Bacterial blight of walnut: a severely re-emerging disease

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The genus *Juglans* consists of approximately 20 species grouped taxonomically into four sections:

1. *Rhysocaryon* (black walnuts, 16 species present in North and South America. *Juglans nigra* commonly used as rootstock for Persian walnut)
2. *Cardiocaryon* (CN, JP, KR, rootstocks)
3. *Trachycaryon* (forest butternut in CND, USA)
4. *Dioscaryon (Juglans regia* L., Persian walnut*)*.

Walnuts species are important both as nuts and as timber (Regulation 2080/92/CE)

Walnut (*Juglans regia*, Persian walnut) is the third most important nut worldwide: its production is approx. 2.950.000 tons (average), over a cultivation area of approx. 950.000 ha, with an increase of 23.3% during 2008-12 (FAO, 2014).

Rank	Country	Production (Tonnes)
1	China	1,700,000
2	Iran	450,000
3	United States	425,820
4	Turkey	194,298
5	Mexico	110,605
6	Ukraine	96,900
7	India	40,000
8	Chile	38,000
9	France	36,425
10	Romania	30,546
—	*World*	3,282,398

LEFT:
Top ten walnut producing countries: (shelled walnuts and walnuts with shell).

Top exporting countries:

USA – 160.000 tons
Mexico, Ukraine, France and Chile

Top importing countries:
China, Italy, Turkey, Spain, Gemany

Italy:

Increase of walnut consumption:
58,3% (years 2000-2010)

Import – approx 26.500 tons/year, for an estimated cost of 114 Mio$/year and an increase in costs of 53% (2007-2011) (ISTAT, 2014)

Main pathogens:

Blackline (Cherry Leaf Roll Virus, CLRV)

Anthrachnose (*Gnomonia leptostyla*)

Root rot (*Armillaria mellea*)

Root and Crown rot (*Phythopthora* spp.)

Thousand canker disease (*Geosmithia morbida*)

Crown gall (*Agrobacterium tumefaciens*)

Shallow bark canker (*Brenneria nigrifluens*)

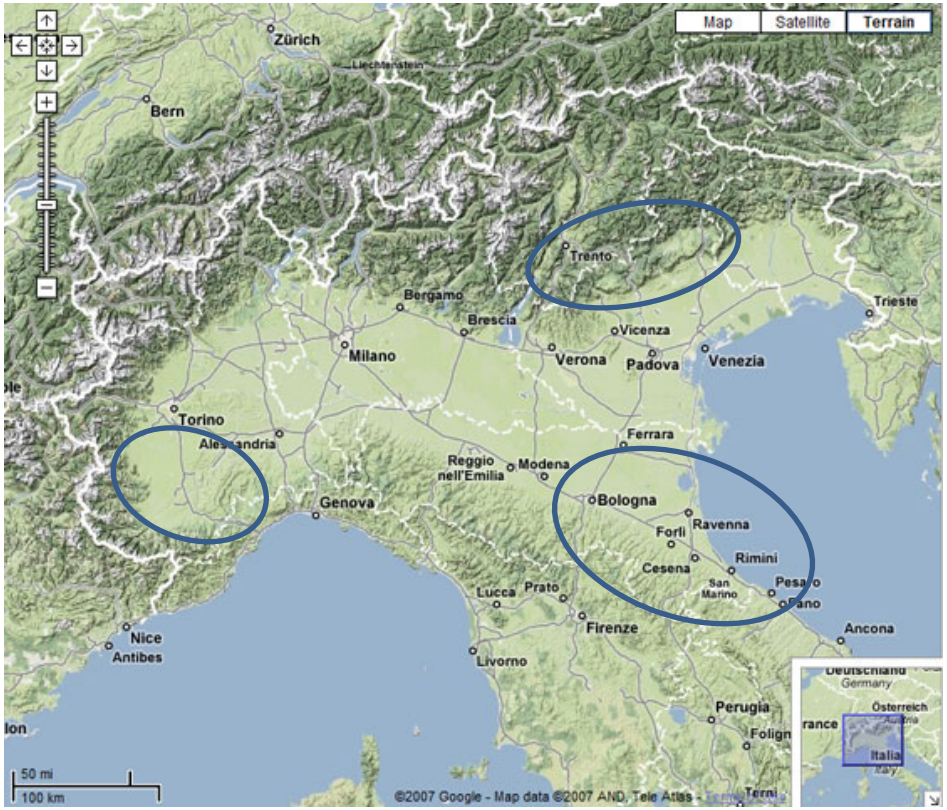
Deep bark canker (*Brenneria rubrifaciens*)

Walnut blight (*Xanthomonas arboricola* pv. *juglandis*)

We summarise research carried out in 2008-2014 in Romagna, one Italian region where walnut production is increasing.

Other regions in northern Italy:

Veneto
Trentino
Piedmont



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Research was focused on the bacterial blight:

1. **Monitoring the disease (Incidence, Severity, Losses);**
2. **Collect isolates and analyse them;**
3. **Understand disease epidemiology;**
4. **Implement control strategies.**

Disease monitoring and sample collection: 2008-2012

Symptoms of bacterial blight on walnut

The brown apical necrosis

In 1999 Belisario et al., described the brown apical necrosis (BAN), as a disease casused mainly by different fungi, i.e. *Fusarium* spp., *Alternaria* spp., *Phomopsis* and *Colletotrichum*. More recently, BAN was mainly associated with the presence of Xaj alone or together with *Fusarium* spp. or *Alternaria* spp. (Arquero et al, 2005; Moragrega et al., 2010).

Monitoring the disease: RESULTS

1. All the aerial parts might be affected;
2. In most cases, 80-100% of trees in walnut groves visited showed symptoms;
3. Severity of symptoms variable but … fruits drop in high number between mid May and end of June (up to 60% of dropped walnuts);
4. Shallow bark canker also observed on several trees.

Shallow bark canker (*Brenneria nigrifluens*)

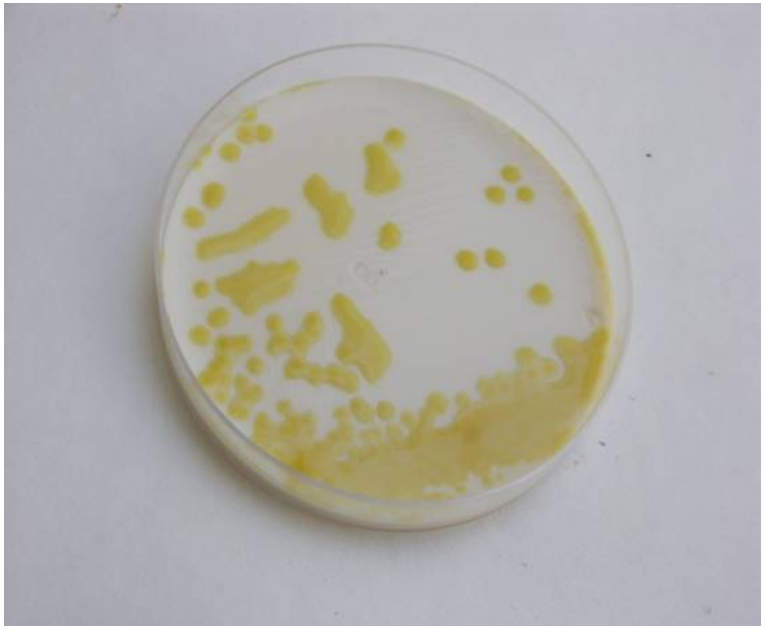
Different severity of the shallow bark canker (Giorcelli & Gennaro, 2014)

Isolation of the causal agent (*Xanthomonas arboricola pv. juglandis*)

Isolation of Xaj was easy on GYCA.
An isolate collection was established, identified on the basis of a positive HR on bean pods and pathogenicity assays on walnut fruitlets.

Isolation was done from different symptoms (spots on fruits, lesions on leaves, cankers on twigs and branches).

Symptomless material allowed isolation as well:
Buds,
Catkins,
Female flowers,
Leaf washings,
Fruitlets
Dust on machineries



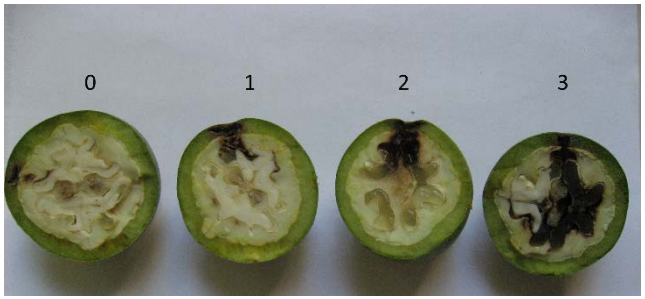
Characterisation of a Xaj collection

Surveys allowed to collect 136 putative Xaj isolates: 78 from symptoms, 58 from symptomless material

77 of them (from both symtomatic and symptomless material) confirmed their identity as Xaj, after a pathogenicity test.

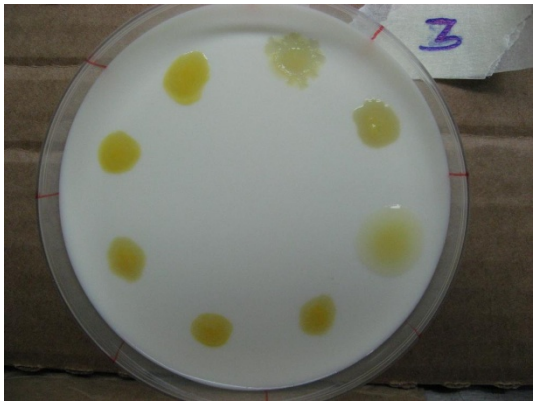
Pathogenicity was assayed (each strain tested three times, in independent tests, on a set of 10 fruitlets) and evaluated .

11 isolates to class 1
29 isolates to class 2
37 isolates to class 3



Three morphotypes described:

M1 = bright yellow, mucoid, glistening
M2 = dark yellow, mucoid, glistening
M3 = bright yellow, buttery, wrinkled



Characterisation of a Xaj collection

Metabolic assays:

All isolates were able to use arabinose, cellobiose, fructose, sucrose, trhalose as the only carbos source (Ayers et al., 1957).

Copper resistance

On MGYA amended with Cu++:

50 ppm: all isolates grow well
100 ppm: **68** isolates grow well
200 ppm: **58** isolates grow well
300 ppm: **52** isolates grow well
500 ppm: **20** isolates grow well

CopABL gene cluster present in all isolates, and sequence homology >90%, when compared with those from other phytopathogenic xanthomonads.

BUT, an additional Cu++ detoxifying system should be present in the most resistant isolates.

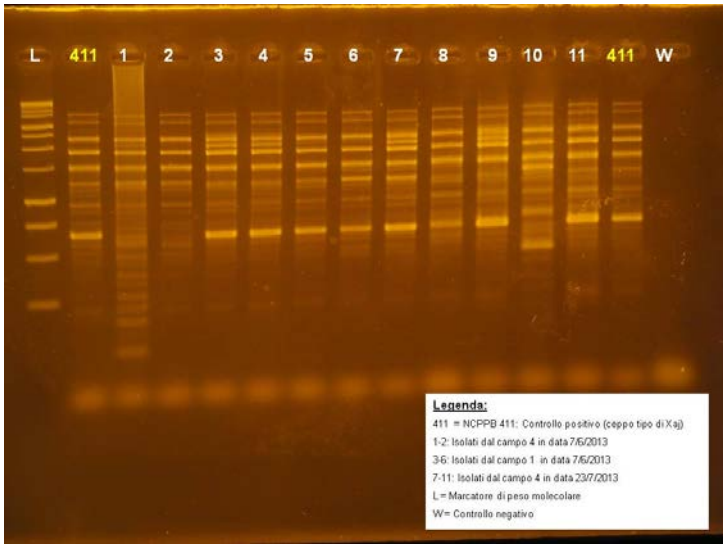
Genetic characterisation of a Xaj collection

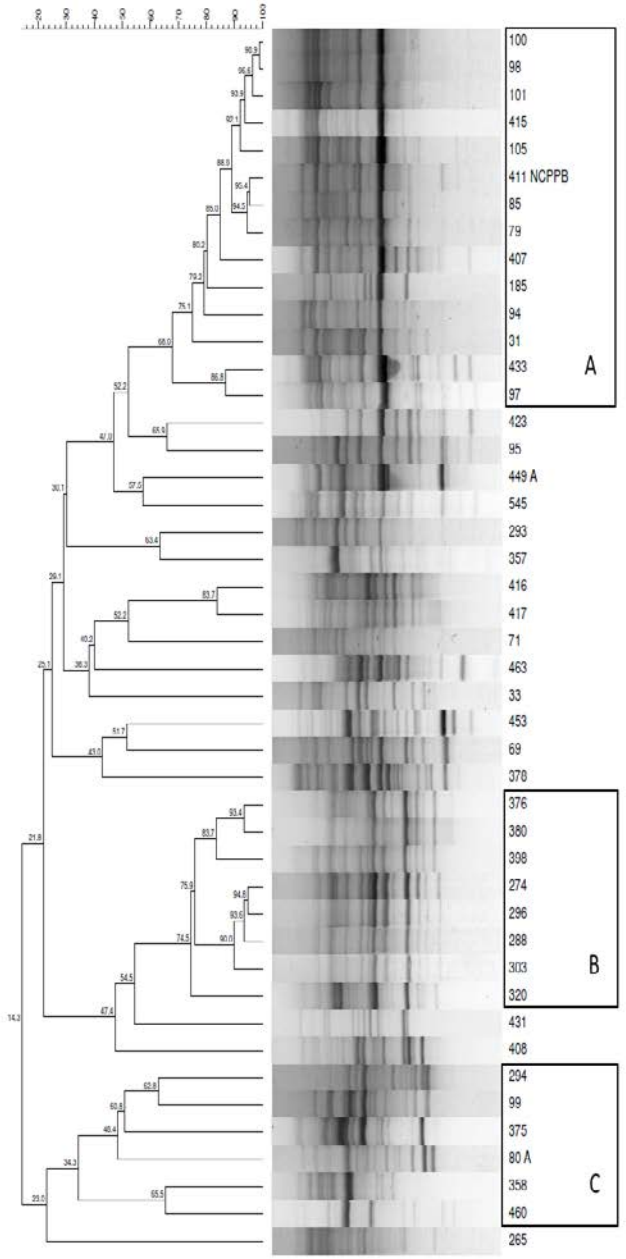
BOX profiles:

UPGMA revealed high intrapathovar variability

According Pearson's index: three main clusters

According Dice's index : one loose main clusters
Plus several outgroups.





Genetic characterisation of a Xaj collection

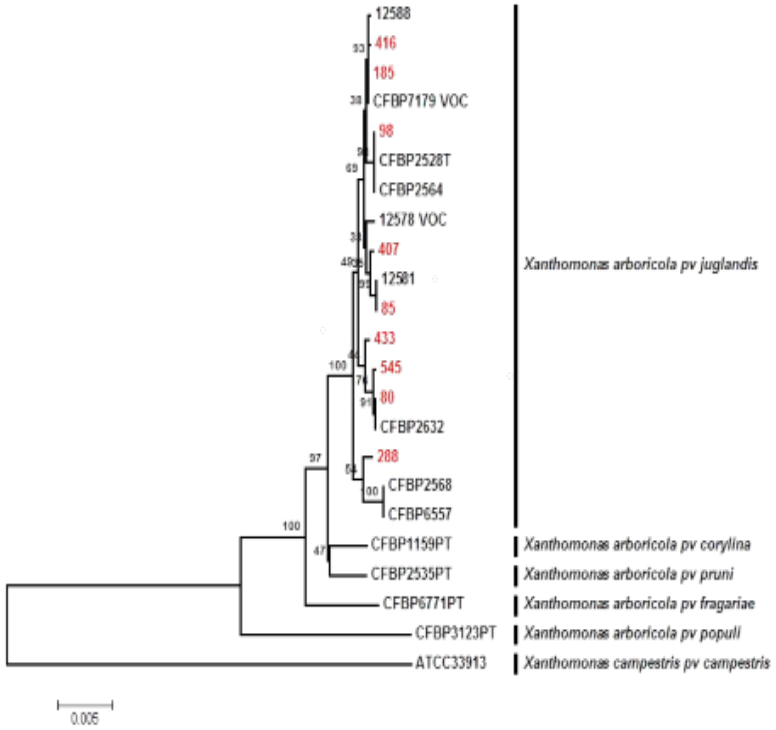
VNTR analysis:

The TR5b locus was used as tandem repeat and analysis revealed **five different haplotypes**, characterised by the presence of 4, 5, 7, 9, 11 tandem repeats units.

MLSA

Following 7 housekeeping genes were chosen:
atpD, dnaK, efP, fyuA, glnA, gyrB, rpoD

Following *X. arboricola* pvs. were also compared: *corylina, fragariae, populi, pruni.*

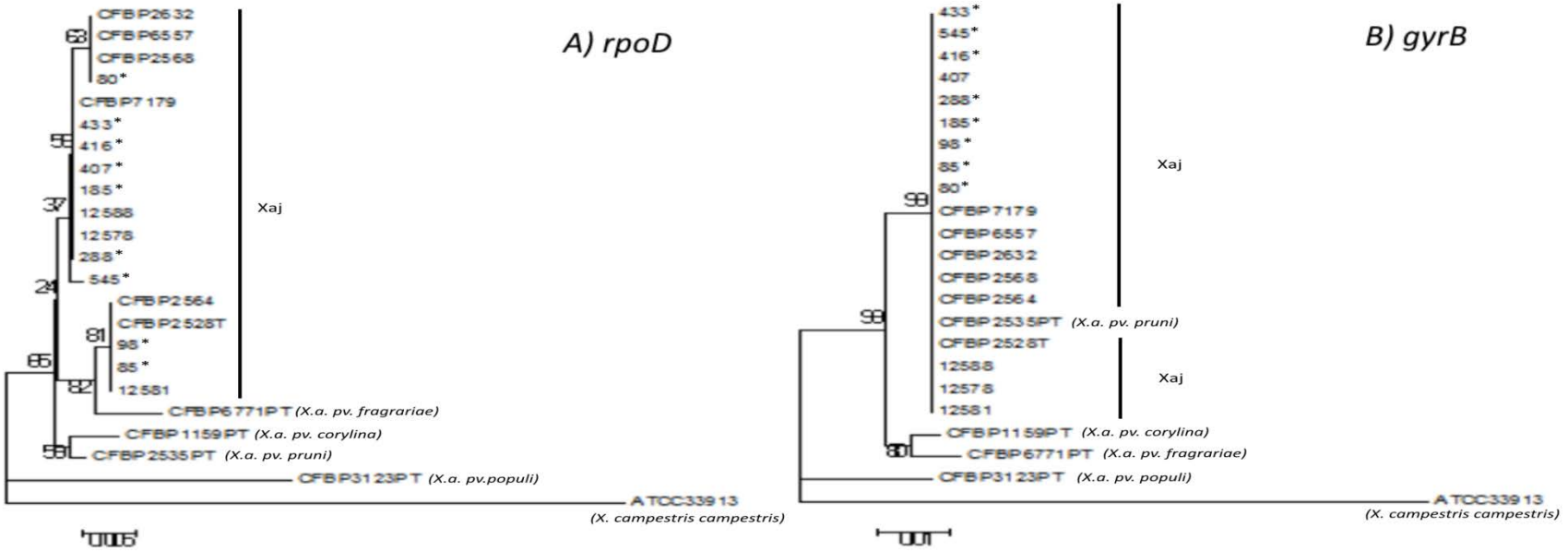


Genetic characterisation of a Xaj collection

MLSA

Seven housekeeping genes were chosen: *atpD, dnaK, efP, fyuA, glnA, gyrB, rpoD*

Highest stability for *gyrB*, highest variability for *rpoD*



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Characterisation of a Xaj collection: summary

- **Different morphotypes present, with different virulence and different copper tolerance: differences not correlated to the source of isolation or the walnut grove**

- **Different haplotypes present, even in a small region**

- **Sequence of housekeeping genes revealed possible environmental adaptability: for instance, *rpoD* is transcribed in the polymerase rpoD primary sigma factor.**

  (*Bacterial RNAPs are composed of a core enzyme for a RNA synthesis and a* σ^{70} *sub-unit for recognition. Transcriptional switching in bacteria might be achiebed by modulation of* σ^{70} activity**)**

Epidemiology

Isolation of Xaj in late winter-early spring led to the finding that primary inoculum is present in buds (overwintering), catkins, female flowers.

Overwintering sites are also small/tiny lesions on twigs.

Epidemiology

Xaj dissemination occurs during all qrowing season: pollen is a vehicle, showers and rain enhance dissemination, harvesters are also inciting spread of Xaj throughout the groves.

Disease control

Local varieties are quite tolerant to the disease, whereas all groves inspected have been planted with a few modern cultivars

From USA: Chandler, Howard, Hartley
From France: Lara, Franquette, Parisienne

1. On such cvs. no chance to control the disease with copper compounds; therefore, Mancozeb is added to copper under derogation.
2. Xaj population diversity might be responsible for environmental adaptation and difficulties encountered in disese management.
3. High susceptibility of cv. Chandler (somewhat less sensitive cv. Howard) to bacterial infection: a few tiny little lesions present of immature fruits where sufficient to cause an intense fruit drop .

Disease control

Search of new agrochemicals:

1. Experiments have been conducted under controlled conditions (2009-2011) with innovative biochemicals, suitable both for conventional IPM strategies and in organic horticulture: disease has been significantly reduced but, nonetheless, fruits fall.