Susceptibility of maize varieties to opposite mating type strains of *Fusarium verticillioides*

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Abstract

Fusarium verticillioides the causal agent of maize ear rot is world wide distributed heterothalic species with dimictic mating system of reproduction. Presented paper summarizes results of fourth years (2007 – 2010) field studies analyzing disease severity of maize plant after inoculation with opposite mating type strains of the fungus. Genotypes of fourth maize cultivars (*Zea mays* var *indentata*, *Zea mays* var *indurata*, *Zea mays* var *everta* and *Zea mays* var *saccharata*) were inoculated with two *F. verticillioides* isolates KFI 2856 and KFI 3011 representing respectively MAT1-1 and MAT1-2 subpopulation of the species. Infection degree was evaluated using six degree (0 – 5) rating scale at seven days intervals after inoculation. Obtained results revealed that mating type of analyzed species had not significant impact on maize ear infection in any of growing seasons. Reaction of particular maize varieties against ear rot due to *F. verticillioides* in particular years were stable. The lowest infection degree exhibited plants of *Zea mays* var *everta* while the highest susceptibility *Zea mays* var *saccharata*. Regardless of cropping seasons significantly less was affected genotypes of sweet maize (*Su1*) than super sweet (*Sh2*).

Keywords: ear rot, *Fusarium verticillioides*

Introduction

Maize is infected by Fusaria at different developmental stages, leading to root rot, seedling blight stem and ear rot, which are considered the most important maize diseases worldwide (White, 1999). Although the etiology of this diseases is complex, the principal causative factors are members of the *Discolor* and *Liseola* section of the *Fusarium* genus (Munkvold, 2003). Occurrence of *Fusarium* species in particular cropping seasons is variable, changes from year to year, and is strongly influenced by weather conditions (Stewart et al, 2002). High temperature and limited precipitation favor especially *F. verticillioides* incidence (Afolabi et al, 2007, Miller et al, 1995, Ochor et al, 1987). Because hot and dry summers have been observed in Poland and some parts of Europe more and more frequently, this work was devoted to risk factors favoring maize ear rot caused by *F. verticillioides*.

The *Fusarium verticillioides* perfect stage *Gibberella moniliformis* is a world-wide distributed species. The fungus is heterothallic (Wineland, 1924) with a dimictic mating system (Kerenyi et al, 1999) typical for numerous Ascomycota and governed by two idiomorphs, MAT1-1 and MAT1-2 (Steenkamp et al, 2000). Although talus of opposite mating type strains with MAT1-1 or MAT1-2 genes are widespread (da Silva, 2006; Venturini et al, 2011), perithecia of this fungus in nature have been recognized only twice, by Voorhees (1933) and Summerell et al (1994). Most often the infected parts of plants developed the mitotic conidial stage only.

Fusarium verticillioides is commonly known as a maize pathogen but in fact it is a polyphagous species and its occurrence has been documented on plants representing over 48 families among monocots and dicots as well. Among monocots, the most important group recognized as a host of this fungus comprises plants of the *Poaceae* family, with over 30 species including *Zea mays*.

In performed studies, the impact of mating type of the fungus on infection degree of maize plants was analyzed. There is evidence that opposite mating type of some pathogens characterize significantly different traits important in pathogenesis. An extreme example of mating type contribution during infection and disease development is *Cryptococcus neoformans*, where only fungal strains of one MAT α type exhibit pathogenicity (Lin et al, 2006). Similarly, the MAT locus controlled pathogenicity in *Ustilago hordei* (Lee et al, 1999). Different virulence of opposite mating types was proved for *Phytophthora ramorum* and significantly higher virulence was exhibited by A1 type of this heterothallic Chromista (Brasier, 2003). Secondly, the paper stresses the role of host susceptibility in disease development and indicated signifi-

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cant differences of that trait among maize plants.

Materials and Methods

The studies were performed during four growing seasons (2007-2010) in an experimental field of the Plant Breeding and Acclimatization Institute at Radzików. The trials were set up in a three factorial, split-plot design (genotype, strain, harvest time) with 8 plants per unit plot.

Plant material

Ten lines and hybrids of four maize varieties were applied in this study. These were genotypes of *Zea mays* var *indentata* (2), *Zea mays* var *indurata* (1), *Zea mays* var *saccharata* (5), and *Zea mays* var *everta* (2).

Fungal strains

Two strains of *Fusarium verticillioides*, KFI 2856 and KFI 3011, from the Collection of the Department of Plant Pathology WULF were applied. They represented MAT1-1 and MAT1-2 mating type of the fungus and were originally derived from rotted maize cob collected at Radzików and Kobierzyce respectively. Two weeks before the planned inoculation term, the strains were seeded on PDA in Petri dishes and incubated for approximately 10 days until mycelium overgrew the microbiological base. Subsequently the mycelium was scraped from the medium surface, suspended in distilled water and after filtration through cheesecloth adjusted to the needed concentration.

Plant inoculations

Inoculations were performed seven days after silk emergence using 2 ml of conidial suspension of fungus strain at a concentration of 10^6 cfu per ml. Inoculum was introduced to the maize cob via a silk channel by a semiautomatic syringe. Infection degree was evaluated according to a 6-degree scale where 0 = no symptoms; 1 = very weak infection with 1–3% damaged kernels; 2 = weak infection with 3–10% damaged kernels; 3 = medium infection with 10–30% damaged kernels, 4 = strong infection with 30–50% damaged kernels; 5 = very strong infection with above 50% infected kernels. Disease ratings were performed eight times during each growing season in seven-day intervals since inoculation time.

Obtained data were analyzed statistically using Statgraphics 4.0 for Windows.

Ergosterol analysis

Samples of 100 mg ground kernels (in triplicates) were suspended in 2 ml methanol in culture tube and treated with 0.5 ml of 2 M aqueous sodium hydroxide. Subsequently obtained suspension were twice irradiated in microwave oven (370 W) for 20 sec. and after cooling neutralized with 1 M aqueous hydrochloric acid. Samples were supplemented with 2 ml MeOH and extracted with pentane (3 x 4 ml). The combined pentane extracts were evaporated to dryness in a stream of nitrogen and before analysis dissolved in 1 ml of MeOH.

Analysis were performed by HPLC method using a 150 x 3.9 mm Nova Pak C-18, 4 μm column and eluted with methanol:acetonitrile (90:10, v/v) at a flow rate of 0.6 ml min^{-1}. Ergosterol was detected with a Waters 2996 Photodiode Array Detector (Waters, Milford, USA) set at 282 nm. The detection limit of the method was 10 ng g^{-1} and standard deviation was below 5%.

Results

Four-year inoculation studies carried out from 2007 to 2010 indicated generally weak pathogenic properties of F. verticillioides. It was found that mating type of the fungus did not have a significant influence on infection level of maize. On average, during the analyzed growing seasons, the infection degree caused by strains KFI 3011 and KFI 2856 representing the MAT1-1 and MAT1-2 population of the fungus was respectively 1.77 and 1.81 on the applied six-degree rating scale. Differences in infection degree of maize between applied strains were not significant in any cropping season during the period 2007–2010 (Figure 1). *F. verticillioides* mating types also had no influence on disease progress in any of the applied maize varieties (Figure 2) in four cropping seasons. The lack of significant differences in pathogenic properties of the both mating strains in particular years confirmed ergosterol analysis (Figure 1). Applied *F. verticillioides* strains had not also significant effect on

Table 1 - Ear rot severity and ergosterol content in kernels of tested cultivars of Zea mays var. Saccharata.

Cultivar	Genotype	Infection degree				Ergosterol content [μg/g]			
		2007	2008	2009	2010	2007	2008	2009	2010
Harvest Gold	*Su1*	2.39	1.27	1.60	1.50	60.98	72.40	53.35	67.32
Jubile	*Su1*	2.53	2.28	2.06	1.88	91.27	166.13	190.23	167.39
Mean for Su1 genotypes		2.46^{a}	1.78^{a}	1.83^{a}	1.69^{a}	76.13^{a}	119.27^{a}	121.79^{a}	117.36^{a}
Trophy	*Sh2*	2.72	2.90	2.59	1.94	184.46	258.23	283.25	185.11
Sheba	*Sh2*	3.83	2.25	2.75	2.36	420.47	214.90	251.20	347.68
Candle	*Sh2*	3.25	2.78	2.65	2.39	451.96	428.44	385.65	412.35
Mean for Sh2 genotypes		3.27^{b}	2.64^{b}	2.66^{b}	2.23^{b}	352.30^{b}	300.52^{b}	306.70^{b}	315.01^{b}

Means with the same letters do not differ significantly.

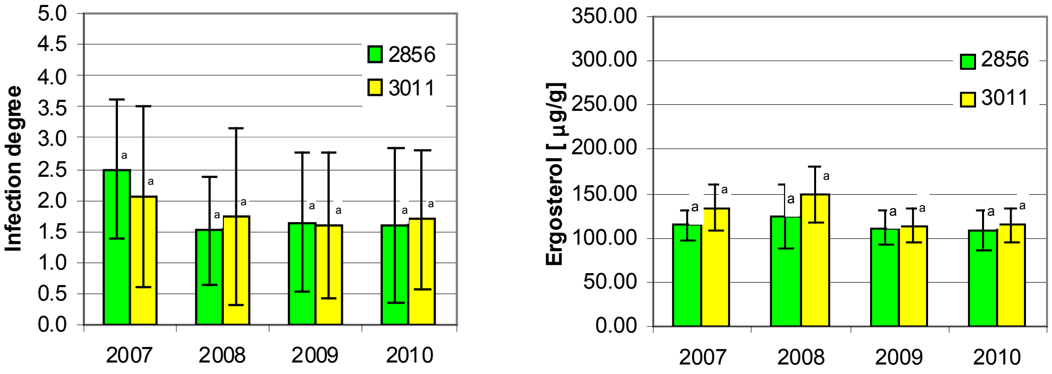

Figure 1 - Ear rot severity and ergosterol content in maize kernels due to MAT1-1 (KFI-2865) and MAT1-2 (KFI-3011) *F. verticillioides* in four cropping seasons 200 - 2010. Error bars express standard error; the same letters indicate lack of significant differences ($p<0.05$).

dynamics of ergosterol accumulation. The rate of this process was strongly related with maize variety and the level of ergosterol content most rapidly increased in kernels of *Z. mays* var *saccharata* (Figure 3).

A factor which exerted a substantial impact on severity of maize ear infection with *F. verticillioides* was cropping season. Significantly the highest (2.28 on a six-degree scale) cob destruction was noted in 2007. During the next years, observed variation of infection was statistically lower and ranged from 1.62 in 2008 and 2009 to 1.66 in 2010. The level of ergosterol occurred in kernels was also dependent on cropping season however significantly higher concentration of this metabolite were detected in 2007 and 2008.

Maize varieties exhibited significantly different susceptibility to *F. verticillioides*. On average, in four years of trials the most sensitive to infection was *Z. mays* var *saccharata* (2.26). A significantly lower infection degree was shown by *Z. mays* var *indentata* (1.80), and the two next varieties, *Z. mays* var *indurata* (1.51) and *Z. mays* var *everta*, with the lowest infection index, 0.88. It is worth stressing that the same ranking of infection degree among particular varieties was observed in each of the four growing seasons. Cobs of *Z. mays* var *saccharata* were infected most intensively and *Z. mays* var *everta* to the lowest extent, regardless of year and *F. verticillioides* strain (Figure 4). The differences in maize susceptibility to *F. verticillioides* were much more distinctly expressed by ergosterol content. This fungal biomass marker was detected in highest concentration in kernels of *Z. mays* var *saccharata* (Figure 5). Among them geno-

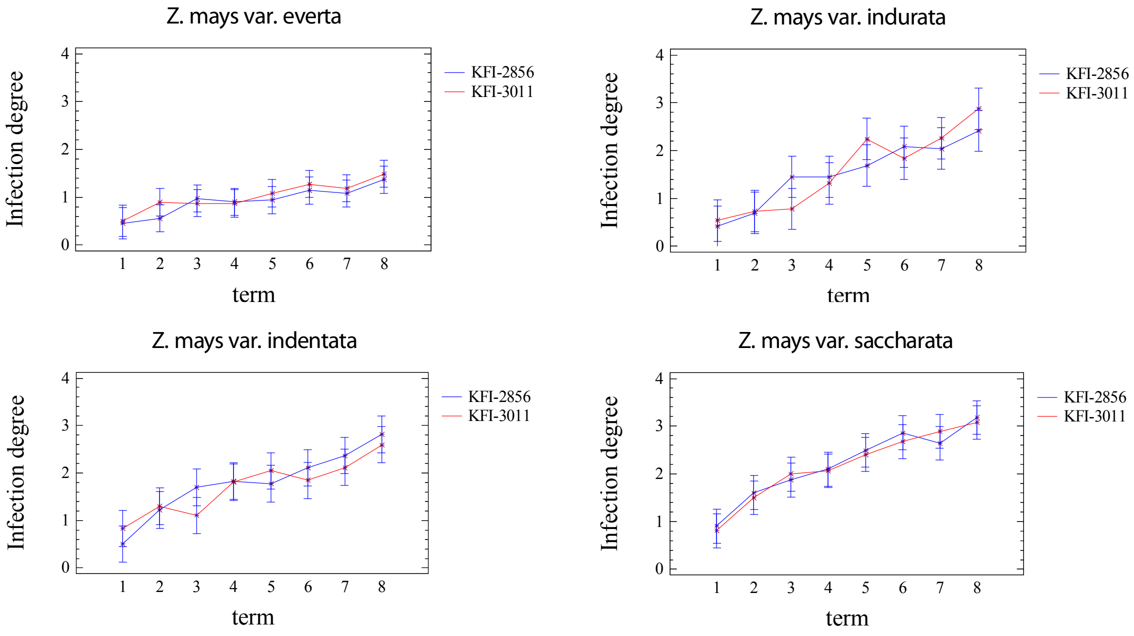

Figure 2 - Disease progress curve on ears of applied maize varieties inoculated with strains of opposite mating type MAT1-1 (KFI-2865) and MAT1- 2 (KFI-3011) *F. verticillioides*. Mean values for the period 2007 – 2010.

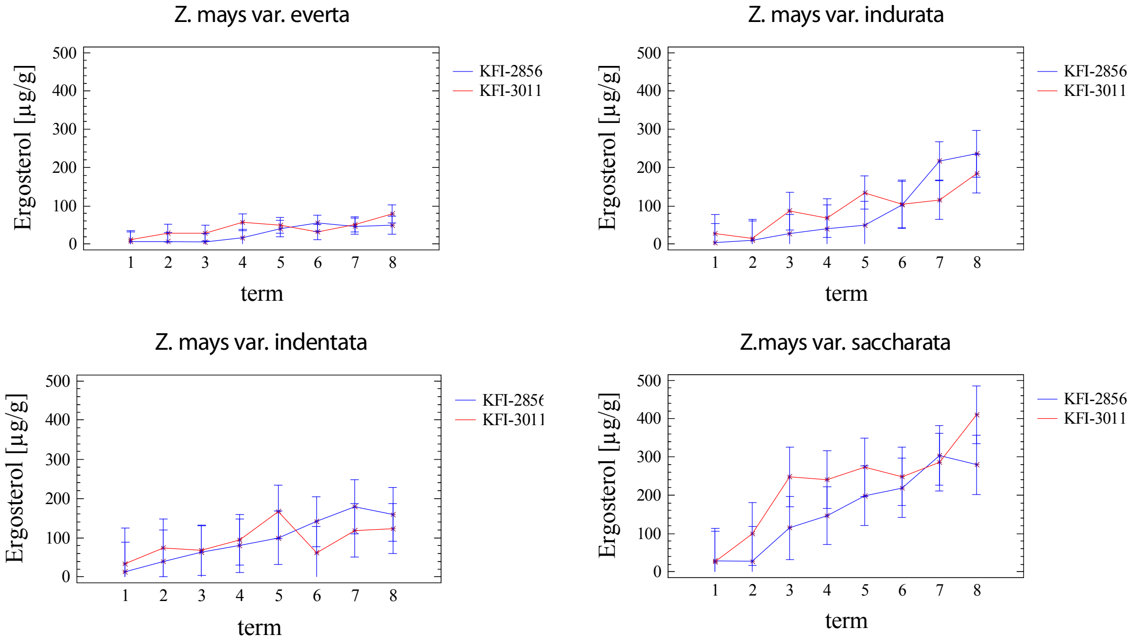

Figure 3 - Dynamics of ergosterol accumulation in kernels of applied maize varieties inoculated with strains of opposite mating type MAT1-1 (KFI-2865) and MAT1-2 (KFI-3011) of *F. verticillioides*. Mean values for the period 2007 – 2010.

types with *Sh2* gene exhibited substantially higher infectoion degree as well as ergosterol concentration than *Su1* genotypes (Table 1). During four years of studies, the mean infection degree of sweet maize ranged from 1.69 in 2010 to 2.46 in 2007, while super sweet ranged from 2.23 in 2010 to 3.27 in 2007, on a six-degree scale. At the same period the level of ergosterol was 2.5 to 3 times higher in kernels of super sweet than sweet maize.

Discussion

Results presented in this paper constitute the first report analyzing the impact of *F. verticillioides* MAT idiomorph on ear maize infection. Although there is

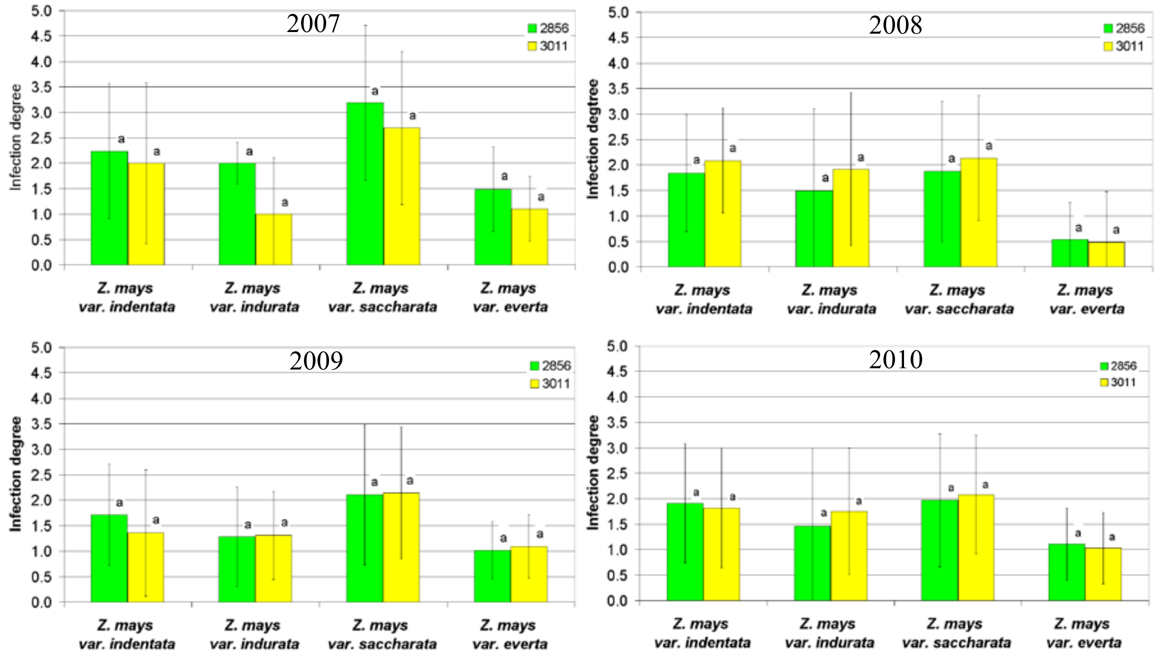

Figure 4 - Infection degree of maize varieties after ears inoculation with strains of opposite mating type MAT1-1 (KFI-2865) and MAT1- 2 (KFI-3011) *F. verticillioides* in four cropping seasons 2007 – 2010. Error bars express standard error; the same letters indicate lack of significant differences ($p<0.05$).

evidence that opposite mating type of some pathogens significantly influences different traits important in pathogenesis (Brasier, 2003; Lee et al, 1999; Lin et al, 1996), tested *F. verticillioides* strains KFI 3011 and 2856, representing MAT1-1 and MAT1-2 respectively, did not differ in pathogenicity. Equal aggressiveness has been confirmed during 4-year field studies but based only on two isolates, and that is why it should not decide definitely on lack of sex significance in pathogenesis of diseases caused by *F. verticillioides*. Mating type is a more complex trait than it could seem. Its particular important element is the mitogen-activated protein (MAP) kinase pathway – common transducers of extracellular signals (Xu, 2000), also those induced by pheromones produced by particular MAT types. Activity of the cascade pathway component is directly associated with pathogenicity of fungi (Zhao et al, 2007). Among fusaria it was proved that disruption of the GMPK1 gene element of mating MAP kinase caused loss of *F. graminearum* virulence (Jenczmionka et al, 2003). Influence of MAT types on fumonisin production is not clear or fully elucidated. In a population of FB1 non-producers, MAT1-1 occurred significantly more frequently than MAT1-2 strains, while the opposite score was observed among fumonisin producing isolates (Mirete et al, 2004).

F. verticillioides is often considered as a fungus of low pathogenic properties towards cereals compared to other *Fusarium* species affecting this crop (Wit et al, 2011). As reported by Yates et al (1997), its occurrence in maize seeds did not have any influence on rate or percentage of kernel germination, but plant height, leaf length, and plant weight analyzed on the 7th day after planting were significantly retarded. Listed differences between infected and non-infected plants gradually faded away and in 4-week old seedlings no noticeable harmful effect of fungus activity was observed. Similarly, there was not observed any effect of seed contamination with *F. verticillioides* on yield and vegetative growth of mature maize plants (Yates et al, 2005). Reports indicating high *F. verticillioides* pathogenicity are rare, but some papers have stressed that mold-infected or symptomless kernels usually did not germinate or produced blighted seedlings. Headrick and Pataky (1989) reported reduced kernel emergence obtained from silk inoculated sweet corn ears. Most recently, Iglesias et al (2010) discovered occurrence of unusual, highly aggressive strains in tested population of *F. verticillioides*.

Data recorded during the presented studies generally confirm low pathogenicity of *F. verticillioides*, although the infection degree was strongly dependent on growing season (Figure 1) and maize variety (Figure 4). Especially high susceptibility was exhibited by genotypes of *Z. mays* var *saccharata*. Regardless of cropping season, significantly less affected were ears of sweet than super sweet maize with the *sugary-1* (*su1*) and shrunken-2 (*sh2)* genes, respectively (Table 1). Both genes *su1* and *sh2*, responsible for sweetness, are recessive mutant genes that slow down starch biosynthesis. *su1* is a mutant of the starch debranching enzyme encoding gene, and *sh2* is a mutated gene encoding the large subunit of ADP-glucose pyrophosphorylase. Besides lower content of starch, which is observed in both

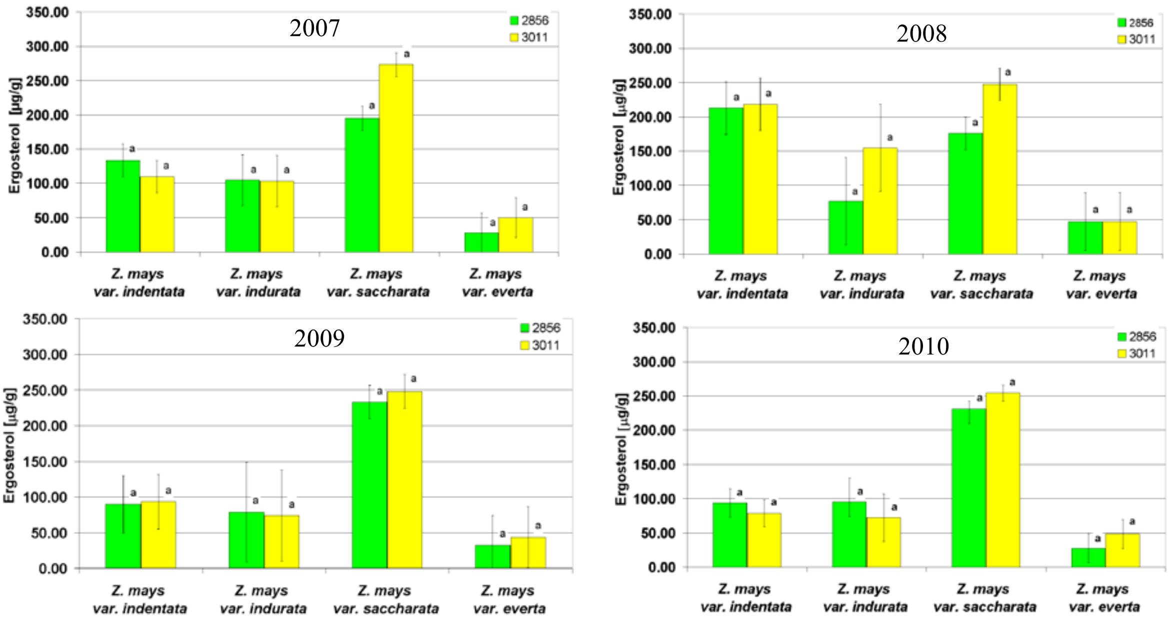

Figure 5 - Ergosterol content in kernels after maize ears inoculation with strains of opposite mating type MAT1-1 (KFI-2865) and MAT1- 2 (KFI-3011) *F. verticillioides* in four cropping seasons 2007 – 2010. Error bars express standard error, the same letters indicate lack of significant differences ($p<0.05$).

cases, plants with the *su* gene are characterized by higher levels of reducing sugars, sucrose and water soluble polysaccharide; moreover, mature kernels are wrinkled. This type of mutation also exhibited a variety of side effects such as thinner pericarp, lower dry weight and higher content of water. Much higher content of reducing sugars and sucrose occurred in plants with *sh2*. Kernels of super sweet corn with *sh2* compared to other maize type are smaller, shrunken, have a thin seed coat and exhibit low germination capacity (Manicacci et al, 2007; Adetimirin et al, 2006). Additionally, weak seed germination may contribute to their higher susceptibility towards soil pathogens (Baird, 1994). Reaction of super sweet maize after infection with other common maize pathogens, i.e. *Erwinia stewartii*, *Puccinia sorghi*, *Exserohilum turcicum*, or *Bipolaris maydis*, did not differ significantly in comparison to the reaction of standard maize type (Pataky et al, 1998). An exception is very high susceptibility of maize with *sh2* to *F. verticillioides*.

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