

FOUNDATION PLANT MATERIALS SERVICE



The **R**ose Clean Stock Program

*A guide to FPMS virus testing and elimination methods
for establishing foundation rose propagating stock*

About FPMS~

Foundation Plant Materials Service (FPMS) is a self-supporting department in the College of Agricultural and Environmental Sciences at the University of California, Davis. The crop programs for grapes, fruit and nut trees, sweet potatoes, strawberries and roses share a common mission: to collect important varieties, test them for viruses, and make the virus tested clean stock available to nurseries and growers. This is the “foundation stock” that becomes the basis for many commercial vineyards, orchards, fields and retail plants. All programs are funded by industries they serve.

FPMS is accredited by the California Department of Food and Agriculture’s registration and certification programs for grapevines, strawberries, and fruit and nut trees. It is home to the only dedicated U.S. grape importation facility, effectively testing and processing foreign grape selections through quarantine.

Foundation-level stock of all University of California-patented strawberry, grape, fruit tree and nut tree cultivars is grown and distributed by FPMS to licensees. FPMS maintains mother blocks of disease-tested sweet potatoes and offers virus testing and elimination services for commercial sweet potato growers. FPMS also produces and distributes UCB-1 pistachio rootstock seed.

The rose program has expanded from a vision by the founder, Dr. George Nyland, to an established program for creating and maintaining a healthy, virus-tested collection available to rose production nurseries and, ultimately, the individual rose enthusiast. Recently developed roses, as well as many long established cultivars, are found in the extensive collection.

Every year, visitors from around the world come to FPMS to view the state-of-the-art facilities and inquire about the clean stock programs. This publication describes just some of the processes and procedures involved in establishing and maintaining healthy rose planting stock.

Assistance from rose growers, breeders and nurseries has been invaluable to FPMS. Their experience, guidance and financial support have made the current collection a reality.



The Rose Clean Stock Program is a publication of Foundation Plant Materials Service

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History of the FPMS Rose Collection

The FPMS rose collection was established during the 1960s by Dr. George Nyland, a UC Davis plant pathologist. Nyland was convinced that rose mosaic disease was a problem in roses that needed to be addressed by a program of virus testing and heat therapy for virus elimination. When he found that an important cultivar was infected with rose mosaic, Nyland employed recently developed virus elimination techniques; propagating from plants that were grown at 100°F until he was able to find a healthy version of the cultivar.



Dr. George Nyland

Jeff Hall

This work led to the establishment of a virus-tested collection at UC Davis. Budwood from these plants was made available to nurseries and growers to serve as a source of propagating stock. Throughout his career, and even after his retirement in 1985, George Nyland was a tireless advocate of virus-tested planting stock for rose nursery propagation.

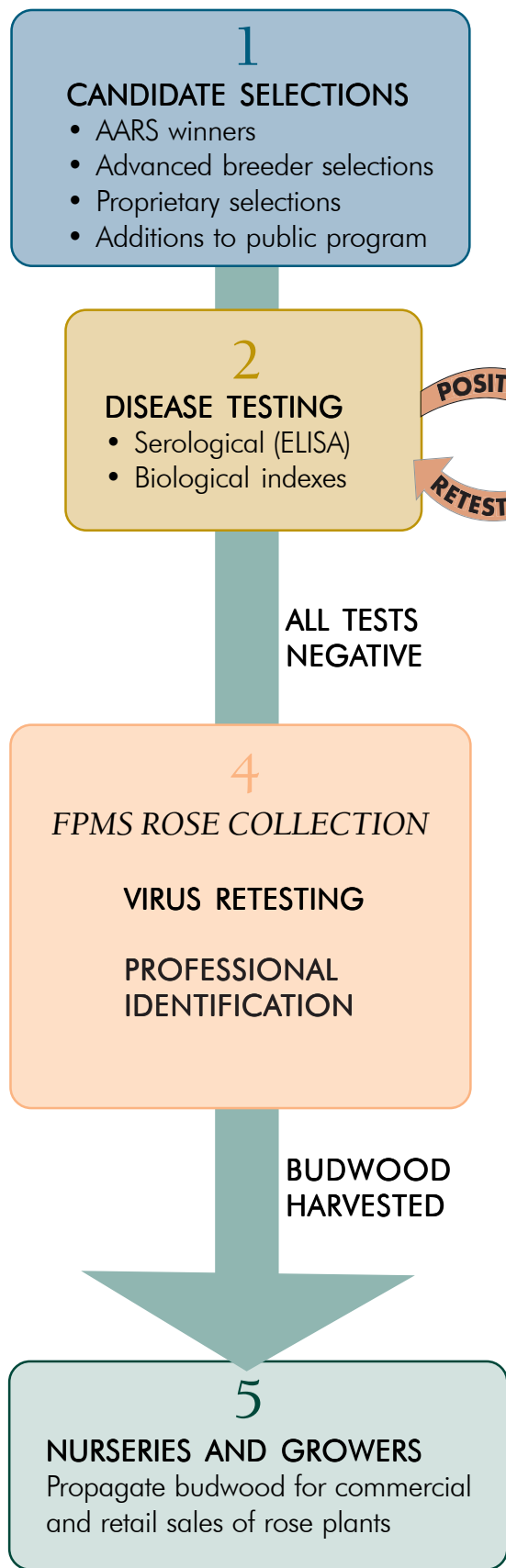
By the early 1990s, the FPMS rose collection was in need of repropagation and retesting. At that time, Mike Cunningham, FPMS rose program manager, was concerned that the plants were aging and less productive, that many of the newer cultivars were not in the collection, and that the plants had not been retested for virus in many years. Cunningham began working closely with the garden rose nursery industry to find funding for this work. His efforts led to planting a new foundation rose collection in 1995, which has become an important resource for nurseries and growers throughout the United States as a reliable source of clean budwood of many major rose cultivars.

The current collection covers eight acres and includes more than 400 rose scion and seven understock cultivars. Each year new cultivars are added to the collection after virus testing and, if necessary, virus elimination treatment. By the rules of the All America Rose Selection (AARS) committee, all new AARS winners enter the FPMS program so that their virus-tested stock becomes readily available.

The FPMS rose collection covers eight acres—the largest public collection of virus-tested roses in the United States.



Overview of the FPMS Rose Program From New



1 New introductions, called “candidate selections,” are typically sent to FPMS as dormant, bareroot plants. Candidate selections may originate as AARS winners, advanced selections of a rose hybridizer, or the proprietary selection of a commercial rose nursery. There is also a growing interest in bringing historic or “heritage” roses into the program to ensure that they are available free of rose mosaic. After potting and labeling, plants are placed outdoors at the FPMS nursery to grow under ambient conditions.

2 Disease testing for viral pathogens is the first step for inclusion of a candidate selection into the collection. In March and April, samples of young leaf tissue from the candidate plants are tested by the Enzyme-Linked Immunosorbent Assay (ELISA), a serological test conducted in the laboratory. Then, after the first flowering, mature budwood from each candidate rose is graft-inoculated onto both Shirofugen cherry and Burr multiflora rose for biological indexing. Combining the results of these tests gives a greater accuracy for virus detection than relying on any single test. Two to three years are required to complete all tests. (Details in *Disease Testing*.)

3 When initial testing demonstrates that a rose cultivar is infected with a viral pathogen, heat therapy or tissue culture procedures are begun for virus elimination. All tissue culture selections and plants that were propagated from heat-treated buds must undergo complete ELISA, Shirofugen cherry and multiflora indexing to determine whether the virus elimination treatment was successful. (Details in *Virus Elimination Therapies*).

Once the ELISA, Shirofugen cherry, and the first year of multiflora tests are negative, rooted cuttings of the candidate selection are propagated in FPMS greenhouses in preparation for establishment in the field collection. During the second spring after the inoculation, the multiflora indicator plants are reinspected.

Selection to Commercial Distribution

4 Only when the candidate selection is found negative for virus by each testing method are the rooted cuttings finally planted in the FPMS rose collection. Whenever possible, new introductions are planted as cuttings on their own roots to reduce the possibility of introducing virus through an infected understock and to prevent the problems associated with rootstock suckering.

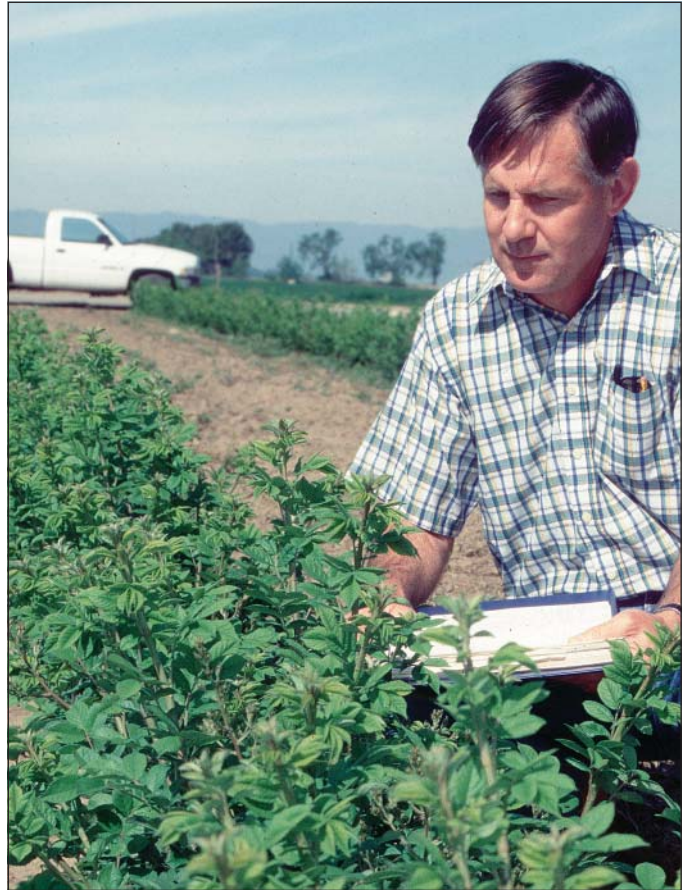
The FPMS rose collection is regularly retested by ELISA for reoccurrence of virus. The plants are also visually inspected in the spring for virus symptoms.

As the selections mature, the rose collection is carefully examined for correct identification and trueness to type. FPMS holds regular meetings with professional rose propagators and breeders who inspect the collection. Any selection that is not up to standard is rogued out. If questions arise about the identity or productivity of a selection, it is flagged in the computer database, restricting distribution until a conclusive determination is made by industry representatives. The quality of the rose program is enhanced as the rose production industry reports back on its experience with the plant materials originating from the FPMS rose program.

5 Dormant budwood is cut and shipped from the FPMS rose collection in early November. Understock canes are distributed in 9-, 18-, or 27-inch lengths, whereas scion varieties are sold by the bud and are distributed as budsticks of varying lengths. After labeling and bundling, the dormant budwood is kept in cold storage until shipped or picked up. Leafy cuttings of scion and understock varieties can be harvested upon request during the summer months for green propagation by customers. (Details in *Ordering Rose Materials and Services*.)



Dormant rose understock is cut and bundled for the November distribution.



Mike Cunningham, FPMS rose program manager, inspects the multiflora index for virus symptoms. Readings take place in early spring, when symptoms such as vein clearing and mottled leaf coloring are most obvious.



Rose industry representatives inspect the entire FPMS rose collection for trueness to type as an assurance that each rose cultivar is represented accurately.

ROSE MOSAIC DISEASE



Question & Answer

Q *What causes rose mosaic disease?*

A Rose mosaic disease is caused by viruses. The most common viruses causing rose mosaic disease are prunus necrotic ringspot virus and apple mosaic virus. Arabis mosaic virus is sometimes also implicated in rose mosaic disease. These viruses may be present alone or in various combinations—accounting in part for the array of symptoms observed on infected plants.

Q *What are the symptoms? Are they distinctive enough to diagnose the disease visually?*

A Rose mosaic disease is named for the leaf symptoms displayed by the rose plant when infected. Ringspots, line patterns, mosaics, and distortion or puckering are typical. Leaf symptoms will vary depending on which virus(es) are present, the rose cultivar, time of year, and growing conditions. Color break on flowers also can be symptomatic of rose mosaic disease. These symptoms can have other causes such as spray damage or naturally occurring genetic variation. Visual symptoms can also be transient; i.e. hot and bright days can cause the symptoms to appear milder or disappear. The virus is still present in this event, and the plant becomes a symptomless carrier. Laboratory tests and biological indexing may be needed for an accurate diagnosis.

Q *How serious is rose mosaic disease?*

A For homeowners, the problem is largely unsightly foliage, decreased plant vigor, and smaller and/or fewer flowers. For cut flower producers, there may be a significant decrease in production and/or quality of the blooms, depending on the rose variety and type of virus(es). Nursery plant producers may face rejection of interstate shipments and the eventual destruction of large numbers of plants as unsalable.

Q *Is there a cure once a plant is infected?*

A For the home rose grower, there is no effective method of eliminating the viruses that cause rose mosaic disease. Foundation Plant Materials Service currently employs two virus elimination techniques, heat therapy and meristem tissue culture, to reestablish a rose cultivar without the virus pathogens. These are slow, time-consuming processes. Use of virus indexed stock (plants that have tested negative for these viruses by laboratory and field methods) is the recommended preventative practice.

Q *How is rose mosaic disease spread?*

A The viruses that cause rose mosaic disease are most commonly spread through propagation procedures such as budding an infected scion onto a healthy understock, or a healthy scion to an infected understock. Disease symptoms are not always obvious, which is why the use of virus-tested planting stock is advantageous. There is some evidence that rose mosaic spreads in commercial rose plantings. UC researchers are presently looking for possible explanations.

Q *How is “virus tested” different from “virus free?” What is “clean stock?”*

A Many rose catalogs and books refer to “virus free” roses. The science of plant virology has shown in recent years that most horticultural plants have cryptic viruses in them, the function and importance of which are not known. As more sophisticated virus testing techniques have been developed, many “virus-free” programs discovered that their stock was not as free of virus as thought. FPMS uses the term “virus tested” or “specific virus tested,” meaning tested for the specific viruses that are known to cause rose mosaic disease. Worldwide, plant material that has been tested for and found free of the viruses known to cause disease symptoms is referred to as “clean stock.”

Disease Testing

Currently, FPMS utilizes biological indexes, serological tests and DNA tests to determine the presence of virus pathogens in rose cultivars. Used extensively to check for virus in plants prior to inclusion in the FPMS rose collection, they are also offered on a fee-for-service basis to private customers.

Biological Indexes

Biological indexes have been used for decades to detect rose viruses. Various sensitive “indicator” plants are inoculated with buds of candidate plants, and the indicators are then monitored for disease symptoms. The development of symptoms on the indicator means that the candidate was virus infected. Indicator varieties are chosen for their ability to display relatively rapid, distinct disease symptoms when infected. Biological tests for rose viruses are very reliable, but they may require up to three years before results are obtained. Two biological tests used at FPMS for rose viruses are the Shirofugen cherry index and the *Rosa multiflora* (Burr) field index.

Shirofugen Cherry Index

Field indexing on Shirofugen cherry trees can be performed from the time the cherry trees are in full leaf until six weeks prior to leaf fall. The use of trees in good vigor is important. Budwood is taken from three sides of each candidate rose plant being indexed, as the virus can be unevenly distributed and accurate detection depends upon a thorough sampling.

Buds from the candidate rose are inoculated, using T-buds, to a branch of the Shirofugen cherry. The candidate buds are checked 10 days later, and the indexing procedure is considered valid if at least two of the three buds are still green.

Thirty days after budding, the entire Shirofugen branch is removed from the tree at least six inches below the rose buds in order to eliminate any possibility of systemically infecting the Shirofugen tree. The budding rubbers are cut and the bark on either side of the rose buds is removed to expose the cambial tissue of the cherry branch. A distinctive gumming and necrosis around the inoculated area indicates that virus is present in the candidate rose. Healthy Shirofugen cherry tissue at the budding site indicates a negative test and the absence of rose mosaic.



Leaf symptoms of rose mosaic disease, left and opposite page, may consist of ringspots, line patterns and mosaic yellow mottling. Vigor and flower production are often decreased.



Shirofugen cherry trees are inoculated with buds from candidate rose plants and labeled. The budded cherry branches are removed 30 to 35 days later and the bark cut away for symptom observation.



Above: (Negative response) The candidate rose buds are visible, surrounded by healthy Shirofugen cherry tissue.

Below: (Positive reaction) Darkening and oozing are responses of Shirofugen cherry to the presence of virus.



Multiflora Field Index

At FPMS, *Rosa multiflora* (Burr) understock is used as an indicator variety for the detection of rose mosaic, rose spring dwarf and rose yellow mosaic. The field procedure requires at least two growing seasons. This indexing procedure can also be performed in a single season in the greenhouse under tightly controlled conditions for light and temperature. Symptom observations require some degree of interpretation, as

abnormalities in plant growth may be caused by environmental factors as well as viral pathogens.

To conduct this field test, buds from a candidate variety of unknown disease status are T-budded into the growing branches or trunk of multiflora canes that were stuck in the field and rooted the previous fall. Two multiflora indicator plants are used to test each candidate rose bush. Each of the two indicator plants receives three buds from the candidate, for a total of six candidate buds. Approximately two weeks after budding, the candidate buds are inspected to determine if they are alive—virus transmission is reduced if buds die, and the inoculation may need to be repeated. The



A bud from a candidate rose plant is cut (left), and T-budded onto the multiflora indicator (right).

indicator plants are visually inspected for mosaic type symptoms several times during the early spring following inoculation. Symptoms may include vein clearing, ringspots, line patterns, mosaics, and leaf distortion and puckering. Symptoms are most clearly expressed in March and April, fading dramatically as seasonal daytime temperatures increase.



Left: Vein-clearing caused by virus is displayed on a multiflora rose. Virus symptoms may fade or disappear in the summer, though the virus remains. Below: Multiflora index: plants in foreground are stunted, a symptom of virus.



Laboratory Tests for Disease

ELISA

Enzyme-Linked Immunosorbent Assay (ELISA) is a sensitive and rapid laboratory serological method for detecting viruses. To develop an ELISA assay for a particular virus, a specific antibody to the virus is required.

Tissue is taken from the plant to be tested (usually the leaf or cambial scrapings) and ground in a buffer solution. This sample from the test plant is pipetted into the wells of a plastic microtiter plate which has previously been incubated with the specific virus antibody. If the sample contains virus, it will be captured by the antibody. After rinsing, another virus-specific antibody conjugated to an enzyme is bound to the captured virus. Finally, a clear substrate buffer, specific to the enzyme, is added.

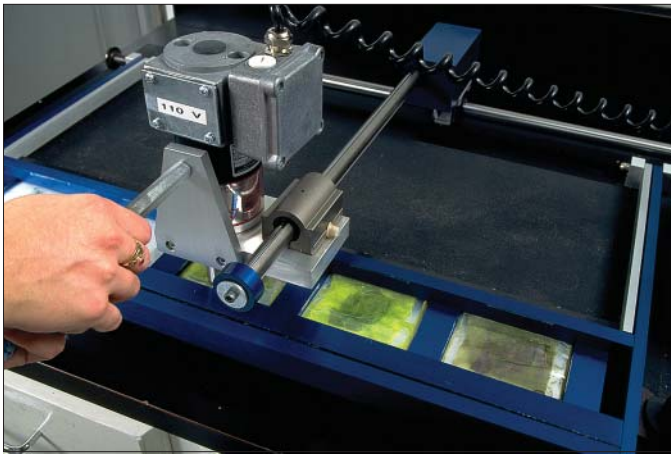
Color change in the final substrate buffer solution is the result of binding of the viral antigens in the plant material to the antibodies, and indicates a positive response. Completion of the test requires only two days; however, plant samples must be collected at specific seasons to obtain accurate results.

ELISA technology is sensitive, but its scope is limited to detecting viruses for which antibodies are available. FPMS laboratory staff routinely uses ELISA to test roses for prunus necrotic ringspot virus (PNRSV), apple mosaic virus (ApMV), arabis mosaic virus (ArMV) and prune dwarf virus (PDV).

PCR

DNA tests rely on knowledge of the genome of a pathogen such as a plant virus. Most promising is Polymerase Chain Reaction (PCR), which involves the selective amplification, using enzymes, of a small part of the virus' genome or unique genetic code. If the virus is present in a plant sample, even in very low amounts, the amplification steps in PCR allow for its detection. It is this amplification that makes PCR such a sensitive test.

PCR only detects specific viruses, but it is much more sensitive than ELISA in most cases. FPMS laboratory staff are exploring the use of PCR for testing roses for PNRSV, ApMV, ArMV and PDV; however, the process is more expensive than ELISA and currently only used in special circumstances.



Jack Kelly Clark

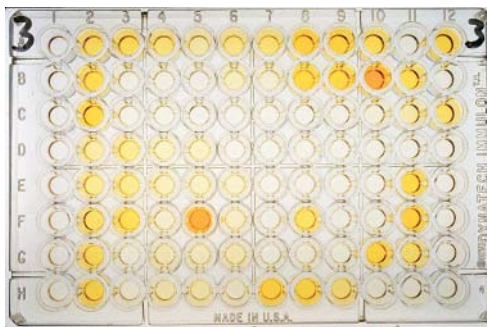
Above: Plant samples are ground in buffer solution for ELISA and PCR tests.

Below: An ELISA plate loaded by pipette with virus antibody.



Jack Kelly Clark

Color changes in the ELISA plate indicate a positive result.



Jack Kelly Clark

Right: A pipette is used to load PCR product from plant samples onto an agarose gel. An electric current is used to move the PCR products across the gel where they separate according to size. This creates banding patterns in the gel which can be used to identify viral pathogens.

FPMS Virus Tests for Roses

Biological Indexes

Shirofugen cherry and Rosa multiflora (Burr)

for detection of:

- Rose mosaic disease
- Rose spring dwarf disease
- Rose yellow mosaic disease

Serological (ELISA)

for detection of:

- Prunus necrotic ringspot virus (PNRSV)
- Apple mosaic virus (ApMV)
- Arabis mosaic virus (ArMV)
- Prune dwarf virus (PDV)
- Tomato ringspot virus (ToRSV)*
- Strawberry latent ringspot virus (SLRSV)*

Nucleic Acid (PCR)

for detection of:

- Prunus necrotic ringspot virus (PNRSV)*
- Apple mosaic virus (ApMV)*
- Arabis mosaic virus (ArMV)*
- Prune dwarf virus (PDV)*

*denotes experimental use only, not regularly performed on the FPMS rose collection.



Virus Elimination Therapies

An ounce of prevention is invaluable compared to a cure when the subject is rose viruses. There is no “cure” for an individual plant once it is infected; however, it is possible to “clean up” a variety, i.e., eliminate the virus from the propagating stock. Two possible techniques for eliminating virus from an infected variety are heat therapy and tissue culture.

Heat therapy

Heat therapy has been used for many decades at FPMS to treat virus-infected rose cultivars and create new foundation stock for the collection. In the heat therapy process, potted bushes of rose cultivars infected with virus are subjected to constant 100°F temperatures in a heat chamber. The condition of each plant in the heat chamber is checked on a weekly basis. Budsticks are removed from the plants as they begin to show the effects of the heat. Many propagations are made, each with an increasing number of days of heat therapy.



Jack Kelly Clark

Temperature, light and humidity are controlled in eight plant growth chambers at FPMS.

Maintenance of the rose plant for four weeks or longer at 100°F is optimal for elimination of rose mosaic virus. Rose selections that cannot tolerate such a high temperature may begin to deteriorate quickly and budwood is removed sooner—sometimes as early as two weeks. Healthy propagation wood may be obtained even with the shorter period of heat therapy, since



Rose plants undergoing heat therapy at 100°F to kill or inactivate the viral pathogens.

viral pathogens are not uniformly distributed within the plant, and buds might be cut that, by chance, have not been infected.

The heat-treated plant material is budded onto *Rosa multiflora* (Burr) understock, which is used simultaneously as understock and as an indicator for disease. The multiflora rootstock will support the bud and, if virus is present in the bud, will develop symptoms of rose mosaic disease.

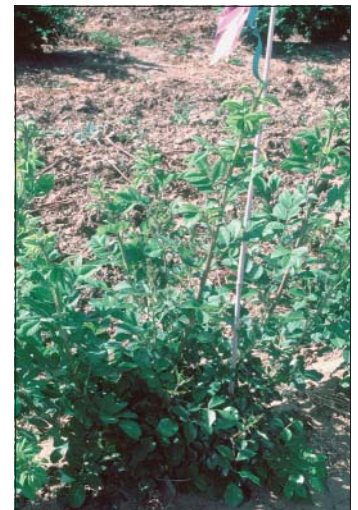
Symptoms are initially observed the spring following a summer’s heat treatment work. This allows time for the bud to heal and virus (if present) to move into the multiflora. Indexing for the viruses in rose plants propagated from heat-treated buds is repeated several times, as one test may not always detect the presence of virus.

After the final observations, multiflora plants that have no observed virus symptoms are pruned back to the heat-treated buds. This

encourages growth of the heat-treated cultivar, which is itself monitored for virus symptoms. Multiflora suckers are not removed entirely, and are observed for symptoms again in the spring.

When the buds of the heat-treated cultivar have grown out and matured sufficiently, budwood is taken and indexed on Shirofugen cherry trees. In addition, leaf tissue is taken from the new growth for ELISA testing for apple mosaic virus, arabis mosaic virus, and prunus necrotic ringspot virus.

The heat therapy is considered successful only when the ELISA test, and Shirofugen cherry and multiflora indexes are all negative for virus. The heat-treated scion cultivar is then propagated by rooting leafy cuttings or by budding onto a healthy understock.



Since this scion from a heat-treated bud and the multiflora understock appear healthy, the plant is flagged in the field. Material from the scion will be further tested for virus by ELISA and Shirofugen cherry tests.

Tissue culture

Tissue culture therapy is an alternative virus elimination technique successfully used with many horticultural crops. At FPMS, heat treatment for virus elimination has been replaced or supplemented by shoot-tip tissue culture therapy for grapevines, strawberries and sweet potatoes. Virus elimination is normally achieved in 90–100 percent of plants that are cultured using our procedures. FPMS researchers are now working on an experimental basis to optimize rose shoot-tip culture.

A shoot tip, essentially a meristematic dome surrounded by a few leaf primordia, is cut from the virus-infected selection. The meristematic tissue has a unique potential to regenerate a new plant with a minimum chance of mutation, or genetic change, in comparison to plants derived from other tissues. A shoot tip length in the range of 0.2–1.0 mm is normally small enough to eliminate virus in other crops. It is not known exactly *why* virus is eliminated, but from a practical point of view, the smaller the explant (the part of a plant used to start an *in vitro* culture) which can be cultured, the greater the chance of eliminating virus. Unfortunately, the smaller the explant, the more difficult it is to regenerate a plant.

The shoot tip is excised under sterile conditions and placed on a special gelatinous mixture – a growth medium – inside a sterile test tube. These cultures are then maintained in a growth chamber which provides



Sam Woo

controlled growing conditions during the critical establishment phase. Depending on the needs of the plant species, several transfers may be made to specialized media for shoot growth, rooting, etc. Once well developed, plants are transferred into soil, acclimated to normal light and air, moved into greenhouses and screen houses and, finally, to outdoor conditions.

FPMS has successfully produced a few rose plants using shoot-tip culture from virus-infected selections. Although there are some difficulties to overcome, it is hoped that this technique becomes routine for roses as FPMS conducts further research and gains experience. If successful, this would greatly increase the availability of virus-free roses throughout the United States.



Jack Kelly Clark

Two sterile culture growth rooms provide the conditions necessary to regenerate plants from microshoot tips. Temperature, humidity and light (type and intensity) are controlled to provide optimum conditions for growth.



Sam Woo

Stages of rose shoot tips growing on sterile culture in test tubes. Shown from left to right are: the initial explant, callus and the formation of leaves, and a plant with leaves and stem ready for rooting.



This rose plant was propagated at FPMS using shoot-tip culture techniques for virus elimination.

Ordering Rose Materials and Services

Rose scion budwood and understock canes from the FPMS disease-tested collection may be purchased for propagation purposes. Because the FPMS program is designed to put virus-tested stock into the hands of professional propagators, sales are made primarily to the rose nursery industry in order to establish healthy sources of propagation materials at the production level. Rose enthusiasts benefit indirectly from the FPMS program when nurseries use disease-tested stock to produce plants of improved disease status for retail sale.

Propagating material is available in the form of unrooted dormant and green cuttings. FPMS does not sell rooted plants. Orders must be received by October 15 of each year to be included in the November allocation. Material can also be requested from late spring through early fall on a first-come, first-serve basis. To receive a list of available rose varieties, prices and an order form, please contact the FPMS office by phone at (530) 752-3590, by e-mail at fpms@ucdavis.edu or on the Web at <http://fpms.ucdavis.edu>.

Custom rose virus testing and/or virus elimination services employing the protocols described in this brochure are available from FPMS on a fee-for-service basis by contract with the University of California. A service request form, including prices and instructions for submitting material, can be obtained by contacting FPMS.

➔ Acknowledgements ➔

The Garden Rose Council (GRC) is a non-profit organization whose members account for more than 95 percent of U.S. garden rose production. The GRC mission is to support agricultural research and issues that will further the production of disease resistant roses in an environmentally sensitive manner. GRC support and funding has enabled FPMS to plant the new rose collection and improve the level of virus testing, and it supports research at FPMS on rose mosaic virus transmission and development of shoot tip culture methods for rose virus elimination.

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