Trkulja N et *al.*, Khan M, Monitoring of *Cercospora beticola* Resistance to Fungicides in Serbia. In: Deising HB; Fraaije B; Mehl A; Oerke EC; Sierotzki H; Stammler G (Eds), "Modern Fungicides and Antifungal Compounds", Vol. VIII, pp. 201-204. © 2017 Deutsche Phytomedizinische Gesellschaft, Braunschweig, ISBN: 978-3-941261-15-0

Monitoring of *Cercospora* beticola Resistance to Fungicides in Serbia

Trkulja N[1], Mitrović M[1], Milosavljević A[1], Khan M[2]

[1]*Ins. for Plant Protection and Environment, Teodora Drajzera 9, 11000 Beograd, Serbia*

[2]*Department of Plant Pathology, North Dakota State University, Fargo, ND, USA*

[3]*University of Minnesota, St. Paul, MN, USA.*

Email: trkulja_nenad@yahoo.com

INTRODUCTION

The most destructive disease of sugar beet (*Beta vulgaris* L.) worldwide is Cercospora leaf spot disease (CLS), caused by *Cercospora beticola* (Holtschulte 2000). In areas with favorable conditions for disease development such as high relative humidity and daily temperatures, CLS can lead to significant yield losses up to 50% (Byford 1996). Over the last two years, sugar beet production in Serbia was faced with dramatic decrease in efficacy of fungicides intended for *C. beticola* control. Cercospora leaf spot disease management over the last years based on intensive use of three groups of fungicides with different modes of action, benzimidazoles (MBCs), triazoles (DMIs), and strobilurins (QoIs) imposing, consequently, *C. beticola* resistance selection pressure. In Serbia, benzimidazoles were used in *C. beticola* management since 1971, but only three years later resistant isolates have been recorded (Marić et *al*. 1976). At the begining of the 1980s, triazoles were successfully introduced in CLS management (Marić et *al.* 1984), but the occurrence of *C. beticola* isolates resistant to DMIs have been recently reported in all sugar beet regions in Serbia (Trkulja et *al*. 2009). Fungicides from the QoI group have been used for CLS managament since 2006, and still without any detection of decreased sensitivity. The aim of this study was to verify a possible decrease in the sensitivity of *C. beticola* populations to MBC, DMI, and QoI fungicides as well as of isolates resistant to two or all three fungicide groups.

MATERIAL AND METHODS

Sampling, pathogen isolation, and sensitivity testing

In this sensitivity monitoring, after apearance of first simptoms of CLS, samples of sugar beet leaves with sporulating lesions of *C. beticola* were collected from commercial sugar beet fields. Collection took place in 2014 in the districts of South Bačka and North Bačka, and in 2015 in the districts of Srem and South Banat (Table 1). After 24h of incubation on potato dextrose agar (PDA), pieces of medium bearing single germinated conidia were excised with the aid of a microscope and transferred onto new PDA plates. The single-conidia isolates were incubated at 25°C in the dark. Fungicides used in the study were commercial formulations of a) the QoIs trifloxystrobin (Zato 50WG, Bayer AG, Germany) and pyraclostrobin (Retengo, BASF, Germany), b) the DMIs flutriafol (Impact 12.5SC, Cheminova, Denmark) and tetra-conazole (Eminent 125 EW, Isagro, Italy), and c) of the MBCs carbendazim (Galofungin 500SC, Galenika Phytopharmacy, Serbia) and thiophanate-methyl (Galofungin T 450SC, Galenika Phytopharmacy, Serbia). Sensitivity testing was set up as a mycelial growth measurement for *C. beticola* isolates at a discriminatory concentration (DC) of 1 mg/l for the MBCs and DMIs, and mycelial growth was measured after 7 days of incubation at 25°C in the dark. Isolates with relative growth >50% compared to the control were considered as resistant. Test of conidial germination was used to detect sensitivity differences of the isolates to both QoIs, trifloxystrobin and pyraclostrobin, at DC= 5 mg/l. Isolates with germination of conidia greater than 50% were considered as resistant.

Table. 1 Number of tested isolates in four different districts of Serbia with sampling year.

District of Serbia	year of sampling	Number of isolates
South Bačka	2014	44
North Bačka	2014	30
Srem	2015	26
South Banat	2015	73

RESULTS AND DISCUSSION

In 2014, frequencies of *C. beticola* isolates resistant to MBC, QoI, and DMI fungicides in the district of South Bačka were 61%, 82%, and 98%, respectively, and in North Bačka 50%, 90%, and 100%. Isolates collected during 2015 in South Banat were resistant to MBC, QoI, and DMI fungicides in frequencies of 38%, 90%, and 96 %, and in Srem in frequencies of 54%, 77%, and 96%, respectively (Figure 1). These results indicate a significant decrease of the sensitivity of *C. beticola* populations to fungicides used today to control the disease. Previous surveys, which have been conducted from 2008 to 2011, recorded high frequencies of resistance to MBCs, only low resistance frequencies to DMI fungicides, and no QoI resistant isolate (Trkulja et *al*. 2009; 2013; 2015). Results evidently show an emergence of *C. beticola* populations resistant to all three groups of fungicides applied to control the pathogen over the years and provides a new insight into the development of highly frequent resistance of *C. beticola* to MBC, QoI, and DMI fungicides which, consequently, had a strong impact on the decline of the compounds´ efficacy in sugar beet fields in Serbia. In other European sugar beet growing regions with favorable disease conditions for CLS, e.g. in Italy or Greece, as well as in the USA (Minnesota and North Dakota), resistance to MBCs was also detected in high frequences (Rossi et *al*. 1995; Karaoglanidis and Ioannidis 2010; Secor et *al*. 2010). Resistance to DMIs is also known in all these regions, but the frequency of resistance detected was significantly lower compared to the resistance frequency found with MBCs. However, a significant decrease in the sensitivity to DMIs has been already detected in Greece more than a

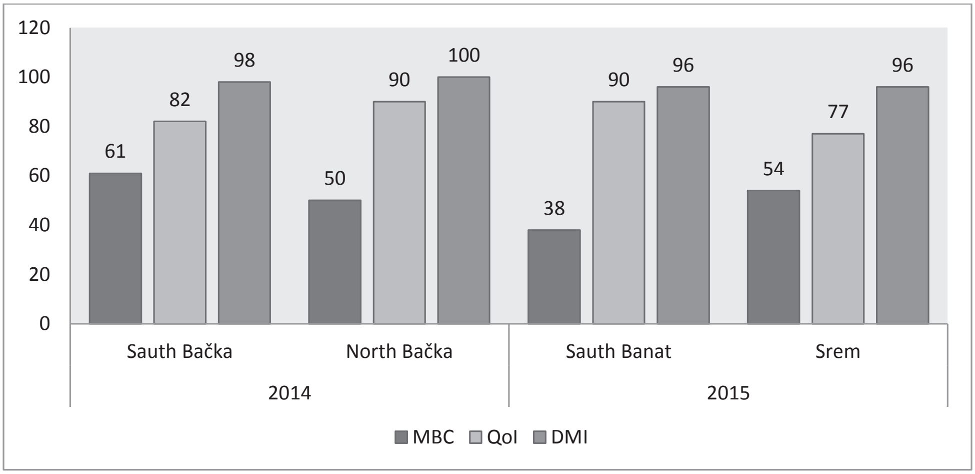

Figure 1 Frequency of resistance (%) of *C. beticola* isolates to MBC, QoI, and DMI fungicides in different districts of Serbia during 2014 and 2015.

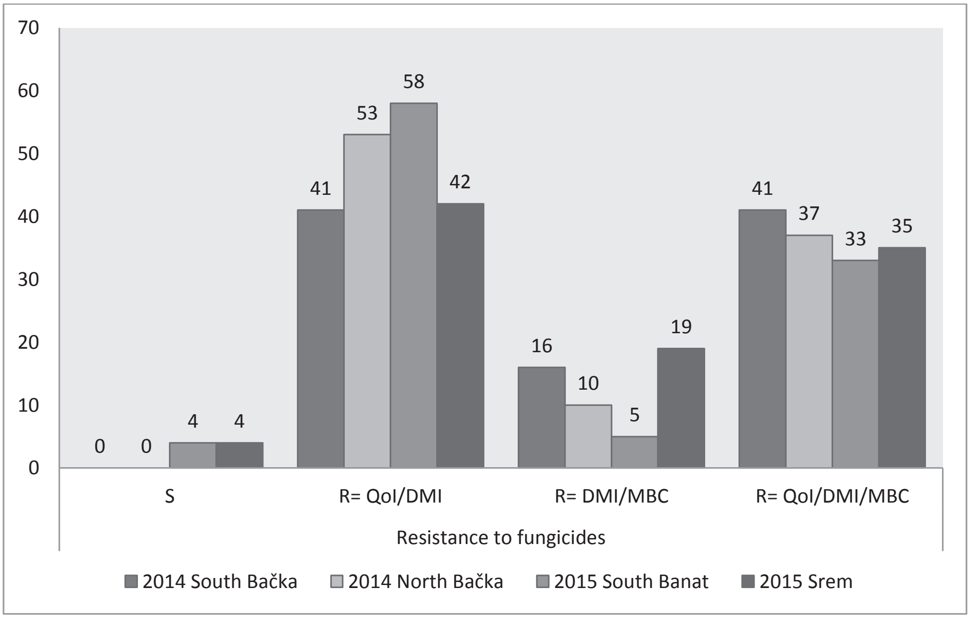

Figure 2 Frequency of resistance (%) of *C. beticola* isolates to QoI and DMI fungicides (R=QoI/DMI); DMIs and MBCs (R=DMI/MBC); QoIs, DMIs, and MBCs (R=QoI/DMI/MBC), and of sensitive isolates (S) to all tested fungicides in different districts in Serbia for the period of 2014-2015.

decade ago, with frequencies of resistance over 70%, causing reduced fungicide efficacy in the field (Karaoglanidis et *al*. 2002). A crucial record of this monitoring is the appearance of field isolates of *C. beticola* resistant to two or all three fungicide groups (MBCs, DMIs, QoIs), as the frequency of *C. beticola* isolates resistant to all three groups of fungicides ranged from

33% to 41%, the frequency of strains being resistant to QoIs and DMIs from 41% to 58%, and the frequency of isolates resistant towards DMIs and MBCs from 5% to 19%. On the other hand, the amount of isolates being sensitive to all three fungicide groups decreases (Figure 2), suggesting a development of multi-resistance of *C. beticola* populations in sugar beet fields in Serbia. These findings indicate the need for intensive changes in the management strategy in accordance to the growing resistance of populations to multiple fungicidal mode of action.

ACKNOWLEDGEMENTS

This research was supported by the grant of the Ministry of Education, Science, and Technological Development of the Republic of Serbia (TR31018).

REFERENCES

Byford WJ (1996). A survey of foliar diseases of sugar beet and their control in Europe. 59th International Institute of Beet Research (IIRB) Conference.

Karaoglanidis SG; Ioannidis PM; Thanassoulopoulos CC (2002). Changes in sensitivity of *Cercospora beticola* populations to sterol-demethylation-inhibiting fungicides during a 4-year period in northern Greece. *Plant Pathology* 51, 55–62.

Karaoglanidis GS; Ioannidis PM (2010). Fungicide resistance of *Cercospora beticola* in Europe. In: *Cercospora Leaf Spot of Sugar Beet and Related Species*, eds. RT Lartey, JJ Weiland, L Panella, PW Crous, CE Windels. American Phytopathological Society, St. Paul, MN.

Holtschulte B (2000). *Cercospora beticola* – worldwide distribution and incidence. In: *Advances in Sugar Beet Research. Vol. 2:* Cercospora beticola *Sacc. Biology, Agronomic Influence and Control Measures in Sugar Beet 2000*, eds. MJC Asher, B Holtschulte, MR Molard, F Rosso, G Steinruecken, and R Beckers, pp. 5-16. International Institute for Beet Research: Brussels.

Marić A; Petrov M; Maširević S (1976). Pojava tolerantnosti kod *Cercospora beticola* Sacc. prema benomilu u Jugoslaviji i mogućnosti suzbijanja ovog parazita. *Zaštita bilja* 27, 227-236.

Marić A; Maširević S; Jercović Z (1984). Increase in resistance of *Cercospora beticola* to benomyl and first occurrence of strains tolerant to fentin acetate in Vojvodini. *Zaštita Bilja* 35, 207-215.

Rossi V; Racca P; Giosue S. (1995). Geophytopathological analysis of Cercospora leaf spot of sugar beet in the Mediterranean area. *Phytopathol Mediterr* 34, 69–82.

Secor GA; Rivera VV; Khan MFR; Gudmestad NC (2010). Monitoring fungicide sensitivity of *Cercospora beticola* of sugar beet for disease management decisions. *Plant Disease* 94, 1272–1282.

Trkulja N; Aleksić G; Starović M; Dolovac N; Ivanović Ž; Živković S (2009). Osetljivost izolata *Cercospora beticola* prema karbendazimu i flutriafolu u Srbiji. *Zaštita bilja* 270, 237-245.

Trkulja N; Ivanović Ž; Pfaf-Dolovac E; Dolovac N; Mitrović M; Toševski I; Jović J (2013). Characterization of benzimidazole resistance of *Cercospora beticola* in Serbia using PCR-based detection of resistance-associated mutations of the ß-tubulin gene. *European Journal of Plant Pathology* 135, 889-902.