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Influence of Selected Fungicides Registered in the Czech Republic for Winter Oilseed Rape on *In Vitro Sclerotinia sclerotiorum* Mycelial Growth

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Abstract

Poslušná J. Plachká E., Mazáková J. (2018): Influence of selected fungicides registered in the Czech Republic for winter oilseed rape on *in vitro Sclerotinia sclerotiorum* mycelial growth. Plant Protect. Sci., 54: 101–110.

The baseline sensitivity of 55 isolates of *Sclerotinia sclerotiorum,* collected from oilseed rape in 6 regions of the Czech Republic, to selected fungicides was determined during the period 2013–2015. One single-component fungicide – Horizon (tebuconazole), and four multicomponent fungicides – Pictor (boscalid, dimoxystrobin), Efilor (boscalid, metconazole), Prosaro 250 EC (prothioconazole, tebuconazole), and Propulse (fluopyram, prothioconazole), were chosen as these are commonly used locally. The effect of each fungicide on the *in vitro* pathogen radial mycelial growth and EC_{50} values for the respective fungicides were determined. The following MIC values were estimated; for the fungicides Horizon 250 EW, Efilor, and Propulse the mean MIC values ranged between 0.125 and 0.250 µl/ml, for Prosaro 250 EC ranged between 0.0625 and 0.125 µl/ml, and for Pictor ranged from 0.00781 to 0.01562 µl/ml. No strains of *S. sclerotiorum* resistant to the tested fungicides were detected and the growth of all isolates was fully inhibited at concentrations corresponding to their registered dose rates. The highest fungicidal efficacy on the collected *S. sclerotiorum* isolates was recorded for Pictor, followed by Prosaro 250 with an EC_{50} value 0.05856 µl/ml and then the remaining fungicides Propulse, Efilor, and Horizon 250 EW (EC_{50} values 0.07277, 0.07221, and 0.08519 µl/ml, respectively).

Keywords: *Brassica napus* L.; *Sclerotinia sclerotiorum*; efficacy; tebuconazole; boscalid; dimoxystrobin; metconazole; prothioconazole; fluopyram

Sclerotinia stem rot of *Brassica napus* L., caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is an important disease worldwide. This disease leads to significant losses in seed quantity and quality (Zhao *et al.* 2004). *Sclerotinia sclerotiorum* is one of the non-specific plant pathogens (Purdy 1979) and attacks over 360 plant species in 61 families (Boland & Hall 1994; Paul 2003). In the Czech Republic this disease attacks many agronomic field crops such as potatoes, oilseed rape, poppy, sunflower, and various vegetable crops (Spitzer *et al.* 2012). Sclerotinia stem rot is considered a local disease in the context of the years studied, but with a growing presence of hosts in crop rotations its harmfulness has been increasing (Bittner 2006). Annual yield losses from Sclerotinia stem rot account for 10–20%, but in years of heavy outbreak it can cause yield losses of 30–50%, though in some years it hardly appears at all (Kazda & Škeřík 2008).

At the present time, the situation in the Czech Republic is focused on a growing proportion of arable

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land sown with winter oilseed rape. In 2008 winter oilseed rape was grown on more than 300 000 ha, in 2012 it was on more than 400 000 ha. In the last two years the area under this crop has decreased to 350 000 ha and the average proportion of oilseed rape during the last 5 years has reached 15.75% (ČSÚ 2016), when the recommended proportion of oilseed rape in crop rotations is 12.5% with a 4–5 year interval in crop rotation (Fábry 2001). On many farms specialising in oilseed rape cultivation the proportion of oilseed rape in the crop rotation is much higher, approaching 25–30% (Spitzer *et al.* 2012); in the most extreme cases farmers grow 50% oilseed rape and 50% wheat (Fábry 2001). An increasing proportion of winter rape in crop rotations brings not only a heightened risk of higher incidences of fungal pathogens like *S. sclerotiorum* (Spitzer *et al.* 2012) but also of *Brassica* pests, which again leads to a higher risk of yield losses.

Protection of winter rape crops against *S. sclerotiorum* is currently provided mostly by applications of fungicides during oilseed rape flowering. Fungicides are applied in a period when no infection by the disease is visible in the stand (Spitzer *et al.* 2012). In connection with the increase in rape sown areas, the higher proportion of oilseed rape in crop rotations and the amount of pesticides sprayed onto oilseed rape stands it can be assumed that there exists a risk of developing resistant populations of the pathogen *S. sclerotiorum*. Recommended usage of lower doses of fungicides with a morph-regulation effect could also promote selection for resistance. Frequent use of synthetic fungicides leads to the development of a resistant population of the fungi (Lobato *et al*. 2010 in Dalilí 2015). According to the Fungicide Resistance Action Committee (FRAC 2013) *S. sclerotiorum* is one of the plant pathogens with a low risk of developing resistance to fungicides or that the risk is of minor commercial importance. Nevertheless, resistant isolates have been detected in many parts of the world. In France, the preventative chemical control of Sclerotinia stem rot at the beginning of flowering has led to the occurrence of resistance of *S. sclerotiorum* to benzimidazole fungicides (MBC). Resistance of *S. sclerotiorum* strains to carbendazim, which is used to be sprayed because of its effectiveness and its cheapness, is widespread in most oilseed rape cropping areas from central to north-eastern parts of France (Penaud *et al.* 2003) and also in China, where carbendazim has been used to control this fungal disease since the 1980s (Ma *et al.* 2009; Xu *et al.* 2015). Resistant populations of *S. sclerotiorum* against benomyl, which belongs to the same fungicidal class, have been detected in Canada (Gossen *et al.* 2001). Generally in China, which has an annual oilseed rape area of more than 7 million hectars (Zhou *et al.* 2014), a lot of resistant mutants of *S. sclerotiorum* have been found; strains resistant to fludioxonil (Kuang *et al.* 2011), dimethachlon (Zhou *et al.* 2014), and boscalid mutants (Wang *et al.* 2015) were mostly obtained under laboratory conditions.

In the Czech Republic screening tests of the growth dynamics of the pathogen *S. sclerotiorum* collected from caraway (*Carum carvi* L.) plants were performed on poisoned PDA plates in 2005 and 2006 by Odstrčilová (2007). Strains resistant to the tested fungicidal products were found and some strains were also able to produce sclerotia at a concentration level close to 0.1 µl/ml of fungicidal products like Rovral Pro, Topsin, Proline, Amistar, Capitan, and Pictor in descending order.

In this study, selected fungicides containing different active ingredients, single or multicomponent types, were tested. For the fungicide tebuconazole (Horizon 250 EW), a representative of DMIs (dimethylation inhibitors) belonging to the class evaluated with low risk of resistance development, fungicidal resistance has not yet been detected in oilseed rape. In China, Li *et al.* (2015) tested another triazole fungicide epoxiconazole, which proved its efficacy against carbendazim- and dimethachlon-resistant strains and was recommended for Sclerotinia stem rot management. The following multicomponent fungicides were tested as representatives of different fungicidal classes: Efilor and Pictor with boscalid (SDHI) plus dimoxystrobin (QoI-fungicides) and/or metconazole (DMI) and finally Prosaro 250 EC and Propulse with prothioconazole (DMI) plus tebuconazole (DMI) and/or fluopyram (SDHI class).

The objectives of this study were to (*i*) investigate the effect of different concentrations of several fungicides commonly used in the Czech Republic to control Sclerotinia stem rot in oilseed rape, on the mycelial growth of *S. sclerotiorum*; (*ii*) to determine the minimum inhibitory concentration (MIC) of the tested fungicides for the collected isolates of *S. sclerotiorum*; and (*iii*) to determine EC_{50} values for the tested fungicides.

MATERIAL AND METHODS

Sampling of sclerotia. Since 2013, pathogen sclerotia have been sampled from infected oilseed rape stems in different localities within the Czech Re-

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Table 1. *Sclerotinia sclerotiorum* isolates collected from different parts of the Czech Republic

No.	Isolate ID	Locality	Region	Sampling year
1	SS-CHC	Chlumec nad Cidlinou	Hradec Kralové	2014
2	SS-SV	Stará voda	Karlovy Vary	2014
3	SS-R	Roudnice	Hradec Kralové	2014
4	SS-LL	Lhota pod Libčany	Hradec Kralové	2014
5	SS-HK	Hradec Kralové	Hradec Kralové	2014
6	SS-TO	Třebechovice pod Orebem	Hradec Kralové	2014
7	SS-M	Moravičany	Olomouc	2014
8	SS-P	Praskačka	Hradec Kralové	2014
9	SS-VJ	Větrný Jeníkov	Vysočina	2014
10	SS-Č	Černuc	Central Bohemian	2014
11	SS-D	Dolany	Central Bohemian	2014
12	SS-H	Horoměřice	Central Bohemian	2014
13	SS-KM	Kamenný most	Central Bohemian	2014
14	SS-T	Tursko	Central Bohemian	2014
15	SS-NL	Unknown locality		2014
16	HN2012	Hněvčeves	Hradec Kralové	2012
17	HN2014a	Hněvčeves	Hradec Kralové	2014
18	HN2014b	Hněvčeves	Hradec Kralové	2014
19	HN2014c	Hněvčeves	Hradec Kralové	2014
20	CH2008	Chlumec nad Cidlinou	Hradec Kralové	2008
21	CH2012	Chlumec nad Cidlinou	Hradec Kralové	2012
22	CHL2014	Chlumec nad Cidlinou	Hradec Kralové	2014
23	KU2012	Kujavy	Moravian-Silesian	2012
24	KU2014	Kujavy	Moravian-Silesian	2014
25	KY2013/22	Opava-Kylešovice field 22	Moravian-Silesian	2013
26	KY2014/40	Opava-Kylešovice field 40	Moravian-Silesian	2014
27	KY2014/47	Opava-Komárov field 47	Moravian-Silesian	2014
28	Havl1_14	Šumperk-Havlas	Olomouc	2014
29	Havl2_14	Šumperk-Havlas	Olomouc	2014
30	Lit1_14	Litovel	Olomouc	2014
31	Lit2_14	Litovel	Olomouc	2014
32	Lit3_14	Litovel	Olomouc	2014
33	Louč1_14	Loučka	Olomouc	2014
34	Louc3_14	Loučka	Olomouc	2014
35	Libv2_14	Libiva	Olomouc	2014
36	Krch1_14	Krchleby	Olomouc	2014
37	Krch2_14	Krchleby	Olomouc	2014
38	Brez1_14	Břevenec	Olomouc	2014
39	Brez4_14	Břevenec	Olomouc	2014
40	Les1_14	Leština	Olomouc	2015
41	Rov1_14	Rovensko	Olomouc	2014
42	Rov2_14	Rovensko	Olomouc	2014
43	Pas1_14	Paseka	Olomouc	2014
44	Stern1_14	Šternberk	Olomouc	2014
45	Stern2_14	Šternberk	Olomouc	2014
46	Spk1_14	Šumperk-Temenice	Olomouc	2014
47	Libn1_14	Libina	Olomouc	2014
48	Kol1_14	Dolní Sukolom	Olomouc	2014
49	Rap1_14	Rapotín	Olomouc	2014
50	Vik1_2015	Vikýřovice	Olomouc	2015
51	Vik2_2015	Vikýřovice	Olomouc	2015
52	Rok1_15	Rokytnice u Přerova	Olomouc	2015
53	Rok2_15	Rokytnice u Přerova	Olomouc	2015
54	Spk1_15	Šumperk-Temenice	Olomouc	2015
55	Rap1_15	Rapotin	Olomouc	2015

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public (Table 1), from farmers' fields with winter rape stands and from experimental plots of several research facilities. The sclerotia were put into paper bags where the locality they were collected from was marked, and stored in the laboratory for testing under dry, dark conditions.

Fungal isolation. The sclerotia were firstly sterilised in a 10% solution of SAVO (sodium hypochlorite 1–5%, sodium hydroxide 0.1–1.0%; Unilever) for one minute and then rinsed three times with distilled water. The following procedures were carried out in sterile conditions. Each sclerotium was placed separately in the middle of a Petri dish (9 cm diameter) with pure PDA agar (HiMedia Laboratories, Mumbai, India) labelled with a description of the pathogen. The Petri dishes containing the sclerotia were incubated in a laboratory chamber under controlled conditions for mycelium growth, at a temperature of 25°C, in the dark, for 3–5 days.

Fungicides used. For the laboratory experiments fungicides registered in the Czech Republic for the control of Sclerotinia stem rot in oilseed rape were used. For this study 5 fungicides were selected: Horizon 250 EW (a.i. tebuconazole 250 g/l; Bayer CropScience AG, Monheim, Germany), Pictor (a.i. boscalid 200 g/l, dimoxystrobin 200 g/l; BASF SE, Ludwigshafen, Germany), Efilor (a.i. metconazole 60 g/l, boscalid 133 g/l; BASF AG, Ludwigshafen, Germany), Prosaro 250 EC (a.i. prothioconazole 125 g/l, tebuconazole 125 g/l; Bayer CropScience AG, Monheim, Germany), and Propulse (a.i. fluopyram 125 g/l, prothioconazole 125 g/l; Bayer S.A.S., Lyon, France), which are widely used for the Sclerotinia stem rot control.

Preparation of PDA and poisoned plates. Briefly, Potato dextrose agar (PDA) was prepared by suspending 39 g in 1000 ml of distilled water, then autoclaved at 121°C for 15 min and cooled to 50°C.

Pure PDA agar was used as a non-amended control. An amended PDA agar enriched with different concentrations of fungicides served as test concentration levels for determining minimum inhibitory concentrations of the selected fungicides.

In the first series of tests a higher scale of fungicide concentration levels was used in accordance with the registered dose rate for each fungicide listed in the Register of Plant Protection Products (ÚKZÚZ 2016) (Table 2) and recommended spray solutions used in local practice 150–400 l/ha.

The tested fungicides were diluted in DMSO (dimethyl sulfoxide; Lachner, Neratovice, Czech Republic) and added to PDA to give the following concentration levels: 16, 8, 4, and 2 µl/ml of PDA (Eckert *et al.* 2009) where 2 µl/ml corresponded to 80% of the registered dose rate of the fungicide Horizon 250 EW (and/or of Efilor, Propulse, and Prosaro 250 EC) and 400 l of water used as a spray solution per hectare. The concentration level 16 µl/ml of fungicide in PDA was very close to the double dose of Horizon 250 EW diluted in 150 l/ha of water, the dose rate used for phytotoxicity testing.

In the second series of tests, lower concentration levels applicable for MIC determination were used. The tested fungicide Horizon 250 EW (a.i. tebuconazole) was diluted in DMSO and added to PDA to give 1, 0.5, 0.25, and 0.1 µl/ml (second series).

In the third series, all the fungicides were diluted in DMSO and added to PDA to give 0.125, 0.0625, 0.03125, and 0.015625 µl/ml of PDA of each fungicide. For Pictor more concentrations were added, 0.00781 and 0.00391 µl/ml.

The content of active ingredients in µg/ml of agar differed only with regard to the active ingredient content of each fungicide. About thirty millilitres of the fungicide enriched agar was poured into Petri dishes (9 cm diameter).

Testing of sensitivity. Pure cultures of each isolate of *S. sclerotiorum* were incubated on pure PDA, and after 3–5 days plugs (5 mm diameter) were taken from the active growing margins of the pathogen colony. One plug was placed in the middle of each

Table 2. Registered dose rates of tested fungicides for the control of Sclerotinia stem rot in oilseed rape (l/ha) and the content of active ingredients in the spray volume 400 l/ha

Reference product	Registration number	Dose rate of product (l/ha)	Content of active ingredients (µg/ml)
Horizon 250 EW	3975-9	1.0–1.5	tebuconazole (625–937.5)
Pictor	4606-0	0.5	boscalid (250), dimoxystrobin (250)
Efilor	5093-0	1.0	metconazole (150), boscalid (332.5)
Propulse	4912-1	1.0	prothioconazole (312.5), tebuconazole (312.5)
Prosaro 250 EC	4561-2	0.75–1.0	fluopyram (234.7–312.5), prothioconazole (234.7–312.5)

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of the three plates representing one concentration level of each fungicide.

To assess the sensitivity level of the tested isolates of *S. sclerotiorum* the radial mycelial growth of the pathogen (cm) was continuously recorded for each plate until the mycelium in the non-intoxicated control fully covered the Petri dish (diameter 9 cm). The percentage of fungal growth inhibition was calculated according to the Pandey *et al.* (1982) formula:

Growth inhibition % = [(growth in the control – growth in the sample)/growth in the control] × 100

Statistical analysis. The data was statistically analysed using Statistica v10 (StatSoft Inc., 2010). The inhibition of radial mycelial growth was examined using analysis of variance (ANOVA). The MIC values were estimated for each fungicide showing the initial fungal growth inhibition higher than 50%. EC_{50} values were determined using linear regression of inhibition values on mycelium growth and the fungicide concentrations used.

RESULTS

In this study 55 isolates of the pathogen *Sclerotinia sclerotiorum* were collected from different localities within the Czech Republic; 29 isolates from the Olomouc region, 13 isolates from the Hradec Kralové region, 5 isolates from the Moravian-Silesian region, 5 isolates from the Central Bohemian region, 1 isolate from the Vysočina region, 1 isolate from the Karlovy Vary region, and one isolate from an unknown locality.

Efficacy of fungicides on in-vitro S. sclerotiorum mycelial growth and determination of MIC values. Pilot tests were carried out to check the efficacy of the selected fungicide Horizon 250 EW. The chosen concentration levels corresponded to the recalculated registered dose rate of Horizon 250 EW recommended for oilseed rape to control Sclerotinia stem rot and using the spray solution commonly used by Czech farmers in local practice. The efficacy of Horizon 250 EW was determined on 34 tested isolates of *S. sclerotiorum*, where all tested concentrations of fungicide (2, 4, 8, and 16 µl/ml) fully limited the growth of the pathogen mycelium. The results showed that even the lowest tested concentration level, corresponding to 80% of the registered dose rate for the tested fungicide Horizon 250 EW (1 l/ha) recalculated for 400 l of spray solution (2 µl/ml of Horizon 250 EW), fully controlled the growth of all tested isolates sampled from the selected localities.

Since this discovery we have continued testing with lower concentration levels of Horizon 250 EW to determine the lowest concentration limiting mycelium growth. It could be assumed that the same concentration levels used in tests on the following selected fungicides would show similar efficacy. This was proved in tests using Efilor and Pictor on small samples of *S. sclerotiorum* isolates, where the mycelial growth of all tested isolates was inhibited at all concentrations (2, 4, 8, and 16 µl/ml).

The binary concentration scale was increased twice to determine the concentration level at which the pathogen mycelial growth on fungicide-amended agar is limited/permitted.

Subsequent testing of the inhibitory effect of Horizon 250 EW at lower concentration levels (1, 0.5, 0.25, and 0.1 µl/ml) was carried out at 3 research facilities in Praha, Šumperk, and Opava. The results showed a similar inhibitory effect of the tested concentration levels of Horizon 250 EW on selected isolates originating from localities in the Czech Republic, with only 2 isolates showing lower sensitivity to the highest concentration level (1 µl/ml). However, more than 95% of tested isolates did not grow on this fungicide-amended agar. Finally, decreasing concentration levels were then used (0.125, 0.0625, 0.03125, and 0.015625 µl/ml) to reveal the concentration limits of Horizon 250 EW which permit the pathogen mycelium to grow. Even so, some isolates were still fully inhibited at 0.125 µl/ml (40% of isolates tested in Praha, and/or 15% of all tested isolates in this series), which shows a high variance of tested isolates in fungicide sensitivity. The means of inhibitory effect calculated for different concentration levels (0.125, 0.0625, 0.03125, and 0.015625 µl/ml) were 86.12, 50.22, 10.97, and 10.93%. Mean MIC values of tested isolates estimated for Horizon 250 EW ranged from 0.125 µl/ml to 0.25 µl/ml.

The highest efficacy was observed in the fungicide Pictor, where the inhibitory effect determined for 25 tested isolates for a concentration level of 0.01563 µl/ml ranged from 83.53% to 100%. The tests were repeated a second time with additional concentration levels (at 0.00781 and 0.00391 µl/ml) with selected isolates only. For a concentration level of 0.00781 µl/ml the inhibitory effect ranged from 74.71% to 100% and for an additionally tested concentration level 0.00391 µl/ml, it ranged from 51.57% to 100%. A high variation in the sensitivity of tested

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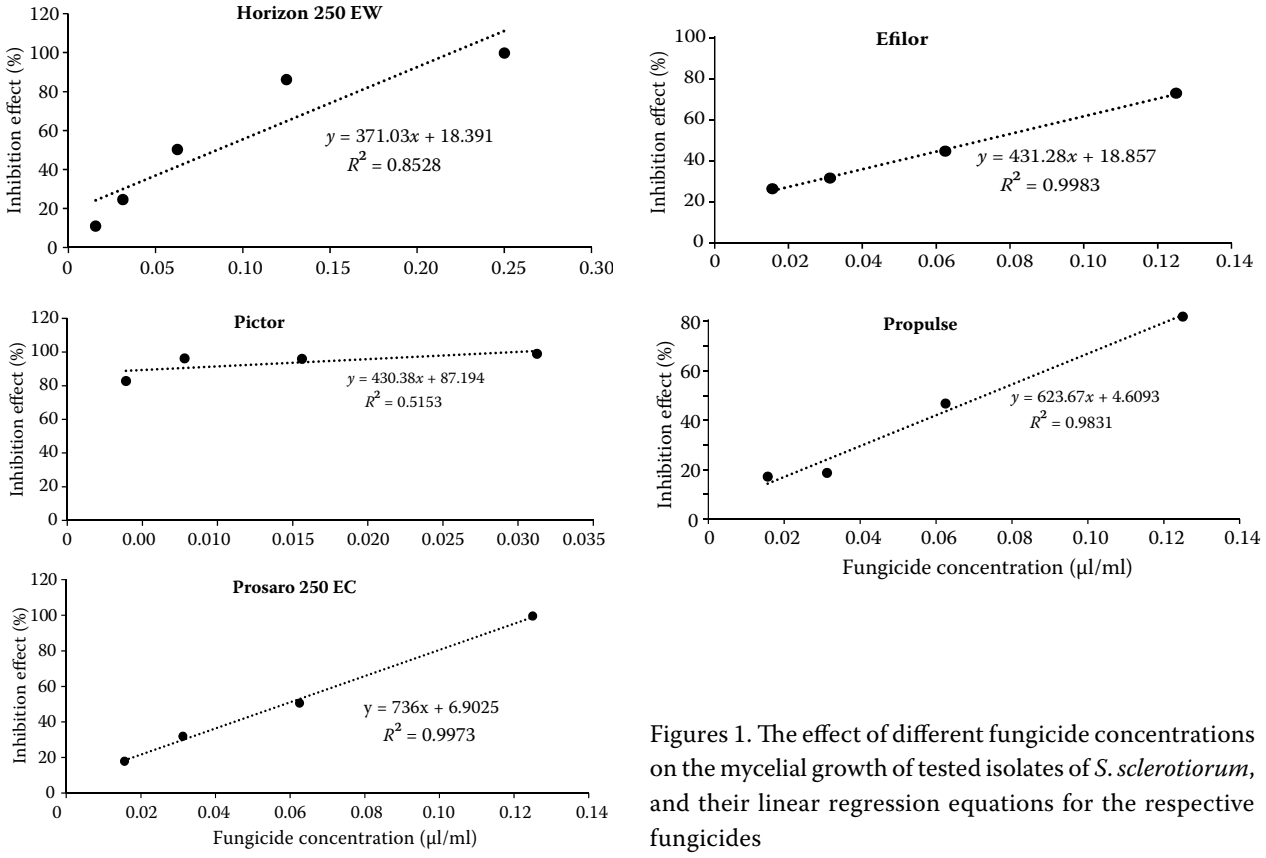
isolates of *S. sclerotiorum* was observed at all tested concentration levels. Two isolates were detected which were not fully inhibited at 0.125 µl/ml and another two isolates at 0.03125 µl/ml. The proportion of inhibited isolates decreased to a concentration level of 0.01563 µl/ml, where higher sensitivity was observed in isolates collected mostly from the Central Bohemian region of the Czech Republic. The respective mean inhibitory effects calculated for different concentration levels (0.125, 0.0625, 0.03125, 0.015625, 0.00781, and 0.00391 µl/ml) were 98.41, 100.00, 98.95, 95.96, 95.76, and 82.81%. Mean MIC values of the tested isolates estimated for the fungicide Pictor ranged from 0.01563 µl/ml to 0.00781 µl/ml.

The inhibitory effect of Efilor was determined for 25 isolates of *S. sclerotiorum*. Only 5 isolates were fully inhibited at 0.125 µl/ml; the rest showed a high variance in sensitivity, with an inhibitory effect ranging from 30.59% to 100%. However, the lowest individual inhibitory effect at the lowest concentration level of 0.01563 µl/ml was determined for one isolate originating from the Hradec Kralové region (isolate SS–R) at 3.73%. Gradual development in the variance of inhibitory effect observed at tested concentrations was contrary to that of Horizon 250 EW. The mean inhibitory effects calculated for different concentration levels (0.125, 0.0625, 0.03125, and 0.01563 µl/ml) were 73.22, 44.96, 31.75, and 26.58%, respectively. The estimated mean MIC values for Efilor ranged from 0.125 to 0.25 µl/ml.

The inhibitory effects of another two fungicides Propulse and Prosaro 250 EC were determined for 15 isolates. From this collection only 3 isolates were recorded with lower sensitivity to the inhibitory effect of Propulse (isolates SS-P, SS-LL, and SS-H) with 0.00, 2.35, and 3.25% inhibition. The means of inhibitory effect calculated for different concentration levels (0.125, 0.0625, 0.03125, and 0.01563 µl/ml) were 81.99, 46.73, 18.73, and 17.16%, respectively. The estimated mean MIC values for tested isolates ranged from 0.125 to 0.25 µl/ml.

In contrast, the fungicide Prosaro 250 EC fully inhibited growth in 14 out of 15 isolates at 0.125 µl/ml. At a lower concentration level 0.0625 µl/ml the results showed higher variance of inhibitory effect, ranging from 29.61% to 90.98%. In general, the inhibitory effect of the fungicide Prosaro 250 EC decreased gradually, the calculated means for different concentrations (0.125, 0.0625, 0.03125, and 0.015625 µl/ml)



Figures 1. The effect of different fungicide concentrations on the mycelial growth of tested isolates of *S. sclerotiorum*, and their linear regression equations for the respective fungicides

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Table 3. Inhibitory effect of selected fungicides (mean ± SE)

Concentration of fungicide (µl/ml)	Horizon 250 EW		Pictor		Efilor		Propulse		Prosaro 250 EC	
	tebuconazole (µg/ml)	inhibitory effect (%)	boscalid + dimoxystrobin (µg/ml)	inhibitory effect(%)	boscalid + metconazole (µg/ml)	inhibitory effect (%)	fluopyram + prothioconazole (µg/ml)	inhibition effect (%)	prothioconazole + tebuconazole (µg/ml)	inhibition effect (%)
1*	0.2500	99.62 ± 1.92								
0.5*	0.1250	99.54 ± 1.91								
0.25*	0.0625	99.82 ± 0.73								
0.1*	0.0250	73.52 ± 9.47								
0.125	0.0313	86.12 ± 8.59	0.0250 + 0.0250	98.41 ± 5.40	0.0166 + 0.0075	73.21 ± 22.50	0.0156 + 0.0156	84.98 ± 12.95	.0156 + 0.0156	99.59 ± 1.51
0.0625	0.0156	50.22 ± 14.69	0.0125 + 0.0125	100.00 ± 0.00	0.0083 + 0.0038	44.96 ± 21.59	0.0078 + 0.0078	46.73 ± 18.08	0.0078 + 0.0078	50.60 ± 17.35
0.03125	0.0078	24.55 ± 13.54	0.0063 + 0.0063	98.95 ± 4.06	0.0042 + 0.0019	31.74 ± 11.79	0.0039 + 0.0039	18.73 ± 9.37	0.0039 + 0.0039	31.94 ± 20.79
0.01563	0.0039	10.97 ± 11.86	0.0031 + 0.0031	95.96 ± 5.53	0.0021 + 0.0009	26.58 ± 10.51	0.0020 + 0.0020	17.15 ± 11.11	0.0020 + 0.0020	17.96 ± 9.46
0.00781	0.0020	10.93 ± 11.27	0.0016 + 0.0016	94.31 ± 8.32						
0.00781**			0.0016 + 0.0016	98.17 ± 2.69						
0.00391**			0.0008 + 0.0008	82.81 ± 13.36						

*first series of tests with higher concentrations with fungicide Horizon 250 EW; **second series of tests with the lowest concentrations with fungicide Pictor

being 99.59, 50.61, 31.95, and 17.96%, respectively. The estimated mean MIC values for tested isolates ranged from 0.0625 to 0.125 µl/ml.

When comparing all the tested fungicides, Pictor with the lowest mean MIC values (0.00781 to 0.01563 µl/ml) was clearly shown to have the highest inhibitory effect, followed by Prosaro 250 EC (mean MIC values 0.0625–0.125 µl/ml). The rest of the fungicides Propulse, Efilor, and Horizon 250 EW showed mean MIC values of 0.125–0.025 µl/ml. A brief overview of obtained results is documented in Table 3.

Determination of EC_{50} for the fungicides tested for the control of Sclerotinia sclerotiorum. After evaluation of the mycelial growth of the pathogen *S. sclerotiorum* in each Petri dish, a linear regression for each fungicide was established using concentrations and the inhibition values of mycelial growth (Figure 1).

For Horizon 250 EW the regression equation (y = 371.03x + 18.391) was determined and the fungicide concentration for 50% inhibition was calculated as 0.08519 µl/ml.

For Efilor the regression equation (y = 431.284x + 18.857) was determined and the fungicide concentration for 50% inhibition was calculated as 0.07221 µl/ml.

For Propulse the regression equation (y = 623.674x + 4.6093) was determined and the fungicide concentration for 50% inhibition was calculated as 0.07277 µl/ml.

For Prosaro 250 EC the regression equation (y = 736x + 6.9025) was determined and the fungicide concentration for 50% inhibition was calculated as 0.05856 µl/ml.

For Pictor the regression equation (y = 430.38x + 87.194) was calculated from tests at lower concentrations, but there was no clear correlation for the EC_{50} value due to a low coefficient of determination.

DISCUSSION

Winter oilseed rape is an important field crop in the Czech Republic with a sown area of around 350 000 ha (ČSÚ 2016). Sclerotinia stem rot caused by the polyphagous plant pathogen *Sclerotinia sclerotiorum* is one of the most serious fungal diseases (together with its related genera like *S. minor* and *S. homeocarpa*) affecting most field crops such as oilseed rape, sunflower, mustard, soya, and other legumes and various vegetable crops (Spitzer *et al.* 2012). Nowadays, when oilseed rape is the third most commonly planted crop (ČSÚ 2016) and the legume proportion

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in crop rotations has been increasing due to greening measures (EU 2013), we can expect an increase in the importance of Sclerotinia stem rot. Although its occurrence is mainly a year to year issue arising from climatic conditions of temperature and relative humidity (Caesar & Pearson 1983), this fungal disease causes economic losses of about 10–20% annually (Kazda & Škeřík 2008). The last year with significantly higher damage was in 2008, when 30–40% of infected oilseed rape plants were affected by primary infection from the soil (Šaroun & Říha 2008).

Sclerotinia stem rot management in the Czech Republic is based on the fungicidal treatment of oilseed rape during flowering. However, there are other methods used in the framework of integrated pest management (IPM) for controlling Sclerotinia stem rot. These include deep tillage, incorporation of infected postharvest residues, land selection, planting of resistant cultivars of oilseed rape or application of the biological fungicide Contans WG (containing spores of *Coniothyrium minitans* strain CON/M/91-08; Bayer S.A.S.). In particular, compliance with crop rotation rules is essential, with oilseed rape being sown in the same field only after a 4-year break (Poslušná & Plachká 2014).

In the Czech Republic 16 fungicides from different fungicidal classes are registered for the Sclerotinia stem rot control. On the Czech market 25 single-component fungicidal products prevail over their multicomponent rivals (14), despite the number of active ingredients contained in single-component products classed as DMI fungicides (tebuconazole, metconazole, prothioconazole) and QoI fungicides (azoxystrobin, picoxystrobin) only (ÚKZÚZ 2016).

From the list of DMI fungicides registered for the control of Sclerotinia stem rot (ÚKZÚZ 2016) there are 7 fungicides available (tebuconazole, metconazole, prothioconazole, cyproconazole, tetraconazole, propiconazole, and prochloraz) which are considered by FRAC (2016) to have a medium risk of resistance development due to the single-site MOA, although no *S. sclerotiorum* resistant strains have been detected yet (Dalili *et al.* 2015; Li *et al.* 2015). From the QoI fungicide class there are 3 fungicides available (azoxystrobin, picoxystrobin, and dimoxystrobin) considered to be a high risk. The fungicides from the SDHI group (boscalid, isopyrazam, and fluopyram) available on the Czech market are a more discussed topic, as these are considered to be of medium to high risk.

Although no reports of resistant strains of *S. sclerotiorum* detected in the Czech Republic have been published, anti-resistant strategies should still be followed. In common practice farmers use a lot of fungicides repeatedly (in autumn, in early spring, and again during flowering) to control other fungal diseases such as Phoma stem canker, Alternaria spot, etc. In order to delay the development of resistance and prolong the effective life of fungicides, FRAC has developed a series of fungicide resistance management guidelines, including e.g. not using a single fungicide exclusively, restricting the number of applications per season, using the fungicides with a different mode of action to control the same pathogen (Brent & Hollomon 2007).

In our study five commonly used fungicidal products were tested: the single-component fungicide Horizon 250 EW (tebuconazole) and 4 multicomponent fungicides Pictor, Efilor (boscalid and dimoxystrobin, and/or boscalid and metconazole), Propulse, and Prosaro 250 EC (prothioconazole and fluopyram, and/or prothioconazole and tebuconazole).

Our results showed that all the tested fungicides and/or final products at their registered dose rates were fully effective in inhibiting the pathogen mycelial growth on fungicide-amended PDA. No resistant strains of *S. sclerotiorum* were detected. Pictor (boscalid, dimoxystrobin) was the most effective fungicidal product, followed by Prosaro 250 EC (tebuconazole, prothioconazole). The precise order of other fungicides cannot be determined, because the other fungicides fell under same position. Although the data appeared to be different, no statistically significant differences were found in results between concentration levels (Tukey, $P = 0.95$). The results supported the widely held theory that the use of multicomponent fungicides probably precludes the selection of resistance by having a different mode of action.

In the present study, the baseline sensitivity to five different fungicidal products was established for 55 *S. sclerotiorum* isolates collected from 6 different regions of the Czech Republic. Screening tests of growth dynamics using decreasing concentration levels performed on fungicide-amended plates showed a wide range of sensitivity through the collection as well as occurrence of some resistant isolates sampled from oilseed rape stands. This confirmed the conclusions of Odstrčilová (2007), where some isolates collected from caraway moderately overcame the inhibitory effect of Pictor at a concentration level close to 0.1 µm/ml and could also produce sclerotia. It also confirms the results of Li *et al.* (2015), who established baseline sensitivity for *S. sclerotiorum*

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isolates collected from oilseed rape, soybean and sunflower to epoxiconazole, where the baseline sensitivity could differ within each host, even if they had similar shapes of frequency distributions of sensitivity to the tested fungicide.

The results would seem to have significant implications with regard to possible sensitivity changes or development of resistance to fungicides used as well as for future monitoring programs.

The obtained results could also be evaluated from an economic aspect. The inhibitory effect of the tested fungicides correlated with the fungicide price on the market. The single-component fungicide Horizon 250 EW is the cheapest tested product compared to Pictor, the most expensive tested fungicide. Fungicides such as Prosaro 250 EC, Propulse, and Efilor are cost comparable and can be purchased for half the price of Pictor. Our results from multi-annual field trials carried out on several localities in the Czech Republic showed that the efficacy of fungicides was influenced by several parameters such as occurrence of the pathogen in the oilseed rape stand, weather conditions during vegetation, locality, year, oilseed rape cultivar and especially date of application (Plachká & Poslušná 2015).

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