

INTERNATIONAL SCIENTIFIC CONFERENCE  
„Sustainable Fruit Growing:  
From Plant to Product”

# Occurrence of viruses in apple and pear orchards in Latvia.

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The economically important virus diseases of apple and pear trees worldwide are Apple mosaic ilarvirus (ApMV), Apple chlorotic leaf spot trichovirus (ACLSV), Apple stem grooving capillovirus (ASGV) and Apple stem pitting foveavirus (ASPV).

The status of occurrence of viral diseases in orchards is not clear, since the 80s research has not been carried out on plant viruses and no certification program has been established in Latvia.

The aim of this study was to evaluate the distribution of ApMV, ASGV, ACLSV and ASPV in different fruit-tree varieties in commercial orchards and to collect virus isolates for further study.



# Apple mosaic ilarvirus

ApMV is named after the disease it causes in apple, the first host in which it was described. ApMV induces bright yellow patterns on leaves.

In previous years pear was known to be susceptible to ApMV, but it was not known as a natural host. ApMV was recently detected in pears.

At least 34 other plant species are susceptible host of ApMV, including *Fragaria*, *Prunus*, *Ribes* and *Rubus*.

The virus is graft-transmissible and it persists in propagative material, which is probably the main source of virus infection. The mechanism of transmission of ApMV is still unknown.



ApMV symptoms on 'Ausma'

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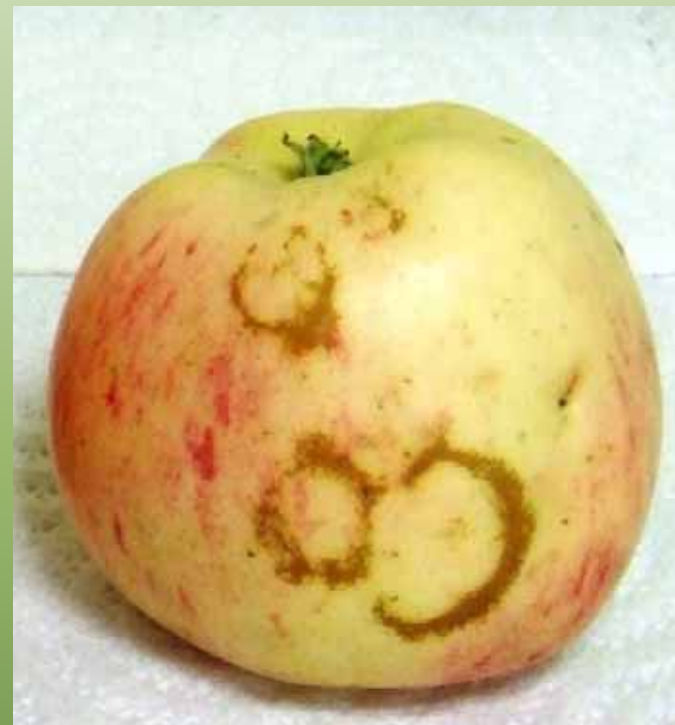


# Apple chlorotic leaf spot trichovirus

The ACLSV infect a wide range of fruit trees: apples, pears, plums and cherries.

It is the most common virus in apple and pear orchards in other countries.

Although most strains are latent, but some strains can cause russet rings on leaves and fruits.



Russet rings caused by ACLSV on 'Mārgerts Skujenieks'



# Apple stem grooving capillovirus

ASGV is distributed worldwide in apple but it also infects pear, apricot and cherry trees. Usually ASGV doesn't cause obvious symptoms but in sensitive cultivars it can develop long grooves on the woody stem.



# Apple stem pitting foveavirus

ASPV is commonly present in commercial apple cultivars worldwide. ASPV frequently occurs in combination with other viruses such as ACLSV and ASGV. ASPV cause xylem pits in the stem of sensitive cultivars. ASPV is also the causal agent of stony pits and vein yellow disease of pear. The green crinkle and star crack diseases of apple are also associated with ASPV.

Transmission of ASPV to woody host plants occurs by grafting only because a vector is not yet known.



ASPV symptoms on pear cv. 'Bergamote'

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# Materials and Methods

## Sample collection

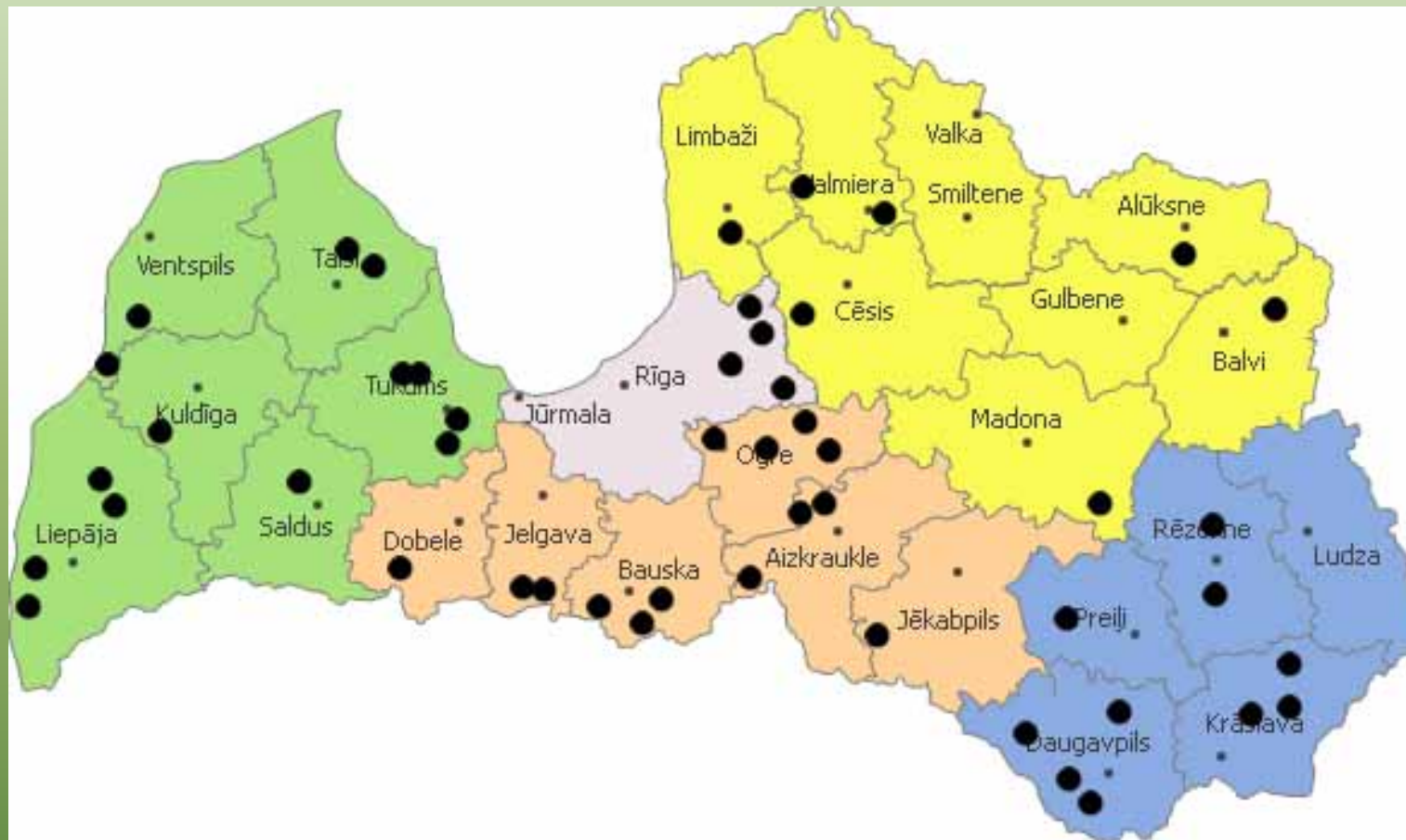
The samples were collected randomly from different cultivars from commercial orchards during June-July of 2007 for ApMV, ACLSV, ASGV and during August-September for ASPV.

Totally 818 samples from 122 apple cultivars in 50 commercial orchards and 238 samples from 47 pear cultivars in 35 commercial orchards were collected .

All collected leaf samples were analysed with ELISA and around 200 apple samples were also analysed with RT-PCR.



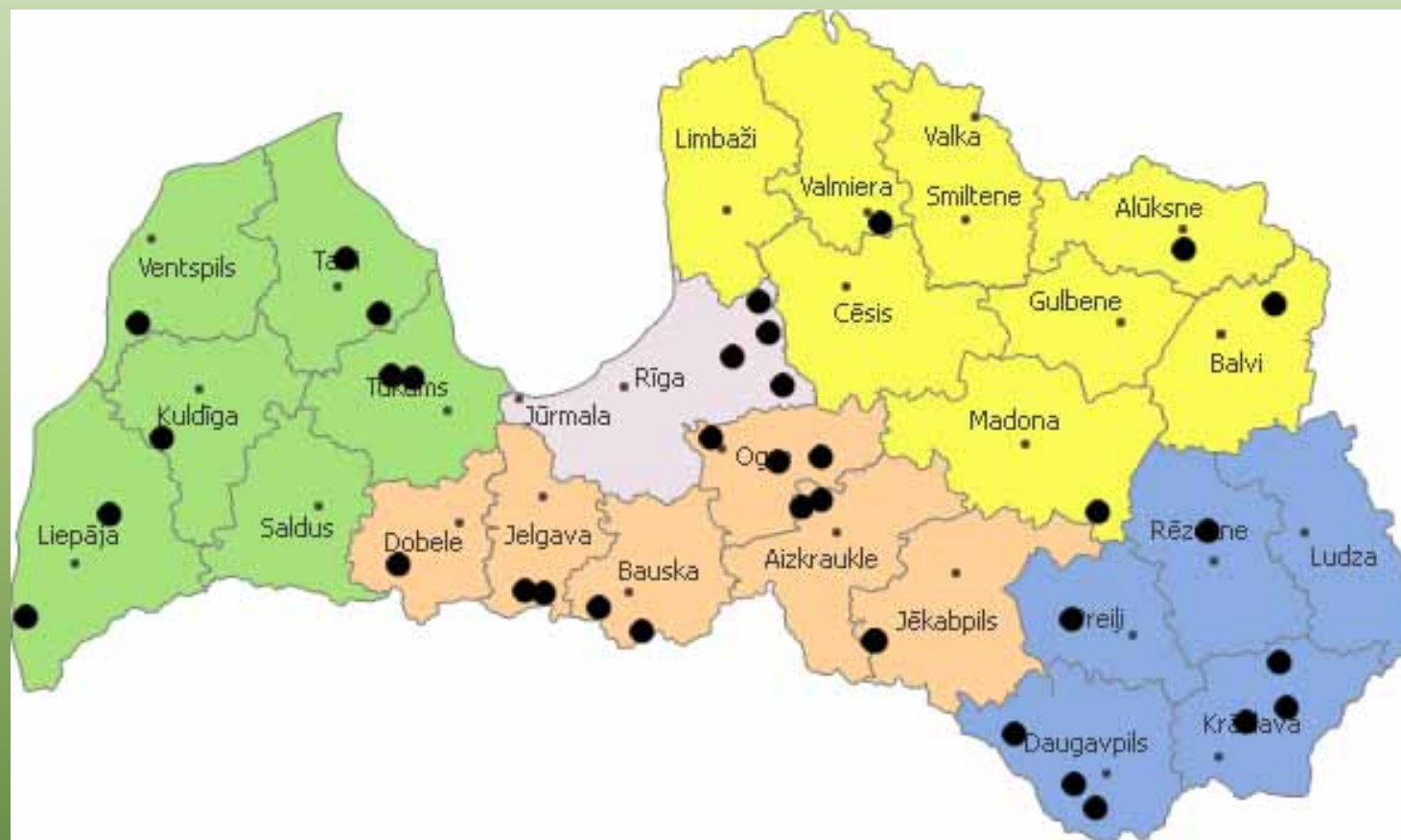
# Map of surveyed commercial apple orchards



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# Map of surveyed commercial pear orchards



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## DAS ELISA – enzyme-linked immunosorbent assay

All samples were tested by DAS ELISA for detection of ApMV, ACLSV, ASGV and ASPV, using commercial kits (Bioreba, Switzerland).

The cut-off value was determined by formula:

$$\text{Cut-off} = \text{mean OD value} + 3 S + 10\%$$

S- standard device



## Pentaplex RT-PCR (according Hassan, Myrta and Polak, 2005)

1. Total RNA extraction was carried out using Rneasy Plant Mini Kit, according to the manufacturer's instructions (Qiagen, Germany)

2. Primers:

ApMV: 5'CGT AGA GGA GGA CAG CTT GG 3' sense  
5' CCG GTG GTA ACT CAC TCG TT 3' antisense

ACLSV:5' TTC ATG GAA AGA CAG GGG CAA3' sense  
5' AAG TCT ACA GGC TAT TTA TTA TAA GTC TAA 3' antisense

ASGV: 5' GCC ACT TCT AGG CAG AAC TCT TTG AA 3' sense  
5' AAC CCC TTT TTG TCC TTC AGT ACG AA 3' antisense

ASPV: 5' ATG TCT GGA ACC TCA TGC TGC AA 3' sense  
5' TTG GGA TCA ACT TTA CTA AAA AGC ATA A 3' antisense

Nad5: 5' GAT GCT TCT TGG GGC TTC TTG TT 3' sense  
5' CTC CAG TCA CCA ACA TTG GCA TAA 3' antisense

3. The Qiagen One step RT-PCR kit (Qiagen, Germany) was used for RT-PCR assays

4. PCR products were separated by electrophoresis in 2 % agarose gels in TAE buffer



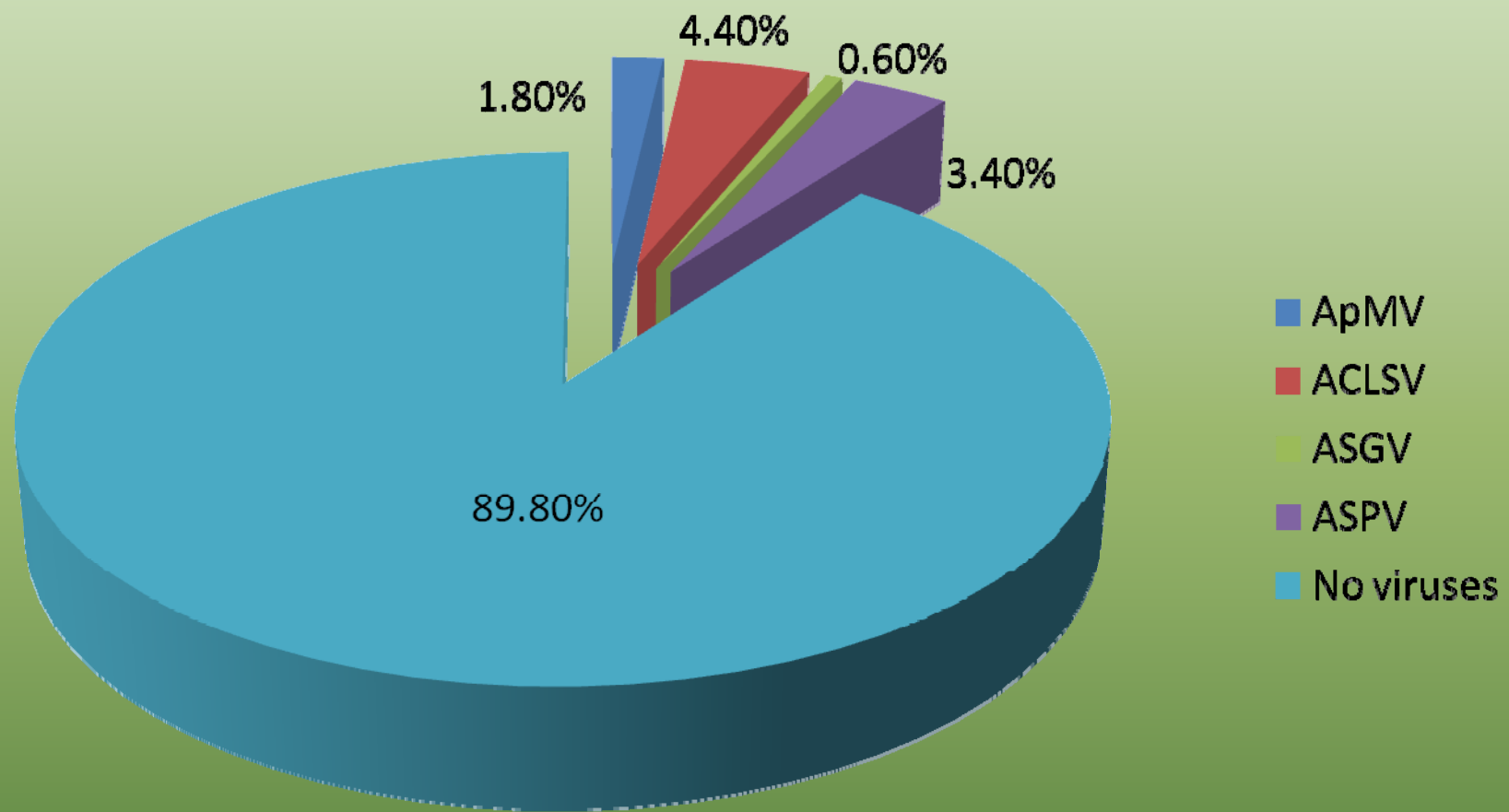
# Results

Three viruses (ACLSV, ASGV and ASPV) were detected in apple and pear orchards during the survey , but ApMV was detected only in apple trees.

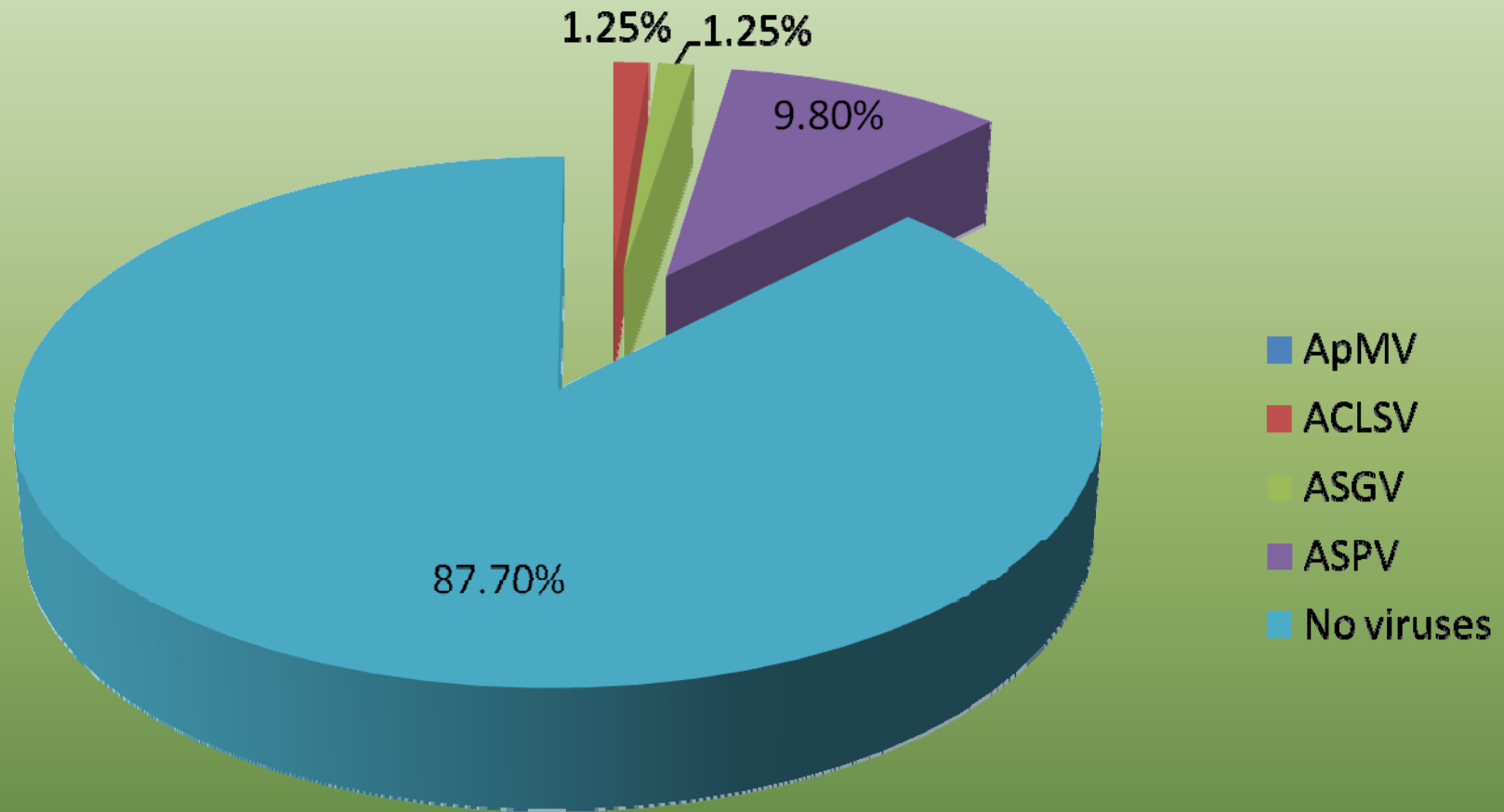
These viral diseases are spread in all apple and pear growing regions in Latvia. Most common virus in apple trees was ACLSV, but in pear trees most common was ASPV.



# The occurrence of 4 viruses in apple samples by DAS-ELISA



## The occurrence of 4 viruses in pear samples by DAS-ELISA



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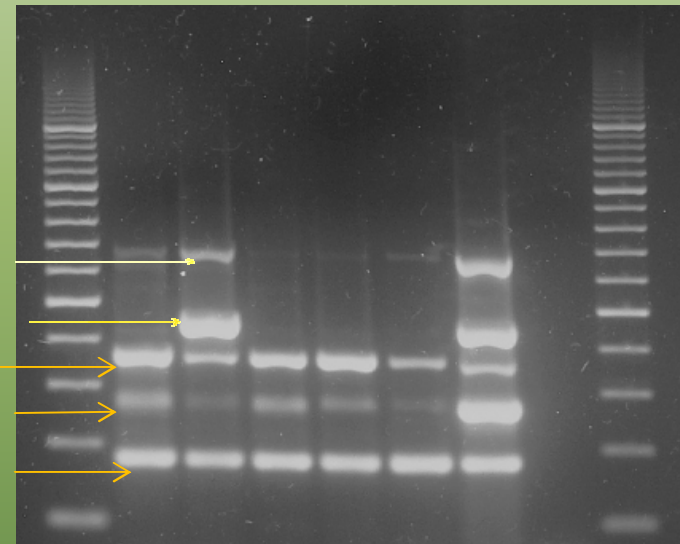
# Pentaplex RT-PCR

RT-PCR is highly specific and sensitive assay for detection of all four viruses in apple trees. Pentaplex RT-PCR allows to detect four target viruses in one assay.

Pentaplex PCR was chosen to detect particular viruses in order to save time, reagents and to reduce costs.

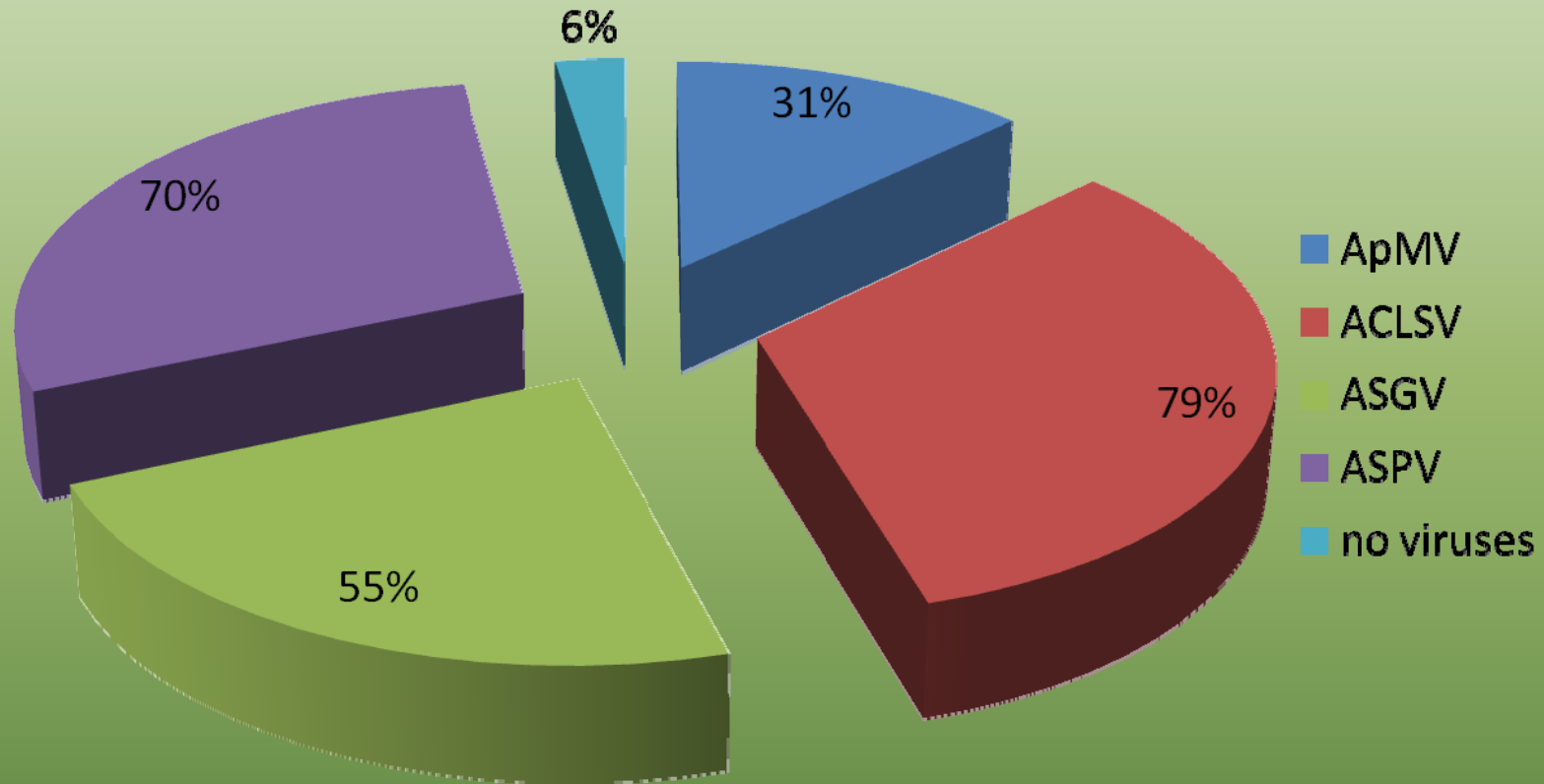
Many samples were infected with more than two viruses in different combinations. Most common combined infection was of three main apple viruses – ACLSV, ASGV and ASPV.

ACLSV 677 bp  
ApMV 450bp  
ASPV 370 bp  
ASGV 273 bp  
Nad5 181 bp



# RT-PCR

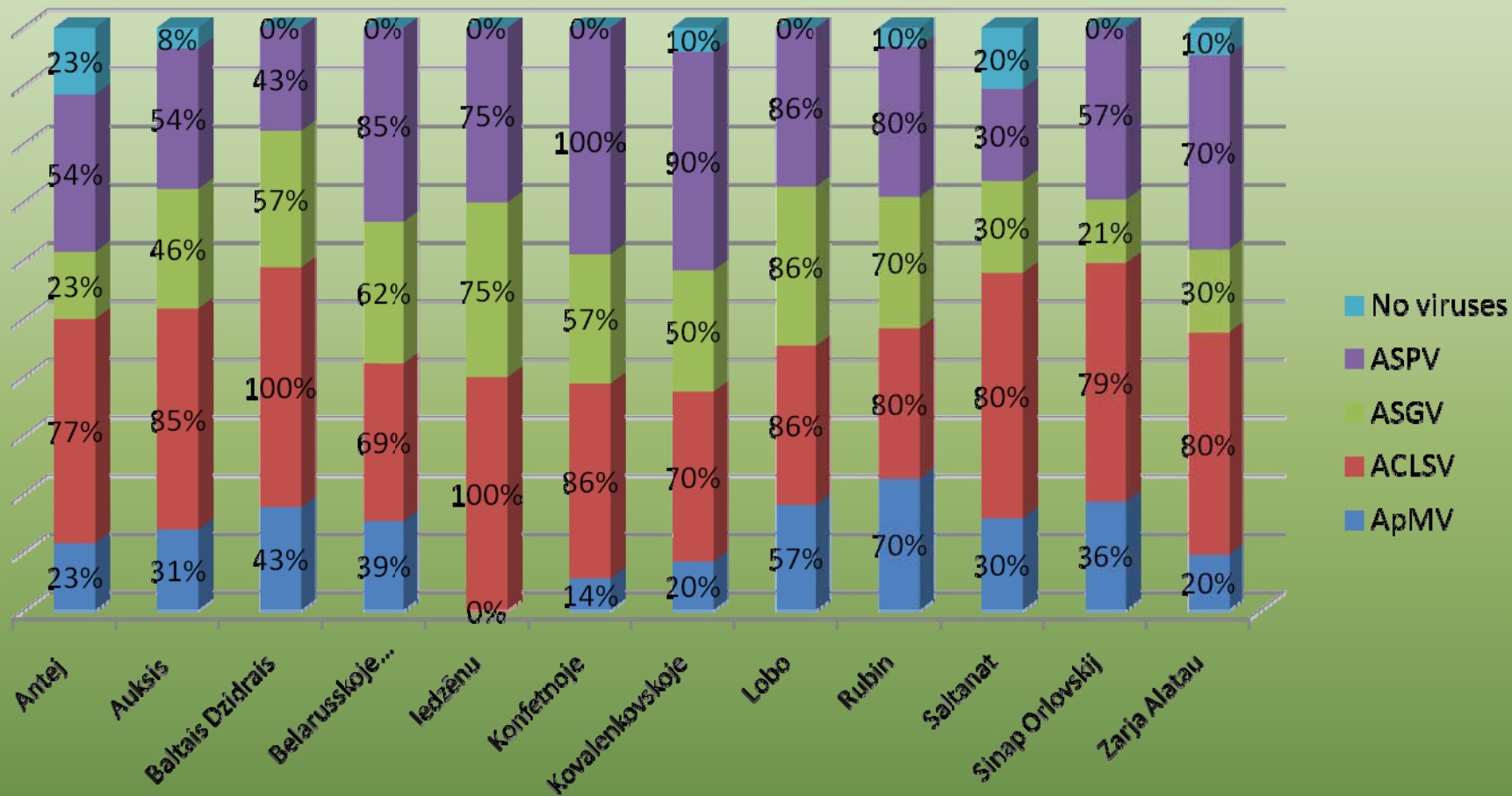
The occurrence of 4 viruses in apple samples by RT-PCR



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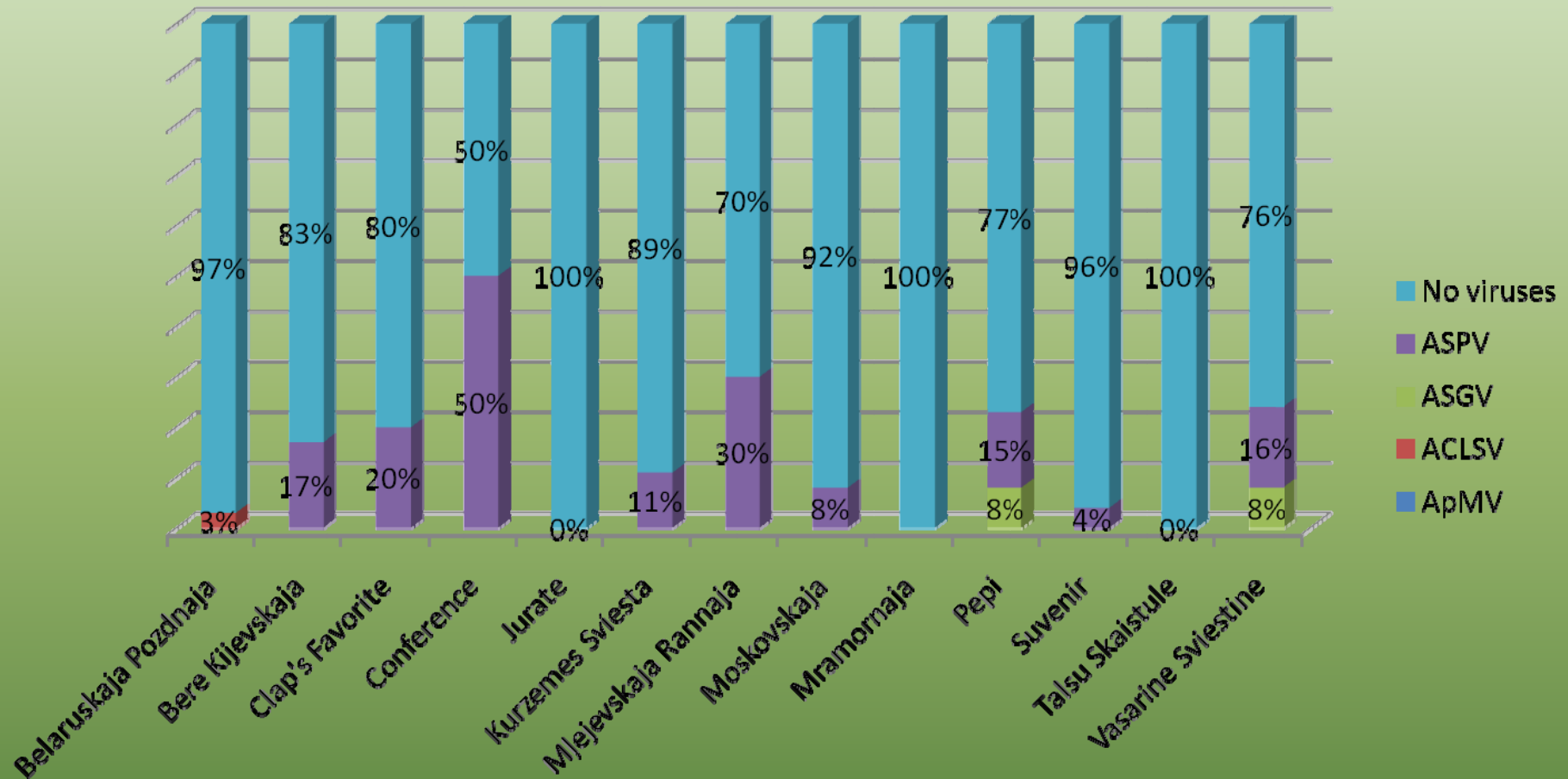
## Occurrence of four viruses in different apple cultivars according to RT-PCR data



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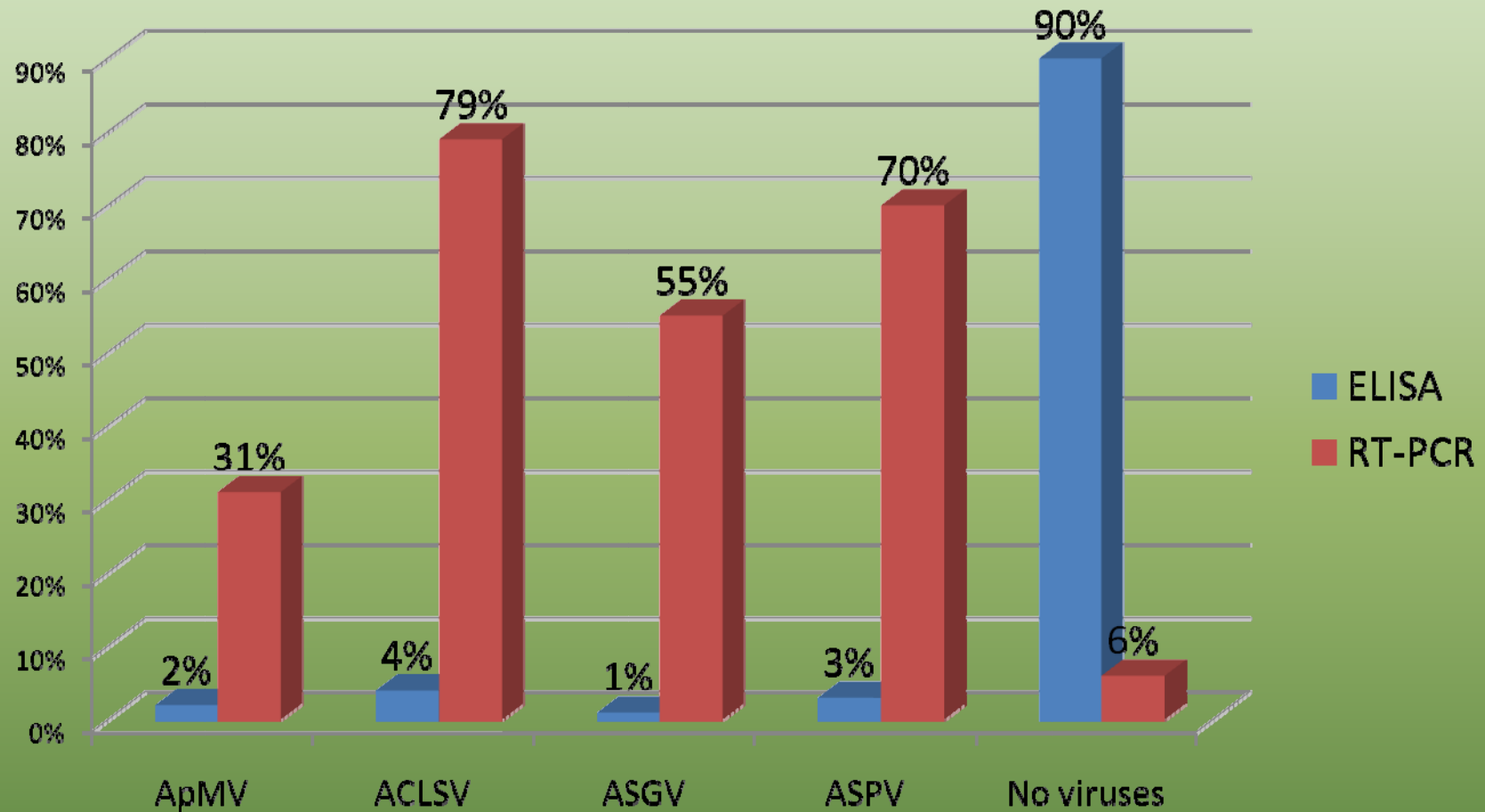
## Occurrence of four viruses in different pear cultivars, according to ELISA data



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## Comparison of ELISA with RT-PCR



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# Conclusions

The ACLSV, ApMV, ASPV and ASGV are very common and wide spread in apple and pear orchards in Latvia and most of the commercially grown cultivars are infected.

The production of certified and virus-free planting material and its usage for the establishment of new plantings is the only measure to stop spreading of these viral diseases in orchards.

ELISA which is commonly used for virus detection is fast and simple assay with relatively low cost, however it becomes unreliable for the detection of woody plant viruses during the summer due to decrease of virus concentration. In several cases it is reliable only during a short period – maximum two months after bud break in spring.

Pentaplex RT-PCR is more effective and reliable for apple virus detection in comparison with ELISA and it is not dependent of time of the year. The internal control in this assay exclude false negatives which could be as a result of RNA degradation or presence of inhibitors, such as, polyphenols and polysaccharides, which can decrease the sensitivity of the detection.



Thank you for attention!



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