Field Screening of Grape Rootstock Selections for Resistance to Fanleaf Degeneration

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ABSTRACT

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Fifty-five grape rootstock selections produced by L. A. Lider, nine *Vitis vinifera* × *Muscadinia rotundifolia* (VR) hybrids produced by H. P. Olmo (both of the University of California, Davis), and three fanleaf degeneration-susceptible grape rootstocks were planted in 1979 in a site in the Napa Valley, California, known to be infested with grapevine fanleaf virus (GFLV) and viruliferous *Xiphinema index*. All of these rootstock selections were field-budded with *V. vinifera* cv. Cabernet Sauvignon. The site was chosen because of the relatively uniform distribution of virus and vector. Shoot tips from the scions were first assessed for the presence of GFLV in 1981 with enzyme-linked immunosorbent assay (ELISA). Yearly ELISA assessments continued through 1991. GFLV was detected in scions on all rootstocks, but not for 10 yr in scions on VR O39-16.

Fanleaf degeneration, also known as fanleaf, is one of the most serious viral diseases of grapevines (8). Foliar symptoms of the disease include leaf deformation, yellow mosaic, and vein-banding. The most damaging consequence of fanleaf is a severe reduction in berry set and subsequent loss of harvestable crop.

Fanleaf is caused by grapevine fanleaf virus (GFLV, nepovirus group) and is spread from root to root by the Longidorid nematode *Xiphinema index* Thorne & Allen. The use of nematicides and fumigants has failed to control the vector and the disease because of depth of the vector in the soil, poor pesticide penetration and distribution, and the long-lived nature of grape roots in the soil which act as a food and virus source for *X. index* (10,11). Thus, the most likely means of controlling fanleaf degeneration is development and utilization of fanleaf-resistant rootstocks.

Efforts to produce fanleaf-resistant rootstocks commenced at the University of California, Davis, with an assessment of *Vitis* species for resistance to *X. index* feeding and reproduction in which *V. rufotomentosa* Small was identified as the most resistant species (5). Hybrids developed with *V. rufotomentosa* were tested in Australia (4), and many were planted in a trial designed to screen for fanleaf degeneration resistance near Rutherford in the Napa Valley, California, in 1979. Also included in the Rutherford trial were crosses of *V. vinifera* L. × *Muscadinia rotundifolia* Small (VR hybrids) bred by H. P. Olmo in 1948 to examine cytogenetic differences between the two genera (9).

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After 9 yr of testing at the Rutherford trial, two selections appeared resistant to fanleaf degeneration and were released: O39-16 and O43-43 (6,7,13). Both of these rootstocks are VR hybrids bred by Olmo. We report here on the field screening of these hybrid rootstocks for susceptibility to GFLV infection.

MATERIALS AND METHODS

The test site near Rutherford in the Napa Valley, California, was chosen because of the relatively uniform presence of fanleaf degeneration and the high populations of *X. index*. The soil type varied from a sandy to gravelly clay loam 1–1.5 m deep overlaying a thick dark clay layer. After establishment, vines in the plot did not receive supplemental irrigation (mean yearly rainfall about 75 cm). The existing vineyard was removed in 1978, and experimental rootstock selections were planted in June 1979. No nematicides were applied and no effort was made to remove nematode-hosting root pieces before planting. The plot was laid out in a randomized complete block design with five single-vine replicates of each selection; not all replicates of each selection survived establishment, however.

Fifty-five Lider selections were chosen for use as rootstocks from seedling populations that had been screened in pots for resistance to *X. index*. Two of these selections (25-19 and 56-14) were chosen to serve as susceptible controls. Nine of Olmo's VR hybrids were chosen for testing as resistant rootstocks, primarily because of their relative ease of propagation. Three rootstocks (AXR#1, St. George, and Harmony) were included as fanleaf-susceptible standards for production comparisons. Table 1 lists the parentages of these selections and rootstocks. The rootstocks were planted in June 1979 and were field-budded with certified virus-tested *V. vinifera* cv. Cabernet Sauvignon the following autumn. The Rutherford trial was concluded in January 1992.

Sampling for GFLV was initiated in 1981. Samples of about 1 g of leaf and stem tissue were taken from growing shoot tips. Shoot tips were taken from vines growing in the test plot and from shoots forced in a greenhouse from canes harvested during the dormant season. Samples were ground in the sample extraction buffer (14) at a 1:10 (w/v) dilution. In the first 6 yr of testing, samples were ground in a chilled mortar and pestle. After 1988, samples were collected in plastic scintillation vials, diluted with extraction buffer, partially frozen to a slurry, ground with a Brinkman Polytron homogenizer, PT10 probe (Brinkman Instruments, Inc., Westbury, NY), on number 6 setting for 20–25 sec, then frozen at −20 C until used. The homogenized samples were tested with double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) following techniques described by Rowhani et al (12) using Dynatech Immulon II flat-bottomed ELISA plates (Dynatech Laboratories, Inc., Alexandria, VA). Antiserum was provided by the Department of Plant Pathology, University of California, Davis. An antiserum produced by G. P. Martelli and W. B. Hewitt was used during the first 3 yr of testing, after which one produced by F. Jimenez (1:8,192 titer) was used.

The samples and GFLV-positive and GFLV-negative controls were loaded into duplicate wells on the ELISA plates until 1987, after which triplicate wells were used. ELISA plates were incubated for 1 hr in the dark at room temperature, and the resulting color reactions were measured at 405 nm. The readings were assigned to six classes: 1 = uninfected (≤0.075 OD 405 nm), 2 = possibly infected (0.076–0.150), 3 = infected at a low level (0.151–0.500), 4 = moderately infected (0.501–1.000), 5 = highly infected (1.001–1.500), and 6 = severely infected (>1.500). The mean of the negative control was 0.042 (SD = 0.034) over the 10 yr of testing and 0.022 (SD = 0.016) over the last 5 yr of testing.

Fanleaf degeneration in the foliage and clusters was visually assessed just before harvest by M. A. Walker and A. C. Goheen in 1982 and 1983 and by Walker and J. A. Wolpert in 1987 and 1989. Foliage and fruit were assessed on a 1 to

4 scale, where 1 = no apparent symptoms, 2 = possible symptoms, 3 = probable symptoms, and 4 = obvious fanleaf symptoms. Foliar symptoms were vein-banding, exaggerated leaf dentation and lobing, misshapen leaves, and widened petiolar sinus openings. Fruit symptoms consisted of poor set and presence of excessive numbers of "shot-berries" (small, green, unfertilized berries).

The ELISA readings and visual assessments of O39-16, O43-43, L171-6, AXR#1, St. George, and Harmony were compared for 1982, 1983, 1987, and 1989. Associations between the two measurements were determined with Kendall's tau coefficient (1) using StatView II (Abacus Concepts Inc., Berkeley, CA).

RESULTS

In the 1982 samples of scions for GFLV, 93 of 226 vines (42% of the test plot) were ELISA-positive, and infected vines were scattered throughout the plot. GFLV was detected in at least one replicate of scions grafted on 42 of the Lider seedling selections. When two replicates of a selection were ELISA-positive for GFLV, the selection was eliminated from consideration. From 1982 to 1991, scions on O39-16, O43-43, and L171-6 and three susceptible control rootstocks (AXR#1, Harmony, and St. George) were assayed. GFLV was not detected in scions on O39-16 until 1989 (Table 2). GFLV was detected in scions on O43-43 and L171-6 in 1984, but data were taken on O43-43 because scions on it produced normal fruit yields while infected and from L171-6 because it had the best performance of the Lider selections.

The data from visual assessments for fanleaf symptoms in fruit and foliage made in 1982, 1983, 1987, and 1989 for scions grafted on each replicate of O39-16, O43-43, L171-6, AXR#1, Harmony, and St. George were compared with the respective ELISA ratings (Table 3). The 1982 comparisons showed that ELISA readings and visual assessments of leaves and fruit were associated, i.e., that visual assessments appeared to predict virus status. ELISA readings were associated with fruit visual assessments in 1983 and with foliage visual assessments in 1987. There were no other significant associations.

DISCUSSION

Results of the 1982 ELISA testing indicated that most selections were susceptible and that viruliferous nematodes appeared evenly distributed throughout the plot. By 1984, the majority of selections had two or more replicates that tested positive for GFLV. Most of the 55 Lider selections (Table 1) were based on species that were judged resistant to *X. index* (5), but the only Lider selection showing potential resistance to fanleaf was L171-6, a *V. rufotomentosa* × *V. vinifera* cv. French Colombard hybrid. Studies in Australia (4) concluded that the 171-seedling series possessed resistance to *X. index* feeding. In this study, however, scions on all replicates of L171-6 were ELISA-positive for GFLV by 1987. A major hindrance to the use of the 171 series as rootstocks is their *V. vinifera* parentage and, therefore, potential susceptibility to grape phylloxera (*Daktulosphaira vitifoliae* Fitch). Other populations and selections of the Lider crosses that did not utilize *V. vinifera* need further study and may provide parental material for future crosses capable of resisting the fanleaf degeneration com-

Table 1. Rootstock selections and their parentage tested at a fanleaf degeneration site in the Napa Valley, California[a]

Seedling number[b]	Parentage
Lider selections	
6-1	(Riparia Gloire × Ramsey) selfed
25-6, -12, -19	*champinii* × (Riparia Gloire × Ramsey)
50-21	*longii* × *rupestris* Metallique
56-14	*longii* × *longii*
88-16	*slavinii* × *rupestris* Metallique
91-64	Riparia Gloire × *candicans*
101-9, -11, -56	*arizonica* × *candicans*
103-45	*longii* × *vinifera* French Colombard
106-38	*longii* × (Riparia Gloire × Ramsey)
112-2, -9, -71	1613 Couderc × (Riparia Gloire × Ramsey)
116-3, -5, -24, -25, -34, -60	1613 Couderc × *candicans*
119-29	1613 Couderc × *longii*
122-4, -16	1613 Couderc × *rupestris* Metallique
142-2, -50	*rufotomentosa* × *candicans*
150-1, -5	*rufotomentosa* × *longii*
171-6, -14, -23, -24, -33, -52, -53	*rufotomentosa* × *vinifera* French Colombard
176-9, -11	*rufotomentosa* × *rupestris* Metallique
182-7, -51	*solonis* × *longii*
187-24	*solonis* × *candicans*
192-32	*solonis* × *rupestris* Metallique
196-48	*solonis* × French Colombard
200-92	*solonis* × Riparia Gloire
508-42	*vinifera* Almeria × *candicans*
513-3, -4, -6, -7, -10, -11, -14, -24, -28, -30	*rufotomentosa* × Riparia Gloire
Olmo selections	
O39-16	*vinifera* Almeria × *rotundifolia* male 1
O41-5, -13, -37	*vinifera* Almeria × *rotundifolia*
O42-35	vinifera Hunisa × *rotundifolia* Trayshed
O43-15, -43	*vinifera* Hunisa × *rotundifolia*
O44-4, -54	*vinifera* Hunisa × *rotundifolia* male 2
Standard rootstock controls	
AXR#1	*vinifera* Aramon × *rupestris* Ganzin
St. George	*rupestris*
Harmony	seedling of 1613 Couderc OP[c] × seedling of Dog Ridge OP

[a] Certified virus-tested Cabernet Sauvignon was used as the scion cultivar.
[b] First digit identifies the cross and digits following hyphens are individual seedlings within that cross.
[c] OP = open pollinated.

Table 2. Detection of grapevine fanleaf virus by ELISA in Cabernet Sauvignon scions on three rootstock selections and three fanleaf-susceptible rootstock controls

Rootstock selections	Yearly ratings per replicate[a]									
	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991
O39-16	1/1/1/1	1/1/1/1	1/1/1/1	1/1/1/1	1/1/1/1	1/2/1/1	1/2/1/1	2/1/3/1	3/3/3/1	6/6/6/1
O43-43	1/1/1	1/1/1	2/2/4	3/1/5	5/5/3	6/6/6	5/3/4	6/6/6	6/6/6	6/6/6
L171-6	1/1/2/2	1/1/2/2	4/2/2/4	5/1/1/5	4/2/2/5	4/5/6/6	4/5/5/6	5/4/5/6	5/4/5/6	6/6/6/6
AXR#1	2/2/6/6	2/2/6/6	2/2/5/5	1/3/5/5	2/3/6/6	3/4/5/5	4/4/6/6	6/4/6/6	6/4/6/6	6/6/6/6
Harmony	1/3/3/6/5	1/3/3/6/5	2/2/3/5/5	1/4/3/5/5	2/2/3/5/5	4/5/4/5/6	5/6/5/5/5	6/6/5/6/5	6/6/5/6/5	6/6/6/6/6
St. George	4/4/4/6/4	4/4/4/6/4	5/5/5/5/5	6/6/6/6/6	6/6/6/6/6	5/5/5/5/5	6/6/6/6/6	6/6/6/6/6	6/6/6/6/6	6/6/6/6/6

[a] Replicate 1/replicate 2/replicate 3/replicate 4/replicate 5; 1 = uninfected (≤0.075 OD 405 nm), 2 = possibily infected (0.076–0.150), 3 = infected at a low level (0.151–0.500), 4 = moderately infected (0.501–1.000), 5 = highly infected (1.001–1.500), and 6 = severely infected (>1.500).

Table 3. Association of ELISA ratings with visual assessments of foliage and fruit for symptoms of fanleaf degeneration in fanleaf-tolerant hybrids O39-16 and O43-43 and fanleaf-susceptible rootstocks L171-6, AXR#1, Harmony, and St. George, using the Kendall tau coefficient ($n = 25$)

	Year			
Assessment	**1982**	**1983**	**1987**	**1989**
ELISA vs. foliage	0.500** [a]	0.260 NS	0.287*	0.200 NS
ELISA vs. fruit	0.394*	0.333*	0.153 NS	0.157 NS

[a] * = Significant at $P = 0.05$, ** = significant at $P = 0.01$, NS = not significant.

plex of nematode feeding and virus infection.

Of the nine VR hybrids tested, only scions on O39-16 and O43-43 were ELISA-negative for GFLV for an extended period. Olmo's VR hybrids were selected because of their potential nematode resistance and because they propagated and grafted with relative ease. *M. rotundifolia* is extremely difficult to propagate from dormant cuttings (2), a trait it brings to many VR hybrid progeny. GFLV was first detected in Cabernet Sauvignon grafted on O43-43 in 1984, and by 1986 GFLV was detected in scions of all its replicates. GFLV was first detected in scions on O39-16 in 1989, but when the trial was concluded in 1992, only one replicate of O39-16 was ELISA-negative for GFLV.

The ELISA readings were categorized on a six-point scale. A value of 2 was cautionary, meaning that the plant might have GFLV but that the absorbance reading was too low to be certain. The ELISA ratings of 1 and 2 varied for some replicates from year to year, as, for example, replicates 2 and 3 of L171-6 during 1984–1986. There was only one example of a replicate testing positive for GFLV one year and questionable the next. Replicate 2 of Harmony registered a 4 in 1985 and a 2 in 1986 but was clearly infected (an ELISA rating of 5) in 1987 (Table 2). This discrepancy may have been the result of poor sample quality in 1986. Rowhani et al (12) found that when shoot tips were growing slowly because of high temperatures, water or nutrient stress, or disease status, GFLV was difficult to detect in known GFLV-positive vines. The variability in ELISA values observed from year to year could have been due to sampling time or tissue quality. However, when all of the data were considered, the predominant trend was toward higher ELISA readings in all selections over time.

Visual assessments of the foliage or fruit were of limited use in detecting fanleaf and can grossly underestimate the actual infection level in a vineyard (M. A. Walker, *unpublished*). Correlations were found between ELISA and visual assessments (Table 3), and when ELISA readings and visual assessments for all 67 selections were compared in 1982 and 1989, the Kendall tau coefficients were highly significant (*data not shown*). There were many grapevines with vein-banding symptoms and high ELISA values. There were also common instances of high ELISA readings from plants without distinct vein-banding or definite fanleaf degeneration symptoms, but plants with vein-banding and low ELISA readings were not seen. It is likely that the large number of grapevines with distinct visual symptoms and high ELISA readings caused the significant correlations mentioned above. Nevertheless, many of the remaining data pairs were poorly associated.

The most promising rootstocks from this trial were O39-16 and O43-43. ELISA testing for GFLV in 1988 determined that the vines surrounding the uninfected VR hybrids were positive, indicating a potential source for GFLV infection and spread. By the conclusion of the trial in 1992, scions on both O39-16 and O43-43 were ELISA-positive for GFLV but produced relatively normal crops. This suggests these scion-rootstock combinations may have tolerance to GFLV. The vegetative and fruiting characteristics of Cabernet Sauvignon grafted on O39-16 and O43-43 over the course of the trial are described elsewhere (15), and O39-16 and O43-43 are being further tested in a series of trials across the state to determine the rate at which these rootstocks become infected with GFLV and, once infected, their tolerance to the disease.

Studies by Granett et al (3) found that O43-43 allowed high levels of phylloxera feeding and reproduction, which cautions against the use of this rootstock in phylloxerated vineyards; O39-16 did not allow phylloxera feeding or reproduction in this excised root bioassay. Given O43-43's potential phylloxera susceptibility, O39-16 is the only rootstock suitable for sites infested with both fanleaf and phylloxera. Furthermore, because the viticultural characteristics O39-16 imparts to scions have not been adequately assessed, the recommendation for its use is restricted to fanleaf degeneration sites.

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