Foundation Plant Materials Service: Production and Distribution of Virus-tested Propagation Materials

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INTRODUCTION

Foundation Plant Materials Service (FPMS), a unit of the University of California at Davis, was created to provide virus-tested plant materials for research and commercial use. Field- and greenhouse-grown plants of new varieties developed at the University, as well as plants from several unique collections from other sources, are maintained by FPMS on the Davis campus. Propagation material in the form of seed, buds, cuttings, rooted plants, and tissue-culture plantlets are supplied to the public. This service is in keeping with the University's policy to release healthy plant material whenever possible. Crops included in the FPMS programs include grapevines, deciduous fruit, and nut trees, roses, strawberries, and sweet potatoes.

Most of the material maintained and distributed by FPMS is propagated vegetatively. Although vegetative propagation has the advantage of creating progeny plants that are genetically identical to the mother, it also has the potential to transmit diseases caused by viruses or other plant pathogens. It is important to avoid propagation of infected material. Virus infection can have various adverse affects on plant growth that can be manifested as reduced vigor, reduced uniformity, shortened productive life, reduced cold hardiness, a lower rate of survival when transplanting, reduced crop yields, and inferior crop quality.

In addition to the distribution of virus-indexed plant materials, FPMS provides custom disease detection and elimination services on a fee-for-service basis and operates a National Grapevine Importation Program for growers who wish to import foreign grape materials into the United States. To meet these missions FPMS must follow the regulations and policies of the California Department of Food and Agriculture, the U.S. Department of Agriculture Animal and Plant Health Inspection Service, and the University of California.

TECHNIQUES FOR VIRUS DETECTION

Disease testing procedures, commonly referred to as indexing, are proscribed by California state regulation and are used to determine the presence or absence of virus diseases before release of plant material. Although foundation materials supplied by FPMS are apparently free of potentially harmful viruses, it is not possible to guarantee that materials are healthy due to limitations of testing methods available.

Detection of plant viruses can currently be accomplished using four distinct methodologies: serological screening, bioassay using *Prunus* 'Shiro-fugen' (syn. *P. serrulata* 'Shirofugen') flowering cherry, graft transmission, and mechanical transmission. For the purposes of this text the plant material that undergoes indexing procedures will be referred to as the "candidate selection" or the "candidate", a description defining the selection's potential for inclusion in a certification program.

Serological testing, using Enzyme-Linked Immunosorbent Assay or ELISA, is a laboratory test that can be performed in as little as one day. To detect a specific virus in a plant, the wells of an ELISA plate are coated with the antibody specific to the virus. The antibody has been produced by the immune system of a warm-blooded animal previously injected with the virus particles purified from infected plant tissue. Sap derived from macerated tissue from the candidate is then added to the coated well and incubated. During the incubation time any virus particles present in the candidate sample will bind to its specific antibody. After washing the plate, an enzyme-conjugated antibody (the same antibody initially used for coating the plate, but conjugated to an enzyme) is added to each well of the plate and incubated. After another wash, a substrate specific to the enzyme is added to the plate and any resultant yellow color development in the wells indicates the presence of virus in the candidate sample. This technology is extremely sensitive and can be used to identify specific viruses in a variety of plant types. However, its scope is somewhat limited as not all viruses present in plants have been successfully purified and characterized.

A second method of indexing involves the flowering cherry *Prunus* 'Shirofugen' which displays a hypersensitive reaction when inoculated with virus-infected buds. Buds from the candidate are inoculated onto the Shirofugen cherry by T-budding to a branch of the cherry tree. Thirty days after budding, the entire branch of the cherry tree is removed from the tree and the bark on either side of the inoculated buds is removed to expose the cambial tissue layer. A distinctive gumming and necrosis around the inoculated site is indicative of the presence of virus. Shirofugen cherry indexing is used primarily for testing of roses and fruit and nut trees in the species *Prunus*.

Virus indexing through graft transmission is a third method of disease detection. For each candidate plant type, a series of indicator varieties of the same genus is selected based on the relatively rapid, distinct disease symptoms they display when infected. Examples of such indicator varieties are as follows.

- *Prunus*: Bing cherry (*Prunus avium* 'Bing'), kwanzan cherry (*P.* 'Kwanzan'), tomentosa cherry (*P. tomentosa*), Tilton apricot (*P. armeniaca* 'Tilton'), Elberta peach (*P. persica* 'Elberta'), Shiro plum (*P.* 'Shiro')
- *Pyrus*: Bartlett pear (*P. communis* 'Williams' Bon Chrétien'), Beurre Bosc pear (*P. communis* 'Beurre Bosc'), Nouveau Poiteau pear (*P. communis* 'Nouveau Poiteau'), Passe Crassane pear (*P. communis* 'Passe Crassane')
- *Rosa*: Burr multiflora, 'Madame Butterfly'
- Strawberry: *Fragaria vesca*, *F. virginiana*
- Grape: *Vitis rupestris* 'Saint George', Cabernet Franc (*V.* 'Cabernet Franc'), LN33, Kober 5BB, Richter 110

Indicator plants are inoculated with buds or leaves of the candidate plants and are observed for disease symptoms over a period of time varying from 6 weeks in strawberry to two entire growing seasons needed to complete the field index for *Prunus*. Symptom expression can consist of the balling, rosetted growth, and veinal chlorosis as occurs in rose spring dwarf, to the extremely serrate leaf margins and widely splayed petiolar sinus found in grape leaves infected with fanleaf virus.

Another method used for virus detection is referred to as mechanical transmission. Herbaceous host indicator plants such as lambsquarter (*Chenopodium*), tobacco (*Nicotiana tabacum*), and cucumber (*Cucumis sativus*) are grown from seed in the greenhouse. For infection to occur, the virus must enter the tissues of the herbaceous indicator through a sublethal wound. This is normally accomplished by the application of a mild abrasive such as carborundum powder, which damages the cuticle and epidermis of the herbaceous indicator as sap extracted from the candidate plant is rubbed on the leaf surface. The virus enters cells of the herbaceous indicator through these wounds. The plants continue to grow in the greenhouse and in 4 to 6 weeks, under proper conditions, symptoms will develop on the hosts inoculated with a positive sample.

VIRUS ELIMINATION

Upon completion of one or more of the indexing tests, results are compiled and a determination of the virus status is made. For those plants infected with virus, disease elimination work becomes an option. The feasibility of virus elimination will depend upon the particular virus present, the importance of the variety to the individual or industry, the possibility of locating another potentially healthy selection of the variety, and availability of funds to perform the work, often running into the thousands of dollars.

Virus disease elimination can be accomplished in two ways. Inactivating or killing the virus particles through thermotherapy can be used for elimination of many viruses that are sensitive to high temperatures. Using this method entire plants, or simply buds of the diseased material budded to a healthy rootstock, are placed in a heat chamber and are held at 100F for a minimum of 60 days or until the plant begins to decline beyond the point from which viable buds can be removed. New plants are propagated from the heat-treated material either through further budding to a rootstock or by rooting the green growing heat-treated material under mist in the greenhouse.

A second method used for virus elimination is tissue culture. In this procedure the apical meristem and the first few leaves below it are excised from the shoot tip and placed in conditions such that roots form at the base to produce a small plantlet. This technique is used to produce a pathogen-free stem cutting, since the tip is usually free of bacteria, fungi, and viruses.

MAINTENANCE OF VIRUS-TESTED PLANTING STOCK

The value of virus-tested stock is widely recognized by the establishment of certification programs in many states and countries. In California, various certification programs are administered by the Department of Food and Agriculture (CDFA). CDFA has designated FPMS as a source of propagation materials for its grapevine, deciduous fruit and nut tree, and strawberry certification programs.

Plant material produced and tested by FPMS is called foundation stock. Prior to its inclusion in the collections at FPMS, prospective foundation level plant material is tested using one or more of the indexing procedures described above. In addition, each plant is checked by experts for trueness-to-type and to variety, confirming that it is correctly identified and labeled. All of the plants are visually inspected by state, county, and university personnel each spring and fall and are subject to annual ELISA testing to monitor disease status.

Foundation-level plant material is used to produce planting stock at the registered level by private nurseries that are participating in the certification program. A third level, certified nursery stock, is produced from the registered increase blocks. It is certified stock that is sold to growers for production purposes. Each level of plant material is identified with a different color tag issued by CDFA—white for foundation stock, purple for registered stock, and blue for certified stock. This source and disease testing information, maintained by FPMS and CDFA, can be used to assure growers of the quality of the plant material they are purchasing, to track the source history of the plants, and to inform growers of any changes in the disease status of the plants. Complete regulations, describing growing conditions, inspection and testing procedures required to produce foundation, registered and certified stock are available from CDFA.

Propagation materials are sold by FPMS to the public in accordance with an allocation policy that ensures that materials are distributed as widely as possible to industry, researchers, repositories, and others. Amounts suitable for establishing increase plantings are distributed as first priority to participants in the California Registration and Certification programs. Remaining materials are sold first to domestic and then to foreign customers.