Research in Plain English: Crown Gall Elimination

Elimination of the Crown Gall Pathogen, *Agrobacterium vitis*, from Systematically Infected Grapevines by Tissue Culture

Research in Plain English provides brief, non-technical summaries of journal articles by Cornell faculty, students, and staff.

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Am. J. Enol. Vitic. 2019, 70(3); DOI: 10.5344/ajev.2019.18083

Summary by Rebecca Wiepz.

The takeaway.

- Crown gall is a bacterial disease that causes the formation of cankers on grapevines throughout the world, leading to production losses, higher vineyard management costs and eventual vine replacement.
- Current management strategies are limited to cultural practices as chemical treatments cannot impact bacteria that reside within the vines.
- Previous diagnostic tests were not sensitive enough to consistently detect crown gall bacteria, and often indicated that vines were clean when they were not. A new diagnostic test was developed that is 10,000 times more sensitive than the previous test, decreasing potential error.
- This study used tissue culture to produce clean vines from infected mother vines.
- Repeated testing of tissue-cultured vines demonstrated that the crown gall bacterium was eliminated.

Background.

Crown gall (a bacterial disease caused by *Agrobacterium vitis*) is a significant problem in vineyards around the world. The formation of cankers on vine trunks inhibits vine growth and production and causes economic losses, both in terms of fruit production and eventual vine replacement.

Management is currently limited to cultural practices, since the systemic nature of the infection protects the bacteria from chemical treatments and no host resistance has been documented. While temperature-based sanitation (heat

treatment to dormant cuttings) has been used to reduce crown gall incidence when propagating cuttings, high temperatures needed to eliminate A. *vitis* have resulted in significant bud death, making this technique for crown gall elimination less than ideal.

While previous attempts have been made to eliminate crown gall through tissue culture in the past, the development of a new, highly sensitive test (MCH-qPCR) showed that many vines previously thought to be 'clean' were, in fact, infected. This experiment aimed, through repeated cycles of propagation and testing, to develop a set of pathogen-free plants that could be grown out in a vineyard.

The procedure.

To produce vines that were free of A. *vitis*, (Figure 1) shoot tips were selected from greenhouse grown vines (A) and trimmed down excess leaves and tissue until they were left with an apical bud ready for culture (B-D). Apical meristems (E) and axillary buds (F) were trimmed and dissected before being propagated.

Plants were then grown on both solid media (G) and liquid media using a filter paper bridge (H) and tested using the robust test procedure called "Magnetic Capture Hybridization" (MCH-qPCR) (I) after at least three subcultures onto clean media. Plantlets that tested negative for A. *vitis* were established in soil and grown in the greenhouse for further testing (J). Plants with negative tests were maintained in the greenhouse and tested over the next two seasons for presence of A. *vitis* (K).

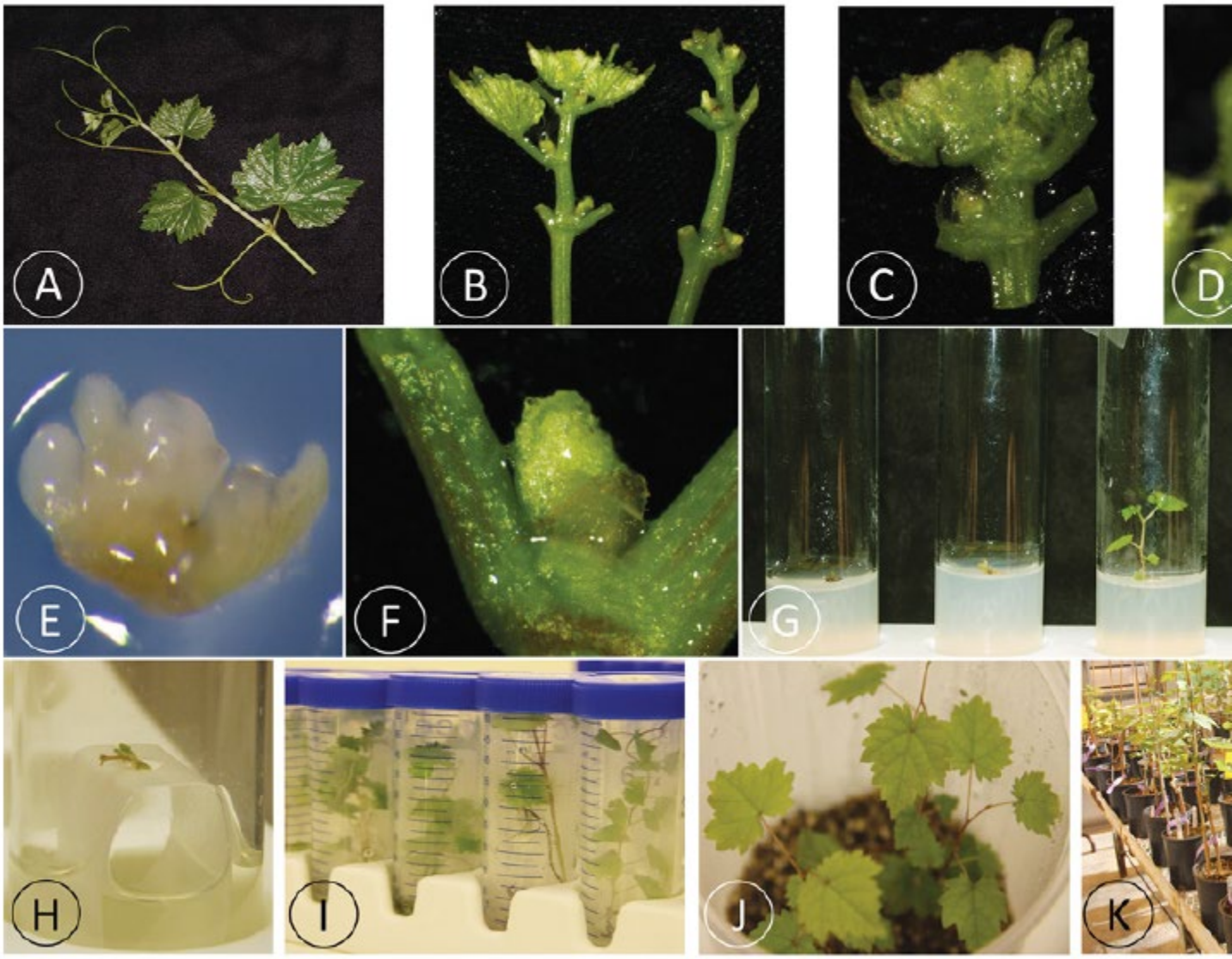

Figure 1
The results.

Even when a vine is known to be infected, some samples will not test positive, due to the mobility of the bacteria throughout the plant. As such, plants selected through this process had to be tested multiple times to ensure the absence of A. *vitis* bacteria.

After two years of selection, replication, and testing, 100% of the plantlets selected and propagated were clean.

Conclusions and practical considerations.

Using a recently developed diagnostic test, which is 10,000 times more sensitive than the previously used test, it was determined that repeated selection and tissue

culture can produce pathogen-free vines that can then be planted out into a vineyard as clean plant material.

Due to the systemic nature of the infection and the pervasive presence of the bacteria in the environment (it has been found living on the surfaces of numerous grapevines and in wild grapes), eventual infection of a vineyard is likely. However, elimination of crown gall from nursery stock would prevent introduction of the pathogen into the graft wound. When coupled with careful cultural practices once in the vineyard, this could delay economic impacts significantly.

The implementation of this procedure and test in the industry has potential to dramatically reduce crown gall injury, reducing costs associated with trunk and vine replacement.

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