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Pseudomonas avellanae causing European hazelnut decline in Serbia

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Summary. The plant pathogenic bacterium *Pseudomonas avellanae*, the causal agent of decline and dieback of hazelnut (*Corylus avellana*), affects vascular tissues of host trees and leads to the rapid wilting of leaves. Severe damage to hazelnut orchards has been reported in northern Greece and central Italy. In 2024, hazelnut trees (cv. Tonda di Giffoni) showed symptoms of rapid leaf wilting and brown discolouration of vascular tissues in the branches. Isolations resulted in convex, levan-positive, mucoid, cream-whitish bacterial colonies on Nutrient agar (plus 5% sucrose), which LOPAT results (+---+) identified the bacteria as *Pseudomonas syringae* group Ia. Preliminary identification of isolates was achieved using specific conventional PCR (cPCR) for *P. avellanae*. Further genetic identification and characterization was carried out using multilocus sequence analysis (MLSA) using five housekeeping genes (*gyrB-gapA-gltA-rpoD-recG*). Repetitive ERIC-PCR fingerprinting of two Serbian isolates from hazelnut, 13 Italian, and one Greek isolate of *P. avellanae* revealed three distinct molecular patterns. The Serbian isolates clustered with the Italian strains, while the Greek strain formed a more distinct group. Pathogenicity assessments, carried out by inoculating 1-year-old hazelnut plants (Tonda di Giffoni), resulted in the development of necrosis at the inoculation sites 10 d post-inoculation, which reached lengths 15-20 cm longitudinally along the stems within 2 months. The inoculated bacterium was re-isolated from the stems of inoculated plants. This study highlights the emerging threat of *P. avellanae* to hazelnut production, with significant implications for the European hazelnut industry. Spread of this pathogen across regions, and the potential impacts on orchard health, emphasize the need for enhanced monitoring and disease management strategies.

Keywords. *Corylus avellana*, canker, decline, MLSA, rep-PCR

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INTRODUCTION

The *Corylus* genus (*Betulaceae*) includes a variety of woody plants, from small, multi-stemmed shrubs to tall trees, all of which yield edible nuts. *Corylus avellana* L. (European hazelnut) is the most well-known and thoroughly studied species, and is the main variety cultivated for commercial purposes (Molnar, 2011). Hazelnuts are rich sources of essential human nutrients, offering high levels of protein, dietary fibre, vitamins, and minerals, so international demand for hazelnuts is steadily increasing (Paunović *et al*., 2020). The leading producer and exporter countries of hazelnuts include Turkey, Italy, Spain, the United States of America, and Greece (Yıldırım *et al*., 2024). In Serbia, hazelnut production is estimated to be approx. 8,910 tons, cultivated across 8,718 ha, and yielding approx. 1 ton ha^{-1} (https://serbia-business.eu/). This planting area is a significant increase compared to previous years, with twice the area and nearly double the production of five years previously, now positioning Serbia among the leading world hazelnut producers.

Hazelnut trees are affected by a range of pathogens, predominantly fungi and bacteria, prevalent across all hazelnut-growing regions. Among bacterial pathogens, *Xanthomonas* spp., particularly *X. arboricola* pv. *corylina* causing bacterial blight, and *Pseudomonas* spp. (*Pseudomonas avellanae*, *P. syringae* pv. *avellanae*, *P. syringae* pv. *coryli, P. syringae* pv. *syringae*), particularly *P. avellanae,* leading to cankers and decline, pose significant concerns for hazelnut production (Scortichini, 1998; Marcelletti and Scortichini, 2015; Nicoletti *et al*., 2022).

Disease symptoms caused by *P. avellanae* include the progressive tip branch dieback, which eventually spreads to whole plants, and these symptoms occur from spring to autumn. In some cases, longitudinal cankers can be observed along tree trunks (Scortichini *et al*., 2006). Diseased bark turns reddish to brown, and when the bark is removed from an affected branch, a brown discolouration of the sapwood becomes visible. In addition to the necrotic leaves, immature (dead) fruits may remain attached to the twigs for several weeks. Infected trees that survive the winter often die the following summer (Range, 2008; Scortichini, 2000). This disease leads to significant economic losses in northern Greece (Psallidas, 1987) and the Viterbo region of Italy (Scortichini, 1998), resulting in death of thousands of trees each year.

In Serbia, symptoms of hazelnut (cultivar Tonda di Giffoni) decline were observed in a 5-year-old plantation during July 2024. Symptoms of rapid wilting of leaves on branches developed when trees started to yield, while diseased stems had brown discolouration of vascular tissues.

The aim of the present study was to identify and characterize the causal agent of hazelnut dieback in Serbia, and then to assess the relatedness of obtained pathogens to infer possible pathways of introduction into this country and assess the current population of the pathogens in Europe.

MATERIALS AND METHODS

Pathogen isolation and biochemical/physiological identification tests

Hazelnut trees of the cultivar Tonda di Giffoni, showing cankers along branches followed by dieback, were observed during July 2024 in a 5-year-old plantation (45 ha) in the locality of Bačka Topola, Serbia (Figure 1). The propagative material for this plantation had

Figure 1. A hazelnut tree (cv. Tonda di Giffoni) naturally infected by *Pseudomonas avellanae*, as observed in Bačka Topola (Serbia).

Figure 2. A diseased hazelnut stem used for pathogen isolation, showing necrotic tissues under the bark and within wood tissues.

been imported from Italy. The proportion of infected trees was up to 0.1%. The hazelnut orchard was not located near other hazelnut orchards.

Five samples, each consisting of diseased branches, were taken from the orchard for pathogen isolations (Figure 2). Samples were first surface sterilized with 1% sodium hypochlorite (NaOCl) solution for 1-2 min, then rinsed with tap water and dried on sterile filter paper. Small fragments (up to 5 mm) were then taken from the margins of healthy and diseased tissues and were soaked in sterile distilled water (SDW) for 2 h hours at room temperature. Obtained extracts were then plated onto Nutrient Agar supplemented with 5% w/v sucrose (NSA), and the inoculated Petri plates were incubated at 26°C for 72 h. Levan-positive colonies were purified and stored in Nutrient Broth containing glycerol (20%) at -80°C. Two isolates, Pa2124 and Pa4224, obtained from two different trees, were characterized by carrying out LOPAT tests (Oxidase reaction, Pectolytic activity, Arginine dihydrolase activity, Tobacco hypersensitivity) (Lelliott *et al*., 1966). The isolates were maintained at -20°C in Luria Bertani (LB) broth containing 20% (v/v) glycerol.

The biochemical properties of two isolates (Pa2124 and Pa4224) from hazelnut were analysed using the API 50 CH system, following the manufacturer's guidelines (bioMérieux, France).

Identification and genetic analyses of isolates

Preliminary identification

Bacterial suspensions (10^8 cfu mL^{-1}) of isolates, Pa2124 and Pa4224, and of *P. avellanae* strain NCPPB 3872, were denatured for 10 min at 95°C, and then centrifuged for 3 min at 8000 g. The supernatants were recovered and assessed by cPCR, using specific primers WA and WC (Loreti and Gallelli, 2002) to confirm their identities.

Genetic fingerprinting

Genomic DNA of *P. avellanae* strains was extracted using the QuickPick™ SML Plant DNA Kit (QRET Technologies) in the KingFisher (Thermo Fisher Scientific) automated platform. Repetitive-sequence PCR using ERIC primer sets (Louws *et al*., 1994) was carried out to compare the two Serbian isolates (Pa4224 and Pa2124), obtained *C. avellana* and presumed to belong to *P. avellanae*, with 13 other *P. avellanae* strains isolated from hazelnut in different countries (Table 1). The procedures described by OEPP/EPPO (2010) were used with minor modifications, consisting of initial denaturation at 95°C for 5 min, and a final extension at 65°C for 15 min. PCR reactions were carried out in 50 µL volumes, using 2 μL^{-1} of DNA extract (20 ng μL^{-1}) and the following reagents at the corresponding final concentration: GoTaq® G2 Flexi DNA Polymerase (Promega) (1.25 U μL^{-1}), 5× GoTaq Flexi buffer (1×), $MgCl_2$ Promega (2 mM), and DMSO was included at a final concentration of 5%. The resulting ERIC-PCR fingerprinting dataset was then analysed using Geneious Prime® 2024.0.5 to generate a phylogenetic tree based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method (Figure 3).

Multilocus sequence analysis (MLSA)

For MLSA, the DNA of the two Serbian isolates Pa2124 and Pa4224 was isolated using the hexadecyltrimethylammonium bromide (CTAB) method described by Popović *et al.* (2019). The DNA was amplified using primers targeting partial sequences of five housekeeping genes, including: *gapA* (glyceraldehyde-3-phosphate dehydrogenase), *gyrB* (DNA gyrase subunit B), *gltA* (citrate synthase), *rpoD* (RNA polymerase sigma factor RpoD), and *recG* (ATP-dependent DNA helicase RecG) (Sarkar and Guttman, 2004; Hwang *et al.* 2005; Iličić *et al*., 2022). The total volume of each PCR mixture (25 µL) included 12.5 µL of Color OptiTaq PCR Master Mix (2×), 9.5 µL of ultrapure DNase/RNase-free water, 1 µL of each primer (10 µM), and 1 µL of the sample DNA (Popović Milovanović *et al*., 2025). PCR amplifications were as follows: initial denaturation step at 94°C for 3 min, then 30 cycles each of denaturation at

Table 1. List of *Pseudomonas avellanae* strains isolated from *Corylus avellana* in different countries, and assessed in the present study.

Isolate code[a]	Origin	Year of isolation
Pa2124	Serbia	2024
Pa4224	Serbia	2024
CREA-DC 1109	Italy	1998
CREA-DC 1113	Italy	1998
CREA-DC 1115	Italy	1998
NCPPB 3872	Italy	1991
NCPPB 3487^{T}	Greece	1976
CREA-DC 1209	Italy	1993
CREA-DC 1210	Italy	1992
CREA-DC 1265	Italy	2003
CREA-DC 1332	Italy	2006
CREA-DC 1341	Italy	2007
CREA-DC 1348	Italy	2007
CREA-DC 1455	Italy	2007
CREA-DC 2106	Italy	2022

[a] CREA-DC: culture collection of the Research Centre for Plant Protection and Certification, Rome, Italy; NCPPB: National Collection of Plant Pathogenic Bacteria, York, United Kingdom.
[T]: type strain of *P. avellanae*.

94°C for 2 min, annealing at 54°C (*gapA*) 56°C (*gltA*, *recG*), 62°C (*gyrB*) and 63°C (*rpoD*), for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The obtained PCR products were separated on a 1% agarose gel and stained with ethidium bromide, and the bands of approx. 610, 634, 556, 521, and 557 bp, corresponding, respectively, to *gyrB*, *gapA*, *gltA*, *rpoD,* and *recG*, were visualized under UV light. PCR products were purified and sequenced commercially (Macrogen Inc. Seoul, South Korea). The obtained sequences were checked for quality and preliminarily identified based on nucleotide Basic Local Alignment Search Tool (BLASTn) analysis of the National Center for Biotechnology Information (NCBI) database. For the final identification of the two isolates, a Maximum likelihood phylogenetic tree was constructed using MEGA12.1 software (Kumar *et al*., 2024; Stecher *et al*., 2025), based on the concatenated sequences of all five genes with 21 comparative strains retrieved from the NCBI database (Table 2). The tree was constructed using the Tamura-Nei model combined with a gamma distribution (Tamura and Nei, 1993) and the Bootstrap method, with 1000 replications. Before tree construction, all sequences of the tested and comparative strains were trimmed to the following sizes: 579 nt (*gapA*), 665 nt (*gltA*), 588 nt (*gyrB*), 499 nt (*rpoD*), 521 nt (*recG*), and concatenated (2852 nt). Sequences of the two Serbian isolates Pa2124 and Pa4224 from hazelnut for all five genes were deposited in NCBI, and their respective accession numbers were obtained.

Pathogenicity assessments

Pathogenicity tests were carried out in early autumn on potted 1-year-old hazelnut (cv. Tonda di Giffoni) plants, using the leaf scar inoculation method of (Scortichini and Tropiano, 1994). Inoculations were carried out by detaching the leaves from plant branches and coating the leaf scar wounds with bacterial suspensions prepared in SDW, and adjusted to concentration of 10^9 CFU mL^{-1}. The inoculated sites were each wrapped with wet wool and aluminium foil to retain the bacterial suspension within the leaf scars for the following 10 d, until the first assessment for symptom development. The potted hazelnut plants were then kept outside for 2 months. SDW was used as the negative inoculation control treatment. Bacteria were re-isolated from hazelnut cankers onto NSA, and their identities were determined using the LOPAT tests (above) and sequencing of the *gyrB* gene, to assess fulfilment of Koch's postulates.

RESULTS

From the five collected hazelnut samples, isolations on NSA were positive from two of the samples, as shown by formation of bacterial colonies, that were whitish, mucoid, convex, shiny, and levan-positive, as assessed after 3 d of incubation. The results of LOPAT tests (+ – – – +) for the two obtained isolates Pa2124 and Pa4224, were positive for HR on tobacco leaves, and were negative for oxidase, pectolytic activity on potato slices, and arginine dihydrolase, matching with the Ia group of fluorescent *Pseudomonas*.

In the API test (50CH), the isolates Pa2124 and Pa4224 were: positive for erythritol, L-arabinose, D-xylose, D-galactose, D-turanose, D-lyxose, D-fucose, L-fucose, D-arabitol; weakly positive for D-glucose, D-fructose, D-mannose; but were negative for glycerol, D-arabinose, D-ribose, L-xylose, D-adonitol, methyl-β-d-xylopyranoside, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-d-glucopyranoside, amygdalin, arbutin, aesculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose (sucrose), D-trehalose, inulin, D-melezitose, D-raffinose, Amidon (starch), glycogen, xylitol, gentiobiose, D-tagatose, L-arabitol, L-sorbose, N-acetylglucosamine, methyl α-d-mannopyranoside, potassium gluconate, potassium 2-ketogluconate, and potassium 5-ketogluconate. Both isolates gave identical results in the applied tests.

Table 2. *Pseudomonas* spp. strains from the NCBI database used for phylogenetic analyses.

Bacterium isolate[a]	Host	Country of origin	Accession number
Pseudomonas avellanae			
NCPPB 4222	Hazelnut	Italy	CP091147
NCPPB 3491	Hazelnut	Greece	CP091143
JCM 11937^T (=NCPPB 3487^T=DSM 11809= CIP 105176=BPI631)	Hazelnut	Greece	BMNO01000071 (*recG*)/ BMNO01000045 (*gltA*)/ BMNO01000048 (*gyrB*)/ BMNO01000030 (*rpoD*)/ BMNO01000084 (*gapA*)
R29095	Sweet cherry	United Kingdom: Warwickshire	MLED01000019 (*recG*)/ MLED01000015 (*gltA*)/ MLED01000032 (*gyrB*)/ MLED01000045 (*rpoD*)/ MLED01000007 (*gapA*)
ICMP 3690	Cherry	New Zealand	LKBV01000079 (*recG*)/ LKBV01000035 (*gltA*)/ LKBV01000238 (*gyrB*)/ LKBV01000259 (*rpoD*)/ LKBV01000001 (*gapA*)/
ICMP 9749	Hazelnut	Greece: Mouries, Kilkis	RBTX01000679 (*recG*)/ RBTX01000462 (*gltA*)/ RBTX01000327 (*gyrB*)/ RBTX01000611 (*rpoD*)/ RBTX01000608 (*gapA*)
Pseudomonas syringae pv. *actinidiae*			
CRAFRU 14.08^T	Kiwifruit	Portugal	CP019732
MAFF212063	Kiwifruit	Japan: Saga	CP024712
ICMP 18708	Kiwifruit	New Zealand	CP012179
FX219	Kiwifruit	China: Jiangxi province	CP186534
Pseudomonas syringae pv. *syringae*			
CFBP4215	Sweet cherry	France	LT962480
CFBP2118	Sour cherry	France	LT962481
Pss9644	Sweet cherry	United Kingdom: Worcestershire	CP066263
B48	Peach	USA: South Carolina	CP125300
Pseudomonas syringae pv. *morsprunorum*			
R15244	Sweet cherry	United Kingdom: Kent	CP026558
Pseudomonas cerasi			
58^T (=LMG 28609^T = CFBP 8305^T)	Sour cherry	Poland	LT222319
PL963	Sweet cherry	Poland	LT963395
Pseudomonas viridiflava			
CFBP 1590^R	Sour cherry	France	LT855380
DSM 6694^T (=625=ATCC 13223)	Runner bean	Switzerland	JRXH01000077 (*recG*)/ JRXH01000067 (*gltA*)/ JRXH01000029 (*gyrB*)/ JRXH01000049 (*rpoD*)/ JRXH01000119 (*gapA*)
Pseudomonas asturiensis			
LMG 26898^T	Soybean	Spain	FRDA01000015 (*recG*)/ FRDA01000011 (*gltA*)/ FRDA01000025 (*gyrB*)/ FRDA01000006 (*rpoD*)/ FRDA01000018 (*gapA*)
Pseudomonas savastanoi pv. *savastanoi*			
NCPPB 3335^T	Olive knot	France	CP008742

[a] : CFBP: Collection Française des Bactéries Phytopathogènes, Angers, France; CIP: Institut Pasteur Collection, Paris, France; CRAFRU: culture collection of the Research Centre for Olive, Fruit and Citrus Crops, Rome, Italy; DSM: Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; JCM: Japan Collection of Microorganisms, Tsukuba, Ibaraki Prefecture, Japan; LMG: BCCM/LMG Bacteria Collection, Ghent, Belgium; MAFF: Ministry of Agriculture, Forestry and Fisheries, Genebank in Tsukuba, Japan; NCPPB: National Collection of Plant Pathogenic Bacteria, York, United Kingdom.
R: designated as the reference genome of the species in NCBI database outgroup strain.
T: type strain of the reference species.

Genetic analyses

Preliminary identification

The two Serbian hazelnut isolates Pa2124 and Pa4224, and the reference strain NCPPB 3872, yielded the expected ~350 bp amplicon in the cPCR assay of Loreti and Gallelli (2002), confirming their identities as *P. avellanae*.

Genetic fingerprinting

Repetitive ERIC-PCR fingerprinting patterns and the related dendrogram of relationships for the Serbian isolates Pa2124 and Pa4224, 13 Italian strains, and one Greek strain of *P. avellanae* are shown in Figure 3, a and b. Three distinct molecular patterns were identified (Figure 3 a). The UPGMA dendrogram (Figure 3 b) identified three different groups. The first group (I) comprised the two Serbian isolates (Figure 3 a, lanes 1 and 2), which overlapped with Italian strains CREA-DC 1109, 1113, 1115, 1210, 1455, and 2106 (Figure 3 a, lanes 3, 4, 5, 9, 14, and 15). A second group (II) included the remaining Italian strains NCPPB 3872, CREA-DC 1209, 1265, 1332, 1341, and 1348 (Figure 3 a, lanes 6, 8, 10, 11, 12, and 13), which clustered separately. The Greek strain NCPPB 3487^{T} (Figure 3 a, lane 7) formed a more distant group (III). The negative control (Figure 3 a, lane 16) produced no amplification products.

Multilocus sequence analysis (MLSA)

Sequences of all five genes for the two Serbian hazelnut isolates Pa2124 and Pa4224 were identical. Preliminary BLASTn analysis of the *gapA*, *gltA*, *gyrB,* and *recG* sequences of the two isolates indicated 100% similarity with *P. avellanae* strains NCPPB 4222, NCPPB 3491, BPIC631 (only *gapA* and *gyrB*), and CFBP 4960 (only *gapA* and *gyrB*). *RpoD* gene sequences showed 100% identity with *P. avellanae* strain BPIC631 (LT622045) and *P. syringae* pv. *morsprunorum* strains IO 77 (HG000022) and R2 (OP834101), and 99.80% with *P. avellanae* strains NCPPB 4222 and NCPPB 3491. The constructed maximum likelihood phylogenetic tree based on the concatenated sequences (*gapA-gltA-gyrB-rpoD-recG*) is presented in Figure 4. This tree showed that the two Serbian isolates were *P. avellanae,* showing the greatest similarity to the *P. avellanae* type strain JCM 11937, as well as to strains NCPPB 3491, ICMP 9749, and NCPPB 4222, all isolated from hazelnut in Greece (strain NCPPB 4222 from Italy). In contrast, *P.*

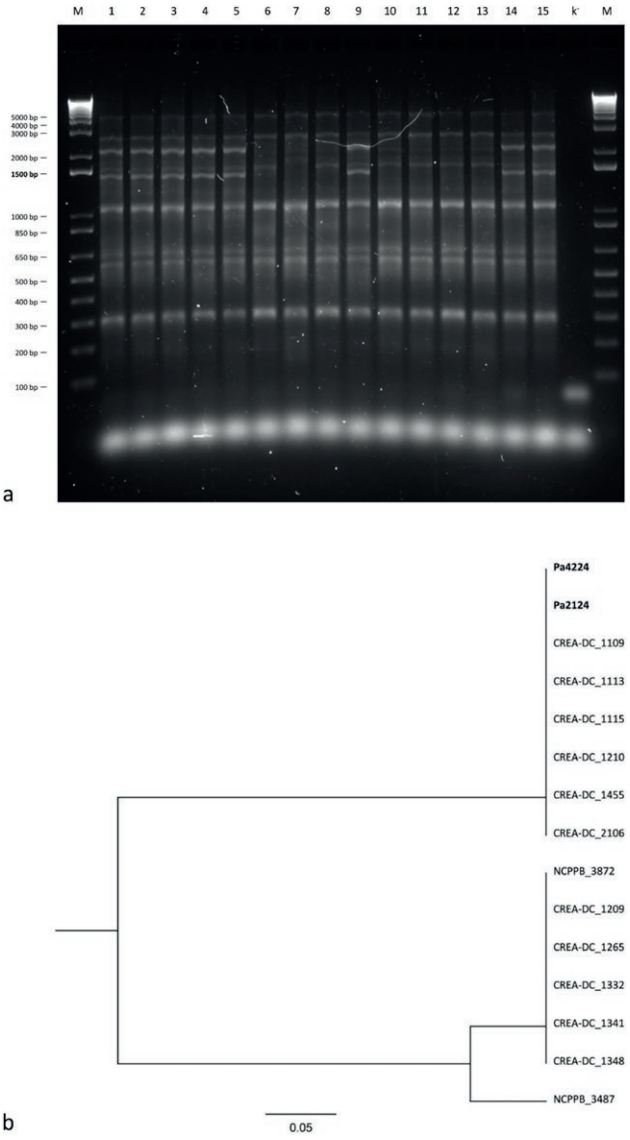

Figure 3. (a) Repetitive ERIC-PCR fingerprinting patterns from genomic DNA of two *Pseudomonas avellanae* isolates obtained in Serbia (lane 1, isolate Pa42242, and lane 2, isolate Pa2124), compared to 13 strains isolated in Italy and Greece in different years (Table 1) (lanes 3 to 15 respectively: strains CREA-DC 1109, CREA-DC 1113, CREA-DC 1115, NCPPB 3487T = BPIC631, NCPPB 3872, CREA-DC 1209, CREA-DC 1210, CREA-DC 1265, CREA-DC 1332, CREA-DC 1341, CREA-DC 1348, CREA-DC 1455, and CREA-DC 1455). Lane k is the negative control, and lane M is the molecular size marker 1 Kb Plus DNA Ladder (Invitrogen™). (b) Dendrogram of relationships between isolates, based on the fingerprinting patterns obtained through the ERIC-PCR analysis for the 15 *P. avellanae* isolates. The ERIC-PCR fingerprinting data set was analysed using Geneious prime 2024.0.5 software to generate a phylogenetic tree based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method.

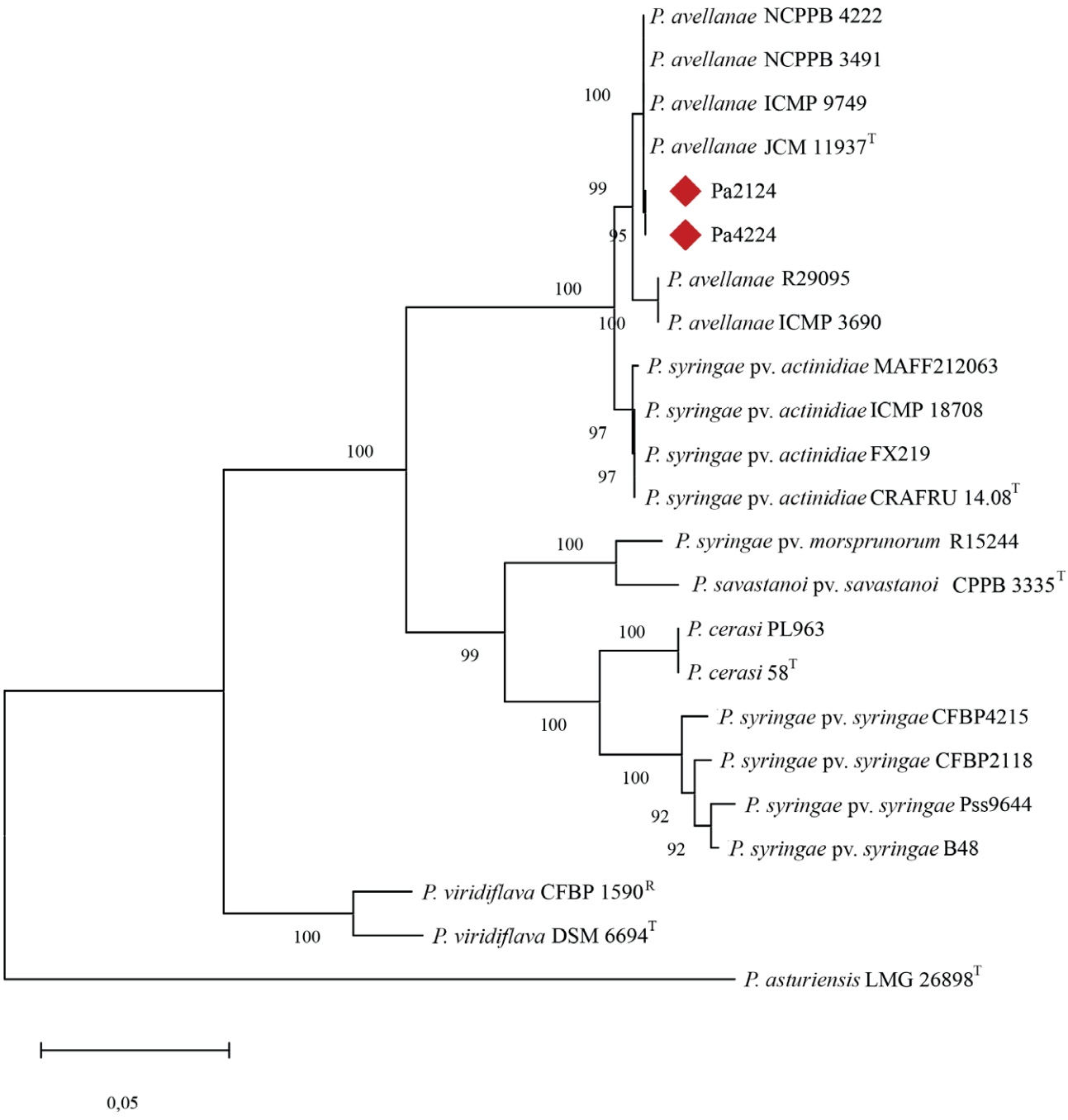

Figure 4. Maximum likelihood phylogenetic tree showing placements of the two Serbian hazelnut isolates (marked with red rhombi) in relation to 21 comparative strains of *Pseudomonas* spp. (*avellanae*, *cerasi, viridiflava,* and *asturiensis*), *P. syringae* pvs. (*actinidiae*, *morsprunorum*, and *syringae*), and *P. savastanoi* pv. *savastanoi* retrieved from the NCBI. The superscript 'T' marks the type strain of each species, while superscript 'R' indicates the reference genome of the species designated by NCBI.

avellanae strains R29095 and ICMP 3690, isolated from cherry in United Kingdom and New Zealand, respectively, were slightly different, although still positioned within the same cluster of the tree. The remaining strains were clearly separated into four distinct tree clusters, each corresponding to a specific species (*P. syringae* pv. *actinidiae*, *P. syringae* pv. *syringae*, *P. viridiflava*, and *P. cerasi*), except for *P. syringae* pv. *morsprunorum* isolate R15244 and *P. savastanoi* pv. *savastanoi* strain CPPB 3335^T, which clustered together due to their high similar-

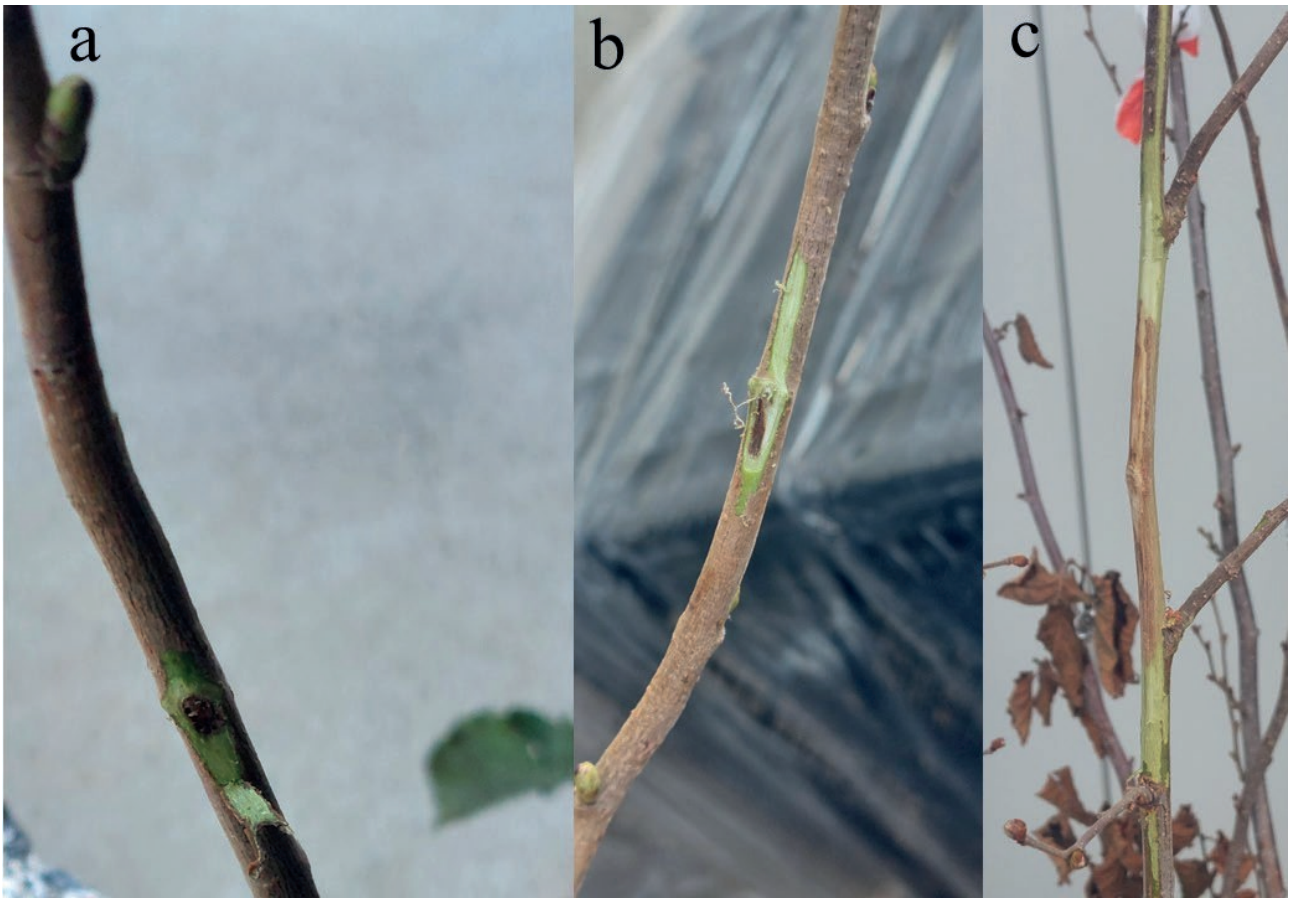

Figure 5. *Pseudomonas avellanae* pathogenicity on potted Tonda di Giffoni hazelnut plants. (a) After ten days, (b) after 21 days, and (c) after 2 months. Necrotic discolourations can be seen along the woody tissue under the bark, as well as twig decline at 2 months post-inoculation.

ity based on the assessed genes. The type strain of *Pseudomonas asturiensis* (LMG 26898) was in a monophyletic tree branch. The respective accession numbers for the sequences of the two Serbian *P. avellanae* isolates Pa2124 and Pa4224 were: *gapA* (PX406174 and PX406175), *gltA* (PX406176 and PX406177), *gyrB* (PX406178 and PX406179), *rpoD* (PX406180 and PX406181), and *recG* (PX406182 and PX406183).

Pathogenicity

The Serbian isolates Pa2124 and Pa4224 both caused brown necroses at the inoculation sites 10 d after artificial inoculations (Figure 5). On the inoculated plants, the bark developed cracking, leading to formation of cankers. Upon removal of the bark, brown discolourations of the sapwood were revealed. After 21 d, necroses had spread longitudinally along the stems, and after 2 months had reach lengths up to 20 cm (Figure 5). Plants treated with SDW were symptomless. Re-isolations from inoculated hazelnut plants produced bacterial colonies on NSA, and LOPAT (+---+) and *gyrB* sequences of these isolates confirmed the identity of the obtained hazelnut re-isolates with the inoculated isolates.

DISCUSSION

The present study is the first to report occurrence of *P. avellanae* in hazelnut orchards of Serbia planted with cv. Tonda di Giffoni. The specific cPCR assay of Loreti and Gallelli (2002) allowed confirmation of the identity of the suspected *P. avellanae* colonies recovered from infected plant material. Rep-PCR carried out with ERIC primer sets clustered the two Serbian isolates in the same group as some of the *P. avellanae* isolates previously obtained in Italy, indicating close relationship between the Serbian and Italian pathogen populations. A second cluster comprised *P. avellanae* strains collected

in Italy in different years, highlighting genetic variability among the Italian strains, while a separate, more distantly related cluster included the Greek strain. Despite the clear separation into three clusters, no evidence was observed of clustering based on either temporal or geographical origins of the bacteria. MLSA indicated similarity between the Serbian and the *P. avellanae* strains obtained from hazelnut cultivations in Greece and Italy, although the Serbian isolates were genetically different.

The present study was the first to assess *P. avellanae* isolates from the cultivar Tonda di Giffoni. Previously, isolates from the hazelnut cultivars Tonda Gentile Romana and Nocchione and from the Greek cultivars have been characterized (Scortichini *et al*., 2000; 2006). This difference could be due either to adaptation of the bacterium to different host cultivars, or to differentiation of *P. avellanae* that has occurred during the last 20 years. Variability within the core genomes of *P. avellanae* strains from Italy was also detected by Turco *et al*. (2022), who assessed seven housekeeping genes in the pathogen.

The young age of the hazelnut orchard from which *P. avellanae* was isolated indicates a possible origin of the pathogen from the hazelnut planting material obtained from Italy. This also indicates expanded occurrence this bacterium in the European Union, and to areas where *C. avellana* cultivation has been recently introduced as a profitable crop. Surveys assessing possible signs of hazelnut decline should be carried out on the plantation examined in the present to possibly prevent further spread of the disease. Testing of propagation hazelnut material before planting for possible presence of *P. avellanae*, would help prevent new outbreaks caused by the movement of latently infected, asymptomatic plant material, before the pathogen becomes established in hazelnut cultivation areas.

Pathogenicity tests have confirmed that hazelnut leaf scars can be colonized by *P. avellanae* during leaf fall (Scortichini and Tropiano, 1994). Copper treatments should protect these points of pathogen entry during autumn, as afterwards, due to systemic spread of the bacterium within tree tissues, it is not possible to effectively control the disease. Particular care should also be taken to disinfect pruning shears to avoid mechanical transmission of *P. avellanae* within and between orchards. Heavily affected trees should be removed from orchards, including removal of tree roots.

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