1.25^{(7)}$	$2.02^{(6)}$
ATX623 x IS8525J	$2.57^{(5)}$	$1.75^{(15)}$	$2.16^{(15)}$
ATX623 x BTX643	$2.75^{(6)}$	$1.85^{(21)}$	$2.30^{(20)}$

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

‡ The superscript number indicates the line ranking in that particular environment.

§ The superscript number indicates the line ranking across environments.

The level of disease observed in the A1TCJ testcross population in the overall population analysis across environments was similar to the trend of the A1TCJ population in the individual analysis.

The ergot vulnerability ratings for the A1TCJ population were higher in WE01, than in CS02 (Table 34). The combined ergot vulnerability distribution of the A1TCJ testcross population was a curve skewed to the right (Fig. 3). Also, significant differences were detected among the combined ergot vulnerability mean of the A1TCJ test cross population and checks (Table 34).

For ergot severity, the same trend as ergot vulnerability was observed with the higher ergot severity rating in WE01. Significant differences were detected between the combined ergot severity mean of the A1TCJ population and the combined ergot severity mean of the checks (Table 35).

Variation Within A3TCJ Population

Weslaco 2001 (WE01)

Ergot vulnerability for the A3TCJ population averaged 2.80 and the distribution of genotypes was strongly skewed to highly susceptible. Very few genotypes were rated as a 4 or less (Fig. 4). Because the majority of hybrids were extremely susceptible, no significant differences in vulnerability were detected among hybrids in the A3TCJ testcross population (Table 40)
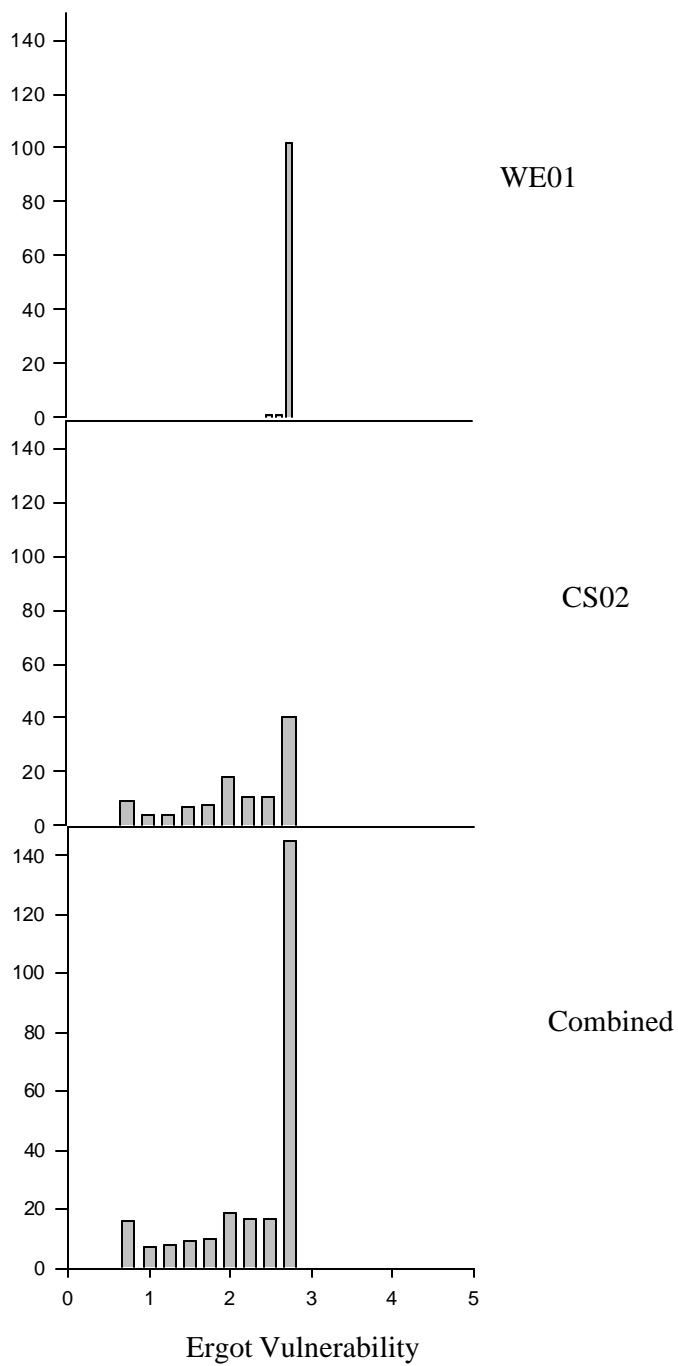
Figure 4. Distribution of ergot vulnerability ratings for the testcross population
derived from the cross of A3TX623/IS8525J recombinant inbred lines (A3TCJ).

Ergot severity in the A3TCJ averaged 4.99 and due to the extreme level of severity, no significant differences in severity were detected among individuals of the A3TCJ testcross population (Table 40). No significant differences were detected between the A3TCJ population and checks for ergot severity (Table 35). For the variable incidence, the A3TCJ testcross population had an average ergot incidence of 100.00 %. No significant differences in ergot incidence were detected among individuals of the A3TCJ testcross population (Table 40). No significant differences were detected among the A3TCJ testcross population and checks for ergot incidence (Table 36).

Table 40. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the A3TX623/IS8525J recombinant inbred lines cross (A3TCJ) at WE01.

		Mean Squares†		
Source	df	Vulnerability	Severity	Incidence
A3TCJ	25	0.00	0.00	0.00
REP	1	0.00	0.00	0.00
Error	77	0.00	0.00	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

College Station 2002 (CS02)

In CS02, the A3TCJ testcross population had an average ergot vulnerability rating of 2.22. However, ergot vulnerability ratings within A3TCJ in CS02 were variable (Fig. 4). The ergot vulnerability ratings distribution in CS02 tended to be normal compared to the one at WE01. Significant differences in vulnerability were detected among individual of the A3TCJ testcross population (Table 41). In addition, transgressive segregation was observed in CS02. There were testcrosses with similar, higher and lower ergot vulnerability ratings than the hybrids created from the original parents. Also, significant differences were detected between A3TCJ and checks for ergot vulnerability (Table 34).

For the variable severity, the A3TCJ testcross population had an average ergot severity rating of 4.17. Significant differences in severity were detected among individuals of the A3TCJ testcross population (Table 41). Also, for ergot severity in CS02, transgressive segregation was observed. There were testcrosses with similar, higher and lower ergot severity ratings than the hybrids created from original parents. Also, significant differences were detected between A3TCJ and checks for ergot severity (Table 34).

For ergot incidence, the A3TCJ testcross population had an average ergot incidence of 100.00% and due to the lack of variation, no differences in ergot incidence were detected among entries or checks in this test (Table 36 and 40).

Table 41. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the A3TX623/IS8525J recombinant inbred lines cross (A3TCJ) at CS02.

		Mean Squares†		
Source	df	Vulnerability	Severity	Incidence
A3TCJ	27	0.61**	1.25**	0.00
REP	1	0.51	1.04	0.00
Error	73	0.33	0.68	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Combined Analysis

The ergot vulnerability ratings for the A3TCJ population were higher in WE01 than in CS02 (Table 34). In addition a significant genotype by environment interaction was detected for ergot vulnerability (Table 42). As mentioned before ergot resistance is not stable across environments indicating that the performance of the A3TCJ hybrids differed across environments. Close evaluation of the performance of individual hybrids indicates that many hybrids were extremely variable in their response across environments (Table 43).

The combined ergot vulnerability distribution of the A3TCJ testcross population was a curve skewed to the right (Fig. 4). Significant differences were detected among the combined ergot vulnerability mean of the A3TCJ test cross population and checks (Table 34). For ergot severity, the same trend as ergot vulnerability was observed with the highest ergot severity rating in WE01 (Table 35). Significant differences were detected between the combined ergot severity mean of the A3TCJ population and the combined ergot severity mean of the checks (Table 35). For ergot incidence, both environments were equally high and therefore, no significant differences were detected between the mean of the A3TCJ testcross population and the ergot incidence mean of the checks (Table 36 and 41).

Table 42. Calculated mean squares for the combined analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the A3TX623/IS8525J recombinant inbred lines cross (A3TCJ) at two environments.

| | | Mean Squares† | | |
Source	df	Vulnerability	Severity	Incidence
Environment	1	52.23**	106.30**	0.00
Rep (Environment)	2	1.13**	2.30**	0.00
Hybrids	27	1.15**	2.35**	0.00
Hybrids * Environment	25	0.31**	0.64	0.00
Error	150	0.22	0.44	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 43. Ergot vulnerability† combined ratings (with their relative rank in parentheses) for the testcross hybrids derived from the A3Tx623/IS8525J recombinant inbred lines cross at two environments.

Testcross Hybrid derived from Entry	Environment		
	WE01	CS02	Combined
35	$2.76^{(2)}$	$1.47^{(1)}$	$2.12^{(1)}$
29	$2.80^{(3)}$	$1.53^{(2)}$	$2.17^{(2)}$
23	$2.80^{(3)}$	$1.56^{(3)}$	$2.18^{(3)}$
8	$2.80^{(3)}$	$1.60^{(4)}$	$2.20^{(4)}$
26	$2.80^{(3)}$	$1.70^{(5)}$	$2.25^{(5)}$
24	$2.80^{(3)}$	$1.75^{(6)}$	$2.27^{(6)}$
A3TX623 x BTX643	$2.80^{(3)}$	$2.18^{(13)}$	$2.49^{(12)}$
A3TX623 x IS8525J	$2.80^{(3)}$	$2.73^{(26)}$	$2.77^{(25)}$

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

‡ The superscript number indicates the line ranking in that particular environment.

§ The superscript number indicates the line ranking across environments.

Variation Within A1TCD Population

Weslaco 2001 (WE01)

The distribution of the ergot vulnerability ratings within A1TCD testcross were strongly skewed toward susceptibility (Fig. 5). Significant differences in vulnerability were detected among individual of the A1TCD testcross population (Table 44). Transgressive segregation was observed as there were testcrosses with similar, higher and lower ergot vulnerability ratings than the hybrids created from the original parents.

For ergot severity, significant differences in severity were detected among individuals of the A1TCD testcross population and transgressive segregation was observed (Table 44). There were testcrosses with similar, higher and lower ergot severity ratings than the hybrids created from the original parents. Significant differences were detected between A1TCD and checks for ergot severity (Table 34). For the variable incidence, the A1TCD testcross population had an average ergot incidence of 100% and no significant differences in ergot incidence were detected (Table 44).
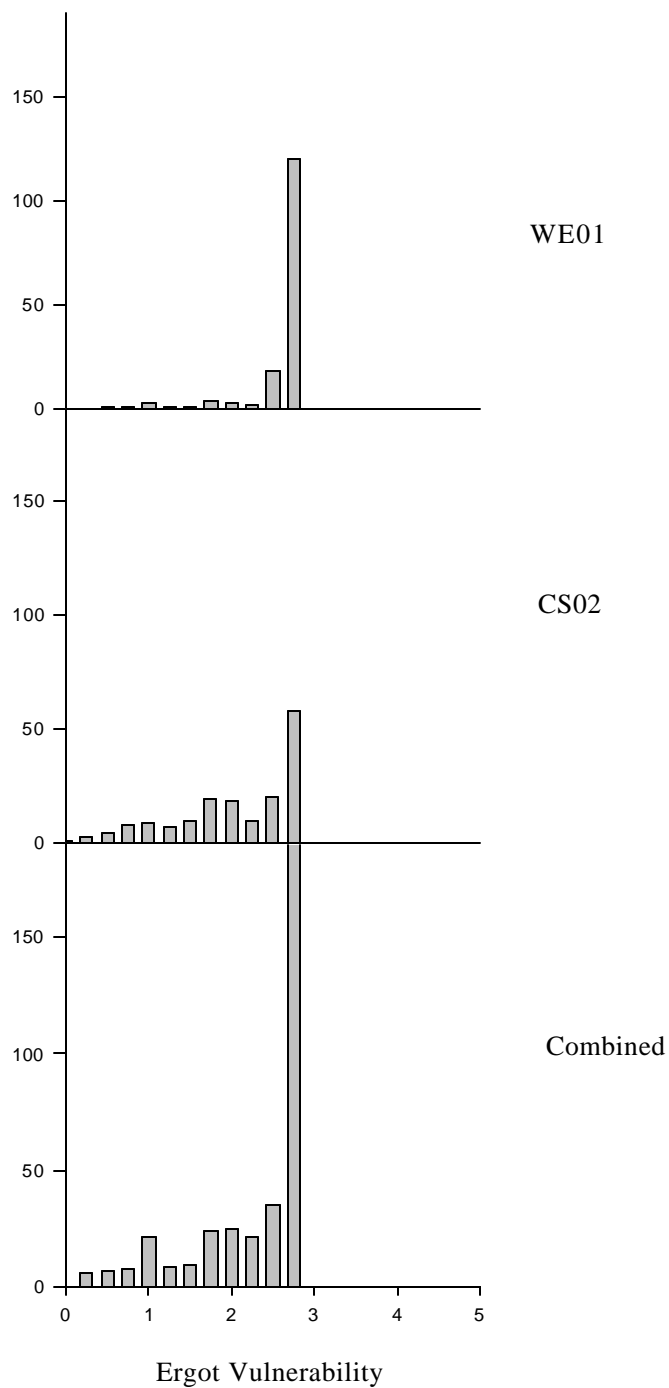
Figure 5. Distribution of ergot vulnerability ratings for the testcross population deived from the cross of ATX623/IS8525D recombinant inbred lines (A1TCD)

Table 44. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the ATX623/IS8525D recombinant inbred lines cross (A1TCD) at WE01.

| Source | df | Mean Squares† | | |
		Vulnerability	Severity	Incidence
A1TCD	40	0.44**	0.90**	0.00
REP	1	0.00	0.01	0.00
Error	121	0.11	0.23	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

College Station 2002 (CS02)

In CS02, the A1TCD testcross population ergot vulnerability averaged 2.08. The distribution of hybrids was slightly skewed toward increased susceptibility (Fig. 5). Significant differences in vulnerability were detected among the hybrids in the A1TCD testcross population (Table 45). In this variation, transgressive segregation with testcrosses with similar, higher and lower ergot vulnerability ratings than the hybrids created from the original parents was observed. Significant differences were detected between A1TCD and checks for ergot vulnerability (Table 34).

For ergot severity, the A1TCD testcross population averaged 3.97. Significant differences in severity, including transgressive segregation, were detected among individuals of the A1TCD testcross population (Table 45). Significant differences were detected between A1TCD and checks for ergot severity (Table 35). Ergot incidence was consistently 100% and thus there was no significant variation (Table 45). No significant differences were detected among the A1TCD testcross population and checks for ergot incidence (Table 36).

Table 45. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the ATX623/IS8525D recombinant inbred lines cross (A1TCD) at CS02.

| Source | df | Mean Squares† | | |
		Vulnerability	Severity	Incidence
A1TCD	39	1.32**	2.69**	0.00
REP	1	0.00	0.00	0.00
Error	115	0.24	0.48	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Combined Analysis

Significant differences (P < 0.05) were detected between environments. In addition a significant genotype by environment interaction was detected for ergot vulnerability (Table 46). As mentioned before ergot resistance is not stable across environments indicating that the performance of the A1TCD hybrids differed across environments. Close evaluation of the performance of individual hybrids indicates that many hybrids were extremely variable in their response across environments (Table 47).

The level of disease observed in the A1TCD testcross population in the overall population analysis across environments was similar to the trend of the A1TCD population in the individual environment analysis.

The ergot vulnerability ratings for the A1TCD population were higher in WE01 than in CS02 (Table 34). The combined ergot vulnerability distribution of the A1TCD testcross population was strongly skewed to increase susceptibility (Fig. 5). Significant differences for ergot vulnerability were detected among the A1TCD test cross population and checks (Table 34).

For ergot severity, trends were similar to those of ergot vulnerability (Table 35). Significant differences were detected between the combined ergot severity mean of the A1TCD population and the combined ergot severity mean of the checks (Table 35). For ergot incidence, both environments were equal with high values of ergot incidence (Table 36) and no significant differences were detected.

Table 46. Calculated mean squares for the combined analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the ATX623/IS8525D recombinant inbred lines cross (A1TCD) at two environments.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
Environment	1	17.68**	35.99**	0.00
Rep (Environment)	2	0.00	0.00	0.00
Hybrid	40	0.98	2.00	0.00
Hybrid * Environment	39	0.77**	1.57**	0.00
Error	236	1.77	0.36	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 47. Ergot vulnerability† combined ratings (with their relative rank in parentheses) for the testcross hybrids derived from the ATx623/IS8525D recombinant inbred lines cross at two environments.

Testcross Hybrid derived from Entry	Environment		
	WE01	CS02	Combined
27	$1.12^{(1)}$	$1.4^{(5)}$	$1.26^{(1)}$
18	$2.31^{(7)}$	$0.42^{(1)}$	$1.37^{(2)}$
15	$2.17^{(5)}$	$1.13^{(3)}$	$1.65^{(3)}$
36	$2.76^{(26)}$	$0.56^{(2)}$	$1.66^{(4)}$
38	$1.85^{(3)}$	$1.75^{(11)}$	$1.80^{(5)}$
16	$2.73^{(25)}$	$1.30^{(4)}$	$2.02^{(6)}$
ATX623 x IS8525D	$2.58^{(13)}$	$1.56^{(7)}$	$2.07^{(7)}$
ATX623 x BTX643	$2.75^{(26)}$	$1.85^{(14)}$	$2.30^{(15)}$

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

‡ The superscript number indicates the line ranking in that particular environment.

§ The superscript number indicates the line ranking across environments.

Variation Within A3TCD Population

Weslaco 2001 (WE01)

Ergot vulnerability ratings were high in WE01, averaging 2.75 and the distribution for the A3TCD population was strongly skewed toward increased susceptibility (Fig. 6). Significant differences in vulnerability, including transgressive segregation, were detected among individual of the A3TCD testcross population (Table 48). Significant differences were detected between A3TCD and checks for ergot vulnerability (Table 34).

For ergot severity, the A3TCD testcross population mean was 4.92. Significant differences in severity were detected among individuals of the A3TCD testcross population (Table 48). Significant differences were also detected between A3TCD and checks for ergot severity (Table 35). For ergot incidence, no variation was present as ergot incidence was 100% (Table 48). No significant differences were detected among the A3TCD testcross population and checks for ergot incidence (Table 36).
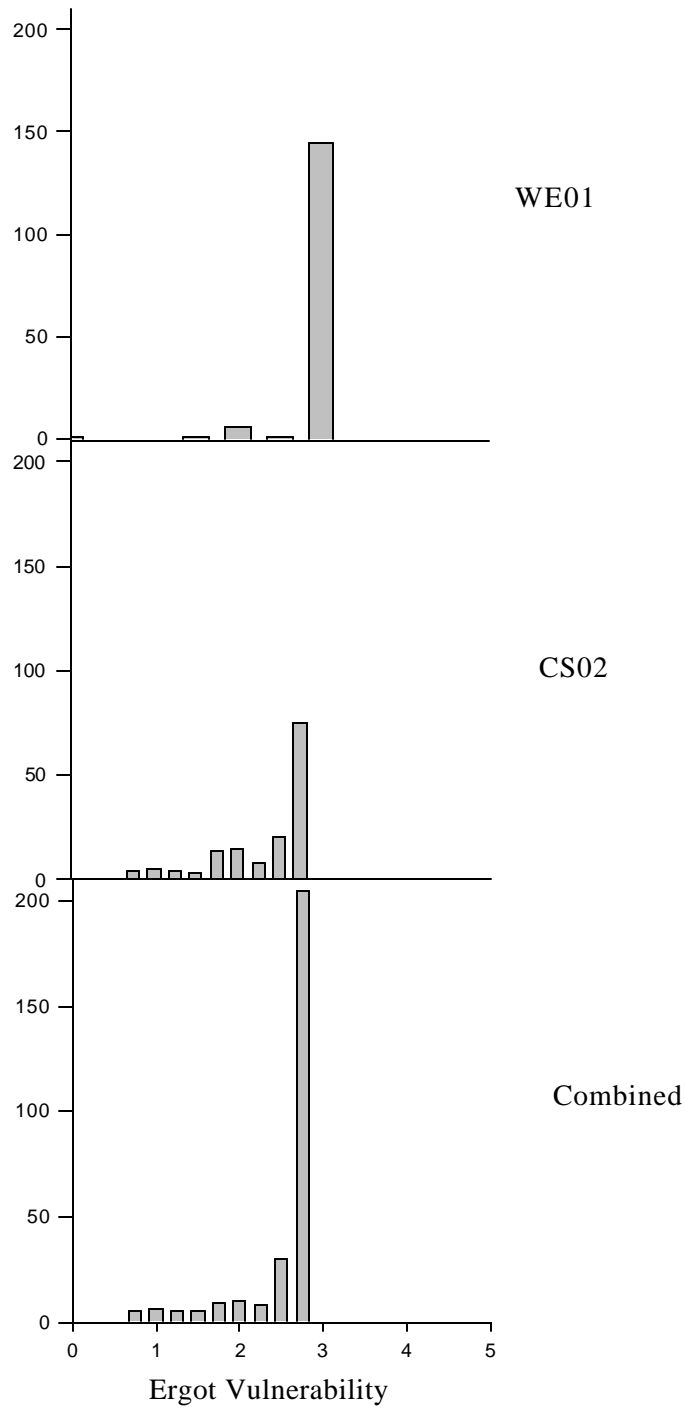
Figure 6. Distribution of ergot vulnerability ratings for the testcross population
derived from the cross of A3TX623/IS8525D recombinant inbred lines (A3TCD)

Table 48. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the A3TX623/IS8525D recombinant inbred lines cross (A3TCD) at WE01.

		Mean Squares†		
Source	df	Vulnerability	Severity	Incidence
A3TCD	38	0.26**	0.54**	0.00
REP	1	0.00	0.00	0.00
Error	114	0.00	0.01	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

College Station 2002 (CS02)

In CS02, the A3TCD testcross population ergot vulnerability rating averaged 2.34 and the distribution of hybrids around the mean was skewed toward increased susceptibility (Fig. 6). Significant differences in vulnerability, including transgressive segregation, were detected among individual of the A3TCD testcross population (Table 49). Significant differences were detected between A3TCD and checks for ergot vulnerability (Table 34). Similar trends were seen in ergot severity (Table 35). No variation existed for ergot incidence as all entries had 100% incidence. Consequently, no differences existed (Table 49).

Combined Analysis

Significant differences (P < 0.05) were detected between environments. In addition a significant genotype by environment interaction was detected for ergot vulnerability (Table 50). As mentioned before ergot resistance is not stable across environments indicating that the performance of the A3TCD hybrids differed across environments. Close evaluation of the performance of individual hybrids indicates that many hybrids were extremely variable in their response across environments (Table 51).

The level of disease observed in the A3TCD testcross population in the overall population analysis across environments was similar to the trend of the A3TCD population in the individual analysis.

Table 49. Calculated mean squares for the analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the A3TX623/IS8525D recombinant inbred lines cross (A3TCD) at CS02.

		Mean Squares†		
Source	df	Vulnerability	Severity	Incidence
A3TCD	37	0.53**	1.07**	0.00
REP	1	0.77	1.57	0.00
Error	106	0.24	0.48	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

Table 50. Calculated mean squares for the combined analysis of variance on ergot vulnerability, ergot severity and ergot incidence for the testcross population derived from the A3TX623/IS8525D recombinant inbred lines cross (A3TCD) at two environments.

Source	df	Mean Squares†		
		Vulnerability	Severity	Incidence
ENV	1	12.71**	25.86**	0.00
REP(ENV)	2	0.38**	0.78**	0.00
A3TCD	38	0.51**	1.04**	0.00
ENV * A1TCD	37	0.28**	0.58**	0.00
ERROR	220	0.12	0.24	0.00

** Significantly different from zero at the 0.05 probability level.

† Based on type III sum of squares.

The ergot vulnerability ratings for the A3TCD population were higher in WE01 (Table 34). The combined ergot vulnerability distribution of the A3TCD testcross population was a curve skewed toward increased susceptibility (Fig. 6). Also, significant differences were detected among the combined ergot vulnerability mean of the A3TCD test cross population and checks (Table 34). Similar trends were observed for ergot severity (Table 35). Significant differences were detected between the combined ergot severity mean of the A3TCD population and the combined ergot

severity mean of the checks (Table 35). All entries had ergot incidences of 100%, therefore, no variation was available for analysis (Table 36).

Table 51. Ergot vulnerability† combined ratings (relative rank in each environment in parentheses) for the testcross hybrids derived from the A3Tx623/IS8525D recombinant inbred lines cross at two environments.

	Environment		
Testcross Hybrid Derived from Entry	WE01	CS02	Combined
10	2.80$^{(4)}$	1.12$^{(1)}$	1.96$^{(1)}$
48	1.98$^{(1)}$	2.2$^{(14)}$	2.09$^{(2)}$
8	2.80$^{(4)}$	1.71$^{(2)}$	2.26$^{(3)}$
A3TX623 x IS8525D	2.80$^{(4)}$	1.75$^{(3)}$	2.27$^{(4)}$
41	2.80$^{(4)}$	1.85$^{(4)}$	2.33$^{(5)}$
22	2.80$^{(4)}$	1.91$^{(5)}$	2.36$^{(6)}$
47	2.80$^{(4)}$	1.92$^{(6)}$	2.36$^{(6)}$
A3TX623 x BTX643	2.80$^{(4)}$	2.18$^{(10)}$	2.49$^{(14)}$

† Ergot vulnerability ratings were on a scale developed by the factor analysis of ergot severity and incidence ratings measured in each environment.

‡ The superscript number indicates the line ranking in that particular environment.

§ The superscript number indicates the line ranking across environments.

Conclusions

Ergot was prevalent throughout the hybrid trials, primarily due to male sterility in the hybrids. The lack of viable pollen increased the incidence to 100%, effectively eliminating this variable as a useful measure. The high incidence also confirmed that the tolerance in IS8525 is mostly pollen mediated, as the even in hybrids of IS8525, ergot incidences were 100%.

Because ergot incidence was not an informative measurement of ergot tolerance, ergot severity and vulnerability ratings were effectively similar in their response. Therefore, there is relatively little value in the vulnerability rating over severity: either rating could be used to measure ergot tolerance. Variation for both severity and vulnerability were detected and measurable, however, both indicated a significant shift of most entries to susceptibility. There were relatively few testcross hybrids with any useful level of ergot tolerance. The ergot tolerance in the A1 testcross hybrids was slightly better than that seen in the A3 testcrosses, primarily due to the presence of some partially fertile A1 testcross hybrids. All findings indicate that the ergot tolerance in IS8525 is predominantly pollen mediated.

Hybrid x environment interactions was significant in the combined analysis, indicating that at least some hybrids performed differently across the two environments. Closer examination of the best testcross hybrids indicated that their responses were different across environments, which indicates selection for stable response across environments would be difficult even if the trait were heritable.

Heritability estimates within an environment were quite variable, but heritability estimates from combining the environments were generally quite low (Table 31). The mixed results in individual environments and low heritability in combined analysis indicate that genotype x environment interactions are substantial and maybe difficult to control. These results agree with results reported by Reed et al. (2002). In addition, this is a broad-sense heritability estimate from testcross hybrids, meaning that this estimate will include both additive and dominant genetic variance. Since dominance variance is not heritable, the actual heritabilities of this trait are even lower. This observation is confirmed by the relatively low correlation between line per se and testcross hybrid ergot severity and vulnerability ratings. These results indicate that selection for ergot tolerance will be difficult and the evaluation of any potential gains in tolerance must be confirmed in male sterile testcrosses.

Nevertheless, transgressive segregants were identified that had increased ergot tolerance in both the line per se and in the testcross population. In a few cases, the line per se and the respective testcross hybrid both showed increased tolerance. This germplasm maybe useful to actually determine the potential gain from selection for ergot tolerance.

CHAPTER V

SUMMARY

Evaluations of the original parents indicate that ergot tolerance in IS8525D parent was consistently better than that in IS8525J parent. As expected, neither parent provided complete resistance, but rather a higher level of tolerance when compared to commonly grown parental lines. Thus, the results of Dahberg et al. (1998) and Reed et al. (2002) are confirmed herein.

In the RILs from each parent, similar trends for the population as a whole were observed. The IS8525J recombinant inbred line population showed significantly more ergot susceptibility than the IS8525D recombinant inbred line population and this trend was consistent across environments. One possible reason for the differences in ergot susceptibility between the two populations is male sterility, which was encountered in the IS8525J population. However, this cannot explain all of the increased susceptibility because IS8525J (which is completely fertile) also showed higher levels of ergot susceptibility.

Variation for ergot vulnerability among recombinant inbred lines for both populations was detected, but the amount of variability was environment dependent. For example, no variation was detected in Puerto Rico and the results from Corpus Christi were inconsistent. In addition, the performance of individual entries in both populations differed across environments as indicated by a significant genotype by environment

interaction.   This indicates that a consistent response for ergot tolerance across environments should not be expected.

Entries with greater levels of ergot tolerance were observed in the evaluation of the recombinant inbred lines, indicating that this germplasm maybe useful to determine the potential gain from selection to ergot resistance. However, the heritability studies in both populations indicated that the level of heritability is highly dependent on the environment.

In the testcross hybrids, all four populations were susceptible to ergot, primarily due to male sterility in the hybrids, confirming that the tolerance shown in IS8525 germplasm is mostly pollen mediated. However, a greater level of tolerance in the IS8525 hybrid checks confirmed the reports of tolerance by Dahlberg et al. (1998) and Reed et al. (2002).

Few testcross hybrids with a useful level of tolerance to ergot were found. The ergot tolerance in the A1 testcross hybrids was slightly better than that observed in the A3 testcross hybrids, primarily due to the presence of some partially fertile A1 testcross hybrids.   Similar to trends observed in the recombinant inbred line populations, a significant hybrid x environment interaction was observed, indicating that some hybrids performed differently across the two environments. A closer examination of hybrids across environments confirmed that selection for stable ergot susceptibility response across environments would be difficult even though the trait is heritable. Finally, the transgressive segregation observed in the hybrid testcross population indicates that variation exists for improvement.

Even though a significant correlation between line per se and testcross hybrid performance was detected, it was too small to be of any practical value. This indicates that while some of the hybrids responded similarly to their respective recombinant inbred line parent, but it was not a frequent occurrence. This indicates that breeding for ergot tolerance in A/B-lines would require testcrosses during the selection process to identify the particular lines which were effective at transmitting higher levels of ergot tolerance to the male sterile.

In conclusion, these results indicate that selection for ergot tolerance could be effective to some extent. However, given that heritability levels were low and variable across environments and the genotype x environment interactions were highly significant and important, it will be difficult to expect that selection for ergot tolerance could produce lines that are consistent in their response to ergot across environments. In addition, it is not likely that IS8525 could provide a level of ergot tolerance in male sterile lines that seed producers would find acceptable. Therefore, we do not recommend the initiation of a breeding program to transfer ergot tolerance from IS8525 to elite sorghum inbred lines.

REFERENCES

Bandyopadhyay, R., D.E. Frederickson, N.W. McLaren, G.N. Odvody, and M.J. Ryley. 1998. Ergot: A new disease threat to sorghum in the Americas and Australia. Plant Dis. 82(4):356-367.

Boon-long, T. 1992. Sorghum diseases in Thailand. p. 41-43. *In* W. A. J. de Milliano, (ed.) Sorghum and millets diseases: A second world review. Proceedings of the International Workshop on Sorghum Diseases, Hyderabad, India. 11-15 Dec. 1978. International Crops Research Institute for the Semi-Arid Tropics. Andhra Pradesh, India.

Cyberspace Farm. 2003. A short history of grain sorghum. http://www.cyberspaceag.com/sorghumhistory.html.

Dahlberg, J. R. Bandyopadhyay, W. Rooney, G. Odvody, D. Fredericksen. 1998. Proceedings for 1998 conference on the status of sorghum ergot in North America. http://www.sorghumgrowers.com/Research/confernc/dahlberg.htm.

De Millano, W. A. J., Tavares Nogueira, M. F. R., Pomela, L. M., Msiska, F. S. Kunene, S., Matalaote, B., Mbwaga, A, M., Kaula, G. M., and Mtisi, E. 1991. New records of ergot of sorghum caused by *Sphacelia sorghi* in southern Africa. Plant Dis. 75: 215.

Food and Agriculture Organization of the United Nations, and International Crops Research Institute of the Semi-arid Tropics. 1996. The world sorghum and millet economies: Facts, trends and outlook. FAO, Rome, Italy.

Food and Agriculture Organization of the United Nations, and International Crops Research Institute of the semi-arid tropics. 2001. The world sorghum and millet economies: Facts, trends and outlook. FAO, Rome, Italy.

Frederickson, D.E., P.G. Mantle, and W.A.J. de Milliano. 1991. Claviceps africana sp. Nov.; The distinctive ergot pathogen of sorghum in Africa. Mycol. Res. 95(9):1101-1107.

Frederickson, D.E., P.G. Mantle, and W.A.J. de Millano. 1993. Windborne spread of ergot disease (*Claviceps africana*) in sorghum A-lines in Zimbabwe. Plant Pathol. 42:368-377.

Frederickson, N.W. McLaren, G.N. Odvody, and M.J. Ryley. 1998. Ergot: A new disease threat to sorghum in the Americas and Australia. Plant Dis. 82(4):356-367.

Frederickson, D.E., 1999. The lifecycle, spread and survival of the sorghum ergot pathogen. p. 135-140. *In* R. Casela (ed.) Proceedings of the Global Conference on Ergot of Sorghum. Sete Lagaos, Brazil. 1-8 June. 1997

Frederiksen, R., and G. Odvody. 2000. Compendium of Sorghum Diseases. Second edition. P. 40-42. Amer Phytopathological Society, St. Paul, MN.

Futrell, M.C. and Webster, O. J. 1966. Host range and epidemiology of the sorghum ergot organism. Plant Dis. Rep. 50:828-831.

House, L.R. 1982. El Sorgo: La Genetica del Sorgo. Grupo Editorial Gaceta. Mexico City, Mexico.

Langdon, R. F. N. and Champs, B. R. 1954. Insect vectors of *Claviceps purpurea* in Queesland. J. Aust. Inst. Agric. Sci. 10:286-289. *In* Bandyopadhyay, R., D.E. Frederickson, N.W. McLaren, G.N. Odvody, and M.J. Ryley. 1998. Ergot: A new disease threat to sorghum in the Americas and Australia. Plant Dis. 82(4):356-367.

Little, T.M. and Hills, F.J. 1978. Agricultural Experimentation. John Wiley and Sons, New York.

McLaren, N.W., and F.C. Wehner. 1992. Pre-flowering low temperatures predisposition of sorghum to sugary disease (Claviceps Africana). J. Phytopathology 135: 328-334.

McLaren, N. W. 1993. Effect of sugary disease exudates on germination, seedling development and predisposition to seedling diseases of sorghum (*Sorghum bicolor*). S. Afr. J. Plant Soil 10: 12-16.

McLaren, N.W. 1994. Efficacy of systematic fungicides and timing of preventative sprays in the control of sugary disease of grain sorghum (*Sorghum bicolor*). S. Afr. J. Plant Soil 11:30-33.

McLaren, N.W. 1999. Host x Pathogen x environment interaction in sorghum ergot disease. p. 122-140. *In* R. Casela (ed.) Proceedings of the Global Conference on Ergot of Sorghum. Sete Lagaos, Brazil. 1-8 June. 1997.

McRae, W. 1917. Notes on some south Indian fungi. Madras Agric. Yearb. 1917: 108-111.

Montes., N, 2000 A Laboratory guide to the identification of Claviceps purpurea and Claviceps africana in grass and sorghum seed samples. Amer Phytopathological Society, St. Paul, MN.

Moran, J.L. 2000. Differences in ergot vulnerability among sorghum genotypes and the relationship between stigma receptivity and ergot vulnerability. Masters Thesis, Texas A&M University, College Station.

Mukuru, S. Z. 1999. The ergot disease of sorghum in Africa. p. 55-58. *In* R. Casela (ed.) Proceedings of the Global Conference on Ergot of Sorghum. Sete Lagoas, Brazil. 1-8 June, 1997.

Murty, D.S. and K.A. Kumar. 1995. Traditional uses of sorghum and millets. *In* D.A.V. Dendy (ed.), Sorghum and Millets: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, MN, pp.185-221.

Musabyimana, T., C. Sehene, and R. Bandyopadhyay. 1995. Ergot resistance in sorghum in relation to flowering, inoculation technique and disease development. Plant Pathol. 44:109-115.

Pazoutova, S., and D. P. Parbery. 1999. The taxonomy and philogeny of clavicpes. p. 57-78. *In* Moran (ed.) 2000 Differences in Ergot Vulnerability among Sorghum Genotypes and the Relationship between Stigma Receptivity and Ergot Vulnerability. Masters Thesis, Texas A&M University, College Station.

Quinby, J. R. 1958. Grain sorghum production in Texas. Texas Agric. Exp. Stn. Bull. 912. Texas A&M University, College Station.

Reed, J. D. B. A. Ramundo, L. E. Clafin, and M. R. Tuinstra 2002. Analysis of Resistance to Ergot in Sorghum and Potential Alternate Hosts. Crop Sci. 42:1135-1138.

Reis, E. M., Mantle, and H.A.G. Hassan. 1996. First report in the Americas of sorghum ergot disease, caused by a pathogen diagnosed as *Claviceps africana*. Plant Dis. 80:463.

Rooney, L. W., M.E. Blakely, F.R. Miller, and D.T. Rosenow. 1980. Factors affecting the polyphenols of sorghum and their development and location in the sorghum kernel. *In* J.H. Hulse (ed.), Polyphenols in Cereals and Legumes. International Development Research Center, Ottawa, Ontario, Canada, pp. 25-35.

Ryley, M.J., Alcorn, J.K. Kochman, G.A. Kong, and S.M. Thompson. 1996. Ergot on sorghum spp. Australia. Aust. Plant Pathol. 25:214.

Smith, C.W., and R.A. Frederiksen. 2000. Sorghum: Origin, History, Technology, and Production, Wiley series in crop Science. New York.

Sorghum bicolor (L.) Moench. Icrisat 2003.

http://www.icrisat.org/text/coolstuff/crops/gcrops2.html

Stack, J., 2000 Sorghum ergot in the northern great plains. . University of Nebraska Cooperative Extension EC00-1879-S. Lincoln, NE.

Steel, R. G. D. and Torrie, J.H. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw-Hill Book Co., New York.

Stephens, J. C., and J.R. Quinby. 1934. Anthesis, pollination, and fertilization in sorghum. Journal of Agriculture Research 49(2): 123-136.

Tenberge, K. B. 1999. Biology and life strategy of the ergot fungi. p. 25-56. *In* Differences in Ergot Vulnerability among Sorghum Genotypes and the Relationship between Stigma Receptivity and Ergot Vulnerability. Masters Thesis, Texas A&M University, College Station.

Williangale, J., Mantle, P. G., and Thakur, R. P. 1986. Post-pollination stigmatic constriction, the basis of ergot resistance in selected lines of pearl millet. Phytopathology 76:536-539.

VITA

Rafael Arturo Mateo Moncada, was born on January 29, 1975 in Tegucigalpa, Honduras. He graduated from the Escuela Agricola Panamericana, Zamorano, Honduras with a B.S. in agronomy in 1995. In December 2003, he received a M.S. in plant breeding from Texas A&M University.

Permanent mailing address:
Rafael Arturo Mateo
Colonia Hato de Enmedio
Sector 4, Bloque 49, Casa 4907
Tegucigalpa, Honduras
Phone: (504) 255-1982