Plant Disease • 2021 • 105:3510-3521 • https://doi.org/10.1094/PDIS-01-21-0037-RE

# *Penicillium* and *Talaromyces* Species as Postharvest Pathogens of Pear Fruit (*Pyrus communis*) in Serbia

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## Abstract

Pears are one of the oldest and the third most important fruit species grown in temperate regions. They are consumed because of their nutritional and health benefits, in fresh form or as various processed products. This article resolves the etiology of the *Penicillium*-like mold symptoms on pear fruits in Serbia. Samples of pear fruits with blue mold and other *Penicillium*-like mold symptoms were collected in Serbia from 2016 to 2019, from four storages. The recovered isolates were identified and characterized according to a polyphasic approach. Morphological and physiological analyses were performed on three media and five temperatures, respectively. Four loci (internal transcribed spacer, beta-tubulin, calmodulin, and DNA-dependent RNA polymerase II second largest subunit) were used for sequencing, genetic identification, and phylogenetic analyses. The results of the identification by conventional and molecular methods were in agreement, and they revealed that the obtained isolates belong to five species: *Penicillium crustosum*, *P. expansum*, *P. italicum*, *Talaromyces minioluteus*, and *T. rugulosus*. In a pathogenicity test, *P. crustosum*, *P. expansum*, *T. minioluteus*, and *T. rugulosus* produced decay on artificially inoculated pear fruits, and *P. italicum* induced tissue response lesions. The results of this study are the first reports of *T. minioluteus* and *T. rugulosus* as postharvest pear pathogens. Also, these are the first world records of *T. minioluteus*, *T. rugulosus*, and *P. italicum* on fruits of European pear. Furthermore, this is the first finding of *P. crustosum*, *P. expansum*, *P. italicum*, *T. minioluteus*, and *T. rugulosus* on pear fruit in Serbia.

*Keywords*: blue mold, identification, multigene phylogeny, pear fruit, *Penicillium*, postharvest pathogens, *Talaromyces*

Pears (*Pyrus* spp.) are fruits from Rosaceae family, widely grown on every continent of the world except Antarctica (Chagné et al. 2014; Wu et al. 2013). After grapes and apples, pears are the third most important temperate fruit species and one of the oldest fruit crops, with cultivation dating to >3,000 years ago. *Pyrus* is a genetically diverse genus, with at least 22 primary species and thousands of cultivars, but only *Pyrus* × *bretschneideri*, *P. pyrifolia*, *P. ussuriensis*, *P.* × *sinkiangensis*, and *P. communis* are used for commercial fruit production (Wu et al. 2013). In 2018 world production of pears was 23,733,772 t on 1,381,923 ha (United Nations Food and Agriculture Organization 2020). European pear (*Pyrus communis* L.) is a commercially cultivated pear species in Serbia, with 52,291 t of total production on 5,703 ha. This ranks pears in the sixth place of all of the fruit types produced in Serbia in 2017 (Statistical Office of the Republic of Serbia 2020).

Pear fruits are an important part of a balanced human diet and are often consumed in fresh form, but they are also used to produce marmalades, purées, and various alcoholic and nonalcoholic drinks (Chen et al. 2007; Kolniak-Ostek 2016). They are a good source of fiber, vitamin C, and potassium (Reiland and Slavin 2015). Pear fruits are also rich in flavonoids and antioxidants, so regular consumption of pears (and apples) can lead to a decreased body mass index and a lower risk of cardiovascular diseases, according to the findings of a meta-analysis conducted by Gayer et al. (2019).

Species of numerous fungal genera are responsible for pear fruit losses, in the orchards and after harvest: *Alternaria*, *Botrytis*, *Cadophora*, *Colletotrichum*, *Diplocarpon*, *Monilinia*, *Neofabrea*, *Penicillium*, *Podosphaera*, *Phytophthora*, *Rhizopus*, *Trichothecium*, and *Venturia* (Fu et al. 2019; Sardella et al. 2016; Živković 2011). Members of penicillia are among the most important and the most destructive postharvest pathogens of pear, which can destroy ≥50% of fruits if storage conditions are inadequate (Sardella et al. 2016; Scholtz and Korsten 2016; Snowdon 1990). *Penicillium*-associated pear fruit postharvest diseases are usually described as blue mold, characterized by the presence of blue-green conidial masses and a specific, musty odor (Jones and Aldwinckle 1990). *Penicillium aurantiogriseum*, *P. aurantiogriseum* var. *aurantiogriseum* (referred as *P. puberulum*), *P. brevicompactum*, *P. commune*, *P. crustosum*, *P. cyclopium*, *P. digitatum*, *P. expansum*, *P. griseofulvum*, *P. polonicum*, *P. spinulosum*, *P. solitum*, and *P. verrucosum* are *Penicillium* species confirmed as pathogens of pear fruits in previous studies (Barkai-Golan 1974; Borecka 1977; Khokhar et al. 2019; Kim et al. 2002; Louw and Korsten 2014; Neri et al. 2006, 2010; Sanderson and Spotts 1995; Shim et al. 2002; Zhang et al. 2006). Species of *Talaromyces* are usually not regarded as spoilage agents of pears. The only record comes from Borecka (1977), who reported *Talaromyces diversus* (referred as *P. diversum*) from decayed pear fruits in Poland.

Besides a major contribution to postharvest losses in pear fruit production, species of *Penicillium* and *Talaromyces* are also important because they are consistent producers of mycotoxins. These substances can compromise human and animal health when contaminated food or feed is consumed. Patulin, ochratoxin A, citrinin, penicillic acid, cyclopiazonic acid, citreoviridin, penitrem, PR-toxin, and secondary metabolites such as chaetoglobosins, communesins, roquefortine C, expansolides, janthitrems, and paxillines, are some of the metabolites produced by *Penicillium* and *Talaromyces* species (Barkai-Golan 2008). Of those listed, only patulin and ochratoxin A are *Penicillium* or *Talaromyces* mycotoxins whose levels are regulated by law in many countries of the world, including Serbia (van Egmond and Jonker 2008). Patulin exhibits acute toxic, teratogenic, cardiotoxic, immunotoxic, and gastrointestinal adverse effects (Frisvad 2018; Saleh and Goktepe 2019). This mycotoxin has been denoted as a possible genotoxic by the World Health Organization (WHO 2005). Ochratoxin A also has a number of negative influences on humans and animals (teratogenic, embryotoxic, genotoxic, neurotoxic, immunosuppressive, carcinogenic, and nephrotoxic), and its harmful effects can be amplified when it is produced with other mycotoxins (Klarić et al. 2013).

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**Funding:** This study was financially supported by Ministarstvo Prosvete, Nauke i Tehnološkog Razvoja Republike Srbije (Ministry of Education, Science and Technological Development of the Republic of Serbia, contract no. 451-03-9/2021-14/200010).

The author(s) declare no conflict of interest.

Accepted for publication 18 May 2021.

To the best of our knowledge, there are no reports of *Penicillium* and *Talaromyces* species as pathogens of pear fruits in Serbia. Pathogenic species of these genera are detected in Serbia on other pomaceous fruits: *P. expansum* and *P. crustosum* on apple fruit (Vico et al. 2014a, b) and *T. minioluteus* on quince fruit (Stošić et al. 2020). Identification of the *Penicillium* and *Talaromyces* species as causal decay agents on pear fruits based on the expressed symptoms is not possible, because different species often can cause similar symptoms, so an integrative (polyphasic) approach is used in this study. A combination of different morphological, physiological, and molecular methods was applied to identify the species of these genera, as recommended by Visagie et al. (2014) and Yilmaz et al. (2014). Therefore, the main objectives of the current study were examination of the etiology of blue mold (*Penicillium*-like) symptoms on pear fruit in Serbia; identification and characterization of isolates of *Penicillium* and *Talaromyces* from pear via a polyphasic approach; sequencing of four molecular markers (internal transcribed spacer [ITS], beta-tubulin [*BenA*], calmodulin [*CaM*], and DNA-dependent RNA polymerase II second largest subunit [*RPB2*]) and their use in exploring the phylogenetic relationships of the *Penicillium* and *Talaromyces* isolates obtained from Serbia with other species of these genera; and pathogenicity assessment of the collected isolates on pear fruit.

## Materials And Methods

**Sample collection and fungal isolation.** Samples of pear fruits (cultivar Williams) with blue mold and other *Penicillium*-like mold symptoms were collected in Serbia in 2016 to 2019, from four privately owned storage facilities that did not have strictly controlled air temperature and relative humidity. The numbers of collected samples/obtained isolates per storage were 58/15 in Ruma, 55/14 in Smederevo, 57/17 in Grocka, and 68/21 in Kraljevo. In total, 238 samples were collected and 67 isolates were recovered. Fruit collection was done during the winter, 3 to 4 months after the harvest. Collected fruits had one or a combination of the following symptoms: brown, round, sometimes sunken spots, watery tissue, fruit skin discoloration, developed white and yellow mycelia, or the presence of blue-green spores. We isolated the fungi by removing small pieces from the margin of the infected and healthy tissue with a sterilized scalpel and disinfecting them in 1% aqueous solution of NaOCl for 2 min. The pieces were then rinsed with sterile distilled water and placed on malt extract agar (MEA) plates, which were incubated for 7 days in the dark at 25°C. Monosporial cultures were subsequently obtained via serial dilution (Dhingra and Sinclair 1995) and conserved on potato dextrose agar slants at 4°C in the fungal collection of the Institute for Plant Protection and Environment, Belgrade, Serbia. Representative isolates were chosen for morphological and molecular identification (ITS and *BenA*), and a subset of isolates was used for further molecular characterization (*CaM*, *RPB2*) and physiological and pathogenicity assays (Table 1).

**Morphological analyses.** The selected isolates were inoculated at three points on 90-mm Petri dishes containing Czapek yeast autolysate agar (CYA), MEA, and creatine sucrose agar (CREA) with methods and media composition described by Visagie et al. (2014). All isolates were inoculated in three replicates and incubated for 7 days in the dark at 25°C. At the end of the incubation period, two perpendicular diameters of each colony were measured and the average was calculated. The colony texture, mycelium and spore colors, sporulation intensity, and production and color of exudates and soluble pigments were also recorded. All plates were photographed with a digital camera (model FE-220/X-785; Olympus Corporation, Japan).

**Micromorphological examination.** We prepared temporary microscopic slides from 7- to 10 day-old cultures on MEA by mounting the sample in 60% lactic acid (Visagie et al. 2014). Morphological features of conidiophores (branching type), phialides, and conidia (shape, cell wall ornamentation) were recorded. Observations were done with a phase contrast microscope (model BX51; Olympus Corporation, Japan) and photographed with a camera from the same manufacturer (model E620). One hundred conidia of each isolate were measured (length and width) in the Quick Photo Camera software program (PROMICRA, Czech Republic).

**Temperature assays.** Selected isolates were inoculated on CYA and incubated at 5, 15, 30, and 37°C to monitor the effect of different temperature incubation conditions (Frisvad and Samson 2004). The inoculation method, incubation duration, and mycelial growth were assessed as previously described.

**DNA extraction.** The cultures were grown for 7 days on MEA at 25°C for DNA extraction. About 100 mg of mycelium was scraped from the plate surface with a sterile blade and transferred to a 2-ml centrifuge tube. Mechanical disruption of the cells was performed with a sterile micropestle used to grind the samples previously frozen in liquid nitrogen. Total DNA was extracted with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, and the extracts were stored at −20°C.

**PCR and sequencing.** PCR and sequencing were performed for four genetic loci: ITS, *BenA*, *CaM*, and *RPB2* (Table 1). These molecular markers were recommended for molecular identification of *Penicillium* and *Talaromyces* species by Visagie et al. (2014) and Yilmaz et al. (2014). A modified version of their protocols used in our previous study (Stošić et al. 2020) was applied in this work.

**Sequence and phylogenetic analysis.** Sequencing of the amplified genomic regions was performed in both directions in an automated sequencer by Macrogen Europe's commercial sequencing service (Macrogen Europe B.V., Amsterdam, the Netherlands) with the same primer pairs used as for the amplification. FinchTV software (version 1.4.0, Geospiza, Inc.) was used to inspect the quality of the sequences, and the ClustalW algorithm (Thompson et al. 1994) in MEGA7 software (Kumar et al. 2016) was applied to compute consensus sequences. The consensus sequences were submitted to the NCBI GenBank database (Table 1).

The sequences of ITS, *BenA*, *CaM*, and *RPB2* generated in this study were compared with the reference and other sequences of *Penicillium* and *Talaromyces* available in the NCBI GenBank database with the BLASTn algorithm search tool. Individual and multilocus gene alignments were performed with the ClustalW algorithm (Thompson et al. 1994) in MEGA7 software (Kumar et al. 2016).

Maximum likelihood (ML) trees were constructed for individual and multilocus aligned datasets of ITS, *BenA*, *CaM*, and *RPB2* (Table 1) in MEGA7 software (Kumar et al. 2016). The best models for ML analyses were chosen based on the Akaike information criterion computed in MEGA7. The initial tree was calculated with the Default-NJ/BioNJ option, and nearest neighbor interchange was selected for the ML heuristic method. The reliability of the constructed phylogenetic trees was evaluated with 1,000 replications for branch stability. Bootstrap confidence values <70% were omitted. Sequences of *Neocosmospora phaseoli* (isolate CBS 102429) were used as the outgroup. Visual preparation and editing of the trees was done in Adobe Illustrator CS6 (Adobe, U.S.A.).

**Pathogenicity assay.** Pathogenicity of the selected *Penicillium* and *Talaromyces* isolates (Table 1) was tested on intact pear fruits (cultivar Williams). The fruit surface was disinfected with 70% ethanol and allowed to air dry. The fruits were punctured with a sterile needle, and 50 µl of spore suspension was injected into the wound. Preparation of the conidial suspensions was done in 1 ml of sterile distilled water with 14-day-old MEA-grown cultures. A hemocytometer (Neubauer chamber) was used to determine and adjust the final spore concentration to $1 \times 10^6$/ml. Pear fruits inoculated with 50 µl of sterile distilled water were used as the control. Three fruits were inoculated per one isolate or control, and each fruit is counted as a replicate. The inoculated fruits were placed in a plastic container and incubated at 25°C and 95% relative air humidity. Seven days after the inoculation, the fruits were photographed, and developed lesions were examined. We assessed decay intensity by measuring the horizontal and vertical (stem–calyx axis vertical) diameters of the lesions. We verified the fulfillment of Koch's postulates by examining the colony and spore morphology of the developed, reisolated MEA cultures. The assay was repeated once, and the arrangement followed a completely randomized design.

**Data analysis.** IBM SPSS Statistics software, version 23 (IBM Corporation, U.S.A.) was used for data analyses. The experiments including macromorphological and physiological properties were

**Table 1.** Isolates of *Penicillium* and *Talaromyces* spp. with corresponding NCBI GenBank accession numbers used in phylogenetic analyses; bolded isolates are from this research

| Species | Isolate[a] | Substrate and origin | GenBank accessions | | | |
|---|---|---|---|---|---|---|
| | | | ITS[b] | *BenA*[c] | *CaM*[d] | *RPB2*[e] |
| *Neocosmospora phaseoli* (=*Fusarium solani*) | CBS 102429 | Tree bark, Australia | KM231808 | KM232069 | KM231381 | KM232376 |
| *P. crustosum* | **KrP/2**[f] | **Pear fruit, Serbia** | **MT872085** | **MW162400** | [g] | [g] |
| | **KrP/5**[f] | **Pear fruit, Serbia** | **MT872086** | **MW162401** | [g] | [g] |
| | **KrP/6**[f,h] | **Pear fruit, Serbia** | **MT872087** | **MW162402** | **MW115930** | **MW145142** |
| | FRR 1669 = CBS 115503 = IMI 091917 | Lemon fruit, Aberdeen, Scotland, UK | AY373907 | AY674353 | DQ911132 | [g] |
| | SFC20140101-M781 = 5501 | Unknown | KJ527442 | KJ527407 | [g] | KJ527372 |
| | CV1267 = DTO182I3 | *Protea repens* infructescence, Riverlands (Malmesbury), South Africa | JX091401 | JX091537 | JX141578 | MK461536 |
| | CV1529 = DTO183C4 | *Protea repens* infructescence, Riverlands (Malmesbury), South Africa | JX091402 | JX091538 | JX141579 | MK461542 |
| | CV0241 = DTO181D2 | *Protea repens* infructescence, Stellenbosch, South Africa | JX091403 | JX091536 | JX141576 | MK461524 |
| | CV0251 = DTO181D6 | Mite from *Protea repens* infructescence, Stellenbosch, South Africa | JX091404 | JX091530 | JX141577 | MK461525 |
| | CNU 6043 | Apple fruit, Yesan, Korea | HQ225711 | HQ225724 | [g] | [g] |
| *P. digitatum* | CBS 112082 | Lemon, Italy | KJ834506 | KJ834447 | KU896833 | JN121426 |
| *P. expansum* | **19-10**[f] | **Pear fruit, Serbia** | **MT872088** | **MW162403** | [g] | [g] |
| | **19-11**[f] | **Pear fruit, Serbia** | **MT872089** | **MW162404** | [g] | [g] |
| | **19-12**[f,h] | **Pear fruit, Serbia** | **MT872090** | **MW162405** | **MW115931** | **MW145143** |
| | **19-13**[f] | **Pear fruit, Serbia** | **MT872091** | **MW162406** | [g] | [g] |
| | **19-14**[f] | **Pear fruit, Serbia** | **MT872092** | **MW162407** | [g] | [g] |
| | CBS 325.48 = ATCC 7861 | Apple fruit, U.S.A. | AY373912 | AY674400 | DQ911134 | JF417427 |
| | SFC20140101-M737 = 5537 | Unknown | KJ527444 | KJ527409 | [g] | KJ527374 |
| | F758 | Sugar beet root, Idaho, U.S.A. | MG714838 | MG714864 | MG714821 | MG714845 |
| | CV2860 = DTO180F6 = CV 407 | Soil, South Africa | FJ230989 | JX091539 | JX141580 | MK450839 |
| | CV2861 = DTO180F7 = CV 432 | Soil, South Africa | FJ230990 | JX091540 | JX141581 | MK450840 |
| | CNU 7003 | Apple fruit, Daejoen, Korea | HQ225715 | HQ225727 | [g] | [g] |
| *P. italicum* | **KrP/3**[f] | **Pear fruit, Serbia** | **MT872093** | **MW162408** | [g] | [g] |
| | **KrP/4**[f] | **Pear fruit, Serbia** | **MT872094** | **MW162409** | [g] | [g] |
| | **KrP/9**[f,h] | **Pear fruit, Serbia** | **MT872095** | **MW162410** | **MW115932** | **MW145144** |
| | CBS 339.48 | Citrus fruit, Riverside, CA, U.S.A. | KJ834509 | AY674398 | DQ911135 | JN121496 |
| | SFC20140101-M724 = 5340 | Unknown | KJ527447 | KJ527412 | [g] | KJ527377 |
| | ATCC 48114 | *Citrus aurantium*, U.K. | AY373920 | [g] | [g] | [g] |
| | CNU 6089 | Apple fruit, Yesan, Korea | HQ225716 | HQ225728 | [g] | [g] |
| *P. polonicum* | CBS 222.28 = NRRL 995 | Soil, Poland | AF033475 | AY674305 | KU896848 | JN406609 |
| | F775 | Sugar beet root, Idaho, U.S.A. | MG714841 | MG714868 | MG714825 | MG714849 |

(*Continued on next page*)

[a] All isolates listed in this column were used in phylogenetic analyses.
[b] ITS, internal transcribed spacer.
[c] *BenA*, β-tubulin.
[d] *CaM*, calmodulin.
[e] *RPB2*, DNA-dependent RNA polymerase II second largest subunit.
[f] Isolates used in macromorphological, micromorphological analyses, molecular identification (ITS and *BenA*).
[g] No corresponding entry exists.
[h] Isolates used in further molecular characterization (*CaM* and *RPB2*), temperature and pathogenicity assays.

**Table 1.** (*Continued from previous page*)

| Species | Isolate[a] | Substrate and origin | GenBank accessions | | | |
|---|---|---|---|---|---|---|
| | | | ITS[b] | *BenA*[c] | *CaM*[d] | *RPB2*[e] |
| *P. solitum* | CBS 424.89 = FRR 937 | Unknown, Germany | AY373932 | AY674354 | KU896851 | KU904363 |
| | CNU 4096 | Apple fruit, Daegu, Korea | HQ213935 | HQ225721 | [g] | [g] |
| *P. viridicatum* | CBS 390.48 = DTO 005-C9 = FRR 963 | Air, Washington DC, U.S.A. | AY373939 | AY674295 | KU896856 | JN121511 |
| *T. flavus* | CBS 310.38 | Unknown, New Zealand | JN899360 | JX494302 | KF741949 | JF417426 |
| *T. islandicus* | CBS 338.48 | Unknown, Cape Town, South Africa | KF984885 | KF984655 | KF984780 | KF985018 |
| *T. minioluteus* | CV0383 | Unknown | JX091487 | JX091620 | JX140693 | [g] |
| | DnjP/2 | Quince fruit, Serbia | MN311448 | MN306504 | MN306512 | MN306520 |
| | **KrP/7**[f,h] | **Pear fruit, Serbia** | **MT872096** | **MW162411** | **MW115933** | **MW145145** |
| | CBS 642.68 | Unknown | JN899346 | KF114799 | KJ885273 | JF417443 |
| | CBS 270.35 | *Zea mays*, U.S.A. | KM066172 | KM066129 | [g] | [g] |
| *T. purpureus* | CBS 475.71 | Soil, Esterel, France | JN899328 | GU385739 | KJ885292 | JN121522 |
| *T. rugulosus* | **KrP/1**[f,h] | **Pear fruit, Serbia** | **MT872097** | **MW162412** | **MW115934** | **MW145146** |
| | **KrP/8**[f] | **Pear fruit, Serbia** | **MT872098** | **MW162413** | [g] | [g] |
| | CBS 371.48 | Rotting potato tubers (*Solanum tuberosum*), U.S.A. | KF984834 | KF984575 | KF984702 | KF984925 |
| | CBS 378.48 = NRRL 1073 | Type of *P. tardum* and *P. elongatum*, decaying twigs, France | KF984832 | KF984579 | KF984711 | KF984927 |
| | CBS 137366 = DTO 61-E8 | Air sample, beer producing factory, Kaulille, Belgium | KF984850 | KF984572 | KF984700 | KF984922 |
| *T. trachyspermus* | CBS 373.48 | Unknown, U.S.A. | JN899354 | KF114803 | KJ885281 | JF417432 |

**Table 2.** Overview of characteristics of *Penicillium* and *Talaromyces* isolates from Serbia on different media

| Isolates | Czapek yeast autolysate agar | | Malt extract agar | | Creatine sucrose agar |
|---|---|---|---|---|---|
| | Colony morphology (obverse) | Colony color (reverse) | Colony morphology (obverse) | Colony color (reverse) | Acid production |
| KrP/1<br>KrP/8 | Compact, with wrinkly center; velvety texture; dark green spores | Light cream yellow | Compact, flat; velvety texture; blue green to dark green | Light cream yellow | Moderate |
| KrP/2<br>KrP/5<br>KrP/6 | Radially sulcate; velvety texture; gray-green spores | Cream yellow | Compact, flat; fasciculate to crustose texture; gray-green spores | Light cream yellow | Strong |
| KrP/7 | Compact, flat; velvety texture; dark green spores | Yellowish green with dark orange center | Compact, slightly raised; strongly funiculose texture; bluish green spores | Yellow with orangeish yellow center | Very weak |
| KrP/3<br>KrP/4<br>KrP/9 | Moderately sulcate (central area); velvety to fasciculate texture; gray-green | Yellow-brown | Compact, slightly craterifrom; loosely funiculose texture in the center, velutinous periphery; gray green | Pale yellowish | None |
| 19-10<br>19-11<br>19-12<br>19-13<br>19-14 | Moderately sulcate (central area); fasciculate texture; green to blue green spores | Beige yellow | Compact, flat; velvety texture; blue-green spores | Cream yellow | Strong |

repeated once. Culture growth on the previously mentioned media and different temperatures was assessed according to basic descriptive indicators (minimal, maximal, and average values, standard deviation). After identifying the isolates to the species level, we calculated total average of culture growth for each species for each medium and temperature. One-way analysis of variance ($P \leq 0.05$) and Tukey's honestly significant difference test were used to compare the average colony growth and lesion diameters. The homogeneity of variance was assessed via Levene's test. The more robust Welch's analysis of variance ($P \leq 0.05$) was used in cases where the equality of the variances was not fulfilled, and a subsequent Games–Howell procedure was used as a post hoc test to identify statistically significant differences between isolates. The spore dimensions were represented in terms of minimal, maximal, and average length and width.

## Results

**Symptoms and isolation of the fungi.** On naturally infected pear fruits the observed symptoms included discolored tissue; brown, round, somewhere sunken, soft, and watery spots; presence of white and yellow mycelia; and blue and green conidial masses on the surface of the fruits. Sixty-seven isolates of *Penicillium* or *Talaromyces* were recovered, and 14 representative isolates were selected for morphological and genetic identification (Table 1).

**Macromorphological characterization.** Macromorphology of the representative isolates was examined by inoculation on three different media (CYA, MEA, and CREA) and incubation for 7 days at 25°C. The observed characteristics are presented in Table 2 and Figure 1. One common trait for all isolates was intensive sporulation on CYA (Fig. 1A, E, I, M, and Q) and MEA (Fig. 1B, F, J, N, and R). Texture of

the cultures varied most on MEA. Clear exudate droplets were noticed on MEA and CREA in isolates 19-10 to 19-14 (later identified as *P. expansum*) and on MEA in cultures KrP/2, KrP/5, and KrP/6 (subsequently determined to be *P. crustosum*).

Statistical analyses showed significant differences ($P \leq 0.05$) between isolates grown on the same type of medium (Fig. 2A). The radial growth of all isolates was the least intensive on CREA (total average 20.40 mm) and the most intensive on MEA (total average 32.32 mm). Isolates KrP/1 and KrP/8 (later resolved as *T. rugulosus*) had the slowest mycelial growth on all three tested media (13.00 mm on CYA, 14.58 mm on MEA, and 9.10 mm on CREA). The fastest growth on CYA had isolates of *P. expansum* (19-10 to 19-14, average

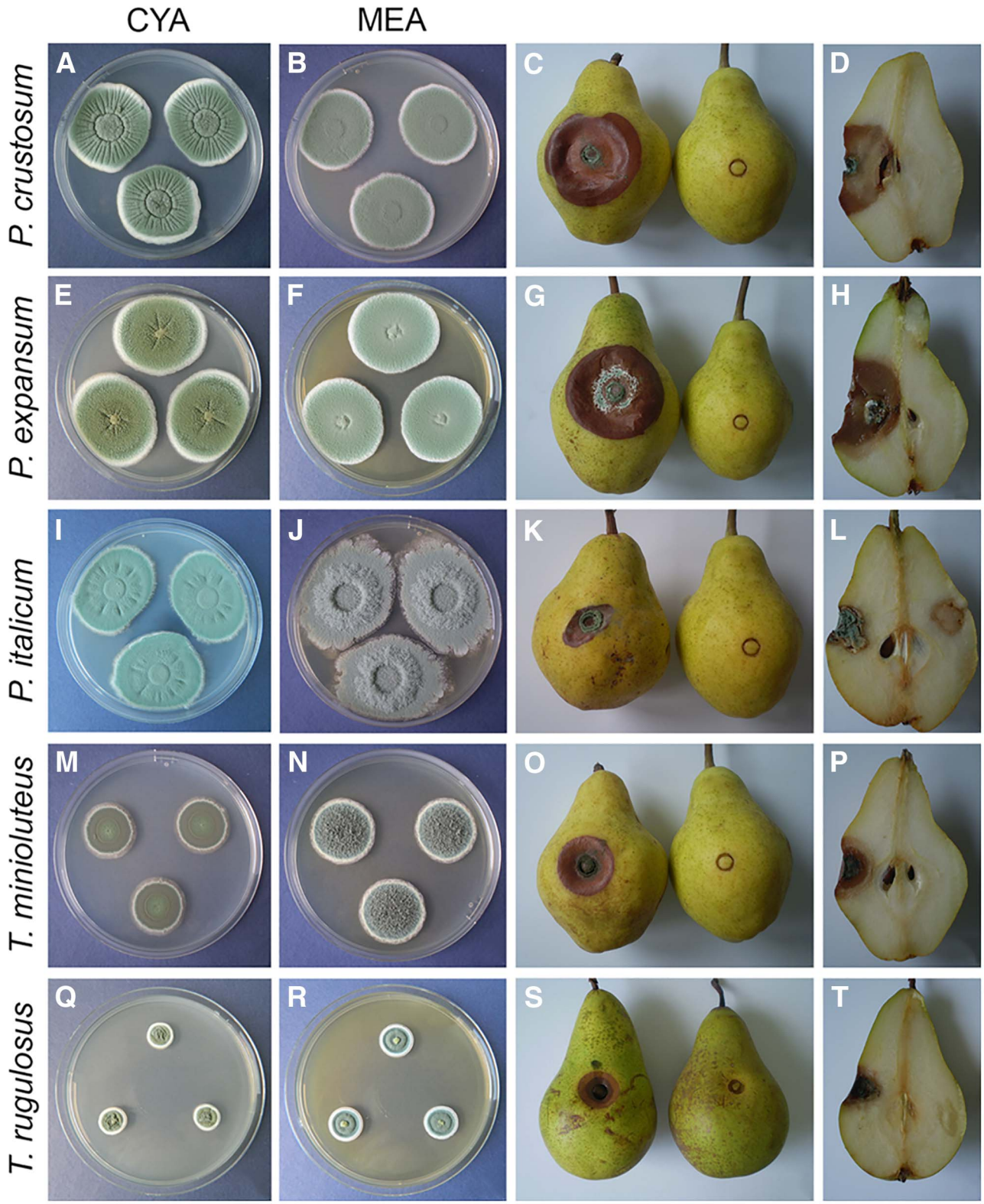


**Fig. 1.** Colony morphology on Czapek yeast autolysate agar (CYA) and malt extract agar (MEA) (obverse) and pathogenicity of Serbian *Penicillium* and *Talaromyces* isolates on pear fruits. **A to D,** *P. crustosum* (isolate KrP/6); **E to H,** *P. expansum* (isolate 19-12); **I to L,** *P. italicum* (isolate KrP/9); **M to P,** *T. minioluteus* (isolate KrP/7); **Q to T,** *T. rugulosus* (isolate KrP/1). Columns, left to right: CYA, MEA, pear fruits inoculated with pathogen (right) and the control (left, without symptoms), cross-sections of the pear fruit.

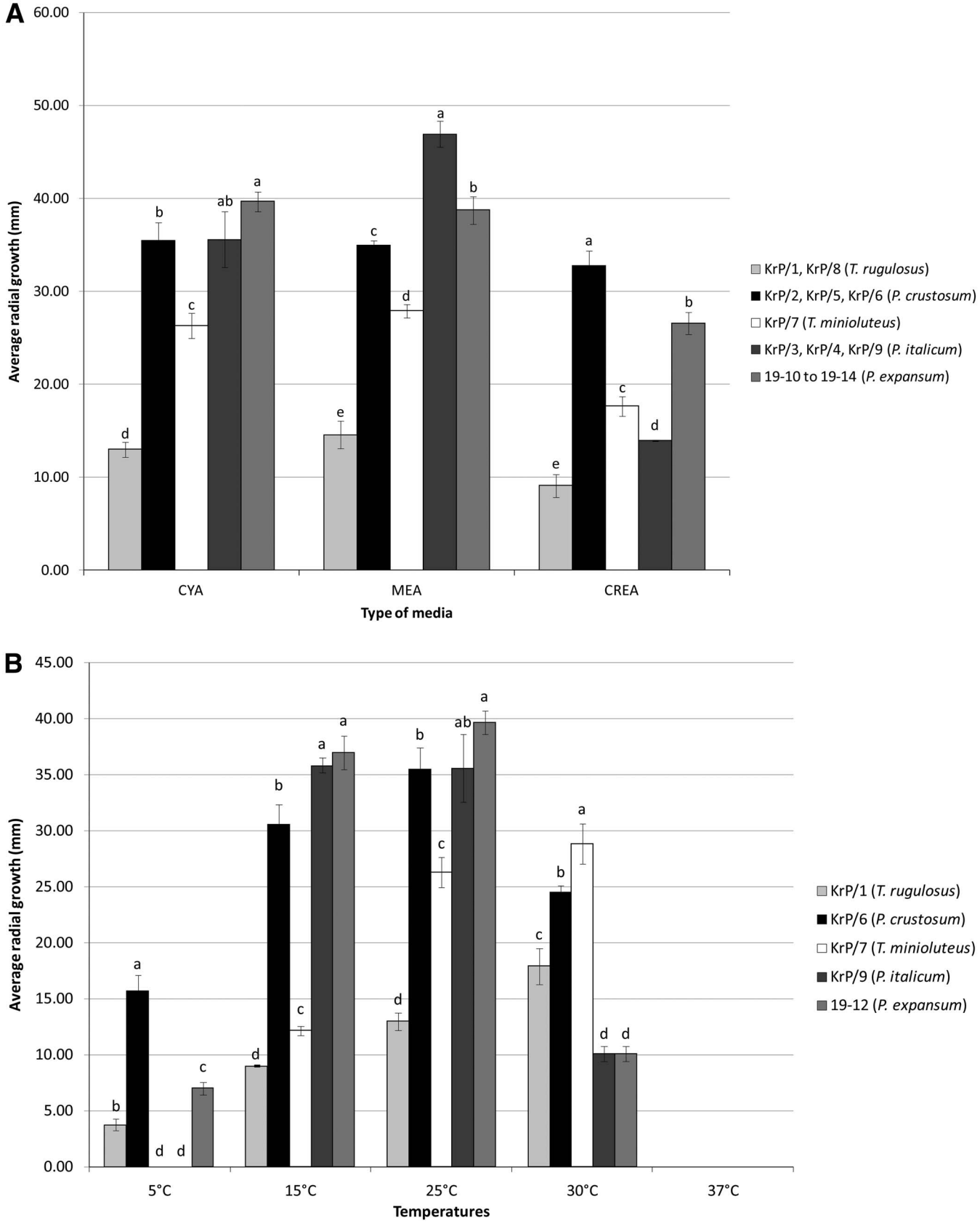


**Fig. 2.** Mean colony diameters of *Penicillium* and *Talaromyces* isolates after 7 days of incubation at 25°C. Vertical error bars indicate standard deviation of the mean (SD). **A,** Colony growth on different media. Bars marked by the same letters for Czapek yeast autolysate agar (CYA) and creatine sucrose agar (CREA) represent values that are not statistically significant according to Games–Howell test ($P \leq 0.05$). Bars marked by the same letters for malt extract agar (MEA) represent values that are not statistically significant according to Tukey's honestly significant difference test ($P \leq 0.05$). **B,** Colony growth on CYA at five tested temperatures. Bars marked by the same letters represent values that are not statistically significant according to Games–Howell test ($P \leq 0.05$).

39.67 mm) and on MEA isolates KrP/3, KrP/4, and KrP/9 (46.92 mm), later identified as *P. italicum*. The most intensive mycelial growth on CREA was observed in isolates of KrP/2, KrP/5, and KrP/6 (total average 32.75 mm), later determined to be *P. crustosum* (Fig. 2A).

**Microscopic investigation.** Variations in characteristics were observed during inspection of conidiophores of the representative isolates (Table 3). Conidiophores were borne on surface or subsurface hyphae of isolates KrP/3, KrP/4, KrP/9, and 19-10 to 19-14. Conidiophores of KrP/2, KrP/5, KrP/6, and KrP/7 isolates were borne on subsurface hyphae, whereas surface and aerial hyphae carried conidiophores on isolates KrP/1 and KrP/8. Biverticillate conidiophores were noticed on isolates KrP/1, KrP/7, and KrP/8, and the rest of the isolates had terverticillate branching, with isolates KrP/3, KrP/4, and KrP/9 having distinctively large regular or irregular terminal terverticillate penicilli. Spore sizes ranged from 2.05 × 2.91 µm (isolate KrP/7) to 4.31 × 4.30 µm (isolate KrP/2). Fruiting bodies (ascomata) were not noticed on any of the studied isolates.

**Effect of the temperature on growth.** Incubation at different temperatures affected mycelial growth on CYA for all tested isolates. There was a statistically significant difference ($P \leq 0.05$) in average growth diameters of all isolates between the five tested temperatures (Fig. 2B). The most intensive growth was recorded at 25°C (total average of all isolates 30.14 mm), and 5°C was the temperature with the least growth (5.28 mm total average). None of the isolates grew at 37°C. Statistical significance ($P \leq 0.05$) was determined between the isolates within each of the examined incubation temperatures where the growth was manifested. At 5°C isolate KrP/6 had the fastest growth, at 30°C isolate KrP/7 grew the fastest, and at 15°C and 25°C the most intensive growth was observed in isolate 19-12 (Fig. 2B).

Based on the data from macromorphology, micromorphology, and temperature assays, isolates KrP/2, KrP/5, and KrP/6 corresponded to the descriptions of *P. crustosum*; isolates 19-10 to 19-14 matched the descriptions of *P. expansum*; and isolates KrP/3, KrP/4, and KrP/9 corresponded to the descriptions of *P. italicum*. Isolate KrP/7 fit the descriptions of *T. minioluteus*, and isolates KrP/1 and KrP/8 were tentatively identified as *T. rugulosus*.

**Molecular identification, characterization, and phylogenetic analyses.** Molecular identification and characterization of the isolates from this study was done with four molecular markers (ITS, *BenA*, *CaM*, and *RPB2*). Genetic identification confirmed the identification achieved via conventional morphophysiological methods. ITS sequences of Serbian isolates belonging to the same species were 100% identical to each other. The comparison of *BenA* sequences for Serbian isolates of the same species had the same result. Sequences of *CaM* and *RPB2* from this study were generated for one representative isolate of each of the five species. Therefore, they were compared only to the previous GenBank records, and most of them were 100% identical to the sequences of the corresponding species from the BLAST search. The exceptions for *CaM* include *P. expansum* (99.4% identical, i.e., 3-bp difference from *P. expansum* isolate F791 from sugar beet, U.S.A., accession number MG714827) and *P. italicum* (98.4% similar, or 8 bp different from the Chinese *P. italicum* isolate AS3.6587 from an unknown source, AY678571). For *RPB2*, the exceptions were *P. crustosum* (99.8% or 2 nt difference with sequence MN149970, isolated from *Protea repens* infructescence in South Africa) and *T. rugulosus* (sequence KX657495 from an unknown source, U.S.A., 98.5% identical or 15-bp difference).

The phylogenetic relationships were examined in ML analyses for all four molecular markers individually and for a multilocus dataset (Figs. 3 and 4, respectively). Multiple sequence alignments were 430, 301, 527, and 610 nt long for ITS, *BenA*, *CaM*, and *RPB2*, respectively. The aligned combined dataset was 1,868 nt long, and it contained 49 sequences from 14 different taxa (representative *Penicillium* or *Talaromyces* species and outgroup sequence of *N. phaseoli*). The best-fitting models for nucleotide substitution were Kimura 2-parameter with gamma distribution (K2 + G) for ITS; Kimura 2-parameter with gamma distribution and certain evolutionary invariable sites (K2 + G + I) for *BenA*, *CaM*, and *RPB2*; and Tamura 3-parameter with gamma distribution and evolutionary invariable sites (T92 + G + I) for a four-gene combined dataset. Tree topologies were similar in a single-locus and multigene ML analyses. All phylogenetic trees (four individual gene trees and a concatenated tree) were divided into two main clades, one encompassing *Penicillium* spp. and the other encompassing *Talaromyces* species (Figs. 3 and 4). Within those clades, Serbian isolates of *P. crustosum*, *P. expansum*, *P. italicum*, *T. minioluteus*, and *T. rugulosus* clustered together with the other GenBank sequences of corresponding species with high bootstrap supports.

**Pathogenicity test.** The pear fruits manifested rot symptoms 7 days after inoculation with the selected isolates of *Penicillium* and *Talaromyces* (Fig. 1C, G, K, O, and S). The symptoms resembled those observed in the originating hosts. Tissue around the inoculation point was brown, sunken, soft, and watery. The sporulation was noticed in fruits infected with all species except in those inoculated with *T. rugulosus*. The spores were dark olive green in *P. crustosum*, *P. italicum*, and *P. expansum*, whereas *T. minioluteus* produced dark green spores on pear fruits. In cross-sections (stalk–calyx axis) it was observed that all species weakened the mesocarp of the pear fruits, with *P. crustosum*, *P. expansum*, and *T. minioluteus* reaching the endocarpal part (Fig. 1D, H, and P, respectively). *P. expansum* affected the pear fruit the most, in terms of the size of the lesions and the intensity of the tissue decay. In the case of *P. italicum*, the infection was constrained, and the fungal progression toward the center was stopped (Fig. 1L), so this could be characterized as a tissue response lesion. It is noteworthy that despite the smaller size of the lesions and its tissue response character, *P. italicum* sporulated at the lesion spots. The fruits in the control group did not manifest any symptoms of decay. The same morphological properties were observed in the reisolates (100% of reisolation frequency) recovered from artificially inoculated fruits, and therefore Koch's postulates were confirmed.

A statistically significant difference ($P \leq 0.05$) was found in the pathogenicity of different species (Fig. 5). In Tukey's test the species were clustered in two groups: The isolates of *P. crustosum* (KrP/6) and *P. expansum* (19-12) belong to the group with greater mean lesion

**Table 3.** Overview of conidial characteristics and dimensions of *Penicillium* and *Talaromyces* isolates from Serbia

| Isolate | Spore dimensions (µm), minimum–(average)–maximum | Spore shape | Spore wall ornamentation | Phialides shape | Metulae shape | Stipe wall ornamentation | Conidiophore branching type |
|---|---|---|---|---|---|---|---|
| KrP/2 | 3.75–(4.31)–5.00 × 3.75–(4.30)–5.00 | Subglobose to globose | Smooth | Cylindrical | Cylindrical | Rough | Terverticillate |
| KrP/5 | 3.00–(3.94)–5.00 × 3.00–(3.51)–5.00 | | | | | | |
| KrP/6 | 3.00–(3.84)–5.00 × 3.00–(3.94)–5.00 | | | | | | |
| 19-10 | 3.00–(3.21)–4.25 × 3.00–(4.15)–4.75 | Subglobose to ellipsoidal | Smooth | Cylindrical | Cylindrical | Smooth or finely roughened | Terverticillate |
| 19-11 | 3.25–(3.34)–4.00 × 3.00–(4.27)–5.00 | | | | | | |
| 19-12 | 3.00–(3.27)–4.00 × 3.00–(4.06)–5.00 | | | | | | |
| 19-13 | 3.00–(3.41)–4.25 × 2.75–(4.34)–5.00 | | | | | | |
| 19-14 | 2.75–(3.29)–4.00 × 3.00–(4.09)–4.75 | | | | | | |
| KrP/3 | 2.75–(3.73)–4.75 × 1.75–(2.83)–3.75 | Cylindrical, ellipsoidal or subglobose | Smooth | Cylindrical | Cylindrical | Smooth | Terverticillate |
| KrP/4 | 3.00–(4.44)–5.00 × 2.00–(3.27)–4.00 | | | | | | |
| KrP/9 | 3.00–(4.50)–6.00 × 2.00–(3.18)–4.00 | | | | | | |
| KrP/7 | 2.00–(2.91)–3.00 × 2.00–(2.05)–3.00 | Subglobose to ellipsoidal | Smooth | Acerose | Cylindrical | Smooth | Biverticillate |
| KrP/1 | 2.50–(2.91)–3.75 × 2.50–(2.88)–4.25 | Ellipsoidal | Rough | Cylindrical | Cylindrical | Smooth | Biverticillate |
| KrP/8 | 2.75–(3.18)–4.00 × 2.75–(3.02)–4.25 | | | | | | |

diameters (37.50 and 42.50 mm, respectively), and the second group encompasses the isolates of *T. rugulosus* (KrP/1), *P. italicum* (KrP/9), and *T. minioluteus* (KrP/7) with lower mean lesion values (18.17, 18.67, and 24.00 mm, respectively) (Fig. 5). Of all tested species from this study, *P. expansum* (isolate 19-12) caused the most severe decay, and *T. rugulosus* (KrP/1) was the least virulent.

## Discussion

This research presents the first results of *Penicillium* and *Talaromyces* spp. isolated from diseased pear fruits in Serbia during a 4-year sampling period. An integrative approach was applied to identify the species associated with *Penicillium*-like mold symptoms observed on pear fruits. Pathogenicity testing was also conducted to evaluate the virulence of the

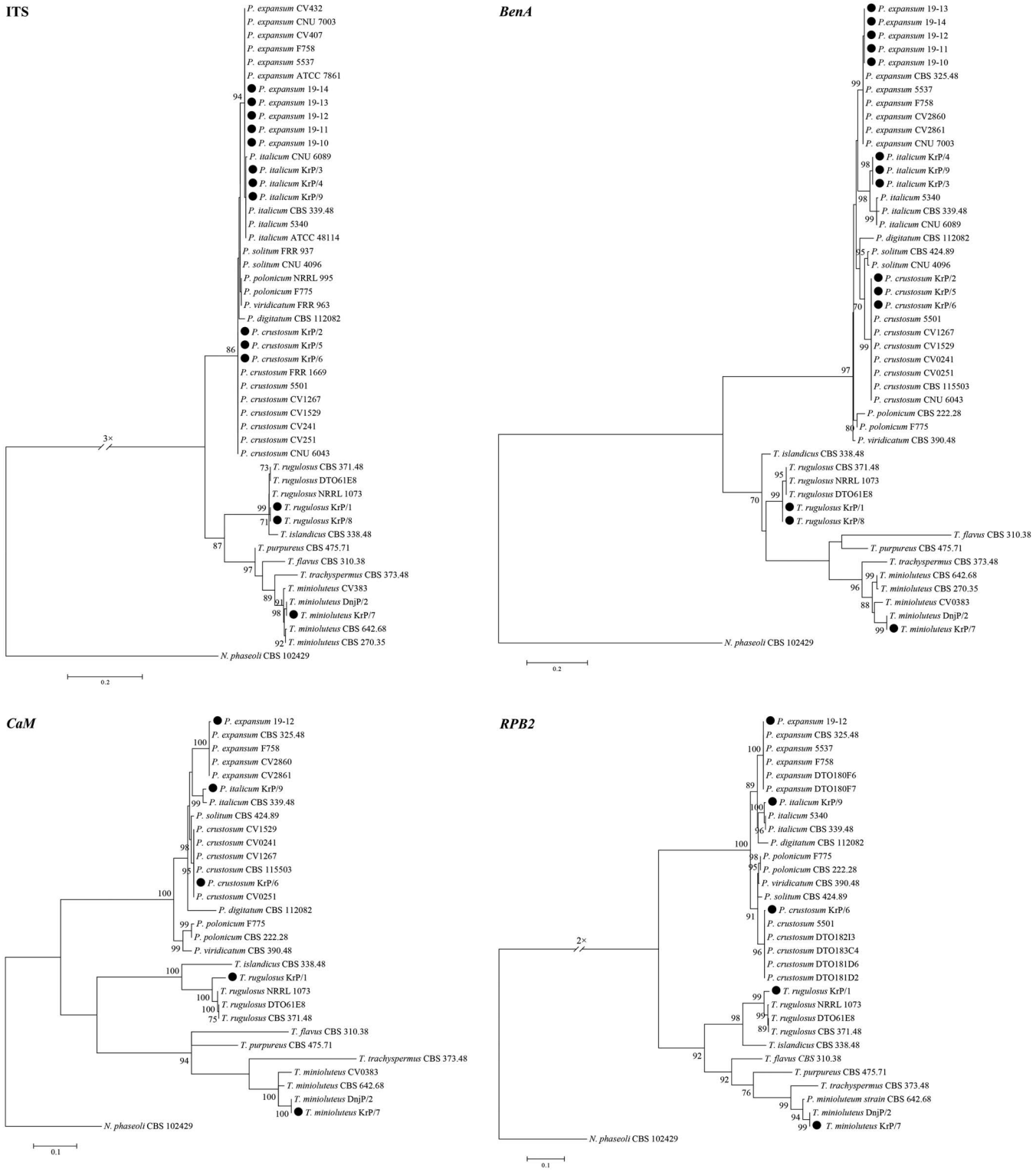


**Fig. 3.** Maximum likelihood phylogenetic trees of internal transcribed spacer (ITS) region, beta-tubulin (*BenA*), calmodulin (*CaM*), and DNA-dependent RNA polymerase II second largest subunit (*RPB2*) genes of isolates of *Penicillium* and *Talaromyces*. *Neocosmospora phaseoli* sequences (isolate CBS 102429) served as the outgroup. Bootstrap tests were performed in 1,000 replicates for each generated tree, and bootstrap support values (>70%) are displayed next to the relevant nodes. The bars indicate the number of substitutions per position. *Penicillium* and *Talaromyces* isolates from this study are marked with black circles.

recovered species and confirm Koch's postulates. The causal agents of the blue mold symptoms on collected pear fruits in our study were identified as *P. crustosum*, *P. expansum*, *P. italicum*, *T. minioluteus*, and *T. rugulosus*. Based on the extensive review of the literature, these are the first records of *T. minioluteus*, *T. rugulosus*, and *P. italicum* on fruits of European pear. This is also the first report in which these two *Talaromyces* species were identified as postharvest pear pathogens. Furthermore, this is the first finding of *P. crustosum*, *P. expansum*, *P. italicum*, *T. minioluteus*, and *T. rugulosus* on pear fruit in Serbia.

In general, phenotypic characteristics of Serbian *Penicillium* and *Talaromyces* spp. isolates on CYA, MEA, and CREA and in microscopic observations were consistent with previous descriptions (Frisvad et al. 1990; Frisvad and Samson 2004; Khodaei et al. 2015; Pitt 1973, 1979; Samson et al. 2010; Van Reenen-Hoekstra et al. 1990; Visagie 2012; Yilmaz et al. 2014). Some degree of variation was noticed regarding their radial growth values on three tested media and temperatures. The mean growth diameters of our *T. minioluteus* isolates were close to the values found by Khodaei et al. (2015) but higher than in the works of other authors (Frisvad et al. 1990; Stošić et al. 2020; Van Reenen-Hoekstra et al. 1990; Yilmaz et al. 2014). Moderately weak acid production of *T. rugulosus* on CREA in this study is slightly different from the results of Yilmaz et al. (2014), which reported little or no acid production on this medium.

In assays evaluating fungal growth at different temperatures, isolates of *P. expansum*, *P. crustosum*, and *T. rugulosus* in this research manifested growth at low temperature (5°C) and therefore can be characterized as psychrotolerant organisms. This finding is consistent with previous results (Gunde-Cimerman et al. 2003; Pitt and Hocking 2009). This result is an important parameter for postharvest pathogens that indicates that pear fruit infection with these species can occur even in cold storage conditions. The optimal temperature for mycelial growth for our *Penicillium* spp. was 25°C, which is consistent with the findings by Pitt and Hocking (2009). Serbian *Talaromyces* species had growth optimums at 30°C that were higher than those reported by Pitt and Hocking (2009). Growth of *T. minioluteus* isolate was consistent with the reports by Visagie (2012) and Stošić et al. (2020).

The variations in phenotypic properties of *Penicillium* and *Talaromyces* spp. are well documented in the literature, and they are a

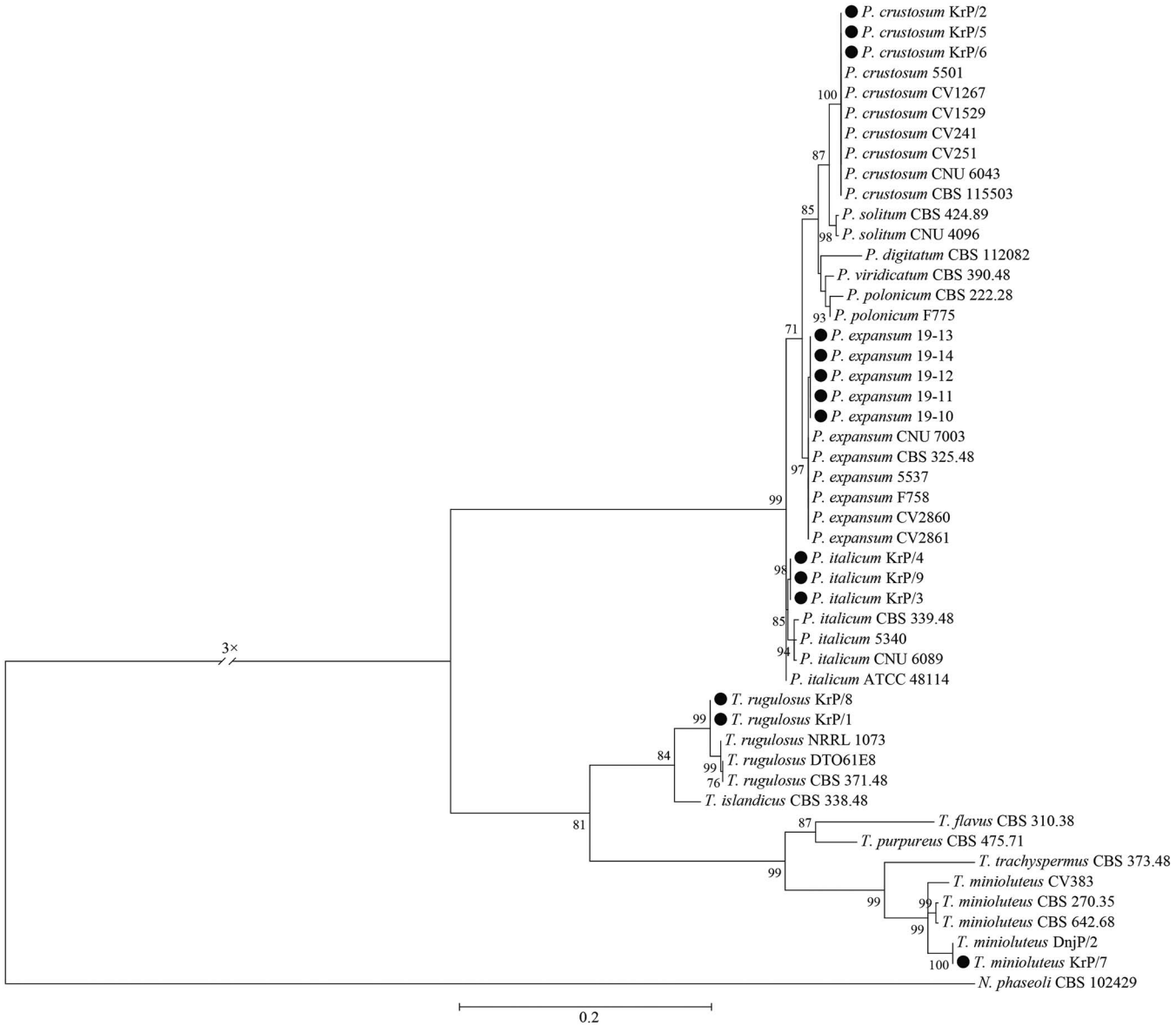


**Fig. 4.** Maximum likelihood multilocus phylogenetic tree of isolates of *Penicillium* and *Talaromyces*. Tree was generated with concatenated sequences of internal transcribed spacer (ITS) region, beta-tubulin (*BenA*), calmodulin (*CaM*), and DNA-dependent RNA polymerase II second largest subunit (*RPB2*) genes. *Neocosmospora phaseoli* sequence (isolate CBS 102429) served as the outgroup. Bootstrap tests were performed in 1,000 replicates for each generated tree, and bootstrap support values (>70%) are displayed next to the relevant nodes. The bars indicate the number of substitutions per position. *Penicillium* and *Talaromyces* isolates from this study are marked with black circles.

complicating factor in their correct identification. The variation occurs because of inherent variability within these genera, different environmental factors affecting morphological and physiological characteristics, and possible degeneration of phenotypic characters during subculturing (Dupont et al. 2006; Pitt 1973, 1988; Visagie et al. 2014).

Multilocus sequence typing and phylogenetic analyses of the selected isolates have been performed based on four genetic loci now commonly used in *Penicillium* and *Talaromyces* identification and characterization: ITS, *BenA*, *CaM*, and *RPB* (Visagie et al. 2014; Yilmaz et al. 2014). Molecular identification confirmed the results of morphological analyses of the isolates recovered in this research. BLAST results of some of our ITS sequences did not always agree with the results of the BLAST search of other markers (especially in comparison with the *BenA* results). The ITS region of ribosomal DNA has been extensively used in fungal phylogenetic analyses and is used as a universal DNA barcoding sequence for fungi (Schoch et al. 2012), but it does not possess enough discriminative power for species-level identification of *Penicillium* and *Talaromyces* spp. (Visagie et al. 2014; Yilmaz et al. 2014). This shortcoming was confirmed in our research and in previous studies (Skouboe et al. 1999; Yilmaz et al. 2016; Yin et al. 2017). Therefore, additional genes must be applied as secondary molecular markers for comprehensive and accurate identification of the species from these genera, with *BenA* being the first choice (Visagie et al. 2014; Yilmaz et al. 2014). The only difficulty we experienced was in the amplification of *BenA* in *T. rugulosus* with Bt2a/Bt2b primers, so the other combination of *BenA* primers were used to obtain satisfying sequences (T10/Bt2b, as recommended by Yilmaz et al. (2014) for *Talaromyces* section *Islandici*). For successful *RPB2* amplification, the protocol used in our previous study of *T. minioluteus* (Stošić et al. 2020) was applied, with conventional PCR (annealing temperature 60°C) instead of touch-up proposed by Yilmaz et al. (2014) and Visagie et al. (2014). Although Yilmaz et al. (2014) warned in their study that the amplification of calmodulin can be problematic, we did not encounter any problems with the primer pair CMD5/CMD6. The isolates of five species identified in this study (*P. crustosum*, *P. expansum*, *P. italicum*, *T. minioluteus*, and *T. rugulosus*) clustered together with the isolates of the corresponding species in all constructed ML phylogenetic trees (single-gene and multilocus). All generated trees had congruent topologies when compared with each other. Also, the overall tree topologies consisted of two main branches separating the two genera, one with *Penicillium* and the other with *Talaromyces* species, which is consistent with the findings of Samson et al. (2011).

In a pathogenicity test, the isolates of *P. crustosum*, *P. expansum*, *T. minioluteus*, and *T. rugulosus* produced decay on artificially inoculated pear fruits. The symptoms induced by *P. italicum* on the pear fruit corresponded to tissue response lesions. Various *Penicillium* spp. were isolated from pear fruit and determined to be pear pathogens in previous studies: *P. aurantiogriseum* and its variant *P. aurantiogriseum* var. *aurantiogriesum* (listed as *P. puberulum*), *P. brevicompactum*, *P. cyclopium*, *P. commune*, *P. crustosum*, *P. digitatum*, *P. expansum*, *P. polonicum*, and *P. solitum* (Barkai-Golan 1974; Khokhar et al. 2019; Kim et al. 2002; Louw and Korsten 2014; Neri et al. 2006; Sanderson and Spotts 1995; Shim et al. 2002; Zhang et al. 2006). Furthermore, pear fruits were determined to be one of the most susceptible to penicillia infection in previous research (Barkai-Golan 1974). *Penicillium* and *Talaromyces* species has not been reported on pear fruit in Serbia.

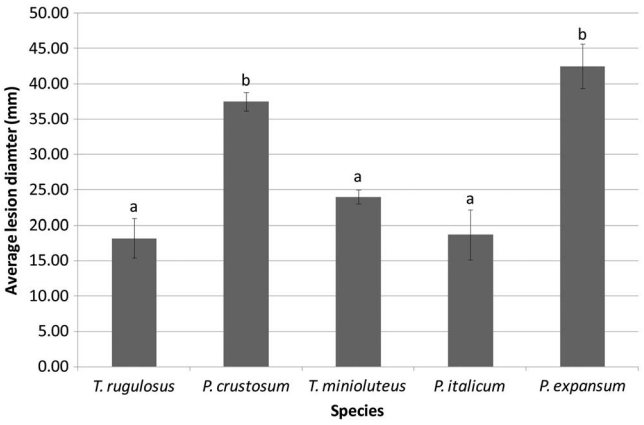


**Fig. 5.** Average lesion diameters of *Penicillium* and *Talaromyces* isolates on pear fruits in the pathogenicity assay. Bars with different letters represent values that are significantly different in Tukey's honestly significant difference test ($P \leq 0.05$), and vertical error bars indicate standard deviation of the mean.

Many authors have noted that *P. expansum* is one of the most common and the most important *Penicillium* species on pear and other pomaceous fruits (Frisvad and Samson 2004; Sardella et al. 2016; Scholtz and Korsten 2016; Snowdon 1990), which is consistent with our results. *P. expansum* was isolated from collected pear fruits in the highest percentage in this study (21/67 isolates, or approximately 31%). Beside pomaceous fruits, this species is an important pathogen of various other plant hosts (Barkai-Golan 1974; Frisvad and Samson 2004; Pitt and Hocking 2009). Broad host range, cosmopolitan distribution, and decay-producing capability coupled with a strong virulence are properties that characterize *P. expansum* as a broad-spectrum pathogen (Neri et al. 2010; Pitt and Hocking 2009). This species was confirmed as the one with the highest pathogenic potential on pear fruit in our study, which is consistent with the results of Barkai-Golan (1974), Kim et al. (2002), and Louw and Korsten (2014). Fast growth rate on the infected fruits and high virulence are the main factors that give *P. expansum* excellent competitive ability (and consequently strong pathogenicity), as Sanderson and Spotts (1995) found. These traits can also contribute to faster overlapping of other *Penicillium* and *Talaromyces* spp. by *P. expansum* on fruits, especially when mixed infections are present (Quintanilla 1985).

Another species isolated in this survey, *P. crustosum*, has been previously reported from pear fruit. In an investigation of the causal agents of postharvest decay of pear fruit in Korea, *P. crustosum* was confirmed as a pear pathogen (Kim et al. 2002). It was classified as a pathogen with weaker virulence, in contrast to our findings. An extensive study performed by Louw and Korsten (2014) determined *P. crustosum* to be a pathogen on several pear fruit cultivars, placing it in a second place in terms of its aggressiveness. This species has been positioned in third place in terms of isolation frequency from the pear fruit environment (Scholtz and Korsten 2016). It is a widely distributed spoilage fungus isolated from a wide range of hosts and substrates, including citrus fruits, melons, and onions (Pitt and Hocking 2009). *P. crustosum* can be characterized as a resilient species, because its ability to tolerate changes in osmotic balance and nutritional deficiency enables it to adapt and survive in environments too harsh for many other living organisms (e.g., Arctic ice) (Gunde-Cimerman et al. 2003). Small spores contribute to more efficient nutrient uptake, and dense conidial production is an advantage in fast spread and colonization of many microenvironments (Sonjak et al. 2006).

In this study *P. italicum* produced lesions on inoculated pear fruit, but it is questionable whether this species could be characterized as a pathogen of pear fruit. We would classify this result as tissue response lesions because the infection was stopped after reaching a certain size. Nevertheless, *P. italicum* was able to sporulate inside the lesions. This result is similar to the *P. crustosum* and *P. expansum* tissue response lesions on citrus hosts reported by Louw and Korsten (2015). *P. italicum* is a species usually associated with citrus hosts, and some of these substrate/host–penicillia associations have been recognized long ago and are well known (Frisvad and Samson 2004). The previously cited authors emphasize that citrus-inhabiting penicillia are not likely to grow successfully on some other substrates, but in the same article it is mentioned that these species are occasionally recorded in soil and on plant roots and stems. Barkai-Golan (1974) even designated *P. italicum* as one of the *Penicillium* species with narrow natural occurrence and selective pathogenicity. However, in the fungal database curated by Agricultural Research Service of the U.S. Department of Agriculture, two pear species (*Pyrus serotina* var. *culta* and *Pyrus ussuriensis*) are listed as hosts for *P. italicum* (Farr and Rossman 2020). In addition, most of the *Penicillium* spp. can be considered generalists in terms of habitat preference, and because they are ubiquitous decomposers of the organic matter in ecosystems (Frisvad and Samson 2004; Visagie et al. 2014), it is plausible that *P. italicum* induced lesions on pear fruit in our assays. *P. italicum* was

also one of the 10 most frequently isolated *Penicillium* species from the pear supply chain (Scholtz and Korsten 2016). This finding indicates that *P. italicum* can reside in storage-linked environments, thus increasing the probability of fruit contamination, which can lead to pear decay and consequently economic losses. On the other hand, pear fruit infection with *P. italicum* might not always cause substantial decay, but it can weaken the fruits, making them susceptible to attack by the other, more aggressive and more virulent *Penicillium* species.

The results of this study are the first confirmations of *T. minioluteus* and *T. rugulosus* as pathogens on pear fruit. To date, only one species of *Talaromyces* was isolated from pear fruit, and that was *Talaromyces diversus* (referred as *P. diversum*), recovered from rotten pears in Poland (Borecka 1977). Of other pome fruit hosts, *T. minioluteus* was determined to be a pathogen on quince fruit (Stošić et al. 2020). *T. rugulosus* was one of the most prevalent fungi isolated from the surface of apple fruits in the study by Radenkovs and Juhnevica-Radenkova (2018), but a pathogenicity evaluation was not performed. Pitt and Hocking (2009) noted that *T. rugulosus* can be a plant pathogen, and it is probably more commonly distributed than the previous findings indicate.

All species detected on pear fruit in Serbia (except *P. italicum*) are known producers of various mycotoxins and other secondary metabolites. *P. expansum* synthesizes patulin, citrinin, chaetoglobosins A and C, communesin B, roquefortine C, and expansolides A and B. Penitrem A, terrestric acid, and roquefortine C are produced by *P. crustosum*. *T. minioluteus* produces secalonic acid D and C, and *T. rugulosus* is a producer of rugulosin (Andersen et al. 2004; Frisvad and Samson 2004; Frisvad et al. 2004; Pitt and Hocking 2009; Yilmaz et al. 2014). Therefore, it is important to minimize contamination of pear fruits with *Penicillium* and *Talaromyces* spp. and the development of those rots.

This study provides the first comprehensive investigation of penicillia-linked rot of pear fruits in Serbia. Characterization of *Penicillium* and *Talaromyces* species was carried out via conventional and molecular techniques, coupled with a pathogenicity assay. Five *Penicillium* and *Talaromyces* spp. were isolated from pear fruits for the first time in Serbia, with four of them confirmed to be pathogenic on pear fruits (*P. crustosum*, *P. expansum*, *T. minioluteus*, *T. rugulosus*) and the fifth being able to cause tissue response lesions (*P. italicum*). The outcomes of our study prove that species of *Talaromyces* cause pear fruit decay, and this is the first record of *T. minioluteus* and *T. rugulosus* on this fruit. They are often overlooked in identification procedures, probably because of the higher growth rates of other, more virulent penicillia. These findings could be a good starting point for fast and accurate identification of *Penicillium* and *Talaromyces* species and for developing efficient control strategies against the decay of stored pear fruits caused by species of these genera.

## Acknowledgment

The authors thank Mr. Slobodan Minić for his help with data analyses.

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