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VIRULENCE OF *COLLETOTRICHUM ACUTATUM* ISOLATES TO SEVERAL HOST PLANTS

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Abstract

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Colletotrichum acutatum belongs to polyphagous fungal pathogens and is widespread in many countries on all continents. *C. acutatum* causes the most serious economic damage in strawberry (*Fragaria x ananassa* Duch.). Considering the wide variability of the pathogen may be assumed spread to other areas which constitutes danger not only for strawberry, but also other economically important fruit crops, vegetables and fruits.

The main objective of our study was to verify the cross infection of eleven *C. acutatum* isolates from different host plants (strawberry, safflower, lupine, pepper and *Hypericum perforatum*) to selected host plants (strawberry, pepper and safflower). Two varieties from each of the experimental plant species were selected and virulence of isolates *C. acutatum* was evaluated.

Based on results of statistical evaluation, virulence of *C. acutatum* isolates was different on strawberry, pepper and safflower. The strawberry variety Pegasus was more susceptible to *C. acutatum* than the variety Elkas. Isolate 710 from *H. perforatum* showed the highest virulence for both varieties in terms of index of infection intensity. The pepper variety Pirouet was more susceptible than the variety Cynthia. The highest degree of virulence was found for isolate 29267 from pepper in the variety Cynthia, the highest virulence was proved for isolate 231 from strawberry in the variety Pirouet. No statistical difference was confirmed between susceptibility of the safflower varieties. Isolate 1209 from safflower showed the most important effect on tested plants of safflower.

Isolates 710 from *H. perforatum*, isolate 1209 from safflower, isolate 29267 from pepper and isolate 231 from strawberry showed different virulence for tested host plants.

Colletotrichum acutatum, safflower, strawberry, pepper, virulence, cross-infection

Colletotrichum acutatum J. H. Simmonds 1968 (syn. *C. simmondsii* R. G. Shives & Y. P. Tan, sp. nov. 2009) belongs to polyphagous fungal pathogens with ubiquitous distribution which causes significant crop losses (Peres *et al*., 2005), such as in strawberry (*Fragaria x ananassa* Duch.), apple (*Malus pumila* Mill. and *Malus sylvestris* Mill.), cherry (*Prunus cerasus* L.), pepper (*Capsicum annuum* L.), tomato (*Lycopersicon esculentum* Mill.), lupine (*Lupinus polyphyllus* Lindl.) (Sreenivasaprasad *et al*., 2005), almond (*Prunus dulcis* Mill.), olive (*Olea europaea* L.), papaya (*Carica papaya* L.) and citrus (*Citrus* spp.). The hosts of *C. acutatum* are also some ornamental plants and conifers (Guerber *et al*., 2003). *C. acutatum* is also the most serious economic pathogen of safflower (*Carthamus tinctorius* L.) (Víchová *et al*., 2011), currently.

Generally, the pathogen infects both young and adult plants and symptoms are apparent on leaves, stems and flower parts. Typical symptoms on fruits are round or square, sunken spots on which orange conidial masses are visible. Under severe disease pressure, lesions may coalesce (Lewis Ivey *et al*., 2004). In plants, where the pathogen infects herbaceous parts, characteristic symptoms are irregular or circular spots on leaves which later dries. Irregular, sunken, brown spots are visible on stem and orange conidail masses also appear on them, eventually. Latent infections were also

described (Šindelková *et al.*, 2008) and can be main cause of dissemination of *C. acutatum* (Debode *et al.*, 2009).

C. acutatum belongs to the *Ascomycetes*. The pathogen reproduces by ellipsoidal or fusiform conidia (size 7–14 × 2.5–3.5 µm, with a thin cell wall) (Than *et al.*, 2008) which are spread by wind and water (leaking, watering and rain). The infection can occur in a broad range of temperatures (18–33 °C), high relative humidity (95–100 %), exposure to moisture and irrigation, dew or rainfall for 13 hours or more. The incubation period is only a few days (Šindelková *et al.*, 2008).

C. acutatum represents a species which is morphologically and genetically variable, so for determination PCR is used to identify genetic differences and possible biologically closed groups between morphologically identical isolates (Guerber *et al.*, 2003). Intraspecific variability could have an effect on virulence of the pathogen (Sreenivasaprasad *et al.*, 2005; Guerber *et al.*, 2003).

In the Czech Republic only one fungicide is registered against *C. acutatum* – Ortiva in strawberry. Preventive control measures (healthy seeds and seedlings, locality selection, nutrition and air circulation) are very important. One possible way of effective protection against *C. acutatum* is breeding for resistance. The knowledge of the pathogen virulence is very important for successful fulfilment of this process.

The main objective of our study was to verify the cross infection of *C. acutatum* isolates from different host plants (strawberry, safflower, lupine, pepper, *H. perforatum*) to selected host plants (strawberry, pepper and safflower) and virulence evaluation of particular pathogen isolates.

MATERIALS AND METHODS

Fungal cultures and growth conditions

Isolates of *C. acutatum* from different host plants were obtained from the microorganisms collection of CBS Fungal Biodiversity Centre and Proefcentrum fruitteelt vzw (PCFruit), isolates from safflower and *H. perforatum* are owned by Department of Crop Science, Breeding and Plant Medicine (Tab. I). Monosporic isolates were prepared from all isolates of the pathogen which were used for virulence tests for strawberry, pepper and safflower. Species identity of isolates was verified by PCR according to methods Garrido *et al.* (2009) and Garrido *et al.* (2008).

Isolates were cultured under conditions suitable for sporulation – temperature 25 ± 2 °C. Obtained samples of cultures were put into distilled water and filtered through a thin cloth to remove pieces of mycelium. The number of spores in suspension was established by Bürker chamber and all samples were adjusted to uniform concentration – 8.1×10^4 conidia/ml.

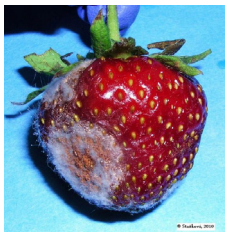
I: *C. acutatum isolates and their origins*

Isolate	Plant	Supplier
CBS 112202	Fragaria x ananassa	CBS
CBS 292.67	Capsicum annuum	CBS
CBS 786.86	Malus sylvestris	CBS
PCF 231	Fragaria x ananassa	PCFruit
PCF 437	Lupinus alba	PCFruit
710	Hypericum perforatum	DCSBPM MENDELU Brno
308	Carthamus tinctorius	DCSBPM MENDELU Brno
508	Carthamus tinctorius	DCSBPM MENDELU Brno
709	Carthamus tinctorius	DCSBPM MENDELU Brno
809	Carthamus tinctorius	DCSBPM MENDELU Brno
1 209	Carthamus tinctorius	DCSBPM MENDELU Brno

Fruit and plant inoculation and evaluation procedures

Methodology for evaluation of isolates virulence of the pathogen on strawberry and pepper was based on the methods of inoculation and assessment of infection described by Lewis Ivey *et al.* (2004) and Freeman *et al.* (2001). Methods for evaluation of isolates virulence on safflower were designed by Víchová (2009).

Fruits of the strawberry varieties Pegasus and Elkas from Jahodárna Bratčice and fruits of the pepper varieties Pirouet and Cynthia from growers Ing. Pilař and Mr. Míča were purchased for testing. Fruits were washed thoroughly to remove post-harvest dirt, surface was disinfected with 3% sodium hypochlorite for 2 min and rinsed in sterile distilled water. Fruits were injured by sterile needle and 10 µl of spores suspension was inoculated by pipetting into the wound. Fruits was laid down on plastic trays, where before textile fabric and filter paper was put down and infused by sterile distilled water – for the subsequent maintenance of the increased humidity in the vicinity of the sample. Plastic trays with fruits were transferred to cultivation room and carefully wrapped in plastic wrap for 48 hours. Incubation temperature was adjusted to 25 ± 2 °C. Fruits were visually evaluated after 4 days from inoculation of strawberry and after 11 days from inoculation of pepper and the reaction of particular isolates was compared with mock inoculated control (inoculation by sterilised water – without the patogen spores). The reaction of strawberry fruits was evaluated according to scale of infection: degree 1 – healthy fruit, degree 2 – to 20% of fruit area attacked, degree 3 – to 50% of fruit area attacked, degree 4 – to 75% of fruit area attacked and degree 5 – to 100% fruit area attacked (Fig. 1). Index of infection intensity and frequency of infection were calculated from degree of infection of particular

Degree 1	Degree 2	Degree 3	Degree 4	Degree 5
				

1: *Particular degrees of strawberry fruits infection by C. acutatum*

Degree 1	Degree 2	Degree 3	Degree 4
			

2: *Particular degrees of safflower plants infection by C. acutatum*

fruits. Diameters of lesions were measured in pepper fruits, consequently surface for statistical evaluation was calculated from these diameters.

Virulence of isolates was also tested on the safflower varieties Sabina and India. Both of these varieties were obtained from Research Institute for Fodder Crops, Ltd. Troubsko. Inoculum (20 ml) was applied by hand spray on the three-week-old safflower plants with two or three pairs of leaves.

Plants were packed for 48 hours in plastic wrap to maintain the necessary humidity and exposure to moisture leaves for growth of the pathogen and placed in cultivation room with controlled temperature of 25 ± 2 °C. The infection of plants were evaluated 4–5 days after inoculation, attention was always focused on the last (the youngest) three leaves. Evaluation was carried out according to created scale: degree 1 – healthy leaf, degrese 2 – single spots on leaf, degree 3 – half of leaf attacked (dry) and degree 4 – whole leaf infected (dry) (Fig. 2).

All experiments were established in three replications, in one replication 10 fruits of strawberry or 8 fruits of peppers or 10 plants of safflower were included. The results were statistically processed by software Unistat, multi-factor ANOVA (factors: variety, isolate, leaf – in safflower) and evaluated by Tukey-HSD test. Then one-factor (factor isolate) analysis was also done for each variety of particular crops. Statistically significantly different groups were evaluated at $\alpha = 0.05$.

RESULTS

Strawberry

Statistically significant differences were found between susceptibility of the strawberry varieties Pegasus and Elkas – the variety Pegasus was more susceptible to the pathogen than the variety Elkas. Between isolates – in testing across varieties – no statistical differences were proved in index of infection intensity, statistical differences were confirmed between isolates 29 267 and 1 209 in frequency of infection, isolate 29 267 showed the lowest (86.6 %) and isolate 1 209 the highest (100 %) frequency of infection (Tab. II).

II: *Index of infection intensity and frequency of infection in regard to particular factors in strawberry*

	Index	Frequency
Variety		
Elkas	2.51 ^{A}	93.89 ^{A}
Pegasus	3.99 ^{B}	99.44 ^{B}
Isolate		
29 267	3.02 ^{A}	86.67 ^{A}
231	3.12 ^{A}	98.33 ^{AB}
1209	3.15 ^{A}	100.00 ^{B}
78 686	3.33 ^{A}	98.33 ^{AB}
437	3.43 ^{A}	98.33 ^{AB}
710	3.47 ^{A}	98.33 ^{AB}

IIa: *Multi-factor ANOVA for Tab. II – index of infection intensity*

Source of variability	Sum of squares	df	Mean Square	F	Sig.
Main Effects	20.82	6	3.47	40.56	0.0000
Variety	19.80	1	19.80	231.46	0.0000
Isolate	1.018	5	0.20	2.38	0.0689
Interaction of the 2nd order	0.70	5	0.14	1.63	0.1912
Variety × Isolate	0.70	5	0.14	1.63	0.1912
Explained	21.52	11	1.96	22.87	0.0000
Error	2.05	24	0.09		
Total	23.57	35	0.67		

IIb: *Multi-factor ANOVA for Tab. II – frequency of infection*

Source of variability	Sum of squares	df	Mean Square	F	Sig.
Main Effects	1 011.11	6	168.52	12.13	0.0000
Variety	277.78	1	277.78	20.00	0.0002
Isolate	733.33	5	146.67	10.56	0.0000
Interaction of the 2nd order	855.56	5	171.11	12.32	0.0000
Variety × Isolate	855.56	5	171.11	12.32	0.0000
Explained	1 866.67	11	169.70	12.22	0.0000
Error	333.33	24	13.89		
Total	2 200.00	35	62.86		

No statistical differences in index of infection intensity were found by the evaluation of isolates in particular varieties (Tab. III). No statistical differences were confirmed between isolates in the variety Pegasus in frequency of infection, for the variety Elkas statistically significant differences were proved between isolate 29 267 (the lowest frequency of infection) and other isolates. Isolate 710 exhibited the highest level of index of infection intensity, on the other hand isolate 231 had the lowest one in the variety Pegasus, in the variety Elkas isolate 710 showed the highest degree of virulence and isolate 29 267 proved the lowest degree, but the statistically significant differences were not proved. Frequency of infection of strawberry fruits almost always reached 100% which led to the conclusion that the strawberry fruits were highly susceptible to several isolates of the pathogen.

III: *Index of infection intensity and frequency of infection in particular strawberry varieties infected by C. acutatum*

	Pegasus		Elkas	
Isolate	**Index**	**Frequency**	**Index**	**Frequency**
231	3.60 ^{A}	96.67 ^{A}	2.63 ^{A}	100.00 ^{B}
29 267	3.87 ^{A}	100.00 ^{A}	2.17 ^{A}	73.33 ^{A}
78 686	4.00 ^{A}	100.00 ^{A}	2.67 ^{A}	96.67 ^{B}
1 209	4.07 ^{A}	100.00 ^{A}	2.23 ^{A}	100.00 ^{B}
437	4.20 ^{A}	100.00 ^{A}	2.67 ^{A}	96.67 ^{B}
710	4.23 ^{A}	100.00 ^{A}	2.70 ^{A}	96.67 ^{B}
MS	0.166	5.556	0.177	321.22

Pepper

Statistically significant differences were found between the pepper varieties Cynthia and Pirouet reaction of *C. acutatum* infection. The area of damaged tissue in the variety Pirouet was proved more larger than in the variety Cynthia.

Between isolates – in testing across varieties – statistical differences were proved between isolate 710, isolate 1 209 (the smallest average of damaged tissue) and isolate 78 686, isolate 231 (the largest average of damaged tissue) (Tab. IV).

Only isolates 710 and 1 209 did not differ statistically in the variety Cynthia in the evaluation of isolates in particular varieties. These isolates showed the lowest degree of virulence. Statistical differences were detected between all isolates in the variety Pirouet. No virulence was proved in isolate 710 in both varieties. Isolate 29 267 in the

IV: *Area of damaged tissue in regard to particular factors in pepper*

	Infected area [mm²]
Variety	
Cynthia	32.84 ^{A}
Piroeut	79.99 ^{B}
Isolate	
710	0.00 ^{A}
1 209	8.22 ^{A}
437	51.98 ^{AB}
29 267	64.07 ^{AB}
78 686	92.11 ^{B}
231	122.11 ^{B}

IVa: *Multi-factor ANOVA for Tab. III*

Source of variability	Sum of squares	df	Mean Square	F	Sig.
Main Effects	87 056.21	6	14 509.37	1 010.16	0.0000
Variety	20 013.15	1	20 013.15	1 393.35	0.0000
Isolate	67 043.06	5	13 408.61	933.53	0.0000
Interaction of the 2nd order	36 785.04	5	7 357.01	512.21	0.0000
Variety × Isolate	36 785.04	5	7 357.01	512.21	0.0000
Explained	123 841.25	11	11 258.30	783.82	0.0000
Error	344.72	24	14.36		
Total	124 185.97	35	3 548.17		

variety Cynthia and isolate 231 in the variety Pirouet showed the highest degree of virulence. Different reactions of particular varieties were confirmed for this set of isolates. In the variety Pirouet it was found that isolate 1 209 had significantly higher virulence compared with the virulence of isolate 710, in the variety Cynthia these differences were not found (Tab. V).

V: *Area of damaged tissue in particular pepper varieties infected by C. acutatum*

	Cynthia	Pirouet
Isolate	**Infected area [mm²]**	**Infected area [mm²]**
710	0.00 A	0.00 A
1 209	4.91 A	11.53 B
78 686	21.50 B	162.72 E
437	37.61 C	66.35 D
231	57.78 D	186.44 F
29 267	75.23 E	52.92 C
MS	2657.621	18108.401

Safflower

The safflower testing was divided into two independent groups. Distribution of isolates into groups was random and depended on space capacity of cultivation room. Isolate 1 209, which is used as a standard for tests of virulence in our department currently, was tested in both groups.

Group A: No statistically significant differences were found between susceptibility of the varieties Sabina and India both in frequency of infection and in index of infection intensity. No statistical

VI: *Index of infection intensity and frequency of infection in regard to particular factors in safflower – Group A*

	Index	Frequency
Variety		
India	2.03 A	45.56 A
Sabina	2.15 A	48.00 A
Leaf		
1	1.82 A	42.00 A
2	2.12 A	48.00 A
3	2.34 A	50.33 A
Isolate		
231	1.12 A	7.78 A
709	1.24 A	17.78 A
437	1.74 B	32.22 B
1 209	2.98 C	87.78 C
308	3.36 C	88.33 C

VIa: *Multi-factor ANOVA for Tab. VI – index of infection intensity*

Source of variability	Sum of squares	df	Mean Square	F	Sig.
Main Effects	79.47	7	11.35	151.15	0.0000
Variety	0.36	1	0.36	4.81	0.0322
Isolate	75.02	4	18.76	249.71	0.0000
Leaf	4.09	2	2.04	27.21	0.0000
Interaction of the 2nd order	5.85	14	0.42	5.56	0.0000
Variety × Isolate	3.55	4	0.89	11.81	0.0000
Variety × Leaf	0.64	2	0.32	4.28	0.0183
Isolate × Leaf	1.66	8	0.21	2.76	0.0114
Interaction of the 3rd order	0.33	8	0.04	0.55	0.8137
Variety × Isolate × Leaf	0.33	8	0.04	0.55	0.8137
Explained	85.65	29	2.95	39.32	0.0000
Error	4.51	60	0.08		
Total	90.16	89	1.01		

VIb: *Multi-factor ANOVA for Tab. VI – frequency of infection*

Source of variability	Sum of squares	df	MeanSquare	F	Sig.
Main Effects	108 914.44	7	15 559.21	112.93	0.0000
Variety	134.44	1	134.44	0.98	0.3272
Isolate	10 7671.11	4	26 917.78	195.37	0.0000
Leaf	1 108.89	2	554.44	4.02	0.0229
Interaction of the 2nd order	6 208.89	14	443.49	3.22	0.0008
Variety × Isolate	5 337.78	4	1 334.44	9.69	0.0000
Variety × Leaf	402.22	2	201.11	1.46	0.2404
Isolate × Leaf	468.89	8	58.61	0.43	0.9013
Interaction of the 3rd order	575.56	8	71.94	0.52	0.8352
Variety × Isolate × Leaf	575.56	8	71.94	0.52	0.8352
Explained	115 698.89	29	3 989.62	28.96	0.0000
Error	8 266.67	60	137.78		
Total	123 965.56	89	1 392.87		

differences were detected between particular leaves both in frequency of infection and in index of infection intensity. Between isolates – in testing across varieties – statistical differences were proved between isolates 231, 709 (the lowest degree of virulence) and isolate 437 and isolates 1 209, 308 (the highest degree of virulence) both in index of infection intensity and in frequency of infection (Tab. VI).

The results of statistical evaluation of the individual varieties showed that statistical differences were confirmed between isolate 231 (the lowest index of infection intensity) and isolates 709, 437 and isolate 1 209 and isolate 308 (the highest index of infection intensity) in the variety Sabina. In the variety India statistical differences were proved between isolates 709 (the lowest index of infection intensity), 231 and isolate 437 and isolates 308, 1 209 (the highest index of infection intensity) (Tab. VII).

VII: *Index of infection intensity and frequency of infection in particular safflower varieties infected by C. acutatum – Group A*

	Sabina		India	
Isolate	**Index**	**Frequency**	**Index**	**Frequency**
231	1.02 ^{A}	2.22 ^{A}	1.23 ^{A}	13.33 ^{A}
709	1.41 ^{B}	28.89 ^{B}	1.08 ^{A}	6.67 ^{A}
437	1.59 ^{B}	23.33 ^{B}	1.89 ^{B}	41.11 ^{B}
1209	2.99 ^{C}	88.89 ^{C}	2.97 ^{C}	86.67 ^{C}
308	3.76 ^{D}	96.67 ^{C}	2.97 ^{C}	80.00 ^{C}
MS	8.057	10 457.177	5.638	8 393.333

Different reactions of particular varieties were showed in index of infection intensity for this group of isolates. Isolate 308 showed significantly higher virulence compared with virulence of isolate 1209 in the variety Sabina, no differences were observed in the variety India. A similar situation was also found in isolate 231 and isolate 709. Different reactions to particular varieties were confirmed in frequency of infection too – isolate 709 showed higher frequency compared with virulence of isolate 231 in the variety Sabina, but not in the variety India.

Group B: No statistically significant differences were proved between susceptibility of the varieties in frequency of infection and in index of infection intensity. Statistical differences were confirmed between the 1st (the lowest attack) and the 2nd–3rd leaves in index of infection intensity. No differences were shown in frequency of infection. There were statistical differences between virulence of particular isolates. The lowest index of infection intensity and the lowest frequency of infection (10 %) were found in isolate 710, whereas isolate 508 proved the highest index of infection intensity and isolate 809 showed the highest frequency of infection (92.22 %) (Tab. VIII).

Statistical differences were identical between isolates in both varieties in index of infection intensity. Isolate 710 showed the lowest virulence

VIII: *Index of infection intensity and frequency of infection in regard to particular factors in safflower – Group B*

	Index	Frequency
Variety		
India	2.23 ^{A}	60.37 ^{A}
Sabina	2.29 ^{A}	63.70 ^{A}
Leaf		
1	1.69 ^{A}	50.28 ^{A}
2	2.33 ^{B}	65.56 ^{A}
3	2.78 ^{B}	70.28 ^{A}
Isolate		
710	1.12 ^{A}	10.00 ^{A}
29 267	1.22 ^{A}	15.00 ^{A}
112 202	2.46 ^{B}	72.22 ^{B}
809	2.83 ^{BC}	92.22 ^{C}
1 209	2.88 ^{BC}	91.11 ^{C}
508	3.07 ^{C}	91.67 ^{C}

VIIIa: *Multi-factor ANOVA for Tab. VIII – index of infection intensity*

Source of variability	Sum of squares	df	Mean Square	F	Sig.
Main Effects	89.65	8	11.21	102.14	0.0000
Variety	0.09	1	0.09	0.81	0.3708
Isolate	68.01	5	13.60	123.96	0.0000
Leaf	21.56	2	10.78	98.23	0.0000
Interaction of the 2nd order	8.58	17	0.51	4.60	0.0000
Variety × Isolate	1.06	5	0.21	1.93	0.0997
Variety × Leaf	0.03	2	0.01	0.13	0.8828
Isolate × Leaf	7.49	10	0.75	6.83	0.0000
Interaction of the 3rd order	0.48	10	0.05	0.44	0.9219
Variety × Isolate × Leaf	0.48	10	0.05	0.44	0.9219
Explained	98.71	35	2.82	25.71	0.0000
Error	7.90	72	0.11		
Total	106.61	107	1.00		

VIIIb: *Multi-factor ANOVA for Tab. VIII – frequency of infection*

Source of variability	Sum of squares	df	Mean Square	F	Sig.
Main Effects	146 020.37	8	18 252.55	64.42	0.0000
Variety	300.00	1	300.00	1.06	0.3069
Isolate	137 851.85	5	27 570.37	97.31	0.0000
Leaf	7 868.52	2	3 934.26	13.89	0.0000
Interaction of the 2nd order	4 981.48	17	293.03	1.03	0.4338
Variety × Isolate	2 711.11	5	542.22	1.91	0.1026
Variety × Leaf	105.56	2	52.78	0.19	0.8304
Isolate × Leaf	2 164.82	10	216.48	0.76	0.6624
Interaction of the 3rd order	1 750.00	10	175.00	0.62	0.7940
Variety × Isolate × Leaf	1 750.00	10	175.00	0.62	0.7940
Explained	152 751.85	35	4 364.34	15.40	0.0000
Error	20 400.00	72	283.33		
Total	173 151.85	107	1 618.24		

and isolate 1 209 proved the highest virulence. The lowest frequency of infection was found in isolate 710 in both varieties, the highest frequency of infection was found in isolate 1 209 (100%) in the variety Sabina and in isolate 508 (97%) in the variety India (see Tab. IX).

It was found that isolate 1 209 in Group A and B had almost identical degree of index of infection intensity in both varieties, on the other hand it differed in frequency of infection in the variety Sabina in Group B. For this reason, it is possible to compare the virulence between isolates of Groups A and B.

IX: *Index of infection intensity and frequency of infection in particular safflower varieties infected by C. acutatum – Group B*

	Sabina		India	
Isolate	**Index**	**Frequency**	**Index**	**Frequency**
710	1.08 ^{A}	7.78 ^{A}	1.17 ^{A}	12.22 ^{A}
29 267	1.19 ^{A}	13.33 ^{A}	1.24 ^{A}	16.67 ^{A}
112 202	2.59 ^{B}	78.89 ^{B}	2.33 ^{B}	65.56 ^{B}
809	2.92 ^{B}	95.56 ^{B}	2.73 ^{B}	88.89 ^{BC}
508	2.94 ^{B}	86.67 ^{B}	2.73 ^{B}	96.67 ^{C}
1 209	3.02 ^{B}	100.00 ^{B}	3.19 ^{B}	82.22 ^{BC}
MS	6.802	11726.455	6.148	9493.122

DISCUSSION

C. acutatum belongs to polyphagous fungal pathogens which is one of the major disease of important crops worldwide. Three species of host plants were chosen for testing. Strawberry – the disease is responsible for up to 80% plant death and yield losses of over 50% in strawberry production (Debode *et al.*, 2009). Pepper – yield losses amount to 100% (Lewis Ivey *et al.*, 2004) and safflower – *C. acutatum* is the most serious economic pathogen of safflower, currently (Víchová *et al.*, 2011).

The major objective was to verify the cross infection of *C. acutatum* isolates from different host plants (strawberry, safflower, lupine, pepper, apple

and *H. perforatum*) to selected host plants. Fruits and plants were inoculated at appropriete concentration of 8.1 x 10^4 conidia/ml, Lewis Ivey *et al.* (2004) used in experiments higher concentration of 4 × 10^5 conidia/ml and Freeman *et al.* (2001) applied inoculum concentration of 5 × 10^6 conidia/ml. In all cases the inoculum infected plants or fruits perfectly.

Based on results of statistical evaluation, the virulence of different isolates of *C. acutatum* on strawberry, pepper and safflower was different. The strawberry variety Pegasus was more susceptible to *C. acutatum* than the variety Elkas. The pepper variety Pirouet was more susceptible than the variety Cynthia. No statistical difference was shown between susceptibility of the safflower varieties. Lewis Ivey *et al.* (2004) also tested tomato. It was found that wounded and nonwounded mature and immature tomato fruits were susceptible to isolate from pepper too.

Comparison of virulence of isolates *C. acutatum* was also carried out on different plant species. Isolates 710 from *H. perforatum*, 1209 from safflower, 29267 from pepper and 231 from strawberry showed different virulence for tested host plant species – while for both strawberry varieties isolate 710 proved the highest index of infection intensity, for both pepper varieties and the safflower varieties showed the lowest virulence. Differences in virulence were also found in isolate 1209 from safflower – while one of the highest virulence was confirmed for both safflower varieties, this isolate was the least virulent for both pepper varieties. On the contrary, isolate 29267 from pepper was the most virulent for the pepper variety Cynthia, but this isolate belonged to the least virulent isolates in both safflower varieties and the strawberry variety Elkas. A similar situation was found in isolate 231 from strawberry – it was the most virulent for the pepper variety Pirouet, but the least virulent for both safflower varieties and the strawberry variety Pegasus.

The cross virulence of *C. acutatum* to different host was proved. Knowledge concerning virulence of this pathogen may be used in breeding of new varieties of host plants and arrangement of agro-technical measures to prevent this pathogen.

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