production of nuclear stock according to the new EPPO Certification scheme for *Ribes* (Anonym 2008) - *Strawberry latent ringspot virus*, *Raspberry ringspot virus*, *Arabis mosaic virus*, and *Cucumber mosaic virus* seem to be of declining importance. Nevertheless, there are still challenges for further research on e.g. no-name rhabdovirus reported first from black currant in the U.K. (Roberts and Jones 1997) and later from the Czech Republic (Přibylová et al., 2002).

A new research programme aimed at the production of 6 certified *Ribes* cultivars fulfilling the criteria of the EPPO Certification scheme started in the Czech Republic in 2009. It is based on co-operation between the Institute of Plant Molecular Biology (IPMB) and Research and Breeding Institute of Pomology Ltd. (RBIP). IPMB´s experience in detection and identification of viruses and phytoplasmas infecting *Ribes* combined with the RBIP´s experience in tissue-culture virus elimination, propagation and maintenance of virus-free clones in technical and field isolates and check of trueness-to-type fruit quality are employed in the project, which is funded by the National Agency for Agricultural Research grant No. QH 91224 and supported by COST 863 Action.

Oral Session II

Disease detection in quality systems for production of nursery stock

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Growers of nursery stock aim to produce a high quality product. Plants are selected and propagated, stocks are indexed and possibly certified through quality-plus systems. Detection tests for plant pathogens e.g. viruses are carried out in order to identify and remove infected plant material.

Although detection tests are reliable they cannot guarantee with a 100% certainty that the plant material is healthy. In recent years, laboratories like inspection services started to obtain accreditation for the detection tests. Validation is the key to describe the reliability of a detection test. It includes the determination of factors like detection limit, selectivity and specificity, repeatability, robustness etc. Besides the quality of the test other factors are important to consider. The successful detection of viruses in plants depends also on the right sampling procedures. Virus titres can vary with season, viruses of viruslike diseases may not be uniformly distributed throughout the plant, etc. Conditions under which plants are kept, disease pressure in the surrounding environment, presence of vectors will influence the disease status of the stock. For quarantine viruses a zero-tolerance is in effect, however, what will it mean when more sensitive detection methods are implemented?

In order to develop a certification system that includes disease testing these aspects need to be addressed. The paper will discuss these aspects using examples from detection of fruit viruses in current certification systems of Naktuinbouw, for instance Plum pox virus (PPV) in Prunus and virus diseases in strawberry.

Introduction of a certification program in a production of a plum planting material

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Certification program for the production of a fruit planting material in not completely established in the Republic of Serbia. Despite this fact, Fruit Research Institute – Čačak started an introduction of a certification in a production of a plum planting material of cultivars developed at the Institute.

Propagate material from pomologically selected trees in commercial and experimental orchards was collected and grafted onto virus-free Myrobalan rootstock. A total of 89 plants of 15 plum varieties are included in this study: Čačanska lepotica, Čačanska rodna, Čačanska najbolja, Čačanska rana, Valjevka, Valerija, Čačanski šećer, Jelica, Timočanka, Boranka, Mildora, Krina, Pozna plava, Požegača, Stenley, and perspective hybrid 14/21.

All tests have been done according to the EPPO recommendations. Selected clones were tested on woody indicators Prunus tomentosa, *P. persica* and *P. serrulata* cv. Shirofugen. ELISA test was performed in

appropriate time for the detection of the following viruses: *Plum pox virus, Prune dwarf virus, Prunus necrotic ringspot virus*, Apple chlorotic leaf spot virus, Apple mosaic virus and Myrobalan latent ringspot virus. To increase the sensitivity of Plum pox virus detection, IC-RT-PCR was used. Material was also tested for the presence of '*Candidatus* Phytoplasma pruni' by nested-PCR method.

The presence of the viruses was found in 8 plants. Four plants of cv. Jelica were found to be positive in ELISA test with Apple chlorotic leaf spot virus. Latent infection with *Plum pox virus* was detected in 4 candidate clones (1 plant per cvs Valerija, Čačanska rodna, Čačanska lepotica and Požegača). The rest of the material was free of all other viruses. Not a single infection with '*Candidatus* Phytoplasma pruni' was found.

Susceptibility of a range of hazelnut cultivars to apple mosaic ilarvirus

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The susceptibility of seven hazelnut cultivars to apple mosaic virus was compared (ApMV) for four years in an experimental orchard. Observations were carried out on different criteria regarding the symptoms severity on leaves as well as the yield and growth. All the cultivars were sensitive to ApMV with different level of symptoms. The yield and the growth were affected by ApMV infection.

Confirmation of the elimination of Apple stem grooving virus from apple trees by *in vitro* chemotherapy

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Apple stem grooving virus (ASGV) is widespread in its distribution in apple trees. The virus causes tree decline and graft union necrosis in certain combinations of scion and rootstock, and attempts are made usually to control the virus where apples are grown commercially. ASGV is difficult if not impossible to eliminate by heat therapy. In 1996, in vitro cultures of apple infected with ASGV were grown for 9-12 weeks on media containing quercetin and ribavirin (10 ug/ml of each), then cultured on media free of these chemicals. Analysis by immunocapture (IC) RT-PCR failed to detect the presence of ASGV, while all untreated controls were positive. Treated and untreated cultures were subcloned, rooted, hardened, and eventually planted in the field. The plants were observed and tested regularly by IC/RT-PCR from 1998 - 2008. The treated plants were consistently negative by IC/RT-PCR, while untreated plants tested positive. After 10 years of testing by the sensitive IC/RT-PCR assay, it is safe to say that in vitro chemotherapy with quercetin and ribavirin is effective for the elimination of ASGV from apple plants.

Detection of four pome fruit viruses by ELISA and RT-PCR and cluster analyses of apple chlorotic leafspot virus (ACLSV) in apple and pear at the Canadian Clonal Genebank

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A survey of apple (*Malus domestica*) and pear (*Pyrus communis*) viruses was carried out at the Canadian Clonal Genebank (CCG), Greenhouse and Processing Crops Research Center (GPCRC), Harrow Ontario, Canada, during the fall/winter of 2007 and spring of 2008. In total, 438 accessions of apple and 122 of pear were tested using randomly collected samples of leaves and/or dormant cuttings and processed using Double Antibody Sandwich – Enzyme Linked

ImmunoSorbent Assay (DAS- ELISA). All samples were tested by ELISA for the presence of *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV) and *Apple mosaic virus* (ApMV). Specific infection rates for apples were ACLSV (48.1%), ASGV (10.0%), ASPV (6.6%) and ApMV (7.1%) and for pears ACLSV (42.6 %), ASGV (0 %), ASPV (91.8 %) and ApMV (90.1%). Seventeen of the apple accessions were also tested by multiplex RT-PCR and the results proved that RT-PCR is more sensitive than ELISA. One-step RT-PCR was used to amplify 22 ACLSV isolates (from apple and pear accessions originated in different countries) and to obtain the nucleotide sequence of the coat protein. Using these data a phylogenetic relationship among the ACLSV isolates was created. The nucleotide homology was 84%-100% among the 22 ACLSV isolates whereas the homology of amino acid was 91%-100%. The homology of nucleotide was 100% among the isolates of MAL0427, MAL0270, MAL0537, MAL0375, MAL0577, MAL0844, MAL0976, MAL0107, PYR0206, PYR0112, and PYR0129.

Keywords: *Malus*, *Pyrus*, DAS-ELISA, multiplex RT-PCR, one-step RT-PCR, cluster analyses.

Effects of thermotherapy temperatures on the growth of in vitro cultured pear plants and the elimination of three viruses

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In vitro-cultured plants of Pyrus pyrifolia cv. Huanghua, widely grown in central and southern China, were treated at different temperatures and the virus presence of regenerated plants was evaluated by ELISA and dot-blot hybridization. Results showed that treatment with a continue high temperature at 37 °C had a serious effect on the growth of in vitro pear plants and resulted in a high death rate, but virus-free plants could be regenerated from tips about 1 mm in length from survived plants. Treatment at 32 °C /38 °C changed every 8 h and 16 h had a mild effect on the survival and could improve the growth speed of in vitro pear plants. However, no virus-free plants were regenerated from shoot tips in size 0.5 mm to 1 mm after a treatment period for 50 days. Therefore, treatment temperatures were raised to 34 °C /42 °C changed every 8 h and 16 h. Although the death rates during the treatment were somewhat higher than that during the treatment at 32 °C /38 °C and no improvement on the growth speed compared to plants under a normal condition, a higher virus elimination efficiency (100%) was achieved by thermotherapy for more than 55 days combined with 0.5 mm to 1 mm shoot tip culture. The results were confirmed with three other pear varieties. Results also indicated that ACLSV was more sensitive to high temperature, which could be eliminated from shoot tips after a treatment period five-day shorter than ASGV and ASPV.

Keywords: thermotherapy, meristem culture, virus, pear

A one-Step Reverse transcription-polymerase chain reaction-based detection of olive trees viruses in Egypt

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Olive (Olea Europea) is a very important crop for the new reclamation land in Egypt. The estimated acreage of cultivated olive trees in Egypt is above 135.00 Feddens of which the total production is 500.000 tons per year (Ministry of Agriculture statistics, 2007). Olive trees are affected by several viruses and virus-like diseases. To date, 15 viruses that belong to eight genera have been isolated from olive trees. In a preliminary survey for the assessment of the sanitary status of olive tree in five different location in Egypt, leaf samples from 300 trees of 9 different cultivars were collected. Using virus-specific primers, a one-step RT-PCR assay was used to detect and identify each of the eight viruses most commonly found in olives. Namely, Cucumber mosaic virus (CMV), Olive latent ringspot virus (OLRSV), Olive latent virus-1 (OLV-1), Olive latent virus-2 (OLV-2), Olive leaf yellowing-associated virus (OLYaV), Strawberry latent ringspot virus (SLRSV), Cherry leaf roll virus (CLRV), and Arabis mosaic virus (ArMV). Among the eight viruses assayed , OLRSV (6.7%), OLV-1 (5.7%), CLRV (4.6%),

OLV-2 (2.7%), SLRSV (2.3%), OLYaV (1.3%) and ArMV (0.7%) were detected. The most common virus found was CMV which was prevailed with a high incidence of 24.7% in olive orchards. The use of one step RT-PCR has revealed efficient and reliable to detect most of the eight olive viruses found in Egypt. Surprisingly, the infection rate found is lower than the expected, if we take into consideratin previous surveys conducted in the Mediterranean area. Last, this technqiue could be useful to be applied in the decetion of olive viruses for production of certified plant along with the certification programs.

Oral Session III

The microarray detecting six fruit-tree viruses

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An oligonucleotide microarray for the detection of *Apple mosaic virus* (ApMV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV) and *Plum pox virus* (PPV) was developed. Specific fragment of viruses were PCR amplified by using Cy3-labelled primers and hybridized onto microarray and detected simultaneously. Advantages and drawbacks of the method will be discussed.

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Validation of a microarrays protocol for detection and genotyping of PPV reference samples

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A genomic strategy for PPV identification has been recently developed (Pasquini et al., 2008). The method is based on using a 70-mer oligonucleotide DNA microarray chip capable of simultaneously detecting and genotyping PPV strains. Universal and specific probes have been identified and used with a sensitive protocol of hybridization using an indirect fluorescent labelling of cDNA product with cyanine that enhanced the sensitivity of the virus detection without the use of the PCR amplification step About 30 samples belonging to a PPV isolates collection, including M, D, EA, and C strains, have been used for the validation and standardization of the protocol. The sensitivity, specificity, repeatability and reproducibility of the protocol have been tested.

Real time PCR quantitative analysis of plant viruses in stone fruit trees tissues

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Real Time PCR assays aiming at quantifying the level of plant infection by pathogens have been increasing for the last few years. Within microbiology, the application of Real Time PCR has had the biggest impact upon the field of virology. However, Real-time PCR application in fundamental plant virology studies is still lagging behind. The use of relative and absolute quantification is discussed in this study. Also, case studies including Plum pox virus, Prune dwarf virus and Apple chlorotic leaf spot virus are presented.

Simultaneous detection of the main stone fruit viruses and viroids by non-isotopic molecular hybridization polyprobe

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