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### Mycoflora on Maize Cobs Infected by *Ustilago maydis* (DC) Corda

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#### ABSTRACT

*Ustilago maydis* (DC) Corda, the causal agent of maize smut disease, can form enormous galls on cobs and cause significant yield losses under favorable conditions. In addition to yield losses, *U. maydis* may have an influence on the kernel quality in smutted cobs. The aim of this study was to determine presence of mycoflora on the cobs infected by *U. maydis*. A 2-year field experiment was conducted in Antalya Province. In the study, some maize cultivars belonging to various maize variety groups including dent corn, flint corn, sweet corn and popcorn were used as host plants. Inoculations were performed by injecting inoculum into ear silk of each cob of the plants in inoculated plots. For each treatment, control plots were also set up. When the kernels of cobs in control plots were mature enough to harvest, kernel samples in inoculated plots were taken from smutted cobs and investigated in terms of fungal flora. A total of 6 genera (*Paecilomyces*, *Aspergillus*, *Penicillium*, *Acremonium*, *Fusarium*, *Rhizopus*) were detected from the kernels in the smutted cobs. However, frequently isolated species were *Aspergillus fumigatus*, *A. niger*, *A. parasiticus* (*A. flavus*), *Paecilomyces lilacinus*, *Penicillium citrinum*, *Fusarium solani*, *F. oxysporum* and *Rhizopus stolonifer*. The most frequently isolated genus was *Aspergillus* (31.4%), while the lowest one was *Penicillium* (6.4%). The study showed that *U. maydis* may affect kernel quality of maize by harboring some fungal microorganisms in smutted cobs.

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#### 1. Introduction

Maize (*Zea mays* L.) is an important crop in terms of both human and animal nutrition and is used in many fields as a raw material in starch, glucose, oil and fodder industry (Kırtok, 1998). Having broad adaptation capability and high yield potential, maize can be grown in almost all the regions in Turkey (Gençtan et al., 1995).

Maize smut caused by *Ustilago maydis* occurs wherever maize is grown in the worldwide. However, it is more prevalent in warm and moderately dry areas. Unlike other cereal smuts, *U. maydis* gives rise to local infection and damages remarkably stalk and ear of maize by forming sizeable galls on them (Tunçdemir and İren, 1980). However, Aktaş (2001) reported that big galls, in particular, located on the ears of corn, could reduce yield up to 40-100%. In addition, du Toit and Pataky (1999) emphasized that smut causes greater economic damage to sweet corn than to other types of

maize. Because an ear of sweet corn with only one or two galls is non-marketable and smutted ears create additional costs during harvest and processing.

Apart from yield losses in the ears, corn smut leads to small kernels in the smutted cobs (Kinacı, 1987). Corn smut also affects kernel quality. The kernels infected by *U. maydis* can be vulnerable to the pathogens causing ear rot (Tunçdemir and İren, 1980). Likewise, Michaelson (1957) stated that plants infected with *U. maydis* were more subject to stalk rot caused by *Diplodia zeae* (Schw.) Lev. and *Gibberella zeae* (Schw.) Petch. than smut-free plants and that ears of smutted plants were more likely to be invaded with ear-rotting fungi than those from smut-free plants.

Although, a number of studies (e.g. Shah et al. 2010; Goertz et al. 2010; Altınok and Dikilitaş, 2011; Covarelli et al. 2011; Maširević et al. 2012; Bii et al. 2012) related to mycoflora on maize kernels are available, little is known about fungal contamination in the smutted cobs.

The objective of the present study was to find out presence of mycoflora on the kernels infected by *U. maydis*.

## 2. Materials and Methods

Galls were obtained from smutty plants in the maize producing areas of Batı Akdeniz Agricultural Research Institute in Aksu district of Antalya in 2009 and in 2010. Potato dextrose agar (PDA, Oxoid) and 20% carrot solution were used to get pure culture of *U. maydis* and propagate sporidia (basidiospores). In the field trials, dent corn (*Zea mays* var. *indentata*) cultivars, Ada-523, Pioneer-3394 and Side, flint corn (*Zea mays* var. *indurata*) cultivars, Karaçay and Karadeniz Yıldızı, sweet corn (*Zea mays* var. *saccharata*) cultivars, Merit, Vega and popcorn (*Zea mays* var. *everta*) variety, Antcin-98, were used as host plants.

### Isolation of *Ustilago maydis*

The galls were chopped and chlamydospores (teliospores) were separated from the gall tissues by sieving through a tea strainer. Afterwards, teliospores were surface-sterilized by immersion in a 1% copper sulfate solution for 20 to 60 h and filtered through two layers of sterile cheesecloth not allowing the teliospores to pass through. Later, teliospores on the cheesecloth were washed in three times in sterile distilled water and dried on sterile filter paper, and transferred on PDA supplemented antibiotic (streptomycin sulphate) in petri dishes. The dishes were incubated at 25 °C for 4 to 5 days until sporidia (basidiospores) of *U. maydis* emerged. When sporidia were about a pinhead in size, they were taken from cultures, and transferred in 500-mL Erlenmeyer flasks containing 20% sterile carrot solution, and incubated at 25 °C for 7 days. At the same time, Erlenmeyer flasks were shaken vigorously for 1 to 2 min once or twice a week. In this way, inoculum required for inoculations was obtained by allowing sporidia to multiply in the carrot solution (Tunçdemir, 1985).

### Preparing of the inoculum

Basidiospore suspensions in the Erlenmeyer flasks were stirred to get a homogeneous solution and basidiospores were counted by using a hemocytometer (Neubauer, Isolab, Germany). Basidiospore suspensions were diluted to appropriate concentrations by using sterile carrot solution and adjusted to  $4 \times 10^6$  sporidia mL<sup>-1</sup>, afterwards, in the same way, teliospore suspensions were arranged to  $1 \times 10^6$  teliospores mL<sup>-1</sup> and added into the basidiospore suspensions (Tunçdemir, 1985).

### Field experiment

The experiment was set up under ecological conditions of Aksu district in Antalya Province, located in the Mediterranean region of Turkey. Field trials were carried out in a randomized complete blocks design

with a factorial arrangement with three replications (Düzgüneş et al., 1987). Each plot consisted of four rows, each of which was 5 m long. Row spacings was 70 cm between the rows and 20 cm within the rows. Control plots were established for each treatment.

### Ecological properties of the research area

General soil texture of the experiment area was a type of heavy textured soil, clayish-loamy. The experiment area was fertilized with nitrogen, phosphor and potassium at the rates of 180, 80 and 80 kg ha<sup>-1</sup> respectively. In addition, meteorological data including temperature, rainfall and relative humidity of the research area during maize growing seasons in 2010 and 2011 were given in Table 1.

Table 1

Meteorological data of the experiment field (2010-2011)

Months	Temperature (°C)		Rainfall (mm)	
	2010	2011	2010	2011
May	21.7	19.9	4.2	107.2
June	25.1	25.1	25.4	5.0
July	27.9	28.6	-	-
August	30.5	29.6	4.2	0.0
September	26.9	26.7	4.8	83.2
October	20.8	19.1	89.0	395.8
May	21.7	19.9	4.2	107.2
June	25.1	25.1	25.4	5.0

Regional Meteorology Station, Antalya

### Inoculation

The ear inoculation method described by Pataky et al. (1995) was used with some modifications: For the ear silk of each emerging plant before pollination, 3 mL inoculum ( $3 \times 10^6$  sporidia mL<sup>-1</sup> +  $1 \times 10^6$  chlamydospores mL<sup>-1</sup>) was injected into the ear of each plant through a hypodermic syringe. Inoculations of ears were performed on the dates of 3, 10 and 20 August in 2010 and 11, 15, 18 and 25 August in 2011 respectively.

### Isolation of the mycoflora

Observing appropriate harvest time for the kernels in the control plots, a total of 30 kernels from each smutted ear in inoculated plots were taken to the small paper bags. Later, the kernels in the paper bags were separated small seed lots and exposed to running tap water. The seeds in the each lot were submerged in 2% NaOCl for 2 min for surface disinfection. Afterwards, the kernels were rinsed three times with sterile water and dried on sterile filter papers. 10 seeds for each petri dish were placed on different media including PDA (potato dextrose agar), MEA (Malt extract agar) CA (cherry agar), and blotter papers and incubated at 25 °C for 5 to 7 days until emergence of any fungal colony.

### Identification

Growing colonies on the media, a pinhead in size medium from each colony were transferred to new media (PDA, MEA, CA) via a loop in order to provide development of each colony purely. This process were repeated a few times and then prepares from pure culture of each fungal colony were examined under a trinocular microscope (Leica DM Z500). Identification of the fungi were performed by Professor Dr. Salih Maden (Ankara University) and Professor Dr. Nuh BOYRAZ (Selçuk University) according to the morphological structures of the fungi. During identification of fungi, several sources (Von Ark, 1970; Barnett and Hunter, 1972; Warham et al., 1996) were used.

### 3. Results and Discussion

Following injection of the inoculum, about 10-12 days later, galls on the ears of the plants in inoculated plots appeared. The galls were observed as semi-fleshy, consisting of the smut fungus intermixed with enlarged cells of the cobs of the hosts. Initially, the young galls were firm with a whitish tint and were covered by semi-glossy peridium, which turned black as the galls matured. When the membrane of the galls died, it usually cracked open, exposing the dry powdery spores (teliospores). In general, the galls varied from minute sizes (0.2 cm in diameter) to 24 cm in diameter. In the ears, the ovaries and glumes were smutted. However, various sizes of galls were observed on the cobs of the hosts. Sometimes, the entire pistillate inflorescence was converted into a huge smut gall due to severe infection of *U. maydis* (Figure 1).



Figure 1

Infection of *U. maydis* on the cob

As a clear example of fungal contamination, whitish mycelium growth around the galls on the smutted ear, is seen in the Figure 2.



Figure 2

Fungal contamination on smutted cob

As a consequences of inoculations performed both in 2010 and in 2011, with development of the galls on the ears, a total of 124 fungal isolates were isolated from 136 samples collected from smutted cobs in the both years.

6 genera (*Paecilomyces*, *Aspergillus*, *Penicillium*, *Acremonium*, *Fusarium* and *Rhizopus*) were detected from the samples. However, totally 7 species, as the most frequently isolated species (*Aspergillus fumigatus*, *A. niger*, *A. parasiticus* (*A. flavus*), *Paecilomyces lilacinus*, *Penicillium citrinum*, *Fusarium solani*, *F. oxysporum* and *Rhizopus stolonifer*) were established among those genera.

The most frequently isolated genus from all the samples collected both in 2010 and in 2011 was *Aspergillus* at a rate of 31.4% whereas the lowest frequently isolated genus was *Penicillium* in the ratio of 6.4%. As for other genera isolated were *Paecilomyces* (21.7%), *Fusarium* (18.5%), *Rhizopus* (12.9%) and *Acremonium* (8.8%) respectively (Figure 3).

Compared the species isolated within their genus, the most frequently isolated species was *Aspergillus fumigatus* with a rate of 10.2% in *Aspergillus* genus. However, *Aspergillus niger* (7.6%) and *Aspergillus parasiticus* (*A. flavus*) (5.1%) were the other *Aspergillus* species determined from the samples. As for *Fusarium* genus, the most frequently isolated species were *Fusarium oxysporum* and *F. solani* at the rates of 13.0% and 8.6% respectively. In addition, within the 6 genera isolated, other species determined were *Paecilomyces lilacinus* (22.2%), *Penicillium citrinum* (12.5%) and *Rhizopus stolonifer* (18.7%) (Figure 4).

In the present study, a total of 8 species belonging to 6 genera were determined. However, in a study conducted by Davoodee and Fahmideh (2003) in Iran, 58 different isolates belonging to 5 genera of fungi were isolated from rotted ears. However, Demirci and Kor-

dalı (2000) reported that a total of 26 fungal species pertaining to 19 genera were isolated from the kernels

in the Eastern Black Sea Region in Turkey.

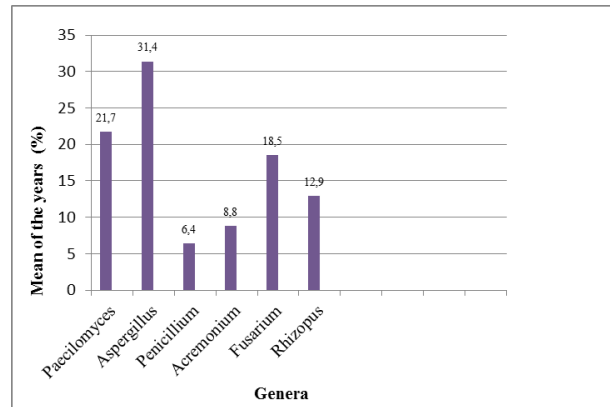


Figure 3  
Fungus genera isolated from the smutted ears (2010/2011)

In our study, when the genera isolated were compared, the most frequently isolated genera were *Aspergillus* (31.4%), *Paecilomyces* (21.7%), *Fusarium* (18.5%), *Rhizopus* (12.9%), *Acremonium* (8.8%) and *Penicillium* (6.4%) respectively. Likewise, Kpoda et al. (2000) reported that *Aspergillus* spp. were dominant fungi (76.4%) followed by *Penicillium* spp. (19.9%) in Ghana. Ařkun (2006) also stated that *Aspergillus* spp. (25%), *Fusarium* spp. (21%), *Rhizopus* spp. (21%) and *Penicillium* spp. (13%) were commonly isolated genera from the disinfected maize samples in Balıkesir province of Turkey.

On the other hand, Orsi et al. (2000) emphasized that a predominance of *Fusarium* spp. followed by *Penicillium* spp., *Aspergillus* spp. in Brasil. In addition, Covarelli et al. (2011) reported that *Fusarium* spp. were the most abundant species detected in maize kernels, followed by *Aspergillus* species of sections *Flavi* and *Nigri* by *Penicillium* spp. in central Italy. However, Krnjaja et al. (2013) presented the highest incidence of *Rhizopus* (56.41%), *Aspergillus* (43.66%) and *Fusarium* (14.97%) genera on maize kernels.

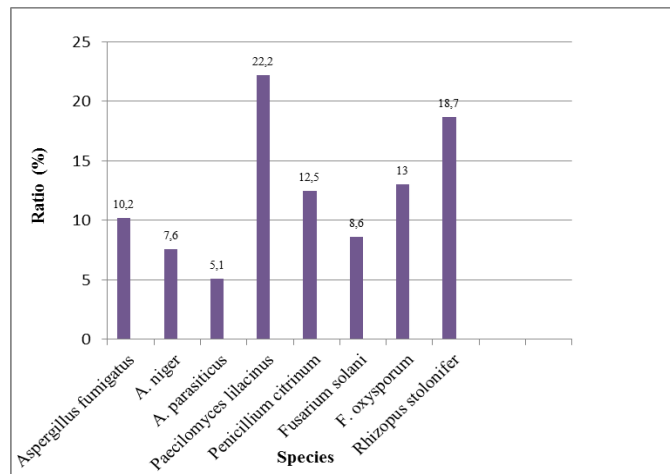


Figure 4  
Fungus species isolated from smutted cobs (2010/2011)

In our study, within the genera isolated, the most dominant species were *Aspergillus fumigatus*, *A. niger*, *A. parasiticus*, *Paecilomyces lilacinus*, *Fusarium oxysporum*, *F. solani*, *Penicillium citrinum* and *Rhizopus stolonifer*. However, Kaaya and Kyamuhangire (2006) reported that *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* were the most predominant fungal genera identified and, among their species, *A. niger* had the highest incidence, followed by *A. flavus*, *F. verticillioides*, *A. wentii*, *A. penicillioides* and *Rhizopus stolonifer* in Uganda. Sitara and Akhter (2007) stated that 7 genera and 11 species of fungi (*Aspergillus niger*, *A. flavus*, *A. wentii*, *Chaetomium* sp., *Drechslera* sp., *Fusarium chlamydosporum*, *F. oxysporum*, *F. moniliforme*, *F. semitectum*, *F. nivale*, *Nigrospora* sp., *Phoma* sp. and *Rhizopus* sp.) were isolated from maize seeds in Pakistan. Maširević et al. (2012) also reported that fungi from genera *Fusarium*, *Penicillium*, *Aspergillus* and *Alternaria* were isolated from maize seeds in Serbia.

During harvest and post-harvest, maize kernels could be infected by several fungi inducing toxins for harmful to humans and animals (Mostafa and Kazem, 2011). In 114 farmsteads in Zambia, concentration of fumonisins from six districts, and aflatoxin from two districts, was 10-fold higher than 2 ppm and far higher than 2 ppb maximum daily intake recommended by the FAO/WHO, indicating high levels of ear rot infections in maize grain (Mukanga et al., 2010). Stored maize and maize products in Kenya were contaminated by various types of toxigenic fungi (e.g. *Penicillium islandicum*, *Aspergillus terreus*, *Aspergillus clavatus*) (Gacheri, 1990). In addition, in a number of studies (e.g. Altıparmak, 2007; Uçkun, 2008; Shah et al., 2010; Goertz et al., 2010; Altınok and Dikilitaş, 2011; Covarelli et al., 2011; Maširević et al., 2012; Bii et al., 2012) were also emphasized that various mycoflora in particular *Aspergillus*, *Fusarium*, and *Penicillium* on maize kernels may exist and produce detrimental toxins. Hence, it can be concluded from our study that the fungal genera detected have the potential for inducing mycotoxin.

During our field experiments, as the galls on the ears were growing, husks of the ears were loosening up and accordingly kernels in the smutted cobs were seen directly. With loosening of the husks, kernels in the smutted cobs can be readily exposed to invasion of any microorganism. Likewise, Sutton et al. (1980) reported that loosening and shredding of husks was a factor in predisposition to fungal growth. Thus, the kernels in smutted cobs might be exposed to miscellaneous fungal contamination and become small and dull appearance as our study. Accordingly, another emerging problem is marketing of such crops due to its appearance.

In conclusion, the kernels in smutted cobs could be low quality due to the galls formed by *U. maydis*. In addition, smutted cobs can be easily exposed to fungi producing mycotoxins. The current study suggested

that forming galls on cobs, maize smut not only reduce yield but also make it possible other fungal contamination of the kernels and accordingly can pose a risk for human and animal health.

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