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THE EFFECTS OF HEAD ROT DISEASE (*Rhizopus stolonifer*) ON SUNFLOWER GENOTYPES AT TWO DIFFERENT GROWTH STAGES

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ABSTRACT

Head rot caused by *Rhizopus stolonifer* reduces sunflower seed yield and quality. The objective of this study was to determine the effects of head rot disease on seed yield in eight sunflower cultivars. The sunflower cultivars were inoculated by *R. stolonifer* at two different growth stages (R5.7 and R6.0) of sunflower. The results revealed that mechanical or physical damage on back of sunflower head results in infection of the head rot disease and significantly reduced the seed yield. The differences among the cultivars were significant for disease severity. The results also significantly varied with growth stages for seed yield of the cultivars. In addition, the cultivars showed significant differences in terms of their responses to *R. stolonifer* at both two growing stages. As a result, mechanical damages on back side of the plant head cause significant increase in disease severity and yield losses in sunflower.

Key words: Sunflower, *Rhizopus stolonifer*, yield, sensitivity.

INTRODUCTION

Rhizopus spp. is well-known to cause soft rotting in fresh fruits, vegetables, flowers, bulbs, tubers and seedlings (Agrios, 2005). Head rotting disease (*Rhizopus stolonifer, R. arrhizus, R. oryzae, R. microsporus*) in sunflower (*Helianthus annuus* L.) is observed in all sunflower growing zones in the world and causes considerable yield and quality losses (Arnan et al., 1970; Shtienberg, 1997; Clarke, 1999). The fungus penetrates into plants via scars on the sunflower heads and then it degrades pectin of middle lamella with pectonic enzymes. It goes through tissues and degrades cell walls with cellulose enzyme, which demolishes entire cell. Finally it causes softening the tissues. Sporangiofors produced by fungus grown in the host across soften epidermis and produce sporangium, stolon and rhizoids (Agrios, 2005). Scars on plants are usually caused by insects, hails, birds or mechanical damage (Arnan et al., 1970; Gulya et al., 1997). Severity of the disease depends on intensity of the damage (Klisiewicz 1979; Harveson 2000; Yıldırım and Kaya 2005). In addition, humidity and temperature increase severity of the disease. For example, optimum temperature and relative humidity for development of the disease was found to be 20-30 °C and 85-90 %, respectively (Wilson et al., 1983; Gulya et al., 1991; Bhutta et al., 1993).

In sunflower, crop yield and quality losses caused by the *Rhizopus* change depending upon growth stages. Although infection does not occur at budding stage, it can severely occur at anthesis. So the disease causes dramatic yield reductions in number of seeds and yield per head in sunflower (Mishra et al., 1972; Shtienberg, 1997). Also the head may drop off within 3-7 days after infection (Yang and Thomas, 1981). Thomson and Rogers (1980) stated that oil quality in sunflower infected by *Rhizopus* decreased with increasing content of saturated fatty acids (e.g., palmitic and stearic). In fungal diseases such *Sclerotinia* and *Rhizopus* developing resistant cultivars is very important (Çetinkaya and Yıldız, 1988).

Although some previous investigations on head root disease have been carried out, limited number of sunflower cultivars was used in those studies. Therefore, the severity of head rot disease and yield losses caused by *R. stolonifer* on sunflower cultivars, widely grown in Turkey, was investigated in this study.

MATERIALS AND METHODS

The experiment was carried out in Experimental Field of Agricultural Faculty at Çanakkale University in 2006. *R. stolonifer* isolate which causes head rot disease was used. In the study, seven sunflower hybrids (Meriç, Sirena, Isera, Vanko, Sanbro, Sanay and Pioneer) and one open pollinated local non-hybrid line were inoculated with *R. stolonifer*.

R. stolonifer was isolated from disease infected sunflower heads. The fungus was cultured on PDA (Potato Dextrose Agar) at 25 °C for seven days. Sterile distilled water (100 ml) was then added on the Petri dishes and gently brushed in order to release the spores into water. The spores was separated from fungal fragments by sieving through sterile cheese clothe. The spore density of the suspension was determined by using hemocytometer. The spore density was

adjusted to 10^5 spore ml^{-1} by diluting with sterile distilled water.

Table 1. Probabilities (P values) of variance analyses for disease severity and seed yield in sunflower inoculated with *Rhizopus stolonifer* at two growth stages.

Source	Disease severity	Seed yield
Growth stage	0.6194	0.0257
Treatment	<.0001	<.0001
Cultivar	0.0290	0.1578
Growth stage x Treatment	0.3310	0.6948
Growth stage x Cultivar	0.4758	0.1727
Treatment x Cultivar Growth stage x Treatment x Cultivar	0.0251 0.0114	0.1162 0.2365

The experimental field was fertilized with 300 kg ha^{-1} (20-20-0 NPK) prior to sowing. Afterwards, the seeds were planted at 70 x 35 cm density. When the plants required, they were watered. No chemical or spray was applied during whole experimental period. Each plot size was 2.8 x 5 m.

In each plot, 28 plants were inoculated by a cork borer (3 mm diameter and 1 cm depth) at R5.7 and R6.0 growth stages. Growth stages in sunflower were previously described by Schneiter and Miller (1981). Growth stage of R5.7 (Reproductive stages) is beginning of flowering, and can be divided into substages dependent upon the percent of the head area (disk flowers) that has completed or is in flowering. On the other hand, R6.0 is that flowering is complete and ray flowers are wilting. For the inoculation without damage, the plants were sprayed with the solution containing the spores. In addition to these two treatments, three plots were left without any treatment for the control. After inoculation, the experimental field was covered with a net in order to prevent any damage from bird or some other pests.

Disease ratio (%) was calculated by proportion of infected area in total area of the head. So susceptibility of the cultivars was expressed with this value. The scale was; < 5%: resistant, 6-20 %: less susceptible, 21-40 %: susceptible, 41-60 %: high susceptible and > 60 %: very high susceptible. The scale used in our study was modified from Becelaera and Miller (2004).

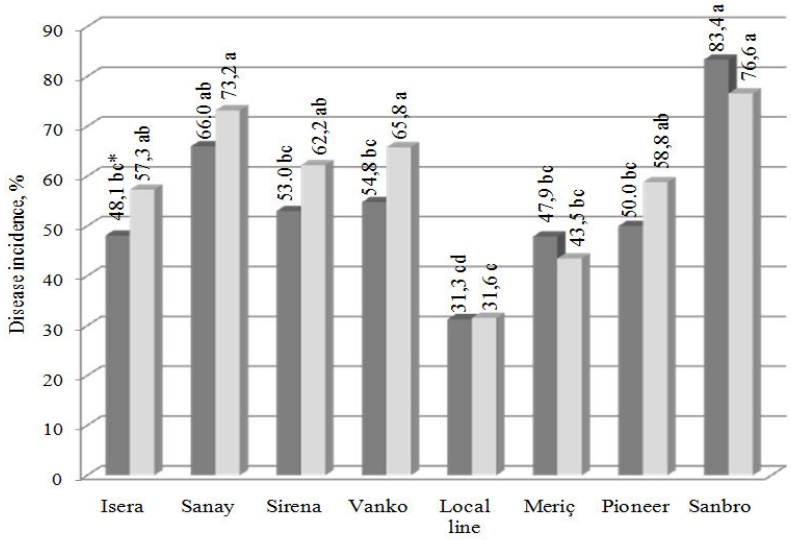
The seed yield was obtained by harvesting each plant separately after the plants totally dried in the field. The experimental design was split-split plot with three replications. The data were analyzed using SAS program (SAS, 1989). LSD test was used for the mean separation.

RESULTS

The results of variance analyses revealed that there were significant differences between treatments (control and inoculated). However, there was no significant difference between growing periods (R5.7 and R6.0) in terms of disease severity. In addition to the effect of cultivars, cultivar*treatment on the disease severity was significant. It is important to note that the plants which were inoculated without wounding did not develop any disease. Therefore, they were not included in the statistical analysis.

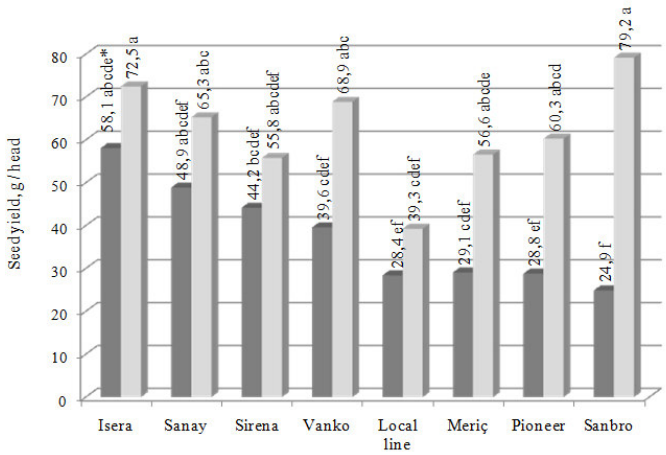
In Table 1, the differences among cultivars were not significant when all statistical parameters such as treatments and growth stages were included in the analysis. However, comparison of only wounded cultivars for disease severity revealed that the differences among the cultivars were significant (Fig. 1).

The highest disease severity was observed in cultivar Sanbro at R5.7., whereas Sanbro, Sanay and Vanko were the highest effected cultivars at R6.0 growth stage (Fig. 1). The lowest disease severity was obtained in local line at R5.7 whereas it was obtained in local line and Meriç at the R6.0 growth stage.



*The means with the same letter were not significantly different LSD 0.05 for R5.7= 27.557 and LSD0.05 for R6.0= 19.492

Figure 1. The susceptibility of some sunflower cultivars for *Rhizopus stolonifer* at two growth stages: ■, R5.7; ■, R6.0.



*The means with same letter were not significantly different LSD 0.05= 31.412

Figure 2. Seed yield of sunflower cultivars inoculated with *Rhizopus stolonifer* at R5.7 growth stage: ■, inoculated; ■, control.

Sunflower showed a variation in susceptibility to *R. stolonifer* (Table 2). Among the cultivars, local line was "susceptible", Vanko, Sirena, Meriç, Pioneer and Isera were "highly susceptible", and Sanbro and Sanay were "very highly susceptible" at the R5.7 stage. At the R6 stage, Sirena was susceptible, Sanay, Isera and local line were highly susceptible, and Sanbro, Vanko, Sirena, Meriç and Pioneer were very highly susceptible.

The results showed that there were significant differences between inoculated plants and control plants in terms of seed yield. The differences among cultivars were also significant for two growth stages (Fig. 2 and 3).

Among control plants, the highest seed yield per plant was obtained from Sanbro (79.2 g plant^{-1}) followed by Isera (72.5 g plant^{-1}) and Vanko (68.9 g plant^{-1}) at the R5.7 growth stage (Fig 2). When the plants inoculated with the pathogen the highest seed yield was obtained from cultivar Isera whereas the lowest yield was obtained from Sanbro (24.9 g plant^{-1}). Similarly, there were also significant differences between control and *R. stolonifer* inoculated plants at R6.0 growth stage (Fig. 3).

Although absolute values presented in Figure 2 and 3 give some information for performances of the cultivars against head rot disease, relative reduction or tolerance of a cultivar would be more appropriate in order to observe actual resistance to the disease. For this reason, relative reduction caused by the disease for each cultivar and growth stage was presented in Figure 4. The values in the figure were calculated based on the means from Figure 2 and 3 using following formula:

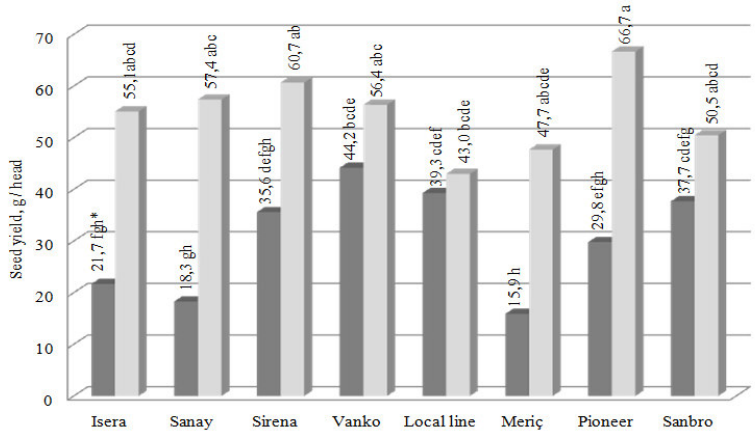
Yield losss = 100 - (100 x seed yield from the inocluated plants / seed yield from the control plants)

The most resistant cultivar in R5.7 growth stage in terms of seed yield was Isera followed by Sirena (Figure 4). In this stage, the most sensitive cultivar was Sanbro followed by Pioneer. In R6.0 growth stage, the most resistant cultivars was the local line followed by Vanko whereas the most sensitive variety was Meriç followed Isera. These results showed that the sensitivity of cultivars changed with the growth stage.

Table 2. Susceptibility of sunflower cultivars infected with *Rhizopus stolonifer* at the R5.7 and R6 growth stages.

Cultivar	R*		LS		S		HS		VHS	
	R5.7	R6	R5.7	R6	R5.7	R6	R5.7	R6	R5.7	R6
Sanbro	-	-	-	-	-	-	-	-	+	+
Sanay	-	-	-	-	-	-	-	+	+	-
Vanko	-	-	-	-	-	-	+	-	-	+
Sirena	-	-	-	-	-	+	+	-	-	-
Meriç	-	-	-	-	-	-	+	-	-	+
Pioneer	-	-	-	-	-	-	+	-	-	+
Isera	-	-	-	-	-	-	+	+	-	-
Local	-	-	-	-	+	-	-	+	-	-

*R: Resistant, LS: Less susceptible, S: Susceptible, HS: High susceptible, VHS: Very high susceptible



*The means with same letter were not significantly different LSD 0.05 = 20.121

Figure 3. Seed yield of sunflower cultivars inoculated with *Rhizopus stolonifer* at R6.0 growth stage: ■, inoculated■ , control.

DISCUSSION AND CONCLUSION

Head rot disease (*Rhizopus* spp.) is a common and an important disease in sunflower fields. In this study, the effects of the head rot disease (*R. stolonifer*) on widely grown sunflower cultivars were investigated. Yield losses in plants increase with high humidity (85-90 %), optimum temperature (20 – 30 °C) and damage on sunflower head by larva of butterflies, birds, hale and wind (Mishra et al., 1972; Yang et al., 1979; Wilson et al., 1983; Bhutta et al., 1993; Shtienberg, 1997). Thus, disease severity and the yield losses in the plants wounded artificially were found to be very high compared to unwounded plants in which no disease incident was observed. The effect of disease on seed yield increased with increasing severity of the disease. Yıldırım and Kaya (2005) carried out a survey study in Marmara Region and found intensity of the disease increases with increasing the damage on sunflower heads by birds, insects, larva of butterflies or mechanical wounding in especially fields with having high humidity.

The disease affects the plants during generative growth stages, thus, other morphological characters rather than seed yield were not significantly affected from the disease. In our study, at R5.7 and R6.0 growth stages, sunflower complete its vegetative growth and also flower pollination. Shtinberg (1997) pointed out that the effect of the *Rhizopus* on seed yield was not significant when the disease infection occurs after formation of seed. On the other hand, the disease could affect seed quality such as color and taste. *Rhizopus* causes aflotoxin accumulation in seeds, consequently reduces seed quality (Begum et al., 2003).

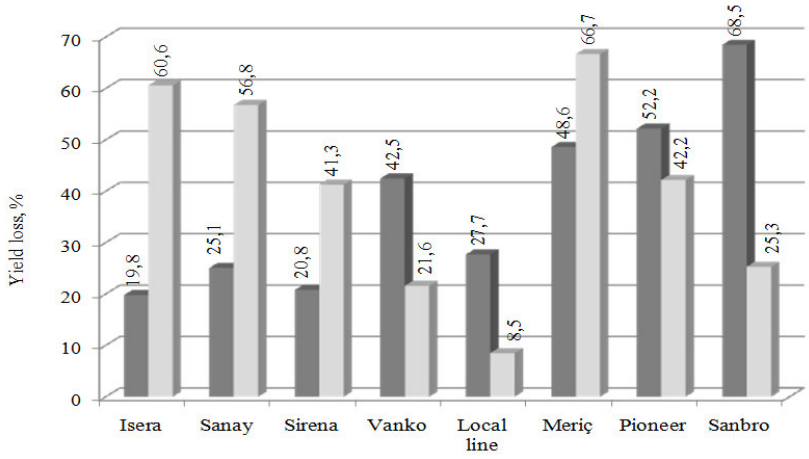


Figure 4. Seed yield loss in sunflower cultivars inoculated with *Rhizopus stolonifer*: ■ , R5.7; ■, R6.0. The values were calculated based on the means from Figure 2 and 3.

The sunflower cultivars used in this study showed significant variation in terms of resistance to the head rot. Although the local line had the lowest seed yield for both growing stages in the control plots, it was the most resistant cultivar as it had the lowest relative reduction which caused by the disease (Fig. 5). These results revealed that genetic factors also involved in the resistance mechanism to the *Rhizopus* in sunflower. Moreover, the disease severity was high in cultivars inoculated at R5.7 growth stage compared to R6.0 growth stage. This indicates the later head rot disease inoculation in sunflower results in less yield losses. As a result, damages on back side of the sunflower head by birds or insects may cause significant increases in disease severity and yield losses. Therefore, in order to reduce damage, it is important that choosing cultivars resistance to head rot disease or less preferred by the birds, carrying out an effective management for the pest which cause damages on sunflower heads and not growing (if its possible) sunflower in the areas with high humidity and bird population, especially near to villages, water resources, fruit plantations or forest.

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