Acta fytotechn zootechn, 23, 2020(3): 147–154
http://www.acta.fapz.uniag.sk

Original Paper

Sensitivity of *Cercospora beticola* to fungicides in Slovakia

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Article Details: Received: 2020-03-04 | Accepted: 2020-05-01 | Available online: 2020-09-30

https://doi.org/10.15414/afz.2020.23.03.147-154

The fungus *Cercospora beticola* Sacc. is the one of the most important pathogens on the sugar beet. The frequent application of fungicides with the same mode of action increase a risk of development of resistant strains of the pathogen. Occurrence of *C. beticola* resistant strains has been never researched in Slovakia. In this work, *C. beticola* isolates were collected from 10 localities of Slovakia and analysed for fungicide resistance in laboratory conditions. Nine fungicides with different mode of action were tested – trifloxystrobin + cyproconazole, kresoxim-methyl + epoxiconazole, azoxystrobin + cyproconazole, thiophanate-methyl + tetraconazole, thiophanate-methyl, prochloraz + propiconazole, picoxystrobin, tetraconazole, and difenoconazole. The results confirmed, that occurrence of fungicide resistance in *C. beticola* population was established in Slovakia. Different criteria of assessment of fungicide resistance (based on EC_{50} and on growth rate – inhibition percentage) showed slightly different results, but both criteria confirmed resistant *C. beticola* strains to thiophanate-methyl, picoxystrobin and difenoconazole. Fields with higher frequency of application of these fungicides significantly supported the development of resistant strains. Assessment of any *C. beticola* strains have not confirmed reduced sensitivity to active ingredients tetraconazole and prochloraz + propiconazole. The lowest level of risk of fungicide resistance was confirmed in the locality Oslany. It is very important to focus on anti-resistant strategy and reduce of using fungicides on localities, where the occurrence of resistant *C. beticola* strains was confirmed – Dolné Saliby (thiophanate-methyl and picoxystrobin) and Senec (picoxystrobin and difenoconazole).

Keywords: sugar beet, *Cercospora beticola*, fungicides, *in vitro*, resistance, Slovakia

1 Introduction

Sugar beet belongs to traditional crops in Europe. In Slovakia, it is mainly grown as a technical crop for the sugar industry. Sugar beet is currently grown on about 22 000 hectares in Slovakia. The farmers accepted the offer of sugar companies to grown more sugar beets, which helped to stabilize sugar production to satisfy consumption in Slovakia (Černý et al., 2019). An important factor in growing of sugar beet is the control of diseases and pests (Almquist et al., 2016; Černý et al., 2018). The already emerging plants may be threatened by pests such as wireworms (Elateridae), mangold flea beatle or brassy flea beatle (*Chaetocnema concinna*, *Ch. tibialis*), beet tortoise beetle (*Cassida nebulosi*) (Hajyieva and Soroka, 2008) and diseases such as damping-off sedlings (*Phoma* sp., *Pythium* sp., *Aphanomyces* sp., *Fusarium* sp., *Rhizoctonia* sp.). Damage by foliar pathogens lead to reduction of assimilative leaves surface. The significant leaf pathogens are *Cercospora beticola*, *Erysiphe betae*, *Uromyces betae* and *Alternaria alternata* (Mahmound, 2016; Mahlein et al., 2012; Hudec and Roháčik, 2002). *Cercospora beticola* causes *Cercospora* leaf spot disease, which is the most important disease worldwide (Tedford et al., 2017). *Cercospora beticola* is a necrotrophic fungus that uses *C. beticola* toxin (CBT) to kill infected plants. CBT causes the typical symptoms of leaf spots and prevents root formation. *Cercospora* leaf spot is economy problem for growers (Khan and Khan, 2009), because it causes decrease of assimilate transport to root. The result of disease damage is lower yield and sugar quality and high storage rots (Harveson and Bolton, 2013).

The term "resistance of pathogens to fungicides" is a phenomenon in chemical control in recent years. The term is used by Fungicide resistance action committee;

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it means gained and heritable reduction in susceptibility of a fungus to fungicide (FRAC, 2017). Fungal resistance represents a serious problem for farmers and may causes significant damage to crops (Setiawan et al., 2000). The terms "reduced sensitivity" or "tolerance" refer for lower fungicide efficacy, while term "resistance" indicates total loss of fungicidal efficacy against pathogens (FRAC, 2016). Resistance of *C. beticola* in sugar beet seems to be controlled by 4–5 pairs of genes with an additive action (Smith and Gaskill, 1970); their expression strongly interacts (66%) with the environment (Van den Bosch et al., 2011).

Fungicidal resistance seems to be actual problem in agricultural practice (Budakov et al., 2014). High level of resistance of *C. beticola* isolates to benzimidazoles (93.3–98.6%) was reported in Serbia, whereas 6.2–42.4% of isolates were resistant to demethylation inhibitors (DMI) fungicides (Trkulja et al., 2015). Pathogen strain CbCyp51 induced several-fold higher in *C. beticola* DMi-resistant strain than *C. beticola* wild type (Bolton et al., 2016). Resistance to azoxystrobin (QoI) was found out in 41% isolates of *C. beticola*, with higher EC_{50} value than 0.2 μg ml^{-1} in New York. The mutation of G143A was identified in these isolates, which is known as a cause of resistance to QoI fungicides (Vaghefi et al., 2016).

This study is focused on monitoring of *Cercospora beticola* sensitivity to the most frequently used fungicides, authorised against *Cercospora* leaf spot in Slovakia. The work is based on hypothesis of possible different fungicide sensitivity in *C. beticola* population, which could results to diverse risk of fungicide resistance in certain localities of Slovakia.

2 Material and methods

Cercospora beticola isolates were obtained from infected sugar beet leaves collected at the end of vegetation (Shrestha et al., 2017) from several localities of Slovakia. The localities and their characteristics are shown in Table 1. The fragments of leaves with pathogen sporulation spots were cut and put into petri dishes with potato dextrose agar (PDA) (Aggarwal et al., 2014). The petri dishes were incubated for 1–2 days in laboratory conditions. Isolates from spores were obtained by transferring the individual germinating conidia by a sterile needle to a nutrient medium. The isolates were determined microscopically according producing of typical conidia (Trkulja et al., 2013). Isolates were incubated at 25 °C and photoperiods 12/12, which are optimal conditions for growing of *Cercospora beticola* (Forsyth, 1963). After 14 days of incubation, each isolate was inoculated into three petri dishes containing PDA and incubated under the same temperature and light conditions.

Table 1 Geographic origin of *Cercospora beticola* isolates (Slovakia)

Samples name	Date of sampling	Locality	Cultivar	Altitude (MASL)*	GPS coordinates
N – 16	26. 9. 2016	Nižná	Natura	186	N 48° 32′ 8.985″ E 17° 39′ 9.095″
CH – 16	26. 9. 2016	Horné Chlebany	KWS	172	N 48° 36′ 16.145″ E 18° 13′ 20.582″
DS – 16	27. 9. 2016	Dolné Saliby	Leopolda	116	N 48° 6′ 33.45″ E 17° 46′ 47.727″
ČS – 16	27. 9. 2016	Senec	Antek	131	N 48° 13′ 1.327″ E 17° 24′ 22.113″
NZ – 16	28. 9. 2016	Nové Zámky	Predátor	117	N 47° 59′ 17.391″ E 18° 9′ 25.595″
M – 16	28. 9. 2016	Mojmírovce	Kosmos	140	N 48° 12′ 26.896″ E 18° 3′ 51.255″
O – 16	2. 10. 2016	Oslany	Plinius	200	N 48° 37′ 49.104″ E 18° 28′ 8.323″
B – 16	3. 10. 2016	Bolešov	Natura	230	N 48° 59′ 8.945″ E 18° 9′ 25.83″
S – 16	3. 10. 2016	Senica	Tatry	211	N 48° 40′ 47.512″ E 17° 21′ 39.6″
H – 16	4. 10. 2016	Hronovce	Primavera	136	N 48° 0′ 14.371″ E 18° 39′ 20.005″

*MASL – meters above sea level

2.1 Test of C. beticola sensitivity to fungicides

Sensitivity test of *C. beticola* to fungicides was performed with several concentrations for each fungicide to determine the EC_{50} (EC_{50} = half maximal effective concentration refers to the concentration of fungicide, which induces a response halfway the baseline and maximum) (Karaoglanidis and Thanassoulopoulos, 2003). Concentration of each active ingredient was based on recommended dose per hectare by the fungicide manufacturers, diluted in 200, 400 and 1000 liters of (spraying) water per hectare. The fungicides represented commercial formulations of those authorised against *Cercospora* leaf spot in Slovakia: trifloxystrobin + cyproconazole (Sfera 525 SC), kresoxym-methyl + epoxiconazole (Juwel), azoxystrobin + cyproconazole (Amistar Xtra), thiophanate-methyl + tetraconazole (Yamato), thiophanate-methyl (Topsin 500 SC), prochloraz + propiconazole (Bumper Super), picoxystrobin (Acanto), tetraconazole (Eminent 125ME), and difenoconazole (Score). Fungicides were aseptically added to the sterile medium prior to inoculation until the agar was still liquid. Sensitivity to fungicides was tested by inoculating 5 mm fragment of pathogens strain, removed from the mycelium edge of 14 days old culture. The fragment was upside down transformed into Petri Dishes with PDA (Karaoglanidis et al, 2002; Russell, 2004). The effect of fungicides and concentrations on mycelial growth was determined by measuring the diameter of colonies mycelium after 14 days (Malandrakis et al., 2006). The percent inhibition (*PI*) of each fungicide was calculated by following formula (Tumbek et al., 2011):

$$PI\ (\%) = \left((a - b)/a\right) \times 100$$

where:

PI – percentage of inhibition
a – average diameter of the nontreated (check) sample colony
b – average diameter of the treated sample

PI of the least 3 concentrations for each fungicide and each strain were subjected to regression analysis against the decadic logarithm of the fungicide dose to determination the EC_{50} value using by MS Excel. Differences between isolates and regions were determined by analysing the *PI* value for all doses by analysis of variance (ANOVA), at $P = 0.05$ to expressed statistically significant differences between fungicides and localities (Gaurilčikienė et al., 2006). Fungi-toxic curve was created from the results as the relationship between relative growth (*RR* = average of the treated sample colony/average of the control colony × 100) and fungicide concentration. Petri dishes with an equivalent amount of agar without fungicide were used as control (check) samples (Malandrakis et al., 2006). The concentrations of the active ingredients used in the tests are given in Table 2.

3 Results and discussion

Results of sensitivity test of *Cercospora beticola* population based on the mean of *PI* values showed that the mean *PI* value of thiophanate-methyl for each concentration achieved less than 27% for 6 of 10 localities. All the isolates from Mojmírovce, Hronovce, Senec, and Oslany localities were not able to grow on thiophanate-methyl concentration from 350 to 1750 µg ml^{-1} (Table 3). Thiophanate-methyl as a single fungicide failed to protect against *C. beticola*, because the combination of thiophanate-methyl +

Table 2 Tested concentrate of active ingredient in ppm

Fungicides	Active ingredients	Concentration (dose of water per ha)*		
		1,000	400	200
		ppm**		
Sfera 525 SC	trifloxystrobin + cyproconazol	187.25	468.125	936.25
Juwel	kresoxim-methyl + epoxiconazol	250	625	1,250
AmistarXtra	azoxystrobín + cyprokonazol	210	525	1,050
Yamato	thiophanate methyl + tetraconazole	454.5	1,136.25	2,272.5
Topsin	thiophanate methyl	350	875	1,750
Bumper Super	prochloraz + propiconazole	490	1,225	2450
Acanto	picoxystrobin	250	625	1,250
Eminent	tetraconazole	100	250	500
Score	difenoconazole	100	250	500

* Concentration of recommended dose by the fungicide manufactures, dilution with 200, 400 and 1000 l water per hectare, ** Concentrations of active ingredients in ppm

Table 3 Inhibition of *C. beticola* mycelium growth by thiophanate-methyl

Locality	Concentration (µg ml^{-1})		
	350*	875	1,750
	mean of *PI*** values (%)		
Dolné Saliby	17.63^{c}***	19.78^{c}	26.62^{c}
Bolešov	14.6^{b}	15.87^{b}	22.22^{b}
Nižná	9.36^{b}	14.05^{b}	18.73^{b}
Horné Chlebany	11.29^{a}	14.11^{a}	17.24^{a}
Senica	9.73^{b}	12.75^{b}	17.45^{b}
Mojmírovce	100.00^{d}	100.00^{d}	100.00^{d}
Hronovce	100.00^{d}	100.00^{d}	100.00^{d}
Senec	100.00^{d}	100.00^{d}	100.00^{d}
Oslany	100.00^{d}	100.00^{d}	100.00^{d}
Nové Zámky	8.57^{a}	16.83^{b}	21.9^{b}

* concentration of active ingredient in the PDA (µg ml^{-1}); ** *PI* – percentage of inhibition (%); *** the differences between the values marked with the same letters in the column are not statistically significant, LSD test, $P = 0.05$

Table 5 Inhibition of *C. beticola* mycelium growth by prochloraz + propiconazole

Locality	Concentration (µg ml^{-1}) *		
	0.1	0.25	0.5
	mean of *PI*** values (%)		
Dolné Saliby	86.69^{bc}***	90.29^{ab}	94.06^{a}
Bolešov	90.79^{c}	94.92^{c}	97.78^{b}
Nižná	100.00^{e}	100.00^{d}	100.00^{b}
Horné Chlebany	94.19^{d}	96.64^{c}	99.39^{b}
Senica	88.62^{bc}	91.72^{b}	92.41^{a}
Mojmírovce	83.83^{a}	88.83^{a}	94.06^{a}
Hronovce	87.66^{bc}	89.61^{a}	92.86^{a}
Senec	85.37^{b}	88.85^{a}	97.21^{b}
Oslany	100.00^{e}	100.00^{d}	100.00^{b}
Nové Zámky	87.46^{bc}	89.77^{a}	92.41^{a}

* concentration of active ingredient in the PDA (µg ml^{-1}); ** *PI* – percentage of inhibition (%); *** The differences between the values marked with the same letters in the column are not statistically significant, LSD test, $P = 0.05$

tetraconazole achieved excellent results compared with single thiophanate-methyl. The lowest average *PI* value on all of localities by the highest concentration was recorded on active ingredient thiophanate-methyl (*PI* = 51.49%) among tested fungicides, but combination of thiophanate-methyl + tetraconazole achieved very high efficacy against *C. beticola* (*PI* = 92.45%) (Table 3). Lower level of sensitivity *C. beticola* was observed also by active ingredient picoxystrobin. Picoxystrobin achieved the lowest inhibitory effect at the lowest (*PI* = 36.40%) and middle concentration (*PI* = 45.62%). Lower susceptibility of pathogen was found by difenoconazole, IP varied from 45.23 to 63.28%. The highest average inhibitory effect was observed by combination of active ingredients prochloraz + propiconazole (Table 4). The fungicide achieved great results, IP ranged from 83.83 to 100% at all isolates (Table 5). The high sensitivity of *C. beticola* isolates was observed also in single-site fungicide with the active ingredient tetraconazole, *PI* ranged from 81.18 to 93.63% (Table 4). *PI* values of tetraconazole were only

Table 4 Average inhibitory effect (*PI*) of tested fungicides to *C. beticola* isolates for all of the localities

Active ingredients	Concentration (L water per ha) *		
	1,000	400	200
	mean of *PI*** values (%)		
Trifloxystrobin + cyproconazole	79.69^{a} ***	87.75^{a}	90.13^{a}
Kresoxim-methyl + epoxiconazole	80.83^{a}	82.22^{a}	83.84^{a}
Azoxystrobin + cyproconazole	81.28^{a}	87.36^{a}	88.95^{a}
Thiophanate methyl + tetraconazole	83.86^{a}	88.56^{a}	92.45^{a}
Thiophanate methyl	46.10^{b}	48.38^{b}	51.49^{b}
Prochloraz + propiconazole	90.42^{a}	93.04^{a}	96.02^{a}
Picoxystrobin	36.40^{b}	45.62^{b}	53.68^{b}
Tetraconazole	81.18^{a}	90.06^{a}	93.63^{a}
Difenoconazole	45.23^{b}	55.12^{b}	63.28^{b}

* concentration of recommended dose by the fungicide manufactures, dilution with 200, 400 and 1,000 l water per hectare; ** mean percentage of inhibition for all of the localities; *** the differences between the values marked with the same letters in the column are not statistically significant, LSD test, $P = 0.05$

slightly lower than for multi-site fungicide prochloraz + propiconazole.

According to work of Karaoglanidis and Thanassoulopoulos (2003), isolates was categorized to 3 groups based on growth rate. These categories of results are presented in table 6. Percentage of categorized isolates presents table 7. The most numbers of sensitive isolates were observed to single-site fungicide tetraconazole (100%) and to multi-site fungicide prochloraz + propiconazole (100%). Resistant strains of *C. beticola* were recorded to all single-site fungicides, except tetraconazole. High percentage of susceptible strains was observed by using of thiophanate-methyl + tetraconazole (90% sensitive, 10% reduced sensitivity). No resistant strains were confirmed to multi-site fungicides. The most numbers of resistant isolates were observed by using of thiophanate methyl (60%), slightly lower numbers of resistant isolates were observed by picoxystrobin (40%). For difenoconazole, it was 80% of isolates determined as "resistant" and 10% as "reduced sensitivity".

EC_{50} values for the isolates were calculated by regression analysis from fungicide *PI* values. Application of multi-site fungicides kresoxim-methyl + epoxiconazole provided excellent inhibitory effect for all of isolates with average EC_{50} value 6.10E-05 ppm. All multi-site fungicides (trifloxystrobin + cyproconazole, kresoxim-methyl + epoxiconazole, azoxystrobin + cyproconazole, thiophanate methyl + tetraconazole, prochloraz + propiconazole) achieved average EC_{50} value <5 ppm. The highest average EC_{50} value was achieved by picoxystrobin (3,622.70 ppm). Average EC_{50} value for the single-site fungicides (thiophanate methyl, picoxystrobin, tetraconazole, difenoconazole), except tetraconazole, varied from 618.71 (difenoconazole) to 3,622.7 ppm (picoxystrobin). Average EC_{50} for tetraconazole achieved 14.03 ppm (Table 6). Ppm of recommended dose of active ingredients in field conditions is compared with EC_{50} values of active ingredients established by laboratory assay (Table 7). Laboratory EC_{50} values of picoxystrobin, thiophanate-methyl and difenoconazole were several times higher than ppm of recommended concentration for field conditions. Based on EC_{50} values, the isolates of *C. beticola* were sorted into three categories – sensitive, medium sensitive and resistant (Giannopolitis, 1978). All the tested isolates were categorized as sensitive to multi-site fungicides. 20% of isolates were resistant to picoxystrobin according EC_{50}, while according growth rate, it was 40% of isolates.

Among tested sites, isolates from the Oslany site were categorized as very susceptible to all active ingredient, all isolates achieved EC_{50} value ≤0.06 μg ml^{-1}. Sensitivity of *Cercospora beticola* isolates to thiophanate-methyl from localities Dolné Saliby, Bolešov, Nižná, Horné Chlebany, Senica, and Nové Zámky was different significantly compared with isolates from localities Oslany, Mojmírovce, Hronovce and Senec. The highest EC_{50} value to thiophanate-methyl was observed on isolates from Horné Chlebany (2,804.82 μg ml^{-1}). EC_{50} value to picoxystrobin varied from <0.01 to 16.71 μg ml^{-1}. Sensitivity of isolates to fungicides from each locality had compared each other on base EC_{50} values, statistically significant difference was found out to three active ingredients – thiophanate-methyl, picoxystrobin and difenoconazole (Table 6).

Assessment of resistance to fungicides, based on the growth rate of isolates is considered as a good indicator (Karaoglanidis and Thanassoulopoulos, 2003). Results

Table 6 Numbers of the *Cercospora beticola* isolates sorted into 3 categories according different criteria

Active ingredients	Categories based on growth rate*			EC_{50}** (ppm)	AD*** (ppm)	Categories based on EC_{50} values****		
	S	*RS*	*R*			S	MS	R
Trifloxystrobin + cyproconazole	70	30	0	3.58	1.25	100	0	0
Kresoxim-methyl + epoxiconazole	50	50	0	0.00103	2.45	100	0	0
Azoxystrobin + cyproconazole	60	40	0	1.09	1.05	100	0	0
Thiophanate methyl + tetraconazole	90	10	0	2.63	2.27	100	0	0
Thiophanate methyl	40	0	60	2,440.60	0.94	40	0	60
Prochloraz + propiconazole	100	0	0	0.0084	0.50	100	0	0
Picoxystrobin	30	30	40	3,622.70	0.50	40	40	20
Tetraconazole	100	0	0	14.03	1.75	100	0	0
Difenoconazole	10	80	10	618.71	1.25	80	10	10

* percentage categories based on daily growth, sensitive (*S*) – no growth; reduced sensitivity (*RS*) = <2 mm growth per day; resistant (*R*) = >2 mm per day (Karaoglanidis et al., 2003); ** average of EC_{50} value of isolates to active ingredients; *** concentration of active ingredients in application dose in field conditions; **** percentage categories based on EC_{50} value, *S* – sensitive (<0.5), MS – medium sensitive (0.5–5.0), *R* – resistant (>5.0) (Giannopolitis, 1978)

Table 7 Sensitivity (in EC_{50} value) of *C. beticola* isolates against fungicides

Active ingredients	Localities									
	DS*	B	N	CH	S	M	H	ČS	O	NZ
Trifloxystrobin + cyproconazole	<0.01^{e**}	<0.01^{c}	<0.01^{c}	<0.01^{b}	<0.01^{c}	<0.01^{d}	<0.01^{d}	<0.01^{d}	<0.01^{b}	<0.01^{d}
Kresoxim-methyl + epoxiconazole	<0.01^{e}	<0.01^{c}	<0.01^{c}	<0.01^{b}	<0.01^{c}	<0.01^{d}	0.03^{c}	<0.01^{d}	<0.01^{b}	<0.01^{d}
Azoxystrobin + cyproconazole	0.02^{e}	<0.01^{c}	<0.01^{c}	<0.01^{b}	<0.01^{c}	0.02^{c}	0.01^{d}	0.02^{c}	<0.01^{b}	<0.01^{d}
Thiophanate methyl + tetraconazole	0.06^{d}	0.01^{c}	0.01^{c}	0.01^{b}	<0.01^{c}	<0.01^{d}	<0.01^{d}	<0.01^{d}	<0.01^{b}	0.01^{d}
Thiophanate methyl	32.18^{a}	199.74^{a}	81.56^{a}	2,804.82^{a}	387.79^{a}	<0.01^{d}	<0.01^{d}	<0.01^{d}	<0.01^{b}	9.93^{a}
prochloraz + propiconazole	<0.01^{e}	<0.01^{c}	<0.01^{c}	<0.01^{b}	<0.01^{c}	<0.01^{d}	<0.01^{d}	<0.01^{d}	<0.01^{b}	<0.01^{d}
picoxystrobin	5.27^{b}	<0.01^{c}	1.58^{b}	<0.01^{b}	<0.01^{c}	3.50^{a}	1.09^{a}	16.71^{a}	0.06^{a}	1.75^{b}
tetraconazole	0.01^{e}	<0.01^{c}	<0.01^{c}	<0.01^{b}	<0.01^{c}	0.01^{d}	0.01^{d}	<0.01^{d}	<0.01^{b}	<0.01^{d}
difenoconazole	0.11^{c}	0.02^{b}	1.41^{b}	0.01^{b}	0.02^{b}	0.11^{b}	0.15^{b}	7.54^{b}	0.01^{b}	0.02^{c}

* DS, Dolné Saliby;B, Bolešov; N, Nižná; CH, Horné Chlebany; S, Senica; M, Mojmírovce; H, Hronovce; ČS, Senec; O, Oslany; **EC_{50} values based on mycelial growth (μg ml^{-1}); the differences between the values marked with the same letters in the column are not statistically significant, LSD test, $P = 0.05$

of this work showed, that 60% of tested isolates was resistant to thiophanate-methyl, based on growth rate. Resistance to thiophanate-methyl was not confirmed in all of the tested localities. Isolates from Mojmírovce, Senec, Hronovce and Oslany were categorized as very sensitive to thiophanate-methyl. This could be caused by agrotechnical measures, anti-resistance strategy or by low frequency of using thiophanate-methyl. According Karaoglanidis and Thanassoulopoulos (2003), the level of resistant strain can be low without using benzimidazole, but frequency of occurrence of resistant strains to benzimidazoles is increasing with use of benzimidazoles on field conditions. Results of Trkulja et al. (2013) study showed that there was no difference in occurrence or frequency of resistant isolates between use and non-use of benzimidazoles fungicides (Trkulja et al., 2013). In our results, the significant difference was found between isolates from localities, where the thiophanate-methyl was used for several years ago (Horné Chlebany, Senica, Bolešov) and those with a very low thiophanate-methyl use (Oslany, Hronovce, and Mojmírovce).

Development of resistant strains could by a consequence of higher using of benzimidazole fungicides. Consumption of benzimidazoles in Slovakia was 35425 litres in 2015, which is 10% more than in 2014 (UKSUP, 2016). Resistance to benzimidazole is caused by mutation at codon 198 in the β-tubulin (Davidson et al., 2006). Results of Groenewald's (2008) study showed high genetic variability of *Cercospora beticola* isolates. According to FRAC (2017), thiophanate-methyl belongs to the group of "high risk for resistance". One of the reasons of high risk for resistance could be a fact that thiophanate-methyl was first registered in 1971 (general information) yet. On the other side, the combination of thiophanate-methyl + tetraconazole achieved excellent results. It could be caused by mixture by other active ingredient – tetraconazole, because it recorded 100% of sensitive isolates to single tetraconazole in our study.

Laboratory results in this study confirmed the presence of resistant *C. beticola* strains to active ingredient picoxystrobin. Picoxystrobin is classified in "high risk for resistance" group, because of their specific mode of action (Grasso et al., 2006; FRAC, 2017). According to Karaoglanidis and Thanassoulopoulos (2003) classification, 40% of tested isolates in our work were categorized as resistant, but according to Giannopolitis (1978) classification, only 20% of our tested isolates were resistant. Diameter of untreated colony for picoxystrobin was slightly smaller than other check colonies. That could be reason of difference in the results. Lower EC_{50} value than 0.01 μg ml^{-1} to picoxystrobin was observed on 3 isolates only (Bolešov, H. Chlebany and Senica). It had the lowest number of isolates with EC_{50} value lower than 0.01 μg ml^{-1} among all tested fungicides.

Quinone outside inhibitors (QoI) fungicides is good combinable with demethylation inhibitors (DMi) fungicides in anti-resistant strategy against *Cercospora* leaf spot (CLS) (Karadimos and Karaoglanidis 2006). Increase of resistance *C. beticola* strain to QoI fungicides in Poland was delayed (Brila et al., 2012). It could be result of low frequent using of chemical control in their climate (Piszczek et al., 2017). DMi fungicides are known for their broad-spectral fungicide, curative and protective effect (Bolton et al., 2012), and have been using more than 20 years and still have a sufficient effect (Nikou et al., 2009).

Resistant *C. beticola* strains to difenoconazole were observed only in Senec in our work. According to FRAC (2017), difenoconazole belongs to the group of medium risk for resistance. Percentage of reduced sensitivity isolates to difenoconazole based on growth rate was 80%, while based on EC_{50} value was only 10%. It was observed difference between assessments based on different criteria, while categorization according Karaoglanidis and Thanassoulopoulos (2003), based on EC_{50} was slight stricter. From Trkulja study (2015) followed, that 20% of resistance isolates to DMI were also resistance to methyl benzimidazole carbamates (MBC). In this study, the cross resistance between DMi and MBC fungicides was not confirmed.

The highest inhibitory effect against *C. beticola* isolates was demonstrated by using of prochloraz + propiconazole. Average *PI* value of prochloraz + propiconazole varied from 90.42 to 96.02%. EC_{50} value achieved <0.01 $\mu g.ml^{-1}$ on all of tested localities. Both active ingredients belong to the group of DMi fungicides, and by FRAC were classified as "medium risk". According to Karaoglanidis and Karadimos (2003), all single-site fungicides had reduced efficacy.

Monitoring of sensitivity of *C. beticola* to fungicides can be an excellent tool to determine the development of *C. beticola* resistance and effective recommendations for sugar beet production areas (Kirk et al., 2012). In this work, the hypothesis of possible different fungicide sensitivity in *C. beticola* population was confirmed. According to the results, the risk of fungicide resistance is different in certain localities of Slovakia.

4 Conclusions

Cercospora beticola is a pathogen with high risk of developing of resistance. Occurrence of fungicide resistance in *C. beticola* population was confirmed in Slovakia. Resistant strains were confirmed for three (thiophanate-methyl, picoxystrobin and difenoconazole) from nine tested fungicides. Different criteria of assessment of fungicide resistance (based on EC_{50} and on growth rate – inhibition percentage) showed slightly different results, but both of the criteria confirmed occurrence of resistant *C. beticola* strains to thiophanate-methyl, picoxystrobin and difenoconazole in Slovakia. Fields with higher frequency of application of these fungicides significantly supported the development of resistant strains. Their use should be reconsidered on critical areas. It is very important to focus on anti-resistant strategy and reduce of using of risk fungicides on localities, where it was confirmed occurrence of resistant *Cercospora beticola* strains. The highest frequency of fungicide resistant strains was confirmed in localities Dolné Saliby (thiophanate-methyl and picoxystrobin) and Senec (picoxystrobin and difenoconazole). The lowest level of risk of fungicide resistance was confirmed in the locality Oslany. Assessment of any *C. beticola* strains have not confirmed reduced sensitivity to active ingredients tetraconazole and prochloraz + propiconazole. These active ingredients achieved the highest efficacy against *C. beticola* isolates from all of the tested localities. The serious risk of fungicide resistance in some localities in Slovakia was confirmed in this work. For farmers, avoid of risk fungicides application in certain localities is recommended.

References

AGGARWAL, N. K. et al. (2014). Mycobiota associated with Parthenium hysterophorus isolated from North India. *Indian Journal of Weed Science*, 46(2), 155–160.

ALMQUIST, C. et al. (2016). Disease risk assessment of sugar beet root rot using quantitative real-time PCR analysis of *Aphanomyces cochlioides*. *European Journal of Plant Pathology*, 145(4), 731–742. https://doi.org/10.1007/s10658-016-0862-5

BOLTON, M. et al. (2012). Characterization of CbCyp51 from field isolates of *Cercospora beticola*. *Phytopathology*, 102(3), 298–305. https://doi.org/10.1094/PHYTO-07-11-0212

BOLTON, M. D. et al. (2016). RNA-sequencing of *Cercospora beticola* DMI-sensitive and-resistant isolates after treatment with tetraconazole identifies common and contrasting pathway induction. *Fungal Genetics and Biology*, 92, 1–13. https://doi.org/10.1016/j.fgb.2016.04.003

BRILA, K. et al. (2012). Characterization of cytochrome b from European field isolates of *Cercospora beticola* with quinone outside inhibitor resistance. *European Journal of Plant Pathology*, 134, 475–488. https://doi.org/10.1007/s10658-012-0029-y

BUDAKOV, D. et al. (2014). Sensitivity of *Cercospora beticola* isolates from Serbia to carbendazimand and flutriafol. *Crop Protection*, 66, 120–126. https://doi.org/10.1016/j.cropro.2014.09.010

ČERNÝ, I. et al. (2018). Crop formation and digestion of sugar beet depending on the year and foliar application of biologically active substances and fertilizers. *Listy cukrovarnické a řepařské*, 134(4), 141–145.

ČERNÝ, I. et al. (2019). Crop formation and digestion of sugar beet depending on the various technology of soil preparation. *Listy cukrovarnické a řepařské*, 135(12), 396–400.

DAVIDSON, R. M. et al. (2006). Analysis of β-tubulin gene fragments from benzimidazole-sensitive and tolerant *Cercospora beticola*. *Journal of Phytopathology*, 154, 321–328. https://doi.org/10.1111/j.1439-0434.2006.01080.x

FORSYTH, F. R. et al. (1963). Cultural and pathogenic studies of an isolate of *Cercospora beticola* Sacc. *Journal of American Society of Sugar Beet Technology*, 12, 485–491.

FRAC. (2016). Definition of fungicide resistance. FRAC. Retrieved 2.11.2016 from http://www.frac.info/resistance-overview

GAURILČIKIENĖ, I. et al. (2006). Epidemic progress of *Cercospora beticola* Sacc. in *Beta vulgaris* L. under different conditions and cultivar resistance. *Biologija*, 4, 54–59. https://doi.org/10.6001/biologija.vi4.698

GIANNOPOLITIS, C. N. (1978). Occurrence of strains of *Cercospora beticola* resistant to triphenyltin fungicides in Greece. *Plant Disease Reporter*, 62, 205–208.

GRASSO, V. et al. (2006). Characterization of the cytochrome b gene fragment of Puccinia species responsible for the binding site of QoI fungicides. *Pesticide Biochemistry and Physiology*, 84(2), 72–82. https://doi.org/10.1016/j.pestbp.2005.05.005

GROENEWALD, M. et al. (2008). Indirect evidence for sexual reproduction in *Cercospora beticola* populations from sugar beet. *Plant Pathology*, 57, 25–32. https://doi.org/10.1111/j.1365-3059.2007.01697.x

HAJYIEVA, H. and SOROKA, S. (2008). Phytosanitary situation in sugar beet crops in Belarus. *Zemdirbyste-Agriculture*, 95(3), 65–73.

HARVESON, R. M. and BOLTON, M. D. (2013). First Evidence of a Binucleate *Rhizoctonia* as the Casual Agent of Dry Rot Canker of Sugar Beet in Nebraska. *Plant Diseaes*, 97(11), 1508. https://doi.org/10.1094/PDIS-04-13-0375-PDN

HUDEC, K. and ROHÁČIK, T. (2002). *Alternaria alternata* (Fr.) Keissler-new pathogen on sugar beet leaf in Slovakia. *Plant Protection Science*, 38(2), 81–82.

KARADIMOS, D. A. and KARAOGLANIDIS, G. S. (2006). Comparative efficacy, selection of effective partners and application time of strobilurin fungicides for control of *Cercospora* leaf-spot of sugar beet. *Plant Disease*, 90(6), 820–825. https://doi.org/10.1094/PD-90-0820

KARAOGLANIDIS, G. S. and THANASSOULOPOULOS, C. C. (2003). Cross-resistance patterns among sterol biosynthesis inhibiting fungicides (SBIs) in *Cercospora beticola*. *European Journal of Plant Pathology*, 109(9), 929–934.

KARAOGLANIDIS, G. S. et al. (2003). Sensitivity of *Cercospora beticola* populations to fentin-acetate, benomyl and flutriafol in Greece. *Crop Protection*, 22(5), 735–740. https://doi.org/10.1016/S0261-2194(03)00036-X

KARAOGLANIDIS, G. S. et al. (2002). Changes in sensitivity of *Cercospora beticola* populations to sterol-demethylation-inhibiting fungicides during a 4-year period in northern Greece. *Plant Pathology*, 51(1), 55–62. https://doi.org/10.1046/j.0032-0862.2001.x-i2

KHAN, J. et al. (2009). Fluctuations in number of *Cercospora beticola* conidia in relationship to environment and disease severity in sugar beet. *Phytopathology*, 99(7), 796–801. https://doi.org/10.1094/PHYTO-99-7-0796

KIRK, W. W et al. (2012). First report of strobilurin resistance in *Cercospora beticola* in sugar beet (*Beta vulgaris*) in Michigan and Nebraska, USA. *New Disease Reports*, 26, 3. http://dx.doi.org/10.5197/j.2044-0588.2012.026.003

MAHLEIN, A. K. et al. (2012). Hyperspectral imaging for small-scale analysis of symptoms caused by different sugar beet diseases. *Plant Methods*, 8(1), 3. https://doi.org/10.1186/1746-4811-8-3

MAHMOUD, A. F. (2016). Suppression of sugar beet damping-off caused by *Rhizoctonia solani* using bacterial and fungal antagonists. *Archives of Phytopathology and Plant Protection*, 49(19–20), 575–585. https://doi.org/10.1080/03235408.2016.1245052

MALANDRAKIS, A. A. et al. (2006). Biological and molecular characterization of laboratory mutants of *Cercospora beticola* resistant to Qo inhibitors. *European Journal of Plant Pathology*, 116(2), 155–166. https://doi.org/10.1007/s10658-006-9052-1

NIKOU, D. et al. (2009). Molecular characterization and detection of overexpressed C-14 alpha-demethylase-based DMI resistance in *Cercospora beticola* field isolates. *Pesticide Biochemistry and Physiology*, 95(1), 18–27. https://doi.org/10.1016/j.pestbp.2009.04.014

PISZCZEK, J. et al. (2017). First report of G143A strobilurin resistance in *Cercospora beticola* in sugar beet (*Beta vulgaris*) in Poland. *Journal of Plant Diseases and Protection*, 125(1), 99–101. https://doi.org/10.1007/s41348-017-0119-3

RUSSELL, P. E. (2002). *Sensitivity baselines in fungicide resistance research and management*. Brussels: Crop Life International FRAC Monograph.

SETIAWAN, A. et al. (2000). Mapping quantitative trait loci (QTLs) for resistance to *Cercospora* leaf spot disease (*Cercospora beticola* Sacc.) in sugar beet (*Beta vulgaris* L.). *Theoretical and Applied Genetics*, 100(8), 1176–1182. https://doi.org/10.1007/s001220051421

SHRESTHA, S. K. et al. (2017). Genetic diversity, QoI fungicide resistance, and mating type distribution of *Cercospora sojina* – Implications for the disease dynamics of frogeye leaf spot on soybean. *Plos One*, 12(5), 1. https://doi.org/10.1371/journal.pone.0177220

SMITH, G. A. and GASKILL, J. O. (1970). Inheritance of resistance to *Cercospora* leaf spot in sugarbeet. *Journal of the American Society of Sugar Beet Technologists*, 16(2), 172–180.

TEDFORD, S. L. et al. (2017). Relationships among airborne *Cercospora beticola* conidia concentration, weather variables, and cercospora leaf spot severity in sugar beet (*Beta vulgaris* L.). *Canadian Journal of Plant Pathology*, 40(1), 1–10. https://doi.org/10.1080/07060661.2017.1410726

TRKULJA, N. et al. (2013). Characterisation of benzimidazole resistance of *Cercospora beticola* in Serbia using PCR-based detection of resistance-associated mutations of the β-tubulin gene. *European Journal of Plant Pathology*, 135(4), 889–902. https://doi.org/10.1007/s10658-012-0135-x

TRKULJA, N. et al. (2015). Occurrence of *Cercospora beticola* populations resistant to benzimidazoles and demethylation-inhibiting fungicides in Serbia and their impact on disease management. *Crop Protection*, 75, 80–87. https://doi.org/10.1016/j.cropro.2015.05.017

TÜMBEK, A. et al. (2011). Sensitivity of *Cercospora beticola* populations in Turkey to flutriafol, mancozeb, and fentin acetate. *Turkish Journal of Agriculture and Forestry*, 35(1), 65–71. https://doi.org/10.3906/tar-0910-24

ÚKSÚP. (2016). List of authorized plant protection products and plant protection products authorized for parallel trade. Bratislava: UKSÚP. Retrieved 15.1.2020 from http://web.uksup.agroinstitut.sk/orp-pripravky-na-ochranu-rastlin-registre-a-zoznamy/?start

VAGHEFI, N. et al. (2016). Genotypic diversity and resistance to azoxystrobin of *Cercospora beticola* on processing table beet in New York. *Plant Disease*, 100(7), 1466–1473. https://doi.org/10.1094/PDIS-09-15-1014-RE

VAN DEN BOSCH, F. et al. (2011). The dose rate debate: does the risk of fungicide resistance increase or decrease with dose? *Plant Pathology*, 60(4), 597–606. https://doi.org/10.1111/j.1365-3059.2011.02439.x