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PHOMA MACDONALDI ON SEED AND ITS IMPORTANCE IN ETIOLOGY OF PHOMA BLACK STEM IN SUNFLOWER

SUMMARY: *Phoma macdonaldi* Boerema, teleomorph *Leptosphaeria lindquistii* Frezzi, is a widespread pathogen of sunflower. The aim of this research was to identify the presence of fungus *P. macdonaldi* in seed of different sunflower hybrids, as well as the correlation between seed and field infection. Phoma black stem assessment was performed on three hybrids grown in six localities in Serbia. Untreated and processed seeds of these hybrids were used in the seed health test. Severity of the disease did not differ between localities. Average disease index for hybrids H7, H9 and H19 was 14.01%, 13.25% and 11.83% respectively, and it shows that there are no significant differences in hybrid susceptibility. The index of disease indicates tolerance of these hybrids to Phoma black stem. Seed analysis showed the presence of fungi from the following genera: *Phoma*, *Alternaria*, *Botrytis*, *Sclerotinia*, *Penicillium* and *Aspergillus*. Seed infection with Phoma (of the untreated seeds) per hybrid ranged from 1.2–3.5%. There is no significant correlation between stem and seed infection.

KEYWORDS: *Phoma macdonaldi*, sunflower, seed and field infection

INTRODUCTION

Diseases are one of the limiting factors in sunflower production worldwide [Škorić et al., 2006]. Sunflower is a host plant for approximately 40 pathogens, but only some of them, depending on region, have a potential to reduce yield [Gulya et al., 1997]. In the favorable conditions, sunflower could be attacked by pathogens in all stages of development.

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Disease severity and yield losses depend on genotype resistance, aggressiveness of pathogen and climatic conditions. Intensity of the most important sunflower diseases such as downy mildew (*Plasmopara halstedii*), sunflower stem canker (*Phomopsis helianthi*), Phoma black stem (*Phoma macdonaldi*) and white rot (*Sclerotinia sclerotiorum*) is related to higher amount of precipitation [Maširević and Forgić, 2000]. *Phoma macdonaldi* Boerema, teleomorph *Leptosphaeria lindquistii* Frezzi, is a widespread and moderately damaging pathogen of sunflower [Maširević and Jasnić, 2006]. Phoma black stem has been spreading rapidly on the global scale in the last 10–15 years [Škorić et al., 2012].

P. macdonaldi overwinters on infected plant debris in form of mycelium and pycnidia and after the third year it could form perithecia as teleomorph *Leptosphaeria lindquistii* [Frezzi, 1968; Marić et al., 1981]. Penetration of fungus into plant tissue is done mechanically through wounds or through natural plant openings such as stomata [Al Fadil et al., 2011]. Symptoms of Phoma black stem could be seen on the all above ground plant organs [Maširević and Jasnić, 2006]. The most typical symptoms appear on the stem as circle shaped, oval or irregular large black spots (5–10 cm in diameter). Number of the spots increases during vegetation, and in some cases could encircle the stem. In conditions favorable for disease development, a stem could be completely covered with black spots [Marić et al., 1988]. Lesions are limited to the surface layer of the stem [Debaeke and Pérès, 2003]. However, in highly susceptible genotypes during later stages of plant development the lesions could sometimes penetrate into the central part of the stem and could cause its breaking. A severe attack of the pathogen causes the diseased plants to wilt and die prematurely [Marić et al., 1988]. Their heads become smaller; seeds are empty or shriveled; seed and oil yields decrease [Darvishzadeh et al., 2008]. The role of seed in the process of fungus transmission is not clear.

The aim of this research was to identify the presence of fungus *Phoma* in seeds of different sunflower hybrids in different localities in Serbia, as well as the correlation between stem and seed infection. The aim was also to show the possibility and importance of seed infection for spreading of the pathogen.

MATERIAL AND METHOD

Disease assessment

Disease assessment (presence of *P. macdonaldi*) was performed on three hybrids grown in six localities. Sunflower hybrids marked H7, H9 and H19 were from the Institute of Field and Vegetable Crops in Novi Sad, and the six localities were as follows: Neštin, Kula, Pančevo, Vrbas, Zaječar and Sombor. The experiment was conducted during 2010.

The evaluation of Phoma black stem natural infections in the field was done according to scale 0-9 (0 - no disease; 1 - 10–20%; 2 - 21–30%; 3 - 31–40%; 4 - 41–50%; 5 - 51–60%; 6 - 61–70%; 7 - 71–80%; 8 - 81–90%; 9 - 91–100%) when sunflower plants were in physiological maturity [Maširević, 1995]. Approximately 150 plants of each hybrid in each locality were assessed. McKinney's disease index was calculated. Fungicide treatments during the vegetation were not applied to the tested sunflower hybrids.

Examination of seed health status

Untreated and processed seeds of sunflower hybrids evaluated in the field were used for testing. Sunflower seeds were sterilized by 1% sodium hypochlorite (NaOCl) for 5 min followed by draining. Sterilized and dried seeds were put on wet filter paper. Seed analysis of every hybrid was done in four replicates with 25 seeds. Sunflower seeds were incubated for 7 days at the temperature between 25 and 26 °C. Presence of the causal agent of Phoma black stem was identified according to morphological characteristics of isolates and forming of pycnidia and pycniospores on the surface of sunflower seeds and in the pure culture isolates [Boerema et al., 2004].

Seed germination data were transformed into Arcsin values and analyzed by factorial ANOVA and Duncan test. Correlation between the level of infection in the field and the level of seed infection in laboratory conditions was calculated. Statistical analysis was performed using Statistica 10 software.

RESULTS AND DISCUSSION

Field evaluation

Severity of Phoma black stem did not vary between five examined localities and it ranged from 12–16%. Significantly lower index of disease, comparing to the other localities, was noticed only in the locality Zaječar (3.3%). Average disease index for hybrids H7, H9 and H19 was 14.01, 13.25% and 11.83% respectively and it shows that there are no significant differences in hybrid susceptibility (Table 1). The index of disease indicates the resistance of hybrids to Phoma black stem. A large majority of sunflower plants had infection intensity 10–20%, although in four out of six localities (Neštin, Kula-Vitovnica, Pančevo and Vrbas) there were individual plants with higher infection level, but not over 40%. Genotypes with disease attack of 20% and less could be classified as resistant [Goian, 1984].

Table 1. McKinney's disease index of Phoma black stem in tested hybrids in different localities

Locality	McKinney's disease index (%)			Average disease index per locality
	H7	H9	H19	
Neštin	23.08	12.5	12.75	16.11
Kula-Vitovnica	11.11	20.76	15.30	15.7
Pančevo	11.11	17.11	12.30	13.5
Vrbas	22.83	12.73	14.77	16.7
Zaječar	4.01	5.30	1.03	3.3
Sombor	11.93	11.11	14.84	12.6
Average	14.01	13.25	11.83	13.0

The amount of precipitation during 2010 was highly above the average in five out of six localities [Republic Hydrometeorological Service of Serbia, 2010] (Figure 1). According to Aćimović [1998] infections in natural conditions usually occur in the first half of July. Optimal conditions for disease development are temperatures around 25 °C, relative humidity 100% and presence of water drops. Zaječar was the locality with the lowest amount of rainfalls during vegetation period and the lowest disease incidence. The amount of rainfalls in Zaječar was at the level of multiannual average of rainfalls in Serbia, with significantly lower precipitation in August and September. Our results are in accordance with Marić et al. [1988] who reported that lower humidity in August and September lead to weaker attack of Phoma black stem. Disease intensity in other tested localities was higher than in Zaječar. During their four-year research, Fayzalla and Marić [1981] found that disease severity was greater in years with less favorable distribution of rainfalls and these factors lead to weakness of plant vitality and increased their

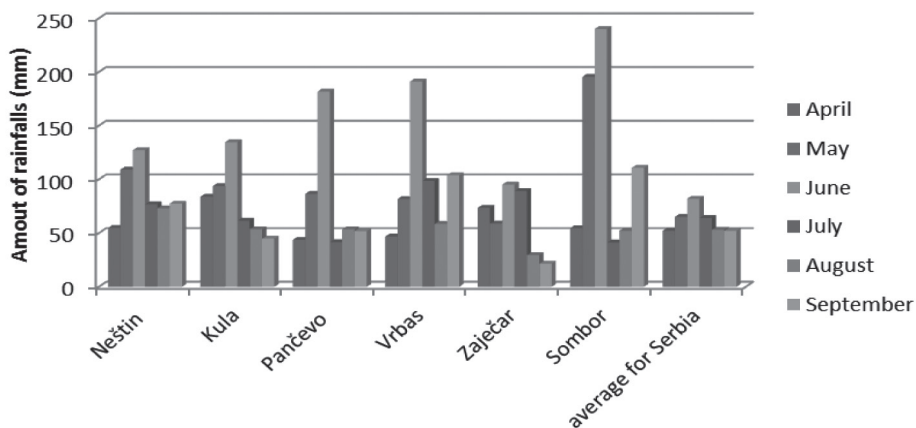


Figure 1. Monthly amount of rainfall per locality

susceptibility to disease. Testing the sunflower inbred lines tolerance to *Phoma* black stem during 2010 in Rimski Šančevi in the conditions of artificial infection, Dedić [2012] obtained higher disease intensity in the non-irrigated fields.

Seed health testing

Analysis of sunflower showed presence of fungi from the following genera: *Phoma*, *Alternaria*, *Botrytis*, *Sclerotinia*, *Penicillium*, and *Aspergillus*. The most frequent were fungi from genus *Alternaria*. The appearance of other fungi in sunflower seed were below 5%. The seed infection with *Phoma* was low, but there were two localities where a significantly higher level of seed infection was detected (Pančevo and Neštin) (Table 2).

There is no significant correlation between stem and seed infection. This could be explained by lower field infection and the presence of symptoms only in stems but not in head.

Table 2. Seed infection depending on locality

Locality	Seed infection with <i>Phoma</i> (%)
Sombor	0.67 ^a
Vrbas	1.00 ^a
Kula-Vitovnica	1.33 ^a
Zaječar	1.67 ^a
Nestin	3.67 ^b
Pančevo	6.33 ^b
F= 7.41* p= 0.0001*	

Seed infection with *Phoma* (identified in untreated seed) per hybrid ranged from 1.2–3.5% (Table 3). Average level of seed infection is different and depends on hybrid in contrary to stem field infections. Hybrid H7 had the lowest percent of seed infection. Darvishzadeh et al. [2007a] reported that the level of seed infection depended on the variability in resistance among the genotypes as well as among the isolates and their interaction.

Table 3. Seed infection depending on hybrid

Hybrid	Seed infection (%)
H7	1.2 ^a
H19	2.7 ^{ab}
H9	3.5 ^b
F=5.77* p=0.008*	

According to some earlier researches, the presence of *P. macdonaldi* was not noticed in sunflower seed and the conclusion was that seed could not serve as a source of inoculum [Fayzalla, 1978]. However, other authors confirmed that pathogen could develop in seed and could be transmitted by seed [El-Sayed and Marić, 1981; Bhutta et al., 1997; Darvishzadeh, 2007b] and that the infection could also lead to reduction of germination [Saharan et al., 2006]. Lević et al. [2012] in a recent study of seed mycoflora (seed samples originated from different localities in Serbia) did not detect *P. macdonaldi* in sunflower seed. Our results showed the presence of *P. macdonaldi* in seed. Similar percent of seed infection (0.25–3.25%) was also confirmed by other authors [Stajić et al., 2001]. Infected seeds could be eliminated through fungicide treatment [Marić et al., 1988]. It is well known that all commercial seed is treated with some fungicides and that is one of the most important reasons why this pathogen could not be widespread from region to region by contaminated seed. These results indicate that this pathogen is seed borne.

BLACK STEM IN SUNFLOWER



CONCLUSION

The tested sunflower hybrids had stem infection intensity from 10–20% and seed infection from 1–3.5%. In the conditions of naturally infected plants such disease incidence indicates that there is no significant correlation between field stem and seed infection, despite the fact that these pathogen can be seed borne.

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PHOMA MACDONALDI НА СЕМЕНУ И ЊЕН ЗНАЧАЈ У ЕТИОЛОГИЈИ ЦРНЕ ПЕГАВОСТИ СУНЦОКРЕТА

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САЖЕТАК: *Phoma macdonaldi* Воерема; телеоморф *Leptosphaeria lindquistii* Frezzi један је од најраширенијих патогена сунцокрета. Циљ овог истраживања био је да се утврди присуство гљиве *P. macdonaldi* на семену различитих хибрида сунцокрета као и корелација између инфекције стабла у пољу и инфекције семена. Процена интензитета болести извршена је на три хибрида која су гајена на шест локалитета. За одређивање здравственог стања коришћено је нетретирано и дорађено семе хибрида испитиваних у пољу. Интензитет напада болести није се значајно разликовао међу локалитетима. Просечан индекс обољења за хибриде Н7, Н9 и Н19 био је 14,01%, 13,25% и 11,83% што показује да не постоје значајне разлике у осетљивости хибрида према црној пегавости. Такође, добијени индекс указује на толерантност испитиваних хибрида према овом проузроковачу болести. Анализом здравственог стања семена сунцокрета утврђено је присуство гљива из следећих родова: *Phoma*, *Alternaria*, *Botrytis*, *Sclerotinia*, *Penicillium* и *Aspergillus*. Зараженост семена гљивом *P. macdonaldi* утврђена је на дорађеном и нетретираном семену и кретала се од 1,2–3,5%. Није утврђена статистички значајна корелација између инфекције стабла и семена у пољским условима. Ово се може објаснити ниским степеном напада патогена и присуством симптома само на стаблу, а не на главици сунцокрета.

КЉУЧНЕ РЕЧИ: *Phoma macdonaldi*, инфекција у пољу, семе сунцокрета