However, just a draft sequence can be provided by this approach for the majority of genome projects with de novo character in contrast to projects with re-sequencing character. Uncertainties resulting from the used technique and/or resulting draft sequences limit the benefit. In consequence, sequence quality has to be increased by the combination of new and old technologies. Examples and preliminary results from running projects will be presented to illustrate the use and benefit of the new techniques in the genome research of plant associated species from the Enterobacteriaceae and Acholeplasmataceae.

## *Oral Session I*

### Elucidation of the roles of blackcurrant reversion virus and phytoplasma in the etiology of full blossom disease in currants

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To determine the roles of phytoplasmas and Blackcurrant reversion virus (BRV) in the etiology of full blossom disease (FBD), we conducted graft and dodder transmission experiments. Scions from FBD affected Ribes rubrum were grafted onto red, white and black currants. Red and white cultivars revealed symptoms of FBD, whereas blackcurrant displayed symptoms of BRV infection. No differences in symptoms were observed between plants infected with BRV only and those infected with BRV and phytoplasma.

Aster yellows phytoplasma subgroup 16SrI-C was transferred from FBDinfected red currants to periwinkle, which symptoms of green and yellow petal were observed. Back transmission of phytoplasma to currant seedlings of red and black currant was not successful.

Scions of periwinkle infected with aster yellows phytoplasmas of subgroup 16SrI-C and 16SrI-B, which were bottle-, bark-, and approach-grafted onto seedlings of red and black currant resulted in positive but symptomless transmission of phytoplasma to red currant. We conclude that FBD symptoms are induced by BRV rather than by phytoplasma, which was originally described as the causal agent of FBD.

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### Association of Tomato Rinsgpot Virus, Tobacco Ringspot Virus and *Xiphinema americanum* with a decline of highbush blueberry in New York

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A survey of highbush blueberry (Vaccinium corymbosum L.), including some cultivars showing virus-like symptoms, i.e. stunted growth, chlorotic spots, purple lesions, distorted leaves and defoliated shoots, was conducted for the occurrence of viruses in New York. Leaves from symptomatic and nonsymptomatic plants were tested for virus infection by enzyme-linked immunosorbent assay using specific serological reagents (Bioreba, Reinach, Switzerland). Several samples reacted positively for Tobacco ringspot virus (TRSV) and Tomato ringspot virus (ToRSV), two virus species belonging to the genus Nepovirus in the family Comoviridae. The occurrence of TRSV and ToRSV was confirmed in blueberry leaf samples by reverse transcription-polymerase chain reaction (RT-PCR) or immunocapture-RT-PCR with appropriate primers to amplify a 310-bp and a 580-bp fragment of the RNA-dependent RNA polymerase gene, respectively. Comparative sequence analysis of the viral amplicons of New York isolates indicated moderate to high nucleotide sequence identities with corresponding ToRSV and TRSV reference strains. Also, analysis of soil samples collected from the root zone of blueberry bushes for the occurrence of nematodes indicated the presence of specimens from the Xiphinema americanum group.

Cucumber bait plants planted in soil samples containing X. americanumgroup nematodes became infected with either ToRSV or TRSV in a greenhouse. Altogether, our findings indicate that ToRSV and TRSV and their vector X. americanum sensu lato are associated with the decline of highbush blueberry in New York.

## A new member of the family Reoviridae may contribute to severe crumbly fruit in 'Meeker' red raspberry

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A virus induced crumbly fruit disease of considerable importance in 'Meeker' and other cultivars of red raspberry has been observed in northern Washington, USA and British Columbia, Canada. Raspberry bushy dwarf virus (RBDV), a pollenborne virus, has been attributed as the causal agent of the disease. Recent dsRNA extractions from symptomatic plants in northern Washington revealed the presence of additional viruses, as evidenced by more than 12 bands on agarose gels. All the bands, except those corresponding to RBDV (2.2 kb and 5.5 kb) were gel-purified and cloned for sequencing. Thus far, sequencing results showed the presence of at least two viruses in addition to RBDV. One has significant amino acid sequence identity (~40%) to 8 genome segments of Rice ragged stunt virus (RRStV), a ten-RNA-segmented oryzavirus that belongs to the family Reoviridae. The complete sequence for the segments that correspond to RNA S1 S4 and S7 of RRStV has been determined. Partial sequences of segments S2, S3, S5, S9, and S10 are also known and are being used to generate the complete genomes using poly A tailing of the 3' ends. In addition, Raspberry mottle virus (RMoV), a recently characterized member of the Closteroviridae, was also identified from raspberries with severe crumbly fruit. These findings along with the lack of severe crumbly fruit symptoms in 'Meeker' red raspberry singly infected with RBDV in Oregon, suggest the existence of a novel virus complex associated with severe crumbly fruit in red raspberries. The complex may involve RBDV, RMoV and/or this new reovirus. Transmission studies are underway to determine the affect of each of these viruses singly and in all combinations on crumbly fruit symptom development in 'Meeker' red raspberry.

## Biology of *Cixius wagneri* the planthopper vector of '*Candidatus Phlomobacter fragariae*' in strawberry production tunnel, and its consequence on the epidemiology of strawberry marginal chlorosis

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«*Candidatus Phlomobacter fragariae*» is the prevalent agent of strawberry marginal chlorosis (SMC) transmitted by the planthopper *Cixius wagneri*. Because the insect biology was unknown, a field experiment was set up to determine if it was able to reproduce on strawberry plants, to determine the number of insect generations per year and the ability of nymphs to transmit SMC. During spring 2004, 80 *C. wagneri* adults were delivered into 4 small insect-proof tunnels containing 30 healthy plants. Fifteen percent of the delivered insect population was carrying the pathogen. At October 2004, only 3 young L1 instar nymphs were found in the first tunnel, demonstrating there was no new insect generation during summer. At April 2005, 430 *C. wagneri* of early L1 to late L5 nymph instars were collected at the roots of the plants. It clearly indicated that a single insect generation had overwintered as larvae and had emerged at the following spring. All instars were proved to carry '*Ca. P. fragariae*' (70 to 75% of the larvae) and were able to transmit SMC as assessed by transmission assays. An insecticide treatment was applied in March 2005 in a third tunnel and a fourth tunnel was kept as a control. More than two hundred *C. wagneri* adults were collected on the control tunnel 4 in June 2005 confirming that an insect generation arose in the tunnel, whereas no insects could be found in the treated tunnel 3. All plants were kept for two years, surveyed for symptoms expression and tested for '*Ca. P. fragariae*' infection by 16S-PCR. Results indicated a reduced mortality and SMC incidence in tunnel 3, and a higher mortality and SMC incidence in tunnel 2 than in tunnel 1, attesting that *C. wagneri* larvae had spread SMC and that an early insecticide treatment could control the disease.

## Production of antisera and evaluation of serology-based techniques for the detection of Blackcurrant reversion virus

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Synthetic peptides based on the amino acid sequence of the coat protein of Blackcurrant reversion virus (BRV) were used as immunogens for the production of polyclonal and monoclonal antibodies. In preliminary studies the polyclonal antibodies were evaluated by plate-traped antigen ELISA, using both herbaceous and woody plants, to determine their affinity and specificity. Subsequently, the polyclonal antibodies were used in combination with the various monoclonal antibodies in a TAS-ELISA assay format for the detection of BCRV. Also, the polyclonal antibodies were purified and used for trapping in immunocapture RTPCR. Immunocapture RT-PCR was found to be more sensitive and more reliable when compared to standard RT-PCR for the detection of BRV

## Molecular diagnostics for the detection of strawberry viruses in Australia

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The supply of high-health, certified strawberry runners throughout Australia is dependent on the collections of nucleus plants maintained in Victoria and Queensland. Both collections are tested annually in spring for several virus associated diseases using a biological indexing method of petiole grafting candidate tissue onto sensitive indicator species. This method is reliable and sensitive only if done in spring or early summer. Biological indexing is also labour intensive, expensive and time consuming, taking 6-8 weeks to generate a result.

Advances in molecular techniques have been published for detection of Strawberry mottle sadwavirus (SMoV), Strawberry crinkle cytorhabdovirus (SCV), Strawberry mild yellow edge potexvirus (SMYEV), Strawberry vein banding caulimovirus (SVBV), Beet pseudos yellows crinivirus (BPYV), and Strawberry pallidosis associated crinivirus (SPaV). We have adopted these tests from international, peer-reviewed literature and trialled them on positive control plants, maintained 12 months of the year under glasshouse conditions. Each PCR test more accurately identified virus infection in graft inoculated indicator plants compared to visual observation for virus associated symptoms. Results of monthly testing of the glasshouse-grown, virus-infected strawberry plants over two years indicated that the best time to detect viruses using molecular methods was in spring and autumn.

The X-tractor Gene™, an automated system for the extraction of nucleic acid, was trialled for nucleic acid extraction from strawberry because of reduced labour costs and consumables. Unfortunately the X-tractor Gene™ was unreliable for nucleic acid extraction from strawberry compared to the RNeasy® Plant Mini Kit and further development is required.

We have partially validated these tests by surveying 100 strawberry plants from Queensland, Western Australia and Victoria. Our results indicate that the tests can detect viruses in a field situation and very few positive results were observed. These results suggest that strawberry viruses may not be widely distributed in Australia, indicating the success of the certification schemes in reducing the incidence of these pathogens.

## Detection of phloem restricted bacteria responsible of strawberry marginal chlorosis (SMC) by real-time PCR in a single assay

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Two uncultured phloem restricted plant pathogens, the γ3 proteobacterium «*Candidatus Phlomobacter fragariae*» and the stolbur phytoplasma (group 16SrXII-A) are associated with SMC in France. As "*Ca. P. fragariae*" and stolbur phytoplasma induce identical symptoms, the only way to identify the pathogen infected a given diseased plant rely on molecular DNA tests such as conventional nested PCR techniques. Because the two nested–PCR techniques targeting each of the two bacteria must be separately performed and are time consuming, a new approach using triplex real time PCR was developed for the routine detection of "*Ca. P. fragariae* " and stolbur phytoplasma. The real time PCR brings the advantage of being faster and present reduced risks of producing false positives. Furthermore, real-time PCR techniques provide the possibility of multiplexing by using probes with different compatible fluorescent dyes. Here, we present a new sensitive Taqman® method which permits the amplification and the differenciation of three DNA targets in one test: the map gene of stolbur phytoplasma, the spoT gene of "*Ca. P. fragariae*" and the cox gene of strawberry chloroplast taken as an internal control. The specificity and the efficiency of this method were determined and its evaluation for routine detection on field samples was assessed.

## Sequencing studies for the identification and characterization of new and old Rubus viruses

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In Europe, raspberry plants are commonly infected with a complex of aphidtransmitted viruses that together cause raspberry mosaic disease (RMD). During the previous 30 years, by grafting and vector transmission to a range of red and black raspberry cultivars, these viruses have been loosely characterized and identified as Raspberry leaf spot virus (RLSV), Raspberry leaf mottle Virus (RLMV), Black raspberry necrosis virus (BRNV) and Rubus yellow net virus. An additional, very widespread virus, Raspberry vein chlorosis virus (RVCV), is spread by a different aphid vector. Recently some sequence data have been obtained for RYNV, BRNV and Raspberry mottle virus (RMoV), a virus found in plants showing RMD symptoms.

We have carried out sequencing studies using random amplification and mass analysis approaches and will present information on the relationship between RMoV, RLSV and RLMV, as well as the first data for RVCV and a novel, possibly segmented minus-strand RNA virus infecting raspberry.

## Emerging strawberry virus and virus-like diseases in the world

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Strawberry production is increasing annually, with the world production exceeding four million tons. Strawberry caused virus diseases are also increasing. A decade ago there were about a dozen viruses known to infect strawberry. Today this number has more than doubled with several new viruses being identified in declining plants in the United States. There are now five aphid transmitted viruses - Crinkle, Mottle, Mild yellow edge, Vein banding and Chlorotic fleck. The former four remain the most common viruses in temperate areas around the world, whereas chlorotic fleck is not as common although it is present in both the United States and Europe. A new group of viruses, members of the genus *Crinivirus* have emerged as a new threat to strawberry in areas where vectors are present. There have been four new criniviruses discovered, with Strawberry pallidosis associated virus being the most common of the four, present in both the Old and New World. The ilarviruses that infect strawberry include Strawberry necrotic shock, Apple mosaic, Tobacco streak and Fragaria chiloensis latent viruses. Strawberry necrotic shock is the predominant ilarvirus in the United States whereas Fragaria chiloensis latent has significant presence in Chile. Tobacco streak is not very common in strawberry - previous reports of Tobacco streak in strawberry were probably misdiagnosis of Strawberry necrotic shock. Modern strawberry cultivation

has minimized the impact of nematode transmitted viruses but the elimination of methyl bromide may lead to the reemergence of this group of viruses in the future. With the knowledge we have acquired over the last decade it is now possible to have robust certification systems, the cornerstone for minimizing the impact and spread of strawberry viruses. With the massive increase of strawberry production in China recently, it will be curious to see if new viruses endemic to the area will infect strawberry.

## Viruses and virus-like diseases in blueberry

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Over the past 15 years, blueberry production has increased dramatically in hectares grown and in the number of countries producing blueberries. It should be expected that as blueberry cultivation continues to expand into new areas, the plants will become exposed to viruses that have not been observed in blueberry previously. Most blueberries are grown in North America and that is where most viruses of the crop have been described. Blueberry scorch, an aphid-transmitted Carlavirus, can be very devastating and is the virus of greatest importance, in terms of management and quarantine. Blueberry red ringspot (BRRSV), a Caulimovirus, has been spreading in the mid and southern atlantic states, but spread has not been observed in the Pacific Northwest. Blueberry shock, a pollen-borne Ilarvirus, is interesting in that it causes a 100% crop loss for one year, and then plants recover to full production in subsequent years with no apparent loss in yield or fruit quality. The nepoviruses Tomato and Tobacco ringspot can be important in some areas, but Peach rosette mosaic has only been observed in experimental blocks. Blueberry shoestring is still an important virus in Michigan, but less so in other areas. Blueberry leaf mottle appears to almost have vanished, or at least has not been detected in the past five years. A new ringspot of blueberry in the southeastern USA has been observed since 2008 and several viruses have been partially cloned from dsRNA extracted from symptomatic plants, a Tobamo, Poty and possible Cilevirus. Whether any of these viruses individually or a in combination cause the disease is still unknown. There is also a phytoplasma, Blueberry stunt that occasionally affects blueberry. Cranberry, another widely planted Vaccinium, has two additional viruses reported, Tobacco streak and Cucumber mosaic. At this point, CMV is associated with a funky flower disease in cranberry.

## Viruses and virus-like diseases in European *Ribes*

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During the last decade there was a tremendous advance in the identification of viruses and phytoplasmas infecting currants. The breakthrough – identification of the *Blackcurrant reversion virus* (BRV) as the causal agent of black currant reversion disease (BCRD) (Lemmetty and Lehto, 1999), economically the most important disease in currants, was followed by BRV genome sequencing (Latvala-Kilby and Lehto, 1999; Pacot-Hiriart et al, 2001) and development of PCR- based detection protocol (Jones and McGavin, 2002) - a prerequisite for effective control of the disease. However, the existing the E- and R-forms of BCRD were not elucidated by the studies on BRV variability (Lehto et al., 2004; Přibylová et al., 2008) and remain obscure. The gall mite (*Cecidophyopsis ribis* Westw.) a vector of BRV, is the most serious pest of black currant causing the 'big bud' damage. Measures to control *C. ribis* in Europe are restricted to sulphur sprays, since previously available chemical agents are now withhdrawn for environmental reasons. Successful resistance-breeding programme against *C. ribis* in black currant continues at the Scottish Crop Research Institute, U.K. and has recently led to the development of a PCR-based marker linked to the resistance (Brennan 2008). Hot water treatment of *Ribes* cuttings is now more widely used for the elimination of *C. ribis* from propagation material of sensitive cultivars.

Phytoplasmas were identified in black currant currants with symptoms of the BCRD (Špak et al., 2004) and in red and white currants with symptoms of the Full blossom disease (Navrátil et al., 2007), always together with BRV infection, but seem to be of less economic importance.

*Gooseberry vein banding virus*, a badnavirus transmitted by aphids and causing gooseberry veinbanding disease in currants was identified and partially sequenced, which enables its PCR detection in germplasm and propagation materials (Jones at al., 2001). Other viruses, which are subjects to testing during the

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