

SANITARY ANALYSIS OF ASYMPTOMATIC AND DAMAGED GRAINS OF MAIZE IN TOCANTINS, BRAZIL

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ABSTRACT

In the climatic conditions of the state of Tocantins, fungi have been considered limiting factors to the production of maize (*Zea mays*), since they favor the pathogens development in all plant parts at different stages, including during the ripening and post-harvest of maize grains. The aim of this study was to identify and quantify the fungi present in asymptomatic and damaged grains of 11 maize cultivars. For sanitary analysis, the filter paper method (Blotter test) was used. In the study, 12 maize-grain-associated genres were found: *Fusarium*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Trichoderma*, *Bipolaris*, *Phoma*, *Curvularia*, *Rizhopus*, *Colletotrichum*, *Papularia*, and *Nigrospora*. There was a little variation in the diversity of genres found in damaged and asymptomatic grains, taking into account the growing seasons. The highest incidence was observed of the *Fusarium* genus, followed by *Cladosporium*, *Aspergillus* and *Penicillium*, respectively, which were associated with all the evaluated cultivars grains. The cultivars showed low percentage of damaged grains in the evaluated conditions.

Keywords: Phytopathogens, Mycoflora, *Zea mays*

ANÁLISE SANITÁRIA DE GRÃOS ASSINTOMÁTICOS E ARDIDOS DE MILHO NO TOCANTINS, BRASIL

RESUMO

Nas condições climáticas do estado do Tocantins, os fungos têm sido considerados fatores limitantes à produção de milho (*Zea mays*), pois o clima da região favorece o desenvolvimento de fitopatógenos em diferentes estágios, inclusive durante a maturação e pós-colheita dos grãos de milho. O objetivo do presente estudo foi identificar e quantificar os fungos presentes em grãos assintomáticos e ardidos de 11 cultivares de milho. Para a análise sanitária utilizou-se o método do papel de filtro (Blotter test). Foram encontrados 12 gêneros associados aos grãos do milho: *Fusarium*, *Aspergillus*, *Penicillium*,

Cladosporium, *Trichoderma*, *Bipolaris*, *Phoma*, *Curvularia*, *Rizhopus*, *Colletotrichum*, *Papularia* e *Nigrospora* com pouca variação quanto à diversidade dos gêneros encontrados em grãos ardidos e assintomáticos. Maior incidência observada foi do gênero *Fusarium*, seguido por *Cladosporium*, *Aspergillus* e *Penicillium*, respectivamente, os quais estavam associados a todos os cultivares avaliados. Os cultivares apresentaram baixa porcentagem de grãos ardidos nas condições avaliadas.

Palavras-chave: Fitopatógenos, Micoflora, *Zea mays*

INTRODUCTION

Different pathogens attack the maize plant (*Zea mays* L.) in all of its vegetative stages, including on the ripening phase of the grains and on the storage, being an important cause of quantitative and qualitative losses (FREIRE et al., 2007).

The use of high-quality seeds on the maize crop is fundamental for the establishment of the crop on the fields. However, the deterioration of the seeds is a serious problem for the agriculture, being responsible for major losses worldwide and especially on the tropics, where broadly, the temperatures are elevated as well as the relative air humidity (OLIVEIRA et al., 1999).

The fungi are microorganisms with vast geographical distribution and are relevant in many economic activities, like the food production, pharmacy, enzymes, and organic acids. However, some fungi are plant pathogenic agents and food-deterioration agents, being able to cause reduction on the nutritional value of food, production of toxic secondary metabolites and diseases on humans and animals (SILVA et al., 2015).

The maize grains, in phytopathological terms, are classified as damaged when infected by fungi and when it suffers discoloration on at least a quarter of the grain surface. The coloration of these grains may vary between light-brown to purple or between light-red to dark-red, depending on the present pathogenic agent (PINTO, 2005).

Damaged grains occur mainly because of the contamination of the cobs in the phase of grain filling and the production of mycotoxins by the pathogens is constituted as one of the main problems of reduction of grain quality; including the fumonisins and aflatoxins, that cause severe health problems on animals and humans (WORDEL FILHO & SPAGNOLLO, 2013).

Most industries tolerate a maximum of 6% of damaged grains as quality standard on the commercial lots of maize (FREIRE et al., 2007). Due to these factors, the fungi that cause cob rotteness and consequently damaged grains must be studied more deeply.

The literature of fungi associated to maize seeds is relatively vast. However, in the state of Tocantins, Brazil, researches related to the identification and quantification of presence of fungi on grains derived from commercial cultivars are still very scarce. Therefore, the objective of this study was to identify and quantify the fungi incidence on damaged and on healthy grains of maize cultivars on this state.

MATERIAL AND METHODS

For the attainment of the grains, three experiments were carried out on field, at the experimental area of EMBRAPA, located on the municipality of Palmas, in the state of Tocantins, Brazil, with the coordinates: Latitude: 10°23'59,87" S and Longitude: 48°21'42,33" W. Sowings occurred on days 03/02/2014, 25/02/2014 and 12/02/2015 with experimental establishing of blocks designed with 11 cultivars (BRS 1060, DKB 310 PRO, 30F53 YH, AS 1596 PRO, CD355, DKB 390 PRO, BRS 2020, RB9308YG, BALU 761, AG7088 PRO, and 2B587 PW) and four repetitions.

Each portion was constituted of two lines of five meters, with 0.5 meter between them, totaling a population of 55000 plants per hectare. After the harvest, 500g-samples of the maize grains were collected from each portion and later forwarded to the Phytopathology Laboratory of the Federal University of Tocantins, located on the municipality of Gurupi.

The damaged grains found in each 500g-sample were visually separated and weighted. The determination of percentage of the damaged grains was based on the weight of the sample and on the weight of the damaged grains. Subsequently, all the damaged grains and a sample with 400 asymptomatic grains from each cultivar were submitted to the sanitary test with four repetitions.

At first, the grains were submitted to a 50%-alcohol asepsis for one minute, submitted to a 1%-hypochlorite solution for one minute right after, and then washed in two parts of distilled water. Subsequently, the grains were submitted to the sanitary test by the method of filter paper (Blotter test), where were placed upon Germitest paper layers moist with sterile water in the interior of a transparent box (Gerbox).

These were initially maintained for 24 hours in incubation chamber, at a temperature of $25^{\circ}\text{C} \pm 2^{\circ}$ and next it was taken to the interior of a freezer at -20°C for 24 hours, in order to inhibit the germination process of the seeds and ease the fungi identification. After that, the grains were placed again in an incubation chamber where it stayed for five days, under an alternated light scheme of 12 hours.

Finally, the process of identification and quantification of the fungi incidence was done based on their morphological characteristics, identified under a magnifying glass and an optical microscope. The identification was done using specialized literature (BARNETT & HUNTER, 1972). When necessary, the fungi that were not identified with the magnifying glass were sent to the potato-dextrose-agar culture (PDA), for posterior microscopic identification, based on the morphological characteristics of the conidia. The fungal genres identified and quantified were expressed in percentage of incidence in each of the cultivars.

The data of the fungi incidence in each cultivar and the percentage of damaged grains were submitted to the variance analysis and the means were compared by Tukey test at 5% of probability, using the ASSISTAT 7.7 software (SILVA, 2015). The data of percentage of the damaged grains were transformed to: $x = \arcsen \sqrt{x/100}$.

RESULTS AND DISCUSSION

Figures 1A and 1B show the incidence of fungi on the damaged and on the asymptomatic maize grains on the first two experiments, corresponding to the crop of 2013/2014. Among the fungi that colonized the damaged and the asymptomatic grains, these genres were more frequently found: *Fusarium*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Trichoderma*, *Bipolaris*, *Phoma*, *Curvularia*, *Rhizopus*, and *Colletotrichum*, which varied differently in incidence. On the two experiments it was observed that the fungi of the genre *Fusarium* had high incidence, infesting more than 80% of the damaged grains studied.

On the Figure 1B, it was verified that the genre *Fusarium* also presented high incidence on the asymptomatic grains, with values varying between 82% and 94% on the first sowing and between 75% and 87% on the second. On the first sowing there was a higher variation on the incidence values when compared to the second one. The diversity of fungi also varied from one sowing to another, in instance *Rhizopus* sp. and *Phoma* sp., which were observed only on the first experiment and the genres *Colletotrichum*, *Curvularia*, and *Nigrospora*, which appeared only on the second experiment.

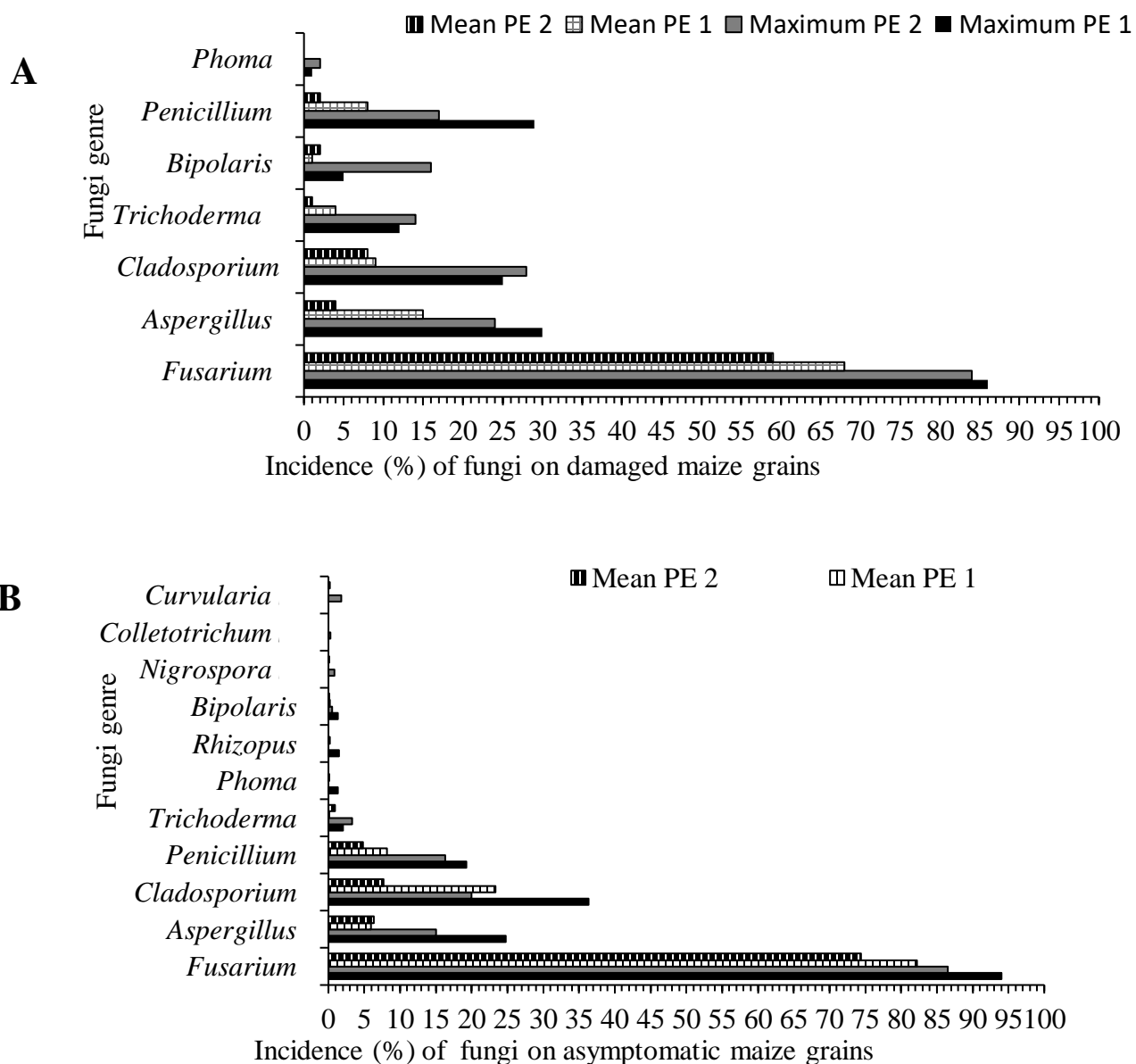


Figure 1. Mean and maximum percentage (%) of fungi associated to damaged maize grains (A) and asymptomatic (B), period (PE1) and 2 (PE 2), Palmas, Tocantins, crop 2013/2014.

The genre *Fusarium* is frequently found colonizing maize grains. Many studies have already been published reporting the problems provoked by this fungus, that has as main inoculum sources the infected rests of the maize crop and also the soil (FESSEL et al., 2003; CASA et al., 2007; RAMOS et al., 2010; HENNING et al., 2011 and HUSSAIN et al., 2013). The spore dissemination occurs because of the wind and insects, and the period of higher susceptibility occurs between seven and ten days after the pollination of the stigmas. Symptomatically, a pink (*F. verticillioides*) or a purple (*F. graminearum*)

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pigmentation may occur on the grains, and the cobs that do not double after the physiological maturity of the grains and those with bad thatch are more susceptible to this effect (PINTO, 2005).

Henning et al. (2011), in studies conducted about sanity of maize grains, found high incidence of the fungi *F. moniliforme*, *A. flavus*, and *Penicillium* sp. Ramos et al. (2010) also reported the presence of the fungi *Penicillium* sp., *Fusarium* sp., *Aspergillus* sp., *Cladosporium* sp., *Cephalosporium* sp., and *Stenocarpella* sp. on damaged grains and on maize seeds.

Several sowings along the year are not positives from the sanitary point of view, since this practice, together with the inadequate management, use of susceptible cultivars, and favorable climate to the development of the pathogens, increase the quantity of inoculum. Also, it should be taken into account that the majority of fungi that are causative agents of diseases remain alive in crop rests.

On the crop of 2014/2015, the presence of fungi was higher than the extracted from the two experiments from the crop of the previous year (Figure 2).

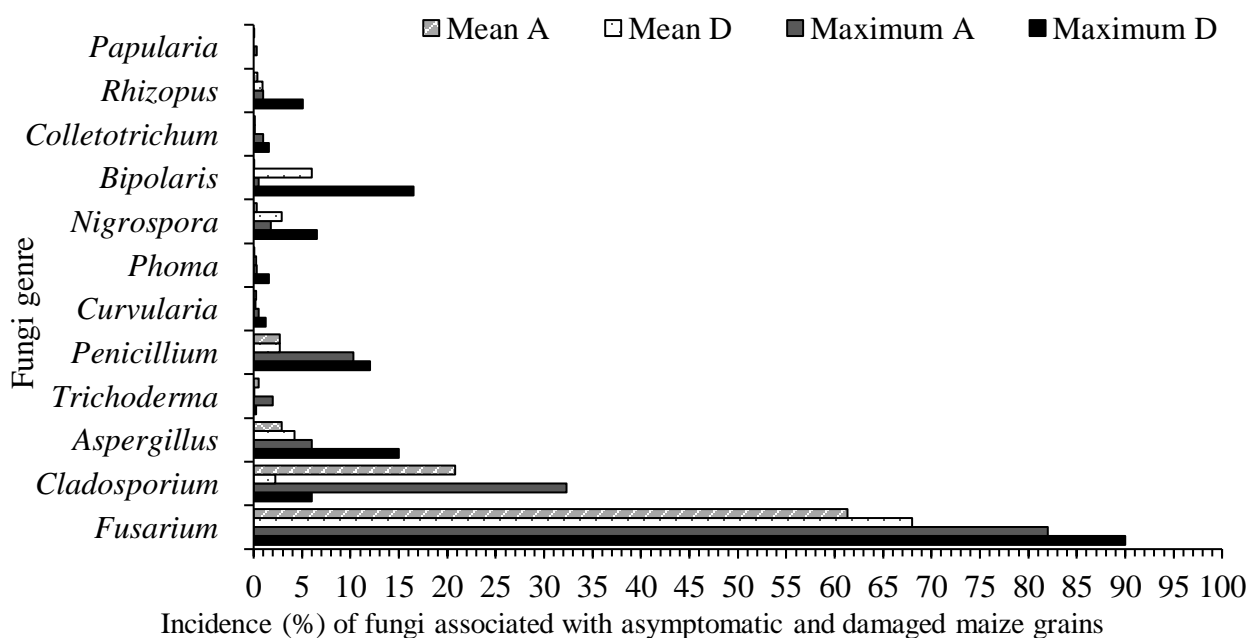


Figure 2. Mean and maximum percentage values of fungi associated with asymptomatic (A) and damaged maize grains (S), Palmas, Tocantins, crop 2014/2015.

Fusarium had mean incidence of 68% and maximum of 90% on the damaged grains. In asymptomatic grains the incidence varied from 61% to 90%. *Cladosporium* genre was the second most incident on the asymptomatic grains, when compared to the damaged grains, with infection percentage

varying from approximately 21% to 32%. Following, the genres *Aspergillus*, *Penicillium* and *Bipolaris* had incidence on the damaged grain of 15%, 12% and 16.5%, respectively (Figure 2).

A low incidence was registered for the genres *Curvularia*, *Nigrospora* and *Rhizopus*, which were already reported associated to maize grains by other authors (BENTO et al., 2012). The genres *Fusarium*, *Cladosporium* and *Nigrospora* found on the present study, are not commonly considered as storage fungi, except if the humidity of the grains is inadequate on the storage facilities, different from the ideal condition of humidity presented on this experiment.

However, differently from these, the genres *Aspergillus*, *Rhizopus* and *Penicillium* are considered storage fungi, having a very low incidence in plants on the fields and in newly-harvested grains (PEZZINI et al., 2005). Other fungi also considered of fields and that has their seeds propagated were also found on this study, for instance the *Trichoderma* sp., *Bipolaris* sp., *Curvularia* sp., and *Colletotrichum* sp.

The mean incidence values (%) in each cultivar evaluated on the three experiments can be observed (Tables 1 and 2). *Fusarium* was present with high incidence on the grains of evaluated cultivars, both on the asymptomatic grains and on the damaged ones. About the efficiency of the genetic resistance of the cultivars on the incidence of fungi on damaged grains and on healthy grains of maize, the obtained results from this study demonstrated variation in the fungal microflora and on the incidence values. Independently of the evaluated crops and sanitary condition of the grains, the tested cultivars presented high susceptibility to *Fusarium* sp.

Considering the damaged and asymptomatic grains on the first experiment (Table 1), crop of 2013/2014, there was the presence of seven and eight fungi genre, respectively. However, it was observed that the contamination percentage varied from one cultivar to another. Each cultivar presented at least two different genre of fungi on the damaged grains and four genres on the initially considered asymptomatic. Regarding the genre that had higher incidence on the damaged grains, *Fusarium* infect all the cultivars, followed by *Cladosporium* sp., *Aspergillus* sp. and *Trichoderma* sp., which infected from six to seven cultivars. Regarding the fungi that were present on the damaged grains, all of them were found colonizing the asymptomatic grains.

The experiment 2 shows the incidence values of the presence of fungi derived from the second evaluation, crop 2013/2014 (Table 2).

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Table 1. Incidence of fungi associated to the damaged and asymptomatic grains of maize cultivars - Experiment 1. Palmas, Tocantins, crop 2013/2014

EXPERIMENT 1											
Incidence of fungi in damaged grains (%)											
Genre	Cultivars ¹										
	1	2	3	4	5	6	7	8	9	10	11
<i>Fusarium</i>	90aA	92aA	92aA	100aA	94aA	93aA	94aA	80bA	90aA	91aA	81bA
<i>Aspergillus</i>	12cB	26bB	0dC	0dB	0,6dB	0dC	0dB	1,2dB	0dB	52aB	3dB
<i>Penicilium</i>	0bC	0,5bC	20aB	0bB	0bB	5,5bB	0bB	0bB	0bB	0bC	0bB
<i>Cladosporium</i>	13aB	1,5bC	0bC	0bB	6bB	11bB	0bB	1bB	2bB	0bC	1bB
<i>Trichoderma</i>	14aB	1bC	0bC	0bB	0bB	1bC	4,5bB	0bB	0bB	1bC	6bB
<i>Phoma</i>	0aC	0aC	0aC	0aB	0aB	0aC	0aB	0aB	0aB	0aC	0aB
<i>Bipolaris</i>	0aC	0aC	0aC	0aB	0aB	0aC	0aB	0aB	0aB	0aC	0aB

Incidence of fungi in asymptomatic grains of maize (%)											
Genre	Cultivars ¹										
	1	2	3	4	5	6	7	8	9	10	11
<i>Fusarium</i>	79dA	86abcdA	93abA	87abcdA	94abA	92abcA	85bcdA	94abA	93abA	82cdA	96aA
<i>Aspergillus</i>	2,3cdC	2cdC	3bcdB	2cdC	10abcBC	0dD	13abC	1cdC	18aC	6bcC	8abcdC
<i>Penicilium</i>	5,5bcC	13abB	4,5bcB	3,5bcC	2cCD	17aC	22aC	0cC	12abC	3bcC	6bcC
<i>Cladosporim</i>	47aB	6eBC	9deB	41aB	17cdB	43aB	23bcB	19cdB	24bcB	41aB	30bB
<i>Trichoderma</i>	4aC	0,3aC	0,6aB	1aC	3aCD	0,3aD	0aD	0,3aC	0,6aD	0,6aC	0aC
<i>Bipolaris</i>	0aC	0aC	0aB	2aC	0aD	0,3aD	0aD	0aC	0aD	0aC	0aC
<i>Phoma</i>	0aC	0aC	0aB	0aC	0aD	0aD	0aD	0aC	0aD	2aC	0aC
<i>Rhizopus</i>	0aC	0aC	0aB	0aC	0aD	0aD	0aD	0aC	2aD	3aC	0aC

¹Cultivars: 1(AS1596PRO), 2 (DKB310PRO), 3(CD35), 4 (AG7088PRO), 5 (2B587PW), 6 (DKB390PRO), 7 (BRS1060), 8 (RB9308YG), 9 (BALU761) 10 (30F53YH) e 11 (BRS2020). Means followed by the same letter, lower case comparing in the row and upper case in the column does not differ statistically by the Scott-Knott test at 5%

The quantity of genres present on the damaged and on the asymptomatic grains were the same observed on the first experiment, with few differences on the diversity of fungi on both experiments. On the second experiment, there was found again the same tendency observed previously, where higher quantity of genres was found on the healthy grains.

Table 2: Incidence of fungi associated to the damaged and asymptomatic grains of maize cultivars - Experiment 2. Palmas, Tocantins, crop 2013/2014

EXPERIMENT 2											
Incidence of fungi in damaged grains (%)											
Cultivars ¹											
Genre	1	2	3	4	5	6	7	8	9	10	11
<i>Fusarium</i>	90aA	92aA	92aA	100aA	94aA	93aA	93aA	79bA	90aA	91aA	81bA
<i>Aspergillus</i>	12cB	26bB	0dC	0dB	0,6dB	0dC	0dB	1,2dB	0dB	52aB	3dB
<i>Penicillium</i>	0bC	0,6bC	20aB	0bB	0bB	5,5bB	0bB	0bB	0bB	0bC	0bB
<i>Cladosporim</i>	13aB	1,5bC	0bC	0bB	6bB	11aB	0bB	1bB	1,7bB	0bC	1,8bB
<i>Trichoderma</i>	13aB	1bC	0bC	0bB	0bB	1bC	4,5bB	0bB	0bB	1bC	6bB
<i>Bipolaris</i>	0aC	0aC	0aC	0aB	0aB	0aC	0aB	0aB	0aB	0aC	0aB
<i>Curvularia</i>	0aC	0aC	0aC	0aB	0aB	0aC	0aB	0aB	0aB	0aC	0aB

Incidence of fungi in asymptomatic grains of maize (%)											
Cultivars ¹											
Genre	1	2	3	4	5	6	7	8	9	10	11
<i>Fusarium</i>	94abA	87bcA	82cdA	86bcA	98aA	76deA	69efA	73eA	59gA	63fgA	93abA
<i>Aspergillus</i>	9aC	6aBC	1,3aBC	3aB	3aC	0,3aB	3aB	1aC	0,3aB	4,3aB	1aB
<i>Penicilium</i>	0,6bD	10aB	4abBC	0bB	0,6bC	0,3bB	2,3abB	0,3bC	1,6abB	1,3abB	4abB
<i>Cladosporim</i>	35aB	7cB	8cBC	3cB	18bB	8cB	2cB	19bB	5cB	2cB	4cB
<i>Trichoderma</i>	0aD	0aC	1aBC	0,5aB	1aC	1aB	0aB	0aC	0aB	0aB	1aB
<i>Bipolaris</i>	0,5aD	0aC	0,5aBC	0aB	0aC	0aB	0aB	0aC	1aB	0aB	0aB
<i>Curvularia</i>	0aD	0aC	0aC	3aB	0aC	0aB	0aB	0aC	0aB	0aB	0aB
<i>Nigrospora</i>	0aD	0aC	0aC	0aB	0aC	0aB	0aB	0aC	0aB	0aB	1,5aB

¹Cultivars: 1(AS1596PRO), 2 (DKB310PRO), 3(CD35), 4 (AG7088PRO), 5 (2B587PW), 6 (DKB390PRO), 7 (BRS1060), 8 (RB9308YG), 9 (BALU761) 10 (30F53YH) e 11 (BRS2020). Means followed by the same letter, lower case comparing in the row and upper case in the column does not differ statistically by the Scott-Knott test at 5%

Regarding the incidence of grains on the crop 2014/2015 (Table 3), it was observed the same tendency on the experiments of the previous crop, with more incidence of the genres *Fusarium*, *Aspergillus*, *Penicillium*, and *Cladosporium* again.

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Table 3. Incidence of fungi associated to the damaged and asymptomatic grains of maize cultivars - Experiment 3. Palmas, Tocantins, crop 2014/2015.

Incidence of fungi in damaged grains (%)											
Cultivars ¹											
Genre	1	2	3	4	5	6	7	8	9	10	11
<i>Fusarium</i>	97abA	93bA	97abA	96abA	93bA	96abA	96abA	100aA	93bA	97abA	73cA
<i>Aspergillus</i>	7bB	4bBC	0cB	0cC	7bBC	0cB	20aB	5bB	7bB	0cC	0,6bcB
<i>Penicillium</i>	0,6bC	13bBC	0bB	2bC	9aB	0bB	1,3bC	1,5bB	0bC	0,3bC	2bB
<i>Cladosporim</i>	4abBC	7aB	0bB	3abBC	6abBCD	1,5abB	0bC	2abB	5,5abC	7aB	0bB
<i>Bipolaris</i>	0aC	1aBC	0,5aB	0aC	0aD	2aB	0aC	1,5aB	0aC	5,6aBC	5aB
<i>Curvularia</i>	0aC	0aC	0aB	0aC	2,5aCD	1aB	0aC	0aB	0aC	0aC	0aB
<i>Phoma</i>	0aC	2aBC	0,5aB	3aBC	0aD	0aB	0aC	0aB	0,5aC	0aC	0aB
<i>Nigrospora</i>	0aC	1aBC	2,6aB	1,3aC	0aD	0aB	0aC	0aB	0,5aC	0aC	0aB
Incidence of fungi in asymptomatic grains of maize (%)											
Cultivars ¹											
Genre	1	2	3	4	5	6	7	8	9	10	11
<i>Fusarium</i>	84abA	91aA	63cA	90aA	86abA	80bA	78bA	63cA	86abA	85abA	92aA
<i>Aspergillus</i>	1aC	4aC	2aB	5aBC	4aC	1aC	3aC	0aC	4aC	4aC	1aC
<i>Penicilium</i>	1aC	6aC	4aB	2aC	2aC	7aC	2aC	0aC	2aC	0aC	4aC
<i>Cladosporim</i>	24abcB	26abB	8dB	14dB	23abcB	17bcdB	16cdB	28aB	12dB	10dB	16cdB
<i>Trichoderma</i>	1aC	2aC	0aB	0aC	0,5aC	0,5aC	0,5aC	0,5aC	0aC	0aC	4aC
<i>Bipolaris</i>	0,5aC	0aC	0aB	0aC	0,5aC	0aC	0,5aC	0aC	0aC	0aC	0aC
<i>Curvularia</i>	1aC	1aC	1aB	0aC	0,5aC	0,5aC	0aC	0aC	0aC	0aC	0,3aC
<i>Nigrospora</i>	0aC	0aC	0aB	0aC	0aC	0aC	1,5aC	0aC	0aC	0aC	0aC
<i>Colletotrichum</i>	0aC	0,5aC	0aB	0aC	0aC	0aC	0,5aC	0aC	0aC	2aBC	0aC
<i>Phoma</i>	0aC	0,5aC	0aB	0aC	0aC	0aC	0aC	0aC	0aC	0,5aC	0aC
<i>Papularia</i>	0aC	0aC	0aB	0aC	0aC	0,5aC	0aC	1aC	0aC	0aC	0aC
<i>Rhizopus</i>	1aC	0,5aC	0aB	0aC	0aC	0aC	0aC	0aC	0aC	1,5aBC	0aC

¹Cultivars: 1(AS1596PRO), 2 (DKB310PRO), 3(CD35), 4 (AG7088PRO), 5 (2B587PW), 6 (DKB390PRO), 7 (BRS1060), 8 (RB9308YG), 9 (BALU761) 10 (30F53YH) e 11 (BRS2020). Means followed by the same letter, lower case comparing in the row and upper case in the column does not differ statistically by the Scott-Knott test at 5%.

Other fungi were identified on the damaged and on the asymptomatic grains in this period, even in lower incidence levels. *Papularia* genre was found only in the last experiment on the asymptomatic grains of cultivars RB9308YG and DKB390PRO with very low incidence (Table 3). *Papularia* sp. and

Nigrospora sp. are also considered intermediate fungi, because they develop on mature or immature grains (FIGUEIRA et al., 2003).

The present study demonstrated that even apparently healthy (or asymptomatic) grains, when placed under humidity and favorable temperature, allow the development of previously present fungi. This fact is preoccupying when the grains are destined to feeding, resulting in food with possible toxins, as well as the seeds, because the germination can transmit the phytopathogens. Like the genre obtained in this study, for instance, the *Bipolaris*, *Colletotrichum*, *Fusarium*, *Aspergillus*, *Penicillium*, etc. are associated to the rottenness of maize seeds and death of seedlings in pre and post-emergence (HENNING et al., 2011).

The percentage values of damaged grains of each cultivar are presented in the table 4. It can be observed that in the experiment 1, crop 2013/2014 (Table 4), the cultivar BALU 761 presented the highest percentage of damaged grains, statistically differing from the cultivar BRS1060. Statistically, nine cultivars did not differ from the cultivars BALU 761 and BRS 1060. None of the cultivars passed the maximum limit of tolerance of 6% established as maximum value for the presence of damaged grains in lots of maize grains in Brazil.

In the second experiment, also, there was no percentage higher than the allowed, but again the cultivar BALU 761 presented highest percentage, differing from the cultivar DKB 390 PRO. Four cultivars did not statistically differ from the cultivar BALU 761. It was observed that in the crop of 2014/2015 (experiment 3), the hybrid 30F53 YH had higher percentage of damaged grains.

The observed values above the allowed of 6% may have occurred due to the presence of plague insects on the samples. These insects, when present, favor the higher contamination by the fungi, due to the damages they cause to the grains when they tear the cell wall to feed themselves, favoring the faster and more efficient penetration of the fungi.

The control and evaluation of the maximum tolerance limits to infected maize grains have high importance for food health of animals and humans, because as said by Hermanns et. al. (2006), the maize is one of the most susceptible grains to contamination by fungi considered toxigenic and to the accumulation of toxins that they produce. These mycotoxins are results of the secondary metabolism of some genres found on this present study, like *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp., which can inappropriately the grains for consumption, resulting in considerable quality and economic losses, fostering risks to health.

SANITARY ANALYSIS OF ASYMPTOMATIC AND DAMAGED GRAINS OF MAIZE IN TOCANTINS, BRAZIL

Table 4 - Percentage of damaged grains in the maize cultivars. Palmas, Tocantins, crop 2013/2014 and 2014/2015.

Cultivars	Experiment 1 ⁽¹⁾	Experiment 2 ⁽¹⁾	Experiment 3 ⁽¹⁾
AS1596 PRO	0,59 ab	0,44 abc	5,4 ab
DKB310 PRO	0,79 ab	0,28 bc	3,8 ab
CD355	0,68 ab	0,37 bc	6,8 ab
AG7088 PRO	2,04 ab	1,41 bc	2,0 b
2B587 PW	0,97 ab	0,29 abc	2,6 ab
DKB390 PRO	0,84 ab	0,19 c	5,8 ab
BRS1060	0,25 b	0,4 abc	3,4 ab
RB9308 YG	0,94 ab	0,35 bc	2,9 ab
BALU761	2,37 a	1,08 a	9,6 ab
30F53 YH	1,29 ab	0,83 ab	21,1 a
BRS2020	1,19 ab	0,32 bc	4,9 ab
CV%	35,02	27,26	8,67

¹ Values represent the original data, which for the analysis of variance were transformed by: $x = \arcsen\sqrt{x} / 100$. Means followed by the same letter do not differ statistically by the Tukey test at 5%

More than 500 types of mycotoxins have already been identified, however the ones with more importance for agriculture, responsible for the highest rates of contamination of grains, seeds and other foods are: the aflatoxins produced by fungi of *Aspergillus* genre, like *A. flavus* and *A. parasiticus*; the ochratoxins produced by the *Aspergillus* and *Penicillium* and the fusarium-toxins that carry as important representants the thricothecenes, zearalenones (*F. graminearum* and *F. poae*), and the fumonisins (*F. verticillioides* and *F. subglutinans*), vomitoxins, (*F. verticillioides*), T-2 toxin (*F. sporotrichioides*) produced by many species of the *Fusarium* genre. In Brazil, the more important mycotoxins, due to their high toxicity and contamination of food are the aflatoxins (MALLMANN & DILKIN, 2007; PINTO, 2007).

With the opening of new markets for exportation and food production, the preoccupation with food risks and with the development of pathologies, the sanitary quality of the produced primary material, like maize, becomes necessary and demands priority (BENTO et al., 2012).

The fungi that cause damaged grains infect the grains in many different periods, from the plants still on field to the post-harvest, however, many environmental, genetic and management factors interfere

directly on this process. Thus, there must have attention for the correct use of the practices for integrated production management and control of diseases on field and post-harvest, like the appropriate storage place with the most adequate phytosanitary conditions.

CONCLUSIONS

The damaged and asymptomatic grains of the cultivars presented a very diverse mycoflora and had as main associated genres the *Fusarium*, *Cladosporium*, *Penicillium*, and *Aspergillus*. The cultivars presented damaged grains percentage under the standards established by the Brazilian regulations. The asymptomatic grains presented high incidence of the genres *Fusarium*, *Penicillium* and *Aspergillus*.

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