Susceptibility of sunflower breeding material to *Alternaria* sp.

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ABSTRACT

- In Croatia the leaf and stem spot disease caused by *Alternaria* species is considered as a disease associated with senescent plants and of minor importance. During 2008, 2009 and 2010 year *Alternaria* sp. isolated in a higher percentage on all sunflower growing location in areas of Eastern Croatia. On some locations except leaf lesions, stem lesions occured more frequently, they coalesced and form large blackened areas, resulting in stem breakage. Management practice in order to control this disease include use less susceptible varieties and hybrids, rotate sunflowers and seed fungicides treatments.
- Since that *Alternaria* disease in recent years occured more frequently on stems the aim of our research was to compare pathogenicity of *A. helianthi*, *Alternaria* sp. and *A. helianthinficiens* to sunflower stem and estimate the tolerance of a eight sunflower lines to the *Alternaria* species. For artificial infection 5 isolates of *Alternaria* species were used. *A. helianthi* (isolate Alh1), *Alternaria* sp. (isolates Al4 and Al5) were isolated from naturally infected sunflower plants in Croatia (locaton Sopot). Two isolates of *A. helianthinficiens* (E.G.S. 36-184 and 50-174, CBS 208.86) courtesy of Emory G. Simmons. Longitudinal lesion length on stems were measured 10 and 20 days after inoculation.
- Average lesion length on inoculated sunflower plants with isolate *A. helianthi* (Alh1) measured 10 days after inoculation were between 2.67cm and 3.25 cm. Other isolates caused significantly smaller lesions. Twenty day after inoculation lesion size on plants inoculated with isolate *A. helianthi* (Alh1) ranged from 4.01 to 4.87 cm. Lesions on plants inoculated with isolates *Alternaria* sp. and *A. helianthinficiens* were smaller and varied from 1.0 cm (isolate E.G.S. 36-184) to 2.07 cm (isolate E.G.S. 50-174). Statistical differences in susceptibility between tested sunflower lines were determined after artificial infection with all tested isolates. Observing the differences in pathogenicity between the isolates, regardless of sunflower lines and days after inoculation the most pathogenic was isolate *A. helianthi*. Mean stem lesion size caused by *A. helianthi* 10 and 20 days after inoculation were 3.01 and 4.07, respectively. Ten days after artificial infection with *A. helianthi* isolate variation in susceptibility of tested sunflower lines were not found, 20 days later on two lines (DP-3 and DP-4) statistically smaller lesions were measured.
- The results of this work indicate that *A. helianthi* compared with other tested *Alternaria* sp. is the most pathogenic for artificialy infected sunflower stem. Other tested *Alternaria* species caused significantly shorter lesion.
- *A. helianthi* can cause leaf and stem lesions, seedling blight and head rot. It has been reported to reduce seed and oil yield up to 84% and 33%, respectively (Balasubrahmanyam and Kolte, 1980, Kolte, 1985), photosynthetic reduction of around 20% (Calvet et al. 2005), 1,000-seed weight and number of seeds produced per head (Balasubrahmanyam and Kolte, 1980). The most economical and effective means to control sunflower diseases and other pests is planting resistant or tolerant hybrids.

Key words: *Alternaria*, sunflower, tolerance

INTRODUCTION

In Croatia the leaf and stem spot disease caused by *Alternaria* species is considered as a disease associated with senescent plants and of minor importance. During 2008, 2009 and 2010 *Alternaria* sp. was isolated in a higher percentage on all sunflower growing areas of Eastern Croatia. On some locations in addition to leaf lesions, stem lesions occured more frequently, they coalesced and formed large blackened areas, resulting in stem breakage. In our country two species of *Alternaria: A. helianthi* (Hansford) Tubaki and Nishihara and *A. alternata* (Fr.:Fr.) Keissl. have been determined (Jurkovic and Cosic, 2004). *A. alternata* is the more prevalent, but *A. helainthi* is more serious. Several other *Alternaria* species have been reported on sunflower worldwide: *A. zinniae* Ellis, *A. tenuissima* (Fries) Wiltshire, *A. leucanthemi* Nelen, *A. helianthicola* Rao and Rajagopalan, *A. longissima* Deighton and MacGarvey, *A. helianthinficiens* Simmons, and *A. protenta* Simmons. Worldwide *A. helianthi* is the most dominant and damaging species. *A. helianthi* showed negative affect on all parameters of yield (Balasubrahmanyam and Kolte, 1980., Allen et all., 1981, Carson, 1985). In Greece Lagopodi and Thanassoulopoulos (1998) reported that *A. alternata* can be as damaging to sunflower as *A. helianthi*. *A. alternata* reduced number of seeds produced per head (16-65%) and seed weight (15 - 79%).

In subtropical sunflower growing areas *Alternaria* leaf blights are considered as a major disease and can cause yield losses from 15 to 90% (Berglund, 2007).

During 1998 and 2000 growing seasons in Korea *A. helianthi*, *A. helianthinficiens* and *A. protenta* caused the leaf spot and blight diseases and were responsible for considerable damage (Hye Sun Cho and Seung Hun Yu, 2000).

In order to control this disease management practice should include less susceptible varieties and hybrids, rotation of cultures and fungicidal treatments of seeds. Due to the fact that *Alternaria* disease in recent years occured more frequently on stems, the aim of our research was to compare pathogenicity of *A. helianthi*, *Alternaria* sp. and *A. helianthinficiens* to sunflower stem and estimate the tolerance of eight sunflower lines to the *Alternaria* species. The lines are considered as potential plant material in sunflower breeding program of the Institute of Agriculture in Osijek.

MATERIAL AND METHODS

The experiment was conducted on the fields of the Institute of Agriculture in Osijek during a 2009. Tolerance of eight sunflower lines to *Alternaria* sp. was evaluated. The lines (DP-2, DP-3, DP-4, DP-5, DP-6, DP-7, DP-8 and DP-15) were created at the Institute of Agriculture in Osijek.

For artificial infection 5 isolates of *Alternaria* species were used. *A. helianthi* (isolate Alh1) and *Alternaria* sp. (isolates Al4 and Al5) were isolated from naturally infected sunflower plants in Croatia (locaton Sopot). Species association was not determined isolates A14 and A15. Based on the symptoms and characteristics of conidia and conidiophores which were discovered on the natural media (there was no sporulation on PDA) we assumed that the species is *A. helianthinficiens*. Since it is not possible to determine species association without molecular analysis we remained with *Alternaria* sp. associations for isolates Al4 and Al5.

Two isolates of *A. helianthinficiens* (E.G.S. 36-184 and 50-174, CBS 208.86) courtesy of Emory G. Simmons. Mycelium of *Alternaria* species used for sunflower stem inoculation were cultured on potato dextrose agar (PDA) at 22±1ºC with 12 h light/12 h dark regime for seven days. From the edge of the developed mycelium small pieces of mycelium were transferred on water agar (WA) with sterile oats seeds. After twelve days oats seed covered with mycelium were used for sunflower inoculation. Sunflower plants in full button stage (R2 stage - Schneiter and Miller, 1981) were inoculated. One seed per plant were placed into a hole made on sunflower stems. Inoculation site was covered with moistened cotton wool and wrapped in aluminium foil. Seed without mycelium were put into holes on control plants. Longitudinal lesion length on stems were measured 10 and 20 days after inoculation. The trial was carried out in four replications, with 5 plants in each repetition. The data for both tests were analyzed statistically by ANOVA using Statistical Analyses System Version 8.2 (SAS Institute). Significance of differences among treatments was tested by Fisher's test.

RESULTS AND DISCUSSIONS

Average lesion length on inoculated sunflower plants with isolate *A. helianthi* (Alh1) measured 10 days after inoculation were between 2.67 cm and 3.25 cm. There was not statistical differences in susceptibility between tested sunflower lines (Table 1). Other isolates caused significantly smaller lesions. After inoculation with isolates *Alternaria* sp. (Al4) and *A. helianthinficiens* (E.G.S. 36-184 and E.G.S. 50-1749) there were differences in tolerance between sunflower lines.

Table 1. Mean value of lesion size (cm) 10 days after inoculation with different *Alternaria* species

Lines	Isolates				
	Alh1	**Al4**	**Al5**	**E.G.S. 36-184**	**E.G.S. 50-174**
DP-2	3.15^{A}	1.62^{AB}	1.07^{A}	0.87^{BC}	1.42^{AB}
DP-3	2.67^{A}	1.12^{B}	0.95^{A}	0.80^{BC}	1.62^{AB}
DP-4	2.92^{A}	1.37^{AB}	1.0^{A}	1.12^{ABC}	1.82^{A}
DP-5	2.90^{A}	1.10^{B}	0.97^{A}	1.13^{ABC}	1.13^{B}
DP-6	3.00^{A}	2.00^{A}	1.12^{A}	1.25^{AB}	1.50^{AB}
DP-7	3.25^{A}	1.37^{AB}	0.89^{A}	1.35^{A}	1.12^{B}
DP-8	3.10^{A}	1.11^{B}	0.87^{A}	0.75^{C}	1.10^{B}
DP-15	3.01^{A}	1.20^{B}	1.12^{A}	1.07^{ABC}	1.25^{B}

$^{A, B, C}$ – different letters mark statistically significant difference according to Fisher's test at the 95% level of probability

Twenty days after inoculation lesion size on plants inoculated with isolate *A. helianthi* (Alh1) ranged from 4.01 to 4.87 cm (Table 2). Lesions on plants inoculated with isolates *Alternaria* sp. and *A. helianthinficiens* were smaller and varied from 1.0 cm (isolate E.G.S. 36-184) to 2.07 cm (isolate E.G.S. 50-174). The experiment was conducted in July in the field conditions. The July was drought and had higher temperatures than a 30-year average. Since temperature and amount of precipitation were unfavorable for artificial infection the length of lesions were lower. Statistical differences in susceptibility between tested sunflower lines were determined after artificial infection with all tested isolates.

Table 2. Mean value of lesion size (cm) 20 days after inoculation with different *Alternaria* species

Lines	Isolates				
	Alh1	**Al4**	**Al5**	**E.G.S. 36-184**	**E.G.S. 50-174**
DP-2	4.87^{A}	2.00^{A}	1.62^{AB}	1.50^{A}	1.87^{AB}
DP-3	3.62^{B}	1.87^{AB}	1.12^{BC}	1.12^{AB}	1.80^{AB}
DP-4	3.59^{B}	1.82^{AB}	1.38^{BC}	1.52^{A}	2.07^{A}
DP-5	4.17^{AB}	1.25^{B}	1.01^{C}	1.20^{AB}	1.27^{C}
DP-6	4.01^{AB}	2.12^{A}	1.36^{BC}	1.37^{AB}	1.86^{AB}
DP-7	4.37^{AB}	1.75^{AB}	1.02^{BC}	1.55^{A}	1.50^{BC}
DP-8	4.12^{AB}	1.50^{AB}	1.12^{BC}	1.00^{B}	1.37^{BC}
DP-15	4.85^{A}	1.62^{AB}	2.12^{A}	1.60^{A}	1.30^{BC}

$^{A, B, C}$ – different letters mark statistically significant difference according to Fisher's test at the 95% level of probability

Observing the differences in pathogenicity between the isolates, regardless of sunflower lines and days after inoculation the most pathogenic was isolate *A. helianthi*. Mean stem lesion size caused by *A. helianthi* 10 and 20 days after inoculation were 3.01 and 4.07, respectively (Table 3).

Table 3. Mean value of lesion size (cm) after inoculation with different *Alternaria* species

Isolate	Lesion size after 10days	Lesion size after 20 days
Alh1	3.01^{A}	4.07^{A}
Al4	1.36^{B}	1.74^{B}
Al5	0.99^{C}	1.34^{C}
E.G.S. 36-184	1.04^{C}	1.35^{C}
E.G.S. 50-174	1.37^{B}	1.66^{B}

$^{A, B, C}$ – different letters mark statistically significant difference according to Fisher's test at the 95% level of probability

In Croatia *Alternaria* leaf spot and blight of sunflower is common disases that appears later in the summer with minimal impact on yield. Recently, under warm, humid conditions disease is also present on the stem. Stem sunflower diseases can be caused by several fungal species. *Diaporthe helianthi* Munt.-Cvetk. and *Sclerotinia sclerotiorum* (Lib.) de Bary occur commonly in the field of Croatia. Each pathogen is favoured by particular environmental condition. The results of this work indicate that *A. helanthi* compared with other tested *Alternaria* sp. is the most pathogenic for artificialy infected sunflower stem. Other tested *Alternaria* species caused significantly shorter lesion. *A. helianthi* can cause leaf and stem lesions, seedling blight and head rot. It has been reported to reduce seed and oil yield up to 84% and 33%, respectively (Balasubrahmanyam and Kolte, 1980, Kolte, 1985), photosynthetic reduction of around 20% (Calvet et al. 2005), 1,000-seed weight and number of seeds produced per head (Balasubrahmanyam and Kolte, 1980). The most economical and effective means to control sunflower diseases and other pests is to plant resistant or tolerant hybrids. Ten days after artificial infection with *A. helainti* isolate variation in susceptibility of tested sunflower lines were not found, 20 days later on two lines (DP-3 and DP-4) statistically smaller lesions were measured.

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