Project Report

American Vineyard Foundation, California Raisin Marketing Order, California Rootstock Commission, the UC-DANR Competitive Grant Program in Viticulture and Enology, and the Viticulture Consortium

April 1, 2000- March 31, 2001

Project Title: The role of single and mixed infections of grapevine leafroll associated virus-2 and grapevine virus B in virus-induced rootstock decline

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Objectives and Experiments Conducted to Accomplish Objectives:

The main objective of these studies is increase our understanding of virus-induced rootstock decline (VIRD) and it's importance in vineyard productivity. Specifically, the objectives are to : 1) measure the effect of VIRD on economially-important rootstocks; 2) to determine the degree of synergism in double and multiple infections as compared to single infections of the corresponding viruses demonstrated to be involved in VIRD; 3) assess the degree of specificity in the virus types which can cause VIRD; and 4) survey field selections from industry for the predictive value of VIRD screening by PCR.

Objective 1) Measure the effect of virus induced rootstock decline on economically-important rootstocks. In the first year of this experiment, seven rootstock and two *V. vinifera* scion varieties were inoculated by chip budding with 6 virus treatments. Rootstock trials grafted in 2000 were: AXR, St. George, Harmony, 101-14 Mgt, SO4, Kober 5BB and 110R. Scions varieties were Cabernet Franc and Chardonnay. Scions were inoculated to test demonstrate our hypothesis that the same virus combination which can cause rootstock decline may be a minor problem on own-rooted *V. vinifera*. If this is true, it will be an important consideration in the choice of planting stocks in those parts of the world where there is an ongoing transition from own-rooted vineyards to vineyards propagated on rootstock.

Treatments, virus types and virus sources found in each source are listed in table 1. Treatments #1 and #2 are negative controls. Treatment #3 is a single infection of GLRV-3. The other three treatments (#4, #5, and #6) are various combinations of two or more viruses that occurred naturally. These three sources cause severe stunting in Freedom rootstock.

Table 1. Virus treatments inoculated to each of nine grape varieties. 40 plants were budded/ treatment/ variety and planted in 5 blocks with 8 plants/block for a total of 240 plants/ variety and 2160 plants.

Treatment #	Virus Type(s)	Virus Source
1	none - negative control	not grafted
2	none - negative control	healthy control
3	GLRV-3	LR101
4	GLRV-1, GLRV-2, GVB	LR102
5	GFkV, GLRV-3, GVC	LR109
6	GLRV-2,GVB	CB100

Chipbudding was done by grafting dormant buds from the virus source vines into potted, green growing rootstock and scion varieties. Treatments were randomized between budders so that variation in budtake due to different technique was controlled for all rootstocks except Kober 5BB. In Kober 5BB, the budding was not randomized correctly, so budtake comparisons aren't valid. However, we will be able to take growth data on Kober 5BB. Two inoculum buds were grafted into each plant to increase the chance of virus transmission to the rootstock. They healed in a greenhouse for 3 weeks and were hardened off in a shadehouse before planting. They were planted in a randomized complete block design (RCBD) with 5 blocks and 8 plants/treatment/block for a total of 240 plants per rootstock. Budtake was read 1 to 2 months after grafting. The reading procedure was to take off budding rubber and tape; cut into the bud to see if it was green and alive or brown and dead. Then, pull it to try to take it off the plant. Buds were rated on a scale of 0 to 4: 0 = bud was dead, 1 = bud alive and attached; 2 = bud dead and attached (some possibility of virus transmission); 3 = bud alive and not attached to rootstock (unlikely to have virus transmission); and 4 = bud was pushing. Total percent budtake was expressed as the total number of buds rated 1,2,3,or 4. After budtake was read, the inoculum buds were removed.

Trunk diameter of each plant was measured approximately 6 cm below top growth with a digital calipers. The rootstock will remain in the blocks for two years, during which shoot growth, trunk growth, pruning weight, leaf and stem symptoms and graft union abnormalities will be measured and observed. Virus diagnosis is being coordinated with ongoing studies of PCR detection of virus in rootstock.

Objective 2) Determine the degree of synergism in double and multiple infections as compared to single infections of the corresponding viruses.

Two rootstocks, Freedom and Kober 5BB, were inoculated with 11 combinations of single and mixed virus infections including GVB and the grapevine leafroll viruses. Virus treatments, sources, and virus types are listed in table 2. As can be seen in the list, there are two negative controls (treatments #1 and #2), two positive controls (#3 and #4), three single infections of different virus types (#4,#5,and #6), three treatments of artificial mixes of single infections to create double infections (#8, #9, and #10) and finally, one treatment of an artificial mix to create a triple infection (#11).

Inoculation was done by chip budding as described above except that when two different virus sources were to be budded into a single plant, only one inoculum bud was budded of each source. This way, no more than two buds were grafted into any one plant.

Forty plants were budded per treatment per rootstock for a total of 440 plants per rootstock. They were planted in the field in an RCBD with 5 blocks and 8 plants/treatment/block. Budtake was read, and trunk diameter was measured as described. .

3) *Assess the degree of specificity in the virus types which can cause VIRD.*

To achieve as accurate diagnosis as possible, virus accessions from the Davis Virus Collection and FPMS collection have been tested by RT-PCR numerous times, as well as variants on the PCR method, such as nested PCR and PCR using RNA extraction. In addition, the standard biological and serological tests, including ELISA, herbaceous host testing and woody indexing was done.

4) *Survey field selections from industry for the predictive value of VIRD screening by PCR.*

We are collaborating with private labs and farm advisors to determine the extent to which PCR results can be used to predict VIRD induced decline of young vineyards.

PCR screening is now available in our FPMS lab for most of the characterized grapevine viruses including grapevine leafroll associated viruses (GLRaV) -1, -2, -3, -4, -5 and -7, grapevine virus A (GVA), grapevine virus B (GVB), grapevine fleck virus (GFkV), grapevine fanleaf virus (GFLV), Rupestris stem pitting (RSP), tomato ringspot virus (TmRSV), tobacco ringspot virus, and arabis mosaic virus (ArMV)(see Table 3). We are also testing variants of the RT-PCR test, including nested PCR and RNA extraction. To date, RNA extraction seems much more sensitive and reliable than the usual method; however, each test is more than ten times as expensive as an ELISA test.

Table 2. Virus treatments, types and virus sources inoculated to compare effects of single and multiple virus infections on growth of Freedom and Kober 5BB. 40 plants/treatment were budded and planted in 5 blocks with 8 plants/block for a total of 440 plants/rootstock and a total of 880 plants.

Treatment #	Virus Type(s)	Virus Source

1	none - negative control	not grafted
2	none - negative control	healthy
3	GVB, GLRV-1, GLRV-2 - positive control	LR102
4	GVB, GLRV-2 - positive control	CB100
5	GVB	CB120
6	GLRV-2	LV93-11
7	GLRV-3	LR101
8	GVB + GLRV-2	CB120 + LV93-11
9	GVB + GLRV-3	CB120 + LR101
10	GLRV-2 + GLRV-3	LV93-11 + LR101
11	GVB, GLRV-2 + GLRV-3	CB100 + LR101

Summary of Research (Major Accomplishments and Results) by Objective:

We have made tremendous progress in the first year of this project. Approximately 2 acres have been planted with 3000 plants that were chip bud inoculated with virus for 11 randomized experiments. Budtake and baseline data was measured and we expect these experiments to give us a a great deal of information over the next two years which will provide grape growers with useful information about screening procedures for field selections and the relative susceptibility of rootstock varieties to virus-induced rootstock decline (VIRD).

Objective 1) Measure the effect of virus induced rootstock decline on economically important rootstocks.

Seven rootstock and two *V. vinifera* scion varieties were inoculated by chipbudding with 6 virus treatments. Rootstocks used were: AXR, St. George, Harmony, 101-14 Mgt, SO4, Kober 5BB and 110R. Scions varieties were Cabernet Franc and Chardonnay. Percent budtake was high (average 98%) across all virus treatments for AXR, St. George, and two *V. viniferas*, Chardonnay and Cabernet Franc. This supports our hypothesis that these varieties are relatively tolerant to viruses (Table 4). In the other 5 varieties, budtake was very variable (range 32% to 100%) and only somewhat indicative of virus treatment effect. In general, as compared to healthy, percent budtake was consistently lower for virus treatment #6, CB100, than other virus treatments. The rootstock 110R was adversely affected by all the multiple-infection virus treatments (#4,#5 and #6). No significant difference in budtake was observed between buds that were grafted above or below the other bud on the plant.

Objective 2) Determine the degree of synergism in double and multiple infections as compared to single infections of the corresponding viruses.

Two rootstocks were inoculated with 11 combinations of virus sources to compare effects of single and multiple virus infections on growth of Freedom and Kober 5BB. 40 plants/treatment were budded and planted in 5 blocks with 8 plants/block for a total of 440 plants/rootstock and a total of 880 plants. Budtake for all 11 treatments was 90 - 100% in Kober 5BB. Budtake for the treatments in Freedom rootstock will be read spring, 2001.

Objective 3) Assess the degree of specificity in the virus types which can cause VIRD

Five new virus sources were evaluated for growth effects on Freedom. Two of these cause severe growth effects on Freedom and are infected with GVB and GLRV-2, two do not cause severe effects on Freedom and are not infected with GVB and GLRV-2. Testing with the fifth source LV93-11, will be repeated because it does not fit our current VIRD hypothesis (Fig.1). It is infected only with GLRV-2 in tests to date, but does cause severe effects on Freedom. This is further evidence for our hypothesis that these two viruses are important in VIRD. We have begun to screen our virus collection by testing with RT-PCR to obtain a representative collection of single strain isolates. Data is being analyzed. At this time, we have found mostly multiple infections; only a few sources are single infections. Work is continuing on this front. This aspect of the work will enable us to determine whether some strains virus are more virulent (pathogenic) than others or whether pathogenic effects are seen only with multiple infections.

Objective 4) Survey field selections from industry for the predictive value of VIRD screening by PCR.

We are collaborating with private labs and farm advisors to determine the extent to which PCR results can be used to predict VIRD induced decline of young vineyards. As documented in Table 3, PCR screening is now available at Davis for most of the characterized grapevine viruses including grapevine leafroll associated viruses (GLRaV) -1, -2, -3, -4, -5 and -7, grapevine virus A (GVA), grapevine virus B (GVB), grapevine fleck virus (GFkV), grapevine fanleaf virus (GFLV), Rupestris stem pitting (RSP), tomato ringspot virus (TmRSV), tobacco ringspot virus, and arabis mosaic virus (ArMV). We are also testing variants of the RT-PCR test, including extracting RNA from tissue before running the PCR reaction. To date, RNA extraction seems much more sensitive and reliable than the usual method; however, each test is more than ten times as expensive as an ELISA test. As we test these samples, we are also running established biological and serological tests to document the relative merits of these methodologies.

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Concise General Summary of Current Years Results:

The first year of this project has been successful in establishing field trials that will enable us to determine susceptibility of rootstock and scion varieties to virus-induced rootstock decline (VIRD); and to correlate virus status with biological effects seen in vineyards. Approximately 2 acres have been planted with 3000 plants that were chip bud inoculated by hand with virus for 11 randomized experiments.

In the first set of trials, 7 rootstocks and two scion varieties were inoculated by chipbudding with 6 virus treatments. Freedom was inoculated as a positive control for the VIRD effect. Nine grape varieties - AXR, St. George, Harmony, 101-14 Mgt, SO4, Kober 5BB and 110R, Cabernet Franc and Chardonnay were inoculated with six virus treatments each. They were planted in a randomized complete block design (RCBD) with 5 blocks and 8 plants/treatment/block for an overall total of 2160 plants. Bud take and baseline data was taken.

In the second set of trials, Freedom and Kober 5BB rootstocks (both susceptible to VIRD) were inoculated with single virus infections and artificial mixes of the single infections to assess the effects of single versus multiple virus infections on growth. They were planted in the field in an RCBD with 5 blocks and 8 plants/treatment/block for a total of 880 plants. Bud take and baseline data was taken.

Extensive PCR testing for 15 grapevine viruses has been done on a selection of virus sources to further identify and characterize naturally-occurring sources of multiple and single virus infections.

Table 3. VIRUS TESTS PERFORMED ON LATENT VIRUS ISOLATES

Field tests

Cabernet Franc (leaf) to test for leafroll *
LN33 (stem) test for corky bark *
St. George (leaf) test for fanleaf degeneration and other NEPO viruses, fleck and asteroid mosaic *
St. George (stem) test for stem pitting *

Herbaceous tests

Chenopodium quinoa for detection of NEPO viruses and mechanically transmitted agents *
Chenopodium amaranticolor for detection of NEPO viruses and mechanically transmitted agents
Cucumber for detection of NEPO viruses and mechanically transmitted agents
Tobacco for detection of NEPO viruses and mechanically transmitted agents

ELISA tests to detect:

Arabis mosaic virus (quarantine materials only)
Fleck virus
Grapevine fanleaf virus
Grapevine leafroll associated virus Type 1, 2, 3, 4, 5
Grapevine virus A
Grapevine virus B
Tomato ringspot virus

PCR tests to detect:

Arabis mosaic virus (quarantine materials only)
Fleck virus
Grapevine fanleaf virus
Grapevine leafroll associated virus Type 1, 2, 3, 4, 5, and 7
Grapevine virus A
Grapevine virus B
Grapevine virus D
Redglobe virus
Rupestris stempitting associated virus
Tomato ringspot virus

An (*) indicates tests which are required by the CDFA Grapevine Registration and Certification Program and the APHIS importation permit.

Table 4. Percent budtake of buds from five different virus treatments chipbudded into nine grape varieties. Varieties listed above the thick line are hypothesized to be relatively tolerant of virus infection.

Grape Variety	**Virus Treatment**				
	#2- Healthy	**#3- LR101**	**#4- LR102**	**#5- LR109**	**#6- CB100**
AXR	99%	100%	100%	100%	98%
St. George	98%	100%	100%	98%	100%
Cab. Franc	86%	99%	100%	96%	100%
Chardonnay	97%	96%	96%	99%	96%
101-14	84%	79%	89%	69%	32%
110R	85%	76%	66%	57%	70%
Harmony	100%	100%	100%	100%	100%
SO4	86%	79%	91%	83%	41%

Fig. 1. Average shoot length of the longest shoot on Freedom rootstock after inoculation by 8 virus treatments. This data is from a previous grant and shows that some virus sources have a severe effect on growth of Freedom rootstock. Of the four sources that caused the most stunting, three are infections that include GVB and GLRV-2. The fourth source, LV93-11, is infected only with GLRV-2 in tests to date, but does cause severe effects on Freedom. Testing is continuing on this source. This is further evidence for our hypothesis that GLRV-2 and GVB are important in virus-induced rootstock decline.