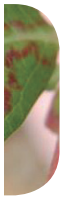

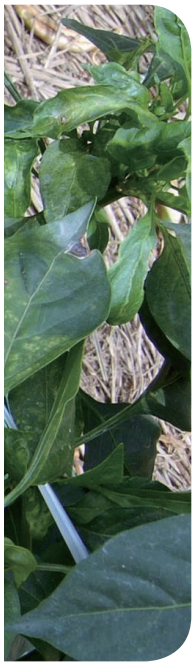

14th IPVE
International Plant Virus Epidemiology Symposium

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International Plant Virus Epidemiology Symposium

Coex, Seoul, South Korea I May 13-17th 2019

Rural Development Administration

National Institute of Horticultural and Herbal Science

IPET Korea Institute of Planningand Evaluation for Technology in Food, Agriculture and Forestry

The Korean Society of Plant Pathology

This conference proceeding was supported by the Korea Institute of Planning & Evaluation for Technology in Food, Agriculture & Forestry.

Welcome Message

We are pleased to invite you to participate in the 14th International Plant Virus Epidemiology Symposium (IPVE 2019) from May 13 to May 17, 2019, COEX, Seoul, South Korea.

IPVE 2019 will bring together a collection of research scientists who are at the forefront of Plant Virology and related scientific fields and will provide opportunities for junior scientists and graduate students to interactively present their work and exchange ideas with established senior scientists. This program will include symposia, poster sessions and special discussions on the wide range of session themes on plant virus epidemiology and related science.

This will be an opportunity to exchange information, engage in stimulating discussions and collaborate with your fellow members from around the world. We are sure that our expert speakers will provide you with the most critically relevant and up-to-date information.

We hope all of you will participate in the 14th IPVE and enjoy new information of plant epidemiology and the taste of dynamic KOREA.

IPVE 2019 has been partially supported by the sponsorship of
the Rural Development Administration (RDA),
the Korean Society for Plant Pathology (KSPP)
and has been convened by Dr. Peter Palukaitis and Dr. Ju-Yeon Yoon.

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14th International Plant Virus Epidemiology (IPVE) Symposium 2019

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Epidemiology and Ecology

Mitigating cassava virus pandemics in an increasingly connected global environment: Lessons from the last 30 years

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Viruses have a long history of association with cassava, but virus disease epidemics have only been recorded relatively recently. In the late 1980s, unusually severe cassava mosaic disease (CMD), caused by cassava mosaic begomoviruses, was first reported in Uganda. This was later recognized as the first report of a devastating pandemic of severe CMD that subsequently spread to affect more than 3 million sq km in 12 African countries. Cassava brown streak disease (CBSD), recognized for many years as being confined to coastal East Africa and the shores of Lake Malawi, began to spread to mid-altitude regions of East and Central Africa from 2004. It now affects 11 countries and continues to advance westwards. Most recently, four countries in South-east Asia are experiencing rapid spread of CMD, following the introduction of the disease from South Asia. These pandemics have generated increasing levels of research interest over time, and there have been major achievements in mitigating their impacts. In this overview, we present some of the key advances in understanding and managing these pandemics, as follows: (i) The ecology and characteristics of viral pathogens, their vectors and interactions with the cassava host are complex, and therefore necessitate fundamental research; (ii) There are important differences in the distribution and epidemiological characteristics of virus strains, species and mixed virus infections, and patterns of spread vary greatly depending on the relative importance of vector- vs. cutting-borne infection; (iii) Increased intensity of cassava cultivation and the greater inter-connectedness of countries and regions heightens the risk of cassava virus spread; (iv) Rapid surveillance is essential, and new ICT tools are greatly improving the speed and coverage of surveillance programmes; (v) Establishing and maintaining 'clean seed systems' is critical to effective cassava virus pandemic management; (vi) Conventional approaches have been highly effective in developing host plant resistance, but are slow; and (vii) Global collaboration and local action have strengthened over the last 30 years, and must be sustained if the negative impacts of current and potential future pandemics are to be overcome effectively.

Association of virus infections with flowering ginger diseases in Hawaii

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Growers of ornamental flowering ginger on the island of O'ahu, Hawai'i, have recently reported outbreaks of a disease causing severe dieback and threatening crop yield and quality. Virus-like symptoms were observed on red ginger [*Alpinia purpurata* (Vieillard) K. Schumann] from several locations. Plant badnaviruses and a potyvirus have previously been reported on flowering ginger in Hawaii (1, 2). Symptoms appear on plants infected with single or multiple combinations of viruses, but only plants infected with the potyvirus, banana bract mosaic virus, show clear, easily identifiable symptoms (1). Symptoms on red ginger infected with the canna yellow mottle virus and banana streak virus are more difficult to assess (2). Therefore, the causal agent(s) of the current dieback disease remains uncertain. We are currently examining the etiology of this disease with virus-specific PCR assays to discern the involvement of the above-mentioned viruses and to develop strategies to manage this disease in ginger.

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First natural crossover recombination of intact ORFs between two distinct species of the family *Closteroviridae*

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A new disease in French bean (*Phaseolus vulgaris*), observed in Spain in 2003, lead to the description of bean yellow disorder virus (BnYDV), which was then recognized as the first crinivirus (family *Closteroviridae*, genus *Crinivirus*) that infects crop species of the Leguminosae family (1). Symptoms resembling nutritional disorders consisted of interveinal mottling and yellowing in leaves, combined with stiffness or brittleness, and were produced on the middle to lower parts of the plant. Affected plants were all observed in greenhouses infested with *Bemisia tabaci*. The virus was frequently observed in commercial bean crops during the following years until 2011 when symptomatic plants turned out negative after testing for BnYDV. Instead, a different virus was found and subsequently identified as a new strain (SP) of lettuce chlorosis crinivirus (LCV) (2). The latter was hitherto only found in California (USA), and surprisingly, the LCV-SP readily infected bean but failed to do so in lettuce. The LCV-SP full-length genome sequence revealed that both RNA1 and RNA2 were similar to the Californian virus, except for the 3'end of RNA1: LCV-SP did not contain the typical P8-P23 coding genes, but instead the corresponding sequence was similar to P26-P6 genes from BnYDV. Results of the analysis using RDP4 and Simplot programs supported the conclusion that LCV-SP is the first recombinant of the family *Closteroviridae* by crossover recombination of intact ORFs, being the LCV RNA1 and BnYDV RNA1, the origin of the new LCV strain (3). Periodic analyses of symptomatic bean plants sampled in commercial greenhouses from South-east Spain revealed that LCV-SP has displaced BnYDV. The existence of LCV-SP changes the perspectives of the possible epidemiological scenarios within the genus *Crinivirus*, an emergent genus and, with more recombination possibilities than previously shown.

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Wild potato mosaic virus infecting pepino (*Solanum muricatum*) crops in Peru: Occurrence, host reactions, and molecular properties

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Pepino (*Solanum muricatum*) is a perennial bush fruit crop first domesticated in the Andean region of South America. It is currently grown there at altitudes ranging from close to sea level up to 3,000 meters and its culture depends entirely on traditional land races. It grows best under warm conditions, is propagated only from cuttings, produces a sweet fruit tasting like melon and is grown mainly by smallholder farmers. There is no healthy stock program. Due to pepino's sensitivity to chilling, pests and diseases, its crops are replanted annually. Several different viruses have been reported infecting the crop (1,2). In 1976, a virus with flexuous filamentous virions typical of *Potyviridae* was isolated from symptomatic pepino (*S. muricatum*) crop plants in two valleys in Peru's coastal desert region. In 2014, a virus with similar shaped virions was isolated from fruits obtained from pepino plants growing in six coastal valleys and a valley in Peru's Andean highlands. Using serology and/or high throughput sequencing (HTS), both were identified as wild potato mosaic virus (WPMV), a virus previously only found infecting the wild potato species *Solanum chancayense* growing in the same region (3). The symptoms caused by the two old and seven new isolates, were examined in solanaceous plant species. These hosts varied considerably in their sensitivities to infection and individual isolates greatly in virulence. However, all seven new isolates killed infected *Nicotiana benthamiana* plants and more than half of them killed infected *Physalis floridana* and WPMV's original isolation host *S. chancayense*, these three species being the most sensitive to infection; the WSMV type isolate did not kill *S. chancayense.* The most virulent isolate was BA as it killed 5/8 solanaceous host species whereas CA was the mildest as it only killed *N. benthamiana*. Using HTS, complete genomic sequences of six WPMV isolates were obtained; only one showing evidence of recombination (FE). The distances between individual WPMV isolates in phylogenetic trees and the geographical distances between their collection sites were unrelated. Their complete ORF sequences were from 80.9 – 99.8% identical in pairwise comparisons, and the most closely related virus, potato virus V (PVV), had ORFs around 75% identical. WPMV, PVV and Peru tomato virus formed clusters of similar phylogenetic diversity, and were distinct but related viruses within the overall potato virus Y lineage, which contained 27 viruses first isolated in the Americas. WPMV infection seems widespread and, especially when present in mixed infection with other viruses, likely to be of considerable economic significance to pepino producers in Peru's coastal valleys. A healthy stock program supplying healthy virus free cuttings to farmers is advocated.

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Geographic distribution and evolution of Cucurbitaceae and Solanaceae viruses in the French Mediterranean basin

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Emerging plant viral diseases represent a significant burden to plant health, and their highest impact in Mediterranean agriculture is on vegetables grown under intensive horticultural practices. The emergence of a new viral disease results from a complex interaction among several factors, including ecological changes of host and vector populations, and genetic changes due to the introduction of new crop varieties and the evolution of the viruses and/or vectors. In order to better understand virus evolution and emergence, viruses and aphid vectors were mapped in Cucurbitaceae/Solanaceae crops and reservoirs in the French Mediterranean area, and virus diversity, evolution and population structure were studied through molecular epidemiology and spatial genetics approaches. Surveys were performed in summer 2016 and 2017, representing a total of 1619 crop samples, 351 weed reservoirs and 1121 aphids. The plant samples were analyzed using serological and molecular diagnostic tools, including next generation sequencing (NGS). The viral species and their frequency in crops were quite similar to those of surveys conducted ten years ago in the same areas. Contrary to other Mediterranean countries, aphid-transmitted viruses remain the most prevalent problems in France whereas whitefly-transmitted ones have not yet emerged. However, NGS analysis of viral evolution revealed the appearance of viral variants undescribed until now, especially for watermelon mosaic virus (WMV) in cucurbits, or variants not present in France before, as for cucumber mosaic virus (CMV) in solanaceous crops. Deep sequencing results also revealed complex virus populations within individual plants. The low spatial structuration of viral variability suggested frequent long-distance exchanges between viral populations even for non-persistently transmitted viruses.

Bipartite network analysis for understanding associations in plant viromes: The sweetpotato virome in Africa

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Metagenomic analysis has supported the discovery and identification of a wide range of new plant viruses. The large datasets generated by high-throughput sequencing also offer opportunities for new types of analyses to understand associations among viruses and their effects on hosts. Descriptive statistics and phylogenetic analysis are helpful to evaluate virus diversity and phylogenetic relationships among species, and network analysis can elucidate the structure of viral communities (1). Just as the community ecology of plant pollinators or host-parasitoid interactions is often studied in networks, we are exploring the association networks of viruses and plant hosts using bipartite networks. Bipartite networks are composed of two node levels, one level corresponding to plant viruses, and a second level corresponding to plant-hosts. Links exist between the two levels, but one characteristic of bipartite networks is that links are not shared between nodes in the same level. We are using a bipartite network framework to analyze the Pan-African sweet potato virome, a dataset consisting of 1168 samples collected from individual plants, from 223 field plots in 13 countries (2). The sweetpotato virome included 102 known and novel viruses from 13 genera, as well as five satellites and one pospiviroid. Mozambique, Ethiopia, Uganda, and Angola had the most diverse virus communities. Only 30 viruses were commonly identified in most countries. Analysis of the sweetpotato virome at the individual plant level found five large virus hub nodes: sweet potato badnaviruses (genus *Badnavirus*), sweet potato feathery mottle virus (genus *Potyvirus*), sweet potato symptomless mastrevirus 1 (genus *Mastrevirus*), and sweet potato leaf curl virus (genus *Begomovirus*). Other node centrality measures included node degree, betweenness, and closeness. Modularity, connectance, and nestedness were also evaluated by country, with low connectance values (0.25) but highly nested (0.61) viral communities. Positive and negative associations between virus species were also evaluated at local, regional, and continental scales.
More information about this analysis and ongoing code development is available at garrettlab.com/vnet

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Ecology of cereal dwarf viruses in an agricultural landscape

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The most important cereal viruses are barley/cereal yellow dwarf virus (B/CYDV, genera *Luteovirus/Polerovirus*, family *Luteoviridae*) and wheat dwarf virus (WDV, genus *Mastrevirus*, family *Geminiviridae*). B/CYDV are a complex of at least nine viral species (including BYDV-PAV, BYDV-MAV and CYDV-RPV) and WDV consists of two main strains. B/CYDV and WDV are transmitted by several aphid species and one leafhopper species, respectively. The host species of these viruses belong to the *Poaceae* family (e.g. wheat, barley and oat). As cereal crops are harvested annually, alternative hosts such as volunteers and/or wild *Poaceae* species are necessary to complete the epidemiological cycle of B/CYDV and WDV. Understanding the role of the wild compartment in the epidemiology of these cereal viruses requires a description of plant species diversity and virus prevalence within the landscape. Twenty-four wild *Poaceae* species were identified during a survey conducted in grasslands, woods and inhabited areas located in a 5 km² site (Annoix, Cher, France). We sampled 4646 single leaves of these plant species and tested the presence of BYDV-PAV/MAV, CYDV-RPV and WDV using serological tools. BYDV-PAV/MAV was the most prevalent (3.9%), followed by CYDV-RPV (1.4%) and WDV (0.7%). We also showed that almost all CYDV-RPV and WDV infections in the wild compartment occurred in five species (*Anisantha sterilis*, *Bromopsis erecta*, *Dactylis glomerata*, *Lolium perenne*, *Poa pratensis*). These data were complemented with inoculations of BYDV-PAV and WDV carried out under controlled conditions. Altogether, these approaches detected the infection of 16 species by BYDV-PAV/MAV, eight species by CYDV-RPV and 11 species by WDV. This study increases the number of known *Poaceae* host species for BYDV-PAV by 2.5% (four new host species) and for WDV by 20% (eight new host species). The next steps to understand the role of the different wild host species in cereal virus epidemiology will include the study of virus flows within and between wild and cultivated *Poaceae* species.

A necrotic disease of cucumber in greenhouses caused by mixed infection of tobacco necrosis virus and olive latent virus 1

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Necrosis in greenhouse-grown cucumber (*Cucumus sativus)* has been observed occasionally. Already in 1972, the causal agent of the disease was identified as (a strain of) tobacco necrosis virus (TNV, genus *Alphanecrovirus* (TNV-A) or *Betanecrovirus* (TNV-D), family *Tombusviridae*) (1). TNV is transmitted by the root-infecting chytrid fungus *Olpidium brassicae* (2), later taxonomically divided into *O. brassicae* and *O. virulentus*. Recently, cucumber plants at a nursery in The Netherlands were found showing small necrotic lesions in the stem and leaves. Investigation by light microscopy revealed the presence of *Olpidium* resting spores in the root cells of the infected cucumber plants. DAS-ELISA using antibodies raised against two serotypes of TNV (B4 and P, PrimeDiagnostics, Wageningen) indicated the presence of TNV in roots and in the leaves showing necrotic spots. Leaf material without symptoms from the same infected plants did not react in DAS-ELISA. RT-PCR with primers designed for the detection of different necroviruses in olive (3) showed that the diseased cucumber plants were co-infected with olive latent virus 1 (OLV-1, genus *Alphanecrovirus*, family *Tombusviridae*). This is the first time that OLV-1 has been reported from cucumber. However, cucumber was earlier reported to be infected after mechanical inoculation with an OLV-1 isolate from tomato (*Solanum lycopersicum*). This isolate was found in Poland in tomatoes showing necrotic foliar symptoms (4). In order to develop measures for eradication and prevention of the infections with both *Olpidium* and the two necroviruses, a survey was conducted in the different crops and materials present on the nursery, as well as the irrigation and draining system. *Olpidium virulentus* resting spores were found in dry root debris, still present on the concrete floors of the greenhouse and in the draining system. *O. virulentus* was also found in high concentrations in potted chrysanthemum, which may be the source of the *Olpidium* infection. Sources of the necroviruses TNV and OLV-1 were not identified.

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Biological characterization of AMVV1, AMPV1 and AMPV2, three new cryptic viruses of black-grass

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Three novel viruses were reported from black-grass (*Alopecurus myosuroides*) populations by Sabbadin *et al.* (1). Alopecurus myosuroides partitivirus 1 (AMPV1) and Alopecurus myosuroides partitivirus 2 (AMPV2) were identified as belonging to the genus *Alphapartitivirus*, while Alopecurus myosuroides varicosavirus 1 (AMVV1) was identified as belonging to the genus *Varicosavirus*. These viruses do not cause apparent disease symptoms and it has been hypothesized that they could potentially have a beneficial effect on their host. Studies have been conducted to investigate the transmission and in plant effects of these viruses. Viral incidence was assessed by RT-qPCR in five UK black-grass populations. AMPV1 and AMPV2 were widespread in all populations while AMVV1 showed variation in spread. Infection of plants was assessed by RT-qPCR using plant sections (leaves, stem, flower, roots) and tillers. Infection was found to be systemic for all three viruses. Analysis of pollen and seeds showed viruses were present following the same pattern of viral incidence in parent plants, strongly suggesting that viral transmission is vertical. Experiments to confirm vertical transmission are underway. Mechanical transmission and vector transmission using *Olpidium virulentus* for AMVV1 were tested and results will be discussed.

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Cassava brown streak disease pandemic continues to spread in South-eastern Democratic Republic of Congo

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Cassava is one of the most important staple food crops in small-holder farming systems in the Democratic Republic of Congo (DRC). Unfortunately, it is attacked by several pests and diseases, of which the most dangerous is cassava brown streak disease (CBSD) caused by two cassava brown streak ipomoviruses. These viruses are spread by whiteflies and infected cuttings. CBSD has been spreading as a pandemic through East and Central Africa since 2004, and DRC provides a route for its spread to the major cassava-producing countries of West Africa, most notably Nigeria which is the country with the most cassava production worldwide. CBSD has been reported in eastern parts of DRC since 2012, but the disease continues to expand its range. The study reported here aimed to examine whether the disease has spread into south-eastern parts of the country. From July to August 2018, 35 cassava fields were inspected in Haut-Katanga Province. A total of 350 (ten per field) leaf samples were collected and dried in an herbarium press. Total RNA was isolated using a cetyltrimethyl ammonium bromide method optimized for cassava. Samples were subsequently analysed with cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) specific real-time PCR TaqMan assays at IITA-Bukavu to identify the species associated with the infections. Typical CBSD symptoms were observed during field inspections in Pweto Territory in the variety *Shilefasi* in Simpweto and 13 other villages. High levels of incidence of foliar symptoms were observed, including some fields in which all plants appeared to be infected. Species-level identifications were made using real-time PCR and revealed that fields were only infected by CBSV. This is the first report confirming the occurrence of CBSV in the South-eastern DRC. It confirms the continued spread of the disease that threatens cassava production in Central and West Africa.

Comparison of different host species with tomato leaf curl New Delhi virus isolated from Italy

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Tomato leaf curl New Delhi virus (ToLCNDV, genus *Begomovirus*, family *Geminiviridae*) is one of the important tomato-infecting viruses in India and neighboring countries. In recent years, substantial losses for cultivated cucurbit crops in Mediterranean regions such as Italy, Spain and Tunisia have been caused by ToLCNDV. To prepare for the damage when this virus is introduced into Korea, its infectious clones were constructed and inoculation assays were carried out using commercial crops in both Korea and Italy. Two infectious clones for DNA-A and DNA-B of an Italian ToLCNDV isolate were constructed by rolling-circle amplification from ToLCNDV-infected pumpkin samples in Italy and cloning into the expression vector pCAMBIA1303. Two infectious clones were agro-inoculated together into different Solanaceae and Cucurbitaceae species for both Korean and Italian cultivars. According to PCR and Southern blot hybridization results with ToLCNDV-specific primers and probes, the Italian isolated ToLCNDV could not replicate in both Korean and Italian tomato and hot pepper. Also typical symptoms such as severe mosaic in leaves could be founded in almost all cultivars of Cucurbita crops. Interestingly, ToLCNDV showed differences in Italian and Korean watermelon. After agro-inoculation, ToLCNDV could amplify in Italian watermelon but could not do so in Korean watermelon. This is the first study experimentally to show and compare different host species of ToLCNDV.

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Cucumber green mottle mosaic virus affects symptom expression and viral accumulation of tomato leaf curl New Delhi virus in cucurbits

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Tomato leaf curl New Delhi virus (ToLCNDV; genus *Begomovirus*) and cucumber green mottle mosaic virus(CGMMV; genus *Tobamovirus*) cause diseases in cucurbit crops and are of increasing importance in many parts of the world. Both viruses also cause significant damage in protected greenhouse crops in Spain (1,2). We examined single and mixed infections of these viruses in cucumber and zucchini. Cucumber plants single infected with CGMMV and co-infected with ToLCNDV, produced tobamovirus-specific symptoms, and had reduced growth and number of fruits when compared with single ToLCNDV infections. Zucchini infected with CGMMV remained symptomless but when infected with ToLCNDV only, most developed severe begomovirus-specific symptoms, and had reduced vegetative development and less fruits. Fewer zucchini plants co-infected with ToLCNDV and CGMMV produced symptoms than those infected with ToLCNDV alone. When inoculated with CGMMV, the virus replicated at similar rates in single and mixed infections with ToLCNDV in cucumber as well as zucchini, whereas the begomovirus accumulated significantly less when co-infected with CGMMV. The results suggest the existence of an antagonistic effect of CGMMV against ToLCNDV accumulation in cucumber. Such effect would also explain similar differences in viral loads, the vegetative and reproductive development, and the reduced symptom expression in zucchini.

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Emergence of new whitefly-transmitted viruses affecting cucurbit production in the Southwestern United States

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Cucurbit production in the Southwestern United States and Northwestern Mexico has been severely impacted by cucurbit yellow stunting disorder virus (CYSDV; genus *Crinivirus*; family *Closteroviridae*) since its emergence in 2006. This has resulted in the elimination of fall production in the Imperial Valley of California. In response, an aggressive effort to identify sources of resistance against CYSDV and to incorporate this resistance into melon cultivars was initiated (1). Management of CYSDV in the region is compounded by high populations of whitefly (*Bemisia tabaci* MEAM1) and the prevalence of weed and alternate crop hosts; e.g., alfalfa (1). Development of cultivars with resistance to CYSDV was progressing well; however, in 2014 squash vein yellowing virus (SqVYV; genus *Ipomovirus*, family *Potyviridae*) was identified in the region (2) and, in 2018, cucurbit chlorotic yellows virus (CCYV; genus *Crinivirus*) was identified and subsequently detected in samples collected since 2014 (3). The presence of CCYV has complicated CYSDV resistance breeding, because both viruses produce similar interveinal yellowing and chlorotic spotting symptoms in melon and watermelon. Although CCYV is often less competitive in mixed infection with CYSDV (4), it remains to be determined if this pattern holds in the US Desert Southwest, and what impact the additional presence of the ipomovirus may have on accumulation of the criniviruses in resistant cultivars and on disease development.

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Field survey of virus diseases in *Ranunculus asiaticus* L. in Japan by newly established multiplex RT-PCR

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Systemic mosaics and distortion were frequently observed in production fields of ranunculus (*Ranunculus asiaticus* L.) in Japan. Ranunculus can be infected with a large number of plant virus species (1). Based on incidence of the virus diseases in ranunculus, ranunculus mild mosaic virus (RanMMV), tomato spotted wilt virus (TSWV) and cucumber mosaic virus (CMV) infections were investigated by epidemiological field survey in Japan. To focus the incidence and distribution of the three viruses, we first established a new multiplex reverse transcription-polymerase chain reaction method (mRT-PCR) for specific and simultaneous detection of RanMMV, TSWV, and CMV in ranunculus. We conducted a survey on virus infection in ranunculus production fields in Tohoku and Kyushu regions for several years. The results revealed that RanMMV was the most prevalent virus among the other viruses in all the fields sampled. Interestingly, the infection rate of RanMMV showed an increasing trend every year, suggesting virus transmission by aphids during the vegetative propagation cycles of ranunculus in the fields. Collectively, mRT-PCR was proven to be effective for specific and simultaneous detection of RanMMV, TSWV and CMV in ranunculus plants, and RanMMV appeared to be one of the most important plant pathogenic viruses to control in ranunculus fields in Japan.

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First finding of cucumber green mottle mosaic virus in *Pisum sativum*

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Wageningen Plant Research (WPR) maintains an extensive collection of virus isolates many of which date back to the 1970s and earlier. High Throughput Sequencing (HTS) is used on particular isolates as one of the methods to verify the correct identification of these isolates in the past. Peas (*Pisum sativum*; cultivar Rondo) were collected in 1959 from a field in the Wieringermeer in the Dutch province Noord-Holland showing distinct symptoms of pea early browning virus (PEBV). The presence of PEBV in these peas was confirmed at that time by indicator plants and serological tests and the PEBV isolate was included in the WPR virus collection as PEBV isolate E116. Field collected peas were stored at -20 ° C.

In 2016 an HTS study was initiated to characterize different tobacco rattle virus (TRV) and PEBV isolates from the WPR virus collection, including isolate E116. Analyses of the RNA-Seq (Illumina HiSeq, 125 paired-end reads) indeed confirmed the presence of PEBV and the (near) full genome of PEBV could be *de novo* assembled.

Interestingly, a Blastn analyses of the contigs obtained from the *de novo* assembly, also identified one contig with a high level of identity to cucumber green mottle virus (CGMMV). A subsequent reference assembly of the reads against the CGMMV reference sequence from NCBI (NC_001801) mapped 2888 reads against this reference genome. The reads were nicely evenly distributed over the CGMMV genome and the resulting sequence of 6404 nucleotides showed all genome characteristics of a tobamovirus. The nucleotide sequence of CGMMV E116 isolate showed 99.7% overall sequence identity with the NC_001801 CGMMV genome.

To our knowledge this is the first description of CGMMV in pea. Strikingly this virus was found in pea seeds collected from a field nearly 60 years ago. It is generally believed that the host range of CGMMV is restricted to the *Cucurbitacea*, although its presence from several weed species has been reported. Our result shows that the natural host range of CGMMV may be broader than assumed.

Incidence and characterization of pepper mild mottle virus (PMMoV) isolates from pepper crops in Greece

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Pepper mild mottle virus (PMMoV) (genus *Tobamovirus*, family *Virgaviridae*) (1) is an important pathogen of pepper and has a worldwide distribution. It is seed-borne and mechanically transmitted under field conditions. Recently, it was identified in pepper crops in the island of Crete, Greece, associated with serious epidemics causing high yield losses due to fruit discoloration and/or necrosis. The purpose of this work was the study of the incidence and the characterization of PMMoV isolates from the pepper crops of our country. In the years 2012-2018, 267 samples from 12 different regions of Greece were gathered from pepper plants showing leaf and fruit mottling and/or fruit necrosis. The samples were molecularly tested by RT-PCR for the presence of the virus with primers that amplify the capsid protein (CP) gene (2). PMMoV was detected in 74 of the 267 samples (27.7%), mainly in Crete (25%) and in very low rates in Imathia (Northern Greece) (0.37%). Also, sequencing of the CP gene from 15 virus isolates from two regions of Greece (Imathia, Crete) was carried out. The results showed 95 to 100% identity between the Greek isolates at the nucleotide level, while their identities with the sequences deposited in the databases ranged from 94 to 100%. Further phylogenetic analysis clustered the Greek isolates of PMMoV in two different branches. Currently, full genome sequencing from virus isolates belonging to both clades is being conducted. Finally, four tomato hybrids were successfully mechanically infected by PMMoV but they did not show any symptoms. The study of the incidence of the virus in other regions of Greece as well as its transmission to other *Solanaceae* hosts is under way.

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Maize lethal necrosis disease: Current research status in Kenya

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Maize is the staple food in Kenya. In 2011, the maize lethal necrosis (MLN) disease was reported in Kenya (1). The disease can cause up to 100% yield loss in farmers' fields (1, 2). MLN is caused by co-infection of two viruses: maize chlorotic mottle virus (MCMV), and a member of the genus potyvirus. Since the identification of the disease in Kenya, work has been going on to understand the epidemiology and the viruses responsible for the disease. This paper presents the advances made to understand and control the spread of the viruses causing MLN. MCMV, a member of the genus *Machlomovirus* (*Tombusviridae* family), was first reported in Kenya in 2011 (1). The local isolate has over 96% similarity to those reported in other parts of the world (3). The main potyvirus identified in diseased plants is sugarcane mosaic virus (SCMV) (3, 4). However, other potyviruses and poleroviruses have been identified in diseased plants (4). Spread of the SCMV and MCMV is by vectors – mainly corn leaf aphids and corn thrips respectively; manual transmission and ongoing work has shown seed transmission. The host plants for MCMV and SCMV include maize, sorghum, napier grass and sugarcane (4, 5) Management of the spread of the viruses includes: identification of MLN tolerant lines for use in the breeding programs (6, 7); and integrated pest management by planting maize with crops that capture corn aphids and avoids infection of maize with SCMV, thus reducing the MLN severity (7). Mathematical models have shown that severity of MLN is affected by temperature, soil moisture, rainfall and slope of the area (8). Much of effort has been input to ensure decrease in the incidences and severity of MLN in Kenya. This review gives a wholesome look at the steps taken to understand and reduce MLN in Kenya and draws research gaps this far.

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Metagenomic analysis leads to the discovery of infective plant viruses in both untreated and treated wastewater

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Aquatic environments represent a potential pathway for plant virus transmission (1). This is especially relevant in the case of reclaimed wastewater used for irrigation purposes in many arid regions worldwide. Our aim was to assess the composition of plant viruses in wastewater, the potential relevance of such virus in terms of infectivity and the efficacy of traditional wastewater treatment for their inactivation. For this purpose, we collected samples of treated and untreated wastewater from a Slovenian wastewater treatment plant and we concentrated the viral fraction using Convective Interaction Media (CIM) monolithic chromatography (2). Using shotgun high-throughput sequencing (HTS), we discovered a high diversity of plant viruses from different taxonomic families in both treated and untreated wastewater. Specific virus detection for the most common plant viruses was done by RT-qPCR and their capsid integrity was confirmed using transmission electron microscopy. Subsequently, we focused on assaying the infectivity of plant viruses from the genus *Tobamovirus*, family *Virgaviridae*, that were abundant according to HTS analysis. Using mechanical inoculation of test plants coupled with sRNA based HTS sequencing analysis (3), we confirmed the infectivity of pepper mild mottle virus and tomato mosaic virus in untreated wastewater samples, and of pepper mild mottle virus and tobacco mild green mosaic virus in treated wastewater samples. Our results demonstrate the presence of infective plant viruses in treated wastewater, confirming the inefficacy of traditional wastewater treatment for plant virus inactivation and rising concerns on the uncontrolled use of such kind of water for irrigation purposes. We highly recommend implementation of additional water disinfection methods that are more focused on virus inactivation for a safe recycling of treated wastewater. CIM concentration of viruses coupled with HTS and infectivity assays on test plants has proved to be an efficient strategy to assess the presence, composition and epidemiological impact of plant viruses in different water samples, including reclaimed wastewater.

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Pollinator-conducive flower strips may act as reservoirs for plant viruses

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Intensifying and upscaling agriculture has led to negative effects on a large scale, e.g. the loss of biodiversity of flora and fauna. Since 1989, in The Netherlands, flower strips have been used to mitigate the negative effects of intensively managed landscapes (1). These flower strips have direct influence on the drift of pesticides and washing out of nutrients (a reduction has been observed for both), and a positive influence on the abundancy and diversity of beneficiary insects and pollinators (1,2). Besides many positive effects, also possible negative effects of using flower strips within crops or at field borders may exist. The first negative effect may be the competition between wild flowers and flowering crops in attracting pollinators. Another described negative effect is the unwanted uptake of pesticides from the flowering field borders by insects. The third possible negative effect is that plants in field borders may be infected with plant diseases and thus can act as source plants from which crop plants can be infected. Experimental research on such negative effects of flowering field borders is scarce. In a pilot study, we focused on possible virus infections in plants (including common weeds) in flowering field borders. We analyzed samples taken from 1, 2 or 3-year old flower strips, located on a blueberry farm in The Netherlands and in a field trial at the experimental station of "Wageningen UR Open field crops" in Lelystad, The Netherlands. Sampled plants were ground in a phosphate inoculation buffer and mechanically inoculated onto a set of four test plants, including *Chenopodium quinoa*, *Nicotiana benthamiana*, *N. glutinosa*, and *N. occidentalis* 'P1'. A number of test plants reacted with mild virus-like symptoms, but analysis with an electron microscope did not lead to positive detection of virus particles. However, some samples induced heavy necrosis in the *Nicotiana* plants and chlorotic or necrotic local lesions in the *C. quinoa* plants. Electron microscopy on samples of these necrotic plants clearly indicated the presence of filamentous particles of approximately 500 nm in length, indicating infections with members of the genus *Potexvirus* (family *Alphaflexiviridae*). Identification of these viruses is currently going on, and will be presented . Several potexviruses have a broad host range. Being transmitted via contact or by mites, the presence and retention in (overwintering) weeds and perennials used in flower strip mixtures are a potential hazard for adjacent crops.

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Predictions of migrations of cereal aphid, a vector of BYDV in Central Europe

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Barley yellow dwarf virus (BYDV) species occur frequently around the world and together with wheat dwarf virus (WDV) they cause the most widely distributed viral diseases of cereals in Central European countries (1). BYDV is primarily harmful in the autumn, when emerging winter cereals are infected. At this time of the year, the bird cherry-oat aphid (*Rhopalosiphum padi* (L.) is the predominant abundant species in the crops (2). In Central Europe, *R. padi* typically has two distinct flight peaks. The first peak occurs in the autumn, when primary BYDV infections take place in winter crops; the second peak occurs in the summer. *R. padi* migration has been monitored since 1992 using suction traps at five sites in the Czech Republic. The count data were subjected to the following different analyses: i) The minimum temperature thresholds for the aphids to take off were determined; ii) A partial redundancy analysis using the minimum, average, and maximum temperatures, as well as the wind speed, the precipitation total, and past aphid migration descriptors was performed to explain the relationship between aphid occurrences and weather patterns; iii) Three types of models from the field of machine learning were used to predict aphid occurrences.

Our analysis showed the following findings:

- In Central Europe, 8 °C is the temperature threshold for *R. padi* migration. The take-off can be delayed, depending on the amount of daylight.
- Autumn temperatures influence the size of the summer migration peak, possibly by controlling the ratio of individual aphid sexual forms.
- In the autumn, large diurnal temperature amplitudes can be beneficial for the sexual forms because their production is stimulated by low night temperatures, whereas high day temperatures enable their long-distance migration.
- During autumns that are warm throughout the day and night, winged viviparous aphids might be produced more readily and the sexual forms tend to be born later in the autumn.
- High precipitation in February, March, and summer will increase the duration of the summer migration.
- An extended summer migration leads to an abundant autumn migration.
- Winter precipitation may play a role in aligning the hatching of aphid eggs with the bud burst. Aphids feeding on the unfurling leaves produce offspring that can prolong the summer migration.

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Seed transmission of tomato leaf curl New Delhi virus Italian isolate in Cucurbita crops

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Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus affecting tomato cultivation in the Indian subcontinent. Recently, however, ToLCNDV has spread into Mediterranean countries such as Spain, Italy and Tunisia, and occurs in Cucurbita crops causing economic damage. Although ToLCNDV is known to be spread by sweet potato whitefly (*Bemisia tabaci*), like other begomoviruses, it is not been clear how ToLCNDV has suddenly spread from the Indian subcontinent to the Mediterranean region. In 2017, ToLCNDV was diagnosed in young seedlings germinated naturally from fruit fallen the previous year in a farm located in Giugliano in Campania, Naples, Italy where ToLCNDV occurred. Because many sweet potato whiteflies had spread naturally in that region, it was necessary to identify whether that resulted in an artificial insect vector-free condition. Seeds were harvested from ToLCNDV-infected zucchini squash plants in Naples in 2017 and 2018 to examine whether ToLCNDV can be transmitted from zucchini squash seeds to young plants. Viral DNA was isolated from harvested seeds and 2-week-old seedlings germinated from them, respectively, and then amplified with a ToLCNDV-specific primer set. According to the PCR results, viral contamination was confirmed from all harvested seeds and dissemination was proven from almost half of tested seedling samples. Virus infection was confirmed by organ-specific PCR from seedling samples. This is the first report demonstrating that the ToLCNDV Italian isolate is a seed-transmissible virus in zucchini squash plants.

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Spatiotemporal spread of yam mosaic virus (YMV) in seed yam fields in Nigeria

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Spatiotemporal spread of yam mosaic virus (YMV, genus *Potyvirus*) was studied in seed yam (*Dioscorea rotundata*) fields in Ibadan, Nigeria. Fields were planted in 2016-17 and 2018-19 with virus-free rooted seedlings at 0.25 m × 1 m spacing at a density of 40,000 plants per ha. Twenty-three plots each of 10 m^2 were marked for observing YMV incidence and severity at monthly intervals from planting to harvest. Aphid populations in each sampling plot were monitored using water traps at weekly intervals. Each study plot was parsed into 2 m^2 quadrants (16 plant per quadrant) to determine index of disease dispersion (χ). Distribution pattern of infected plants were fitted to β-binomial distribution (P_b) and Spatial Analysis of Distance Indices (SADIE) to determine randomness and aggregation pattern. Spatial autocorrelation (3rd order) was assessed to determine relationship between disease incidences among spatially related disease units. Limited increase in YMV incidence was observed in the two trails: 2 to 6% in 2016-17 trials, and 20 to 40% in 2018-19 trials. Early phase of YMV spread showed no significant dispersion (χ; $P>0.05$), while late phase demonstrated significant disease aggregation combined with over dispersion of disease clusters indicated by β-binomial distribution pattern (P_b; $P<0.05$) and dispersion index (χ; $P<0.05$), respectively. Autocorrelogram indicates disease incidence among neighbouring plots were highly significant to 3rd order ($P<0.05$), but YMV incidence and aphid population were not correlated indicating a role of non-colonizing aphids in disease spread within the field. This study points to importance of short and medium range dispersion of YMV within a cropping season on overall YMV incidence in the seed yam fields. Agronomic management including isolation distance and roguing of inoculum sources has high potential to minimize reinfection in the new fields.

Survey for viruses infecting wild, ornamental and vegetable *Allium* species in the Czech Republic

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A survey for viruses infecting *Allium* species was carried out in different regions in the Czech Republic. In total, 850 Samples from 72 different wild and cultivated species were collected in nature, private gardens, botanical gardens, parks, and experimental fields. These samples were tested by ELISA and/or PCR for the presence of 15 virus member of one of the genera *Allexivirus, Carlavirus, Potyvirus, or Tospovirus*. The most frequently detected viruses were leek yellow stripe virus, shallot virus *X* (ShVX) and garlic common latent virus). However, in some materials and localities the incidence of shallot latent virus was also rather high. Surprisingly, onion yellow dwarf virus was found at lower percentage in samples than we originally expected. The occurrence of shallot yellow stripe virus was demonstrated probably for the first time in the country. Besides ShVX all other known allexiviruses were detected in various numbers of samples, as well. On the other hand, the situation with regard to the presence of impatiens necrotic spot virus and iris yellow spot virus, both members of the genus *Tospovirus*, in tested plants remains unclear and further experiments are needed. Garlic was the most heavily infected plant examined. The same was observed for onion despite the absence of symptoms. The situation in ornamental species was highly variable; some were heavily infected while others were completely virus free. Samples from nature were mostly completely healthy despite the fact that some of them are propagated exclusively or predominantly in a vegetative manner, thus ready transmission of viruses by this method might be expected.

Session

02

Epidemiology and Modeling

Modelling and manipulation of aphid-mediated spread of non-persistently-transmitted viruses

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Aphids vector many plant viruses in a non-persistent manner, i.e. virus particles bind loosely to the insect mouthparts (stylet). This means that acquisition of virus particles from infected plants, and inoculation of uninfected plants by viruliferous aphids, are rapid processes that require only brief probes of the plant's epidermal cells. Virus infection alters plant biochemistry, which causes changes in emission of volatile organic compounds and altered accumulation of nutrients and defence compounds in host tissues. These virus-induced biochemical changes can influence the migration, settling and feeding behaviours of aphids (1). Working mainly with cucumber mosaic virus (CMV) and several potyviruses, a number of research groups have noted that in some plants, virus infection engenders resistance to aphid settling (sometimes accompanied by emission of deceptively attractive volatiles). However, in certain other hosts, virus infection renders plants more susceptible to aphid colonisation. It has been suggested that induction of resistance to settling encourages transmission of non-persistently transmitted viruses, while induction of susceptibility to aphid settling retards transmission. However, recent mathematical modelling indicates that both virus-induced effects contribute to epidemic development at different scales (2). We have also investigated at the molecular level the processes leading to induction, by CMV, of feeding deterrence *versus* susceptibility to aphid infestation. Both processes involve complex interactions between specific viral proteins and host factors, resulting in manipulation or suppression of the plant's immune networks

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Plant virus management: Tools for evaluating strategies

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Management of virus epidemics is a challenge, especially in the seed systems of vegetatively-propagated crops in many regions of the world (1,2,3). To address this challenge, we developed scenario analysis tools to evaluate strategies for management of seed health, and for general improvements to seed systems.

The idea of an "integrated seed health strategy" is that farmers can combine use of quality-declared seed, disease resistance, and on-farm management of seed health such as by positive selection (7). To illustrate and evaluate the relative utility of these management components, we have developed a new R package, seedHealth, to implement scenario analyses related to these management decisions. Application of these analyses is illustrated for key viruses important to the health of root, tuber, and banana crops.

Seed health and seed availability are determined by the structure of seed system networks. There is a trade-off as greater seed system connectivity makes it easier for farmers to obtain improved varieties, but also makes it easier for pathogens to spread. Impact network analysis (INA) is a platform for evaluating how technologies affect linked socioeconomic and biophysical networks, with outcomes such as regional disease management success and/or regional crop productivity (4,5,6). Our new R package, INA, supports evaluation of seed system strategies. Its application is illustrated for identifying priorities for managing epidemics, for improving the spread of varieties, and for developing seed systems likely to upscale successfully.

https://www.garrettlab.com/software/ http://www.garrettlab.com/seed-systems/

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Epidemiology of zucchini yellow mosaic virus in a tropical irrigation area in Northwest Australia

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In the remote Ord River Valley, which runs from south to north in tropical Northwest Australia, a diverse range of crops are grown intensively under flood irrigation. The valley's cropping zone, called the Ord River Irrigation Area (ORIA), was established in 1963 following clearing of native bushland, completion of a Diversion Dam across the Ord River and construction of the irrigation scheme. Sandalwood plantations currently occupy 50% of the cropping area. One of the most important annual crops grown in the ORIA's dry season (April to October) is cucurbits (melon, watermelon, pumpkin), but this industry is threatened by severe epidemics of zucchini yellow mosaic virus (ZYMV)'s Southeast Asian strain which is absent elsewhere in Australia. Although an Integrated Disease Management Strategy (IDM) for ZYMV in cucurbits was devised for the ORIA, critical epidemiological information needed to optimize it was lacking and severe annual epidemics still occurred. Research was therefore done to determine: (i) which aphid species are important ZYMV vectors locally; (ii) which aphid vector hosts are present; (iii) how both ZYMV and aphids persist in the absence of commercial cucurbit crops during the annual wet season (November to March), and (iv) which climatic and other factors drive aphid population build-up and ZYMV epidemic development in cucurbit crops. Eleven surveys were undertaken in 2015-2017 to establish how ZYMV and its aphid vectors survive in the wet season and understand the roles ZYMV and aphid host species play in epidemic development during the dry season. All-year-round information on aphid populations was gathered using sticky traps placed at five representative sites in the valley. Early and late planted annual data collection blocks in the middle of the valley provided detailed information on aphid vector populations and ZYMV epidemic development during each growing season and a field experiment studied spatio-temporal patterns of ZYMV spread.

ZYMV survived the wet season by occurring at a low infection incidence in wild *Cucumis melo* and *Citrullus lanatus* var. *citroides* plants, and in crop cucurbits growing as volunteer plants in wet places or in back garden plantings. No alternative ZYMV hosts were detected in other plant families, and no ZYMV-infected hosts were found within sandalwood plantations. Only six aphid species were found in the ORIA, three of which were key ZYMV vectors, *Aphis craccivora, A. gossypii* and *A. nerii*. In both the wet and dry seasons, colonies of all three vector aphids were found readily. *A. nerii* colonised only the convolvulaceous weed *Caltropis procera, A. gossypii* colonised legumes and species in several other families, whereas *A. gossypii* was more polyphagous and the only aphid that colonised cucurbits. *A. craccivora* often colonised sandalwood host trees and leguminous plantation understory weeds. Numbers of winged aphids caught throughout the year peaked in July-August (i.e. mid growing season) and were greatest in the valley's north, but numbers caught varied widely with trap site reflecting local host abundance factors and year. In data collection blocks, time of aphid arrival, subsequent melon aphid population build-up and ZYMV spread in 2015 and 2016, resembled that in commercial cucurbit crops throughout the valley. In 2017, delayed aphid time of arrival and subsequent aphid population build-up in data collection blocks mirrored those in most of the valley with ZYMV only reaching 3% incidence, but not those in its southern end reflecting its greater local abundance of aphid and ZYMV sources rather than climatic differences. The ZYMV IDM strategy for the ORIA was revised based on these findings, especially by placing more emphasis on effectively managing reservoirs of ZYMV and aphids, and avoiding planting cucurbit crops next to sandalwood plantations.

Detection of a previously unknown virus associated with lettuce dieback disease

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Lettuce dieback disease causes necrosis, stunting, and death of lettuce plants throughout western U.S. lettuce production regions in California and Arizona each year. The disease is most frequently found in low lying areas with poor drainage, areas near rivers, or on recently flooded land. Lettuce dieback was demonstrated to be caused by either of two tombusviruses, tomato bushy stunt virus (TBSV) and Morrocan pepper virus (MPV) (1,2); however, the presence of TBSV or MPV is insufficient to cause disease, rather additional environmental factors associated with high soil moisture are believed to contribute to infection and symptom development. A dominant resistance gene, *Tvr1*, prevents tombusvirus infection and disease development in lettuce varieties carrying the gene. Most iceberg varieties have carried this resistance gene since the 1940s, and it has been increasingly incorporated into other lettuce types since its identification and characterization 15 years ago (3,4). However, testing of symptomatic field samples over the past four years has demonstrated an absence of either tombusvirus in a large percentage of symptomatic samples from which an infectious agent was mechanically passaged, suggesting the presence of an additional virus that may cause lettuce dieback disease. Development of degenerate primers for detection of a broad array of tombusviruses failed to amplify additional viruses; however, high throughput sequencing of symptomatic lettuce field samples and passages from symptomatic lettuce consistently identified a virus with limited (31-36%) homology to a virus recently characterized from watermelon, associated with the family *Phenuiviridae*, order *Bunyavirales*. Primers were developed to a portion of the polymerase protein, and RT-PCR analysis of older archived samples indicates a consistent association of the unknown virus with plants exhibiting lettuce dieback symptoms. Continuing molecular and biological studies are determining the full genome of the new virus and clarifying the relationship of this new virus with disease development and with TBSV and MPV.

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A new virus of Arabidopsis widespread in geographically dispersed ecotypes

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A transcriptome study of Illumina HiSeq RNA-seq datasets of different *Arabidopsis thaliana* ecotypes revealed the presence of virus-like sequences. *De novo* assembly and subsequent Blastn and Blastx analyses showed high levels of identity of some contigs to RNA1 and RNA2 of several viruses from the genus *Comovirus*, family *Secoviridae*. Two RNA sequences were assembled that indeed show the typical genome arrangements of a comovirus including a 3' poly-A tail. The assembled RNA1 sequence consists of 5950 nucleotides (nt), encoding one open reading frame (ORF) of 1850 amino acids (aa). The assembled RNA2 sequence consists of 3600 nt, encoding one ORF of 1046 aa. Both viral RNAs show the highest level of overall nucleotide and amino acid sequence identity to the comovirus turnip ringspot virus with 58% and 52% for RNA 1 and 58% and 52% for RNA2, respectively.

RT-PCR and SYBR-green based detection protocols were developed for both RNA1 and RNA2 of the virus. Tests of seeds batches from various ecotypes from the *A. thaliana* HAPMAP collection present in Wageningen showed the presence of the virus in different ecotypes. Seeds from several ecotypes, positive for the virus were sown and progeny plants were assessed for possible virus infections and phenotype. Vertical transmission of the virus was confirmed by low virus titres in the F1 plants. Virus-infected plants did not show obvious symptoms and could visually not be distinguished from uninfected plants.

Analyses of additional Illumina RNA-Seq data sets, including over 2200 Sequence Read Archives (SRAs) from different Bioprojects in the NCBI SRA database, revealed the presence of this newly discovered virus in over 60% of all analysed SRAs. These SRAs were derived from over 220 different *A. thaliana* ecotypes, originating from various geographical regions worldwide. Phylogenetic analyses of the nucleotide and amino acid sequences of near full length RNAs 1 and 2 extracted from various SRAs indicated distinct clades. These data indicate a global distribution of this so far undiscovered virus and suggests that ArLV1 and *A. thaliana* have an ancient relationship.

Widespread occurrence of a new yellow dwarf virus in cereals and grasses of Northern Europe

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Yellow dwarf viruses (YDVs) of the family *Luteoviridae* constitute a complex of ssRNA viruses that is the most widespread group of cereal-infecting viruses. Previously, an isolate of a new tentative YDV species was identified in oat (*Avena sativa*) in Latvia by sequencing of the coat protein (*cp*) gene (1): barley yellow dwarf virus-OYV (BYDV-OYV). In this study, virus surveys identified the presence of BYDV-OYV also in Estonia and Sweden. In 2012-2015, a virus survey of 47 cereal fields in Estonia was performed using high throughput sequencing (HTS). Among the identified viruses, complete genome sequences were recovered for seven isolates of BYDV-OYV from oat and wheat (*Triticum aestivum*) and the 5'-end of the genome was confirmed by 5′-RACE. In 2010-2011, a survey of YDVs was carried out in Sweden identifying BYDV-OYV in Triticale, couch grass (*Elytrigia repens*) and meadow fescue (*Festuca pratensis*) by sequencing of the *cp* gene. The genome sequence was determined for one Swedish isolate of BYDV-OYV from the forage grass meadow fescue and it shared 93% nucleotide identity with the Estonian BYDV-OYV isolates. The genome organization of BYDV-OYV was as for a typical member of the genus *Luteovirus* and sequence comparisons of genome sequences revealed highest nucleotide identity to BYDV-PAV-CN (83%) and BYDV-PAS (82%). In a phylogenetic analysis, the genome sequences of BYDV-OYV isolates formed one well-supported clade between BYDV-PAV-CN and BYDV-PAS. Sequence comparisons and recombination detection analyses revealed recombination between isolates of BYDV-OYV and also that BYDV-OYV is a putative parent for BYDV-PAV-CN and BYDV-PAS. Our results show that BYDV-OYV is a distinct species within the genus *Luteovirus* and indicate that this virus is commonly occurring in both grasses and cereals in northern Europe.

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Molecular characterization of the genetic diversity within whitefly populations in Northern Nigeria

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Adult whiteflies *Bemisa tabaci* (Genn.) were collected from different plant species grown in irrigated vegetable-growing areas of Kaduna State (N 9 03' 11 32', E 6 05' 8 48'), Nigeria. Total DNA was purified from three whiteflies individually per host plant population using the CTAB method. The whitefly mtCOI gene (850 bp fragment) was amplified by polymerase chain reaction using primers C1-J-2195 MTD-10 and L2-N-3014 MTD-12 (1). The PCR products were cloned and sequenced bidirectionally for three individual whiteflies, per collection. The sequences (n= 59) were assembled using DNASTAR software and manually edited to achieve 0% conflicts between forward and reverse reads. The mtCOI mitotype group affiliations were determined by phylogenetic analysis using selected references downloaded from GenBank and the Brown Laboratory archive. The sequences (n=208) were aligned using MUSCLE, implemented in Mesquite v2.75 (http://mesquiteproject.org). The alignment was manually edited and visually inspected for NUMTS per Song et al. (2). The aligned sequences were trimmed to 742 bp, and identical sequences were collapsed to obtain one representative haplotype using FaBOX (http://users-birc.au.dk/biopv/php/fabox/), yielding a final alignment containing 145 unique haplotypes, of which 26 were obtained herein. The mtCOI for *Bemisia afer*, Accession no. AF418673, was included as the 'outgroup'. The phylogenetic tree was reconstructed using MrBayes v3.2.6 (3). The log-likelihood scores of sampled points were plotted against the generations sampled using Tracer v1.6 (http://beast.bio.ed.ac.uk/Tracer) to verify stationarity of the chain and determine burn-in. The Effective Sample Size (EES) value per sample was >200. The MCMC runs were summarized using sumt command in MrBayes v3.2.5 (3). The resultant 50% majority-rule consensus tree was visualized using the FigTree v1.4.2 software (http://tree.bio.ed.ac.uk/software/figtree/). Within and between pairwise nucleotide divergence was calculated in Mega 6 (4). One mitotype each clustered within the North Africa-Mediterranean-Middle East (NAF-MED-ME) [III] and Sub-Saharan Africa-East, South and West (SSA) [I] major clades. Two Q mitotype subclades were distinguished within the NAF-MED-ME major clade. One putatively exotic mitotype group was nearly identical to the introduced Arizona Q prototype (5) and grouped with references from Burkina Faso, Morocco, Spain, Sudan, and Turkey, at 0-0.93% nt divergence. The uniquely African Q-like mitotypes from Nigeria were most closely related to Cameroon, Ivory Coast, and Zimbabwe references, at 0-1.2% nt divergence. The two Q-like subclades were 2.5-3.4% divergent. The Sub-Saharan I major clade mitotypes grouped with references from East and West Africa, including Cameroon, Kenya, and Uganda, with within sequence divergences, at 0-1.4%. The most predominant mitotype was the putatively exotic Q, at 75% (relative frequency), and was collected from *Gossypium sp.*, and to a lesser extent from *Cucumis sativus*, *Helianthus annuus*, *Phaseolus lunatus*, and *Solanum lycopersicum*. The putatively endemic African Q-like mitotypes were represented by 15% of samples collected from *Gossypium sp.*, *H. annuus*, and *S. lycopersicum*. The Sub-Saharan group occurred with a frequency of 10%, and was associated primarily with cassava. The diversity of mitotypes varied by collection site. Representatives of all of the mitotypes were found in Gungurufa, a multi-cropped agro-ecosystem, whereas both Q-like mitotypes were found exclusively in Samaru and Zaria, where cotton is the dominant crop.

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Monitoring aphid population dynamics: towards a better understanding of virus epidemics in melon crops

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In France, open field melon crops are regularly impacted by four aphid-borne viruses: cucurbit aphid-borne yellows virus (CABYV), cucumber mosaic virus (CMV), watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV). The efficiency of control methods is likely to be enhanced with an accurate knowledge of epidemic drivers in particular those linked with aphid vectors. Field experiments were conducted in Southeastern France between 2010 and 2018 to investigate the relationships between aphid population dynamics and virus epidemics. Winged aphids visiting melon crops were sampled daily using non-biased suction traps and aphid species were identified under a stereomicroscope. Viruses were monitored weekly by DAS-ELISA. Gompertz models were fitted to virus incidence data sets by nonlinear regression and AUDPCs (Area Under the Disease Progress Curve) were calculated. A statistical analysis was performed to explore the relationships existing between several "aphid" variables (total aphid abundances and specific abundances over different periods of time) and several "virus" variables (cumulative total of infected plants over different periods of time, newly infected plants per week, AUDPCs, Gompertz model parameter estimates). No significant relationship was highlighted between aphids and non-persistent viruses (CMV, WMV, ZYMV). Interestingly, a predictive relationship was established between *Aphis gossypii* population dynamics and CABYV epidemics suggesting that an early control of the population of *Aphis gossypii* could impact favourably the epidemic onset and progress of this persistent virus in melon crops.

Minor plant species can be key players of the *Poaceae* virome at the agroecosystem scale

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Prior to the domestication of plants, it is hypothesized that plant viruses were only co-evolving with uncultivated plants growing in mixed species communities, which probably resulted in complex interactions (antagonism, commensalism, mutualism). The development of agriculture further deeply modified natural ecosystems and land use, creating agroecosystems comprised of both cultivated and uncultivated areas. It is postulated that the advent of agriculture has modified the dynamics of virus-plant interactions, which has fostered the occurrence of virus disease emergence events. At the level of natural ecosystems, the relationships between plant communities' diversity and plant virus diversity, the distribution of plant viruses and the interactions between viruses and their uncultivated hosts have just started to be explored. In this context, we are conducting a study in the Natural Park "Burdinale-Mehaigne" (Belgium) using high throughput sequencing technologies in order to characterize the virome of *Poaceae* communities, including minor species, in contrast to agricultural ecosystems (cereal monocultures, grazed pastures and natural grasslands). We adapted a virion-associated nucleic acids (VANA) metagenomics protocol to sequence at high throughput pools of 50 plant samples per ecosystem (50 samples reflecting plant species composition) and per plant species. Over two years, about 4,300 *Poaceae* plants (corresponding to 24 species) were sampled and processed using the VANA metagenomics approach. The bioinformatic analyses revealed the presence of diverse viral communities in wild and cultivated *Poaceae*, even though they did not present any symptoms. These viruses belong to diverse families (e.g. *Endornaviridae, Luteoviridae, Partitiviridae, Potyviridae, Reoviridae, Secoviridae*), infecting a large range of hosts within the *Poaceae* and transmitted by different vectors (aphids, planthoppers, mites, nematodes) or seed-borne. Interestingly, minor *Poaceae* species studied in grasslands contained a wide virus richness, sometimes greater than ecosystem pools containing 8-11 major plant species. Moreover, some virus genera (e.g. *Amalgavirus, Alphaendornavirus, Potyvirus, Sobemovirus*) were found only in minor species, underlining the importance of minor plant species as virus reservoirs in wild ecosystems such as grasslands.

Harnessed plant viruses: Towards a universal framework for infectious clone assembly and biological characterization of plant viruses

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High-throughput sequencing and metagenomics allow unprecedented opportunities for plant viral and subviral agent discovery (1,2). Compared to their discovery, technological advances to characterize newly identified and known viruses lag behind. Infectious clones are powerful tools to validate (whole) genome sequencing results and to examine biological impacts of viruses (3,4). Generation of infectious clones is however a major bottleneck in plant virology.

Agrobacterium-mediated inoculation is the most efficient and universal way of delivering to plants DNA or RNA viruses and subviral agents. Based on current synthetic biology advances, we developed the pLX series, a set of mini binary T-DNA vectors (~3 kb). The pLX vectors are suitable for advanced cloning methods, autonomously replicate in *Escherichia coli* and *Agrobacterium*, and enable transient and stable transformation of plants (5). To ease the generation of infectious clones, we designed a workflow employing the pLX vectors, overlap-based cloning and plasmid verification by Illumina sequencing (6). The workflow allowed one-step assembly of clones of ssRNA, ss- and dsDNA viruses of the families *Potyviridae*, *Virgaviridae*, *Geminiviridae*, and *Caulimoviridae* (5,6). These were agro-inoculated successfully into plants. Of note, we established the first reverse genetic systems for wasabi mottle virus (genus *Tobamovirus*), a pathogen of the condiment crop wasabi, and for Ugandan cassava brown streak virus (*Ipomovirus*), a major threat to the staple food crop cassava.

To summarize, we describe a flexible framework for the assembly of infectious clones that facilitates the biological characterization of known and new plant viruses. The knowledge derived from the use of this technological framework will be valuable for epidemiology and applied research studies of emerging viral diseases in major and neglected crops.

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Modeling cassava mosaic disease in Southeast Asia for regional real-time decision making

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Cassava mosaic viruses, causal agents of cassava mosaic disease (CMD), are a major threat to cassava yield globally. Sri Lankan cassava mosaic virus (SLCMV) was first reported in Cambodia in 2015 (1) and more recently in China (2) and Vietnam (3) It is vectored by whiteflies and spread by infected cassava planting material. There is evidence that planting material, in the form of stakes, has been transported long distances across Cambodia and throughout Vietnam, with movement regularly across national boundaries (4). We present a computational framework for combining social and environmental geospatial data layers in a multilayer network model, to forecast and map regional disease risk and outcomes of management scenarios. Data for parameterizing the farmer trade network model include household surveys conducted in Cambodia and Vietnam in 2016 and 2017, and associated surveys of SLCMV incidence. We identified priority regions, both for pathogen surveillance and for epidemic management (through phytosanitary regulation, deployment of new varieties, farmer education). Interestingly, locations that were optimal for surveillance were not necessarily optimal for management, a practical finding for epidemic mitigation. We present potential patterns of introduction and spread of SLCMV, regional risk maps, and management recommendations from scenario analysis. Additionally, we discuss risk pathways that may be important for the introduction of additional strains of cassava mosaic virus and other cassava pathogens that may pose a threat to the region. More information about this analysis and ongoing code development is available at garrettlab.com/ugsweets.

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Preliminary characterization of potato virus Y (PVY) populations in Algerian potato fields

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To date, only limited data are available concerning the viral pressure present in potato crops in Algeria. For three consecutive years, surveys were conducted in potato fields in the main Algerian potato-growing regions during the mid-season crop cycle (January to March). A total of 285 potato samples were characterized to define the prevalence of the five most common virus species: potato virus Y (PVY), potato leaf roll virus, potato virus X, potato virus A and potato virus S. The results showed a higher incidence of PVY compared to the other four viruses. Because of this predominance of PVY and its distribution in all of the scouted regions, an analysis was carried out on the diversity of PVY populations. From a panel of 185 samples, serologically confirmed as being solely infected by PVY, 96.7% were found to be of serotype-N, and only 3.2% of serotype-O. A set of 31 PVY isolates was further analyzed by biotyping on tobacco and by molecular typing (RT-PCR, sequencing), targeting the nucleotide sequence polymorphism in the 5'NTR/P1 region and the three recombination junctions within the HC-Pro/P3 (RJ2), VPg/NIa (RJ3) and CP (RJ4) regions. All 28 PVY isolates from serotype-N inducing vein necrosis on tobacco were recombinant PVYNTN isolates. Among the three PVY isolates of serotype-O, two were typed as PVYN-Wi and induced vein necrosis on tobacco. This is the first report of the identification of NTN and Wilga type isolates in Algeria.

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Session

03

Virus Evolution

Variability in resistance genes conditions the evolution of emerging plant RNA viruses

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It is assumed that host genetic variability for susceptibility to infection conditions the evolution of viruses, either by driving them to diversification into strains that track the different host defense alleles (*e.g.*, antigenic diversity), or by canalization to infect only the most susceptible genotypes. Associated to these processes, virulence may or may not increase.

To test these hypotheses, we performed evolution experiments with tobacco etch (TEV) and turnip mosaic (TuMV) potyviruses in different genotypes of *Arabidopsis thaliana*. In a first set of experiments, we explored the role of genetic variability for susceptibility in TEV virulence. Results showed a pattern of local adaptation, characterized by a higher virulence on the ecotype(s) encountered during evolution. However, local adaptation did not always pay a cost in foreign ecotypes: viral lineages evolved in more susceptible ecotypes evolved as mild specialists, whereas adaptation to the less susceptible ecotypes resulted in virulent generalists.

In a second set of experiments, TuMV evolved in *A. thaliana* genotypes that differ in mutations in genes involved in resistance pathways and in genes whose products are essential for potyviruses infection. Plant genotypes classified into three categories: hypersusceptible, equal to wild-type and hyposusceptibles. TuMV induces severer symptoms in hypersusceptible genotypes than those observed in wild-type. By contrast, it induces weaker or no symptoms in hyposusceptible genotypes. When all evolved TuMV lineages were tested for fitness in all host genotypes used in the experiments, we found that the infection matrix had an interesting structure. On the one side, in agreement with the above TEV results, the matrix was significantly nested, suggesting the evolution of generalist viruses selected by the most restrictive mutant genotypes. On the other side, a modular pattern was also observed, which suggests that genotypes with mutations affecting related resistance pathways were selecting for viruses of increased fitness in all these related genotypes.

The Peruvian potato virome I: Potato virus Y

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Potato virus Y (PVY) is the type member of the genus *Potyvirus*, in the family *Potyviridae,* and one of the most damaging viruses of potatoes and other solanaceous crops. The Andean region, which includes Peru, is the center of potato diversity and therefore likely also of some potato viruses such as PVY. During 2016 we collected potato samples from farmers' fields at altitudes of 2443-3916 masl in four departments in the northern (Cajamarca), central (Huanuco, Junin) and southern (Cuzco) Andean highlands of Peru and analyzed them by small RNA sequencing and assembly. All known potato viruses as well as several new ones were identified in this survey and PVY was amongst those most commonly found. PVY was detected in 235 out of 552 samples considered in this study, but many of these could not be unambiguously resolved into individual or complete sequences, due to mixed infection by multiple genotypes or limited coverage by small RNAs respectively. We report the analysis of the complete genomic sequences of 32 Peruvian isolates of PVY which, together with 428 published genomic sequences, gave an alignment of 460 sequences. Of these 190 (41%) were non-recombinant, corresponding to the N, O and C phylogroups. However, analysis suggested that isolates of the C phylogroup, that mostly infected crops other than potatoes, was evolving faster and were therefore not considered in the analysis, leaving 162 sequences that provided a dated phylogeny. This allowed the likely history of PVY evolution in potato to be discussed. The current PVY population originated around 156 CE. It was probably first taken to Europe in the 16th century in tubers, which subsequently established the potato crop in other continents. Much of the present PVY diversity probably emerged after the mid 19th century, when potato breeding was stimulated by the late blight (*Phytophthora infestans*) epidemics of the mid 19th century, breeding lines were imported and shared, quarantine was absent, and eventually N phylogroup isolates joined the European O and C phylogroup population, and generated the recombinant R1 and R2 population of damaging necrogenic strains.

The HAM1 proteins of cassava brown streak virus and Ugandan CBSV determine the severity of the cassava brown streak disease and the course of cassava infection processes

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The two species, cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) causing cassava brown streak disease in Africa have distinct genomes and biological features. While causing a disease with similar symptomatology and outcome CBSV isolates are more rapid to invade its cassava host and replicate at high levels while UCBSV movement in infected cassava is delayed and virus replication remains at a level significantly lower than that of CBSV. In *N. benthamiana* symptoms of UCBSV infections consist of leaf curling and blistering while in contrast CBSV infections are associated with local lesions in inoculated leaves followed by systemic necrosis eventually leading to plant death. The genomes of U/CBSV are unique in particular because of a HAM1 sequence with unknown biological function inserted between NIb replicase and CP. We present results from biological experiments using recombinant infectious cDNA clones of UCBSV and CBSV isolates carrying various variants and mutations of HAM1 to infect cassava. Following infections of recombinant viruses, we demonstrate that HAM1 is crucial for the pathogenicity of the viruses in cassava determining the rate of replication and movement as well as the severity of symptoms. Differences in virulence between both viruses in light of the cassava brown streak disease and the epidemic will be discussed.

Evolution of plant and fungal viruses, and impacts on plant health

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Fungi have been crucial to plant health since the emergence of plants on land over 450 million years ago. Although fungi also interact with animals, these relationships are not so ancient, and are less frequently symbiotic. The intimacy between plants and fungi is reflected in the significant number of virus families that infect both plants and fungi, and in some cases the virus phylogenies are not congruent with the hosts, implying transmission of viruses across the kingdoms of plants and fungi. In plants these viruses are mostly double-stranded RNA viruses, and are in the category of persistent plant viruses. In at least one case the probable introduction of a persistent plant virus can be traced in the lineage of the plant host.

Many fungal viruses appear to be commensal or beneficial, and the viruses of plant-interacting fungi also can have beneficial effects on the plants that the fungus colonizes. The plant viruses in families shared between the two kingdoms are also frequently commensal. In a few cases these viruses are clearly beneficial, although they are generally very poorly studied. The frequency of persistent viruses in crop plants implies selection for these viruses during domestication, and it seems likely that this would have occurred due to their positive effects.

Host growth temperature and mixed strain infections alter the population genetic diversity of a plant RNA virus

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Ecological factors can shape the genetic structure and evolutionary dynamics of plant virus populations, resulting in important epidemiological consequences. With the accumulating evidence for climate change and the common detection of multiple viral infections in nature, there is an increasing need to understand how changes in host growth temperature, combined with the presence of viruses infecting the same host, may affect the genetic variability in the viral populations. Here, we addressed this issue by using two molecularly-cloned isolates of pepino mosaic virus (PepMV; genus *Potexvirus*) that belong to the European (EU) and Chilean (CH2) strains. Both PepMV clones were used in long-term single and mixed infections under different plant growth temperature conditions, and thereafter performing whole genome deep-sequencing of the viral populations. We found that all genetic changes corresponded to single-nucleotide polymosphisms that were randomly distributted throughout their genomes compared to the initial sequence of both PepMV genomes. However, this nucleotide variation was higher in the CH2 populations than in its counterpart, and mixed infections had a differential effect when plants were grown at high temperatures. The viral accumulation at the end of the experiment was not correlated for the genetic mutation supply rate, but a viral competition experiment showed that the relative fitness of the CH2 clone was affected significantly in the presence of the EU clone and was temperature-dependent as well. These results suggest that while the nucleotide variation of the virus population may remain similar in single and mixed infections in plants grown at low temperatures, higher average temperatures may alter the genetic variability of the virus populations. This study may therefore contribute to the understanding of the emergence and prevalence of viral infections in agricultural contexts of climate change.

Mechanisms underlying intraspecies differentiation of plantago asiatica mosaic virus isolated from a variety of host plants

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Plantago asiatica mosaic virus (PlAMV), a member of the genus *Potexvirus*, has an exceptionally wide host range within the genus and has been isolated from various weeds including *Plantago asiatica*, *Nandina domestica*, and *Viola grypoceras*. PlAMV also infects ornamental lilies and particularly causes severe necrotic symptoms. We have previously reported that these various PlAMV isolates were divided into multiple clades in the phylogenetic tree (1). However, the mechanisms of this intraspecies diversification have not been revealed yet. In this study, we examined the evolutionary processes shaping the intraspecies diversification of PlAMV using the full-length genome sequences of all available 26 isolates of PlAMV including several new isolates in Japan. Phylogenetic analysis revealed that PlAMV isolates were divided into five clades, which do not completely correspond to their host plants. For example, a new isolate from *Rhemannia glutinosa* in Japan (2) belongs to the same clade with nandina isolates, but unexpectedly, this clade does not include the *R. glutinosa* isolate from Korea (3). Based on these five clades, we then performed population genetic analyses. Genetic diversity in all PlAMV isolates (π = 0.181) was higher than those reported in other plant viruses. Consistent with the previous study (4), the ornamental lily clade has a low degree of sequence variability with π = 0.007, which was much lower than the other four clades (π = 0.090 to 0.300). Fst, a measure of genetic differentiation, of the ornamental lily clade was higher than the other clades, indicating that it is more differentiated compared with the other clades. We further calculated Tajima's D and dN/dS, and collectively suggested that natural selection is not an important force leading to intraspecies differentiation of PlAMV.

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Helper component protein of onion yellow dwarf virus evolved by losing N-terminal ~100 amino acids for viral adaptation to Japanese garlic

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Onion yellow dwarf virus (OYDV), a common potyvirus in *Allium* species, causes a great deal of damage in onion- and garlic-cultivation. OYDV is transmitted by several aphid species in a non-persistent manner. The genome of OYDV consists of a positive-sense single-stranded RNA, and it encodes the HC-Pro protein, an RNA silencing suppressor (RSS). We found that Japanese isolates of OYDV always co-infected garlic plants with leek yellow stripe virus (LYSV). Previously, it has been reported that the N-terminal 92 amino acids (aa) of HC-Pro was lacking in a Japanese OYDV strain (1). We sequenced the HC-Pro proteins of several Japanese OYDV isolates. Notably, all seven HC-Pro sequences of the Japanese OYDV isolates had a ~100-aa deletion in their N-terminal region, which contains a putative aphid-transmission determinant (2). Our phylogenetic analysis based on the amino acid sequences revealed two distinct groups suggesting that the N-terminal deletions in HC-Pro may have been created independently between the two groups. To obtain a clue for the molecular mechanism of the N-terminal deletion, we analyzed the secondary structures of the HC-Pro transcripts. The analysis showed that the start and end positions of the N-terminal deletion were distributed close to each other, suggesting possible template-switching to create a long deletion. To determine whether the lack of the N-terminal region affects the function of OYDV HC-Pro as an RSS, we examined the RSS activity of several HC-Pro proteins: OYDV HC-Pro from the USA (Ame-HC-Pro), China (Chi-HC-Pro) and Tokoro (Toko-HC-Pro). Ame- and Chi-HC-Pro showed RSS activity in onion tissues by agro-infiltration, whereas Toko-HC-Pro did not show RSS activity. Therefore, some OYDV HC-Pro proteins may not have RSS activity anymore. Because OYDV must have lost its aphid-transmission ability due to the N-terminal deletion of HC-Pro, we assume that another potyvirus, LYSV may play an important role in OYDV epidemiology.

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The Peruvian potato virome: mapping virus diversity to understand current and future threats under a changing climate

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The Andean Region, which includes Peru, is the center of potato diversity and therefor likely also of potato viruses. Under global warming, the emergence of new viral diseases can be expected due to changes in the population of virus and their vectors as affected by temperature. Here we report the viruses detected in potato samples collected throughout Peru by using small RNA sequencing and assembling approach. The viruses detected with the highest incidence were PVX, PVY, PVS, PVV, and PVB. Other viruses detected with lower incidence were PLRV, PVA, PMTV, PYV, APLV, APMoV, and PBRSV. New strains corresponding to the viruses PVX, PVY, PVV, PVA, PVB, PBRSV, APMoV, APLV, PVS, APMMV, and PYV were identified as well as of several novel viruses in the genera *Potexvirus*, *Potyvirus*, *Nepovirus*, *Comovirus*, *Tymovirus*, *Carlavirus*, *Ilarvirus*, *Badnavirus*, *Torradovirus*, *Enamovirus*, *Ophiovirus*, *Polerovirus*, *Fabavirus*, *Tobravirus*, and *Pomovirus*. Viruses PVB, PVA and a torrado-like virus (coded as SB26/SB29) were found more widespread than expected. This variability gives a snapshot of the viral diversity in potato in its center of domestication and was higher in Cusco and Junin than in Cajamarca, Huánuco, and Huancavelica. This could be the result of higher variability of domesticated and wild potato species in these regions, but may also imply that there is a greater risk for potato cultivation in Cusco and Junín, considering that greater variation means a greater possibility that new variant viruses could emerge as a result of a changing climate.

Session

04

Virus and Vector Interactions Ⅰ

The ecology of host and vector manipulation by plant viruses: new perspectives on a rapidly expanding field

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Dependence on vectors for transmission shapes the evolution of plant virus adaptations for facilitating acquisition, retention, and inoculation. Until recently, it was hypothesized that these adaptations are limited to virus proteins that enable virion binding to vector mouthparts or invasion of internal tissues. However, we now have evidence that viruses can manipulate host plant phenotypes in ways that enhance transmission by vectors. Viruses influence vector behavior through alteration of plant cues that mediate vector orientation, feeding, and dispersal behaviors (1). Effects are not uniform, but exhibit convergence depending on the specific frequency and duration of probing and feeding required to transmit distinct types of plant viruses (1). The induction of similar phenotypes by phylogenetically divergent viruses transmitted via the same sequences of vector behavior supports the hypothesis that virus effects are not just by-products of infection. Instead, effects are purportedly induced by multifunctional proteins that, in addition to primary roles in host plant exploitation, have evolved secondary functions as effectors of host phenotypes (2). The question of whether viruses can evolve manipulative functions in natural or agricultural settings is central to our understanding of the ecological and epidemiological importance of host and vector manipulation (3). The context for this question necessarily includes consideration of molecular and environmental constraints on virus evolution, limitations of existing studies, and prospects for future research (3). For this keynote presentation, I will combine empirical results from several pathosystems under study in my laboratory with meta-analytical outputs, recent theoretical work, and applied agricultural research, with the goal of stimulating greater interest in pursuing a holistic approach to understanding manipulation by viruses in real-world contexts.

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Novel insights into the transmission of phloem-limited viruses by aphids

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Barley yellow dwarf virus (BYDV; genus *Luteovirus*) is a phloem-limited, persistently transmitted virus that infects over 150 species within the family *Poaceae*. Once BYDV is acquired from an infected plant by the aphid vector, virus particles pass into the hemolymph and are transported into the accessory salivary glands to be released together with the watery saliva during the inoculation process (1). Conversely, beet yellows virus (BYV; genus *Closterovirus*) is also phloem-limited, but transmitted in a semipersistent manner by *Myzus persicae* among other aphid species. BYV is retained in the cuticle presumably associated to the foregut and released during salivation or egestion. Previous studies using the electrical penetration graph (EPG) technique revealed that the inoculation of BYDV is mainly associated with continuous salivation of *Rhopalosiphum padi* L. (Hemiptera: Aphididae) aphid into the phloem sieve elements (E1 waveform) (2). However, the same work showed that BYDV inoculation can occur at a low rate during undefined brief stylet intracellular punctures (potential drop waveform: 'pd'), presumable produced in phloem cells. Recently, a new distinct 'phloem-pd' pattern was associated with the inoculation of BYV by *M. persicae* (3). The aim of our study was to investigate if these newly described 'phloem-pd' patterns produced by *R. padi* were related to the transmission of BYDV (PAV serotype) to barley (*Hordeum vulgare* L.). The feeding process of viruliferous *R. padi* was monitored by EPGs and terminated after the observation of specific waveforms: 1) standard-pds, 2) standard-pds + 'phloem-pd', and 3) standard-pds + 'phloem-pd' patterns + a single E1 phase. Our EPG-controlled transmission tests revealed that *R. padi* produced two distinct 'phloem-pd' patterns prior to the E1 phase resulting in successful BYDV infection. Interestingly, the rate of transmission of BYDV increased gradually with longer periods of salivation in phloem cells. However, such gradual increase in transmission rate was not observed for the cuticle-borne BYV. Thus, our results suggest that phloem-limited viruses, either semipersistently (BYV) or persistently (BYDV) transmitted, are inoculated during brief intracellular punctures in the phloem cells by their aphid vectors but there are some distinct and quantitative differences in the way each virus is released/inoculated into their host plants. The specific aphid mechanisms associated to 'phloem-pd' patterns produced by *R. padi* and *M. persicae* and their role in the delivery/inoculation of persistently and semipersistently transmitted virus particles are discussed on the basis of our findings.

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ADP ribosylation factor 1 facilitates spread of wheat dwarf virus in its insect vector, *Psammotettix alienus*

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Many plant viruses are vectored by insects in a persistent circulative manner (1). The insect gut and salivary gland are important barriers limiting virus spread, but the mechanisms by which viruses are able to cross the gut escape barriers of the insect remain largely unknown (2). Wheat dwarf virus (WDV), transmitted by *Psammotettix alienus* in a persistent, circulative and nonpropagative manner, causes the most economically important virus disease in wheat. In this study, ADP ribosylation factor 1 (ARF1) was found to interact with the coat protein (CP) of WDV in a yeast two-hybrid, pull-down assay and to colocalize with virus in the gut and salivary glands of *P. alienus*. When expression of ARF1 was suppressed by RNA interference, the WDV titre decreased in the hemolymph and salivary glands, and transmission efficiency decreased, but titre in the gut did not differ from that of the control. These data suggest that ARF1 of *P. alienus* binds to the WDV virion and helps virus spread from gut to hemolymph. Our study provides direct experimental evidence that WDV can use the existing membrane trafficking mechanism to aid its spread within the insect vector. This first analysis of the molecular interaction between WDV and its vector *P. alienus* contributes to understanding the mechanisms involved in circulative transmission of the virus by the leafhopper vector.

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Plant rhabdoviruses-origins, diversity and vector interactions

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Rhabdoviruses are a large family of ecologically diverse viruses that infect vertebrates, invertebrates and plants. Plant rhabdoviruses are taxonomically classified in the family *Rhabdoviridae*, order *Mononegavirales* [1,2]. 'Classical' rhabdoviruses infect monocot and dicot plants, have enveloped bacilliform virions and a non-segmented negative-sense RNA genome, and are transmitted by hemipteran insects (leafhoppers, planthoppers, aphids) in a persistent-propagative fashion. Cytorhabdoviruses replicate in the cytoplasm while nucleorhabdoviruses replicate in the nuclei of infected cells [2]. All plant rhabdovirus genomes feature five canonical structural protein genes that may be overprinted, overlapped or interspersed with novel and diverse accessory genes, making their genome organization complex. New plant rhabdoviruses are increasingly reported, aided by high throughput sequencing studies. Their phylogenies reveal increasingly complex clusters of rhabdoviruses from diverse plant species. Some evidence suggests that plant-infecting rhabdoviruses are actually derived from insect viruses [3]. Plant-virus interaction studies are beginning to reveal conserved and unique biological features of these plant viruses in their plant hosts and invertebrate vectors. New molecular biology tools and infectious clones for plant rhabdoviruses are increasing our understanding of their lifestyles. Dichorhaviruses and varicosaviruses (transmitted by chytrid fungi) on the other hand have bi-segmented genomes. Dichorhaviruses are bacilliform, nuclear (like nucleorhabdoviruses), non-enveloped, although often seen associated with cellular membranes. They infect citrus, coffee and ornamental plants and are transmitted by *Brevipalpus* mites in which they also replicate. They generally cause local rather than systemic infections, but this may be temperature-dependent [4]. The epidemiology of plant-rhabdovirus-vector interactions will be illustrated using cytorhabdovirus, nucleorhabdovirus and dichorhavirus model systems.

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Cucumber mosaic virus Y satellite turns tobacco yellow to attract aphids in favour of its survival

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Y satellite RNA (Y-sat) is a satRNA which depends on cucumber mosaic virus (CMV) for its replication and encapsidation (1). The presence of Y-sat in CMV-infected *Nicotiana* plants (CMV+Y-sat) modifies the mosaic symptoms turning leaves bright yellow. Y-sat specifically down-regulates the *ChlI* mRNA, impairing the chlorophyll biosynthesis in *Nicotiana* plants (1) and thus causing bright yellow symptoms. We tested the effect of yellow colour on the epidemiology of CMV+Y-sat. A pairwise aphid attraction bioassay and Y-tube olfactory bioassay were conducted using healthy, CMV-infected and [CMV+Y-sat]-infected *Nicotiana* plants to observe the colour-dependent and odour-dependent aphid attraction, respectively. The CMV accumulation levels in CMV-infected and [CMV+Y-sat]-infected *Nicotiana* plants were determined by RT-q-PCR. The ability of the aphid to transmit the virus from either CMV-infected or [CMV+Y-sat]-infected *Nicotiana* plants was tested using *Myzus persicae* (green peach aphid), a natural vector of CMV. The results showed a significantly higher number of aphids was attracted by intense yellow of [CMV+Y-sat]-infected plants (27.63%) compared to that of CMV-infected plants (12.5%). The olfactory bioassay showed that there was no significant difference in aphid attraction among all plant types, showing that neither CMV infection nor Y-sat infection can induce odour-dependent attraction of aphids. Our RT-q-PCR results revealed that the level of CMV in CMV-infected plants was nearly 13x higher than that in [CMV+Y-sat]-infected plants. The transmission experiments resulted in 85% infection when aphids were transferred from CMV-infected to healthy plants, whereas we obtained 55% infection for [CMV+Y-sat]-infected plants. Therefore we found that the CMV transmission rate was not strongly affected by the CMV level in [CMV+Y-sat]-infected plants although Y-sat normally reduces the CMV level down to less than 1/10 of the level in CMV-infected plants. It is thus evident that the Y-sat-mediated yellow symptom specifically attracts aphids. Taken together, we concluded that Y-sat dominates the epidemiology of the helper virus by attracting a significantly higher number of aphids ensuring Y-sat survival in nature.

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The relationships between aphid numbers, numbers of aphids carrying virus and virus incidence in plants

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The numbers of peach-potato aphids (*Myzus persicae*) caught in Rothamsted Insect Survey suction traps and yellow water traps (YWTs) adjacent to cabbage (*Brassica oleracea*) plots, at two sites in the UK (Lincolnshire and Warwickshire) was monitored, during three crop growing seasons (2015-2017). The presence of *Turnip yellows virus* (TuYV) in the aphids was also determined using a real-time TaqMan PCR assay. Cabbage plants in the plots (two different varieties) were tested for the presence of TuYV by triple antibody sandwich (TAS) ELISA, at two time points each year.

There were two peaks in *M. persicae* numbers, the first and highest in June/July and the second between August and November, depending on the year. Total numbers of winged *M. persicae* were similar in the three years in Warwickshire. In Lincolnshire, total numbers differed considerably (10-fold) between the three years; the highest abundance was in 2015 and the lowest in 2016. Intermediate numbers were caught in 2017. Numbers of winged *M. persicae* in Warwickshire were higher than in Lincolnshire in all three years of the field experiments. The relationship between the numbers of *M. persicae* caught in YWTs and numbers in suction traps could be described by site-specific equations.

The percentage of winged *M. persicae* that carried TuYV fluctuated during the season. Higher incidences were seen in the first flight (summer) than in the second flight (autumn) and this was consistent over the three years of the experiments. The relationship between the numbers of aphids carrying TuYV and the incidence of TuYV in the cabbage plants is being investigated.

Function of the CP and CRP genes of garlic viruses in viral pathogenicity and insect-transmission

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Allexiviruses are the garlic viruses that cause a reduction in garlic production. In the viral genome, the cysteine-rich protein (CRP) gene is located just downstream of the coat protein (CP) gene. They are transmitted by eriophyid mites, and CP has been suggested to be a vector determinant. The genus *Allexivirus* consists of eight species: *Garlic virus A, B, C, D, E* and *X*, *Shallot virus X* and *Garlic mite-borne filamentous virus*. The natural hosts of garlic virus A, B, C, D, E and X (GarVA, B, C, D, E and X), shallot virus X and garlic mite-borne filamentous virus of are limited to *Allium* plants: garlic, shallot and onion. The CRP of GarVX functions as an RNA silencing suppressor (RSS) (1). On the basis of the sequences of the CP and CRP genes, allexiviruses are clearly divided into two major groups (group I and group II) (2). Moreover, group II members contain distinct inserted sequences (ISs) between the CP and CRP genes, while group I members do not. The roles of CP and CRP in the viral pathogenicity are not clear. To analyze the biological significance, if any, of ISs, we selected the CP and CRP gene of GarVB and GarVD, which belong to group II and I, respectively. *CP-CRP* and *CRP* were cloned into the potato virus X (PVX) vector. The PVX accumulation levels were quantified by qRT-PCR. The PVX level in the plant infected by PVX:GarVB-CP-CRP was significantly higher than that in the PVX:GarVB-CRP-infected plant. Such a significant difference was not observed when the GarVD constructs were examined. By western blotting, we could not detect GarVB-CRP in the PVX-GarVB-CP-CRP-infected plant. In addition, by the Agrobacterium transient-expression assay, we found that CP + CRP had less RSS activity than CP alone. On the other hand, we detected an RSS activity in GarVB CRP but did not in GarVD CRP. These results together suggest that CRP can somehow inhibit CP, and that ISs between CP and CRP control the CRP expression. The CP-CRP interaction and the ISs-mediated control of the CRP expression may affect not only the RSS activity of CP and CRP but also CP-mediated insect-transmission.

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Mixed infections of tomato yellow leaf curl virus and tomato mottle virus: Effects on virus titers, transmission rates and viral genetic diversity

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Mixed infections of plant viruses can be important factors influencing disease severity, genetic diversity of the co-infecting viruses, and transmission rates. The role of the vector in propagating mixed infections has been largely ignored. In this study we investigated the influence of whitefly transmission on the acquisition and transmission of tomato yellow leaf curl virus (TYLCV) and tomato mottle virus (ToMoV) (members of the family *Geminiviridae,* genus *Begomovirus*) by *Bemisia tabaci* (MEAM1) in mixed infections. Additionally, we examined the effect of acquisition sequence of each virus on inoculation and establishment of mixed infections. Data will be presented on whitefly acquisition of both viruses, symptoms, virus titers, and transmission efficiency.

Plant-mediated effects of potato virus Y on the zebra chip pathosystem

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Coexistence of two or more pathosystems is likely to occur in agricultural settings. In the Pacific Northwest, the main potato production region of the United States, both potato virus Y (PVY) and Zebra Chip (ZC) can impact tuber yield and quality. PVY is the most significant issue in seed potato production. It can be spread by seed pieces and by aphids in a non-persistent manner. ZC is associated with the bacterium '*Candidatus* Liberibacter solanacearum' (Lso), that is transmitted by the potato psyllid, *Bactericera cockerelli* Šulc. ZC reduces potato yield and makes tubers unmarketable. In Idaho, PVY is likely to establish in potato fields before ZC appearance since potato psyllids tend to appear later in the growing season. Little is known about potential effects of PVY infection on Lso development and/or its psyllid vector. We evaluated the impact of PVY infection on the transmission success of Lso, and on the fecundity and behavior of the potato psyllid. In the greenhouse, tomato seedlings were first inoculated with PVY. Three weeks later, the PVY-inoculated and mock-inoculated control plants were infested with Lso-positive potato psyllids. Lso transmission success was quantified as the percentage of Lso-positive plants three weeks after infestation. The effect of PVY on psyllid fecundity was evaluated in a no-choice experiment. PVY-inoculated or mock-inoculated plants were exposed to four Lso-positive psyllids for 48 h. The number of deposited eggs was recorded upon psyllid removal, and one week later, egg hatch rate was determined. Free choice experiments were conducted to evaluate psyllid preference for PVY-inoculated or mock-inoculated plants; vector preference was determined after 24 h. Lso transmission success (GLMM, $F_{1,73}$=0.039; $P > 0.05$) and Lso titer (GLMM, $F_{1,75}$=0.641; $P > 0.05$) were not significantly affected by the presence of PVY. Although oviposition of Lso-positive psyllids was significantly reduced on PVY-infected hosts (Mann − Whitney, $U = 0.008$, $P < 0.05$), hatch rate was not influenced by PVY presence (GLMM, $F_{1,63} = 0.033$; $P > 0.05$). Preliminary results indicated that Lso-positive psyllids may prefer to settle on PVY-inoculated plants at least within the first 24 h. Additional experiments are needed to confirm this pattern. Our study demonstrated that PVY has a negative impact on fecundity but may favor the relative preference of the vector of ZC for PVY-inoculated plants.

Session

05

Other Vector-borne Disease

Ecology of the 'other' vector-borne plant diseases: Similar questions and challenges?

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While there are important differences between viruses and other plant pathogens, I would argue that the ecology and epidemiology of vector-borne plant pathogens are remarkably similar, regardless of disease etiological agents. I will use the sharpshooter leafhopper transmitted bacterial pathogen *Xylella fastidiosa* as an example of a non-viral system, and present a combination of experimental manipulations and disease dynamics simulation models to highlight questions and challenges of relevant to most if not all vector-borne plant disease systems.

Analysis of "*Candidatus* Liberibacter solanacearum" haplotype effect on the feeding behaviour of its insect vector, *Bactericera cockerelli*

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The potato psyllid, *Bactericera cockerelli* Šulc (Hemiptera: Triozidae), is a significant pest of solanaceous crops in North America. Crucially, this psyllid species is the sole vector of the fastidious bacterium "*Candidatus* Liberibacter solanacearum" (Lso), which is the causative agent of diseases in several solanaceous crops, including the economically important zebra chip disease of potatoes. Currently, five Lso haplotypes have been identified, two of which (LsoA and LsoB) are exclusively transmitted by *B. cockerelli*. However, relatively little is known about the interactions of either Lso haplotype with its plant and insect hosts, due to its fastidious nature. In these studies, we elucidated the feeding behavior of Lso-infected (LsoA or LsoB) and Lso-free *B. cockerelli* using electrical penetration graphs to better understand the effects of this bacterium in its insect vector that ultimately leads to the successful infection of plant hosts by the manipulative parasite. Critically, these data provide new information regarding Lso-*B. cockerelli* interactions.

Flight behaviour and patterns of directional movement on *Philaenus spumarius*

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The recent emergence of *Xyllela fastidiosa* in Europe is a major threat for key crops such as olive, almond or grapevines. *Philaenus spumarius* was identified as the predominant vector involved in the spread of *X. fastidiosa* in southern Italy. This meadow spittlebug is distributed among most of the Palearctic and Nearctic regions. Understanding vector movement is critical to develop effective control measures and limit the spread of the diseases caused by *X. fastidiosa.* This insect species was reported to travel as much as 100 m within 24 hours in the field (1). However, both *P. spumarius* and *Neophilaenus lineatus*, another potential vector, can vertically displace up to the planetary boundary layer so they may passively travel long distances by laminar air currents (2). Our goal was to determine the duration and speed of flight of *P. spumarius* under laboratory conditions using a modified commercial flight mill. Thus, insects were collected from the field and separated by gender and age. Individuals were anaesthetized by applying CO_2. Then, a pinhead was attached to the insect pronotum using a drop of glue and connected to the flight mill. About 200 individuals were tested so far to estimate the number of flights, flight duration and number of turns. Experiments were carried out under controlled temperature, light and humidity. The atmospheric pressure and relative humidity was also recorded. The number and duration of turns were recorded using two different procedures: 1. Ethovision XT (Noldus), placing a video camera above the flight mill. 2. Mill_recorder, a computer-based device programmed to register the number and duration of each turn. The distance travelled and the average speed was calculated using the above information. Our data available to date show that *P. spumarius* is able to fly a distance of 1.99 km (in a single 1 h 40min continuous flight), that is much higher than it was previously thought. Furthermore, our preliminary results show that there are differences in the flight potential between males and females that vary throughout the year. This knowledge on the flight potential of *P. spumarius* will be critical to improve the mathematical modelling and management actions against *P. spumarius* and the spread of *X. fastidiosa* in Europe.

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Session

06

Virus and Vector Interactions Ⅱ

Identification of plant virus receptor candidates in the stylets of their aphid vectors

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Plant viruses transmitted by aphids cause tremendous losses around the world. The primary mode of virus transmission is the noncirculative manner where viruses are retained on specific receptors located in aphid stylets. Viruses are transported on the stylet's surface, while the aphid moves from plant to plant. They are inoculated often within a single puncture, thereby promoting viral outbreaks. In this context, the identification of receptors of viruses within their insect vectors is a key challenge to understanding the mechanisms of transmission, and offers an avenue for future alternative control strategies to limit viral spread. We developed several approaches to characterize and identify plant virus receptors in aphid stylets. These approaches include, amongst others, fine-tune dissection of insect mouthparts for *in vitro* interaction assays with viruses, immunolabeling with specific antibodies, high-resolution microscopic or proteomic analyses and RNAi silencing and targeted mutagenesis in aphids to validate gene candidate receptors. All this work has first been developed using cauliflower mosaic virus as model, and should be of benefit to other vector-transmitted noncirculative viruses. We discovered the acrostyle at the tip of the aphid maxillary stylets (1), an organ that displays plant virus receptors all over its surface (2). We determined the first proteome of insect stylets and identified the proteins present at the surface of the acrostyle, named stylins. And more importantly, we mapped peptides at the interface of virus-vector interactions. Our results allowed the identification of the first receptor of noncirculative plant viruses, the protein Stylin-01 shown to play a key role in cauliflower mosaic virus transmission (3), and provide novel insights into virus-vector interactions. They also revealed that the acrostyle is a multifaceted organ with various binding properties and functions.

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Genetic differentiation of tomato spotted wilt virus isolates sequenced from source plants and *Thrips tabaci*

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Efficiency of transmission of different isolates of tomato spotted wilt virus (TSWV) (family *Bunyavirales,* genus *Orthotospovirus)* by *Thrips tabaci* isolines has been shown to vary in a manner that is specific to the isoline-isolate combination tested. Evidence for coevolution of the vector and virus is supported by higher average transmission rates among isolate-isoline combinations collected from the same geographic location, and geographic population genetic structuring of vector populations across the sampled area. This study further examined vector-virus relationships underlying transmissibility of TSWV by characterizing the genetic structuring of TSWV isolates and the influence of vector transmission on viral populations. Next-generation sequencing was used to generate whole-genome sequences of TSWV isolates from source plants and adult *T. tabaci* that acquired and transmitted these isolates in the previously reported transmission assays. Analyses of genetic diversity, polymorphism, recombination, and phylogenetic relationships among TSWV nucleocapsid (N), glycoproteins (Gn and Gc), non-structural movement protein (NSm), non-structural silencing suppressor (NSs) and the RNA-dependent RNA polymerase (RdRp) genes identified differences among TSWV genes sequenced from source plants and vectors. Sequences originating from the vectors grouped separately from the sequences originating from the source plants, and increased genetic diversity was observed in the vectors.

Higher bacterial diversity of gut microbiota in different leafhopper (*Psammotettix alienus*) populations and the association with virus transmission

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The bacterial communities in the gut of an insect have important ecological and functional effects on the insect (1). However, the community composition and diversity of the gut microbiota in insects that vector plant viruses are poorly understood. As an important insect vector, *Psammotettix alienus* transmits various viruses including wheat dwarf virus (WDV) (2). Here, we used the combination of leafhopper and WDV as model to survey the influence of gut microbiota on virus transmission characteristic of insect vector and vice versa. We have characterized 22 phyla and 249 genera of all gut bacterial communities in the leafhopper populations collected from six geographic regions in China. Community composition and diversity varied across different geographic populations. However, WDV transmission efficiencies of these six field populations were all greater than 80% with no significant difference. Interestingly, the transmission efficiency of WDV by laboratory reared insects with decreased gut bacterial diversity was similar to that of field populations. Furthermore, we found that the composition of the leafhopper gut bacteria was dynamic and could reversibly respond to WDV acquisition. Higher bacterial diversity and abundance of gut microbiota in different leafhopper populations did not influence their WDV transmission efficiency, while the acquisition of WDV changes gut microbiota by a dynamic and reversible manner. This report provides insight into the complex relationship between the gut microbiota, insect vector and virus.

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The effects of mixed-viral infections in host plants and in the vector (whitefly) on vector fitness and implications for epidemics

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A plethora of studies have examined the effects of single-virus infections on their vectors; however, very few have assessed the impacts of mixed-virus infections on their vector/s. The primary reason being that mixed-infections can tremendously increase the complexity of the pathosystem. Our earlier studies clearly demonstrated that mixed-infections in host plants can differentially alter the plant phenotype, influence virus acquisition and transmission, and vector fitness, than single-virus infections (1,2). Our current whitefly-virus pathosystem in the Southern United States is incredibly complex. This pathosystem has two facets: 1. Mixed-infection in host plants due to multiple viruses transmitted by the same vector, and 2. Mixed-infection in the vector (whitefly) due to acquisition of multiple viruses from multiple host plants in the farmscape. For the first facet, we examined the effects of a begomovirus (cucurbit leaf crumple virus, CuLCrV) and a crinivirus (cucurbit yellow stunting disorder virus, CYSDV) infecting squash, on whitefly (*Bemisia tabaci* Gennadius MEAM1) preference and fitness. Mixed-infection of CuLCrV and CYSDV in squash drastically altered its phenotype and affected whitefly settling, wherein whiteflies seem to prefer non-infected plants, and the magnitude of such preference varied between viruliferous and non-viruliferous whiteflies. Mixed-infected plants, despite their altered phenotype (increased symptom severity), had fewer viral copies of at least one of the component viruses than singly-infected plants, and this difference affected virus acquisition by whiteflies. For the second facet, we evaluated the combined acquisition (mixed-infection) of tomato-infecting tomato yellow leaf curl virus (TYLCV) and squash-infecting CuLCrV by whiteflies. Mixed-infection of CuLCRV and TYLCV in whiteflies seems to enhance settling towards non-infected tomato and squash plants. The fitness study involving whiteflies infected with CuLCrV and/or TYLCV was conducted on a virus non-host (cotton), and results revealed that the mere presence of the viruses in the vector alone influenced its fecundity positively. The effects on fecundity also varied between singly-and dually-infected whiteflies. Taken together, the results indicate that mixed-infections of viruses in host plants and within the vector could differentially influence vector preference and fitness, and virus epidemics, more than single-virus infections.

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Session

07

Climate Change

Climate change and plant virus epidemiology

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Climate change and its effects on societies, ecosystems and agriculture, including plant viruses and their vectors, is one of the great challenges that requires immediate action. Ever increasing burning of fossil fuels, deforestation and other anthropogenic greenhouse gas emissions, i.e., carbon dioxide (CO_2), have increased global levels of pre-industrial revolution CO_2 from around 300 ppm to current levels of over 400 ppm. Global atmospheric CO_2 concentration is predicted to double by the end of the century, causing temperatures to rise and therefore to collectively change many biological functions (1).

Plant viruses and their vectors are an integral part of the environment, therefore highly conducive to change as a result of temperature and CO_2 increases. Currently, we have insufficient data on plant viruses and their vectors to be able to describe the general direction of change, but based on a few case studies, both viruses and vectors are highly sensitive to increasing CO_2 and temperature. Incidence and titre of barley yellow dwarf virus (BYDV) have been shown to increase under elevated CO_2 and temperature (2). Virus vectors, including the bird cherry-oat aphid (*Rhopalosiphum padi*) and the green peach aphid (*Myzus persicae*), are highly sensitive to changes in temperature and, indirectly via changes to plant phenology and biochemistry, to elevated CO_2 (3). Climate changes will open new frontiers for expansion of the viruses and vectors, as some regions, especially those at higher latitudes, will be more suitable in terms of temperature and length of the season.

It is reasonable to think that due to the recent rapid CO_2 increases, which had not been seen over the last thousands of years, viruses and their vectors are going to be more important within the environment and agriculture. Their short generation time when compared to their hosts can be adventitious, through quicker adaptation, taking greater advantage of the hosts and their greater expansion, which in turn should be a concern for future agriculture and global food security.

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A process-based model of the potato yellow vein virus - *Trialeurodes vaporariorum* (greenhouse whitefly) - potato pathosystem to support risk assessment and surveillance under changing climate

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Viruses cause major crop losses throughout the world. Sustainable control methods would be particularly effective if supported by predictive epidemiological models that can efficiently and accurately forecast disease spread and enable taking appropriate decision about the pre-emptive deployment of different control measures. Whereas several different modeling approaches have been described for insect pests, specific models for predicting impacts of climate change on virus epidemics have to date not been developed.

Potato yellow vein virus (PYVV) can reduce potato yields by 30% to 50%. PYVV is semi-persistently transmitted by the whitefly *Trialeurodes vaporariorum* (Westwood). It has sporadically caused problems on potato crops in Northern South America for over 60 years but has during the last 20 years started spreading from Venezuela and Colombia southwards along the Andes, where it has currently reached central Peru, while at the same time increasing in prevalence in Colombia. We developed a model to predict virus transmission risk by combining a vector phenology models with virus transmission efficiencies determined at different temperatures. Controlled laboratory experiments for virus transmission under different temperatures were performed and showed a clear temperature dependent transmission rate of the virus by the vector with a narrow range for efficient transmission between 12-18 °C. A non-linear equation including stochastic functions was fit to describe the temperature-dependent virus transmission by the insect vector and validated by transmissions under fluctuating natural temperatures in a screenhouse. The transmission function was then combined with a life cycle model of *T. vaporariorum* using a new module within the ILCYM 4.0 software to develop a specific virus transmission risk index. GIS maps produced by the model using ILCYM reflected very accurately the current occurrence of the virus in its endemic area and showed a 74% correlation with actual virus occurrence from survey data collected in Colombia, Ecuador and Peru between 2007-2010. The maps also predict new areas at high risk of transmission, one of which was targeted for surveillance and where the virus was subsequently confirmed to be present in a new country. This modeling approach could likely be applied to other insect vectored viruses as temperature dependent transmission has been reported in several cases.

Session

08

Disease Control

Development of plant virus resistance by a genome editing approach

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Developing plants resistance to viruses is an important feature in reducing crop losses. To date, the key strategy to cope with plant viruses is by classical breeding for cultivars containing plant-encoded viral resistance genes. Precise genome editing in plants has been boosted tremendously by development of the CRISPR/Cas9 technology (1). This powerful tool allows substantial improvement of plant traits in addition to those of classical breeding. Editing for viral resistance has been shown by precise cleavage via knockout of host viral susceptibility genes (2). Nonetheless, the opportunities for genome editing are expanding; e.g., increasing host defense gene expression levels by editing promoter and gene sequences, which soon will be adopted for the development of virus resistance. Here we demonstrate the development of virus resistance to potyviruses and tobamoviruses in cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.) by utilizing Cas9/sgRNA technology to disrupt the recessive gene function. The Cas9/sgRNA constructs were designed to disrupt specific target sequences in *CseIF4E*, *CseIFiso4E*, *SleIF4E1* and *SleIF4E2* genes for potyviridae resistance, and CsTOM1, *CsTOM3*, *SlTOM1* and *SlTOM3* genes for tobamovirus resistance. INDEL mutations were observed in the gene-targeted sites of transformed plants, but not in putative off-target sites. Non-transgenic mutant plants were generated by cross and self-pollination to segregate-out the transgene from the mutant plants. Homozygous mutant progenies with targeted sites exhibited variable resistance to different potyviruses and tobamoviruses. In addition to the knockout of susceptibility genes, we were able to increase the expression level of *CsRDR1* genes by promoter targeting using CRISPR/Cas9. In summary, CRISPR/Cas9 based precise genome editing is a "dream technology" which paves the way to improve plants for virus resistance.

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The challenge of yam mosaic virus control: recent advances to contain a persistent virus threat to yam in West Africa

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Yam mosaic virus (YMV, genus *Potyvirus*) responsible for the mosaic disease of yam (*Dioscorea* spp.) was first reported in 1970s from Cote d'Ivoire. This virus is known to infect several species of edible yams resulting in mild to severe mosaic symptoms depending on the cultivars. There were no records of YMV epidemics in West Africa, but the virus is highly pervasive and recognized as the most important constraint reducing yields by 30 to 50% especially in white yam (*D. rotundata*) cultivars which dominates production area in West Africa. Annual surveys between 2012 and 2016 indicated >80% incidence and prevalence of YMV in all yam production zones in Nigeria and Ghana. Vegetative propagation, frequent reuse of virus-infected seed, uncontrolled exchange of planting materials and lack of regulated seed production systems have been recognized as the plausible causes for this situation. Field experiments between 2016 and 2019, using virus-free planting material, indicated high reinfection rate of up to 40% in one season, most likely due to feeding of non-colonizing, migratory aphid vectors. Systematic efforts since 2012 have contributed to mapping of YMV spread and characterization of over 120 YMV isolates. This knowledge was used to develop versatile diagnostic tools necessary for YMV indexing and production of virus-free planting materials. Efforts to understand the rate of virus infection offered clues to YMV epidemiology and this knowledge has been employed for on-farm management to reduce YMV incidence and severity. Integration of multiple approaches is expected to result in the comprehensive management of the most persistent virus threats to yam in West Africa.

Exchanging cassava germplasm for the regional management of two major viral disease epidemics in Eastern and Southern Africa

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Epidemics of two viral diseases, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD), have been the major constraints for cassava production in Eastern and Southern Africa for the last two decades. In this work led by the IITA, we set out assembling the best cassava germplasm resistant to the two diseases from the five most affected countries of Kenya, Malawi, Mozambique, Tanzania and Uganda with the objective of cleaning them from viral infections and supplying them back to the farmers for cultivation. Thirty-one cassava varieties were assembled from the five countries and sent to NRI in the UK and KEPHIS Kenya for tissue culture and virus-indexing. At NRI, we developed a rigorous method for cleaning the cassava varieties from viral infections by cassava node bud culture in a tissue culture media followed by treatment with thermo- and chemo-therapies and subsequent diagnosis of viruses by PCR and RT-PCR. This method proved highly successful with 87% of the varieties [25 var.] cleaned in the first cycle of cleaning while the remaining four varieties took additional two attempts (1). Virus-free, tissue-cultured cassava plantlets were then shipped back to Kenya, where they were further multiplied and distributed to all five country partners. The plants were hardened in poly-houses and then further multiplied in fields in all countries. A strict quarantine regime was followed for removing CMD and CBSD-infected plants at 'primary multiplication' plots from where NGOs and progressive farmers purchased planting material for bulking up in secondary and tertiary sites for sale of cassava cuttings to farmers. This was the first cross-boundary effort to exchange cassava germplasm within the region and important lessons learnt in the project will be shared (2).

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Plant virus taxonomy: why is it so confusing?

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Epidemiological modelling of control strategies against the spread of cassava virus diseases

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Cassava production can be improved and secured by minimising losses due to both cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) through effective disease management and control strategies (1). Using a multi-disciplinary approach that combines biology, epidemiological modelling and economic factors, we develop disease control strategies to manage the dispersal of vector-mediated cassava virus diseases when the resources available for control are limited. We account for interactions between multiple factors that may contribute to the spread of the disease such as the host availability, the epidemiology of the disease, the nature of dispersal, and the nature, timing and scale of the control options. Our research provides a framework to optimise the management of insect-mediated disease dispersal when the availability of control resources is limited.

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Correlations between chemical structure of systemic acquired resistance inducers and their biological efficacy

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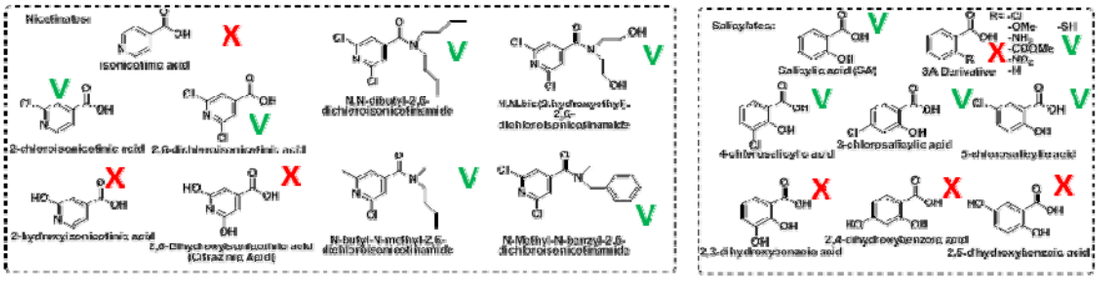
Modern agriculture is facing many challenges, which are undoubtedly focused on increasing productivity with limited usage of pesticides. In this situation, scientists are looking for alternative methods of plant protection which exhibit high efficacy at low doses. One of them is based on the systemic acquired resistance (SAR) phenomenon that can be triggered by inducers (elicitors), such as 2,6-dichloroisonicotinic or salicylic acids stimulating the natural mechanisms of plants resistance. Known inducers have a structure with carboxylic acids based on a substituted aromatic ring. The presented work describes the correlation between chemical structure of the elicitor, especially the type or the positioning of the group attached to the ring or carboxylic and its biological activity (Fig. 1). This relation may be linked to the affinity to hydrolysis, size, or possibility to protein binding by the resultant compound (1). The methodology of the presented research will include SAR induction tests on the model tobacco infected by tobacco mosaic virus and structural analysis describing possible behavior due to the mechanism of action. The presented data and the following conclusions will allow for finding the relationship between the structure of plant resistance inducers and their biological efficacy. This relationship may result in the ability to design and purposefully synthesize these types of compounds in the future, with a high efficiency, as well as the possible basis for a better understanding of the mechanisms of induction of resistance in the plant.



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Bacterial volatile compound 2,3-butanediol protects pepper against virus polyinfection in the field

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The volatile compound 2,3-butanediol, which is produced by certain strains of root-associated bacteria, consists of three stereoisomers, namely, two enantiomers (2R,3R- and 2S,3S-butanediol) and one meso compound (2R,3S-butanediol). The ability of 2,3-butanediol to induce plant resistance against pathogenic fungi and bacteria has been investigated; however, little is known about its effects on induced resistance against viruses in plants. To investigate the effects of 2,3-butanediol on plant systemic defense against viruses, we evaluated the disease control capacity of each of its three stereoisomers in pepper. Specifically, we investigated the optimal concentration of 2,3-butanediol to use for disease control against cucumber mosaic virus and tobacco mosaic virus in the greenhouse and examined the effects of drench application of these compounds in the field. In the field trial, treatment with 2R,3R-butanediol and 2R,3S-butanediol significantly reduced the incidence of naturally occurring viruses compared with 2S,3S-butanediol and control treatments. In addition, 2R,3R-butanediol treatment induced the expression of plant defense marker genes in the salicylic acid, jasmonic acid, and ethylene signaling pathways to levels similar to those of the benzothiadiazole-treated positive control. This study reports the first field trial showing that specific stereoisomers of 2,3-butanediol trigger plant immunity against multiple viruses.

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Field resistance as a strategy to control cassava brown streak disease in Tanzania

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Cassava brown streak disease (CBSD) causes the most significant food security threat throughout East and Central Africa. CBSD is caused by two virus members the genus *Ipomovirus*, family, *Potyviridae*; viz. cassava brown streak virus and Ugandan cassava brown streak virus, collectively referred to as cassava brown streak ipomoviruses. This study aimed at evaluating field-resistance of a selected set of promising, CBSD-resistant cassava varieties under contrasting disease pressure environments in Tanzania. Resistance was assessed by the absence of visual foliar and root symptoms in varieties planted over repeated planting cycles at a CBSD hot-spot site in coastal Tanzania. Environment x variety interaction effects on disease development and root yield production were determined by evaluating varieties across diverse growing environments. Varieties differed significantly in symptom expression over repeated cropping cycles where some of the tested varieties remained symptomless at least in the foliar parts over four planting cycles. Environment x variety effects were significant for both CBSD development and root yield production. Whilst ensuring food security, field evaluation of cassava varieties provides potential information on how crops interact with the environment under contrasting CBSD inoculum pressure scenarios.

Tackling maize lethal necrosis (MLN), a complex disease in Eastern Africa

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Maize (*Zea mays* L.) is the most important cereal crop in sub-Saharan Africa (SSA), covering over 35 million ha, largely in smallholder farming systems that produce over 70 million metric tons (MMT) of grain. Maize lethal necrosis (MLN) disease first appeared in Kenya in 2011, and became a major threat to maize production in Eastern Africa in subsequent years. In Eastern Africa, MLN is caused mainly by synergistic interaction between two viruses, maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV). MLN can cause up to 100% yield loss in susceptible maize varieties. The disease poses a complex challenge as the MLN-causing viruses are transmitted by insect vectors, and also through contamination of the seed, especially by MCMV. CIMMYT implemented a multipronged strategy in partnership with several international and national partners to tackle the MLN challenge. These efforts included: a) establishing a state-of-the-art MLN Screening Facility in partnership with Kenya Agriculture and Livestock Research Organization (KALRO) in Naivasha for identifying sources of resistance to MLN, MCMV and SCMV under artificial inoculation; b) accelerated breeding and deployment of MLN-tolerant/resistant maize varieties with other relevant traits preferred by African smallholders; c) creating awareness among the maize seed sector institutions on SOPs for producing and exchanging MLN-free commercial seed; d) disseminating information on farming practices for minimizing MLN incidence; e) establishing an MLN Phytosanitary Community of Practice involving various stakeholders, including national plant protection organizations (NPPOs), seed companies, regional/sub-regional organizations, etc.; and f) probing the epidemiology of the disease, especially the factors underlying seed contamination by MCMV. These comprehensive efforts have led not only in preventing the further spread of MLN into other major maize-growing countries in sub-Saharan Africa, especially Southern and West Africa, but also minimized the incidence of the disease in the MLN-endemic countries in Eastern Africa.

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Antiviral candidates screening using a viral-GFP vector-based antiviral agent screening system (VAASS) for developing biopesticides

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A pepper mottle virus (PepMoV)-based vector systemically expressing a *turbo GFP* in infected plant was successfully developed. A Viral-GFP vector-based Antiviral Agent Screening System (VAASS) was developed using leaf discs of plants infected with the plant viral-GFP vector. The VAASS was used for early and rapid detection of the antiviral activity of the extract library derived from plants and fungal resources. In the VAASS, leaf discs were treated with the extract library and dimethyl sulfoxide (positive control). The discs of healthy plants were used as a negative control. All leaf discs were observed under UV and visible light for 7 days. Subsequent candidates detected in the VAASS were investigated using systemic host method to confirm antiviral effects against PepMoV-Vb1/GFP. As a results, the extracts (including compounds: brusato, bruceantin, brucein A, bruceantinol, and brucein B) isolated from *Brucea javanica* exhibited inactivation effects against PepMoV-Vb/GFP. The extracts (including compounds: trichodermin and trichoderminol) isolated from *Trichoderma albolutescens* exhibited inactivation effect against PepMoV-Vb/GFP. These results were confirmed by RT-PCR and western blot analyses. Additionally, the extracts of *Aspergillus cervinus*, *Penicillium expansum*, *Polyporales* spp., and *Psoralea corylifolia* were detected that showing antiviral effects in the VAASS. To find efficacious antiviral agents, using the VAASS might be necessary in the future. In conclusion, this study demonstrated that some antiviral candidates derived from plants or fungi showed antiviral activity against PepMoV-Vb1/GFP in plants.

Using insect-proof net tunnels to reduce virus-related seed degeneration in sweetpotato

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Virus-related degeneration constrains production of quality sweetpotato seed, especially under open field conditions. Once in the open, virus-indexed seed is prone to virus infections whose accumulation lead to a decline in performance. Insect-proof net tunnels have been proven to reduce virus infections under researcher management. However, their effectiveness under farmer-multiplier management is not known. This study investigated the ability of net tunnels to reduce virus-related seed degeneration in sweetpotato under farmer-multiplier management. Infection and degeneration were assessed for two cultivars, Kabode and Polista, grown in net tunnels and open fields at two sites with varying virus pressures. There was zero virus incidence at both sites during the first five growth cycles. Sweet potato feathery mottle virus (SPFMV) and sweetpotato chlorotic stunt virus (SPCSV) were present in the last three generations, occurring singly or in combination to form sweet potato virus disease (SPVD). Virus infection increased successively with higher incidences recorded at the high virus pressure site. Seed degeneration modeling illustrated that for both Kabode and Polista, degeneration was reduced by the maintenance of vines under net tunnel conditions. The time series of likely degeneration based on a generic model of yield loss indicated that a potential economic threshold of 25%, under the conditions experienced during the experiment period, would not be reached in net tunnels within 10 generations, but might be crossed in the open field after four generations. Adopting the technology at the farmer-multiplier level can increase availability of clean seed, particularly in high virus pressure areas.

Application of a loop-mediated isothermal amplification protocol in an early warning system for epidemics of an externally sourced plant virus

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Previously, turnip yellows virus (TuYV; Family *Luteoviridae*, Genus *Polerovirus*) diagnostic protocols were used to test already symptomatic plants, limiting their practical value to post hoc grower support. A reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay protocol was developed to detect TuYV in crude extractions of its principal aphid vector *Myzus persicae* (1). The protocol was able to detect a single viruliferous aphid in 99 non-viruliferous aphids. Furthermore, detection was achieved in aphids stored in 30% ethylene glycol, sticky trap glue and 70% ethanol for at least four weeks. In 2017 and 2018, this protocol was field-validated at 30 South-west Australian grainbelt field sites sown to oilseed rape by testing aphids caught on yellow sticky traps and leaf samples from the crop. The data collected was used to develop an early warning system by examining the relationships between detection of viruliferous aphids before and throughout the growing season and TuYV crop incidence. Detection of TuYV-carrying aphids was a strong predictor for subsequent spread in the crop. In all scenarios in which TuYV reached ≥60% crop incidence by growth stage 30 (GS30; beginning of bolting), TuYV-carrying aphids were detected on >30% of trap sides in a six week period from pre-emergence until the five leaf stage (GS15). Conversely, TuYV detection on ≤15% trap faces during this period was associated with ≤6% TuYV spread at GS30. Although the presence of aphids during this period was a prerequisite for spread to occur, there were six scenarios in which a large number of aphids were caught but no TuYV was detected in them, with minimal subsequent TuYV spread by GS30 in the crop. Therefore, the RT-LAMP TuYV protocol can provide early warning for TuYV epidemics and enable proactive TuYV management, predominantly non-prophylactic, precisely timed and thereby highly effective systemic insecticide applications. These will eliminate initial *M. persicae* crop colonisation, protect vulnerable plants from future infestations, prevent TuYV epidemics in pre-flowering oilseed rape and minimize subsequent seed yield and quality losses.

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Aboveground application of the leaf-associated *Pseudozyma churashimaensis* and *Bacillus amyloliquenfaciens* elicits induced resistance against pepper virus complex

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Yeast associates with many plant parts including the phyllosphere, where it is subject to harsh environmental conditions. Few studies have reported on biological control of foliar pathogens by yeast. Here, we newly isolated leaf-colonizing yeasts from leaves of field-grown pepper plants in a major pepper production area of South Korea. The yeast was isolated using semi-selective medium supplemented with rifampicin to inhibit bacterial growth and its disease control capacity against *Xanthomonas axonopodis* infection of pepper plants in the greenhouse was evaluated. Of 838 isolated yeasts, foliar spray of *Pseudozyma churashimaensis* strain RGJ1 at 10^8 cfu/mL conferred significant protection against *X. axonopodis* and unexpectedly against cucumber mosaic virus, pepper mottle virus, pepper mild mottle virus, and broad bean wilt virus under field conditions. Direct antagonism between strain RGJ1 and *X. axonopodis* was not detected from co-culture assays, suggesting that disease is suppressed via induced resistance. Additional molecular analysis of the induced resistance marker genes *Capsicum annuum Pathogenesis-Related (CaPR) 4* and *CaPR5* indicated that strain RGJ1 elicited plant defense priming. To our knowledge, this study is the first report of plant protection against bacterial and viral pathogens mediated by a leaf-colonizing yeast and has potential for effective disease management in the field.

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Antiviral activity of a commercial extract from seaweed against cucumber mosaic virus

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Cucumber mosaic virus (CMV), one of major viruses in chili pepper (*Capsicum annuum*), causes economical losses yearly in South Korea (1). Seaweeds have been used as potential plant growth stimulants. With their copious nutrients they also contain plant growth-promoting (PGP) compounds. This PGP activity of seaweed products can be direct (as nutrients for plants) or indirect (by providing nutrients for beneficial PGPR) to plants (2). In a recent study, the components of *Ascophyllum nodosum* seaweed extracts were found to boost plant immunity in *Arabidopsis thaliana* and protect the plant against bacterial pathogens (3). Based on this study, we surveyed the antiviral effects of six commercial extracts from seaweeds against CMV in chili pepper using ELISA. Each seaweed extract was sprayed on to whole plants once a day for three days and then CMV was inoculated mechanically on two leaves of of the seaweed extract-treated pepper plants. Among them, pepper plants treated with *A. nodosum* seaweed extracts, Algaus® (J-Agro), showed a significant decrease of about 40.6% in CMV accumulation compared with distilled water as a control. Upper leaves of Algaus®-treated pepper plants inoculated with CMV showed delayed CMV symptoms or symptomless 14 days after inoculation. We confirmed that the expression of the pathogenesis-related 1a gene (PR-1a) gene, a key gene for systemic acquired resistance (SAR), and other SAR-associated genes were significantly increased on pepper seedlings treated with Algaus® using RT-qPCR. In this study we clearly showed that seaweed extract, Algaus®, induced antiviral responses to CMV and could be effective in reducing the severity of CMV infection and systemic spread of the virus in pepper. Further analysis for antiviral activities by Algaus® implicated in SAR will be discussed.

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Biological efficacy of salts based on systemic acquired resistance inducers combined with phytotoxicity reducing cholinium and betainium cations

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Systemic acquired resistance (SAR) induction is a very promising method of fighting against viral plant diseases. Using chemical SAR elicitors to trigger natural plant immunity has been proved on many plant/pathogen models. However use of those compounds are burdened by their phytotoxic impact on plants. Fortunately, SAR inducers can be modified into ionic form and combined with protective counterions into bifunctional salts. There are many compounds which can be used as enhancements against biotic and abiotic stress in plants. Some of these are compounds based on the cholinium structure that increase the tolerance of plants to adverse environmental factors. High biological efficiency of the elicitors combined with protective properties in one chemical compound may be an interesting possibility to show a new concept for design of plant protection agents. We have synthesized new salts (ionic liquids) composed of the anion of plant resistance inducers and betainium and cholinium cations. The biological properties of the salts obtained were successfully determined in the field of SAR induction efficacy by monitoring inhibition of the viral infection on tobacco (*Nicotiana tabacum* var. *Xanthi*) plants infected by TMV. Also phytotoxicity assessments were performed on tobacco *N. tabacum* var *Xanthi* plants (by spraying) and *Raphanus sativus* (sprouting efficacy). The results obtained show that the presence of betainium and cholinium cations decreased the phytotoxic effect of SAR inducer compounds. Moreover modification of elicitors in way presented did not change the SAR-inducing properties of such inducers as 2,6-dichloroisonicotinic acid, 7-carboxybenzo[1,2,3] thiadiazole acid, saccharinate, salicylic acid, 4-aminobutyric acid derivatives.

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Characterization of a new resistance related gene against soybean mosaic virus

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Soybean mosaic virus (SMV), a member of the genus *Potyvirus*, is the most common virus that infects soybean plants [*Glycine max* (L.) Merr]. In this study, we characterized *Glyma12* from soybean cultivar L29, which is an *Rsv3* related gene, located in chromosome 6, and putatively encoding a Metallo-dependent phosphatase-like protein. To observe the effect of the *Glyma12* on SMV strain G5H, we expressed Glyma12 protein into an SMV vector expressing GFP (pSMV-GFP). The expression clone (pSMV-GFP:Glyma12) was then inoculated into soybean cultivar Lee 74 (*Rsv3*-free). To observe SMV replication and movement, GFP observations under UV light and quantitative real-time RT-PCRs, using SMV CP specific primers, were carried out at 14 and 21 days post-inoculation (dpi). In inoculated leaves, expression of the Glyma12 protein along with SMV-GFP showed a weak expression of GFP and a decreased level of viral replication up to seven times lower than the vector control (pSMV-GFP). Interestingly, neither GFP expression nor viral RNA replication was detected in the systemic leaves of pSMV-GFP:Glyma12 inoculated Lee 74 plants at both 14 and 21 dpi. Taking together, these results suggested that Glyma12 might function as a potential resistance factor against SMV by inhibiting the viral replication, and preventing the movement of the SMV into systemic leaves.

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Correlations between chemical structures of systemic acquired resistance inducers and their biological efficacy

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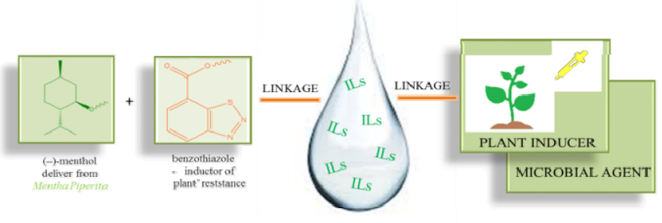
Inspired by one of the major problem in the environmental industry, which is plant protection using ecologically and human friendly plant resistance inducers, we advantageously used the plant sources and substance responsible for the induction of plant resistance as the starting materials to synthesize new ionic liquids (ILs). The designed ammonium and imidazolium salts where obtained with excellent yields (≥98%), via a "green" energy-free procedure, using the economically viable and readily available natural origin (−)-menthol moiety widely used in many industries. This monoterpene alcohol is derived from renewable raw resources and was introduced to the cation part of the synthesized ILs. As a counterion benzo[1.2.3]thiadiazole-7-carboxylate is presented, which has SAR immunity induction properties. Herein we demonstrate that the careful design of the cation and anion of IL leads to new bifunctional compounds. This methodology manifests in high microbiological activity of the salts obtained that allow the prepared compounds to function as antimicrobial agents. On the other hand, the described ILs of natural terpene origin present excellent properties as plants inducers. The tested ammonium and imidazolium ILs exhibited microbial activities higher than the ones shown by commonly used ingredient in biocides, benzalkonium chloride and very high SAR induction properties verified on tests on TMV inhibition on tobacco plants. It was noted that the length of the alkyl chain, presence of the natural occurring substituent, type of the anion, size of the cation, but also steric hindrance of cyclic groups are the principal factors in the antimicrobial properties when analyzing ILs.



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Development and use of predictive molecular markers for efficient selection of maize lines resistant to maize streak virus disease

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Developing and releasing new maize cultivar in SSA entails incorporation of maize streak virus (MSV, genus *Mastrevirus*) resistance as the disease is rampant in the region. Selection for resistant lines is conventionally done by artificially infecting plants in a screenhouse. To circumvent this cumbersome and time-consuming procedure, SNP markers linked to a major MSV resistance gene (1) were developed and used in high throughput early generation selection. Assessment of the robustness of the markers in 160 lines tested in controlled environment indicated that the markers have more than 90% accuracy in predicting the phenotype of the plants. Lines selected with msv1 markers initially exhibit higher disease score and gradually recover with newly formed leaves post-infection showing moderate to very mild streaks (recovery type of resistance). Recovery resistance to MSV is known to be controlled by one major QTL, *msv1*, and several minor QTLs. Validation of genotypes selected as resistant or susceptible were validated by MSV phenotyping under screenhouse conditions, suggests that SNP-based markers offer advantage in eliminating susceptible lines. Further work is underway to identify more genetic factors associated with recovery resistance by using bi-parental mapping populations for QTL analysis. QTL analysis using $F_{2:3}$ families genotyped with DArT markers and evaluated under artificial inoculation, identified 15 QTLs distributed over five chromosomes, accounting for 2.6% to 23.1% of the phenotypic variance, were identified suggesting that several loci are probably required for resistance to MSVD in maize. One major QTL on chromosome 5 was in the region harboring clusters of genes influencing resistance to viral diseases in maize including MSVD. Further investigation and validation of this QTL may lead to discovery of additional and powerful markers for MAS in maize as well as provides insight into the mechanism of resistance.

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Development of a management program through biological control of both western flower thrips and tomato spotted wilt virus in chrysanthemum

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Western flower thrips (*Frankliniella occidentalis*) are a major worldwide pest of vegetables and ornamental crops. In addition, thrips-transmitted tospoviruses cause severe yield losses to several economically important horticulture crops in South Korea and worldwide (1). Recently, predatory mites have been used for biocontrol of thrips, pest mites and nematodes in eco-friendly management (2). Essential oils-based insecticides are low toxicity, environmentally persistent and eco-friendly (3). Therefore, they have been used in biological control programs with indigenous natural enemies of pests. To determine a selective effectiveness for specific pesticides on biological control with predatory mites (*Stratiolaelaps scimitus*), we evaluated the contact toxicity of 41 essential oils as bio-insecticides for insect management in laboratory condition. Among them, four essential oils were effective from 87.3 to 100%. To apply to chrysanthemum field, *S. scimitus* were treated sequentially in soil, and then mixed oil combined with four essential oils applied as a foliar treatment every two weeks. The effects of treatments were assessed by counting the number of thrips captured on the yellow sticky traps. The mean percentage of thrips that emerged as adults from the soil was very low (1.2 to 8.5 %) in the chrysanthemum field when surveyed every month. Tomato spotted wilt virus (TSWV), transmitted by western flower thrips, was detected by RT-PCR in the individual thrips and upper leaves of chrysanthemum. The incidence of TSWV was reduced significantly (0.9 to 2.7 %) compared with those in non-treated fields (32 to 45 %). These findings will facilitate the selection of eco-friendly insecticides for effective control of western flower thrips and for developing insecticide resistance management strategies. Further management strategies for both thrips and TSWV will be discussed.

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Farmer knowledge in potato virus epidemiology and control in Kenya

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Potato is an important food security crop in Kenya, however its production is low because of the high prevalence of potato viruses in farmers' fields. Previous intervention strategies have not been successful because of a lack of information on farmer's perceptions and practices in managing spread of such diseases. To understand the farmer's knowledge and practices in virus control and set the pathways for intervention, a farmer household survey was conducted in two major potato growing regions in Kenya. The study revealed that the lack of technical information is the main hindrance to virus control among the farmers. Potato viruses were found to be among the least understood among the common diseases in the farmer's fields. Based on farmer's knowledge and practices, a system sustainably approach of spider diagram was used to analyze a framework for priority setting for farmer training on virus control. Among the different indicators of potato virus control, the use of host resistance in potato virus control was found with the lowest indicator scores (0/10) and was identified for prioritization in farmer training on potato virus management. We proposed for specialized farmer training based on analysis of farmer specific knowledge needs which varied between the two regions. Improved farmer knowledge and practices is expected to lower virus prevalence in farmers' fields, improve potato production and enhance food security in the country.

Interaction between two transcription factors following recognition of tobacco mosaic virus (TMV) by the N resistance protein

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Hypersensitive resistance to tobacco mosaic virus (TMV) in tobacco is conferred by the *N* gene which encodes the N resistance protein. Through recognition of TMV by the N protein, multiple resistance responses are activated for inhibiting the infection of TMV. Most of the responses are activated by salicylic acid (SA), but the transcription factor (TF) signaling hub effector1 (SHE1), an AP2/ERF TF, was shown to be independent of SA as well as jasmonic acid and ethylene phytohormone regulation (1). We found that SHE1 is upstream of and regulates production of an inhibitor of virus replication (IVR), the latter molecules acting independent of the salicylic acid (SA)-dependent pathway. The kinetics of gene expression of various SA-activated, defense response-involved genes in tobacco plants, along with IVR, were examined by real-time RT-PCR, as were the kinetics of gene expression of SHE1 and MYB1, which is an SA-activated TF. The results indicate complex regulation of expression of the various defense genes, with silencing of SHE1 or MYB1 affecting the expression of genes in the other pathway. In the yeast two-hybrid system, IVR and the TF SHE1 interacted with each other, which was confirmed by bimolecular fluorescent complementation and co-immunoprecipitation. This suggests that a feedback mechanism occurs between TF SHE1 and MYB1 for expression of IVR.

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Managing cassava virus diseases through clean seed systems in Tanzania

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Cassava is an important food security crop for over 300 million people in Africa. However, pests and diseases adversely hamper its productivity. Cassava virus diseases are especially important in Eastern, Central and Southern Africa, where cassava brown streak disease (CBSD) has devastated the crop causing unprecedented root quality and yield losses. The second most important disease is cassava mosaic disease (CMD), which continues to affect farmers wherever cassava is cultivated, although the effects of this disease have been partially mitigated through the development and widespread dissemination of CMD-resistant varieties. The viruses causing CBSD and CMD − cassava brown streak ipomoviruses and cassava mosaic begomoviruses − are transmitted by a whitefly vector, although long distance movements occur through the transport of infected planting material (= seed). To ensure the availability of quality cassava seed in Tanzania, a cassava seed system was piloted through the project: "New Cassava Varieties and Clean Seed to Combat CMD and CBSD" (5CP) using available CBSD/CMD-resistant varieties to bulk early generation seed of clean planting material. The early generation planting material was certified by the Tanzania Official Seed Certification Institute (TOSCI) and multiplied through a nationwide network of commercial seed producers at basic, certified and quality-declared seed levels. This initiative is currently being scaled through the project: "Building an Economically Sustainable Seed System in Tanzania" (BEST). Commercialising the system means that seed for each of the certification categories is produced in a sustainable way by cassava seed entrepreneurs (CSEs). In turn, this approach ensures that rural cassava producers have timely access to superior, disease- and drought-resistant cassava varieties. The careful management of this system for the production of high-quality cassava seed is expected to have a significant positive impact in controlling the spread of cassava viruses in Tanzania. This will have the twin benefits of reducing levels of cassava virus inoculum, as well as greatly minimising long distance movements of these viruses resulting from the transport of infected stems. In view of the successes achieved in virus management in Tanzania through implementing the seed system described, IITA is working with national partners to set up similar systems in other countries affected by CBSD and CMD, including Burundi, the Democratic Republic of Congo, and Rwanda.

New bifunctional salts based on SAR inducers as a highly efficient, eco-friendly weapon against viral and bacterial plant diseases

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The biggest unsolved problems in agriculture involve viral plant diseases. At present, there are no chemical methods with the ability to inhibit the spread of viral diseases that lead to huge losses in plant production worldwide every year. However, during the evolutionary process, plants developed many mechanisms allowing them to defend against pathogens. One of those is the systemic acquired resistance (SAR) phenomenon, induced by pathogen attack or artificially, by using an elicitor (immune inducer). Elicitors are a modern group of compounds that imitate interactions between pathogens and plants. This functionality leads to activation of natural signaling pathways and induction of plant immunity. We have synthesized salts based on SAR-inducer anions combined with quaternary ammonium cations possessing different properties, such as antimicrobial action or/and water solubility enhancement. The biological properties of the salts obtained were determined by monitoring inhibition of the viral infection in tobacco (*Nicotiana tabacum* var. Xanthi) plants against TMV infection. The salts obtained were also tested as to their antibiocidal activity by determination of minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values in *in vitro* tests against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Salmonella typhimurium*. The results obtained showed that the presence of the quaternary ammonium cations increased the SAR-inducing properties of the obtained salts. Antimicrobial analysis of all the salts obtained indicate lower values of MIC and MBC in comparison to reference substances.

This work was supported by the National Science Centre (Poland), project OPUS (No. UMO-2015/17/B/NZ9/01676) - "Systemic Acquired Resistance (SAR) of plants against viruses: new elicitors and biological and molecular characterization of their mechanism of action"

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The most dangerous diseases of vegetable crops transmitted by seeds

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In recent years, the production of vegetables has been growing in the Russian Federation, but there is a high share of imported planting material and seeds. Thus, the import of vegetable seeds is about 80%. Seeds are a source of latent infection by dangerous phytopathogens, including viruses and bacteria. In most cases, the infection titer in the seeds is at a very low level, and therefore, there is a need to use highly sensitive diagnostic methods and specific sample preparation. The main problem on tomatoes is pepino mosaic virus (PepMV), a member of the family *Potexvirus*, causes a decrease in yield and quality of tomato fruits. In some European countries, the virus infected 70% of greenhouse tomatoes. Methods of diagnosis of PepMV currently are well represented. For laboratories, available commercial kits for ELISA and lateral flow immunoassays of different producers and also a number of primers and probes for conventional RT-PCR, RT-qPCR have been published.

In addition, in recent years, another dangerous phytopathogen, *Candidatus* Liberibacter solanacearum, has appeared in Europe, affecting mainly potatoes and carrots, and causing serious damage to the production of these crops. Initially it was believed that Lso damages only the cultures of the *Solanaceae* family and is spread by *Bactericera cockerelli*. However, in 2008, *Ca*. L. solanacearum was first found on carrots in Finland. Five geographic haplotypes of Ca are currently described. L. solanacearum. Two haplotypes (LsoA and LsoB) are associated with diseases of potatoes and other solanaceous crops, three others (LsoC, LsoD and LsoE) - with diseases of carrots and celery. In 2018 haplotype C was detected in symptomless potato plants in Finland. All daughter tubers of the CLso-positive potato plants were all CLso negative but it is necessary to continue these studies to confirm the results.

Tobacco ringspot nepovirus (TRSV) is a quarantine virus for the EAEU. Has spread in many regions of the world by infected seeds and planting material, where is caused serious diseases of soybean, tobacco, grapes and *Cucurbitaceae*. Methods of diagnosis of the virus include ELISA and various PCR variants. In 2017, we developed primers for qPCR (TRSV-lab2-F / TRSV-lab2-R / TRSV-lab2-P), allowing to identify all TRSV isolates from the All-Russian Plant Quarantine Center collection and recommended to use for quarantine diagnostic.

In recent years in All-Russian Plant Quarantine Center were sent more and more samples from producers and importers of vegetable seeds for the purpose of determining the presence of infection. Due to the necessity and importance of quarantine and dangerous diseases, the scientific department of the All-Russian Plant Quarantine Center has been developed, validated and used various fast and high sensitivities methods of diagnosis.

Session

09

Diagnostics and Surveillance

Impact of new diagnostic methods in revealing plant virus transmission pathways and diagnostics

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The development of new virus concentration methods and in particular, of extremely sensitive detection methods such as real-time PCR and its digital version droplet digital PCR (ddPCR), has opened new possibilities for studying unexplored plant virus transmission pathways. Several decades ago, the almost impossible task of detecting low concentrations of viruses both in plants and outside their hosts, in insect vectors or even in the environment, has now become much easier (1). ddPCR is a method that, in addition to being extremely sensitive, also allows the absolute quantification of virus copy numbers skipping the need of a standard curve. It is significantly less sensitive to different PCR inhibitors, as we demonstrated using pepper mild mosaic virus as a model (2). A similar situation applies to the LAMP method (3), which allows for the amplification reaction on-site even without prior nucleic acid extraction, allowing us to detect viruses in highly complex samples.

All of these methods have already found their place in routine diagnostics labs worldwide. However, the real revolution in virology during the last years is undoubtedly best actualized in the high-throughput sequencing (HTS) method, which is the only truly effective generic method for detecting viruses. HTS enables detection of all nucleic acids in the investigated sample. Using HTS, we can rapidly find new unknown viruses, identify viruses in new hosts, and unravel the responsible causative viral agents behind symptoms of unknown etiology. The latter is demonstrated by our recent discovery of henbane mosaic virus infecting tomato as a new host (4). HTS also helps us in determining new viral genotypes and studying within-host virus evolution (5). The rapidly evolving nanopore-based HTS platform allows us to sequence longer viral genomic sequences and is therefore effective for the assembly of genome components containing repeating regions and for searching for recombination events. Due to the high benefit that HTS offers, several “user-friendly” pipelines have been developed, adapted to diagnostics requirements, which enable easier bioinformatics processing of the data. At the same time, more complex protocols focused on exploiting this technology at its maximum, both in diagnostics and research, are being developed also in the frame of VALITEST and several Euphresco projects. The applications of the mentioned methods in virus research, tracking viruses in complex samples and examples of their application for diagnostic purposes will be presented.

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Identification of novel cereal infecting tenuivirus in three Northern European countries indicates it may represent a "forgotten" species, European wheat striate mosaic virus (ESWMV)

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The development of high-throughput sequencing (HTS) technologies has changed the paradigm in pathogen identification. Nowadays, over hundred plant viruses have been identified by the HTS data analysis. Whereas the majority of these viruses are novel and data on biology of these viruses is absent, another category of viruses is now rediscovered. Indeed, some viral species are actually biologically well-characterized by earlier scientists before the advent of molecular methods: their host range, symptomatology and transmission pathways are well characterized but there is no information on their genome sequence. Sequencing the genomes of these species is particularly useful as it will enable the linkage of novel molecular data with the existing scientific knowledge and to start collecting new data on their current distribution, economic and environmental impact. Moreover, it will also provide the first insights into molecular biology and phylogeny of these species.

In the current study, the use of HTS technologies led to the identification of a novel tenuivirus genome that consists of four RNA segments, from three Northern European countries: Estonia, Sweden and Norway. Interestingly, our host range and vector studies show it may represent a tenuivirus called European wheat striate mosaic virus (EWSMV) that was found to be widespread in Europe in 1950-60s but forgotten later as the attempts to purify virus particles for antisera production did not succeed and the sequence of the virus was unknown. Our study indicates it can infect different cereal crops (wheat, oats, triticale, barley and rye) and it is transmitted by the planthoppers belonging to family *Delphacidae* genus *Javesella* (Fennah) like EWSMV. Sequence identity between the novel virus isolates is higher than 92 % for any genome segment. A phylogenetic analysis places the novel virus between rice grassy stunt virus (RGSV) and the "core group" of other cereal-infecting tenuiviruses.

Multiplex detection of pospiviroid plant pathogens

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The genus *Pospiviroid* currently contains nine single-stranded, naked circular RNA plant pathogens which can be transmitted mechanically and through seed (1,2). Many countries have established phytosanitary regulations for potato spindle tuber viroid because it can cause serious diseases in potato and tomato crops (3), and various ornamentals. For routine testing and certification of propagation material, efficient and reliable diagnostic tools were developed (3). In general the available tests detect only a part of the pospiviroid complex. Therefore, different tests are needed to detect all pospiviroids. This study describes the development of a true multiplexed diagnostic method for the detection and identification of pospiviroids using the Luminex MagPlex xTAG technology. This bead based application is able to detect and identify members of all nine currently recognized pospiviroid species, as well as a generic internal control and a generic pospiviroid signal simultaneously. The method proved to be very specific, sensitive and reproducible. The multiplexed array described here is robust, easy to use, displays unambiguous results and has strong potential for use in routine pospiviroid indexing to improve disease management strategics.

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Role of diagnostics and quarantine regulations in biosecurity against plant viral diseases in South and East Asia: Challenges

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Plant viral diseases are known to cause serious yield losses. The global trade of agricultural commodities and exchange of germplasm has the potential to introduce new viruses which may pose potential risk to the agriculture of importing country. The increasing numbers of plant viruses discovered in the last 15 years has increased the requirement for countries and regions to protect their crops from exotic viruses. The National Plant Protection Organizations assume responsibility for protecting their countries from the unwanted entry of new pests including viruses. The combination of regulatory and technical approaches would ensure biosecurity of crops against pests for a region. In India, the Directorate of Plant Protection, Quarantine and Storage (DPPQS) under the Ministry of Agriculture and Farmers' Welfare is responsible for enforcing quarantine regulations and for inspection/ disinfestation of agricultural commodities meant for commercial purpose. The imported germplasm material including transgenics are subjected to quarantine at ICAR-National Bureau of Plant Genetic Resources. The Plant Quarantine (Regulation of Import into India) Order, 2003 (PQ Order), requires Additional Declarations to be included in the Phytosanitary Certificate by the exporting country for seeds and other planting material as free from pests. As per the PQ Order, 264 viruses are regulated pests which are of quarantine significance for India. Early, sensitive and accurate diagnosis is necessary for detection of viruses in quarantine. The challenges in virus detection in quarantine include availability of antisera, viral genome sequences in Genbank, detecting an unknown / exotic virus etc. Also strengthening of infrastructure, capabilities and methodologies for detection of viruses in bulk samples is essential. During the last two decades, at ICAR-NBPGR, New Delhi, using techniques ranging from biological to serological / molecular, a large number of viruses including 19 viruses not reported and 21 viruses not known to occur on particular host(s) in India have been intercepted. The introduction of 19 exotic viruses into India was averted. The pest risk analysis revealed absence / presence of viruses in certain countries in South and East Asia region. Establishment of a South and East Asia Diagnostic Network for Plant Viruses would be the backbone for strengthening the programme on plant biosecurity. Also South and East Asia Regional Working Group of Experts for Diagnosis of Plant Viruses thus need to be formed to explore cooperation in terms of sharing of expertise and facilities, especially where the borders are contiguous. Designating A1 pests (not present in South and East Asia) and A2 pests (present in South and East Asia but not widely distributed and being officially controlled) in the region in line with EPPO would help in preventing the introduction of plant viruses not known in the region as well as the movement of plant viruses within the region.

The Peruvian potato virome II: Potato yellowing virus

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Potato yellowing virus (PYV), a member of the Genus *Ilarvirus* Family *Bromoviridae*, has been reported infecting potatoes in Peru, Ecuador and Chile. It is associated to symptomless infections, however yellowing of young leaves has been observed in some potato cultivars. However, to date, no complete sequence is yet available for this virus and it's prevalence in field grown potatoes is unknown. The Andean region, which includes Peru, is the center of potato diversity and therefore likely also of some potato viruses such as PYV. During 2016 and 2018 we collected potato samples from farmers' fields at altitudes of 10-4286 masl throughout Peru and analyzed them by small RNA sequencing and assembly. All known potato viruses as well as several new ones were identified in this survey and PYV like viruses were detected in about 2.6% of all samples. Ten additional samples from Ecuador, Peru and Bolivia (including two from 'yacon': *Smallanthus sonchifolius*) from CIPs genebank collection, found previously to by positive to PYV by ELISA, were included. Here we report the sequence characterization of the complete genomes of thirty-one related Ilarvirus isolates found infecting Solanum *tuberosum*, *Solanum phureja* in Peru, and partial sequences of RNA 2 and 3 of isolates infecting *Smallanthus sonchifolius* (yacon) in Peru and Bolivia. At the sequence level PYV isolates found in Ecuador clustered in a distinct phylogenetic clade when compared to Peruvian isolates. Similar grouping could be observed by symptoms induced by isolates of these two groups in *Physalis floridana.* These viruses were closely related to Fragaria chiloensis latent virus (FCiLV) reported in strawberry from Chile which were readily detected by ELISA with PYV antiserum, and they thus may represent strains of the same virus. Although these ilarviruses are not serologically related to alfalfa mosaic virus (AMV) they share several characteristics with AMV such as the transmission by aphids, thus supporting the previous suggestion of AMV as being a member of the Ilarvirus genus.

Making better use of historic isolate collections to give context to novel detections from high throughput sequencing

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High throughput sequencing (HTS) technologies have revolutionized the field of plant virus detection. However, as HTS is applied to a broader range of plants, such as niche crops where with little information on the viruses present, the number of novel viruses discovered is increasing. This has created a challenge for plant protection agencies and regulatory authorities in assessing the risks associated with these novel pathogens (1). Conventional approaches to investigating the risks of invasive plant pathogens can be laborious and time-consuming, including host range studies, transmission studies, and impact assessments that can take years to complete and potentially hinder timely action.

Samples of *Ullucus tuberosus,* a tuber forming crop originating from South America, were submitted by plant health inspectors for testing at Fera Science Ltd. These were found to be infected with multiple novel viruses as well as characterized viruses which have not been previously reported from Europe (2). The suite of viruses previously reported from this host were biologically or serologically characterized but do not have associated sequence data. Investigations into similar crops, such as mashua (*Tropaeolum tuberosum*) and yacon (*Smallanthus sonchifolius*) have also resulted in the detection of novel viruses which appear similar to those previously reported infecting these hosts. By sequencing historic isolates, connections can be made between those isolates and viruses found today, e.g. (3). These studies will be presented and discussed in the context of the forthcoming Euphresco network research topic 'VirusCurate'.

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Potential risk of introducing potato (quarantine) viruses by flight passengers

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In 2016 ten small lots of potato tubers from Peru were intercepted at Schiphol airport, Amsterdam, The Netherlands. The tubers were found in suitcases entering the European Union (EU). Since it is not allowed to import *Solanum tuberosum* and other tuber-bearing *Solanum* species into the EU, the tubers were sent to the National Reference Centre (NRC) of the NPPO for screening on the presence of plant pathogens. Tubers were planted in the quarantine facilities and plants inspected for visual symptoms. In addition, samples were tested for the presence of viruses and viroids by mechanical inoculation of test plants, DAS-ELISA (1) and real-time RT-PCR (2). The results of these tests revealed that all lots were infected by at least one virus species, all being considered quarantine viruses in the EU. No viroids were found. Virus confirmation and identification was performed by high-throughput sequencing (HTS) using a ribosomal RNA (rRNA)-depleted total RNA approach. A CLC-based pipeline for detection of viruses developed at NRC was used to *de novo* and reference assemble raw data into consensus sequences followed by BLASTn, BLASTx and conserved viral protein domain searches. The results of HTS-analyses confirmed the identity of the viruses detected by ELISA. HTS revealed, however, additional viral sequences indicating the presence of several potentially novel viruses. Verification of these results is ongoing. The (biological) characterization is necessary to assess the potential risk and impact of these novel viruses for potato production in the EU. Moreover, these interceptions raise the question to which extend these imports serve as a pathway for introducing harmful pathogens into new areas. Illegal imports of potato tubers or other potato material may pose one of the main risks of introduction of pathogens in potato, because no guarantees on the phytosanitary status of this material can be provided.

Footnotes

(1) Antisera to: Andean potato latent virus, Andean potato mild mosaic virus, Andean potato mottle virus, arracacha virus B, potato black ringspot virus, potato latent virus, potato leaf roll virus, potato viruses A, M, S, T, V, X, Y, potato yellowing virus, and tomato spotted wilt virus.
(2) Generic pospiviroid test detecting all currently ICTV-recognized pospiviroid species.

Area wide management of vegetable virus diseases and bacterial diseases in Australia: Tospoviruses

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Vegetables are grown in many temperate, sub-tropical and tropical regions throughout Australia, allowing for year-round production. A wide range of vegetable crops are grown but some of the more common species include potatoes, tomatoes, onions, carrots, pumpkin, cucumbers, capsicum, broccoli, cabbages, cauliflower and celery. The size of farms varies considerably throughout Australia and within specific growing regions, but most farms are between 5-70 hectares. Growers often specialize in a few crop species that suit the region, but some are highly diverse and may grow according to market demand. Within a region, many farms occur side by side, but disease management often occurs at the individual farm level. This traditional management strategy has been ineffective in controlling outbreaks and spread of virus and bacterial diseases within vegetable growing regions. Area wide management (AWM) has potential for controlling plant diseases, particularly those with aerial dispersal mechanisms such as insect-vectored viruses and wind dispersed bacteria and fungi. Rather than focussing at the individual farm level, control tactics are co-ordinated amongst growers, incorporating multiple premises over a broad area to maintain pest populations below economic impact levels.

This project aims to develop an AWM strategy to address emerging and high priority viral and bacterial diseases affecting vegetable crops and to develop effective, innovative and rapid diagnostics for major endemic and exotic viral and bacterial pathogens. Tospoviruses are among the most widespread and damaging plant viruses causing major losses both in field grown and glasshouse crops worldwide, including Australia. Tomato spotted wilt virus (TSWV), capsicum chlorosis virus (CaCV), impatiens necrotic spot virus (INSV) and iris yellow spot virus (IYSV) are a continuing problem for the Australian vegetable industry. Diseases of vegetable crops associated with these tospoviruses will be presented and the prevalence and genetic diversity of each virus species will be discussed in context to an AWM approach for Australian vegetable growers.

Survey and molecular detection of cassava mosaic disease in Thailand

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The quantity of cassava (*Manihot esculenta* Crantz) produced in South East Asia is more than 55 million tons per year and worth more than 10 billion dollars. Currently, the cassava products in Cambodia and Vietnam decreased last year due to outbreak of the cassava mosaic disease (CMD). CMD has been reported and Sri Lankan cassava mosaic virus (SLCMV) has been reported in 2015 in Northeast Cambodia (Wang et al. 2015). SLCMV is caused by a number of distinct begomoviruses in the family *Geminiviridae*. SLCMD is naturally transmitted by the whitefly (*Bemisia tabaci*) and infected stem cuttings. In Thailand, the survey was conducted from May 2018 – January 2019 in fields located in the border between Thailand and Cambodia (Sa Kaeo, Prachin Buri, Buri Ram, Surin and Sisaket province). CMD were found in Prachin Buri, Surin and Sisaket province. Mean CMD incidence varied across three provinces but was greatest in Prachin Buri province (0.009%). The infections appeared in several varieties, such as Huai Bong 80, Rayong 9, Rayong 72 and CMR 89. On a scale of 1 to 5, the severity of infection was characterized as moderate and very uniform - 3. The CMR 89 variety showed severer infection more than the other variety - 3 to 4 (1). Typical symptoms of SLCMV were mosaic, mottling, misshapen and twisted leaflets. The infections appear to have taken place approximately 2 to 2.5 months ago. In polymerase chain reaction (PCR) discrimination of 146 infected plants, all the sample were positive, using two specific primers for SLCMV (forward: 5’ GTT GAA GGT ACT TAT TCC C 3’ and reverse: TAT TAA TAC GGT TGT AAA CGC 3’) and (forward: 5’ TAT AAT TCT CAA AAG TTA CAG TC 3’ and reverse 5’ ATA TGG ACC ACA ATC GTG TC 3’) (1). The size of the PCR products was approximately 950 and 600 bp, respectively. The sequence analyses of PCR fragments showed >90% homology with the SLCMV strain Sri Lankan by BLAST searches against GenBank. Furthermore, all the infected samples were double checked by the rolling circle amplification (RCA) method using primer: 5’ ATA TAT GTG TCT CCA AAT GGC ATT 3’. The result showed that all the incidences had product size similar to the positive control. However, infected plants were eradicated immediately after discovering and are being closely monitored to prevent the spread of virus.

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Developing virus assays to detect common lily viruses in Northwest China

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Lanzhou lily (*Lilium davidill* var. *unicolor*) is also an important edible bulb crop, which is mainly grown in Central Gansu province in Northern China (1). And in recent years, the ornamental lily industry in Gansu has also been developing rapidly. Unfortunately, lilies are easily infected with viral diseases causing degeneration in the quality of many elite varieties. Among the most important lily-infecting viruses, in Gansu they are lily symptomless virus (LSV), cucumber mosaic virus (CMV), lily mottle virus (LMoV) and Arabis mosaic virus (ArMV) (2). Reliable and precise detection systems have been developed for virus identification. A sensitive immunocapture (IC) reverse transcription (RT) loop-mediated isothermal amplification (LAMP) assay was developed for detection of LSV, CMV, LMoV and ArMV, respectively. In this test, virus antigens are captured with the help of specific antibodies followed by detection using RT-LAMP, without the need to isolate RNA. This relatively simple and sensitive technique showed excellent potential with field-collected samples. Furthermore, we have also developed a specific quintuplex RT-PCR assay to simultaneously detect LSV, CMV, LMoV, ArMV and using the lily 18S rRNA gene as an internal control. We are starting to use the convenient and reliable detection methods now routinely for production of high health bulbs, or crop health monitoring of lily.

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Checking the spread of maize lethal necrosis (MLN) in Sub Saharan Africa (SSA) using modern surveillance and viral diagnostics tools

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Since 2011, maize lethal necrosis (MLN) has emerged as a major threat to food security in the Eastern Africa region. There are several strategies in the management and in preventing further spread to Southern Africa countries where maize is a staple food and the seed maize industry is vibrant. One of the approaches is to have a robust disease monitoring structure and appropriate diagnostics tools within the phytosanitary systems of the NPPOs from the countries in the region.

CIMMYT through the MLN Diagnostics and Management project has worked with NPPOs from the five MLN endemic countries in eastern Africa (Kenya, Uganda, Tanzania, Rwanda and Ethiopia) and the three countries in Southern Africa (Zambia, Malawi and Zimbabwe) in strengthening their phytosanitary systems towards this end. For surveillance, an effective MLN surveillance and monitoring system was established, including web-based information exchange amongst relevant institutions. Using the latest technology in field surveillance, the android based Open Data Kit (ODK), the MLN surveillance protocols were designed and uploaded in the application for use. Further, an MLN data Toolbox was also set up to manage the surveillance data generated from the massive regional surveillance exercise from the region. The data generated is published in MLN web portal, a product of the project. Harmonized surveillance, sampling and diagnostics protocols for detecting MLN-causing viruses especially MCMV in farmers' fields, seed fields and in commercial seed lots were designed and implemented. Various stakeholders have been trained on the ODK surveillance tool, field testing for MCMV using immunostrips and testing for MLN viruses in seed using ELISA. This has assisted the NPPOs in the region to monitor the movement of seed, grain and germplasm for research to limit the spread of MLN viruses in the region. The establishment of the MLN Phytosanitary Community of Practice, understanding seed transmission of MCMV, appropriate surveillance and diagnostic protocols have contributed immensely in the management strategies for MLN in Eastern Africa and hence prevented its spread to Southern Africa

A real-time monitoring system for the migratory pests between countries regions using the smart airborne net trap

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Rice plant-hoppers (RPHs) are severe damaging pest of rice in the Asian region that can migrate long-distance from country to country. RPHs also transmit viruses that are detrimental to rice plants. In order to catch migratory insect pests moving in air currents, a net was installed at 10 meters above the ground and the person responsible carried out the monitoring task to check the net trap regularly. The accuracy and promptness of the monitoring varied according to person's expertise and diligence. Another problem is tha it is impossible to monitor in case of rainfall. This problem is critical to the monitoring of migratory pests, especially considering the tendency for pest migration to occur before and after rainfall. SANT (Smart Net Airborne Trap) has been developed with high resolution imaging, networking and managing functionalities while maintaining the proven method of traditional traps. A real-time monitoring system for the arrivals of migratory pests (plant-hoppers) has been implemented also. The SANT system can do quick and accurate monitoring tasks based on objective image information anywhere and is not affected by the rainfall problem; real-time precise monitoring can be done under any conditions. Since 2016, we have been doing real-time precise monitoring of the migratory pests that fly into Korea with the installation of SANT in 40 regions nationwide. The verification has been completed by real-time detection of middle scale arrival of small brown planthoppers at the end of May 2017. The downsized and improved SANT has been developed for overseas delivery and installation. It is currently installed and operated in Vietnam, in the Mekong Delta region, and in Thailand, in the central region. This allows the implementation of a monitoring system for migratory pests between regions of different countries. These systems can contribute to global food security through real-time sharing of actual and objective information on the departure, stops and arrival of migratory pests.

Application of next generation sequencing (NGS) for detection of viruses and viroids infecting grapevines in Korea

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Grapevine (*Vitis* spp.) is one of the major fruit crops, covering an area of 12,795 ha with an annual grape production of about 162,000 tonnes in South Korea (http://krei.re.kr, 2018). Approximately 65 viruses and 6 viroids have been reported from grapevine and the viral diseases cause economic losses. In 2018, symptoms observed in some grapevine plants of cultivar 'Kyoho' showed scattered yellow spots on leaves and poor color development of berries. Using next generation sequencing (NGS), the presence of grapevine fleck virus, grapevine ruspestris stem pitting-associated virus, grapevine Pinot gris virus, grapevine geminivirus A, hop stunt viroid and grapevine yellow speckle viroid 1 (GYSVd-1) were detected in the symptomatic samples. All of the viruses and viroids have been previously reported in Korea and GYSVd-1 is recognized as the causal agent of grapevine yellow speckle disease. The complete genome sequences of GYSVd-1 (367 nt) shared 96 to 100% identity with GYSVd-1 GenBank isolates. NGS can be used effectively for detection of viruses and viroids from grapevines. Further studies are needed to clarify an association between viral diseases and symptoms on grape.

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Evaluation of seed transmission of pepper mottle virus in *Capsicum annuum*

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Pepper (*Capsicum annuum*) is known to be infected by over 40 species of virus in Korea. Mixed infections were prevalent over single infections in pepper. Seed transmission of cucumber mosaic virus (CMV) and pepper mild mottle virus (PMMoV) has been relatively well reported, but pepper mottle virus (PepMoV) has been scarcely studied. For visualizing the PepMoV infection in pepper, we inoculated pepper with a PepMoV construct expressing the green fluorescent protein (GFP). We observed the symptoms on each part in the pepper plant and green fluorescent illuminated by GFP under UV light in pepper infected with PepMoV. PepMoV was detected by RT-PCR from leaves, flowers, pedicles, pericarps, capsaicin glands and whole seeds. Additionally, RT-PCR was carried out with seed coats, embryos and seedlings harvested from PepMoV-infected peppers. The seed coat infection rate of PepMoV was 2% - 20%, but embryos and seedlings were not detected. To confirm the seed transmission of PepMoV in mixed-infected pepper, we inoculated the pepper with the PepMoV, CMV and PMMoV. Mixed infection slowed the growth of pepper plants and the maturation of fruits compared to single infection. In mixed-infected pepper plants, seed coat infection rate of PepMoV was 6%-60%, but PepMoV was not detected in embryos and seedlings. This is the first report on seed transmission test for PepMoV in mixed-infected pepper. Consequently, PepMoV invades the seed coat, but not the germinated embryos in pepper. Infection by mixed viruses may lead to higher infection of the seed coat than single virus infection in pepper. These results suggested that mixed viruses provide enough time for PepMoV to infect the seed coat of pepper.

Genetic diversity and rapid detection of pear viruses

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Field surveys to investigate the incidence and occurrence pattern of viruses in pear leaves and fruits were conducted during 2017 in Korea. In total, 158 pear leaf and 35 pear fruit samples that showed disease symptoms were collected during June to September from farmers' fields in five major pear producing areas in Korea (Sangju, Namyangju, Ulsan, Cheonan, and Naju). Multiplex reverse transcription-polymerase chain reaction (Multiplex RT-PCR) was used to test the samples for the presence of one or more of the following viruses: apple stem pitting virus (ASPV), apple stem grooving virus (ASGV), and apple chlorotic leaf spot virus (ACLSV). Disease incidence was 95.6% in 2017, and ASGV was detected in almost all samples across the different regions studied. The percentage of virus incidence in the collected leaf samples was as follows: ASGV, 95.6%; ASPV, 34.2%; and ACLSV, 19.0%, while that in fruit samples was as follows: ASGV, 100%; ASPV, 31.4%; and ACLSV; 0%. In addition, the percentage of virus incidence in the collected regions was as follows: Sangju, 92.5%; Namyangju, 100%; Ulsan, 93.3%; Cheonan, 94.1%; and Naju, 100%. Furthermore, we investigated the degree of infection of the three viruses in commercial nursery trees of pear cultivars such as 'Shinwha', 'Niitaka', 'Whasan', 'Chuwhang', and 'Manpung'. The ACLSV, ASGV, and ASPV infection rate in the scion of pear cultivars was 10%, 56%, and 25%, respectively. Furthermore, the development of rapid and sensitive diagnostic methods to verify that the planting material is virus-free is of paramount importance to control the spread of pear viruses. A novel reverse transcription recombinase polymerase amplification (RT-RPA)-based method was developed for rapid detection of ASGV and ASPV. Sensitivity analysis showed that the detection limit of RT-RPA in ASPV-infected samples was 150 pg/μl of RNA, while in ASGV-infected samples it was at least 470 pg/μl of RNA. The major improvement in the assay is the reduction in the reaction time for the target viruses to as little as 1 min and the assay can be easily performed in the laboratory. This assay is a promising alternative method for pear breeding programs or virus-free certification laboratories.

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Genome characterization of two highly divergent isolates of cycas necrotic stunt virus from two different plant species

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Cycas necrotic stunt virus (CNSV), which is a member of the genus *Nepovirus* (subfamily *Comovirinae*, family *Secoviridae*, order *Picornavirales*), was first identified from *Cycas revoluta* in Japan (1). Some other CNSV isolates have been detected in various plant species, such as *Daphne odora* (2), *Gladiolus* spp. (3), *Lilium longiflorum* (4), *Paeonia lactiflora* (5), and *Primula sieboldii* (6). However, the genome sequence of a CNSV isolate from *C. revoluta* is the only complete one known so far, and the genomic RNA2 sequence of a CNSV isolate from *L. longiflorum* is available. In this study, complete genome sequences of two highly divergent isolates of CNSV from *Paeonia suffruticosa* and *D. odora* collected in South Korea were determined using high-throughput RNA sequencing. Phylogenetic analyses and pairwise comparisons using complete RNA1- or RNA2-encoded polyproteins showed that the two CNSV isolates are very divergent (83.19% - 89.42% in polyprotein 1 and 73.61% - 85.78% in polyprotein 2). However, comparative analysis based on taxonomic criteria for species demarcation of nepoviruses confirmed that the two isolates are not new species but highly divergent variants. This is the first report on complete genome sequences of CNSV identified in *P. suffruticosa* and *D. odora*. In addition, this is, to the best of our knowledge, the first report of CNSV infecting *P. suffruticosa* in the world.

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Incidence and occurrence pattern of viruses on paprika on Jeju Island

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Field surveys was conducted to investigate the incidence and occurrence pattern of viruses in paprika. Between 2014 and 2018, there were 8 types of virus, including BWYV, detected in paprika grown on Jeju island. PepMoV is known to be an aphid-borne virus, and PepMoV was detected in green peach aphids collected from greenhouses at two farms in 2018. PepMoV is considered an important virus, with the highest prevalence in Jeju Island during the 5 years of the previous survey, and more research will be required to estimate the initial routes of infection and methods of prevention, accounting for the peak seasons for vectors such as aphids. ToCV was first detected in tomatoes on Jeju Island in 2013, but had not been detected in paprika. However, in 2017, ToCV single infection and mixed-infection were observed across the whole survey area. Following self-prevention measures, no infections were detected in 2018, indicating that ToCV infection was not permanent. BWYV was observed as single infections in 2014, and mixed infections between 2015 and 2018. Although there was no particular increase in BWYV infections, given that infections are occurring continually, elimination of the infection source or aphid control will be required to avoid permanent aphid-borne infection. INSV was detected in the form of mixed-infections in two samples from a single farm on Jeju Island in 2015, but the infection did not spread or persist. In 2018, in Hangyeong-myeon, Jeju-City, a paprika showed round yellow spots on the leaves and abnormal fruit growth; a specimen from the greenhouse with severe thrips damage was analyzed and showed TSWV infection. The infection was caused by the farm's own seedlings, rather than seedlings purchased from another region, and TSWV was detected in western flower thrips, which act as a vector. The TSWV outbreak in Jeju Island did not occur sporadically via seedlings purchased from another region and the damage did not spread, but occurred in seedlings raised directly at the farm during the 2018 survey, and virus was also detected in the vector, western flower thrips. Since Jeju Island has favorable conditions for western flower thrips to overwinter, unlike other regions, there is a risk of TSWV settling and spreading that will require continuous monitoring.

Incidence of viruses occurring in *Rehmannia glutinosa* in Korea

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Recently, five viruses including rehmannia mosaic virus (ReMV), youcai mosaic virus (YoMV), broad bean wilt virus 2, Plantago asiatica mosaic virus and rehmannia virus 1, all of which infect rehmannia (*Rehmannia glutinosa* Libosch), have been identified in Korea. Rehmannia infected with these viruses appears on leaves with mosaic, yellowing, discoloration and poor growth. To investigate the incidence of the above five species viruses on rehmannia, field surveys were conducted during 2017-2018. A total of 145 samples were collected from fields in major cultivation areas. Molecular diagnosis assays showed that all collected samples were mixed infection of two or more viruses. Particularly, two species of tobamovirus, ReMV and YoMV, were detected in all collected samples. Since rehmannia is propagated by vegetative propagation, tuberous roots were examined for virus diagnosis. Similarly, two or more viruses were detected. It is considered that the production of virus-free roots should be needed for high quality rehmannia production.

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Occurrence pattern of viral infection on pear in Korea and genetic characterization of apple scar skin viroid isolates

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Apple chlorotic leaf spot virus (ACLSV), apple stem pitting virus (ASPV), apple stem grooving virus (ASGV), and apple scar skin viroid (ASSVd) are economically important viral agents infecting pear trees worldwide. A large-scale survey was carried out in five major pear producing area of Korea in 2017 and 2018 to investigate the occurrence pattern of these viruses in pear leaves, fruits, and commercial nursery trees. A total of 363 pear leaf samples, 109 pear fruit samples, and 72 pear nursery trees were collected randomly and tested for infection using multiplex RT-PCR. The accuracy of detection of the viruses was confirmed by sequencing amplified PCR fragments. More than 95% of the tested samples were infected with one or more pear viruses. ASGV was the most prevalent virus in leaves, fruits, and nursery trees. The virus incidence rate in collected leaf samples was ACLSV, 13.8%; ASPV, 31.7%; ASGV, 95.3%; and ASSVd, 3.6%; and in fruit samples was ACLSV; 2.8%; ASPV, 28.4%; ASGV, 99.0%; and ASSVd, 13.8%. The infection ratio of ACLSV, ASPV, ASGV, and ASSVd for the scion of pear cultivar Niitaka was 10%, 45%, 77%, and 50%, respectively. From the scion of pear cultivar Chuwhang, infection ratios of ASPV, ASGV, and ASSVd were found to be 70%, 50%, and 60%, respectively. From the scion of pear cultivar Whasan, infection ratios of ACLSV, ASPV, ASGV and ASSVd were found to be 40%, 60%, 93%, and 20%, respectively. From the root stock of pear cultivar Wonwhang, infection ratios of ACLSV, ASPV, ASGV, and ASSVd showed 28%, 57%, 100%, and 14%, respectively. Mixed infections occurred in leaves, fruit, and nursery trees, and the most common combination was ASGV+ASPV (22%, 23.9%, and 20.8%, respectively). Furthermore, the ASSVd genome was completely sequenced. The ASSVd isolate showed 99% sequence identity at a nucleotide level to other apple isolates.

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On-field detection of banana bunchy top virus (BBTV) using exo-recombinase polymerase amplification (Exo-RPA)

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Banana bunchy top virus (BBTV, genus *Babuvirus*) is an emerging disease of banana and plantain (*Musa* spp.) in West Africa. A simple and sensitive diagnostic method usable in the field is necessary for regional surveillance programs focused on early detection and eradication of infected plants. In this study we optimized the exo-recombinase polymerase amplification (exo-RPA) method for the detection of BBTV in plant tissues and the banana aphid (*Pentalonia nigronervosa*) vector. RPA is an isothermal method for rapid amplification (10 to 20 min) of nucleotide segments of up to 300 bp at temperatures set between 38°C to 42°C. Two primers in opposite orientation were designed for amplification of 146 bp region of the BBTV-R segment, which were used together with a probe labelled with Fam-dT (thymine nucleotide carrying fluorescein) and BHQ1 quencher for real-time detection using a mobile fluorophore detector in the field. A method was standardized for rapid release of virus particles by soaking tissue samples in alkaline PEG buffer and 2 µl of the extract was used as a template for virus detection. The assay has an analytical sensitivity to detect BBTV in total nucleic acids diluted up to 10 ng/µl, and the results were comparable with conventional PCR detection. This assay was successfully used for the detection and eradication of BBTV in Togo in a surveillance programs conducted in 2018. The simple workflow of this method makes it an ideal option for BBTV detection in non-laboratory settings.

Prevalence, incidence and yield losses associated with yellow dwarf viruses in Australia

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Yellow dwarf viruses (YDVs) are economically important pathogens of cereals, reducing yields worldwide. YDVs are transmitted from plant to plant exclusively by aphids; the most common aphid vectors of YDVs in Victoria (Australia) are the bird cherry-oat aphid (*Rhopalosiphum padi*) and the corn leaf aphid (*Rhopalosiphum maidis*). Environmental factors such as temperature and rainfall play an important part in the epidemiology of YDVs by influencing aphid populations, aphid activity and the prevalence of virus and aphid reservoirs, both within and between growing seasons. Across Victoria, climatic conditions are generally hotter and drier in the north and cooler and wetter in the south of the state. In this study, the prevalence and incidence of YDVs in cereal fields from three distinct regions (north, central and south) across the climatic gradient in Victoria were assessed during September/October for four consecutive years (2014-2017). One hundred plant samples were collected from each field and tested for the presence of three species of YDV using tissue blot immunoassay. YDVs were detected in fewer fields and with lower mean incidence in the north, which was the hottest and driest region, than the central and southern regions (1). There were no overall differences in prevalence or incidence between the central and southern regions. YDVs were also more prevalent and occurred with higher incidence than had previously been reported in Victoria. Additionally, field trials showed significant yield reductions of up to 83% in cereals as a result of YDV infection, further stressing that yield losses associated with YDV infection in Victoria have been underestimated.

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Reverse transcription-Loop mediated isothermal amplification (RT-LAMP) assay to diagnose pepper mild mottle virus

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Pepper is one of the most economically important seasoning vegetables. Sixty plant viruses infecting pepper have been described worldwide whereas about sixteen viruses infecting pepper are known in South Korea, including cucumber mosaic virus, pepper mottle virus, pepper mild mottle virus (PMMoV) and tobacco mild green mosaic virus. PMMoV is a member of the genus *Tobamovirus*. PMMoV infection of pepper plants causes huge economic losses in the pepper industry. Plant viral diseases cannot be treated directly using chemical agents. It is important to diagnose early and accurately for the management of plant viral diseases. Since loop-mediated isothermal amplification (LAMP) was developed in 2000 (1), it has been applied in many plant virus diagnostics, because it has several advantages over RT-PCR, such as short amplification time, high sensitivity and high diagnostic specificity. In this study, an RT-LAMP assay was developed to diagnose PMMoV. The optimum reaction temperature was 65°C. The RT-LAMP assay was about 100 times more sensitive than the conventional one-step RT-PCR. When the external primer concentration was 5 pmol and the internal primer concentration was 30 pmol, the brightest ladder-like band appeared on the agarose gel. At least 0.6 mM dNTP was required for RT-LAMP, and 6 mM MgSO4 was needed for a sufficient RT-LAMP assay. Addition of the loop primers reduced the assay time from 28 min to 15 min. Although plant sap extract was used, the PMMoV RNA was successfully amplified by RT-LAMP. This study shows that RT-LAMP is highly sensitive and rapid for detecting PMMoV, suggesting that LAMP can be a good tool for field diagnosis.

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Screening for plant viruses and viroids in *Alstroemeria* spp. by next generation sequencing

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Alstroemeria is a genus endemic to South America, with a diversification center in central Chile. Alstroemerias have acquired great importance as ornamental plants and cut flowers, reaching in some cases great commercial value. However, vegetative reproduction by rhizome has been the main form of asexual propagation for years, leading to the transmission and spread of viruses. In Chile, symptoms in commercial production ranging from necrotic striations in the leaves and stems, leaf mosaic to mild systemic venal chlorosis have been observed. Therefore, the main objective of this work was to use next generation sequencing (NGS) to identify possible viruses and viroids infecting commercial and endemic alstroemerias in Chile. Viral small RNA (vsRNAs) purified from alstroemeria leaves collected in the central area of Chile were submitted to deep sequencing and then assembled to reference genomes. The presence of Alstroemeria mosaic virus, lily symptomless virus and lily mottle virus were determined, presenting a coverage of 98%, 88.3% and 83.6% to the reference sequences (DQ295032, JQ710691 and AJ516059.1). In addition, Alstroemeria with coverage of 98% and 97% respectively, the presence of Peru tomato mosaic virus (EU495235.1) and a pospiviroid (DQ318794.1) was determined. These results are important to be considered in programs of breeding, cultivation and production of these ornamental plants and flowers.

Virus surveillance in flower bulbs in The Netherlands

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The Flower Bulb Inspection Service (Bloembollenkeuringsdienst; BKD) has been tasked by the Ministry of Agriculture, Nature and Food Quality with inspecting the quality of all flower bulb crops in The Netherlands on both quality defects and quarantine pathogens. This inspection comprises visual inspection by field inspectors and molecular test in our laboratory. Annually, the BKD laboratory performs approximately 6 million ELISA reactions and 200.000 PCR reactions. Since our laboratory tests are mandatory, comparison of our test results over the years give an impression of common health of Dutch flower bulbs. Our results show that, on average, virus pressure has decreased in lily and tulip over the last five years.

Viruses naturally infecting a fenugreek crop in Australia

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Fenugreek (*Trigonella foenum-graecum*) is a cool season legume crop; in Australia, it is a minor crop grown mainly for seed production. A field of fenugreek cv. Sungold located in South Australia was devastated by virus infection showing a range of symptoms such as red discoloration, mild red discoloration, yellow discoloration and leaf tip necrosis (1). One hundred samples were collected randomly from the infected field and tested for a range of viruses by tissue blot immunoassay then confirmed by PCR. Three viruses were detected: cucumber mosaic virus, pea seed-borne mosaic virus and turnip yellows virus, with 90%, 20% and 75%, respective levels of incidence. These three viruses were also detected in volunteer field pea (*Pisum sativum*), lentil (*Lens culinaris*), burr medic (*Medicago polymorpha*) and barrel medic (*Medicago truncatula*) plants present in and around the field. As a result of the virus infection, total crop losses were observed. This is a first record of natural virus infection in a fenugreek crop in Australia.

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Session

10

Plant Virology in East Asia

Discovery of Hibiscus latent Singapore virus - a familiar tobamovirus that possesses an unfamiliar genome

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Hibiscus latent Singapore virus (HLSV) is a member of the genus *Tobamovirus* (1). It was first discovered in Hibiscus shrubs in Singapore. Its genome structure is similar to other tobamoviruses with a 5' untranslated region (UTR), four open reading frames (ORFs) and a 3' UTR. In most other tobamoviruses, their 3' UTRs consist of two parts: an upstream pseudoknot domain (UPD), followed by a tRNA-like structure (TLS). HLSV, on the other hand, does not possess a UPD. Instead, it contains an internal poly(A) tract that ranges from 77-96 nucleotides. We have purified the virus and determined the coat protein (CP) structure using x-ray fiber diffraction to 3.5Å resolution. His-122 on its CP contributes to major structural stability. HLSV also contains a hepta-adenosine stretch located in its 5' genome that is responsible for programmed -1 ribosomal frameshifting. Mutant HLSV-22A which contains a short 22 nucleotides internal poly(A) could not express CP nor infect plants systemically (2). A serine- and threonine-rich motif TTTSTTT is located at the C-terminus of HLSV CP. The motif was found to be involved in virus replication and systemic movement. Deletion of the last amino acid residue in HLSV-22A led to more rapid virus replication, but with delayed systemic movement. When the RNA structure in TTTSTTT motif was altered, while keeping its amino acids unchanged, two mutants, HLSV-22A-mmSL and HLSV-87A-mmSL, showed no change in virus accumulation. These results indicated that the unique TTTSTTT motif is associated with virus replication and systemic movement. Deletion but not substitution of amino acid(s) at the C-terminus of TTTSTTT motif of HLSV CP with a short internal poly(A) track (IPAT) of 22A enhanced virus replication, whereas the virus with a long IPAT of 87A showed delayed systemic movement (3).

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Geographical spread of cassava mosaic disease (CMD) and Sri Lankan cassava mosaic virus in South East Asia

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Global trade and crop intensification, combined with poor phytosanitation measures, can significantly increase the invasive potential of emerging diseases. Among those affecting cassava cultivation is paramount the case of cassava mosaic disease (CMD) in Africa and South India, where multiple co-introduction and spread of different and recombinant geminivirus species, the causal agents, are associated to dramatic yield losses. Therefore, since its first report in Southeast Asia (SEA) the research community realized the potential of CMD to spread widely throughout this region. To obtain a more complete picture of CMD distribution and the range of cassava-infecting geminiviruses occurring, we organized and supported field surveys in the region since early January 2016, using a wide range of standardized methods including farmer participatory research, molecular diagnostics, virus sequence analysis and information and communication technologies (ICT) tools for field data collection. As a result, we detected a widespread occurrence of a single geminivirus (Sri Lankan cassava mosaic virus, SLCMV) and a significant number of symptomless infections. Three years after its first report in Cambodia (1), CMD occurred also in Vietnam (2), Thailand and China (3), with no significant sequence diversity found among SLCMV isolates from these separate regions. Because of the role cassava plays in the agriculture commodity trading in SEA, urgent coordinated actions involving local governments and agricultural development agencies and programs (FAO, ACIAR, SATREPS, IFAD), should be strengthen and continued. Delays in sharing information can only aggravate the problem, as this facilitates the inadvertent spread of infected material to neighbouring territories and limits the interaction with the research community. We stress the importance of standard, validated diagnostic tests for early confirmation of new diseases as an essential part of disease management to ensure smallholder farmers access to adequate disease management packages and agricultural extension support.

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Epidemiology and disease control of tomato spotted wilt virus in a chrysanthemum field in South Korea

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Tomato spotted wilt virus (TSWV) (a member of the genus *Orthotospovirus*; family *Bunyaviridae*) is one of most threatening viruses in many vegetable and ornamental crop production in the world. TSWV infects over 1000 plant species and causes significant economic damage. In 2003, TSWV was found in paprika, showing necrotic ringspot symptom on leaves and fruits in Yesan, Korea (1). It occurred with a high incidence rate in a field cultivating 14 vegetable crops and commercial chrysanthemum in the northwest of Korea, in 2004-2005 (2). Subsequently, TSWV was identified in the southwest of Korea 2006, and in the northeast and southeast of Korea in 2009. The virus then spread to Jeju Island in 2010 (3). At present, TSWV has become a prevalent virus in vegetable crops including chili pepper, tomato and lettuce, etc., in Korea. TSWV is transmitted exclusively by at least seven thrips species in a persistent manner. Among those, *Frankliniella occidentalis* (western flower thrips) and *F. intonsa* (flower thrips) are the most efficient vectors in the field in Korea. To control the thrips, chemical control was usually used in the field, but thrips are very difficult to control and have developed resistance to pesticides in all major chemical classes. The most recent effective strategy is to incorporate biological control into management. Predatory mites used for biocontrol feed on the thrips lavae or pupae in soil. The eco-friendly management with predatory mites (*S. scimitus*) and essential oils-based insecticides as a foliar treatment was developed and applied in chrysanthemum fields. The mean percentage of thrips that emerged as adults from the soil was very low in the chrysanthemum fields when surveyed every month. The incidence of TSWV was reduced significantly (0.9% to 2.7 %) compared with those in non-treated fields (32% to 45 %). In this study, we report that our strategy for management of thrips and TSWV was effective and facilitated the selection of eco-friendly insecticides.

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Emergence and management of viruses and viroids in 21st century, Japan

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In accordance with the expansion of globalization, international trade develops progressively. Because of the rapid decrease in number and aging of Japanese farmers, Japan is depending more and more on foreign countries for seed and nursery production. In the transition of this social situation, new pathogens never found in the past have been identified in Japan. Leaf chlorosis and yellowing symptoms suddenly appeared in cucurbit greenhouses in a southern area in 2004. Its causal agent turned out to be a new crinivirus, cucurbit chlorotic yellows virus (CCYV). CCYV is now widely distributed in the western half of the country. Though the origin of CCYV in Japan is still unknown, the rapid spread of the virus is apparently correlated with the previous invasion and expansion of the whitefly vector, *Bemisia tabaci*. After the occurrences of CCYV in Japan, it has also emerged in many countries of East Asia and Middle East. There are also other occurrences of quarantine pests never seen earlier increased in Japan. Chlorotic dwarf diseases of tomato occurred in 2006 and 2009, whose causal agents were identified as tomato chlorotic dwarf viroid and potato spindle tuber viroid, respectively. Those pospiviroids were assumed to be introduced into Japan through seeds and/or seedlings from overseas. Occurrences of the viroids have been restricted in only a few places so far by applying appropriate control measures such as diagnosis and disinfection techniques, newly developed. In 2009, Plum pox virus (PPV) was detected in Japanese apricot (*Prunus mume*) and was distributed in various places. Phylogenetic analysis of the complete nucleotide sequences from those isolates revealed that all isolates were strain D. PPV invasion into Japan might have resulted from import of infected plant materials. An eradication program for PPV, led by the Japanese government, is in progress, which includes prohibition of movement of the host plants and elimination of PPV-infected plants from designated areas. Since the beginning of the 21st century, the worldwide distribution of old and new pests has increased. Measures to prevent invasion of such viruses and viroids and protect agricultural production in not only Japan but also others from alien pests are discussed.

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The immunity regulation of bacteriophage cf

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Phages are viruses that infect bacteria. A filamentous phage, cf, isolated from *Xanthomonas campestris* pv. *citri*, a bacterial pathogen that causes citrus canker, was found to set up a stable lysogenic state with its genome integrated into the host chromosome (1). Cf has a genome of 7.3 kb, which is almost 1 kb larger than those of Ff phages. Open reading frame analysis indicated the presence of a *PT* gene encoded from the minus strand of the cf RF DNA. Mutations located up-strand of and in the coding region of the *PT* gene resulted in superinfection of the cf mutants on the cf lysogen (2). To understand the immunity determinants of bacteriophage cf, we constructed cf immunity mutants through the DNA shuffling technique and found that rather than superinfection inhibition, both PT protein and its up-strand cis elements function as positive regulators of cf superinfection immunity. Our preliminary data suggested the coexistence of this to predict immunity determinants for mutated phages to superinfect the cf lysogen. This work may help us to gain further insight in the mechanism of cf superinfection immunity.

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Biological properties and sequence variation among 10 new radish isolates of turnip mosaic virus showing different pathologicity in *Nicotiana benthamiana*, *Brassica rapa* and *Raphanus sativus*

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Radish (*Raphanus sativus* L.) is consumed as one of important vegetables for Kimchi and other Korean traditional dishes in South Korea. Recently, virus-like symptoms in radish plants have been observed from many cultivation field of radish in southern and north-eastern areas in South Korea. *Turnip mosaic virus* (TuMV) was identified as the causal agent for most radish diseases using serological and molecular diagnostic methods. Ten TuMV isolates from radish plants showed virus-like symptoms in cultivated farms in Jeolllabuk-Do, South Korea were individually isolated and propagated in radish. Nine TuMV radish isolates induced necrotic spots in the inoculated leaves and finally caused systemic necrosis in *Nicotiana benthamiana*. A few TuMV radish isolates caused chlorotic spots and yellowing in Kimchi cabbage (*Brassica rapa* subsp. *pekinensis*) and turnip (*B. rapa* subsp. *rapa*). One TuMV isolate induced typical dark and green mosaic symptoms in Kimchi cabbage and turnip. In particular, all TuMV isolates could infect systemically F1 cultivars of Kimchi cabbage containing the TuMV resistant gene (so called C4 gene). All TuMV isolates caused severe mosaic and stunt symptoms in commercial F1 hybrids of radish. Interestingly, all the isolated TuMV isolates could infect systemically radish cultivar Daikon, but neither TuMV-JPN1 or TuMV-UK1 TuMV could infect systemically the radish cultivar Daikon (1).

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Comparison of host range and nucleotide sequences of two hordeiviruses

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Lychnis ringspot virus (LRSV) and Poa semilatent virus (PSLV) are members of the genus *Hordeivirus* in the family *Virgaviridae*. These viruses cause several different symptoms in different host plants. The purpose of this study is to define the host range of the two different species of hordeivirus. From previous studies, LRSV has been reported to be infectious in several dicot plants but not in monocot plants. By contrast, PSLV has been reported in several monocot plants but not in dicot plants. In this study, we inoculated these two viruses to four monocot plants. The LRSV strain (LRSV-SN) used in this study showed no infectivity in *Avena sativa, Hordeum vulgare Sorghum bicolor,* and *Zea mays*. But PSLV showed systemic chlorosis in *A. sativa* and *H. vulgare*, was asymptomatic in *Zea mays*, but was not infectious in *S. bicolor*. The result of comparison of the coat protein sequences of LRSV and PSLV showed that these two viruses are grouped within the genus *Hordeivirus*.

First nanovirus in Korea: Milk vetch dwarf virus, an emerging threat to world agriculture

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Milk vetch dwarf virus (MVDV), an important member of the genus Nanovirus, consists of several ssDNA components of about 1 kb each along with two or three alpha-satellite molecules (1). MVDV infects mainly plants of the legume family Fabaceae, induces symptoms such as leaf rolling, crinkling, and stunting, and is transmitted by the Aphis craccivora. Papaya (Carica papaya) is an evergreen flowering tree valued for its antioxidant nutrients rich fruit. Papaya plants with symptoms of leaf yellowing and dwarfism were collected from Yesan, Korea. Total DNA was extracted from papaya leaves and circular DNA was amplified with extracted total DNA as template through rolling circle amplification before being digested with the restriction enzyme Sac I (2). The amplified product was visualized by gel electrophoresis and determined to be 1kb. The amplicon was cloned and sequenced. The results revealed 97% nucleotide identity with segment M of MVDV. Polymerase chain reaction (PCR) amplification of segment R was conducted using newly designed specific primers. Sequencing result showed 95% nucleotide identity with MVDV DNA-R. We isolated and characterized six more DNA components and three satellites DNA from the papaya sample. Furthermore, Southern blot hybridization was carried out using a PCR product of segment R as a probe; this showed multiple bands of dsDNA of different conformations as well as of ssDNA. This is the first report of MVDV or any other nanovirus in papaya in Korea. To examine the geographical distribution of MVDV, some symptomatic papayas were harvested in Vietnam and Taiwan in 2018 along with tomatoes and peppers grown in adjacent papaya fields. PCR results using the specific primer sets for segment M and S of MVDV revealed the presence of MVDV in papaya, pepper and tomato. In addition, the vector transmission analysis of MVDV has been confirmed from infected papaya to healthy tomatoes and peppers. Our studies, revealed that MVDV can infect plants in the family Solanaceae along with Fabaceae and Caricaceae. The reports of MVDV presence in economically and nutritionally important families are alarming.

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Occurrence and eradication of plum pox virus on ornamentals in Korea, 2016-2018

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Plum pox virus (PPV) is a significant viral disease in *Prunus* spp. worldwide. Many countries have controlled PPV through a survey and eradication program. A nationwide survey was started in *Prunus* spp. orchards, since PPV was first detected from peach in Korea, in 2015 (1). During 2016-2017, samples were collected from 30,333 trees in 1,985 orchards of stone fruits in eight provinces and four cities in Korea and were tested by reverse transcription polymerase chain reaction using a specific PPV primer set (PPV-DetFw2/PPV-R). As a result, 21 trees including peach (nine trees), Japanese apricot (four trees), plum (one tree) and apricot (seven trees) in 10 orchards were infected and controlled by an eradication program. Amplicons of the expected size (547 bp) were obtained from total RNA of seven peach trees in 2016, and directly sequenced. Blast analysis revealed the highest nucleotide identity (99%) with a PPV D isolates (LC331298, LT600782) in Genbank. The seven isolates shared nucelotide sequence identities of 98% to 100% with one another. Phylogenetic analysis showed the seven isolates in peach clustered closely with the PPV D isolates (LC159485, AY912057, AF401295 and AY912057) from Korea, Japan, USA and Canada. No positive samples were found in 2018; the percentage of positive finds decreased during the three years of the survey and eradication. Our results and efforts will contribute to effective measures for eradication of PPV.

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Occurrence of cymbidium chlorotic mosaic virus in *Cymbidium goeringii* in Korea

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Orchid is one of the most important potted floricultural crops in Korea. Among *Cymbidium* spp., *Cymbidium goeringii* Reichenbach ill (Spring Orchid) grows in the wild in China, Japan, South Korea, and Southeast Asia. In 2017, typical viral symptoms, including chlorotic mosaic and streaks, were observed on the leaves of *C. goeringii* in Nonsan, South Korea. Typical spherical particles, with a diameter of approximately 28 nm, were observed by transmission electron microscopy (TEM) in the leaf samples of *C. goeringii* plants. Subsequently, total RNA was extracted for transcriptome analysis of *C. goeringii* with the TruSeq RNA Library Preparation Kit v2 and Illumina HiSeq 2000 Sequencing System. The assembled sequences were aligned with viral reference genomes by searches using BLAST for identifying any viruses infecting these orchids, and sequences corresponding to Cymbidium chlorotic mosaic virus (CyCMV) were identified by a *de novo* transcriptome assembly. The complete genome sequence of the CyCMV isolate Kor (CyCMV-Kor) was determined to be 4,084 nt and was deposited to GenBank (Accession no. LC381945), after confirmation of the 5′- and 3′-end of the CyCMV genome using the SMARTerTMRACE kit. A BLAST search revealed that the nucleotide and amino acid sequence of the *coat protein* genes of CyCMV-Kor were closely related (91.3−91.6% similarity) with those of Cym92-20. To the best of our knowledge, this is the first report on the occurrence of CyCMV in Cymbidium plants in Korea.

Production of infectious cDNA clones for two BR pathpotypes of turnip mosaic virus isolates infecting Brassicaceae plants

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A few field surveys for virus-like symptoms of Kimchi cabbage (also called Chinese cabbage; *Brassiaca rapa* subsp. *pekinensis*) and radish (*Raphanus sativus*) in South Korea showed that approximately 60% of infections in Kimchi cabbage plants and 50% of infections in radish plants were caused by turnip mosaic virus (TuMV) or TuMV plus another virus in farms, resulting in economical losses annually (1). From the field survey from 2017 to 2018, we singly isolated a few TuMV isolates from Kimchi cabbage plants or radish plants and maintained each TuMV isolate in commercial F1 hybrids of Kimchi cabbage and radish. Sequence analysis of *coat protein* genes of each TuMV isolate showed that all the maintained TuMV isolates were classified into Asian BR pathotype that infects both Kimchi cabbage and radish. Interestingly, the pathogenicity of TuMV isolates could be distinctly differentiated from each isolates in *Nicotiana benthamiana*, showing that most TuMV isolates induced systemic necrosis and a few TuMV isolates induced mild mosaic symptoms in *N. benthamiana*. Infectious cDNA clones of the representative Asian BR isolates of TuMV were constructed using RT-PCR with a pair of primers containing the T7 RNA promoter sequence. TuMV transcripts transcribed by T7 RNA polymerase from cDNA clones were successfully infectious in *N. benthamiana.* These results indicate that the infectious cDNA clones of TuMV isolates are useful for TuMV-resistance assessment in the family Brassicaceae.

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Profiling of differentially expressed genes in *Chenopodium quinoa* during pepper mottle virus infection

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Pepper mottle virus (PepMoV) is a member of the genus *Potyvirus*, family *Potyviridae*. PepMoV was first identified in Arizona, USA, in 1969, as a new strain of a potyvirus that infected peppers (1). PepMoV infects species of the *Solanaceae* family, including *Capsicum annuum* and *Solanum lycopersicum* (2). *Chenopodium amaranticolor* exhibits chlorotic local lesions on the inoculated leaves against PepMoV, without systemic infection. *Chenopodium* spp. have a special defense system, called the hypersensitive response, which causes cell death at the infection site to restrict the spread of pathogen. Thus, this study was carried out to identify and understand that the differentially-expressed genes related to symptoms and defense reaction to PepMoV in the inoculated leaf of *Chenopodium* species by subtractive hybridization, and to profile the related genes according to their functions. As a result, functional categories were generated according to KEGG orthology, and expression levels of several ESTs from the library, such as defense- and stress-associated genes were evaluated using RT-PCR and qRT-PCR. Evaluating expression level of ESTs identified from subtractive library showed several pathogenesis-related proteins were differentially expressed between mock and PepMoV-inoculated *C.quinoa*.

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Synergistic interaction in pathogenicity and disease symptoms among turnip mosaic virus, youcai mosaic virus and cucumber mosaic virus in radish plants (*Raphanus sativus* L.)

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Simultaneous infection of a plant by two viruses generally shows more severe disease than is caused by infection with either virus alone (1). *Youcai mosaic virus* (YoMV), the genus Tobmovirus, itself causing imperceptible symptoms in radish (*Raphanus sativus* L.) exacerbated symptoms of infection by *Turnip mosaic virus* (TuMV), the well-studied member of the genus *Potyvirus* in the family Potyviridae. The synergy in symptom expression was most evident in a reduced size of leaves and accumulation of YoMV was slighly increased in the systemic leaves. Similarly, we found that synergistic interactions occurred in mixed infection of TuMV and *Cucumber mosaic virus* (CMV) that causes very mild symptoms in radish. CMV accumulation is significantly enhanced by TuMV infection in radish plants. Furthermore, long-distance movement of both YoMV and CMV was facilitated in the presence of TuMV in the radish plants. Synergistic interaction among the three viruses will be discussed.

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Systemic infection on four legumes determined by two amino acids in the 2a protein of cucumber mosaic virus

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Cucumber mosaic virus (CMV) Pa3 isolate, CMV-Pa3, was isolated from *Vigna angularis* in Chuncheon, Korea. CMV-Pa3 induced mild symptoms on *Nicotiana benthamiana* and *N. tabacum* (cv. Xanthi nc), and infected systemically on four legume species (*V. unguiculata*, *V. angularis*, *Phaseolus vulgaris* and *Pisum sativum*). In a previous study, it was reported that systemic infection in cowpea induced by a CMV bean strain (CMV-B) was determined by two amino acids of the 2a protein (viral replicase gene) on RNA2 (1). Sequence analysis revealed that the two amino acids (631 and 641 of the 2a protein) of CMV-Pa3 were identical to CMV-B with Tyr and Ser, respectively. We constructed point-mutants (R1/R2-F631Y/R3 and R1/R2-A641S/R3) in which Phe and Ala (amino acids at position 631 and 641) in the 2a protein of CMV-Rs1, only inducing the necrotic spots on inoculated leaves of cowpea, were substituted with Tyr and Ser. These mutants also induced systemic infection in four species of legume. It suggested that the two amino acids are determinants of systemic infection not only in cowpea but also in other legumes such as red bean, kidney bean and pea. In further works, it is necessary to study the host factors that interact with viral proteins in four legumes.

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Threatening of seed transmissible pepper yellow leaf curl Indonesia virus

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Virus members of the family are *Geminiviridae* are among the most threatening plant viruses in the world. Recently, chili pepper (*Capsicum annuum*) plants in Indonesia have a serious problem with infection of a geminivirus named pepper yellow leaf curl Indonesia virus (PepYLCIV), a member of the genus *Begomovirus*, which causes disease that lead to reduction in the amount of chili production. In 1999, pepper yellow disease by a geminivirus in West Java was first described. Since then, research on this virus has continued to develop. Around 2003, the virus was found to be spread in Central Java and this virus was found to infect chili pepper in Sumatra Island around 2005. The spreading of this virus is very worrying. Pepper yellow disease could reduce chili pepper yields by 100% in Indonesia (1). Until now, there is still no suitable prevention method for this virus. Even recently, this virus could reduce the yield of chili from 15 tons per ha to 9 tons per ha in a region. As previously known, begomoviruses can be transmitted through whiteflies. Previous studies determined that several plants from the family *Solanaceae*, *Compositae*, and *Leguminosae*, are hosts of virus causing pepper yellow leaf curl disease due to whitefly transmission. Efforts to overcome this disease have been carried out by demolition of the soil and eradication of white flies, but chili pepper plants still have yellow disease. Thus, it is suspected that seed transmission can occur in chili plants, since based on previous research, tomato yellow leaf curl virus, another begomovirus, can be transmitted through seeds to several plant hosts (2). This study was conducted using seeds and seedlings taken from peppers and tomatoes infected with PepYLCIV from Java Island, Indonesia. To detect PepYLCIV and to prevent laboratory carry-over contamination of PCR product, DNA extraction and amplification were carried out through UDG-PCR. Results showed that seeds and seedlings of PepYLCIV infected peppers and tomatoes were also infected by PepYLCIV DNA-A (AB267838.1) and DNA-B (LC314795.1). Taken together, the possibility of seed transmission of PepYLCIV on peppers and tomatoes should be investigated.

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DNA free Taq DNA Polymerase	DNA free Fast Taq DNA Polymerase	DNA free DuoTaq DNA Polymerase	DNA free HotTaq DNA Polymerase	DNA free DuoLR Taq DNA Polymerase	Pfu DNA Polymerase	DNA free DuoLR Taq DNA Polymerase	Mplex 2XMultiplex PCR Master Mix
DNA free DuoTaq DNA Polymerase	DNA free Fast HotTaq DNA Polymerase		DNA free Fast HotTaq DNA Polymerase		Fast Ton DNA Polymerase	Quantum Pfu DNA Polymerase	
	Fast Ton DNA Polymerase		DNA free DuoHotTaq DNA Polymerase		Quantum Pfu DNA Polymerase		

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QGreen 2X SybrGreen qPCR Master Mix	Fast QGreen 2X Fast SybrGreen qPCR Master Mix	QPlex 2X Probe qPCR Master Mix

CellSafe

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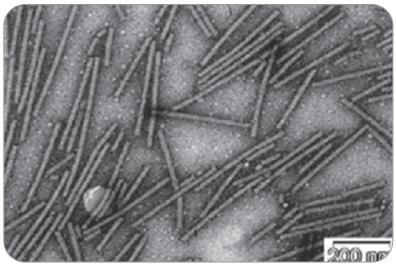
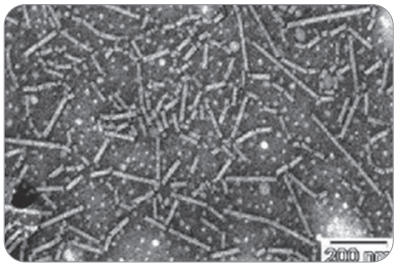
Characteristics of product

- Product made from oriental herb medicine.
- The virus is inactivated within 20 minutes after contacting of the product.
- Both foliar and soil treatment are possible.
- Possible to respond to the virus infection pattern.
- Excellent efficacy on various viruses.
- Suitable for high quality organic agricultural product.

TMV particle change after Anti-VⓈ treatment (Mode of action)



Untreated



20mins after Anti-VⓈ treatment

Anti-V Gold SL

Anti-V Gold GR

Anti-VⓈ WP

Organic Virucide

V-cut Scissors

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