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Genetic, phenotypic and pathogenic diversity of *Xanthomonas arboricola* pv. *corylina* strains question the representative nature of the type strain

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A collection of 31 *Xanthomonas arboricola* pv. *corylina* strains isolated from *Corylus maxima* and *C. avellana* of different countries were assessed by means of repetitive PCR using ERIC, BOX and REP primer sets and analysis of whole-cell protein extracts; pathogenicity tests to three hazelnut (*C. avellana*) cultivars; and some key biochemical tests. From these studies, the *X. arboricola* pv. *corylina* strains were clustered into five and three groups by repetitive PCR and protein analysis, respectively, and by using UPGMA cluster analysis, with two strains forming an outlier group to these. The groups showed a high degree of similarity. Strain membership between the groups designed by the two methods exhibited a high degree of congruence, and diversity between the groups was low. Surprisingly, the two strains originating from *C. maxima*, that include the type strain NCPPB 935, formed the most distinctive group. No relationship to geographic origin of the strains was evident. All strains proved pathogenic towards three different hazelnut cultivars, although the strains obtained from *C. maxima* did not incite any significant symptoms on buds and twigs. No other relationships between rep-PCR and whole-cell protein groups and pathogenicity were evident. The distinctiveness of the *C. maxima* strains was supported further by atypical negative gelatin liquefaction test and reduced quinate metabolism results.

Keywords: bacterial blight, *Corylus* spp., repetitive PCR, type strain, whole-cell protein analysis, *Xanthomonas arboricola* pv. *corylina*

Introduction

Xanthomonas arboricola pv. *corylina* (synonym: *X. campestris* pv. *corylina*), the causal agent of bacterial blight of hazelnut (*Corylus avellana*), has a worldwide distribution and is a European Plant Protection Organisaton (EPPO) A2 quarantine microorganism (Smith *et al.*, 1997). It is particularly damaging to young (1–4-year-old) hazelnut orchards, where it can kill up to 10% of plants (EPPO, 1986). The pathogen was initially described by studying strains obtained from *C. maxima* (Miller *et al.*, 1940), a minor species of the nut-producing crops which is rarely cultivated. Recently, on the basis of DNA hybridization of the type and some reference strains with other xanthomonads, Vauterin *et al.* (1995) placed the pathogen in the new species *X. arboricola* as pathovar *corylina*. Apart from reports concerning the occurrence of the pathogen in different geographical areas of *C. avellana* cultivation (Smith *et al.*, 1997), there are only a few epidemiological studies (Gardan & Devaux, 1987; Pruvost & Gardan, 1988). Studies on the characterization of the pathogen are limited to a single phenotypic comparison between the biochemical and nutritional characteristics of different strains isolated in France and the type strain isolated in Oregon, USA (Luisetti *et al.*, 1976).

To date, a comparative study on the genetic, phenotypic and pathogenic features of strains isolated from different geographical origins is lacking. The aim of this study was to assess the diversity of *X. arboricola* pv. *corylina* strains obtained from different countries by means of repetitive polymerase chain reaction (PCR), whole-cell protein analysis, pathogenicity and biochemical tests.

Materials and methods

Bacterial strains and growth conditions

The *X. arboricola* pv. *corylina* strains used in this study are listed in Table 1, and reference strains of *X. arboricola* pv. *juglandis* NCPPB 411, *X. arboricola* pv. *pruni* NCPPB 416, and *X. campestris* pv. *campestris* NCPPB 528 were included for comparative purposes. The strains were

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Table 1 List of *Xanthomonas arboricola* pv. *corylina* strains used in this study

Strain	Country	Year of isolation	Host
NCPPB 935[T]	USA (Oregon)	1939	*Corylus maxima*
NCPPB 984	USA (Oregon)	1941	*C. maxima*
Fil 6	USA (Oregon)	unknown	*C. avellana*
Fil 19	USA (Oregon)	unknown	*C. avellana*
Fil 29	USA (Oregon)	unknown	*C. avellana*
Fil 85	USA (Oregon)	unknown	*C. avellana*
NCPPB 2896	United Kingdom	1976	*C. avellana*
NCPPB 3037	United Kingdom	1977	*C. avellana*
PD 1896	the Netherlands	1991	*C. avellana*
PD 1897	the Netherlands	1991	*C. avellana*
NCPPB 3339	France	1984	*C. avellana*
PD 3657	Germany	1999	*C. avellana*
ISF Nc 1	Italy	1996	*C. avellana*
ISF Nc 2	Italy	1996	*C. avellana*
ISF Nc 3	Italy	1997	*C. avellana*
ISF Nc 4	Italy	1997	*C. avellana*
ISF Nc 5	Italy	1997	*C. avellana*
ISF Nc 6	Italy	1998	*C. avellana*
ISF Nc 7	Italy	1998	*C. avellana*
ISF Nc 8	Italy	1998	*C. avellana*
ISF Nc 9	Italy	1999	*C. avellana*
ISF Nc 10	Italy	1999	*C. avellana*
ISF Nc 11	Italy	1999	*C. avellana*
ISF Nc 12	Italy	1999	*C. avellana*
ISF Nc 13	Italy	1999	*C. avellana*
ISF Nc 14	Italy	2000	*C. avellana*
ISF Nc 15	Italy	2000	*C. avellana*
ISF Nc 16	Italy	2000	*C. avellana*
ISF Nc 17	Italy	2000	*C. avellana*
ISF Nc 18	Italy	2000	*C. avellana*
ISF Nc 19	Italy	2000	*C. avellana*

[T]Type strain; NCPPB, National Collection of Plant Pathogenic Bacteria, York, UK; PD, Culture Collection of Plant Protection Service, Wageningen, the Netherlands; ISF, Culture Collection of Istituto Sperimentale per la Frutticoltura, Roma, Italy.

cultured on glucose–yeast extract–calcium carbonate (GYCA) medium, at 25–27°C.

Biochemical tests

With all *X. arboricola* pv. *corylina* strains, the following biochemical tests were performed following the methods described by Lelliott & Stead (1987): starch and esculin hydrolysis, and gelatine liquefaction. In addition, the ability of strains to grow on brilliant-cresyl blue starch (BS) semiselective medium (Mulrean & Schroth, 1981) and to metabolize quinate on succinate-quinate (SQ) medium (Lee *et al.*, 1992) was also checked.

Repetitive PCR analysis

For total genomic preparation, a slight modification of the technique used by Smith *et al.* (1995) was used. A loopful (3 mm) of a single colony of each strain grown for 24 h on NSA at 25–27°C was suspended in sterile saline (SS: 0·85% of NaCl in distilled water) and centrifuged at 8900 *g* for 2 min. After discarding the supernatant, the pellet was suspended in SS at an optical density corresponding to 10^8 CFU mL^{-1}. The suspension was placed in boiling water for 10 min and then stored at –20°C. The repetitive PCR method used was that of Louws *et al.* (1994). The Enterobacterial Repetitive Intergenic Consensus (ERIC); Repetitive Extragenic Palindromic (REP); and BOXA subunit of the BOX element of *Streptococcus pneumoniae* (BOX) primer sets were synthesized by Eurogentech (Seraing, Belgium). Amplification was performed on an MJ Research PTC 100 programmable thermal controller (MJ Research, Watertown, MS, USA) in a 25 μL reaction mixture containing 200 μM deoxynucleoside triphosphate, 2 mM $MgCl_2$, primers at 60 pmol, *Taq* polymerase 1·0 U and 4 μL of cell preparation sample, overlaid with 25 μL of mineral oil. After thermal cycling (Louws *et al.*, 1994), PCR products were separated by vertical gel electrophoresis on 6% acrylamyde gel in 1 × TBE buffer, at 160 V and 4°C for 30 min, in a Bio-Rad Mini Protean apparatus (Bio-Rad, Hercules, CA, USA). The gels were stained with ethidium bromide and visualized under a UV transilluminator and photographed with a Polaroid film type 55. The PCR amplifications were performed in triplicate. Gel analyses were made as described by Smith *et al.* (1995), where bands common to all three amplifications were recorded. For each primer and for each strain, bands were scored as present (1) or absent (0) and the readings were entered in a computer file as a binary matrix. Similarity coefficients for all pairwise combinations were determined using Dice's coefficients (Dice, 1945) and clustered by unweighted paired-group using arithmetic averages (UPGMA) by means of NTSYS software, version 1·80 (Exeter Software, New York, NY, USA).

Whole-cell protein analyses

From each strain, the soluble whole-cell protein extracts were collected according to Vauterin *et al.* (1991). SDS–PAGE electrophoresis was performed using a Bio-Rad Mini Protean apparatus, in a 12% (wt/vol) polyacrylamyde gel, run vertically (40 mA constant current, 4°C) until the bromophenol blue tracking dye had migrated to the bottom of the gel. Destained gels were photographed and readings of the gels were taken. Alignments of the gels were performed with GELCOMPARE II. Each strain was run in triplicate. For protein analysis, the method previously described for PCR fingerprinting analysis was adopted.

Pathogenicity tests

All strains were inoculated in hazelnut cultivars Tonda Gentile Romana, Tonda Gentile delle Langhe and Nocchione, at the beginning of October 2000, in open-field conditions, following the method suggested by Gardan & Devaux (1987). Inocula were prepared from 48 h cultures grown on GYCA, and a loopful of the bacterial colony was suspended in SS and photometrically adjusted to an optical density corresponding to $1–2 \times 10^8$ CFU mL^{-1}. Buds were punctured with a sterile needle and 10 μL of

the bacterial suspension placed onto the wound. In addition, twigs were longitudinally wounded (1·0 cm) with a sterile scalpel, and 15 μL of the suspension placed along the wound. For each strain, 10 buds and 10 twigs were inoculated. Sterile saline, inoculated in the same way, served as control. Symptoms were checked 40 days after inoculation. The incidence of the disease was measured as the percentage of necrotic buds and symptomatic twigs, whereas the severity of the disease was measured as the length of necrotic lesions developed upwards and downwards from the wound. Data expressed as percentage underwent arcsin transformation, and subsequently were subjected to ANOVA and Duncan's multiple range test. Data concerning the severity of disease were analysed using Student's *t*-test.

Results

Biochemical and pathogenicity tests

All strains hydrolysed starch and esculin, and all strains except the type strain NCPPB 935 liquefied gelatine within 21 days of inoculation. After this period, NCPPB 935 induced only a faint liquefaction on the surface of the gelatine. In addition, all strains grew on BS medium producing the characteristic hydrolysis of starch, and metabolized quinate on SQ medium, although NCPPB 935 metabolized quinate more slowly (1 week after the other strains). Statistical analysis of incidence and severity of disease clearly identified two groups of strains (Tables 2 and 3). With respect to bud inoculation, strains NCPPB 935 and 984, isolated from *C. maxima*, incited only a light necrosis around the site of inoculation and without bud necrosis. By contrast, all strains obtained from *C. avellana* incited wilting of buds to a varying degree. ANOVA significantly separated the strains obtained from *C. avellana* from those isolated from *C. maxima* ($P = 0{\cdot}01$). Similar results were obtained with inoculation through the twig (Table 2). In this case, incidence of the disease was evident only for strains isolated from *C. avellana*, and no visible sign of infection was observed along twigs inoculated with NCPPB 935 or 938. The severity of disease varied among the strains isolated from *C. avellana*, although no significant difference was observed (Table 3).

Table 2 Pathogenicity tests (incidence of disease) performed with *Xanthomonas arboricola* pv. *corylina* strains towards *Corylus avellana* cultivars

	Necrotic buds (%)			Positive reactions on twigs (%)		
Strain	TGR	TGL	N	TGR	TGL	N
NCPPB 935[T]	0a[a]	0a	0a	0a	0a	0a
NCPPB 984	0a	0a	0a	0a	0a	0a
Fil 6	50b	60b	60b	20b	30b	40b
Fil 19	40b	60b	50b	20b	30b	30b
Fil 29	40b	50b	60b	30b	30b	30b
Fil 85	40b	60b	60b	30b	40b	40b
NCPPB 2896	50b	70b	60b	40b	50b	40b
NCPPB 3037	40b	50b	50b	30b	40b	40b
PD 1896	40b	50b	50b	40b	40b	40b
PD 1897	50b	50b	50b	30b	30b	40b
NCPPB 3339	40b	40b	50b	40b	40b	40b
PD 3657	50b	40b	50b	40b	40b	50b
ISF Nc 1	40b	40b	50b	30b	40b	30b
ISF Nc 2	50b	40b	50b	40b	50b	40b
ISF Nc 3	40b	40b	50b	40b	40b	50b
ISF Nc 4	40b	50b	50b	40b	50b	50b
ISF Nc 5	60b	50b	60b	50b	40b	50b
ISF Nc 6	60b	40b	50b	40b	40b	50b
ISF Nc 7	50b	50b	50b	40b	50b	50b
ISF Nc 8	60b	60b	70b	40b	40b	40b
ISF Nc 9	50b	60b	60b	40b	40b	50b
ISF Nc 10	60b	60b	70b	50b	50b	40b
ISF Nc 11	60b	50b	50b	60b	50b	50b
ISF Nc 12	50b	60b	50b	60b	50b	40b
ISF Nc 13	60b	60b	40b	50b	50b	50b
ISF Nc 14	50b[a]	50b	60b	50b	40b	40b
ISF Nc 15	60b	50b	60b	50b	50b	40b
ISF Nc 16	50b	60b	60b	50b	50b	40b
ISF Nc 17	50b	50b	60b	50b	40b	30b
ISF Nc 18	50b	60b	60b	50b	50b	40b
ISF Nc 19	60b	60b	50b	40b	40b	30b

[a]Data followed by the same letter in each column are not significantly different at $P = 0{\cdot}01$ according to Duncan's multiple range test.
[T]Type strain; TGR, Tonda Gentile Romana; TGL, Tonda Gentile delle Langhe; N, Nocchione.

Repetitive PCR analysis

ERIC, BOX and REP primer sets gave reproducible genomic PCR profiles consisting of bands ranging from around 100–1700 base pairs. Polyacrylamide gels allowed a very clear differentiation of the bands. For UPGMA analysis, a total of 26 reproducible, clearly resolved bands were scored: 10 with ERIC primers, eight with the BOX primer and eight with REP primers. ERIC and BOX primers were more discriminatory than REP in differentiating *X. arboricola* pv. *corylina* strains. Representative genomic patterns are shown in Figs 1 and 2. UPGMA analysis revealed genetic diversity among the strains isolated from different geographical areas of hazelnut cultivation, and they were divided into five groups with two strains, NCPPB 935 (type strain) and NCPPB 984 forming outliers to these groups (Fig. 3). The grouping identified did not relate to the geographic origin of the strains. In some cases, strains isolated from different continents or different European countries showed the same profile (NCPPB 3339 isolated in France and Fil 85 isolated in Oregon; PD 1896 isolated in the Netherlands and ISF Nc 6 isolated in Italy; Fig. 1b). In other cases, strains isolated in the same country showed different profiles (strains Fil 29 and Fil 85, both isolated in Oregon; Fig. 1b). However, the groups did not relate to the virulence of the strains. The two outlying *X. arboricola* pv. *corylina* strains were both from *C. maxima*. The other xanthomonads used in the UPGMA comparison showed the following similarities to the *X. arboricola* pv. *corylina* strains: *X. arboricola* pv. *juglandis* NCPPB 411, 86%; *X. arboricola* pv. *pruni* NCPPB 416, 80%; *X. campestris* pv. *campestris* NCPPB 528, 64%.

Table 3 Pathogenicity tests (severity of disease) performed with *Xanthomonas arboricola* pv. *corylina* strains towards *Corylus avellana* cultivars

Strain	Length of necrosis (mm)		
	TGR	TGL	N
Fil 6	13 ± 2·0a[a]	11 ± 3·0a	10 ± 1·0a
Fil 19	14 ± 2·0a	13 ± 1·0a	12 ± 1·0a
Fil 29	14 ± 1·0a	15 ± 2·0a	12 ± 2·0a
Fil 85	16 ± 2·0a	12 ± 3·0a	13 ± 3·0a
NCPPB 2896	12 ± 3·0a	14 ± 2·0a	15 ± 3·0a
NCPPB, 3037	16 ± 4·0a	12 ± 3·0a	15 ± 1·0a
PD, 1896	13 ± 2·0a	11 ± 2·0a	13 ± 2·0a
PD, 1897	17 ± 2·0a	15 ± 3·0a	18 ± 3·0a
NCPPB 3339	16 ± 2·0a	13 ± 3·0a	12 ± 2·0a
PD 3657	13 ± 4·0a	18 ± 2·0a	17 ± 4·0a
ISF Nc 1	16 ± 1·0a	18 ± 3·0a	13 ± 2·0a
ISF Nc 2	15 ± 4·0a	12 ± 2·0a	15 ± 3·0a
ISF Nc 3	14 ± 1·0a	15 ± 3·0a	17 ± 3·0a
ISF Nc 4	11 ± 1·0a	14 ± 2·0a	13 ± 2·0a
ISF Nc 5	14 ± 2·0a	13 ± 2·0a	15 ± 3·0a
ISF Nc 6	15 ± 3·0a	16 ± 3·0a	13 ± 2·0a
ISF Nc 7	19 ± 3·0a	14 ± 2·0a	18 ± 3·0a
ISF Nc 8	21 ± 3·0a	18 ± 3·0a	16 ± 4·0a
ISF Nc 9	18 ± 3·0a	13 ± 2·0a	15 ± 3·0a
ISF Nc 10	16 ± 3·0a	12 ± 3·0a	17 ± 3·0a
ISF Nc 11	19 ± 3·0a	15 ± 3.oa	20 ± 3·0a
ISF Nc 12	16 ± 4·0a	12 ± 3·0a	19 ± 2·0a
ISF Nc 13	16 ± 3·0a	17 ± 3·0a	16 ± 4·0a
ISF Nc 14	17 ± 3·0a	12 ± 3·0a	16 ± 3·0a
ISF Nc 15	17 ± 3·0a	14 ± 4·0a	15 ± 3·0a
ISF Nc 16	12 ± 2·0a	16 ± 3·0a	13 ± 2·0a
ISF Nc 17	13 ± 4·0a	15 ± 3·0a	20 ± 3·0a
ISF Nc 18	16 ± 3·0a	18 ± 3·0a	19 ± 4·0a
ISF Nc 19	19 ± 3·0a	17 ± 3·0a	18 ± 3·0a

[a]Means followed by the same letter in each coumn are not significantly different at $P = 0{\cdot}05$ using Student's *t*-test. TGR, Tonda Gentile Romana; TGL, Tonda Gentile delle Langhe; N, Nocchione. Data concern the mean length of necrosis observed in symptomatic twigs.

Whole-cell protein analysis

UPGMA analysis performed on SDS–PAGE profiles of protein extracts also revealed the existence of different groups of strains. However, by this method it was possible to define only three groups with two outlying strains, NCPPB 935 and 984 (Fig. 4). *Xanthomonas arboricola* pv. *juglandis* NCPPB 411 and *X. arboricola* pv. *pruni* NCPPB 416 were closely related to *X. arboricola* pv. *corylina* strains, whereas *X. campestris* pv. *campestris* NCPPB 528 showed less than 80% of similarity. Strain membership to the grouping identified by rep-PCR and whole-cell protein analysis showed a high degree of congruence (Table 4). Accordingly, no relationship to geographic location or strain virulence was evident and the outlying strains were common to both methods.

Discussion

This study ascertained that genetic, phenotypic and pathogenic diversity exists among *X. arboricola* pv. *corylina* strains isolated from different geographical areas and from different *Corylus* species. Based on SDS–PAGE and repetitive PCR fingerprints, UPGMA analysis indicated that the strains could be split into groups – but that these groups had a high degree of similarity, and strain membership between the groups showed good congruence. However, the groups were not related to the area of hazelnut cultivation. In some cases, strains obtained from different continents showed the same profile, for example, NCPPB 3339 isolated in France and Fil 85 isolated in Oregon. A possible explanation for the observed commonality of strains across continents is that in Oregon the cultivation of hazelnut was introduced only a century ago with *C. avellana* planting material (cultivar Barcelona; synonym: Fertile de Coutard) from France, although the first outbreak of the pathogen in France was only recently recorded in a young hazelnut orchard established with propagative material imported from Oregon (Gardan & Devaux, 1987). A strain isolated in the Netherlands and another obtained from Sardinia (Italy) showed the same profile with repetitive PCR.

With respect to the relationship between *X. arboricola* pathovars, the geographical area of crop cultivation and the association to fruit tree species, the following features are evident. The *X. arboricola* pv. *juglandis* strains are related to the area of cultivation of their host plant, namely *Juglans regia* (Scortichini *et al*., 2001), whereas this is not apparent in *X. arboricola* pv. *pruni* (Zaccardelli *et al*., 1999) and *X. arboricola* pv. *corylina* strains

Table 4 Composition of *Xanthomonas arboricola* pv. *corylina* groups on repetitive PCR, and whole-cell protein UPGMA analysis (see Figs 3 and 4)

Group	Repetitive PCR	Group	Whole-cell proteins
I	NCPPB 2896, NCPPB 3037, ISF Nc 16, ISF Nc 18	I	NCPPB 2896, NCPPB 3037, ISF Nc 7, ISF Nc 8, ISF Nc 11, ISF Nc 12, ISF Nc 16, ISF Nc 18
II	ISF Nc7, ISF Nc 8, ISF Nc 11, ISF Nc 12		
III	PD 1896, PD 1897, NCPPB, 3339, Fil 85, ISF Nc 5, ISF Nc 6	II	PD 1896, PD 1897, NCPPB 3339 Fil 85, ISF Nc 1, ISF Nc 2, ISF Nc 3, ISF Nc 4, ISF Nc 5, ISF Nc 6, ISF Nc 13, ISF Nc 14, ISF Nc 19
IV	ISF Nc 1, ISF Nc 2; ISF Nc 3, ISF Nc 4, ISF Nc 19		
V	PD 3657, Fil 6, Fil 19, Fil 29, ISF Nc9, ISF Nc 10, ISF Nc 15, ISF Nc 17	III	PD 3657, Fil 6, Fil 19, Fil 29, ISF Nc 9, ISF Nc 10, ISF Nc 15, ISF Nc 17

X. arboricola pv. *corylina* type-strain NCPPB 935 and NCPPB 984 isolated from *Corylus maxima* showed different and unique profiles.

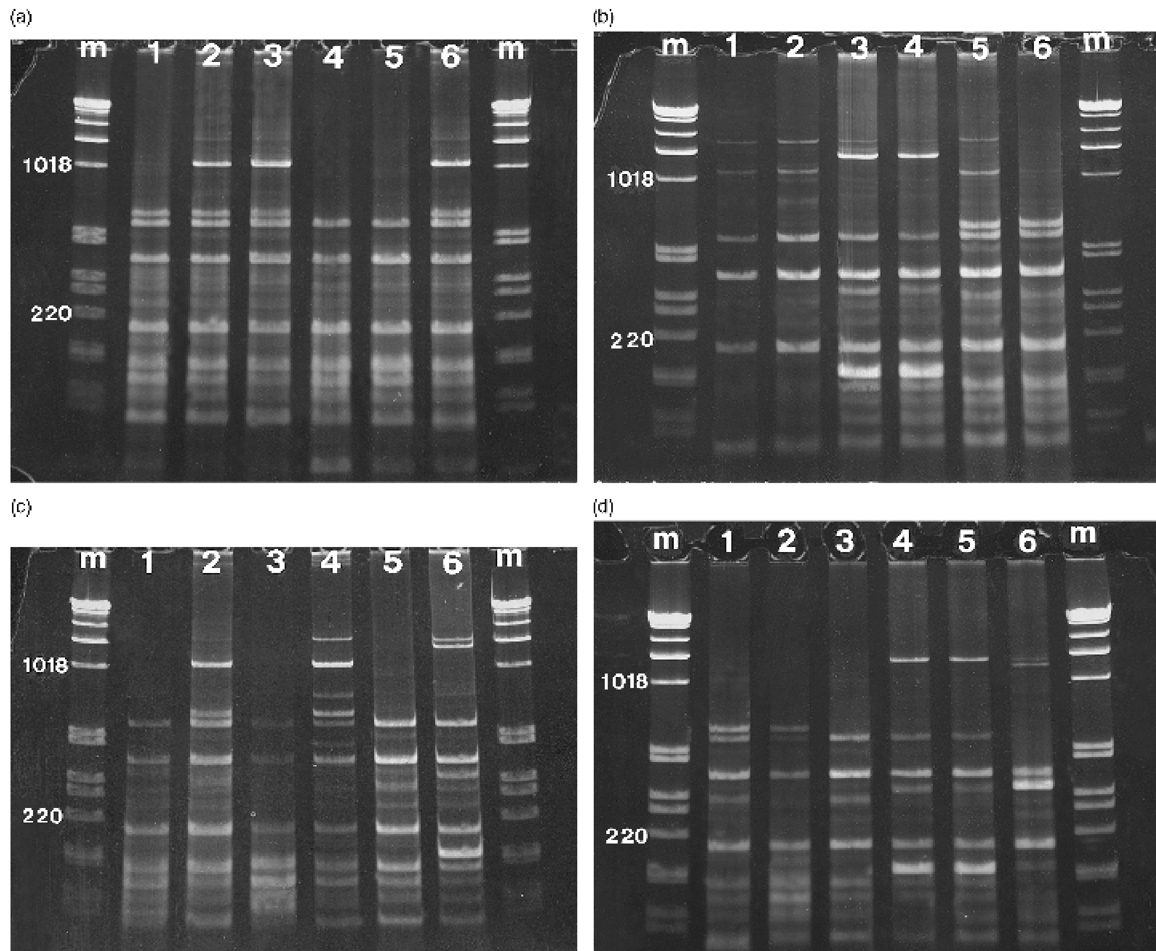

Figure 1 PCR fingerprinting patterns from genomic DNA of *Xanthomonas arboricola* pv. *corylina* strains, obtained by using ERIC primer sets. m, Molecular size marker (1 kb ladder; Gibco BRL, Life Technologies, Milan, Italy); sizes are indicated in base pairs. (a) Lanes 1, NCPPB 935 (type strain); 2, Fil 6; 3, Fil 19; 4, PD 1896; 5, ISF Nc 6; 6, PD 3657. (b) Lanes 1, PD 1897; 2, Fil 29; 3, Fil 85; 4, ISF Nc 1; 5, NCPPB 3339; 6, NCPPB 3037. (c) Lanes 1, ISF Nc 3; 2, ISF Nc 4; 3, NCPPB 3037; 4, NCPPB 2896; 5, Fil 19; 6, Fil 6. (d) Lanes 1, NCPPB 3657; 2, NCPPB 935 (type strain); 3, NCPPB 3339; 4, NCPPB 3037; 5, NCPPB 2896; 6, NCPPB 984.

(present study), as strains isolated from different countries show the same profile. For *X. arboricola* pv. *pruni*, this is not surprising considering the intense international and national traffic of propagative material; however, for *X. arboricola* pv. *corylina* such a characteristic is more intriguing, as in Europe each country and/or region has its own predominant hazelnut cultivar.

A major finding of this study has been to verify that *X. arboricola* pv. *corylina* type strain NCPPB 935 (= ATCC 19313; LMG 689, PD 991), isolated in Oregon in 1939 from *C. maxima*, deviates from the other strains studied which, with the exception of NCPPB 984, form largely homogeneous groups. It may be significant that NCPPB 984 was also isolated from *C. maxima*. The other *X. arboricola* pv. *corylina* strains isolated more recently in Oregon from *C. avellana* clustered in groups III and V on rep-PCR and UPGMA analysis, and in group III with SDS–PAGE and UPGMA analysis. The distinctive nature of NCPPB 935 brings into question the validity of this being the type strain for *X. arboricola* pv. *corylina*.

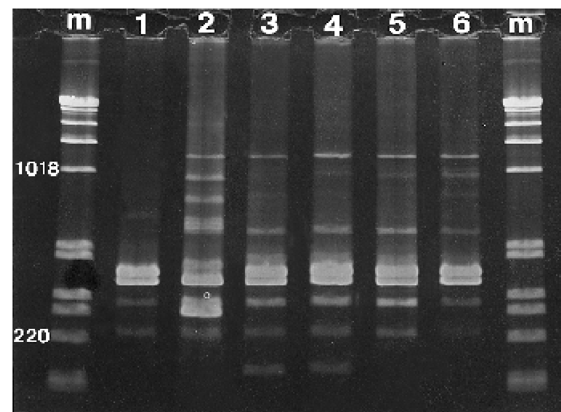

Figure 2 PCR fingerprinting patterns from genomic DNA of *Xanthomonas arboricola* pv. *corylina* strains, obtained by using BOX primer. m, Molecular size marker (1 kb ladder; Gibco BRL); sizes are indicated in base pairs. Lanes 1, NCPPB 935 (type strain); 2, NCPPB 934; 3, NCPPB 2896; 4, NCPPB 3037; 5, NCPPB 3339; 6, ISF Nc 5.

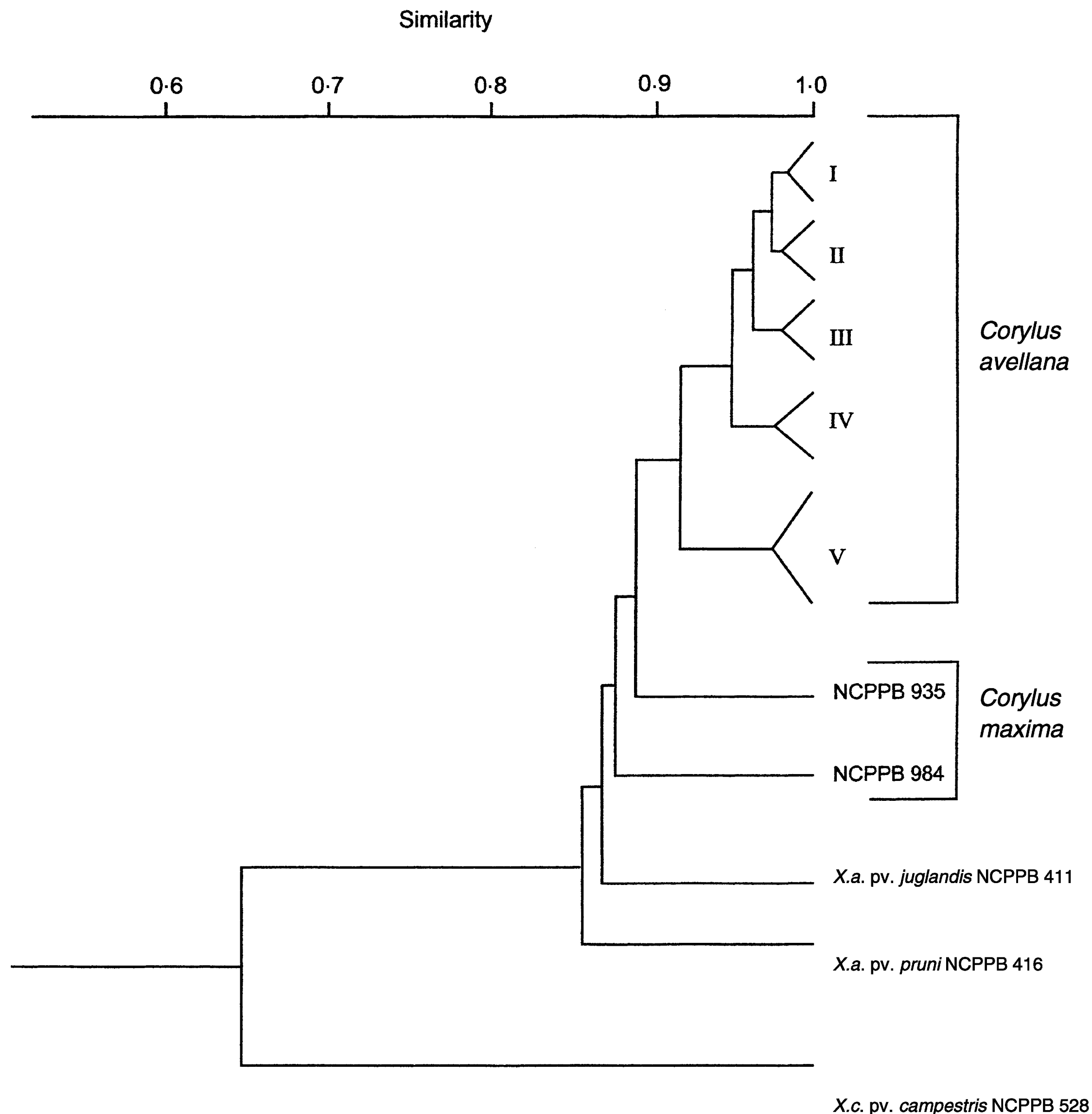

Figure 3 Dendrogram of *Xanthomonas arboricola* pv. *corylina* strains, obtained by means of UPGMA after repetitive PCR using ERIC, BOX and REP primer sets. Matrices were calculated with Dice's coefficients. For group composition see Table 3.

Particularly distinctive is the atypical negative gelatin liquefaction character of this strain, a parameter on which bacterial plant pathogens are commonly identified (Lelliott & Stead, 1987). It should be noted that the gelatin-negative reaction of NCPPB 935 may be due to variation in the assessment technique used by Miller *et al.* (1940), when the pathogen was first described, where the inoculated gelatine was incubated at 22°C and complete liquefaction was observed 6 weeks after inoculation. The different nature of these strains was confirmed by the pathogenicity tests reported here, and clearly indicated that these strains were not virulent towards *C. avellana* germplasm. On the contrary, all strains obtained from *C. avellana* induced a high incidence of bud necrosis and lesion development in inoculated twigs. Whether the strains isolated from *C. maxima* exhibit host specificity was not studied here, and this possibility deserves further investigation. Taking into consideration that hazelnut cultivation worldwide is currently based on *C. avellana* (Bozoglu, 2001), and observing that the type strain of the pathovar *corylina* is phenotypically and pathogenically deviating from all strains isolated from *C. avellana*, it may be prudent to designate NCPPB 2896 as more typical of isolates from *X. arboricola* pv. *corylina*. NCPPB 2896 was isolated in the UK in 1976, and is the oldest extant strain from *C. avellana*.

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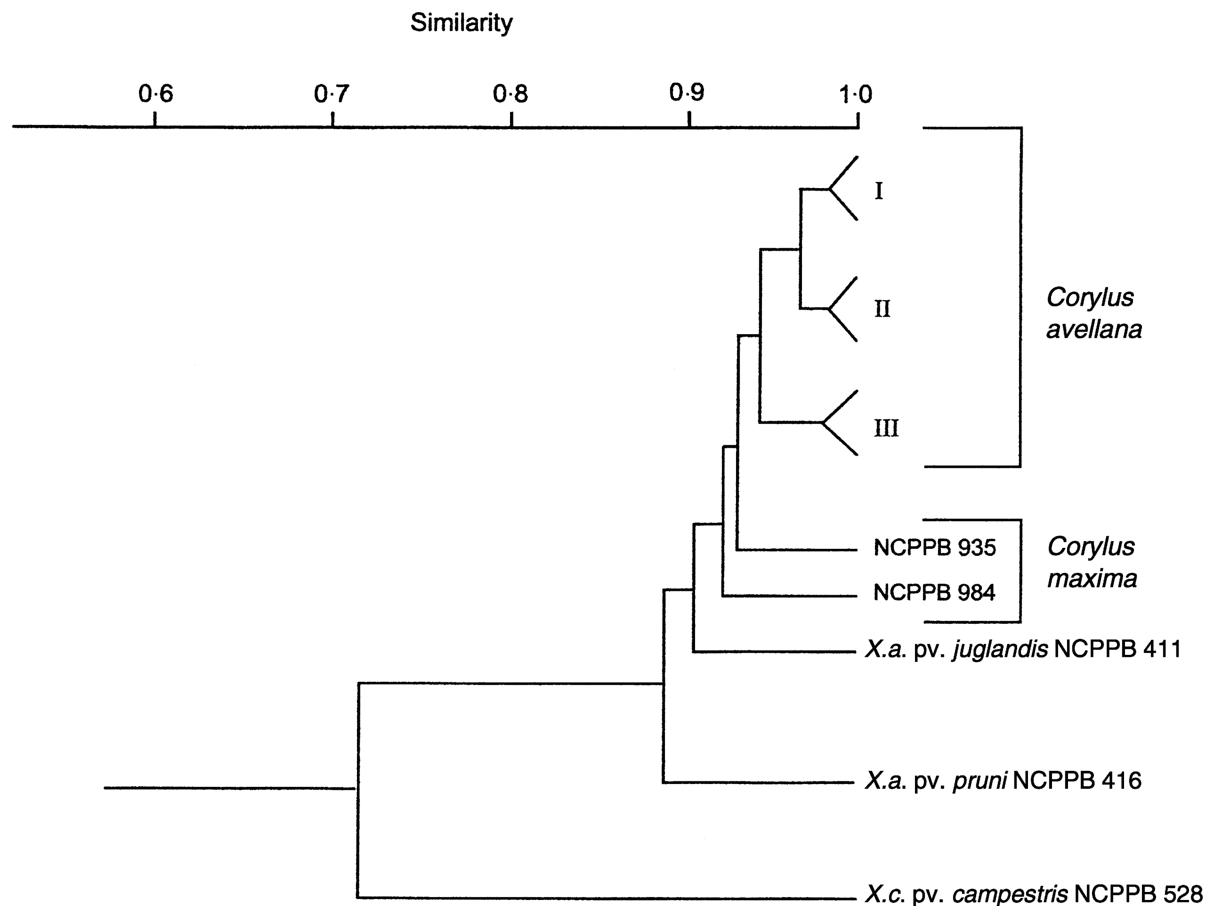

Figure 4 Dendrogram of *Xanthomonas arboricola* pv. *corylina* strains, obtained by means of UPGMA after whole-cell protein extract analysis. Matrices were calculated with Dice's coefficients. For group composition see Table 3.

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