

Oregon Wine Advisory Board Research Progress Report

1998 - 1999

Development and Testing of Bacteria Free Grapevines for Prevention of Crown Gall in Oregon Nurseries and Vineyards

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Agrobacterium vitis is a bacterium that causes crown gall in grapevines. Plant injury from freezing temperatures or mechanical damage during cultivation often provide wounds required for infection to occur. Crown gall may kill grapevines and a new shoot is often brought up from the root to replace dead vines. Discovery of phylloxera in Oregon vineyards has led to replacement plantings of grapevines grafted onto phylloxera resistant rootstocks. Grafted plants are also at risk for crown gall because the grafting wound allows infection by bacteria present within the plant vascular tissues (2, 5). Several nursery owners have reported a high incidence of crown gall at the graft site. Heat treatments at temperatures 50°C for 30 minutes have been reported to reduce disease incidence without impairing a graft (1, 4). However, pathogenic bacteria may not be completely eradicated at 50°C. When bacterial suspensions of isolates from Oregon vineyards have been subjected to 50°C for 30 and 45 minutes, most strains were killed after a 45 minute treatment, but two of 13 biovar 1 strains survived after a 60 minute treatment, as did one biovar 2 strain of 12 tested. This indicates that some bacterial strains could survive in heat treated dormant grapevines.

One strategy for protecting plants from crown gall is to use grapevines that are resistant to the disease. Rootstocks suitable to Oregon soils and climatic conditions were evaluated for various horticultural and wine characteristics at several sites in Oregon. Steve Price and Bernadine Strik reported that there was a high incidence of crown gall in three of these rootstocks, T5C, 420A Mgt and Harmony (8). Those found to be most resistant were 3309 C, 101-14 Mgt and S04.

Tests for resistance to crown gall were conducted in our laboratory over the past two years using an *in vitro* system on the rootstocks *Vitis riparia* Gloire, *V. rupestris* Saint George, *V. rupestris* X *V. riparia* 3309C and 101-14 Mgt, *V. riparia* X *V. berlandieri* S04, T5C, and 420A Mgt and *V. californica*. *A. vitis* and other agrobacterial strains that have been isolated from 21 sites in Oregon over the past three years were used to screen the plant material. These tests showed that 3309C and 101-14 MGT were the most tolerant to crown gall bacteria, yet there were many strains that could infect these rootstocks (6). The wild grape, *V. californica*, was the most tolerant and may be useful in a breeding program to develop new rootstocks. The rootstocks currently in use may reduce but not eliminate the threat of crown gall. Even in rootstocks that are tolerant to crown Gall bacteria, pathogenic agrobacteria within their vascular system may be transmitted to susceptible scion wood. A study completed this past year resulted showed that over 50% of all grafted plants developed crown gall at the graft union when dormant rootstocks had been infiltrated with pathogenic agrobacteria prior to grafting. Thus, even though the rootstocks were tolerant to the pathogenic agrobacteria strains used for infiltration, the susceptible scion wood still

developed tumors.

One alarming discovery made during our survey of Oregon vineyards and nurseries was the presence of biotype 2 agrobacteria that are highly virulent on grapes. This biotype usually infects nursery crops and hosts other than grape. These biotype 2 bacteria were found at six different sites, including three nurseries, and were highly virulent on ALL eight rootstocks tested.

To summarize our results on crown gall in grape so far: 1) we have detected pathogenic agrobacteria in grapevines from 11 different sites. Other sites that we sampled may also have pathogens that were undetected during this survey, 2) while the bacteria that were isolated are predominantly biotype 3 (*A. vitis*), the presence of biotype 2 strains that are highly virulent is cause for concern, 3) heating at 50°C for 30 minutes eliminates most, but not all, pathogenic agrobacteria and has no effect on grafting, 4) rootstocks 3309C and 101-14 Mgt are the most tolerant to crown gall bacteria, but are easily infected by the biotype 2 strains and 5) rootstocks infiltrated with pathogenic bacteria may not develop tumors within the rootstock section, but up to 50% formed tumors at the graft union on susceptible scion wood. While selection of tolerant rootstocks and use of heat treatments of dormant grape plants may reduce crown gall of grape, these treatments alone are not sufficient to prevent crown gall completely. The most desirable method may be to start with clean plant material propagated by tissue culture.

Objectives of the current research are: **1) to test the effectiveness of tissue cultured grape vines to eliminate systemically infected propagating materials, 2) to determine the rate of reinfestation and crown gall incidence, if any, after outplanting and 3) to determine the effects, if any, on grape production and wine quality.**

Grapevine cuttings were taken from the Lewis-Brown Horticultural Farm at OSU by Steve Castagnoli and rooted under mist in the greenhouse. Rooted plants were transferred to one gallon pots and grown in the greenhouse. New shoot tips from the potted grapevines were used as a source of plant material to establish grape in tissue culture. The tips were rinsed in running tap water for one hour, then surface sterilized in 10% clorox solution for 15 minutes on a shaker. Tips were then transferred to a modified Murishige and Skoog propagation medium for initiation. The plant tips were placed in a growth room at 25°C with a photoperiod of 16h. Transfers were made every two weeks onto fresh medium. Once plants were established, they were transferred to a richer growth medium for propagation. After propagation was established, plants were transferred to a medium known to stimulate rooting. Once roots were developed, the plants were placed in small pots in the greenhouse with plastic covers to keep the humidity high until the plants had developed functional stomates. The plastic tops were gradually elevated to allow the plants to adapt to the greenhouse atmosphere.

So far plants established in the greenhouse are: rootstocks 101-14 and 3309C. Scion wood for Chardonnay (DJN 96), Merlot (UCD03), Pinot Gris (Pol 1406), and Pinot Noir (DJN 115) are all established in tissue culture as clean plants. Micropropagation can be used to multiply the plants, then they can be rooted in the greenhouse and planted out at cooperating vineyards and at a planting site in southern Oregon to be established as a mother block to continue growing the plants. One group of plants will be tested in pots in the greenhouse to see if reinfection can occur from soil using marked strains of agrobacteria. Plants will be observed for crown gall symptoms and tested by sap extraction for the presence of the marked strains. Another group of clean plants will be planted in vineyards at sites known to have had a high incidence of crown gall to see if reinfection occurs.

REFERENCES

1. Bazzi, C., E. Stefani, R. Gozzi, T. J. Burr, C. L. Moore and F. Anaclerio. 1991. Hot-water

treatment of dormant grape cuttings: Its effects on *Agrobacterium tumefaciens* and on grafting and growth of vine. *Vitis*. 30:177-187.

2. Burr, T. J. and B. H. Katz. 1983. Isolation of *Agrobacterium tumefaciens* biovar 3 from grapevine galls and sap, and from vineyard soil. *Phytopathology* 73:163-165.
3. Burr, T. J., B. H. Katz, A. L. Bishop, C. A. Meyers, and V. L. Mittak. 1988. Effect of shoot age and tip culture propagation of grapes on systemic infestations by *Agrobacterium tumefaciens* Biovar 3. *Am. J. Enol. Vitic.* 39:67-70.
4. Burr, T. J., K. Ophel, B. H. Katz, and Kerr A. 1989. Effect of hot water treatment on systemic *Agrobacterium tumefaciens* Biovar 3 in dormant grape cuttings. *Plant Disease*. 73:242-245.
5. Lehoczky, J. 1968. Spread of *Agrobacterium tumefaciens* in the vessels of the grapevine, after natural infection. *Phytopath. Z.* 63:239-246.
6. Miller, Marilyn L., Katie Tooley, Kathleen Ferguson, Clara Schindler and Larry Moore. 1997. Resistant of grape rootstocks to crown gall. OSU Winegrape Research Wine Advisory Board Progress Reports 1996-1997. OSU Dept. of Food and Technology. 100 Wiegand Hall. Corvallis.
7. Moore, L. W., Marilyn L. Miller and TJ White. 1996. Crown gall in Oregon grapevines: biology and treatment of planting stock with hot water dips. OSU Winegrape Research Wine Advisory Board Progress Reports 1995 - 1996. OSU Dept. of Food Science and Technology. 100 Wiegand Hall. Corvallis.
8. Price, Steve, and Bernadine Strik. 1992. Rootstock trials in Oregon, in Grape Rootstock Meeting Proceedings, edited by B. Strik and Viki S. Freeman. Department of Horticulture. OSU. Corvallis, Oregon.
9. Tarbah, F. A. and R. N. Goodman. 1986. Rapid detection of *Agrobacterium tumefaciens* in grapevine propagating material and the basis for an efficient indexing system. *Plant Disease* 70:566- 568.