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Morphological and ecological features as differentiation criteria for *Colletotrichum* species

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Abstract

Colletotrichum acutatum and *Colletotrichum gloeosporioides* are important post harvest apple fruit pathogens in temperate regions. Seven *Colletotrichum* isolates obtained from apple fruits with anthracnose symptoms, and two reference isolates *C. gloeosporioides* AVO 37 4B (avocado, Israel) and *C. acutatum* TUT 137 A (strawberry, Israel) were investigated. Pathogenicity of all nine isolates was demonstrated on wound-inoculated apple fruits by fulfilling Koch's postulates. Investigated morphological characteristics involved shape, colour and margin of the colony, as well as conidial shape. Observed ecological parameters were growth and sporulation of isolates after 4, 7 and 11 days of incubation at temperatures of 15, 20, 23, 25, 27, 30, 32 and 35°C and after 10 days of incubation under conditions of day light regime and dark. The isolates were identified by PCR using species-specific primers. The data regarding growth were processed by factorial and one way *ANOVA* using software *Statistica 10*.
All seven isolates obtained from apple fruits and reference *C. acutatum* isolate formed velvety, gray colonies with slightly wavy margin, and one celled fusiform conidia on PDA medium. Mycelium of the reference isolate *C. gloeosporioides* AVO 37 4B was snow-white and conidia were cylindrical. Maximum mycelial growth rate for isolates obtained from apple fruits was at temperature of 23°C, for reference isolate *C. acutatum* at 27°C and for *C. gloeosporioides* at 23–27°C. Temperature of 35°C proved to be restrictive for *C. acutatum* isolates. Isolate *C. gloeosporioides* had the fastest growth rate compared to all other isolates. Using PCR with species-specific primers all eight isolates were identified as *C. acutatum* (fragments sized 490 bp were amplified), and one as *C. gloeosporioides* (fragments sized 450 bp were amplified).
The results suggest that differentiation between the two *Colletotrichum* species based on several morphological and ecological parameters is possible.

Key words: apple, anthracnose, identification, *Colletotrichum* spp.

Introduction

Fungi of the genus *Colletotrichum* and their teleomorph *Glomerella* spp. are causal agents of the plant disease known as anthracnose. They cause significant economic damage to crops in tropical, subtropical, and temperate regions. Cereals, legumes, ornamentals, vegetables and fruit trees can be severely affected by pathogens of this genus (Freeman et al., 1998; Peres et al., 2002; Lewis Ivey et al., 2004; Gregori et al., 2010).

Fruit species of temperate region are frequently susceptible to diseases caused by *Colletotrichum* spp., particularly by *C. acutatum* J.H. Simmonds and *C. gloeosporioides* (Penz.) Penz. i Sacc (Wharton, Dieguez-Uribeondo, 2004). The presence of the two mentioned species in Serbia has been registered on various fruit and vegetable species (Ivanović et al., 2005; Živković, 2011). Sour cherry, apple and pear are the most common hosts among fruit species (Ivanović et al., 2005; Grahovac et al., 2010; Živković, 2011).

Disease outbreaks can occur rapidly and losses can be severe especially under prolonged warm and wet weather conditions (Biggs, Miller, 2001). Two distinct types of anthracnose disease can occur: the type that affects developing fruit in the field (preharvest) and the type that affects mature fruit during storage (postharvest). Due to ability to cause latent or quinsent infections, *Colletotrichum* spp. are considered as very important postharvest pathogens. Infection can occur at all developmental stages of the plant, but in most cases, the most severe economic losses are recorded in postharvest fruit infections. Typical symptoms of postharvest anthracnose are observed as circular, dark, sunken lesions that produce mucilaginous, pink to orange conidial masses. Under se-

vere disease pressure, the lesions can coalesce (Freeman et al., 1998; Lewis Ivey et al., 2004).

Morpho-taxonomic criteria such as conidial shape and size, apressoria morphology and size, setae morphology, temperature response on potato dextrose agar medium (PDA) and host specificity, as well as molecular identification techniques, are currently in use for identification of *Colletotrichum* spp. (Sutton, 1992; Freeman et al., 1998). Given that morphological features vary considerably with environmental conditions (Wasantha Kumara, Rawal, 2008) and that isolates have overlapping ranges of conidial and colony characteristics and because variation in morphology is accepted for isolates within a species (Sutton, 1992), molecular confirmation of identification results is desirable.

Concerning that both species are well known as postharvest pathogens of apple fruit, the aims of the study were to identify the causal agent of apple fruit rot to the species level, and to determine if colony or conidial morphology, as well as isolates growth rate at different temperatures can be used as identification criteria for *Colletotrichum* species.

Materials and methods

The investigation was conducted during summer 2011.

Pathogen isolation. The isolates were obtained from infected apple fruits with anthracnose symptoms, collected from storages at four localities (Radmilovac, Arilje, Uzići and Belgrade) in winter 2010–2011 and spring 2011. The pathogen was isolated using standard phytopathological isolation techniques. Infected apple fruits were surface-sterilized with 96% ethyl alcohol, cut at the turn of diseased to healthy tissue, and tissue fragments were aseptically placed on sterile potato dextrose agar medium (PDA) and incubated at 25°C for seven days. After seven days, the obtained mycelium was subcultured to sterilized PDA medium to obtain a pure culture. The obtained pure cultures were incubated for three days on PDA slant at 20°C and afterwards kept in a refrigerator at 4°C until use (Dhingra, Sinclair, 1995).

C. gloeosporioides AVO 37 4B (avocado, Israel) and *C. acutatum* TUT 137 A (strawberry, Israel) were used as reference islolates.

Pathogen identification. The isolates were identified according to pathogenic, morphological and ecological characteristics, and the identification was confirmed by polimerase chain reaction (PCR).

Pathogenicity test and reisolation. The obtained isolates were tested for pathogenicity by artificial inoculation of injured apple fruits (Vignutelli et al., 2002). Mycelial fragments from PDA cultures were placed on previously surface-sterilised and injured healthy apple fruits. Fruits inoculated with sterile fragments of PDA medium were used as a control. Inoculated fruits were incubated in a wet chamber at room temperature, and the symptom occurrence was observed daily during 10 successive days. After rot symptoms occurred, the pathogen was reisolated from inoculated fruits on PDA medium, and the obtained re-isolates were compared with the isolates used for artificial inoculation.

Morphological characteristics of isolates. Morphological characteristics of the isolates were investigated after 11-day incubation on PDA medium at 25ºC. The following parameters were observed: colony shape, colour, margin and conidial shape. Conidia were observed under light microscope.

Mycelial growth rate assay. Three-millimeter-diameter mycelial plugs were cut from the margin of seven days old colony, placed on PDA medium and incubated at temperatures of 15, 20, 23, 27, 30, 32 and 35°C to test the influence of temperature on isolates growth. Colony diameter was measured in two perpendicular directions, after incubation of four, seven and eleven days. The effect of light was investigated by incubation of the isolates at room temperature under day light regime and in dark. Colony diameter was measured in two perpendicular directions, after incubation of ten days. The trials were conducted independently in three replicates, the data were processed by factorial and one way *ANOVA* using software *Statistica 10* (2010). Duncan's multiple range test was used to test significance of difference between means of colony diameters at 5% significance level.

DNA extraction. Total amount of deoxyribonucleic acids (DNA) was extracted from seven days old mycelia of the isolates grown on PDA medium according to method described by Harrington and Wingfield (1995).

Molecular identification of isolates. Species-specific primers for *C. gloeosporioides* (CgInt) and *C. acutatum* (CaInt2) from the ITS1 region of the ribosomal DNA gene in combination with the conserved primer ITS4 were used for the reaction, according to protocol described by Sreenivasaprasad et al. (1996) and Lewis Ivey et al. (2004). Primers are shown in Table 1. Each of 25 µl reaction mixture contained: 2.5 µl of DNA (50 ng μl^{-1}), 0.12 µl of each 10 µM primer, 0.08 µl of 10 mM dNTP, 0.5 µl of *Taq* polymerase (5 U μl^{-1}), 1.5 µl of 25 mM MgCl, 2.5 µl of 10 × polymerase buffer and 16.9 µl of sterile milli-Q water. Reaction PCR mix without added DNA served as a negative control. PCR reactions were performed in Eppendorf master cycler and the reaction conditions were as follows: 5 min at 94°C, 30 cycles of 1.5 min at 94°C, 2 min at 55° and 3 min at 72°C, and then a 10-minute final extension at 72°C. PCR products (7 µl) were separated by horizontal gel electrophoresis in 1.5% agarose gel 0.5 × TBE buffer at 100 V constant voltage for 60 minutes. Gels were stained in ethidium bromide solution (2 µg ml^{-1}) and visualised under UV light. Molecular weight of the obtained PCR product was determined according to its position in relation to 1 kb DNA marker ("Fermentas", Lithuania). The occurrence of amplicons about 490 bp in size was considered as positive reaction for *C. acutatum*, and of 450 bp for *C. gloeosporioides*.

Table 1. Primers used for detection of *C. acutatum* and *C. gloeosporioides*

Primer set	Primer name	Sequence (5'-3')
Reverse primer	ITS4	TCCTCCGCTTATTGATATGC
Forward primer for *C. acutatum*	CaInt2	GGGGAAGCCTCTCGCGG
Forward primer for *C. gloeosporioides*	CgInt	GGCCTCCCGCCTCCGGGCGG

Results and discussion

Pathogen isolation, pathogenicity test and morphological characteristics of the isolates. Seven isolates were obtained from collected apple fruits with anthracnose symptoms. All tested isolates, including reference isolates, caused anthracnose symptoms on inoculated apple fruits after incubation of three days. Anthracnose concentrically spread from inoculation site, and dark sunken lesions were formed (Fig. 1).

Figure 1. Pathogenicity test: anthracnose symptoms on artificially inoculated apple fruit by mycelial fragments of the isolate IS200 (left) and by sterile PDA fragments, after ten days of incubation

The symptoms were not observed in the control. The fungus was re-isolated onto PDA from the lesions on the inoculated apples. After seven days of incubation, the colonies were the same as those of the original isolates. All seven isolates and the reference isolate *C. acutatum* TUT 137 A, formed velvety, gray colonies with slightly wavy margin on PDA medium. At the beginning, the mycelium was snow-white and with culture aging it became pale gray. The underside of the colony was cream coloured or pale gray to tan, never dark, which is in agreement with *C. acutatum* colony appearance described by Strandberg and Chellemi (2002). However, contrary to colonies described by the mentioned authors, pale pink or orange-pink pigment surrounding the colony was not formed within agar media. Mycelium of the reference isolate *C. gloeosporioides* AVO 37 4B remained snow-white during the whole incubation period (Fig. 2).

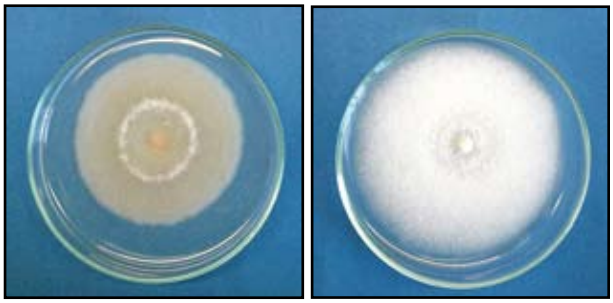


Figure 2. Colony of the isolate IS1 (left) and isolate AVO 37 4B (right) incubated at 25°C for eleven days

All seven isolates as well as the isolate TUT 137 A formed orange acervuli with hyaline one-celled fusiform conidia (Fig. 3). Given that colony appearance and conidia shape of tested isolates agreed with those of reference *C. acutatum* isolate, they could be identified as *C. acutatum*. The reference isolate AVO 37 4B formed yellow acervuli with one-celled cylindrical conidia.

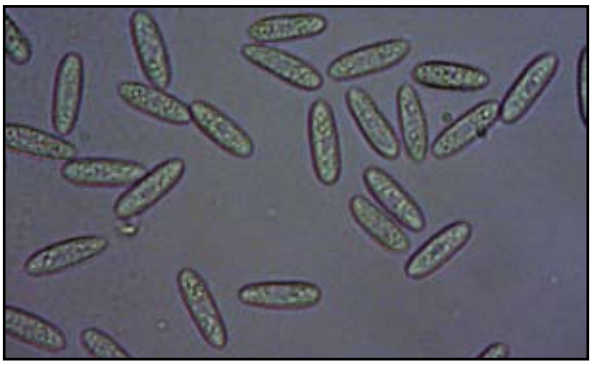
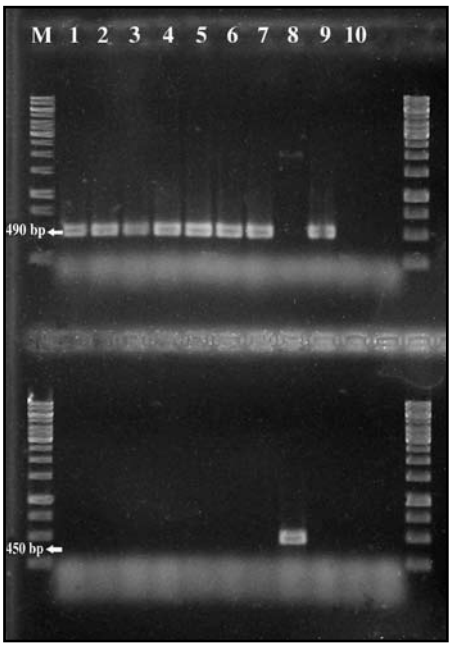


Figure 3. Conidial morphology of *C. acutatum* isolate IS200 grown at 25°C for seven days – microscopic view (40^{x} magnification)

Molecular identification. *C. acutatum* specific primer – CaInt2, in conjunction with ITS4 primer, amplified a 490 bp fragment from genomic DNA from isolates IS1, IS2, IS21, IS23, IS154, IS166 and IS200 and from reference *C. acutatum* isolate, but not from isolate AVO 37 4B. Contrary, a 450 bp fragment was amplified from genomic DNA of reference *C. gloeosporioides* isolate AVO 37 4B with species-specific primer CgInt and primer ITS4. CgInt did not amplify a product from DNA of all seven apple isolates and reference TUT 137 A isolate. In PCR reaction, no amplicons occurred in negative control (Fig. 4). According to Lewis Ivey et al. (2004), molecular tools such as PCR with species-specific primers are useful in distinguishing species of *Colletotrichum* that cannot be distinguished using morphological methods. However, identification of isolates by PCR reaction in our study was in agreement with the identification based on morphological characteristics.



Note. Columns 1–7 isolates from apple fruits (IS1, IS2, IS21, IS23, IS154, IS166 and IS200), column 8 reference isolate *C. gloeosporioides* AVO 37 4B, column 9 reference isolate *C. acutatum* TUT 137 A, column 10 negative control.

Figure 4. Visualisation of the amplicons obtained by species-specific primer pairs for *C. acutatum* CaInt2 and ITS 4 and for *C. gleosporioides* CgInt and ITS4 in 1.5% agarose gel

Mycelial growth rate assay. Among environmental factors which affect growth of fungi, temperature plays an extremely important role. Temperature affects almost every function of the fungi (Agrios, 2005).

The results of the study indicate that there were significant differences between isolates, temperatures and their interaction when observed four days after inoculation. The biggest source of variation in isolates growth was temperature (Table 2).

Growth of isolates after four days of incubation at different temperatures is shown in Figure 5. Maximum mycelial growth rate was recorded at 23°C for isolates IS1, IS21 and IS154, and at 23–25°C for isolates IS2, IS23 and IS166. Reference isolates TUT 137 A showed maximum growth at 27°C and AVO 37 4B at 25–27°C. No growth for isolates identified as *C. acutatum* was recorded at temperatures of 32°C and 35°C, while isolate *C. gloeosporioides* grew at these temperatures, and showed significantly higher growth rate at 32°C than at 35°C. Isolate AVO 37 4B had the fastest growth rate compared to all other isolates, except for IS1 and IS200 which at 20°C had growth rate at the same level of significance as AVO 37 4B. The isolate IS1 at 15°C had significantly higher growth rate compared to AVO 37 4B.

Table 2. Results of analysis of variance for mycelial growth of *C. acutatum* and *C. gloeosporioides* isolates after four days of incubation at different temperatures

Source of variation	SS	Degree of freedom	MS	*F-value*	*p-value*
Isolate	8203.6	8	1025.4	865.1	0.00
Temperature	62710.1	7	8958.6	7557.3	0.00
Isolate × temperature*	8273.9	56	147.7	124.6	0.00
Error	426.8	360	1.2		

**ANOVA* – factorial analysis of variance, SS – sum of squares, MS – mean square

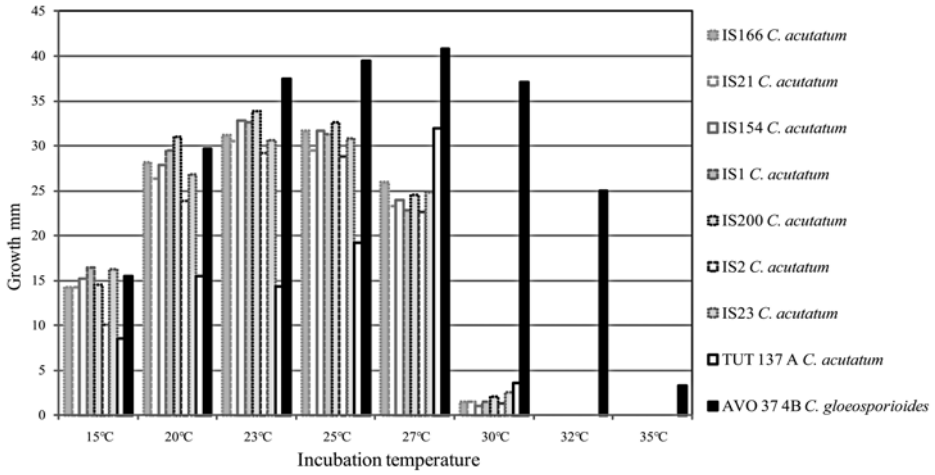


Figure 5. Growth of the isolates on PDA medium after four days of incubation at different temperatures

After seven days of incubation, temperature was still the biggest source of variation for isolates growth, although isolates and temperature × isolate interaction also had significant effect on growth (Table 3).

Table 3. Results of analysis of variance for mycelial growth of *C. acutatum* and *C. gloeosporioides* isolates after seven days of incubation at different temperatures

Source of variation	SS	Degree of freedom	MS	*F-value*	*p-value*
Isolate	34331.3	8	4291.4	3273.4	0.00
Temperature	193504.7	7	27643.5	21085.9	0.00
Isolate × temperature*	26197.5	56	467.8	356.8	0.00
Error	472.0	360	1.3		

**ANOVA* – factorial analysis of variance, SS – sum of squares, MS – mean square

Seven days from inoculation the isolates IS1, IS2, IS21, IS23, IS200 had maximum mycelial growth rate at 23°C, while IS154 and IS166 had maximum growth at 23–25°C. Temperature of 27°C provided maximum mycelial growth for both reference isolates. Significantly higher growth rate was observed for reference isolate *C. gloeosporioides* (AVO 37 4B) compared to all other isolates at temperature of 23°C and higher. After seven days of incubation, growth of all tested isolates was recorded at 32°C, although the growth of AVO 37 4B was the most significant. However, at 35°C no growth was observed for the isolates identified as *C. acutatum* (IS1, IS2, IS21, IS23, IS154, IS200, TUT 137 A), while *C. gloeosporioides* (isolate AVO 37 4B) still grew at this temperature, but the growth was significantly lower compared to its growth at 32°C (Fig. 6).

Temperature remained the biggest source of variation for isolates growth after eleven days of incubation. Isolates and temperature × isolate interaction also had significant effect on growth (Table 4).

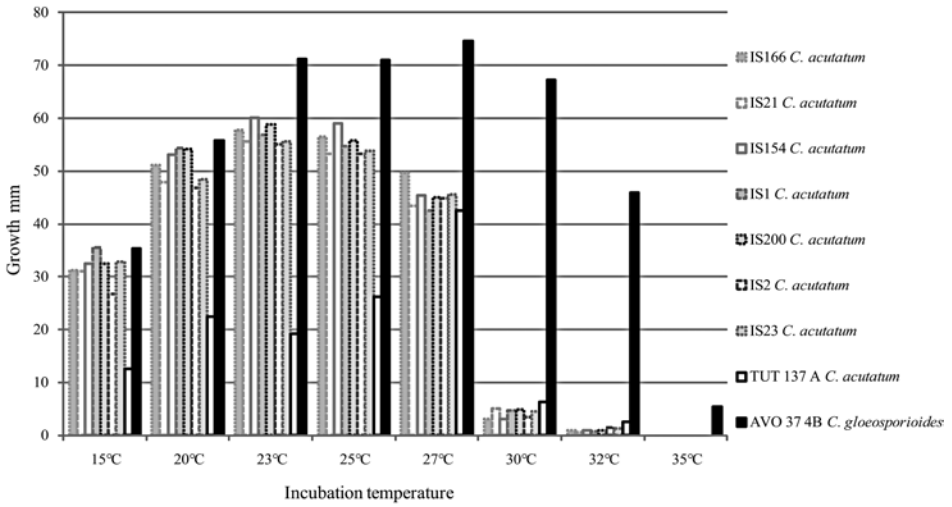


Figure 6. Growth of the isolates on PDA medium after seven days of incubation at different temperatures

Table 4. Results of analysis of variance for mycelial growth of *C. acutatum* and *C. gloeosporioides* isolates after eleven days of incubation at different temperatures

Source of variation	SS	Degree of freedom	MS	*F-value*	*p-value*
Isolate	61349.5	8	7668.7	2299.6	0.00
Temperature	369559.1	7	52794.2	15831.1	0.00
Isolate × temperature*	47890.3	56	855.2	256.4	0.00
Error	1200.5	360	3.3		

**ANOVA* – factorial analysis of variance, SS – sum of squares, MS – mean square

As shown in Figure 7, all isolates derived from apple fruits (IS1, IS2, IS21, IS23, IS154, IS166, IS200) had maximum mycelial growth rate at 23°C after eleven days of incubation. Reference isolates had maximum growth at 27°C (TUT 137 A) and 23–27°C (AVO 37 4B). After eleven days of incubation at 35°C, no growth was recorded for *C. acutatum* isolates, while reference isolate *C. gloeosporioides* (AVO 37 4B) still grew at this temperature, yet at the lowest significance level compared to its growth at other temperatures. Reference isolate *C. gloeosporioides* (AVO 37 4B) had the highest growth rate at all investigated temperatures compared to all other tested isolates (*C. acutatum*), except at 20°C where its growth was at the same level of significance with isolates IS1 and IS200.

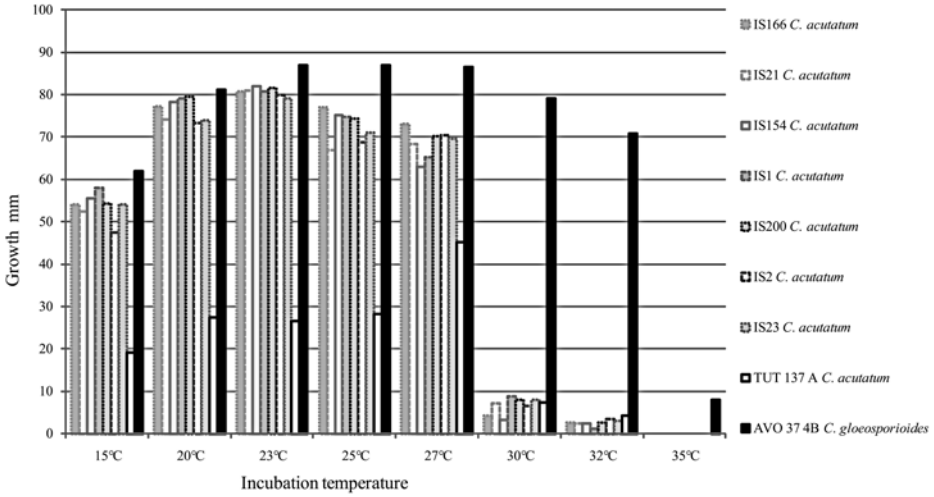


Figure 7. Growth of the isolates on PDA medium after eleven days of incubation at different temperatures

A number of investigations indicate that isolates of the species *C. acutatum* have significantly slower growth rate compared to isolates of *C. gloeosporioides* (Smith, Black, 1990; Bernstein et al., 1995; Shi et al., 1996; McKay et al., 2009), which was confirmed in our study. However, according to Freeman et al. (1998), difference in growth rate and optimal growth temperature is not always a reliable criterion for species identification. The same author stated that the use of species-specific primers for PCR amplification of unique rDNA fragments seems the most promising for differentiation among the species of *Colletotrichum.* In our study, differentiation

between *C. acutatum* and *C. gloeosporioides* based on mycelial growth rate was consistent with the results of PCR reaction. Differentiation between the mentioned *Colletotrichum* species was not possible on the basis of optimal growth temperature in our investigation. However, optimal growth temperature of all islolates originating from apple fruits from temperate region storages was 23°C (Fig. 7), while the isolate of the same species originating from subtropical region (Israel) from strawberry had optimal growth temperature of 27°C. This difference in optimal growth temperature between the isolates of the same species may be in connection with climate zone from which the isolates originate (Freeman et al., 1998; Bardas et al., 2009). Therefore, it may be expected that isolates of the same species and climate zone will have the same optimal growth temperature which could be a reliable criterion for their differentiation from other *Colletotrichum* species. In the study conducted by McKay et al. (2009), isolates of *C. acutatum* from almond in Australia grew the fastest at 25°C, except one isolate for which the mycelial growth rate at 20°C was the same as at 25°C. Likewise, the maximum mycelial growth rate for isolates representative of the Californian pink and gray subpopulations of *C. acutatum* occurred at 25°C, whereas an isolate representative of Israeli *Colletotrichum* spp. from almond, grew more rapidly at 20°C and 25°C than at other temperatures. In the same study, the only isolates to grow at 35°C were two *C. gloeosporioides* from orange in Australia and one *C. acutatum* isolate from almond in Australia. In our study, the temperature of 35°C showed to be a restrictive temperature for *C. acutatum*, but not for *C. gloeosporioides* and proved as reliable differentiation criterion. However, in our study, the growth of only one *C. gloeosporioides* isolate was investigated, therefore, future investigations are needed to confirm that the temperature of 35°C is restrictive to *C. acutatum* and can serve as a reliable criterion for differentiation between *C. acutatum* and *C. gloeosporioides*.

At all investigated temperatures below 30°C, eleven days after inoculation, all isolates identified as *C. acutatum* sporulated on PDA medium. For the *C. gloeosporioides* isolate AVO 37 4B no sporulation was observed at temperature of 20°C and lower, as well as at temperatures above 30°C. In the study of temperature effect on sporulation of *C. gloeosporioides* isolates, Wasantha Kumara and Rawal (2008) recorded no spore production at 15–20°C and Sangeetha and Rawal (2010) at 15–20°C and 30°C, which is in agreement with our results.

After incubation of 10 days at room temperature under conditions of day light regime and dark, the isolate AVO 37 4B had significantly higher growth rate compared to all other isolates, as shown in Figure 8. This confirmed once more that *C. gloeosporioides* have significantly higher growth rate than *C. acutatum* species (Smith, Black, 1990; Bernstein et al., 1995; Shi et al., 1996) when grown under the same conditions.

The isolates IS2, IS23, IS166, TUT 137 A and AVO 37 4B had significantly higher growth rate in conditions of day light regime, while the isolates IS200 and IS1 had higher growth rate in conditions of day light regime. Only two isolates had higher growth rate in dark (IS21 and IS154), but not significantly compared to growth under day light regime. Differences in growth under conditions of day light regime and dark cannot be used for reliable differentiation between the two *Colletotrichum* species

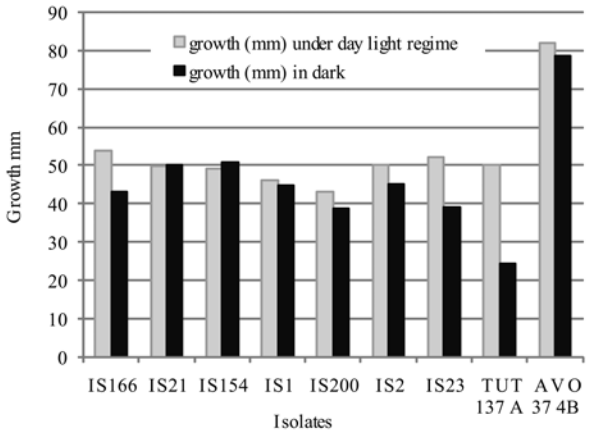


Figure 8. Growth rates of tested and reference isolates after ten days incubation at room temperature under conditions of day light regime and dark

As indicated in Table 5, the biggest source of variability in growth under conditions of day light regime and dark at room temperature were isolates, yet conditions of growth (light/dark) as well as their interaction (isolate × condition light/dark) had also significant impact on isolate growth.

Table 5. Results of analysis of variance for mycelial growth of *C. acutatum* and *C. gloeosporioides* isolates after ten days of incubation at room temperature in conditions of day light regime and dark

Source of variation	SS	Degree of freedom	MS	*F-value*	*p-value*
Isolate	9686.3	8	1210.8	195.32	0.000000
Condition light/dark	754.0	1	754.0	121.63	0.000000
Isolate × condition light/dark*	1110.6	8	138.8	22.39	0.000000
Error	334.8	54	6.2		

**ANOVA* – factorial analysis of variance, SS – sum of squares, MS – mean square

Conclusions

1. All tested isolates (*Colletotrichum acutatum* and *C. gloeosporioides*) caused dark, sunken lesions on the infected fruit tissue, and were hard to differentiate.

2. Differentiation between *C. acutatum* and *C. gloeosporioides* based on colony shape, margin and colour, colour of acervuli, conidia shape, growth rate, restrictive temperature for growth and sporulation proved to be possible, which was confirmed by PCR reaction using species-specific primers.

3. Isolates identified as *C. acutatum* formed velvety, gray colonies with slightly wavy margin, orange acervuli and one celled, fusiform conidia. Reference isolate of *C. gloeosporioides* formed snow white colony, yellow acervuli and one celled, cylindrical conidia.

4. Temperature of 35°C proved to be restrictive for all tested *C. acutatum* isolates.

5. All tested *C. acutatum* isolates sporulated at temperature below 30°C, while for *C. gloeosporioides* no sporulation was observed at temperature of 20°C and lower, and above 30°C.

6. *C. acutatum* had significantly lower growth rate compared to *C. gloeosporioides*.

7. Optimal growth temperature, as well as growth in conditions of day light regime and dark did not prove to be reliable criteria for differentiation of these two species.

8. Optimal growth temperature for *C. acutatum* isolates ranged from 23°C (isolates derived from apple fruits from storages in the Republic of Serbia) to 27°C (reference isolate from strawberry, Israel). For *C. gloeosporioides* (avocado, Israel) optimal growth temperature was in the same range.

9. Given that temperature affects almost every function of the fungi and that favourable environmental conditions are needed for the outbreak of the disease, the results of this study regarding temperature requirement are valuable for practice.

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Deguliagrybių (*Colletotrichum* spp.) atskyrimo morfologiniai ir ekologiniai kriterijai

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Santrauka

Colletotrichum acutatum ir *Colletotrichum gloeosporioides* yra svarbūs nuskintų obuolių patogenai vidutinio klimato regionuose. Tirti septyni *Colletotrichum* izoliatai, išskirti iš obuolių su antraknozės simptomais, ir du kontroliniai izoliatai *C. gloeosporioides* AVO 37 4B (avokadai, Izraelis) bei *C. acutatum* TUT 137 A (braškės, Izraelis). Visų devynių izoliatų patogeniškumas nustatytas ant obuolių, žaizdas inokuliuojant pagal Kochą. Tirtos šios morfologinės savybės: kolonijos spalva bei kraštai ir konidijų forma. Analizuoti šie ekologiniai rodikliai: izoliatų augimas ir sporuliacija po 4, 7 ir 11 dienų inkubacijos 15, 20, 23, 25, 27, 30, 32 ir 35° C temperatūroje ir po 10 inkubacijos dienų šviesoje ir tamsoje. Izoliatai identifikuoti PGR, naudojant rūšiai specifinius pradmenis. Izoliatų augimo duomenys analizuoti naudojant faktorinę analizę ir vieno veiksnio dispersinę analizę, taikant *ANOVA* bei kompiuterinę programą *Statistica 10*.

Visi septyni izoliatai, gauti iš obuolių, ir kontrolinis izoliatas *C. acutatum* ant PDA terpės formavo pilkai aksomines kolonijas šiek tiek banguotais kraštais ir vienaląstes fuzines konidijas. Kontrolinio izoliato *C. gloeosporioides* AVO 37 4B micelis buvo sniego baltumo, o konidijos cilindrinės. Maksimalus micelio augimo greitis izoliatų, gautų iš obuolių, buvo esant 23° C, kontrolinio izoliato *C. acutatum* – 27° C, o *C. gloeosporioides* – 23–27° C temperatūrai. Tačiau 35° C temperatūra pasirodė ribojanti *C. acutatum* izoliatams. *C. gloeosporioides* sparčiausiai augo, palyginti su kitais tirtais izoliatais. Naudojant PGR su rūšims specifiniais pradmenimis, buvo identifikuoti aštuoni *C. acutatum* (buvo amplifikuoti 490 bp fragmentai) ir vienas *C. gloeosporioides* (buvo amplifikuoti 450 bp fragmentai) izoliatai.

Tyrimų rezultatai parodė, kad remiantis keliais morfologiniais ir ekologiniais rodikliais galima atskirti dvi tirtas *Colletotrichum* rūšis.

Reikšminiai žodžiai: obuoliai, antraknozė, identifikavimas, *Colletotrichum* spp.