Maize Grains Infected with *Fusarium* spp. in Relation to Toxin Production

BY

El-Said Mohamed El-Shabrawi
B.Sc. (Plant Pathology), Fac. of Agric., Tanta Univ., Egypt, 1996
M.Sc. (Plant pathology), Fac. of Agric., Tanta Univ., Egypt, 2001

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APPROVAL SHEET

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Approved by:

Dr. Eed Mohamed Abou-Taleb ……………………………...
Professor of Plant Pathology, Fac. Agric., Alexandria University.

Dr. Fawzy Abd El Zaher Khalil …………………………….
Professor of Plant Pathology, Fac. Agric., Cairo University.

Dr. Kamel kamal Ali Sabet ……………………………………
Professor of Plant Pathology, Fac. Agric., Cairo University.

Dr. Dr. Ahmed Mohamed Abdel-Kader Ashour………….
Assistant Professor of Plant Pathology, Fac. Agric., Cairo University.

Date: / / 2007

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Dr. Kamel kamal Ali Sabet
Professor of Plant Pathology, Fac. Agric., Cairo University.

Dr. Ahmed Mohamed Abdel-Kader Ashour
Assistant Professor of Plant Pathology, Fac. Agric., Cairo University.

Dr. Elhamy Mostafa El-Assuity
Chief of Research, Plant Path. Res. Instit., ARC., Giza.

Name of Candidate: El-Said Mohamed El-Shabrawi Dgree: Ph.D.
Title of Thesis: Maize Grains Infected With *Fusarium* Spp. In Relation To Toxin Production
Supervisors: Prof. Dr. Kamel K. Sabet, Dr. Ahmed M.A. Ashour and Prof. Dr. Elhamy M. El-Assuity
Department: Plant Pathology
Branch: Plant Pathology APPROVAL: / /2007

ABSTRACT

This study was carried out to throw the light on the importance of ear and kernel rot of maize in Egypt.

Isolations and identifications done for fungal pathogens which attack maize ears in the fields of Lower Egypt and Beni-Suef revealed that *Fusarium verticillioides* was the most dominant pathogen among the recovered isolates from all localities. The toxigenic strains of this pathogen were found to be common within this population. Fumonisin B1 (FB1) was detected in significant amounts from grain samples collected from farmer`s fields and stores as well. Samples of Nubariya were highly contaminated with FB1 comparable to the other locations.

Two mating populations were only identified as MAT-A (*F. verticillioides*) and MAT-D (*F. proliferatum*). MAT-A produced considerable amounts of fumonisin compared to those belonging to MAT-D. Female fertile strain could produce higher amounts of the toxin than female sterile. Studying the effective population number in biological species showed that the female sterility was lesser than 50% indicating the prevalence of the sexual reproduction in *F. verticillioides* population. MAT-A strains were shown to be highly virulent to maize ears than MAT-D strains. A clear correlation was found between the virulence of isolates and MAT-A, Mat-1, fertility and efficiency in FB1 production.

Varietal resistance to infection with ear rot under natural and artificial infestation was studied and found that resistance differed from maize genotype to another. Production of FB1 also differed according to the genotype. Maize single crosses were more resistant than the other cultivars.

Rot onset and toxin accumulation were observed earlier in Baladi cultivar of maize than TWC-310. Disease severity and FB1 production increased gradually by increasing the plant age. Kernel moisture content seemed to be critical factor for infection with ear rot and toxin production.

Field trials were carried out to manage infection of maize with ear rot and minimize FB1 production. Infection could be significantly controlled by applying plants with some organic and inorganic elicitors, where acetyle salicylic, ethephon & ascorbic acid were highly effective in reducing infection. Dipel 2X as a biological insecticide was efficient in controlling stem borers and decreasing, in turn infection with ear rot. A synergistic effect of using the two methods of application together was obtained. This trial significantly reduced infection with ear rot and production of fumonisin better than the single treatment with each one of the elicitors or Dipel 2 X alone.

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Name of Candidate: El-Said Mohamed El-Shabrawi **Dgree: Ph.D.**
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CONTENTS

INTRODUCTION

Maize is attacked by several serious diseases that affect different parts of the crop. Ear and kernel rots are considered the most destructive group of diseases that cause direct damage to the crop yield. In addition, man and animal health are affected due to the hazardous mycotoxins which produced by the causal organisms of these group of diseases. Numerous fungi are affecting maize ear producing different types of rots allover the world. In Egypt, *Fusarium* spp., particularly *F. verticillioides* (El-Sayed, 1996) and *Aspergillus* spp. especially *A. flavus,* are the most common and seriously affecting maize ears causing severe damage to the crop (El-Shabrawi, 2001). Fumonisins and aflatoxins are quite known to be produced by *F. verticillioides* and *A. flavus*, respectively. Survey done in the field and market revealed that maize grains and their products are contaminated with significant amounts of these mycotoxins (Mohamed, 1999). These groups of mycotoxins are of concern due to their toxicological implications to man and animal (Franceschi *et al.*, 1990 and Abbas *et al.*, 1999). Therefore, the present study concerned with fumonisin-producible isolates of *Fusarium* spp. which predominate on maize ears.

The main objective of the present study was to identify the principal and prevalent *Fusarium* species that cause ear rot in maize growing fields of the country. Identifying *Fusarium* strains which produce the dangerous and hazardous fumonisin (FB1) in relation to their efficiency to infect maize ears was taken in consideration. Evolution of new strains from the toxigenic *Fusarium* spp. due to the probability of occurrence of sexual reproduction was studied. Some field trials were also carried out to control maize ear rot by using natural products rather than the hazardous pesticides that affect public health and pollute the surrounding conditions.

Discussion

Isolations and identifications done throughout the present study revealed that the common and prevalent fungal isolates that attack maize ears in most of the maize grown fields belonged to *Fusarium* spp. followed by *Aspergillus* spp. It is well known that these two groups of pathogens are cosmopolitan fungi that could be recovered from infected or even healthy maize ears and considered as dangerous toxigenic pathogens of maize ears (Miller *et al.*, 1995; Musser & Plattner, 1997; Munkvold & Desjardins, 1997 and El-Shabrawi, 2001). This study concentrated on *Fusarium* spp., in particular *F. verticillioides;* and other toxigenic fusaria belonging to section *liseola* which are common on maize ears and kernels in Egypt. Samples collected from different regions of Egypt revealed that maize grown in Kaf El-Sheikh, Menufiya, Beheira and Nubariya was the most highly contaminated with *Fusarium* spp. compared to those of the other locations. Fusarium ear rot is favoured by hot and dry conditions at flowering coupled with high humidity as stated by White (1999). It is known that these conditions are prevalent at above mentioned regions of Egypt.

Survey for fumonisin B1 (FB1) contamination in maize grains showed that samples collected from farmer’s stores contaminated with higher levels of the toxin if compared with those obtained at harvest from the fields. This finding agrees with that of Ngoko *et al.* (2001) who found that fumonisin was increased with storage period in maize collected from different zones of Cameroon. Also, many investigators stated that fumonisins concentration is affected by the prevailing environmental conditions during the storage of maize grains (Ono *et al.,* 2002 and Fandohan *et al.,* 2005). It is worth to mention that fumonisins have been occurred in significant levels in maize and maize products of food stuffs (Mohamed, 1999), where fumonisins are not destroyed by many methods that used for food processing (Troxell, 1996 and Jackson *et al.*, 1997). It was also found that yellow grain samples contained higher amounts of FB1 than white grains. This result is in harmony with that of Nelson *et al.* (1993) who stated that yellow grains of maize are vulnerable to contamination with fumonisins more than white grains. Samples taken from Nubariya, either from farmer's fields or stores, showed the highest level of FB1 contaminations. On the other hand, grain samples from Beni-Suef, which located in middle Egypt where the high temperature and low humidity prevail, exhibited the lowest level of FB1 contamination. Low temperature and high relative humidity which prevail in Nubariya are favoured for toxin accumulation as reported by Hennigen *et al.* (2000).

Visual screening for FB1 production, using UV revealed that out of 288 Fusarium isolates, 177 ones recovered from rotted maize ears collected from different locations of Egypt were shown to be toxigenic produced FB1. Higher numbers of toxigenic Fusarium isolates were obtained from Beheira, Kafr El-Sheikh, Menufiya and Nubariya. Unfortunately, these localities are the major productive areas of maize in Egypt. Whereas, lesser number of toxigenic isolates were found in Beni-Suef. This fact explains and confirms the above mentioned results of toxin contaminated grains from Lower Egypt where low or moderate temperatures and high relative humidity prevail during summer season.

Among the toxigenic isolates of Fusarium, 120 isolates representing the surveyed locations were chosen for biological identification. Two biological species MAT-“A” (*F. verticillioides*) and MAT-“D” (*F. proliferatum*) were identified throughout this work. Moreover, MAT-“A” was found to be the most common compared with MAT-“D”. This is in conformity with those obtained by Leslie (1995) and Kedera *et al.* (1999), who stated that *F. verticillioides* and *F. proliferatum* are the most prevalent strains within Fusarium section *liseola* that affect maize. Isolates belong to MAT-“A” were able to produce considerable amounts of FB1 if compared with isolates of MAT-“D”. The same findings were found by Thiel *et al.* (1991) who stated that *F. verticillioides* is considered the most dangerous strain in section *Liseola* as a toxigenic agent threatening man and animal health.

Dealing with the toxigenic Fusarium isolates the present study showed clear correlation between the levels of fumonisin produced, expressed as intensity of the fluorescence under UV in visual scanning, location from which these isolates were recovered, mating population (A or D) and the female fertility in the population. It could be concluded from results obtained throughout the present study that strains belonging to MAT-A and mating type (MAT-1) as well as female fertile strains were able to produce higher amounts of FB1 in comparable to that of MAT-D, MAT-2 and/or female sterile. This fact is in harmony with those found by Nelson *et al*. (1991) who stated that the potential exists for production of fumonisins by such strains in agricultural commodities and other substrates are widespread in geographic areas. Also, the same trend was reported by Leslie *et al.* (1992b) who found that members of the biological species MAT-A could produce an average of 1,786 ppm of the FB1, much higher than members of the MAT-D population which averaged 636 ppm when grown on maize grains. Moreover, it could be emphasized by Leslie *et al*. (1992a) that the female fertile strains produced considerable amounts of FB1 comparable to that produced by the female sterile ones of the same species. They also reported that strains belonging to MAT-A mating type 1

(MAT-1) produced higher amounts (665-5206 ppm) of FB1 than that (422-4744 ppm) of MAT-2 strains.

In case of the effective population number, based on the mating types, obtained results cleared that (MAT-1/MAT-2) ratios were differed in both mating populations (A and D) from 1:1, resulting in large decreases in the inbreeding effective population number to 50.4% of the count for MAT-A and 96% in MAT-D. Concerning the effective population number, based on FS/hermaphorditism, obtained results revealed that it was high in both mating types, where it was 95.6% in MAT-A and 93.8% in MAT-D. This result could be explained because of the low percentage of FS proportion in both mating types, where it was 34.7 and 40% in MAT-A and MAT-D, respectively. This low sterility increased the inbreeding effective population number *Ne*(*f*) and decreased the variance effective population number *Ne*(*v*) which did not differed in both mating populations. These findings are suggesting the high probability of evolution of new strains due to occurrence of sexual reproduction in Egyptian maize ear habitat of the toxigenic Fusarium population. Break down in hybrid resistance of maize to ear rot is anticipated in the localities of Nubariya, Menufiya, Gharbiya, Kafr El-Sheikh and Beheira where the effective population size was shown to be higher than that in other surveyed localities. The possibility of inducing new strains expected as discussed above is supported by the findings of Leslie and Klein (1996) who stated that the female fertility is at a selective advantage every time sexual reproduction occurs, and have disadvantage during vegetative propagation.

It could be concluded from results obtained throughout the present study that strains within the mating population (MAT-A) in all of the surveyed locations have the advantage to sexual reproduction occurs and the new recombination would be found, generally. As what emphasized by Leslie (1999), mating population “D” usually resembles mating population “A” in most of these features, but is usually recovered in smaller quantities making such analyses more difficult. Hence, incomplete data derived during executing this work may be regarded to the insufficient number of the recovered isolates during this study in MAT-D.

As regards to the effect of locations from which the field strains of *Fusarium* spp. were recovered, the effective population number (*Ne*) based on the ratio of MAT-1/MAT-2 differed obviously from location to another, where it was higher in Daqahliya population and lesser in that of Kafr El-Sheikh. Whereas, *Ne* based on FS/hermaphroditism, except in Daqahliya, were always very high. FS/hermaphroditism was always <50%. It could be concluded from results derived from this study that, in general, sexual reproduction in mating population 'A' (*F. verticillioides*) recovered from affected maize ears throughout the surveyed locations, is common in higher frequencies. Although, the frequency of female sterility reached 100% in Daqahliya populations, sexual reproduction was not common, but widely dispersed clones would be expected in this locality.

In conclusion, there is a complex dynamic among populations of *Fusarium* spp. associated with grain moulds of maize as suggested by Mansuetus *et al.* (1997). Successful protection of maize from fungi associated with ear and grain mould will probably require an approach that combines host resistance mechanisms with other control measures concerned with the diversity and behaviour of the pathogen.

To study the virulence of the recovered toxigenic isolates of *F. verticillioides* and *F. proliferatum* in infecting maize ears, 37 isolates were chosen according to their ability to produce FB1and fertility from different locations of study. It was found that isolates belonging to MAT-A (*F. verticillioides*) were more virulent to maize ears than those of MAT-D (*F. proliferatum*). Also, the highly producing FB1 strains within *F. verticillioides* population were shown to be more virulent and causing high degrees of severity to maize than the low producing ones. This result is coincided with the findings found by Lampreckt *et al.* (1994) and Munkvold & Desjardins (1997) who stated that fumonisins play an important role in pathogenesis on maize. The present investigation emphasized also that female fertile strains of *F. verticillioides* were higher in toxin production and virulence. Leslie *et al.* (1992a) reported that female fertile strains of mating population "A" produce one fold of FB1 as female sterile strains. Thus, female fertility appears to be associated with the production of higher levels of FB1, but there are exceptions in this general rule.

Study of varietal resistance showed that reaction of maize accessions to infection with ear rots greatly differed from cultivar to cultivar. Concern over the quality of maize for human and animal consumption has led to breeding efforts aimed at developing maize genotypes with resistance to *F. verticillioides* and subsequent fumonisin accumulation as suggested by Bush *et al.* (2004). Although, heritable resistance has been identified in maize (King and Scott, 1981 and Shelby *et al.,* 1994), no highly resistant genotypes are known. Therefore, mechanisms of resistance to local infection of maize kernels by *Fusarium* spp. are not fully understood. However, explaining the mechanisms of resistance may open an avenue for the benefit of maize breeding program to improve maize varieties and hybrids. Resistance to local infection has been associated with the silks, pericarp, aleurone, pedicel and black layer of kernel (Headrick and Pataky,