ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 63 122 Number 4, 2015
http://dx.doi.org/10.11118/actaun201563041111

SENSITIVITY OF *COLLETOTRICHUM ACUTATUM* ISOLATES TO SELECTED FUNGICIDES

Barbora Jílková[1], Jana Víchová[1], Radovan Pokorný[1], Karel Vejražka[2]

[1] Department of Crop Science, Breeding and Plant Medicine, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic
[2] Agricultural Research, Ltd. Troubsko, Zahradní 1, 664 41 Troubsko, Czech Republic

Abstract

JÍLKOVÁ BARBORA, VÍCHOVÁ JANA, POKORNÝ RADOVAN, VEJRAŽKA KAREL. 2015. Sensitivity of *Colletotrichum acutatum* Isolates to Selected Fungicides. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 63(4): 1111–1119.

Laboratory tests of six isolates of the pathogen *Colletotrichum acutatum* from different host plants demonstrated the varying sensitivity of pathogen with regard to mycelium growth and conidial germination after treatment with seven fungicides containing various active ingredients. None of the evaluated isolates was tolerant to the selected active ingredients in the fungicides. In tests of mycelium growth sensitivity, isolates from lupin and strawberry were most frequently identified as the most sensitive of all evaluated fungicides. The safflower isolate, on the other hand, most frequently exhibited the lowest reaction to fungicides. Differences in conidial germination of individual isolates were not detected in fungicides with the active ingredients dithianon, folpet and mancozeb, for which inhibition reached 100% in almost all isolates. The most significant differences in sensitivity among individual isolates were recorded in fungicides with the active ingredients azoxystrobin and metiram. In the case of the fungicide with active ingredient azoxystrobin, the highest inhibitory effect was achieved in the safflower isolate and the lowest in the white lupin isolate. After treatment with the fungicide with active ingredient metiram, the lowest germination rate was recorded in isolates from safflower and strawberry and the highest in isolates from hypericum and lupin.

Keywords: azoxystrobin, captan, dithianon, folpet, mancozeb, metiram, thiram, fungal pathogen

INTRODUCTION

Colletotrichum acutatum J. H. Simmonds 1968 is a fungal pathogen with ubiquitous distribution and a very broad range of plant hosts. This organism infects fruit plants, vegetables, ornamental plants, oil crops, fodder crops, conifers and weeds (Freeman *et al*., 2001; Mari *et al*., 2012; Peres *et al*., 2008; Sreenivasaprasad and Talhinhans, 2005). Incidence of *C. acutatum* on strawberry, lupin, safflower, cherry, tomato, apple trees, and gooseberry has been confirmed in the Czech Republic (Novotný *et al*., 2006; Víchová *et al*., 2011, 2012, 2013).

C. acutatum causes economically significant losses especially in strawberry (Howard *et al*., 1992), while marked damages in quality and quantity of yield are recorded also on other host crops (Agostini *et al*., 1992; Kim *et al*., 2008). In the Czech Republic, this pathogen is among the most serious pathogens of safflower in terms of its economic impact, causing losses of up to 100% (Víchová *et al*., 2011). Staňková *et al.* (2011) have demonstrated, however, that isolates of the pathogen may also infect host plants other than those from which they were isolated, thus increasing the risk of the pathogen's spread.

Effective defence against *C. acutatum* is based on appropriate agronomic measures, resistant varieties, and direct chemical or biological protection (Wharton and Diéguez-Uribeondo, 2004). In the Czech Republic, only one fungicide against *C. acutatum* is authorized. With the commercial name *Ortiva* (active ingredient azoxystrobin), it is registered exclusively for strawberry. Given the wide range of hosts, this level of chemical protection availability is insufficient. In countries where incidence of *C. acutatum* has longer been established, a number of fungicides with such active ingredients as captan, thiram, benomyl, propiconazole, cyprodinil, azoxystrobin and pyraclostrobin are

authorized (MacKenzie and Peres, 2012; Peres *et al*., 2010). Other active ingredients are also being sought, because strains of pathogen with tolerance for fungicide preparations long in use can be selected within the pathogen population. The tolerance of pathogen to certain fungicide preparations from the benzimidazole group has been demonstrated (Hwang *et al*., 2010; Kim *et al*., 2007; Peres *et al*., 2004). In the case of benomyl, tests have shown the pathogen to be insensitive to this active ingredient (Goes and Kimati, 1998).

The objective of our study was to test the effectiveness of a selected range of fungicides against several isolates of *C. acutatum* under laboratory conditions, which could subsequently be verified in the field.

MATERIALS AND METHODS

Isolates and Cultivation

Seven different single-component fungicides were used for testing (Tab. I). These were selected on the basis of preliminary results of *in vitro* tests at Agricultural Research, Ltd. Troubsko.

I: *Fungicides used in testing – basic concentration 1:1*

Active ingredient	Basic concentration
azoxystrobin	0.1 mg.ml^{-1}
captan	1.2 mg.ml^{-1}
mancozeb	0.32 mg.ml^{-1}
thiram	0.32 mg.ml^{-1}
dithianon	0.07 mg.ml^{-1}
folpet	0.32 mg.ml^{-1}
metiram	0.315 mg.ml^{-1}

The effectiveness of the selected fungicides was tested under laboratory conditions on six isolates (Tab. II). Monosporic isolates of the pathogen were cultivated on potato dextrose agar (PDA) at a temperature of 25 ± 2 °C.

Mycelium Growth Sensitivity Test

Fungicides were diluted using sterile distilled water. Three concentrations were prepared from each preparation: basic 1:1 (i.e. concentration recommended by the manufacturer; ratio of fungicide to amount of water applied to a unit of treated area), 1:5 and 1:10. For testing, the requisite amounts of mycelium and orange conidial mass were collected from the pathogen isolates and suspensions were prepared in sterile distilled water and the suspension was homogenized. The suspension was streaked cross-wise using a sterile inoculation loop onto Petri dishes 10 cm in diameter containing PDA. Four sterile filter paper discs 8 mm in diameter were placed into each dish, one in each quarter. With a pipette, 12.5 µl of fungicide in the given concentration was applied to three of the discs, each disc represents one replication. The fourth disc was used as a control, to which 12.5 µl of sterile distilled water was applied. The Petri dishes were placed in a cultivation room and held at a temperature of 25 ± 2 °C under a 12-hour light-dark alternating cycle. Inhibition zones in the vicinity of the discs were measured 4 and 7 day (d) after the start date of experiment.

Results as to the effectiveness of individual fungicides on individual isolates were statistically processed in the UNISTAT program by one way ANOVA (factor: isolate) and subsequently using Tukey's HSD test. Level of significance was $\alpha = 0.05$.

Conidial Germination Test

The methodology was based on the procedure of Kloutvorová and Kupková (2009) and was adjusted according to actual conditions. Fungicides were diluted in potato dextrose broth (PDB), and twofold concentration in comparison with basic concentration used in mycelium growth sensitivity test was prepared. Using an inoculation loop, we took from the orange conidial masses of the individual isolates such amount so that 25–35 conidia were visible in the microscopic field under 400× magnification using an Olympus CX41 microscope. Suspensions of the individual isolates in PDB were prepared. Into each well of a 3-well microscope slide there were pipetted 10 µl of fungicide and 10 µl of the conidia suspension, and thus the resulting concentration of the fungicide returned to the basic concentration. Each well was evaluated as a single replication. A single-well microscope slide was considered as the control, with 10 µl of PDB and 10 µl of the conidia suspension pipetted into the well. The microscope slides were

II: *Isolates of C. acutatum and their origin*

Isolate	Host plant	Supplier
CBS 786.86	*Malus domestica* Borkh.	CBS
PCF 231	*Fragaria × ananassa* Duch.	PCFruit
PCF 437	*Lupinus albus* L.	PCFruit
710	*Hypericum perforatum* L.	DCSBPM MENDELU
1109	*Carthamus tinctorius* L.	DCSBPM MENDELU
1209	*Carthamus tinctorius* L.	DCSBPM MENDELU

Note: CBS – Fungal Biodiversity Centre; PCFruit – Proefcentrum Fruitteelt vzw; DCSBPM MENDELU – Department of Crop Science, Breeding and Plant Medicine, Mendel University in Brno

placed individually into sterile Petri dishes 10 cm in diameter, with filter paper sufficiently soaked with sterile distilled water placed on the bottom to prevent dehydration of the experimental material. The dishes were cultivated at a temperature of 25 ± 2 °C under a 12-hour light-dark alternating cycle. After 48 h from the start of the experiment, using an Olympus CX41 microscope under 400× magnification, we counted the number of germinating conidia in a randomly selected field of view in the total set of 150 conidia in each tested fungicide preparation and respective control.

The results were statistically processed using single-factor ANOVA (factor: isolate) and subsequently using Tukey's HSD test in the UPAV program. The level of significance was established at $\alpha = 0.05$.

RESULTS

Mycelium Growth Sensitivity Test

Fungicide with Active Ingredient Azoxystrobin

In the first measurement, the width of the inhibition zones decreased due to decreasing concentration of the fungicide. Exceptions were the isolates 1109, CBS 786.86 and PCF 231, for which identical values were recorded between concentrations (Tab. III). In the strongest concentration (1:1), differences were confirmed between isolate PCF 231 and the other isolates. Differences between the isolates, however, were not recorded at concentrations of 1:5 and 1:10.

In the second measurement, the average widths of inhibition zones decreased with decreasing concentration of the fungicide only in the variant with isolate PCF 437 (Tab. III). Differences were detected between isolates in all tested concentrations. At concentration 1:1, there were differences between isolate 1209, isolate 1109 and isolate PCF 231. At concentrations 1:5 and 1:10, no inhibition zones were measured in isolates 1209 and 710. Such occurrence was not recorded in any of the remaining isolates.

After evaluating the average inhibition zones of isolates at all three concentrations and both measurements, we concluded that isolate 1209 was the least sensitive to azoxystrobin, while isolate PCF 231 was the most sensitive.

Fungicide with Active Ingredient Captan

In the first measurement, the average widths of inhibition zones for individual isolates decreased with decreasing concentration of the fungicide preparation (Tab. IV). It was confirmed that the isolates responded differently.

III: *Average inhibition zones in cm for pathogen isolates in the case of fungicide with active ingredient azoxystrobin*

	Concentration					
	1st measurement			2nd measurement		
Isolate	1:1	1:5	1:10	1:1	1:5	1:10
1109	0.80 ^{A}	0.80 ^{A}	0.60 ^{A}	0.80 ^{B}	0.80 ^{B}	0.27 ^{AB}
1209	0.83 ^{A}	0.80 ^{A}	0.53 ^{A}	0.17 ^{A}	0.00 ^{A}	0.00 ^{A}
710	0.87 ^{A}	0.53 ^{A}	0.47 ^{A}	0.70 ^{AB}	0.00 ^{A}	0.00 ^{A}
CBS 786.86	0.80 ^{A}	0.80 ^{A}	0.80 ^{A}	0.67 ^{AB}	0.80 ^{B}	0.57 ^{AB}
PCF 231	1.70 ^{B}	0.80 ^{A}	0.80 ^{A}	1.67 ^{C}	0.80 ^{B}	0.80 ^{C}
PCF 437	1.17 ^{A}	0.93 ^{A}	0.87 ^{A}	1.10 ^{BC}	0.83 ^{B}	0.57 ^{AB}
M. S.	0.383	0.052	0.082	0.753	0.523	0.328

Note: M. S. – mean squared; statistically significant differences with level of significance of $\alpha = 0.05$ are marked with different upper-case letters

IV: *Average inhibition zones in cm for pathogen isolates in the case of fungicide with active ingredient captan*

	Concentration					
	1st measurement			2nd measurement		
Isolate	1:1	1:5	1:10	1:1	1:5	1:10
1109	2.10 ^{A}	2.07 ^{AB}	1.67 ^{AB}	2.03 ^{A}	1.83 ^{A}	1.60 ^{AB}
1209	2.87 ^{B}	2.70 ^{B}	2.33 ^{C}	2.83 ^{B}	2.67 ^{B}	2.33 ^{C}
710	2.20 ^{A}	1.87 ^{A}	1.43 ^{A}	2.00 ^{A}	1.77 ^{A}	1.27 ^{A}
CBS 786.86	1.93 ^{A}	1.73 ^{A}	1.53 ^{AB}	1.87 ^{A}	1.60 ^{A}	1.20 ^{A}
PCF 231	2.27 ^{AB}	2.17 ^{AB}	2.07 ^{BC}	2.00 ^{A}	2.00 ^{AB}	2.00 ^{BC}
PCF 437	2.10 ^{A}	2.03 ^{AB}	1.63 ^{AB}	1.83 ^{A}	1.63 ^{A}	1.37 ^{A}
M. S.	0.317	0.335	0.362	0.413	0.468	0.611

Note: See Tab. III

Differences were also demonstrated between isolates in the second measurement. Identical widths of inhibition zones were measured in all concentrations of PCF 231 isolate (Tab. IV).

Isolate 1209 exhibited the highest sensitivity to captan in both measurements and at all tested concentrations. Isolates 710 and CBS 786.86 were the least sensitive to the fungicide.

Fungicide with Active Ingredient Dithianon

No differences between isolates were confirmed in either of the two measurements. Concentration showed no effect on width of inhibition zones in isolates CBS 786.86 and 1209 (Tab. V). Isolate CBS 786.86 showed identical average inhibition zones (0.80 cm) at all three concentrations in both measurements. Isolate 1209 had identical values of average inhibition zones at concentrations 1:5 and 1:10 (i.e., 0.93 cm in the first measurement and 0.87 cm in the second).

Fungicide with Active Ingredient Folpet

Significant differences were recorded between isolates, with the exception of the 1:5 concentration in the first measurement (Tab. VI).

In the variant with isolate 710, concentration showed no effect on the width of inhibition zones. In the first measurement this isolate reached

V: *Average inhibition zones in cm for pathogen isolates in the case of fungicide with active ingredient dithianon*

	Concentration					
	1st measurement			2nd measurement		
Isolate	1:1	1:5	1:10	1:1	1:5	1:10
1109	1.23 A	0.97 A	0.90 A	1.20 A	0.93 A	0.80 A
1209	1.00 A	0.93 A	0.93 A	1.00 A	0.87 A	0.87 A
710	1.47 A	1.17 A	0.93 A	1.20 A	1.10 A	0.90 A
CBS 786.86	0.80 A	0.83 A	0.80 A	0.80 A	0.80 A	0.80 A
PCF 231	1.27 A	1.03 A	1.00 A	1.23 A	0.97 A	0.90 A
PCF 437	1.17 A	1.07 A	0.83 A	1.10 A	1.00 A	0.73 A
M. S.	0.160	0.040	0.016	0.082	0.033	0.013

Note: See Tab. III

VI: *Average inhibition zones in cm for pathogen isolates in the case of fungicide with active ingredient folpet*

	Concentration					
	1st measurement			2nd measurement		
Isolate	1:1	1:5	1:10	1:1	1:5	1:10
1109	1.83 AB	1.80 A	1.47 A	1.83 AB	1.73 A	1.53 AB
1209	2.20 BC	2.10 A	1.77 AB	1.98 B	1.87 AB	1.70 AB
710	1.63 A	1.70 A	1.50 A	1.47 A	1.60 A	1.33 A
CBS 786.86	1.87 AB	1.83 A	1.67 AB	1.77 AB	1.73 A	1.43 A
PCF 231	2.23 BC	1.97 A	1.73 AB	2.08 BC	1.77 AB	1.73 AB
PCF 437	2.43 C	2.13 A	1.97 C	2.43 C	2.13 B	1.90 B
M. S.	0.273	0.090	0.102	0.318	0.099	0.133

Note: See Tab. III

VII: *Average inhibition zones in cm for pathogen isolates in the case of fungicide with active ingredient mancozeb*

	Concentration					
	1st measurement			2nd measurement		
Isolate	1:1	1:5	1:10	1:1	1:5	1:10
1109	2.30 A	1.23 A	0.90 AB	2.20 A	1.20 A	0.80 A
1209	2.37 A	1.30 A	0.62 A	1.87 A	1.17 A	0.83 A
710	2.67 A	1.63 AB	1.20 ABC	2.27 A	1.37 AB	0.97 AB
CBS 786.86	2.08 A	2.17 AB	1.87 BC	1.90 A	1.80 BC	1.63 BC
PCF 231	2.27 A	1.57 AB	1.20 ABC	1.67 A	1.37 AB	1.07 AB
PCF 437	4.10 B	2.37 B	2.17 C	4.00 B	2.33 C	2.17 C
M. S.	1.663	0.637	1.029	2.189	0.607	0.886

Note: See Tab. III

similar values in concentrations 1:1 and 1:5, while in the second measurement the 1:1 concentration showed a lower average inhibition zone than did the 1:5 concentration.

Overall, the highest sensitivity to the fungicide was detected in isolate PCF 437, while the lowest sensitivity was exhibited in isolate 710.

Fungicide with Active Ingredient Mancozeb

The average widths of the inhibition zones of isolates decreased with decreasing concentrations of the fungicide preparation in both the first and second measurements (Tab. VII). Differences were established between the isolates. Isolate PCF 437 differed from the remaining isolates and was determined to be the most sensitive to the tested fungicide as measured by width of inhibition zone. This fungicide had a relatively low effect especially on isolate 1209.

Fungicide with Active Ingredient Metiram

In the first measurement for isolate 1109, average inhibition zone width was identical at 0.97 cm for concentrations 1:1 and 1:5 (Tab. VIII). A similar case was recorded in the case of isolate CBS 786.86, for which the width was identical (1.23 cm) for concentrations 1:5 and 1:10. Isolate PCF 437 demonstrated similar sensitivity at concentrations 1:5 and 1:10. In a statistical evaluation, differences between isolates were detected only for concentration 1:1, at which isolate PCF 231 was significantly more sensitive.

In the second measurement, it was determined that the average inhibition zones of isolates 1109, 710 and PCF 437 at concentration 1:10 were of zero width (Tab. VIII). Differences between isolates were determined at all concentrations.

Isolate 1109 proved least sensitive to the fungicide, while the highest sensitivity was detected in isolate PCF 231.

Fungicide with Active Ingredient Thiram

In the first measurement, significant differences were determined between isolates at all evaluated concentrations. At concentrations 1:1 and 1:5, isolate 1109 showed almost identical inhibition zone widths while those of isolate 710 were exactly the same (Tab. IX).

In the second measurement, significant differences between isolates were detected at all concentrations. An identical average inhibition zone was detected for isolate CBS 786.86 at concentrations 1:5 and 1:10 (Tab. IX).

Overall, isolate 1209 exhibited the lowest sensitivity to the fungicide with active ingredient thiram. This fungicide preparation was the most effective on isolates PCF 437 and PCF 231.

VIII: *Average inhibition zones in cm for pathogen isolates in the case of fungicide with active ingredient metiram*

	Concentration					
	1st measurement			2nd measurement		
Isolate	1:1	1:5	1:10	1:1	1:5	1:10
1109	0.97 A	0.97 A	0.80 A	0.93 A	0.53 A	0.00 A
1209	1.60 A	1.13 A	0.83 A	1.43 A	0.97 AB	0.80 B
710	1.60 A	1.07 A	0.80 A	1.33 A	1.03 AB	0.00 A
CBS 786.86	1.73 A	1.23 A	1.23 A	1.53 A	1.27 AB	1.00 B
PCF 231	3.40 B	1.83 A	1.40 A	3.20 B	1.27 AB	0.53 B
PCF 437	1.73 A	1.50 A	1.53 A	1.33 A	1.30 B	0.00 A
M. S.	2.001	0.313	0.328	1.905	0.258	0.610

Note: See Tab. III

IX: *Average inhibition zones in cm for pathogen isolates in the case of fungicide with active ingredient thiram*

	Concentration					
	1st measurement			2nd measurement		
Isolate	1:1	1:5	1:10	1:1	1:5	1:10
1109	2.43 AB	2.47 B	1.80 AB	2.00 AB	1.63 B	1.13 AB
1209	1.60 A	1.40 A	1.00 A	1.13 A	0.77 A	0.80 A
710	3.00 B	3.00 B	2.77 C	3.00 C	1.70 B	1.60 BC
CBS 786.86	3.53 B	2.80 B	2.60 BC	2.67 BC	1.60 B	1.60 BC
PCF 231	3.53 B	3.13 B	2.60 BC	2.93 BC	2.13 B	1.57 BC
PCF 437	3.40 B	2.23 AB	1.93 BC	3.40 C	2.13 B	1.80 C
M. S.	1.781	1.214	1.362	2.034	0.751	0.418

Note: See Tab. III

X: *Inhibition of conidial germination rates of isolates for individual active ingredients of fungicides in %*

	Active ingredient						
Isolate	**azoxystrobin**	**captan**	**dithianon**	**folpet**	**mancozeb**	**metiram**	**thiram**
CBS 786.86	28.37 ^{AB}	100.00 ^{A}	100.00 ^{A}	100.00 ^{A}	99.80 ^{A}	55.03 ^{AB}	96.40 ^{B}
PCF 231	25.63 ^{AB}	100.00 ^{A}	100.00 ^{A}	100.00 ^{A}	99.83 ^{A}	61.84 ^{A}	100.00 ^{A}
PCF 437	22.97 ^{B}	100.00 ^{A}	98.43 ^{A}	100.00 ^{A}	100.00 ^{A}	20.13 ^{C}	100.00 ^{A}
710	36.10 ^{AB}	98.53 ^{B}	96.77 ^{A}	99.20 ^{A}	100.00 ^{A}	16.90 ^{C}	95.70 ^{B}
1109	42.07 ^{A}	100.00 ^{A}	97.87 ^{A}	100.00 ^{A}	99.87 ^{A}	29.37 ^{BC}	96.60 ^{AB}
1209	29.27 ^{AB}	100.00 ^{A}	96.50 ^{A}	100.00 ^{A}	100.00 ^{A}	76.73 ^{A}	100.00 ^{A}

Statistically significant differences with level of significance $\alpha = 0.05$ are marked with different capital letters

Conidial Germination Test

All evaluated active ingredients in fungicides demonstrated an inhibitory effect on the conidial germination of pathogen (Tab. X). No differences in the conidial germination rates of the isolates were detected for fungicides with active ingredients dithianon, folpet and mancozeb. More significant differences between individual isolates were recorded for fungicides with active ingredients azoxystrobin and metiram. The fungicide with active ingredient azoxystrobin demonstrated the greatest inhibitory effect in isolate 1109 and the lowest inhibition in isolate PCF 437. The lowest conidial germination rates for the fungicide with active ingredient metiram was recorded in isolate 1209 and isolate PCF 231, while isolates 710 and PCF 437 had the highest germination rates.

DISCUSSION

In our experiments, a comparison of the first and second measurements of isolate mycelia growth sensitivity at concentration 1:1 clearly showed that the average inhibition zone widths decreased over the course of 3 d. The only exceptions were the isolates 1109 and PCF 437 after treatment with the fungicide with active ingredient folpet and isolates 710 and PCF 437 in the case of the fungicide preparation with active ingredient thiram, and for which the average inhibition zone widths remained identical also in the second measurement.

The varying sensitivity of fungal pathogen isolates to the active ingredients of fungicides can affect their survival and spread to host plants even when chemical protection is used. Isolate 1209 from safflower exhibited a different response to the individual active ingredients of fungicides within the set of tested isolates. Of all isolates tested, this isolate was the least sensitive to preparations with active ingredients azoxystrobin, mancozeb and thiram, but the most sensitive to the effect of the fungicide preparation with active ingredient captan.

In general, in terms of sensitivity of mycelium growth the isolates PCF 437 from lupin and PCF 231 from strawberry can be considered the most sensitive isolates from the group of tested isolates at concentration 1:1. Isolate PCF 437 demonstrated the greatest mycelium growth inhibition for the fungicides with active ingredients folpet, mancozeb and thiram. Isolate PCF 231 was the most sensitive after treatment with the fungicides with active ingredients azoxystrobin and metiram. Of all isolates tested, isolate 1209, on the other hand, most often exhibited the lowest reaction at this concentration with fungicide preparations with active ingredients azoxystrobin and thiram. We also tested in our laboratory the sensitivity of the same six isolates to dual-component fungicides. Novotná *et al.* (2011) determined that fungicides with active ingredient combinations of folpet–azoxystrobin, mancozeb–cymoxanil and mancozeb–metalaxyl-M had the highest inhibitory effect on mycelium growth of isolate PCF 437. This also corresponds with our finding for single-component fungicides containing active ingredients folpet and mancozeb. Isolate 1209 was determined to be the least sensitive for fungicide preparation with active ingredients folpet–azoxystrobin, isolate 1109 for fungicide with active ingredients mancozeb–cymoxanil, and isolates PCF 231 and 710 for fungicide with active ingredients mancozeb–metalaxyl-M. Apart from isolate 1209, which in our tests was also the least sensitive to the single-component fungicide preparation with active ingredient azoxystrobin, the above mentioned results differed from ours. In our tests, isolate 1209 was the most sensitive in the case of single-component fungicide with active ingredient mancozeb. Moreover, Novotná *et al.* (2011) demonstrated in her tests that the additional component azoxystrobin markedly increased the effectiveness of the fungicide with active ingredients folpet–azoxystrobin, but components added to the basic active ingredient mancozeb did not increase the effectiveness of the tested fungicides.

None of our tested pathogen isolates was tolerant to the selected fungicides. Other authors, have examined fungicide protection against *C. acutatum*, too. Kloutvorová and Kupková (2009) evaluated mycelium growth and conidial germination under laboratory conditions. Those authors determined that the active ingredient azoxystrobin had a significantly lesser effect on *C. acutatum* mycelium growth than did other active ingredients, while the greatest inhibition of mycelium growth

occurred on agar with the addition of a fungicide with the active ingredient thiram – whereby average colonies were reduced by almost 87.2% in comparison with a control sample. Those authors had confirmed a good inhibitory effect on pathogen conidial germination for fungicide with active ingredient azoxystrobin but recorded complete suppression of conidial germination for fungicides containing active ingredients captan and thiram. Peres *et al.* (2010) noted that under field conditions a fungicide with active ingredient captan had the highest effect in the early stages of infection (application within 8 h after artificial inoculation) and with shorter period of plant wetting. In the case of active ingredient mancozeb, Goes *et al.* (2008) determined that if orange tree buds were preventively treated only with a fungicide with this active ingredient then protection against *C. acutatum* was not ensured, but if this was followed by spraying with a fungicide containing the active ingredient folpet or carbendazim the number of symptomatic flowers was markedly reduced. The active ingredient mancozeb also exhibited effectiveness against other pathogens from genus *Colletotrichum*, such as *C. capsici* (Shukla *et al.*, 2010) and *C. dematium* (Machowicz-Stefaniak and Zalewska, 2011). The knowledge of the reaction of different *Colletotrichum acutatum* to treatment with fungicides in necessary from the possible isolate resistance point of view. This resistance is known in several species of genus *Colletotrichum*. Xu *et al.* (2014) founded some *C. gloeosporoides* isolates with lower sensitivity to fungicide active ingredients tebuconazole and prochloraz and Zhang *et al.* (2013) isolates resistant to carbendazim. Some isolates of *C. cereale* were resistant to thiophanate-methyl (Young *et al.*, 2010) and benzimidazole (Wong *et al.*, 2008).

Efficacy of fungicides is usually tested in field tests. Laboratory tests with different pathogen isolates may be used only for preliminary determinations. On the other hand, these tests are necessary to exclude fungicides with no efficacy on particular isolates of pathogen. It is also appropriate to perform artificial inoculations using more isolates of the respective pathogen during field testing in order to avoid the risk of a fungicide decreasing effectiveness of preparation due to the incidence of a more tolerant population of the pathogen. In this way the risk of environment damage with pesticides can be decreased.

CONCLUSION

The sensitivity of *Colletotrichum acutatum* isolates was tested in *in vitro* laboratory experiments. Seven different single-component fungicides were used for testing and their effectiveness was tested on six isolates. Fungicides were diluted using sterile distilled water. Three concentrations were prepared from each preparation (1:1, 1:5 and 1:10). The suspension of particular *C. acutatum* isolates was streaked cross-wise using a sterile inoculation loop onto Petri dishes 10 cm in diameter containing PDA. Sterile filter paper discs were placed into each dish and they were soaked with particular concentration of fungicides. The inhibition zones were measured in two terms. None of our tested pathogen isolates was tolerant to the selected fungicides in our experiments. A comparison of the first and second measurements of isolate mycelia growth sensitivity at concentration 1:1 clearly showed that the average inhibition zone widths decreased over the course of 3 d. Isolate PCF 437 demonstrated the greatest mycelium growth inhibition for the fungicides with active ingredients folpet, mancozeb and thiram. Isolate PCF 231 was the most sensitive after treatment with the fungicides with active ingredients azoxystrobin and metiram. On the other hand, isolate 1209 exhibited the lowest reaction to fungicide preparations with active ingredients azoxystrobin and thiram. The inhibition of conidial germination was also tested. All evaluated active ingredients in fungicides demonstrated an inhibitory effect on the conidial germination of pathogen. No differences in the conidial germination rates of the isolates were detected for fungicides with active ingredients dithianon, folpet and mancozeb. The fungicide with active ingredient azoxystrobin demonstrated the greatest inhibitory effect in isolate 1109 and the lowest inhibition in isolate PCF 437. The lowest conidial germination rates for the fungicide with active ingredient metiram was recorded in isolate 1209 and isolate PCF 231, while isolates 710 and PCF 437 had the highest germination rates.

Acknowledgement

This study was supported by NAZV MZe No. QH81029: "Innovation protection of safflower (*Carthamus tinctorius* L.) against important fungal pathogens".

REFERENCES

AGOSTINI, J. P., TIMMER, L. W., MITCHELL, D. J. 1992. Morphological and pathological characteristics of strains of Colletotrichum gloeosporioides from citrus. *Phytopathology,* 82(11): 1377–1382.

FREEMAN, S., HOROWITZ, S., SHARON, A. 2001. Pathogenic and nonpathogenic lifestyles in Colletotrichum acutatum from strawberry and other plants. *Phytopathology,* 91(10): 986–992.

GOES, A., KIMATI, H. 1998. Colletotrichum acutatum, the causal agent of postbloom fruit drop: resistant or insensitive to benomyl? *Summa Phytopathologica,* 24(3/4): 246–253.

GOES, A., GARRIDO, R. B. O., REIS, R. F., BALDASSARI, R. B., SOARES, M. A. 2008. Evaluation of fungicide applications to sweet orange at different flowering stages for control of postbloom fruit drop caused by Colletotrichum acutatum. *Crop Protection,* 27 (1): 71–76.

HOWARD, C. M., MAAS, J. L., CHANDLER, C. K., ALBREGTS, E. E. 1992. Anthracnose of strawberry caused by the Colletotrichum complex in Florida. *Plant Disease,* 76(10): 976–981.

HWANG, S.-Y., KIM, H.-R., KIM, J.-H., PARK, J.-H., LEE, S.-B., CHEONG, S-R., KIM, H.-T. 2010. Sensitivity of Colletotrichum spp. isolated from Grapes in Korea to carbendazim and the mixture of carbendazim plus diethofencarb. *Plant Pathology Journal,* 26(1): 49–56.

KIM, S. G., KIM, Y.-H., KIM, H.-T., KIM, Y. H. 2008. Effect of delayed inoculation after wounding on the development of anthracnose disease caused by Colletotrichum acutatum on chili pepper fruit. *Plant Pathology Journal,* 24(4): 392–399.

KIM, Y.-S., MIN, J. Y., KANG, B. K., BACH, N. V., CHOI, W. B., PARK, E. W., KIM, H. T. 2007. Analyses of the less benzimidazole-sensitivity of the isolates of Colletotrichum spp. causing the anthracnose in pepper and strawberry. *Plant Pathology Journal*, 23(3): 187–192.

KLOUTVOROVÁ, J., KUPKOVÁ, J. 2009. Laboratorní testy citlivosti houby Colletotrichum acutatum k fungicidům. *Vědecké práce ovocnářské,* 21: 47–52.

MACHOWICZ-STEFANIAK, Z., ZALEWSKA, E. 2011. Occurrence of Colletotrichum dematium on selected herbs species and preparations inhibiting pathogen's growth and development in vitro. *Ecological Chemistry and Engineering S,* 18(4): 465–478.

MACKENZIE, S. J., PERES, N. A. 2012. Use of leaf wetness and temperature to time fungicide applications to control anthracnose fruit rot of strawberry in Florida. *Plant Disease,* 96(4): 522–528.

MARI, M., GUIDARELLI, M., MARTINI, C., SPADONI, A. 2012. First report of Colletotrichum acutatum causing bitter rot on apple in Italy. *Plant Disease,* 96(1): 144.

NOVOTNÁ, K., STAŇKOVÁ, B., VÍCHOVÁ, J.,VEJRAŽKA, K., POKORNÝ, R. 2011. Účinnost dvousložkových fungicidů na vybrané izoláty Colletotrichum acutatum. *Úroda,* 59(12): 243–246.

NOVOTNÝ, D., KŘÍŽKOVÁ-KUDLÍKOVÁ, I., KRÁTKÁ, J., SALAVA, J. 2006. The presence of Colletotrichum acutatum in the Czech Republic. In: *Sborník abstraktů "XVII. česká a slovenská konference o ochraně rostlin"*, Česká zemědělská univerzita v Praze, 12.–14. září. Praha: Česká zemědělská univerzita, 252.

PERES, N. A., MACKENZIE, S. J., PEEVER, T. L., TIMMER, L. W. 2008. Postbloom fruit drop of citrus and Key lime anthracnose are caused by distinct phylogenetic lineages of Colleotrichum acutatum. *Phytopathology,* 98(3): 345–352.

PERES, N. A., SEIJO, T. E., TURECHEK, W. W. 2010. Pre- and post-inoculation activity of a protectant and a systemic fungicide for control of anthracnose fruit rot of strawberry under different wetness durations. *Crop Protection,* 29: 1105–1110.

PERES, N. A., SOUZA, N. L., PEEVERT, T. L., TIMMER, L. W. 2004. Benomyl sensitivity of isolates of Colletotrichum acutatum and C. gloeosporioides from citrus. *Plant Disease*, 88(2): 125–130.

SHUKLA, R. S., KHALIQ, A., ALAM, M. 2010. Chemical control of blossom blight disease of sarpagandha caused by Colletotrichum capsici. *African Journal of Biotechnology,* 9(38): 6397–6400.

SREENIVASAPRASAD, S., TALHINHAS, P. 2005. Genotypic and phenotypic diversity in Colletotrichum acutatum, a cosmopolitan pathogen causing anthracnose on a wide range of hosts. *Molecular Plant Pathology*, 6(4): 361–378.

STAŇKOVÁ, B., VÍCHOVÁ, J., POKORNÝ, R. 2011. Virulence of Colletotrichum acutatum isolates to several host plants. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 59(3): 161–169.

VÍCHOVÁ, J., VEJRAŽKA, K., CHOLASTOVÁ, T., POKORNÝ, R., HRUDOVÁ, E. 2011. Colletotrichum simmondsii causing anthracnose on safflower in the Czech Republic. *Plant Disease,* 95(1): 79.

VÍCHOVÁ, J., STAŇKOVÁ, B., POKORNÝ, R. 2012. First report of Colletotrichum acutatum on tomato and apple fruits in the Czech Republic. *Plant Disease,* 96(5): 769–770.

VÍCHOVÁ, J., STAŇKOVÁ, B., POKORNÝ, R. 2013. First report of Colletotrichum acutatum sensu lato causing anthracnose on gooseberry fruits in the Czech Republic. *Plant Disease*, 97(9): 1249.

WHARTON, P. S., DIÉGUEZ-URIBEONDO, J. 2004. The biology of Colletotrichum acutatum. *Anales del Jardín Botánico de Madrid,* 61(1): 3–22.

WONG, F. P., CERDA, K. A., DE LA HERNANDEZ-MARTINEZ, R. MIDLAND, S. L. 2008. Detection and characterization of benzimidazole resistance in California populations of Colletotrichum cereale. *Plant Disease*, 92(2): 239–246.

XU, X. F., LIN, T., YUAN, S. K., DAI, D. J., SHI, H. J., ZHANG, C. Q., WANG, H. D. 2014. Characterization of baseline sensitivity and resistance risk of Colletotrichum gloeosporioides complex isolates from strawberry and grape to two demethylation-inhibitor fungicides, prochloraz

and tebuconazole. *Australasian Plant Pathology*, 43(6): 605–613.

YOUNG, J. R. TOMASO-PETERSON, M. CERDA, K., DE LA WONG, F. P. 2010. Two mutations in beta -tubulin 2 gene associated with thiophanate-methyl resistance in Colletotrichum cereale isolates from creeping bentgrass in Mississippi and Alabama. *Plant Disease*, 94(2): 207–212.

ZHANG, L. H., LI, M., GAO, Z. Y., ZHANG, Z. K., YANG, F. Z., XIE, Y. X., HU, M. J., YANG, Y. 2013. Screening and cross-resistance analysis of alternative fungicides against carbendazim-resistant Colletotrichum gloeosporioides Penz. from mango (*Mangifera indica* L.). *Acta Horticulturae*, 992: 415–421.

Contact information

Barbora Jílková: jilkova.barca@gmail.com
Jana Víchová: jana.vichova@mendelu.cz
Radovan Pokorný: radovan.pokorny@mendelu.cz
Karel Vejražka: vejrazka@vupt.cz