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Original Paper

Diversity and fungicidal resistance of *Cercospora beticola*

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The fungus *Cercospora beticola* Sacc. belongs to the most important pathogens on sugar beet. The *Cercospora* leaf spot disease is important problem for growers in all growing areas of sugar beet. This study was focused on the morphological diversity of *C. beticola* isolates and occurrence of their fungicidal resistance in Slovakia. Isolates involved in this work were collected from sugar beet leaf during 2016–2018. Average growth rate of the tested *C. beticola* isolates on five different media showed the major role of cultivation temperature. The fastest growth was measured by 30 °C on TE medium, followed by PDA, SBLEA, AWSBL, and V8. The colour of aerial mycelium varied from olive-green to grey with white powdery appearance, per grey colour with wrinkled texture, to black colour. There is no significant correlation among isolates origin, colony morphology parameters and growth rate. The highest sporulation rate was recorded at cultivation temperature 25 °C by using of TE growth medium, the lowest one by V8 medium. The inhibition effect of azoxystrobin + cyproconazole was significantly different among the tested isolates from different localities and years. The results showed reduced sensitivity of *C. beticola* population in Slovakia strictly depending of the locality. The most reduced sensitivity was measured on localities Hronovce and Nové Zámky, followed by localities Mojmírovce, Senec, and Dolné Saliby with similar values. There is no positive correlation between inhibition effect and mycelial growth rate. The results showed increasing fungicidal resistance of *C. beticola* to azoxystrobin + cyproconazole in some localities of Slovakia.

Keywords: *Cercospora beticola*, sporulation, fungicides, resistance

1 Introduction

Sugar beet belongs to traditional crops in Europe. In Slovakia, it is mainly grown as a technical crop for sugar production. *Cercospora* leaf spot (CLS), caused by the air-borne fungus *Cercospora beticola* Sacc. could cause a decreasing of sugar beet leaf mass and the consequent regrowth of leaves, which results in lower yields and sugar contents (Balandžić et al., 2020). The fungus *C. beticola* belongs to the most important pathogens on sugar beet worldwide, causing high yield losses, ranging from 25 to 50% (Shane & Teng, 1992). The disease leads to premature death of leaves, and to reduction of assimilation area. Finally, the content of sucrose is diminished (Skaracis et al., 2010). This crop damage is partially mediated by mycotoxin cercosporin, which is produced during CLS development and enhance virulence of the pathogen (Upchurch et al., 1991). In Slovakia, the *Cercospora* leaf spot is frequently controlled with single-site fungicides which can lead to development of resistance in pathogen population (Ma & Michailides, 2005).

Morphological characters of fungi are commonly used in the taxonomy. The most distinguish ones for identification of *C. beticola* are the size and shape of the conidia, conidiophores, conidial scars and hyaline image of the conidia. Important tool for identification and characterization of phytopathogenic fungi is the dynamics of mycelial growth by different cultivation temperature (Groenewald et al., 2005). Groenewald (2008) mentioned high genetic variability into and among population of *C. beticola*. It indicates that sexual recombination within this species is highly possible. This finding is supported by Moretti et al. (2006), who found high variability between isolates from the same lesion from one sugar beet leaf.

Due to the meaning of sugar beet in agricultural praxis, it is very important to assess the occurrence of fungicide resistance. Survey of pathogen sensitivity to fungicides is the most important aspect of the anti-resistance strategy (Karaoglanidis et al., 2003).

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The aim of this study was to analyse the morphological diversity of *C. beticola* isolates, to estimate the most appropriate media and cultivation for laboratory survey, and the presence of resistance of *C. beticola* strains to azoxystrobin + cyproconazole in Slovak republic.

2 Material and methods

2.1 Sampling and isolation of *C. beticola*

Samples for pathogen isolation were collected from 10 localities in geographically different areas of Slovakia (Nižná, Chlebany, Dolné Saliby, Senec, Nové Zámky, Mojmírovce, Oslany, Bolešov, Senica, Hronovce). Sugar beet leaves with typical symptoms of CLS were collected during September of 2016, 2017, and 2018. Leaves were examined directly under a stereo-microscope to observe sporulation. Those with sporulating spots were used for direct isolation of *C. beticola*. The others without sporulation were placed on Petri dishes with wet filter paper for inducing of sporulation. Petri dishes were incubated at 23 °C by photoperiod period of 24 hours. Single-conidial isolates of *C. beticola* were obtained by transferring individual germinating conidia by the needle to potato dextrose agar (PDA) plates. For each sample, conidia from only one lesion per leaf were transferred to the PDA. After four days of incubation the growing fungal colonies were transferred to new PDA and incubated for the next 14 days. From each locality and year, one isolates group were selected for this study (300 isolates totally). Each isolates group contains 10 isolates, and each isolate originated from different plant.

2.2 Morphological and cultural characterization

For the morphological and cultural characterization, the isolates (one isolate per each group of isolates) were cultured on different nutrition media: PDA (potato dextrose agar), V8, SBLEA (sugar beets leaf extract agar), TE (tomato extract), AWSBL (agar with sugar beet leaf). Mycelial rings (5 mm in diameter) were separated from the colony margins of 14-day-old cultures and were placed upside down on new culture media and incubated at different temperatures (20, 25, and 30 °C) under 12/12 photoperiods. Colony diameters were measured in 3–4 days interval, and finally when the isolate with the fastest growth filled the whole Petri dish (p = 80 mm). A total of three replicates were realised for each isolate on each medium.

The intensity of sporulation was estimated by counting of spore number in 1 ml of water splashing of the colony surface by 10 ml of distilled water. The experiments included three replicates for each isolate. The total 30 isolates was used, each one per locality (10 localities) and year (3 years).

For the estimation of origin influence on fungal growth, 300 isolates were placed on PDA and cultivated under 25 °C. The growth rate was measured on 25th day and calculated as mm day^{-1}. Aside the growth rate, the colour and mycelium characteristics of the above and reverse side of the colony, and form and intensity of sporulation was determined.

All data obtained were subjected to the analysis of variance (ANOVA) and means were compared using Fisher's least significant difference (LSD) procedure (p = 0.05).

2.3 Estimation of fungicide sensitivity

Sensitivity test of *C. beticola* to fungicide azoxystrobin + cyproconazole was performed with several concentrations to determine inhibition of mycelial growth (Karaoglanidis & Thanassoulopoulos, 2003). Concentration of the fungicide (210, 525 and 1050 ppm)

Table 1 Origin and characterisation of *Cercospora beticola* isolates

Isolates* groups	Year of sampling	Locality	Altitude
N16, N17, N18	2016, 2017, 2018	Nižná	186
CH16, CH17, CH18	2016, 2017, 2018	Horné Chlebany	172
DS16, DS17, DS18	2016, 2017, 2018	Dolné Saliby	116
ČS16, ČS17, ČS18	2016, 2017, 2018	Senec	131
NZ16, NZ17, NZ18	2016, 2017, 2018	Nové Zámky	117
M16, M17, M18	2016, 2017, 2018	Mojmírovce	140
O16, O17, O18	2016, 2017, 2018	Oslany	200
B16, B17, B18	2016, 2017, 2018	Bolešov	230
S16, S17, S18	2016, 2017, 2018	Senica	211
H16, H17, H18	2016, 2017, 2018	Hronovce	136

* isolates groups – each isolates group contains 10 isolates

was aseptically added to the sterile PDA medium prior to inoculation of the fungus. The sensitivity 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). Petri dishes with an equivalent amount of agar and those without fungicide were used as control (check) samples (Malandrakis et al., 2006). The effect of fungicides on mycelial growth was determined by measuring the diameter of colonies mycelium after 14 days (Malandrakis et al., 2006). The percentage of inhibition (*PI*) 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 non-treated (check) sample colony; *b* – average diameter of the treated sample

Differences between isolates and regions were determined by analysing the *PI* value for all doses by analysis of variance (ANOVA), at $p = 0.05$.

3 Results and discussion

3.1 Morphological characterization of *C. beticola* isolates

Average growth rate of 30 tested *C. beticola* isolates on five different media showed the major role of cultivation temperature. Isolates expressed significant differences in growth on all tested media and temperatures (Table 2 and 3).

The fastest growth on 25th day was measured by TE medium at temperature 30 °C (76.81 mm), followed by PDA at 30 °C (75.98 mm), SBLEA at 25 °C (73.25 mm), and AWSBL at 25 °C (58.76 mm), while the lowest average growth was measured on the V8 at 30 °C (17.48 mm). The optimal temperature for mycelium growth on majority of the cultivation media (AWSBL, V8, SBLEA) was 25 °C. At 30 °C, the fastest growth was measured by using of PDA and TE media. The lowest growth rate was measured at 20 °C by using of all cultivation media, excepting V8. In general, the growth rate of all tested isolates was the lowest by using of 20 °C temperature and V8 medium.

Specific results were measured by using of V8 medium. The growth intensity on V8 medium was comparable with other cultivation media during first 11 days of cultivation. After 11th day, the fungus growth slowed and stopped, while on other media the isolates continue in growth (Table 2).

The isolates of *C. beticola* formed several types of mycelia (Table 4). The colour of aerial mycelium varied from olive-green to grey with white powdery appearance (PDA), per grey colour with wrinkled texture (TE), to black colour (V8). The colour of colony reverse was black by all the isolates and on all the growth media. According to the data shown in the Table 5, there is no significant correlation among isolates origin, colony morphology parameters and growth rate measured on PDA at 25 °C.

The variability in morphology of *C. beticola* isolates of a wide geographic origin is known, but recent studies have shown that even *C. beticola* isolates originating from the same spot may vary in morphology (Moretti et al., 2006). A wide range variability was found in the *C. beticola* population tested in our study. The results of morphological characteristics on PDA showed that the *C. beticola* strains have market different texture and colour of mycelium. The morphological characteristics

Table 2 Influence of temperatures on average growth rate (mm) of *C. beticola* isolates on AWSBL, PDA, and V8 cultivation media

Days of cultivation	Growth medium								
	AWSBL2			PDA			V8		
	20 °C	25 °C	30 °C	20 °C	25 °C	30 °C	20 °C	25 °C	30 °C
0	5.00[a 1, 3]	5.00[a]	5.00[a]	5.00[a]	5.00[a]	5.00[a]	5.00[a]	5.00[a]	5.00[a]
4	13.42[h]	10.40[n]	10.15[o]	14.86[a]	14.00[e]	14.31[c]	13.55[g]	14.55[b]	11.54[m]
7	18.03[m]	18.61[k]	21.49[g]	20.94[h]	24.32[c]	24.96[b]	18.56[l]	22.21[f]	15.67[n]
11	27.95[k]	30.03[h]	34.93[f]	28.69[j]	36.97[c]	38.99[b]	21.64[n]	26.47[m]	15.49[o]
15	36.49[k]	38.84[h]	47.27[e]	36.59[j]	46.38[f]	52.53[b]	22.81[n]	27.71[m]	16.57[o]
19	45.02[j]	48.33[h]	53.22[f]	44.50[k]	54.79[e]	63.67[b]	23.15[n]	28.21[m]	17.16[o]
22	50.53[j]	53.15[i]	56.41[g]	50.42[k]	58.76[e]	70.88[b]	23.25[n]	28.32[m]	17.28[o]
25	54.75[j]	58.76[h]	58.53[i]	54.20[k]	63.38[f]	75.98[b]	23.32[n]	28.34[m]	17.48[o]

1 – different letters indicate significant differences according to Fisher's LSD test ($p \leq 0.05$); 2 – AWSBL (agar with sugar beet leaf), PDA (potato dextrose agar), V8 (V8 Juice agar); 3 – diameter of colony – mm

Table 3 Influence of temperatures on dynamics of average growth rate (mm) of *C. beticola* isolates on TE and SBLEA cultivation media

Days of cultivation	Growth medium					
	TE			SBLEA		
	20 °C[3]	25 °C	30 °C	20 °C	25 °C	30 °C
0	5.00[a]	5.00[a]	5.00[a]	5.00[a]	5.00[a]	5.00[a]
4	14.24[d]	11.83[l]	13.90[f]	12.73[k]	12.80[j]	13.18[i]
7	20.39[j]	20.81[i]	26.45[a]	18.03[m]	22.60[e]	24.02[d]
11	29.55[i]	32.39[g]	41.67[a]	26.65[l]	36.63[d]	35.97[e]
15	38.30[i]	42.11[g]	55.42[a]	34.60[l]	48.98[d]	49.01[c]
19	47.05[i]	51.69[g]	65.55[a]	42.55[l]	60.35[c]	56.06[d]
22	53.61[h]	57.81[f]	71.96[a]	48.51[l]	67.23[c]	60.38[d]
25	58.77[g]	64.39[d]	76.81[a]	54.11[l]	73.25[c]	64.03[e]

1 – different letters indicate significant differences according to Fisher's LSD test ($p \leq 0.05$); 2 – TE (tomato extract agar), SBLEA (sugar beets leaf extract agar), 3 – diameter of colony – mm

Table 4 Average morphological characteristics in *Cercospora beticola* isolates on different growth media

Characteristics		Growth medium				
		AWSBL[1]	PDA	V8	TE	SBLEA
Aerial mycelium	texture	cottony 30/30[2]	cottony 29/30	cottony 30/30	cottony wrinkled 25/30	cottony 27/30
	colour	olive-green 26/30	olive-green-grey 22/30	black 30/30	grey 26/30	dark grey, white centre 27/30
	colour of mycelium border	none 30/30	dark green 22/30	none 30/30	none 28/30	none 24/30
Reverse of mycelium		black 30/30	black 30/30	black 30/30	black 30/30	black 30/30

1 – PDA (potato dextrose agar), V8 (V8 Juice agar), SBLEA (sugar beets leaf extract agar), TE (tomato extract agar), AWSBL (agar with sugar beet leaf); 2 – the number of isolates with showed parameter/total number of tested isolates

divided the isolates collection into several different groups depending on colour of aerial mycelium and other characteristics (Table 4). The major colony characteristic of most of the isolates was cottony aerial mycelium with olive green to dark colour, with black reverse, which is in agreement with results of Esh and Moghaieb (2011). Colonies are morphologically divergent depend on growth media (Table 4) with several different colours ranged from olive-green, olive-green grey, grey, dark grey with white centre to black. In morphology of *C. beticola* isolates, there were no differences in dependence of locality and used cultivation media. The presented results establish that the naturally occurring population of *C. beticola* is variable phenotypically for mycelial growth, as previously being reported (Moretti et al., 2006). Presented results of morphological tests showed the presence of different populations of *C. beticola* in Slovakia.

The influence of cultivation media and cultivation temperatures on sporulation of *C. beticola* isolates is showed in Figure 1. The results showed that the highest rate of sporulation was recorded at cultivation temperature 25 °C in all growth media. The lowest sporulation was assessed by 20 °C. The rate of sporulation varied from 4.10^4 conidia ml^{-1} to $22.5.10^4$ conidia ml^{-1}. The highest rate of sporulation was observed by using of TE growth medium, the lowest one by using of V8 medium. The results of morphological analysis and sporulation of *C. beticola* isolates created important criteria for choosing of appropriate growth medium for testing of sensitivity to fungicides, especially in field of sporulation. Incubation temperature and medium pH directly affect the growth and sporulation of *Cercospora* species (Silva et al., 2016). In general, the *C. beticola* sporulation on growth media is very sporadically. In our experiment, the highest sporulation rate was recorded by using of TE medium at 25 °C, followed by SBLEA. Medium sporulation rate was recorded by using of PDA and AWSBL at 25 °C, the lowest one was observed by using of V8 medium at 20 °C. Opposite to our results, positive effect of V8 medium on

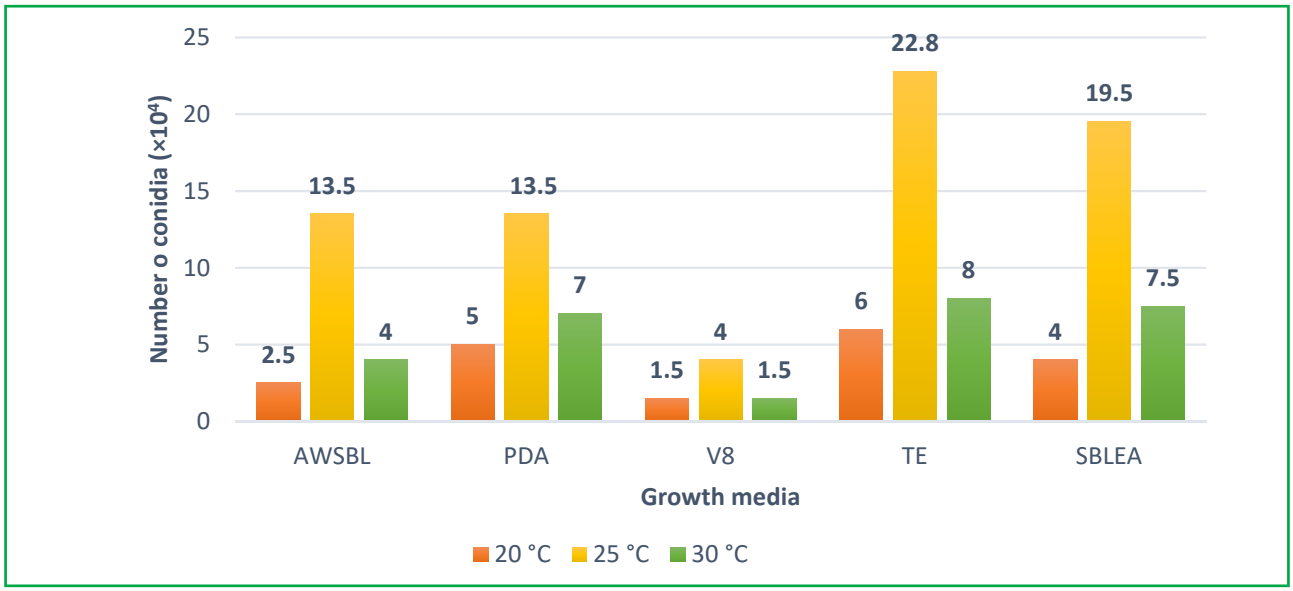

Figure 1 Sporulation of *Cercospora beticola* isolates on different cultivation media and temperatures
*PDA (potato dextrose agar), V8 (V8 Juice agar), SBLEA (sugar beets leaf extract agar), TE (tomato extract agar), AWSBL (agar with sugar beet leaf); sporulation – number of conidia ml^{-1}

sporulation was observed by other species of *Cercospora* genus (Uppala et al., 2019). Our results confirmed that the cultivation media and cultivation temperature have the direct influence on growth and sporulation of *C. beticola* isolates. The morphologic diversification of *C. beticola* isolates provide useful information for testing of inhibition effect of fungicide resistance on mycelial growth. Our results shoved, that the most appropriate media for growth rate estimation were TE and PDA at cultivation temperature 30 °C, followed by SBLEA and AWSBL at 25 °C.

Table 5 Comparison of origin of the *C. beticola* isolates with morphological characteristics and mycelial growth of isolates

Isolates[1] groups		Mycelium texture	Mycelium colour	Colour of mycelium border	Reverse of mycelium	Growth Rate[4] (mm.day^{-1})
N16, N17, N18	Nižná	C[2] (300[a])[3]	OGG (300[a])	DG (300[a])	B (300[a])	2.5[a5]
CH16, H17, CH18	Horné Chlebany	C (300[a])	OGG (300[a])	DG (300[a])	B (300[a])	2.3[a]
DS16, S17, DS18	Dolné Saliby	C (300[a])	OGG (300[a])	DG (288[a]) N (12)	B (300[a])	2.8[a]
ČS16, S17, ČS18	Senec	C (275[a]) CW (25)	OGG (282[a]) B (18)	DG (300[a])	B (300[a])	2.4[a]
NZ16, Z17, NZ18	Nové Zámky	C (300[a])	OGG (300[a])	DG (300[a])	B (300[a])	2.1[a]
M16, M17, M18	Mojmírovce	C (300[a])	OGG (291[a]) G (9)	DG (300[a])	B (300[a])	2.6[a]
O16, O17, O18	Oslany	C (300[a])	OGG (300[a])	DG (300[a])	B (300[a])	2.4[a]
B16, B17, B18	Bolešov	C (300[a])	OGG (300[a])	DG (300[a])	B (300[a])	2.3[a]
S16, S17, S18	Senica	C (300[a])	OGG (266[a]) OG (29), DG (5)	DG (300[a])	B (300[a])	2.8[a]
H16, H17, H18	Hronovce	C (300[a])	OGG (300[a])	DG (291[a]) N (9)	B (300[a])	2.5[a]

1 – each isolates group contains 10 isolates (e.g. the group N16 contains isolates from N16-1 to N16-10); 2 – C – cottony, CW – cottony wrinkled, OG – olive-green, OGG – olive-green-grey, G – grey, DG – dark grey, B – black, N – none; 3 – the number of isolates with showed parameter from total number of tested isolates (300); 4 – average growth rate of all tested isolates on 25th day after inoculation on PDA at cultivation temperature 25 °C; 5 – different letters indicate significant differences between values according to Fisher's LSD test ($p = 0.05$)

3.2 Inhibition effect of azoxystrobin + cyproconazole

The inhibition effect of azoxystrobin + cyproconazole on mycelial growth of *C. beticola* isolates is shown in the Table 6. The inhibition was significantly different among the tested isolates from different localities and years.

In year 2016, significantly the lowest inhibition of azoxystrobin + cyproconazole on C. isolates growth rate was measured by isolates originated from locality Hronovce, Mojmírovce, Dolné Saliby, Senec, and Nové Zámky. In year 2017, the lowest inhibition was measured by isolates originated from Nové Zámky, Hronovce, and Dolné Saliby locality. In the year 2018, the reduced inhibition effect was assessed by all the localities excluding Horné Chlebany. In three years average of inhibition effect, the lowest one was assessed in locality Hronovce and Nové Zámky, followed by localities with similar values (Mojmírovce, Senec, and Dolné Saliby). In the others localities, the average inhibition was very high, near or equal 100%.

The relationship between average (2016–2018) inhibition effect of azoxystrobin + cyproconazole on mycelial growth and pathogen growth rate on PDA cultivation medium is shown in Figure 2. According to presented data, there is no positive correlation between inhibition effect and mycelial growth rate. The inhibition effect of azoxystrobin + cyproconazole was the lowest in locality Hronovce, whereby the growth of *C. beticola* isolates originated from the same locality was one of the highest among the localities.

In case of the meaning of sugar beets in agricultural production, it is very important to monitor the occurrence of QoI-resistant isolates of *C. beticola* (Piszczek et al., 2018). Survey of pathogen sensitivity to fungicides is the most important aspect of the anti-resistance strategy (Karaoglanidis et al., 2003). Assessment of resistance to fungicides, based on the growth rate of isolates is considered as a good indicator (Karaoglanidis & Thanassoulopoulos, 2003). However, QoI resistance is developing in many sugar beet growing areas (Bolton et al., 2012). In this work, the occurrence of different sensitivity in *C. beticola* population to azoxystrobin + cyproconazole was confirmed. Our results showed reduced sensitivity of *C. beticola* population in Slovakia strictly depending of the locality. The most reduced sensitivity of the *C. beticola* isolates was measured on localities Hronovce and Nové Zámky. The results indicate that none of the isolates was moderately or completely resistant to the tested fungicide (Balandžić et al., 2020). These localities are areas with frequent growing of sugar beet crops, where the risk of resistance is expected. The reduced sensitivity is probably enhanced by frequent using of fungicides based on azoxystrobin + cyproconazole (information obtained from the farmers from these localities). The increasing occurrence of

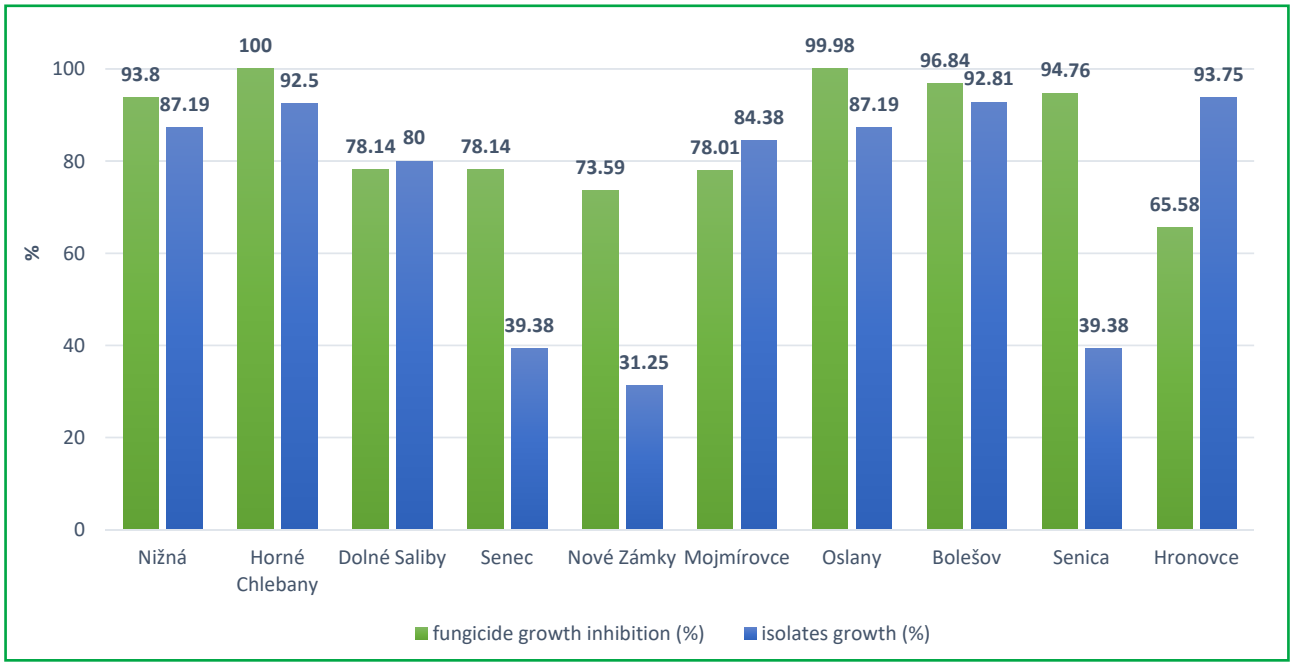

Figure 2 Relationship between inhibition of azoxystrobin + cyproconazole on *Cercospora beticola* isolates growth and the isolates growth on PDA medium

average inhibition (%) – the means of growth rate inhibition from years 2016–2018 by different ppm (210, 525 and 1050) of azoxystrobin + cyproconazole; Growth (%) – the means of growth rate of the tested *C. beticola* isolates (collected from years 2016–2018) expressed as a percentage of whole Petri dish (*P* = 80 mm) surface covered by mycelium on 14th day of cultivation

Table 6 Inhibition effect of azoxystrobin + cyproconazole on *Cercospora beticola* isolates growth rate in years 2016–2018

Localities	ppm	2016[2]	2017	2018	Average
Nižná	210	92.95[b1]	100.00 [c]	73.54 [b]	88.83 [b]
	525	100.00 [b]	100.00 [c]	84.13 [b]	94.71 [b]
	1050	100.00 [b]	100.00 [c]	93.54	97.85 [b]
	average	97.65 [b]	100.00 [c]	83.74 [b]	93.80 [b]
Horné Chlebany	210	100.00 [b]	100.00 [c]	100.00 [b]	100.00 [b]
	525	100.00 [b]	100.00 [c]	100.00 [b]	100.00 [b]
	1050	100.00 [b]	100.00 [c]	100.00 [b]	100.00 [b]
	average	100.00 [b]	100.00 [c]	100.00 [b]	100.00 [b]
Dolné Saliby	210	60.76 [a]	73.01 [c]	58.96 [a]	64.25 [a]
	525	77.99 [a]	78.69 [c]	93.79 [b]	83.49 [b]
	1050	77.18 [a]	86.35 [c]	96.50 [b]	86.68 [b]
	average	71.98 [a]	79.35 [c]	83.08 [b]	78.14 [ab]
Senec	210	65.34 [a]	85.03 [c]	41.58 [a]	63.99 [a]
	525	78.56 [a]	91.79 [c]	75.39 [b]	81.91 [b]
	1050	85.33 [ab]	97.30 [c]	82.95 [b]	88.53 [b]
	average	76.41 [a]	91.38 [c]	66.64 [ab]	78.14 [ab]
Nové Zámky	210	74.08 [a]	44.16 [a]	71.29 [b]	63.18 [a]
	525	82.12 [ab]	54.98 [a]	87.84 [b]	74.98 [ab]
	1050	86.16 [ab]	63.25 [ab]	98.45	82.62 [b]
	average	80.79 [ab]	54.13 [a]	85.86 [b]	73.59 [ab]
Mojmírovce	210	60.83 [a]	100.00 [c]	42.56 [a]	67.80 [a]
	525	66.59 [a]	100.00 [c]	75.02 [b]	80.53 [b]
	1050	72.99 [a]	100.00 [c]	84.13 [b]	85.71 [b]
	average	66.80 [a]	100.00 [c]	67.23 [ab]	78.01 [ab]
Oslany	210	100.00 [b]	100.00 [c]	99.85 [b]	99.95 [b]
	525	100.00 [b]	100.00 [c]	100.00 [b]	100.00 [b]
	1050	100.00 [b]	100.00 [c]	100.00 [b]	100.00 [b]
	average	100.00 [b]	100.00 [c]	99.95 [b]	99.98 [b]
Bolešov	210	100.00 [b]	100.00 [c]	86.36 [b]	95.45 [b]
	525	100.00 [b]	100.00 [c]	88.88 [b]	96.29 [b]
	1050	100.00 [b]	100.00 [c]	96.36 [b]	98.79 [b]
	average	100.00 [b]	100.00 [c]	90.53 [b]	96.84 [b]
Senica	210	100.00 [b]	95.74 [c]	64.46 [ab]	86.73 [b]
	525	100.00 [b]	98.69 [c]	95.33 [b]	98.01 [b]
	1050	100.00 [b]	100.00 [c]	98.65 [b]	99.55 [b]
	average	100.00 [b]	98.14 [c]	86.15 [b]	94.76 [b]
Hronovce	210	56.58 [a]	50.95 [a]	64.16 [ab]	57.23 [a]
	525	64.94 [a]	63.31 [ab]	75.29 [b]	67.84 [ab]
	1050	65.43 [a]	64.94 [ab]	84.61 [b]	71.66 [b]
	average	62.32 [a]	59.73 [a]	74.68 [b]	65.58 [ab]

1 – inhibition of the growth of mycelium (%); 2 – year of *C. beticola* isolation; different letters indicate significant differences between values according to Fisher's LSD test ($p \leq 0.05$)

C. beticola resistance to azoxystrobin reported Balandžić et al. (2020) too. Out results confirmed the presence of low sensitive *C. beticola* strains to active ingredient azoxystrobin + cyproconazole, which is in conformity with findings of Piszczek et al. (2018). Quinone outside inhibitors (QoI) fungicides, including azoxystrobin used in our study, are good combination partners with other fungicides to final formulation for in anti-resistant strategy against *Cercospora* leaf spot in the praxis (Karadimos & Karaoglanidis 2006). Despite the combination with other fungicides, there were recorded the occurrence of field resistance of *C. beticola* to QoI fungicides, but still now it is in low range (Piszczek et al., 2018). 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). Increasing of pathogen resistance to fungicide leads generally to decreased efficacy of a given fungicide. Therefore, the predominance of isolates resistant to QoIs in the field population can result in decreasing of yields (Budakov et al., 2014).

The relationship between average inhibition effect of azoxystrobin + cyproconazole on mycelial growth and pathogen growth rate on PDA cultivation medium was not confirmed. No positive correlation between inhibition effect and mycelial growth rate was observed.

4 Conclusions

Presented study showed the presence of different populations of *C. beticola* fungus in Slovakia. The *C. beticola* strains have market different texture and colour of mycelium. The results of morphological analysis of *C. beticola* isolates created important criteria for choosing of appropriate growth medium for testing of sensitivity to fungicides, especially in field of sporulation. Our results confirmed that the cultivation media and cultivation temperature have the direct influence on growth and sporulation of *C. beticola* isolates. According to this finfings, the most appropriate media for growth rate estimation were TE and PDA at cultivation temperature 30 °C, followed by SBLEA and AWSBL at 25 °C. In this work, the occurrence of different sensitivity in *C. beticola* population to azoxystrobin + cyproconazole was confirmed in Slovakia. The most reduced sensitivity of the *C. beticola* isolates was measured on localities Hronovce and Nové Zámky. These localities are areas with frequent growing of sugar beet crops, where the risk of resistance is expected. The reduced sensitivity is probably enhanced by frequent using of fungicides based on. For these localities, it is important to reduce the using of azoxystrobin + cyproconazole, to avoid the decreased efficacy in the field. The relationship between average inhibition effect of azoxystrobin + cyproconazole on mycelial growth and pathogen growth rate on PDA cultivation medium was not confirmed. The study shoved the need to continue in the monitoring of fungicidal resistance of *C. beticola* in Slovakia, concerning to anti-resistance strategy for the fungicides with high resistance potential.

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