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Influence of Pollination on Smut Disease and Yield in Maize

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ABSTRACT

Ustilago maydis, causal agent of smut disease in maize, induces significant yield losses by forming colossal galls (tumours) on cobs. Since infection process of *U. maydis* is parallel with natural pollination of maize, an interaction between maize pollination and the smut fungus is probable. To reveal this interaction perceptibly, a 2-year field study was carried out in Antalya province of Turkey. As host plants, 8-maize-cultivars belonging to different maize variety groups [dent (Ada-523, Pioneer-3394 and Side), flint (Karaçay and Karadeniz Yıldızı), sweet (Merit and Vega) and popcorn (Antcin-98)] were used in the experiment. Inoculations were performed by injecting inoculum into cob silks in pre- and post-pollination periods in plots. In addition, control plots were set up for each treatment. In conclusion, average disease severity, incidence and yield losses of all the maize cultivars in pre-pollination inoculations (PrePI) were 3.8, 20.7 and 45.5%, whereas in post-pollination (PostPI) inoculations, they were 0.9, 15.7 and 35.9%, respectively. It was found that in both years, disease values of the PrePI were higher than those of the PostPI. This study suggested that pollination period of maize is an important factor affecting *U. maydis* infection in cobs and accordingly yield losses.

Keywords: Maize pollination; *Ustilago maydis*; Yield loss

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1. Introduction

Maize is a staple crop for human and animal nutrition as well as fodder industry (Kırtok 1998). In 2013, total maize production in the world exceeded one billion tons (FAO 2015). However, *Ustilago maydis* (DC) Corda, called also as corn smut, is one of the primary constraint to maize yield. The disease occurs wherever maize is grown. Unlike other cereal smuts, *U. maydis* gives rise to local infections and severe damages to cobs through formation of huge galls on them (Tunçdemir & Iren 1980). In addition, it particularly causes great economic damage to sweet corn (du Toit & Pataky 1999).

When maize seedlings are infected by the fungus, small galls appear on the leaves and stems, and the seedling may remain stunted or may be killed. On older plants, infections occur on the young, actively growing tissues of axillary buds, individual flowers of the cob and tassel, leaves and stalks. Infected areas are permeated by the fungus mycelium, which stimulates the host cells to divide and enlarge, thus forming galls. Galls are first covered with a greenish white membrane. Later, as the galls mature, their interior darkens and turns into a mass of powdery, dark olive-brown spores (Agrios 2005). *U. maydis* infects stigmas in maize cobs via silks at the tip of the cob. Sporidia of the fungus accumulate on silks (Shurtleff 1980). Infection process of stigma by the fungus is as follows: when fungus sporidia contact with stigma, sporidia mate in pairs and each pair forms a dikaryotic infection hypha and then enter the stigma. Similarly, fertilization of stigma by pollens occurs as follows: pollens moving from tassels pass through the silk channel and reach to stigma and fertilize ovaries. Following pollination, an abscission zone consisting of layer of dead cells in pollinated silks occurs. *U. maydis* is not able to grow across this abscission zone (Snetselaar & Mims 1993; Snetselaar et al 2001). This phenomenon suggests that when the pollens reach to the ovary earlier than the infective hypha of the fungus, *U. maydis* infection in the cobs become formidable. Correspondingly, plant may escape from the infection.

The main objectives of the study were to perceptibly examine this phenomenon by injecting inoculum into cob silks of different maize varieties in pre and post pollination periods and determine yield losses arising from the fungus infection during pollination period.

2. Material and Methods

Galls on cobs were obtained from naturally infected plants in maize-producing areas of Batı Akdeniz Agricultural Research Institute in 2010. Flint corn varieties [Karaçay (Batı Akdeniz Agricultural Research Institute) (BAARI) and Karadeniz Yıldızı (Black Sea Agricultural Research Institute)], dent corn cultivars [Ada-523 (Maize Research Institute), Pioneer-3394 (Pioneer Firm) and Side (BAARI)], sweet corn cultivars [Merit (May Firm) and Vega (May Firm)], and popcorn variety [Antcin-98 (BAARI)] were used as host plants.

2.1. Isolation

Teliospores of *U. maydis*, also named as chlamydospores, were obtained by crumbling and filtering out the galls. Teliospores were exposed to 1% copper sulfate solution for 20 to 60 h. Afterwards, they were dried up using blotting paper, and transmitted on PDA medium and incubated at 25 °C. Within a week, basidiospores (sporidia) of the fungus appeared. Later, they were transmitted to a 20% carrot solution in 500 mL flasks and incubated at 25 °C for one week. In this way, necessary inoculum was obtained.

2.2. Inoculum

With gently shaking the flasks, sporidia were moved to ensure homogeneity of the spore solution. By means of a hemocytometer, sporidia and teliospore suspensions were arranged to 3×10^6 basidiospores mL^{-1} and 1×10^6 teliospores mL^{-1}, respectively. In this manner, inoculum was arranged according to Tunçdemir (1985).

2.3. Experiments

Experiments were conducted in completely randomized block factorial design with three replicates. Plots, 5 m long, were set up as four rows including controls. Seeds of the each cultivar were sown on 7 June in 2010 and on 3 June in 2011. Mean number of the plants in every plot were one hundred. Furrow irrigation was used and irrigation, a total of 9 times, was done at 15 to 18 days intervals depending on the moisture in the soil. To manage with weeds, the herbicide, foramsulfuron 22.5 g L^{-1} (active substance), was used at 2 to 6 leaf stage of maize. However, deltamethrin 25 g L^{-1} (active substance), was applied at silking stage against earworms. Picking ears by hand from the each plot, harvest was done. Harvest times were on 26 to 29 October in 2010, while they were on 10 to 12 October in 2011.

2.4. Ecological properties of the research area

General soil texture of the research area was clay loam. In sowing-time, the area was fertilized with compound fertiliser (NPK 18:8:8) at the rates of 180 nitrogen, 80 phosphorus and 80 potassium kg ha^{-1}, respectively. Field studies were established in Aksu location of Antalya. The total monthly rainfall when the inoculations of the maize ears were performed in August of 2010 was 4.2 mm, whereas no measurable rainfall was recorded in the same period in 2011. However, the average temperature and relative humidity in August 2010 and 2011 were 30.5 ºC, 59.1% and 29.6 ºC and 50.0%, respectively (Anonymous 2013).

2.5. Inoculation

The cob inoculation method of Pataky et al (1995) was modified as follows: In total, 3 mL mixed inoculum, consisted of 3×10^6 basidiospores mL^{-1} and 1×10^6 teliospores mL^{-1}, was syringed in primary cob in pre-pollination period (3 days before natural pollination of maize) and in the post-pollination period (3 days after silk browning) per plant. In 2010, inoculations of PrePI and PostPI were performed on August 10th and 20th, while in 2011, those inoculations were applied on August 15th and 25th, respectively. In the control plots, cobs were injected with sterile water.

2.6. Disease assessments

Using 0 to 5 scale of Johnson & Christensen (1935), severity of the disease was determined. Rating of the scale was as follows: 5 = big galls (>10 cm diam); 2.5 = medium galls (5 to 10 cm diam); 1 = small galls (2.5 to 5 cm diam); and 0 = very small galls (<2.5 cm diam). Comparing the number of infected and non-infected cobs, incidence of the disease was detected. Twenty cobs were assessed in each plot for each treatment. In 2010, disease incidence and severity ratings of PrePI and PostPI were done on September 2nd and 10th, whereas in 2011, those ratings were assessed on September 6th and 14th, respectively. To determine yield losses of the hosts, cobs collected from the plots were peeled from the cob leaves and dried. Leaving kernels at 72 °C for 72 h, moisture contents were found. Adjustment of yield was done in compliance with 15% moisture ratio and using Equation (1) of Poehlman (1987) below.

Adjusted weight= Plot weight × (100 – moisture%) / 85 × (kernel/cob) / 100 (1)

Ratio of kernel/cob and plot yield were detected using Equation (2) of Yanıkoğlu et al (1999).

Plot yield (kg ha^{-1})= Adjusted weight × 10000 / Plot area (m^2) (2)

In conclusion, yield losses were designated by comparing yields of inoculated plots with non-inoculated plots.

2.7. Statistical analysis

Analysis of variance was done using *JMP program* (SAS Institute Inc., Cary, North Carolina, USA) and average values established as different were categorized considering $LSD_{0.01}$.

3. Results and Discussion

Average disease incidence (DI) of all the cultivars (hosts) in PrePI was 20.7%, whereas in PostPI, that was 15.7%. However, the highest DI in the PrePI and PostPI were found in cv. Karadeniz Yıldızı and cv. Side, whereas the lowest DI was in cv. Antcin-98 (Table 1). Likewise, average disease severity (DS) of all the hosts in the PrePI was found at the rate of 3.8, while in the PostPI, it was 0.9 (Table 2). In both years, average DS and DI of the hosts in the PrePI were higher than that of the PostPI. In 2010, average yields of the cultivars in the control, PrePI and PostPI plots were 8180, 5240 and 5950 kg ha^{-1}, respectively. However, in 2011, they were 7510, 3060 and 3730 kg ha^{-1}, respectively. In addition, as an average of both years, mean yields of the hosts in the control, PrePI and PostPI were 7850, 4150 and 4850 kg ha^{-1}, respectively. Interactions of the year, cultivar, and year × cultivar × disease in the PrePI and PostPI were significant ($P<0.01$) in both years (Table 3).

In 2010, average yield losses of the hosts in the PrePI and PostPI were 36.5% and 28.0%, while in 2011, they were 55.5% and 44.7%, respectively. Mean yield loss of the hosts over the two years in the PrePI was determined at the rate of 45.5%, whereas in the PostPI, it was 35.9%. Compared all the hosts, the highest yield losses in the PrePI (52.4%) and PostPI (43.8%) were established from Ada-523 (dent corn variety). However, the lowest yield losses in the PrePI (38.4%) and in the PostPI (26.2%) were found in the popcorn variety, Antcin-98 (Table 4).

Table 1- Disease incidence of the cultivars in pre and post pollination inoculations (%)

Cultivar (host)	Disease incidence in PrePI[1]			Disease incidence in PostPI[2]		
	2010	2011	Mean	2010	2011	Mean
Ada-523	13.3*	26.6	19.9	8.3	16.6	12.4
Pioneer-3394	15.0	36.6	25.8	10.0	30.0	20.0
Side	13.3	50.0	31.6	8.3	36.6	22.4
Karaçay	3.3	33.3	18.3	3.3	28.3	15.8
Karadeniz Yıldızı	20.0	38.3	29.1	18.3	31.6	24.9
Merit	16.6	20.0	18.3	10.0	15.0	12.5
Vega	13.3	15.0	14.1	10.0	15.0	12.5
Antcin-98	6.6	11.6	9.1	3.3	8.3	5.8
Mean	12.6	28.9	20.7	8.9	22.6	15.7
	Year LSD (0.01)= 2.5 Cultivar LSD (0.01)= 5.1 Year x cultivar LSD (0.01)= 7.3			Year LSD (0.01)= 1.9 Cultivar LSD (0.01)= 3.8 Year x cultivar LSD (0.01)= 5.4		

*, data are means of three replications; PrePI[1], pre-pollination inoculations; PostPI[2], post-pollination inoculations

Table 2- Disease severity of the cultivars in pre and post pollination inoculations

Cultivar (host)	Disease severity in PrePI**			Disease severity in PostPI**		
	2010	2011	Mean	2010	2011	Mean
Ada-523	1.5*	6.8	4.1	0.5	2.0	1.2
Pioneer-3394	1.7	4.2	2.9	0.3	0.8	0.5
Side	2.9	10.5	6.7	0.6	2.6	1.6
Karaçay	0.8	7.4	4.1	0.3	2.2	1.2
Karadeniz Yıldızı	3.2	11.4	7.3	0.8	3.7	2.2
Merit	2.4	2.3	2.3	0.6	0.5	0.5
Vega	2.5	1.7	2.1	0.3	0.6	0.4
Antcin-98	0.5	1.4	0.9	0.1	0.3	0.2
Mean	1.9	5.7	3.8	0.4	1.5	0.9
	Year LSD (0.01)= 0.2 Cultivar LSD (0.01)= 0.4 Year x cultivar LSD (0.01)= 0.6			Year LSD (0.01)= 0.1 Cultivar LSD (0.01)= 0.2 Year x cultivar LSD (0.01)= 0.3		

**, the highest disease severity value was accepted as 10.0; *, data are means of three replications

In both years, average DS, DI and yield losses of all the maize cultivars in the PrePI were higher than those in the PostPI (Table 1 and 2). In the study, inoculations performed in the pre-pollination period induced a higher DS, DI and yield losses on the cultivars than those in the PostPI treatments. These findings revealed that irrespective of the host, injection of *U. maydis* during pre-pollination of maize caused more severe smut infection in cobs than those in the post-pollination period. The results of the present study corroborated the earlier findings of Snetselaar et al (2001). These authors reported that an abscission layer appeared at the bottom of pollinated cob silks, which may preclude ovaries from infection of *U. maydis* in maize cobs. Similarly, du Toit & Pataky (1999) underscored that

Table 3- Yield of the cultivars in control, pre and post-pollination inoculation

	2010				2011				Mean (Cultivar × disease)			
Cultivar (host)	Yield control (kg ha^{-1})	Yield inoculated (kg ha^{-1}) (PrePI)[1]	Yield inoculated (kg ha^{-1}) (PostPI)[2]	Mean (cultivar × year)	Yield control (kg ha^{-1})	Yield inoculated (kg ha^{-1}) PrePI	Yield inoculated (kg ha^{-1}) PostPI	Mean (cultivar × year)	Yield control (kg ha^{-1})	Yield inoculated (kg ha^{-1}) PrePI	Yield İnoculated (kg ha^{-1}) PostPI	Mean cultivar
Ada-523	11260*	6220	7530	8340	11360	4540	5170	7020	11310	5380	6350	7680
Karaçay	11230	8170	8880	9430	9430	3380	3990	5600	10330	5780	6440	7520
Pioneer-3394	10140	6730	7330	8070	10260	4260	5240	6590	10200	5500	6290	7330
Side	10510	6910	7940	8450	9730	3140	3720	5530	10120	5030	5830	6990
K. Yıldızı	10420	6580	7770	8260	8100	2760	3540	4800	9260	4670	5660	6530
Merit	4010	2410	2530	2980	3870	2280	3060	3070	3940	2350	2800	3030
Antcin-98	3900	2560	2930	3130	4050	1930	2520	2830	3980	2250	2730	2990
Vega	3940	2300	2710	2980	3300	2160	2620	2690	3620	2230	2670	2840
Mean (year × treatment)	8180	5240	5950		7510	3060	3730		7850	4150	4850	5610
Mean		6460				4770						

Year LSD (0.01)= 236; Cultivar LSD (0.01)= 473; Disease LSD (0.01)= 290; Year × cultivar LSD (0.01)= 669

Year × disease LSD (0.01)= 410; Cultivar × disease LSD (0.01)= 820; Year × cultivar × disease LSD (0.01)= 1160

PrePI[1], pre-pollination inoculations; PostPI[2], post-pollination inoculations; *, data are means of three replications

Table 4- Mean yield losses of the cultivars tested

Cultivar (host)	2010		2011		Mean	
	Yield loss (%) PrePI[1]	Yield loss (%) PostPI[2]	Yield loss (%) PrePI	Yield loss (%) PostPI	Yield loss (%) PrePI	Yield loss (%) PostPI
Ada-523	44.7	33.1	60.0	54.4	52.4	43.8
Pioneer-3394	33.6	27.7	58.4	48.9	50.2	42.3
Side	34.2	24.4	67.7	61.7	44.0	37.6
Karaçay	27.2	20.9	64.1	57.6	46.0	38.3
Karadeniz Yıldızı	36.8	25.4	65.9	56.2	49.5	38.8
Merit	39.9	36.9	41.1	20.9	40.3	28.9
Vega	41.6	31.2	34.5	20.6	43.4	31.4
Antcin-98	34.3	24.8	52.3	37.8	38.4	26.2
Mean	36.5	28.0	55.5	44.7	45.5	35.9
	32.2		50.1		40.7	

PrePI[1], pre-pollination inoculations; PostPI[2], post-pollination inoculations

maize cobs were susceptible to the smut fungus from the beginning of silk arising until the two week after silking period of maize. In the course of this vulnerability period, amount of infected cobs reduced with silk aging. In addition, Snetselaar & Mims (1993) postulated that unpollinated cobs were more vulnerable to *U. maydis* infection than pollinated ones.

Vulnerability of maize to fungi colonizing cobs via silks alternates depending on silk maturity e.g., *Fusarium graminearum,* causes ear rot in maize, was highest if silks were inoculated soon after beginning of silk arising and diminished drastically with silk maturity (Enerson & Hunter 1980; Reid et al 1992; du Toit & Pataky 1999). Furthermore, several researchers (Marsh & Payne 1984; Headrick et al 1990) revealed similar results regarding *Fusarium moniliforme* and *Aspergillus flavus*, another cob infecting fungi. Considering all of these, the findings of the present study and aforementioned authors have been suggested that occurrence of maize pollination prior to *U. maydis* infection in cobs renders ovaries more resistant to the fungus.

Of all maize varieties, the highest DS in the PrePI and PostPI was found in Karadeniz Yıldızı (flint corn variety), whereas the lowest DS was in Antcin-98 (popcorn variety) (Table 2). As it had more severe smut gall on its cobs, Karadeniz Yıldızı was more vulnerable to infection of *U. maydis* than the other varieties. However, the cobs of Antcin-98 were less affected by the fungus than the others. In the present study, among the varieties, the DS values were ranked according to host's gall size (from largest to smallest) as follows: Flint, dent, sweet, and popcorn, respectively. It can be concluded that the bigger the cobs, the higher the development of large smut galls on them; flint and dent corn varieties showed higher DS values than the others. In addition, DI values in the study were similar to DS values. These results supported the earlier findings of Pataky & Snetselaar (2006). These authors reported that incidence of smut infection was greater than 50% in 1976 in several areas of Germany where hybrids derived from European flint corn were prevalent. Similarly, in a study conducted by Bojanowski (1969) in Poland, U12 (a flint corn inbred) was identified as susceptible to corn smut. Pataky (1991) stated that extremely susceptible genotypes may exist among dent, flint, floury and other types of corn. In addition, Aydoğdu (2015) was reported that flint and dent corn cultivars were susceptible to *U. maydis* infection.

The average DS, DI and yield losses of all the hosts in 2011 were higher than those in 2010 (Tables 1, 2, 3 and 4). Year-to-year variation found in the present study could be explained as follows: it is known that environmental conditions can influence the development of diseases, in particular during penetration and infection of the host. Tunçdemir & Iren (1980) reported that the most favorable temperature for development of maize smut ranges between 18 °C and 21 °C. In this regard, in 2010, the average daily temperature on the inoculation day of PrePI and PostPI were 30.2 °C and 31.6 °C, whereas in 2011, they were 27.2 °C and 27.3 °C, respectively (Table 5). Therefore, those temperatures may have adversely affected germination and penetration of the fungus in 2010. However, mean relative humidity (RH) of the August in 2010 was 59.1%, while it was 50.0% in 2011. In addition, the environmental conditions in 2010 may have been favorable for the host. Because, in 2010, average yield of all the hosts in the control plots was 8180 kg ha^{-1}, while in 2011, it was 7510 kg ha^{-1} (Table 3). Depending on these factors, in 2010, average yield loss of all the maize varieties was 32.2%, but in 2011, it was 50.1% (Table 4). Kyle (1929) emphasized that when environmental factors are in favor of the host during the maize growing season, smut infection levels are reduced. In a two-year study conducted in Germany, Görtz et al (2008) stated that in 2006, the frequency of kernel infected by *Fusarium* spp. ranged from 0.7% to 99.7%, while in 2007, the highest incidence of *Fusarium* ear rot was 64%. The authors expressed that the year-to-year variability in the overall infection rate may be explained by significant differences in temperature and precipitation during the growth periods.

Apart from these factors, plant nutrition can affect smut infection. Aydoğdu & Boyraz (2011) reported that nitrogenous and organic fertilization may affect the severity of the disease. Additionally, physiology and morphological structure of the host have an impact on colonization of *U. maydis*. Since maize cultivars tested have specific physiology and morphological features, different disease values were determined in the present study. Pataky & Richter (2007) emphasized that leaves surrounding cobs may influence silks and accordingly infection of *U. maydis*. Pataky & Chandler (2003) also emphasized that gall size induced by *U. maydis* varies depending on maize genotypes, virulency of the pathogen, and climatic conditions. A cultivar-trial in the Columbia Basin in the U.S showed marked differences in susceptibility to corn smut between field corn hybrids (Mohan et al 2013).

Table 5- Daily mean temperature of the research area during inoculations

Inoculation	*Inoculation date 2010*	*Daily average temperature (°C)*	*Inoculation*	*Inoculation date 2011*	*Daily average temperature (°C)*
PrePI	10 August	30.2	PrePI	15 August	27.2
PostPI	20 August	31.6	PostPI	25 August	27.3

Source: Regional Meteorology Station, Antalya

4. Conclusions

Corn smut is a devastating disease of maize when environmental conditions are favourable for the fungus. In our study, for the first time, yield losses on the cobs, stemming from *U. maydis* infection, were revealed perceptibly based on interactions between maize pollination and *U. maydis*. The present study also suggested that pollination has an influence on *U. maydis* infection in cobs and correspondingly yield losses. Considering maize phenology and this host-pathogen interaction in cobs during maize pollination, new control methods can be developed. Taking into account all, further studies are needed to establish management strategies against the fungus.

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