AWRI Australian Wine Research Institute

Fact Sheet
VITICULTURE

Crown gall in Australian vineyards

Background

Crown gall, a significant plant disease globally, is caused by two bacterial genera: *Agrobacterium* and *Allorhizobium*, both of which belong to the *Rhizobiaceae* family. These gram-negative, motile, rod-shaped bacteria do not form spores and are closely related to the nitrogen-fixing rhizobium bacteria. Historically, in Australian vineyards, the main causal organism of crown gall disease has been *Allorhizobium vitis* (syn. *Agrobacterium vitis*) in the biovar 3 group. However, since mid-2022, there have been reports that an *Agrobacterium* species has been detected in vines exhibiting symptoms resembling crown gall, with preliminary testing suggesting this strain may belong to the biovar 1 group. Prior to this, *Agrobacterium* species in the biovar 1 group were seldom linked with grapevines (Portier et al. 2006, Argun et al. 2002, Genov et al. 2015).

Allorhizobium vitis (*All. vitis*) typically exhibits a narrow host range, primarily infecting *Vitis vinifera*, whereas *Agrobacterium* biovar 1 species can infect more than 140 species of dicotyledons, including pome fruit, stone fruit and nut trees. While the most severe outbreaks of crown gall on grapevines have been documented in cooler climate regions, occurrences of the disease have also been reported in Mediterranean regions (Burr et al. 1998).

Agrobacterium and *Allorhizobium* species are commonly found in soils and water worldwide, where they typically lead saprophytic lives, surviving independently of a host. However, when they are found in plants, they can either be non-pathogenic or act as pathogens, causing diseases. Pathogenicity is linked to virulence genes on a tumour-inducing plasmid (Ti plasmid). The Ti plasmid

is a small DNA molecule within the cell of some *Agrobacterium* and *Allorhizobium* species but is separate from the bacterial chromosome.

Agrobacterium classification

The classification of *Agrobacterium*, which has changed over the years, was initially based on symptoms in host plants. Originally, strains causing galls were classified as *Agrobacterium tumefaciens,* those causing hairy roots as *Agrobacterium rhizogenes*, and non-pathogenic strains as *Agrobacterium radiobacter*. However, these classifications are no longer valid, as the Ti plasmid can transfer to non-pathogenic strains, making them pathogenic.

In the 1970s, *Agrobacterium* species were divided into three biovars (biovar 1, 2, and 3), based on physiological and biochemical analyses. Biovar 2 was later reclassified as a genus in *Rhizobium* spp. and *Agrobacterium vitis* (biovar 3) was reclassified as *Allorhizobium vitis (All. vitis)* (Ophel and Kerr 1990). As more biological and genetic information became available, the classification and naming of species across the three biovars evolved. Biovar 1 is now considered a complex of *Agrobacterium* species, including *A. tumefaciens;* biovar 2 contains species in the genus *Rhizobium;* and biovar 3 contains *Allorhizobium vitis,* which was previously known as *Agrobacterium vitis.* The biovar 1 group of *Agrobacterium* species contains multiple *Agrobacterium* strains, which can cause galling when hosting a Ti plasmid, including *Agrobacterium tumefaciens* (Figure 1).

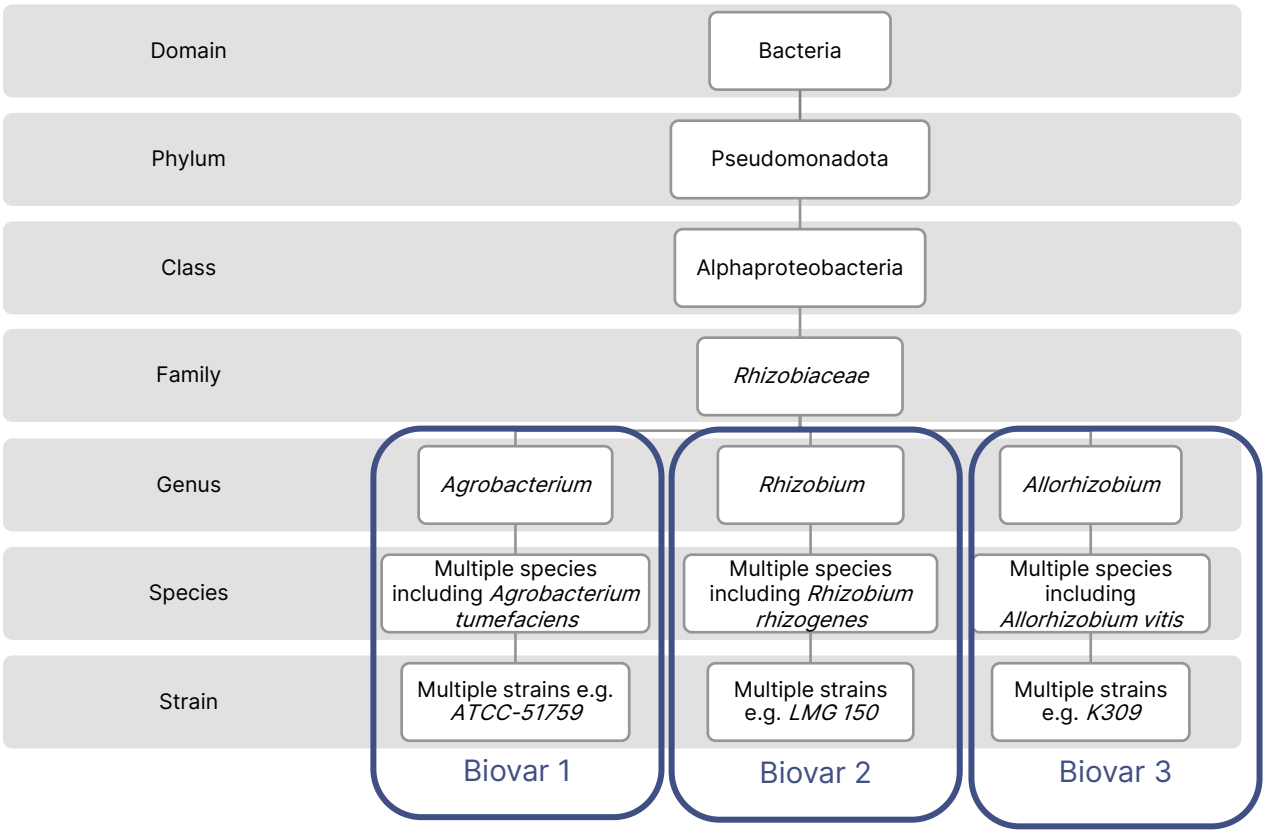

Figure 1. Taxonomic classification of bacteria in the *Rhizobiaceae* family

Symptoms

The most distinct characteristic of crown gall disease is the formation of galls on the crown of vines, where the main roots join the trunk, and also on roots. Galls may also form on the trunk above the soil line or on shoots and canes. Beyond the presence of galls, the disease may not cause any other visible symptoms. Galls tend to evolve from fleshy, white tissue to dry, cork-like structures over time. Peeling and cracking of bark, as well as a gradual decline in vine vigour due to girdling and root necrosis, may occur as the disease progresses. Young vines are especially susceptible, as galls can impede water and nutrient flow, potentially resulting in vine death in severe cases. Aerial root formation has also been associated with crown gall disease. Frost injury causing cracking of the bark can lead to small tumours forming in a line along the cracks.

Figure 2. Crown gall-like symptoms on young vine

Figure 3. Crown gall-like symptoms on young vine. Note the reddening of the leaves in response to girdling.

Figure 4. Crown gall-like symptoms on a young vine. Note the cracking and peeling of bark.

Figure 5. Crown gall-like symptoms on a young vine. Note the cracking and peeling of bark and gall formation.

Symptoms of crown gall disease can sometimes be mistaken for those of vine strangulation (Figure 6) and root knot nematodes (Figure 7).

Figure 6. Young vine with tightly tied string around trunk causing 'strangulation'.

Figure 7. Grapevine roots with root knot nematode (Reproduced from State of New South Wales. Department of Primary Industries NSW (2013) licensed under CC BY 4.0).

Disease cycle

The disease cycle for *Agrobacterium* and *Allorhizobium* species encompasses several stages: initial injury to host plant, subsequent secretion of plant wound exudates that serve as attractants for the bacteria, adherence of bacteria to the plant cell surface, activation of virulence genes and synthesis of the Ti plasmid within the bacteria, transfer of the Ti plasmid into the plant cell, and finally, integration of Ti-DNA into the plant cell genome. This then triggers the production of plant hormones, leading to tissue overgrowth. Additionally, Ti-DNA genes encode enzymes that produce compounds called opines, which serve as energy sources for the bacteria. Opines also aid in the spread of Ti plasmids among bacteria within tumours, promoting their proliferation.

The bacteria may be spread during the grafting process via contaminated tools, water or soil/media and some varieties and rootstocks can be asymptomatic carriers of the pathogen (Dodds and Fearnley 2023).

Potential sources of *Agrobacterium* species and *Allorhizobium vitis* in vineyards include infected planting material, soil, and plant debris (Krimi et al. 2002, Burr et al. 1995, Dodds and Fearnley 2023). The bacteria can spread from infected vines to healthy vines via pruning equipment and tools and through the movement of infected soil (Dodds and Fearnley 2023). Irrigation, rainfall, and flooding may further aid the dissemination of the bacteria and they can also survive on the roots of

vineyard weeds or the remnants of previous crops (including the roots of previously planted vines) (Burr et al. 1995, Dodds and Fearnley 2023, Smith 2019).

In established vines, spread may be caused by pruning with contaminated secateurs or through wounds to roots (e.g. by soil-dwelling insects like nematodes, mechanical damage or from waterlogging). In already affected vines, galls can start to develop in any part of the vine from wounds caused by mechanical damage, hail, pruning and frost (Burr et al. 1995, Dodds and Fearnley 2023, Smith 2019).

Control measures in the vineyard

There are currently no effective and practical methods available for completely eradicating *Agrobacterium* and *Allorhizobium* bacteria from a vineyard, primarily due to their ability to persist in the soil on roots or grape debris for extended periods. Diligent monitoring for symptoms and prompt removal and destruction of diseased plants helps to reduce the pathogen load. Because the bacteria that cause crown gall can persist in residual roots after vine removal, it is important to remove as much of the plant root system as possible when removing vines. Replanting in the same spot where the infected vine was growing is not advised, as sloughed off galls serve as an abundant source of the bacteria.

Site selection

Site selection plays a crucial role in reducing the risk of crown gall disease. Sites that have a history of crown gall in grapevine and/or other horticultural and agricultural crops. For new vineyard developments, warmer regions with low risk of winter injury and good water drainage are favourable.

Farm-gate hygiene

Agrobacterium and *Allorhizobium* bacteria can easily spread from infected to healthy areas through various means such as contaminated soil, water, and equipment. Enforcing strict hygiene measures is essential to curb disease transmission. This involves initiating vineyard activities in clean blocks and concluding in diseased blocks, regularly disinfecting tools and equipment in contact with soil or vines, preventing the transfer of soil or plant debris from diseased to clean blocks, and ensuring contaminated water does not flow from infected areas to clean blocks or water storage areas.

Vinehealth Australia provides protocols on footwear and small tool disinfection, harvester cleaning, and the Top 10 farm-gate hygiene activities to minimise the potential transmission of pests and diseases from infected vines to healthy ones. However, it is important to note that the disinfestation protocols were designed specifically for phylloxera and not for *Agrobacterium* and *Allorhizobium* bacteria. Vinehealth Australia's current recommendation for disinfecting footwear and small hand tools to prevent the spread of *Agrobacterium* and *Allorhizobium* bacteria is to use undiluted methylated spirits (95% ethanol). For best results, growers are advised to securely place a lid over the ethanol tub during daylight hours to prevent breakdown due to exposure to sunlight, replace the ethanol daily, or more frequently if it becomes soiled, as soil may diminish the efficacy of the

ethanol, and ensure footwear is immersed for at least 60 seconds in the ethanol and not rinsed after immersion. Caution is advised as 95% ethanol is highly flammable.

Cultural practices

Retaining multiple trunks does not prevent crown gall but it may reduce the damage caused by the disease, since it is unlikely that all trunks of a vine will succumb to the disease simultaneously and provides a spare if one trunk does get affected.

Resistant rootstocks

Vitis vinifera is highly susceptible to crown gall, whereas certain American and French-American hybrids demonstrate genetic resistance. Grafting a susceptible scion onto a resistant rootstock significantly reduces the risk of infection from contaminated vineyard soil. However, susceptibility levels of grapevine rootstocks and scions vary globally, with studies often yielding divergent results. This suggests that genetically variable strains of *Agrobacterium* and *Allorhizobium* bacteria from different regions may induce differing tumorigenic reactions on grape cultivars and rootstocks. Wine Australia's Grapevine rootstock selector tool provides information about susceptibility of rootstocks to *Allorhizobium vitis.* In Australia, the rootstock Paulsen 775 is considered very highly resistant to *Allorhizobium vitis*, while rootstocks such as 101.14, 3309C, Kober 5BB and Schwarzmann are considered to have high resistance. Rootstocks Paulsen 1103 and SO4 exhibit low to moderate resistance, whereas rootstocks Ramsay, Richter 110, Richter 99, Ruggeri 140 and Teleki 5C show low resistance. It is important to note that these rootstocks have not been assessed for resistance towards *Agrobacterium* from the biovar 1 group.

Fallow period

Implementing a fallow period may reduce carry-over of bacterial inoculum into new plantings, but the outcomes are variable. While some studies indicate that pathogenic strains can still be isolated after a 16-year fallow period, others show no detection of pathogenic strains after just a one-year fallow period. It has been reported that the survival success is influenced by various factors, including soil texture, pH and water content. A further study demonstrated fluctuations in population numbers throughout the year, with levels being lowest in autumn and winter and highest in spring.

Soil fumigation

Soil fumigation has been shown to reduce the population of *Agrobacterium* and *Allorhizobium* bacteria in the soil, reducing infection of grapevines. However, soil fumigation does not eliminate the pathogen from the soil, and it is a challenging and environmentally destructive practice.

Currently, no chemicals are registered for control of crown gall disease in vineyards. Antibacterial compounds, such as copper products, can kill crown gall pathogens on the vine's exterior but do not affect the survival of the pathogens inside the vine tissue.

Control measures in the nursery

Agrobacterium and *Allorhizobium* bacteria can be spread readily via planting material, which serves as a potential source of infection in new plantings. If these bacteria are present in propagation source blocks, they can persist through the grafting and other nursery processes. Contaminated grafting and pruning equipment can also serve as a source of bacterial inoculum, as can contaminated water used in the propagation process. Additionally, not all infected vines exhibit symptoms, making detection difficult.

Hot water treatment

In Australia, grapevine nurseries employ a long-duration hot water treatment regimen, specifically at 50°C for 30 minutes, to treat propagation material. This process aims to minimise the risk of transmitting specific insect pests, as well as targeted bacterial and fungal pathogens, into new vineyards through propagation materials. When executed correctly, this method proves highly effective in controlling Phylloxera infestations and reducing *All. vitis* infections. However, it is important to note that this treatment has not been tested against *Agrobacterium* Biovar 1 strains (Ophel et al. 1990, Mahmoodzadeh et al. 2003, Burr et al. 1998).

Tissue culture

Tissue culture can effectively eliminate *Agrobacterium and All. vitis* from severely affected *Vitis vinifera* vines. However, the process is both expensive and time-consuming.

Biocontrol

Non-pathogenic *Agrobacterium* strains (described as *A. radiobacter* K84 and K1026) have been used effectively as a biocontrol agent for *Agrobacterium* species of the biovar 1 group in stone fruit trees. However, these strains are not effective against *All. vitis* (Asghari et al. 2020). Other non-pathogenic *Agrobacterium* strains are currently being assessed in Australia for efficacy in controlling *Agrobacterium* species of the biovar 1 group in grapevines. Research globally has shown the potential of endophytic bacteria to induce resistance against *Agrobacterium* and *Allorhizobium* in grapevines (Asghari et al. 2020).

What to do if you observe crown gall-like symptoms

- Photograph and record the location of affected vines
- For new vine plantings, contact your nursery supplier and the Vine Industry Nursery Association (VINA)
- Contact the AWRI helpdesk on helpdesk@awri.com.au or 08 8313 6600 for advice on testing and identification.

Testing and identification

The diagnostic techniques for identifying *Agrobacterium* and *Allorhizobium* bacteria are rapidly advancing. Detection of *Agrobacterium* and *Allorhizobium* species can be difficult because the bacteria may be in low concentration or unevenly distributed. Detection of the bacteria in asymptomatic material is less reliable than detection from diseased material.

Polymerase chain reaction (PCR) can be used to detect the presence of *Agrobacterium* and *Allorhizobium* species within the plant. PCR, however, does not identify the specific strain and does not provide information on bacterial viability or the pathogenicity of the organism detected (unless the Ti plasmid DNA is targeted). There is a risk that PCR may detect bacteria present on the surface of samples, not just the bacteria residing within the plant material.

Whole genome sequencing is required for *Agrobacterium* and *Allorhizobium* strain identification.

To evaluate bacterial activity, a second test involves isolating the bacteria from the symptomatic vine and culturing it in artificial media.

To determine the pathogenicity of the organism, a third test—specifically, a pathogenicity test—is necessary to confirm if the bacterium can cause galls. This test entails infecting clean planting material, or an indicator species such as tomato seedlings or carrot discs, with the suspected agent and observing whether galls are produced.

Testing soil for *Agrobacterium* presents challenges due to low concentrations. Culturing prior to PCR to isolate the *Agrobacterium* offers more accurate detection.

Agrobacterium and *Allorhizobium* species are known to reside systemically within grapevines, migrating to new shoot tissue during early spring. Their presence typically diminishes as spring progresses, only to surge again during autumn. These bacteria are most commonly detected in roots and fresh galls. Therefore, it is advisable to collect samples of roots and fresh galls during early spring or autumn.

Conclusion

The complexity of *Agrobacterium* and *Allorhizobium* induced crown gall disease in grapevines is underscored by the evolving classification and testing methods of these pathogens, adapting with technological advancements. Effectively managing the disease once established poses significant challenges. Research on potential biological controls is currently underway. It is crucial to note that *Agrobacterium* and *Allorhizobium* bacteria in propagation source blocks can persist through the grafting process, serving as a potential source of infection in new vineyards. Additionally, not all vines infected with *Agrobacterium* and *Allorhizobium* exhibit crown gall symptoms, and while hot water treatment can reduce the titre of *Allorhizobium*, it does not eliminate the bacteria and has no impact on some *Agrobacterium* species in the biovar 1 group.

Diagnostic facilities

Collaborative efforts are currently underway between the Plant Health Diagnostic Service of NSW DPI, Crop Health Services (CHS) of Agriculture Victoria and Affinity Labs in South Australia, with the goal of standardising testing methodologies. Growers are strongly encouraged to discuss the advantages and limitations of each testing option, to identify the best option for their needs.

Plant Health Diagnostic Service of NSW DPI

Contact: Dr Toni Chapman
Phone: 1800 675 623
Email: laboratory.services@dpi.nsw.gov.au
Website: https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/plant-health
Address: Plant Health Diagnostic Service (PHDS)
EMAI
Woodbridge Road
Menangle NSW 2568

Tests offered:

- PCR to detect the presence of DNA from *Agrobacterium* species of the biovar 1 or 2 group or *Allorhizobium vitis* of the biovar 3 group in plant material.
- Culturing to isolate live *Agrobacterium* biovar 1 group or *Allorhizobium vitis* biovar 3 group from the plant material to evaluate bacterial activity.
- Partial sequencing of target genes.

Crop Health Services (CHS) of Agriculture Victoria

Contact: Dr David Lovelock
Phone: (03) 9032 7170
Email: chs.reception@agriculture.vic.gov.au
Website: https://agriculture.vic.gov.au/support-and-resources/services/diagnostic-services
Address: AgriBio Specimen Reception
Main Loading Dock
5 Ring Road
La Trobe University
Bundoora VIC 3083

Tests offered:

- PCR to detect the presence of DNA from *Agrobacterium* species of the biovar 1 or 2 group or *Allorhizobium vitis* of the biovar 3 group in the plant material.
- Culturing to isolate live *Agrobacterium* biovar 1 group or *Allorhizobium vitis* biovar 3 group from the plant material to evaluate bacterial activity.
- Partial sequencing of target genes.

Affinity Labs

Contact: Dr Regina Baaijens
Phone: 08 8313 0444
Email : customerservice@affinitylabs.com.au
Website: https://affinitylabs.com.au/expertise/expertise-wine-grapes/
Address: Affinity Labs
Corner of Hartley Grove and Paratoo Rd
Urrbrae (Adelaide), SA 5064

Tests offered:

- PCR to detect the presence of DNA from *Allorhizobium vitis* of the biovar 3 group and the Ti plasmid in the plant material.

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Resources

An extensive list of resources on crown gall is available in the AWRI's crown gall information pack.

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