Foundation Plant Services

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UCDAVIS

FPS FRUIT & NUT TREE PCR PATHOGEN TESTING SERVICE

for

almonds, apricots, cherries, nectarines, peaches, plums, prunes and walnuts

As a part of its custom disease-testing services, FPS offers pathogen testing using polymerase chain reaction (PCR) technology on a fee-for-service basis for five important pathogens of fruit and nut trees. The full panel consists of tests for all five pathogens listed below. Descriptions of these pathogens and their impacts on fruit and nut trees are included in this information packet. This service is currently available only for plant samples originating in California—no samples from outside California or the U.S. will be accepted.

Cherry leaf roll virus-walnut isolate (CLRV-W)
Phytoplasmas (Phyto)
Prune dwarf virus (PDV)
Prunus necrotic ring spot virus (PNRSV)
Tomato ring spot virus (ToRSV)

Price Schedule and Billing

Minimum setup fee of $200.00 per submission plus $42.00 per test for each sample

An invoice for testing services will be sent to you along with your results when testing is complete. Payment is due upon receipt of invoice.

Agreement For Provision of Plant PCR Testing Services

To initiate PCR testing services, please complete and sign both of the attached copies of the "Agreement For Provision Of Plant PCR Testing Services", being sure to specify in Attachment A which tests you would like performed on which samples. ***Both of the completed and signed originals of the Agreement and Attachment A must be submitted to FPS along with your samples.*** After the Agreement is signed on behalf of the University in the UCD Business Contracts Office, you will receive a fully-executed original in the mail for your records.

Sample Collection and Submission

- FPS will accept woody dormant cuttings for Prune dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV), Tomato ringspot virus (ToRSV), and Cherry leaf roll virus-walnut isolate (CLRV-W) from October to March. Young leaf tissue may be submitted for detection of the above viruses in the spring (March-May) only. ***Because CLRV-W distribution in mature walnut trees is erratic, FPS recommends submitting samples from very young seedlings of English walnut or pollen samples for testing. Please consult with Dr. Adib Rowhani or Lori Leong before submitting samples.*** Testing of samples for phytoplasmas will require submission of mature leaf samples in summer months (July – September) only. Please make arrangements directly with Lori Leong ahead of time if you require submission of leaf samples for testing.

- Bundle six to eight 10-inch dormant cuttings for each sample. For young leaf tissue, collect six to eight 10-inch succulent shoot tips. For phytoplasmas testing, collect six to eight 10-inch cuttings with mature leaves. Cuttings for each sample (tree) should be collected from random locations around a tree.

- Each sample should be labeled with a consecutive number, starting with 1. Please do not include variety names or field locations when labeling samples; keep such records for your own information only.

- Each sample must be placed in its own plastic bag (such as Ziploc©) with a moist paper towel to prevent sample from drying out. Keep samples cool or refrigerated until shipping if possible. Do not place samples directly on ice.

- Samples should be collected and shipped on the same day via overnight carrier to assure accurate PCR results.

Receipt and inspection of samples

All samples will be inspected and properly labeled and stored upon receipt at FPS. Any samples received in poor condition (samples were dried out, moldy or decayed) will not be tested. Customers will be notified of any samples received in unsatisfactory condition, and arrangements can be made to have replacement samples delivered to FPS for testing. PCR testing will be done in the time frame agreed upon in the testing service agreement. All testing will be completed by qualified technicians at FPS, and results will be reviewed by the FPS plant pathologist prior to being reported to the customer. The customer will receive a written report of all results along with an invoice for performed services.

Who To Contact For More Information

If you have questions about this service or would like more information about fruit and nut tree PCR testing at FPS, please direct your inquiries to Dr. Adib Rowhani or Ms. Lori Leong in the FPS lab:

Dr. Adib Rowhani, FPS Plant Pathology Specialist
Phone: (530) 752-5401 Email: akrowhani@ucdavis.edu

Ms. Lori Leong, FPS Disease Testing Coordinator
Phone: (530) 754-8189 Email: leongll@ucdavis.edu

USE OF PCR IN DISEASE DETECTION

The most common method of disease diagnosis for trees is based on visual inspection of indicator trees that have been graft-inoculated with budwood from a suspected diseased tree. While this method is reliable and sensitive enough to detect a variety of pathogens, it is slow and very time consuming. Additionally, graft-inoculation of indicator plants does not identify a single pathogen infection. Recently, new molecular methods have become available for detection of viruses and other pathogens in plants. Unlike visual observation of disease symptoms on indicator plants, these new methods are capable of identifying single viruses, even in trees that appear healthy. One of the most sensitive methods for disease detection currently available is Polymerase Chain Reaction (PCR).

PCR refers to the process of amplification of a small, select part of genetic code of a particular organism. PCR can be used for the detection of viral pathogens in trees because each virus has its own unique genetic code. In the past decade, molecular scientists have been identifying the genetic code of various plant viruses. Once the genetic code of a virus is known, PCR detection of that virus in plant tissue is possible. Currently, FPS has developed PCR testing for six different pathogens of fruit and nut trees. The pathogens, their disease symptoms, and severity of disease are described below.

Although PCR is currently one of the most sensitive tests and will identify a single pathogen infection in a plant, it has its limitations. The tests performed at FPS are only for the pathogens listed below. There may be other unidentified pathogens or strains of known pathogens, for which PCR tests have not been developed. Therefore, we cannot determine whether a plant is healthy. Rather, we determine whether a plant is free of the pathogens we can test for at this time. For this reason, it is possible to observe disease symptoms in a plant that has tested negative by PCR for all the pathogens listed below.

Disclaimer

Although PCR is a very powerful and sensitive detection tool, it has its limitations. The PCR tests performed by FPS are for specific pathogens that have been associated with causing certain symptoms in plants. However, these may not be the sole agents of a particular disease. There may be other agents that produce the same disease symptoms. Customers should understand that it is possible that a plant which tests negative for a certain pathogen by PCR may still show symptoms of the disease caused by that pathogen. Another factor that may affect test results is the distribution of a particular pathogen in the plant. Some pathogens may replicate and move slowly in a plant, and may not be uniformly distributed by the time of sample collection. Therefore, it is possible for a sample to test negative by PCR even though the pathogen is present in the plant. Sample condition also affects PCR test outcome. Sample condition is the sole responsibility of customer.

PATHOGEN DESCRIPTIONS AND ASSOCIATED DISEASE SYMPTOMS OF FRUIT & NUT TREES

Adapted from *Compendium of Apple and Pear Diseases*, *Compendium of Stone Fruit Diseases*, and *Compendium of Nut Crop Diseases in Temperate Zones*; published by APS Press

Cherry leaf roll virus-Walnut isolate (CLRV-W)

The walnut strain of cherry leafroll virus causes blackline disease of English walnuts. Symptoms, observed on English walnut grafted on black walnut or paradox rootstocks, include gradual decline in tree vigor, small holes and cracks at the graft union, and a dead black line or black canker on the wood at the graft union when the bark is removed. CLRV-W titer exhibits very erratic distribution in mature trees; sometimes only one limb or part of a limb may contain virus. The virus, belonging to the family of nepoviruses, is spread by propagation of budwood and by pollen.

Phytoplasmas

Phytoplasmas (formerly known as mycoplasma-like organisms or MLOs) are known causal agents of hundreds of diseases in various plants. They reside primarily in the phloem tissues and are transmitted naturally by phloem-feeding insects such as leafhoppers and psyllas. Phytoplasmas are also graft-transmissible. In fruit and nut trees, they are the causal agents of many yellows diseases described in these crops. Diseases caused by phytoplasmas are named after the symptoms they produce on a particular tree crop. Some of the most common diseases found in various stone fruit and almond varieties are briefly described below.

Phytoplasmas in Almonds

Phytoplasma disease is sporadic in California and has only been confirmed in a few cases. There are two known strains of phytoplasma causing disease in the United States. Almond trees affected by **X-Disease phytoplasma** appear normal in overall growth and fruit production, although the disease can spread to stone fruits where it can be more damaging (see below). **Peach yellow leafroll (PYLR) phytoplasma** is identical to the pear decline (PD) phytoplasma and causes two diseases, depending on the rootstock used: ***Almond brown line and decline (ABLD***) is characterized by quick decline of tree scion, whereas ***Almond kernel shrivel (AKS***), causes a slow decline of the scion in the affected trees.

Phytoplasmas in Cherries

X-Disease is an economically important disease of cherries in the eastern and western fruit-growing regions of the United States. X-Disease causes either rapid or slow decline of affected trees, depending on the type of rootstock used and the climate of a particular growing region. X-Disease causes a hypersensitive reaction in Mahaleb rootstocks, resulting in girdling at the scion union, and a fast decline of infected trees. The disease is more severe in long, hot growing seasons, such as in the California Central Valley. In contrast, Mazzard, Colt, and Stockton Morello rootstocks show slower decline of scionwood with development of small, red-tinged leaves on affected scaffolds. In addition, fruit maturation is delayed and color-development is incomplete in affected trees.

Other diseases of cherries caused by phytoplasmas have been identified, but are rare and of minor importance. These include ***Cherry blossom anomaly***, a disease characterized by green coloration along veins and midribs of white petals, and fruit that is more pointed with a rough skin, and ***Cherry albino disease*** that was first observed in the late 1930's in Oregon and has not been found since.

Phytoplasmas in Peaches

X-Disease is the most prevalent phytoplasma disease found in peaches in California. Symptoms develop in the inoculated scaffold first, and after two years the whole tree will show symptoms. Usually, chlorosis appears on leaves in mid-June and progresses to necrotic lesions that later abscise to shot hole lesions. Fruit development is delayed with reduced yield and smaller fruit that lacks flavor. Chronically-infected trees show dieback and tree death may occur after five years.

Peach yellow leaf roll (PYLR) is caused by genetically distinct phytoplasmas. The disease is more virulent than X-Disease and produces symptoms that contrast with those of X-Disease. Symptoms on affected trees include chlorosis and downward curling of leaves as well as swelling of midribs. Necrotic lesions and shot hole symptoms are absent in PYLR infections. Another distinguishing symptom is premature fruit drop caused by PYLR infections.

Peach yellows (also known as little peach) is currently of very low incidence and is confined to the Southeastern U.S. (from Maryland to South Carolina). The phytoplasmas that cause Peach yellows produce the following symptoms on affected trees: buds break two weeks earlier than normal, the leaves are small and narrow and develop chlorotic and red spots. Terminal buds develop dense tufts of leaves while interior canopy produces slender shoots. Fruit set is premature and fruit tastes bland or bitter. As the disease progresses, affected limbs die back, and within five years the tree will die.

Peach rosette is a phytoplasma disease of minor importance and is confined to the Southeastern U. S. and Texas. Symptoms of peach rosette are similar to those of peach yellows, with leaves becoming chlorotic, but being nearly normal in size and morphology. Shoot internodes are short and leaves are in tight clusters called brooms. In addition, very few fruits develop and those that do drop prematurely. Peach rosette also appears to be more virulent than peach yellows phytoplasma.

Peach red suture is a minor disease caused by phytoplasmas. Its distribution closely follows that of peach yellows. Symptoms are most apparent on fruit and include a rough, bumpy, swollen suture with intense coloring. The suture area ripens early while the opposite side remains green. The trees may survive for several years and will eventually exhibit open centers due to reduced shoot growth.

Prune dwarf virus (PDV)

PDV, an ilarvirus, is the causal agent of several economically important diseases, including yellows of sour cherry, blindwood and narrow leaf of sweet cherry, and dwarf of Italian prune. Mixed infections of PDV and another ilarvirus, Prunus necrotic ringspot virus (PNRSV), cause stunt of peach. PDV is similar to

PNRSV, causing mosaic symptoms in almonds and necrosis and shot holes in stone fruit trees. PDV is found wherever stone fruits are grown. Symptoms include chlorotic rings and mottle on young leaves that become milder as leaves expand; symptoms do not seem to recur after the first year of infection and symptomless infections are common. Like PNRSV, PDV is spread by vegetative propagation, pollen and seed.

Prunus necrotic ringspot virus (PNRSV)

PNRSV affects most *Prunus* species and is the causal agent of almond calico, apricot line pattern, cherry necrotic ringspot, cherry rugose mosaic, prunus ringspot, plum line pattern, and tatter leaf of cherry or peach. PNRSV is often found in mixed infection with Apple mosaic virus (ApMV). Although symptoms are not often apparent, they may include necrotic spots and shot holes on young leaves during the first year of infection. In almonds, the virus causes calico and mosaic symptoms and can also cause infectious bud failure disease. The virus belongs to the ilarvirus family, and is spread by routine plant propagation practices and naturally by pollen or seed.

Tomato ringspot virus (ToRSV)

Various strains of ToRSV are associated with several diseases of the *Prunus* species, including peaches and nectarines, plums, cherries, and almonds. Diseases caused by this virus are given various names that describe the symptoms in the affected species. For example, ToRSV has been associated with prunus (peach) stem pitting, peach yellow bud mosaic, cherry eola rasp leaf, and prune brown line diseases of stone fruit crops. The various diseases caused by ToRSV in stone fruit trees and almonds are described in more detail below. The ToRSV virus is spread by propagation practices, by its nematode vector, from infected tree to other trees, or from some weed hosts in which the virus is seedborne. Severity of symptoms is dependent on the length of time of infection, cultivar of host species, and virus isolate.

ToRSV in Almonds

In almond, ToRSV is the causal agent of ***peach yellow bud mosaic***, a disease with a scattered distribution that is confined to the Sacramento Valley of California. Symptoms include chlorotic ring spots and mosaic. Young buds develop tufts of yellow leaves that later die. In addition, terminal shoots do not grow, fruit set is reduced and fruit has thick, wrinkled hulls. Symptoms are most severe in Mission cultivars, while the Nonpareil and Ne Plus Ultra cultivars do not show symptoms.

ToRSV in Peaches

ToRSV causes ***Prunus stem pitting*** in peaches in orchards in the eastern United States. Symptoms include premature yellowing or reddening of leaves and leaf abscission, delayed unfolding of leaf buds in spring, and most notably abnormally thickened, spongy bark, and pits and grooves in the wood underneath. These symptoms are usually most severe in the trunk area immediately above and below the soil line. Symptom severity varies with cultivar. ToRSV also causes ***Peach yellow bud mosaic (PYBM)*** in orchards of California. The symptoms of PYBM contrast with those described above. Leaf bud growth is severely limited, and young leaves are small and yellow. Affected leaves die as soon as seasonal temperatures rise. In the first year of infection (mosaic phase), leaves have chlorotic spots, rings, and oak-leaf pattern. In the second year (yellow bud phase), shoots have yellow buds that die during the fruiting season. Fruit production is decreased.

ToRSV in Cherries

Prunus stem pitting disease in cherries causes symptoms similar to those described in peaches. Severity of symptoms depends on the scion-rootstock combination. For example, Mahaleb roots develop short and shallow pits while 'Stockton Morello' roots develop deep, extensive grooving and cambial necrosis. 'Bing' also exhibits severe pitting, while the virus remains asymptomatic in ''Royal Ann' cultivars in which pitting is only found on the rootstock portion of the trees. ***Peach yellow bud mosaic*** **(PYBM)** symptoms in cherries closely resemble those in peaches, however pitting is minimal with scattered pockets of shallow pits.

ToRSV in Prunes

ToRSV causes poor growth and sparse foliage in prune trees. 'Stanley' prune grafted onto Myrobalan rootstock exhibits the most severe symptoms, including necrotic tissue (brown line) at the scion-rootstock union. The area below the graft union grows more slowly than the scion, giving the appearance of overgrowth. In California, ToRSV causes ***Prune brown line*** in French and Italian prune grafted onto Myrobalan and peach rootstocks.

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