FPMS GRAPE PROGRAM NEWSLETTER

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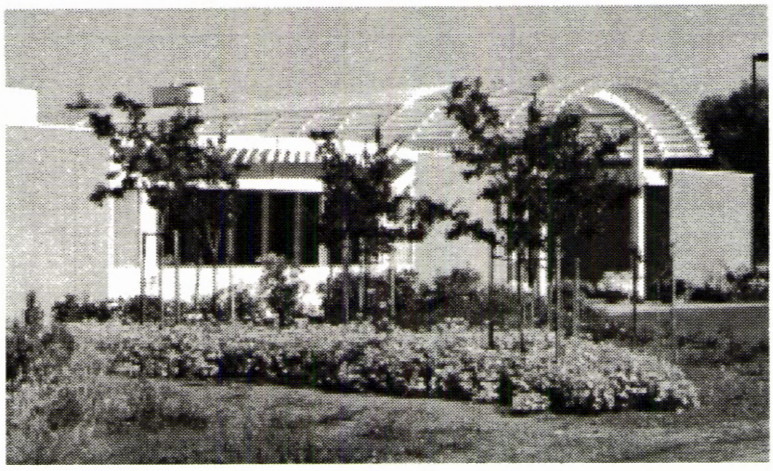


1999-2000 Grape Orders

Ordering information for grape materials available from FPMS in the 1999-2000 season has just been updated to include the new materials and newly registered selections.

Please see the handout entitled "Registered Grape Selections Offered by FPMS in the 1999-2000 Dormant Season" to make hardwood cutting selections. Note that there are 20 selections on this list that have just been reregistered or registered for the first time this year. Please order before November 15th to be included in the allocations for selections in short supply. Dormant wood is shipped to customers in February and March. No current-season dormant cutting orders are accepted after the end of February.

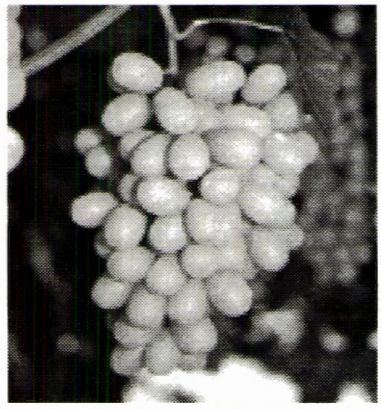
The list entitled: "New materials available from FPMS in the 1999-2000 season" shows the newest selections planted into the Foundation block at FPMS. The mother vines for these selections are too small to produce hardwood cuttings. However, customers who want to establish the newest selections in their nurseries may order mist propagated plants (MPP) of these selections.

Mist propagated plants may be ordered at any time of the year for any of the grape selections in the collection at FPMS. It usually takes 6 to 12 months to fill MPP orders depending on the amount of materials available to start from and other requests for the same material. A minimum order of 16 MPPs/selection is required. Priority is given to filling orders for the first 500 MPPs per nursery to be used to plant California registered increase blocks. However, orders for larger amounts and for other purposes are accepted on a greenhouse space available basis.

All grape materials lists, price lists and order forms are available from the FPMS office and from the FPMS Web site at: http://fpms.ucdavis.edu.

New Grape Materials

Three new table grape varieties released by USDA Agricultural Research Service (ARS) in February 1999 are now available from FPMS as mist propagated plants. Melissa is a white seedless grape with naturally large sweet berries (5-6 grams). It ripens at the end of the Thompson seedless season. Summer Royal is a mid season black seedless grape with medium size berries. Summer Muscat is an early season white seedless grape that can be dried on cut canes. The clusters are small and



Melissa

Summer Royal

the berries are medium sized. The berries have a sweet strong muscat flavor that remains in the raisin.

There are now three selections of the Mt. Eden Chardonnay clone available from FPMS. Two of these selections (FPMS selection numbers 27 and 28) were generously donated to the public collection last year by Matanzas Creek Winery. Registered hardwood cuttings will be available in the dormant season and registered MPP will be produced for customers on a custom order basis. A third Mt. Eden Chardonnay selection (FPMS #66) now available is a heritage clone that was donated to the public collection by the Simi Winery. It is available only as provisional MPPs on a custom order basis this year because the mother vines are too young to produce many hardwood cuttings.

Another heritage clone released this year for the first time is a Martini Pinot noir clone identified as Pinot noir–66 at FPMS. It has been known as the V clone in the Carneros Creek Winery Pinot noir experimental plot. Frank Mahoney of Carneros Creek has generously donated a number of clones from this trial.

One generic French clone reported to be from the French clone Sauvignon blanc 316 is being released this year. It is identified at FPMS as Sauvignon blanc–14.

Provisional Materials

Customers who receive Provisional Foundation grape materials from FPMS may request Foundation tags for those materials as soon as the source mother vines in the FPMS Foundation block become registered. Source mother vines advance from Provisional to Registered when they are determined to be correctly identified by a grape variety expert. This usually occurs two or three years after being planted in the block.

A fast way for customers to find out which selections have recently advanced to registered status is to review the underlined selections on the "Registered Grape Selections Offered by FPMS in the 1999-2000 Dormant Season" list. If you have received materials from any of the selections on the list, then it is time to contact FPMS to request retroactive Foundation stock tags.

039-16 Report

A survey completed for FPMS by Andy Walker at the end of June, 1999 showed that an apparent mix-up of the rootstock 039-16 distributed in the late 1980s may have limited impact because of the relatively low numbers of mislabeled vines. The survey examined more that 14,000 rootstock vines in nursery vineyards and found that about 8 percent thought to be 039-16 were misidentified. It is anticipated that all of those misidentified plantings will be eliminated before the 1999-2000 grafting season.

The 039-16 rootstock is used primarily in North Coast vineyards that are infected with grapevine fanleaf virus and the nematode that carries it. Very little use of 039-16 is reported outside of Napa and Sonoma counties. Some 039-16 plantings have been found to be contaminated with the phylloxera susceptible rootstock, 043-43, and phylloxera is a pest that occurs in the North Coast areas. Contamination rates ranged from zero to 50% at nurseries surveyed. The purity of the statewide 039-16 nursery stock is probably higher than found in the nursery survey because newer nursery plantings and those propagated from cuttings rather than whole plants are likely to be the correct rootstock variety, and the some of the new nurseries were not checked in the survey. No rootstock mix-ups were found in nursery plantings propagated after 1990 or in blocks propagated from cuttings.

DNA tests were used in the survey to determine the identity of some of the vines, but this type of test is too expensive (about $100/vine) to use at the grower level. Growers are advised to contact their nurseries to find out the source of the vines in their fields. If the vines came from an uncontaminated source, it is unlikely there will be future problems. Growers whose vineyards are planted with 039-16 vines affected by the mix-up should keep an eye on their vines and contact their farm advisor if vines appear to be failing. "We, on campus, will be prepared to work in concert with the farm advisors

to assist growers in any way we can," said Jim Wolpert, Chair of the UCD Viticulture and Enology Department.

Procedures used by FPMS to propagate and distribute rootstock were changed dramatically in 1993. "It's highly unlikely that a mix-up like this could occur with the multiple checks we have built into our current operations," according to Deborah Golino, FPMS Director.

Retesting Foundation Mother Vines

Regular retesting of the Foundation mother vines is part of the ongoing quality control at FPMS. This year one third of all the established mother vines and all the new vines planted in the vineyard in 1998 and 1999 were tested by ELISA for grapevine fanleaf virus, tomato ring spot virus, and leafroll-associated viruses.

Funds provided by the California Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board (IAB) are being used to completely retest about 20 Foundation mother vines per year. Complete retesting consists of all the woody indexes (field tests), herbaceous, and ELISA tests used to qualify new materials for the Foundation block (see chart of tests used). Eight of the tests used in this process have never been used to check these vines before and are not recognized in the current regulations for the California Grapevine Registration and Certification program.

Complete retesting is advisable periodically to make sure that disease has not moved into the mother vines since they were last tested and to recheck the accuracy of the old tests. Retesting now is advisable because some of the vines currently planted in the Foundation block have been propagated from materials that have not been woody index tested since the 1960s.

Results from the 1997-98 tests, which were reported to the California Department of Agriculture (CDFA) and presented at an FPMS Grapevine Advisory Committee meeting in the Spring of 1999, are shown on page 4. Also shown are the vines for which complete testing is in progress in 1998-99 and 99-2000. Results for tests in progress will be reported to CDFA and the industry the year after the end of the test.

To date, CDFA has taken no action in response to the 1997-98 test results, even though some of the vines tested positive for Rupestris Stempitting (RSP) and RSP is one of the diseases excluded under the current California Grapevine Registration and Certification Program. Kathleen Harvey, Program Supervisor for Nursery, Seed and Cotton at CDFA, recently announced that CDFA will file a petition with the Office of Administrative Law to delete RSP from the California Grapevine Registration and Certification Program Regulations. A public notice asking for comments regarding this change will be published sometime in the Fall of 1999.

Test Panel Used to Recheck Foundation Mother Vines

Field tests

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

Herbaceous tests

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

ELISA tests to detect:

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

Results from retesting of foundation mother vines in 1997-98

Variety		sel#	vine location	test result (no results = all negative)
Cabernet Sauvignon	04	BKN	B2 V5	LN33 stem repeat (buds died)
Chardonnay	04	BKN	C5 V5	
Dolcetto	01	BKS	H 4 V7	
Merlot	06	BKN	A13 V7	
Merlot	09	BKS	H4 V9	RSP+; LN33 stem repeat
Pinot noir	09	BKN	C15 V7	
Pinot noir	16	BKN	A16 V1	RSP+
Pinot noir	23	BKN	A16 V5	RSP+
Pinot noir	37	BKS	J6 V3	St. George leaf and stem repeat
Primitivo	03	BKS	G12 V5	St. George stem repeat
Primitivo	05	BKS	K6 V7	
Primitivo	06	BKS	K6 V9	RSP+
Semillon	05	BKN	A18 V9	St. George stem repeat; Cab Franc repeat (buds died)
Shiraz	07	BKS	H12 V5	St. George stem repeat
Thompson seedless	02A	BKN	A19 V5	RSP+
Thompson seedless	02A	BKN	A19 V6	RSP+
Zinfandel	01A	BKN	C19 V9	

6 RSP+/17 total vines tested
4 St+Repeat

Foundation mother vines being retested in 1998-99:

Variety/selection#	Source Plant Location
Cabernet Sauvignon 04	BKN B2 V6
Cabernet Sauvignon 06	BKN B2 V10
Cabernet Sauvignon 07	BKN C2 V1
Cabernet Sauvignon 15	BKN A3 V11
Grenache 03	BKN A11 V4
Malbec 04	BKS G3 V9
Malbec 06	BKN B12 V9
Petit Verdot 01	BKN B15 V2
Petit Verdot 02	BKN B15 V8
Pinot noir 32	BKS H2 V3
Pinot noir 39	BKS G13 V7
Sangiovese 02	BKS G16 V3
Sangiovese 04	BKS H9 V10
Semillon 05	BKN A18V10
Shiraz 01	BKN B18 V7
Tempranillo 02	BKS H10 V7
Tinto Cao 01A	BKN B19 V1
White Riesling 09	BKS H14 V1
White Riesling 12	BKN C19 V8
Zinfandel 06	BKS H13 V1

Foundation mother vines being retested in 1999-2000:

Variety/selection#	Source Plant Location
Couderc 3309 02	BKS N3 V2
Freedom 01	BKS C3 V7
Harmony 05	BKS C5 V9
Kober 5BB 06	BKS C7 V7
LN33 01	BKN AA3 V6
M.G. 101-14 01	BKS N2.5 V1
M.G. 420A 04	BKS N2 V31
Malegue 44-53 01	BKS N .25 V7
Malegue 44-53 01	BKS N .25 V3
Oppenheim 4 (SO4) 09	BKS M1 V5
Paulsen 1103 02	BKS M3 V2
Richter 110 01	BKS L8 V9
Richter 110 01	BKS M8 V2
Richter 99 01	BKS D2 V7
Riparia Gloire 03	BKS N1 V3
Riparia Gloire 04	BKS N1 V6
Ruggeri 140 02	BKS C1.5 V5
Schwarzmann 01	BKS N1 V25
Saint George 15	BKS D2.5 V7
Teleki 5C 08	BKS E1 V1

New Technology

by Dr. Deborah Golino, FPMS Director and Susan Nelson-Kluk , FPMS Grape Program Manager

One of the important roles of this newsletter is to keep FPMS customers updated about the newest technological developments for grapevine disease detection, disease elimination, variety identification, and the creation of new grapevine materials. A number of articles about emerging new technologies with broad potential application are featured in this edition. These technologies drive the changes that continually reshape the FPMS grape program, the California Grapevine Registration and Certification Program, and the grape nursery industry. Refinements and improvements in technology are expected to occur with frequency in the future due to the tremendous power of molecular biology applications.

As new technology is developed, difficult and sometimes expensive decisions need to be made about implementation of the available tools. What diseases and pests are relevant to a clean stock program? What diseases and pests are better managed by nurseries or growers? What criteria should be used to determine if a selection is true to type? How do we make sure the nursery industry has access to the new technology? Should CDFA or the University provide these technologies to nurseries? Who will pay the expenses?

The article by Dr. Rowhani, "PCR for the Future" (page 9), is a good example of new technology being used to generate new information about Rupestris stem pitting (RSP) in FPMS Foundation mother vines. PCR tests are showing that RSP appears to exist in many of the Foundation mother vines that we thought were free of this disease. Recent testing of Foundation mother vines using the old field test (two-year St. George woody index) was initiated to check the PCR data. Field test results also show RSP in Foundation mother vines. These results are described in "Retesting Foundation Mother Vines"(page 3). It is now clear that our traditional woody indexing tests for RSP are inadequate and the disease is more widespread than previously believed. At the request of FPMS, Kathleen Harvey, Program Supervisor for Nursery, Seed, and Cotton, is planning to formally request that Rupestris stem pitting be removed from the list of diseases excluded by the Registration and Certification Program. Without the new PCR test developed by Dr. Rowhani, the data to understand this long standing situation would not be available.

Plant Pathologist discovering a new disease

Dr. Rowhani and his colleagues around the world have developed PCR tests for 11 different viruses that infect grapevine including RSP. These tests are useful for fast field diagnosis of grapevines. They were among the tests used to detect the virus combinations described in Dr. Golino"s article on latent viruses (page 8). FPMS is now collaborating with AgriAnalysis, a private lab in Davis, to determine whether the PCR tests can be used to predict latent virus problems in field selections.

Dr. Carole Meredith's articles about grapevine variety identification also represent cutting edge science that is being used to characterize Foundation mother vines at FPMS. Her data increases our confidence in the varietal names assigned to the majority of the vines she tested. In most cases, she has confirmed that the vines are correctly identified. In one case, she identified a young vine of Pinot gris-06 which was misidentified and has since been put on hold at FPMS. Two years ago she was able to determine that a misidentified Cabernet Franc selection is actually the newly popular variety Carmenere, so Carmenere is now among the RSP+ varieties now offered for sale by FPMS.

Dr. Andy Walker's new test for distinguishing between 039-16 and 043-43 (see article page 2) has been key in sorting out mixed plantings of these varieties in registered increase blocks and in confirming the correct identity of the Foundation mother vines at FPMS.

The evolution of the grape program is a continual process. FPMS and our clients need to stay informed about new technological developments and discuss which technologies are relevant to our program. Decisions regarding the timing and precise methods for implementing new technology at FPMS need to be made jointly with the nursery industry, grape growers, the University and CDFA. Experience has shown that this is the best way to ensure that there is continuity in delivery of our programs and to avoid unnecessary surprises. We hope that the articles in this newsletter help explain some of the new tools available to us. Please let us know if you have suggestions about managing this important area.

Family Ties

By Dr. Carole Meredith, Professor Viticulture and Enology, UCD

In 1996, when our database of DNA profiles contained only about 50 varieties, graduate student John Bowers and I realized that our DNA data was not only useful for identifying varieties but might also be ablé to tell us something about how varieties are related to each other, much as DNA is used to establish paternity in humans. John developed a way to analyze our data with this in mind and discovered the parentage of Cabernet Sauvignon (a natural cross between Sauvignon blanc and Cabernet franc). This made us wonder what other important grapes might also be the progeny of other varieties.

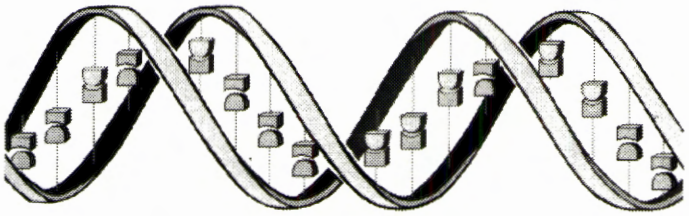
We knew we couldn't look at everything—there are just too many grape varieties—so we decided to concentrate on French grapes, since we have more French varieties in California than any other kind. We enlisted the collaboration of Jean-Michel Boursiquot and Patrice This in Montpellier, France because the variety collection in Montpellier is the best and most complete in France and because Boursiquot, with his unrivaled knowledge of the French grapes, could help us decide which varieties to look at. In June 1997, John Bowers went to Montpellier and extracted DNA samples from over 300 varieties that we had chosen as likely candidates based on the historical French grape literature and discussions with Boursiquot. Bowers then returned to Davis to generate DNA profiles from each of the 300 and to then look for parental relationships among them. It is this large collection of DNA profiles that now serves us well in verifying the identity of FPMS vines and identifying questionable vines in California vineyards.

As we had hoped, we did indeed find some close family relationships. The most interesting finding was that almost all the varieties grown in northeastern France are very close relatives. We found that 16 varieties, including Chardonnay, Melon, Gamay noir, Aligote and Auxerrois are all full siblings. They all arose as individual seedlings from natural crosses between the same pair of parents—Pinot and a now-obscure variety called Gouais blanc. Both parents were very widely grown in northeastern France long ago. Pinot (in all its forms) is still widely grown there but Gouais blanc was scorned by the landowners and nobility. It was banned and is now no longer grown. It seems quite probable that Gouais blanc is originally from eastern Europe and was brought to France by the Romans.

Gouais blanc

The important role that Gouais blanc has played in the development of Chardonnay and other important French varieties was completely unsuspected. Our findings suggest that other grape varieties may also be very close relatives. This knowledge will help grape breeders in selecting parents to use in crosses. It also teaches us that a variety that is itself not highly regarded may in fact be a very valuable genetic resource and argues for the preservation of variety collections.

A detailed report of this work was published in the journal *Science* on September 3, 1999. Copies are available on request from Carole Meredith's office.



1998-99 DNA Testing of FPMS Vines

By Dr. Carole Meredith, Professor, Viticulture and Enology, UCD

We have been verifying the varietal identity of FPMS vines by comparing their DNA profiles to those of authentic references. During the 1998 growing season, we took leaf samples from each vine and extracted DNA from them. We then generated DNA profiles by analyzing specific regions of the DNA with SSR DNA markers. These markers are now internationally accepted as the most reliable and objective way to identify grape varieties. We compared our results with DNA profiles we had previously obtained with vines known to be correctly identified or, in some cases, with profiles provided to us by European colleagues. One of the great advantages of using SSR DNA markers is that researchers in different countries can easily compare their results, without the need to exchange DNA or plant material. Although six DNA markers is generally regarded as sufficient to uniquely identify every variety, we prefer to use eight to add an extra measure of confidence.

Shiraz and Syrah– To reassure anyone who still questions whether Shiraz and Syrah are the same variety, we compared all seven Shiraz selections, as well as selections called Syrah and Sirah, to four Syrah accessions from the French national variety collection in Montpellier. All the FPMS vines have exactly the same DNA profile as the French Syrah.

Charbono– Anna Schneider from Italy told us some time ago that the FPMS vines labeled Charbono are not the same variety as Charbono in Italy. Jean-Michel Boursiquot agreed and thought they were probably an old variety called Corbeau. One of Corbeau's many synonyms is Charbonneau. We compared all six FPMS Charbono selections to DNA profiles in our database from Montpellier vines of Corbeau, Courbu, Courbu noir and Petit Courbu. Boursiquot was right—the FPMS Charbono matched the DNA profile of Corbeau but not that of the others. Contrary to Galet's opinion, we found that Corbeau is not the same as Dolcetto.

Sauvignon musque– The question has come up time and again. Is Sauvignon musque a separate variety or is it simply an aromatic clone of Sauvignon blanc? We compared them and found that Sauvignon musque and Sauvignon blanc have the same DNA profile, thus Sauvignon musque should be considered a form of the variety Sauvignon and not a separate variety.

Cabernet Sauvignon

Cabernet Sauvignon Heritage selections– Fourteen vines representing five Heritage selections from highly reputed California Cabernet Sauvignon vineyards were planted in March 1998. We produced DNA profiles for these vines in order to confirm for the record that these individual vines, when planted, were verified as being Cabernet Sauvignon. Our reference was our own database profile for Cabernet Sauvignon 08 and also profiles independently generated by colleagues in Austria and Greece.

Barbera– Barbera 06 was derived from Barbera 01 by way of a private vineyard. The original Barbera 01 vines in the old Foundation Vineyard are now leafroll infected, so it was important to confirm that

no errors occurred in the transition from FPMS and back again to FPMS. We confirmed that Barbera 06 is Barbera by comparison to two recently imported Barbera clones from Torino, Italy (CVT 171 and CVT 84). We also confirmed that Barbera 02 is really Barbera although it has a somewhat different appearance than Barbera 01.

Pinot gris– We tested several selections of Pinot gris. One of them (Pinot gris 06) had originally been labeled Rulander and was re-named Pinot gris because Rulander is a known synonym for Pinot gris. We compared the DNA profiles of Pinot gris 01, 04, 05 and 06 to Pinot gris S1 (a recent introduction from France) and also to several recent introductions of Pinot noir (which has the same DNA profile as Pinot gris and Pinot blanc). We also referred to data produced by a colleague in Austria. All of the Pinot gris accessions were confirmed as Pinot with the exception of Pinot gris 06. Of the two Pinot gris 06 vines tested (both in the Tyree Vineyard), one of them is correct but the second is another variety, as yet unidentified.

Gamay noir, Gamay Beaujolais, Napa Gamay, Valdiguié– We compared the three registered selections of Gamay noir (02, 03 and 05) to selection S3 (an introduction from France via Oregon State) and to two Gamay clones from Montpellier, France. All were confirmed as true Gamay noir. Only one selection of Napa Gamay has so far been analyzed (Napa Gamay) and it was confirmed to be the same as a sample of Valdiguié from Montpellier. Napa Gamay 02 and 03 are heat treatments of Napa Gamay 01 so are expected to produce the same results. Only one of the "Gamay Beaujolais" selections of Pinot noir (Pinot noir GB18) has so far been analyzed and it was confirmed to be a Pinot and not a Gamay.

IAB-funded retesting of selected mother vines
The IAB has been providing funding each year for the ongoing re-indexing of individual vines of important selections. The process was begun on 17 vines in 1997-98 and 20 vines in 1998-99. We have also checked the DNA profile of each of these vines. All but a few have been confirmed as correctly identified. The limitation for the vines we have not yet confirmed (Petit Verdot, Tinta Cao, Tempranillo, Thompson Seedless) is that we do not yet have reference DNA profiles from authentic and independent vines for them. We are continuing to work with colleagues in other countries to obtain this data.

Latent Virus Progress

by Dr. Deborah Golino, FPMS Director

Determining the cause of failure of young vines in newly planted vineyards can be a difficult task because numerous causal agents can contribute to problems with vineyard establishment. Symptoms, vineyard case histories, and my field trials strongly suggest that in some cases, young vine decline can be caused by grapevine latent viruses. New results from industry funded research strongly support this theory. We report on this work here because it can be so important for nurseries to recognize these types of problems since a large part of the scion wood used by growers for field budding and by nurseries for bench grafts is uncertified and, therefore, more likely to be virus infected.

Over the years, we have selected samples from sites in vineyards in Napa, Sonoma, San Joaquin, Merced, and King counties where replant failure might have been caused by latent viruses. Wood was collected, propagated, and planted in a permanent site on the Davis campus. We have done extensive studies of the viruses present in this collection and also used wood from the virus infected plants to set up experiments to determine the effects of these viruses on rootstocks.
This included common stock which was diseased and also "Heritage" sections of grapes for the FPMS public program. The heritage selections are well respected grape field selections which were not previously available in the public grape certification programs. Many of them came to FPMS because winemakers and grape growers knew that they were virus infected and asked us to create healthy, virus tested stock for their use.

Research in our laboratory in this area has three goals: 1) to determine if virus disease can be

responsible for young vineyard failures and which virus or combination of viruses may be the causal agent(s); 2) to test selected rootstocks for response to latent viruses; and 3) To apply new molecular tests to our characterized latent virus selections in hope of developing reliable, fast lab procedures to screen field selections. We believe that accomplishing these goals will improve the ability of growers to avoid replanting problems as well as increasing our basic knowledge of grapevine viruses in ways which will ultimately produce better disease control strategies.

We can now report on the results of two sets of experiments which have provided us with clear evidence that vine failure can be caused by latent viruses. First, we have documented that different

Two year old Freedom rootings inoculated with latent virus in the foreground and healthy controls in the background.

Freedom rootstock grafted with Cabernet Sauvignon. Healthy vines on the left; vines inoculated with a virus profile including GVB, GLRV-1 and GLRV-2 on the right.

rootstocks have varying sensitivity to some of the virus found in California field selections. Second, we have discovered that many latent virus sites and Heritage selections are infected with a combination of Grapevine Leafroll Virus 2 and Grapevine Virus B. We believe that PCR testing to screen for these viruses might help propagators avoid the most severe virus problems.

If you are interested in seeing these disease trials at UC Davis, you would like more information about this work, or a copy of the poster we presented at the ASEV meetings in June, 1999, please call Deborah Golino 530-754-8102.

PCR for the Future

by Dr. Adib Rowhani, FPMS Plant Pathologist

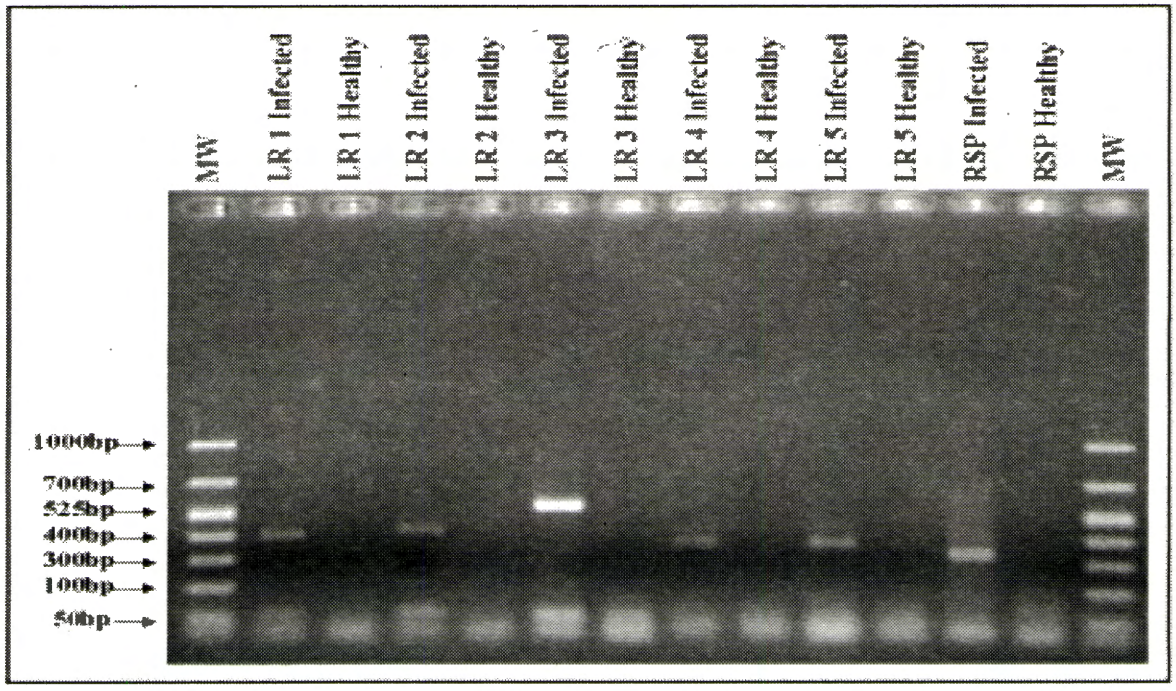
Control of viral diseases in woody crops is best accomplished by establishing new plantings from virus-tested plants. Programs for the certification of nursery stock require fast, sensitive, inexpensive screening methods for detection of these pathogens. Screening for some diseases can be accomplished by inoculating indicator plants in the field or in the greenhouse. These procedures are not ideal however, since they require considerable time for symptom development, are labor-intensive, and require significant amounts of greenhouse or field space. In addition, the reliability of such indexing programs can suffer from technical difficulties commonly encountered when transmitting viruses from woody plants to new hosts. A second approach to screening plants for disease is to develop assays to quickly detect the causal agent or agents. The enzyme linked immunosorbent assay (ELISA) is a rapid, cost effective means for detecting viruses in woody plants. However, ELISA has its own limitations, lacking the sensitivity to reliably detect viruses when they occur at low titers. Additionally, the highly purified virus preparations required to initially produce, and then to restock the antisera needed for this test are difficult, sometimes impossible, to obtain. This is especially true in cases where singly infected plants cannot be obtained.

Reverse-transcription-polymerase chain reaction (RT-PCR) has the potential to be an extremely sensitive alternative to ELISA, providing a means

for potentially detecting viruses in woody plants throughout the year, even during seasons of low titer. The design of nucleic acid primers for RT-PCR is a demanding task, and primer sequences may require revision in the advent of evolved strains of a particular virus. Nonetheless, PCR primers are more easily produced than antiserum for ELISA. As a detection technique, RT-PCR requires extensive manipulation of each sample prior to the RT-PCR reactions. We have developed a simple extraction protocol for preparing samples for RT-PCR. Now it is no more complex or labor intensive to prepare woody plant samples for RT-PCR than it is to prepare them for ELISA.

PCR primers, required for this assay, can be designed to specifically detect a particular virus, or a specific strain of that virus. This can be extremely useful to plant pathologists attempting to trace down the origins of an outbreak of a particular virus. Conversely, more general primers can be designed for general detection of a virus or class of viruses by targeting conserved regions. Applications of this technique should be especially useful to clean stock programs and regulatory agencies worldwide. With the ability to run large numbers of samples, from diverse tissue types and in all seasons, it should be possible to improve the reliability of current disease testing protocols. Ultimately, this could lead to significant improvements in the quality of certified nursery stock, streamlining of importation and quarantine programs, and facilitating international trade in plant materials.

In our laboratory, we have developed RT-PCR methodology for different viruses in grapevine. These viruses include: grapevine leafroll associated viruses 1 to 5, grapevine fanleaf virus, tomato ringspot virus (causal agent of grapevine yellow vein virus), rupestris stem pitting associated virus, grapevine virus A (a virus associated with Kober stem grooving), grapevine virus B (a virus associated with corky bark disease), and grapevine fleck virus. The work is continuing to optimize the procedure by investigating the strain variability for each virus and developing universal primers for their detection.

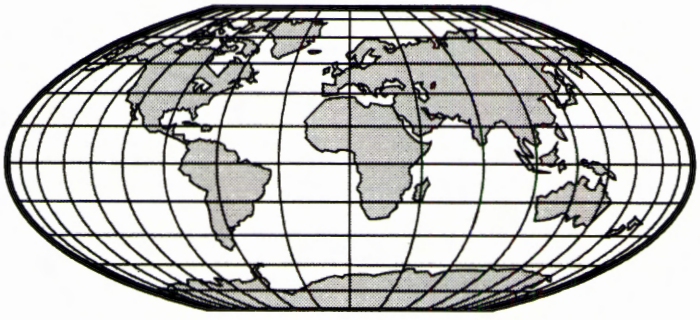


Petiole samples prepared, using PCR techniques, from healthy and infected grapevines are analyzed on an agarose gel. The different banding patterns are characteristic for different leafroll associated viruses as indicated across the top of the gel. The size of the molecules in the bands is shown to the left of the gel.

This year we started using RT-PCR to test part of the FPMS Foundation vineyards for rupestris stem pitting associated virus. We tested 248 vines in NYL Foundation vineyard and 55 vines (22%) tested positive for this virus. We are planning to test the rest of the Foundation vineyards for this virus by PCR in upcoming years. Starting this year, we will test all quarantine and newly introduced vines by RT-PCR for the viruses listed above. This practice will assure the quality of grapevines which will be released as Foundation plants in the future and also validates the reliability of the RT-PCR test used for the detection of these viruses.

NAPPO and the Grape Nursery

by Dr. Deborah Golino, FPMS Director



In March, 1999, the Grape working group of the North American Plant Protection Organization (NAPPO) met at FPMS. Grape industry members on the FPMS-NAPPO mailing list were all invited. This working group has been meeting for several years to develop a grape standard that will provide guidelines for the movement of grape nursery stock within the United States, Canada, and Mexico. These guidelines will also set an important precedent for standards for the movement of grape nursery stock into the United States from the rest of the world. This meeting provided an opportunity for grape nurseries, growers, and vintners to understand the trade issues that are emerging as NAPPO attempts to develop a grape nursery standard.

NAPPO is a regional plant protection organization that is represented by members from the national plant protection organizations of Canada, the United States and Mexico. It is one of many regional plant protection organizations whose primary responsibility is to develop regional plant protection standards which would protect the member states from the entry and establishment of pests, while facilitating trade. The Animal and Plant Health Inspection Service (APHIS), a regulatory branch of the United States Department of Agriculture (USDA), represents the United States in NAPPO.

NAPPO is engaged in the process of creating regional trade standards for North America in a number of important nursery crops. A potato standard has recently been approved. The grape panel has been meeting for several years. In 1999, panels began meeting to develop standards for *Citrus* and fruit trees (*Malus* and *Prunus*). Panels for additional crops are planned for the near future. These standards are intended to meet new international guidelines for free trade. The American Nursery and Landscape Association is coordinating efforts between commodities to help provide industry input for U.S. participation.

As the NAPPO panels have worked to develop a standard for these crops, a common problem has arisen for U.S. panel members attempting to follow new global standards while protecting U.S. growers. U.S. clean stock programs depend heavily on the "umbrella" of our current U.S. quarantine regulations which are very strict. In addition, we have excellent voluntary certification programs which are run on a statewide basis. But we do not have national nursery certification programs for any horticultural crops.

For grapevines, this creates a serious problem. Most participants in NAPPO are doubtful that our existing voluntary programs will constitute sufficient control to allow the existing state programs to set a standard for foreign nursery material entering the U.S. and provide U.S. growers with the level of protection they now enjoy against disease.

Discussions are just beginning about possible solutions to this dilemma. As work continues on NAPPO Standards for these crops, removing non-quarantine damaging diseases from the regional lists, and ultimately national quarantine lists, the grape industry faces possible importation of damaging pests and diseases resulting in a degradation of quality and a loss of farm productivity. Many growers, regulators, and researchers find this prospect unacceptable.

A national program of regulation, either mandatory certification programs or official control programs for target diseases for each commodity, could allow classification of these economically important diseases as regulated non-quarantine pests, according to international standards. State or domestic regional regulations might also serve this purpose. By establishing domestic regulations, only imported nursery stock meeting high standards of freedom from specific domestic diseases could enter the country. However, the idea of a national mandatory certification program has no existing model in the U.S. Many nurserymen and growers find the idea intrusive and contrary to American ideals of free choice, trade and competition. Further, any program would require funding to enforce; this could come from industry, state or federal funds but is likely to be far more expensive than our current exclusionary system.

In the meantime, the current system has served us well. National standards under the voluntary system for grape nursery stock are very high; U.S. grape nursery products have ranked at the top of testing done by independent regulatory agencies. Although grape nursery stock does not enter the country directly from foreign countries, many foreign nurseries have invested in the U.S. and brought new plant materials, techniques and ideas to our industry. The current system is inexpensive; because very little stock enters the U.S., a large regulatory infrastructure to supervise imports is not needed.

It is unlikely that the international pressures on the U.S. nursery industry to clarify and harmonize standards will subside. Although it might be a number of years before a change in our current practices are forced by either a World Trade Organization (WTO) challenge or changes in U.S. regulations as a result of international agreements, it would be wise for the grape industry to begin discussions of the issues, solutions and implementation before that time comes.

The FPMS-NAPPO industry sub-committee held a follow up meeting in Reno, Nevada, during the 1999 ASEV meetings to discuss impressions of the NAPPO process. At that meeting, a motion was unanimously passed by the industry members present. It proposed that a meeting be held in the next year between USDA-APHIS and U.S. grape industry representatives to dicuss the preservation of the health of the national grape industry.

A comprehensive Web site about NAPPO is found at www.nappo.org. For additional information or to be included on the FPMS-NAPPO mailing list, please call FPMS.