SENSITIVITY OF COLLETOTRICHUM ACUTATUM ISOLATES TO SELECTED FUNGICIDES

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Abstract


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; Sreenivasasprasad and Talhinhas, 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-Urbeondo, 2004). In the Czech Republic, only one fungicide against C. acutatum is authorized. With the commercial name Oríteá [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.

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Basic concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>azoxystrobin</td>
<td>0.1 mg.ml⁻¹</td>
</tr>
<tr>
<td>captan</td>
<td>1.2 mg.ml⁻¹</td>
</tr>
<tr>
<td>mancozeb</td>
<td>0.32 mg.ml⁻¹</td>
</tr>
<tr>
<td>thiram</td>
<td>0.32 mg.ml⁻¹</td>
</tr>
<tr>
<td>dithianon</td>
<td>0.07 mg.ml⁻¹</td>
</tr>
<tr>
<td>folpet</td>
<td>0.32 mg.ml⁻¹</td>
</tr>
<tr>
<td>metiram</td>
<td>0.315 mg.ml⁻¹</td>
</tr>
</tbody>
</table>

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 α = 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

### Table I: Fungicides used in testing – basic concentration 1:1

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Host plant</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS 786.86</td>
<td><em>Malus domestica</em> Borkh.</td>
<td>CBS</td>
</tr>
<tr>
<td>PCF 231</td>
<td><em>Fragaria × ananassa</em> Duch.</td>
<td>PCFruit</td>
</tr>
<tr>
<td>PCF 437</td>
<td><em>Lupinus albus</em> L.</td>
<td>PCFruit</td>
</tr>
<tr>
<td>710</td>
<td><em>Hypericum perforatum</em> L.</td>
<td>DCSBPM MENDELU</td>
</tr>
<tr>
<td>1109</td>
<td><em>Carthamus tinctorius</em> L.</td>
<td>DCSBPM MENDELU</td>
</tr>
<tr>
<td>1209</td>
<td><em>Carthamus tinctorius</em> L.</td>
<td>DCSBPM MENDELU</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1st measurement</th>
<th>2nd measurement</th>
<th>1st measurement</th>
<th>2nd measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate</td>
<td>1:1</td>
<td>1:5</td>
<td>1:10</td>
<td>1:1</td>
</tr>
<tr>
<td>1109</td>
<td>0.80 A</td>
<td>0.80 A</td>
<td>0.60 A</td>
<td>0.80 A</td>
</tr>
<tr>
<td>1209</td>
<td>0.83 A</td>
<td>0.80 A</td>
<td>0.53 A</td>
<td>0.17 A</td>
</tr>
<tr>
<td>710</td>
<td>0.87 A</td>
<td>0.53 A</td>
<td>0.47 A</td>
<td>0.70 AB</td>
</tr>
<tr>
<td>CBS 786.86</td>
<td>0.80 A</td>
<td>0.80 A</td>
<td>0.80 A</td>
<td>0.67 AB</td>
</tr>
<tr>
<td>PCF 231</td>
<td>1.70 B</td>
<td>0.80 A</td>
<td>0.80 A</td>
<td>1.67 C</td>
</tr>
<tr>
<td>PCF 437</td>
<td>1.17 A</td>
<td>0.93 A</td>
<td>0.87 A</td>
<td>1.10 BC</td>
</tr>
<tr>
<td>M. S.</td>
<td>0.383</td>
<td>0.052</td>
<td>0.082</td>
<td>0.753</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1st measurement</th>
<th>2nd measurement</th>
<th>1st measurement</th>
<th>2nd measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate</td>
<td>1:1</td>
<td>1:5</td>
<td>1:10</td>
<td>1:1</td>
</tr>
<tr>
<td>1109</td>
<td>2.10 A</td>
<td>2.07 AB</td>
<td>1.67 AB</td>
<td>2.03 A</td>
</tr>
<tr>
<td>1209</td>
<td>2.87 B</td>
<td>2.70 B</td>
<td>2.33 C</td>
<td>2.83 B</td>
</tr>
<tr>
<td>710</td>
<td>2.20 A</td>
<td>1.87 A</td>
<td>1.43 A</td>
<td>2.00 A</td>
</tr>
<tr>
<td>CBS 786.86</td>
<td>1.93 A</td>
<td>1.73 A</td>
<td>1.53 AB</td>
<td>1.87 A</td>
</tr>
<tr>
<td>PCF 231</td>
<td>2.27 AB</td>
<td>2.17 AB</td>
<td>2.07 BC</td>
<td>2.00 A</td>
</tr>
<tr>
<td>PCF 437</td>
<td>2.10 A</td>
<td>2.03 AB</td>
<td>1.63 AB</td>
<td>1.83 A</td>
</tr>
<tr>
<td>M. S.</td>
<td>0.317</td>
<td>0.335</td>
<td>0.362</td>
<td>0.413</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Concentration</th>
<th>1st measurement</th>
<th>2nd measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1:1</td>
<td>1:5</td>
</tr>
<tr>
<td>I109</td>
<td>1.23 A</td>
<td>0.97 A</td>
<td>0.90 A</td>
</tr>
<tr>
<td>1209</td>
<td>1.00 A</td>
<td>0.93 A</td>
<td>0.93 A</td>
</tr>
<tr>
<td>710</td>
<td>1.47 A</td>
<td>1.17 A</td>
<td>0.93 A</td>
</tr>
<tr>
<td>CBS 786.86</td>
<td>0.80 A</td>
<td>0.83 A</td>
<td>0.80 A</td>
</tr>
<tr>
<td>PCF 231</td>
<td>1.27 A</td>
<td>1.03 A</td>
<td>1.00 A</td>
</tr>
<tr>
<td>PCF 437</td>
<td>1.17 A</td>
<td>1.07 A</td>
<td>0.83 A</td>
</tr>
<tr>
<td>M. S.</td>
<td>0.160</td>
<td>0.040</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Note: See Tab. III

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

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Concentration</th>
<th>1st measurement</th>
<th>2nd measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1:1</td>
<td>1:5</td>
</tr>
<tr>
<td>I109</td>
<td>1.83 AB</td>
<td>1.80 A</td>
<td>1.47 A</td>
</tr>
<tr>
<td>1209</td>
<td>2.20 B</td>
<td>2.10 A</td>
<td>1.77 AB</td>
</tr>
<tr>
<td>710</td>
<td>1.63 A</td>
<td>1.70 A</td>
<td>1.50 A</td>
</tr>
<tr>
<td>CBS 786.86</td>
<td>1.87 A</td>
<td>1.83 A</td>
<td>1.67 AB</td>
</tr>
<tr>
<td>PCF 231</td>
<td>2.23 B</td>
<td>1.97 A</td>
<td>1.73 AB</td>
</tr>
<tr>
<td>PCF 437</td>
<td>2.43 C</td>
<td>2.13 A</td>
<td>1.97 C</td>
</tr>
<tr>
<td>M. S.</td>
<td>0.273</td>
<td>0.090</td>
<td>0.102</td>
</tr>
</tbody>
</table>

Note: See Tab. III

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

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Concentration</th>
<th>1st measurement</th>
<th>2nd measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1:1</td>
<td>1:5</td>
</tr>
<tr>
<td>I109</td>
<td>2.30 A</td>
<td>1.23 A</td>
<td>0.90 AB</td>
</tr>
<tr>
<td>1209</td>
<td>2.37 A</td>
<td>1.30 A</td>
<td>0.62 A</td>
</tr>
<tr>
<td>710</td>
<td>2.67 A</td>
<td>1.63 AB</td>
<td>1.20 ABC</td>
</tr>
<tr>
<td>CBS 786.86</td>
<td>2.08 A</td>
<td>2.17 AB</td>
<td>1.87 BC</td>
</tr>
<tr>
<td>PCF 231</td>
<td>2.27 A</td>
<td>1.57 AB</td>
<td>1.20 ABC</td>
</tr>
<tr>
<td>PCF 437</td>
<td>4.10 B</td>
<td>2.37 B</td>
<td>2.17 C</td>
</tr>
<tr>
<td>M. S.</td>
<td>1.663</td>
<td>0.637</td>
<td>1.029</td>
</tr>
</tbody>
</table>

Note: See Tab. III
Sensitivity of *Colletotrichum acutatum* Isolates to Selected Fungicides

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.

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

<table>
<thead>
<tr>
<th>Isolate</th>
<th>1st measurement (Concentration)</th>
<th>2nd measurement (Concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:1</td>
<td>1:5</td>
</tr>
<tr>
<td>1109</td>
<td>0.97 A</td>
<td>0.97 A</td>
</tr>
<tr>
<td>1209</td>
<td>1.60 A</td>
<td>1.13 A</td>
</tr>
<tr>
<td>710</td>
<td>1.60 A</td>
<td>1.07 A</td>
</tr>
<tr>
<td>CBS 786.86</td>
<td>1.73 A</td>
<td>1.23 A</td>
</tr>
<tr>
<td>PCF 231</td>
<td>3.40 B</td>
<td>1.83 A</td>
</tr>
<tr>
<td>PCF 437</td>
<td>1.73 A</td>
<td>1.50 A</td>
</tr>
<tr>
<td>M. S.</td>
<td>2.001</td>
<td>0.313</td>
</tr>
</tbody>
</table>

Note: See Tab. III

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

<table>
<thead>
<tr>
<th>Isolate</th>
<th>1st measurement (Concentration)</th>
<th>2nd measurement (Concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:1</td>
<td>1:5</td>
</tr>
<tr>
<td>1109</td>
<td>2.43 AB</td>
<td>2.47 B</td>
</tr>
<tr>
<td>1209</td>
<td>1.60 A</td>
<td>1.40 A</td>
</tr>
<tr>
<td>710</td>
<td>3.00 B</td>
<td>3.00 B</td>
</tr>
<tr>
<td>CBS 786.86</td>
<td>3.53 B</td>
<td>2.80 B</td>
</tr>
<tr>
<td>PCF 231</td>
<td>3.51 B</td>
<td>3.13 B</td>
</tr>
<tr>
<td>PCF 437</td>
<td>3.40 B</td>
<td>2.23 AB</td>
</tr>
<tr>
<td>M. S.</td>
<td>1.781</td>
<td>1.214</td>
</tr>
</tbody>
</table>

Note: See Tab. III
and thiram, but the most sensitive to the effect of active ingredients azoxystrobin, 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 were recorded in isolate 1209 and isolate PCF 231, while isolates 710 and PCF 437 had the highest germination rates.

**DISCUSSION**

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 were recorded in isolate 1209 and isolate PCF 231, while isolates 710 and PCF 437 had the highest germination rates.

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

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Isolate</th>
<th>azoxystrobin</th>
<th>captan</th>
<th>dithianon</th>
<th>folpet</th>
<th>mancozeb</th>
<th>metiram</th>
<th>thiram</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS 786.86</td>
<td>28.37</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>99.80</td>
<td>55.03</td>
<td>96.40</td>
<td></td>
</tr>
<tr>
<td>PCF 231</td>
<td>25.63</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>99.83</td>
<td>61.84</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>PCF 437</td>
<td>22.97</td>
<td>100.00</td>
<td>98.43</td>
<td>100.00</td>
<td>100.00</td>
<td>20.13</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>710</td>
<td>36.10</td>
<td>98.53</td>
<td>96.77</td>
<td>99.20</td>
<td>100.00</td>
<td>16.90</td>
<td>95.70</td>
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</tr>
<tr>
<td>1109</td>
<td>42.07</td>
<td>100.00</td>
<td>97.87</td>
<td>100.00</td>
<td>99.87</td>
<td>29.37</td>
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<tr>
<td>1209</td>
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<td>100.00</td>
<td>96.50</td>
<td>100.00</td>
<td>100.00</td>
<td>76.73</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant differences with level of significance $\alpha = 0.05$ are marked with different capital letters.
occurred on agar with the addition of a fungicide with the active ingredient thiiram – 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. gloeosporioides 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 thiiram. 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 thiiram. 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.

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