Research News from Cornell's Viticulture and Enology Program Research Focus 2017-5

Research Focus

What We have Learned about Crown Gall

Thomas Burr

Professor, Section of Plant Pathology and Plant Microbe Biology, School of Integrative Plant Science, Cornell University,

New York State Agricultural Experiment Station, Geneva, NY 14456

Tom Burr

I appreciate having the opportunity to write this review in which I summarize research accomplishments made by members of my laboratory on grape crown gall research that I feel are most relevant to the NY grape industry.

Starting point. A key discovery made in Hungary in the late 1960's was that *Agrobacterium vitis* (at that time called *A. tumefaciens*) survives systemically in grapevines and therefore is spread in dormant cuttings (Lehoczky, 1971) (**Figure 1**). I was fortunate in the 1980's to meet Dr. Janus Lehoczky and collaborate throughout my career with Hungarian scientists as well as those from several other regions of the world where grape crown gall occurs. Our overriding goals were to advance the understanding of crown gall biology in vineyards as well as develop management tools for the disease.

Early on, we found that both gall-forming and non-gall-forming strains of *A. vitis* are common in grapevines. The gall-forming strains causes crown gall whereas both types cause a necrosis (tissue death) that is most easily observed on grape roots. The significance of necrosis on early graft strength and vine growth are currently being researched, as discussed below.

Genetic diversity. A collaborative research project with Dr. Leon Otten revealed that *A. vitis* is highly diverse genetically (Otten et al. 1990). We determined this by examining the variability of a genetic region in the bacterium that is required for causing crown gall infections. An extension of this study was the opportunity to study crown gall and strains of *A. vitis* from Turkey. This work, done by a Turkish graduate student who did a study leave in Geneva, characterized strains of the pathogen from central Turkey that were isolated from "local varieties" that had been planted in the region for many years (Argun et al. 2001). These results are important when considering how the pathogen has evolved and how the dis-

Photo byTom Burr

Figure 1. *Crown gall on trunk of vines. Wounds from grafting and from freeze injuries are main points for initiation of infections.*

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Figure 2. *Cherie Reid (left) and Kameka Johnson collecting samples in vineyard to assay for the presence of A. vitis. Cherie has been the lead technician on crown gall research for over 25 years and Kameka Johnson developed the MCH, RT-PCR assaying method.*

ease might be managed. They also shed light on why differences might be observed in grape species resistance to crown gall and how the diversity impacts development of biological controls.

Wounds and crown gall expression. It is well-known for all crown gall infections that a plant wound is necessary. The importance of the wound is not to provide an entry point for the pathogen but rather to stimulate the plant wound response, which initiates growth of plant cells that are susceptible to crown gall infection. Our lab demonstrated that auxin flow to the wound site—which is associated with wound healing—stimulates growth of cells that are susceptible to infection (Creasap et al 2005).

We also demonstrated that *A. vitis* may persist in grape root debris in soil for years (Burr et al. 1995). This research helped us to better understand why site selection and cultural practices that help to avoid grapevine wounding are key considerations for the management of crown gall.

Minimizing propagation-related transmission of crown gall. We studied procedures to minimize the presence of pathogen in propagation material.

- **Hot water treatment.** A procedure for employing hot water dips for controlling internal *A. vitis* in dormant grape canes was developed together with colleagues from Cornell, Australia, Italy and Hungary as well as commercial partners. We demonstrated that the pathogen was significantly reduced in hot water treated cuttings but was not eradicated with this approach (Burr et al. 1989).
- **Tissue culture.** Another procedure focuses on the production of *A. vitis*-free vines through tissue culture propagation as a means to eliminate the pathogen. This work is ongoing but has shown that "clean" vines can be produced as determined using our most efficient detection method, however additional research on this aspect is still underway and will be necessary. Specifically the possible survival of very low numbers of that pathogen in shoot tips and meristems needs to be addressed.

Improved diagnostic testing methods. For a long time, studies on the biology of *A. vitis* in the environment were limited because we lacked a sensitive and efficient method for detecting the pathogen. In 2013, Kameka Johnson, who was a postdoc in my lab, developed a method based on a technology called Magnetic Capture Hybridization (Johnson et al. 2013) **(Figure 2)**. This technology used together with real-time polymerase chain reaction (MCH, RT-PCR) (*see description in Appellation Cornell article How close are we to crown gall-free nursery stock*) has greatly enhanced our understanding of *A. vitis* biology. By employing this method, we have been able to greatly improve our knowledge on the following topics:

- **Within-vine distribution.** We determined that *A. vitis* is randomly distributed in nodes and inter-nodes from the base to apical ends of dormant canes. We also discovered that the pathogen persists in dormant buds, on green shoot tips, and on leaf surfaces during the growing season (Johnson et al. 2016, Orel et al. 2017). Therefore, *A. vitis* persists both internally and externally on grapevines and is not restricted to internal tissues of the vine.
- **Presence in wild grapevines in New York and California.** We found that pathogenic forms of the

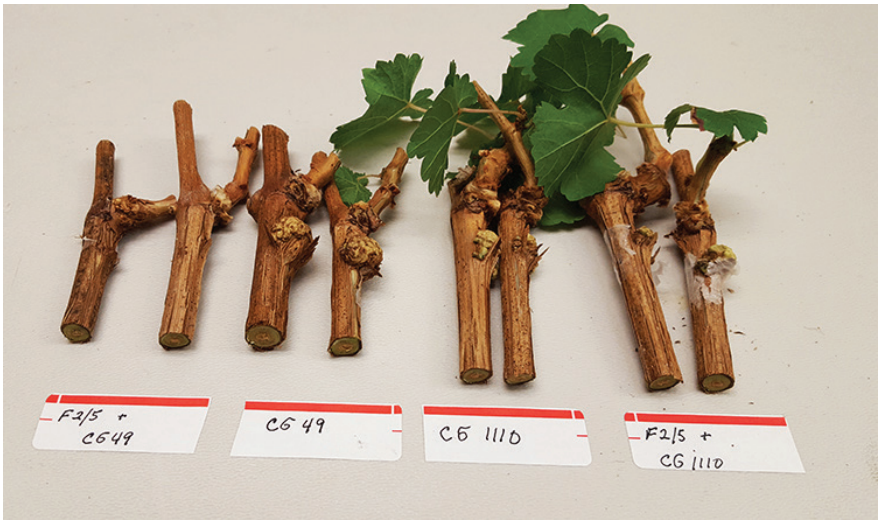

Photo by Tom Burr

Figure 3. *Strain F2/5 inhibits grape crown gall caused by A. vitis pathogen (CG49) but not by other species of Agrobacterium that typically do not infect grapes (A. tumefaciens strain, CG1100)*

pathogen persist in wild grapevines (*V. riparia*) in NY as well as in feral vines collected in California. These findings increase the likelihood of disease spread in managed vineyards and indicate that there are additional potential sources of the pathogen that could infect clean vines (Orel et al. 2017).

- **Screening vines for grape foundation plantings.** We have assayed grape foundation material using the MCH, RT-PCR method and found that some of it carries the pathogen. This was a relatively new discovery made possible by this sensitive assay method. It points out the need for additional assessment of methods to produce clean plants, to determine how they can be managed most effectively in nurseries and vineyards. It also points out the need for research on the economic impacts of crown gall on vines that become infected at different ages.
- **Evaluating tissue culture for crown gall elimination.** The MCH, RT-PCR method has been extremely valuable for evaluating the effectiveness of tissue culture propagation for producing *A. vitis*-free grapevines. However, as mentioned above, additional research is needed to verify the effectiveness of tissue culture and factors that could lead to contamination of vines in nurseries and vineyards.

Effect of *A. vitis* on graft unions. Determining that the pathogen is randomly distributed in dormant canes and that wounds are necessary for infection to occur led us to ask the question of whether the presence of the pathogen at grafting sites could be detrimental to graft healing and vine growth. This first paper on this research was just published (Hao et al. 2017) and further research is needed to determine more long-term impacts of grafts that become infected with *A. vitis*.

Biological control. Biological control of crown gall on many plants species has been highly successful; however the control is not effective against *A. vitis* on grapevines. Since there are no effective chemical controls for grape crown gall, our lab and others have investigated other potential biological control candidates. We have extensively evaluated a non-pathogenic strain of *A. vitis* for its ability to inhibit crown gall on grape. The strain (F2/5) was originally isolated from grape in South Africa (Staphorst et al, 1985). Our research led to discoveries that:

- If applied to grape wounds prior to initiation of infection by tumorigenic strains, F2/5 inhibits crown gall caused by gall-forming strains of *A. vitis* but not by other *Agrobacterium* species (Figure 3).
- Concentration of F2/5 on the grape wound must be equal to or greater than that of pathogen.
- Gall production by some tumorigenic strains is inhibited more than others by F2/5.
- The pathogen is not killed by F2/5 but is prevented from causing crown gall on grape.
- Gall inhibition is due to ability of F2/5 to inhibit the pathogen's virulence system.

F2/5, like other *A. vitis* strains, causes necrosis of grapevine tissue, which can affect graft healing and plant growth (**Figure 4**). For this reason, we developed necrosis-negative, gall inhibition-positive strains derived from F2/5 that are currently being developed as a potential commercial product for managing crown gall of grape. The derivatives were made by disrupting single genes that are essential for necrosis (necrosis-negative) but not for gall-inhibition. Once additional laboratory and greenhouse research is completed large scale nursery trials will be conducted.

Photo by Tom Burr

Figure 4. *Necrosis on grape roots caused by A. vitis strain F2/5. Other strains of A. vitis also cause similar necrosis that is specific to grape.*

Support. Research in my laboratory was possible because of the talented and dedicated technical staff, students and postdoctoral associates that I had the pleasure to work with over the years.

Their strong interest and support along with the productive collaboration I received from Geneva and Ithaca Cornell and USDA colleagues provided the diverse expertise and the ability to implement new technologies that were required for success in research and extension. Together with a network of international collaborators the research has been incredibly exciting and rewarding.

Equally important has been the interest and support received from the NY wine, grape and nursery industries. From across the state the NY growers have always been partners in our work and have provided not only funding but also vineyard sites and plant material and have been interested in the development of new discoveries and a means to implement them on their farms (**Figure 5**).

Photo courtesy Tom Burr

Figure 5. *Partnerships with growers such as Fred Frank of Konstantin Frank Vinifera Wine Cellars in Hammondsport have been most important to carry out our research.*

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Cornell University
College of Agriculture and Life Sciences
Cornell Cooperative Extension