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Research Papers

First report of *Rhodococcus fascians* causing leafy gall on *Iberis sempervirens* in Hungary

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Summary. In spring of 2023, leafy gall symptoms were detected on plants of evergreen candytuft (*Iberis sempervirens* 'Pink Ice') in Hungary. Bacteria isolated from gall-like tissues of short, stunted shoots, and showing a characteristic appearance on selective culture media were investigated using bacteriological and molecular methods, and phylogenetic analysis. Nucleotide sequences of the 16S rRNA gene, *fasD* and *vicA* genes were determined. Pathogenicity of selected isolates was confirmed on garden pea (*Pisum sativum* 'Tristar'). Characterization of the investigated isolates indicated the presence of *Rhodococcus fascians* in *I. sempervirens*. This is the first report identifying the causal agent of leafy gall from this plant in Hungary.

Keywords. Bacterial plant disease, evergreen candytuft, fasciation.

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INTRODUCTION

Evergreen candytuft (*Iberis sempervirens* L., *Brassicaceae*) is a popular ornamental subshrub plant which is native to the Mediterranean basin (POWO, 2024). One of its bacterial pathogens (Putnam and Miller, 2007) is the Gram-positive *Rhodococcus fascians* (Tilford 1936) Goodfellow 1984 [syn. *Rhodococcoides fascians* (Tilford 1936) Val-Calvo and Vázquez-Boland 2023], the only known phytopathogenic *Rhodococcus* species. This pathogen impairs development and growth of a wide range of host plants, including developmental abnormalities, many of which can be mimicked by application of the plant hormone cytokinin (Jameson *et al.*, 2019). These

growth disorders include hyperplasia, stunting and formation of leafy galls, which are masses of differentiated tissues compacted into small spaces (Cornelis *et al.,* 2001; Putnam and Miller, 2007; Stes *et al.,* 2013). These galls can reduce host plant vigour and make affected plants unmarketable. *Rhodococcus fascians* infections are often not obvious due to the bacterium's ability to be latent or to induce mild symptoms, which may lead to rapid spread by vegetative host propagation material (Putnam and Miller, 2007). To avoid severe economic losses, sanitation and prevention are the primary means of disease management, because the pathogen can spread in plant sap and on cultivation tools (Gordon *et al.,* 2024).

Leafy gall was first reported nearly 100 years ago from the United States of America on sweet peas (*Lathyrus odoratus* L.) (Brown, 1927). The disease is now widely distributed (CABI, 2022), and *R. fascians* is a regulated non-quarantine pest based on Regulation 2019/2072 of the European Union concerning *Rubus* plants, with a 0% threshold for the planting material (EU, 2019). Accordingly, a policy in the Netherlands requires abnormal *Lilium* bulbs to be destroyed during flower bulb production (de Best *et al.,* 2000). During regular visual assessments of Hungarian perennial plant nurseries in 2023, unusual, leafy gall-like symptoms were observed on some *Iberis* plants in container production.

MATERIALS AND METHODS

Sources of plant material, visual assessments and isolation of the potential pathogen

In spring of 2023, a general visual assessment of several *Iberis sempervirens* cultivars was carried out at four major Hungarian perennial plant nurseries, which produce approx. 50% of the total perennial plant output of the country. At each nursery, all potted plants were separated from the soil by a drainage layer and weed barrier cloth. The *Iberis* cuttings had originated from Germany and the Netherlands in autumn 2022. During spring of 2023 plants of *I. sempervirens* 'Pink Ice', a popular *Iberis* cultivar with narrow leaves and racemes of pale pink flowers, showed characteristic symptoms of leafy gall in two nurseries. The symptomatic plants had leafy galls, which developed primarily at the plant bases (Figure 1). Based on these symptoms, plant material was collected and tested for the presence of *R. fascians*.

Symptomatic stems and leaves of the collected plant samples were homogenized without surface disinfection using mortar and pestle in phosphate buffered saline (PBS) pH 7.4, prior to dilution plating onto cycloheximide-amended (100 ppm) D2 agar (Kado and Heskett, 1970) plates. This medium favours growth of *Rhodococcus* spp. (syn. *Corynebacterium* spp.). Inoculated plates were incubated in the dark at 27°C (Klement *et al.,* 1990) for 4 days, after which small, circular, convex, mucoid

Figure 1. Symptoms of leafy gall detected on *Iberis sempervirens* 'Pink Ice' plants. A: Dense clusters of deformed leafy shoots (red arrows), compared to symptomless *I. sempervirens* 'Fischbeck' plants (B and C).

and glistening orange shade colonies were selected for purification and organism identification.

Characterization of pathogenic isolates

Phenotypic characterization of selected isolates was carried out according to standard methods (Klement *et al.,* 1990; Schaad *et al.,* 2001) using the *R. fascians* strain NCAIM B.01614 isolated by W.J. Dowson from chrysanthemum as a reference strain.

Genomic DNA was extracted from 23 colonies by suspending them in 50 µL sterile nuclease-free water, then boiling (10 min at 99°C) and centrifuging (10 min at 4°C, 16700 *g*) each bacterial suspension separately. Supernatants were used as templates for PCR-amplification of the partial 16S rRNA gene using universal primers 27F/1492R (Lane, 1991), while the *fasD* (encoding isopentenyltransferase) gene fragment amplified with fasD-F/FasD-R primer pair, and the *vicA* (encoding malate synthase) gene fragment amplified with the vicA44-F/vicA737-R primer pair were used according to Park *et al.* (2021) (Table 1).

DNA sequencing of the PCR amplified products of the three loci were carried out for species identification of the bacterial isolates. The isolated bacteria were identified mainly on the basis of 16S rRNA and the virulence gene *fasD* sequence analyses, while *vicA* was used to compare the relationships among the *Rhodococcus* strains.

Amplified DNA products of two isolates (IsHu1 and IsHu2) and the strain NCAIM B.01614 were selected for sequence analysis and were purified using a high pure PCR product purification kit (Roche) according to the manufacturer's protocol. The sequences obtained (Macrogen Europe BV, Amsterdam, The Netherlands) were compared with publicly available sequences of plant-associated *Rhodococcus* strains derived from NCBI databases by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and were used to construct a phylogenetic tree of 26 *Rhodococcus* isolates and a *Streptomyces* strain as the outgroup. Phylogenetic analysis was carried out using MEGA software version 11 (Tamura *et al.,* 2021) after multiple alignments of sequence data were achieved using the ClustalW algorithm. The amplified 16S rRNA, *fasD*, and *vicA* gene regions were concatenated into a single data set of 2406 sites, and were incorporated into a single phylogenetic tree using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993).

Pathogenicity tests

Pathogenicity tests were carried out according to Dhaouadi *et al.* (2021), with modifications. Germinated seeds of garden pea (*Pisum sativum* L. 'Tristar') were inoculated with 19 bacterial isolates carrying the *fasD* gene. Following surface disinfection, the pea seeds were placed between moistened sterile blotting papers in 9 cm Petri dishes in the dark at constant 20°C for 4 days. The germinated seeds were then inoculated by shaking in bacterial suspensions (10^8 CFU mL^{-1}) for 2 h at 125 rpm at room temperature. Treatment with PBS served as a negative control. Three seeds inoculated with each isolate were then sown into autoclaved soil-peat mixture (1:1), and were incubated in a growth chamber at 25°C/20°C 16 h/8 h light/dark cycles for 14 days. Re-isolations of *R. fascians* from symptomatic seedlings inoculated with isolates IsHu1 or IsHu2, or from a PBS-treated negative control plant, were carried out following the isolation procedure described above, and pathogen identity was assessed by colony PCR using *fasD*-specific primers.

Table 1. Oligonucleotide primers and PCR conditions applied in the present study.

Primer	Nucleotid sequence (5' → 3')	Target gene	Amplicon length (bp)	Cycling conditions denaturation (initial and repeated), annealing, extension, cycle number, final extension	Reference
27F	AGAGTTTGATCMTGGCTCAG	16S rRNA	1517	95°C / 5 min, 95°C / 30 s, 59°C / 30 s, 72°C / 90 s, 35, 72°C / 5 min	Lane, 1991
1492R	GGTTACCTTGTTACGACTT				
fasD-F	ATTGTTGTTGCCGACCGTATC	*fasD*	573	95°C / 3 min, 95°C / 20 s, 55°C / 30 s, 72°C / 60 s, 40, 72°C / 10 min	Park *et al.,* 2021
fasD-R	AAGGACGCCGTGCTCGACATAC				
vicA44-F	TCCTATTCGATTTCGTCGAGAAG	*vicA*	694		
vicA737-R	GGGTCGATCTGGATCTCGAA				

A further pathogenicity test was carried out with the three selected isolates (IsHu1, IsHu2, and IsHu3), which had caused characteristic symptoms. The strain NCAIM B.01614 was used as a positive control and PBS was used as the negative control.

The numbers and lengths of plant shoots grown from ten treated seeds per isolate were measured at 14 days post-inoculation. Means and standard deviations for these data were calculated and statistical analysis was performed using the Kruskal-Wallis test with Statistica software (StatSoft, Inc.).

RESULTS

During surveys conducted in 2023 in nurseries in Hungary, abnormal growth of *Iberis sempervirens* plants was observed at two different locations (Table 2).

Table 2 shows marked susceptibility of the cultivar 'Pink Ice', as compared to other cultivars assessed. During the 2023 growing season, the symptomatic plants became severely weakened and they were unable to overwinter.

Following isolations of bacteria from symptomatic plant samples, colonies typical of *R. fascians* formed on D2 agar, nutrient-broth yeast extract agar (NBY), and 1% glucose nutrient agar (GNA). The isolated bacteria were Gram-positive, aerobic, non-motile, with urease activity, were unable to grow at 36°C, and did not tolerate 7% sodium chloride, in line with data outlined by Klement *et al.* (1990) and Schaad *et al.* (2001).

Nineteen isolates tested were positive by PCR for the plasmid-associated virulence gene *fasD*, which is present in the fasciation (fas) operon in *R. fascians*, while the chromosomal malate synthase gene *vicA* was found in all 23 isolates. Sequence analyses showed that isolates IsHu1 and IsHu2 had 100% sequence identity within 16S rRNA, *fasD* and *vicA* gene fragments. Comparison with sequences available in GenBank indicated that isolate IsHu1 belonged to *R. fascians*. The sequences of IsHu1 and NCAIM B.01614 were deposited in NCBI GenBank under accession numbers PP125720 and PP125739 for the partial 16S rRNA gene, PP130585 and PP130586 for *fasD*, and PP130584 and PP130587 for *vicA*.

Rhodococcus isolates used for sequence analyses and construction of a phylogenetic tree are shown in Table 3.

The 16S rRNA gene sequence comparisons in NCBI GenBank revealed 100% identity between isolate IsHu1 and the plant-associated *R. fascians* strains D188, 15-508-1b and YWS4-1 (Table 4). The *fasD* gene sequence obtained from isolate IsHu1 showed 100% identity with the type strain *R. fascians* D188, and with several other *Rhodococcus* isolates (Table 4). The sequence of the *vicA* gene of isolate IsHu1 shared >99% identity with *R. fascians* strains NBRC 12155 = LMG 3623 (99.83%), YWS8-2 (99.66%), A78 (99.49%) and YWS3-1 (99.49%) (Table 4).

Sequences of 16S rRNA, *fasD* and *vicA* genes were concatenated into one combined alignment, which was used for Maximum Likelihood tree inference. IsHu1 was closely related to strains D188 and 15-508-1b in the phylogenetic tree (Figure 2). The strain NCAIM B.01614, which served as a positive control in the artificial inoculation experiments, clustered in the same clade as IsHu1,

Table 2. Leafy gall incidence (assessed visually) for different *Iberis* cultivars at different nurseries in Hungary in 2023.

Location	Assessment date	Cultivar	Disease incidence (%)	Total number of plants assessed
Northwest Great Plain	April 17	Appen-Etz	0	1200
		Fischbeck	0	1200
		Golden Candy	0	520
		Nevina	0	1200
Western Transdanubia	April 21	Appen-Etz	0	1600
		Fischbeck	0	3200
		Pink Ice	100	408
		Schneeflocke	0	1680
Northwest-Transdanubia	April 24	Absolutely Amethyst	0	520
		Appen-Ezt	0	1040
		Snowsurfer Compact	0	1040
Southeastern Great Plain	May 5	Appen-Etz	0	9360
		Golden Candy	0	1560
		Pink Ice	10.22	74880

Table 3. *Rhodococcus* isolates used for sequence analyses and construction of a phylogenetic tree.

Strain	Accession number (NCBI GenBank)			Host/Source	Pathogen	Reference
	16S rRNA	*vicA*	*fasD*			
Rhodococcus fascians IsHu1	PP125720	PP130584	PP130585	*Iberis sempervirens* 'Pink Ice', Hungary, 2023	+	present study
R. fascians D188	CP015235	CP015235	CP015236	*Chrysanthemum* × *morifolium*, UK, 1946	+	Desomer *et al.*, 1988
R. fascians 15-508-1b	NPFO01000033	NPFO01000016	NPFO01000019	*Petunia* 'Flash Mob Pinkceptional', USA, 2015	nt	Savory *et al.*, 2017
R. fascians YWS4-1	MW394218	MW394214	MW394222	*Lilium* sp., South Korea, 2020	+	Park *et al.*, 2021
R. sp. 05-2221-1B	NOZL01000044	NOZL01000021	NOZL01000042	*Verbascum* 'Sierra Sunset', USA, 2005	nt	Savory *et al.*, 2017
R. sp. 14-2496-1d	NPFV01000050	NPFV01000029	NPFV01000030	*Scabiosa* sp., USA, 2014	nt	Savory *et al.*, 2017
R. fascians A76	JMEV01000015	JMEV01000004	JMEV01000021	*Veronica spicata* 'Royal Candles', USA, 2002	+	Creason *et al.*, 2014a
R. fascians A21d2	CP049748	CP049748	None	*Oenothera speciosa*, 'Siskiyou', USA, 2002	+	Serdani *et al.*, 2013
R. sp. 14-2483-1-1	NPFX01000030	NPFX01000022	NPFX01000024	*Geranium* sp., USA, 2014	nt	Savory *et al.*, 2017
R. fascians NCAIM B.01614	PP125739	PP130587	PP130586	*Chrysanthemum* sp., UK	+	present study
R. fascians A78	JMEU01000039	JMEU01000016	JMEU01000002	*Leucanthemum* × *superbum*, 'Becky', USA, 2002	+	Creason *et al.*, 2014a
R. fascians YWS8-2	MW394219	MW394215	MW394223	*Lilium* sp., South Korea, 2020	+	Park *et al.*, 2021
R. fascians YWS1-1	MW394216	MW394212	MW394220	*Lilium* sp., South Korea, 2020	+	Park *et al.*, 2021
R. fascians A3b	JMEY01000022	JMEY01000029	JMEY01000009	*Heliopsis helianthoides* 'Loraine Sunshine', USA, 2005	+	Creason *et al.*, 2014a
R. fascians NBRC 12155 = LMG 3623	JMEN01000010	JMEN01000030	JMEN01000005	*Lathyrus odoratus*, USA, Ohio, 1936	+	Tilford, 1936
R. fascians YWS3-1	MW394217	MW394213	MW394221	*Lilium* sp., South Korea, 2020	+	Park *et al.*, 2021
R. sp. 05-2254-3	NOZI01000017	NOZI01000027	NOZI01000005	*Veronica* 'Sunny Border Blue', USA, 2005	nt	Savory *et al.*, 2017
R. fascians A25f	CP049744	CP049744	CP049745	*Nemesia* 'Natalie', USA, 2002	+	Creason *et al.*, 2014a
R. fascians 05-339-2	NOYW01000001	JMFC01000021	NOYW01000019	*Hosta* 'Blue Umbrellas', USA, 2005	+	Creason *et al.*, 2014a
R. fascians A73a	JMEW01000018	JMEW01000014	JMEW01000015	*Aster amellus* 'Violet Queen', USA, 2003	+	Creason *et al.*, 2014a
R. fascians 02-815	JMFF01000017	JMFF01000006	JMFF01000005	*Campanula* 'Sarastro', USA, 2002	+	Creason *et al.*, 2014a
R. fascians A44A	JMEX01000006	JMEX01000005	JMEX01000009	*Veronica spicata* 'Minuet', USA, 2002	+	Creason *et al.*, 2014a
R. sp. 06-156-4	NPGB01000004	NPGB01000015	NPGB01000038	*Campanula* sp., USA, 2006	nt	Savory *et al.*, 2017
R. sp. 15-1189-1-1a	NPFQ01000064	NPFQ01000035	NPFQ01000039	*Leucanthemum* sp., South America, 2015	+	Savory *et al.*, 2017
R. kyotonensis DS472	AB269261	FZOW01000009	None	soil, Japan	nt	Li *et al.*, 2007
R. corynebacterioides DSM 20151	LPZL01000057	LPZL01000030	None	air contaminant	nt	Yassin and Schaal, 2005
Streptomyces sp. NEAU-BLH26	JBFBOR010000023	JBFBOR010000006	JBFBOR010000010	*Adonis amurensis* 'Regel', China, 2021	nt	unpublished

Abbreviation: nt, not tested.

Table 4. Sequence identities (%) for the 16S rRNA, *fasD* and *vicA* genes between *Rhodococcus fascians* IsHu1 and other isolates used for construction of the phylogenetic tree.

Isolate	Homology (%)		
	16S rRNA	*fasD*	*vicA*
R. fascians D188	100.00	100.00	98.62
R. fascians 15-508-1b	100.00	100.00	98.97
R. fascians YWS4-1	100.00	100.00	97.04
R. sp. 05-2221-1B	99.76	100.00	87.19
R. sp. 14-2496-1d	99.76	100.00	87.39
R. fascians A76	99.76	100.00	88.40
R. fascians A21d2	99.76	None	87.39
R. sp. 14-2483-1-1	99.76	100.00	87.59
R. fascians NCAIM B.01614	99.44	100.00	98.62
R. fascians A78	99.36	100.00	99.49
R. fascians YWS8-2	99.36	100.00	99.66
R. fascians YWS1-1	99.36	100.00	97.92
R. fascians A3b	99.36	100.00	98.97
R. fascians NBRC 12155=LMG 3623	99.36	100.00	99.83
R. fascians YWS3-1	99.36	100.00	99.49
R. sp. 05-2254-3	99.20	100.00	88.59
R. fascians A25f	99.12	97.05	86.38
R. sp. 05-339-2	99.04	100.00	86.99
R. fascians A73a	98.08	100.00	75.14
R. fascians 02-815	98.08	100.00	77.02
R. fascians A44A	98.08	100.00	75.38
R. sp. 06-156-4	98.08	100.00	77.02
R. sp. 15-1189-1-1a	98.01	100.00	76.08
R. kyotonensis DS472	97.93	None	75.85
R. corynebacterioides DSM 20151	96.33	None	70.73
Streptomyces sp. NEAU-BLH26	89.71	45.76	45.39

along with YWS strains isolated from symptomatic lilies, as well as strains A3b, A78, and LMG 3623 (Figure 2).

During the pathogenicity test with 19 bacterial isolates, all of the inoculated plants showed characteristic symptoms of shoot proliferation, stunting, and hypertrophy, as compared to the control plants, that grew normally (Figure 3). There were no differences among severity of disease symptoms caused by these 19 isolates. Therefore, two groups of plants displaying characteristic symptoms (inoculated respectively with IsHu1 or IsHu2) were selected for re-isolation to assess fulfilment of Koch's postulates. *Rhodococcus fascians* was re-isolated from symptomatic seedlings that had been artificially

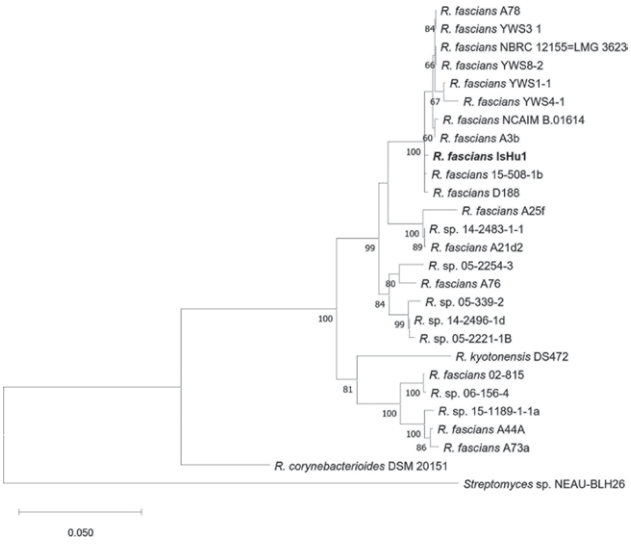

Figure 2. Evolutionary analysis of homologous sequences of concatenated 16S rRNA, *fasD* and *vicA* genes in *Rhodococcus* spp., which was conducted with the Maximum Likelihood algorithm in MEGA 11 using the bootstrap method and the Tamura-Nei model. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches; only values >50% are shown at branch points. *Rhodococcus fascians* IsHu1 is shown in bold type. The scale bar represents the number of substitutions per site.

inoculated with isolates IsHu1 or IsHu2, and its identity was confirmed through colony PCR using the *fasD*-specific primers. The bacterium was not present in PBS-treated control plants.

Inoculations of pea plants with the selected bacterial isolates IsHu1, IsHu2, and IsHu3 increased the numbers of shoots by 5.6- to 7.1-fold, and reduced shoot lengths by 72 to 79%, as compared to the untreated controls. The Kruskal-Wallis non-parametric test revealed no differences ($P < 0.05$) among mean numbers and lengths of shoots 2 weeks after inoculation with different isolates, including the positive control inoculation with strain NCAIM B.01614 (Table 5).

Table 5. Mean shoot lengths and numbers for pea plants inoculated 14 days previously with different *Rhodococcus fascians* isolates.

Inoculation treatments	Mean shoot lengths (mm) (± SE)	Mean numbers of shoots (± SE)
IsHu1	27.4 ± 5.6 b	5.6 ± 0.6 a
IsHu2	26.8 ± 4.5 b	5.7 ± 0.6 a
IsHu3	19.8 ± 2.9 b	7.1 ± 0.5 a
NCAIM B.01614	25.7 ± 4.5 b	6.0 ± 0.8 a
PBS control	96.5 ± 8.8 a	1.0 ± 0.0 b

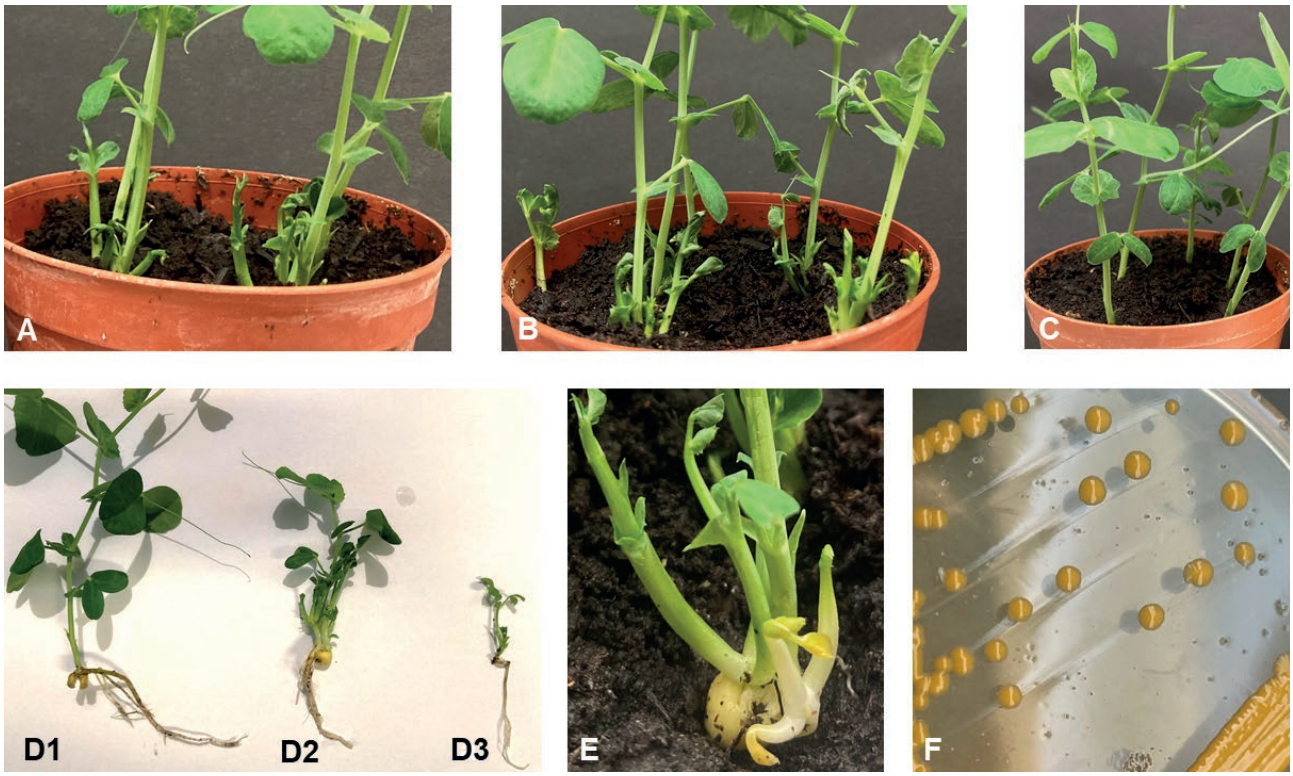

Figure 3. Pathogenicity assessments for *Rhodococcus fascians* isolate IsHu1. Typically small, distorted shoots developed from artificially inoculated *Pisum sativum* 'Tristar' seeds (photos A, D2, D3, and E), and similarly after inoculation with isolate NCAIM B.01614 used as a positive control (B). The negative control plants (C, and D1) developing from seeds treated with PBS showed normal growth. These photos were taken at 14 days post-inoculation. Typical orange *R. fascians* colonies formed on D2 agar plates (F).

Inoculations were carried out with three selected *R. fascians* isolates (IsHu1, IsHu2, IsHu3) and strain NCAIM B.01614 on pea (*Pisum sativum* 'Tristar') seedlings raised from ten seeds per isolate. The number and lengths of shoots were recorded 2 weeks after inoculation. Means and standard errors (SE) were calculated from ten replicates. Statistical analyses were carried out using the Kruskal-Wallis test and Statistica software (StatSoft Inc.). Different lowercase letters in each column indicate differences ($P < 0.05$) between means.

DISCUSSION

Of the *Iberis sempervirens* cultivars assessed in the nurseries, only 'Pink Ice' plants had leafy gall symptoms. Each nursery growing this cultivar had symptomatic plants at the time of the visual assessment. The affected plants were unmarketable and had to be destroyed to prevent further spread of the infections.

The present study showed that the severe leafy gall symptoms on *I. sempervirens* 'Pink Ice' in 2023 in Hungary were caused by *R. fascians*. Phylogenetic analysis of the pathogen was employed on the aligned 16S rRNA, *fasD* and *vicA* gene sequences. The virulence gene *fasD* is usually present in pathogenic *R. fascians* isolates (Savory *et al.*, 2020). Pathogenicity of *R. fascians* requires a cluster of three loci present on a linear plasmid, of which the fasciation (*fas*) operon plays a core role in virulence (Creason *et al.*, 2014b) through local and persistent secretion of an array of synergistically operating cytokinins which bring about continuous tissue proliferation (Pertry *et al.*, 2009; 2010). The *fasD*-coded isopentenyltransferase protein is a pivotal factor in symptom initiation (Pertry *et al.*, 2010). On the other hand, *vicA* is a chromosomal gene encoding malate synthase G of the glyoxylate shunt of the Krebs cycle. Although this gene is not associated with the pathogenic phenotype, it is a suitable marker for phylogenetic reconstructions in *Rhodococcus* (Savory *et al.*, 2017), as it exhibits greater sequence variation than the 16S rRNA gene, allowing for better discrimination among bacterial strains.

The present study results showed that the nucleotide sequences of *R. fascians* isolate IsHu1 obtained had high

overall similarity with those of *R. fascians* 15-508-1b and the well-characterized virulent *R. fascians* model strain D188, based on sequence homology of 16S rRNA, *fasD* and *vicA* gene fragments.

To date in Hungary *R. fascians* has been identified only from geranium (*Pelargonium* × *hortorum* L. H. Bailey) (Süle, 1976). This bacterium is known for its ability to infect a variety of plant hosts (Dhaouadi *et al.,* 2020), including two species of *Iberis* (*I. gibraltarica* L. and *I. sempervirens* L.) (Putnam and Miller, 2007). However, the present study is the first to report *R. fascians* from a cultivar of *I. sempervirens*, but also from this plant in Hungary.

The severe outbreak of *R. fascians* reported here may imply that the propagation material used could have been contaminated with the pathogen. Putnam and Miller (2007) suggest that non-pathogen-free propagating material was probably the primary means by which *R. fascians* can be introduced into non-infested areas. The present report may contribute towards further research on the control of *R. fascians*, which is still based on pathogen prevention.

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LITERATURE CITED

Brown N.A., 1927. Sweet pea fasciation, a form of crown gall. *Phytopathology* 17: 29–39.

CABI, 2022. *Rhodococcus fascians* (fasciation: leafy gall). PlantwisePlus Knowledge Bank. Technical factsheet. Available at: https://doi.org/10.1079/pwkb.species.15332

Cornelis K., Ritsema T., Nijsse J., Holsters M., Goethals K., Jaziri M., 2001. The plant pathogen *Rhodococcus fascians* colonizes the exterior and interior of the aerial parts of plants. *Molecular Plant-Microbe Interaction* 14: 599–608. https://doi.org/10.1094/MPMI.2001.14.5.599

Creason A.L., Davis E.W., Putnam M.L., Vandeputte O.M., Chang J.H., 2014a. Use of whole genome sequences to develop a molecular phylogenetic framework for *Rhodococcus fascians* and the *Rhodococcus* genus. *Frontiers in Plant Science* 5: 406. https://doi.org/10.3389/fpls.2014.00406

Creason A.L., Vandeputte O.M., Savory E.A., Davis E.W., Putnam M.L., … Chang J.H., 2014b. Analysis of genome sequences from plant pathogenic *Rhodococcus* reveals genetic novelties in virulence loci. *PLoS One* 9:e101996. https://doi.org/10.1371/journal.pone.0101996

de Best A.L.I.C., Zwart M.J., van Aartrijk J., van den Ende J.E., Peeters J.M.M., 2000. *Ziekten en afwijkingen bij bolgewassen: Liliaceae* (Vols. 1–2, Vol. 1). Lisse: Laboratorium voor Bloembollenonderzoek.

Desomer J., Dhaese P., van Montagu M., 1988. Conjugative transfer of cadmium resistance plasmids in *Rhodococcus fascians* strains. *Journal of Bacteriology* 170(5): 2401–2405. https://doi.org/10.1128/jb.170.5.2401-2405.1988

Dhaouadi S., Mougou A.H., Rhouma A., 2020. The plant pathogen *Rhodococcus fascians*. History, disease symptomatology, host range, pathogenesis and plant–pathogen interaction. *Annals of Applied Biology* 177(1): 4–15. https://doi.org/10.1111/aab.12600

Dhaouadi S., Mougou A.H., Rhouma A., 2021. Isolation and characterization of *Rhodococcus* spp. from pistachio and almond rootstocks and trees in Tunisia. *Agronomy* 11(2): 355. https://doi.org/10.3390/agronomy11020355

European Union (2019). Commission Implementing Regulation (EU) 2019/2072 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No. 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019. *Official Journal of the European Union* L 319: 1–279. Available at: https://eur-lex.europa.eu/homepage.html

Goodfellow M., 1984. Reclassification of *Corynebacterium fascians* (Tilford) Dowson in the genus *Rhodococcus*, as *Rhodococcus fascians* comb. nov. *Systematic and Applied Microbiology* 5(2): 225–229. https://doi.org/10.1016/S0723-2020(84)80023-5

Gordon M.I., Thomas W.J., Putnam M.L., 2024. Transmission and management of pathogenic *Agrobacterium tumefaciens* and *Rhodococcus fascians* in select ornamentals. *Plant Disease* 108(1): 50–61. https://doi.org/10.1094/PDIS-11-22-2557-RE

Jameson P.E., Dhandapani P., Song J., Zatloukal M., Strnad M., … Novák O., 2019. The cytokinin complex associated with *Rhodococcus fascians*: Which compounds are critical for virulence? *Frontiers in Plant Science* 10: 674. https://doi.org/10.3389/fpls.2019.00674

Kado C.I., Heskett M.G., 1970. Selective media for *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. *Phytopathology* 60(6): 969–976. https://doi.org/10.1094/phyto-60-969

Klement Z., Rudolph K., Sands D.C. (eds.), 1990. *Methods in phytobacteriology*. Akadémia Kiadó, Budapest, Hungary, 568 pp.

Lane D.J., 1991. 16S/23S rRNA sequencing. In *Nucleic acid techniques in bacterial systematics* (E. Stackebrandt, M. Goodfellow, eds.), John Wiley and Sons, New York, United States of America, 115–176.

Li B., Furihata K., Ding L.X., Yokota A., 2007. *Rhodococcus kyotonensis* sp. nov., a novel actinomycete isolated from soil. *International Journal of Systematic and Evolutionary Microbiology* 57(9): 1956-1959. https://doi.org/10.1099/ijs.0.64770-0

Park J.M., Koo J., Kang S.W., Jo S.H., Park J.M., 2021. Detection of *Rhodococcus fascians*, the causative agent of lily fasciation in South Korea. *Pathogens* 10(2): 241. https://doi.org/10.3390/pathogens10020241

Pertry I., Václavíková K., Depuydt S., Galuszka P., Spíchal L., … Vereecke D., 2009. Identification of *Rhodococcus fascians* cytokinins and their modus operandi to reshape the plant. *PNAS* 106(3): 929–934. https://doi.org/10.1073/pnas.0811683106

Pertry I., Václavíková K., Gemrotová M., Spíchal L., Galuszka P., … Vereecke D., 2010. *Rhodococcus fascians* impacts plant development through the dynamic fas-mediated production of a cytokinin mix. *Molecular Plant-Microbe Interaction* 23(9): 1164–1174. https://doi.org/10.1094/MPMI-23-9-1164

POWO, 2024. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet. Available at: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:324670-2

Putnam M.L., Miller M.L., 2007. *Rhodococcus fascians* in herbaceous perennials. *Plant Disease* 91(9): 1064–1076. https://doi.org/10.1094/PDIS-91-9-1064

Savory E.A., Fuller S.L., Weisberg A.J., Thomas W.J., Gordon M.I., … Chang J.H., 2017. Evolutionary transitions between beneficial and phytopathogenic *Rhodococcus* challenge disease management. *eLife* 6: e30925. https://doi.org/10.7554/eLife.30925

Savory E.A., Weisberg A.J., Stevens D.M., Creason, A.L., Fuller S.L., … Chang, J. H., 2020. Phytopathogenic *Rhodococcus* have diverse plasmids with few conserved virulence functions. *Frontiers in Microbiology* 11: 1022. https://doi.org/10.3389/fmicb.2020.01022

Schaad N.W., Jones J.B., Chun W. (eds.), 2001. *Laboratory guide for identification of plant pathogenic bacteria.* 3rd ed. APS Press, St. Paul, MN, United States of America, 373 pp.

Serdani M., Curtis M., Miller M.L., Kraus J., Putnam M.L., 2013. Loop-mediated isothermal amplification and polymerase chain reaction methods for specific and rapid detection of *Rhodococcus fascians*. *Plant Disease* 97(4): 517–529. https://doi.org/10.1094/PDIS-02-12-0214-RE

Süle S., 1976. Bacterial fasciation of *Pelargonium hortorum* in Hungary. *Acta Phytopathologica Academiae Scientiarum Hungaricae* 11(3–4): 223–230.

Stes E., Francis I., Pertry I., Dolzblasz A., Depuydt S., Vereecke D., 2013. The leafy gall syndrome induced by *Rhodococcus fascians*. *FEMS Microbiology Letters* 342(2): 187–194. https://doi.org/10.1111/1574-6968.12119.

Tamura K., Nei M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10(3): 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tamura K., Stecher G., Kumar S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11, *Molecular Biology and Evolution* 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

Tilford P.E., 1936. Fasciation of sweet peas caused by *Phytomonas fascians* n. sp. *Journal of Agricultural Research* 53(5): 383–394.

Val-Calvo J., Vázquez-Boland J.A. 2023. Mycobacteriales taxonomy using network analysis-aided, context-uniform phylogenomic approach for non-subjective genus demarcation. *mBio* 14: e02207-23. https://doi.org/10.1128/mbio.02207-23

Yassin A.F., Schaal K.P., 2005. Reclassification of *Nocardia corynebacterioides* Serrano et al. 1972 (Approved Lists 1980) as *Rhodococcus corynebacterioides* comb. nov. *International Journal of Systematic and Evolutionary Microbiology* 55(3): 1345-1348. https://doi.org/10.1099/ijs.0.63529-0