Potyvirus Plum Pox Virus

Scientific Name
Potyvirus Plum pox virus (PPV)

Common Name(s)
Plum pox virus, Plum pox, sharka

Type of Pest
Plant pathogenic virus

Taxonomic Position
Class: RNA Virus, **Family:** *Potyviridae* **Genus:** *Potyvirus*

Reason for Inclusion in Manual
Program Pest

Pest Description
Plum pox virus (PPV) is a RNA virus with flexuous filamentous particles approximately 750 nm in length x 15 nm in diameter. PPV particles are composed of one molecule of RNA (positive sense, single-stranded RNA) and a protein envelope. The genome is expressed as a 350 kDa polyprotein precursor that is proteolytically processed by viral and host proteases into ten smaller functional proteins (Garcia et al., 1994; Lopez-Moya et al., 2000; Schneider et al., 2011). An additional protein is predicted to be produced by a translational frameshift (Garcia et al., 2014). PPV is the causal agent of plum pox disease. Currently, eight strains are recognized for PPV, which may be more than for any other potyvirus (Garcia et al., 2014). One or more of these strains can infect all cultivated stone fruit species including plum, peach, nectarine, apricot, almond, and cherry, as well as wild and ornamental *Prunus* species.

Figure 1. PPV symptoms on a plum leaf and fruit. Photos courtesy of Dr. Laszlo Palkonvics, Corvinus University, Budapest, Hungary.

Last update: November 28, 2016

Eight strains of PPV (D, M, El-Amar, C, CR, W, T, and Rec) (Kerlan and Dunez, 1979; Crescenzi et al., 1997a; Bodin et al., 2003; James et al., 2003; James and Varga, 2005; Glasa et al., 2005; Serce et al., 2009; Glasa et al., 2013; Garcia et al., 2014), have been identified worldwide based on their biological, serological and molecular properties to date (Table 1). A putative ninth PPV strain (PPV-An) could be represented by a recently identified isolate from eastern Albania (Garcia et al., 2014). PPV-M and PPV-D are the most widespread. All occurrences in the United States have been identified as strain D (PPV-D) (Damsteegt et al., 2001; Schneider et al., 2011); whereas strains D, W, and Rec have been reported from Canada (Rochon et al., 2003; Thompson et al., 2009). Strain D naturally infects peach, nectarine, apricot and plum; almond and cherry are not natural hosts, although they can be infected experimentally (Damsteegt et al., 2007). Epidemics of PPV-D generally progress slowly in peach, and this virus strain is not seed-transmitted.

Table 1: Strains or Serotypes of *Plum pox virus*

Strain	Originally Described From	Notes
PPV-M (Marcus)	Greece (peach)	Present in many European countries but absent from the Americas. Causes rapidly spreading epidemics in peach, but less frequently found in plums. Efficiently transmitted by aphids.
PPV-D (Dideron)	France (apricots)	PPV-D can be considered as the most epidemiologically competitive and most widespread strain of PPV. Present in all areas where PPV has been reported, including the United States and Japan. Infrequently found in peach in Europe. PPV-D isolates are less efficiently transmitted by aphids than PPV-M.
PPV-Rec (Recombinant)	Serbia	A group of isolates from a single homologous recombination event between PPV-M and PPV-D (*Nlb* gene). Widespread in several central and eastern European countries. Recently found in Turkey and France. Frequently associated with plums and very rarely with peach in nature. Efficiently transmitted by aphids.
PPV-EA (El Amar)	Egypt (apricots)	Not reported outside of Egypt at this time.
PPV-C (Cherry)	Moldova (sour cherry)	Reported in Moldova in 1980's. Reported and eradicated in Italy. Sporadically present in central and eastern European countries. Recently reported from Belarus, Russia and Croatia. Able to infect other *Prunus* spp. under experimental conditions.
PPV-CR (Cherry Russian)	Russia (sour cherry)	Unusual PPV isolates recovered from naturally infected sour cherries in several regions of Russia have been characterized and proposed

		to form a second cherry-adapted strain, PPV-CR (Cherry Russian). The epidemiology of this strain remains to be determined.
PPV-W (Winona)	Canada (plum)	Reported and eradicated from two infected plum trees in Canada. Recently reported to be present in Latvia and Ukraine, and to be widespread in Russia.
PPV-T (Turkey)	Turkey (apricot)	Recognized only recently through the use of improved strain typing methods. These isolates have a recombination event in the *HC-Pro* gene. This strain occurs in Turkey and was identified also in Albania.

Figure 2: Fruit deformation caused by plum pox virus infection in sensitive plum. Photo courtesy of P. Gentit, Ctifl, France.

Plum pox is a major viral disease of *Prunus* species and is the most important and destructive viral disease of stone fruit trees in Europe and the Mediterranean region (Roy and Smith 1994). Plum pox virus (PPV) is an aphid transmitted disease and was first reported from plums in Bulgaria around 1917-1918. Its viral nature was not known until 1932 (Atanasoff, 1933). Also widely known around the world by its Slavic name, sharka, the virus spread slowly through eastern Europe, reaching western Europe in the 1970's. In Canada, plum pox was found in Ontario and Nova Scotia in 2000 (Thompson et al., 2001). In the United States, the disease was recorded in Pennsylvania in 1999 (Levy et al., 2000a), followed by New York and Michigan in 2006 (Gottwald, 2006; Snover-Clift et al., 2007). The disease has since been eradicated in Michigan and Pennsylvania (NAPPO Phytosanitary Pest Alert, 2009) and Nova Scotia (Ministry of Agriculture, 2010). Eradication efforts are continuing in New York, and containment efforts are ongoing in Ontario.

Biology and Ecology

Short distance spread of PPV is the result of aphid transmission in a non-persistent manner (Cornell University, 2008). Aphids test leaf and fruit surfaces by probing them. When an aphid test probes a leaf or a fruit cell, the aphid's sap-sucking mouthpart, a stylet, penetrates the tissue and draws up cell contents. Test probes last as little as 30 seconds. During probing of an infected host, virus particles can be pulled into the stylet and stick to the lining of the food canal. Once acquired, PPV remains in the stylet for up to three hours. During this time the virus can be transferred to healthy trees when viruliferous (virus carrying) aphids expel their stylet contents during new probes. The virus does not persist in the aphid after it has been expelled into new tissue (Cornell University, 2008).

Spring aphid flights are important for spread within and between orchards. About twenty aphid species can transmit PPV but only a few of them are considered important vectors in the northeastern United States: the black bean aphid (*Aphid fabae*), the spirea aphid (*Aphid spiraecola*), the black peach aphid (*Brachycaudus persicae*), and the green peach aphid (*Myzus persicae*) (Gildow et al., 2004). *Toxoptera citricida* (brown citrus aphid) is also an efficient vector, but does not occur in major stone-fruit growing areas (Gildow et al., 2004). All infected trees, even when not showing symptoms, are sources of possible PPV transmission to healthy trees. Aphids can spread PPV from several yards to about 2 km (1 ¼ miles), and transmission is unlikely to occur over long-distance as the lifespan of the virus within an aphid is generally less than an hour. Long-distance spread of PPV occurs primarily by movement of infected plants or plant parts. Virus infection can spread through infected nursery stock or infected buds collected from infected trees. Spatial analysis of PPV-infected trees in orchards suggests a preferential virus spread several tree spaces away from infected trees, rather than to neighboring trees (Dallot et al., 2003). Thus, secondary infections can be widely scattered from the original infection site if the primary virus sources are not controlled.

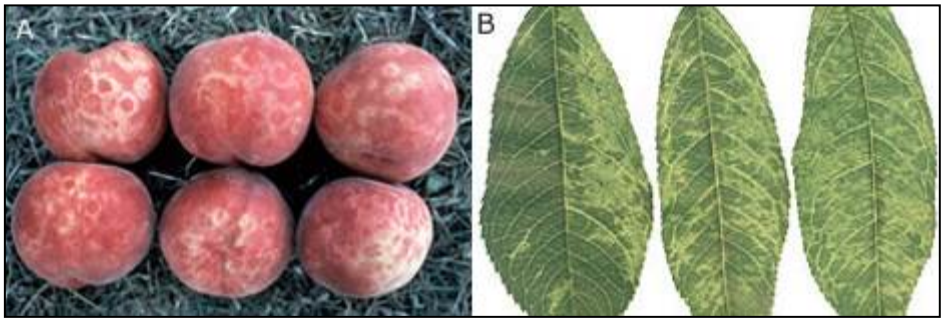

Figure 3: Symptoms of plum pox virus. **A)** Chlorotic ring patterns in peach fruit; **B)** Chlorotic blotches in peach leaves. Photos courtesy of P. Gentit, Ctifl, France.

Figure 4: Yellow rings caused by PPV on a yellow-fleshed peach cultivar (top), color break symptoms induced by PPV in peach flowers. Photos courtesy of European and Mediterranean Plant Protection Organization Archive, www.bugwood.org and P. Gentit, Ctifl, France.

The presence of other viruses, such as *Plum dwarf virus*, *Prunus necrotic ringspot virus*, and *Apple chlorotic leaf spot virus*, can increase the severity (synergistic effect) of plum pox symptoms. Capote et al. (2006) inoculated Japanese plum plants with either PPV-D or PPV-M and then one year later challenge inoculated with the other strain. The presence of PPV-D did not cross-protect the tree against PPV-M infection. In PPV-D-infected plants, the PPV-M strain used as challenge inoculation behaved differently depending upon the plum cultivar assayed. In cv. Black Diamond, PPV-M invaded the plant progressively, displacing the previous PPV-D population; whereas in cv. Sun Gold, both PPV isolates coexisted in the plant. In contrast, the PPV-D isolate was unable to infect plants of both cultivars in which a PPV-M population was already established.

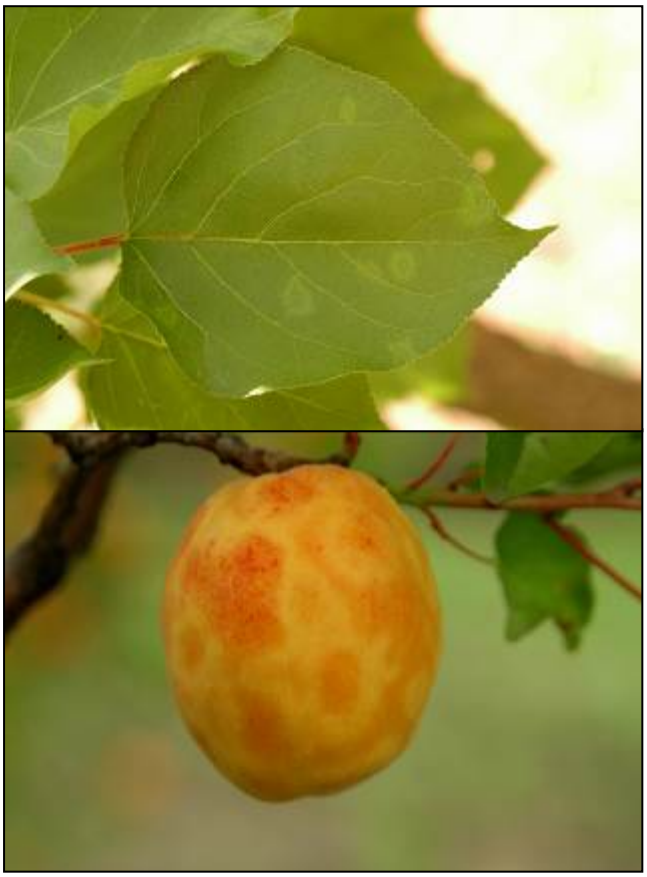

Figure 5. PPV symptoms on an apricot leaf and fruit. Photos courtesy of Dr. Laszlo Palkovics, Corvinus University, Budapest, Hungary.

There are presently some effective control measures against plum pox virus. The use of certified planting material, removal of infected trees and wild hosts, and the control of aphid vectors will all help to prevent any outbreaks of the disease and reduce the risk of the disease spreading.

Symptoms/Signs

Symptoms of PPV can be conspicuous (especially on susceptible cultivars) or very subtle on stone fruit trees. Symptoms vary in type and severity with the host, cultivar, environmental factors, and the timing of infection. Diagnostic symptoms occur mainly on leaves and fruits in the United States. In general, leaf symptoms include vein yellowing or light green to yellow rings. Foliar symptoms may develop during the cooler temperatures of spring and fall but fade during the hot summer months. Symptoms of PPV occur sporadically and often are not apparent until three or more years after infection. Newly infected trees are rarely symptomatic. It is critical that symptomless trees be regarded very seriously as they will act as a silent virus source for further infections.

Figure 6: Rings on the stone of apricot caused by PPV. Photo courtesy of Miroslav Glasa.

Plums: Pale green or light yellow chlorotic spots, blotches, bands, rings, or line patterns (Fig. 1) may occur on the leaves. They are difficult to see in the bright sunlight. Leaf symptoms are most easily seen on the fully expanded leaves from late May/early June. These symptoms are often irregularly distributed and may appear on only a few branches or leaves. Plum fruit symptoms depend on the original color of the fruit. Dark-skinned fruits show bluish, necrotic rings, which may be sunken (Fig. 1). Pale-skinned fruit show uneven ripening, blotching, and rings. Necrotic tissue may extend through the flesh to the stone, on which a reddish necrotic ring may develop. Plum fruits are often deformed (Fig. 2). Also, some plum cultivars can drop fruit prematurely.

Peach: The leaf symptoms of PPV on peach are distinctive. Affected leaves are distorted when they first unfold, having a wavy edge and a slight twist, and the veins show pale green or bright yellow flecks or lines (Fig. 3). These symptoms disappear as the leaves mature. Peach fruit may develop lightly pigmented rings (Fig. 3, 4) or line patterns that result from the convergence of several rings. Peach fruit, however, may have paler colored rings and lines than those found in plums. Flowers on PPV-infected peach trees may exhibit color breaking (Fig. 4) but only on cultivars with large showy flowers. Color-breaking appears as darker pink stripes on the flower petals.

Almonds: Show few leaf symptoms. Infection is often symptomless.

Apricot: Show lighter symptoms than plum or peach (Fig. 5). Apricot fruits may be misshapen, turn brown or become necrotic and may have rings (Fig. 6) on the surface of the seed.

Cherry: Pale green patterns and rings appear on the leaves. Fruits may be slightly deformed with chlorotic and necrotic rings, notched marks, and premature fruit drop**. Note: PPV strain D, which occurs in the United States, is not known to naturally cause infection in cherry.**

The visual symptoms accompanying the reduction in sugar content make the affected fruit unmarketable.

Pest Importance

PPV is the most widespread disease of stone fruits in Europe. This virus reduces fruit yield and quality. It also shortens the productive lifespan of orchards and can render stone fruit trees useless for fruit production. Even symptomless trees produce reduced quantities of fruit. The economic impact of PPV to the peach, plum, and apricot industry worldwide is estimated at $600 million per year. Nemeth (1986) estimated that losses due to the disease of some susceptible plum cultivars could be as high as 80 – 100% crop loss.

Plum pox is economically important because it causes fruit to be unmarketable, it weakens infected trees, and it decreases fruit yield. A wide-scale outbreak of PPV could lead to a decrease in stone fruit exports and higher prices for domestic consumers. The presence of PPV can also enhance the damaging effects and increase

the economic losses caused by other endemic viruses infecting various species of the genus *Prunus.* These include the *Prune dwarf virus*, *Prunus necrotic ringspot virus* (causes browning), and *Apple chlorotic leaf spot virus* (causes yellowing).

PPV is listed as a harmful organism in 73 countries worldwide, including Canada, China, and numerous other large trading partners (USDA-PCIT, 2014). There likely will be trade implications with some of these countries if this virus becomes further established in the United States.

Known Hosts

Plum pox virus has a broad experimental host range, although it has a rather restricted natural host range within the genus *Prunus* (Damsteegt et al., 2007). Wild and weedy *Prunus* spp. can serve as reservoirs of the virus in European countries (Polak, 2004, 2006). This has not been shown, however, in Canada or the United States (Stobbs et al., 2005). Virus isolates vary in their reaction to different hosts, and not all strains or isolates infect the same hosts.

Natural hosts: *P. amygdalus* (almond), *Prunus armeniaca* (apricot), *P. avium* (sweet cherry, only for cherry-adapted C and CR strains), *P. blireana* (blireana flowering plum), *P. cerasifera* (Myrobalan plum), *P. cerasus* (sour cherry, only for cherry-adapted C and CR strains), *P. domestica* (plum), *P. glandulosa* (dwarf flowering almond, cherry almond), *P. insititia* (damson plum), *P. japonica* (Korean cherry/ Japanese bush cherry), *P. mume* (Japanese apricot), *P. nigra* (Canada plum), *P. persica* (peach/ nectarine), *P. salicina* (Japanese plum), *P. serotina* (black cherry), *Prunus spinosa* (blackthorn), and *P. tomentosa* (Nanking cherry) (Nemeth, 1986; Polak, 1997; Labonne et al., 2004; Polak, 2004; Stobbs et al., 2005; James and Thompson, 2006; Polak, 2006; Damsteegt et al., 2007; Maejima et al., 2010).

Prunus species that have been proven to be hosts to Pennsylvania PPV-D strains in nature or by aphid and/or graft inoculation trials (most followed by back transmissions) (Damsteegt et al., 2007) are given in Table 2.

Table 2: *Prunus* susceptibility to Pennsylvania isolates of *Plum pox virus* (PPV-D) as assessed by either or both aphid and graft inoculation*

Species	Common Name	Visual Symptoms**	ELISA***	PCR (aphid, graft)****
P. americana	American plum	(8/23)	(11/23)	(+, +)
P. andersonii	Desert peach	(0/9)	(1/9)	(+, +)
P. angustifolia	Chickasaw plum	(14/21)	(9/21)	(+, +)
P. armeniaca	Apricot	(15/31)	(11/31)	(+, +)
***P. avium* 'Mazzard'**	Sweet cherry	(10/54)	(11/54)	(+, +)
P. cerasifera	Cherry plum	(12/14)	(8/14)	(+, NA)
***P. cerasifera* 'Myrobalana'**	Myrobalan plum	NA	NA	(NA, +)
***P. cerasifera* 'Thundercloud'**	Myrobalan plum	NA	NA	(NA, +)

P. cerasus	Sour (tart) cherry	(0/19)	(0/19)	(-, NA)
P. cistena	Purple leaf sand cherry	(0/65)	(3/65)	(+,+)
P. davidiana	David's peach	(0/13)	(5/13)	(+, +)
P. domestica **'Brompton'**	Garden plum	(2/2)	(2/2)	(+, +)
P. domestica **subsp. *insititia***	Bullace plum	(1/2)	(1/2)	(NA, NA)
P. dulcis **'Butte' and 'Mission'**	Almond	(3/30)	(17/30)	(+, NA)
P. emarginata	Bitter cherry	(4/32)	(7/32)	(+, +)
P. fruticosa	European dwarf cherry	NA	NA	(NA, +)
P. glandulosa **'Rosea Plena'**	Dwarf flowering almond	NA	NA	(NA, +)
P. hortulana	Wild goose plum	NA	NA	(NA, +)
P. humilis	Humble bush cherry	(10/18)	(12/18)	(+, NA)
P. ilicifolia	Holly leaf cherry	NA	NA	(NA, +)
P. incam **'Okame'**	Flowering cherry	(0/13)	(2/13)	(-, +)
(P. incam **'Okame') – OP 'Dream Catcher'**	Flowering Cherry	NA	NA	(NA, +)
P. incisa		NA	NA	(NA, +)
P. laurocerasus **'Otto Luyken'**	'Otto Luyken' cherry laurel	NA	NA	(NA, +)
P. laurocerasus **'Schipkaensis'**	'Schipkaensis' cherry laurel	(0/29)	(3/29)	(+, NA)
P. lyonii	Catalina Isl. cherry	NA	NA	(NA, +)
P. maackii	Manchurian cherry	NA	NA	(NA, +)
P. mahaleb	Mahaleb cherry	(6/74)	(19/74)	(+, +)
P. maritima	Beach plum	(3/3)	(3/3)	(+, +)
P. mexicana	Mexican plum	NA	NA	(NA, +)
P. mume	Japanese apricot	(12/12)	(12/12)	(+, NA)
P. nigra	Canadian plum	(0/3)	(1/3)	(+, +)
P. padus	European bird cherry	(4/45)	(14/45)	(+, +)
P. pensylvanica	Pin cherry	(2/44)	(13/44)	(+, +)
P. pumila **var. *besseyi***	Western sand cherry	(6/39)	(14/39)	(+, +)
P. pumila **var. *depressa***	Eastern sand cherry	(0/35)	(22/35)	(+, +)
P. salicina	Japanese plum	(3/21)	(5/21)	(+, NA)
P. sargentii	Sargent's cherry	NA	NA	(NA, +)
P. serotina	Black cherry	(11/78)	(35/78)	(+, +)
P. serrulata	Japanese flowering cherry	(9/15)	(9/15)	(+, NA)
P. serrulata **'Kwansan'**	Kwansan cherry	(0/13)	(0/13)	(+, +)

***P.* × 'Snofozam' (Snow Fountains)**	Snow Fountain cherry	(0/17)	(0/17)	(-, +)
P. spinosa	Blackthorn, sloe	(1/1)	(1/1)	(NA, NA)
***P. subhirtella* 'Pendula'**	Equinox cherry	NA	NA	(NA, +)
P. tenella	Dwarf Russian almond	NA	NA	(NA, +)
P. triloba	Flowering almond	(3/5)	(3/5)	(+, +)
P. virginiana	Chokecherry	(10/35)	(11/35)	(+, +)
P. virginiana* var. *demissa	Western chokecherry	(3/21)	(4/21)	(+, +)
P. yedoensis	Yoshino flowering cherry	NA	NA	(NA, +)

* Data from Damsteegt et al. (2007)

** Visual symptoms: number of plants with symptoms/total number of plants

*** ELISA: number of plants with 405 nm absorbance levels 4 x higher than negative controls/total plants

**** PCR: NA=not attempted, + = positive, - = negative

Experimental hosts (all strains):

The virus has been transmitted to many *Prunus* species including: *Prunus americana* (American plum), *P. armeniaca ansu* (ansu apricot), *P. besseyi* (western sandcherry), *P. besseyi* x *munsoniana* x *salicina*, *P. brigantina* (alpine, Briancon apricot), *P. cerasifera* x *munsoniana* x *angustifolia*, *P. cerasifera* x *spinosa*, *P. cistena* (purple leaf sand cherry), *P. cocomilia* (Italian plum), *P. kurdica*, *P. dasycarpa* (black apricot), *P. davidiana* (David's peach), *P. holosericea* (apricot), *P. hortulana* (wild goose plum/Hortulan plum), *P. laurocerasus* (cherry laurel), *P. mahaleb* (mahaleb cherry), *P. mandshurica* (Manchurian apricot), *P. maritima* (beach plum), *P. mexicana* (Mexican plum), *P. microcarpa* (Japanese apricot), *P. munsoniana* (wild goose plum), *P. munsoniana* x *triloba*, *P. pensylvanica* (pin cherry), *P. pseudoarmeniaca* (Italian plum), *P. pumila* (sand cherry), *P. serrulata* (Japanese flowering cherry), *P. sibirica* (Siberian apricot), *P. simonii* (apricot plum), and *P. triloba* (flowering almond) (Hamdorf, 1975; Nemeth, 1986; Polak, 2001; Labonne et al., 2004).

Many non-*Prunus* species, in at least sixteen plant families, have been experimentally infected with one or more strains of the *Plum pox virus*, and in some cases found to be naturally infected (**shown in bold**) in the field. The reports of natural infection, however, were the result of tentative screening methods without the use of confirmatory techniques and should be considered suspect (T. Candresse, personal communication, 2014). Herbaceous hosts infected experimentally by Pennsylvania isolates of PPV are shown in blue (Schneider et al., 2011). Most of these are herbaceous annuals but a few are perennial or woody and could serve as overwintering sources of the virus. Hosts include: *Agrostemma githago* (common corncockle), *Ajuga genevensis* (blue bugleweed), *Arabidopsis thaliana* (arabidopsis), *Amni majus* (laceflower), *Borago officinalis* (common borage), ***Campanula rapunculoides*** (rampion bellflower), *Capsella bursa-pastoris* (shepherd's purse), *Celosia* spp. (cock's comb), *Chenopodium amaranticolor* (lambsquarters), *Chenopodium foetidum* (lambsquarters), *Chenopodium murale* (nettleleaf goosefoot), *Chenopodium quinoa* (quinoa), *Chenopodium* spp.

(lambsquarters), *Chrysanthemum* spp. (chrysanthemum), *Cichorium* spp. (chicory, endive), *Cirsium arvense* (Canada thistle), *Clematis* spp. (clematis/virgin's bower), *Convulvulus arvense* (field bindweed), *Coreopsis* spp. (beggarticks/tickseed), *Cyamopsis tetragonoloba* (guar), *Digitalis lanata* (Grecian foxglove), ***Dimorphotheca aurantiaca*** (cape marigold), *Emilia sagittata* (tasselflower), ***Euonymus europea*** (euonymous), *Galeopsis segetum* (downy hempneedle), *Gladiolus* spp. (gladiolus), *Gomphrena globosa* (common globe amaranth), *Humulus lupulus* (hop), *Hyoscyamus niger* (black henbane), *Lactuca serriola* (prickly lettuce), ***Lamium album*** (white deadnettle), ***L. amplexicaule*** (henbit, deadnettle), ***L. purpureum*** (purple deadnettle), *Lathyrus odoratus* (sweet pea), *Linaria cymbalaria* (Kenilworth ivy), ***Ligustrum vulgare*** (European privet), *Lithospermum arvense* (corn gromwell), ***Lupinus albus*** (white lupine), *Lupinus luteus* (European yellow lupine), *Lycium barbarum* (matrimony vine), *L. halimifolium* (matrimony vine), ***Medicago lupulina*** (black medic), ***Melilotus officinalis*** (yellow sweet clover), *Meliotus* spp. (sweet clover), *Mimulus variegates* (monkey flower), *Nicandra physaloides* (shoo-fly plants/apple of Peru), *Nicotiana benthamiana* (tobacco), *N. megalosiphon* (tobacco), *N. occidentalis* #37 B (tobacco), *N. tabacum* (tobacco), *Nicotiana* spp. (tobacco), ***Oenothera biennis*** (evening primrose), *Papaver somniferum* (opium poppy), *Passiflora foetida* (fetid passionflower), *Petunia hybrid* (petunia), *Pisum sativum* (pea), *Pisum* spp. (pea), *Physalis* spp. (groundcherry), ***Ranunculus acer*** (buttercup), ***R. arvensis*** (buttercup), *R. repens* (buttercup), *Ranunculus* spp. (buttercup), *Rorippa sylvestris* (creeping yellow cress), *Rumex crispus* (curled dock), *Senecio* spp. (groundsel), *Sesbania exaltata* (bigpod sesbania), *Sesbania vulgaris* (Colorado river hemp), ***Silene inflata*** (maidenstears), ***Silene vulgaris*** (maidenstears), ***Solanum dulcamara*** (climbing nightshade), *Solanum lycopersicon* (tomato), *Solanum* spp. (nightshades), *Sonchus* spp. (sowthistle), ***Sorbus domestica*** (service tree), *Stachys recta* (stiff hedgenettle), *Stellaria media* (common chickweed), *Symphitum officinale* (common comfrey), *Taraxacum officinale* (common dandelion), *Trifolium incarnatum* (crimson clover), ***T. pretense*** (red clover), *T. repens* (white clover), *Trigonella foenum-graecum* (fenugreek), *Torenia fournieri* (bluewings), *Verbena officinalis* (herb of the cross, prostrate verbena), *Veronica* spp. (speedwell), *Vicia* spp. (vetch), ***Zinnia elegans*** (elegant zinnia), and *Z. violacea* (zinnia) (Sutic, 1972; Nemeth,1986; Polak, 2001; Llacer, 2006; Wang et al., 2006; Schneider et al., 2011)

Known vectors (or associated organisms)

Plum pox virus has been transmitted by at least 20 aphid species, although only four to six are considered important vectors (Table 3). The efficiency of transmission is dependent on the virus isolate, host cultivars, age of the host cultivars, aphid species, and time of year. The most important aphid vectors reported from several countries are *Brachycaudus cardui, B. helichrysi, Hyalopterus pruni, Myzus persicae,* and *Phorodon humuli.* Although reports vary from country to country, the natural virus spread is low in July and August but high in spring and autumn. Spring flights of *B. helichrysi, M. persicae,* and *P. humuli* are most important for spread within and between orchards (Levy et al., 2000b). Analysis of spatial distribution of PPV by Gottwald et al. (1995) suggest a lack of movement by aphid vectors to immediately adjacent trees and a preference for movement several tree spaces away.

Aphids can acquire the virus in probes as short as 30 seconds and can transmit for up to one hour. Aphids that have been starved before feeding can transmit for up to three hours after acquisition. There is no correlation between the ability to transmit PPV and the ability to colonize *Prunus*. PPV can be spread in orchards by transient aphids as efficiently as by aphids colonizing *Prunus* (Labonne et al., 1995). Aphids can also acquire PPV from harvested infected fruits (Gildow et al., 2004).

Aphids were found to transmit PPV within 100 to 120 meters of the source plants, but they have been shown to carry the virus on their stylets for several kilometers if starved before feeding.

Table 3: Aphid species shown to be vectors of *Plum pox virus*.

Aphid Species	Colonizes *Prunus*	Host
Aphis arbuti	No	*Arbutus unedo*
A. craccivora*	No	Polyphagous
A. fabae	No	Polyphagous
A. gossypii*	No	Polyphagous
A. hederae	No	*Hedera helix*
A. spiraecola*	Occasionally	Polyphagus; Apple; Citrus
Brachycaudus cardui	Yes	*Prunus;* Compositae
B. helichrysi**	Yes	*Prunus;* Compositae
B. persicae*	Yes	*Prunus*
Dysaphis plantaginea	No	Apple; Plantago
D. pyri	No	Pear; *Gallium*
Hyalopterus pruni*	Yes	*Prunus; Fragmites*
Macrosiphum rosae	No	*Rosa;* Dipsaceae
Megoura rosae	No	Leguminoseae
Myzus persicae**	Yes	Polyphagous
M. varians	Yes	Peach; *Clematis*
Phorodon humuli**	Yes	*Prunus; Hop*
Rhopalosiphum padi	No	*Prunus padus;* Gramineae
Sitobion fragariae	No	*Rosa;* Gramineae
Ureleucon sonchi	No	*Lactuca; Sonchus*

*Recognized aphid vectors, ** Most important vectors. Data from Levy et al. (2000b).

Known Distribution

Africa: Egypt and Tunisia. **Asia:** China, India, Iran, Israel, Japan, Jordan, Kazakhstan, Lebanon, Pakistan, South Korea, Syria, and Turkey. **Europe:** Albania, Austria, Belarus, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, France (including Corsica), Germany, Greece, Hungary, Italy, Latvia, Lithuania, Luxembourg, Moldova, Netherlands, Norway, Poland, Portugal (including Azores), Romania, Russia, Serbia and Montenegro, Slovakia, Slovenia, Spain, Switzerland, Ukraine, and the United Kingdom. **North America:** Canada and the United States. **South America:** Argentina, and Chile (Staniulis et al., 1998; Reyes et al., 2003; Boulila et al., 2004; Spiegel et al., 2004; Navratil et al., 2005; Dal Zotto et al., 2006; Kollerová et al., 2006; Mumford, 2006; Candresse et al., 2007; Papayiannis et al., 2007; Maejima et al., 2010; Salavei et al., 2012; EPPO, 2014).

The disease has been found in Denmark, Estonia, and Georgia, but did not establish or is no longer found (Roy and Smith, 1994; Levy et al., 2000b, EPPO, 2014).

In 2014, a plum tree in Finland was found to be infected with PPV-D (Santala and Soukainen, 2015). This tree, originally from Russia, was used as a mother tree. This tree and all six infected progeny trees were destroyed, and PPV is currently considered to be under eradication in Finland.

In 2014, symptomatic peach fruit in Brazil was found to contain PPV (strain not reported) (Rezende et al., 2016). This fruit was imported from Chile, where PPV is known to occur. It is unclear if PPV is established in Brazil or not.

Pathway

According to Federal Order DA-2013-18, effective May 20, 2013, the import of *Prunus* spp. propagative material (all propagules except seeds) is currently prohibited from all countries except Canada and the Netherlands to restrict import of host material of *Anoplophora chinensis* (Chinese Longhorned Beetle) and *Anoplophora glabripennsis* (Asian Longhorned Beetle) (USDA, 2014). Prior to this Federal Order, import of *Prunus* spp. propagative material was allowed from the following countries known to have PPV: Belgium, Canada, France, Germany, Netherlands, and the United Kingdom (USDA, 2014). Import of seeds of *Prunus* spp. susceptible to PPV is currently allowed from Europe and Canada if they are accompanied by a phytosanitary certificate (USDA, 2014).

Since 2004, there have been shipments of *Prunus* spp. plant material to U.S. ports of entry from the following countries known to have PPV: Canada (573), Czech Republic (2), France (31), Germany (1), Hungary (1), Italy (1), Netherlands (13), Spain (2), and the United Kingdom (7). The largest of these shipments was from Canada and contained 49,222 plant units. Another shipment from the Netherlands contained 46,000 plant units (AQAS, 2014). There have also been a total of 137 interceptions at U.S. ports of entry of *Prunus* spp. plant material intended for propagation from 25 different countries where PPV is present since 2004 (AQAS, 2014).

Of the major known vectors of PPV, only one, *Phorodon humuli*, is reportable at U.S. ports of entry. There have been two known interceptions of this aphid since 1985, but neither of these interceptions were from known host countries. The remaining important aphid vectors (Table 3) are not reportable (AQAS, 2014). However, due to the relatively short infectivity period of the aphid vectors, the chance of PPV entry through infected vectors is low.

Potential Distribution within the United States

In the United States, *Plum pox virus* was first recorded in Pennsylvania in 1999, followed by reports from New York and Michigan in 2006. The virus is now considered to be eradicated in Pennsylvania and Michigan. It has the potential to occur wherever susceptible hosts are grown. A recent host risk analysis by USDA-APHIS-PPQ-CPHST

indicates that most of the eastern United States and portions of Washington, Oregon, and California have a moderate risk rating for *Plum pox virus* establishment based on host availability within the continental United States.

Survey

CAPS-Approved Method*: The CAPS-approved survey method is to collect samples utilizing the National PPV Sampling Plan that uses a hierarchical sampling method (Hughes et al., 2002).

From National PPV Survey Plan:

"Detection survey for PPV in orchards is based on the use of a hierarchical sampling method. This involves collecting 8 leaf samples from each of 25 percent of the trees in an orchard. Trees to be sampled are selected in groups of 4, with 32 leaves that are collected from the four trees being ELISA tested as four 8 leaf samples. To determine the total number of samples to be collected in an orchard being surveyed, the following formula can be used:

$$\frac{\text{Number of trees per acre x number of acres of in the orchard}}{4}$$

Thus, in a 9 acre orchard with a planting rate of 140 trees per acre, 315 eight leaf samples would be submitted for ELISA testing. Sampling at lower rates significantly reduces the likelihood of detecting PPV, and sampling at higher rates is only warranted if there are unusual risk factors present.

APHIS recommends that the selection of trees or orchards to be sampled and the rate at which sampling is conducted should be based on the risk associated with the material being sampled. At the present time, outside of NY, there is no reason to think that any orchards or budwood source trees in any state presents a particular risk associated with having been exposed to PPV. Thus, decisions on which trees and orchards are selected for inclusion in the survey are more based on the risk that might be present should PPV be found. Foundation trees used to produce budwood for the production of registered nursery stock trees or certified budwood source trees present the highest risk, and all of these trees should be sampled at the level of 8 leaves per tree.

The second level of risk are individual trees outside of orchard settings, or orchards from which budwood is or recently has been cut – either registered or certified budwood, or common budwood. Individual trees outside of orchards should be sampled at the level of 8 leaves per tree. All of the orchards containing trees from which budwood has been cut should be sampled at the 25 percent level using the hierarchical sampling model. Once orchards containing trees used for budwood production have been sampled, the remaining funds may be used to conduct hierarchical surveys of a representative group of orchards throughout the state.

The best time to collect samples is in the spring - from the time new leaves are fully expanded until it becomes too hot for reliable leaf samples to be collected. Once average daily temperatures exceed 35°C (95°F) the virus titer in leaves declines significantly and samples from infected trees would be likely produce false negative results. In some southern areas this means that there is a relatively narrow window in which samples can usefully be collected – while in other more northern areas the window in which samples can be collected may be as long as several months.

In Summary:

- All foundation trees should be sampled.
- All other trees used as budwood sources and not in orchards should be sampled.
- Any orchards containing trees form which budwood is cut should be sampled at a 25 percent level, using a hierarchical sampling model.
- Any remaining funds can be used to survey a representative group of orchards, with sampling being conducted at the same 25 percent level.

States can either process their own samples using ELISA kits purchased from AgDia, or can have samples processed by another state or by AgDia."

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at https://caps.ceris.purdue.edu/approved-methods.

Literature-Based Methods:

Hughes et al. (2002) and Gottwald (2006) discuss the use of hierarchical sampling in the surveillance program for *Plum pox virus* in the United States. The method was adopted by the Pennsylvania Department of Agriculture, as well as Canada, to survey for PPV. Virus incidence may be assessed by sampling groups of orchard trees, recording the groups as 'virus-positive' (one or more infected trees) or 'virus-negative' (no infected trees), and then calculating disease incidence at the individual tree scale by means of a formula involving incidence at the group scale and the number of trees per group. Differences in spatial aggregation characteristics of various pathosystems can be accounted for by adjusting the apparent group size in the formula to predict disease incidence more accurately at the individual plant scale from incidence at the group scale.

Following the confirmation of PPV infection in Adams County, an initial survey was conducted by the Pennsylvania Department of Agriculture in autumn 1999. In this survey, a number of orchard blocks with visual symptoms of PPV infection were located before leaf fall made further sampling impossible (Gottwald, 2006). Plots consisting of 400 trees in a 20 by 20 rectangular pattern were established in nine of these blocks (*i.e.,* all those of sufficient size). In these plots, the location and PPV status, determined by ELISA using 5B-IVIA monoclonal antibodies (Cambra et al., 1994), of each tree were

recorded in the form of a 'map'. Missing trees were also recorded. More orchard blocks with visual symptoms of PPV infection were located when the survey was continued in the spring and summer of 2000.

In the hierarchical sampling scheme, the sample covers 25% of the trees in a block. The sampling unit is a group of four trees in a two-by-two rectangular arrangement. In practice, three to four leaves are taken from each tree; one leaf from each main unit is kept as a bulked sample. For subsequent laboratory assay, this bulked sample is divided into two subsamples of six to eight leaves each. If neither of the subsamples provides a PPV-positive ELISA result, the group is recorded as PPV-negative; otherwise the group is recorded as PPV-positive. Since only PPV incidence at the group scale is assessed in this way, PPV incidence at the scale of the individual tree is then calculated from the equation:

$$\tilde{p}_{low} = 1(1 - \check{p}_{\text{high}})^{\frac{1}{\check{\nu}}}$$

Where:

$\check{p}_{\text{high}}$ = the probability that the group contains at least one PPV-positive tree

$\tilde{p}_{low}$ = the probability that an individual tree is positive.

$\check{\nu}$ = effective sample size

Where the disease has been reported previously, surveys are centered at and near locations where positive trees were found the previous year. Within a one mile radius of a site that tested positive, all trees have one sample taken (a sample consists of eight leaves per tree). Because distribution of PPV in infected trees is not uniform, each sample consists of eight leaves taken from multiple locations on a tree. This strategy increases the probability that the virus, if present, will be detected. Out to five miles from former sites of infection, every tree is sampled at four leaves per tree, two trees combined per sample. Beyond five miles, all the orchards are sampled and each orchard has 25% of the trees sampled at four leaves per tree. Leaf samples are placed in plastic bags, barcode labeled, and stored on ice until shipped to a laboratory. Once orchard and homeowner samples are delivered, they are scanned into a database and tracked by their barcode number. No information other than the barcode is available to technicians (blind testing procedure).

Key Diagnostics

CAPS-Approved Method*: The approved screening protocol for the field is the PPV Enzyme-Linked ImmunoSorbent Assay (ELISA). The work instruction is available upon request from Ashlee Barth (301-504-7100 x 9227, ashlee.k.barth@aphis.usda.gov). The work instruction describes detection of PPV using the ELISA kit from Agdia Inc., which detects six known PPQ strains/subgroups: PPV-C, PPV-D, PPV-EA, PPV-M, PPV-Rec, and PPV-W in leaves, fruit, and flowers.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at https://caps.ceris.purdue.edu/approved-methods.

Literature-Based Methods:

Indicator Hosts: Indicator hosts are given in Table 4 with diagnostic symptoms (Bernhard et al., 1969; Damsteegt et al., 1997; Kegler et al., 2001; Glasa and Candresse, 2005; Gentit, 2006). Herbaceous hosts, however, are difficult to manage, and get a good sensitivity with (T. Candresse, personal communication, 2014).

Table 4: Plum pox virus indicator host plants.

Indicators	Symptoms
Woody Plants:	
***Prunus persica* cv. GF305**	Vein clearing and distortion of leaves
P. tomentosa	Chlorotic mottle, vein chlorosis, leaf deformation, and necrotic spots
***Prunus marianna* cv. GF8.1**	Diffuse chlorotic spots on leaves
***Prunus instititia* cv. St. Julien no.2**	Chlorotic spots and rings
***Prunus domestica* K4 (Kirke x Persikovaja)**	Hypersensitive hybrid, pale green leaf mottling, necrotic leaf spots, shoot tip necrosis and/or eventual decline, depending on the isolate
Herbaceous Plants:	
Chenopodium foetidum	Chlorotic, chloro-necrotic, or necrotic spots depending on the viral isolate
Nicotiana benthamiana	Stunting, chlorotic mosaic with dark green islands, leaf puckering
***N. clevelandii* or *N. clevelandii* x *N. glutinosa* hybrid**	Chlorotic or necrotic local lesions, systemic chlorotic mottling – some isolates induce very mild or no symptoms
***Pisum sativum* cv. Colmo, Express Genereux or Serpette d' Auvergne**	Light green mosaic, chlorotic mottling

Staniulis et al. (1998) inoculated plants of *Chenopodium foetidum* (chlorotic local lesions) and *Pisum sativum* cvs. Rainiai and Citron (mottling). Damsteegt et al. (1997) reported that *P. tomentosa* was generally useful as a diagnostic indicator of PPV and showed different symptoms when infected with PPV-D or PPV-M. This report, however, has not been validated with more isolates.

Antibodies: Monoclonal antibody 5B-IVIA (Cambra et al., 1994) allows for the universal detection of PPV. Strain-specific monoclonal antibodies have also been developed for both the D and M strains/serotypes (Cambra et al., 1994; Boscia et al., 1997). These antibodies, however, are not suited for differentiating other serotypes (*e.g.*, El-Amar and Cherry) (Candresse et al., 1998). Polyclonal and monoclonal antibodies have also been produced for the PPV-C and PPV-EA strain (Boscia et al., 1997; Crescenzi et al., 1997b; Myrta et al., 1998; Myrta et al., 2000).

ELISA: Clark and Adams (1977) developed the first ELISA test and included *Plum pox virus* as an application of this technology to plant viruses. Strain-specific antibodies are available (see section above).

Individual state Department of Agriculture laboratories work in conjunction with USDA-APHIS to screen *Prunus* trees for the presence of *Plum pox virus* (PPV). The protocol described below is used in New York State and is provided as an example of how PPV is detected. The PPV Lab in Geneva, NY, tests approximately 2,500 to 3,000 leaf samples daily during their survey operations period.

Upon delivery to the testing lab, leaf samples are unpacked, scanned into a database, and tracked by a barcode assigned to them by the collection team. Duplicate barcode labels are then printed and stored with the samples in a cold room until processing. Leaf samples are stacked with the petioles aligned and 0.5 grams of tissue is cut from the base of the leaves, avoiding petioles and midribs. Leaf tissue weights are checked every 10 samples or whenever leaf types change or a new *Prunus* species is tested.

Excised leaf material is placed in a plastic bag, which is labeled with the corresponding barcode. Grinding buffer (5 ml) is added to the bag and the sample is ground with a tissue homogenizer. Crude leaf extracts are then tested for PPV by ELISA) in microtiter plates using specific antibodies. Remaining leaf material is stored in a cold room until the sample has gone through the entire ELISA procedure with no indication of PPV infection.

For ELISA, microtiter plates consist of 96 wells, six of which are designated as controls (positive: PPV-infected material, negative: healthy plant material and grinding buffer). Every sample is replicated, so that a total of 45 samples can be run on one microtiter plate. Sample testing is a three-day process. On the first day, 96-well microtiter plates are coated with an antibody specific to PPV and incubated overnight in a cold room, allowing the antibodies to adhere to the surface of the wells. On the second day, microtiter plates are rinsed, loaded with ground leaf samples, and incubated overnight in a cold room. If PPV is present in the leaf sample to be tested, virus particles will adhere to the antibodies coated on the microtiter plate wells. On the third day, microtiter plates are rinsed and treated with an antibody specific to PPV that has an enzyme tag. If the coating antibody captured PPV, the second antibody will adhere to it, sandwiching the PPV particle between the two antibodies. Microtiter plates are rinsed and a solution that reacts colorimetrically with the enzyme tag on the secondary antibody is added. After one hour of incubation in the dark, any well, in which the virus is present, will turn yellow. In contrast, wells that do not contain the virus will remain colorless. Plates are scanned on a microplate reader and any sample that reads 2.0 times higher than the negative control is flagged as a positive suspect.

Lateral Flow Device: Mumford et al. (2001) describe a lateral flow device for on-site detection of PPV. The on-site kit, which contains a one-step lateral flow device and a simple, bottle extraction system, can give a result in three minutes.

Immunochromatographic Assay: Byzova et al. (2010) raised two monoclonal antibodies that recognized strains PPV-D, M, and C. The authors developed a 10-minute immunochromatographic assay for PPV with a detection limit of 3 ng/ml. The assay demonstrated good compatibility with the data obtained via ELISA.

Molecular: Sequence analysis of PCR fragments corresponding to the C-terminal part of the PPV coat protein gene has allowed identification of a molecular polymorphism correlated to serotype of the PPV isolates (Candresse et al., 1994, 1995). Initial results have indicated that an *Rsa*I restriction fragment length polymorphism (RFLP) located in this region could be used for PCR amplification, to discriminate between D and M serotypes of PPV (Bousalem et al., 1994; Wetzel et al., 1991). More recently, a cluster of non-coding, third-base mutations on five consecutive codons located around the *Rsa*I RFLP site was found to show excellent correlation with the viral serotype (Candresse et al., 1995). This observation was used as a basis for direct PCR typing of isolates belonging to the D and M serotypes of PPV (Candresse et al., 1994). Levy and Hadidi (1994) utilized a simple and rapid procedure for processing PPV infected plant tissue (Gene Releaser) for use with a specific 3' non-coding region RT-PCR assay. Immunocapture PCR(IC-PCR), reverse transcriptase PCR (RT-PCR), RT-PCR with RFLP, PCR-ELISA, print-capture PCR, and integrated RT-PCR/nested PCR have been used to detect PPV and to type strains/serotypes of PPV (Wetzel et al., 1991; Wetzel et al., 1992; Olmos et al., 1996; Olmos et al., 1997; Olmos et al. 1999; Poggi Pollini et al., 1997; Hammond et al., 1998; Nemchinov et al., 1998; Staniulis et al., 1998; Szemes et al., 2001; Glasa et al., 2005; Papayiannis et al., 2007; Olmos et al., 2008). Szemes et al., (2001) developed a RT-PCR/nested PCR technique for the simultaneous detection of PPV-D, M/Rec, EA, and C. Subr et al., (2004) developed a strain-specific RT-PCR with binding sites located on both sides of the recombination crossover situated in the C-terminal part of NIb, applicable for direct identification of PPV-M, D, and Rec isolates.

Due to a high genetic variability of PPV isolates, a broad intra-strain and intra-isolate diversity (Jridi et al., 2006), and the potential existence of divergent forms on one hand and the possibility of a mixed infection of the tree by 2 or more PPV strains, the ultimate typing and identification of strain/isolate present in the tree is made by partial or complete sequencing of the viral genome.

Faggioli et al. (1998) compared three different techniques to prepare PPV viral RNA for RT-PCR: 1) an immunocapture technique using a specific antiserum, 2) a silica-capture method using a non-specific matrix, and a simple and rapid RNA extraction. All three techniques allowed for the successful amplification and detection of PPV, but the silica capture method was less effective.

Candresse et al. (1998) compared an indirect double antibody sandwich ELISA using monoclonal antibodies for PPV-D and PPV-M with specific PCR assays or RFLP analysis of PCR fragments. Overall, the authors found an excellent correlation between the results of the ELISA and PCR assays for PPV-D and PPV-M. Adams et al. (1999) compared the detection effectiveness of immunocapture PCR (IC- PCR) and ELISA in dormant plum trees. IC-PCR has been shown to be about a thousand times more

sensitive than ELISA (Candresse et al., 1995). ELISA was shown to be effective for the detection of PPV in bark samples throughout the winter; 71-80% of samples were ELISA positive compared to 85-86% in the same samples by IC-PCR. In one-year old shoots taken from infected branches of orchard trees, 66-81% were positive by ELISA compared with 81-87% by IC-PCR. It was not recommended that sampled be bulked for ELISA detection, because only 38-65% were positive by ELISA compared with 92-100% by IC-PCR due to the uneven distribution of the virus in plant tissues.

Sanchez-Navarro et al. (2005) developed a multiplex RT-PCR for the detection of eight stone fruit viruses: *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Plum pox virus* (PPV), *Apple mosaic virus* (ApMV), *American plum line pattern virus* (APLPV), *Apple chlorotic leaf sport virus* (ACLSV), *Apricot latent virus* (ApLV), and *Plum bark necrosis stem pitting associated virus* (PBNSPaV). Jarosova and Kundu (2010) used a single-tube multiplex RT-PCR to detect PPV, PDV, and PNRSV. Both methods included an internal control.

Real-Time PCR: Schneider et al. (2004) developed a real-time, fluorescent, RT-PCR reaction assay for the detection of PPV in the Smart Cycler (Cepheid). Varga and James (2005) developed real-time multiplex assay utilizing SYBR Green technology to detect and differentiate PPV-D and PPV-M types in woody and herbaceous plants. Olmos et al. (2005) used Taqman technology in real-time assay for the universal detection and quantification of PPV in plant material and aphid vectors. The sensitivity of the real-time RT-PCR assay was 100 times higher than nested RT-PCR and 1000 times higher than ELISA and conventional RT-PCR.

Easily Confused Species

PPV symptoms are sometimes difficult to distinguish from other diseases and may be confused with rusty spot (*Podosphaera* spp.) of peaches and nectarines and bacterial canker as well as insect-related problems such as damage from thrips, white apple leafhopper, and San Jose scale. Symptoms from other viruses-in particular *Apple chlorotic leaf spot* in plum and *Prunus necrotic ringspot* in cherry-can also cause confusion. Nutritional deficiencies and pesticide damage can also be confused with PPV symptoms.

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May 2011: Original version written and posted to CAPS Resource and Collaboration site in the Stone Fruit Manual

May 2013: Minor updates to host and distribution sections.

April 2014: Added Israel and Belarus to distribution. Updated Pest Importance section with PCIT data. Added Pathway section.

July, 2014: Incorporated comments and corrections from Thierry Candresse. Added the newly identified strain PPV-CR to table 1.

October, 2014: Incorporated comments from Miroslav Glasa and Delano James. Updated the Pest Description, Known Hosts, and Key Diagnostics sections. Replaced Figure 6 with a better image.

March, 2015: Minor update to distribution section. Rec strain found in France.

August, 2015: Added Finland to distribution section.

July, 2016: Updated mapping data

November, 2016: Updated distribution list. Strain D found in South Korea, and PPV found in Brazil

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