Oregon Wine Advisory Board
Research Progress Report

1996 - 1997

Resistance of Grape Rootstocks to Crown Gall

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Crown gall can develop on grapevines wounded by freezing temperatures, mechanical damage or grafting. Increased demand for grape varieties grafted to phylloxera resistant rootstocks has led to increased incidence of crown gall at graft unions. Therefore a search for plant material that is resistant to crown gall has been undertaken. Previous studies on resistance have produced confusing and in some cases contradictory results, probably due to the difference in methods and agrobacterial strains used in each investigation (5, 11).

Characteristics of Agrobacteria

At OSU resistance testing was conducted using agrobacteria isolated from Oregon nurseries and vineyards. There were 6,112 strains isolated from 18 vineyards and 3 nurseries in western Oregon. Among 3,617 isolates screened with DNA probes for pathogenic agrobacteria, 216 pathogens were detected and characterized. The isolates were typed according to biochemical and physiological responses on a variety of media. They were also screened for sensitivity to three biological control agents (7,8).

Table 1. Pathogenic agrobacteria isolated from Oregon grapevines

Biotype	%
1	5
2	11
3	82
Unknown	2

Sensitivity	%
K84	18
HLB-2	44
E-26	13
None	25

RESISTANCE TESTING

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 107 colony forming units per ml and 10 microliters were applied to the wounded tips. Each bacterial strain was inoculated to 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. Initial testing was conducted using eight strains of pathogenic agrobacteria isolated from Oregon vineyards and nurseries.

Table 2. Pathogenic *Agrobacterium* strains used to test grape rootstocks for resistance to crown gall

Strain	Source[1]	Biotype	Sensitivity[2]
AL9/95	Chardonnay	3	HLB-2
AV11/95	Chardonnay	3	HLB-2
BR26/95	Gewurtztrameiner	3	HLB-2
D4/95	Teleki 5C	2	HLB-2, E26
I309/95	Pinot gris/101-14	2	K84
O508/93	Pinot noir/101-14	3	HLB-2
P3/93	Pinot gris/3309C	1	HLB-2
T60/94	Muscat	3	HLB-2

1. Host plant from which bacteria were isolated
2. *A. radiobacter* strains that produce antibiotics against pathogenic agrobacteria

There are several factors that contribute to damage from crown gall. Agrobacteria are known to reside within the vascular tissue of most grapevines (6), unless they have been heat treated or propagated through tissue culture (1, 3, 4, 9). If highly virulent strains of agrobacteria are living within the plant, they will quickly infect plant wounds to induce tumor formation. Some rootstocks or grape varieties form abundant callusing and these usually develop larger tumors that can disrupt vascular flow, leading to the death of vines. Biotype 3 agrobacteria *(Agrohacterium* vitis) produce an enzyme that will produce necrosis of plant tissue (2). This allows bacteria to provide their own wounds for entry into the plant. If necrosis is severe, it can damage roots, but is also a method of walling off areas of infection so that bacteria do not spread within the plant. In that sense, it is a hypersensitive response by the plant and serves to protect against extensive crown gall development. All of these factors were taken into consideration in evaluating resistance of grape rootstocks.

Table 3. Rootstocks tested for resistance to crown call disease

Rootstock	Description
Riparia gloire	*Vitis riparia*
St. George	*V. rupestris*
Selection Oppenheim 4 (SO4)	*V. riparia* X *V. berlandieri*
5 C Teleki (T5C)	*V. riparia* X *V. berlandieri*
420 A Millardet aet De Grasset	*V. riparia* X *V. berlandieri*
Kober 5BB	*V. riparia* X *V. berlandieri*
3309 Couderc	*V. riparia* X *V. rupestris*
101-14 Millardet et De Grasset	*V. riparia* X *V. rupestris*

Onset of symptoms was scored using a point scale: onset during days 1-7 = 6 points per plantlet, days 8-14 = 4 points per plantlet, days 15-21 = 2 points per plantlet. Therefore, those rootstocks with the lowest total point scores would be considered the most resistant.

Table 4. Onset of symptoms on eight rootstocks after inoculation with eight pathogenic agrobacteria strains

Rank	Rootstock	Score
1	101-14	32
2	3309C	39
3	5BB	51
4	SO4	52
5	Riparia Gloire	56
6	420A	62
7	T5C	62
8	St. George	64

After each experiment was completed, tumors were removed and weighed. Those with the lowest mean weight for tumors were considered the most resistant.

Table 5. Mean weight (mg) of tumors and largest tumors

Rootstock	Mean Tumor Weight	Rank	Weight of Largest Tumor	Rank
420A	50	1	207	1
5BB	96	2	330	2
3309C	156	3	386	4
T5C	225	4	415	5
101-14	256	5	491	6
SO4	394	6	379	3

A total of 32 plants were inoculated (4 plants with each of 8 bacterial strains) in testing for resistant. The most resistant plants were those with the fewest plants that developed tumors.

Table 6. Number and percentage of rootstocks that developed tumors after inoculation with eight individual pathogenic agrobacteria strains.

Rank	Rootstock	Number of plants with Tumors (of 32 plants inoculated)	%
1	101-14	19	60
2	3309C	22	69
3	420A	22	69
4	SO4	28	88
5	5BB	29	91
6	T5C	29	91

Another way of assessing resistance of rootstocks was to see how many of the pathogens chosen infected all eight hosts. This gives an indication of the host range of each strain, since those infecting all rootstocks would be considered to be the greatest threat in producing crown gall.

Table 7. Strain X Rootstock

Strain	BT	101-14	St.Geo.	Rip.G.	3309C	SO4	420A	T5C	5BB
AI9/95	3	-	-	-	-	-	+	-	-
AV11/95	3	-	+	+	+	+	+	+	+
BR26/95	3	+	+	+	+	-	+	+	-
D4/95	2	+	+	+	+	+	+	+	+
I309/95	2	+	+	+	+	+	+	+	+
O508/93	3	-	+	-	-	-	+	-	+
P3/93	1	-	-	-	-	-	+	-	+
T60/95	3	+	+	-	+	-	+	-	+

The agrobacteria with the widest host range were biotype 3 strains AV11/95 and BR26/95 and biotype 2 strains D4/95 and I309/95. Biotype 2 strains are not usually isolated from grape tumors but in this study biotype 2 strains were found at two nurseries and three field sites. They seem to be unusually aggressive in that they form tumors earlier and the tumors are much larger than those caused by biovar 3 strains.

Comparision of the weights between two biotype 2 and two biotype 3 strains shows a remarkable difference.

Table 8. Weight (mg) of tumors produced by Biotype 2 and Biotype 3 pathogenic agrobacteria on two rootstocks

	3309C		101-14	
	mean wt.	largest tumor	mean wt.	largest tumor
Biotype 2 D4/95 + I309/95	233	628	220	935
Biotype 3 BR26/95 + AV11/95	80	144	37	45

To estimate rootstock resistance to the eight bacterial strains chosen, the criteria above were combined to give an overall ranking. In each category, the largest number was assigned the value of 100% and all others ranked by percentage of the largest number. Then percentages were totaled across all categories.

Table 9. Resistance of six grape rootstocks to eight pathogenic agrobacteria strains

Rootstock	onset	hosts	mean wt	largest tumor	Plants infected	TOTAL
3309C	60	63	40	78	76	**317**
101-14	50	50	65	100	65	**330**
420A	100	100	13	42	76	**331**
5BB	79	75	24	67	100	**345**
SO4	81	38	100	77	97	**393**
T5C	96	50	57	84	100	**483**

Although 420A would appear to be among the three most resistant of the rootstocks tested, it is the only rootstock in the group to be infected by all the pathogens tested so far. Further tests have been conducted on the first three rootstocks listed in the group above using additional Oregon isolates.

Table 10. Screening of three rootstocks with eight additional pathogenic agrobacteria

Strain	BT	Host	3309C	101-14	420A
CE12/95	3	Reisling	0/4	1/4	4/4
CG53/95	1	R. gloire	0/4	0/4	2/4
CH27/95	3	P.N./101-14	0/4	0/4	4/4
D1/94	2	T5C	4/4	3/4	4/4
P84/93	3	PG/3309C	3/4	0/4	4/4
Q164/93	3	Grenache/5C	0/4	0/4	4/4
T325/94	3	Mueller-Thur.	0/4	0/4	4/4
V90/94	3	Mueller-Thur.	1/4	0/4	4/4
Total			**8/32**	**4/32**	**30/32**
%			**25**	**13**	**94**

These data show that 3309C and 101-14 are resistant to infection from many more strains than 420A. It also shows that another biotype 2 strain infects all three rootstocks as in previous experiments. Tests will continue with 56 more strains on 3309C, 101-14 and 420A to see if this pattern is consistent.

Based on the above experiments, selection of rootstocks 3309C and 101-14 would be the best choice for minimizing the risk of crown gall. However, it will not guarantee disease free plants and other practices are equally important. Heat treatments of planting material at 50 C for at least 30 minutes(l, 4, 9), elimination of bacteria through tissue culture propagation (3), use of biological control agents (7,8) when grafting and planting into vineyards, and planting onto clean soil (10) are also beneficial and would further reduce the risk.

Literature cited

1. Bazzi, C., E. Stefani, R. Gozzi, T. I Burr, C. L. Moore and F. Anaclerio. 1991. Hot-water treatment of dormant grape cuttings: Its effects on Agrobacterium lumefaciens and on grafting and growth of vine. Vitis 30:177-187.

2. Burr, T. J., A. L. Bishop, B. H. Katz, L. M. Blanchard and C. Bazzi. 1988. A root-specific decay of grapevine caused by Agrobacterjum tumefaciens and A. radiobacter biovar a3. Phytopathology 77:1424-1427.

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 Agobacterium lumefaciens biovar 3. Am. I Enol. Vitic. 39:67-70.

4. Burr, R. I , 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 73:242245.

5. Ferreira, I H. S., and F. G. H. van Zyl. 1986. Susceptibility of grapevine rootstocks to strains ofAgrobacterium tumefaciens biovar 3. S. Afr. I Enol. Vitic. 7:101-104.

6. Goodman, R. N., D. Butrov and F. Tarbah. 1987. The occurrence of Agrobacterium tumefaciens in grapevine-propagating material and a simplified indexing system. Am. I Enol. Vitic. 38:189-93

7. Jifeng, You, Me Xuemei, Chen Peimin and Guo Junmei. 1990. Control of grape crown gall disease with BLB-2 strain of Agrobacterium radiobacter. Chinese J. of Biol. Control. 6:35-37.

8. Kerr, A. 1972. Biological control of crown gall: seed inoculation. I Appl. Bacteriol. 35:4937.

9. Ophel, K., P. R. Nicholas, P. A. Magery and A. W. Bass, 1990. Hot water Treatment of dormant cuttings reduces crown gall incidence in a field nursery. Am. I Enol. Vitic. 41:325-329.

10. Pu, Xin-An and R. N. Goodman. 1993. Effects of ftunigation and biological control on infection of indexed crown gall free grape plants. Am. I Enol. Vitic. 44:241-248.

11. Sule, S., I Mozsar and T. I Burr. 1994. Crown gall resistance of Vitis spp. and grapevine rootstocks. Phytopathology 84:607-611.