Oral Presentations
Viruses

O VIR 3
Emergence of plum pox virus, the most damaging viral pathogen of stone fruits, in Japan
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Sharka disease, caused by plum pox virus (PPV, genus *Potyvirus*), is the most serious viral disease of stone fruits (*Prunus* spp.). Among the eight known strains of the virus, PPV-D is the most important due to its recent spread from Europe to North and South America and Asia. In 2009, we found PPV (which belongs to the D-type strain) in Tokyo, Japan, for the first time. PPV was found in a new natural host, *Prunus mume* (Japanese apricot), which is one of the most popular fruit and flowering trees in East Asia, including Japan. For the control of PPV, we developed rapid test kits for the detection of PPV based on immunochromatography and reverse transcription-loop mediated isothermal amplification (RT-LAMP), which can detect PPV within 15 and 60 minutes, respectively. Using these kits, the plant protection station of Japan has conducted nationwide surveys for six years, and found more than 20,000 PPV-positive trees across 11 prefectures (Tokyo, Ibaraki, Saitama, Kanagawa, Aichi, Mie, Shiga, Nara, Wakayama, Osaka, and Hyogo). However, the viral transmission routes in Japan are poorly understood, which is hindering the eradication of PPV. Therefore, we collected geographically-diverse PPV isolates and performed a molecular epidemiological analysis. Despite the low genetic diversity, the phylogenetic tree based on the complete genome sequences enabled precise estimations of the transmission routes of the PPV-D strain at the national and international level.

O VIR 4
Infection of grasses and cereals by wheat dwarf virus and a diverse set of luteoviruses
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To understand the ecology of viruses infecting grasses and cereals, we have carried out virus screens and sequence analyses. Barley yellow dwarf virus (BYDV)/Cereal yellow dwarf virus (CYDV) constitute a group of viruses in the family Luteoviridae that infect grasses and cereals. They have a genome of single-stranded RNA and are classified into several species, which are transmitted by different aphids in a persistent manner. The different virus species can be difficult to discriminate using serological methods, and the high viral diversity is only now beginning to be revealed. In a survey of B/CYDVs in cereals and grasses from different regions of Sweden and Estonia, we have detected a high virus incidence and sequence analyses have revealed infection with BYDV-PAV, BYDV-MAV, BYDV-PAS, BYDV-OYV, BYDV-GPV and BYDV-RMV. The four latter species were found for the first time in Sweden and BYDV-GPV for the first time outside China. Several species of BYDV/CYDV could be found in the same field with up to three species in the same plant. Different viruses were detected in forage grasses and cereals growing next to each other, suggesting that the forage grass was not the virus source for the infection in cereals. BYDV-OYV has previously only been described as a single isolate from an oat plant in Latvia. We could now find isolates of BYDV-OYV in different parts of Sweden. The first complete genome sequencing of BYDV-OYV shows that it is related to viruses within the genus Luteovirus, but sequence comparisons reveal that it is distinct from the other species and should tentatively constitute a new species. Wheat dwarf virus (WDV; genus Mastrevirus; family Geminiviridae) is also infecting grasses and cereals. It has a genome of single-stranded circular DNA and is transmitted in a persistent manner by leafhoppers. In our studies to identify virus reservoirs for cereal-infecting viruses, WDV was detected at a low frequency in randomly sampled ryegrass plants. Nucleotide sequence analyses revealed that the ryegrass isolates were closely related to those from wheat. Infection tests with WDV isolates from wheat resulted only in low infection rates and low virus titers in different ryegrass species and cultivars, while wheat plants were very susceptible. The results suggest that WDV may persist at low levels in ryegrass and other grasses.

O VIR 5
Establishment of an *in-vitro* assay for functional characterization of the viral proteinase and processing of RNA2-encoded polyprotein P2 of *Cherry leaf roll virus* (CLRV)
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Question: The bipartite genome of *Cherry leaf roll virus* (CLRV, Genus *Nepovirus,* subgroup C, family *Secoviridae*) consists of two positively orientated single-stranded RNAs, which encode for two polyproteins (P1 and P2). P1 harbors characteristic domains for a proteinase-cofactor (PCo), a helicase (Hel), a genome-linked protein (VPg), a proteinase (Pro), and an RNA-depending

polymerase (Pol). P2 encodes, besides a region at the 5`-end that has not been functionally assigned by now, the movement protein (MP) and the coat protein (CP). The polyproteins are processed into their functional units by the viral proteinase. *In-silico* analysis of the full-length sequence revealed putative processing sites similar to already proven sites of related nepoviruses. Prerequisite for the functional characterization of viral gene-products is the elucidation of their processing into the mature subunits. Aim of the project is therefore to establish an *in-vitro* assay to prove the proteolytic activity of the proteinase and to identify the cleavage sites of CLRV.
Methods: The polypeptide constituting the putative proteinase of CLRV was heterologuesly expressed in *E. coli* and purified under native conditions. Regions surrounding putative cleavage sites were cloned and expressed *in-vitro*, using biotinylated lysins added to the nascent protein as a label. Activity assays were performed by subjecting the *in vitro* translation products as substrates to the proteinase. By western blot and streptavidin-AP conjugates, processing at the putative cleavage sites was monitored.
Results: The proteinase of CLRV was successfully expressed in *E. coli* und was purified by affinity-chromatography. The *in-vitro* assay for the testing of the proteolytic activity of the proteinase was successfully established. Translation products comprising the putative processing-sites of P2 were subjected to the *in-vitro* assay for their experimental verification.
Conclusion: The established *in-vitro* assay is suitable for experimental confirmation of cleavage sites utilized by the proteinase of CLRV responsible for processing of P1 and P2.

O VIR 6

MicroRNA-like fragments from Turnip mosaic virus targets host gene *HVA22D*: a new opportunity to develop resistant crops

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Plant viruses are notorious for their rapid evolution, enabling them to overcome host resistance and making them highly successful. We focus on a specific Australian strain of *Turnip mosaic virus* (TuMV), first sequenced in our laboratory. Previous research found that this strain differed from other previously sequenced strains of TuMV and that its genome also coded for viral miRNA-like fragments which were shown to act in a similar manner to plant miRNA in regulating gene expression. The virus-derived miRNA-like fragments were found to target the *HVA22D* gene in *Arabidopsis*. The T-DNA mutant *hva22d* shows increased susceptibility to TuMV. By introducing silent mutations in the viral miRNA binding site of *HVA22D* we were able to interfere with miRNA-host gene binding. In addition, we have developed a decoy construct that specifically binds to TuMV miRNA. Both of these approaches provide new strategies to create TuMV-resistant plants.
To better understand the mechanism of TuMV-plant host gene interaction, we focus on studying the function of *HVA22D* in more detail. HVA22D is an abiotic stress inducible protein and is also thought to have a role in controlling autophagy. HVA22D is one of five homologs found *Arabidopsis*, and is most tightly regulated by abscisic acid. The gene is highly conserved with homologs identified in diverse eukaryotes; the *YOP1* gene in yeast as well as *TB2* and *DP1* in humans have a role in tubule-forming proteins. The yeast homolog and those found in *Arabidopsis* are also thought to regulate autophagy in a negative manner. HVA22D may be involved in changes of membrane composition due to environmental stresses. We hypothesise that the plant may alter its lipid and membrane composition in an attempt to prevent the spread of the virus via their plasmodesmata or to inhibit virus replication. By using *HVA22D*-overexpressing viral miRNA-resistant mutants we will determine whether the plant does alter the lipid composition of its membranes of the mutant and wild type plants both challenged with the virus. It is hope that this work will provide new plant viral resistance strategies and hopefully generate more durably resistant crop varieties to increase food security for the future.