Health Status of California Grapevine Planting Material

Pathogenic viruses, fungi and bacteria presence in CDFA-certified nursery stock

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ANY LATE AUGUST OR SEPTEMBER trip through Napa or Sonoma vineyard country illustrates in vivid red and purple the consequences of pathogen contamination in young and mature grapevine plants. Visitors to the region marvel at the wonderful display of color in these red-fruited varieties, but those in the know understand that this phenomenon is a harbinger of weak-flavored, difficult to ripen fruit. Grapevine Leafroll Associated Virus (GLRaV) (PHOTO 1) is usually at work here, affecting white varieties too, albeit without the visual spectacle as they do not synthesize red pigments, either in the berry or the leaf.

PHOTO 1: Sonoma Valley Alicante Bouschet with GLRaV-3 and Fanleaf virus

Over the last few seasons, the incidence of reddened harvest-time foliage in California vineyards has increased substantially, and it is considered that this may be a response to low winter rainfall testing newly popular rootstock varieties under conditions of stress not previously experienced. Red- and white-berried varieties succumb equally to stress, but the symptoms are far more striking in those with red fruit. Gophers and voles, mechanical damage from tilling, crown gall and fungal infections all induce symptoms similar to those associated with leafroll and corky bark diseases.

The expression of these factors is influenced by the physical and physiological integrity of the vine. Physical defects in plant materials, such as poorly healed graft unions, inadequate root systems and the presence of rootstock shaft lesions, all impact the ability of the plant to deal with situations of stress. Consequently, purchasing physically sound plant materials with a clear pedigree of physical and pathogen status should be the cornerstone of any new vineyard development.

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CDFA Nursery Certification of Grapevine Stock

Although not required by any regulatory authority, it is likely that 100 percent of all rootstock used in the production of California nursery stock is propagated under the auspices of the **California Department of Food and Agriculture** nursery certification program (*www.cdfa.ca.gov/phpps/PE/Nursery/pdfs/nipm_5_regs_grades_stds.pdf*).

Nurseries that voluntarily participate in the program must purchase *registered* stock from **UC Davis' Foundation Plant Services** (FPS). These materials have been tested for economically important grapevine viruses through immunological (ELISA) and RNA fingerprinting (PCR) techniques after screening by grafting to indicator plants that are extremely sensitive to specific viruses. In addition to testing at FPS, the Foundation vines are routinely examined and re-tested while program employees examine registered stock under cultivation at CDFA-certified nurseries annually.

While for all intents and purposes all rootstock materials available for purchase in California are derived from the CDFA program, the propagation of scion materials is a different matter. There are no records available, but experience suggests that up to 40 percent of all scion materials used either in nursery or field-grafted vines may be derived from non-CDFA sources. These sources include some selections from ENTAV California partner nurseries (ENTAV in Montpellier is the French equivalent of UC Davis' FPS) and field selections derived from prized California vineyards.

CDFA-certified rootstock increase blocks are the backbone of the California nursery business and wine industry. Because these materials are so important, non-certified scion materials so popular and virus symptoms evident statewide in certified plantings, an increasing number of growers elect to test nursery stock before planting.

The substance of this article reports on the independent testing of a wide range of California-certified rootstock blocks from 2000 to 2010. A comprehensive database has been amassed detailing the viral status of CDFA-certified rootstock and scion increase blocks throughout California. A broad selection of non-certified wine varieties has also been tested.

Considerations When Testing Vine Samples

There are several commercial laboratories in California offering virus diagnostic services (SIDEBAR 1). It may be valuable to test increase and source blocks "blind" so that laboratories are unaware of the provenance of the submitted samples.

SIDEBAR 1. **Commercial diagnostic laboratories**
Virus and fungal pathogen testing in grapevines

name, website	location	contact, phone, email
Agri-Analysis *www.agri-analysis.com*	Sacramento, CA	Alan Wei, Ph.D. 800-506-9852, *apwei@agri-analysis.com*
ALL Crop Solutions *www.allcropsolutions.com*	Davis, CA	Anna-Liisa Fabritius, Ph.D. 530-759-9460, *info@allcropsolutions.com*
CA Seed & Plant Lab *www.calspl.com*	Elverta, CA	Parm Randhawa, Ph.D. 916-655-1581, *randhawa@calspl.com*
FPS *fpms.ucdavis.edu*	Davis, CA	Lori Leong, MS 530-752-3590, *leongll@ucdavis.edu*
Eurofins STA Laboratories *www.stalabs.com*	Gilroy, CA	Judit Monis, Ph.D. 888-782-5220, *juditmonis@eurofinsus.com*

When choosing a laboratory, it is important to consider the following:

1. Because virus is unevenly distributed within the vine, it is important that a representative sample include tissues from different parts of the plant—for example, cordons and canes. Although sampling of an established vine might entail collection of six or more cane segments, some laboratories will discard all but two to avoid potential dilution of viral RNA.
2. Check with the laboratory to ensure that the sample tissues submitted are appropriate for the virus and season of collection. Generally, leafroll viruses and *Vitiviruses* (GVA, GVB, GVD) are best detected in lignified tissues (the older the better) from September through break of dormancy in the spring. Grapevine fanleaf virus is most easily detected in fresh spring shoot growth but can also be detected in dormant woody tissue.
3. Some laboratories feel comfortable compositing samples from more than one vine while others do not. However, there are no California or international standards specifying best methods to test grapevines for viruses. This really comes down to budget and the proportion of a block that is to be used as source material. Rootstock vines generally do not show symptoms of virus infection. However, it is relatively easy to see the effects of leafroll, corky bark (Grapevine Vitivirus B) and fanleaf viruses on scions. If sampling a block, research its history and examine for symptoms of disease in the fall.
4. Sample testing fees vary widely between laboratories as do turnaround times. It is always best, if possible, to select a laboratory that appears to be offering the most technically proficient service. The only way to make this decision is to keep abreast of industry developments and occasionally submit "control" samples to competing laboratories. TABLE 1 illustrates the results from testing 10 samples each of Cabernet Sauvignon ENTAV/INRA 341 dormant wood, a clone infected by GLRaV-2. Only one laboratory detected the virus.

TABLE 1. **Virus testing of identical stock at two laboratories**

Clone tested	No. of Samples submitted per lab	Samples positive for GLRaV-2	
		Lab 1	Lab 2
Cabernet Sauvignon ENTAV/INRA 341	10	0%	100%

Virus Status of CDFA-certified Rootstock Increase Blocks

The number of CDFA-certified nursery rootstock increase blocks that were independently tested for economically important viruses since 2000 is presented in TABLE 2. Additional diagnostic tests have become available since 2000, however, notably for grapevine leafroll associated virus species, and so not all blocks were tested for all viruses. Samples consisted of mature woody basal cane segments taken from increase block plants during the dormant season for leafroll and vitivirus detection and shoot tips gathered in spring for fanleaf analysis. Samples were submitted to whichever commercial laboratories were considered best at the time of collection. Samples and increase blocks testing positive for important viruses were not re-tested.

TABLE 2. **Virus detected in CDFA-certified nursery stock**

Virus	Blocks with virus: Total with virus	% total blocks tested	Virus type as % of total positive
GLRaV-1	1	1%	6%
GLRaV-2	10	15%	56%
GLRaV-3	1	1%	6%
GLRaV-4	0	0%	0%
GLRaV-5	4	6%	22%
GLRaV-6	0	0%	0%
GLRaV-7	1	1%	6%
GLRaV-9	0	0%	0%
GVB	4	6%	22%
GLRaV-2 and GLRaV-5	3	4%	17%
GLRaV-2 and GVB	2	3%	11%

LEGEND:

Total blocks tested	68
Total blocks positive	18
Blocks testing positive for any virus	26%

DATA COLLECTED 2000-2010

As a result of consolidation and the changing market, fewer grapevine nurseries exist today than 10 years ago. Most of the increase blocks from 2000, however, are still in use but some may belong to different nurseries. Many nurseries, especially the larger ones, have multiple increase blocks for individual rootstock and even scion varieties. It is important, therefore, to pay attention to the specific rootstock and scion block when testing or ordering plant materials. Furthermore, several CDFA-certified grapevine nurseries do not sell plants—but cuttings only—to nurseries that sell finished product. Again, it is important to note the source location of materials from these cutting operations because their product is used widely. And lastly, it is common practice for nurseries to exchange plants and cutting materials to help fill orders.

VIRUSES IN CERTIFIED NURSERY BLOCKS

Over the last 10 years, 68 CDFA-certified increase blocks from nine nurseries have been tested (TABLE 2). All blocks were analyzed for what are considered economically important grapevine viruses (SIDEBAR 2). Additionally, all samples were tested for Rupestris Stem Pitting virus (RSP), Grapevine Fleck virus and Grapevine Vitivirus A and C. More recently, samples have been tested for RSP-Syrah, the Syrah strain of RSP.

- Eighteen (26 percent) of all tested blocks were infected by either GLRaV (leafroll virus) or GVB (corky bark virus).
- Seven percent of all sampled blocks (5 of 68) tested positive for more than one virus: 4 percent for GLRaV-2 and GLRaV-5, and 3 percent for GLRaV-2 and GVB.
- The most commonly detected viruses were GLRaV-2 (15 percent of all tested blocks, 56 percent of all blocks infected with virus), GLRaV-5 and GVB (TABLE 2).
- Fanleaf virus (GFLV) has not been detected in CDFA-certified increase blocks.

SIDEBAR 2. Grapevine viruses for which samples were tested

GLRaV-1	Grapevine leafroll associated virus-1
GLRaV-2	Grapevine leafroll associated virus-2
GLRaV-2RG	Grapevine leafroll associated virus-2RG
GLRaV-3	Grapevine leafroll associated virus-3
GLRaV-4	Grapevine leafroll associated virus-4
GLRaV-5	Grapevine leafroll associated virus-5
GLRaV-6	Grapevine leafroll associated virus-6
GLRaV-7	Grapevine leafroll associated virus-7
GLRaV-9	Grapevine leafroll associated virus-9
GVA	Grapevine virus A
GVB	Grapevine virus B
GVD	Grapevine virus D
GSy	Grapevine Syrah virus -1
FK	Grapevine fleck virus
RSP	Rupestris stem pitting associated virus
RSP-Sy	Rupestris stem pitting associated virus/Syrah strain
GFLV	Grapevine fanleaf virus

FREQUENCY OF CONTAMINATION OF CERTIFIED BLOCKS

Of the nine nurseries from which samples were collected, seven sell finished grafted vines and rootstock rootings while two sell rootstock and/or scion cuttings only to the other seven. Increase blocks were re-tested every two to three years; and when clean blocks were identified, they were used as the source for new vineyard development projects. The data in TABLE 3 is arranged by nursery from which the most blocks were tested. The results are varied.

- Overall, 26 percent of blocks tested positive for a virus considered economically important.
- All but two nurseries had at least one block that tested positive for viruses.
- The proportion of virus-infected blocks at some nurseries is staggeringly high: 22 to 67 percent of blocks at nurseries where nine or more blocks were tested were positive for economically important viruses (TABLE 3).

TABLE 3. Virus-contaminated CDFA stock by nursery

Nursery	Type of nursery*	Total blocks Tested	Total blocks Positive**	%
A	Vine product	21	1	5%
B	Vine product	11	6	55%
C	Vine product	9	2	22%
D	Vine product	9	0	0%
E	Supplier	9	6	67%
F	Vine product	4	1	25%
G	Vine product	3	0	0%
H	Vine product	1	1	100%
I	Supplier	1	1	100%
Total		**68**	**18**	**26%**

DATA COLLECTED 2000-2010

*Vine product: seller of finished products
*Supplier: supplier of cuttings to vine nursery
**Positive for economically important viruses (Table 2)

ROOTSTOCK VARIETIES TESTING POSITIVE FOR VIRUS

The data collected from sampling 68 increase blocks reflects the rootstock choices most frequently requested by growers in the premium winegrowing regions of the North and Central coasts in California (TABLE 4). Rootstocks, such as 101-14 MG, 420A and 3309C, were most frequently requested and the available data reflect this.

- Examining the data from varieties where at least four blocks were tested shows that all except 110R possessed some virus infection (TABLE 4).
- 1616C was infected most frequently with 3 of 5 (60 percent) of the blocks testing positive for economically important viruses.
- Riparia Gloire (43 percent of blocks) and 420A (38 percent of blocks) followed in frequency of virus infection.
- No virus infection was found in varieties where only one or two blocks were tested (44-53M, 140R, 5C, Freedom and Schwarzmann). Of course, these are less commonly used rootstocks and so it was provident that the first or second blocks tested were virus negative.

TABLE 4. Certified rootstock blocks with virus

Rootstock	Total blocks Tested	Total blocks Positive	%
420A	16	6	38%
3309C	10	2	20%
101-14 MG	8	1	13%
Riparia Gloire	7	3	43%
1103P	5	1	20%
1616C	5	3	60%
110R	4	0	0%
St. George	4	1	25%
VR039-16	3	1	33%
44-53M	2	0	0%
140R	1	0	0%
5C	1	0	0%
Freedom	1	0	0%
Schwarzmann	1	0	0%
Total	**68**	**18**	**26%**

DATA COLLECTED 2000-2010

Spread of Virus Disease in California and Washington State

It is difficult to imagine that this widespread infection of CDFA-certified rootstock could not be related to the alarming spread of leafroll virus in many California premium winegrowing regions. It is not clear, however, how the nursery increase block vines become infected unless directly from FPS or via a vector at the nursery.

It is common practice for nurseries to propagate certified and non-certified materials within close proximity—and it is not that unusual to see virused scion materials growing adjacent to certified stock in the nursery row. However, only GLRaV-1 and -3 and Vitiviruses GVA and GVB are known to be vectored by mealybug and/or soft scale insects in the vineyard, and so it seems likely that some other mechanism is involved in virus spread. In addition to spread of virus disease by infected nursery stock, it is considered possible that root grafting, pruning, equipment and even groundwater may be responsible for spread from infected to clean vines although there is no evidence of this.

CDFA nursery regulations permit participating nurseries to graft over existing certified blocks with new scion selections. In addition to the methods discussed above, it is possible that this practice may also contribute to the dispersal of virus pathogens in certified stock.

These observations are not restricted to California. GLRaV-3 was detected in nearly 7 percent of samples collected from a certified Washington state nursery (Mekuria, T, 2010). In addition to this study, Mekuria found that approximately 60 percent of samples collected from more than 40 Washington vineyards were positive for one or more of the leafroll viruses GLRaV-1, -2, -3, -4, -5 and -9 (Mekuria *et al*, 2009, Mekuria *et al*, 2010).

Virus Infection in CDFA-certified Scion Increase Blocks

Fewer CDFA-certified scion increase blocks have been subjected to independent virus analysis. Over the last two years, however, 15 percent of certified scion blocks examined (6 of 40 blocks) have tested positive for economically important viruses. GLRaV-2 and/or GLRaV-3 have been detected in Pinot Noir, Chardonnay and Cabernet Sauvignon clones (PHOTO 2). A recent third party survey of certified, grafted, field-finishing product from a California nursery showed that 3 of 3 samples each of Cabernet Sauvignon FPS 04 and Sauvignon Blanc FPS 01 grafted to 101-14 MG were infected with GLRaV-2.

PHOTO 2: Leafroll symptoms in CDFA-certified Cabernet Sauvignon increase block

Contamination of Nursery Stock by Fungal Pathogens and Crown Gall

It is understood that certified and non-certified stock alike are infected with fungal and bacterial species of varying pathogenicity. *Phaeomoniella* and *Phaeoacremonium* are considered opportunistic species of moderate pathogenicity. These organisms may induce vine decline (think Young Vine Decline/Esca from the late 1990s) and disease under stress but go unnoticed when the conditions are good. Under the high temperature and humidity conditions of nursery propagation, however, these species along with *Cylindrocarpon* and other more virulent trunk pathogens, such as *Botryosphaeria* and *Phytophthora*, can induce characteristic trunk and graft union lesions which are frequently difficult to detect, especially in green potted vines (PHOTOS 3, 4).

PHOTO 3: Incomplete root system with substantial rootstock lesion in potted vine

PHOTO 4: Transverse section through severe rootstock shaft lesion

There is a correlation between the physical integrity of nursery stock and pathogen activity. In the same way that healthy vines are required to produce good fruit, healthy rootstock and scion increase block tissues are required to produce strong grafted vines. Highly contaminated propagation materials tend to produce less callus at the healing graft union, rootstock base and disbudding sites. This leads to weak vines with incomplete root systems and defective graft unions. These poorly healed tissues are prime targets for pathogen-induced lesion development in the nursery, an environment that supports strong vine development from physically and pathogenically compromised plants (PHOTO 5). When these vines are planted in vineyards, however, they are extremely susceptible to stress, be it caused by improper planting, insufficient irrigation or native pests and diseases—for example, nematodes.

PHOTO 5: Severe rootstock shaft lesion in dormant vine product. Most of the rootstock shaft tissues have collapsed. Graft union at right.

As with fungal pathogens, *Agrobacterium vitis*, the bacterium responsible for Crown Gall disease, is not regulated by the CDFA nursery certification program. Crown gall is much less a problem in California than in colder climates such as Washington state and the East Coast where winter temperatures may damage the trunk and stimulate gall development.

The crown gall pathogen is widely distributed in nursery stock. As with fungal pathogens, the crown gall bacterium is found in increase block vines, field-finishing dormant product and potted plants. Some rootstocks, in particular 420A, are more frequently infected with crown gall. This is particularly problematic because 420A has a reputation for being difficult to benchgraft. Consequently, the vast majority of 420A vines are planted as rootings prior to field grafting. This process results in severe wounding of the plant, which encourages crown gall development, frequently, at the point of bud insertion (PHOTO 6). This may be avoided by using benchgrafted vines. However, it is very important that high quality propagation materials are selected and that the nursery graft sufficient overage to allow for elimination of defective vines at harvest time.

PHOTO 6: Crown gall at point of bud insertion in field grafted 420A rooting

The Importance of Nursery Selection: Cleanliness is Next to Godliness

A recent study demonstrated the importance of careful nursery selection for production of the cleanest stock (TABLE 5). Selection of independently virus-tested blocks should be the first consideration. And over time, it becomes clear that certain nurseries are more willing to work with an interested client than others.

Given these two factors, the next most important aspect in grapevine propagation is cleanliness. Think twice about ordering vines from a nursery where you can barely see the greenhouses or cold storage facility through the dust and dirt swirling around the operations center. This milieu is highly contaminated with fungal and bacterial spores, fallen to the ground from countless field-lifted vines or cutting materials being moved from the field finishing row or increase block to the nursery. Instead, look for nursery operations that more closely resemble a hospital environment than a garbage yard. Clean working surfaces and asphalted roadways are good indicators.

TABLE 5. **Pathogen status of 420A rootings**

Nursery	Nursery		Total positive per 5 samples					Nursery row status
	IB	Lot #	Pal	Pch	Cyl	CROWN GALL	Any pathogen	
A	A	1	0	0	2	3//1	5	1 yr fallow
A	E	2	0	0	1	2//1	3	1 yr fallow
A	B	3	0	0	0	1//0	1	Virgin
B	B	4	0	0	3	5//0	8	Virgin
C	C1	5	1	0	5	1//0	7	Virgin
C	C2	6	2	0	3	2//0	7	Virgin
D	D1	7	0	0	0	2//1	2	Virgin
D	D2	8	0	0	0	4//2	4	Virgin

5 vines tested per lot • IB: increase block TESTED IN OCTOBER 2008

Vines tested for following pathogens:
CG: Crown gall present//pathogenic strain present **Cyl:** Cylindrocarpon spp. **Pal:** Phaeoacremonium aleophilum
Pch: Phaeomoniella chlamydospora **Total positive:** Sum number of positive readings per lot of 5 samples

The pathogenic status of 420A rootings from several different sources was examined in October 2008 (TABLE 5). These rootings were derived from cuttings grown at four nurseries and one cutting material supplier. The cuttings were all derived from virus-tested blocks. All nurseries but one finished vines in land not previously planted to grapevine stock. Vines were callused and then planted in the nursery row in May 2008. Plants were examined twice during the growing season, and in October 2008, strong vines were removed from the field and examined. Imperfect plants were discarded while five randomly selected high quality vines from each lot were tested for trunk disease pathogens. Nursery A grew vines from its own increase block (lot 1), that of supplier E (lot 2) and nursery B (lot 3). Nursery B produced vines from its own block (B) while nurseries C and D both grew vines from two of their own increase blocks. All lots were grown in land not previously planted to grapevines except for lots 1 and 2 at nursery A.

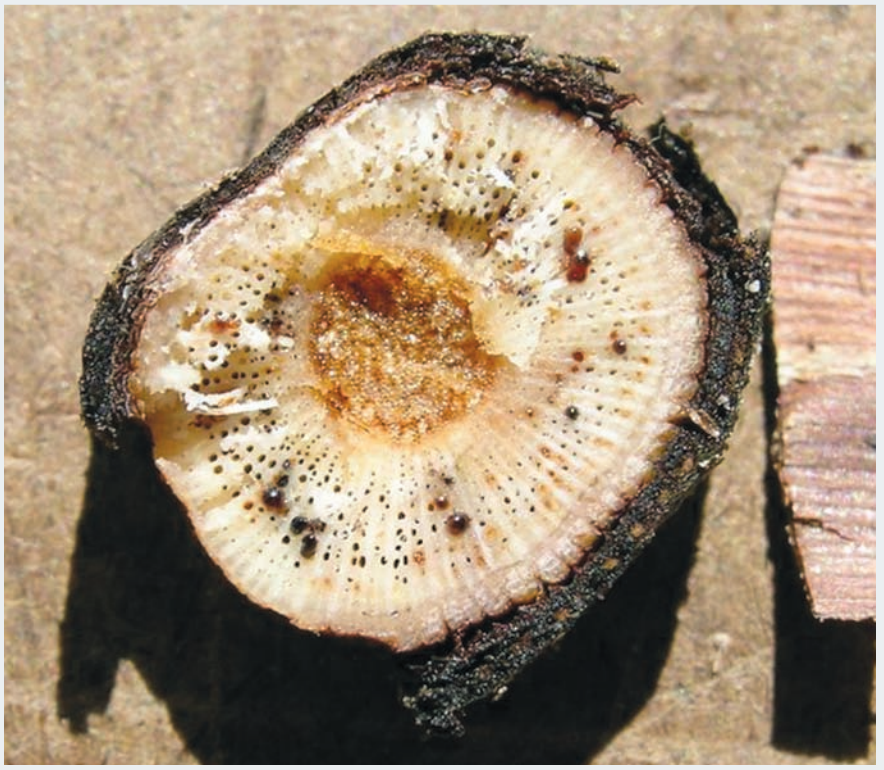

PHOTO 7: Tyloses symptomatic of fungal pathogen activity in transversely sectioned rootstock shaft

Vines were tested for three fungal pathogens and two strains of crown gall (see TABLE 5 for details). The results highlight the importance of materials source and handling and field nursery row condition on the pathogenic status of maturing product (PHOTO 7). The number of vines testing positive for individual and total pathogens is listed in the table and summarized below:

- Plants with the highest infection were grown at nursery B from nursery B increase block materials (lot 4).
- Nursery B cuttings grown at nursery A produced the least contaminated vines (lot 3). Unlike lots 1 and 2 at nursery A, these vines were grown in virgin soil.
- Nursery A (lot 1) and E (lot 2) cuttings grown at nursery A were moderately contaminated.
- Both lots of nursery C cuttings (lots 5 and 6) were highly contaminated.
- Lot 7 from nursery D was minimally contaminated while lot 8 was moderately contaminated.

This study illustrates the importance of using land not previously or recently planted to grapevines for finishing dormant product. Nursery B cuttings were very clean when grown in virgin land at nursery A but highly contaminated when grown in the field at nursery B. This suggests that post-harvest handling of the cuttings at nursery B introduced contamination. The most likely source for this infection is contaminated callusing media and cutting preparation (disbudding, etc.) under non-hygienic conditions (such as those described above).

This study demonstrates the importance of purchasing stock from nurseries that practice common sense farming techniques (field rotation) and illustrates the value of testing nursery stock toward the end of the growing season.

Choosing a Grapevine Nursery

- Research the nursery: location of operations, vineyards and fields
- Inspect facilities
- Use only independently virus-tested rootstock and scion increase blocks
- Be present at propagation to ensure specified materials are used
- Inspect vines during the growing season; evaluate for physical and pathogenic condition
- In mid-summer of the year prior to delivery of dormant products, inquire about the likelihood of your order being filled.

The Future: The Next Generation of Foundation Plant Materials

The materials currently available from certified nurseries are derived from FPS, UC Davis, Foundation stock. These materials have been tested, released and re-tested many times during the last 30 years. Most of these materials were generated using pathogen identification, treatment and elimination techniques that are now outdated.

As reported in October 2009 (**Golino**), FPS, UC Davis recently received a $20 million grant from the **National Clean Plant Network** (NCPN) to establish new, pathogen-free Foundation blocks of the highest quality technically feasible. The NCPN is a national body whose mission is to "provide high quality asexually propagated plant material free of targeted plant pathogens and pests that cause economic loss to protect the environment and ensure the global competitiveness of specialty crop producers" (NCPN mission statement, *http://groups.ucanr.org/ncpn/IMPORTANCE*).

Plants to be produced for this program will be planted at a new FPS ranch dedicated to this "next generation" of grapevines located near Davis, California, in a carefully quarantined environment. Vines will be rendered "disease free" through micro shoot tip tissue culture and the latest plant pathogenic DNA and RNA detection techniques (**Al Rwahnih, M. 2009**). It is estimated that nurseries will be able to supply finished vines derived from this next generation stock in 2015.

In these times of dismal economic news, the plant material basis for new vineyards scheduled for planting in five to 10 years looks promising. But don't hold your breath—by the time these materials come online it is more than likely that a whole array of previously uncharacterized pathogens will rise to meet the challenge. In the meantime, pay close attention to the plants you purchase for your vineyard. Plant materials are frequently considered an afterthought or commodity when it comes to planting a new vineyard. They are anything but, they are the most important part of your vineyard. **wbm**

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