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Programme


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13-17 janvier 2013
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POUR LE DÉVELOPPEMENT

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Role of mosquito vectors in dengue virus evolution

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Arthropod-borne viruses (arboviruses), such as dengue viruses,cause a substantial impact on human health at a global scale. The vast majority of arboviruses are RNA viruses that typically display extensive genetic diversity and frequent lineage turnover. Elucidating the evolutionary forces driving arbovirus evolution is crucial to improve our understanding of disease epidemiology and to design adequate control interventions. Whereas most studies about arbovirus evolution focus on the influence of their vertebrate hosts, the potential role of arthropod vectors has generally been overlooked. In this talk I will (1) review current knowledge on the role of arthropod vectors in arbovirus evolution; (2) describe an example consistent with the idea that mosquito vectors can play a role in adaptive evolution of dengue viruses; and (3) highlight how studying the role of vectors in arbovirus evolution can be complicated by specific interactions between vector and virus genotypes.

Multipartism in viruses allows differential control of gene copy number

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Multipartite viruses are enigmatic entities for both evolutionary biology and system biology. Their genome is divided into several segments, each encapsidated separately. As opposed to monopartite viruses with a genome made of a single nucleic acid molecule, segmentation has been proposed to provide benefits of different kinds, such as a higher stability conferred to viral particles by small genome segments, or faster replication. However, the most popular view portrays multipartism as a spectacular example of the evolution of sex in viruses, intensifying genetic exchanges through easy shuffling of segments. We here provide experimental evidence negating these theories and suggesting that multipartism's major benefit for the virus is the formidable possibility to control differentially the "copy number variation" (CNV) of its genes, a phenomenon increasingly recognized as a key regulator of phenotypic changes in any organism. Whatever the benefits, multipartism decreases the probability to gather at least one copy of each gene within individual cells, thus inducing a cost on the success of cells infection. For a given number of segments, this cost is maximized when their relative frequencies diverge, some being highly frequent and others being rare. Thus, unless otherwise selected, the frequency of each segment should tend to even in order to minimize the cost of genome segmentation. We have monitored *in planta* the relative frequency of the 8 single-gene segments composing the genome of *Faba bean necrotic stunt virus* (FBNSV). We demonstrate that all genes reproducibly adjust to a specific frequency value, ranging from 5% to 40% (so a ratio 1/8) depending on the gene considered. When inoculated with varying segment proportions, the virus rapidly converges to the same "genome formula" where each gene is associated to a specific relative copy number. We further show that the genome formula is host-specific, that its regulation is a systemic property. These results clearly indicate that the system FBNSV stabilizes with diverging segment frequency, a situation consistent with the differential regulation of viral gene copy numbers, but totally incompatible with previous theories where benefit is linked to segmentation itself, but not to frequencies of the segment, which should thus evolve to be equal.

Mots clés : Copy number variation, gene regulation, nanovirus, *Faba bean necrotic stunt virus*, genome formula

Breeding *Musa balbisiania* genitors devoid of infectious eBSV alleles

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Banana streak viruses (BSV) infect bananas and plantains worldwide. They are naturally transmitted by mealybugs; however infections can also occur in the absence of vector-mediated transmission, through the activation of infectious endogenous BSV sequences (eBSVs). Infectious eBSVs are present in the genome of *Musa balbisiana* spp, which are important progenitors for breeding improved banana varieties. Once activated by biotic or abiotic stresses, these viral sequences cause spontaneous infection in both natural and synthetic interspecific hybrids harbouring the *M. balbisiana* genome, denoted B [1]. Therefore, the presence of infectious eBSVs within B genomes is currently the main constraint for breeding banana and plantain interspecific hybrids and for exchanging *Musa* germplasm.

The sequence and organization of eBSVs in the diploid *M. balbisiana* genitor Pisang Klutuk Wulung (PKW) was elucidated [2], showing that integration of infectious eBSGFV [3] and eBSOLV is di-allelic, with one infectious and one non-infectious allele, whereas that of infectious eBSImV is monoallelic [2]. Taking advantage of the development of allele-specific molecular markers [2; 4], eBSV signatures were established for all *M. balbisiana* genitors of the CIRAD Guadeloupe *Musa* collection. This work unveiled important differences between accessions. All combinations of infectious and non-infectious alleles were observed for the three BSV species, as well as complete and uncomplete integrants when compared to those described in PKW. Breeding improved *M. balbisiana* progenitors devoid of infectious eBSGFV and/or eBSOLV alleles was undertaken through self-pollination and chromosome doubling of haploid lines. Both approaches successfully lead to *M. balbisiana* cultivars devoid of infectious eBSOLV and/or eBSGFV resulting from the segregation of eBSOLV and eBSGFV alleles. Improved lines of one particular *M. balbisiana* cultivar, cv. Honduras, originally free of eBSImV, were shown to be free of infectious eBSV. These results pave the way to the safe use of *M. balbisiana* in breeding programs, and open new perspectives for breeding improved banana and plantain hybrid varieties.

Keywords : endogenous pararetrovirus; infectious; Banana streak virus; segregation; breeding

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Profiling of phloem specific gene expression upon infection by a systemic and a phloem-restricted virus

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Plant viruses exploit the vascular phloem system of their hosts to promote their systemic infection. Several viral factors involved in virus long distance movement have been identified, but few plant proteins required for this function have been described yet. Moreover viruses trigger host gene deregulations that may be part of plant defence pathways or of an offensive strategy to exploit cellular mechanisms to promote systemic viral propagation.

To address this issue a cell-type specific transcriptomic approach has been undertaken in *Arabidopsis thaliana* with two viruses belonging to different genera: the *Potyvirus Lettuce mosaic virus* or LMV (filamentous virions, infecting almost all cell types) and the *Polerovirus Turnip yellow virus* or TuYV (icosaedric particles, restricted to phloem cells). Despite their differences in virion morphology, genome organisation and tissue tropism, both viruses use the phloem vasculature to traffic over long distances through the host.

Transgenic *A. thaliana* plants expressing the GFP under the phloem companion-cell specific *AtSUC2* promoter (Imlau et al., 1999) were infected with either LMV or TuYV and used to isolate protoplasts. Fluorescent protoplasts were then sorted by FACS (fluorescence-activated cell sorting) and RNA was extracted and processed by genome-wide micro-arrays (CATMA) or RNA Seq technology (Illumina).

Despite variability between experiments, a set of up or down-regulated genes has been identified for each virus. The number of genes deregulated following infection with each virus was surprisingly low: 95 for LMV and 57 for TuYV. Moreover, very few common genes were found deregulated with both viruses. For each virus a specific set of co-deregulated genes was identified belonging to the same biological pathway. Alteration of gene expression upon virus infection was confirmed by quantitative RT-PCR.

To assess the potential role of these genes in virus long distance movement, *A. thaliana* knock-out (KO) mutant lines were selected for the candidate genes and are currently challenged with either virus. As some of the candidate genes belong to small gene families with potentially functional redundancy, production of double or triple mutants is in progress to further test their behaviour upon infection.

Mots clés: transcriptome, phloème

Characterization of new endogenous geminiviral elements in yam (*Dioscorea* spp.) genomes

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Geminivirus sequences integrated into the genomes of various plant species (*Nicotiana* species, *Lactuca sativa*, *Malus domestica* and *Populus trichocarpa*) have been reported. The best studied of these integrated geminivirus sequences are the so-called "geminivirus-related DNA" or GRD elements within the genomes of various *Nicotiana* species. Our study aimed at assessing whether such integrated geminiviral sequences are present within the genomes of yam species. We have used a polyphasic approach combining a first step of plant genome screening (*in silico* EST analysis and viral metagenomics) followed by a second step of molecular characterization of the integrated sequences (Long-PCR and Inverse-PCR). We have found two new phylogenetically related endogeneous geminivirus (EGV) elements within the genome of the majority of Asian *Dioscorea* spp. section *Enantiophyllum*. Based on multiple lines of evidence, such as (i) seed transmission of the two EGV sequences, (ii) no amplification by rolling circle amplification, (iii) worldwide presence of the two EGV sequences in a range of asympomatic Asian *Dioscorea* species and (iv) lack of sequences of coat protein genes (i.e. they are not likely to be insect transmissible), we hypothesized that those two EGV elements were likely to have become integrated within an ancestor of the Asian *Dioscorea* species. We then confirmed the presence of the two EGV elements within *D. alata* and *D. nummularia* genomes by fluorescence *in situ* hybridization. The predicted proteins expressed by the two EGV sequences had detectable homology to begomovirus replication enhancer and replication associated proteins but only one out of the two EGV sequences contains a GC rich sequence resembling the conserved hairpin structures found at geminivirus virion strand origins of replication (including the TAATATTAC sequence in the likely loop region) and is organized as 2.6 Kb tandem repeats. The discovery and analysis of such fossilized geminivirus sequences within plant genomes would certainly help to more accurately date events deep in the evolutionary history of the *Geminiviridae* family such as the most recent geminivirus common ancestor, and the origins of the various geminivirus genera.

Mots-clés: Integrated viral sequences, Geminivirus, *Dioscoreacae*, *Enantiophyllum*, Phylogeny, FISH

Constitutive and induced defence responses against plant viruses

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To successfully infect a plant, a virus must usurp the cell host machinery and overcome plant defense mechanisms. A major mechanism of constitutive antiviral immunity is ensured by RNA silencing which relies on the recognition and degradation of viral double-stranded RNA into virus-derived small RNA (vsRNA) by DICER-like enzymes. Once incorporated into complexes containing members of the Argonaute (AGO) family of endonucleases, these vsRNA act as guides to target viral RNA for degradation or inhibition of translation. Induced resistance to viruses is afforded by the products of plant disease resistance (*R*) genes encoding NB-LRR proteins. Using several viruses, including potato virus X (PVX), we have investigated the role of different AGO family members in both constitutive and induced anti-viral defenses. We find that induced anti-viral responses are dependent on AGO4 and that these mechanisms appear to inhibit the translation of viral transcripts. In agreement, with this, we find that induced defenses induce massive formation of RNA processing bodies (P-bodies) to deal with viral transcripts. At the same time, we find that constitutive defenses against viruses are directed by AGO2, as well as at least one additional AGO, which belong to a different clade from AGO4-like proteins. Furthermore, we show that RNA silencing components, including AGO2, function both in host and non-host resistance to viruses. Our results indicate a specialization in AGO function in different types of anti-viral defenses and provide new insights into the mechanisms of induced defense responses.

Genetic structure, diversity and selection of the *L* resistance gene toward Tobamoviruses in *Capsicum annuum* var. *glabriusculum* wild populations in Mexico.

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Understanding host-pathogen co-evolution is a central question in Biology as it is at the root of pathogen emergence, host switch and host range expansion, and the composition anddynamics of ecosystems. However, contrary to agroecosystems, evidences for plant-pathogen co-evolution in wild environments are almost non-existent for plant viruses. Thus, we propose to use as a model system the wild pepper *Capsicum annuum* var. *glabriusculum* (chilechiltepin), which is considered as the wild ancestor of the domesticated*C. annuum* species. Wild populations of chiltepin are found in the tropical dry forests of Mexico (1), where they are exploited for their small pungent fruits, and their cultivation by traditional local farmers has recently started in severalregions.A large collection of samples representing wild and cultivated chiltepin populations in Mexico has been obtained (2), and the infecting viruses have been identified. Resistance to tobamoviruses in *Capsicum* spp. is controlled by alleles at the *L* locus. The *L* resistance gene has recently been cloned and characterised (3). This resistance is based on a single-gene locus encoding a CC-NB-LRR protein, which targets the coat protein (CP) of tobamoviruses. This recognition initiates signalling pathways leading to resistance by hypersensitive response (HR).

In this study, the *L* gene frequency was assessed in each chiltepinpopulations. Interestingly, the frequency of this resistance gene is significantly higher in the wild populations than in the cultivated ones, which suggests that the pre-domestication of this species was not focused on the tobamoviruses resistance.Furthemore, the analysis of more than 90 full-length *L* gene sequences showed a high variability and a strong geographical structure of this gene. Local adaptations of chiltepin populations toward different tobamoviruspathotypes and/or different environmental conditions could explain the genetic structure of the *L* genein Mexico.In addition, several sitesunder positive selection and co-variations were detected, mainly located in the domain involved in the CP recognition.The roles of these sites on the recognition of viral pathotypesat different environmental conditions will be further investigated.Altogether, these results suggest that the *L* resistance gene is a target for selection, which could support the hypothesis of co-evolution between chiltepin populations and tobamoviruses in wild ecosystems.

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Viral determinant of the *Grapevine fanleaf virus* capsid protein involved in virus movement *in planta.*

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Grapevine fanleaf virus (GFLV) isresponsible for a severegrapevine degeneration, adisease present in vineyards worldwide. This virus belongs to the genus *Nepovirus* within the *Secoviridae* family. Structurally, it is anicosahedral virus of 30nm in diameter with a pseudo *T = 3* symmetry, composed of 60 identical subunits. GFLV is specifically transmitted from grapevine to grapevine by the ectoparasitic nematode *Xiphinemaindex*. Into the plant, GFLVmoves from cell to cell as entire virions through viral encodedtubulesformed by the self-assembly of the movement protein (MP) and embedded in plasmodesmatas (Amari K. et al., 2010). Recently, reverse genetic experiments carried out on the GFLV coat protein (CP) allowed us to characterize surface-exposed structural motifs essential for GFLV transmission by its nematode vector (SchellenbergerP. et al., 2010). This functional genomics approach also resulted in the production of two other GFLV mutants named R3 and R4, able to replicate in protoplasts, but unable to trigger a systemic infection in whole plants. While (R4) is able to protect its viral genome from RNase degradation R3 is unable to do it(Schellenberger P. et al., 2011). This suggests that R3 fails to encapsidate correctly and, as a consequence, to move from cell-to-cell, while the mutated CP region of the R4 particle may contribute to viral movement, possibly via interactions with the MP.

To gain further insights into GFLV CP determinants involved in virus movement *in planta*and based onthe atomic structure of GFLV obtained in our laboratories, further mutations affecting surface-exposed residues were performed in the CP of GFLV. The different mutated CPs were introduced into a GFLV recombinant RNA2 encoding the Enhanced Green Fluorescent Protein. Biological properties including cell-to-cell movement and long distance movementof the recombinant RNA2in the presence of wild-type RNA1 were evaluated after inoculation of *Chenopodium quinoa* plants. Results will be discussed in relation to the capacity of the CP and MP of GFLV to promote movement as a ribonucleoprotein complex or via entire particles.

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Molecular characterization of *Euphorbia caput-medusae stunt virus*: evidence for the existence of a new genus within the family *Geminiviridae*

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Studies focusing on phytoviruses isolated from the wild are rare. Nevertheless, even if those studies remain scarce, it is increasingly accepted that viruses coming from wild plants might play a role on disease emergence and in the functioning of ecosystems. Geminivirus are a major cause of disease on plants of agronomic interest. We hypothesize that strengthening our knowledge of the geminivirus diversity coming from the wild could help reconstructing the evolutionary history of the *Geminiviridae* family but also could help us understanding and predicting future epidemics.

Over the past two decades, rolling circle amplification (RCA) has been more and more employed for the detection of small circular single-stranded DNA viruses, including geminivirus coming from the wild [1]. We have used this method for detecting the presence of ssDNA from 236 plants collected in the South African fynbos. We have obtained amplified DNAs from 36% of the plants (85 out of 236 plants). Using classical cloning and sequencing methods, we have obtained ten sequences of which one was identified as a plant virus. This viral sequence corresponds to a new geminivirus, which infects a wild spurge (*Euphorbia caput-medusae*). This geminivirus is highly divergent from the current known members of the family *Geminiviridae* and is likely to represent a new previously unknown genus of this agriculturally highly relevant family of viruses. The virus, which we have named *Euphorbia caput-medusae stunt virus* (EcmSV) is not obviously a recombinant of viruses in the known geminivirus genera, has features most similar to viruses in the genus *Mastrevirus* (the presence of a *repA* gene and the production transcripts that are almost certainly spliced), but it also has unique features among geminiviruses (potential product of spliced V2-V3 ORFs). Besides EcmSV providing new information on the evolutionary history of geminiviruses, its discovery stresses the need to better assess viral diversity at the interface between wild and cultivated areas (by *in situ* sampling) and to study viruses isolated from wild hosts for their potential to infect crop species and *vice-versa* (by *in vitro* experimentation).

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Distribution and diversity of *Barley yellow dwarf virus*-PAV inthe sub-Antarctic Kerguelen Islands and characterization of two new *Luteovirus* species

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With the perspective to study plant virus ecology in sub-Antartic ecosystems, a systematic search for viral infection in symptomatic and asymptomatic plants (native and alien species) has been initiated in the Kerguelen Islands, using a range of polyvalent genus-specific PCR assays.

This first screening indicated the presence of both novel viral agents and of well-known viruses such as *Barley yellow dwarf luteovirus* (BYDV) on samples collected during three surveys carried out between 2008 and 2011. BYDV isolates were detected in the introduced grasses *Dactylis glomerata* and *Poa annua* and in the native grasses *Agrostis magellanica*, *Festuca contracta* and with a strong prevalence, *Poa cookii*.

In order to describe the geographical distribution of BYDV and the prevalence of the virus on *P. cookii*, 378 samples were collected from 25 sites (41 collection points) and analyzed for the presence of BYDV by immunoprinting and/or by specific RT-PCR. The average prevalence of the virus has been estimated at 24.9% in the 16 sites where infection was detected, with nevertheless an important variability between sites (10.5% to 100%). The genetic diversity of BYDV was assessed by analyzing 59 isolates and sequencing two genomic regions: the P3 gene (encoding the viral coat protein) and the hypervariable P6 gene. The phylogenetic analysis in the P3 region showed the segregation of BYDV sequences into three major lineages in the *Luteovirus* genus, all supported by strong bootstrap values. The first lineage (Ker-I cluster) comprises the majority of isolates (80% on 11 sites) and shows close homology with BYDV-PAV-I isolates (average divergence 2.2%) and a low intra-lineage diversity of 0.6%. A similar level of diversity was recorded in the hypervariable P6 region (0.9%), probably indicating that Ker-I isolates derive from a recent introduction event of BYDV-PAV followed by a colonization phase. It is likely that BYDV was introduced in the Kerguelen environment with the same time frame as its aphid vector, *Rhopalosiphum padi*. The distribution of this aphid species shows good overlapping with the distribution of BYDV-PAV in Kerguelen Islands. The two other lineages (Ker-II and Ker-III) show respectively 24.8-25.1% and 22.5-28.4% amino acid divergence in the P3 region with the other BYDV clusters/species, strongly suggesting they represent distinct BYDV species. Phylogenetic analyses and detailed sequence comparisons on whole genome sequences are currently ongoing to further define the taxonomic position of these two clusters of isolates. Specific amplification primers, targeting the variable part of the P5 gene of each cluster have been developed, allowing their specific detection even in conditions of mixed infection. These species-specific assays have permitted the analysis of the distribution of isolates of these novel species in the Kerguelen Islands. Considering the high prevalence of BYDV on native *Poaceae* and the presence of the vector *R. padi* in the Kerguelen Island, it will be interesting to evaluate more precisely the potential for epidemic spread for BYDV on the vulnerable plant communities of this remote ecosystem.

The 140K : a key player for the targeting of the TYMV replication complexes to the chloroplasts

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The Turnip yellow mosaic virus (TYMV) is a positive single-stranded RNA virus belonging to the alphavirus-like supergroup and the Tymovirus genus. The TYMV genome encodes three proteins, the 206K polyprotein being the only one required for the viral replication. This protein is cleaved into 140K and 66K encompassing the RNA-dependent RNA polymerase domain [Jakubiec *et al.*, 2007]. Replication complexes are localized at invaginations of the chloroplastic envelope membrane and include both proteins [Prod'homme *et al.*, 2003]. We have previously reported that the viral 140K protein is targeted to chloroplasts where it recruits the RNA-dependent RNA polymerase [Jakubiec *et al.*, 2004].

To delineate the region of 140K protein involved in its targeting to the chloroplast envelope, several constructs encoding mutated versions of 140K fused to GFP were expressed in Arabidopsis protoplasts, and their subcellular localization was determined by fluorescence microscopy.

The domain of 140K involved in the chloroplast targeting was delimitated to a central region within the protein. Secondary structure predictions indicated the presence of several putative alpha-helices, whose contribution was assessed by site-directed mutagenesis. One mutant bearing mutations in a amphipathic helix appeared affected in its targeting to the chloroplast envelope, suggesting that this helix may serve to anchor 140K in the chloroplast envelope membrane.

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Association mapping of quantitative responses to *Turnip mosaic virus* (TuMV) infection trait loci in *Arabidopsis thaliana* through evaluation of biomass, viral accumulation, and metabolic profiles.

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Viruses are obligatory biotrophic pathogens which use their host's components and pathways to complete their cycle. Therefore, they deeply disturb host metabolism and can cause symptoms according to the susceptibility of the host. In the pathosystem *Turnip Mosaic Virus* - *Arabidopsis thaliana*, we were first interested in quantitative responses to the virus. We described plants' responses at a macroscopic scale- biomass evaluation, symptoms scores- and we evaluated viral accumulation and metabolomic profiles in 182 worldwide and french genotypes. Multivariate analysis showed that the infection can trigger the development of symptoms– which are correlated to the viral accumulation -, a reduction of biomass and an increase of all tested primary metabolites concentrations. These disruptions are related to a reduction of photosynthesis and an increased storage of carbon. Using genome wide mapping analysis, we identified some genetic determinants acting in the control of these phenotypes. We mapped genes previously shown involved in responses to Potyvirus. This strategy also allowed us to highlight *de novo* candidate genes involved in *Arabidopsis thaliana-TuMV* interaction.

Characterization of the serological diversity of *Potato virus Y* (PVY): impact on the diagnosis

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Potato virus Y belongs to the *Potyviridae* family and is the type member of the genus *Potyvirus*. It is a virus present in all continents, in all regions producing potato where it is responsible of yield and quality losses, inducing symptoms on potato leaves and necrotic ring spots on the tuber surface. This omnipresence is the consequence of a large capacity of adaptation and evolution of the virus, which results in a significant diversity of PVY, divided into strain groups and variants according to their biological properties (pathogenicity). A wide range of diagnostic tools exist to detect and / or identify the virus. Serological diagnosis, through ELISA, is the most used tool to follow viral populations or to assess the seeds sanitary quality by certification laboratories. Serological tools rely on the use of monoclonal antibodies (Mabs), most of which have been developed for over 20 years. So far it is assumed that these markers differentiate PVY isolates into two serogroups: isolates of serotype-N (PVY^N and PVY^{NTN}) and isolates of serotype O (PVY^O, PVY^C and PVY^N-W). But the recent emerging of isolates with atypical serological profile (non-detection, both O-N serotype, misidentification) shows the weaknesses of this characterization and the risks associated with the use of such tools to identify natural populations of PVY. Therefore, the objectives of our study were to describe the immunological diversity of PVY isolates, select a panel of specific MAbs for detection of different PVY populations and characterize the antigen / antibody interactions via the surface plasmon resonance approach (SPR-Biacore technology). To do that, a bank of 77 Mabs anti-PVY was developed following immunization of mice with a mixture of selected isolates according to their serological characteristics (N, O, or undetermined) and molecular (peptide sequence of the capsid protein). The confrontation of this bank of Mabs over 150 PVY isolates from different countries highlights a serological variability much broader than commonly accepted and puts in question the reliability of a diagnosis based on a single serological marker. Data of reactivity and interaction parameters (affinity, steric hindrance) of selected antibodies will be presented and discussed.

East African cassava mosaic-like viruses from Africa to Indian Ocean Islands: molecular diversity, evolutionary history and geographical dissemination of a bipartite begomovirus

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Cassava (Manihot esculenta) is a major food source for over 200 million sub-Saharan Africans. Unfortunately, its cultivation is severely hampered by cassava mosaic disease (CMD). Caused by a complex of bipartite cassava mosaic geminiviruses (CMG) species (Family: Geminivirideae; Genus: Begomovirus) CMD has been widely described throughout Africa and it is apparent that CMGs are expanding their geographical distribution. Determining where and when CMG movements have occurred could help curtail its spread and reveal the ecological and anthropic factors associated with similar viral invasions. We applied Bayesian phylogeographic inference and recombination analyses to available and newly described CMG sequences to reconstruct a plausible history of CMG diversification and migration between Africa and South West Indian Ocean (SWIO) islands.

The isolation and analysis of 114 DNA-A and 41 DNA-B sequences demonstrated the presence of three CMG species circulating in the Comoros and Seychelles archipelagos (East African cassava mosaic virus, EACMV; East African cassava mosaic Kenya virus, EACMKV; and East African cassava mosaic Cameroon virus, EACMCV). Phylogeographic analyses suggest that CMG's presence on these SWIO islands is probably the result of at least four independent introduction events from mainland Africa occurring between 1988 and 2009. Amongst the islands of the Comoros archipelago, two major migration pathways were inferred: One from Grande Comore to Mohéli and the second from Mayotte to Anjouan. Numerous re-assortments events were detected between EACMV and EACMKV, which seem to almost freely interchange their genome components.

Rapid and extensive virus spread within the SWIO islands was demonstrated for three CMG complex species. Strong evolutionary or ecological interaction between CMG species may explain both their propensity to exchange components and the absence of recombination with non-CMG begomoviruses. Our results suggest an important role of anthropic factors in CMGs spread as the principal axes of viral migration correspond with major routes of human movement and commercial trade.

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Long-distance transport device of *Turnip yellows virus*

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Turnip yellows virus (TuYV) (*Polerovirus*, *Luteoviridae* family) genome is a single stranded positive RNA of 5.6 kb protected by an icosahedral virus particle. TuYV is strictly transmitted by aphids, and in host plants, its replication and movement are restricted to phloem cells.

Polerovirus movement in the plant is divided into two phases: (i) a transport from cell to cell via plasmodesmata connecting nucleate and enucleate phloem cells (companion cells, vascular parenchyma and sieve elements); and (ii) a long-distance movement taking place in the sieve elements that allows the virus to infect the whole plant. Virus particles were observed in phloem sap and in the branched plasmodesmata connecting companion cells and sieve elements (Mutterer *et al.,* 1999), suggesting that virions are responsible for polerovirus long-distance transport. However, sap is also a virion reservoir for aphid transmission, and virus particles in sieve elements could be dedicated to aphid acquisition. The purpose of this work was to investigate the potential role of ribonucleoprotein (RNP) complexes in TuYV systemic transport in the plant.

A set of TuYV mutants modified in the major capsid protein gene, the CP, were analyzed for their ability to systemically infect host plants. We observed a true correlation between an efficient long-distance movement and the capacity of forming virus particles, suggesting that virions are indeed necessary for polerovirus systemic transport. However, this observation does not preclude an essential role of the CP in the formation and movement of RNP complexes. Therefore, we inoculated transgenic *Arabidopsis thaliana* Col0 plants expressing the wild-type TuYV-CP with different TuYV mutants unable to produce systemic infections. Interestingly, one of them was detected in newly developed leaves of the CP-expressing plants. Experiments are in progress to analyze if this mutant moves through RNP complexes or virus particles as no virus particles were observed in the non-inoculated plants.

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Functional analysis of aphid effectors that modulate plant processes in a host specific manner

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Insect herbivores are often highly selective in their plant host choices for feeding and reproduction. The majority of chewing insect herbivores such as lepidopterans and sap-feeding insects of the order Hemiptera are able to colonize single or few related plant species. Relatively few insect species are polyphagous with wide host ranges consisting of plant species of multiple distantly related plant families. There is a growing body of evidence that insects produce virulence factors (effectors) that modulate host processes. These effectors are likely to act in a host-specific manner. However, few investigations have focused on effector identification and function. To elucidate effectors involved in modulation of plant processes of the polyphagous green peach aphid (GPA), *Myzus persicae*, we sequenced the ~400 Mb genome of GPA and utilized comparative RNA-seq and functional genomics approaches. Expression of several GPA candidate effector genes in *Arabidopsis thaliana* promoted colonization of GPA, while silencing of these effector genes in GPA by plant-mediated RNA interference (RNAi) reduced GPA colonization. Intriguingly, orthologs of these effectors of the legume-specialist pea aphid (PA), *Acyrthosiphon pisum*, do not promote GPA colonization on Arabidopsis consistent with Arabidopsis being a host for GPA but not for PA. We found that aphid effectors are fast evolving and that sites under positive selection promote aphid colonization on plants. Taken together, these results provide evidence that aphid effectors are under positive selection to promote aphid colonization on specific plant species.

Cauliflower mosaic virus uses the host sensory system for instantaneous transmission by an insect vector

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Many plant and animal viruses are spread by insect vectors. Cauliflower mosaic virus (CaMV) is aphid-transmitted, with the virus being taken up from specialized transmission bodies (TB) formed within infected plant cells. However, the precise mechanism of TB mediated virus acquisition by aphids is unknown. We have shown that TBs react instantly to the presence of the vector by ultra-rapid and reversible redistribution of their key components onto microtubules throughout the cell. Enhancing or inhibiting the TB reaction pharmacologically or by using virus mutants led to enhanced or inhibited transmission, respectively, confirming the requirement of this TB phenomenon for efficient virus-acquisition. Beyond that our results reveal a fascinating, and hitherto unforeseen mechanism whereby CaMV shares the host's perception of the aphid, translating it into an independent response. The unattended capability of viruses to react, via the host, to the outside world opens new research horizons, i.e. investigating the impact of “perceptive behaviors” on other steps of the infection cycle. We try to understand which mechanisms and signalization pathway are involved in this phenomenon.

Quantitative resistance loci reduce the breakdown frequency of a major resistance gene. A relevant way for durable resistance breeding.

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The combination of major resistance genes with quantitative resistance factors is hypothesized as a promising breeding strategy to preserve the durability of resistant cultivars. In three pathosystems, experimental data demonstrate that the durability of a major resistance gene depends on the plant genetic background but the genetic factors involved are still unknown.

Using the pepper (*Capsicum annuum*)/*Potato virus Y* (PVY) pathosystem,we aimed to identify genetic factors directly involved in *pvr2*^{3} resistance breakdown frequency and to compare them with genetic factors affecting quantitative resistance. For QTL mapping experiments, 156 doubled haploid lines carrying the*pvr2*^{3} resistance allele were tested for *pvr2*^{3} resistance breakdown frequency, virus accumulation and symptoms intensity.

Four loci including additive QTLs and epistatic interactions explained together 70% of the variance of *pvr2*^{3} breakdown frequency. Comparative mapping of the different traits showed that three of the four QTLs controlling the breakdown frequency of the *pvr2*^{3} allele are also involved in quantitative resistance, indicating that QTLs for quantitative resistance have a pleiotropic effect on the durability of the major resistance gene.

This study provides the first mapping of QTLs directly affecting resistance durability and opens the way for sustainable resistance breeding.

Keywords: resistance breakdown, eIF4E, potyvirus, QTL analysis, durability

Post-transcriptional gene silencing suppression study of *Beet soil-borne mosaic virus*: characterization of p14 and production of chimeric isolates of Benyviruses

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Beet soil-borne mosaic virus (BSBMV) belongs to the *Benyvirus* genus, together with *Beet necrotic yellow vein virus* (BNYVV). Both viruses possess a multipartite genome formed by four ssRNAs(+). BSBMV and BNYVV are closely related since they possess the same host range, vector and genome organization. Recent studies demonstrated a possible amplification and transmission of BSBMV RNAs by BNYVV. In the United States of America, both benyviruses are frequently present in the same cultivated field, infecting the same plant but no chimeric forms have been described from field isolates so far.
Chenopodium quinoa infection has been carried out using *in vitro* infectious transcripts of both BNYVV and BSBMV RNA-1 and -2 and the behavior of BSBMV/BNYVV combinations and wild type isolates has been compared. In parallel, the properties of the BSBMV VSR, a cysteine-rich protein (CRP) of 14 kDa expressed by RNA-2, have been investigated and compared to the BNYVV p14 RNA silencing suppressor. P14 has a zinc-finger domain able to bind nucleic acids and agroinfection of *Nicotiana benthamiana* plants demonstrated that p14 is able to suppress the PTGS downstream of the Dicer proteins, without interfering with the transitivity. Moreover, both p14 are localized in the nucleolus, forms homodimers and binds the "coremin" sequence, a stretch of 20 nucleotides present in the RNA-3 of Benyviruses and necessary for the systemic spread of viruses in the plant.
Experiments performed to investigate relationships between BSBMV/BNYVV p14s and VSR activity, "coremin" sequence, long distance movement and absence of natural chimeras of Benyviruses will be presented.

Pathology and viral metagenomics, a recent history

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Human, animal and plant viral pathologies have greatly benefited from recent metagenomics development. Viral metagenomics is a culture-independent approach used to investigate the complete viral genetic populations of a sample. The last decade, metagenomics concepts and techniques that were first used by ecologists, progressively spread into the scientific field of viral pathology. The sample, which has been a fraction of ecosystems for ecologists, became for pathologists organisms that host millions of microbes and viruses. This new approach, providing without *a priori* high-resolution qualitative and quantitative data on the viral diversity, is now revolutionizing the way pathologists decipher viral diseases. This review describes the very last improvements of the high throughput next generation sequencing methods and discusses the applications of viral metagenomics in viral pathology, including discovery of novel viruses, viral surveillance and diagnostic, large-scale molecular epidemiology, and viral evolution.

Original mutational pathways as an alternative to high genetic constraints: Involvement of the ORF2a/ORF2b overlapping region of the *Rice yellow mottle virus* in the resistance-breakdown of an eIF(iso)4G-mediated resistance

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Rice yellow mottle virus (RYMV) reaches high virus content in plants [1], evolves rapidly [2], and is able to overcome the high resistance of rice mediated by the gene *rymv1* [3-5]. Nevertheless, a strong genetic constraint identified in the RYMV genome highly modulates its ability to overcome the high resistance of rice [5].

The resistance alleles *rymv1-2* and *rymv1-3* have been characterized by a single substitution or a short deletion in the central domain of eIF(iso)4G1, respectively [6, 7]. The resistance-breaking (RB) process involved systematically mutations in the central domain of VPg (Viral Protein genome linked). The VPg/eIF(iso)4G1 direct interaction has been demonstrated to correlate with the RYMV/rice infection and the resistance-breaking process. Comparison of the RB processes of *rymv1–2* and *rymv1-3* showed similarities in the mode of adaptation but revealed converse virulence specificity of the isolates. The resistance-breaking ability was related with the polymorphism glutamic acid (E) / threonine (T) at codon 49 of the VPg [4] [1, 5]. Only virus isolates with a T49 broke *rymv1–3* resistance, those with E49 did not. Conversely, the T49 was demonstrated to be the major constraint to overcome the *rymv1-2* allele. The direct influence of the E/T polymorphism at position 49 in the *rymv1-2* RB ability was demonstrated with the WT isolate CIa (T49) and the artificial mutant CIa49E [8]. However, during this last experiment, the resistance-breaking phenotype was not systematically associated with mutations in the VPg.

In this study, a new coding region involved in the overcoming of the high resistance of rice was identified. Surprisingly, the RB mutations emerged in the most conserved region of the RYMV genome, which is corresponds to ORF2a/ORF2b overlapping region. The role of these mutations in the resistance-breaking ability was validated by site-directed mutagenesis of the infectious clone of CIa isolate (T49). The viral accumulation of these RB genotypes in resistant and susceptible hosts was compared with genotypes carrying RB mutations in the VPg. Finally, the functional role of the mutations and the domain involved in the *rymv1-2* resistance-breaking was discussed. Altogether, this study showed for the first time the implication of overlapping regions in the adaptation toward host resistance and demonstrated that, in spite of high restriction by genetic constraints, the non-adapted viral genotypes could adopt original mutational pathways to efficiently overcome strong selective pressure.

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Plant virus ecology: challenges and opportunities

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As a discipline, plant virus ecology examines the influence of plant viruses and their vectors on managed and natural ecosystems, as well as the reciprocal influence of ecosystem properties on the distribution and evolution of viruses and vectors. Building on the achievements of virology and epidemiology, plant virus ecology offers opportunities to examine the full range of virus-host interactions—from negative to positive—within the complexities of natural ecosystems and agricultural-wildland interfaces. Key issues include the nature of virus interactions with perennial plants, which have been underrepresented in virological studies, and the roles of ecological diversity in driving virus dispersal and evolution. Recent efforts in the USA to develop perennial bioenergy crops from native species offer useful insight about the effects of human selection on host growth/defense trade-offs, as well as the influence of ecological diversity on vector and virus pressure in different vegetation types.

Sustainable plant resistance management in agricultural landscapes

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The deployment of virus resistant crops often leads to the emergence of resistance-breaking (RB) pathogens that suppress the yield benefit provided by the resistance. The theoretical model-based analyses presented here are designed to provide guidelines for farmers aiming altogether to optimise the deployment of a resistant cultivar in a landscape over several years. We consider management strategies willing either to minimise the overall yield losses due to the virus (economical strategy) or to keep the frequency of the RB virus in the reservoir hosts under a preset threshold (patrimonial strategy, preserving resistance durability).

Assuming a gene-for-gene type of interaction, virus epidemics are modelled in a landscape composed of a mosaic of resistant and susceptible fields, subjected to seasonality, and of a reservoir hosting viruses year round. The model links the genetic and the epidemiological processes shaping at nested scales the demo-genetic dynamics of viruses. Seasonality leads us to use a semi-discrete model, *i.e.* a hybrid dynamical system that undergoes continuous dynamics most of the time (describing the in-season epidemic dynamics in fields) and that experiences discrete dynamics at some time instants (mimicking pathogen overwintering in reservoirs).

We explored how time-constant optimal cropping ratio (i.e. the proportion of resistant cultivar deployed in a given landscape) defined according either to economical or patrimonial strategiesdepends on the choice of the resistant cultivar and on the epidemiological context. The epidemiological context is defined by the combination of a landscape structure (*i.e.* connectivity between the fields and the reservoir hosts) and an epidemic intensity (*i.e.*mean proportion of plants infected during a seasonin the absence of the resistant cultivar).

Analyses indicated that the choice of the resistance gene (characterized bythe equilibrium frequency of the RB virus at mutation-selection equilibrium in a susceptible plant) is a major determinant of the optimal cropping ratio for both strategies.Epidemiological contextsare also important. For some of them,both strategies have close economical efficiencies(*i.e.* they provide close yield benefits), implying that patrimonial and economical strategies are compatible. For others, the patrimonial strategyhasweak economical efficiency(*i.e.* lower yield benefits compared to those obtained with the economical strategy), meaning that the two strategies are incompatible in such epidemiological contexts.

Such an incompatibility can be lessened by using time-varying management strategies where the proportion of resistant fields in the landscape can change. We show that such strategies can comply with patrimonial objectives while substantially restoring the economical efficiency of time-constant strategies. They can even over-perform the time constant strategiesin landscapes characterized by high between fields connectivity.

Fabre, F., Rousseau, E., Mailleret, L.& Moury, B. 2012. *New Phytologist*, 193, 1064-1075.

Keywords: Deployment strategy; Durable resistance; Evolutionary epidemiology; Gene-for-gene model; Landscape epidemiology.

Mechanisms for disease dilution: effect of plant species community composition and differences in host competence for virus and vector reproduction

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The loss of biodiversity in communities can degrade ecosystem services including productivity, stability, resilience and disease regulation. While high species richness has been often correlated with reduced infection and transmission risk, multiple interacting mechanisms could underlie this dilution effect. The effects of biodiversity on disease prevalence can depend on both the species loss order in community disassembly and the traits of species left in low richness communities. If host communities decay to a predictable subset of hosts in low richness communities, infection risk by generalist, vector-borne parasites may be elevated because the remaining hosts are highly susceptible as well as highly competent to transmit parasites to vectors and/or to support vector reproduction.

Using grassland communities along the US West Coast as our model system, we measured the distribution and local abundance (% cover) of grass species among eleven sites across a latitudinal gradient of 15 degrees and 2000 kilometers. At each site, we monitored the prevalence of a group of generalists aphid-vectored pathogens, the *Barley* and *Cereal yellow dwarf* viruses (B/CYDVs), usingtwo widespread grass species as sentinel hosts (*Elymus glaucus* and *Bromus hordeaceus*). We assessed in controlled conditions the ability of twenty common grass hosts to support i) viral infection after inoculation of a BYDV-PAV isolate, ii) secondary infection of new hosts and iii) reproduction of the aphid vector *Rhopalosiphum padi*. Finally, using a reduced set of six grass hosts, we quantified the reproductive rate of three aphid vectors of B/CYDVs (*R. padi*, *R. maidis* and *Sitobion avenae*), in field and/or lab experimental settings.

Using these data, we show that the change in plant community composition due to biodiversity loss is associated with an altered overall competence of host species assemblages to sustain the reproduction and transmission of infectious agents, as well as the reproduction of their aphid vectors.

Key words: disease dilution effect, vector-borne pathogen, grasslands, B/CYDV, nestedness

Involvement of a calcineurin B-like protein-interacting protein kinase (CIPK) in *Turnip yellows virus* cycle

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Turnip yellows virus is a polerovirus (*Luteoviridae* family) which cycle is restricted to phloem cells namely the companion cells, the phloem parenchyma cells and the enucleate sieve elements. In order to identify cellular partners of TuYV, a companion cell-specific cDNA library from *Arabidopsis thaliana* was constructed from FACS-sorted fluorescent protoplasts and screened by yeast two-hybrid system (Y2H) using viral protein domains as baits.

Among the TuYV putative partners, we found a CIPK (calcineurin B-like protein-interacting protein kinase) interacting with the non-structural C-terminal part of the readthrough protein of TuYV involved in virus movement. The CIPK identified belongs to a multigenic family in *A. thaliana* comprising 26 different CIPK that can interact with 10 CBL isoforms to decode calcium signals. We confirmed by Y2H that the identified CIPK does interact with different CBLs (calcineurin B-like proteins). When co-expressed in *Nicotiana benthamiana* both proteins co-localized in different sub-cellular compartments (cytoplasm, nucleus, nucleolus and filaments). Preliminary assays confirmed by FLIM the *in vivo* interaction between both partners in the cytoplasm. Experiments are underway to assess potential interactions in the other sub-cellular organelles. In addition, we showed that expression of the CIPK mRNA is transiently up-regulated early during infection and that transient expression of the kinase in *N. benthamiana* induces an increase in virus accumulation. These results suggest that the CIP kinase may be involved in TuYV cycle but the mechanism by which the kinase is acting is still unknown and needs further investigations.

Nucleocytoplasmic shuttling of two proteins encoded by *Grapevine fanleaf virus*

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Grapevine fanleaf virus (GFLV) is a bipartite positive-sense RNA virus that replicates on endoplasmic reticulum membranes (Ritzenthaler et al., 2002). Each of the two viral RNAs encodes a single polyprotein. Polyprotein P1, encoded by RNA 1, is processed by the viral proteinase into five proteins of which protein 1A is of unknown function. Polyprotein P2, encoded by RNA 2, gives rise to protein 2A involved in RNA 2 replication, protein $2B^{MP}$ necessary for tubule-guided movement and the capsid protein $2C^{CP}$.

To get insight into the subcellular localization and the function of proteins 1A and 2A, we tagged them with fluorescent proteins. Fusions of protein 1A at its N-terminus as well as fusion of protein 2A at its C-terminus are compatible with local and systemic infection on *Chenopodium quinoa*, *Nicotiana benthamiana* and *Arabidopsis thaliana*, thus indicating that functionality of 1A and 2A is not hindered in this context. This important result allowed us to study the subcellular localization and show the interaction of both proteins using confocal microscopy and Fluorescence Lifetime Imaging (FLIM). Upon ectopic expression, fluorescent protein-tagged 1A exclusively accumulates in the nucleus, whereas fluorescent protein-tagged 2A is exclusively cytoplasmic. When transiently coexpressed as well as in viral context, both recombinant proteins colocalize in both cellular compartments, further demonstrating their interaction.

In order to get a better understanding of the intrinsic properties of each protein as well as the interplay between them, a collection of deletion mutants of 1A and 2A proteins has been generated. These mutants have been studied by transient expression in fusion to fluorescent proteins. This resulted in the identification of two domains in 2A involved in its particular subcellular localization upon ectopic expression and two interaction domains, each of them being sufficient to interact with 1A. One particular mutant of 2A also appeared to mimic 1A subcellular localization even when expressed alone. This mutant also colocalizes and possibly interacts with the structural protein of Cajal bodies, coilin.

Together these results suggest a nucleocytoplasmic shuttling of 1A and 2A. Possible functions for this interplay will be discussed.

Ritzenthaler, C., Laporte, C., Gaire, F., Dunoyer, P., Schmitt, C., Duval, S., Piequet, A., Loudes, A.M., Rohfritsch, O., Stussi-Garaud, C., and Pfeiffer, P. (2002). Grapevine fanleaf virus replication occurs on endoplasmic reticulum-derived membranes. Journal of Virology 76: 8808–8819.

Interests of Next Generation Sequencing, gene synthesis and codon

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The talk will be divided into two parts:
- Gene synthesis and codon optimization: expression of heterologous genes in plant cells.
Following a short introduction on the main applications of synthetic genes, the talk will focus on sequence optimization and codon usage adaptation, demonstrating how these latter can be useful, or even necessary, for the expression of genes in heterologous organisms, notably in plant cells. The main optimization algorithms will be reviewed, describing their respective advantages and drawbacks.
- Use of high-throughput sequencing in plant genomics.
The second part of the talk will be dedicated to high-throughput sequencing technologies in plant genomics, notably plant genome sequencing via multi-libraries approaches (shotgun + Long Jumping Distance).

High-throughput sequencing for the diagnosis of viral diseases: example of ornamental plants

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The production of ornamental plants (cut flowers, plants in pots or for gardens) concerns a far greater number of plant species than those used in agriculture, and the value of ornamental plants imported into France is six times that the value of the corresponding exports. Ornamentals can harbor viruses that can infect and be disseminated in more valuable species, in particular vegetable and fruit crops, and thus present a potential source of damaging new viral diseases. The currently used serological and molecular tests are effective for screening for known viruses, but considering the ever increasing number of ornamental species and the scope of their trade worldwide, it is critical to develop means to also detect plant viruses that are not current targets of sanitary surveillance in a manner as exhaustive as possible. Further, viruses under quarantine restriction are of particular concern.

Two techniques of high-throughput sequencing that can detect unknown viruses have been used to characterize viruses of cultivated plants and also of plants growing in natural environments. One strategy is based on 454 pyrosequencing of cDNAs synthesized from viral double-stranded RNAs (dsRNAs), and the other is based on Illumina HiSeq sequencing of small RNAs (siRNAs) of 21-24 nt. In the first phase of a project of high-throughput sequencing of viruses in ornamental plants of unknown sanitary condition, we have used both sequencing strategies in a model study of petunia plants infected either singly or in mixed infection by *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY) and *Tobacco mosaic virus* (TMV).

We present here the results of this pilot study, in which these two sequencing strategies have been compared according to several criteria: 1) number, size and identity of contigs, 2) quality of the sequences obtained compared to the reference sequences of the viruses inoculated, 3) completeness of coverage of the viral genomes, 4) sensitivity of detection of minor viral components, and 5) the presence of contaminating sequences. Globally, the sequences generated by Illumina sequencing of siRNAs were of better quality; in fact they were nearly always exactly identical to the reference sequences. Both techniques allowed detection of viral genomes that were not detectable by RT-PCR, but Illumina sequencing of siRNAs was even more sensitive than pyrosequencing. Further, siRNA sequencing detected the presence of a viral genome integrated in the petunia genome. However, the sensitivity of both techniques revealed contaminating viral sequences that probably arose by contamination between samples. Neither strategy allowed assembly of complete viral genomes, and in particular the 5'- and 3'-terminal sequences were not present in the contigs, except in some cases when a full-length reference sequence was provided.

In spite of its high cost, Illumina sequencing of siRNAs seems to be superior to pyrosequencing of dsRNAs according to several criteria. We are currently collecting samples of ornamental plants from producers in order to determine by siRNA sequencing to what extent known and unknown viruses are present. This type of study should contribute to our understanding of the potential importance of ornamental plants as sources of emerging viruses in Europe, and more globally of the respective contributions of crops, wild plants and ornamentals to plant virus epidemiology.

Potato virus Y infecting tobacco crops adapts rapidly to the recessive resistance gene *va*: evolutionary pathways to breakdown the resistance

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Since the deployment of tobacco cultivars carrying one of the three alleles (0, 1 and 2) of the PVY recessive resistance gene *va*, symptoms associated to PVY infection have been reported. In 2007, a field survey was performed in different French tobacco growing areas to determine how the selective pressure imposed by the *va* alleles drives the evolution of PVY populations. As first results highlighted both a high frequency of virulent isolates in natural PVY populations and a heterogeneous distribution of pathotypes in susceptible and resistant tobacco cultivars, we focused our researches on the analysis of evolutionary process(es) leading to the emergence of PVY populations adapted to *va*. Results obtained under controlled conditions suggested that the ability of PVY to breakdown the resistance encoded by the va^2 allele depends to mutations in the VPg protein.

An analysis of VPg sequences of PVY isolates collected in susceptible and resistant cultivars and a comparative study between these VPg sequences and *va* pathotypes of some corresponding PVY isolates revealed a rapid evolution of PVY populations to gain virulence toward *va* alleles. Wild-type isolates with known VPg sequences and mutated versions of PVY infectious cDNA clones were tested for their virulence against *va* alleles. This procedure allowed *i)* to validate the VPg protein as the PVY virulence factor corresponding to the *va* resistance gene, *ii)* to highlight that virulence gain in PVY^N and PVY^O occurs rapidly and preferentially by amino acid substitutions at position 105 in the VPg protein as suggested by the detection analysis for selection signature in PVY VPg, and *iii)* to show that the 101G substitution in the PVY^C VPg is responsible of cross-virulence toward two resistances sources. Moreover, it appears that the evolutionary pathway of PVY adaptation to *va* depends on both virus and host genetic backgrounds.

Finally, the competitiveness of the VPg mutants clones, derived from the avirulent infectious PVY^N-605 clone, was tested to determine possible fitness costs due to the acquisition of mutations at position 105 in the VPg. Such type of data should help us to to better estimate the durability of the *va* resistance.

Keywords: *Potato virus Y*, Nicotianae, resistance, virulence gain, mutations, competitiveness.

Ubi-or-not-Ubi: importance of reversible ubiquitylation events in the control of viral replication

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Selective protein degradation via the ubiquitin-proteasome system (UPS) plays an essential role in many major cellular processes, including host-virus interactions (1). We previously reported that the tightly regulated viral RNA-dependent RNA polymerase (RdRp) of Turnip yellow mosaic virus (TYMV) is degraded by the UPS in plant cells, a process that affects viral infectivity (2). Recently, we have shown that the TYMV 98K replication protein can counteract this degradation process thanks to its proteinase domain. *In vitro* assays revealed that the recombinant proteinase domain is a functional deubiquitylating enzyme (DUB), as is the 98K produced during viral infection. Importantly, we also demonstrate that 98K mediates *in vivo* deubiquitylation of TYMV RdRp – its binding partner within replication complexes – leading to its stabilization. Finally, we show that this DUB activity contributes to viral infectivity in plant cells (3). The identification of viral RdRp as a specific substrate of the viral DUB enzyme thus reveals the intricate interplay between ubiquitylation, deubiquitylation and the interaction between viral proteins in controlling levels of RdRp and viral infectivity.

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Diverse translational control mechanisms of plant viruses

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All viruses are parasites of their host's translational machinery. We investigate translation mechanisms used by viruses that have no 5' cap on their RNAs. Normally, the 5' cap must be recognized by the dimeric translation initiation complex eIF4F (eIF4E+eIF4G) to initiate translation. Plant viruses in the Luteovirus genus and the Tombusviridae family contain cap-independent translation elements (CITEs) in their 3' untranslated region that facilitate ribosome binding and entry at the 5' end of the viral RNA. The unrelated Barley yellow dwarf virus-like (BTE) and Panicum mosaic virus-like (PTE) cap-independent translation elements bind and require eIF4G and eIF4E, respectively in novels ways that obviate the need for a 5' cap structure. To understand this high affinity binding, we used chemical probing todetermine the structures of these elements and the initiation factor binding sites. It is likely that viruses have evolved different RNA structures that bind different surfaces of the eIF4F complex to recruit the ribosome and bypass host regulation. Viruses also employ non-canonical translation elongation mechanisms such as ribosomal frameshifting. I will report our recent observations on a novel frameshift event on potyvirus RNAs necessary for translationof the small open reading frame, pipo. In summary, this research reveals how plant viral RNAs "play" with the host's translational machinery using previously unknown types of interactions to generate viral proteins at the levels and timing necessary to sustain virus replication.

Sha3, a host factor indispensable for *Plum pox virus* long distance movement in *Arabidopsis thaliana*

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Sharka is the most devastating disease affecting stone fruit production worldwide. It is caused by a potyvirus, the *Plum Pox Virus* (PPV). Sources of resistance to PPV in its natural hosts are limited; therefore, we are using the model plant *Arabidopsis thaliana* to identify and characterize various resistance mechanisms that could potentially be transferred back to crop species.

Our goal is to identify PPV susceptibility genes. For this purpose, a disease resistance screening was conducted in two core-collections of 24 and 20 Arabidopsis accessions. Genetic analysis and linkage mapping in biparental and multiparental populations confirmed the existence of recessive resistance genes that do not co-localize with already known susceptibility factors. By combining results of linkage mapping in F2 populations and recombinant inbred lines with association mapping among 147 worldwide accessions, we identified a locus containing a cluster of 13 genes of which 7 encode TRAF-like proteins. This major locus, named *sha3* for sharka resistance, is located at the bottom of linkage group 3 and encompasses the *RTM3* gene (Pagny et al., 2012). By using plasmid cloned infectious viruses tagged with GFP and GUS, we showed that SHA3 is essential for PPV long distance movement leading to viral systemic infection through the vascular bundles. In this communication, we present evidence for a major host factor involved in long distance movement of potyvirus. Mutation(s) at this locus that impair(s) systemic infection of the host by the virus are not rare; they are encountered in a significant number of Arabidopsis accessions originating from different geographical niches. Since *rtm3* loss-of-function mutants display susceptibility to PPV and RTM3 confers a dominant form of resistance to PPV, we are not considering *RTM3* as a candidate gene for *sha3*.

We are currently screening a population (> 1,500 individuals) issued from backcrosses between PPV resistant and susceptible recombinant inbred lines in order to refine and confirm the size of the *sha3* locus. Subsequently, we will validate candidate genes present in the interval by PPV infecting Arabidopsis knock-out mutants of each candidate gene then by scoring resistance.

In parallel, we are performing grafting experiments to describe the sha3 phenotype and identify at the cellular level the step in the viral infectious cycle that is impaired in the Arabidopsis PPV resistant accessions.

Pagny, G. et al. (2012). Family-based linkage and association mapping reveals novel genes affecting Plum pox virus infection in Arabidopsis thaliana. New Phytol.196(3):873-86

Host-directed selection of *Cucumber mosaic virus* (CMV) reassortants in plants co-infected with different isolates of CMV subgroups IA and IB

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Reassortment is thought to constitute an advantage for multipartite viruses, facilitating their adaptation to varying environmental conditions through exchange of genomic segments in plants co-infected by different isolates. However, for CMV, natural reassortants were rarely found in the open field until recently, when an in-depth survey of CMV in Tunisian pepper (*Capsicum annuum*) crops revealed that most of the isolates were reassortants between subgroups IA and IB (Ben Tamarzizt *et al*., submitted). These isolates were composed of an RNA1 from subgroup IB, an RNA2 from subgroup IA and an RNA3 from either subgroup. In order to evaluate the role of pepper in the selection of reassortants, we analysed the progeny virus during serial passages in greenhouse-grown pepper plants co-infected by isolates of both subgroups. The results showed a strong and rapid (first or second passage) selection of subgroup IB RNAs 1 and 3. In contrast, the RNA2 of both subgroups was present even after four passages, after which subgroup IB RNA2 progressively supplanted its IA counterpart. Genetically diverse subgroup IB strains collected on different hosts were then tested. Similar results were obtained, with strong and rapid selection of RNAs1 and 3 and a slower selection of RNA2, except that, for RNA1, which RNA was selected differed according to the IB strain. When infections of melon and tobacco were tested, results indicated a similar tendency: quicker selection of RNAs 1 and 3 than RNA2, but which RNAs were selected differed also according to the host species. In all cases, the progeny identified in the systemic leaves of plants infected with a subgroup IA and a subgroup IB strain corresponded to either the IB strain or reassortants between the two strains; the complete IA strain was not recovered. These results showed that CMV is prone to reassortment under experimental conditions, and are coherent with the prevalence of reassortants in the field in Tunisia, although the reassortants we observed under experimental conditions were not strictly the same as those in the Tunisian pepper fields. Perhaps the strong bottleneck exerted on virus populations during natural transmission by aphids could account for a selective dissemination of reassortant populations. However, these results also illustrate a rather complicated situation, since the subgrouping of each genomic segment of the progeny virus depended not only on the strain but also on the host species. These results also raise the question of the low prevalence of reassortants in the open field elsewhere than in Tunisia. That reassortants did not emerge in the two other countries where in-depth surveys were carried out will be discussed. Finally, it would be interesting to analyse carefully the genetic features of CMV populations in countries where all subgroups are abundant, as is the case in Asia.

Cleavage of the C-terminal domain of the readthrough protein of *Cucurbit aphid-borne yellows virus* and involvement in systemic infection of plants

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Cucurbit aphid-borne yellows virus (CABYV) is a polerovirus (*Luteoviridae* family) restricted to phloem tissue where it replicates in nucleated cells and translocates over long distances through sieve elements. Polerovirus capsid is composed of the major coat protein (CP) and of a minor component referred to as the readthrough (RT) protein because it is produced by a bypass of the CP stop codon. Two forms of the RT protein of poleroviruses were reported: a full-length protein of 74 kDa detected in infected plants and a truncated form of about 55 kDa (RT*) incorporated into virions. In CABYV-infected plants, the RT protein was detected in total protein extracts and in sap collected from infected cucumbers, whereas the RT* being was only observed in sap. In order to better understand the specific roles of both proteins in viral life cycle, we studied the *in vivo* RT protein processing and its consequences on systemic movement of CABYV mutants. Using a collection of point mutations introduced in the central domain of the CABYV RT protein, we approached the site of the RT processing and proposed that this process is affected by the secondary structure around the cleavage site. We also reported for the first time the generation of a polerovirus mutant able to synthesize only the RT* protein and to incorporate it into the particle. This mutant was unable to move over long-distance. Conversely another mutant producing a full-length RT protein impaired in correct processing and incorporating a shorter version of the RT* protein showed very weak systemic infection. These data are strongly in favor of a role of both RT proteins in efficient CABYV movement. An inefficient virus transport was still maintained in the absence of RT proteins suggesting an RT-independent movement pathway. Based on these results, we propose a model for CABYV long-distance transport in which the complete RT protein, or its C-terminal part, acts *in trans* on wild-type virions to promote their efficient long-distance transport.

Key Mutations in the Cylindrical Inclusion of *Lettuce mosaic virus* (LMV) are involved in the breakdown of eIF4E-mediated resistance

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The *Potyvirus* genus is the largest genus of plant viruses, including *Lettuce mosaic virus* (LMV) of worldwide distribution in lettuce crops. To date, the major sources of resistance to potyviruses are components of the eukaryotic translation initiation complex. In particular, recessive allelic lettuce genes *mo1*[1] and *mo1*[2], used to protect lettuce crops against LMV, were shown to correspond to mutant alleles of the gene encoding the translation initiation factor eIF4E [1]. Previous work showed that the LMV resistance-breaking determinants mapped not only to the VPg encoding region (the main potyvirus virulence determinant) but also to the C-terminal region of the CI (Cylindrical Inclusion helicase), providing the first example of a potyvirus CI acting as a determinant for eIF4E-mediated resistance breaking [2]. Simultaneously, a study of the LMV natural diversity was conducted in our laboratory. The behavior of non-lettuce LMV isolates towards *mo1* genes, along with determining their CI-VPg sequence led to the identification of 4 candidate amino acids in the CI C-terminal region, potentially involved in *mo1* resistance breaking.

Mutations at these positions (*e.g.* A602V, G617S, S621T and A627T) were introduced in a LMV-0 (non resistance-breaking isolate) background, alone or in combination. The behavior of these mutants towards *mo1* genes was then analyzed. We hence demonstrated that a single substitution at position 621 in the CI is sufficient to confer the ability to overcome the resistance, while single substitutions at the other positions are not. Nevertheless, the combination of mutation 602V and 617S also confers resistance-breaking capacity. Finally, mutation at position 627, although spontaneously appearing in some experiments, does not trigger resistance-breaking in a LMV-0 background.

The potential fitness cost associated with these mutations was investigated through competition tests in susceptible plants. Mutation A602V is associated with a high competitiveness cost for the virus, which is not compensated by the addition of G617S, whereas mutations 621T or 617S seem respectively to be slightly beneficial or neutral for the virus. .

To better understand the viral cycle step impaired by the *mo1* resistance and restored by the resistance-breaking mutations, we are currently monitoring cell to cell movement of LMV-0 and its derived GFP tagged-mutants in resistant plants.

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Natural resistance of the diploid *Musa balbisiana* Pisang Klutuk Wulung (PKW) to banana streak virus is probably driven by transcriptional gene silencing

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The genome of banana (*Musa* sp.) harbours multiple integrations of *Banana streak virus* (eBSV), whereas this badnavirus does not require integration for the replication of its ds DNA genome. Some endogenous BSV sequences (eBSV), only existing in the *Musa balbisiana* genome, are infectious by releasing a functional viral genome following stresses such as those existing in in vitro culture and interspecific crosses context. The structure of these eBSV is much longer than a single BSV genome, composed of viral fragments duplicated and more or less extensively rearranged.

Wild *M. balbisiana* diploid genotypes (BB) such as Pisang Klutuk Wulung (PKW) harbour such infectious eBSV belonging to three widespread species of BSV (*Goldfinger* -BSGFV, *Imové* – BSImV and *Obino l'Ewai* - BSOLV) but are nevertheless resistant to any multiplication of BSV without any visible virus particles. In collaboration with the group headed by M. Pooggin (Basel, Switzerland), a deep sequencing of total siRNAs of PKW was performed using the Illumina ultra-high-throughput technology. We obtained for the first time, experimental evidence of virus-derived small RNA (vsRNA) from eBSOLV, eBSGFV and eBSImV by blasting sequences against the 3 BSV species genomes. vsRNA are enriched in 24-nt class thus eBSV in PKW genome are likely silenced at the transcriptional level. Interestingly, we show that hot and cold spots of vsRNA generation do not target similar viral sequences from one eBSV species to the other but are directly correlated with the structure of the integration. All together, those data seem indicate these eBSV induce a natural resistance driven by gene silencing mechanisms based on their complex molecular re-arranged structure which could lead to dsRNA formation.

Mots Clés : Banana Streak Virus, Musa sp., Silencing, Resistance, Endogenous Pararetrovirus

Genomic of a zoonotic RNA virus : the case of hepatitis E

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The hepatitis E virus (HEV) is responsible for large epidemics of viral hepatitis in many tropical and subtropical countries. In most cases, acute hepatitis, clinically similar to hepatitis A, is observed, but in 1-2% of cases, the infection leads to fatal fulminant hepatitis. This percentage can reach up to 20% in pregnant women in endemic areas. During epidemics, HEV is mainly transmitted through the consumption of contaminated water or contaminated food.

In addition to these outbreaks, HEV is also responsible for sporadic cases of hepatitis in non-endemic areas such as USA, Europe and Japan. In those cases the contamination pathways remain unknown. Unlike other hepatitis viruses, human are not the only natural host of this virus and its propagation in many animal species, especially pigs, suggests that it is a zoonotic agent. Several cases of direct transmission of HEV from animals to humans have been reported after consumption of raw or undercooked meat, infected with HEVHEV belongs to the family of Hepeviridae and is the only representative of the genus Hepevirus. It is a small non-enveloped virus of approximately 30 nm in diameter, with a capsid of icosahedral symmetry composed of a single protein. HEV capsid contains a single-stranded RNA molecule of positive polarity, that is capped and polyadenylated. There are four major genotypes of HEV (1 to 4) divided into 24 subtypes. The four genotypes of HEV have distinct geographical distributions depending on the endemicity of the disease. In endemic areas, genotypes 1 and 2 are present in humans and genotypes 3 and 4 in the animal reservoir. In areas of sporadic indigenous genotypes 3 and 4 are both responsible for acute hepatitis in humans and present in animal reservoirs.

Biology of HEV is still poorly known, firstly because of its recent discovery in 1990 and secondly, because of the absence of in vitro culture model. Very little information is available regarding the presence of a species barrier between strains of genotype 3 and 4, and, on the determinants of virulence. Variability of HEV has been studied during an inter-species transmission using high-throughput sequencing. Furthermore, genomic regions potentially involved in restricting hosts were characterized by analysis of codon usage bias.

Estimating sharka dispersal function by stochastic spatiotemporal modelling

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Plant viral diseases, and especially the ones transmitted by aerial vectors, can cause considerable yield losses. A good knowledge of the distances of spread is key to the understanding of disease dynamics. Exploratory approaches aiming at characterizing the spatiotemporal distribution of diseased plants are often used to get an insight into the distances of spread. A more powerful approach is based on stochastic spatiotemporal modelling in order to estimate the dispersal function of the disease (probability density function describing the probability for an infectious plant to infect a healthy plant at distance *d*). In this study, we implemented a method for estimating the dispersal function of the sharka disease.

Sharka is one of the most serious diseases of stone fruit trees (*Prunus* sp.). It is caused by *Plum pox virus* (PPV, genus *Potyvirus*), transmitted by at least twenty different aphid species in a non persistent manner. Due to the inefficiency of insecticides and the very rare sources of resistance against the virus available in the host species, prophylactic disease control is based on the removal of the diseased trees in the orchards. Thus, a very good knowledge of the dispersal function of sharka is crucial for building epidemiological models and optimizing the strategies of surveillance and control.

We adapted the methodology published by Gibson (1997) based on a Markov chain Monte Carlo (MCMC) algorithm in order to estimate sharka dispersal function from the maps of 157 contiguous peach orchards reporting the exact location and the sanitary status (asymptomatic/symptomatic) of each of the trees during six consecutive years. An estimation method based on the Gibbs sampling algorithm was developed taking into account the specificities of the dataset (more than two dates of observation, annual removal of diseased trees). This estimation algorithm was validated on simulated data and was proved to be more powerful and better adapted to large datasets than the one proposed by Gibson. Moreover, the influence of latency on the estimation of the dispersal function was quantified. This methodology was then used to estimate the dispersal function of the disease from a subset of the real dataset.

The methods developed in this study are generic enough to be used and adapted for the estimation of dispersal functions of any disease transmitted in a non persistent manner, and even for diseases with similar characteristics.

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Biosensors as novel tools to study *Grapevine fanleaf virus* movement and transmission

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Numerous icosahedral plant viruses including *Grapevine fanleaf virus* (GFLV) move through plasmodesmata (PDs) as entire viral particles. This phenomenon leads to profound modifications in PD architecture through the formation of so-called tubules that assemble via the polymerization of movement protein (MP) subunits encoded by the virus. The lumen of the tubules is occupied by aligned virus particles that transit from cell to cell.

PDLP (*Plasmodesmata Located Proteins*) are 30 to 35 kDa membranous proteins specifically located in PDs that are conserved among higher plants and for which 8 isoforms exist in Arabidopsis. Our recent work revealed PDLP proteins that regulate PDs permeability and are delivered to PDs via the secretory pathway and are necessary for the formation of tubules and the movement of GFLV as well as other viruses employing tubule-guided movement. PDLP seem to act as receptor proteins that interact specifically with tubule-forming MPs, thereby promoting the docking of MP subunits to PDs and the nucleation of tubules. In contrast, the absence of PDLP accumulation at PDs leads to the cytosolic retention of the MP and inhibition of virus movement. More recently, we showed that specific myosins XI are involved in the transport of PDLP to PDs and that inhibition of this transport pathway strongly inhibits tubule formation and GFLV movement. Our work represents a major advance in our understanding of GFLV virus movement and in our general knowledge of plant virus movement mechanism. However, we still don't know how virus particles are delivered intracellularly from their site of synthesis, the viral factory, to PDs. Similarly, we do not understand how they are delivered into root cells upon nematode feeding (see abstract of Kamal HLIEBIEH). To answer these questions we developed a novel tool that allows direct visualization of GFLV particles *in vivo*

Contrarily to rod shaped viruses that easily accommodate the production of viral particles consisting of fluorescent protein (FP) - coat protein (CP) fusions, icosahedral viruses are incompatible with such an approach probably due to steric hindrance that totally prevents virion formation and dissemination. As an alternative, we produced single-domain antibody fragments also named Nanobodies that specifically recognize GFLV. When fused to FP and expressed *in planta*, these so-called chromobodies act as biosensors that allow the spatio-temporal visualization of viral particles. Results illustrating this phenomenon will be presented.

Biochemical properties of the viral suppressor/activator of RNA silencing P1 encoded by the *Rice yellow mottle virus*

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Viral suppressors of RNA interference (VSRs) are remarkable multifunctional proteins that play important roles in the viral cycle as well as in the host cells. In particular, VSRs are key components of viral machineries for counteracting host innate immune responses by targeting different components of the host gene silencing pathways (*1, 2*). However the biochemical and structural properties that allow VSRs to ensure several functions remain poorly understood. We analyzed the biochemical features of the multifunctional and dual viral suppressor/activator of RNA silencing (VSR) P1 encoded by the *rice yellow mottle virus* (RYMV) (*3-4*). *In silico* analyses of P1 suggested common features with zinc finger proteins. Using proteomic-based mass spectrometry approaches and SDS-PAGE redox shift assays on recombinant P1 protein, we unambiguously demonstrated that P1 reversibly binds two zinc atoms with different strengths. A zinc-binding domain mapping using recombinant truncated P1 proteins allowed us to precisely locate the position of zinc atoms in P1. We next found that P1 oxidation by H2O2 lead to disulfide bond formation along with zinc release and oligomerization. Zinc release and subsequent conformational changes occurred in an H2O2 dose-dependent manner, and was also observed using truncated P1 or other oxidant compounds. More strikingly, zinc release in P1 was found to be reversible, a redox-dependent zinc binding/release change only reported for a limiting number of Zinc fingers and never report for VSR. Spectroscopy and SDS-PAGE redox shift assay on truncated P1 proteins indicated that the two zinc binding domains exhibit differential sensitivity towards oxidant molecules, consistent with structural differences suggested by *in silico* analyses. Finally, we found that P1 undergoes complex oligomerization under oxidative conditions in vitro and identified key determinants in responsible for conformational change in P1structure. Consistent with our *in vitro* data, complex oxidized oligomeric forms of P1 evolving throughout infection by RYMV were also detected in rice tissues**.** Our results provide a first link between P1's redox-dependent flexibility and complex P1 state in planta.

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The chloroplast phosphoglycerate kinase is involved in *rwm1*-mediated resistance to *Watermelon mosaic potyvirus* in Arabidopsis

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With the aim to characterize plant factors required for the infectious cycle of potyviruses, the *Watermelon mosaic virus* (WMV)-*Arabidopsis thaliana* pathosystem was developed. The exploration of the *Arabidopsis* natural diversity led to the identification of a complete resistance to WMV in the *Cape Verde islands* (Cvi-0) accession. Genetic analysis demonstrated that WMV resistance in Cvi-0 is controlled by a single recessive gene designated *rwm1* (*resistance to watermelon mosaic virus 1*). The *rwm1*-mediated resistance acts at an early stage of the infectious cycle, by impairing viral RNA accumulation in initially infected tissues. Using a positional cloning strategy, *rwm1* was mapped to a 114-kb region of *Arabidopsis* chromosome 1 that contains 30 annotated genes. Based on the literature, the chloroplast phosphoglycerate kinase (cPGK) was selected as the candidate gene for *rwm1*. Sequence analysis allowed the identification of a single amino acid substitution localized in the N-terminal region of the cPGK protein that correlates with resistance to WMV. Virus-induced silencing of *cPGK* in *Nicotiana benthamiana* induced a significant reduction in WMV RNA and protein accumulation. Functional validation in *Arabidopsis* using stable genetic transformation is currently underway. These results supporting a role for cPGK in *rwm1*-mediated resistance will be discussed. The identification of this new host factor required for the potyvirus infectious cycle opens a challenging research area to provide novel insights on plant resistance mechanisms and opportunities to promote the genetic control of plant virus diseases.

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A first comparison of siRNA and dsRNA next-generation sequencing for the detection and characterization of RNA viruses and viroids in stone fruit trees

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Next-generation sequencing (NGS) technologies offer a novel and attractive avenue for the characterization, without any prior knowledge, of all viruses present in a plant sample (viral indexing). Schematically, this strategy is based on the massive generation of sequence data from the sample under study followed by the bioinformatics screening of the sequence data to recover viral sequences. Several nucleic acids (NA) populations can be targeted by these efforts, from total or messenger RNAs to NA populations that should be enriched in viral sequences such as small interfering RNAs (siRNAs) or double stranded RNAs (dsRNAs). In particular, siRNAs produced from viral genomes by the plant post-transcriptional gene silencing antiviral machinery can be sequenced with an extremely high throughput using the Illumina technology. Analysis of such siRNAs populations has been reported to allow the efficient detection of plant viruses in samples of unknown sanitary status (Kreuse *et al.*, 2009). Using multiply infected stone fruit tree (*Prunus*) samples, we have compared this strategy with the 454 pyrosequencing of cDNAs prepared from purified dsRNAs for their efficiency in viral indexing. While high numbers of siRNAs could be readily sequenced, only a low proportion of siRNAs of viral origin could be identified. On the contrary, high amounts of siRNAs derived from the genome of *Peach latent mosaic viroid* (PLMVd) were detected, allowing the reconstruction of the whole genome of the isolate present. Various strategies were used to analyse the virus-derived siRNAs, allowing an effective assessment of the sanitary status of the *Prunus* samples analysed but showing only a partial ability to reconstruct viral genomes without a priori information. In contrast, dsRNA sequencing did not allow the efficient detection of PLMVd but allowed a more efficient reconstruction of RNA viruses genomes and, ultimately, the easier detection of previously uncharacterized agents absent from databases. Using a multiplexing strategy, the cost of this approach was evaluated at a few hundreds of euros per sample, which already compares favourably with the cost of the current reference method, full biological indexing by grafting on woody indicator hosts.

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Intracellular and intercellular movement of *Turnip mosaic virus*-induced vesicles

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Replication by plant (+) RNA virusesinduces the formation in the infectedcell of elaboratemembranous, organelle-like, platformsthatsustain viral RNA synthesis and cell-to-cellmovement. Confocal and electronmicroscopy images show thatTurnipmosaic virus (TuMV) infection leads to significantrearrangements of the earlysecretorypathway. Infection isassociatedwith the formation of at least two distinct types of sub-cellular compartmentsinduced by the viral protein $6K_2$: aperinuclearglobular structure and cortical endoplasmicreticulum (ER)-associated and motile vesicular structures. The perinuclearglobular structure contains ER, Golgi, and COPII cotamers, alongwith viral RNA and viral replicationproteins. The motile vesicular structures are derivedfrom the globular structure and move alongtransvacuolar ER tubules toward the plasma membrane to becomeassociatedwithplasmodesmata. Once atplasmodesmata, the vesiclescan move over into the neighboringcells. $6K_2$isasmall membrane associatedprotein and mutations show that the N-terminal soluble tail has a targeting signal thatisrequired for cell-to-cell infection. We propose that the TuMVperinuclearglobular structure islargely for viral replication and the mobile vesiclesprovide the vehicle for moving viral RNA fromcell-to-cell.

Sequential integrations of badnaviruses into the *M. acuminata* and *M. balbisiana* genomes

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Banana streak virus (BSV) is a double stranded DNA pararetrovirus belonging to the genus *Badnavirus* and triggering necrotic mosaic lesions on banana plants. BSV exhibits a higher biodiversity than other badnaviruses as a consequence of two phenomena: the epidemic process of the disease through contamination of plant to plant by vector and the release of infectious viral genomes by the banana genome harboring endogenous sequences. BSV sequences described so far are spread among the three groups of the badnavirus diversity. BSV-like sequences belonging to group 2 seem to be all integrated into the banana genome since no infectious episomal particles have been identified so far. We describe here the characterization of such integrations in thirty-four plant samples belonging to different families of the Zingiberale order with a banana sampling representative of the *Musa* diversity. We have firstly looked for group 2 sequences using PCR with 7 sets of primers which are specific of the 7 BSV-like species identified so far in this group. We then used Southern blot approach with viral probes corresponding to each BSV-like species. Sequenced PCR products of the RT-RNase H region of the viral genome have been used for the construction of a phylogenetic tree. We found all the 7 BSV-like species within the A genomes whereas only 4 species were within B genomes. Interestingly, two new species have been discovered in *M. balbisiana* genomes only. Additionally, four BSV-like species seem to be integrated in *M. schizocarpa*, *M. basjoo*, *M. ornata* and *M. itinerans* but these results have to be confirmed. The integration patterns observed by Southern blot analysis show multiple and different integrations of BSV-like species into *M. acuminata* plants conversely to *M. balbisiana* plants where patterns are highly conserved. The data suggest that BSV-like integrations likely occurred for some of them before the speciation *M. acuminata/M. balbisiana* whereas others occurred afterwards.

CDC48, a novel interaction partner of TMV MP: Regulator of MP fate and function during infection

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Protein quality control and the removal of misfolded proteins are central cellular processes to avoid severe cellular damage. As virus infection affects the proteostatic state of the host cell we use *Tobacco mosaic virus* (TMV) to investigate the interference of plant virus infection with cellular protein quality control and protein degradation pathways.
TMV replicates in association with the endoplasmic reticulum (ER) and exploits this membrane network for intercellular spread through plasmodesmata (PD), a process depending on virus-encoded movement protein (MP). During infection, this multifunctional protein is overproduced and rearranges the ER network to accumulate in ER-associated inclusions. We show here that infection induces ER-stress and the expression of CELL DIVISION CYCLE PROTEIN 48 (CDC48) an essential, conserved ATP driven chaperone which functions in diverse cellular processes including the targeting of misfolded or aggregated proteins for degradation. We further demonstrate that CDC48 functions in maintaining ER-integrity upon ER-stress and interacts with MP in ER-associated inclusions to promote degradation of the protein. Interaction of CDC48 with MP depends on the MP N-terminus, which is required for degradation of the protein, for PD localization and microtubule accumulation of MP and for function of MP in cell-to-cell transport of the viral RNA. Our data suggest that CDC48 extracts MP from ER-inclusions to the cytosol, where it subsequently accumulates on and stabilizes microtubules. We show that virus movement is impaired upon overexpression of CDC48, indicating that CDC48 further functions in controlling virus movement by removal of MP from the ER-transport pathway and by promoting interference of MP with microtubule dynamics. In conclusion, our data indicate that a CDC48-dependent pathway leading to the clearance of ER-associated protein inclusions exists in plants, that plant viral MPs are substrates for this pathway and that this pathway determines viral protein fate and function during infection. As viruses often exploit host pathways for replication and spread, we propose a model in which CDC48 functions in the degradation of overaccumulating viral protein and also participates in the regulation of TMV replication and cell-to-cell movement.

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Long-term viral competition monitoring: a case of epidemiological rescue

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Biological invasions are major threats to biodiversity and the main causes of emerging viral diseases. The ongoing spread of *Tomato yellow leaf curl virus* is a major concern to the sustainable tomato production throughout the world. The two main strains of TYLCV have been successively introduced in Reunion Island providing a fortuitous field experiment to study the invasion and competition of these two emerging strains in a tropical and insular environment. In this study, a seven-year field survey was performed following the introduction of the Israel strain of TYLCV (TYLCV-IL) into a niche occupied by the Mild strain of TYLCV (TYLCV-Mld). A displacement of the resident TYLCV-Mld by the newcomer TYLCV-IL was observed in this short period. To understand the factors associated with this displacement, biological traits related to fitness were measured to compare these strains. Besides demonstrating a better ecological aptitude of TYLCV-IL, which explains its rapid spread and increasing prevalence, the first estimate of the number of viral particles efficiently transmitted by an insect vector for a circulative virus was obtained. However, TYLCV-Mld persistence in the field (especially in mixed infections with TYLCV-IL) spurred further experiments regarding the effects of the mixed infections on these biological traits. Our study revealed complex interplay between these two strains of one of the most emergent plant virus following their successive introductions in the insular and tropical environment of Reunion Island. This rare case of unilateral facilitation between two pathogens led to the epidemiological rescue and maintenance of the less fit strain.

Influences of RDRs and alternative oxidase on basal and salicylic acid-induced resistance to viruses

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Salicylic acid (SA) triggers multiple anti-viral mechanisms, including mechanism(s) regulated by a mitochondrial enzyme, alternative oxidase (AOX), which may exert its effects via signaling mediated through changes in levels of reactive oxygen species. However, viruses differ in the extent to which AOX-regulated resistance affects them. Thus, both basal and SA-induced resistance to potato virus X (PVX) can be manipulated by altering the expression of wild-type or mutant versions of AOX in transgenic plants but for tobacco mosaic virus (TMV) the effects are more limited[1]. Host RNA-dependent RNA polymerases (RDRs) contribute to antiviral silencing in plants. RDR6 is probably the most important RDR conditioning plant-virus interactions and in *Nicotiana benthamiana* limits the spread of PVX, and prevents infection of the meristem[2]. RDR1 is inducible by SA but although it contributes to basal resistance to TMV, it is dispensable for SA-induced resistance to that virus[1]. *N. benthamiana* is a natural *rdr1* mutant but RDR1 activity is restored in transgenic plants expressing *Medicago truncatula* RDR1[3]. Utilizing *MtRDR1*-transgenic *N. benthamiana* and transgenic plants expressing diminished levels of RDR6 (line *RDR6i*) we have generated double transgenic lines with defects in AOX-regulated signaling and RDR1- or RDR6-mediated silencing to investigate the relative importance of these factors in SA-induced and basal resistance. We found that the spread of TMV into upper, non-inoculated, leaf tissue is not affected, adversely or otherwise, by the expression of *MtRDR1* in *N. benthamiana*, but that recovery from severe TMV disease is. Additionally, MtRDR1 expression and SA treatment act additively to promote recovery from disease symptoms in *MtRDR1*-transgenic plants. Results with *RDR6i*/*Aox* double transgenic plants indicate that both systems (AOX-regulated resistance and RDR6-dependent silencing) are equally important in maintaining basal resistance to PVX but that only AOX-regulated signaling plays a role in SA-induced resistance in *N. benthamiana*.

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Specificity of eIF4E factors towards resistance to potyviruses in *Arabidopsis thaliana*

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The translational initiation factors eIF4E represent the main resistance to *Potyvirus* as well as to other families of plant RNA virus. Although those factors are encoded by small multigenic families, it has been shown that each virus can only use specific eIF4E to perfom its cycle in plants. Often, this coincides with the ability of the virus VPg to interact with those host proteins. Using *Arabidopsis* transgenic approaches, we aimed at better understanding the basis of the virus specificity for eIF4E.

First, we looked at the interaction pattern of the *Clover Yellow Vein Virus* (ClYVV) VPg with all five *Arabidopsis* eIF4E proteins and found that it interacts with three of them. However, *Arabidopsis* mutant studies have shown that only *eIF4E1* is necessary to allow ClYVV infection. This suggests that unlike what has been shown for other plants (as pepper and tomato), not only the eIF4E protein sequence but also its gene expression pattern may have a key role in susceptibility, as we investigated by studying various eIF4E over-expressions.

Then we looked at a case in which the same virus can use very different eIF4E in separate species: the *Tobacco Etch Virus* (TEV) relies on eIF4E1 in tomato and pepper but on its isoform eIFiso4E in *Arabidopsis*. To see whether this specificity depends on those eIF4E isoforms only, or rather on a more general host protein complex, we over-expressed pepper eIF4E in TEV-resistant *Arabidopsis* to see whether it can restore host susceptibility to TEV.

As a result from those idiosyncratic eIF4E requirements, broadening the resistance spectrum of plants to viruses may require the inactivation of different *eIF4E* genes, a process which may be detrimental to the plant development. Indeed, we show that the double mutant *eif4e1*; *eifiso4e* is lethal in *Arabidopsis*. As an alternative, we aimed at creating new resistance alleles in *Arabidopsis* by mimicking natural –and possibly functional- resistance alleles that have been characterized in other species. We hope this may help developing new resistances alleles to *Potyvirus,* including in plants species for which none has been described so far.

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Symptoms determinant of *Grapevine fanleaf virus* in *Nicotiana* species

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The mechanisms of symptoms development following plant infection with viruses remain poorly understood although specific virus-host interactions in terms of replication, movement and cellular reprogramming are involved. Moreover, suppression of resistance by the crosstalk between the miRNA pathway and viral suppressors of RNA interference (VSR) and the induction of a hypersensitive response of the plant affectdisease display (1). So far, little is known about viral sequences responsible for symptom development and no VSR has been identified for members of the genus *Nepovirus* on thefamily *Secoviridae*.

To get insights into nepoviral sequences involved in symptom development, we took advantage of two *Grapevine fanleaf virus* (GFLV) strains that systemically infect *Nicotiana* species with distinct symptoms type: strain GFLV-GHu induces a vein clearing on *N. benthamiana* and chloroticlesions on *N. clevelandii* while strain GFLV-F13 causes asymptomatic infections on bothspecies (2,3). A reverse genetics approach was used to identify GFLV determinants of symptomatology with infectious cDNA clones of strains F13 and GHu, which were already available (4) or developed over the course of this study, respectively. *In vitro* transcripts of homologous combinations of RNA1 and RNA2 induced systemic infection in both *Nicotiana* species with identical symptoms compared to wild-type viruses. We then combined RNA1 from one strain with RNA2 from the other strain and showed that the GFLV-GHu symptoms on *N. benthamiana* and *N. clevelandii* mapped to RNA1. Further experiments were undertaken to identify the coding region responsible for symptom development by swapping cDNA regions of RNA1 between the two GFLV strains. Eight chimeras were produced which allowed us to delineate a coding sequence responsible for typical GFLV-GHu symptoms on *Nicotiana* species. This is the first identification of a nepoviral coding sequence determining symptoms in a plant host.

The GFLV-GHuRNA1 coding sequence that elicits symptoms on *Nicotiana* species was then tested in transient expression assays to determine whether it is able to suppress RNA silencing or induce a symptomatic response on its own. Neither silencing suppression nor symptoms were observed, suggesting that the mechanism underlying the induction of GFLV-GHu symptoms on *N. benthamiana*and *N. clevelandii* is a complex phenomenon.

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Phylogenetic analysis of a worldwide collection of *Cauliflower mosaic virus* isolates to understand the within-host viral accumulation patterns

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The transmission–virulence trade-off hypothesis is one of the few adaptive explanations of virulence evolution, and assumes that there is an overall positive correlation between parasite transmission and virulence. A positive correlation between each of these two traits and within-host growth is often suggested to underlie the relationship between virulence and transmission. However, there are only few experimental tests of this hypothesis exposed in litterature. In this study, we report the first empirical test on a plant pathogen. We infected *Brassica rapa* and *Arabidopsis thaliana* plants with nine wild isolates of *Cauliflower mosaic virus* (CaMV) and then estimated three traits: transmission, virulence, and within-host viral accumulation. As suggested by the trade-off hypothesis, we observed a positive correlation between transmission and virulence on the two hosts [1,2]. We also discovered the unexpected existence of two groups of within-host accumulation on both hosts, differing by at least an order of magnitude. When accumulation groups were not accounted for, within-host accumulation was correlated neither to virulence nor transmission [1,2]. Moreover, isolates were assigned to the same within-host accumulation group on both *B. rapa* and *A. thaliana*, suggesting that the mechanism determining the accumulation group does not depend on the host plant. To understand the existence of these groups, we sequenced the full genome of 25 isolates of CaMV. These 25 isolates correspond to the nine isolates previously described and 16 isolates newly characterized for their accumulation group. Phylogenetic analyses on each open reading frame (ORF) or the entire concatenated genome always indicate that the accumulation groups are polyphyletic. Results will help us to identify the genetic determinants distinguishing the two accumulation groups.

Key-words: CaMV, virulence, transmission, within-host viral accumulation, phylogeny.

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Analysis of plant colonization by TuMV unveils different cell colonization strategies among viruses

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The colonisation of a territory is a crucial step in the life cycle of many organisms. Colonisation strategies have been shown to be rather diverse among species, being the size of the colonising group one of the main parameters defining such strategies. Basically, colonisation can be performed either by a single individual or by a group with variable size and relatedness among the group individuals. Whether group size is one or many can define to a large extent the intensity of interactions among individuals among and within territories, for example influencing gene flow or allowing for the evolution of social behaviours.

Since viruses are intracellular parasites, cells can be considered as their main territory. It is during cell infection that the viral diversity infecting the cell can multiply and interact through genetic exchange, competition or complementation. Hence, the multiplicity of cellular infection (MOI) - i.e. the number of genomes that infect the cell - is equivalent to the size of the colonising group and it largely defines, among others, the intensity of these major phenomena in virus evolution.

It is surprising to verify that viruses are rarely considered to have evolved colonisation strategies with different MOIs despite the potential effects of such strategies on their evolution and epidemiology. The latter is probably explained by the dramatic lack of data on the MOI of viruses. During my talk, I will present a detailed characterisation of the MOI of a plant virus, *Turnip mosaic virus* (TuMV), during the infection of turnip plants. Our results show that primarily-infected cells in a leaf are mainly colonised by a single viral genome even during high viremia peaks. The originally clonal populations generated in those primary sites showed a territorial behaviour during subsequent organ colonisation that severely limited coinfection of cells by different populations. This colonisation pattern completely differs from the pattern we have previously observed with another plant virus in the same host, supporting thus the existence of different MOI strategies in viruses.

Using viral genetic polymorphism to gain insights into sharka dispersal at multiple scales

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Spatially explicit simulation models of disease epidemics and management can be particularly helpful for the definition of optimal strategies of disease surveillance and control as they allow testing various alternative strategies (see poster by Rimbaud *et al.*). However, an appropriate parameterization of such models requires good knowledge of the dispersal function of the pathogen. Stochastic spatiotemporal models using maps of diseased individuals enable estimating such dispersal functions (see talk by Lefort *et al.*). With fast evolving pathogens like RNA viruses, the genetic polymorphism combined with epidemiological data can help differentiating sources of inoculum, reconstructing transmission chains and estimating the dispersal function (Ypma *et al.*, 2012; Morelli *et al.*). The aim of our study was to investigate if the genetic polymorphism of the *Plum pox virus* (PPV, genus *potyvirus*) could be used to gain insights into the spatial dynamics of the corresponding disease, sharka, one of the most devastating diseases of stone fruit trees. PPV is disseminated by the plantation of contaminated material. Once introduced, at least 20 different aphid species can transmit this virus in a nonpersistent manner.

Under a geographical information system, we built a database reporting the exact location of 1157 symptomatic trees detected during exhaustive visual inspections and dispersed over a 450 ha peach production area comprising 215 inter-connected susceptible orchards. We sampled 996 symptomatic trees and the corresponding viral isolates were characterized either by CE-SSCP or by partial genome sequencing. A preliminary approach targeting the Cter-P3/6K1 and Nter-CP genomic regions confirmed that the PPV genetic polymorphism could be informative both within and between orchards. The reconstruction of the haplotype network as well as the strong spatial structure of the viral genetic diversity suggested that disease spread in this area was related to at least two independent introduction events. In a second step, full length genome sequencing of 232 PPV isolates (including 41 isolates from other French outbreaks) was carried out to further the understanding of sharka dispersal in the studied area. The reconstructed phylogeny and sequence genealogies uncovered additional introduction events. Linking spatial information with sequence genealogies provided a first estimate of the distances of PPV dispersal by aphid vectors within and between orchards.

The next step will be to explicitly model viral evolution and epidemiological data within a single framework in order to formally reconstruct transmission chains and estimate the related epidemiological parameters.

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Keywords: dispersal, molecular epidemiology, transmission chains, viral genealogies.

Narrow bottleneck affect a plant virus population during vertical seed transmission

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Estimating the relative intensity of selection and drift in viral population requires determining viral population size. Indeed, if selection acts more intensely in large populations, random effects of genetic drift are more severe in small ones. Although viral population size in their hosts can be tremendous, they also endure narrow population bottlenecks bothduring infection of their hosts and during horizontal transmissionbetween host individuals. Estimating the size of such bottlenecks for plant virus populations has been the focus of several studies in the last decade,but no estimates are available for the vertical transmission of plant viruses (*i.e.* infection of plant progenies by the parental plants) although virus seed transmission is economically significant in at least 18% of plant viruses inat least one plant species.

The goal of this work was to determine, using *Pea seedborne mosaic virus* (PSbMV), genus *Potyvirus*, family Potyviridae, the size of bottlenecks during vertical seed transmission. Twelvepea plants (*Pisumsativum*L.) of the cultivar 'Vedette' were co-infected with 2 isolates of PSbMV, DPD1 and DPD1-R. At 22 and 61 days after inoculation, corresponding to the anthesis of the first flower in the plant population and to the end of flowering respectively, the mean and standard deviation of the relative frequency frequencies of virus isolates were assessed by analyzing 3 leaves at each date and in each plant. Next, pods were harvested, seeds were sown and analyzed 22 days after sowing to determine, for each parental plant the number of seeds not infected, infected only by DPD1 isolate, only by DPD1-R isolate or by both isolates. In all, 823 seeds were analyzed.

In order to estimate the number of viruses infecting a seed and to explore the potential mechanisms underlying the seed infection process, we developed dedicated stochastic models including different sensible operating assumptions. The modelssplitseed infection mechanism into two main successive steps (number of virus entering into a seed and number of virus required to efficiently infect a seed), all steps being described by Poisson processes. The models alsotookexplicitly into account the variation of the proportion of viral isolates within each plant.A simple model, assuming that a single virus particle is enough to infect a seed and that virus isolates jointly caused seed infection fitted the datavery satisfactorily. Parameter inferencesnotably indicated that the mean number of virus entering a pea seed is of the order of the unit, ranging from 0.6 to 1.3. Thus, this study reveals that vertically-transmitted viruses endure bottlenecks as narrow as those imposed by horizontal transmission, which are therefore likely to slow down virus adaptation but to accelerate the differentiation of virus populations.

Posters


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1-Molecular analysis of a German isolate of *Apple stem pitting foveavirus*

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The *Apple stem grooving virus* (ASGV, *Capillovirus*) and *Apple stem pitting virus* (ASPV, Foveavirus) are widely spread in apple growing regions. As they cause no symptoms on most cultivated apple varieties and rootstocks, they are considered as latent in *Malus* x *domestica*. Recently, *M. sieboldii* and its hybrids have gained new interest in Europe as they confer resistance to apple proliferation (AP) disease caused by *Candidatus* Phytoplasma mali. A new breeding program aiming to develop AP-resistant rootstocks of agronomic value reported unexpected tree decline which was found to be associated with ASGV and/or ASPV. As little information is available on the variability of these latent virus isolates, the complete genome of a German isolate of ASPV associated with tree decline was cloned and sequenced.

The comparison between the sequence of the german isolate and those of other ASPV isolates from the databases revealed a high degree of variability between these isolates, which is spread over the entire genome. Overall, the most conserved gene was the first gene from the triple gene block (between 89 and 95% identity between isolates), the most variable one being the coat protein gene (up to 61% diversity between isolates). The replicase gene showed overall identity levels of 65-95% between isolates. The distribution of this variability in the replicase gene was however uneven, some regions being conserved, and others showing a higher degree of variability.

2-The *Cauliflower mosaic virus* reinitiation factor TAV controls the TOR signalling pathway

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Protein kinase TOR (target-of-rapamycin) signalling positively controls translation initiation via phosphorylation of downstream targets in response to integrating signals from growth factors, hormones, energy sufficiency and nutrients. The viral reinitiation factor TAV from Cauliflower mosaic virus (CaMV) activates polycistronic translation reinitiation through a mechanism involving recruitment of eIF3 and the host factor RISP to translating ribosomes that ensures recruitment of the ternary complex and 60S-complexes onto reinitiating 40S. Here we uncover the essential role of the TOR signalling pathway in TAV-mediated reinitiation after long ORF translation. We demonstrate that the TOR/S6K1 pathway, which promotes TAV-mediated reinitiation, is hyperactivated in plants either constitutively expressing TAV or infected by CaMV. Activation of TOR depends on its direct physical interaction with TAV, with mutations in the TOR binding site of TAV abolishing both TOR binding to TAV and TOR activation. Only functionally active TOR was able to stimulate TAV-mediated reinitiation efficiency, and knockdown of TOR abolishes TAV function in plant protoplasts and CaMV infection in plants. TAV binding to TOR is critical for both the reinitiation event and viral fitness. Furthermore, we identified a novel substrate of TOR signalling within reinitiation supporting factors: RISP is a putative substrate of TOR/S6K1 in *Arabidopsis*. Upon activation, TOR binds to polyribosomal complexes concomitantly with polysomal accumulation of eIF3 and RISP in a TAV-dependent manner. In polysomes, TOR signalling leads to phosphorylation of RISP. According our recent finding, the subunit h of eIF3—essential plant factor for translation of upstream open reading frame-containing mRNAs—is phosphorylated in response to TOR activation. Our data support a model whereby TOR functions to ensure the phosphorylation state of factor(s) recruited to translating ribosomes to enable reinitiation at a downstream ORF.

3-Analysis of *Amalgamaviridae* diversity in the Kerguelen Islands ecosystem using two different strategies for metagenome analysis

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The development of novel sequencing techniques (NGS) allows for unprecedented access to viral metagenomes and, in particular, to the communities of phytoviruses in plant populations. Several strategies have been envisioned to gain access to this phytoviral metagenome, each with advantages and limitations. We are currently running such approaches in the constrained and paucispecific ecosystem of the Kerguelen Islands, the second most isolated archipelago on earth. While pyrosequencing of samples and bioinformatics analysis of data is still in progress, we have performed a preliminary analysis of the diversity of *Amalgamaviridae*, a poorly known and recently discovered family of plant-associated double stranded RNA viruses which seems to be quite represented in the Kerguelen. Using a strategy in which 225 plant samples representing 56 species and 18 sampling sites were analysed as a megapool, a total of 19 OTUs (Operational Taxonomy Units, defined using as cut-off a 10% amino acid sequence divergence in a short, highly conserved region surrounding the viral RNA polymerase active site). Nine of these OTUs have been identified in plants analyzed in an ongoing ecogenomic approach which has so far analyzed over 1000 individual plants belonging to 35 species, 29 of which were represented in the megapool. Given the higher number of plants per species and the deeper sequencing effort, this second approach represents an over 20-fold deeper effort on a per species basis. It has identified 17 OTUs, of which 9 are novel and 8 had been previously identified, indicating the deeper effort has doubled the OTUs identified. These preliminary results provide pointers when trying to decide which approach to use and the sampling and sequencing intensity when trying to describe a phytoviral metagenome. Compared to the three *Amalgamaviridae* species described so far, 28 OTUs likely to represent *bona fide* species have been identified in the Kerguelen islands, associated with a wide range of host plants, particularly in the graminaceae. Of particular interest is the observation of widespread infection of a native grass, *Poa cookii* (Cook's bluegrass) which appears to hosts at least 5 different OTUs, several of which appear to be shared with the introduced annual bluegrass, *Poa annua*.

4-Characterization and diagnostic of Yam virus X (YVX) and Yam necrosis virus (YNV), two novel viruses infecting yams in Guadeloupe

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Several viral species infecting cultivated yams (*Dioscorea* spp.) are known. They include viruses belonging to the families *Alphaflexiviridae* (genus *Potexvirus*), *Betaflexiviridae* (genus *Carlavirus*), *Caulimoviridae* (genus *Badnavirus*), *Cucumoviridae* (genus *Cucumovirus*) and *Potyviridae* (genera *Macluravirus* and *Potyvirus*). However, it is widely acknowledged that yet uncharacterized viral species are present in yam germplasm collections worldwide and could be propagated through the distribution of infected germplasm. Therefore, viruses are currently the major constraint for much needed exchanges and distribution of yam germplasm.
In order to promote the safe exchange of yam germplasm conserved in the Guadeloupe Biological Resources Center of Tropical Plants (CRB-PT), searches for new virus species in yams were undertaken. *In silico* analyses of ESTs of *Dioscorea alata* were performed and unveiled the existence of sequences corresponding to several known genera of yam viruses, such as *Badnavirus* and *Macluravirus*, and also to families of unknown yam-associated viruses, including *Geminiviridae* and *Secoviridae*. RT-PCR were performed on crude extracts of symptomatic yams (*D. alata*, *D. trifida*) following direct binding of viral particles and using degenerate primers. Amplification products were cloned and sequenced. Some of them displayed significant levels of homology with potexviruses and with viruses of the family *Secoviridae.* The 3' ends of the corresponding viral genomes were successfully amplified by 3' RACE, cloned and sequenced. Phylogenetic analyses confirmed the existence in yams of one new viral species within the genus *Potexvirus* (tentatively named Yam virus X, YVX) and one within the family *Secoviridae* (tentatively named Yam necrosis virus, YNV). The experimental host range of both viruses was explored through mechanical inoculation on various herbaceous plants.
Molecular diagnostic was developed for both YVX and YNV using direct binding reverse transcription PCR (DB-RT-PCR) and used to perform a prevalence study of both viruses in the Guadeloupe CRB-PT yam germplasm collection.

Keywords: yams; *Potexvirus*; *Sadwavirus*; diagnostic

5-Les conservatoires des maladies végétales : un outil au cœur de la station de Quarantaine

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La station de quarantaine (SQ) est une unité du Laboratoire de la Santé des Végétaux de l'Agence Nationale de Sécurité Sanitaire – Alimentation, Environnement, Travail (Anses).

Les missions et travaux confiés à la station de quarantaine :

- Assurer la quarantaine de végétaux pour le compte des introducteurs
- (recherche scientifique et création variétale)
- Laboratoire National de Référence pour le PPV, les virus des agrumes, les virus de la pomme de terre
- Méthodologie (appropriation, adaptation et mise au point de méthodes, validation de réactifs) en tant que Laboratoire National de Référence

Expertises dans le cadre de ses domaines de compétences (confinement, analyses, …)

Les collections :

Afin de répondre à ces différentes missions, un conservatoire toujours en évolution s'est constitué (environ 80 espèces de virus/viroïdes/phytoplasmes et autres bactéries endophytes).

Différents types de collection existent :

- in vitro pour la collection nationale de référence des maladies de la pomme de terre
- in vivo en milieu confiné NS3 pour les fruitiers, la vigne et les agrumes

Une gestion dynamique est appliquée, avec une maîtrise des différentes techniques de production de plants : le greffage, l'indexage et la culture in vitro.

La conservation de la collection nécessite un entretien dans des conditions climatiques optimisées, et des séries d'analyses pour s'assurer du statut de matériau de référence.

Des échantillons sont également conservés congelés, lyophilisés ou sous forme d'extraits d'acides nucléiques pour constituer une réserve supplémentaire.

Un travail de recherche bibliographique est effectué pour développer des contacts et compléter la collection.

Les partenariats

La collection est à disposition des autres unités du Laboratoire de la Santé des Végétaux.

Du matériel végétal peut également être transféré vers des laboratoires agréés ou centres de recherche pour des besoins spécifiques.

La SQ coopère avec de nombreux acteurs du domaine scientifique et technique.

Les collaborations avec les instituts techniques professionnels (IFV, CTIFL, EPR, …), les centres de recherche français (INRA, CIRAD, ...) et étrangers (Station de Changins, IVIA, SASA, Institut de Bari, …) permettent l'échange de protocoles et de matériel végétal infecté pour les collections.

6-Identification of new molecular determinants of *Potato virus Y* (PVY) involved in the expression of necrotic symptoms in tobacco

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Potato virus Y (PVY, *Potyviridae* family, genus *Potyvirus*) is known as one of the most damaging viruses on potato and tobacco plants by inducing on these two crops significant yield and/or quality losses. For nearly 10 years, these agronomic impacts were strengthened by i) the increase in the field of PVY isolates frequency responsible for potato tuber necrosis, ii) the presence of isolates able to overcome the recessive resistance gene *va*, generally used as the main strategy against PVY on tobacco plants. Considering the importance of this biological property, groups and PVY variants have been defined on the basis of the PVY isolate ability to induce or not vein necrosis in tobacco, to overcome some potato hypersensitive genes and to cause tuber necrosis. Beyond the interest establishing an official classification of the PVY species based on their necrotic ability, the understanding of the biological and molecular basis of the necrosis constitute a major scientific issue associated to the study of the PVY pathosystem. In addition, studies over the past decade allowed the identification of several viral molecular determinants (i.e. G_{205}, K_{400} and E_{419} residues of HC-Pro protein) involved in the necrotic symptom on tobacco (Tribodet *et al*., 2005; Hu *et al.*, 2009). However, non-necrotic isolates encoding the triplet $G_{205}K_{400}E_{419}$ have also been recently described. These data suggest that other molecular determinants might exist within the viral genome. In order to identify them, chimeric viruses resulting from substitution of genomic sequences of a necrotic infectious clone (PVY^N-605) with homologous regions from a non-necrotic isolate (PVY^O-139) and mutated necrotic infectious clone were built and tested in tobacco and potato plants. The combined analysis of molecular and biological characteristics of these chimeric viruses revealed the importance of the N339 residue of the HC-Pro protein and two regions overlapping CI-6K2-NIa region (nucleotides 5496-5932 and 6233-6444) in the expression of tobacco vein necrosis. The precise role of these determinants in the ability to induce necrosis on potato tuber is under analysis. Results from the most recent experiments will be presented.

Tribodet M., Glais L., Kerlan C. and Jacquot E. (2005). Characterization of *Potato virus Y* (PVY) molecular determinants involved in the vein necrosis symptom induced by PVY^N isolates in infected *Nicotiana tabacum* cv. Xanthi. *Journal of General Virology* 86: 2101-2105.

Hu X.J., **Meacham T., Ewing L., Gray .S.M, Karasev A.V.** (2009). A novel recombinant strain of *Potato virus Y* suggests a new viral genetic determinant of vein necrosis in tobacco. *Virus Research* 134 : 68-76.

7-Stratégie combinée de NGS et de Bulk Segregant Analysis pour accélérer le clonage positionnel et identifier des gènes impliqués dans la résistance au *Potato virus X* chez la tomate.

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Le gène de résistance *Rx* cloné chez la pomme de terre code une protéine de résistance de type CC-NBS-LRR et confère une résistance extrême (ER) au *Potato virus X* (PVX) (Bendahmane et al., 1999).Le PVX est un virus à ARN simple brin du genre *Potexvirus*. Son génome code 5 protéines virales. L'une d'entre elles, la protéine de capside (CP) est impliquée dans de nombreux processus viraux mais est également l'éliciteur de la résistance liée à *Rx* (Bendahmane et al., 1995). La tomate (*Solanum lycopersicum*) est une espèce cultivée de grand intérêt dont le génome est maintenant totalement séquencé (Tomato Genome Consortium, 2012). Le gène de résistance *Rx* a été introduit par transgénèse dans un cultivar nain : le Micro-Tom. Une population mutagénéisée à l'EMS a été créée à partir de cette plante transgénique. Le criblage de cette population avec une souche partiellement contournante de PVX, la souche KH2, a permis l'identification de 5 familles de mutants (#1179, #2064, # 2135, #3141 et #3467) présentant une résistance liée à Rx altérée (Sturbois et al., 2012). La caractérisation génétique de ces mutants a montré que les 5 mutations sont indépendantes (3 récessives et 2 dominantes), sont affectées dans des gènes différents et qu'aucune mutation ne se situe dans le gène de résistance *Rx* ou dans des gènes déjà connus pour être impliqués dans la résistance liée à Rx. Afin d'identifier les gènes mutés, une stratégie combinant le séquençage haut débit et la BSA (Bulk Segregant Analysis) a été mise en place, comme cela avait été fait chez *Arabidopsis thaliana* (Schneeberger et al., 2009). La première famille de mutants analysée est la famille #1179, une des mutations dominantes, pour laquelle la population F2 issue d'un croisement 1179 X *Rx* a été phénotypée après inoculation avec la souche PVX-KH2. Les pools résistant et sensible ainsi que les parents et la lignée Micro-Tom sauvage ont été séquencés en utilisant la technique Illumina. Cette approche globale a pour but de détecter des SNP différentiellement présents entre le pool résistant qui contient la mutation responsable du phénotype et le pool sensible sans mutation. Le séquençage haut débit a permis d'obtenir plus de 40 millions de lectures de 80pb en paired-end par pool. Le traitement des données par le logiciel CLCBio a permis d'identifier pour le moment, plusieurs gènes candidats qui vont maintenant être étudiés de manière plus approfondie. Le même type de stratégie sera employée afin d'identifier la mutation récessive dans la famille # 2135.

Bendahmane et al. 1995. Plant J. 8:933-941 - Bendahmane et al. 1999. Plant Cell 11:781-792. - Schneeberger K et al. 2009 6:550-1. - Sturbois B et al. 2012 Mol Plant Microbe Interact. 25:341-54. - Tomato Genome Consortium. 2012 Nature 485:635-41.

8-Comparative study of the prevalence and diversity of Banana streak viruses (BSV) in plantain interspecific hybrids in the Dominican Republic

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Banana and plantain (*Musa* spp.) are major staple food and cash crops in the Dominican Republic. However, production suffers from Black Sigatoka disease (BSD), a severe foliar disease caused by the fungus *Mycosphaerellafijiensis*Morelet. Several BSD-resistant interspecific *Musa acuminata*(A) x *Musa balbisiana*(B) bananaand plantain hybrid varieties were introduced in the late 1990sin the Dominican Republic as an alternative to chemical control of *M. fijiensis*, especially plantain hybrid species FHIA21.However, it was later shown that *M. balbisiana*(B) progenitors harbor infectious endogenous sequences of Banana streak virus (eBSV) [1] and that these sequences can lead to spontaneous infections in created and natural interspecific hybrids, following abiotic stresses including cell culture and temperature differences [2].
In order to assess the risk of spreading BSV in the Dominican Republicthrough large scale distributions of created plantain interspecific hybrids, apreliminary survey was conducted in order to compare the prevalence and diversity of BSV species in hybrid FHIA21 (AAAB) and a local plantain cultivar, Macho X Hembra (AAB), which is a natural interspecific hybrid. Samples were collected in a selection of plots located in the main production area of plantain in the Dominican Republic and representative of the diversity of environmental and growing conditions (temperature, rainfall).All samples were indexed for the four main BSV species, including BSOLV, BSImV and BSGFV for which the existence of infectious eBSVs has been demonstrated. eBSV signatures were established for each sample group and correlated to indexing results. The presence of mealybugs (*Planococcus* ssp.), which are BSV natural vectors, was monitored on each sampling site and taken into account in a multifactor analysis. Results show that the level of prevalence of some BSVspecies is significantly higher in FHIA21 than in Macho X Hembra, and suggest that this difference results from increased activation of infectious eBSVs in FHIA21.

Keywords: banana streak viruses;eBSV; activation; plantain; banana;

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9-L’analyse de risque phytosanitaire (ARP) : un outil d’aide à la décision pour faire évoluer la politique phytosanitaire

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L’analyse de risque phytosanitaire (ARP) est un processus consistant à évaluer les données biologiques, environnementales ainsi qu’économiques et sociales, pour déterminer si un organisme est nuisible, pour mesurer les risques qu’il représente dans une zone géographique déterminée et pour recenser les méthodes de lutte disponibles pour limiter son impact dans la zone géographique concernée et d’évaluer leur efficacité. Dans le cadre des activités réglementaires, les ARP sont couvertes par une norme internationale (NIMP 11) produite par le Secrétariat de la Convention internationale pour la protection des végétaux (CIPV) de l’organisation des nations unies pour l'alimentation et l'agriculture (FAO). Il s’agit d’une obligation internationale pour la mise en place de mesures phytosanitaires qui se doivent d’être harmonisées, transparentes, justifiées et compatibles avec le niveau de risque.

L’ARP se déroule en trois étapes successives. Elle débute par une étape d’initiation (caractérisation de l’organisme nuisible ou de la filière), se continue par une étape d’évaluation du risque (évaluation de la probabilité d’entrée, d’établissement, de dissémination et de l’impact socio- économique dans la zone géographique considérée). Elle s’achève si le risque est jugé comme non acceptable par une étape d’évaluation des mesures de gestion du risque.

Les ARP sont réalisées par les organismes nationaux de protection des végétaux, les organisations internationales tel que l’Organisation européenne et méditerranéenne de protection de plantes (OEPP) et par l’Autorité européenne de sécurité alimentaire (EFSA) quand il s’agit d’une demande commanditée par l’Union européenne (UE). En France, les ARP sont réalisées par l’Anses au travers d’un système d’expertise collective qui permet la confrontation des différentes opinions, l’expression et l’argumentation d’éventuelles positions divergentes afin d’assurer une expertise indépendante et transparente. Un comité d’experts spécialisé (CES) « Risques biologiques pour la santé des végétaux » a été créé en janvier 2012. Il regroupe 18 membres dont les compétences couvrent les domaines de la phytopathologie, l’entomologie, la nématologie, la malherbologie et l’agronomie.

Mots clés : Analyse de risque phytosanitaire, Organismes nuisibles, Expertise

10-Is the central part of CI (Cylindrical Inclusion) involved in LMV chimera adaptation to eIF4E-mediated resistance in lettuce?

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Lettuce mosaic virus (LMV, *Potyvirus)* constitutes a major pathogen in commercial lettuce crops and can infect ornamentals and weeds. We previously showed that recessive allelic genes *mo1*[1] and *mo1*[2] used to protect lettuce crops against LMV correspond to mutant alleles of the gene encoding the eukaryotic translation initiation factor eIF4E [1]. We further showed that the LMV resistance-breaking determinants map not only to the VPg-encoding region (the main potyvirus eIF4E virulence determinant) but also to the C-terminus of the CI (Cylindrical Inclusion) helicase [2], [3].

During a global survey of the biological and molecular diversity of LMV isolates, we showed that propagation of several non-lettuce isolates of LMV in *mo1*[1] plants was accompanied by a gain of virulence correlated with the accumulation of mutations in the CI C-terminus at positions 602 and/or 627. The native isolates were stable when propagated in susceptible lettuce while the *mo1*[1]-evolved isolates had simultaneously gained the ability to overcome the *mo1*[2] resistance. Whole genome sequencing of native and evolved isolates is under process to determine if other mutations could be associated with adaptation to *mo1* resistance. A similar propensity to evolve towards resistance-breaking was observed for synthetic chimeras between two parental LMV infectious cDNA clones (LMV-0 avirulent and LMV-E virulent) upon passaging in *mo1* lettuce. A recombinant derived from LMV-0, containing the central part of the CI from LMV-E, was able to adapt to *mo1* resistance, with mutations accumulating in the CI C-ter at positions 602, 617 and/or 621. The symmetric recombinant derived from LMV-E, containing the central part of the CI from LMV-0, was able to overcome *mo1* resistance, as predicted by the fact that it contains CI-C-ter and VPg of the LMV-E resistance-breaking isolate. However, this recombinant accumulated mutations not only in the CI C-terminus at positions 617 and/or 627, but also at several positions of the VPg (27, 60, 104, 115, 118, 119 and 161) depending on the individual variant analyzed.
Whole genome sequencing revealed the systematic presence of another mutation, G104C, appearing in the N-terminus of the CI in the LMV-0-based chimera and associated with adaptation to *mo1*[2]. The Glycine residue at position 104 is conserved among LMV isolates, with the exception of phylogenetically highly divergent isolates Yar and Es16, which respectively have an Histidine and an Isoleucine at this position. No other mutation was identified in the reverse recombinant based on LMV-E. The effect of this mutation at position 104 of the CI on the capacity of LMV-0 to evolve towards *mo1*[2] resistance-breaking is under investigation, together with the mutations corresponding to differences between LMV-0 and LMV-E in the CI exchanged region.

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11-Marker-assisted genotyping of eBSV alleles in banana

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The genome of banana (*Musa* sp.) harbours multiple integrations of *Banana streak viruses*(eBSVs), although integration is not required for the replication of cognate viruses. In the past 20 years, BSV outbreaks monitored in banana-producing areas resulted mostly from the activation of infectious eBSVs in newly created interspecific banana hybrids. Recently, we established that these problematic infectious eBSVs are present in the*Musa balbisiana* genomes,one of the main group of progenitors used for breeding hybrid varieties [1-4]. We also elucidated the sequence and organization of eBSVs in the model diploid *M. balbisiana* cultivar PisangKlutukWulung (PKW), showing that integration of infectious eBSGFV[5] and eBSOLV is di-allelic, with one infectious and one non-infectious allele, whereas that of infectious eBSImV is monoallelic[6].Based on the sequences and the structure of these eBSV we have developed several PCR and Derived Cleaved Amplified Polymorphic Sequences (deCAPS) markers for genotyping eBSVs[4 ; 6].To this aims, different markers were developed: (i) integration markers targeting eBSV insertion sites, (ii) structure markers targeting internally reorganized eBSV structures and (iii) allelic markers allowing the distinction between alleles of the same eBSV species.
Markers were assessed by screening the same banana genotypes in two different laboratories located in Montpellier and Guadeloupe respectively. A larger scale validation was undertaken through the screening ofall *Musa balbisiana* genotypes available fromCIRAD *Musa* collection in Guadeloupe, newly created hybrids from CARBAP in Cameroon and germplasm collection provided by Bioversity International in Belgium.
The use of these molecular tools is now a prerequisite not only for future crop-oriented breeding programmes aimed atproducing safe interspecific banana hybrids but also for assessing the risk of BSV outbreaksresulting from the activation of infectious eBSVs in natural hybrids that have been widely distributed in developing countries.

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12-Identification and characterization of host genes implicated in *Potexvirus* resistance in Solanacae

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Plants are continually challenged by various pathogens. The *Rx*-mediated resistance against *Potato virus X* (PVX) illustrates a defense mechanism known as the gene-for-gene model ([1]), in which the recognition of the PVX coat protein (CP) the elicitor, by the product of the *Rx1* gene, a NBS-LRR protein determines the outcome of the interaction ([2]). Moreover, previous studies have shown that the CP of other *Potexviruses* (NMV, CymMV, WClMV and PepMV) can induce the *Rx*-mediated resistance ([3]).The minimal fragment elicitor of PVX CP has been characterized and used as bait in a Yeast Two Hybrids (Y2H) screening. Two potential interactors have been identified: a transcription factor and a chloroplastic protein.

Therefore, the resistance mechanism and the two PVX-CP identified interactors, will be analyzed regarding an endemic pathogen of tomato crop: the Pepino Mosaic Virus (PepMV).

In order to go one step further, five tomato (*Solanum lycopersicum*) cDNA libraries have been constructed after PepMV inoculation with various time conditions. After using the CP, the Triple Gene Block protein 1 and 2 (TGBp1, TGBp2) of PepMV as baits in an Y2H screening, a list of host proteins interacting with viral proteins have been established. Functional validation experiments of these potential interactors such as transient gene silencing (VIGS- viral induced gene silencing) or over/under-expressing lines are about to be established. The Tomato TILLING platform held at URGV will be also exploited for validation purpose.

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13-Diverse circular single-stranded DNA viruses discovered from research greenhouses and agro-ecosystems.

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This study focuses on the diversity of circular single-stranded DNA (ssDNA) viruses associated with *Poaceae* growing in the fynbos ecosystem and its neighbouring agro-ecosystems in the Cape floristic region of Southern Africa. We have developed a sequence-independent approach that combined two methods, including a first step of rolling circle amplification (RCA) followed by a second step of whole genome amplification (WGA). We have first tested and validated this approach using controls from our laboratory, including 4 *Geminiviridae* (sugarcane/*Sugarcane streak Egypt virus*; *Datura stramonium*/*Tomato leaf curl virus*, *Datura stramonium*/*Tomato yellow leaf curl virus* and *Euphorbia caput-medusae*/*Euphorbia caput-medusae stunt virus*) and 2 double-stranded DNA *Caulimoviridae* (banana/*Banana streak Obino l'Ewai virus* and *Arabidopsis thaliana*/*Cauliflower mosaic virus*). The RCA/WGA approach was successful for unambiguously detecting the 4 *Geminiviridae* tested but failed to detect both *Caulimoviridae*. In addition to the 4 expected *Geminiviridae*, 2 Mastrevirus (located within the sugarcane plant), two Circovirus (banana and turnip) and three mycoviruses (banana, turnip and *Euphorbia caput-medusae*) were detected by performing Blast searches. This approach was then used for detecting the presence of circular ssDNA viruses within 23 South African *Poaceae*. Again, several ssDNA viruses were detected, including Mastrevirus, Begomovirus, Circovirus, mycoviruses and unknown ssDNA viruses. This study confirms the presence of a wealth of ssDNA viral sequences within plants that blur the boundaries between previously well-defined groups and stresses the need to better understand the evolutionary history of circular ssDNA viruses and to revisit current taxonomic classification schemes (Rosario et al., 2012).

Rosario,K., Duffy,S., and Breitbart,M. (2012) A field guide to eukaryotic circular single-stranded DNA viruses: insights gained from metagenomics. *Archives of Virology* **157:** 1851-1871.

14-Synergism or antagonism between *Rice grassy stunt virus* and *Rice ragged stunt virus* ?

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The recent emergence of rice viruses seriously damaged rice production of Vietnam, a country ranked second of the world rice exporters. Two viruses from different taxonomic families are responsible of the high yield losses in the Mekong Delta region: *Rice grassy stunt virus* (RGSV, *Tenuivirus*) and *Rice ragged stunt virus* (RRSV, *Oryzavirus, Reoviridae*). The two viruses are transmitted after a multiplication step in the vector insect, *Nilaparvata lugens* (brown planthopper, BPH). Already reported in other southeastern Asian countries, these two viruses have never emerged at the same time in the same rice fields. The role of coinfection on the recent emergence of RGSV and RRSV in Vietnam was investigated.
Several greenhouse experiments were performed to evaluate the impact of single and dual infection on the acquisition rate by the insect and on the transmission rate to the plant. The intra-host relationships (synergism or antagonism between viruses) were characterized at each step.
Mathematical modeling of the experiment allowed to estimate parameters (maximum likelihood) and thus to quantify main epidemiological traits, including interaction between the two viruses in the plant host and the vector. Epidemiological consequences in terms of crop loss of such interactions was assessed and compared to field data.

15-Efficiency of thermotherapy applied to whole plant as a sanitation method against three grapevine viruses (GLRaV-1, GVA and GRSPaV).

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Viral diseases are reported to cause several detrimental effects on grape and wine production. In the viticulture industry viruses are disseminated mainly through vegetative propagation and grafting. Propagating of healthy material is one of the main purpose of certification programmes. In Switzerland, clones must be free of 6 to 7 viruses (GFLV, ArMV, GLRaV-1,-3, KSG and CB, plus GFkV for rootstocks) to be certified. Rèze is a white grape cultivar confined to Valais (Switzerland). It is an old autochthonous grapevine variety since it was mentioned for the first time already in 1313. It has been even argued that Rèze could be the old cultivar Raetica already cultivated during the roman period (Vouillamoz et al. 2007). A survey to preserve the genetic diversity of local cultivars was carried out in old Swiss vineyards. It came out that all the 60 collected clones of Rèze were infected by different viruses. Thus, none of those clones was suitable to enter the certification programme. Various approaches have been applied to eliminate viruses in grapevine (Martelli 2010): chemotherapy, cryotherapy, micro-shoot tip tissue culture, micrografting and somatic embryogenesis. The objective of this work is to clean-up one clone of Rèze using whole plant thermotherapy in order to add this variety to the certification programme.

Two plants of Rèze 171 were cultivated in pots and submitted to sanitation process. Dormant plants were grown in green house at 20° C for two months. Then, at BBCH 55, they were put in growth chamber for 4 months. The growth conditions were: 16 hours of light at 36 °C; 8 hours of darkness at 36 °C. Terminal und auxiliary buds (circa 1 cm) were collected once a month. The buds were rooted and then transplanted in pots to generate full plants. Virus detection was done on leaf petioles of 6 months old cuttings using DAS-ELISA for grapevine leafroll-associated virus 1 (GLRaV-1) and RT-PCR for grapevine Rupestris stem pitting-associated virus (GRSPaV) and grapevine virus A (GVA).

The clone 171 of Rèze is infected by GLRaV-1, GVA and GRSPaV. After sanitation, 155 plantlets were obtained. The efficiency of virus elimination was evaluated and results are given in Table 1.

Table 1. Efficiency of elimination of 3 viruses by in vivo thermotherapy for Rèze cultivar.

Viruses	Plantlets tested	Plantlets negative	Efficiency
GLRaV-1	155	116	75%
GVA	30	13	43 %
GRSPaV	30	28	93 %

These preliminary results seem to indicate that in-vivo thermotherapy is effective in eliminating 3 important viruses in grapevine. However, further tests are needed to confirm on older cuttings the elimination of those viruses. Furthermore, the heat-treated Rèze has to be first evaluated during woody indexing before entering the certification programme. The advantage of the in-vivo thermotherapy is its simplicity and, unlike other tissue culture methods, does not present any risk of somaclonal variation.

16-Agro-infiltration of grapevine plantlets with artificial microRNAs directed against GFLVand evidence for target recognition and activity

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Grapevine fanleaf virus (GFLV, genus *Nepovirus*, family *Secoviridae*[1]) is the major causal agent of the grapevine fanleaf disease, leading to severe crop losses and fruit quality damage worldwide. Unfortunately, producers are lacking efficient technologies to control this infection. Recently, scientists modified precursors of microRNA (miRNA) in order to express artificial miRNAs (amiRNAs). These will recruit the natural miRNA pathway to silence endogenous or viral RNAs showing sequence complementarity [2, 3]. This approach has been chosen to target GFLV in grapevine, for virus-host studies.
Two amiRNAs were designed with a view to target the coat and the movement proteins of GFLV [4]. The recognition and the cleavage of the 21-nt long targets of these amiRNAs were studied using a GUS-sensor system as developed by Jelly and colleagues [5]. These GUS-sensors consist of the GUS gene fused to the 21-nt target of a particular amiRNA.
In vitro plantlets of grapevine were transiently transformed with amiRNAs and GUS-sensors by agro-infiltration. Recently, a vacuum-infiltration system has been established inour laboratory, based on Stephan and colleagues [6].
Extinction of the GUS-sensors was observed, showing the efficient targeting of the 21-nt GFLV sequences fused to the GUS gene by both amiRNAs. This is a primary step towards the use of this technology in transgenic grapevines, and the first evidence of amiRNAs activity in a plantlet.

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17-Assessment and characterization of the genetic diversity of viruses infecting cultivated yams (*Dioscorea* spp.) in Haïti

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Several viral species are known to infect yams (*Dioscorea* spp.) and to generate important yield losses on this vegetative propagated crop. Within the framework of the DEVAG project (Caribbean network for the development of agroecological horticultural systems) funded by the INTERREG IV Caribbean program, the inventory, the characterization and the prevalence of viruses infecting yams in Haïti have been undertaken to assess their impact on culture and to suggest appropriate control methods.

Approximately four hundred samples belonging to five yam species cultivated in Haïti (*D. alata, D. bulbifera, D. cayenensis, D. rotundata, D. trifida*) were collected in 2009 in farmers' fields in the five growing regions of yam. Virus detection was performed using broad spectrum PCR and RT-PCR tests targeting badnaviruses, CMV (genus *Cucumovirus*), potexviruses and potyviruses, after direct binding of viral particles on PCR tubes. Ninety four samples were infected by at least one of those viruses. The *Badnavirus* genus represents the most prevalent viral type. Hence, badnavirus RnaseH gene fragment (528 bp) was detected and amplified from most of the *D. bulbifera* samples (15/18) and *D. trifida* samples (6/6), and in half of the *D. alata* samples (61/132). Genetic diversity of these sequences is high (50-97%) and these sequences belong to six out of thirteen groups of yam badnaviruses identified by Kenyon *et al.* (2008). Analysis of the potyvirus sequences (CP - 282 bp) obtained from ten samples revealed the existence of YMMV on *D. alata* (7/132) and *D. trifida* (1/6) samples. Similarly, two strains of a new potyvirus were found in *D. trifida* samples (2/6) with a nucleotide identity of 74-79% with two viral species, ALiMV and PTV. Furthermore, three sequences (245 bp) related to the RdRp gene of potexviruses, with 71-78% of nucleotide identity with PAMV and PepMV were found in two *D. bulbifera* and *D. rotundata* plants.
This is the first inventory of yam viruses in Haïti which highlights a high viral prevalence rate with large amount of diversity within each virus species tested. Excepted for badnaviruses for which impact on yam production is misunderstood, the pressure of RNA viruses appears to be very low for the most common varieties, but strong for the rarer cultivars suggesting that a dramatic varietal genetic erosion is underway.

Kenyon L., Lebas B.S.M., Seal S.E. (2008). Yams (*Dioscorea* spp.) from the South Pacific Islands contain many novel badnaviruses: implications for international movement of yam germplasm. *Archives of Virology* 153: 877-889.

Mots-clés:
Epidemiology, Badnavirus, Potexvirus, Potyvirus

18-Detection of *Sugarcane yellow leaf virus* by a novel reverse transcription loop-mediated isothermal amplification method from three sugarcane production regions in Kenya.

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Sugarcane yellow leaf is a disease caused by *Sugarcane yellow leaf virus* (SCYLV). It is a major emerging disease of sugarcane that has been reported worldwide the last two decades. Efficient tools have been developed for detecting SCYLV but their use requires sophisticated facilities and still remains expensive. Our partners from developing countries cannot easily use them, which hamper an efficient and early diagnostic of the disease in sugarcane production regions. The main goal of this study was to develop an "easy-to-use" method for detecting SCYLV at the level of partner laboratories and potentially at the field level. We develop a novel reverse transcription loop-mediated isothermal amplification method (RT-LAMP) for detecting SCYLV. This method was compared to the classical methods routinely used at CIRAD Montpellier sugarcane quarantine facilities (RT-PCR and Tissue Blot Immunoassay). The three methods were then used for detecting SCYLV in three sugarcane production regions from Kenya, for which no data of prevalence of the disease existed so far. The first results showed that the RT-LAMP is more efficient than the reference methods. SCYLV is present in the three sugarcane production regions with prevalence rate ranging from 5% to 20%. This is the first report of sugarcane yellow leaf disease in Kenya. Furthermore, this is the first report on the application of the LAMP assay for early diagnostic of sugarcane yellow leaf disease from sugarcane production regions. Due to its simplicity, sensitivity and cost-effectiveness for common use, we believe that this assay should be used as an early diagnostic tool by our partners at the field level.

19-What prediction can be done from the consequences of an encounter between highly recombinogenic viruses

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Begomoviruses (Family Geminiviridae) have a circular single-stranded DNA genome (2.8 kb) and are transmitted by the whitefly *Bemisia tabaci* by the persistent modal. Tomato yellow leaf curl virus (TYLCV) is a begomovirus from the Middle East that has been disseminated in numerous regions of the world. This is the most invasive and damaging begomoviruses on tomato crop. In most regions where TYLCV was introduced, indigenous begomoviruses infecting tomato have been identified. The risk of emergence of recombinant forms of TYLCV is considered to be high as begomoviruses are known to be highly recombinogenic. The outcome of the meeting of TYLCV with Tomato yellow leaf curl Sardinia virus has been described in Spain (1) and Italy (2) and recombinant viruses with a wider host range and higher virulence than the parents were identified in field. The introduction of TYLCV in Reunion Island has created a dangerous situation because indigenous begomoviruses infecting tomato occurred in several islands of the South West Indian Ocean. The risk of the meeting of these indigenous begomoviruses with TYLCV species was estimated by testing recombinant generated artificial (3) or natural recombinants between TYLCV and Tomato leaf curl Mayotte virus (ToLCKMV). Considering their infectivity, relative accumulation and vector transmission, some recombinants genomes appeared to be serious candidates for the emergence.

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20-Identification, chez les plantes cultivées, de protéines partenaires impliquées dans la résistance au Potato Virus X (PVX)

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Le gène de résistance *Rx*, dont deux loci, *Rx1* et *Rx2* ont été identifiés, confère à la pomme de terre une très forte résistance (résistance extrême) vis à vis des souches communes du PVX (*Potato virus X*) [1]. Dans ce système biologique, l'étape de reconnaissance
implique une interaction sûrement indirecte entre la protéine RX et la protéine de capside, CP, du PVX (molécule élicitrice) [2]. Dans le pathosystème RX/PVX, la cascade de signalisation et les mécanismes conférant la résistance demeurent obscurs, même si il a été montré, par différentes technologies, que la protéine RX1 interagit avec la protéine RanGAP2 et que son accumulation nécessite la présence d'autres protéines telles que HSP90, SGT1 ou encore RAR1 [3-6]
Afin d'identifier les protéines hôtes impl.iquées dans la résistance liée au PVX, une stratégie de double hybride a été menée. L'appât a consisté en un fragment de 108 acides aminés de la protéine de capside du PVX, fragment identifié grâce à une série de délétions réalisées selon un protocole déjà utilisé pour d'autres capsides de Potexvirus [7]. Ce fragment a été montré, par expression transitoire chez *Nicotiana*, comme étant suffisant pour éliciter la réaction de défense médiée par *Rx1*. Une banque d'ADN complémentaires codant les proies, a été réalisée à partir de plants de *Nicotiana* de type sauvage ou exprimant le gène *Rx1*, infiltrées ou non avec le fragment éliciteur de 108 acides aminés de la CP du PVX.
Le criblage de plusieurs millions de clones a permis de mettre en évidence 12 interacteurs. Nous nous sommes plus particulièrement intéressés à deux interacteurs : un facteur de transcription et une protéase chloroplastique. Les interactions avec la CP ont été confirmées en levure, *in vivo* grâce à la technologie de BiFC et leur implication dans la voie de signalisation de la résistance liée à Rx 1 a été analysée par sous ou surexpression et par génétique inverse (TILLING). L'hypothèse de garde, selon laquelle il existerait un complexe protéique impliqué dans la résistance liée à Rx1, a été étudiée en tenant compte des interacteurs mis en évidence lors du crible double hydride.

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21-Effect of environment on plant architecture and virus-tolerance in *Arabidopsis thaliana*

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Plants have developed a variety of mechanisms to compensate for the cost of biotic or abiotic stresses. Parasites are important agents that can induce changes in host-life traits via multiple actions. Upon parasite infection, host may respond differently, which may be categorized into different strategies [1]: hosts can modify their behavior to avoid contact with parasites; hosts may develop mechanisms that prevent the establishment of infection and trigger defense responses; or host can go through life-history modifications that compensate for the negative effects of parasitism. The last tactic is considered part of tolerance mechanisms, since tolerance is defined as the host ability to reduce the effect of infection on its fitness [2]. In other systems than plant-virus model, various host life-history traits have been reported to be modulated consequently to pathogen infection. Life-history theory makes predictions for the adjustment of resource investment by organisms, based on the notion that trade-offs exist between resources allocated to different fitness components: growth, reproduction and survival [3]. Thus, parasite infection may modify optimal resource distribution and consequently induce plastic modifications to the host. Models for evolution of resource allocation predict that parasitized organisms will allocate more resources to reproduction, subtracting them from those dedicated to growth and survival.Our group tested predictions of life-history evolution theory by establishing the plant-parasite system of *Arabidopsis thaliana* and the generalist virus *Cucumber mosaic virus* (CMV) in order to study the effect of virus infection on plant growth and reproductive effort. It was shown that plastic modifications upon CMV infection were substantial compared to mock inoculated plants, with a general reduction of growth and reproductive effort detected following infection as well as a tendency to increase the age at maturity [4]. More specifically, such modification in allocation of resources due to infection and life-history responses was different depending on the allometric features of *Arabidopsis* genotypes and two groups were distinctively significant [4]. Nevertheless, since tolerance is a quantitative trait based on phenotypic plasticity upon infection, the optimal amount of resources allocated to each of these components may be corrected according to environmental conditions in order to maximize the organism's fitness. Hence, tolerance might be environment-dependent. To test this hypothesis, four Arabidopsis genotypes were inoculated with CMV and grown in conditions that differed in temperature and light intensity. In Llagostera (Ll-0) ecotype, the previously described resource allocation, from vegetative growth to seed production, was modified in various environmental conditions tested. Hardy conditions, naturally affecting life-history traits and resource allocation, may hide precise virus-effect on the architecture of the plant and on defense. However, such resource allocation is required to be present if we want to fully characterize the genetic determinants of virus-tolerance in *Arabidopsis*.

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22-Improvement of three nucleic acid isolation protocols for an overall diagnosis of viruses on six vegetative propagated plants

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Biological Resources Center (BRCs) must be able to guarantee the sanitary status of the resources they distribute, in order to prevent the spread or emergence of diseases. However, BRCs' vegetatively propagated crops do not benefit from the partial sanitation occurring through a seed cycle. This is particularly a problem for viral diseases, which have an overall high prevalence in vegetatively propagated crops. Variously effective sanitation methods exist for recovering virus-free plants but their successful implementation depends on on the availability of sensitive, polyvalent and reliable diagnosis tests for all relevant virus species.
The main objective of the SafePGR project is to improve the knowledge of the diversity of viruses infecting the vegetatively propagated crops addressed by the partners' BRCs (Universidad do Açores, Universidad da Madeira, INRA-CIRAD Guadeloupe and CIRAD La Réunion). Among the various issues addressed in achieving the goals of the SafePGR project, we need to develop new tools for an overall diagnosis of viruses. Thus, recent metagenomics methods associated with high-throughput sequencing will be tested. For this purpose, we started to develop and adapt three different nucleic acids extractions on six plants species: banana, garlic, sugarcane, sweet potato, vanilla and yam. First, we succeeded to extract small RNAs using Trizol or phenol:chloroform methods on these six species. Then, we have developed a protocol to semi-purify viral particles. The third protocol consisted in an enrichment of double-stranded RNAs. The quality and quantity of extracted nucleic acid varied among plant species. Overall, the extracted RNAs from garlic, sugarcane, sweet potato and vanilla were fulfilling criteria of quality and quantity for being used for metagenomic approaches whereas the ones from banana and yam were not adequate. These preliminary results tend to indicate that it would be probably difficult to develop a universal nucleic acid isolation method that could be routinely used by our partners' BRCs.

23-Couplage entre résistance génétique et aménagement parcellaire : vers une gestion efficace et durable du puceron *Aphis gossypii* et des épidémies virales en cultures de melon

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Le gène *Vat* confère au melon une résistance à la colonisation par le puceron *Aphis gossypii* ainsi qu'une résistance aux phytovirus non persistants (CMV, WMV,…) transmis par ces pucerons (Lecoq *et al*., 1979 ; Pitrat & Lecoq, 1982 ; Boissot *et al*., 2010). Il est toutefois inefficace pour bloquer la transmission des virus portés par les autres espèces de pucerons. L'utilisation de *Vat* est donc généralement couplée à des traitements aphicides afin de limiter la transmission virale par les pucerons non colonisateurs du melon. Or, la réduction progressive de l'usage des produits phytosanitaires dans la protection des cultures imposée par l'évolution de la réglementation (plan Eco-Phyto 2018) conduit à rechercher de nouvelles stratégies permettant d'accompagner la lutte génétique pour la gestion des bio-agresseurs.
La bibliographie suggère que l'implantation de bandes de plantes non hôtes (enherbées ou fleuries) à la périphérie des cultures pourrait être une option efficace pour diminuer 1) la pression puceron (et donc le risque de contournement de *Vat*) en favorisant le développement des ennemis naturels (Pfiffner & Wyss, 2004) et 2) la pression virus en constituant notamment un filtre réduisant la charge virale des pucerons avant qu'ils n'atteignent les cultures (Hooks & Fereres, 2006).
L'hypothèse testée dans ce projet est qu'un aménagement adéquat de l'environnement parcellaire peut contribuer à réguler les populations de pucerons et/ou leur potentiel virulifère et ainsi à réduire le risque d'épidémies virales. L'effet de trois types d'aménagement (sol nu, bandes enherbées et bandes fleuries) sur l'efficacité de la résistance médiée par le gène *Vat* est évalué au champ.

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Mots-clés : aménagement parcellaire, *Aphis gossypii*, Cucumis melo, protection intégrée, *Vat*

24-Mise au point de deux tests PCR multiplexes pour détecter simultanément des formes recombinantes et non-recombinantes du *Tomato yellow leaf curl virus* au Maroc

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Le Tomato yellow leaf curl virus (TYLCV, genre Begomvirus, famille Geminiviridae) a été isolé pour la première fois au Maroc en 1998 (Peterschmitt et *al*., 1999) et le Tomato yellow leaf curl Sardinia virus (TYLCSV) en 1999 (Monci et *al*., 2000). La gravité des attaques de la tomate par ces virus a obligé les agriculteurs à cultiver la tomate sous serre insectproof et à progressivement remplacer les variétés traditionnelles par des variétés tolérantes.

Le TYLCV et le TYLCSV étant des virus hautement recombinogènes (Garcia Andres *et al.*, 2007), le risque d'une émergence de recombinants TYLCV/TYLCSV dans une zone infestée par les deux virus était considéré comme très élevé. Pour confirmer ce risque, nous avons mis au point deux tests PCR multiplexes permettant l'identification de tels recombinants. De plus, pour obtenir une première estimation du potentiel d'émergence de ces recombinants ─ les recombinants sont-il détectés seuls ou en co-infection avec les parents ? ─ les tests ont été conçus pour cibler simultanément les recombinants potentiels et les virus parentaux. Outre la description de la technique, nous présenterons aussi quelques résultats préliminaires qui ont permis de valider la technique.

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Monci F, Navas-Castillo J, Cenis JL, *et al.* (2000) Spread of *Tomato yellow leaf curl virus* Sar from the Mediterranean Basin: Presence in the Canary Islands and Morocco. *Plant Disease* **84**, 490.

Peterschmitt M, Granier M, Aboulama S (1999) First report of Tomato yellow leaf curl geminivirus in Morocco. *Plant Disease* **83**, 1074.

25-Development of tools for the analysis of systemic infection of *Prunus sp* by *Plum pox virus*

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Plum pox virus (genus *Potyvirus*, family *Potyviridae*), the agent responsible of the sharka disease, is one of the main pathogens of stone fruits (apricots, peaches, plums…) production. This virus is transmitted in a non persistent manner by at least 20 different aphid species. The strategy used to control sharka in France includes the visual screening of trees and the removal of symptomatic individuals from infected orchards. However, due to the latency between inoculation of the virus and expression of symptoms, this control strategy is not optimal to limit the spread of the disease in orchards. To improve this control method a simulation model of epidemic is under construction (for more information, see the poster by Rimbaud *et al*.). The data currently implemented in our model are related to 'orchard' and 'landscape' scales. However, PPV spread results from both environmental parameters and plant-virus interactions. To grasp these interactions, we decided to focus a part of our researches on PPV at the plant scale. We want to investigate i) the accumulation of viral populations during single or multiple (simultaneous or delayed) inoculations of the host, ii) the dynamic of the production of viral genomes and particles in different parts of the infected perennial hosts, and iii) the impact of the inoculation site(s) on the dynamic of intra-host movement of virus particles. In the first step of this project, several tagged viruses derived from a PPV infectious clone and methods for the specific quantitation of each tagged virus were developed, tested and calibrated. Characteristics of these tools and next steps of the experimental procedure will be presented.

Keywords : PPV, infectious clone, real-time PCR, kinetic of infection

26-Towards the identification of plant factors involved in Potyvirus long distance movement

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Plant viruses exploit the long distance movement system of plant to promote systemic infection. Compatible interactions between phloem proteins and viral factors are expected to occur during this process. Several viral factors involved in this biological process have already been identified, while very few plant factors required for or inhibiting this mechanism have been described. In order to identify such factors in *Arabidopsis thaliana*, screenings of a companion cell (CC, cell-type constitutive of the phloem tissue) cDNA library using the yeast two hybrid (YTH) system were carried on with different baits, in particular with *Lettuce mosaic potyvirus* proteins (CP, VPg, HC-Pro) known to be involved in viral long distance movement and with the Restricted TEV Movement (RTM) proteins, phloem specific factors inhibiting long distance movement of some potyviruses.

Several candidates able to bind potyvirus CP and HC-Pro as well as arabidopsis RTM1 and RTM3 have been identified.. The functional validation has been undertaken for some of them. Fluorescent tagged proteins are being engineered in order to confirm interaction with viral or RTM partners *in planta* using confocal microscopy and FRET technique. Knock-out (KO) mutant lines have been selected to assess a virus long distance movement in plants affected in the expression of the candidate genes. .

In addition, subcellular localisations of the RTM factors were performed in phloem tissues using Arabidopsis transgenic lines expressing RTM genes fused to GFP under the control of their own promoter and in *Nicotiana benthamania* by agroinfiltration of fluorescent tagged RTM under the control of the 35S promoter.

27-Detection of *Potato virus Y* (PVY) in potato tubers: comparison of the reliability of five diagnosis methods

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Potato virus Y (PVY, *Potyviridae* family, genus *Potyvirus*) is one of the most widespread and damaging virus on potato crop mainly due to the predominance in the field of PVY^{NTN} variants that can induce severe necrotic symptoms on potato tubers. Little is known so far to explain the pathogenicity of these isolates. Only the potato variety and the type of PVY isolate are known to be critical in the expression of such symptoms. In the past decades, reverse genetic approaches have allowed the identification of several viral molecular determinants involved in PVY pathogenicity on tobacco plants. However, no molecular marker has been identified so far as a putative determinant of this necrotic process on potato tubers. Apart from the virus molecular characteristics, movement and replication dynamics of PVY^{NTN} isolates in potato tubers could be significant factors triggering this necrotic phenotype on tubers. In order to understand this process, it is essential to develop a reliable PVY diagnosis tool directly suitable on potato tubers. Based on the serological and/or molecular PVY properties, a whole range of diagnosis tools (antibodies, probes, PCR primers …) is available to detect the presence of the virus in a biological sample. However, depending on the nature of the tested material, the storage conditions and the viral concentration in the analysed plant tissues/organs, different techniques may have contrasting levels of reliability. Although the choice of the PVY detection tool in potato tubers is critical for diagnosis efficiency, it is surprising that only few studies have simultaneously attempted to compare ELISA, IC-RT-PCR, RT-PCR, real-time PCR and SNaPshot methodologies for their reliability on tubers. To address this comparison, tubers from 4 physiological stages (haulm destruction, harvesting, 1 and 3 months of storage at 4°C) were used as starting biological material. In addition, several extraction methods were tested to assess the quantity and the quality of RNA viral extracts. Results showed that regardless of the tuber physiological state, a TriZol®-based extraction protocol associated with a real-time PCR diagnosis proved to be the most reliable combination of methodologies to efficiently detect the presence of PVY in tuber samples. The increased sensitivity of this method could thus offer new opportunities for seed certification potato professionals by allowing a faster assessment of seeds sanitary quality.

28-How eBSV polymorphism could enlighten BSV and banana evolution story?

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The nuclear genome of banana plants is invaded by numerous viral sequences of banana streak viruses (BSV), a DNA virus belonging to the family *Caulimoviridae.* These integrations are mostly defective as a result of pseudogenisation driven by the host genome evolution. Conversely some named infectious, can release a functional viral genome following activating stresses. We characterized the infectious endogenous BSV (eBSV) for three BSV species (BSOLV, BSGFV and BSImV) present within the *Musa balbisiana* B genome of the seedy diploid Pisang Klutuk Wulung (PKW). Our aim is to study PKW-related BSV integrations among the diversity of the banana B genomes in order to retrace the evolutionary BSV and banana stories

We extended on purpose the *M. balbisiana* diversity by the addition of interspecific hybrids with *M. acuminata* showing different levels of ploidy for the B genome (ABB, AAB, AB) of the banana sample in order to include unsampled or extinct *M. balbisiana* ressources. We also based the analysis referring to the two areas of sympatry between *M. acuminata* and *M. balbisiana* and the centers of origin for the most largely cultivated AAB cultivars. One was in India and the other one in East Asia going from Philippines to New Guinea (Perrier et al, 2009).

We characterized the PKW-related eBSV allelic polymorphism using PCR markers (described in poster Galzi & Duroy et al.) and Southern blots on 77 accessions. We codified the results of Southern blot and PCR in order to calculate a common dissimilarity matrix and interpret the eBSV distribution. As a result, three dendrograms of PKW-related eBSV made with the Neighbor Joining (NJ) method on the 77 banana accessions, for each BSV species, are presented as well as one dendogram resulting of NJ analysis for the three BSV species all together . We show that the known phylogeny of banana accessions can enlighten the eBSV structure diversity and that eBSV polymorphisms can help to understand the particularly unresolved *M. balbisiana* diversity. An evolutionary scheme of BSV/eBSV banana evolution will be proposed.

Keywords: Banana Streak Virus, *Musa sp.*, Phylogeny, Viral Evolution, Endogenous Pararetrovirus.

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29-Phage Display : a track to decipherplant cellhighjacking during Potyvirus infection ?

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Les Potyvirus, parmi lesquels le PlumPox Virus (PPV) infectant les arbres fruitiers et le LettuceMosaic Virus (LMV) infectant la laitue, occasionnent des dégâts socio-économiques considérables dans le monde entier. Les virus de plantes sont des parasites obligatoires, ce qui entraine la mise en place d'un réseau complexe d'interactions entre les facteurs de l'hôte et du virus lors de l'infection. De ce dialogue découle la sensibilité ou la résistance de la plante à l'infection virale. La recherche d'interactions entre protéines du virus et facteurs de l'hôte a permis d'identifier des facteurs de résistance et représente une bonne alternative aux méthodes de génétique. Jusqu'à présent, le facteur d'initiation de la traduction 4 E (eIF4E) a été identifié comme un interacteur de la protéine des potyvirus liée au génome viral (VPg) et s'est révélé être le produit d'un gène candidat codant pour une résistance récessive aux potyvirus.Mais l'adaptabilité virale rend difficile l'élaboration d'une résistance durable de la plante. Un grand nombre d'interactions encore inconnues conditionnent le cycle infectieux. Pour accroitre la durabilité de la résistance, il est envisagé de combiner dans une même plante deux gènes codant pour des protéines défectueuses pour les interactions nécessaires au virus. Nous avons criblé par phage display une banque de peptides aléatoires en utilisant comme appâts plusieurs composants viraux. On a recherché dans le génôme d'Arabidopsis les séquences peptidiques communes avec celles des peptides sélectionnés pour leurs interactions avec le virus. Un criblage réalisé contre la capside d'un potyvirus a permis d'identifier un nouvel interacteur candidat. Les tests de validation fonctionnelle de cette protéine sont en cours.

Remerciements à Agathe URVOAS et Philippe MINARD pour leur aide à la mise en place de la technique phage display

30-Analysis of key mutations in the VPg of *Lettuce mosaic virus* involved in eIF4E-resistance breaking.

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Previous studies have shown that eIF4E (eukaryotic initiation factor 4E) controls lettuce susceptibility to the potyvirus *Lettuce mosaic virus* (LMV) (1). Indeed, the widely used *mo1*^{1} and *mo1*^{2} resistance alleles of the *mo1* gene correspond to allelic mutant forms of eIF4E in lettuce. Reverse genetics and immunochemical studies (2, 3) revealed the central role of CI and VPg viral proteins, in eIF4E-resistance breaking. Mutations in the VPg allow only the overcoming of *mo1*^{1}, while mutations in the C-terminal part of the CI helicase confer the ability to overcome both resistance alleles.
The alignment of VPg sequences derived from several virulent and avirulent LMV isolates allowed us to identify 5 candidate amino acids in the VPg, potentially involved in *mo1*^{1} resistance breaking. Furthermore, a recombinant derived from LMV-E (virulent), containing the central part of the CI from LMV-0 (avirulent), was able to overcome *mo1* resistance, as predicted by the fact that it contains the VPg of the LMV-E resistance-breaking isolate. However, individual variants of this recombinant accumulated mutations not only in the CI C-terminus but also at some of the 5 candidate positions of the VPg upon propagation on *mo1*^{1} lettuce plants.
Site directed mutagenesis was used to obtain single or double mutant LMV-0 cDNA infectious clones, for each of the 5 candidate amino acids. The analysis of their biological behavior towards the *mo1* alleles will be reported.

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31-Analysis of the diversity of the virus and the vector involved in wheat dwarf disease

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The wheat dwarf disease, described on cultivated (wheat, barley, oat,…) and wild *Poaceae*, is induced by the *Wheat Dwarf Virus* (WDV, genus *Mastrevirus*, family *Geminivirus*). This plant virus is transmitted by species (e.g. *alienus*) of the leafhopper *Psammotettix* in a persistent manner. Since the first description of WDV in the 1960s, this pathogen has been considered as a homogeneous viral species. However, recent studies have assigned WDV isolates in at least two strains (i.e. WDV and WDV-bar strains). Even if WDV and WDV-bar are mainly described in wheat and barley, respectively, the host ranges of WDV strains overlap. Thus, a cereal field can be infected by isolates from the two strains. Molecular characteristics of WDV strains have been recently described but haven't been used to upgrade epidemiological data associated to this disease. Consequently, to collect new data on the epidemiology of wheat dwarf, it is important i) to consider isolate from each WDV strain as a pathogen responsible, alone or in association with isolate from the other strain, of wheat dwarf disease and ii) to set up tools to specifically identify the presence of each WDV strain in field collected samples.

Leafhoppers of the genus *Psammotettix* have been caught from French cereal fields and individually maintained on wheat plantlets in growth chamber under controlled conditions. This biological material has been used to determine both the viruliferous status of each insect and the capability of viruliferous leafhoppers to transmit WDV to healthy wheat plantlets. Then, strain-specific molecular tools have been developed and used to characterize the WDV strain(s) present in viruliferous *Psammotettix*. Finally, the viral load of these insects has been estimated by real-time PCR. To complete the work performed on WDV, characterization of leafhoppers has been initiated. Aedeagus of males have been measured and vibrational communication, known to be species-specific, produced by *Psammotettix* males and females have been recorded.

The analysis of produced data allows to acquire new information on the leafhopper species/viral strains involved in the epidemiology of wheat dwarf in France. In addition to scientific knowledge resulting from this study, the presented work produces important data for future estimation of risks associated to the presence of the virus (WDV strain(s)) and the vector (*Psammotettix sp*) involved in wheat dwarf disease in cereal growing areas. This will be helpful for future managements of plant protection.

Keywords : WDV, Psammotettix, epidemiology, detection, vibrational communication

32-Combining experimental assays with epidemiological surveys to assess indicators of *Plum pox virus* epidemicity

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The huge increase in sequence data those last decades has allowed defining better demarcation criteria for viral taxonomic assignments. For the epidemiologists, such demarcation based on molecular properties, especially at the strain level, is particularly meaningful if one can link the different strains to specific phenotypes (host range, pathogenicity,…) and can thus identify specific epidemic risks for a given host or for a given agro-ecological context.

Plum pox virus (PPV, genus *Potyviru*s, family *Potyviridae*) is responsible for the sharka disease, the most serious disease of stone fruit trees (*Prunus* species). In the framework of a collaborative European research program, an important effort has been made to increase the knowledge of PPV genetic diversity (www sharco.eu). Based on molecular criteria (genetic distances and successful recombinant genomes), eight different strains have been distinguished but very little is known about their specific biological and epidemiological properties. Such a situation is due to different reasons: (i) the quarantine pest status of PPV does not allow experiments unless carried out under a strict confinement; (ii) the perennial nature of the *Prunus* hosts makes the experiments generally long and complicated; and (iii) the epidemiological behaviour of a given strain (or even isolate) can be influenced by specific agro-ecological conditions occurring at a given location.

In this study, we combined biological experiments with epidemiological surveys to gain a better knowledge on PPV epidemic properties. A first approach was thus developed for the three most prevalent PPV strains (M, D and Rec) in Europe, which have different geographical distributions. The experimental assays were designed to allow measuring indicators of the key steps of the infection cycle on three *Prunus* species (apricot, peach and plum): (i) ability for a given strain to infect the host after aphid-mediated (using a clone of *Myzus persicae*) inoculation, (ii) dynamic of infection in each *Prunus* host, and (iii) efficiency of the infected *Prunus* as virus sources (acquisition-transmission assays carried out with *M. persicae* as vector). In parallel to these experimental assays, surveys have been carried out in Serbia in order to assess (i) the relative prevalence of these three PPV strains known to spread in this country, and (ii) their *Prunus* host preference.

These two approaches bring different but complementary information. The experimental assays did not evidence strict host specificity (pathotype) but rather several cumulative advantages (shorter latency in apricot, better generalization in peach, higher overall aphid transmission rates) that should confer a higher epidemicity to the PPV-M strain. The results from surveys evidenced a strong host specificity (PPV-M on peach), frequent co-infections and independence between infections by the three PPV strains in plum.

Keywords: epidemic properties, experimental assays, epidemiological surveys, sharka

33-Do viruses sense the vector?

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The DNA virus *Cauliflower mosaic virus* (CaMV) and the unrelated RNA virus *Turnip mosaic virus* (TuMV) use the non-circulative mode for transmission by aphids: virus particles bind to a receptor located at the tip of the aphid stylets (needle-like mouthparts) when the aphids insert the stylets into cells while feeding on infected plants. When aphids change the plant, the viruses are transported in the stylets to a new host and inoculated into it.
We have shown that CaMV forms an intracellular transmission body (TB) that is specialized for transmission and that transmission requires living cells. This indicates that transmission of CaMV is not by accidental contamination of the vector mouthparts but results from specific interactions between the virus and the vector during the acquisition process. Our results (see also communication by Aurélie Bak) show that the TB reacts specifically on the arrival of the aphid vector and disintegrates rapidly and reversibly, thereby distributing transmissible virus complexes on microtubules throughout the cell and enhancing greatly transmission.
Two major questions arise: 1.) Is CaMV the only virus able to anticipate the vector's arrival and prepare accordingly for transmission? 2.) How does the virus sense the vector?
Here we show that like for CaMV, also vector acquisition of TuMV requires living cells and thus does not result from simple vector contamination. This indicates that CaMV is not the only virus to prepare purposely for transmission a the right time. A pharmacological analysis shows that calcium signalling is important for aphid sensing by both viruses. Taken together, we propose that vector sensing by viruses might be a general phenomenon enabling efficient transmission.

34-Banana plants use post-transcriptional gene silencing to control banana streak virus infection.

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Banana streak virus (BSV), the causative agent of banana streak disease, is a plant pararetrovirus belonging to the family *Caulimoviridae*, genus *Badnavirus*. The genome of BSV is a circular double-stranded DNA of 7.4 kbp made of three ORFs and like other pararetroviruses replicates via reverse transcription of viral pregenomic RNA (Lockhart, 1990). While the first two ORFs encode two small proteins of unknown function, the third ORF (~210 kD) encodes a polyprotein that can be cleaved to yield the viral coat protein and proteins with homology to aspartic protease, reverse transcriptase and RNaseH. Little information is available about antiviral defense response of the host plant on BSV or other members of *Caulimoviridae*. RNA silencing, also known as RNA interference (RNAi), is an ancient gene regulation and cell defense mechanism, which exists in most eukaryotes (Xie and Qi, 2008). Plants have adapted the RNA silencing machinery into an antiviral defense system. Interestingly, Arabidopsis plants infected with *Cauliflower mosaic virus* (CaMV), a type member of the genus *Caulimovirus* in the family *Caulimoviridae*, accumulate siRNAs of 21, 22 and 24 nt size classes, where the 24 nt species are the most predominant ones (Blevins et al., 2006; Moissiard and Voinnet, 2006). Further analysis showed that, the leader region (600 nt) of CaMV pregenomic RNA produces massive amounts of siRNAs with several hot and cold spots of siRNA generation (Blevins et al., 2011) to function as a decoy for the RNA silencing defense system of the plant. To determine whether the viral decoy strategy was universally used among viruses belonging to the family *Caulimoviridae*, we have performed a deep sequencing of total siRNAs of 6 Cavendish banana plants infected independently with one of the 6 BSV species we own in the laboratory. We obtained for the first time, experimental evidence of virus-derived small RNA (vsRNA) from those 6 BSV species by blasting sequencing data against the 6 BSV species genomes. vsRNA are enriched in 21-nt class thus BSV are likely silenced at the post-transcriptional level. Besides, our data unequivocally show that the decoy strategy used by the CaMV is not employed by the BSV since most of the hot spots of siRNA production are located in ORF1 and 2.
Information generated about siRNAs derived from BSV genome could help us to design silencing-based transgenic and non-transgenic (RNA vaccination) approaches to obtain BSV resistance in banana crop.

Keywords : banana, silencing, BSV, siRNA

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35-Generic detection and identification of pospiviroids

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The genus Pospiviroid comprises 9 species which are all damaging to tomato, potentially damaging to potato and that could cause symptoms on chrysanthemum and citrus trees. *Potato spindle tuber viroid* (PSTVd) and *Chrysanthemum stunt viroid* (CSVd) are considered as quarantine organisms for the EU and the risks posed by the other pospiviroids have been recently analyzed by the EFSA. A simple method of detection and identification of all pospiviroids is then needed to manage these pathogens and further understand their epidemiology. A generic method based on a single RT-PCR and direct sequencing is proposed here.

36-Intrinsic Disorder of viral proteins, mutational robustness and host adaptation using the potyviral VPg as a reporter.

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Introduction: Intrinsic Disorder Domains (IDD) into proteins are regions devoid of highly populated secondary and tertiary structure under physiological conditions. The genome of RNA viruses codes for proteins containing an important proportion of IDDs.

It has recently been proposed that IDDs, with their low topology requirements, could confer mutational robustness allowing viruses to tolerate high mutational rates and to accumulate more mutations within proteins without extensive loss of fitness. An exploration by the virus of a broader sequence space without compromising its survival could afford a faster adaptation to various hosts.

PVY (Potato Virus Y) a member of the genius potyvirus, possesses a viral protein linked to its genome (VPg), which contains three IDDs. The VPg interacts with well-identified host proteins (translation initiation factors). This interaction is required for virus replication. The interaction domain has already been mapped to the central IDD.

In susceptible plants, infection is associated with an interaction of the VPg with the eukaryotic Initiation Factor 4E (eIF4E) [1]. In resistant plants, mutations in eIF4E do not permit association with the VPg. A strict molecular relationship has thus been identified between biological infectivity and a physical interaction of VPg with plant translation initiation factors.

We will use the PVY-pepper model system to assess whether there is a correlation between intrinsic disorder in viral proteins, mutational permissiveness and host adaptation.

Results: We first analyzed the possible correlation between disorder and evolutive selection pressure. Resolution of dN/dS at each codon position from 44 PVY VPg was used to localize sites of diversifying selection from site of purifying selection pressure. Similar to some results from eukaryotic studies [2], we found through this predictive analysis that, although IDD are strictly conserved in the VPg, they display two kinds of evolutive behaviors : some domains (N-terminal and NTP-binding) seem to be highly conserved at the amino acid sequence level ("constrained disorder"), whereas some others, and especially the central domain, are not ("flexible disorder"). The VPg central domain interacts with several proteins which imply some kind of structural plasticity. Whether this feature correlate with mutational permissiveness remains to be analyzed.

Perspectives: We will use libraries of VPg (a disordered protein) and eIF4E (ordered) mutants to build a graph of the VPg-EIF4E binary interaction versus mutation accumulation in a view to compare the robustness evolution of two types of proteins (ordered and disordered).

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37-A 6.5Å cryo-EM structure of the *Arabis mosaic virus* reveals new insights into capsid function

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Arabis mosaic virus (ArMV) and *Grapevine fanleaf virus* (GFLV), two closely related *Nepovirus* belonging to the *Secoviradae*family,are the major agents responsible for a severe degeneration of grapevines that occurs in most vineyards worldwide. Both viruses are naturally transmitted by ectoparasitic*Xiphinemae*nematodes from plant to plant. Their bipartite RNA genomes are encapsidated into a 30 nm icosahedral viral particle formed by 60 copies of a single capsid protein (CP). Although sharing a high level of sequence identity and a similar genetic organization, these two closely related viruses are transmitted bydifferent vectors:ArMV is specificallytransmitted by the *Xiphinema diversicaudatum* whereas *X. index*is the vector for GFLV (1).Moreover, the structural determinants involved in the transmission specificity of both viruses map solely to their respective CP(2,3,4). Recently, reverse genetic experiments targeting the GFLV CP combined with GFLV crystallographic structures solved in our laboratories, revealed that a positively charged pocket in the CP B domain located on the surface of the GFLV particlemay be responsible of vector specificity (4,5). However, none of the structural determinant of ArMV CPhas been identified so far (4,5). To gain further insights into the ArMVCP determinants involved in transmission specificity, we aimed to determinethe structure of ArMV. As all ArMV crystallization trials failed, we therefore decided to conduct a cryo-EM study. A 6.5 Å resolution cryo-electron microscopy structure of ArMVwas obtained by using a molecular dynamics flexible fitting approach. This study allowed us to propose a pseudo-atomic structure and highlights connections between ArMV capsid shell and its RNA. Based on the comparison betweenArMV and GFLV structures, new insights regarding the transmission specificity of the *Nepovirus*will be presented.

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38-Insights into early events of GFLV transmission by the dagger nematode *Xiphinema index*

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Grapevine fanleaf virus (GFLV) is a major pathogen on grapes and the causal agent of the grapevine fanleaf degeneration disease (court-noué de la vigne in french and Reisigkrankheit in german) that affects vineyards worldwide and in particular in the Upper Rhine region. No natural resistance to this virus is known in grapevine. Under natural conditions, the virus is transmitted specifically and exclusively on grapevines by the dagger nematode *Xiphinema index*. Transmission is a two-step process: first the virus is acquired by nematodes upon feeding on roots from infected plants and specifically retained in the nematode feeding apparatus. In a second step, the virus is inoculated to healthy plants during subsequent feeding events on roots from by viruliferous nematodes. So far little is known about this transmission process by nematodes and a better understanding of these events could provide new means to control the disease.

In the frame on the Upper Rhine Interreg Bacchus Project that includes the Staatlichen Weinbauinstituts and the Albert-Ludwigs-Universität Freiburg, the Julius Kühn-Institut, the DLR Rheinpfalz, the Université Haute-Alsace and the Institut de la Recherche Agronomique Colmar and the Centre National de Recherche Scientifique Strasbourg, we are aiming at studying the early events of GFLV transmission to grapevine by *X. index*. In particular, our main goal is to understand how GFLV manages to initiate infection upon feeding of viruliferous nematodes on grapevine roots.

To do so *in vitro* nematode transmission tests on grapevine involving recombinant GFLV-encoding fluorescent proteins will be developed to study GFLV multiplication in primary infected root cells from grapes. This involves (i) the production of viruliferous nematode populations carrying different recombinant GFLV encoding fluorescent proteins, (ii) the optimization of *in vitro* GFLV transmission tests with nematode-sensitive or partially nematode-resistant grapevine rootstock varieties, (iii) the monitoring of GFLV in primary infected cells using the fluorescently tagged viral proteins, (iv) the detection of GFLV in living nematodes using GFLV-specific chromobodies (peptides of 12 to 15 kDa derived from camelid single-chain immunoglobulins fused to fluorescent proteins) and (v) the spatio-temporal analysis of the actomyosin network upon GFLV multiplication in primary infected cells by confocal microscopy and real-time life-cell imaging techniques. Preliminary results will be presented.

39-*In vitro* recombination based on « Chew Back Annealing » (CBA): a new strategy to facilitate the construction of full-length infectious cDNA clones of plant RNA viruses?

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The avaibility of full-length infectious cDNA clones (FLcDNAs) is crucial for reverse genetics studies on plant RNA viruses. Approaches to simplify and accelerate the construction of FLcDNAs for plant viruses have been recently described, based on cloning strategies involving homologous recombination in yeast rather than more classical cloning approaches such as *in vitro* ligation (Youssef et al., 2011 ; Desbiez et al., 2012).
In the present study, we used recombination in yeast to generate within less than two weeks FLcDNAs clones derived from *Lettuce mosaic virus* (LMV, potyvirus) that can be inoculated either biolistically or by agroinoculation. The genomic RNA of LMV is about 10.5 kb in length. Using high fidelity long distance PCR and recombination in yeast, we were able to obtain point mutants and GFP-tagged LMV FLcDNAs. The percentage of infectious clones obtained was at least 75% in the conditions used. The fact that recombination in yeast is very efficient, even with short, 30-40 nucleotides-long overlap regions between fragments created using PCR primers, highly facilitated the construction of such recombinant LMV genomes.
In parallel, we are developing an even more rapid and efficient *in vitro* recombination system based on assembling and repairing overlapping PCR-amplified DNA molecules in a single isothermal step. This process involves the concerted action of a 5' exonuclease, a DNA polymerase and a DNA ligase. This approach has been used to join DNA molecules as large as 583 kpb and to clone up to 300 kbp joined products in *Escherichia coli* (Gibson et al., 2009). Named chew back annealing (CBA), this strategy could therefore be used to construct entire synthetic viral genomes. The first results obtained for the engineering of the LMV genome will be presented.

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40-Detection and identification of chrysanthemum tospoviruses

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Impatiens necrotic spot virus (INSV) and *Tomato spotted wilt virus* (TSWV) are *Thripidae* transmitted pathogens known to infect, among a wide range of host, chrysanthemum. More recently a third tospovirus closely related to TSWV, *Chrysanthemum stem necrosis virus* (CSNV) has been identified. Because these three viruses induce similar symptoms and a lack of a robust detection method of discrimination, their actual distribution and epidemiology is not well known. A generic detection and an identification based on a single RT-PCR with degenerate primers followed by a direct sequencing are proposed here to address this problem.

41-Grapevine Vein Necrosis syndrome is not exclusively associated to GRSPaV group I and II molecular variants

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Grapevine Rupestris stem pitting-associated virus (GRSPaV), member of the genus *Foveavirus* within the family *Betaflexiviridae,* is one of the most common viruses in grapevine and is currently detected in all grapevine areas in the world**(1)**. This flexuous virus is composed of a single-stranded positive-sense RNA of 8725 nucleotides (variant GRSPaV-1) and 5 open reading frames (ORF). Six GRSPaV variants **(5)** have been fully sequenced to date: GRSPaV-1, -SG1, -BS,-SY, -PN, -MG. They are divided into five main clusters defined as: cluster I (1), cluster II (SG1, MG), cluster III (BS), cluster IV (SY) and cluster V (PN)**(4)**GRSPaV is the putative causal agent of the Rupestris Stem Pitting (RSP) syndrome and some of its variants are also supposed to be involved in diverse syndromes on *Vitisvinifera*such as Vein Necrosis (VN).

In the present study we aimed to better delineate the relationships between GRSPaV variants and VN syndrome. Therefore, we analyzed the genetic diversity of GRSPaVinfecting fifteen grapevine accessions (ten rootstock and five scion varieties) showing different behaviors towards VN.

Our results lead to moderate the strict etiological relationship previously proposed by other scientists**(2, 3)** between VN syndrome and molecular variants belonging toGRSPaV group I and II. We showed that vein necrosis symptoms can develop in grapevine either in the absence of variants of these groups I or II, or when these variants are in minority. By contrast, some plants remain free of symptoms even in the presence of group I or II variants. The etiology of VN syndrome proves therefore to be more complex than currently thought and may involve so far overlooked factors.

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42-Impact assessment of transgenic grapevine rootstocks expressing viral coat protein and bacterial *nptII*genes,on viral populations and on the soil microflora, using new generation sequencing

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Grapevine fanleaf virus (GFLV) is responsible for fanleaf degeneration, which is the most severe virus disease of grapevine. GFLV causes important economic losses by reducing grape yield, lowering fruit quality, and shortening the longevity of vines. It belongs to the genus *Nepovirus* in the family *Secoviridae* and is exclusively transmitted by longidoridectoparasitic nematodes. Genetic engineering by the development of the genetically modified rootstock (GM) relying on the concept of pathogen-derived resistance (PDR),is the most promising strategy to develop GFLV-resistant grapevines. Regarding PDR, we are interested in determining if GM rootstocks expressing GFLV coat protein gene had any impact on the genetic variability of GFLV isolates and on the structure of GFLV field populations. We also aim to assess if this strategy could contribute to the emergence of viable recombinants involving integration of viral transgene transcripts fragments from GM rootstocks in viral RNAs of the indigenous GFLV populations. Using a conventional RT-PCR approach, we studied the molecular variability of GFLV isolates from 190 transgenic and 157 non-transgenic plants in an open-field trial. Our results indicate that transgenic grapevines did not contribute to the emergence of viable GFLV recombinants to detectable levels and did not affect the molecular diversity of indigenous GFLV populations during the trial period (1). Interestingly, five GFLV recombinants were identified in conventional grapevines from this experimental field site (1), in keeping with recent studies demonstrating that both purifying selection and recombination are important evolutionary mechanisms in the genetic diversification of GFLV(2). We aim to pursue the in-depth evaluation of the environmental impact of transgenic rootstocks by a metagenomic approach. Impact studies will be expanded on the potential environmental consequences of the two transgenes (GFLV coat protein gene and *nptII* gene) expressed in GM rootstocks. Our research project (ANR IMA-GMO), aims at a better evaluation of the consequences of the growth of GM plants in the field on the associated microflora, bacteria and plant viruses. IMA-GMO will take advantage of next generation sequencing (NGS) technologies, to analyse the potential impact of GM plant growth either via expression of transgenes or via possible transgene transfer events (horizontal gene transfer) to microbial populations (including viruses) from a reference or a vineyard soil, irrespective of the cultivability of these microbes. Preliminary results show no significant major modifications in the structure of the virus and bacterial populations, but highlightthe influence of the environment (greenhouse versus field conditions) on modifications of the soil metagenome. Our results further stress the necessity to perform environmental impact assessment in long-term experiments underreal agronomical conditions.

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43-The *Grapevine fanleaf virus* interactome approached in a yeast-two-hybrid system allowing N- and C-terminally fused proteins.

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Grapevine fanleaf virus (GFLV) is responsible of fanleaf degeneration, one of the most severe virus diseases of grapevines worldwide.
GFLV is a bipartite RNA virus of positive polarity, which is translated into two polyproteins, named P1 and P2. The cleavage of P1 by the viral proteinase $1D^{Pro}$ gives rise to five mature proteins 1A, $1B^{Hel}$, $1C^{Vpg}$, $1D^{Pro}$ and $1E^{Pol}$ whereas the cleavage of P2 leads to three final proteins $2A^{HP}$, $2B^{MP}$ and $2C^{CP}$. To date, little information is available on the interactions between these eight viral proteins. In order to confirm probable interactions and gain insights into how viral proteins contribute to a given function, we used a global approach in a yeast two-hybrid system to establish the viral interactome.
Using the recently described Gateway vectors (Stellberger and *al.*, 2010), the eight viral proteins were fused at both their N- and C-terminus to the activation domain (AD) and the binding domain (BD) of the Gal4 transcription factor, allowing four different bait-prey combinations per protein pair: NN, CC, NC, and CN. The transmembrane domains of the $1B^{Hel}$ protein were deleted to include this protein in the yeast two-hybrid test. The 32 expression vectors were then introduced in a mat a or a mat α yeast strain. Western blot analyses were performed to check the expression of the recombinant proteins prior to mating. To test the 64 pairwise interactions, all 256 possible diploid yeasts were generated and plated on different media. A complete map of the interactions and their redundancy in different permutations will be presented.
In order to validate our results and to include the complete membrane protein $1B^{Hel}$, we tested a few of these interactions in *Nicotiana benthamiana* by Fluorescence Lifetime Imaging (FLIM). Confocal images as well as FLIM results will be presented.
To our knowledge this is the first global study of a plant virus interactome. These results open the way to a better understanding of GFLV. The interactions identified in this work will now be further investigated at a functional level and domains of interaction will be mapped. Finally, the yeast vectors containing GFLV genes will be used to screen an *Arabidopsis thaliana* library to identify cellular partners.

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44-Plant biotechnology to develop new plant–virus resistance and production of vaccines or targeted biomolecules : case of Rice yellow Mottle Virus (RYMV)

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Viruses display a large panel of opportunities to be both targets and tools for plant biotechnology. First of all, efficient resistance against viruses can be developed using RNAi strategy to express viral specific artificial siRNA and miRNA. The success of this strategy needs to combine knowledge about virus diversity, identification of gene silencing suppressors and accurate transgene constructions to release multiple small RNA targeting several viral genes. In addition, plants display considerable potential to be used as bio-systems for the economic production of a high level of natively folded proteins of interest. Recent experiments of biopharmaceutical production from transient expression in plants are promising. This is because plants have several advantages over other production systems including: (1) low cost of production, (2) production of multimeric proteins, such as antibodies, (3) they are safer, not being hosts of human pathogens, and (4) glycosylation occurs in plants making vaccines stable. However, the low level of recombinant proteins produced by this technology remains a serious constrain to its development. Basically, the low level of expression results of the activity of PTGS (*Post transcriptional gene silencing*) against the transgene. To circumvent this problem, we proposed to improve the yield of recombinant protein production by identifying (i) a cocktail of viral silencing suppressors that allowed us to neutralize PTGS and to express transiently a high level of recombinant protein in *Nicotiana benthamiana* leaves using a classical 35S-based vector. (ii) by developping a *rice yellow mottle virus* (RYMV)-based expression vector to increase transgene copy number. First application has been done for the production of an anti-*leishmania* vaccine with the expression of the Promastigote Surface Antigen (PSA) of *Leishmania* in *N. benthamiana* leaves. This parasite causes a wide spectrum of human diseases in many tropical and subtropical regions. Vaccine development is the only way to control this disease since there is no efficient therapeutic treatment available.

45-Model-based design and assessment of management strategies for epidemics in a heterogeneous landscape

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Epidemics are often managed by strategies mainly based on expert opinions, although the spread of a disease results from complex interactions between biological processes, to which may be added human interventions. Thus, it is no easy task to optimize disease management, especially when alternative strategies cannot be tested experimentally.

Therefore, this project aims to develop a comprehensive spatiotemporal model which simultaneously simulates the propagation of an epidemic in a heterogeneous landscape and the impact of several innovative management strategies. The key parameters of the model will first be identified by a sensitivity analysis and, when possible, estimated through experiments or statistical analyses of epidemiological data. Then, a wide range of potential management strategies will be tested through the model by Monte Carlo simulations.

The model developed in this project will be applied to sharka, caused by *Plum pox virus* (PPV, genus *Potyvirus*), a quarantine pathogen in the European Union. Indeed, this model is interesting to circumvent the difficulty in carrying out experiments due to the sanitary status of this pathogen. Besides, the improvement of management strategies for this disease is vital because it is the most devastating viral disease of stone fruits (e.g. peach, apricot and plum trees), with a cost of more than 10 billion Euros between 1975 and 2005 in the world. In France, the management of the epidemics is based i) on regular visual inspections of the orchards and ii) on the removal of symptomatic trees. The first step of the project is to make the model flexible enough to include this current management strategy, by taking into account the mandatory observation, sampling and control processes.

Finally, the general approach of this Ph.D project may be used to help managing other sanitary crises due to epidemics.

Key words: model, epidemics, disease management, sharka, *Plum pox virus*

46-Towards a better characterization of endogenous badnavirus sequencesofyams (*Dioscorea* spp.)

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Yams, and more generallytubers, are very important crops for food security in tropical and subtropical countries.They are propagated vegetativelytherefore they accumulate viruses over long periods of time. Viruses are currently the main constraint for yam production and yam germplasm conservation and distribution.
A wide range of badnavirus sequences belonging to 13 distinct viral species were amplified from genomic DNA of severalyam species when using badnavirus degenerate primers [1; 2].However, we consistently observed that the proportion of amplification products raised by PCR performed on total genomic DNA is significantly higher than that raised by direct binding PCR, which has been designed to detect episomal forms of yam badnaviruses.Both observations have fueled suspicion that yams might host endogenous badnavirus sequences, and possibly infectious ones like bananas [3]. Therefore, search for endogenous badnavirus sequences was undertaken in yam accessions conserved in the germplasm collection of the Guadeloupe Tropical Plant Biological Ressource Center (CRB-PT) and the yam quarantine facility in Montpellier (France).

Southern blots performed on genomic DNA extracted from uninfected *Dioscoreatrifida*and using parts of yam badnavirus genomes as probes confirmed the suspicion of endogenous badnavirus sequences in yam genomes. Furthermore, PCR performed on genomic DNA extracted from healthy seedlings of *D. alata* and *D. rotundata* using badnavirus degenerate primers raised amplification products whose sequences fit in the current phylogeny of badnaviruses. Amplification products raised from several of these DNA samples by long-PCR displayedrearrangements such asduplications andreversions which are reminiscent of endogenous badnavirus sequences encountered in the genome of other crops such as banana. Similarly rearranged sequences were raised by rolling circle amplification, which is known to sometime amplify chromosomal sequences.
These results suggest that yams do host endogenous sequences of several distinct badnavirus species.

Keywords: yams; badnavirus; endogenous viral sequence;

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47-Activation of viral integrations following chromosome redistribution during an interspecific cross

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Most of present day edible bananas (*Musa* sp.) are the result of inter-specific hybridizations of two wild species *Musa acuminata* (A genome) and *Musa balbisiana* (B genome). *M. balbisiana* presents interesting agronomical characteristics such as resistance to biotic and abiotic stresses that are targeted during banana breeding programs. However, since the mid-90s, many *Banana streak virus* (BSV) outbreaks have occurred in banana-producing areas resulting from the activation of infectious BSV integrations (eBSVs) in newly created interspecific banana hybrids. Recently, we established that these problematic infectious eBSVs are present in the *M. balbisiana* genomes only. Over the last 10 years, we characterized the sequence and organization of eBSVs of four BSV species present in the seedy *Pisang Klutuk Wulung* (PKW) *M. balbisiana* diploid (Gayral et al., 2008 ; Chabannes et al., 2012). We showed for instance that eBSV Goldfinger (eBSGFV) and eBSV Obino l'ewai (eBSOLV) are di-allelic at one locus, with infectious and non-infectious alleles for each species. They are both present on chromosome 1 of PKW and up to now there is a lack of knowledge on genetic regulation of such infectious eBSV.
We monitored the distribution of infectious eBSV sequences among a F1 triploid AAB population produced by inter-specific cross between the tetraploid CRBP39 (AAAB) female parent and the male diploid *M. acuminata* (AA) Pahang. CRBP39 is a plantain carrying both infectious alleles of eBSGFV and eBSOLV while Pahang do not possess any eBSV for BSOLV and BSGFV. Results showed a strong bias in favor of hybrids containing chromosome 1 of *M. balbisiana* and demonstrated that interspecific chromosome recombination occur between *M. balbisiana* and *M. acuminata* genomes at least for chromosome 1. We are currently analyzing the distribution of chromosomes A and B in the resulting hybrids using microsatellites markers. This characterization is in progress and should allow getting an overview of the genetic structure of each offspring. This will help determined if the presence of particular chromosome segments could influence eBSV activation and thus whether the recombination and/or redistribution events of *M. Balbisiana* chromosomes in these hybrids could impact the recovery of a functional viral genome from eBSV. This should at term allow the safety re-introduction of B genome in banana breeding programs based on a reliable and early selection of risky hybrids.

Keywords: Breeding, plantain, BSV, eBSV, chromosome distribution, microsatellites, selection.

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48-Diversification of host targets to promote resistance against Potyvirus and Begomovirus in tomato

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Tomato (*Solanum lycopersicum*) is one of the most cultivated vegetable in the world, but suffers from important yield losses caused by viral diseases. Therefore, the development and use of cultivars that are genetically resistant to viruses has become a critical factor of competitiveness for both breeders and producers and one of the key stakes for sustainable agriculture. In this context, generating new resistance alleles using biotechnological approaches (e.g., TILLING) appears as a powerful tool to diversify host targets to promote resistance against viruses. Most characterized recessive resistances to potyviruses so far are natural variant of the translational initiation factor eIF4E. Those variants often encode functional eIF4E proteins but have lost the ability to interact with the viral protein VPg (Charron et al., 2008). In tomato, a broad-spectrum resistance to Potyvirus is associated with the natural resistance allele *pot1-eIF4E1* from *Solanum habrochaites* PI247087 (Ruffel et al., 2005). More recently null *eIF4E* alleles were obtained by TILLING (Piron et al., 2010) but strikingly, the resistance spectrum associated with the null *eif4e1* allele is considerably narrower than the one associated with the natural resistance allele *pot1-eIF4E1*. Understanding the apparent discrepancies between those two –natural and induced- resistances could be important to help developing more efficient TILLING-based resistances to pathogens. Therefore, we are investigating the differences between lines harbouring those alleles, focusing both on putative background effects as well as on the effect that the eIF4E1 knock-out might have on the eIF4E family redundancy.

Begomoviruses are much more damaging to tomato culture. However, even if some resistance QTL from wild species and several candidate genes have been characterized so far, the genes underlying those resistances remain unknown. We focused on three candidate genes that encode proteins interacting with the viral proteins Rep and REn as they might be hijacked by the virus to perform its replication. Protein-protein interactions with the viral proteins were checked and 3 to 4 null-allele or alleles showing change in amino-acid were isolated by TILLING for all each candidate. Among the 10 alleles assessed for resistance to Begomoviruses, a single one appears to confer partial resistance to TYLCV. These results have to be confirmed during further assays. We are further characterizing a transcription factor (TF) because it co-localized with the recessive resistance QTL *ty-5* (Anbinder et al, 2009), using a combination of protein-protein interaction studies, gene polymorphism analysis as well as generating dominant-negative TF-expressing plants using the CRES-T technology (Hiratsu et al., 2003).

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49-Comparison of methods for detection of pospiviroids infecting *Solanaceae*

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Since the end of the 1980s, new *Pospiviroids* such as *Tomato Chlorotic Dwarf Viroid* (TCDVd) and *Columnea Latent Viroid* have emerged in the European territory. Often they are latent on ornamental *Solanaceae* which become reservoirs of these pathogens and which facilitate their spread (Verhoeven *et al*., 2004).
The method used at the plant health laboratory at Anses aims to detect PSTVd and TCDVd using RT-PCR developed by Shamloul *et al*. (1997). To estimate the presence of the other viroids on *Solanaceae* , different methods were evaluated based on 2 techniques of end-point RT-PCR (Verhoeven *et al*., 2004; Spieker *et al*., 1996) and one technique of real-time RT-PCR (Monger *et al*., 2010) towards a panel of target samples (*Pospiviroids*) and non-target samples (*Solanaceae* and viruses). The criteria of relative sensitivity, relative specificity, repeatability and reproducibility allowed a scheme to be elaborated for detection of *pospiviroids* including confirmation by genetic characterization.

Reference: Shamloul AM, Hadidi A, Zhu SF, Singh RP, Sagredo B (1997) Sensitive detection of potato spindle tuber viroid using RT-PCR and identification of a viroid variant naturally infecting pepino plants. Can. J. Pl. Pathol. 19: 89-96.

50-Detection and molecular characterization of viruses infecting *Actinidia* spp.

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Kiwifruit (genus Actinidia) is an economically important crop, with an industry in continuous expansion, grown in temperate regions. Italy (415,000 tons), New Zealand (378,000 tons) and Chile (229,000 tons) are the world's largest producers with a harvested area of 25,000, 12,800 and 10,900 Ha, respectively. In Italy the main areas of kiwifruit production are localized in Lazio, Piedmont, Emilia Romagna and Veneto regions.
More than 50 species are recognized in the genus Actinidia but in New Zealand and Italy A. deliciosa and A. chinensis are the most widely grown cultivars.
Kiwifruit has been considered to be relatively disease free for more than 30 years however, since 2003, several viruses and virus-like diseases have been identified and more recent studies demonstrated that Actinidia spp can be infected by a wide range of pathogens. Currently eleven different viral species have been identified on kiwifruit plants.
In order to evaluate and prevent potential risks related to viral diseases spreading, several studies to investigate the presence of viruses on kiwifruit orchards and nurseries have been initiated.
Kiwifruit plants showing symptoms attributable to viral infection, as yellow mosaic, chlorotic or necrotic rings, curled or laciniated leaves and wood pitting, have been identified during inspection of commercial orchards in the Emilia Romagna region. Suspected samples were collected and analyzed in order to determine the etiology of the observed symptoms.
In this work we describe identification and characterization of two viral species: Cucumber Mosaic Virus (CMV) and Pelargonium zonate spot virus (PZSV) from kiwifruit plants showing, respectively, yellow mosaic and chlorotic ring spots. Moreover transmission electron microscopy of partially purified extracts from kiwifruit plants showing laciniated leaves and wood pitting, detected both rod-shaped and flexuous virus particles.
Large-scale sequencing of collected samples, for molecular characterization of new viral isolates using next generation sequencing platforms as Ion Torrent, is also presented.

51-Plant basal immunity against pathogenic microbes

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Over the past 10 years, a concept revolutionizing our understanding of plant-pathogen interactions emerged. This new concept stems from the ability for each organism to discriminate between self and non-self molecules through the action of pattern recognition receptors (PRRs) perceiving specific microbial molecular signatures, conventionally named pathogen-associated molecular patterns (PAMPs). Perception of PAMPs by PRRs activates a range of fast, efficient and multi-layered defense responses including the production of reactive oxygen species, the activation of MAP kinases, defense-related transcription factors and the expression of defense genes, leading to PAMP-triggered immunity (PTI), also called basal immunity. To counteract this defense strategy, successful pathogens deploy effectors proteins, the primary function of which is to evade/interfere with PTI.

Although progress was made in the understanding of PTI through the identification of components involved in the intracellular events, PTI-related signaling cascade and its regulation remain largely unknown in plants. In this context, we are focusing on the immune receptors FLS2 and EFR, as the best characterized PRR model from plants (perceiving bacterial flagellin and the EF-Tu factor respectively). Our results highlight that PTI pathways display many regulation levels during the infection process, including phosphorylation events, the ER-Golgi quality control machinery, the association with cellular adaptors, the role of specific RNA-binding proteins and the suppression of PTI responses by bacterial effectors.

Interestingly, data accumulated recently indicate that pathogenic microorganisms induce similar intracellular signalling in plants or in animals, suggesting that the downstream cascades of basal immunity against different classes of microorganisms could be shared in the same organism-host. More strikingly, animal and plant PRR pathways display impressive structural and strategic similarities. Therefore, based on the understanding of host-virus interactions in the animal field, the question of the basal immunity concept as a novel plant defense layer against plant viruses will be posed, hypothesis supported by many converging elements that emerged recently.

52-RNAi-mediated resistance to *Grapevine fanleaf virus* and *Arabis mosaic virus* in transgenic *Nicotiana benthamiana*

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Grapevine fanleaf virus (GFLV) and *Arabis mosaic virus* (ArMV), two *Nepoviruses*, are the major viruses responsible for a grapevine degeneration worldwide affecting vine performance and grape quality. Both viruses are naturally transmitted from plant to plant by ectoparasitic nematodes of the *Xiphinema* genus. As no natural source of resistance to GFLV and ArMV has been identified in *Vitis spp* species, genetic engineering to trigger virus resistance through RNA interference (RNAi) might be a promising strategy to control this disease.

For this purpose, one hairpin construct targeting both nepoviruses responsible of fanleaf degeneration was generated. Fragments from coat protein and polymerase of GFLV and ArMV were concatenated and cloned into a binary vector. Transgenic *Nicotiana benthamiana* were obtained using *Agrobacterium tumefaciens* mediated transformation.

Twelve primary transformants (T0) were regenerated. Plants were selfed and T1 seeds collected. The transgenic status and resistance level were investigated in some T1 lines by PCR analyses followed by mechanical inoculation of plants with ArMV and GFLV isolates. ELISA analyzes were performed at 14 dpi. Among the 4 initial transgenic lines tested, two did not show any resistance for both tested viruses. However, two other transgenic lines named VC38 and VC40 harboring one and two T-DNA inserts respectively, showed various resistance levels to the tested viruses. Indeed, VC38 did not show resistance when challenged with GFLV and only 24% of transgenic plants exhibited resistance towards ArMV, while 12% and 68% of plants issued from line VC40 showed resistance against GFLV and ArMV, respectively. Further molecular studies are currently underway to refine these preliminary results such as siRNA detection and to locate the insertion of the transgene in the genomic DNA. Given these first promising results on the herbaceous host-model *N. benthamiana*, stable transformation of different grapevine rootstock genotypes with this hairpin construct has been undertaken.

Keywords: *Grapevine fanleaf virus*, genetic engineering, Pathogen Derived Resistance, RNAi, *Nicotiana benthamiana*

53- Phloem specific Virus-Induced Gene-Silencing using Recombinant poleroviruses

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Virus-induced gene silencing (VIGS) has proven to be a powerful tool to study gene functions in plants. We have taken advantage of polerovirus phloem restriction to engineer chimeric viruses carrying a cDNA fragment from two endogenous plant genes to specifically silence the corresponding gene in vascular tissues. Sense (S), antisense (AS) and/or inverted-repeat (IR) sequences of the (i) *AtCHLI1* gene required for chlorophyll biosynthesis or (ii) *AtRTM1* gene restricting *Tobacco etch virus* (TEV) long-distance movement were inserted into the 3' non-coding region of *Turnip yellows virus* (TuYV). The chimeric viruses were agroinoculated to *Arabidopsis thaliana* Col-0. Silencing of the endogenous genes was monitored by symptom development (only for TuYV-CHLI1) and analysed by measuring endogenous mRNA accumulation by RT-qPCR.

(i) TuYV-CHLI1 virus:
A vein chlorosis phenotype was observed in *A. thaliana* as early as nine days after inoculation of all *AtCHLI1*-chimeric viruses. The symptoms further intensified with plant growth but remained strictly restricted to the vasculature. At a later time of infection (4 weeks) vein clearing started to disappear in plants infected with the IR containing viruses. By contrast the phenotype was more durable in plants inoculated with S and AS constructs. Molecular analysis of the viral progeny revealed a lower stability of the insert in the IR recombinant viruses. When similar constructs were introduced into a TuYV virus carrying a null mutation in the silencing suppressor P0 gene, a similar phenotype was observed following agroinfection. Virus titer was however strongly reduced. This confirms that P0 has no major impact on generation, propagation and action of short distance silencing signals.

(ii) TuYV-RTM1 virus:
This virus was constructed to silence *RTM1* in the vasculature in order to allow AF199 strain of *Lettuce mosaic virus* (LMV) that is unable to move systemically in Col-0, to overcome the resistance and to accumulate in upper non-inoculated leaves. Therefore, two weeks post-agroinfection with TuYV-RTM1, Col-0 plants were mechanically inoculated with LMV and analysed 3 weeks later. Expression of *RTM1* was reduced by a factor of six in all TuYV-RTM1 infected plants, which enabled systemic movement of LMV AF199 in two-thirds of the plants.

This study shows that a recombinant polerovirus TuYV containing a host gene sequence can be used to efficiently silence homologous genes specifically in phloem tissues.

54-Impact of mineral oil on *Potato virus Y* disease

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Mineral oil spray has been considered for several decades as an effective mean to protect crops against pathogens and insects. Weekly sprays control the spread of non-persistent viruses such as the *Potato virus Y,* one of the main potato diseases, involving aphids as vector *(*Bradley et al., 1962, Wrobel., 2012). Nevertheless, the mechanism by which mineral oil prevents viral outbreaks is still poorly understood. Some authors reported that the mineral oil somehow interferes with virus attachment to aphid mouthparts (Wang and Pirone., 1996, Wrobel., 2009). Others proposed that it affects aphid feeding behavior or causes a disruption of the host selection process (Simons et al., 1977, Ameline et al., 2010). However, to date, mineral oil effect on plant responses has not been investigated further than the observation of necrosis on leaves.
In our study we assessed Vazyl mineral oil (CCL, France) impact on each partner of the potato plant (*Solanum tuberosum*) – aphid (*Myzus persicae*) – virus (PVY ^{NTN}) system.
We confirmed that the treatment of potato plant by Vazyl prior to viruliferous aphid infestation, led to significantly reduced virus Y^{NTN} transmission. We showed that mineral oil treatment of plant slightly modifies the vector feeding behavior at the intracellular punctures activity, but it has no direct effect on aphid fitness. It has no effect on the virus itself or its infectious proprieties too. At the opposite, it affects both the virus acquisition step by the aphid and the plant immunity. Mineral oil induces plant defense responses similar to pathogen infection thought the reduction of the viral infection could not be explained by systemic resistance.
Thus we confirmed that the phytoprotection brought by mineral oil treatment against viruses mainly results from an alteration of the interaction between aphid mouthparts and the virus during the acquisition process.

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