

Oregon Wine Advisory Board Research Progress Report

1997 - 1998

Crown Gall of Grape Rootstocks

Marilyn L. Miller, Katie Tooley, Kathleen Ferguson, Kyle Toma
Jessica Mentzer and Larry Moore
Department of Botany and Plant Pathology
Oregon State University

INTRODUCTION

Crown gall can develop on grapevines wounded by freezing temperatures, mechanical damage or grafting. Pathogenic agrobacteria residing within the vascular system are able to initiate tumor growth in damaged plant cells. Sampling from 21 sites in Oregon vineyards and nurseries yielded diverse populations of pathogenic agrobacteria. Most of the pathogenic strains isolated were biotype 3 (*Agrobacterium vitis*), but there were some biotype 2 strains isolated from five sites that were highly virulent.

Several methods have been proposed for minimizing crown gall in grapevines. These include: 1) heat treatments to kill bacteria within plants, 2) planting grafted grapevines growing on rootstocks that are tolerant to pathogenic agrobacteria and 3) planting clean grapevines into disease-free soil. Research at Oregon State University over the past few years has resulted in progress in these three areas. 1) Heat treatments. agrobacteria subjected to 54°C (129°F) for 30 minutes killed 97 different strains isolated from grape. These same heat treatments were used on dormant cuttings of rootstocks and scion wood infiltrated with antibiotic resistant strains of pathogenic agrobacteria. No pathogenic agrobacteria were detected from sap extractions of heat-treated cuttings. The plants showed no adverse growth effects from the heat treatments when compared to non-heated controls, but one grower reported that the heat-treated rootstocks did not graft well. 2) Crown gall tolerant rootstocks. Two hybrid rootstocks (*V. riparia* X *V. rupestris*) 101-24 Millardet et de Grasset and 3309 Couderc were the most tolerant to pathogenic agrobacteria grape isolates, while 420 A Milardet et de Grasset (*V. riparia* X *V. berlandieri*) was the most susceptible to crown gall of six rootstocks tested. 3) Clean grapevines in clean soil. Shoot tip culture and propagation is one way to acquire bacteria free plants (2) and soil solarization has been shown to reduce the populations of pathogenic agrobacteria in planting sites (4).

1997 RESEARCH 1. Heat treatments on viability of 80 pathogenic agrobacteria at 50°C (122°F) for 30, 45 and 60 minutes.

This lower temperature has had no effect on grafting of grapevines in previously reported studies (1,3). Bacteria were suspended in water at concentrations of 10^8 - 10^9 cfu ml⁻¹, then samples placed in a water bath at 50°C for 30 and 45 minutes. Suspensions were immediately streaked to plates with appropriate growth media. A second sample was streaked one week after heating. Those strains that survived the 45 minute treatment were tested for 60 minutes (Table 1).

Table 1. Heat treatments at 50 C of pathogenic agrobacteria isolated from grape

Biovar	Number of strains	Strains killed					Strains not killed	
		Time in minutes					45	60
		30	45	60	45	60		
1	13	1	1	2	7	2		
2	12	5	2	4	0	1		
3	38	31	3	4	0	0		

The biovar 1 strains were the most heat resistant but they represent a very small number of total pathogens isolated from grape and were weakly virulent. The biovar 3 strains were the most heat sensitive and represent the largest group of pathogenic isolates from grape. Heating grape canes for 1 hour at 50°C would be the most effective way to reduce internal pathogens based on these results. However, the fact that some biovar 1 strains resist even this long heat treatment indicates that not all pathogens may be eliminated by heat treatments.

2. Expanded resistance testing of 101-14, 3309C and 420A to confirm preliminary results with 8 pathogenic isolates.

Only pathogenic agrobacteria isolated from Oregon vineyards and nurseries were used in the screening. Expanded testing with 65 strains is important due to the variability found among the strains. Cuttings of rootstocks were collected from the OSU Horticultural Farm, rooted under mist, then grown in the greenhouse to provide a year round source of material. For testing, cuttings were removed from the greenhouse plants and brought to the laboratory where they were surface sterilized then cut into 5 cm segments including one leaf and one node. The segments were then transferred to a tissue culture medium without plant hormones. The top of each segment was cut vertically to provide a reservoir for bacterial inoculum. Bacterial suspensions were prepared to give a final concentration of 10^7 cfu units ml^{-1} and 10 microliters were applied to the wounded tips. Each bacterial strain was inoculated to at least four plantlets. Observations were made weekly for three weeks and evaluations were made according to date of onset of symptoms, number of plants infected, and weight of tumors.

The sensitivity to pathogens was consistent with the earlier results using 8 strains but does show that none of the three rootstocks is completely resistant to crown gall bacteria. Especially troublesome is the high infection rate of biovar 2 strains to the three rootstocks (Tables 2 and 3).

Table 2. Pathogenic agrobacteria infecting grape plants *in vitro*

Biovar	Rootstock		
	3309C	101-14	420A
1	8/12 (66%) ^a	4/12 (33%)	6/12 (50%)
2	12/12 (100%)	11/12 (92%)	10/12 (83%)
3	27/41 (66%)	22/41 (54%)	39/41 (95%)

a) Number of strains that induced crown gall/total number tested (%).

Table 3. Number of plants infected *in vitro* after inoculation with crown gall bacteria

Biovar	Rootstock		
	3309C	101-14	420A
1	14/64 (22%) ^a	8/60 (13%)	13/56 (23%)
2	52/56 (93%)	34/52 (65%)	42/52 (81%)
3	51/96 (26%)	44/180 (24%)	134/196 (68%)

a) number of plants developing crown gall/total number of plants inoculated with pathogenic agrobacteria

Rootstock 101-14 had the lowest incidence of infection by all three biovars strains of the three rootstocks tested. However, it was still infected by one-third of the biovar 1 strains, 11 of the 12 biovar 2 strains and over half of the biovar 3 strains. Even though it is the most tolerant of the three rootstocks, it is still susceptible to infection by many grape isolates in Oregon. While using this rootstock may reduce the incidence of crown gall compared to the other two rootstocks, it is still likely to get infected when the conditions are favorable.

For long term considerations in developing resistance to crown gall, it might be well to use the wild gape, *Vitis californica*, in a breeding program to develop new hybrid rootstocks. The tolerance of *V. californica* to pathogenic agrobacteria was compared to three other rootstocks currently being used in Oregon by inoculating them with 16 of the most virulent strains isolated from grape (Table 4).

Table 4. Strains infecting *V. californica* and three other grape rootstocks

Biovar	<i>V. californica</i>	Rootstock		
		3309C	101-14	420A
1	0/2 ^a	1/2	0/2	2/2
2	4/8	8/8	8/8	8/8
3	0/6	3/6	3/6	6/6

a) Number of strains infecting grape *in vitro*/total tested

Only four of the sixteen most virulent strains tested were pathogenic on *V. californica* indicating that it has a high level of natural resistance to Oregon grape pathogens. It will be screened against the remaining 49 grape pathogens used on the other three rootstocks to see if the resistance is broad based.

3. Tests for survivability and transmission of pathogenic agrobacteria. in tolerant rootstocks.

Even though tolerant plants may not develop crown gall when inoculated with pathogenic agrobacteria, they could still harbor the pathogens and transmit them to susceptible scion wood. Two pathogenic *Agrobacterium* strains from grape were selected for testing this hypothesis. Spontaneous rifampicin resistant mutants of AL9/95 and O508/93 (Table 5) were infiltrated into dormant cuttings of 3309C and 101-14 at a concentration of 10^8 cfu ml⁻¹. The canes were grafted to Chardonnay, Pinot Gris and Pinot Noir scion wood and placed in plastic bags in the dark until rooting was initiated. There were 30 plants of each rootstock/scionwood/bacterial train combination.

Table 5. Pathogenic *Agrobacterium* strains used to test survivability and transmission to roots, soil and scion wood

Strain	Source ¹	Biotype		Pathogenicity			
		101-14	3309C	Chardonnay	P. Gris	P. Noir	
AL9/95	Chardonnay	3	0/8	0/8	0/4	4/4	N.T.
O508/93	Pinot noir/101-14	3	0/8	0/8	4/4	4/4	3/4

1. Host plant from which bacteria were isolated

Plants were placed into pasteurized soil in plastic cones after roots appeared and kept under mist in the greenhouse until shoots had developed. They were then transferred to larger pots and kept in the greenhouse for six months. Some intermediate sampling was conducted during this time period (Table 6). At the end of the six-month period, plants were removed from pots and washed. Scion wood was either sap extracted if the diameter was large enough, or alternatively, cut into small pieces and sonicated for 10 minutes (1 gram of tissue in 9 ml sterile distilled water) to release bacteria from plant cells. One hundred microliters of sap or plant-bacteria suspension was streaked to three plates of Mannitol-Glutamate-Yeast Extract medium (MGY) amended with 100 microliters L⁻¹ of rifampicin. Plates were incubated for six days at 28⁰C then counts taken of the bacterial colonies. One gram root and soil samples were also tested using 10⁻² dilution streaked to three plates for each sample. Bacteria were removed from the rootstocks by sap extractions and 100 microliters streaked to three plates per plant. At least 10 plants of each combination were sampled except in cases where less than 10 plants survived (Table 7).

Table 6. Interim Detection of Agrobacteria and Tumor Development

Bacterial strain ¹	AL9/95				O508/93			
	8/4/97	8/25/97	9/10/97	10/14/97	8/4/97	8/25/97	9/10/97	10/14/97
Rootstock								
Scionwood								
101-14								
P. Noir							R	
P. Gris			T, R ²				T, R, Rt	
Chardonnay	R				T, R, Rt			
3309C								
P. Noir				T, R, Rt	R, T(2)	T, R, S, Rt		T, R, Rt rt
P. Gris	T, Rt	T, R		T, R, Rt				
Chardonnay				R, Rt		T, R, Rt		T, R, S, Rt

1) Bacterial strain used

2) T=tumor, R= Rootstock, Rt = roots, S= Scion. One plant per sample unless designated otherwise

Table 7. Detection of Pathogenic Agrobacteria in Grafted Grapevines Eight Months Post-Infiltration

Bacterial strain ¹	AL9/95					O508/93				
	Rootstock	Roots	Shoots	Soil	Tumors Graft Base	Rootstock	Roots	Shoots	Soil	Tumors Graft Base
Rootstock Scion wood										
101-14										
P. Noir	1/10	0	0		7/10 0/10	10/10	0	0		5/10 0/10
P. Gris	8/10	0	0		6/10 1/10	3/3	0	1/3		2/3 2/3
Chardonnay	0/10	0	0		2/10 0/10	1/1	0	0		0/1 0/1
3309C										
P. Noir	5/10	0	0		2/10 0/10	9/10	1/10	0		9/10 8/10
P. Gris	3/10	0	0		9/10 5/10	3/10	0	0		0/10 0/10
Chardonnay	6/10	0	0		9/10 0/10	10/10	0	0		10/10 0/10

1. *Agrobacterium* strain used for infiltration

Bacteria free plants have been established in tissue culture and will be rooted and grown in the greenhouse. Clean plants will be planted at sites where crown gall has been known to occur to see if they become infected and develop tumors over time.

LITERATURE CITED

1. Bazzi, C., E. Stefani, R. Gozzi, T. J. Burr, C. L. Moore and F. Anaclerio. 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., 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.
3. Burr, R. J., K. Ophel, B. H. Katz, and A. Kerr. 1989. Effect of hot water treatment on systemic *Agrobacterium tumefaciens* biovar 3 in dormant grape cuttings. *Plant Disease* 73:242-245.
4. Raio, A., A. Zoina, and L. L. Moore, 1997. The effect of solar heating of soil on natural and inoculated agrobacteria. *Plant Pathology* 46:320-328.