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OCCURRENCE OF STONE FRUIT VIRUSES IN PLUM ORCHARDS IN LATVIA

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To evaluate the occurrence of nine viruses infecting Prunus *a large-scale survey and sampling in Latvian plum orchards was carried out. Occurrence of* Apple mosaic virus *(ApMV),* Prune dwarf virus *(PDV),* Prunus necrotic ringspot virus *(PNRSV),* Apple chlorotic leaf spot virus *(ACLSV), and* Plum pox virus *(PPV) was investigated by RT-PCR and DAS ELISA detection methods. The detection rates of both methods were compared. Screening of occurrence of* Strawberry latent ringspot virus *(SLRSV),* Arabis mosaic virus *(ArMV),* Tomato ringspot virus *(ToRSV) and* Petunia asteroid mosaic virus *(PeAMV) was performed by DAS-ELISA. In total, 38% of the tested trees by RT-PCR were infected at least with one of the analysed viruses. Among those 30.7% were infected with PNRSV and 16.4% with PDV, while ApMV, ACLSV and PPV were detected in few samples. The most widespread mixed infection was the combination of PDV+PNRSV. Observed symptoms characteristic for PPV were confirmed with RT-PCR and D strain was detected. Comparative analyses showed that detection rates by RT-PCR and DAS ELISA in plums depended on the particular virus tested. The results obtained in this study revealed that commonly grown plum cultivars in Latvia are infected with economically important stone fruit viruses and highlight the need to implement a programme to produce and propagate virus-free planting material.*

Key words: Prunus *viruses, DAS-ELISA, RT-PCR, detection rates.*

INTRODUCTION

Stone fruits are hosts for a large number of viruses that can cause substantial economic losses (Nemeth, 1986; Desvignes, 1999; Myrta *et al*., 2003). Among the viruses affecting plums, *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), and *Plum Pox virus* (PPV) are the most significant with worldwide distribution. PNRSV, PDV and *Apple mosaic virus* (ApMV) are members of the genus *Ilarvirus*, family *Bromoviridae* (Roosinck *et al*., 2005). These viruses, alone or in combination, can severely affect fruit yield, fruit maturity, and tree growth (Uyemoto *et al*., 1992). *Apple chlorotic leaf spot virus* (ACLSV) (*Trichovirus*, *Betaflexiviridae*) is economically important due to its high prevalence and to the detrimental effects caused in some infected stone fruit species and rootstocks. Infection of these viruses is often symptomless in most of the stone fruit cultivars. ACLSV, together with PNRSV, may give rise to chlorotic "oak leaf" like patterns on plum leaves (Myrta *et al*., 2011).

PPV (*Potyvirus, Potyviridae*) is the causal agent of Sharka disease on *Prunus* spp. worldwide, including economically important stone fruit crops, such as peach, apricot and plum (García and Cambra, 2007). PPV is considered as the most damaging virus affecting stone fruits and causing yield losses up to 80–100% (Nemeth, 1986; Cambra *et al*., 2006). PPV is transmitted by vegetative propagation and natural vector aphid species. The use of infected plant material for propagation is the main cause of long-distance spread, whereas at a site, spread is mediated by a number of aphid species in a non-persistent manner (Labonne *et al*., 1995; Isac *et al*., 1998). PPV isolates are currently grouped into seven distinct strains, which differ in their pathogenicity, host range, serological and molecular characteristics, and geographical distribution (Glasa and Candresse, 2005; García and Cambra, 2007; Barba *et al.,* 2011). Most PPV isolates belong to the D, M and Rec types. PPV-M is mostly known in Southern, Eastern and Central Europe, while PPV-D mostly in Western Europe (Sochor *et al.*, 2012).

Other less prevalent viruses that infect *Prunus* species belong mainly to the families *Secoviridae* and *Tombusviridae*, such as *Strawberry latent ringspot virus* (SLRSV, *Sadwavirus*), *Arabis mosaic virus* (ArMV, *Nepovirus*) and *Tomato ringspot virus* (ToRSV, *Nepovirus*). *Petunia asteroid mosaic virus* (PeAMV, *Tombusvirus*) belongs to the family

Tombusviridae. These viruses are listed in the European and Mediterranean Plant Protection Organisation (EPPO) standards as a requirement to be tested in the certification scheme for cherry varieties and rootstocks (Anonymous, 2004). In *Prunus,* ToRSV can cause serious diseases including stem pitting and decline in peach and cherry, yellow bud mosaic in peach and almond and brown line and decline in plum. PeAMV infection may cause 5–30% loss of the marketable value of the fruits due to malformations with necrotic spots and sunken and circular pits (Hadidi and Barba, 2011).

Plum plantations in Latvia include cultivars derived from *Prunus domestica* L., such as traditionally grown cultivars 'Victoria', 'Julius', 'Experimentalfältets Sviskon', 'Perdrigon', 'Stanley', and the more recently established diploid cultivars derived from crosses between *P. salicina* Lindl.*, P. cerasifera* Ehrh.*, P. simonii* Carrière *and P. americana* Marshall (e.g. cultivar 'Kubanskaya Kometa') (Skrīvele *et al*., 2008; Kaufmane *et al.,* 2010). Viral diseases in stone fruit orchards were not been studied until 2008, when survey and screening on occurrence was initiated in national research programmes (Pūpola *et al*., 2010). In other previous studies on stone fruit viruses in Latvia (Turka *et al*., 1984) only visual observations have been recorded and noted as infections by ACLSV and ApMV. Since a certification system for planting material has not been established in Latvia, the risk for continuous spread of viral diseases in orchards is high.

Timely monitoring of virus infections and elimination of infected trees from orchards is a key issue in preventing the spread of viruses. For routine diagnosis, reliable, fast and inexpensive procedures are essential. Despite wide use in routine and screening diagnosis, ELISA tests may fail due to low viral titre and the inhibitory effect of woody plant sap compounds (Kinard *et al.,* 1996; MacKenzie *et al.,* 1997). Therefore, PCR based techniques, which are more sensitive and accurate, can provide an alternative for virus detection in woody plants (Kinard *et al*., 1996; Sánchez-Navarro *et al*., 2005; López *et al*., 2008; Jarošová *et al*., 2010; Çevik *et al*., 2011).

The aim of the research presented here was to determine the occurrence of nine viruses of *Prunus* in Latvian plum orchards, as well as to identify the PPV strains present and to compare the detection rates by ELISA and RT-PCR for the most significant viruses.

MATERIALS AND METHODS

Sampling and plant material. Samples of European plum (*P. domestica* L.), cherry plum (*P. cerasifera* Ehrh.), *Prunus* inter-specific hybrids and other *Prunus* species were collected in May 2008. Ten fully expanded leaves were randomly collected around the canopy from each individual tree and represented one sample. In total 654 leaf samples (one sample/one tree) from 92 different genotypes, including different cultivars, hybrids and species, were collected from 28 commercial plum orchards and germplasm collections in five regions of Latvia. The samples were collected from randomly selected trees without symptoms, except in a few cases when typical viral infection symptoms were present. Samples were placed in plastic bags in an ice box and transported to the laboratory where they were immediately analysed or frozen in liquid nitrogen and stored at –80 °C until use. Before analyses or preservation each leaf in the sample was divided in two portions to equally represent the sample for DAS ELISA and RT-PCR.

DAS ELISA. For the initial screening and detection of ACLSV, PPV, ApMV, ArMV, PeAMV, PDV, PNRSV, SLRSV, ToRSV commercially available DAS ELISA kits (Bioreba AG, Switzerland) were used according to the manufacturer's instructions with some modifications. The coating and conjugate conditions were changed to an overnight incubation in a refrigerator at 4–6 °C to increase sensitivity of the test. The absorbance was read at 405/492 nm with a dual filter microplate reader Asys Expert 96 (Hitech, Austria) after 30 min, 1 h and 2 h of incubation. Lyophilised samples supplied in the kit for each virus were used as positive and negative controls. A "cut-off" value was calculated according to manufacturer recommendations (Bioreba AG, Switzerland).

RNA extraction. For total RNA isolation, collected leaves were ground in liquid nitrogen until fine powder and approximately 100 mg of each sample were suspended in 200 µl of TE buffer. The extraction of RNA was carried out with the Genomic DNA Purification Kit (Thermo scientific, Lithuania) following the manufacturer's recommendations with minor modifications adapted for RNA isolation. Nucleic acid precipitation was done with cold 96% ethanol overnight at –20 °C instead of 10 min at –20 °C. After extraction DNase I (Thermo Scientific, Lithuania) was used to obtain DNA free RNA. The quantity and quality of the RNA was measured using a spectrophotometer NanoDropR ND-1000 (Thermo Scientific, USA). RNA was directly used for RT-PCR assays or stored at –80 °C until analysis.

RT-PCR. RT-PCR assay was applied for detection of ACLSV, ApMV, PDV, PNRSV and PPV. RT-PCR assays were carried out with a One-Step RT-PCR kit (Qiagen AG, Germany) in 50-µl reactions according to the manufacturer's instructions. All samples for ACLSV and the majority of the samples for the other viruses were tested twice. Primers used in this study are presented in Table 1. RT-PCR was carried out in a Mastercycler® thermocycler (Eppendorf AG, Germany). Reverse transcription was carried out 30 min at 50 ^{o}C, activation of the HotStart Taq DNA polymerase was at 95 ^{o}C for 15 min, followed by 40 cycles of 94 ^{o}C for 30 s, 55 ^{o}C for 45 s, 72 ^{o}C for 1 min, and a final extension step at 72 ^{o}C for 10 min. Positive samples for each virus repeatedly detected by DAS ELISA were selected and used as positive controls in RT-PCR. PCR grade water was used as a quality control. PCR products were separated by electrophoresis in 2% agarose gel in 1x TAE buffer, stained with ethidium bromide, and visualised under UV light. Approximate sizes of RT-PCR products

Table 1

LIST OF THE PRIMERS USED IN THIS STUDY AND EXPECTED SIZES OF RT-PCR PRODUCTS FOR EACH PRIMER PAIR

Primer	Sequence 5'to 3'	Amplicon size, bp	Reference
ApMVs	CGTAGAGGAGGACAGCTTGG	450	Hassan *et al*., 2006
ApMVa	CCGGTGGTAACTCACTCGTT		
ACLSVs	TTCATGGAAAGACAGGGGCAA	677	Menzel *et al*., 2002
ACLSVa	AAGTCTACAGGCTATTTATTATAAGTCTAA		
PDV-17F	CGAAGTCTATTTCCGAGTGGATGC	303	Massart *et al.*, 2008
PDV-12R	CACTGGCTTGTTTCGCTGTGAAC		
PNRSV-10F	TTCTTGAAGGACCAACCGAGAGG	348	Massart *et al.*, 2008
PNRSV-10R	GCTAACGCAGGTAAGATTTCCAAGC		
P1	ACCGAGACCACTACACTCCC	243	Wetzel *et al.*, 1991b
P2	CAGACTACAGCCTCGCCAGA		
PM*	CTTCAACAACGCCTGTGCGT	198	Olmos *et al*., 1997
PD*	CCTCAACGACACCCGTACGG		

* Used as a reverse primer in pair with P1 primer.

were determined with an O'RangeRuler 100 bp DNA ladder (Thermo scientific, Lithuania).

Data analyses. The data were analysed by Fisher's exact test using SPSS 15.0 computer software (SPSS Inc. USA). The two-tailed Fishers's exact test was used to determine significance at $P < 0.05$ of the associations between variables. The occurrence of viruses was calculated as percentage of positive samples out of the total tested samples. The significance level of virus occurrence among cultivars was calculated by mean value (μ) ± standard deviation (σ).

The part of the data presented here on detection of ApMV, PDV, PNRSV by DAS ELISA and of ACLSV by RT-PCR has been previously reported by Pūpola *et al.* (2010; 2011).

RESULTS

Detection of stone fruit viruses. Based on DAS ELISA and RT-PCR diagnosis, all of the studied viruses were detected in plum orchards. The most widespread stone fruit viruses in plum trees were PNRSV and PDV. The occurrence of stone fruit viruses in plums tested by both diagnostic methods is shown in Figure 1. The other viruses tested by DAS ELISA were detected only in a few cases. ArMV, ToRSV and SLRSV were detected in 0.6% of the trees and PeAMV in 0.5% of the analysed samples. During the orchard surveys no obvious symptoms were observed on plum trees, except in a few cases when characteristic chlorotic rings, necrotic spots and shot holes of PPV infection on plum leaves were observed (Fig. 2). The observed PPV symptoms were confirmed by RT-PCR and in all positive samples only the PPV-D strain was detected by use of strain-specific primers.

Positive analyses by RT-PCR resulted in almost a two times higher number for PNRSV and PDV than those obtained by DAS-ELISA (Fig. 1). In contrast, for ApMV, ACLSV and PPV, the detection rate with DAS ELISA was slightly higher and the data obtained by both methods were in concordance for these viruses. In total, 38% of the tested samples by RT-PCR and 24% by DAS ELISA were detected positive for at least one of the tested viruses. Moreover, when DAS ELISA and RT-PCR results were directly compared one by one, not all samples that tested positive by ELISA were detected positive by RT-PCR and *vice versa*.

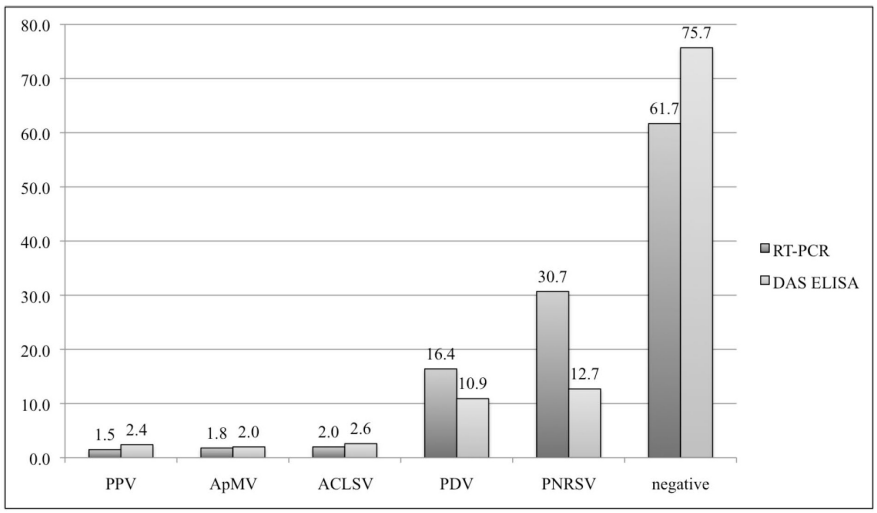

Fig. 1. Occurrence (%) of stone fruit viruses in plums in Latvia assessed with one step RT-PCR and DAS ELISA. ACLSV, *Apple chlorotic leaf spot virus*; PDV, *Prune dwarf virus*; PNRSV, *Prunus necrotic ringspot virus*; ApMV, *Apple mosaic virus*; PPV, *Plum pox virus*.

Fig. 2. Chlorotic rings and necrotic spots characteristic for *Plum pox virus* infection on plum hybrid 1256E.

Direct comparison of positive and negative detection results obtained by DAS ELISA and RT-PCR are presented in Table 2.

Occurrence of mixed infections. Mixed infections were detected in 13.8% of the tested plum samples. The most common co-infection was PNRSV with PDV (10.9%). In few cases ApMV was detected in combination with PDV (0.5%), ApMV with PNRSV (1.2%) and ACLSV with PNRSV (1.2%). Mixed infections of three viruses were detected only in few samples (0.2–0.3%). The detected combinations of three viruses were PNRSV+PDV+ACLSV, PDV+ApMV+PNRSV or PPV+ACLSV+PNRSV. Single-virus infections were detected approximately in one-third of the tested plum samples. Single infection with PNRSV was predominant (17.4%) in comparison to other infections and co-infections. Among the other tested viruses, mainly only one virus was detected in the samples, except in cultivar 'Kubanskaya Kometa' where infection with ArMV, ToRSV, SLRSV and PeAMV was detected. In cultivar 'Skoroplodnaya' ArMV, SLRSV and PeAMV were detected by DAS ELISA.

Occurrence of viruses in relation to orchards and geographical regions. The most widespread virus, PNRSV, was detected in 16 out of 28 surveyed plum orchards. The virus was detected in all regions, but its occurrence was significantly higher in the eastern part of Latvia. Data on occurrence of stone fruit viruses in different geographical regions in Latvia are shown in Table 3. A high PNRSV occurrence was detected in all regions of Latvia, except in the western part, where PDV was the predominant virus. PDV was detected in 13 out of 28 surveyed orchards and

Table 2

DIRECT COMPARISONS AND CONFORMITY OF POSITIVE AND NEGATIVE RESULTS OBTAINED WITH DAS ELISA AND RT-PCR

Virus	Method				Conformity	
	ELISA*		RT-PCR*		%**	
	positive	negative	positive	negative	positive	negative
ACLSV	17	637	13	641	76.5	98.9
PDV	71	583	107	547	66.4	93.8
PNRSV	83	571	201	453	41.3	79.3
ApMV	13	641	12	642	92.3	99.8
PPV	16	638	10	644	62.5	99.1

* Number of samples.

** The conformity of positive and negative results obtained by DAS ELISA and RT-PCR, calculated in percentages.

ACLSV, *Apple chlorotic leaf spot virus*; PDV, *Prune dwarf Virus*; PNRSV, *Prunus necrotic ringspot virus*; ApMV, *Apple mosaic virus*; PPV, *Plum pox virus.*

Table 3

OCCURRENCE OF VIRUSES IN PLUMS IN THE GEOGRAPHICAL REGIONS OF LATVIA

Region	Total number of samples	Number of positive samples, %**				
		ApMV	PDV	PNRSV	PPV	ACLSV
Western part	52	2	40.4	30.8	0.0	2.0
Eastern part	149	4.7	43.6	**51.0***	2.0	1.3
Southern part	195	2.1	0.5	26.7	0.5	**2.6***
Northern part	69	0.0	2.9	7.2	4.3	1.4
Central part	189	0.0	9.5	27.5	1.6	2.1

* Indicates significant difference among the geographical regions by Fishers exact test ($P < 0.05$)

** Detected with RT-PCR

Abbreviations as in Table 2.

found in all regions. ApMV was not found in the northern and central parts and was detected only in four orchards. PPV was not detected in the western part and was found in three out of 28 surveyed orchards; its occurrence in general was low. ACLSV was found in six orchards and its occurrence was significantly higher in the southern part of the country than in the rest. SLRSV was detected in samples from four orchards in the central and western parts, ToRSV in four orchards in central and southern parts, ArMV in three orchards in central, eastern and western parts, and PeAMV was detected in two orchards in the central and southern parts of the country.

Occurrence of viruses in relation to host genotypes. Among different plum genotypes a high variability in the occurrence of the viruses was observed (Table 4). PNRSV was detected in almost all commonly grown plum genotypes, except in the case of Estonian origin cultivar 'Julius'. Cultivars 'Aleynaya', 'Perdrigon' and *P. cerasifera* Ehrh, were found significantly more infected with PNRSV than the other plum genotypes. Similarly, cultivars 'Aleynaya',

Table 4

THE OCCURRENCE OF STONE FRUIT VIRUSES IN MOST COMMONLY GROWN PLUM GENOTYPES

Genotype	Total number of samples	Number of infected samples (%)**				
		PDV	ApMV	ACLSV	PNRSV	PPV
Aleynaja	12	41.7*	0	0	91.7*	0
Ave	14	0	0	7.1	14.3	0
Āženas***	11	9.1	0	18.2*	9.1	0
Latvijas Dzeltenā Olplūme	31	21.2	3.0	0	36.4	0
Prince of Wales	16	6.3	6.3	0	25.0	0
Experimentalfältets Sviskon	24	20.8	0	16.7*	25.0	0
Jubileum	10	40.0*	0	0	40.0	0
Juliu	19	5.3	0	0	0	0
Kārsavas	9	0	33.3*	0	44.4	0
Kubanskaya Kometa	64	20.3	1.6	1.6	23.4	0
Lāse	28	0	3.6	3.6	28.6	3.58
Mirabelle de Nancy	9	22.2	11.1	0	44.4	0
Perdrigon	15	6.7	0	6.7	60.0*	13.3*
Prunus cerasifera Ehrh.	9	55.6*	11.1	0	55.6*	0
Skoroplodnaya	31	19.4	0	0	16.1	0
Stanley	18	5.6	0	0	16.7	0
Tragedy	11	0	0	0	18.2	0
Reine Claude d'Oullins	19	0	0	0	42.1	0
Victoria	56	21.4	0	3.6	21.4	0
Mean		15.6	3.7	3.0	32.2	0.89

* Indicates significantly higher occurrence over mean value among most common plum genotypes ($\mu+\sigma$).

** Detected with RT-PCR.

*** Cultivar of unknown origin is grown under this name in Latvia (Kārkliņš *et al*., 2007).

Abbreviations as in Table 2

'Jubileum' and *P. cerasifera* Ehrh were found significantly more infected with PDV. PDV was less spread in plum genotypes than PNRSV and it was not detected in several local and introduced cultivars, such as 'Ave', 'Kārsavas', 'Lāse', 'Tragedy' and 'Reine Claude d'Oullins'. The occurrence of ApMV, ACLSV and PPV was rather low in all common plum cultivars, except in the cases of cultivars 'Kārsavas', 'Āženas' and 'Experimentalfältets Sviskon'. PPV was detected only in two cultivars among the most common plum genotypes in the orchards and in the introduced cultivar 'Perdrigon' its occurrence was significantly higher.

The other tested viruses by DAS ELISA were detected in a limited number of genotypes. They were detected in the introduced cultivars 'Prince of Wales' (SLRSV), 'Kubanskaya Kometa' (SLRSV, ToRSV, ArMV and PeAMV), 'Skoroplodnaya' (SLRSV, ToRSV and ArMV), 'Washington' (ToRSV), 'Reine Claude d'Oullins' (ArMV) and in one local cultivar 'Lāse' (PeAMV).

DISCUSSION

The obtained results confirmed the presence of all tested viruses in plum orchards in Latvia and showed that the majority of commonly grown cultivars are infected either with PNRSV, PDV, ApMV, PPV and ACLSV or with their combinations at various degrees. ArMV, PeAMV, SLRSV and ToRSV were also detected in few cases by DAS ELISA. Although plums are known as a host of these viruses in Europe (Lovisolo, 1990; Diekmann and Putter, 1996; Kommineni *et al*., 1998), they are not listed in EPPO standards as requirements to be tested in certification schemes of plums. Although detection results of these viruses are indicative and need to be further verified with another test method, e.g. RT-PCR, possibly they also need to be considered in certification schemes of plums to prevent their further spread.

In general, when compared to the occurrence of viral diseases in apple and pear in Latvia (Pūpola *et al.*, 2011), the occurrence of viruses in plum orchards was relatively low, as revealed in this study. The most widespread viruses detected in plum orchards were PNRSV and PDV. Both viruses are seed and pollen transmitted (Desvignes, 1999). In Latvia mainly seedlings of *P. cerasifera* Ehrh are used as rootstocks for plum propagation (Kārkliņš *et al.*, 2007). Our data on the occurrence of stone fruit viruses in different plum genotypes revealed that more than 50% of the tested *P. cerasifera* trees are infected with PNRSV and/or PDV. This indicates that most likely the main sources of inoculum for the spread of PNRSV and PDV in plum cultivars have probably been infected rootstocks.

Although in other countries PDV is reported as the most common *Prunus* virus (Gümüș, 2007; Suchá, 2010), in our study on plums PNRSV was the most widespread. However, according to the data from other studies, the occurrence of PDV and PNRSV in different regions is highly variable, ranging from 0.4% to 17% for PDV (Domínguez *et al.,* 1998; Jarrar *et al.,* 2001; Herrera and Madariaga, 2002) and 6% to 46% for PNRSV (Domínguez *et al.,* 1998; Jarrar *et al*. 2001; Herrera and Madariaga, 2002; Myrta *et al.,* 2002).

The graft-transmitted viruses ACLSV and ApMV were not commonly detected, however, some of the plum cultivars showed more prevalence of these viruses than others. The local cultivar 'Kārsavas' frequently appeared to be infected by ApMV. The Swedish origin cultivar 'Experimentalfältets Sviskon' and an unknown cultivar grown under the name of 'Āženas', showed a high ACLSV prevalence, suggesting that the original source of the material was probably already infected when introduced and that ApMV and ACLSV spread by the use of this infected propagation material.

Although PPV has spread throughout several European countries (Sochor *et al*., 2012), our study revealed that PPV is not widely spread in Latvia and is currently present only in a limited number of genotypes and geographical localities. One of the main reasons for the limited spread of PPV in plum orchards in Latvia could be the low prevalence of aphid species in Northern European countries (Verhoeven *et al.,* 1998; Blystad and Munthe, 2006; Wijkamp and Gaag, 2011). Most likely, the entry and establishment of the virus occurred by infected bud-wood as suggested by the case of the Western European origin cultivar 'Perdrigon', which is one of the oldest introductions in Latvia (Kārkliņš *et al.*, 2007). Moreover, in our study one of the most common PPV strains in Western Europe, the strain PPV-D, was detected in all of the PPV positive samples. The most recently discovered strain PPV-W (Winona), and also PPV-D, have been reported in Latvia (Glasa *et al*., 2011; Malinowski *et al*., 2012). Our data confirm that actions should be taken and eradication continued to prevent further spread of this detrimental viral disease, which currently is not widely spread in the country.

Mixed infections of two or more viruses have frequently been reported in stone fruit trees in different regions of the world (Mytra *et al*., 2003; Çevik *et al.*, 2011). In our study mixed infections of two or three viruses were detected in less than half of the positive samples. The combination of three viruses also occurred, but it was not common. The most commonly detected mixed infection was the combination of PNRSV with PDV as a logical consequence of their transmission modes and of their wide distribution among the rootstock species sources and orchards.

Comparison of DAS ELISA and RT-PCR as diagnostic methods for detection of economically important stone fruit viruses in plums resulted in considerable differences only for PNRSV and PDV. For PNRSV and PDV, RT-PCR was more effective and detected more positive samples. For ACLSV, ApMV and PPV results obtained by both methods were in agreement with previously reported comparisons between ELISA and RT-PCR methods for PPV detection in terms of specificity and sensitivity (Capote *et al*., 2009; Vidal *et al*. 2012a). Our results agree with data obtained in other studies (Sánchez-Navarro *et al*., 1998; 2005; Çevik *et al.*, 2011) suggesting that RT-PCR seems to be more effective than ELISA, giving more positive reactions for the detection of PNRSV and PDV. However, in direct comparisons one by one, not all positive ELISA samples were confirmed with RT-PCR and *vice versa,* probably due to the presence of both false positives and negatives. Our study confirms that the use of only one diagnostic method is not reliable and should be treated with caution due to the drawbacks of both methods, as discussed by Vidal *et al*., (2012a and b). As pointed out by MacKenzie *et al*. (1997) and Capote *et al*. (2009), despite the potentially higher sensitivity and specificity of PCR than ELISA, several factors, such as PCR inhibitors, RNA purity and primer specificity can influence diagnostic performance and could give false-negative results. Similarly, the sensitivity of ELISA can be affected by the low concentration of viruses in host plant and the sampling time in the season (Torrance and Dolby, 1984; Desvignes, 1999).

Our study demonstrated that stone fruit viruses are relatively not widespread in plum orchards in Latvia and that the commonly grown genotypes are infected with one or more of the tested viruses. In the future, the implementation of a programme to produce and propagate virus free planting material and the establishment of virus free planting material collections and certification programmes, which are currently not established in the country, will play the key role for the containment of the spread of these viruses in the orchards.

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KAULEŅKOKU VĪRUSU IZPLATĪBA PLŪMJU STĀDĪJUMOS LATVIJĀ

Veikts pētījums, lai izvērtētu tādu deviņu vīrusu, kuri ir sastopami *Prunus* ģints augiem, izplatību plūmju stādījumos Latvijā. Ābeļu mozaīkas vīrusa (ApMV), plūmju pundurainības vīrusa (PDV), kauleņkoku nekrotiskās gredzenplankumainības vīrusa (PNRSV), ābeļu hlorotiskās lapu plankumainības vīrusa (ACLSV) un plūmju virālo baku vīrusa (PPV) diagnostika veikta, izmantojot RT-PCR and DAS ELISA noteikšanas metodes, kā arī veikts šo abu metožu salīdzinājums. Zemeņu latentā gredzenplankumainības vīrusa (SLRSV), *Arabis* mozaīkas vīrusa (ArMV), tomātu gredzenplankumainības vīrusa (ToRSV) un petūniju asteroīdās mozaīkas vīrusa (PeAMV) diagnostika veikta, izmantojot vienīgi DAS ELISA testu. Kopumā 38% no visiem ar RT-PCR pārbaudītajiem paraugiem bija inficēti vismaz ar vienu no testētajiem vīrusiem. No tiem 30.7% bija inficēti ar PNRSV un 16.4 ar PDV, savukārt ApMV, ACLSV un PPV diagnosticēti nelielā skaitā paraugu. Visbiežāk sastopamā vairāku vīrusu infekcija bija PNRSV kombinācijā ar PDV. Novērotie raksturīgie PPV simptomi tika apstiprināti laboratoriski ar RT-PCR un visos pozitīvajos paraugos noteikts PPV-D celms. Metožu salīdzinājums parādīja, ka pielietoto RT-PCR un DAS ELISA metožu efektivitāte vīrusu noteikšanai plūmju paraugos atkarīga no nosakāmā vīrusa. Pētījuma rezultātā noteikts, ka plašāk audzētās plūmju šķirnes ir inficētas ar ekonomiski nozīmīgiem kauleņkoku vīrusiem un norāda uz nepieciešamību ieviest vīrusbrīva stādāmā materiāla audzēšanas sistēmu, lai novērstu šo vīrusu tālāku izplatīšanos augļu dārzos.