

# INTERNATIONAL WORKING GROUP ON FRUIT TREE VIRUSES

## DETECTION OF VIRUS AND VIRUS-LIKE DISEASES OF FRUIT TREES

### Laboratory Assays, Bioassays and Indicators

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#### **INTRODUCTORY NOTES**

The tables of laboratory assays, bioassays, and indicator hosts were last presented in *Acta Horticulturae* 550: 473-493, 2001. As a result of the discussions of the Fruit Tree Virus Working Group during the business meetings of the 1994 Rome, the 1997 Bethesda, and the 2000 Canterbury Symposia, only few modifications were made to the lists of woody and herbaceous indicators since the publication in *Acta Horticulturae* 309: 407-418, 1992. Changes decided at the 1997 Bethesda Symposium and published in *Acta Horticulturae* 472: 759-783, 1998, included a different layout of the approved herbaceous and woody indicators, and updated nomenclature and knowledge about the causal agents of diseases. As there had been rapid progress in the development of polymerase chain reaction (PCR) based assays in the 1990's, it was decided in 1997 to compile a new list of laboratory assays. Each list for the individual pome and stone fruit species contained the previously approved ELISA tests and, in addition, nucleic acid based tests. Due to the constant development in methodology and often the existence of different probes, primers and protocols, it was attempted to list only key references. It was not feasible for the Fruit Tree Virus Working Group to compile detailed protocols. The nucleic acid based assays previously suggested are mostly well adapted for equipped laboratories, however, in many cases, validation of the tests on a broader range of isolates was required. This situation had not fundamentally changed at the 2000 Canterbury and the 2003 Valencia Symposia. Therefore, the list of molecular assays and their references was only updated in some cases and expanded where tests for additional viruses, viroids and phytoplasma diseases had become available.

Some detailed comments on the potential of the PCR tests are listed under technical notes.

#### **TECHNICAL NOTES**

**Molecular and Serological Assays that Have Been Applied to Temperate Fruit Trees Are Listed-** For details see the appropriate reference

*Abbreviations* - Enzyme linked immunosorbent assay = ELISA, reverse transcription-polymerase chain reaction = RT-PCR, immunocapture-RT-PCR = IC-RT-PCR, reverse transcription-polymerase chain reaction - enzyme linked immunosorbent assay = RT-PCR-ELISA.

**PCR Assays Need Validation** - PCR assays for an increasing amount of viruses and viroids have been validated for many isolates of each pathogen. However, PCR assays for other selected pathogens were conducted only on one or a few isolates of a given pathogen. A

broader range of isolates of some pathogens need to be assessed before the tests can be recommended for certification or quarantine purposes. In addition to the selection of references presented, the newest literature available should be sought for improvements in methodology and reliability.

**Herbaceous Indicators** - The widely used susceptible herbaceous indicators are *Cucumis sativus* and *Chenopodium quinoa*. They can be infected with viruses of the *ilarvirus* and *nepovirus* genera but not all the viruses of these groups or all strains can infect these indicators. Furthermore, success and reliability of transmission depend on the: origin of the inoculum, type of tissue sampled, season, composition of extraction buffer, etc., consult: Németh, M. (1986).

**Woody Indicators** - In general, tests on woody indicators are still considered to be the most reliable ones to use for establishing the health of individual plants (e.g. nuclear stock plants). No indication is given on the superiority of individual test plants where more than one is available. Effectiveness is likely to vary considerably with factors such as virus strain and environmental conditions.

*Abbreviations* - Number of replicates (rep.), temperature conditions for greenhouse indexing (°C), duration of the test (in days = d, weeks = w, years = y, crops = c).

## MOLECULAR ASSAYS FOR VIRUSES, VIROIDS, AND PHYTOPLASMAS OF TEMPERATE FRUIT TREES

### VIRUSES

Pathogen Name	Acronym	Molecular Assay	Reference
American plum line pattern virus	APLPV	RT-PCR	Scott and Zimmerman, 2001
Apple chlorotic leafspot virus	ACLSV	IC-RT-PCR RT-PCR  RT-PCR (multiplex)	Candresse et al., 1995 Nemchninov et al., 1995 Kinard et al., 1996 Menzel et al., 2002
Apple stem grooving virus	ASGV	RT-PCR  IC-RT-PCR RT-PCR-ELISA RT-PCR (multiplex)	Kinard et al., 1996 MacKenzie et al., 1997 Marinho et al., 1998 Crossley et al., 1998 Daniels et al., 1998 James, 1999 Menzel et al., 2002
Apple stem pitting virus	ASPV	IC-RT-PCR  RT-PCR  RT-PCR (multiplex)	Jelkmann and Keim-Konrad, 1997 Schwarz and Jelkmann, 1998 Nemchninov et al., 1998 Malinowski et al., 1998 Menzel et al., 2002

Apple mosaic virus	ApMV	RT-PCR RT-PCR-ELISA RT-PCR (multiplex)	Rowhani, et al., 1995 Candresse et al., 1998b Saade et al., 2000 Menzel et al., 2002
Arabis mosaic virus	ArMV	RT-PCR	MacKenzie et al., 1997 Nassuth et al., 2000
Little cherry virus-1	LChV-1	RT-PCR	Rott and Jelkmann, 2001a Vitushkina et al., 1997
Little cherry virus-2	LChV-2	RT-PCR	Rott and Jelkmann, 2001a
Cherry green ring mottle virus	CGRMV	RT-PCR	Zhang et al., 1998 Rott and Jelkmann, 2001a
Cherry leafroll virus	CLRV	RT-PCR	Rowhani et al., 1995
Cherry mottle leaf virus	CMLV	RT-PCR	James et al., 1999 James and Upton, 1999
Cherry necrotic rusty mottle virus	CNRMV	RT-PCR	Rott and Jelkmann, 2001b
Cherry raspleaf virus	CRLV	RT-PCR	James et al., 2001
Cherry virus A	CVA	RT-PCR	James and Jelkmann, 1998 Eastwell and Bernardy, 1998
Peach mosaic virus	PMV	RT-PCR	James and Upton, 1999
Plum pox virus	PPV <b>PPV-D or</b> <b>PPV-M</b> <b>PPV-C</b>	IC-RT-PCR Strain specific – RT-PCR Strain specific – RT-PCR	Wetzel et al., 1992 Candresse et al., 1998a Nemchinov and Hadidi, 1998
Prune dwarf virus	PDV	RT-PCR RT-PCR-ELISA RT-PCR (multiplex)	Parakh et al., 1995 Rowhani et al., 1998 Saade et al., 2000
Prunus necrotic ringspot virus	PNRSV	RT-PCR  RT-PCR-ELISA  Strain specific – RT-PCR RT-PCR (multiplex) IC-RT-PCR (nested)	Spiegel et al., 1996 MacKenzie et al., 1997 Candresse et al., 1998b Rowhani et al., 1998 Hammond et al., 1998 Saade et al., 2000 Helguera et al., 2001
Tomato ringspot virus	ToRSV	RT-PCR RT-PCR-ELISA	Griesbach, 1995 Rowhani et al., 1998

## ***VIROIDS***

Apple scar skin viroid	ASSVd (DAVd)	Hybridization RT-PCR RT-PCR (multiplex)	Hurt et al., 1996 Desvignes et al., 1999 Shamloul and Hadidi, 1999 Faggioli and Ragozzino, 2002 Di Serio et al., 2002
Hop stunt viroid	HSVd	RT-PCR Hybridization	Hadidi et al., 1992 Astruc et al., 1996 Romero-Durban et al., 1995 Cañizares et al., 1998 Amari et al., 2001
Peach latent mosaic viroid	PLMVd	RT-PCR Hybridization	Shamloul et al., 1995 Ambros et al., 1995 Loreti et al., 1995
Apple dimple fruit viroid	ADFVd	Hybridization RT-PCR RT-PCR (multiplex)	Di Serio et al., 1996, 2001 Faggioli and Ragozzino, 2002 Di Serio et al., 2002
Pear blister canker viroid	PBCVd	Hybridization RT-PCR	Ambros et al., 1995 Loreti et al., 1995 Faggioli and Ragozzino, 2002

## ***PHYTOPLASMAS***

Apple proliferation phytoplasma	AP	PCR	Lorenz et al., 1995 Smart et al., 1996 Carraro et al., 1998
Pear decline phytoplasma	PD	PCR	Lorenz et al., 1995 Smart et al., 1996 Carraro et al., 1998 Davies et al., 1998
European stone fruit yellows phytoplasma	ESFY	PCR	Smart et al., 1996 Carraro et al., 1998 Jarausch et al., 1998

## Woody Indicators

<i>Malus platycarpa</i>	3 rep	20°C	8 w	ACLSV, scaly bark
<i>Malus pumila</i> -Virginia Crab	3 rep	26°C	24 w	ASPV, ASGV (cut back at 12 w, keep for 12 more w)
-R 12740 7 A	3 rep	22°C	4 w	ACLSV
-Spy 227	3 rep	22-25°C	12 w	ASPV, ACLSV
-Kola, Radiant	3 rep	26°C	8 w	ASPV
<i>Malus micromalus</i> -GMAL 273	3 rep	22-26°C	12 w	ACLSV, ASGV
<i>Cydonia oblonga</i> -C 7/1, Pigwa	4 rep	22°C	5 w	ACLSV
<i>Pyronia veitchii</i> (IVP)*	3 rep	22°C	10 w	ACLSV, ASPV

\* IVP = in vitro propagated

## FIELD INDEXING

<i>M. platycarpa</i>	3 rep		2 y	ACLSV, scaly bark, <i>Platycarpa</i> dwarf
<i>Pyronia veitchii</i>	3 rep		2 y	ASPV
<i>M. pumila</i> -Virginia Crab -Spy 227 -R 12740 7 A -Lord Lambourne	3 rep 3 rep 3 rep 5 rep		3 y 2 y 2 y	ASPV, ASGV ASPV ACLSV mosaic (2y), rubbery wood (3y), flat limb (3y), chat fruit (3c)
-Gravensteiner -Stayman -Golden Delicious	3 rep 3 rep 3 rep		3 y 2 c	flat limb Stayman blotch ApMV (2y), proliferation (use root grafting or side-grafting and 5 rep. [2y]); horseshoewound, leaf pucker, star crack, rough skin, ring spot, russet ring, green crinkle, flat apple (CRLV[3c])
-Red Delicious (Red clones e.g. Starkrimson)	3 rep		3 c	ASSVd (DAVd), ADFVd

## VIRUS AND VIRUS-LIKE DISEASES OF PEAR

### LABORATORY ASSAYS

Agent / Disease	Serological Assay	Molecular Assay
ACLSV / pear ring pattern mosaic	ELISA	IC-RT-PCR RT-PCR RT-PCR (multiplex)
ASGV/ pear stem grooving	ELISA	RT-PCR IC-RT-PCR
ASPV/ pear vein yellows		IC-RT-PCR RT-PCR RT-PCR (multiplex)
ASSVd		RT-PCR Hybridization
PBCVd		RT-PCR Hybridization
Pear decline phytoplasma (PD)		PCR

### GREENHOUSE INDEXING

#### Herbaceous indicators

<i>Nicotiana occidentalis</i> 37B	3 rep	20°C	28 d	ACLSV, ASPV (vein yellows)
<i>Chenopodium quinoa</i>	3 rep	20°C	21 d	ACLSV

#### Woody indicators

<i>P. veitchii</i> <i>P. veitchii</i> (IVP)	3 rep	22°C	8 w 10 w	quince sooty ring spot, vein yellows (ASPV), ACLSV
<i>Pyrus communis</i> -Nouveau Poiteau	3 rep	22°C	10 w	pear ring pattern mosaic (ACLSV), vein yellows (ASPV), quince sooty ring spot
-Jules d'Airolles	3 rep	22°C	8 w	ASPV
<i>Malus micromalus</i> -GMAL 273	3 rep	26°C	8 w	ASGV 4 wk then cut back and 4 w more
<i>M. pumila</i> -Virginia Crab	3 rep	26°C	10 w	ASPV, ASGV
<i>C. oblonga</i> -C 7/1 Pigwa	4 rep	22°C	12 w	PBCVd
<i>Pyrus communis</i> -Fieud 37	4 rep 4 rep	22°C 22°C	5 w 12-16 w	ACLSV PBCVd

## FIELD INDEXING

<i>P. veitchii</i>	3 rep		2 y	vein yellows (ASPV), bark necrosis, pear stem grooving (ASGV), quince sooty ringspot
<i>P. communis</i>				
-Beurre Hardy	3 rep		3 y	ring mosaic (ACLSV), bark necrosis, bud drop, bark split, stony pit
-Beurre Bosc	3 rep		3 c	stony pit, freckle pit, corky pit
-Williams (Barlett)	3 rep		3 y	bark split, rough bark, blister bark (PBCVd),
-Doyenne du Comice	3 rep		2 y	bark split, bud drop
-D. du Comice*	5 rep		3 y	pear decline phytoplasma
-A 20	3 rep		3 y	ACLSV, vein yellows (ASPV), blister canker viroid (PBCVd)
-Jules d' Airoles	3 rep		2 y	vein yellows (ASPV)
<i>C. oblonga</i>				
-C 7/1 Pigwa				quince sooty ring spot, quince yellow blotch, quince stunt, ACLSV
<i>M. pumila</i>				
-Lord Lambourne	5 rep		3 y	apple rubbery wood
-Virginia Crab	3 rep		2 y	ASPV, ASGV

\*Preferably on *Pyrus ussuriensis* or *P. calleryana* rootstocks

## VIRUS AND VIRUS-LIKE DISEASES OF CHERRY

### LABORATORY ASSAYS

Agent / Disease	Serological Assay	Molecular Assay
ACLSV	ELISA	IC-RT-PCR RT-PCR
ApMV	ELISA	RT-PCR RT-PCR-ELISA
ArMV	ELISA	RT-PCR Hybridization
LChV-1, LChV-2		RT-PCR
CGRMV		RT-PCR
CIRV	ELISA	
CLRV	ELISA	RT-PCR
CMLV	ELISA	RT-PCR
CNRMV		RT-PCR
CRLV	ELISA	

CVA		RT-PCR
MLRSV	ELISA	
PetAMV	ELISA	
PPV	ELISA	RT-PCR IC-RT-PCR PPV-C specific PCR
PDV	ELISA	RT-PCR RT-PCR-ELISA
PNRSV	ELISA	RT-PCR RT-PCR-ELISA Strain specific RT-PCR
RpRSV	ELISA	
SLRSV	ELISA	
TRSV	ELISA	
TBRV	ELISA	
ToRSV	ELISA	RT-PCR Hybridization

## GREENHOUSE INDEXING

### Herbaceous Indicators

<i>C. quinoa</i>	5 rep	20°C	20 d	PNRSV, genus <i>Nepovirus</i> , ACLSV
<i>C. sativus</i>	3 rep	20°C	20 d	PNRSV, PDV
<i>N. occidentalis</i>	3 rep	20°C	28 d	CTLV

### Woody Indicators

<i>Prunus persica</i> -Seedlings GF 305 (or Elberta)	5 rep	20-25°C	8 w	PNRSV, PDV, ACLSV, European rasp leaf, CLRV, Eola rasp leaf (= stem pitting = ToRSV), SLRSV, mottle leaf (CMLV), CLRV, MLRSV
<i>Prunus tomentosa</i> -IR473 x IR474 hybrid or selfed (Cut back after 6 w and monitor for another 6 w)	3 rep	22°C	12 w	PNRSV, PDV, Eola rasp leaf (ToRSV), ACLSV, green ring mottle (CGRMV)
<i>Prunus serrulata</i> -Shirofugen	5 buds	22-26°C	8 w	PNRSV, PDV, CGRMV



<i>Prunus</i> hybrid -Shiro plum	3 rep	18°C	6 w	peach wart
<i>P. avium</i> -Bing	3 rep	18°C	8 w	CMLV, CRMV, CTLV, CRLV
<i>P. avium</i> -Sam	3 rep	18°C	8 w	CNRMV
<i>P. serrulata</i> Kwanzan	3 rep	18°C	8 w	CGRMV

### FIELD INDEXING

<i>P. serrulata</i> -Shirofugen	5 buds		6 w-1 y	PNRSV, PDV, Shirofugen stunt (bud into 1 y-old trees)
-Kwanzan	3 rep		2 y	green ring mottle (CGRMV)
<i>Prunus avium</i> -Bing	3 rep		3 y	European rasp leaf, Hungarian rasp leaf, CRLV*, rusty mottle, SLRSV, ArMV, twisted leaf, short stem, black canker, detrimental canker, cherry mottle leaf (CMLV), rosette, spur cherry
-Sam	3 rep		3 y	European rusty mottle, necrotic rusty mottle, black canker, detri- mental canker, rusty spot, little cherry disease (LCD)
-Canindex I	3 rep		3 y	LCD
<i>Prunus persica</i> -Seedlings GF 305	3 rep		3 y	ArMV

\* Do not double-bud onto Colt rootstocks as they are hypersensitive and CLRV will probably not move from the inoculum bud to the indicator.

## VIRUS AND VIRUS-LIKE DISEASES OF PEACH

### LABORATORY ASSAYS

Agent / Disease	Serological Assay	Molecular Assays
APLPV	ELISA	RT-PCR
ACLSV	ELISA	IC-RT-PCR RT-PCR
ApMV	ELISA	RT-PCR RT-PCR-ELISA
European stone fruit yellows phytoplasma		PCR
HSVd		RT-PCR Hybridization
PLMVd		RT-PCR Hybridization
PPV	ELISA	RT-PCR IC-RT-PCR PPV-D or PPV-M specific PCR
PDV	ELISA	RT-PCR RT-PCR-ELISA
PNRSV	ELISA	RT-PCR RT-PCR-ELISA IC-RT-PCR (nested)
SLRSV	ELISA	
TBRV	ELISA	
ToRSV	ELISA	RT-PCR Hybridization

### GREENHOUSE INDEXING

#### Herbaceous Indicators

<i>C. quinoa</i>	5 rep	20°C	20 d	PNRSV, ACLSV, genus <i>Nepovirus</i>
<i>C. sativus</i>	3 rep	20°C	20 d	PNRSV, PDV

## Woody Indicators

<i>P. persica</i> -Seedlings GF 305 (or Elberta)	5 rep	20°C	8 w	PNRSV, PDV, ACLSV, PPV, ApMV yellow bud mosaic (ToRSV), stem pitting (ToRSV), willow leaf rosette, enation, asteroid spot, calico, purple mosaic, mottle, yellow mottle, blotch, oil blotch, mule's ear, apricot chlorotic leaf roll phytoplasma, seedling chlorosis, star mosaic, mosaic, yellows, rosette, PLMVd (cross protection test), yellow mosaic, MLRSV, stocky prune, SLRSV, PYLR, American plum line pattern virus, European stone fruit yellows,
	5 rep	20°C	12 w	
<i>P. tomentosa</i> -IR473 x IR474 hybrid	3 rep	22°C	12 w	PNRSV, PDV, ACLSV, PPV, yellow bud mosaic (ToRSV), stem pitting (ToRSV)
<i>P. serrulata</i> -Shirofugen -Shirofugen (IVP)	5 buds or chips 3 rep	22-26°C 22°C	4 w 15 w	PNRSV, PDV green ring mottle (CGRMV), mottle leaf (CMLV)
<i>Prunus</i> hybrid -Shiro plum	3 rep	18°C	6 w	peach wart
<i>P. serrulata</i> -Kwanzan	3 rep	18°C	8 w	CGRMV
<i>P. avium</i> -Bing	3 rep	18°C	8 w	CMLV

## FIELD INDEXING

<i>P. persica</i> -Seedlings GF 305 (or Elberta)	3 rep		4 y	same diseases as in the greenhouse and: phony peach, X disease phytoplasma, peach wart
<i>P. serrulata</i> -Shirofugen -Kwanzan	5 buds 3 rep		6 w- 1y 2 y	PNRSV, PDV green ring mottle
<i>P. avium</i> - Sam	3 rep		2 y	necrotic rusty mottle

<i>Prunus armeniaca</i> -Tilton	3 rep		2 y	apricot ring pox (possibly cherry twisted leaf)
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## VIRUS AND VIRUS-LIKE DISEASES OF PLUM

### LABORATORY ASSAYS

Agent / Disease	Serological Assays	Molecular Assays
APLPV	ELISA	RT-PCR
ACLSV	ELISA	IC-RT-PCR RT-PCR
ApMV	ELISA	RT-PCR RT-PCR-ELISA
European stone fruit yellows phytoplasma		PCR
HSVd		RT-PCR Hybridization
MLRSV	ELISA	
PPV	ELISA	IC-RT-PCR RT-PCR PPV-D or PPV-M specific PCR
PDV	ELISA	RT-PCR RT-PCR-ELISA
PNRSV	ELISA	RT-PCR RT-PCR-ELISA
ToRSV	ELISA	RT-PCR Hybridization

### GREENHOUSE INDEXING

#### Herbaceous indicators

<i>C. quinoa</i>	5 rep	20°C	20 d	PNRSV, ACLSV, genus <i>Nepovirus</i> ,
<i>C. sativus</i>	3 rep	20°C	20 d	PNRSV, PDV, ApMV
<i>N. benthamiana</i>	3 rep	20°C	28 d	PPV

## Woody indicators

<i>P. persica</i> -Seedlings GF 305	5 rep	20°C	8 w	PNRSV, PDV, ACLSV, PPV, SLRSV, pseudopox (ACLSV), European plum line pattern, ApMV, American plum line pattern (APLPV), ToRSV, MLRSV, European stone fruit yellows phytoplasma
<i>P. tomentosa</i> -IR473 x IR474 hybrid	3 rep	22°C	12 w	PNRSV, PDV, ACLSV, bark split (ACLSV), pseudopox (ACLSV), stem pitting (ToRSV), PPV
<i>P. serrulata</i> -Shirofugen	5 buds	22-26°C	4 w	PNRSV, PDV
<i>Prunus</i> hybrid -Shiro plum	3 rep	18°C	6 w	peach wart

## FIELD INDEXING

<i>P. serrulata</i> -Shirofugen -Kwanzan	5 buds 3 rep		6 w-1 y 2 y	PNRSV, PDV green ring mottle
<i>Prunus</i> hybrid -Shiro plum	3 rep		2 y	APLPV
<i>P. domestica</i> -Ersinger -Prune d'Ente 707	3 rep 3 rep		2 y 2 y	European plum line pattern, ApMV bark split, ACLSV
<i>P. persica</i> -Seedlings GF 305	3 rep		2 y	apricot chlorotic leaf roll phytoplasma

## VIRUS AND VIRUS-LIKE DISEASES OF APRICOT

### LABORATORY ASSAYS

Agent / Disease	Serological Assay	Molecular Assay
ACLSV	ELISA	IC-RT-PCR RT-PCR
ApMV	ELISA	RT-PCR RT-PCR-ELISA
European stone fruit yellows phytoplasma		PCR
PPV	ELISA	IC-RT-PCR RT-PCR PPV-D or PPV-M specific PCR
PDV	ELISA	RT-PCR RT-PCR-ELISA
PNRSV	ELISA	RT-PCR RT-PCR-ELISA

## GREENHOUSE INDEXING

### Herbaceous Indicators: Not Reliable

### Woody Indicators

<i>P. persica</i> -Seedlings GF 305 (or Elberta)	5 rep 5 rep	20°C 20°C	8 w 12 w	PNRSV, PDV, ACLSV, PPV, SLRSV MLRSV, ApMV, mosaic, ring pox, pucker leaf, chlorotic leaf mottle, asteroid spot, European stone fruit yellows phytoplasma
<i>P. tomentosa</i> -IR473 x IR474 hybrid	3 rep	22°C	12 w	PNRSV, PDV, ACLSV, PPV, CTLV (apricot ring pox) (26°C)
<i>P. serrulata</i> -Shirofugen	5 buds	22-26°C	4 w	PNRSV, PDV
<i>P. armeniaca</i> -Tilton	3 rep	26°C	4 w	CTLV (apricot ring pox)

## FIELD INDEXING

<i>P. serrulata</i> -Shirofugen -Kwanzan	5 buds 3 rep		6 w-1 y 2 y	PNRSV, PDV green ring mottle
<i>P. armeniaca</i> -Tilton -Moorpark	3 rep 3 rep		3 y 3 y	apricot ring pox moorpark mottle, chlorotic leaf mottle, stone pitting
-Luizet or Priana	3 rep		2 y	European stone fruit yellows phytoplasma
-Wenatchee	3 rep		3 y	apricot ring pox
<i>P. persica</i> -Seedlings GF 305	3 rep		2 y	European stone fruit yellows phytoplasma

## VIRUS AND VIRUS-LIKE DISEASES OF ALMOND

### LABORATORY ASSAYS

Agent / Disease	Serological Assay	Molecular Assay
ACLSV	ELISA	IC-RT-PCR RT-PCR
ApMV	ELISA	RT-PCR RT-PCR-ELISA
PPV	ELISA	IC-RT-PCR RT-PCR PPV-D or PPV-M specific PCR
PDV	ELISA	RT-PCR RT-PCR-ELISA

PNRSV	ELISA	RT-PCR RT-PCR-ELISA
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### GREENHOUSE INDEXING

#### Herbaceous Indicators

<i>C. quinoa</i>	5 rep	20°C	20 d	genus <i>Nepovirus</i> ACLSV
<i>C. sativus</i>	3 rep	20°C	20 d	PNRSV, ApMV, PDV

#### Woody Indicators

<i>P. persica</i> -Seedlings GF 305	5 rep 5 rep	20°C 20°C	8 w 12 w	PNRSV, PDV, ACLSV, SLRSV, PPV, yellow bud mosaic (ToRSV), stem pitting (ToRSV), mosaic, bud failure (mule's ear)
<i>P. tomentosa</i> -IR473 x IR474 hybrid	3 rep	22°C	12 w	PNRSV, PDV, ToRSV, stem pitting (ToRSV), yellow bud mosaic (ToRSV), ACLSV, PPV
<i>P. serrulata</i> -Shirofugen	5 buds	22- 26°C	4 w	PNRSV, PDV

### FIELD INDEXING

<i>P. serrulata</i> Shirofugen	5		6 w-1 y	PNRSV, PDV
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## VIRUS AND VIRUS-LIKE DISEASES OF WALNUT

### LABORATORY ASSAYS

Agent / Disease	Serological Assay	Molecular Assay
CLR V	ELISA	RT-PCR

### GREENHOUSE INDEXING

<i>C. quinoa</i>	5 rep	20°C	20 d	CLR V
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**FIELD INDEXING**

<i>Juglans regia</i> grafted onto <i>J. hindsii</i> or onto <i>J. hindsii</i> x <i>J. regia</i> cv. Paradox	3 rep		3 inoculum batches just above union, 2 y	Black line (CLRv)
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**VIRUS AND VIRUS-LIKE DISEASES OF CHESTNUT**

**GREENHOUSE INDEXING**

Hybrid Maraval Ca74 (IVP)			12 w	mosaic
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**REMARKS**

- In general, *Chenopodium quinoa* and *Cucumis sativus* can be used as indicators to detect the following viruses in fruit trees:

*Chenopodium quinoa*: ACLSV, ASGV, ToRSV, TRSV, ArMV, CIRV, CLRv, CRLV, TBRV, RpRSV, SLRSV, MLRSV, TMV, TNV, PetAMV, PNRSV, and TBSV.

*Cucumis sativus*: PNRSV, PDV, CLRv, SLRSV, CRLV, TRSV, ToRSV, RpRSV, and TMV.

- For classification of viruses see: M.H.V. Van Regenmortel, C.M. Fauquet, D.H.L. Bishop, E.B. Carstens, M.K. Estes, S.M. Lemon, J. Maniloff, M.A. Mayo, D.J. McGeoch, C.R. Pringle, and R.B. Wickner. *Virus Taxonomy - Seventh Report of the International Committee on Taxonomy of Viruses*, San Diego, San Francisco, New York, Boston, London, Sydney, Tokyo: Academic Press, 2000.
- Indexing of PNRSV and PDV on *P. serrulata* Shirofugen: severe strains induce visible typical symptoms within 6 weeks. Nevertheless, it is recommended to continue observations (till following spring for trees inoculated in summer) for the detection of mild strains and for Shirofugen stunt.
- Elberta can replace GF 305 when GF 305 seeds are not available.
- Use of Bing grafted onto F 12/1 allows indexing on two indicators with the same tree.
- Mild strains of peach latent mosaic viroid can be detected by biological indexing in peach by cross protection against a severe mosaic-inducing strain (Desvignes J.C., *Acta Phytopathologica Academiae Scientiarum Hungaricae* 15: 183-190, 1980).