Estimation of sunflower breeding material tolerance to *Diaporthe/Phomopsis helianthi*

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ABSTRACT
Phomopsis of sunflower [caused by *Phomopsis helianthi* Munt.-Cvet. et al. (teleomorph *Diaporthe helianthi* Munt.-Cvet. et al.)], is one of the principal sunflower diseases in the Republic of Croatia and Europe, and has a great influence on grain and oil yield. Hence, in the framework of the sunflower breeding program at the Agricultural Institute Osijek, one of the main objectives is to work on the resistance to this and other principal pathogens. Although sunflower (*Helianthus annuus* L.) has a narrow genetic variability, the source of genetic resistance to this pathogen is found among wild *Helianthus* species, and differences among cultivated genotype tolerance are observed as well. This paper presents only one segment of the work on tolerance by artificial infection under field conditions with the aim to investigating the level of tolerance to this pathogen of a wide range of breeding materials (e.g. cms and restorer lines). The most tolerant material will be used in the creation of new commercial sunflower hybrids.

Key words: artificial infection – *Diaporthe/Phomopsis helianthi* – sunflower – tolerance.

INTRODUCTION
Sunflower (*Helianthus annuus* L.) is one of the most important oil crops in world production and the area under sunflower is in constant increase. Same trend is in Europe, where area under sunflower increased in the period 1995-2005 by 2.3 million ha (FAOSTAT, 2005). Major sunflower producers in Europe are: Russia, Ukraine, Romania, France, Bulgaria, Spain, Hungary and Moldova.

In the Republic of Croatia, sunflower production is characterized by significant oscillations in areas, grain and oil yield. These oscillations significantly depend on the occurrence and intensity of diseases, which, in some years, lead to significant decreasing in grain and oil yields. Different diseases are dominant in different production areas and significantly depend on agroecological conditions. It is known that over 30 different pathogens (among them fungi are predominant) attack sunflower and cause diseases which can produce important economic damage (Škorić et al., 2002.).

Phomopsis helianthi Munt.-Cvet. et al. (teleomorph *Diaporthe helianthi* Munt.-Cvet. et al.), is one of the most important sunflower pathogens in Europe. It causes a disease named gray stem spot (stem canker). It was first described in the former Yugoslavia in 1981 (Mihaljčević et al., 1982.) and from then on it spread all over the world and became one of the most prevalent diseases of cultivated sunflower (Degener et al., 1999). In environmental conditions favorable to disease development (Laville, 1986), it could cause significant grain yield losses (10-50%) and oil content decrease.

Growing resistant hybrids is the most effective measure for disease control. However, there are no completely resistant genotypes and the main challenge to the breeders represents searching for sources of resistance and introducing them into genotypes with valuable agronomic traits. Sources of resistance could be found in some wild species, first of all in some populations of *H. tuberosus* (Škorić et al., 2002). According to Deglene et al. (1999), sunflower resistance in breeding programs could be improved by using inbred lines, which have high values of general combining abilities. In sunflower breeding aimed at disease tolerance, artificial infection in controlled (laboratory) or uncontrolled (field) conditions is essential. There are a few methods of artificial infection and some authors use the least aggressive ones, which are closer to natural infection. Also, there are differences regarding a place of infection (Vear et al., 1997). Sunflower breeding programs in Croatia have a long tradition and have been carried out through scientific projects and programs in the framework of The Agricultural Institute Osijek (Vratarić and Sudarić, 2004; Mijić et al., 2004; Krizmanić et al., 2006). The main goal is the creation of new, superior hybrids, with a high grain yield (above 5 t/ha), oil content (above 50%), and high, stable oil yield. Special attention is given to creation lines with an emphasized tolerance to predominant pathogens. Sunflower breeding with resistance/tolerance to main diseases is the best way to control them and represents the most ecologically acceptable way to do so (Fick and Miller, 1997; Miller and Fick, 1997; Škorić et al., 2002; Vratarić and Sudarić, 2004).

The aim of the investigation was to estimate the tolerance to the pathogen *D. helianthi* of a wide spectrum of inbred lines, including cytoplasmic male sterile (cms, A lines), male fertile (mf, B lines), restorers of fertility (rf, R lines) and two-way sterile hybrids or single cross (SC), by artificial infection method in the field. Inbred lines of good combining abilities for the most important agronomic traits (grain yield, oil content), which show the lowest level of susceptibility, will be considered as potential parents for hybrid development in the framework of the Agricultural Institute Osijek sunflower breeding program.

MATERIALS AND METHODS

The research was conducted during two consecutive years (2006 and 2007) at the experiment field of The Agricultural Institute Osijek (Croatia). Tested breeding material involved 19 different sunflower genotypes, 5 of which were cytoplasmatic male sterile (cms) inbred lines (L-301 A, L-271 A, L-G/04 A, L-205 A, L-101 A), four male fertile lines (L-302 B, L-14 B, L-190 B, L-272 B), 6 sterile single-crosses (female component for three-way hybrids, G/04 A x L-104 B, G/04 A x L-14 B, G/04 A x L-282 B, G/04 A x L-272 B, G/04 A x L-190 B, G/04 A x L-302 B), and four restorer-fertility lines (PI 12/99 R, O3G R, L-Š 89 R, O3 MR). Tested material was developed at the Agricultural Institute Osijek sunflower breeding program. Each genotype was sown in two 5-m long rows, in three replications. One row of each genotype represented the control, while the other was artificially infected. In each replication, 7 plants of each genotype were artificially infected in full button stage (R2, according to Schnieter and Miller, 1981). Sunflower stems were infected on 11th July 2006 and 15th July 2007, with fungal mycelium grown in the laboratory. Previously, during 2004 and 2005, the patogenicity was tested in a considerable number of strains in the location of Osijek, a location of large-scale sunflower production in Croatia. The most aggressive one was used for this investigation. Circular plug of mycelia was placed on a leaf stalk intercept (2-3 cm long) from one of mid-stem leaves. Infection spot was covered with a piece of wet cotton wool and aluminum foil to prevent mycelial dryness and create favorable micro-climate conditions for pathogen development. Susceptibility estimation was performed by weekly measurements of the length of lesions during three weeks after infection. Analysis of variance (ANOVA) and LSD test were processed by Statistical Analysis System for Windows software (SAS Institute, 2003).

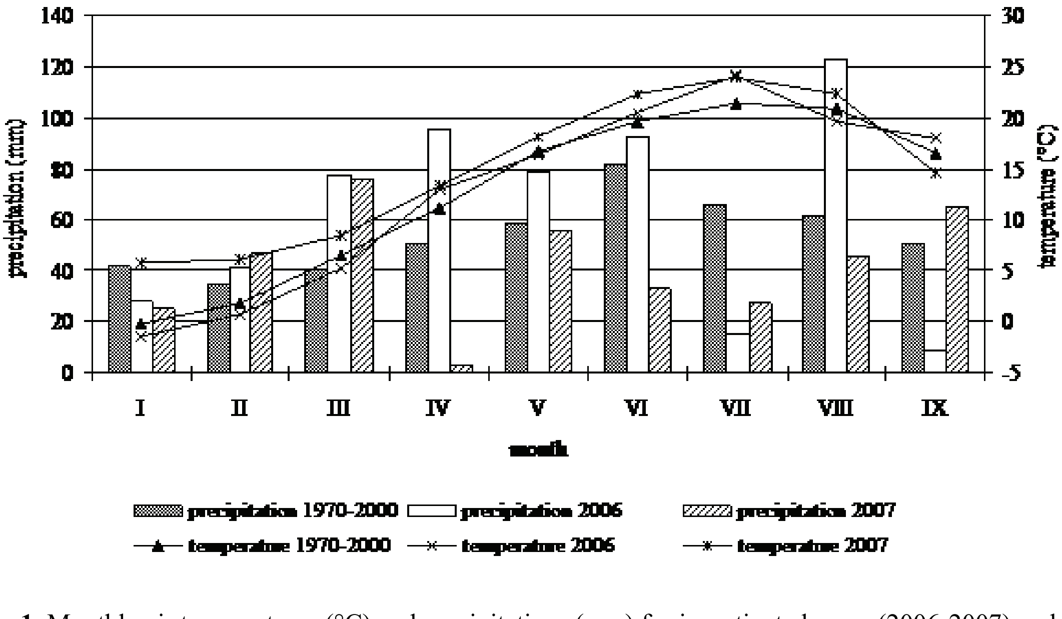

Fig. 1. Monthly air temperatures (°C) and precipitations (mm) for investigated years (2006-2007) and 30-year average (1970-2000), Osijek.

RESULTS AND DISCUSSION

During the first lesion measurement, artificial infection success was clearly visible. Most of the infected plants showed disease symptoms. Occurrence of symptoms was perceived clearer in the second and particularly in the third measurement in both years. Lesion length per measurement as well as the average length of lesions for both years is shown in Table 1. On average, the highest tolerance to the pathogen was shown in SC, then A lines, and B lines, while the lowest resistance recorded was in fertility restorer lines (R). The lowest average value was recorded for the single cross hybrid L-G04 A x L-14 B (2.48), and the highest average value for mf (B) line L-302 B (5.06). The lowest susceptibility to the pathogen corresponded to the cms lines L-101 A and L-205 A. From mf (B) lines, L-272 B and L-190 B were more tolerant. The most tolerant two-way hybrids to artificial infection in this investigation were L-G/04 x L-14 and L-G/04 x L-282. These results should be examined in a further investigation, particularly after developing hybrids from tolerant lines. Although the procedure of creating three-way hybrids is longer and more complex, some authors (Giriraj et al., 1988; Bochkovoy et al., 2000) give these hybrids a certain advantage regarding grain yield stability. Fertility restorers L-O3 M R and L-O3 G R showed the lowest susceptibility to infection (Table 1).

Table 1. Average lesion length (cm) of sunflower inbred lines after infection with *D. helianthi* at Osijek in 2006-2007.

No	Lines	Lesion length (cm)		
		2006	2007	Average
1	L- 271 A	3.94	5.87	4.91
2	L-G/04 A	3.72	5.23	4.48
3	L-301 A	1.84	6.53	4.19
4	L- 205 A	2.58	3.20	2.89
5	L-101 A	2.01	3.39	2.70
	Average	3.88	3.80	3.83
6	L-302 B	4.87	5.25	5.06
7	L-14 B	3.76	4.03	3.90
8	L-190 B	2.33	4.31	3.32
9	L-272 B	2.04	4.23	3.13
	Average	3.41	4.30	3.90
10	L-G/04 x L-104 SC	3.66	5.48	4.57
11	L-G/04 x L-272 SC	1.79	5.29	3.54
12	L-G/04 x L-190 SC	1.51	5.51	3.51
13	L-G/04 x L-302 SC	2.10	4.19	3.14
14	L-G/04 x L-282 SC	0.92	4.43	2.68
15	L-G/04 x L-14 SC	0.97	3.99	2.48
	Average	1.83	4.82	3.32
16	L- 12/99 R	3.52	5.59	4.56
17	L-Š 89 R	1.65	7.07	4.36
18	L-O3 G R	1.44	6.08	3.76
19	L-O3M R	2.22	4.97	3.59
	Average	2.90	5.24	4.10
	LSD 0.05	0.89	1.17	0.72

Legend: A – cytoplasmatic male sterile lines; B – male fertile lines; SC – single cross sterile hybrids; R – restorer of fertility

It is important to emphasize that, besides their genetic potential, the environment has a strong influence on genotype tolerance level. Regarding the fact that these results were obtained in field trials, all data should be observed through climate conditions during investigation (Fig 1). During a two month period (July, August) the amount of precipitation in a 30-year average recorded at the Agricultural Institute Osijek experimental field was 128.2 mm. In 2006, the same period of time recorded a little more than the 30-year average (134.9 mm), while in 2007 this amount was significantly lower (72.4 mm). Observing only July, the month when the artificial infection was carried out, the amount of precipitation was lower in 2006 (15.3 mm) in comparison with 2007 (27.4 mm) and the 30-year average (66.3 mm). In August 2006, this value was 122.6 mm, significantly above the 30-year average (61.9 mm) or the same month in 2007 (45 mm). However, artificial infection and measurements were, in both years investigated, conducted during the second and the third ten day's period of July, when only 15.3 mm of precipitation (2006) was measured, which makes this period drought and unsuitable for artificial infection and pathogen development. In 2007, the rainfall in July was almost double (27.4 mm), but still under the 30-year average (66.3 mm). Air temperatures for 2006 (21.8 °C) in these two months were on average on the same level as the 30-year average (21.1 °C). In 2007, the two month average was 23.1 °C, which made that period more suitable for pathogen development.

Comparing these meteorological data with the results of lesion length for investigated sunflower lines given in Table 1, it could be concluded that precipitation and air temperatures in July are most important for artificial infection as well as for pathogen development. Regarding that fact, in 2007 all investigated lines have longer lesions in comparison with 2006. Also, it can be concluded that, in this investigation, the precipitation had a stronger influence than temperatures on artificial infection as well as on pathogen and disease development. It is known that years with lower air temperatures and a higher precipitation are extremely suitable for white head rot development (Vratarić and Sudarić, 2004; Jurković and Ćosić, 2004; Duvnjak et al., 2006), while higher temperatures and moisture are suitable for Stem canker development.

Although these results were obtained in two-year trials, they could be a good indicator and guideline in further sunflower breeding work related to disease resistance on *D. helianthi.* The research should be continued in following years, including new genotypes. Additionally, testing important agronomic traits in combination with resistance to this pathogen will give a more objective estimation of selecting material for new sunflower hybrid development.

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