
Crown gall of berries: an old, emerging disease

Nemanja Kuzmanović

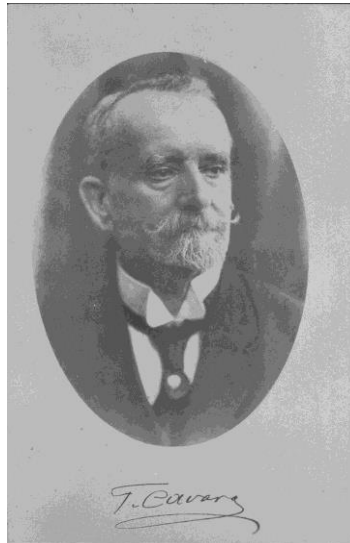
Crown gall disease

- **1853. First written report of crown gall disease**

Fabre, E. and Dunal, F. (1853): Observations sur les maladies régnantes de la vigne. Bull. Soc. Cent. Agric. Dep. Herault. 40, 46.

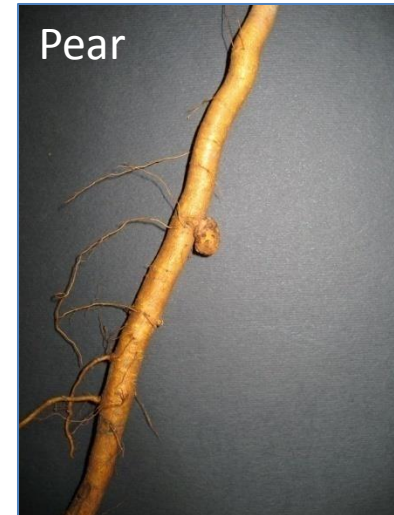
- **1897. F. Cavara identified bacteria, termed *Bacillus ampelopsorae*, as the causal agent of crown gall of grapevine**

Cavara, F. (1897) Tubercolosi della vite. Intorno alla eziologia de alcune malattie di piante coltivate. Stazioni Sperimentali Agrarie Italiane 30, 483–487.



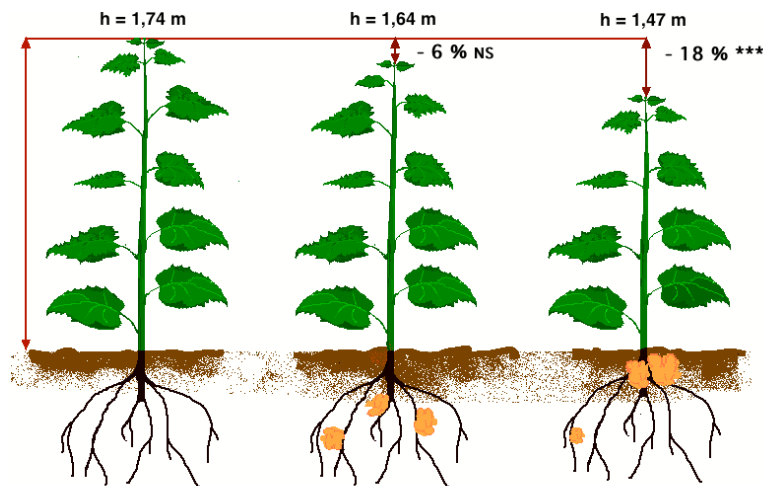
Fridiano Cavara

Symptoms



Economic significance

- Reduces growth, vigor and yield of infected plants
- Severe disease may cause partial or complete death of infected plants
- In some cases, no significant differences between diseased and healthy plants were found



- In USA, annual losses from *Agrobacterium* spp. estimated to several tens of millions of dollars
- High losses occur especially in nurseries

**Tumorigenic *Rhizobiaceae*
species**



**Tumorigenic
agrobacteria**

***Rhizobiaceae* members**

- **Plant pathogens**
 - Crown and cane gall disease
 - Hairy root
- Symbiotic bacteria
- Biological control agents of plant diseases
- Plant-growth promoting bacteria
- Bioremediating bacteria
- Producers of industrially important biomaterials
- Opportunistic human pathogens
- Vectors for plant genetic engineering

Rhizobiaceae members

Diverse bacteria



Plant tumors and hairy-roots
Xylem sap
Soil surrounding infected plants



Root nodules



Soil and rhizosphere
Decaying organic matter
Saprophytes



Sea water



Ditch water



Human-associated
samples



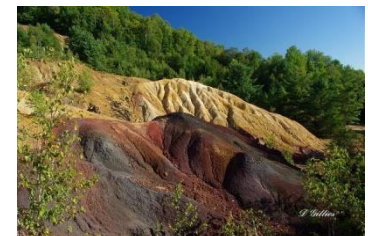
Caves



Endophytes and epiphytes

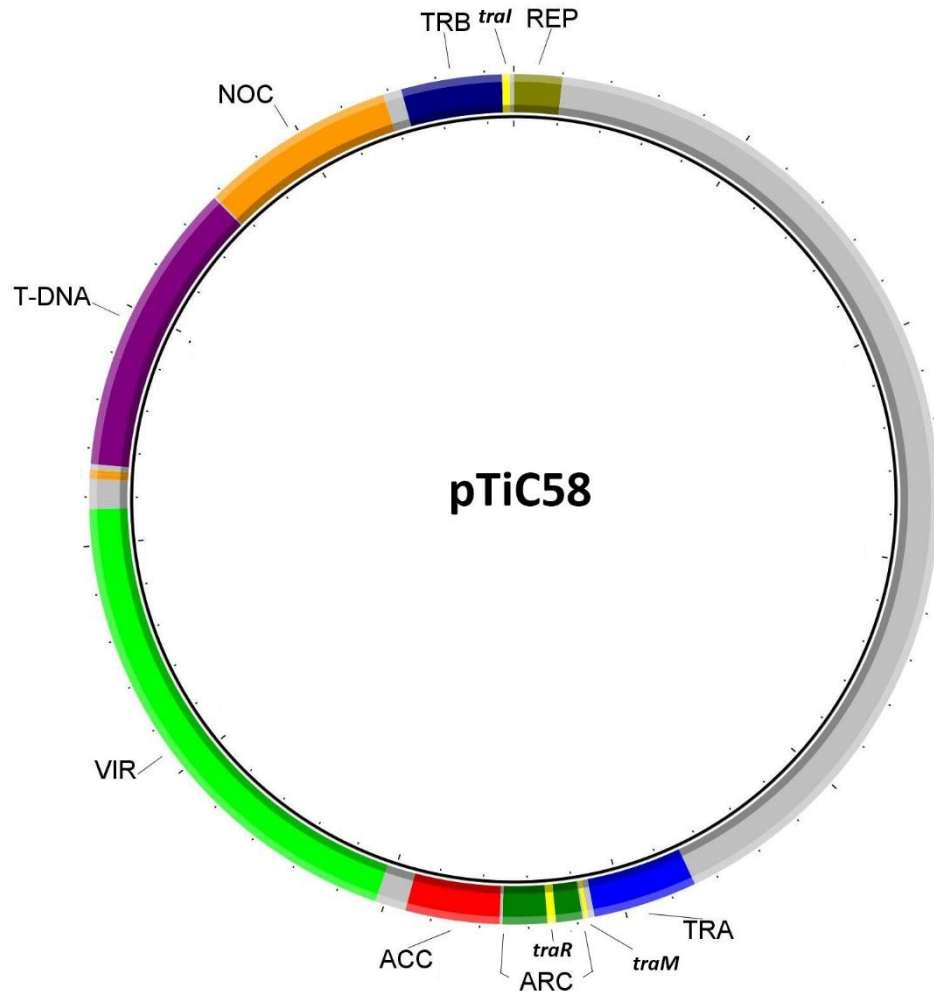


Animal-associated samples



Metal-contaminated
environment

Tumor-inducing (Ti) plasmid



- Transferable/mobile
- Essential for pathogenicity
- Not essential for survival of bacteria

Ti plasmid



Epidemiology of crown gall disease

- Tumorigenic agrobacteria can survive in soil, surrounding infected plants or in plant debris remaining in soil
- Tumorigenic agrobacteria do not generally inhabit soils of the natural environment, that is not used for agriculture
- Tumorigenic agrobacteria are almost exclusively associated with infected plants
- Tumorigenic agrobacteria systemically colonize host plant – they inhabit vascular tissue
- Tumorigenic agrobacteria can be latently present in asymptomatic plant material
- **Main means of dissemination: infected plant material**

Taxonomy of agrobacteria

- Extensively debated through the history and is still under discussion
- Dynamic field

Classification based on phytopathogenic features

- *Agrobacterium tumefaciens*
- *Agrobacterium rhizogenes*
- *Agrobacterium radiobacter*
- *Agrobacterium vitis*
- *Agrobacterium rubi*



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- *Agrobacterium tumefaciens* genomic species G1 = ***Agrobacterium fabacearum*** (*Agrobacterium tumefaciens*)
 - *Agrobacterium tumefaciens* genomic species G2 = ***Agrobacterium pusense*** (NT)
 - *Agrobacterium tumefaciens* genomic species G3
 - *Agrobacterium tumefaciens* genomic species G4 = ***Agrobacterium radiobacter***
 - *Agrobacterium tumefaciens* genomic species G5 (NT)
 - *Agrobacterium tumefaciens* genomic species G6
 - *Agrobacterium tumefaciens* genomic species G7 = “***Agrobacterium deltaense***”
 - *Agrobacterium tumefaciens* genomic species G8 = “*Agrobacterium fabrum*”
 - *Agrobacterium tumefaciens* genomic species G9 = ***Agrobacterium salinitolerans***
 - *Agrobacterium tumefaciens* genomic species G13 (NT)
 - *Agrobacterium tumefaciens* genomic species G14 = ***Agrobacterium nepotum***
 - ***Agrobacterium arsenijevicii***
 - ***Agrobacterium bohemicum*** (NT)
 - ***Agrobacterium larrymoorei***
 - ***Agrobacterium rubi***
 - ***Agrobacterium rosae***
 - ***Agrobacterium skierniewicense***
 - “***Agrobacterium viscosum***” (NT)
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- ***Allorhizobium vitis*** (*Agrobacterium vitis*; *Agrobacterium* biovar 3)
 - ***Rhizobium rhizogenes*** (*Agrobacterium rhizogenes*; *Agrobacterium* biovar 2)
 - ***Rhizobium tumorigenes***
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Why is the taxonomy relevant for nursery growers and farmers?

- Knowing the enemy!
- Epidemiology of the disease
- Disease management - diagnostics!
 - developing new control strategies



Biocontrol

Crown gall and cane gall of berries

- Berries have been identified as natural hosts of tumorigenic agrobacteria
- Early reports on cane gall disease on berries (USA - Bailey, 1895; Sweden – Wulff, 1907)
- *Agrobacterium rubi* (Hildebrand, 1940)
- Crown gall and crown gall of berries have been frequently reported in the last 20 years
- Economical losses varied

Crown gall and cane gall of blackberry-raspberry hybrid in Italy

- Serious epidemic in Italy in 2000 (Weller et al., 2004)
- Blackberry-raspberry (*Rubus occidentalis*-*Rubus idaeus*) hybrid of cv. Lochness
- Tumors on crown of the plants beneath the superficial layer of soil
- The nursery material, imported from France, was planted in soil where no *Rubus* species had previously been cultivated
- *Agrobacterium rhizogenes*/*Rhizobium rhizogenes*/biovar 2



Crown gall of raspberry in Serbia



- Raspberry production and export is of a strategic importance for agriculture in Serbia
- Serbia is one of the world's leading raspberry producers (96,078 t in 2012 [<http://faostat3.fao.org/>]) and exporters

Top producers of raspberries in tons (2019)	
Russian Federation	174,000
Mexico	128,848
Serbia	120,058
United States of America	102,510
Poland	75,660
Source: UN FAOSTAT (http://faostat3.fao.org/)	

Crown gall of raspberry in Serbia



- Crown gall was reported in Serbia before (Arsenijević, 1989)
- *Agrobacterium tumefaciens* (biovar 1) occurring on raspberry plants cv. Villamette collected from plantation in Western Serbia (Milijašević et al., 2007)



Crown gall on raspberry

- Milijašević et al., 2007)

Crown gall of raspberry in Serbia

2011-2013



Cultivars:

- **Polka**
 - **Tulameen**
- Stunted growth
 - Significantly decreased yield
 - Considerable economic losses



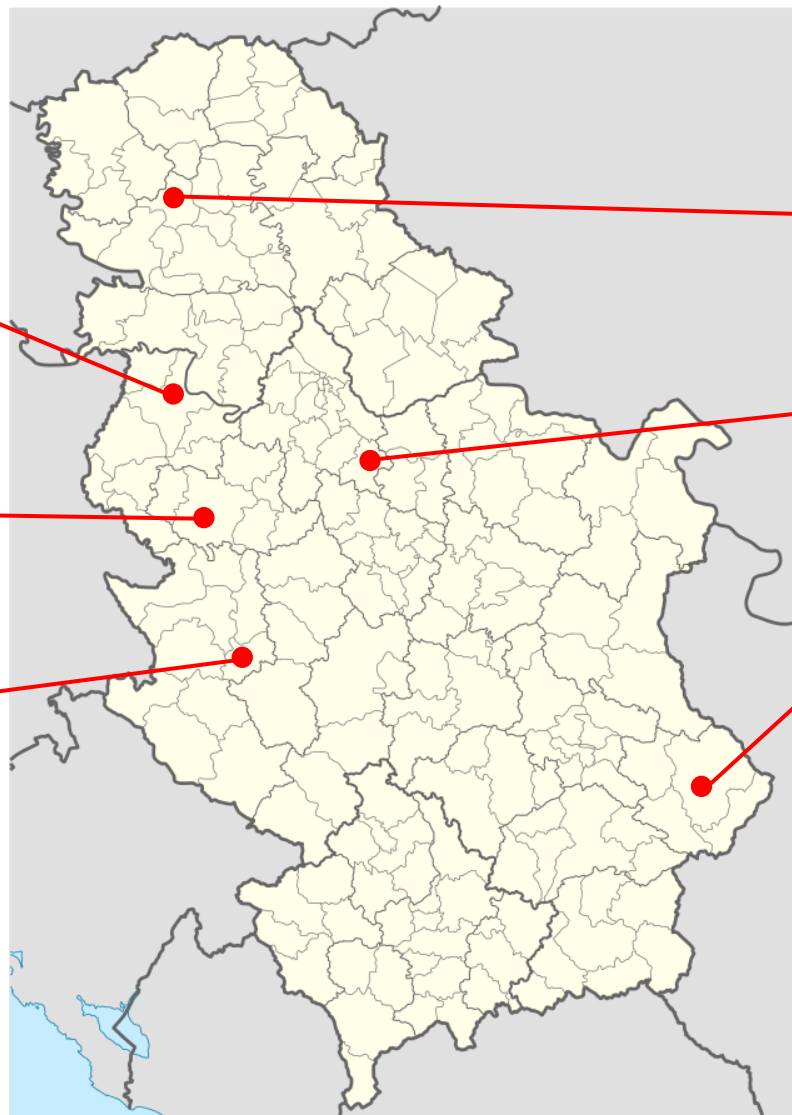
Aim of the study



- to gain an insight into population structure of tumorigenic strains occurring on raspberry in Serbia
- to isolate and identify tumorigenic bacteria from the diseased plants originating from several localities in Serbia, and investigate their pathogenic properties

Insight into population structure of phytopathogenic *Agrobacterium/Rhizobium* spp. may be particularly important for better understanding of epidemiology and ecology of crown gall disease and for developing strategy for rapid, reliable and efficient detection and identification of the pathogen.

Origin of samples (2011-2013.)



Šabac

Vrbas

Valjevo

Mladenovac

Pirot

Arilje



Crown gall of raspberry in Serbia



Plantation



Nursery



Crown gall of raspberry in Serbia

- As a causal agents of crown gall disease in Serbia at least two different *Agrobacterium/Rhizobium* species were found
 - *R. rhizogenes* was prevalent species isolated from raspberry tumors
 - *A. tumefaciens* genomospecies G8
 - New *Agrobacterium* species



Prof. Momčilo
Arsenijević

***Agrobacterium arsenijevicii* sp. nov.**

Summary of results and conclusions



- At least two different *Agrobacterium/Rhizobium* species caused raspberry crown gall disease in Serbia
 - *R. rhizogenes* was prevalent species isolated from raspberry tumors (12 strains)
 - *A. tumefaciens* genomospecies G8 (1 strain)
 - **New bacterial species – *Agrobacterium arsenijevicii***



Complexity of population structure of tumorigenic bacteria associated with crown gall disease of raspberry

- The presented data also highlight the importance of crown gall bacteria on raspberry and contribute to taxonomic studies of agrobacteria.

Cane gall of thornless blackberry in Serbia

- Two localities in western Serbia
- Recurrent outbreaks
- Not lethal
- No significant losses in yield



Cane gall disease of *Rubus* spp.
(Hildebrand, 1940)

Agrobacterium rubi



Cane gall of thornless blackberry in Serbia

- New tumorigenic bacterial species discovered

Rhizobium tumorigenes

- Different than all tumorigenic agrobacteria discovered so far
- Epidemiology of cane gall caused by *R. tumorigenes*?
- Is it systemic in infected plants?
- Does it survive in soil?

Distribution of the novel pathogen („tumorigenes“ group)

- Blackberry in Serbia
- Rhododendron in Germany
- **Blueberry?**



Crown gall is one of the most important bacterial diseases in blueberry production!

***Rhizobium tumorigenes* on blueberry in Germany?**

- Two plantations in Northern Germany
- Cultivars – Duke, Draper
- Causal agent not isolated
- *Rhizobium tumorigenes*-like bacteria detected in tumor tissue by means of molecular methods
- Plant material unavailable!

Crown gall of blueberry in Argentina

- Investigation performed in Argentina, 2006-2008 (Allipi et al., 2012)
- Blueberry plants originating from nurseries and commercial farms
- Tumors at the base of the shoot or on the root
- *Agrobacterium tumefaciens* (biovar 1), *Agrobacterium rhizogenes* (biovar 2) and *Agrobacterium rubi*

Crown gall of blueberry in Poland

Pulawska and Kuzmanovic, 2018

- Three types of symptoms were recorded
- Pathogen could not be identified!



Crown gall of blueberry in Serbia

- Several localities
- Young plantations (1-4 years old)
- Several cultivars (Duke, Hannah's Choice)
- Imported plant material



- In 2017, a severe outbreak of stem gall on stems of mature 'Draper' blueberry plants was observed in several fields in Oregon
- Classical isolation of the pathogen was unsuccessful
- Tissue culture to recover tumorigenic strains
- Pathogen similar to *Rhizobium tumorigenes* was identified



Control of crown gall disease

The use of healthy planting material in areas with no history of the crown gall represents the crucial measure of disease management.

- Production of healthy plant material
- Phytosanitary control of mother plants used for propagation in nurseries
- Control of imported plant material
- Growing resistant cultivars?

Control of crown gall disease

- **No chemical control measures available!**
- **Removing infected plants**
- **Crop rotation**
- **Re-planting?**



Control of crown gall disease

- **Analysis of soil before establishing plantation**



Control of crown gall disease

Biological control

R. rhizogenes, strain K84

R. rhizogenes, strain K1026

