Grape Crown Gall Biology and Strategies for Control

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Crown gall is a disease of worldwide importance on grape that is caused by a bacterium, *Agrobacterium vitis*. Crown gall occurs on many different fruit and ornamental crops, but in those cases it is caused by *A. tumefaciens,* a related bacterium. *A. vitis*, which is only found on grape, most commonly causes the development of galls at graft unions or on lower trunks and to a lesser extent on canes (Figure 1). When young vines develop crown gall at their graft unions they often die, whereas older vines may show stress depending on the severity of the gall and will usually survive the infections. *A. vitis* lives internally in grapevines and can be isolated from bleeding sap; therefore it is frequently disseminated in apparently-healthy propagation material.

Galls are generally not observed on grape roots; however, the bacterium causes necrotic lesions on roots (Figure 2). Recently we also found that *A. vitis* can cause necrosis of the cambium in wounded woody canes, thereby preventing wound-healing and adversely affecting graft take. Further research to elucidate the potential negative effects of *A. vitis* at graft unions is underway.

Infection

The infection process of *Agrobacterium* represents the only known case of natural inter-kingdom transfer of DNA (bacterial DNA is transferred to and expressed in the plant). Thus crown gall infections can be considered a form of natural genetic engineering of plants. The infections are initiated at injury sites; on grapes,

Figure 1. Crown gall infection caused by *Agrobacterium vitis*. Infections are first visible in mid-July following injury that occurred during the winter from cold temperatures.

Photos courtesy of Thomas J. Burr

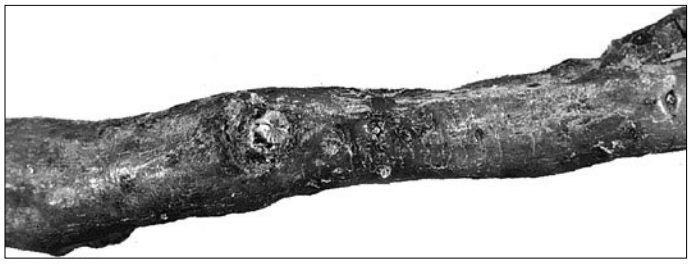

Figure 2. Localized necrosis of a grape root caused by *Agrobacterium vitis*. The bacterium causes necrosis of roots on all rootstock and scion cultivars that have been tested.

these are most commonly caused by freezing temperatures. Another common infection site is the graft union where that bacterium may cause galls, or completely prevent graft take, as mentioned above. Other types of wounds—such as those made from cultivation and during pruning—generally do not stimulate the development of crown gall. Wounds release chemicals (sugars and phenolics) that stimulate the crown gall infection process once they are detected by the bacterium. They cause the bacterium to migrate toward the wound where it attaches to the plant cells. The chemicals also trigger the expression of genes in *A. vitis* that are necessary for infection. Once *A. vitis* transfers a portion of its DNA (genes) to the plant, the genes encode enzymes that stimulate production of abnormal levels of plant hormones (auxin and cytokinin) that cause the plant cells to proliferate and form galls.

Wounding of plants probably plays another role by stimulating the development of plant cells, during wound healing, that are highly susceptible to infection by the pathogen. We are continuing research to identify specifically which cells in grape wounds become infected and the role of auxin in stimulating the development of the cells.

Pathogen variability

Strains of *A. vitis* are variable with regard to their genome, including the number and types of pathogenicity genes they carry. Strains collected from commercial vineyards and from wild *V. riparia* vines collected in New York State were grouped based on genetic profiles. It was discovered that all of the strains isolated from wild *V. riparia* were non-gall forming types and appear to represent a separate group of the bacterium.

Sources of inoculum

Thus far, *A. vitis* has not been detected on plants other than grape, or in soils collected from sources other than vineyards. Once introduced to soil, however, the bacterium was found to survive for at least a two-year period on infested grape root debris. Grape roots are known to persist in soils for long periods after vines have been removed, and it is likely that *A. vitis* will persist with them. Although survival on root debris comprises a source of inoculum for infection of new vines, we believe that the most common and important means of introducing the pathogen to a vineyard is by carrying it along with new vines.

Control practices

Strategies for management of crown gall include water management, maintaining multiple trunks in crown gall-conducive vineyards, considering scion and rootstock susceptibility, obtaining "clean" plants when possible, and selection of sites that are not prone to winter injury. As mentioned above, injuries from cold temperatures stimulate the development of crown gall. The practice of multiple-trunking will not eliminate the bacterium from vines; however, it often allows a grower to establish trunks that do not develop crown gall and from which a crop can be produced. Obviously, if injury occurs yearly on trunks, they will get repeated crown gall infections and will not produce a suitable yield. Where possible, managing vine vigor through water management also affects crown gall. Vines in wet sites that grow vigorously late in the season will typically be more prone to injury and subsequently crown gall.

Because the bacterium can survive internally in cuttings, methods have been developed to index them for *A. vitis*. A standard method that we use involves callusing cuttings and then assaying the callus tissue for the pathogen. If *A. vitis* is present, it will multiply to levels in the callus that allow its detection on a selective culture medium. Isolated colonies are then characterized with antibodies (ELISA) and with other methods such as PCR. Although the methods to identify the bacterium are very accurate, it is difficult to know the sensitivity of indexing methods; i.e., how many bacteria in a cutting can be detected? If *A. vitis* is not detected in a cutting, we cannot be entirely certain that it is free of the pathogen. Research is continuing to improve the sensitivity of indexing methods.

Attempts have been made to produce *A. vitis*-free grapevines. One approach was to submerge grape cuttings in water at 50º–52º C for 30 to 60 minutes. This treatment resulted in little or no bud injury if treatments were done in January and February, when they were fully dormant. Later treatments sometimes resulted in bud death or delayed bud growth. The treatments were shown to significantly reduce the levels of *A. vitis* in the cuttings, but even the higher temperature for 60 minutes did not eradicate all of the bacterium from the cuttings. Therefore, the procedures evaluated thus far are not recommended as means of eliminating *A. vitis* from cuttings.

It is possible to produce *A. vitis*-free vines by initiating the plants from shoot-tips in tissue culture. It was determined that *A. vitis* is not present in tips of grape shoots and therefore vines propagated from them are free of the bacterium. Subsequently, such vines have been established in mother blocks in sites that were not previously planted to grapes. Vines in the mother blocks are being indexed yearly to determine if they remain free of *A. vitis*. Thus far this approach has been successful in three different test cases for providing sources of propagation material that are free of the pathogen. In another mother block that was planted immediately adjacent to a crown gall-infected vineyard, the bacterium could be detected in the mother block vines within four years. Therefore the bacterium can move from vine to vine possibly in water or by roots from different plants that come in contact with each other.

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Scion and rootstock cultivars should also be considered in the management of crown gall, as they differ in their susceptibility. In general, all *Vitis vinifera* cultivars are highly susceptible as compared to *V. labrusca* and hybrids. However, especially within the hybrids, the range of susceptibility can be quite great. Rootstocks also differ greatly in their susceptibility to crown gall. Highly resistant are Couderc 3309 and Mgt 101-14, whereas more susceptible are Richter 110 and Teleki 5C. A general correlation can be made between the efficiency of rootstock callusing and their susceptibility to crown gall. As mentioned above, callus cells are highly conducive to infection and therefore these rootstocks are more susceptible to infection. Although scion and rootstock genotypes differ in susceptibility to crown gall, this does not necessarily mean that "resistant" cultivars are free of the bacterium. Previous studies indicated that resistant cultivars such as C3309 still harbor systemic populations of *A. vitis*. Complicating the determination of cultivar susceptibility to *A. vitis* is the fact that different cultivars respond variably to different diverse strains of the pathogen. Such considerations must be taken into account when evaluating germplasm for susceptibility to crown gall.

There are no effective chemical controls for crown gall. Although antibiotics and copper bactericides are able to kill the bacterium on contact, they do not penetrate the vine and come in contact with bacteria residing systemically. Painting of galls with anti-bacterial mixtures may kill bacteria in the gall (and in some cases gall tissues) but will not eliminate the bacterium from the vine.

Biological control of crown gall on fruit and ornamental plants has been very effective, and commercial preparations of a non-pathogenic *Agrobacterium* (K84) are sold in many regions of the world. Unfortunately, K84 is not effective against *A. vitis* on grape. However, it has been shown that when a non-pathogenic strain of *A. vitis*, F2/5, is applied to wounded grape tissue in advance of gall-forming pathogen, crown gall is prevented. The mechanism by which F2/5 prevents crown gall is unknown. Two interesting points are: that it only inhibits crown gall on grape, and that it must arrive at the wounded grape tissue before the pathogen.

Although F2/5 has been shown to be highly effective for controlling crown gall in greenhouse experiments, its effectiveness has not yet been proven in the field. Several experiments are underway and in these cases vines are soaked in suspensions of F2/5 prior to planting. The objective is to allow F2/5 to colonize wounds on the roots and crown and to ideally establish itself in the grapevine. Thus far, variable success in control of crown gall with F2/5 in the field has been noted.

The incidence of crown gall and its importance in vineyards throughout the U.S. appears to be on the increase. This trend seems to be occurring regardless of winter temperatures. Possible reasons may be related to new vineyards being established in many regions of the U.S. and the possibility that some sites are not environmentally suitable for growing certain cultivars or rootstocks. Other reasons for increased crown gall may have to do with vineyard (crop) management. Over-cropping can lead to vine stress and subsequent sensitivity to cold injury and thus crown gall. In California, the changing of rootstocks in recent years has influenced the number of samples expressing crown gall that have been sent to my laboratory. In almost every case, vines showing crown gall were grafted on highly-susceptible rootstocks.

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