Effect on Yield Losses on Maize (*Zea mays L.*) Caused by Smut Disease (*Ustilago maydis* (DC) Corda)

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ABSTRACT

Ustilago maydis,the causal agent of maize smut disease, can cause significant yield losseson maize under favorable conditions for the fungus. The aim of thestudy was to determine yield losses due to*U. maydis* insome cultivars belonging to different maize variety groups. Thus,a 2-year field experiment was conducted in Antalya Province in 2010 and in2011.Inoculations of *U. maydis* were performed by injecting inoculum into apical nodes of plants, 40- 60 cm high, and ear silk in inoculated plots. For each treatment, control plots were also set up.Mean yield losses of the cultivars tested in 2010 and 2011 were at the rates of 22,3% and 46,3%, respectively. However, of all the varieties, mean yield losses of the two-year varied from 23,1% to 41,4%. The highest yield losseswere found on dent corn cultivars (Ada-523, Pioneer-3394 and Side), whereas, the lowest yield losses were on sweet corn variety, Merit.

Key words: Corn, yield, losses, fungus

Mısır Rastığı Hastalığının (*Ustilago maydis* (DC) Corda) Mısırda (*Zea mays L.*) Verim Kayıplarına Etkisi

ÖZET

Mısır rastık hastalığının etmeni olan *Ustilago maydis,* uygun koşullarda mısır bitkilerinde önemli verim kayıplarına neden olabilmektedir. Çalışmanın amacı, farklı mısır varyete gruplarına ait bazı mısır çeşitlerinde *U. maydis* nedeniyle oluşan verim kayıplarının tespit edilmesiydi. Bu nedenle, 2010 ve 2011 yıllarında Antalya'daiki yıllık tarla denemesi yürütülmüştür. İnokulasyonlar parsellerdeki 40-60 cm boydaki mısır bitkilerinin en uç boğumu ve ipeklere inokulumun enjekte edilmesiyle yapılmıştır. Her muamele için ayrıca kontrol parselleri de teşkil edilmiştir. 2010 ve 2011 yıllarında test edilen mısır bitkilerinde ortalama verim kayıpları sırasıyla % 22,3 ve % 46,3 oranında olmuştur. Bununla birlikte, tüm çeşitler arasında iki yılın ortalama verim kayıpları % 23,1 ile % 41,4 arasında değişmiştir. En yüksek verim kayıpları at dişi mısır çeşitlerinde(Ada-523, Pioneer-3394 ve Side) bulunurken, en düşük verim kayıpları ise Merit şeker mısır çeşidinde belirlenmiştir.

Anahtar kelimeler: Mısır, verim, kayıplar, fungus

INTRODUCTION

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

Maize smut, caused by *Ustilago maydis* (DC) Corda., occurs wherever maize is grown all over the world.However, it is more prevalent in warm and moderately dry areas. Corn smut reduce yields by forming galls aboveground parts of plants. The number, size and location of smut galls on the plant affect the amount of the yield loss.Unlike other cereal smuts, *U. maydis* gives rise to local enfection and damages stalks and ears with its colossal galls (tumors) on them. In case of severe infectionat early development stage of plant, the pathogen also can cause either death or infertility of the plants(Tunçdemir and Iren, 1980).

At the end of the nineteenth century, several publications regarding yield losses due to corn smut were reported in the U.S.A.Henry (1881) stated that losses from corn smut in Wisconsin in 1881 were 5-15% for individual fields. In 1884, the loss for 1 field of sweet corn was at a rate of 66% (Bessey, 1884). However, Selby and Hickman (1897) reported that loss was about 4,4% of the crop in Ohio.

At the twenty century, some authors (Immer and Christensen, 1928; Jorgensen, 1929; Immer and Christensen, 1931; Johnson and Christensen, 1935) indicated that yield of corn from infected plants varied with the number, size and location of the galls. However, Christensen (1963) stated that on the average, a single gall reduced ear yield about 25%. In addition, a medium or large gall and multiple small galls located on a main stalk frequently caused the maize plant to be barren (Garber and Hoover, 1928; Jorgensen, 1929; Immer and Christensen, 1931; Christensen and Johnson, 1935; Stringfield and Bowman, 1942).For twenty-first century, various publications as regards yield losses ofcorn smutwas reported by several authors (Aktaş, 2001; Sade, 2001; Agrios, 2004; Aydoğdu and Boyraz, 2006).

In general, in the studies regarding corn smut in the past, yield losses were evaluated in terms of natural infections of the pathogen. Thus, little is known about yield losses due to the disase, appears via artificial inoculations. The purpose of the study was to determine yield lossesofcorn smut of some maize cultivars through artificial inoculations. Accordingly, the maize cultivars regarding todifferent maize variety groups including dent corn, flint corn, sweet corn and popcorn were tested under the ecological conditions of Aksu district in Antalya Province of Turkey.

MATERIALS AND METHODS

Materials

Galls (smutty ears) were obtained from smutty plants in maize-producing areas of Batı Akdeniz Agricultural Research Institute, located on Mediterranean region of Turkey in 2009 and 2010. Potato dextrose agar (PDA, Oxoid) and 20% carrot solution were used to get pure culture of *U. maydis* and for propagation of sporidia (basidiospores), respectively. 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 and Vega; and popcorn (*Zea mays* var. *everta*) variety; Antcin-98 were used as host plants.

Methods

Isolation of *U. 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 changes of sterile distilled water and dried on sterile filter paper, and transferred on PDA supplemented by 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).

Preparation 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 × 10^6 sporidia mL^{-1}, afterwards, in the same way, teliospore suspensions were arranged to 1 × 10^6 teliospores mL^{-1} and added into the basidiospore suspensions (Tunçdemir, 1985).

Field experiments

Field trials were carried out in a randomized complete blocks design with a factorial arrangement with three replications. Each plot consisted of four rows, 5 m long. The 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

Soil texture of the research area was clayish and loamy. The area was fertilized with nitrogen, phosphor and potassium at the rates of 180, 80 and 80 kg ha^{-1} respectively. Field experiments were set up in Antalya province of Turkey. When inoculations of the maize ears were done in Augustin2010, monthly rainfall in total was 4.2 mm whereas in the same period of 2011 no measurable rainfall was recorded. However, mean temperature and relative humidity of August in 2010 and 2011 were 30.5 º C, 59.1% and 29.6 º C and 50.0%, respectively (Anonim, 2013).

Table 1. Meteorological data of the experiment field during maize growing seasons

Months	Monthly total rainfall (mm)		Monthly mean relative humidity (%)	
	2010	2011	2010	2011
May	4,2	107,2	64,7	65,7
June	25,4	5,0	61,8	57,1
July	-	-	66,0	60,1
August	4,2	0,0	59,1	50,0
September	4,8	83,2	58,1	50,3
October	89,0	395,8	57,2	54,6

Regional Meteorology Station, Antalya

Table 2. Inoculation time and daily mean temperature of the research area

Inoculation time (2010)		Temperature* (2010)		Inoculation time (2011)		Temperature (2011)	
July	August	July (°C)	August (°C)	July	August	July (°C)	August (°C)
16	3	34,1	29,8	18	11	30,0	27,9
17	4	35,6	29,5	19	15	29,7	27,2
19	5	32,4	29,7	20	16	29,7	27,5
	10		29,0	21	17	27,9	28,0
	11		29,2		18		28,9
	12		29,4		25		29,4
	20		29,9		26		28,4
	21		32,0		27		27,6
	22		31,8		28		26,8

*Regional Meteorology Station, Antalya

Inoculations

Inoculations were performed in two growth stages of maize plants as follows:

1. When the plants height were about 40-60 cm, 2 mL inoculum (4 × 10^6 sporidia mL^{-1} + 1 × 10^6 chlamydospores mL^{-1}) was injected into apical node of the plant by means of a hypodermic syringe (Tunçdemir, 1985). The inoculations of the plants were performed between at 6 pm and 8:30 pm on the dates of 15 July and 18 July in 2010 and in 2011 respectively. Mean daily temperature of the ensuing 3-day was 34,0 °C in 2010 whereas it was 29,1 °C in 2011 (Table 2).

2. The ear inoculation method as described by Pataky et al. (1995) was used with some modificatios: For the ear silk of each emerging plant before pollination, 3 mL inoculum (3×10^6 sporidia mL^{-1} + 1×10^6 chlamydospores mL^{-1}) 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. Mean daily temperatures of the inoculation days and the ensuing 3-day in 2010 and in 2011 were recorded as 30,0 °C and 27,9 °C respectively (Table 2).

Yield loss

All ears from both inoculated and non-inoculated plots were separately collected at harvest. The ears collected were husked and left to dry under open air for 3 days. Ratio of kernel/cob as percentage was calculated according to Yanıkoğlu et al. (1999).

Afterwards, moisture content of kernels for each treatment were separately determined by keeping the kernels at 72 °C for 72 h, and yield was adjusted for 15% moisture content according to following formula (Poehlman, 1987).

$$\textbf{Adjusted weight} = \text{Plot weight} \times \frac{(100 - \text{moisture \%})}{85} \times \frac{(\text{kernel/cob})}{100}$$

Yield of the plots in the experiments was determined by using the formula shown below (Yanıkoğlu et al. 1999).

$$\textbf{Yield of plot (kg/da)} = \text{Adjusted weight} \times \frac{1000}{\text{Plot area } (m^2)}$$

In conclusion, yield losses from *U. maydis* was calculated by comparing the yield of inoculated plots with the yield of control plots.

Statistical analysis

JMP statistical software (SAS Institute Inc., Cary, North Carolina, USA) was used for variance analysis. Differences between factors were determined by F test and the mean values determined as different were grouped according to $LSD_{0.05}$ test (Düzgüneş et al., 1987).

RESULTS

The galls varied from minute sizes (0.2 cm in diameter) to 20 cm diam. The leaf galls displayed difference in their size and texture. However, the galls on leaves generally developed as small along the midrib of the leaves. Most of the tiny leaf galls remained firm and frequently contained few teliospores. The galls occuring on the main stalk usually appeared just above the nodes, however, they were observed on any part of the main stalk. The galls located on the main stalk were rather large, 10 to 20 cm in diameter, and varied greatly in size and shape. In the ears, the ovaries and glumes were smutted. Sometimes, the entire pistillate inflorescence was converted into a huge smut gall due to severe infection of *U. maydis*.

Table 3. Yield values determined in maize cultivars tested

Variety	2010			2011			Mean Variety x disease		Mean of the variety
	Yield control (kg/da)	Yield (*inoc.) (kg/da)	Mean (variety x year)	Yield control (kg/da)	Yield (inoc.) (kg/da)	Mean (variety x year)	Yield control (kg/da)	Yield (inoc.) (kg/da)	
Ada-523	**1140 a	879 ef	1010 a	1067 ab	487 gh	777 c	1104 a	683 d	894 a
Karaçay	1130 a	925 de	1028 a	930 cde	437 g-ı	684 d	1030 b	681 d	856 ab
Pioneer-3394	1028 bc	796 f	912 b	1049 ab	506 g	778 c	1039 ab	651 de	845 ab
Side	1086 ab	837 ef	962 ab	1009 b-d	390 h-j	700 d	1048 ab	614 de	831 b
Karadeniz Yıldızı	1057 ab	801 f	929 b	801 f	381 ı-k	591 e	929 c	591 e	760 c
Merit	412 g-j	335 j-l	374 f	400 h-j	288 kl	344 f	406 f	312 g	359 d
Ant-cin	410 g-j	344 ı-l	377 f	407 g-j	252 l	330 f	409 f	298 g	354 d
Vega	404 h-j	268 l	336 f	401 h-j	271 l	336 f	403 f	270 g	337 d
Mean of the year x treatment	833 a	648 c	-	758 b	377 d		796 a	513 b	-
Mean of the year	741 a			568 b					
Mean	655								
Year LSD (0.01)= 33,3 Variety LSD (0.01)= 66,6									
Disease LSD (0.01)= 33,3 Year x variety LSD (0.01)= 94,2									
Year x disease LSD (0.01)= 47,1 Variety x disease LSD (0.01)= 94,2									
Year x variety x disease LSD (0.01)= 133,3									

*: inoculated ** : Data are means of three replicates

Yields of the maize cultivars tested

Differences among yield values of cultivars were significant (P<0.01). Mean yield of the maize varieties, as average of inoculated and control plots, was 741 kg/da in 2010, whereas, this value in 2011 was 568 kg/da (Table 3).

The highest (1140 kg/da) and lowest yield (404 kg/da) in control plots were found on Ada-23 (dent corn variety) and Vega (sweet corn variety), respectively, in 2010. However, in inoculated plots, the highest yield (925 kg/da) and lowest yield (268 kg/da) were on Karaçay (flint corn variety) and Vega (sweet corn variety), respectively. Mean yield of the cultivars was 833 kg/da in control plots while this value was 648 kg/da in inoculated plots in 2010. The highest (1067 kg/da) and the lowest yield (400 kg/da) in control plots were on Ada-523 (dent corn variety) and Merit (sweet corn variety), respectively, in 2011. In inoculated plots, these values detected on Pioneer-3394 (dent corn variety) and Antcin-98 (popcorn variety) with the amount of 506 kg/da and 252 kg/da, respectively in 2011. However, mean yield of the maize cultivars in control plots were 758 kg/da, while, this one in inoculated plots was 377 kg/da in 2011. Average yield of the two year-study in all the cultivars was 796 kg/da in control plots but this value in inoculated plots was 513 kg/da (Table 3).

Yield losses due to corn smut

Yield losses of the maize cultivars in 2011 were higher than the ones in 2010, except for Vega (sweetcorn variety). However, the highest yield loss (33,6%) was found on the Vega while the lowest yield loss (16,0%) was on Antcin-98 (popcorn variety) in 2010 (Table 4).

The highest (61,3%) and the lowest yield loss (28,0%) were found on Side (dent corn variety) and Merit (sweet corn variety), respectively, in 2011. Of all the cultivars, as an average of the two year-value of the study, the highest yield loss (41,4%) was detected onSide (dent corn variety) whereas, the lowest yield loss (23,1%) wason Merit (sweet corn variety). In addition, as an average of the both years, corn smut disease reduced yields of the cultivars with a rate of 33,7% (Table 4).

Table 4. Yield losses from *U. maydis* in the maize cultivars tested

Variety	2010	2011	Mean
	Yield loss(%)	Yield loss(%)	Yield loss(%)
Ada-523	22,8	54,3	38,1
Pioneer-3394	22,5	51,7	37,3
Side	22,9	61,3	41,4
Karaçay	18,1	53,0	33,8
Karadeniz Yıldızı	24,2	52,4	36,3
Merit	18,6	28,0	23,1
Vega	33,6	32,4	33,0
Antcin-98	16,0	38,0	27,1
Mean	22,3	46,3	33,7

*Data are means of three replicates

DISCUSSION

In the present study, with artificial inoculations, galls were observed on any aboveground part of the maize varieties. The size, the shape and the number of the galls on the plants varied depending on susceptibility of the maize cultivars and location of the host. The authors (Christensen, 1963; Kınacı, 1987) reported similar findings in theirstudies. In our study, the galls formed on the ears caused significant yield losses, statistically. In this regard, Christensen (1963) emphasized that large galls on the ear or tassel usually caused barren stalks or severe reduction in ear yield. As for, Aktaş (2001) reported that big galls, in particular, located on ears, could reduce yield up to 40-100 %. The authors (Sade, 2001; Agrios, 2004) also stated that the galls on the ears may lead to serious yield losses in the maize plants.

Mean yield losses of the cultivars, as a mean of the two-year, ranged from 23,1% to 41,4%. However, Aydogdu and Boyraz (2006) reported that yield losses from *U. maydis* varied from 26,4% to 51,7% among 10-dent corn-varietyinKonya Province. Tunçdemir and Iren (1980) also stated that mean yield losses in Samsun Province and its vicinity was 25.504 tons annually due to corn smut. As for, Kınacı (1987) emphasized that smut galls, < 5 cm in diameter, 5-7.6 cm in diameter, and, 7.6 cm < in diameter, in a maize plant caused to yield losses at the rates of 9%, 14% and 40%, respectively.

In our study, yield losses of both dent corn (Ada-523, Pioneer-3394, Side) and flint corn (Karaçay, Karadeniz Yıldızı) varieties were higher than the ones of sweet corn (Merit, Vega) and popcorn (Antcin-98) varieties. This could be explained by evaluating morphological features of the maize cultivars tested. Accordingly, having bigger cobs, both dent corn and flint corn varieties had bigger galls on their cobs than the ones on the other varieties. Therefore, yield losses of the both cultivars were higher than the ones of the others. In our study, average yield of all the varieties decreased at a rate of 33,7% due to corn smut (Table 4). However, Aydogdu and Boyraz (2006) reported that mean yield loss from corn smut of 10-dent corn-variety was 38,1% in Konya.

In addition, in our study, yield losses of all the maize cultivars in 2011 were higher than the ones in 2010 (Table 4). The variation between the years can be attributed to the more severe ear infection in 2011 than the ones in 2010. However, mean yield values of the cultivars in 2010, as a mean of both inoculated and control plots, were higher than the ones of 2011. In this regard, it could be inferred that environmental factors in 2010 were more favorable for the maize cultivars than the ones in 2011 (Table 3). Accordingly, corn smut averagely reduced yield of the cultivars at the rate of 22,3% in 2010 whereas the disease decreased the yield of the varietiesup to 46,3% in 2011 (Table 4). In addition, interactions of year, variety, and year x variety x disease were significant ($P<0.01$) (Table 3). Year-to-year variation of our study could be clarified as follows: as is known that immediately after the inoculation, hours and a few days could play an important role in disease development. Tunçdemir and Iren (1980) reported that the most favorable temperature for development of corn smut rests between 18 °C and 21°C. In our study, in 2010, following inoculation, the mean daily temperature of the ensuing 3 days was 34,0 °C whereas it was 29,1 °C in 2011 (Table 2). The mean daily temperatures during the inoculation in 2010 were about 5 °C higher than the one in 2011. Accordingly, this significant difference may have adversely affected penetration of the pathogen in 2010. However, it was appeared that environmental conditions of 2010 were favorable for the host. Thus, maize plants in 2010 were more resistant to *U. maydis* than the ones in 2011. Kyle (1929) emphasized that when environmental factors continue in favor of the host in maize growing season, smut enfections appear minimum level. As for, Walter (1935) reported that maize plants rapidly-developing in the period between seedling stage and adult plant were resistant to *U. maydis* or escaped from the pathogen.

The year-to-year variability in our study could particularly be attributed to significant discrepancies of the mean daily temperatures during the inoculation periods and other environmental factors. In a two-year-survey, Görtz et al. (2008) stated thatfrrequency of kernels infected by *Fusarium* spp. ranged from 0,7% to 99,7% in 2006 while the highest incidence of *Fusarium* ear rot was 64% in 2007 and the year-to-year variability in the frequency of *Fusarium* species and in the overall infection rate may be explained by significant differences in temperature and precipitation during the growth periods.

Physiology and morphological structure of host can also play an important role in disease development. Since the maize cultivars tested have specific physiology and morhological features, different yield losses from *U. maydis* were determined in the present study. In addition, several authors indicated that both morphological and physiological structure of maize could affect development of smut disease (Walter, 1935; Christensen, 1963; Tunçdemir and Iren, 1980; Yanıkoğlu et al., 1999).

Our study revealed that infection of maize plants by*U. maydis* was significant factor affecting the yield in the maize cultivars tested under ecological conditions of Aksu district in Antalya.

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