Building bridges between disciplines for sustainable management of plant virus diseases

13th International Plant Virus Epidemiology Symposium

6-10 june 2016, Avignon, FRANCE

Programme and Abstracts

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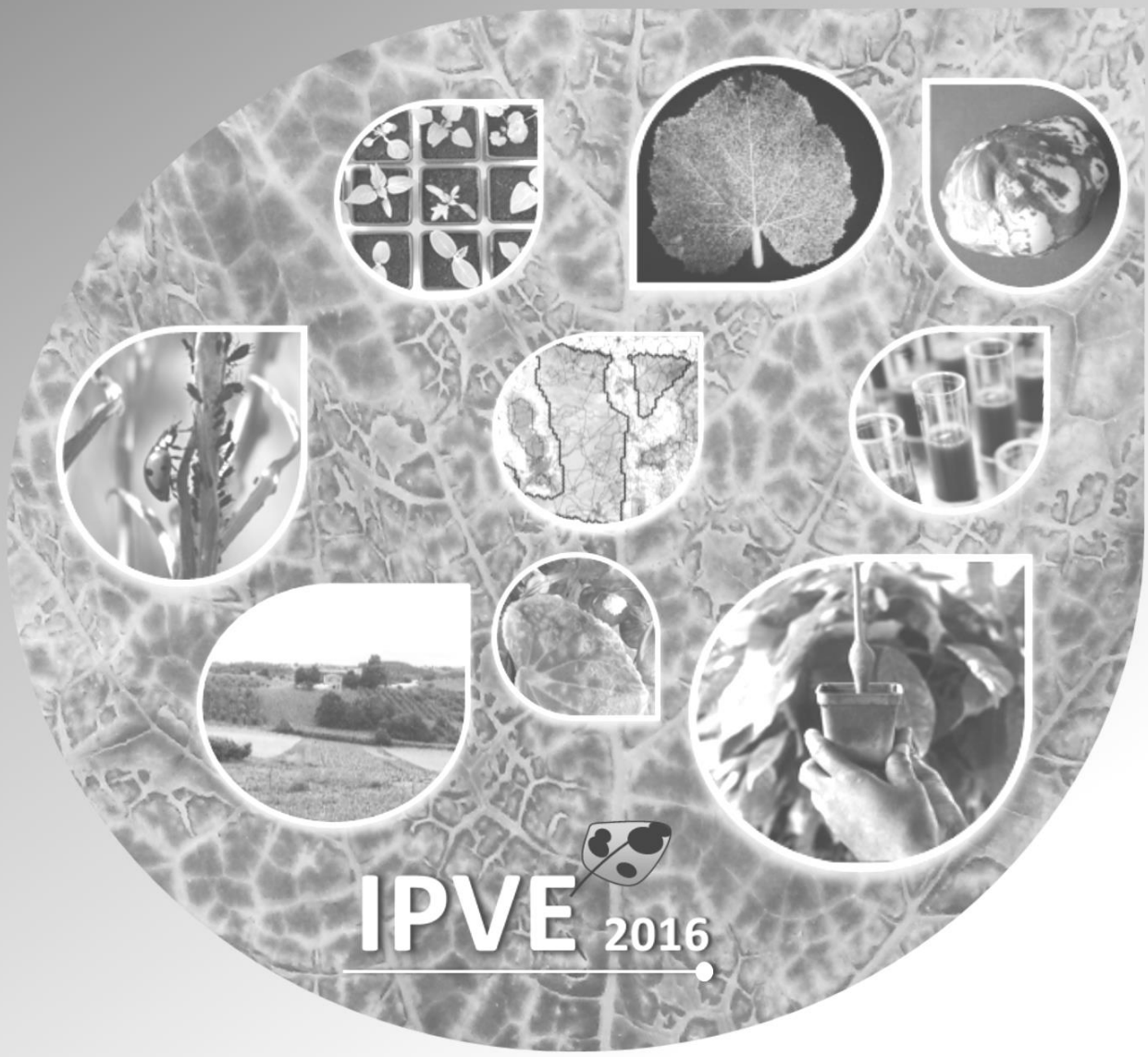

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Programme and Abstracts

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13th International Plant Virus Epidemiology Symposium

- Meeting venue
- Entrance of the University of Avignon
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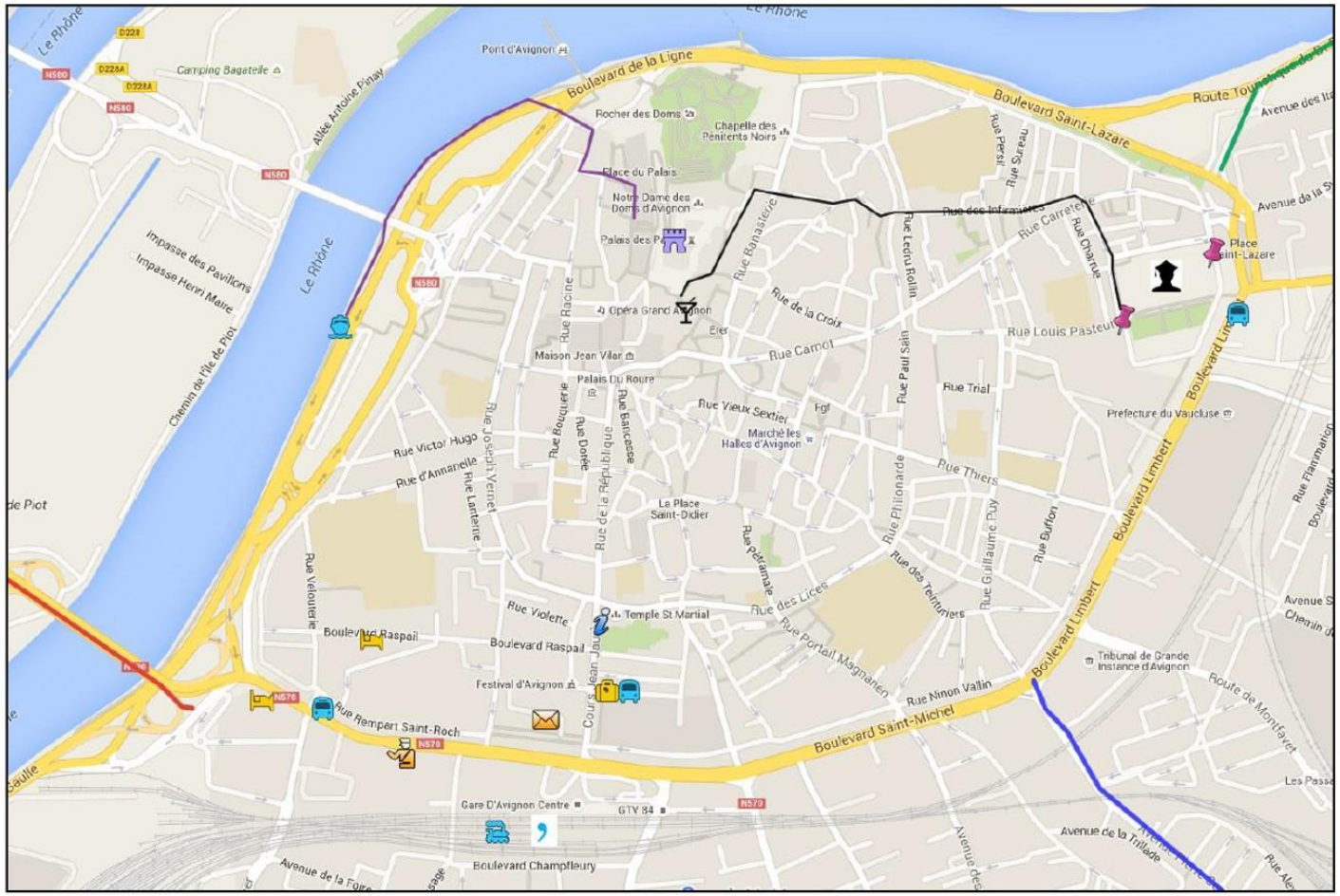

Université d'Avignon - Sainte Marthe
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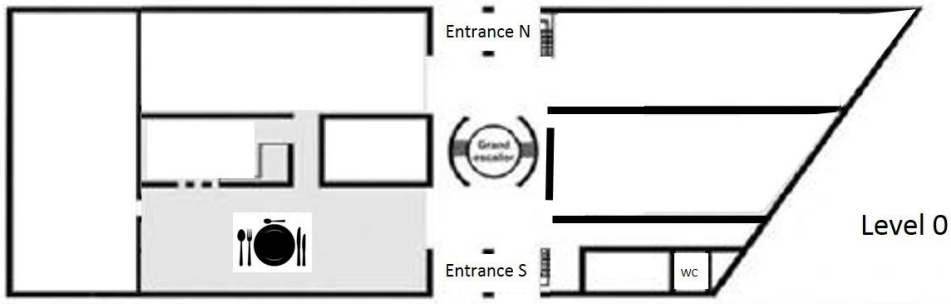
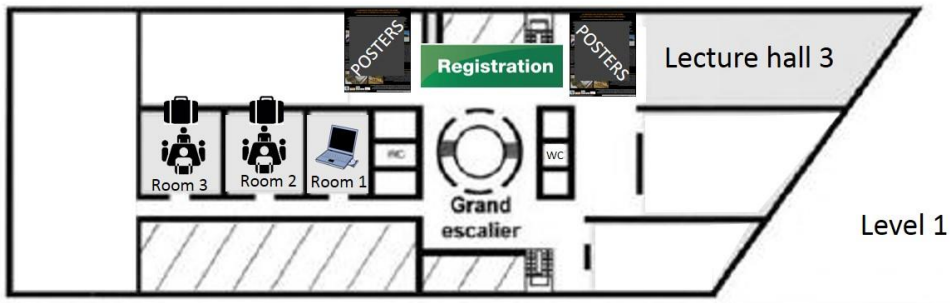
Amphithéatre 3 (lecture hall 3)





Library Level 2

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Monday, 6 June 2016

8:00 - 10:00: Registration, poster hanging, coffee

10:00 - 10:20: Opening

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Moderator - Alberto Fereres

11:20 **Keynote 1: J.K.Brown** - Considerations in molecular diagnostics development for emergent ssDNA-begomoviruses: a case study

12:00 **S1.1: M.Tepfer** - Deep sequencing of siRNAs for detection of known and unknown viral genomes in ornamental plants

12:20 **S1.2: E.Palanga** - Metagenomic-based screening and molecular characterization of cowpea-infecting viruses in Burkina Faso

12:40 - 14:20: Lunch

Moderator - Jean-Michel Lett

14:20 **S1.3: L.Lotos** - New insights into the etiology of pepper yellows disease

14:40 **S1.4: A.G.Blouin** - It is time to take advantage of that complementary strand

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15:20 **S1.6: M.N.Pearson** - Characterisation and transmission of New Zealand cherry leaf roll virus isolates

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Tuesday, 7 June 2016

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Moderator - Claude Bragard

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9:30 **S2.2: C.Lacomme** - Genetic diversity of PVY in potato: strain competition and ability to overcome resistance mechanisms

9:50 **S2.3: L.Tamisier** - Impact of selection, genetic drift and viral accumulation on the evolution of a plant RNA virus

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10:40 **S2.4: Z.Belabess** - Agroecological and evolutionary factors driving the success emergence of a non-canonical tomato yellow leaf curl virus recombinant

11:00 **S2.5: W.J.Cuellar** - Molecular and biological characterization of a novel group of potexviruses lacking a TGB3 gene

11:20 **S2.6: D.Kutnjak** - Deep sequencing of small RNAs for studies of plant virus diversity and evolution

11:40 **S2.7: S.M.Gray** - Evolving disease dynamics of the potato virus Y complex affecting the U.S. potato crop: a group effort between selection pressures and farming practices.

12:00 **S2.8: S.Dallot** - Exploiting viral genetic diversity to uncover sharka dispersal at regional and local scales

12:30 - 14:00: Lunch

Session 1 (part 2) – Etiology and diagnostic

Moderator - Varvara Maliogka

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14:20 **S1.9: H.Lecoq** - Two new whitefly-transmitted cucurbit viruses from Sudan with contrasting distribution patterns

14:40 **S1.10: A.Fox** - Are wild *Apiaceae* a reservoir of carrot virus epidemics?

15:00 **S1.11: L.T.T.Tran-Nguyen** - Cucumber green mottle mosaic virus in Australia- the story so far

15:20 **S1.12: R.A.A.Van der Vlugt** - A multiplex luminex xTAG-assay to distinguish between infectious and non-infectious cucumber green mottle mosaic virus on cucumber seeds

15:40 **S1.13: G.Loebenstein** - Starting a plant virus department and its development a personal experience – from test plants to molecular biology

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Wednesday, 8 June 2016

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Moderator - Stewart Gray

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9:00 **S3.1: P.V.Pinheiro** – Increased lysosomal exocytosis in the aphid midgut decreases circulative virus transmission

9:20 **S3.2: J.Doumayrou** - Domains in the pea enation mosaic virus major coat protein required for virion assembly and aphid transmission

9:40 **S3.3: A.Fereres** - Visual and olfactory cues involved in the selection of virus-infected tomato plants by *Bemisia tabaci*

10:00 - 10:30: Coffee break

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10:50 **S3.5: K.De Jonghe** - Evaluating pospiviroid-host-vector interactions in Belgium

11:10 **S3.6: M.van Munster** - Water-stress can enhance the transmission of plant viruses by insect vectors

11:30 **S3.7: P.Trębicki** - Climate change: will plant viruses and insect vectors threaten future food production?

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10:10 **S4.4: V.Moodley** - Disease mapping and risk assessment of whitefly transmitted viruses infecting vegetable crops in South Africa

10:30 **S4.5: C.M.Malmstrom** - Crop-associated virus infection in a native perennial grass: aster model assessment of fitness effects

10:50 - 12:30: Posters - drinks

12:30 - 14:00: Lunch

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Moderator - Nilsa Bosque Perez

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15:20 **S5.3: S.J.Castle** - Insecticide effects on whitefly transmission and incidence of cucurbit yellow stunting disorder virus

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Friday, 10 June 2016

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Session 4 (part 2) – Quantitative epidemiology and modelling

Moderator - Hanu Pappu

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10:20 **S4.7: B.Moury** - Determinants of host species range in plant viruses

10:40 **S4.8: R.A.C.Jones** - Epidemiology of *Potato spindle tuber viroid* in a remote subtropical irrigation area

11:00 **S4.9: N.A.Bosque-Pérez** - Virus infection in an endangered grassland habitat

11:20 **S4.10: P.Bernardo** - Virus accumulation in a native perennial prairie grass under development as a bioenergy feedstock

11:40 **S4.11: C.Lacroix** - Plant nutrient resources differentially alters two virus species dynamics

12:00 **S4.12: W.M.Wintermantel** - Reservoir hosts of cucurbit yellow stunting disorder virus and development of resistant melon

12:30 - 14:00: Lunch

Session 5 (part 2) – Virus control

Moderator - Hervé Lecoq

14:00 **S5.5: P.R.Campbell** - Area wide management of insect vectored viruses in tomato crops

14:20 **S5.6: G.Thébaud** - Using sensitivity analyses to identify and optimize key parameters of sharka management strategy

14:40 **S5.7: M.Delêtre** - Going viral: using social network theory to explore the social dynamics of the cassava mosaic virus pandemics

15:00 **S5.8: J.P.Legg** - Community phytosanitation and the epidemiology of cassava viruses

15:20 - 15:45: Student awards

15:45 - 16:00: Farewell

ORAL SESSIONS

JOHN MICHAEL THRESH, FATHER OF PLANT VIRUS EPIDEMIOLOGY: MAKING THE CASE

Irwin, M. E. (1), and Fereres, A. (2)

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(2) Instituto de Ciencias Agrarias-CSIC, C/Serrano 115 dpdo, 28006 Madrid, Spain

We propose that Professor John Michael Thresh be honored with the title, “Father of Plant Virus Epidemiology.” We examine his many attributes: his farming background, his reverence of family and the simple life, his athleticism, his love of the arts, his mastery of plant virus epidemiology, his skill at focusing on the problems at hand, his holistic and deep aptitude for problem solving, his demeanor, and his enormous influence on those around him. He is a broad and profound thinker, a true pioneer, innovator, and champion. His approaches to the manifest problems are straightforward and accomplishable, and the outcomes of his solutions are inevitably resounding economic successes and boons to the health and welfare of the people of both advanced and lesser-developed countries. At his core, he is a humanitarian and that, perhaps, is Mike’s most potent and indispensable attribute, the one that drove him to such heights. It is difficult to exaggerate the enormity and value of the collective contribution of his writings to the advancement of the discipline. These all speak to the sterling qualities that make him so exceptional and provide overwhelming evidence that Professor John Michael Thresh be honored with the title, “Father of Plant Virus Epidemiology.” We hereby propose that the ICPVE officially confer on him this title at a business meeting during the 13th International Plant Virus Epidemiology Symposium in Avignon, France.

Prof Mike Thresh, Scientist Emeritus, Natural Resources Institute, UK

CONSIDERATIONS IN MOLECULAR DIAGNOSTICS DEVELOPMENT FOR EMERGENT SSDNA-BEGOMOVIRUSES: A CASE STUDY

Brown, J.K.

School of Plant Sciences, University of Arizona, Tucson, AZ USA; jbrown@ag.arizona.edu

BACKGROUND and OBJECTIVES

Viruses exhibiting minimal genome sizes, greater than average genetic variation, high inter-specific diversity, and broad host ranges are ideal for addressing testable evolutionary hypotheses using effective modeling, and formal inference, without the computational overhead of large, genomically complex viruses. The small genome size, rapid replication rate, and putatively extensive intraspecific genetic variation acted on by frequent recombination and reassortment, a potentially expanding host range, and large number of sequences available for analysis, make the 'emerging' *Begomovirus* genus ideal for the proposed study. Our strategy is to analyze viral genomes from cultivated plants (<100 years, monoculture crops) and uncultivated eudicot species with which the genus, *Begomovirus,* has co-evolved in three habitats, globally consisting of cotton and vegetable poly/monocultures in the African Sahel and Asia, and cotton-vegetable monoculture (SW-US-Mexico) cropping systems, and the proximal endemic/uncultivated flora. Here, the results are reported for a two-year, intensive study of the diversity of begomovirus-satellite complexes in mixed crop-wild host interfaces for which viral sequences were determined for cloned standard PCR amplicons/cloned, rolling circle amplification-enriched, full-length genomes, and by next-generation (Illumina) sequencing. Sequences determined here and available in GenBank were aligned and subjected to various analyses to evaluate extent of diversity in relation to the number of each represented by host and geographic locale. Based on alignments, the design of sequence-specific molecular diagnostics was explored to arrive at effective, sensitive surveillance tools with group- and species-specific detection capabilities.

MATERIAL and METHODS

All available DNA sequences for begomoviruses endemic to Africa, Asian, and the Americas were downloaded from GenBank. Sequences were aligned (Muscle) and haplotypes were removed (96-100% identity), and subjected to SDT analysis. The result was used evaluate and adjust working cut-offs for viral species and betasatellites to achieve workable groups amenable to the design of primers having group- or species-specific specificity. Also, begomoviral-beta/alphasatellite sequence sets were determined for field-collected samples using rolling circle-enrichment and PCR amplification from cotton, vegetables, and prospective wild host species. Sequences were sorted by host, location, and year and subjected to various analyses, including frequency of distribution by host and location, and phylogenetic analysis (ML). Primers were designed around conserved and unique regions, and evaluated as potential targets for broad- spectrum and species-specific viral detection.

RESULTS

Begomoviral and betasatellite primers were designed based on the ten and five groups resolved by pairwise analysis (SDT), respectively. Primers were tested by standard PCR, and primers-probe combinations using isothermal PCR, and qPCR platforms over a range of concentrations of cloned viral or betasatellite genomes for begomovirus complexes endemic to Asia, Africa, and the Americas. Promising primers were identified a) broad group-specificity and b) species-specific detection. Next steps involve validation of platforms using DNA isolated from representative field-collected samples.

CONCLUSIONS

Designing DNA sequence-specific molecular diagnostics platforms that require sequence-specific primers or primer-probe combinations presents an enormous challenge for emergent viruses, because their genome sequences are in a state of flux owing to selection imposed by the combined outcomes of changing virus-vector, vector-plant host, and virus-plant host interactions, perhaps themselves affected by changing weather and climatic. Further, begomoviruses from Asia exhibit particularly high genomic variability, a propensity to recombine, and represent an enormous number of different species and species complexes often found in mixed infections, and recently, together with mastreviruses and introduced New World genomes and African begomoviral genomes.

DEEP SEQUENCING OF siRNAs FOR DETECTION OF KNOWN AND UNKNOWN VIRAL GENOMES IN ORNAMENTAL PLANTS

Verdin, E.(1), Wipf-Scheibel, C.(1), Aller, F.(2), Jacquemond, M.(1) and Tepfer, M.(1,3)

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(3) INRA, UMR1318, Institut J.P. Bourgin, 78026 Versailles cedex, France (Mark.Tepfer@versailles.inra.fr).

BACKGROUND and OBJECTIVES

Among horticultural crops, ornamental plants represent the most diversified sector in terms of genetic diversity (species, cultivars) and markets (cut flowers, plants in pots or for gardens, bulbs, shrubs). In France, the value of imported ornamental plants is 14 times greater than the value of the corresponding exports. These plants can harbor viruses that could cause serious damage in more economically important species, in particular vegetable and fruit crops, and thus present a potential source of new viral diseases. Serological and molecular diagnostic tools are effective for detection of known viruses, but considering the ever increasing number of ornamental species, their rapid turnover, 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. In this study, we present a novel strategy for detecting known and unknown viruses of various genome types, including viroids, in a diverse panel of ornamental plants.

MATERIALS and METHODS

First, we compared several strategies for simplifying the preparation and pooling of samples in order to minimize costs before Illumina Miseq deep sequencing of 21-24 nt siRNAs. We then analyzed 55 field samples representing 39 ornamental species of diverse origins (French and imported). The siRNAs were sequenced in 5x5 matrices of pools of 5 samples, and the reads assembled *de novo* into contigs.

RESULTS

Assembly of the sequencing reads into contigs allowed the identification of viruses of various genome types (positive and negative ssRNA viruses, dsRNA viruses, dsDNA viruses), as well as viroids and viral sequences integrated in the host genome (EPRVs). Of the genomes observed, some were nearly identical to known viruses, but many had only moderate to weak sequence identity with known viral genomes. These potentially new viruses include badnaviruses, begomoviruses, caulimoviruses, ilarviruses, nepoviruses, partitiviruses, tymoviruses and several potyviruses. For many of the virus-positive samples, the sequencing data were confirmed by molecular amplification of the expected viral sequences.

CONCLUSIONS

These analyses contribute to our understanding of the potential importance of ornamental plants as sources of emerging viruses in Europe, and more globally cast a new light on the respective contributions of crops, wild plants and ornamentals to plant virus epidemiology.

METAGENOMIC-BASED SCREENING AND MOLECULAR CHARACTERIZATION OF COWPEA-INFECTING VIRUSES IN BURKINA FASO

Palanga, E. (1,2,3,4), Filloux, D. (3), Fernandez, E. (3), Galzi, S. (3), Ferdinand, R. (3), Neya, B.J. (2,4), Sawadogo, M. (1), Traore, O. (2,4), Peterschmitt, M. (3) and Roumagnac, P. (3)

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BACKGROUND and OBJECTIVES

Cowpea, (*Vigna unguiculata* L. (Walp)) is an annual tropical grain legume, which is one of the most important subsistence legume cultivated in West Africa. Cowpea has been often referred to as « poor man's meat » because the seeds and the leaves of the cowpea plant have high protein content (25-30%). However, African cowpea production is dramatically constrained by viral diseases that can considerably affect cowpea yield. While 11 viruses have been reported so far from Africa, only three viruses have been reported from Burkina Faso to date using conventional approaches, i.e. Cowpea aphid borne mosaic virus (CaBMV, potyvirus), Blackeye cowpea mosaic strain of Bean common mosaic virus (BCMV-BlCMV, Potyvirus) and Cowpea mottle virus (CPMoV, Carmovirus). The diversity of cowpea viruses in Burkina Faso was studied further with a metagnomic-based approach.

MATERIAL and METHODS

Leaf samples were collected in 2013 from 312 symptomatic and non-sympthomatic cowpea plants from three agro-climatic zones of the country, i.e. Sahel, Sudan-Sahel and Sudan. Potential virion-associated nucleic acids were extracted from each sample and sequenced by 454 pyrosequencing (1). The sequence-based virus identification was confirmed and completed with Sanger sequencing of RT-PCR products.

RESULTS

Ten viruses were identified with the metagenomics approach, comprising the three viruses which were previously reported from Burkina Faso, the Southern cowpea mosaic virus (SCPMV) which was never reported from Burkina, and the following six new viruses: two Polerovirus-like viruses (*Luteoviridae*), a Tymoviridae-like virus and three Tombusviridae-like viruses. The complete sequence of the most prevalent Polerovirus-type was determined and RT-PCR primers were designed to routinely detect the six novel cowpea viruses.

CONCLUSIONS

This study supports the relevance of the VANA metagenomic approach to deepen virus diversity studies of cultivated plants. The new viruses need to be further characterized for their biological features, their virulence on cowpea, and their prevalence can be accurately monitored with the designed RT-PCR tests.

REFERENCES

1. Candresse T, Filloux D, Muhire B, Julian C, Galzi S, et al. 2014. Appearances Can Be Deceptive: Revealing a Hidden Viral Infection with Deep Sequencing in a Plant Quarantine Context. *PLoS ONE* 9 (7): e102945.

NEW INSIGHTS INTO THE ETIOLOGY OF PEPPER YELLOWS DISEASE

Lotos, L. (1), Olmos, A. (2), Orfanidou, C. (1), Efthimiou, K. (1), Avgelis, A.D. (3), Katis, N.I. (1) and Maliogka, V.I. (1)

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BACKGROUND and OBJECTIVES

Pepper yellows disease (PYD) appears to have spread in almost every pepper producing region of the world and is related with symptoms such as interveinal yellowing, upward leafroll and stunting. So far, two poleroviruses have been associated with PYD, pepper vein yellows virus (PeVYV) [1] and pepper yellow leaf curl virus (PYLCV) [2]. In Greece the first major outbreak of PYD was observed in 2013 on the island of Crete with disease incidence ranging from 40 to 60%. Initial screening revealed the presence of a PeVYV/PYLCV-like isolate which however exhibited sequence variability from these two species. The goal of this study was to fully characterize this new virus isolate and to define its evolutionary relationships with the other pepper infecting poleroviruses.

MATERIAL and METHODS

Thirty nine (39) pepper samples exhibiting PYD typical symptoms, collected in three subsequent years from Crete and other regions in Greece, were screened using polerovirus generic and “PeVYV-group” specific primers. One of the infected samples (PX3) was used for an NGS analysis and one more (Pi21) was fully sequenced using Sanger sequencing. Maximum likelihood trees constructed with aa and nt sequences of different regions of the genome and sequence similarity analysis were used in order to investigate the relationships of these isolates with other viruses in the genus *Polerovirus*.

RESULTS

Twenty seven out of 39 samples, all originating from Crete, were found positive for the presence of a “PeVYV-group”-like isolate. The complete genomic sequence of PX3 and Pi21 revealed the presence of a polerovirus exhibiting close phylogenetic relationships with PeVYV and PYLCV. However both PX3 and Pi21 exhibited sequence variability in every ORF of their genome from these two viruses. In fact sequence diversity in ORFs 0, 4 and 5 exceeded the species demarcation threshold set for the genus. Therefore, the isolates from Crete represent a putatively new polerovirus species provisionally named pepper yellows virus (PeYV).

CONCLUSIONS

Similar to the other diseases caused by poleroviruses, PYD is caused by a group of different, but closely related viral species. Based on our results the three PYD-related viruses characterized so far have probably originated from a TVDV-like common ancestor. A host jump of the ancestor followed by selection and recombination events shaped each species to its present form.

REFERENCES

1. Murakami et al. (2011). *Archives of Virology*: *156*, 921-923
2. Dombrovsky et al. (2013). *PloS one*: *8* (7), e70722

IT IS TIME TO TAKE ADVANTAGE OF THAT COMPLEMENTARY STRAND

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BACKGROUND and OBJECTIVES

Virus diagnostics has never been so simple with the ease of generating Next Generation Sequencing (NGS) data. Despite this dramatic advance, untargeted large scale plant virus surveys remain rare. Regardless of their impact on plant physiology, plant viruses represent an insignificant quantity of genetic signal. As a consequence, we vastly over-sequence before asserting bioinformatic analyses to find the minute proportion of virus sequences. One alternative is to enrich for the virus, but methods available are arguably as expensive in time as the NGS savings. Here we present a novel approach to enrich the double-stranded RNA (dsRNA) replicative form of RNA viruses. This method is cheap, simple, and rapid can be used to quantify virus replication.

MATERIALS AND METHODS

We used anti-dsRNA monoclonal antibodies (mAbs) (1) bound to protein L magnetic beads (Thermo Scientific) in a pulldown experiment. The dsRNA captured was amplified by RT-PCR (random primers) and then sequenced. Three infected plants were selected in a pilot study. The process used from dsRNA to NGS is similar to the one described by Roossinck *et al.* (2).
The differences in replication of a virus throughout a plant was quantified by RT-qPCR of the dsRNA captured using the above tool in a woody host (grapevine) infected with phloem-limited viruses. The tissue selected was compared, and amount of tissue required adjusted.

RESULTS

From the pilot NGS study five viruses were detected including one new to science; 30 to 74% of the reads obtained were viral (3).The RT-qPCR confirmed that phloem scrappings are the best source of dsRNA in a grapevine, but older leaf petioles represent a practical alternative with sufficient dsRNA to detect the viruses present.

CONCLUSIONS

The method developed represents a simplification of virus enrichment. The dsRNA is captured in less than 2 hours and the virus recovery is comparable to that obtained from the traditional cellulose method. The method opens new prospects for large scale virus ecological research.
The advantages of the method to experimentally measure virus replication in specific tissues or time points was demonstrated. Along with immunocapture, we now have tools to quantify virus replication and accumulation at one time in different tissues.

ACKNOWLEDGMENTS

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THE "PRSV CLUSTER" OF CUCURBIT-INFECTING POTYVIRUSES: MOLECULAR CHARACTERIZATION, GEOGRAPHIC DISTRIBUTION AND POTENTIAL EPIDEMIOLOGICAL IMPACT

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BACKGROUND and OBJECTIVES

More than 70 viruses infect cucurbits worldwide. Among the most common and agronomically important are several potyviruses: ZYMV, WMV, PRSV. Besides these viruses that have a worldwide distribution and present distinct biological, serological and molecular features, several viruses closely related to PRSV that could constitute highly divergent strains of this virus or related species have been defined, and could represent new threats for agriculture.

MATERIALS and METHODS

Biological, serological and molecular studies, as well as analysis of database sequences, were performed in order to characterize the different species in the PRSV cluster and study their geographic distribution and population structure (1,2). Isolates from worldwide origins were tested serologically (DAS-ELISA) or molecularly (RT-PCR with specific or generic primers) for the presence of the different viruses.

RESULTS

Based on their molecular properties, at least 10 distinct species exist in the "PRSV cluster" (3). Their geographic range, field prevalence and agronomic impacts are highly contrasted: PRSV and to a lesser extent ZTMV have a worldwide distribution, MWMV is widespread in Africa and emerging in the Mediterranean Basin but not reported elsewhere, while the other viruses appear endemic to one country even though they can be maintained locally over several decades.

CONCLUSIONS

The number of different potyviruses in the "PRSV cluster" is probably still underestimated. Excepting PRSV and to a lower extent ZTMV and MWMV, species in the "PRSV-cluster" seem to have a narrow geographic range and low epidemiological impact so far. At least two species appear endemic to Sudan, with contrasted frequencies. These results show that currently unimportant viruses may emerge if the local conditions change or if they are introduced in a more favourable environment.

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CHARACTERISATION AND TRANSMISSION OF NEW ZEALAND CHERRY LEAF ROLL VIRUS ISOLATES

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BACKGROUND and OBJECTIVES

Cherry leaf roll virus (CLRV: *Nepovirus*, *Secoviridae*) is globally distributed, infects plants from 36 families, and is transmitted by grafting, seed, and pollen. Sequencing and phylogenetic analysis of the 3´UTR identified five genotype groupings, with particular genotypes being associated with specific plant species, leading to the hypothesis that host specificity is primarily the result of (vertical) pollen transmission of individual CLRV genotypes (Rebenstorf et al., 2006). The aim of this research was to characterize New Zealand CLRV isolates, develop specific detection methods and identify the mode of transmission (horizontal and/or vertical) for these isolates.

MATERIALS and METHODS

1. Six CLRV isolates from *Actinidia chinensis*, *Malus domestica*, *Ribes rubrum*, *Rubus idaeus*, *Rumex obtusifolius* and *Vaccinium darrowii* were sequenced as described by Woo and Pearson (2014).
2. The ability of a high resolution melt (HRM) assay to detect and differentiate CLRV genotype groups as defined by Rebenstorf et al. (2006) was evaluated.
3. CLRV transmission was tested in indicator plants to determine the mode (horizontal and/or vertical) and rate of successful transmission in pollen and seed respectively.

RESULTS

1. The six RNA1 sequences share 92.0-99.7% nt and 81.2-99.4% aa similarity and fall into two clusters with the woody species isolates -Ac, -Ma, -Ri, -Rb and -Va, forming one group and CLRV-Rm forming another. RNA2 sequences share 91.4-99.8% nt and 72.6-99.6% aa similarity with isolates -Ma, -Ri, -Rb and -Va forming one group and -Ac & -Rm forming another.
2. Preliminary results indicate that HRM can differentiate between NZ CLRV isolates from different phylogenetic groups.
3. In *Nicotiana occidentalis* seed transmission was >80% for CLRV-Ma, -Ri, -Va, and -Rm, with no seed transmission observed for CLRV-Rb.

CONCLUSIONS

1. CLRV-Ma, -Ri, -Rb, and –Va genomes are >99% identical, which contrasts with the results of Rebenstorf et al. (2006) where isolates were host specific.
2. HRM shows promise for the detection and differentiation of New Zealand CLRV isolates from different groups and will be further developed to differentiate within group variants.
3. Future work will address the ability of New Zealand CLRV isolates to transmit to pollen-incompatible host species, as was observed for pollen-transmissible *Raspberry bushy dwarf virus* (Isogai et al., 2014).

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EPIDEMIOLOGICAL ASPECTS OF ROSE ROSETTE VIRUS

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BACKGROUND and OBJECTIVES

Rose rosette in the most important virus disease in North America, caused by rose rosette virus (RRV), an emaravirus (Laney et al., 2011). Given the recent fulfilment of Koch's postulates (Di Bello et al., 2015), there is significant anectodal information that, in some cases, contradicts established knowledge on viruses and emaraviruses altogether. In this study we developed a new, sensitive and universal detection protocol and tested the hypothesis of RRV moving systemically in rose. We tested twenty of the major genotypes planted in the United States for resistance to the mite and/or the virus and evaluated the acquisition and transmission access periods (AAP and IAP respectively).

MATERIALS and METHODS

The new detection protocol is based on all available RRV genomic information as of January 2016. For all studies, other than resistance screening, the 'Julia Child' genotype was used. The AAP was performed using time points spanning from 1h to 5 days whereas IAP extended to 14 days. All evaluations on resistance and transmission where based on a >25 viruliferous mites/plant load.

RESULTS

The new, more sensitive test can consistently detect the virus in less than 20 PCR cycles. RRV can move systemically to rose. The mite needs at least 5 days AAP whereas it can transmit the virus after feeding on plants for an hour. One genotype, 'Stormy Weather' has proven resistant to the virus after mite inoculation and grafting experiments. There was no genotype that showed resistance to the mite.

CONCLUSIONS

The detection protocol developed not only can detect all studied RRV isolates but is also superior to the previous available protocols when it comes to sensitivity. This potentially allows for RRV detection even when there are no visual symptoms and elimination of infected material from the propagation pipeline, minimizing the risk of virus dissemination to area that may not be present. As RRV moves systemically, pruning, used by many rosarians to eliminate disease is an inappropriate control method and carries the risk of spreading the virus to adjacent material. The transmission attributes of the virus point to a persistent propagative transmission mode.

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LANDSCAPE HETEROGENEITY AND THE EVOLUTION OF PLANT-VIRUS INTERACTIONS

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Viruses are the major group of emergent plant pathogens, accounting for about 47% of emergence events. Virus emergence is a complex process determined by both ecological and evolutionary factors. It has been proposed that high prevalence and high virulence of viruses in crops would be the result of ecological changes associated with agriculture, which would determine the population dynamics and evolution of plant viruses. This hypothesis rests on scant evidence as, in contrast with abundant information from agroecosystems, little is known about the infection dynamics and the virulence of viruses in non-agricultural ecosystems. Hence, to understand the ecological and evolutionary factors that drive the evolution of plant-virus interactions, analyses should include non-agriculatural.

We are interested in understanding if ecosystem simplification determines: (i) infection patterns and virus prevalence, and (ii) host range and virulence evolution. To address these questions we have analysed virus infections in two sets of habitats that represent a gradient of human management. The first set included a series of wild-to-agricultural habitats of a Mesoamerican plant species currently undergoing incipient domestication. Here, we analysed if human management of the focal host populations was a factor determining the prevalence of virus infection, its virulence, and host-virus co-evolution. The second set was formed by a series of peri-agricultural habitats in a Mediterranean landscape, where we monitored the incidence of 10 generalist virus species. This data set was used to analyse the relationship between virus infection dynamics in each habitat and its biodiversity. Infection networks, and their variation across habitats, were analysed to test if biodiversity decline would favour virus adaptation to the remaining host plant species, as well as to hosts with a higher reservoir potential.

Our data show that human-driven ecosystem simplification may modify patterns of plant-virus interactions leading to virus emergence. We have also found evidence that anthropisation of the habitat results in more virulent infections, expressed as more severe symptoms. Last, we found that habitat anthropisation impacts on both the evolution of viruses and of host defences to virus infection.

JOINT ESTIMATION OF EFFECTIVE POPULATION SIZE AND SELECTION COEFFICIENT WITHOUT NEUTRAL MARKERS: METHOD VALIDATION AND APPLICATION TO EXPERIMENTAL EVOLUTION OF VIRUSES

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BACKGROUND and OBJECTIVES

Experimental evolution studies deserve considerable attention to the estimation of basic evolutionary forces such as selection and genetic drift. With the advent of high-throughput sequencing techniques, these studies gained a renewed attention. However the joint estimation of selection and genetic drift still remain challenging when no neutral markers are available, a common situation with many microbes, such as viruses, due to their small tightly packed genomes.

MATERIAL and METHODS

We have developed a mechanistic-statistical model for estimating effective population size *Ne* and selection coefficient *s* from genetic time series data generated in evolve-and-resequence experiments. Numerical simulations of the dynamics of competing genotypes in asexual haploid Wright-Fisher populations subjected to contrasting genetic drift ($Ne \in [20, 5000]$) and selection ($|s| \in [0, 0.1]$) regimes were used to test the model. This model was then applied to data for competition between five variants of *Potato virus Y* (PVY, genus *Potyvirus*) in 15 doubled haploid lines of pepper (*Capsicum annuum*, family *Solanaceae*) that constituted different host environments. The frequencies of the variants were determined at six time-points, in eight plants per time point for each host environment.

RESULTS

The numerical experiments validated the method and made it possible to obtain bias-adjusted estimators of *s* for each competing genotype and of the dynamics of *Ne* over the time course of the experiment ($R^2 \in [0.93, 0.95]$, slopes $\in [0.98, 1.07]$ and intercepts $\in [-0.14, 0.06]$ for the best linear fit between true and estimated parameters). The real-life experiment showed that the virus populations experienced either stochastic or deterministic evolution, depending on host genotype and that *Ne* and *s* were genetically controlled by the host genotype.

CONCLUSIONS

This method constitutes an advance in the joint estimation of key population genetic parameters from time-sampled data in conditions in which no neutral markers are available and both selection and genetic drift are strong. This is a situation frequently encountered in studies of microorganisms. The observation of highly contrasted genetic drift between plant genotypes may have practical implications for slowing viral emergence through the careful choice of plant cultivars.

GENETIC DIVERSITY OF PVY IN POTATO: STRAIN COMPETITION AND ABILITY TO OVERCOME RESISTANCE MECHANISMS.

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BACKGROUND and OBJECTIVES

Potato virus Y (PVY) is the most important viral pathogen affecting potato crops worldwide (Valkonen 2007). PVY is transmitted non-persistently by non-colonising aphids, resulting in a rapid acquisition and transmission of the virus between plants. PVY exists as a complex of strains that can be distinguished according to their pathogenicity, serology and genome analysis. While virus incidence is low in Scottish seed potato crops, PVY has become the most prevalent virus. A drift in the PVY population structure from PVY^{O}, PVY^{C} to PVY^{N} to recombinant PVY^{NTN} strains (N-Tuber Necrosis) that can cause Potato Tuber Necrotic Ringspot Disease (PTNRD) is currently being observed worldwide. We studied the population dynamics of PVY by studying the molecular nature of PVY field isolates and the epidemiology of PVY^{N} isolates to identify which potential genetic and/or environmental factors are driving their prevalence.

MATERIAL and METHODS

Characterization of PVY isolates was undertaken by assessing symptoms elicited on indicators plants following mechanical passage inoculation with infectious sap. The serology of PVY isolates was as previously described (Davie et al, 2012) using SASA monoclonal and polyclonal antibodies. Genome sequencing of PVY isolates collected from potato fields, phylogenetic analysis and field transmission studies were as previously described (Davie et al, 2012).

RESULTS

A survey of the biological and molecular diversity of PVY field isolates indicated that while a wide range of variants can be identified, the vast majority belong predominantly to the European (EU)-NTN ($PVY^{EU\text{-}NTN}$) molecular group and have the ability to overcome *Nc*, *Ny* and *Nz* PVY resistance genes in potato. Field trials transmission studies were undertaken using plants infected with each of the three main molecular groups identified in seed potato crops (PVY^{O}, $PVY^{EU\text{-}NTN}$, $PVY^{NA\text{-}NTN}$). Bait plants were monitored on a weekly basis to assess aphid transmission rate and at post-harvest to assess PVY incidence in tuber progeny over a 3 year period. The results showed that the PVY isolate representing the most prevalent molecular group $PVY^{EU\text{-}NTN}$ was more efficiently transmitted to the bait plants than $PVY^{NA\text{-}NTN}$ and PVY^{O}. In addition, post-harvest assessment of PVY incidence in tubers showed a higher proportion potato plants infected with $PVY^{EU\text{-}NTN}$. Furthermore, PVY incidence in plants infected at different times after emergence, suggest that $PVY^{EU\text{-}NTN}$ has the ability to infect older plants in comparison to PVY^{O}.

CONCLUSIONS

Altogether our results suggest that while a wide range of molecular variants can be identified in the field, $PVY^{EU\text{-}NTN}$ is largely prevalent. Transmission studies suggest that $PVY^{EU\text{-}NTN}$ might out-compete other PVY strains perhaps by overcoming host resistance mechanisms including mature plant resistance, potentially explaining $PVY^{EU\text{-}NTN}$ prevalence in our environmental conditions.

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IMPACT OF SELECTION, GENETIC DRIFT AND VIRAL ACCUMULATION ON THE EVOLUTION OF A PLANT RNA VIRUS

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BACKGROUND and OBJECTIVES

Genetic control is an efficient way to fight against crop disease, but this method is limited by the pathogen ability to evolve and break the resistance down. In the pepper-*Potato virus Y* (PVY) pathosystem, studies have demonstrated that the plant genetic background can increase the durability of the major resistance gene (Palloix et al., 2009), especially by constraining pathogen evolution (Quenouille et al., 2013). We conducted an experimental evolution to (i) measure the pathogen ability to adapt to plants combining major resistance gene and partially resistant genetic background and (ii) identify the role of the evolutionary forces induced by the genetic background on virus evolution.

MATERIAL and METHODS

We used six doubled-haploid (DH) lines of *Capsicum annuum* carrying the PVY resistance allele *pvr2*^{3} and differing in their genetic background. All these lines were selected for their contrasted effects on viral accumulation, selection and genetic drift imposed to the viral population. Three variants of PVY isolate SON41p showing different levels of adaptation to the DH lines were chosen. Each variant carried one substitution in the VPg cistron, named 101G, 115K or 119N, allowing them to overcome the *pvr2*^{3} resistance. We performed an experimental evolution by passaging serially the PVY variants on the six DH lines. The VPg of the viral lineages was sequenced at each evolutionary cycle and their final level of adaptation and aggressiveness was assessed.

RESULTS

We found that different evolutionary process occurred between the lineages resulting in fitness gains, extinctions or no evolution at all. The fitness gains were always observed in lineages showing at least one non-synonymous mutation in the VPg cistron. Genetic drift had a significant impact on virus evolution, since all the lineages that evolved in plants inducing a strong genetic drift did not adapt or became extinct.

CONCLUSIONS

Using experimental evolution, we demonstrated that genetic backgrounds inducing strong genetic drift reduced or prevented the virus adaption to the major resistance gene.

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AGROECOLOGICAL AND EVOLUTIONARY FACTORS DRIVING THE SUCCESS EMERGENCE OF A NON-CANONICAL TOMATO YELLOW LEAF CURL VIRUS RECOMBINANT

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BACKGROUND and OBJECTIVES

Recombination is one of the driving forces for viral evolution, and particularly for the Tomato yellow leaf curl (Tylc) associated begomoviruses (family *Geminiviridae*). TYLCV-IS76 (IS76) is a peculiar recombinant from Morocco generated between representatives of the Israel strain of *Tomato yellow leaf curl virus* (TYLCV-IL) and the Spanish strain of *Tomato yellow leaf curl Sardinia virus* (TYLCSV-ES). Unlike the previously reported TYLCV/TYLCSV recombinants, IS76 has a non-canonical recombination profile and has entirely displaced its parental viruses, possibly because of its positive selection by tomato cultivars bearing the *Ty-1* tolerant gene (see the sister communication of Belabess et al.(1)). This prediction was tested by comparing the fitness of IS76 to those of representatives of parental viruses, in tomato plants harboring or not the *Ty-1* gene. As the fitness results were consistent with the selective hypothesis, a second question had to be addressed: as all the Mediterranean used tomato cultivar tolerant to Tylc associated viruses are bearing the *Ty-1* gene, why has such recombinant not been reported elsewhere?

MATERIAL and METHODS

The fitness of IS76 was estimated in a Ty-1 tolerant cultivar, and in a susceptible nearly isogenic line, in single or mixed infections with parental viruses TYLCV-IL and TYLCSV-ES. Viral DNA intra plant accumulation was measured with real time PCR (qPCR) and used as a proxy of fitness. An artificial TYLCV-IS76 recombinant (IS76') was constructed by site-directed mutagenesis to analyze the molecular determinant of the potential fitness advantage. The probability of emergence of IS76 type recombinants was estimated in 10 *Ty-1* tolerant and 10 nearly isogenic susceptible plants co-infected with parental viruses, by monitoring the generated recombination patterns over time up to 240 days post inoculation (dpi).

RESULTS

IS76 DNA accumulation was significantly higher than that of TYLCV-IL and TYLCSV-ES in the tolerant cultivar, both in single or mixed infections. The fitness advantage of IS76 in the tolerant plants did not pay any accumulation cost in the susceptible plants in which IS76 accumulation was similar or higher than that of both parental viruses. Interestingly the IS76 selective advantage was associated with a dramatic negative impact on TYLCV-IL accumulation, which was shown to be determined by the recombination nature of IS76. Indeed, the TYLCV-IL parent dropped below the qPCR detection level at 120 dpi and the whitefly transmissibility level from 60 dpi. Recombinants exhibiting the typical IS76 recombination breakpoint were generated in plants of both cultivars co-inoculated with the parental viruses but very unexpectedly they remained at an extremely low frequency even up to one year after infection.

CONCLUSIONS

Our results support the positive selection of IS76 by the *Ty-1* gene and validate the selective hypothesis to explain its emergence and the entire displacement of its parents in the Souss. It is expected that the fitness advantage of IS76 may involve gene silencing because the *Ty-1* gene codes for an RdRp (2) and because the short TYLCSV derived region of IS76 is located in a region which has been reported for two begomoviruses to be one of the favored target of siRNA and methylation(3). The fact that IS76 type recombinants did not dominate in co-inoculated plants in which they were generated may suggest that their emergence may need a highly improbable combination of circumstance which may have occurred by chance in the Souss.

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MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF A NOVEL GROUP OF POTEXVIRUSES LACKING A TGB3 GENE

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BACKGROUND and OBJECTIVES

Different potexviruses have been reported infecting cassava in the Americas, all associated to severe disease symptoms and mixed infections but most of them symptomless in single infections (1). However genome information is available only for one of them. Therefore classification as distinct species remains unresolved, which affects disease diagnostics, cassava germplasm exchange certification and epidemiological studies. Using biological assays, generic potexvirus primers and ELISA tests we studied the diversity of potexviruses and their genome organization for improving diagnostics and monitoring of diseases associated to these viruses in cassava.

MATERIAL and METHODS

More than 500 field-collected samples and greenhouse maintained plantlets were screened for potexviruses using potexvirus generic primers (2). Total RNA extractions (CTAB) and RT-PCR were carried out using standard techniques (1). DAS-ELISA for CsCMV and *Cassava virus X* (CsVX). Amplified PCR fragments were cloned into pGEM-T plasmids (PROMEGA) for Sanger sequencing (MACROGEN). We used MEGA for sequence analysis. Biological tests included bud grafting into cassava control plants and mechanical transmission to and from *Nicotiana benthamiana,* under greenhouse conditions.

RESULTS

Available antisera to detect cassava potexviruses did not recognize a groups of PCR-positive infected plants. After comparison with sequencing results we could identify 3 mayor phylogenetic groups of sequences. One group was formed by members reacting to an antiserum raised against CsCMV and the second corresponded to CsVX isolates. The third phylogenetic group of sequences was closer related to CsVX isolates but did not reacted to the CsVX antiserum. Upon mechanical inoculation to *N. benthamiana*, efficient transmission (Ten plants infected out of ten inoculated, 10/10) was attained only with CsCMV. CsVX and CsNAV could not be efficiently transmitted to *N. benthamiana* (3/10 and 0/10). When further sequence characterized, we observed that isolates of CsVX and CsNAV lacked the gene 3 of the characteristic potexviral Triple Gene Block (TGB). A gene involved in virus movement and symptom expression (3). All isolates of CsCMV induced typical symptoms of leaf mosaic when single infecting cassava (after using *N. benthamiana* as a filter plant for CsCMV purification).

CONCLUSIONS

Although six distinct potexviruses have been reported to infect cassava (1), we could identify three major phylogenetic groups. Serological and biological tests further confirmed the identity of these groups. Only isolates of *Cassava common mosaic virus* (CsCMV) conserves a typical potexvirus genome organization. Members of the other two groups identified in this study, associated to symptomless infections and a lower efficiency of mechanical transmission to indicator plants, lacked the gene 3 of the characteristic potexviral TGB region. This is in agreement with other studies where TGB3 is mutated and the virus (infectious clones) are affected in movement and symptom expression. To our knowledge this is the first report of naturally occurring potexviruses lacking a TGB3 gene and the first sequence identification of CsVX.

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DEEP SEQUENCING OF SMALL RNAs FOR STUDIES OF PLANT VIRUS DIVERSITY AND EVOLUTION

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BACKGROUND and OBJECTIVES

In recent years, next generation sequencing technologies provided unprecedented options for generic studies of viral sequence diversity. Small RNA (sRNA) deep sequencing was established as one of the most efficient options for detection and characterisation of new or unknown viruses in plants. However, little have been known about the diversity of virus-derived small RNA (vsiRNA) sequences. In our research we have used sRNA sequencing to characterise new viruses, but have also tested if the method can be used for in-depth studies of within-plant virus population structure. Finally, we have applied the method to study the evolutionary dynamics of a plant virus within a plant.

MATERIAL and METHODS

Using deep sequencing of sRNAs and assembly of short reads we have first characterised several isolates of Potato virus X. In the next step, to use sRNA data for in-depth studies of within-plant virus population structure, we have first tested how variability inferred from sRNAs can be compared to the one inferred from RNA isolated from purified viral particles, using Potato virus Y(PVY)-potato as a model system. We have then applied sRNA deep sequencing to study evolutionary dynamics of PVY during serial passages on potato cultivars with different sensitivity to PVY. Time sampled single nucleotide polymorphism (SNP) data was analysed using Approximate Bayesian Calculations (ABC) to estimate selection coefficients of the observed SNPs (3). SNP trajectories were clustered to reveal groups of co-occurring SNPs. Evolution of PVY in three different cultivars was compared by comparing the position of SNPs and the magnitude of inferred selection coefficients.

RESULTS

Using sRNA sequencing we have discovered a new phylogenetic group of PVX in South America and were also able to discriminate between two strains of the same virus in the mixed infection (1).
With PVY-potato as a model, we have first showed that sRNAs and RNA isolated from viral particles show highly similar mutational spectrums (2). This allowed us to use the sRNA sequencing in a PVY evolution experiment. In this experiment several groups of co-fluctuating SNPs were observed in virus population by SNP trajectories clustering. The results of the selection analyses showed slight differences in patterns of virus evolution in some of the cultivars.

CONCLUSIONS

All together, the results of our research show that sRNA deep sequencing presents a valuable tool for investigation of new viral strains, studies of viral diversity within a host and evolutionary dynamics of viral populations in different hosts.

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EVOLVING DISEASE DYNAMICS OF THE POTATO VIRUS Y COMPLEX AFFECTING THE U. S. POTATO CROP: A GROUP EFFORT BETWEEN SELECTION PRESSURES AND FARMING PRACTICES.

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BACKGROUND and OBJECTIVES

Potato virus Y (PVY) is the major disease affecting the U.S. seed potato crop. In the past decade we have documented a rapid shift in the PVY strains; the ordinary strain, PVY^{O}, was most common in the 20th century, but now the recombinant strains are emerging. Our overall objective was to identify the factors contributing to this emergence of recombinant strains. Comprehensive efforts have focused on PVY strain incidence and distribution, the influence of potato cultivars, as well as the impacts of farming and regulatory practices on PVY epidemic development.

MATERIALS and METHODS

Comprehensive surveys of post-harvest tuber samples from most U.S. seed potato production states have been conducted since 2004. Sprouted tuber or leaf samples were tested by ELISA to determine PVY infection status; strains were identified using immunocapture, multiplex RT-PCR. Isolates from the most prevalent PVY strains were used to challenge most North American potato cultivars to determine the type and severity of foliar and tuber symptoms. These studies were done under controlled greenhouse conditions using mechanically inoculated plants. Foliar symptoms were assessed every other week, tuber symptoms were assessed at harvest and after 60-90 days in storage. All these data are evaluated in conjunction with data from seed certification programs on the overall health of the seed potato crop as well as data from a multitude of other PVY research activities to better understand the major factors contributing to PVY epidemics and PVY strain shifts.

RESULTS

Early on, PVY^{O} predominanted in all production areas. PVY^{NTN}, the strain causing potato tuber necrotic ringspot disease (PTNRD), was present at low levels in one state. PVY^{NO} was found in all regions with the closely related PVY^{NWi} being a small percentage of the recombinant population. Since 2009, PVY^{O} has continued to decline; PVY^{NTN} is increasing in incidence and distribution, but remains a minority component of the total PVY population. The recombinant $PVY^{NO/NWi}$ population now dominates throughout the U.S., but PVY^{NWi} has nearly displaced PVY^{NO}. Other recombinant strains and genome variants have been detected, but their incidence and distribution changes over years and geographic regions.

Only a handful of the major North American cultivars are susceptible to PTNRD. While PTNRD is induced by most PVY^{NTN} isolates, some isolates from nearly all strains of PVY can induce the disease. Interestingly, most cultivars express one or more *Ny* resistance genes manifested as a foliar hypersensitive-like response to infection by PVY^{O} and some PVY^{NTN} isolates. These reactions often lead to plant death or severe impairment of tuber production. Additionally, aphids do not efficiently transmit virus from these plants. The unintended *Ny* gene deployment is contributing to the disappearance of PVY^{O} and maintenance of PVY^{NTN} at low levels, but also to the selection of $PVY^{NO/NWi}$ strains.

CONCLUSIONS

In general, the recombinant strains induce milder foliar symptoms in most widely grown North American cultivars. This has challenged seed certification programs that rely on symptoms to assess the health of the crop. PVY is the main reason for seed lots failing to be certified. On-farm roguing operations are also less effective at removing diseased plants contributing to increased inoculum in the seed potato crop. A lack of coordination between breeding and pathology programs has resulted in the release and widespread acceptance of numerous PVY tolerant potato cultivars that express limited or transient foliar symptoms; a further a challenge to seed certification. Climate change is expanding growing seasons and pushing aphid flights later in the season especially in northern U.S. production areas. This contributes to a reduction in foliar symptoms due to late season infection and there is a greater overwintering of tubers left in the field resulting in a higher number of volunteer potatoes emerging in the spring. All of these factors are contributing to more virus inoculum in the potato crop and a selection for recombinant strains/isolates that induce milder foliar symptoms, but have a propensity to induce PTNRD.

Many factors are driving the transformation of PVY populations and PVY epidemiology in the U.S. potato crop, but most can be traced back to short comings in the science of developing better potatoes and in the business of producing the potato crop.

EXPLOITING VIRAL GENETIC DIVERSITY TO UNCOVER SHARKA DISPERSAL AT REGIONAL AND LOCAL SCALES

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BACKGROUND and OBJECTIVES

A good understanding of disease spatiotemporal dynamics is essential for the definition of optimal strategies of surveillance and management. Plant RNA viruses are fast-evolving pathogens for which molecular evolution and dispersal occur at a similar time scale. Thus, spatial epidemiological processes leave measurable imprints on viral genomes that can be used to gain insights into, for example, the origin and dissemination pathways of emerging viruses at global or regional scales (Lefeuvre et al., 2010). More locally, viral genetic polymorphism combined with epidemiological data can help differentiating introduction events and reconstructing transmission chains within a given outbreak (Morelli et al., 2012; Ypma et al., 2012).
The aim of this study is to improve our knowledge of the spatial dynamics of sharka, one of the most devastating viral diseases of stone fruit trees. Its causal agent, *Plum pox virus* (PPV, genus *Potyvirus*), is disseminated by the plantation of contaminated material and by many aphid species in a non-persistent manner. We investigated if PPV genetic diversity could be used (i) to reconstruct dissemination routes at a regional scale and (ii) to assess the relative influence of introduction events and secondary aphid dispersal in a local outbreak.

MATERIAL and METHODS

This study focused on a peach production region of southern France where epidemics related to PPV strain M have been documented since the early 1990's. A recently discovered outbreak encompassing 215 susceptible peach orchards was intensively surveyed and 843 geo-referenced PPV isolates were genotyped by CE-SSCP and/or partially sequenced. We obtained whole genome sequences for 16 isolates representative of the PPV diversity in this area, as well as for 43 isolates collected over 17 years in 10 other PPV outbreaks distant from 1.5 to 40 km. Phylogeographic analyses were carried out under a Bayesian framework (BEAST package).

RESULTS and CONCLUSIONS

Our data set displayed a strong temporal signal allowing the reconstruction of the evolutionary history and dispersal of PPV in the study region. Distinct clades were found spreading in northern and southern areas separated by a series of hills that may have prevented further PPV dispersal by aphids. In both areas, gene flow was evidenced between nearby outbreaks. The PPV diversity spreading in the intensively surveyed outbreak was found to stem from multiple introductions events from nearby inoculum sources as well as from more distant ones, suggesting that both aphid dispersal and plantation of contaminated material may have contributed to the epidemic. The next step will be to formally reconstruct the transmission chains and to get direct estimates of the dispersal function within and between orchards.

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CURRENT STATUS OF OCCURRENCE, GENETIC DIVERSITY AND EPIDEMIOLOGY OF BANANA (*Musa* spp.) VIRUSES IN DEMOCRATIC REPUBLIC OF CONGO

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BACKGROUND and OBJECTIVES

Viruses are major constraints to the production and exchange of banana and plantain (*Musa* spp.) germplasm in the world. Several viruses are recognized to affect *Musa* spp: *Banana bunchy top virus* (BBTV*), Banana streak GF virus* (BSGFV), *Banana streak OL virus* (BSOLV*), Banana streak IM virus* (BSIMV)*, Banana streak MY virus* (BSMYV), *Cucumber mosaic virus* (CMV)*, Banana bract mosaic virus,* (BBrMV). To date, in Democratic Republic of Congo (DRC), except BBTV, others banana viruses are not yet reported. The main aim of this work was to elucidate the epidemiological situation, genetic variability and origin of *Musa* spp. viruses in DRC.

MATERIALS and METHODS

Epidemiological surveys were conducted from 2011-2013, in DRC, to search main viruses of *Musa* spp. Up to 666 samples collected from 122 locations; Samples were analysed using PCR based method. Detected viruses were sequenced and phylogenetic studies were performed.

RESULTS

BBTV is the most widespread virus (frequency=44,8%) in DRC. Molecular analyses support infrequent BBTV dispersal events between continents. This study reveals a low genetic variability of DRC BBTV isolates. They are close, gather in the South Pacific group and are linked with isolates of other sub-Saharan Africa (SSA) countries, but no with Egyptian isolates. Since the earliest BBTV report in SSA was in DRC in 1950, it is likely that BBTV spread from DRC to other SSA countries rather than from Egypt and that at least two BBTV introductions occurred in Africa. Haplotypes analysis based on the coding region m-Rep protein showed 38 haplotypes (haplotype diversity value of 0.944±0.013).
In Bas Congo, symptoms of Banana streak disease were associated to the presence of BSV species (frequency=19,9%); the most widespread were BSGFV (frequency=67,2%) and (BSOLV) (frequency=24,2%). The sequenced BSOLV isolate from *Musa acuminata*, gather in the clade I recognized to gathering the BSV species with endogenous counterpart. CMV was also detected (frequency=9,3%). Genetic diversity analysis reveals the high similarity between DRC CMV isolates with a South African isolate). BBrMV was not detected in collected samples.

CONCLUSION

DRC is a place of high prevalence of banana viruses, indicating the need for an improved quality of planting material. DRC seems to be the primary center of BBTV spread in Africa. BSV species data revealed the activation of endogenous BSV and the transmission of BSV species from plantain (B genome) to banana (A genome), in natural conditions. While for CMV, data suggest the hypothesis of a unique introduction through infected planting material and the distribution of a single strain of CMV. The BBrMV still absent in DRC.

TWO NEW WHITEFLY-TRANSMITTED CUCURBIT VIRUSES FROM SUDAN WITH CONTRASTING DISTRIBUTION PATTERNS

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BACKGROUND and OBJECTIVES

Surveys were conducted from 1992 to 2012 in the major cucurbit growing regions of Sudan to determine the relative incidence of viruses infecting these crops, in order to define adapted control strategies and to identify appropriate resistance sources in germplasm collection. During 2012 survey two new whitefly-borne virus species were identified, a tentative torradovirus from squash: squash chlorotic leaf spot virus (SCLSV) (1) and a tentative ipomovirus from a wild cucurbit *Coccinia grandis*: coccinia mottle virus (CocMoV) (2).

MATERIAL and METHODS

Using specific primers developed for SCLSV and CocMoV (1,2), RT-PCR experiments were conducted on extracts kept at -20°C of samples collected during 1992-2012 surveys in order to determine the occurrence of these two viruses in different cucurbits and growing regions.

RESULTS

SCLCV was detected in 9.5% of the 380 samples tested. It was found in all cultivated cucurbits tested (snake melon, melon, squash and watermelon) and in some wild *Cucumis* sp. Interestingly, SCLSV was detected as early as 1992 in Fadasi (near Wad Medani, Gezira State), and it was detected in samples collected in the same farm in 2003 and 2012, indicating that the virus is well established in the environment. In contrast, the tentative ipomovirus CocMoV was found only in another sample from the same host *C. grandis* collected in 1996, in the same area as the sample collected in 2012.

CONCLUSIONS

The striking differences in the distribution patterns of SCLSV and CocMoV will be discussed in relation to the local abundance of vectors and the availability of virus sources. Although presently limited to a specific location and host, CocMoV which causes severe symptoms in cultivated cucurbits under experimental conditions may emerge in crops, assuming more favourable dissemination conditions occur.

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ARE WILD APIACEAE A RESERVOIR OF CARROT VIRUS EPIDEMICS?

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BACKGROUND and OBJECTIVES

Between 2009 and 2011 a team from The Warwick Crop Centre (UK) collected 1555 samples of wild and cultivated *Apiaceae* from 10 locations across the UK. Samples were tested for the presence of *Parsnip yellow fleck virus* (PYFV), *Anthriscus yellows virus* (AYV) and *Carrot red leaf virus* (CtRLV). The virus isolates detected through this survey were sequenced to give insight into the relationships between the specific viruses detected and plant hosts. In most cases CtRLV genotypes originating from cultivated carrot were quite different from those found in any of the wild hosts suggesting weeds are not a major factor in the epidemiology of this virus (Defra project IF0188).
During an investigation into a symptom of unknown aetiology, carrot root necrosis, Next-Generation Sequencing identified several novel or unusual viruses (Adams et al., 2014). *Carrot yellow leaf virus,* not previously reported from UK carrots, was found to be associated with carrot root necrosis. Novel virus findings included Carrot torrado virus-1 (CaTV1), and Carrot closterovirus-1 (CtCV1). Although these viruses are present in carrot crops across the UK and Northern Europe, little is known about reservoirs of these viruses.

MATERIAL and METHODS

RNA extracts of samples previously tested during Defra IF0188 were transferred to the laboratory at Fera, York, UK. As these had been stored for an extended period of time, they were checked for quality using primers for COX samples were then tested for the presence of *Carrot yellow leaf virus*, Carrot closterovirus-1 (Adams, et al.2014); and Carrot torradovirus-1 using real-time PCR assays developed specifically for this project.

RESULTS

Over 1000 samples had both a sufficient quality extract to allow further testing to be carried out, and could be tied to sample origin virus data. CtCV1 was the virus most commonly found, present in all weed species tested. Half of all cow parsley (*Anthriscus sylvestris*) samples were infected with CtCV1. There were multiple novel host records including (*Aegopodium podagraria*) and hemlock (*Conium maculatum*) for both viruses. As CtCV1 had only previously been recorded on carrot during an NGS study, novel records were obtained for all species in the study. CaTV1 was not detected from any weed sample in the study.

CONCLUSIONS

Work is ongoing to confirm the role of weeds as a source of CYLV and CtCV1. CaTV1 infections do not appear to originate from weed sources and work is ongoing to identify sources of this virus including seed and alternate hosts. Latest results of this work will be discussed.

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CUCUMBER GREEN MOTTLE MOSAIC VIRUS IN AUSTRALIA- THE STORY SO FAR

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BACKGROUND AND OBJECTIVES

Cucumber green mottle mosaic virus (CGMMV) is a Tobamovirus that can infect cucurbit plants and is responsible for significant economic losses worldwide (CABI Crop Protection Compendium, October 2015). CGMMV was first detected in Australia in the Northern Territory (NT) in September 2014 (Tesoriero et al 2015) and Queensland (QLD) in April 2015. In the NT, the virus was initially detected in commercial watermelons (*Citrullus lanatus*) expressing severe mosaic and stunted foliar development. By early 2016, CGMMV was identified on 26 properties in the NT and on one commercial watermelon property in QLD. CGMMV has not been detected elsewhere in Australia. Molecular identification based upon the sequence data of the coat and movement protein (1.2 kb total) indicated that the Australian strain (NT and QLD) had greater than 99% sequence similarity to the Indian bottle gourd isolate (DQ767631) and Canadian cucumber isolate (KP772568). Quarantine measures have been in place since September 2014 where CGMMV infested properties have been unable to grow cucurbit crops. Our objectives were to determine the host range of CGMMV in Australia and obtain the whole CGMMV genome sequence for the NT and QLD isolates using next generation sequencing.

MATERIALS AND METHODS

Total RNA was extracted from infected plant material and bee products using the Isolate RNA plant kit (Bioline, Australia). Conventional one step RT-PCR was performed using coat protein (Reingold 2014) and movement protein primers (Ling et al 2014). The PCR products were purified and direct sequencing conducted in both directions. Bioinformatics were conducted using the Geneious software. NGS libraries were conducted using the NEBNext Ultra RNA library prep kit for Illumina (New England Biolabs, Australia) and sequencing conducted on the MiSeq. Bioinformatics analyses of the NGS include de novo assembly using CLC genomics, metagenomics using MGRast and Geneious for contig constructions and reference mapping.

RESULTS

In addition to watermelons, CGMMV has been detected in other cucurbit crops such as pumpkin, cucumber, squash, Asian vegetables (*Benincasa hispida*, *Luffa* sp and *Momordica charantia*) as well as weed hosts (*Amaranthus*, *Cucumis*, *Portulaca* and *Solanum*) and honey bee hive products such as bread, brood, honey, wax and bees in the NT. Near full genome coverage was obtained for both the NT and QLD CGMMV isolates.

CONCLUSION

Cucumber green mottle mosaic virus is widespread in the NT in cucurbit production areas with 26 properties infested. Next generation sequencing data has shown that the Australian CGMMV strain is closesly related to the Canadian cucumber and the Indian bottle gourd strains.

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A MULTIPLEX LUMINEX xTAG-ASSAY TO DISTINGUISH BETWEEN INFECTIOUS AND NON-INFECTIOUS *CUCUMBER GREEN MOTTLE MOSAIC VIRUS* ON CUCUMBER SEEDS

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BACKGROUND and OBJECTIVES

Cucumber green mottle mosaic virus (CGMMV; genus *Tobamovirus*) is currently causing significant problems worldwide. The virus is mainly restricted to *Cucurbitacae* however, several weed species have been reported as possible host plants. CGMMV is clearly seed transmitted which is the suspected cause for recent outbreaks in the USA and Australia.
Several serological and molecular tests are available to detect the virus on seeds however none can distinguish between infectious ('alive') and non-infectious ('dead') virus. Only an elaborate, time-consuming and relatively insensitive bioassay can make its distinction.
We set out to develop a test that can make a more reliable distinction between infectious and non-infectious CGMMV on cucumber seeds.

MATERIALS and METHODS

Different seed batches from various origin and naturally infected with CGMMV were contributed by H. Koenraadt (Naktuinbouw, Roelofarendsveen, The Netherlands). A true multiplex Luminex xTAG assay, employing multiple RT-PCR primer sets in combination with 11 TSPE primers (Van Brunschot et al, 2014) was designed and tested to detect CGMMV RNA from different CGMMV isolates and subjected to an internationally accepted validation protocol.

RESULTS

All TSPE primers gave clear positive signals on the untreated seed batches. A standard dry-heat treatment on the different batches of CGMMV-infected seeds (B. Woudt, Syngenta Seeds, Enkhuizen, The Netherlands) reduced all TSPE signals to nearly background levels, indicating a significant breakdown of the viral RNA.

CONCLUSIONS

The developed multiplex xTAG assay successfully detected different CGMMV isolates in both leaf material and cucumber seeds and was capable to distinguish intact from degraded CGMMV RNA. It was also validated with respect to various performance criteria. Bioassay experiments to confirm the correlation between the absence of TSPE signals with the abolishment of virus infectivity will be reported.

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STARTING A PLANT VIRUS DEPARTMENT AND ITS DEVELOPMENT A PERSONAL EXPERIENCE – FROM TEST PLANTS TO MOLECULAR BIOLOGY

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In the mid 1950ties Israel's agriculture underwent a transition from dry farming to irrigated crops. This was accompanied by outbreaks of virus diseases in vegetable and ornamental crops. . In 1960 the Director of The Volcani Institute, decided that the agricultural needs required a separate research unit for plant virus research. The philosophy of the new department determined that it is not sufficient to identify the pathogen, but that the research has to lead to a practical solution. This was quite in contrast to ideas prevalent in some departments of plant pathology, where the scientists considered that their research was only to identify and classify and uncover the etiology of the causal agent. The research in the department was focused on two aspects: Viruses in seed propagated crops and viruses in vegetative crops – mainly citrus, ornamentals, sweet potato and potato It was also thought from the beginning that the research efforts within the department should be balanced between straightforward applied research, and that leading of an understanding of the viral particle, and the interaction with the vector and the plant and also to understand resistance mechanisms of plants The first projects on viruses in vegetables were centered on viruses in cucurbits resulting after a relative short period in cucumbers resistant to Cucumber mosaic virus. Subsequent projects were on PVY in sweet peppers, viruses in tomato as TMV, and Tomato yellow leaf curl virus (TYLCV), the later remaining an important subject till today. In addition, many viruses infecting vegetable and field crops were identified. Vector transmission studies were an integral part of research projects. ELISA could detect CMV in aphids. We showed that the coat protein of CMV determines its transmissibility by aphids. ZYMV was cloned, and control methods using oil sprays and yellow sticky sheets were evaluated. In the vegetative propagated crops, viruses in citrus, irises, carnation, sweet potato and strawberries were important areas of research. The major challenge was Tristeza as almost all citrus was grafted on sour orange rootstocks. A method for purification of the virions was developed, enabling study of the particles' morphology and the adaptation of a method combining grafting on K-lime with EM for indexing. This was instrumental in operating the Tristeza suppression program, until a serological method based on ELISA was developed. Another thrust entered on ornamental plants as irises, carnations, lilies, pelargonium and roses. This required *inter alia* identification of the viruses, vectors, virus tested propagation. With spray carnations exports of flowers increased from 0 in1960 to 240 million flowers in 1977/78. A major interest were resistance mechanism- local lesions and induced resistance. Using protoplasts we showed that an inhibitor of virus replication (IVR) is associated. The IVR gene was cloned. Transformation of tomatoes with this gene induced resistance to several fungal pathogens.

TRANSMITTED PLANT VIRUSES CAN AFFECT PERFORMANCES OF STARVING APHID VECTORS

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BACKGROUND AND OBJECTIVES

Manipulation of insect vectors by transmitted plant viruses is intensively studied in our scientific community. Viruses operate this manipulation either directly in insect vectors, or indirectly via physiological changes induced in the infected host plant. In all cases, the analyzed parameters are the altered attractiveness of the infected host plant, the settlement of insect vectors onto these plants, their feeding behavior, and their growth rate estimated through generation time and number of offspring. It is remarkable that all analyzed parameters describe the relationship between the insect vectors and the host plants, and that the phase when vectors are starving “off plant” is seldom considered. Yet, the capacity of the vector to successfully find another plant -- its survival time during starvation and the distance or surface area the vector can explore -- is obviously key for the virus epidemiology.

MATERIAL AND METHODS

We thus decided to explore whether a virus (FBNSV, *Nanoviridae*) can modify the performance of its aphid vectors (*A. pisum*), when starving away from host plants (*Vicia faba*). We primarily targeted the survival time, since it is obviously an important property that could be manipulated by the virus. Indeed, surviving longer when starving would increase the chances of the vector finding a new host and the distance at which this host could be found.

RESULTS

Our results demonstrate that aphids previously fed onto infected plants are very significantly longer lived when removed from these plants and kept away from new ones. Long-lived aphid survivors are perfectly able to transmit the virus and to found new colonies when finally transferred onto host plants.

CONCLUSIONS

Though at this stage we do not understand the underlying mechanisms, our results reveal that viruses may manipulate the individual performances of their insect vectors when travelling off host plants. This aspect of vector manipulation by transmitted viruses has thus far been largely overlooked and deserves increased attention, as it might be an important trait for epidemiology.

INCREASED LYSOSOMAL EXOCYTOSIS IN THE APHID MIDGUT DECREASES CIRCULATIVE VIRUS TRANSMISSION

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BACKGROUND and OBJECTIVES

Circulative virus transmission by aphids involves virus entry into the polarized epithelial cells of the gut. When a clonal lineage of *Myzus persicae* was reared on physalis plants (referred to as P-Myzus), it was an efficient vector of the polerovirus *Potato leafroll virus* (PLRV). In contrast, when the same clone was reared on turnip (T-Myzus), it poorly transmits PLRV. These data reveal a surprising phenotypic plasticity in vector competency in a clonal lineage of aphids that is host plant-dependent. We sought to understand the molecular basis for this plasticity in PLRV transmission.

MATERIAL AND METHODS

Mass spectrometry and gel-based quantitative proteomics were used to compare the proteomes and phosphoproteomes of P and T-Myzus. Phosphopeptides were enriched using iron-immobilized metal affinity chromatography. Proteins and phosphoproteins were identified and quantified using a database composed of aphid and bacterial symbiont proteins in Mascot, Proteome Discoverer and Scaffold. Co-immunolocalization using anti-human cathepsin B and PLRV antibodies was performed using confocal microscopy. Cysteine protease inhibition in aphids was performed using the inhibitor E64 orally delivered via membrane sachets. Transmission assays were performed using PLRV-infected hairy nightshade as the source of virus and potato as the recipient plants.

RESULTS

Quantitative proteomics revealed a specific upregulation in lysosomal protein expression in T-Myzus as compared to P-Myzus, including cathepsin B, cathepsin B-16, beta-glucuronidase, peroxidasin, legumain-like, and aminopeptidase-N. Consistent with altered lysosomal function, proteins involved in lysosome-mediated proteolysis and cell proliferation were among those differentially phosphorylated. In P-Myzus midguts, cathepsin B was localized in lysosomes and no-colocalization with virus was observed. In contrast, a relocalization of cathepsin B to the cell membranes in T-Myzus was observed, where it co-localized with PLRV. Inhibition of cathepsin and other cysteine proteases with E64 restored the ability of T-Myzus to transmit PLRV in a dose-dependent manner, showing that the activities of lysosomal enzymes at the cell membrane in T-Myzus is responsible for the change in virus transmission phenotype in these aphids. T-Myzus individuals weighed more and had more progeny than P-Myzus individuals. These data are all consistent with an induction of lysosomal exocytosis by turnip sap in the aphid midgut.

CONCLUSIONS

Lysosomes regulate the degradation and recycling of proteins. They also function in a secretory pathway known as lysosomal exocytosis. Our data support the hypothesis that turnip sap induces lysosomal exocytosis in aphid midguts, which results directly or indirectly in inhibiting the transmission of PLRV. Molecular pathways involved in the regulation of lysosomal exocytosis represent a promising target for the control of circulative plant virus transmission by aphids

DOMAINS IN THE *PEA ENATION MOSAIC VIRUS* MAJOR COAT PROTEIN REQUIRED FOR VIRION ASSEMBLY AND APHID TRANSMISSION

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BACKGROUND and OBJECTIVES

The coat proteins (CP) of plant virus capsids are multifunctional and have been implicated in practically every stage of the viral infection cycle. *Pea enation mosaic virus* 1 (PEMV1, *Luteoviridae* family) and *Pea enation mosaic virus* 2 (PEMV2, *Umbravirus* genus) are two RNA viruses in an obligate symbiosis causing the pea enation mosaic disease mainly in the *Fabaceae* plant family. PEMV1 requires PEMV2 movement protein for long distance movement in the plant while PEMV2 uses the CP encoded by PEMV1 for encapsidation and aphid transmission. Plant viruses have evolved for highly specialized molecular interactions with their aphid vector. A greater understanding of the mechanisms behind the transmission process is important for development of effective control measures for these epidemic plant diseases. The long term goal of this project is to identify amino acids in the CP that interact with the aphid receptor (1) to facilitate PEMV transmission.

MATERIALS and METHODS

After computational prediction of the CP structure based on known structures of similar icosahedral virions, we identified amino acids predicted to be on the surface that may interact with the aphid gut receptor (2). Sixteen mutations were introduced into the CP gene of the full-length infectious clone of PEMV1. *Pisum sativum* plants were co-infected with the CP mutants and an infectious clone of PEMV2 and the following traits were observed: *(i)* Replication of both viruses in inoculated leaves, capacity for long distance movement, viral accumulation at 15 and 21 day post-inoculation were all assessed by RT-qPCR. *(ii)* Translation of CP and the capacity to assemble in new leaves were determined by western blot analysis and transmission electron microscopy (TEM), respectively. *(iii)* The capacity for transmission of each mutant by pea aphids *(Acyrthosiphon pisum)* was evaluated, and a 3D model of the PEMV capsid structure proposed.

RESULTS

All of the 16 CP mutants infected plants in the inoculated leaves, and surprisingly all mutant RNAs could move long distances. The CPs of 14 mutants were detected in upper pea leaves by western blot using specific antibodies, while only four of those were 100% positive by both western blot and TEM (i.e. virions observed). Two mutants containing amino acid changes in the basic amino-terminal R domain lost the capacity for transmission by aphids. Only one of two mutations localized in one epitope of the S domain was impaired in the efficiency of aphid transmission. None of the ten remaining mutants (RNAs, partial detection of CP but no virions) were transmitted by aphids. Mutations in surface localized epitopes prevented virion assembly.

CONCLUSIONS

Three-dimensional modeling of the PEMV CP, combined with biological assays for virion assembly and aphid transmission, allowed for a model for assembly of PEMV coat protein subunits. Thus, the PEMV mutants that assemble and mutants with mutations on the surface of the virion could be tested to evaluate the relationship between their capacity for aphid transmission and binding affinity to the aminopeptidase N receptor in the aphid gut (1). Increased understanding of the molecular interactions between luteovirids and their aphid vectors will facilitate development of management tools for both vector and plant viral disease.

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VISUAL AND OLFACTORY CUES INVOLVED IN THE SELECTION OF VIRUS-INFECTED TOMATO PLANTS BY BEMISIA TABACI

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BACKGROUND and OBJECTIVES

Virus infection may change not only the plant phenotype, but can modify the behaviour and performance of their insect vectors in various ways. Some plant viruses can manipulate the behaviour and fitness of their insect vectors in a way that transmission and spread is enhanced, but this may not always be the case. Here we investigated whitefly response to tomato plants infected by *Tomato chlorosis virus*, ToCV, a semipersistently-transmitted crinivirus and *Tomato severe rugose virus*, ToSRV, a persistently-transmitted begomovirus. More precisely, we tested the role of visual cues and/or volatile emissions of tomato plants infected by ToSRV and ToCV in the host plant selection process of both viruliferous and aviruliferous *Bemisia tabaci* (MEAM1).

MATERIAL and METHODS

Visual cues alone were assessed by placing leaves of virus-infected and mock-inoculated plants underneath a plexiglass plate as targets for whitefly landing. A dual-choice arena (no active air flow) was used to assess whitefly response to headspace volatiles emitted by virus-infected and mock-inoculated tomato leaves (not excised) under light and dark conditions. Similarly, we tested whitefly response to odours using a Y-tube olfactometer with active air flow. Finally, volatiles emitted by virus-infected and mock-inoculated tomato plants were chemically characterized using gas chromatography coupled to mass spectrometry.

RESULTS

Visual stimuli in three-choice test showed that whiteflies preferred to land on virus-infected than on mock-inoculated leaves or blank (no leaf), suggesting that visual cues play a key role during landing. Furthermore, the results of the dual choice leaf arena show that whiteflies had no preference for either virus-infected or mock-inoculated leaves under dark conditions, but preferred virus-infected leaves in the presence of light, suggesting that visual are more important than olfactory cues. However, when the active air flux Y-tube olfactometer was used whiteflies discriminated between virus-infected and mock-inoculated plants. In fact, ToSRV-viruliferous whiteflies preferred volatiles from mock-inoculated than ToSRV-infected plants, while non-viruliferous insects showed no preference. Conversely, non-viruliferous whiteflies preferred volatiles from mock-inoculated than ToCV-infected plants, while ToCV-viruliferous whiteflies showed no preference. No qualitative differences were found in virus-infected (ToSRV or ToCV) relative to mock-inoculated plants. However, some tomato plant volatiles were significantly suppressed 2wks after ToSRV infection, while no significant differences in the concentration of volatiles were found between ToCV-infected and mock-inoculated plants.

CONCLUSIONS

Our results suggest that *B. tabaci* is first attracted to visual cues of virus-infected plants. However, whiteflies also distinguish between odours emitted by virus-infected and non-infected plants, and react to volatiles in a different way depending on virus type (circulative or noncirculative) and whitefly state (viruliferous or non-viruliferous). Viruliferous ToSRV whiteflies prefer volatiles emitted by mock-inoculated than ToSRV-infected plants, a conducive behaviour to enhanced virus spread. However, the opposite happens for ToCV because sources of inoculum are avoided by aviruliferous whiteflies, a behaviour that will be detrimental to the secondary spread of the virus.

LETTUCE NECROTIC LEAF CURL VIRUS, A MEMBER OF A NEW SUBGROUP IN THE GENUS TORRADOVIRUS?

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BACKGROUND and OBJECTIVES

Lettuce necrotic leaf curl virus (LNLCV) was described as the first non-tomato-infecting member of the genus *Torradovirus*. Until today, the virus was only found in The Netherlands in two different areas in an open field crop of lettuce (Verbeek et al, 2014a). In 2015 LNLCV was accepted by the ICTV as a new member of the genus *Torradovirus.* The tomato-infecting torradoviruses *Tomato torrado virus* (ToTV), *Tomato marchitez virus* (ToMarV) and Tomato chocolàte virus (ToChV) are transmitted by at least three whitefly species in a semi-persistent and stylet-borne manner (Verbeek et al, 2014b). As LNLCV seems to be transmitted in the field in The Netherlands, where whiteflies are not present in high numbers, we were wondering if this virus was whitefly-transmitted as well. The objective of our study was to identify the natural vector of LNLCV, and to give insight in the epidemiology of LNCV in The Netherlands.

MATERIALS and METHODS

The reference strain of LNLCV (isolate 5317015) was maintained in lettuce cv. White Boston or in *Nicotiana occidentalis* 37B by mechanical inoculation. Colonies of whiteflies *Bemisia tabaci*, *Trialeurodes vaporariorum*, *T. abutilonea* and *Aleyrodes proletella* were kept on their host plants in insect cages, as well colonies of the aphid species *Myzus persicae*, *Nasonovia ribisnigri*, *Cavariella aegopodii* and *Macrosiphum euphorbiae*. Insects were assessed for their ability to transmit LNLCV by giving cohorts of 50-100 whiteflies or 20-50 aphids an Aquisition Access Period (variable lengths) on a LNLCV-infected host plant and subsequently transferring them to a healthy test plant (lettuce or *N. occidentalis* 37B) for an Inoculation Access Period. Inoculated plants were evaluated for symptom development and/or tested by a LNLCV-specific RT-PCR.
In order to determine the retention site of LNLCV in the various insects, the probosces, including the stylets, were removed using micro-scissors or an ophthalmic scalpel. Stylets and rest bodies (with stylets removed) were tested separately in RT-PCR.

RESULTS

None of the tested whitefly species was able to transmit LNLCV. Transmission was observed only with the aphid species *N. ribisnigri* when AAPs and IAPs were used fitting a semi-persistent or persistent mode of transmission . We were able to localize LNLCV in viruliferous aphids in the stylets, suggesting a stylet-borne transmission, as was observed in whiteflies viruliferous for tomato-infecting torradoviruses. Moreover, LNLCV was detected in the stylets of *N. ribisnigri* and *C. aegopodii* but not in *M. persicae* and *M. euphorbiae*.

CONCLUSIONS

LNLCV was transmitted only by aphids (the lettuce-currant aphid, *N. ribisnigri*) and not by various whitefly species. Based on the type of insect vector, LNLCV might form a new subgroup within the genus *Torradovirus,* together with Carrot torrado virus 1 (CaTV1), which is also aphid-transmissible (A. Fox, FERA, personal communication).

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EVALUATING POSPIVIROID-HOST-VECTOR INTERACTIONS IN BELGIUM

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BACKGROUND AND OBJECTIONS

During the past decade, pospiviroids have been repeatedly reported in many ornamentals and crops all over Europe, implying major phytosanitary risks for the commercial trade of plants. In order to gain a better insight into viroid-vector-host interactions, different kinds of experiments were organized for each part of this trilateral interaction.

MATERIAL AND METHODS

In the context of pathogen-host interactions it was unknown whether naturally occurring weeds can play a role as reservoirs, from which pospiviroid transmission occurs. To evaluate this risk, a survey in commercial greenhouses, a contact-experiment and an inoculation-experiment were organized. To investigate the origin of pospiviroid infections and host specificity in vulnerable crops, like tomatoes, we also performed a phylogenetic analysis using more than 30 recent Belgian pospiviroid isolates. To study pathogen-vector interactions, fluorescence *in-situ* hybridization (FISH) experiments were conducted to localize and quantify viroids in/on insects. The interaction of viroids with other plants viruses was also investigated, and more specifically the phenomenon of “transencapsidation”, where viroids are incorporated within a viral particle. Lastly, in the framework of vector-host interactions, we investigated the effect of viroid ingestion through contaminated pollen; as well as the effect of volatile emissions of pospiviroid-infected plants, on bumblebee behaviour.

RESULTS

The percentage of each insect species that tested positive for pospiviroids in a specific q(RT)-PCR was 100% for *M. persicae*, 4% *B. terrestris*, and 0% for *M. pygmaeus*. The transmission rate of pospiviroids was 0% for *M. persicae* and *M. pygmaeus*, and 2.6% for bumblebees. The results from these experiments show that only 5% of the inoculated weed species are potential hosts for viroids, and that most of the pospiviroid “wild” hosts are species belonging to the families of the *Solanaceae* and the *Asteraceae*. In aphids (*Myzus persicae*) that were fed on pospiviroid-infected plants, viroid signals were observed in the aphid’s stylet and digestive system, but not in the embryos. In subsequent transmission experiments, *M. persicae* was not able to transmit the viroid to healthy host plants. Also insects from other functional groups were tested as potential vectors: i.e. *Bombus terrestris* (pollinators) and *Macrolophus pygmaeus* (biological control agents). While previous reports indicate that virus-assisted transmission of pospiviroids (through transencapsidation) between plants could happen, this has not been observed during our experiments. No negative, nor positive effects on bumblebee behaviour were observed.

CONCLUSIONS

Transmission studies on the risk of pospiviroids being spread through several insect pathways all indicate that this possibility exists, but is rather limited. This comprehensive study will be broadly discussed in the context of EU phytosanitary measures for pospiviroids.

WATER-STRESS CAN ENHANCE THE TRANSMISSION OF PLANT VIRUSES BY INSECT VECTORS

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BACKGROUND and OBJECTIVES

Drought is one of the major abiotic stresses threatening crop production worldwide and is predicted to be accentuated by global warming. In nature, plant viruses constitute another major constraint and are responsible for tremendous agronomic and socio-economic impacts due to damages and yield losses in almost all cultivated crops. Of particular relevance then are analyses on how climate change will affect the future distribution of infectious disease outbreaks in plants. As a matter of fact, a number of recent studies showed the influence of climate change on plant vector-borne diseases and on their spread, mainly focusing on the effects on the vector biology (e.g. developmental time, fecundity, migration,…) and ecology [1, 2]. However, to our knowledge, there is no study reporting a direct impact of water-stress on changes in virus transmission. In this study, we investigated the effect of a severe water deficit on the transmission rate of two non-circulative transmitted plant viruses.

MATERIAL and METHODS

The aphid-transmissible *Cauliflower mosaic virus* (CaMV) and *Turnip mosaic virus* (TuMV) were propagated on 2 week old-turnip (*Brassica rapa*) plants by mechanical sap inoculation. Two weeks after inoculation, infected source plants were subjected to water withholding during eight days before transmission assays (n = 3 experimental replicates) for each virus tested.

RESULTS

For both viruses assayed, we showed that the transmission rate was significantly increased from source plants subjected to water deficit compared to well-watered plants. A 30-percent increase was observed in CaMV, while in the case of TuMV, transmission results were even more spectacular with a doubled transmission rate in water-stressed plants compared to well-watered plants. However, in both cases the increase in transmission rate could not be explained by an increased virus accumulation in water-stressed source plants.

CONCLUSIONS

Evidences that infected plants subjected to water stress are more likely to transmit viruses may have significant consequences for viral epidemiology in a globally changing environment. While we are currently investigating the biological mechanisms involved in the patterns observed, the results reported provide new insights for the modeling of virus transmission and disease dynamics.

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CLIMATE CHANGE: WILL PLANT VIRUSES AND INSECT VECTORS THREATEN FUTURE FOOD PRODUCTION?

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BACKGROUND and OBJECTIVES.

Understanding how future climate conditions and increasing atmospheric carbon dioxide will shape agriculture is essential in order to sustain or improve current crop production levels (Trębicki et al 2015). Pests and diseases are major contributors to the reduction of crop yield and are responsible for raising input costs. *Barley yellow dwarf virus* (BYDV-PAV) can cause serious crop disease and is transmitted by many aphid species. This virus causes yellowing and stunting of plants and adversely affects the yield and quality of wheat, barley and oats as well as other economically important crops in Australia and worldwide (Nancarrow et al 2014; Trębicki et al 2015).

MATERIAL and METHODS.

In this study, we used Australian Grains Free Air CO_2 Enrichment (AGFACE) facility and plant growth chambers to understand the aphid, wheat and BYDV interactions. We exposed infected and uninfected wheat and the bird cherry-aphid (*Rhopalosiphum padi*), the main vector of BYDV, to ambient and eCO_2 (550ppm AGFACE, 650 ppm chambers) levels. We examined the severity of BYDV-PAV in plants and aphid biology using electrical penetration graph (Dader et al 2016) in both virus free and BYDV infected plants, as well as plant and virus response under these two CO_2 scenarios.

RESULTS.

Despite the positive effect of eCO_2 on yield and wheat growth, our study showed detrimental CO_2 effects on grain quality and BYDV severity. We found that BYDV titre significantly increased in the leaves of wheat plants grown under eCO_2 conditions, leading to a decrease in yield (Trębicki et al 2015). We also found a decrease in aphid fecundity on uninfected plants; however, no changes in aphid reproduction were detected on BYDV infected plants. In addition, changes to wheat physiology mediated by eCO_2, resulted in significantly extended aphid feeding activity on uninfected plants. Other experimental data from both the FACE facility and environmental growth chambers will be described and the results from these experiments presented.

CONCLUSIONS.

Our findings highlight the complexity of interactions between plants, insects and viruses under future climate with implications for plant disease epidemiology and crop production.

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THE ROLE OF VECTOR SPATIAL DYNAMICS, PATHOGEN DISPERSAL BY TRADE AND GROWER BEHAVIOUR ON THE SUCCESS OF REGIONAL DISEASE CONTROL EFFORTS.

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Many pathogens have such extensive dispersal distances that effective disease control relies on the collaborative efforts of individual growers on a regional scale. There are thus two key processes to consider in the development of regional disease control methods:

(i) The movement of pathogens by vectors and through planting material.
(ii) The decisions made by growers regarding their control efforts.

In this presentation we explore these by considering two example cases.

Cassava Brown Streak Disease symptoms

Huanglongbing symptoms

Cassava brown streak:
In the first case, the spatial dynamics of cassava brown streak virus, we discuss the effect of vector dispersal and trade of planting material on the regional dynamics.
It will be shown that trade of planting material is the key large-distance pathogen dispersal mechanism, and that vector dispersal is of key importance in local amplification. Furthermore we show that when growers consistently use the same supplier of planting material, and when growers have a small number of suppliers, they have a lower risk of disease. We will show how this is related to the trade-network structure.
Next we turn to the effect of these dispersal patterns on the introduction of systems where clean planting material is distributed amongst growers. These 'clean seed systems' are currently being implemented. Our work provides guidance for these introductions. We will show how different spatial organisations of the introduction of clean seed systems determine the effectiveness of disease control. A community-based approach to the implementation of the clean seed system (as proposed by Legg et al 2015 and Patil et al 2015) is the most effective.
Funded by the Bill and Melinda Gates Foundation through a grant to Chris Gilligan.

Huanglongbing (HLB) disease of citrus:
Next we turn to grower behavioural components in the success of a regional disease control campaign. HLB has been introduced in citrus plantations in the US and is currently threatening the economic viability of citrus production in Florida. This disease is caused by a bacterial species but resembles the epidemiology of viruses as it is vector dispersed, develops systemically throughout the citrus tree and pesticidel control is not possible.
First we will discuss how grower perception can be modelled. The model is based on the two key aspects of a grower's willingness to apply disease controls, (i) the perception of the risk of their groves to get infected by the pathogen and (ii) their perception of the effectiveness of the control options. The opinion dynamics model is then coupled with an epidemiological model.
We have found that the success of an area wide HLB control campaign depends on a combination of the effectiveness of the control methods available and grower perception of the problem. Surprisingly, the grower's initial perception of the level of disease-risk is of far less importance to the success of a regional control campaign than their perception of the efficacy of the available control options. This implies that early information on the efficacy of the available control options is more important than prematurely alarming growers.
This work is funded by the USDA through a grant to Dr Tim Gottwald.

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SPREAD OF GRAPEVINE RED BLOTCH-ASSOCIATED VIRUS IN VINEYARDS

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BACKGROUND AND OBJECTIVES

Red blotch is a recently recognized viral disease of grapevines (Sudarshana et al. 2015). The causal agent, Grapevine red blotch-associated virus (GRBaV) has a single-stranded, circular DNA genome (3,206 nt), and is a proposed member of the family *Geminiviridae*. GRBaV has been detected in all major grape-growing regions of the United States and Canada, likely as a result of the dissemination of infected propagation and planting material (Krenz et al. 2014). Because little information is available on the ecology of GRBaV, we addressed spread in vineyards and carried experiments to identify a vector of epidemiological significance.

MATERIAL AND METHODS

Spread of GRBaV was monitored in diseased vineyards in New York and in California based on visual observations of symptom development and on virus detection by multiplex PCR (Krenz et al. 2014). Spatiotemporal dynamics of virus spread were characterized by stochastic modeling. To identify insect vectors of GRBaV in a selected California vineyard, traps were placed on diseased and healthy vines during the growing season. Insects caught on traps were used for a detailed census of hemipteran insects visiting the vineyard. A sub-set of each insect species caught on traps was collected and tested by PCR for GRBaV.

RESULTS

No indication of GRBaV spread was obtained in most vineyards where the virus was detected. Spatiotemporal patterns of GRBaV spread in a selected California vineyard showed a combination of aggregated and scattered, infected vines, suggesting within-vineyard virus transmission, likely due to an insect vector. Of more than 50 species of insects caught on traps and screened, the majority of specimens of only four species of the families Cicadellidae, Membracidae, and Cixiidae consistently carried genetic elements of GRBaV, as shown by multiplex PCR. The ability of these vector candidate species to transmit GRBaV to healthy grapevines is being assessed in the greenhouse.

CONCLUSIONS

The presence of GRBaV in free-living grapevines (Perry et al. 2016) and the spatiotemporal patterns of GRBaV spread in a vineyard in California suggest the involvement of an insect vector. Four vector candidates were identified and their ability to transmit GRBaV to healthy grapevines in a greenhouse will inform their role in transmission. Characterizing the vector and understanding the mechanism of transmission are critical to devise proper management strategies and minimize spread of GRBaV.

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PEA SEED-BORNE MOSAIC VIRUS EPIDEMIOLOGY IN A MEDITERRANEAN-TYPE ENVIRONMENT

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BACKGROUND AND OBJECTIVES

Pea seed-borne mosaic virus (PSbMV) epidemics in field pea crops cause serious seed yield and quality losses (Coutts et al 2008, 2009). Despite this, its presence in crops often remains unnoticed due to the subtlety of its foliar symptoms. An improved understanding of factors that drive PSbMV epidemics is crucial to enable their prediction so that they can be managed effectively.

MATERIALS AND METHODS

To collect aphid occurrence and PSbMV incidence data that represented a diverse range of climatic scenarios, 23 field pea data collection blocks were sown over a six year period (2010-2015) at five locations in south-west Australia. They were sown in late autumn-early winter with cultivars Kaspa and Twilight. Seed-stocks with 0.1-13% PSbMV infection level were sown and time of sowing varied with location and year. Yellow sticky traps were used to monitor aphid arrival and numbers. Every 2 weeks, 100 randomly collected shoot tip samples each from an individual plant within the blocks were tested for PSbMV by enzyme-linked immunosorbent assay (ELISA). Representative 1000 seed samples of the harvested seed from each block were sown in trays and the seedlings tested for PSbMV seed transmission by ELISA.

RESULTS

Aphid and virus spread data from the blocks provided a diverse range of aphid population and virus incidence scenarios. These ranged from early aphid arrival and high levels of PSbMV spread to late aphid arrival and low levels of PSbMV spread. The aphids caught were all transitory migrants as no field pea colonization ever occurred. The principal aphid species caught was *Myzus persicae*. Autumn rainfall significantly influenced the date of aphid arrival with high rainfall in this period resulting in their early arrival due to the generation of background vegetation which supported increased aphid populations. Additionally, PSbMV infection levels in the seed sown determined the magnitude of infection foci for transmission by aphid vectors and early wind-mediated contact spread.

CONCLUSION

Collection block data from this study is being used to calibrate and validate a forecasting model for PSbMV epidemics which would allow growers to make informed management decisions before sowing. Predicting a high risk year can enable growers to obtain a healthy seed source, plant a PSbMV-resistant pea cultivar and employ other management strategies to avoid losses from PSbMV infection. Predicting a low-risk year permits growers to sow seed of higher yielding cultivars PSbMV-susceptible cultivars with <0.5% seed infection and avoid wasting resources implementing control strategies.

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MAPPING AVERAGED PAIRWISE INFORMATION (MAPI): A NEW TOOL FOR LANDSCAPE GENETICS ON PLANT VIRUS

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BACKGROUND and OBJECTIVES:

Visualisation of spatial networks based on pairwise metrics such as (dis)similarity coefficients provides direct information on spatial organization of biological systems. However, for large networks, graphical representations are often unreadable as nodes (samples), and edges (links between samples) strongly overlap. We present a new method, MAPI (Mapping Averaged Pairwise Information), allowing translation from spatial networks to variation surfaces.

MATERIALS and METHODS:

MAPI relies on i) a spatial network in which samples are linked by ellipses and, ii) a grid of hexagonal cells encompassing the study area. Pairwise metric values are attributed to ellipses and averaged within the cells they intersect. The resulting surface of variation can be displayed as a colour map in GIS, along with other relevant layers, such as land cover. The method also allows the identification of significant discontinuity in grid cell values through a nonparametric randomisation procedure.

RESULTS.

To illustrate our method we analysed *high- throughput* sequencing data of the Endive Necrotic Mosaic Virus virus obtained from 343 wild salsify collected over ~1500 km2 in South Eastern France. The MAPI analysis revealed a strong spatial genetic structure that could mostly result from host plant population dynamics due to habitat heterogeneity.

CONCLUSIONS:

MAPI is freely provided as a PostgreSQL/PostGIS database extension allowing easy interaction with GIS software and other programming languages. Although developed for spatial and landscape genetics, the method can also be useful to visualise spatial organisation from other kinds of data from which pairwise metrics can be computed.

DISEASE MAPPING AND RISK ASSESSMENT OF WHITEFLY TRANSMITTED VIRUSES INFECTING VEGETABLE CROPS IN SOUTH AFRICA

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BACKGROUND AND OBJECTIVES

In recent months, South Africa has experienced climatic extremes that resulted in the most severe drought in thirty years which was heightened by strong El Niño effects. Much of the country's major crop producing provinces were declared drought disaster agricultural regions. The onset of emerging pests and disease spawned across many parts of the country as a result of the driest rainy season on record. These climatic extremes have intensified the whitefly epidemic in South Africa and their subsequent spread of disease. Crop vulnerability to abiotic stress in the presence of an overwhelming attack by pests and disease such as whiteflies has left many farmers destitute. Therefore, the identification, epidemiology and distribution of whitefly transmitted viruses were studied across various provinces in South Africa using molecular analysis and climate risk modeling.

MATERIAL AND METHODS

An extensive survey of vegetable farms was conducted throughout the country. Samples were collected from plants exhibiting typical virus-like symptoms in the presence of whitefly populations. In addition, whiteflies were randomly collected from fields and tunnels for DNA fingerprinting to determine the predominant species/biotypes inhabiting various agro-ecological areas within South Africa's nine provinces. The level of infestation, altitude and GPS coordinates were recorded for each whitefly sampling site. Spatial distribution, population density and the spread of these vectors to previously uninhabited parts of the country using several limiting factors including temperature, altitude and rainfall were investigated using ArcGIS and R statistical software.

RESULTS

A risk assessment map was derived using climatic variables and the density of whitefly populations recorded from various sampling points. Emerging whitefly transmitted torrado, crini and begomo viruses were identified countrywide, including *Tomato torrado virus* (ToTV) which was reported for the first time from the mainland African continent. Furthermore, weeds played a significant role in the epidemiology of whitefly transmitted viruses.

CONCLUSIONS

Farmers in South Africa agree that agriculture in South Africa requires a climate smart approach to cope with issues such as emerging vector-borne disease and extreme weather events. Risk assessment and climate prediction models can provide valuable information to farmers and reduce the overall yield loss. Understanding environmental thresholds which limit the distribution of pests and disease can be used to predict possible future epidemics. Although environmental conditions essentially influence whiteflies and virus epidemiology, anthropogenic activity and pesticide treadmills significantly contribute to epidemic outbreaks in South Africa and abroad.

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CROP-ASSOCIATED VIRUS INFECTION IN A NATIVE PERENNIAL GRASS: ASTER MODEL ASSESSMENT OF FITNESS EFFECTS

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BACKGROUND and OBJECTIVES

In contemporary landscapes, native plants are exposed to multiple sources of infection, including crop-associated viruses dispersed from agricultural fields. Despite growing interest, there has been only limited quantification of the effects of crop-associated viruses on native plant fitness. Because the majority of native plants are perennials, such fitness assessments must consider the integrated and cumulative effects of infection on host performance across several years. The need to examine virus effects on novel perennial species is heightened by current efforts to domesticate native grasses for bioenergy production. To address this challenge, we present the first application of aster models for life history analysis to plant – pathogen interactions.

MATERIAL and METHODS

We examined the effects of *Barley yellow dwarf virus* (BYDV, Family Luteoviridae) on switchgrass (*Panicum virgatum* L.), a native perennial North American prairie grass now under development as a bioenergy feedstock. From a set of BYDV viruses collected in winter wheat in Kansas (USA), we selected a representative BYDV-PAV isolate and used it to infect seedlings of two switchgrass cultivars: Shawnee, a northern upland ecotype, and Kanlow, a southern lowland ecotype. Virus-inoculated and mock-inoculated individuals with ELISA-verified virus status were then planted into an existing outdoor prairie restoration in Kansas and monitored for three years. Plant growth metrics, virus status, and fitness components were measured annually. We evaluated virus influence on switchgrass performance by analyzing individual response variables and by assessing cumulative multi-year fitness with aster models.

RESULTS

Aster modelling found that virus infection reduced switchgrass fitness in the field by 30% over the first two years, largely through multi-year effects on inflorescence production. Individual fitness components and plant size metrics were generally influenced to a lesser degree, with the integrated aster assessment producing the clearest evidence of virus effects. In the third year, unrestricted rodent herbivory became the dominant environmental influence on switchgrass fitness. Cultivars differed in response to infection with the southern lowland cultivar showing more evidence of resistance.

CONCLUSIONS

Crop-associated virus infection can negatively impact the cumulative fitness of native perennial plants, even when the host demonstrates some resistance. Experimental field studies combined with integrative analytical procedures, such as the aster models used here, offer robust approaches for fitness analyses. As evidenced by our findings, viruses merit attention in development of sustainable perennial bioenergy crops.

THERE IS STILL MUCH TO LEARN ABOUT THE EPIDEMIOLOGY OF COMMON PLANT VIRUSES

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The key to developing robust management strategies to control plant viruses in economically important crops is a complete and sound understanding of their epidemiology. In recent times, traditional epidemiology studies with plant viruses have declined considerably worldwide due to: (i) an increasing focus at the molecular level often at the expense of more traditional biological approaches with whole plants and plant populations; (ii) declining numbers of trained plant virologists with traditional biological skills; and (iii) declining funding and high-cost associated with field-based research. Despite this trend, traditional epidemiology research still needs to be done, even with common, well-known viruses. This is especially so when work on their epidemiology was done many years ago and ceased well before the advent of increasingly numerous, improved labour saving methodologies available today. This is so even when many of such viruses are assumed to have been well studied and their epidemiology to be well understood. However, such viruses often remain difficult to control which suggests that the assumption that their 'disease cycles' are sufficiently studied may be unwarranted. This presentation will provide examples demonstrating that what was established about the epidemiology of well-known viruses many years ago is not necessarily the whole story. There is a need to go back and re-examine their epidemiology to establish an improved understanding of how they survive and spread. The chosen examples of re-examined epidemiology where this has proved to be so will include the critical role of seed transmission with *Wheat streak mosaic virus* in wheat crops (1); the role of contact transmission in the epidemiology of *Zucchini yellow mosaic virus* in cucurbit crops (2); the role of water-mediated transmission in the spread of *Potato virus Y* (3). The aim of such research is to fill information gaps likely to provide critical knowledge required to effectively target vulnerabilities in their 'disease cycles'. Revisiting traditional epidemiology findings and combining the information obtained with molecular epidemiology studies enables a greater understanding of how common viruses survive and spread, which, in turn, makes possible the development of improved integrated control procedures that manage their spread effectively.

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THREE DECADES OF MANAGING *TOMATO SPOTTED WILT VIRUS* IN PEANUT IN SOUTHEASTERN UNITED STATES

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BACKGROUND AND OBJECTIVES

Southeastern states such as Georgia, Florida, and Alabama produce >70% of peanuts in the United States. Thrips-transmitted *Tomato spotted wilt virus* (TSWV), which causes spotted wilt disease, continues to be a major impediment to peanut production since the mid 1980s. The cultivars grown at that time were extremely susceptible to TSWV. Early yield losses extended to tens of millions of dollars each year (up to 100% loss in many fields). This situation led to the creation of an interdisciplinary team "Spotted Wilt Action Team –SWAT." Initial efforts focused on risk mitigation using a combination of chemical and cultural management practices along with a strong investment in breeding programs. Beginning in mid 1990s, cultivars with field resistance were developed and integrated with cultural and chemical management options.

RESULTS

A Risk Mitigation Index (Peanut Rx) was made available to growers to assess risks, and mitigate such risks by planting field resistant cultivars with in-furrow insecticides, planting after peak thrips incidence, planting in twin rows, and increasing seeding rates. These efforts helped curtail losses due to spotted wilt. The Peanut Rx continues to be refined every year based on new options. Breeding efforts, predominantly in Georgia and Florida, continue to develop cultivars with incremental field resistance. The present day cultivars (third-generation TSWV-resistant cultivars released after 2010) possess substantial field resistance than second-generation (cultivars released from 2000 to 2010) and first-generation (cultivars released from 1995 to 2000) TSWV-resistant cultivars. Despite increased field resistance, these cultivars are not immune to TSWV and succumb under high thrips and TSWV pressure. Therefore, they cannot serve as 'stand-alone' options and have to be integrated with other management options. The mechanism of resistance is also unknown in field-resistant cultivars. Recent research in our laboratory evaluated field resistant cultivars against thrips and TSWV. Results revealed that resistant cultivars marginally influenced thrips and accumulated fewer viral copies than susceptible cultivars. Transcriptomes developed with the aid of Next Generation Sequencing (Illumina platform) revealed differential gene expression patterns following TSWV infection in susceptible than field resistant cultivars. Results also revealed that plant defense responses in TSWV-resistant cultivars were more robust than susceptible cultivars. On the flipside, the long-term effects of using such resistant cultivars on TSWV were assessed using virus population genetics studies. Initial results suggest lack of positive selection pressure on TSWV, and that the sustainable use of resistant cultivars is not threatened. Follow up research is being conducted.

CONCLUSIONS

Improvements in TSWV management has enhanced sustainability and steadily increased yields from < 2500 lbs/acre before 1995 to ~4500 lbs/acre in 2015.

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EDITING VIRUS RESISTANCE IN CUCUMBER USING CRISPR/CAS9 TECHNOLOGY

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BACKGROUND and OBJECTIVES

Genome editing in plants has been boosted tremendously by development of the clustered, regularly interspaced, short palindromic repeat (CRISPR/Cas9) technology [1]. This powerful tool allows substantial improvement of plant traits in addition to those of classical breeding. Viral recessive resistance genes have been identified naturally for several crops and mutagenesis or silencing of those genes has been shown to develop resistance. Recently, a flexible targeted genome editing technology based on the bacterial CRISPR-associated protein 9 nuclease from *Streptococcus pyogenes* (Cas9) has been developed. In this system, Cas9 complexed with a synthetic guide RNA can induce sequence specific genomic DNA double-strand breaks. This leads to the introduction of nucleotide changes at the break site. To test the efficacy of CRISPR/Cas9 in cucumber and develop a new strategy to generate virus resistance, we chose to disrupt *eIF4E* gene [2]

MATERIAL and METHODS

Two Cas9/sgRNA binary vectors were made with sgRNA1 and sgRNA2 designed to target the first and the third exons of the *eIF4E* gene. *Agrobacterium tumefaciens*-mediated transformation of cucumber was performed. The transgenic lines were genotyped for indel polymorphisms by PCR and sequence analysis. Non-transgenic heterozygous *eIF4E* mutant plants were selected for production of non-transgenic homozygous T3 generation plants.

RESULTS

Small deletions and SNPs were observed in the *eIF4E* gene targeted sites of the T1 generation transformed cucumber plants, but not in putative off-target sites. Homozygous T3 progeny following Cas9/sgRNA that had been targeted to both *eIF4E* sites exhibited immunity to *Cucumber vein yellowing virus* and resistance to the potyviruses *Zucchini yellow mosaic virus* and *Papaya ring spot mosaic virus-W*. In contrast, heterozygous-mutant and non-mutant plants were highly susceptible to those viruses.

CONCLUSIONS

Here we show for the first time that CRISPR/Cas9 is an efficient tool for genome editing in cucumber. Disruption of the *eIF4E* gene in cucumber by Cas9-sgRNA led to development of virus-resistant plants without otherwise affecting the plant genome. For the first time, virus resistance has been edited into cucumber crop, non-transgenically, not visibly affecting plant development, without long-term backcrossing, via a new technology that can be expected to be applicable to a wide range of crop plants. We believe that this novel technology has the potential to expedit development of pest resistance in many crops without the need for regulation, as it has been accepted for many mutant crops.

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INSECTICIDE EFFECTS ON WHITEFLY TRANSMISSION AND INCIDENCE OF *CUCURBIT YELLOW STUNTING DISORDER VIRUS*

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BACKGROUND and OBJECTIVES

Cucurbit yellow stunting disorder virus (CYSDV) is a cosmopolitan virus disease transmitted by *Bemisia tabaci* that infects cucurbit crops. As a phloem-limited virus that is vectored in a semi-persistent manner by *B. tabaci*, CYSDV has transmission characteristics that may be conducive to suppression by select insecticides. Better management practices and a greatly expanded repertoire of insecticides with new modes of action were essential to recovering agricultural productivity in the southwestern U.S. following the first outbreaks of the B biotype in the early 1990s. However, the subsequent invasion of CYSDV in the region beginning in 2006 has renewed the urgency for a management solution to this disease in cantaloupes and other cucurbits. Our objective with this study was to examine various foliar and systemic insecticides for their effects on transmission of CYSDV by *B. tabaci*, and also to determine their relative efficacies at suppressing disease incidence in the field.

MATERIAL and METHODS

Three virus transmission experiments were carried out in the greenhouse that involved different groups of foliar- and soil-applied insecticides. Multiple tests replicated over time and using three densities of whitefly adults were conducted for each experiment. Cantaloupe plants served as virus source and test plants and acquisition and inoculation access periods were standardized across all transmission experiments. Knockdown activity of five foliar- and five soil-applied insecticides was also compared in a separate experiment. Field trials using soil-applied insecticides for suppression of CYSDV were conducted at the University of Arizona Yuma Agricultural Center in in 2014 and 2015.

RESULTS

There were clear differences among insecticide treatments in their respective capacities to suppress CYSDV infection in cantaloupes grown in the greenhouse or under standard production conditions in the field. Many compounds limited virus transmission to <10% infected plants even when challenged by 30 viruliferous whiteflies. Differences among treatments were also apparent in the survivorship of adult whiteflies on treated plants in the greenhouse. Although time of survival does not necessarily equate to the ability to transmit virus, it is possible that longer exposure times increase the probability of transmission. Foliar formulations had greater knockdown activity than their soil-applied analogs and resulted in lower virus transmission. In field trials, insecticides that had the greatest effect on reducing virus transmission in the greenhouse also showed the lowest incidence of CYSDV.

CONCLUSIONS

Select insecticides can significantly reduce transmission of CYSDV. However, insecticide management of CYSDV incidence in cantaloupes has limitations in chronically high infestation areas such as the southwestern U.S, and is often only able to delay disease onset rather than prevent its occurrence. Continuing investigations to identify insecticides and treatment regimens that suppress incidence of CYSDV are crucial to maintaining productivity of cucurbit crops. Complimentary studies on virus transmission under controlled conditions may provide relevant information and improve confidence in treatment choices made by pest and disease managers.

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COMBINING GENETIC RESISTANCE AND MANAGEMENT OF FIELD MARGINS TO CONTROL VIRUS EPIDEMICS IN MELON CROPS

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BACKGROUND and OBJECTIVES

The melon *Vat* gene confers resistance to the aphid species *Aphis gossypii* and resistance to non-persistent viruses (CMV, WMV…) when inoculated by this species (1). It is however ineffective in blocking the transmission of viruses inoculated by other aphid species. Its effect on non-persistent virus epidemics is thus expected to be limited when *A. gossypii* is not the main aphid species visiting the melon crop. On the contrary, although not documented, its effect on the persistent virus CABYV mainly vectored by *A. gossypii* is expected to be strong.
The first objective of the study was to evaluate the effect of *Vat* on virus epidemics and on the genetic structure of virus populations in field conditions.
The second objective was to investigate the benefit of combining *Vat* resistance and an appropriate management of field margins to regulate the populations of aphids and/or their virus load. Indeed, literature suggests that flower strips can participate in pest biological control by favoring natural enemies (2), and strips of non-host plants can protect crops from non-persistent viruses by allowing aphids to probe on healthy plants and thus to lose their virus load before reaching the crops (3).

MATERIALS and METHODS

A five-year field experiment was conducted to compare two types of field margin management (bare soil and flower strip) on the efficiency of *Vat*. Virus epidemics were monitored by DAS-ELISA and genetic structures of virus populations characterized by RT-PCR with specific primers and sequencing. The effect of *Vat* and field margin management on the infection probability of a plant by a given virus was calculated with a generalized linear model (binomial distribution and logit link function).

RESULTS

Vat reduced the development of CABYV epidemics significantly every year. Concerning non-persistent viruses, it had no effect on WMV epidemics, but allowed to reduce CMV epidemics some years, confirming the role of *A. gossypii* in CMV transmission. The genetic structure of virus populations was not affected by *Vat*.
The presence of flower strips had a dual effect on virus epidemics: mostly beneficial for WMV but sometimes detrimental for CMV.

CONCLUSIONS

Combining genetic resistance and management of field margins appeared generally as a promising way to decrease the risk of viral epidemics. Nevertheless, the presence of natural enemies enhanced by flower strips may also have a detrimental effect on non-persistent virus dispersion through a modification of aphid behaviour.

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THE EVOLUTION OF PLANT VIRUS TRANSMISSION PATHWAYS

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BACKGROUND and OBJECTIVES

The evolution of plant virus transmission pathways is studied through transmission via seed, pollen, or vector. Under what circumstances does vector transmission make pollen transmission redundant? Can evolution lead to the coexistence of multiple transmission pathways?

MATERIAL and METHODS

A semi-discrete model with pollen, seed, and vector transmission is formulated for an annual plant population. An ecological stability analysis is performed for the semi-discrete model and used to inform an evolutionary study of trade-offs between pollen and seed versus vector transmission.

RESULTS and CONCLUSIONS

Evolutionary dynamics critically depend on the shape of the trade-off functions. Assuming a trade-off between pollen and vector transmission, evolution either leads to an evolutionarily stable mix of pollen and vector transmission (concave trade-off) or there is evolutionary bi-stability (convex trade-off); the presence of pollen transmission may prevent evolution of vector transmission. Considering a trade-off between seed and vector transmission, evolutionary branching and the subsequent coexistence of pollen-borne and vector-borne strains is possible. This study contributes to the theory behind the diversity of plant-virus transmission patterns.

DETERMINANTS OF HOST SPECIES RANGE IN PLANT VIRUSES

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BACKGROUND and OBJECTIVES

Prediction of pathogen emergence is an important issue, both in human health and agronomy. Strikingly, most studies of pathogen emergence have focused on the responsible ecological or anthropogenic factors rather than on the role of intrinsic pathogen properties.

MATERIAL and METHODS

By focusing on a plant virus database (Brunt et al. 1996), we looked at the relationships between different virus characteristics (genome type, number of segments, type of vertical and horizontal transmissions, vector type, vection type) and the virus plant species range breadth.

RESULTS

We found that four main viral characteristics were strongly linked to virus host range breadth. Viruses (i) with single-stranded genomes (either RNA or DNA), (ii) with bipartite or tripartite genomes, (iii) that were seed transmitted and/or (iv) transmitted by nematodes presented a broader host range than others.

CONCLUSIONS

Viruses harboring these characteristics thus seem to be more prone to emerge in new host species. A statistical model was built that predicts host range breadth based on these four characteristics and could be used to focus quarantine processes and surveillances.

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EPIDEMIOLOGY OF *POTATO SPINDLE TUBER VIROID* IN A REMOTE SUBTROPICAL IRRIGATION AREA

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BACKGROUND & OBJECTIVES

Potato spindle tuber viroid (PSTVd) epidemiology was studied in the Gascoyne Horticultural District (GHD) in arid, central coastal Western Australia (WA). The GHD's plentiful irrigation water and subtropical climate support intensive temperate, tropical and subtropical crop production. The GHD's market gardens mostly occur in close proximity to each other, crop residues and volunteer crop plants often remain after harvest, and large weed populations often occur. PSTVd was detected in field grown tomatoes in 2006, and thereafter frequent PSTVd detections occurred in field grown tomato, pepper and chilli crops. After each PSTVd detection, affected crops were burnt or buried deeply, and roadside verges where PSTVd-infected volunteer crop plants or weeds were found were sprayed with herbicide, but these eradication efforts were unsuccessful.

MATERIAL & METHODS

During 2007-2012, samples from volunteer crop, naturalised weed and native plant species growing mostly in roadside verges were tested for PSTVd by RT-PCR, and 21 viroid isolates were sequenced.

RESULTS

PSTVd was detected infecting volunteer plants of tomato, pepper and chilli; introduced weed species *Solanum nigrum* (blackberry nightshade), *Datura leichhardtii* (thornapple), *Nicandra physalodes* (apple-of-Peru) (*Solanaceae*), and *Conyza bonariensis* (flaxleaf fleabane) (*Asteraceae*); and Australian native species *Atriplex semilunaris* (annual saltbush), *Rhagodia eremaea* (thorny saltbush) (Chenopodiaceae), and *Streptoglossa* sp. (*Asteraceae*) (1). PSTVd was also detected infecting *Physalis angulata* (wild gooseberry) in the Ord River Irrigation Area (ORIA) in north-west WA. Comparison of three complete and 18 partial RNA nucleotide sequences obtained from 20 GHD and one ORIA isolates with those of published sequences showed their highest nucleotide sequence identities were to a PSTVd Chittering strain isolate from south-west WA.

CONCLUSIONS

These results show the GHD has a large naturally occurring PSTVd reservoir. As PSTVd is readily contact transmitted, it's infectivity is stable on diverse surfaces, and, in the GHD, farm machinery often moves over roadside verges before turning back into crops, this reservoir explains the repeated PSTVd detections in its solanaceous field crops. These findings have implications concerning PSTVd spread and management in intensive field production systems in subtropical regions of the world.

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VIRUS INFECTION IN AN ENDANGERED GRASSLAND HABITAT

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BACKGROUND and OBJECTIVES

The *Barley* and *Cereal yellow dwarf virus* (B/CYDV) species complex includes some of the most economically important disease-causing agents of small grain cereal crops. BYDV plays an important role in the competitive dynamics of native and invasive grasses in nonmanaged systems (Malmstrom et al. 2005, Borer et al. 2007). B/CYDV are known to infect over 150 *Poaceae* species, and BYDV was recently found to infect the invasive species *Ventenata dubia* in grassland habitats (Ingwell and Bosque-Pérez 2015). The Palouse region of northern Idaho and eastern Washington in the USA is one of the most productive wheat-growing regions of the world, but little is known about viral prevalence in grassland habitats of the region. In order to manage and conserve endangered grasslands a better understanding of the interactions among members of an ecological community, including pathogens, is required. The objectives of this study were to determine the prevalence of B/CYDV among grass hosts in endangered Palouse Prairie remnants and Conservation Reserve Program (CRP) lands, identify B/CYDV species present in the region, and examine the factors that influence virus prevalence.

MATERIAL AND METHODS

Field surveys of *Poaceae* species in five Palouse Prairie and six CRP sites, were conducted to examine for the presence of B/CYDV among potential grass hosts from 2010-2012. Aphid vectors were sampled to identify species present in each habitat and tested to determine virus presence. Molecular techniques were used to determine virus presence among grass and aphid samples and to identify viral species present.

RESULTS

B/CYDV infection was detected in grass hosts at every CRP and prairie remnant sampled, documenting for the first time virus infection in the endangered prairie remnants. Aphids were encountered at only one sampling time at one site. Virus prevalence was influenced by host life history, host tribe, location, and proportion of grain cover in the surrounding landscape.

CONCLUSIONS

Results demonstrate that B/CYDV infection is common among CRP and prairie habitats in this region. The low number of aphids collected suggests that non-colonizing aphids might be responsible for disease spread in these habitats. The identity of grass species in and grain crop acreage around prairie sites may play an important role in disease dynamics in nonmanaged systems.

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VIRUS ACCUMULATION IN A NATIVE PERENNIAL PRAIRIE GRASS UNDER DEVELOPMENT AS A BIOENERGY FEEDSTOCK

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BACKGROUND and OBJECTIVES

Switchgrass (*Panicumvirgatum*L.) is a perennial native to North American prairie with potential as a bioenergy feedstock.It is infected by crop-associated viruses but also harbors *Switchgrass mosaic virus* (SwMV), a recently discovered leafhopper-transmitted virus that infects wild grasses. Almost nothing is known about SwMVepidemiology or about the epidemiology of native-plant-associated viruses in general.

MATERIAL and METHODS

To examine the influence of plant traits, community composition, and landscape context on virus accumulation, we established a landscape-scale experiment across lower Michigan (USA) in 2012 (Yr 1). At twelve 1-ha sites previously cropped with soybeans, we sowed 10 large plots (20 m x 20 m) of switchgrass, either in monoculture or diverse communities. Treatments included five different switchgrass populations, representing northern upland and southern lowland ecotypes, and a diversity gradient (monoculture, S; monoculture + additional grasses, S+G; monoculture + grasses + forbs, S+G+F). After two establishment years, insect and plant samples were collected in Yrs 3 and 4. Infection was analyzed with RNA extraction and RT-PCR for specific viruses.

RESULTS

We detected notable SwMV infection in native perennial switchgrass at 11/12 sites distributed within a 20,000 km^2 region, four years after seeding.Infection confirmed with molecular tests was consistently associated with notable fine white streaks on leaves. Initial evaluation indicates that the number of tested plots per site with detected infectionwas 0/5-5/5 (mean 3.5). Virus accumulated rapidly: At one site, mean prevalence increased from 9.38% in Yr 3 to 31.25% in Yr 4, with per-plot prevalence reaching 75% (S+G stand). Infection was associated with vector distribution. In Yr 3 leafhopper abundance predicted SwMV prevalence at this site. Across all sites, the nature of switchgrass plantingssignificantly influenced leafhopper abundance. Northern upland ecotypes harbored more leafhoppers on average than did Southern lowland ecotypes, and diversity plots with forbs held fewer leafhoppers than comparable monocultures.

CONCLUSIONS

Our results demonstrate that native-plant-associated viruses may cause notable symptom expression in native perennial hosts, contrary to common assumptions. Furthermore, even in agriculture-dominated landscapes, vectors are able to quickly spread such infection to isolated patches of native plants. Once established, infection prevalence can increase rapidly within a stand, withinfection accumulation influenced by stand diversity and the genetics of host populations.Landscape experiments of this nature are essential for sustainable management of bioenergy feedstocks and development of virus-resistant cultivars.

PLANT NUTRIENT RESOURCES DIFFERENTIALLY ALTERS TWO VIRUS SPECIES DYNAMICS

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BACKGROUND and OBJECTIVES

Competitive interactions among free living organisms such as plants and algae are known to be regulated by rates and ratios of environmental nutrient supplies[1]. Micro-organisms that rely on a host for their reproduction cycle hijack host metabolic machinery, as well as host resources in metabolites, enzymes and proteins. As the synthesis of these molecules requires nitrogen and phosphorus, altered host nutrients supplies have been hypothesized and thereafter shown to influence microbial dynamics, based on the response of single and a group of micro-organism species[2]. Yet, rates and ratios of nutrients supplied to hosts could also mediate the interactions among co-occurring micro-organisms. Understanding these effects would be an important advance because interactions among co-occuring microbes can alter both host population dynamics and epidemiological processes[3]. The objective of our study was to determine the outcomes of nitrogen and phosphorus addition to plant hosts on infection rates of and interactions among two plant virus species.

MATERIALS and METHODS

We singly- and co- inoculated two virus species, (Barley yellow dwarf virus- PAV and Cereal yellow dwarf virus - RPV) to plants of the cultivated crop species *Avena sativa*. In each condition, hosts received one of four different nutrient treatments. The latter represented a full factorial combination of two levels of nitrogen and of phosphorus that created a gradient of N:P supply ratios, one being replicated at low and high nutrient supply. Each plant was tested 19 days after inoculation for virus infection using RT-PCR and specific primers for each virus species.

RESULTS

P addition lowered CYDV-RPV prevalence. N addition altered the interaction strength among viruses. The co-inoculation of BYDV-PAV lowered CYDV-RPV infection rate. However, this antagonistic interaction only occurred at low nutrient supply rates and was alleviated at high N supply rate. BYDV-PAV infection rates were unaffected by nutrient supply rates and by the co-inoculation of CYDV-RPV. Infection rates after co- and -single inoculations were better predicted by nutrient supply rates while coinfection rates were better predicted by a joint probability of successful inoculation of both virus species (i.e. product of infection rate of each virus species in single inoculations) calculated by N:P ratio.

CONCLUSIONS

The results of our study are one of the first empirical demonstrations that plant nutrient resources can mediate interspecific interactions among co-occurring parasites. In the context of elevated nitrogen and phosphorus addition to global biochemical cycles caused by human activities, these results provide insights on how nutrient-host-virus interactions might alter virus prevalence and risks of co-infection, with potential effects on virus epidemiology.

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RESERVOIR HOSTS OF CUCURBIT YELLOW STUNTING DISORDER VIRUS AND DEVELOPMENT OF RESISTANT MELON

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BACKGROUND AND OBJECTIVES

Cucurbit yellow stunting disorder virus (CYSDV), emerged in the Sonoran Desert region of the southwestern USA in 2006 and has become established. The virus is transmitted by the MEAM1 cryptic species of *Bemisia tabaci,* which has been present in the region since the early 1990s. CYSDV results in late-season infection of spring melon crops with limited economic impact; however, all summer and fall cucurbits become infected shortly after emergence due to high *B. tabaci* populations and abundant host plants. Other viruses infecting melon in the region include *Squash leaf curl virus*, *Cucurbit leaf crumple virus*, several potyviruses, and *Squash vein yellowing virus*, which was first identified in the fall of 2014. CYSDV is the most significant of the virus threats to cucurbit production in the region, and previous studies demonstrated an extensive host range among crops and weeds prevalent in the region.

MATERIALS AND METHODS

In order to identify important reservoir hosts for overwintering of the virus and transmission to spring cucurbit crops, a select set of weed and crop hosts that are cultivated in the Imperial Valley were evaluated as CYSDV reservoir hosts. For each host species we determined the capacity of CYSDV to accumulate, the relationship between virus titer in these source plants and transmission by whiteflies, as well as subsequent accumulation in inoculated cucurbit plants. Vector management efforts alone are insufficient to reduce the impact of CYSDV. Therefore, approximately 500 melon accessions of India origin were evaluated in Imperial Valley, CA for reaction to natural infection by CYSDV in open field tests from 2007 to 2013. F_2 progeny and testcrosses with susceptible melon were evaluated under high whitefly feeding pressure in naturally infected field tests.

RESULTS

Among the host plants evaluated as potential reservoirs, there was considerable variation in virus accumulation and transmission rate. Cucurbit hosts had the highest CYSDV titers, were efficient sources for virus acquisition, and showed a positive correlation between titer in source plants and transmission. Non-cucurbit hosts had significantly lower CYSDV titers and varied in their capacity to serve as sources for transmission. Six accessions were heterogeneous for apparent resistance to CYSDV, and CYSDV resistance was confirmed in TGR 1937 and PI 614479. F_1 generations did not significantly differ from their respective susceptible parents, indicating recessive control of resistance to CYSDV in TGR 1937 and PI 614479.

CONCLUSIONS

Experiments demonstrated that multiple factors influence the efficiency with which a host plant species will be a reservoir for vector transmission of CYSDV to crops. Resistance evaluations found significant differences in symptom severity and virus titer at 10 weeks post planting (WPP) between the resistant parents and their susceptible mates, and that CYSDV resistance is a recessive trait.

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AREA WIDE MANEGMENT OF INSECT VECTORED VIRUSES IN TOMATO CROPS

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BACKGROUND and OBJECTIVES

The emergence of tomato yellow leaf curl virus in the largest of Northern Australia's tomato and capsicum growing areas, as well as other problematic viruses lead to the formation of an area wide virus and vector management study. It is a large, wide ranging project including important hosts of viruses and vectors, virus strain diversity and population dynamics and management of vectors, as well as evaluation of tomato germplasm for virus resistance. The study is in the Bowen area of northern Queensland, the major winter supplier of tomatoes and capsicum for the Australian market, with farm gate sales up to $245M per annum. The three main viruses, occasionally causing severe economic losses in tomato in the district are tomato yellow leaf curl virus (TYLCV), tomato spotted wilt virus (TSWV) and potato leaf roll virus (PLRV).

MATERIALS and METHODS

Viral surveys were conducted within the ~130 km^2 of the Bowen cropping area. Ten separate locations that were sampled from three times a year – early (May) mid (July) and late (September) season. Plants were rated on visual symptoms and 300 random individuals were checked for virus presence by PCR. Silver-leaf whiteflies (*Bemisia tabaci*) were collected from the sampling area and tested for the presence of TYLCV. Different weed species in and around the crops were tested for the presence of the three viruses at multiple times.

RESULTS

Over four seasons there has been a large variation in the virus and vector populations due to weather events. Unsuitable conditions for aphids has seen PLRV infection drop from average of 33 % infection across the district in 2012 to 0.05 % over the last two seasons. Similarly, the lack of a decent wet season for the past two years has resulted in reduction of flowering of the weed hosts of TSWV, and virus incidences in the crops. In contrast, the low rainfall kept a high whitefly population, with numbers in one area at the beginning of the season being similar to the levels that normally occur by the end of the season. Only occasionally did high levels of TYLVC in the crop equate to high levels of virus in the whitefly. Since beginning this work, on our recommendation, most growers across the district have moved to dual TSWV/TYLCV resistant lines. The TYLCV resistance still allows virus replication, and at the end of the latest season we detected an average of 29 % infection from one area, with one site 100 % infected with TYLCV, and only ~ 8 % of whitefly carrying the virus

CONCLUSIONS

Throughout the area, there are large differences in the viral loads, mainly due to local differences in soil types and the weeds that are present as well as prevailing winds. Evidence is that the growers in some areas will have to completely forgo non-resistant lines regardless of the planting time.

USING SENSITIVITY ANALYSES TO IDENTIFY AND OPTIMIZE KEY PARAMETERS OF SHARKA MANAGEMENT STRATEGY

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BACKGROUND and OBJECTIVES

Strategies for disease control are often based on expert opinions rather than on the formal demonstration that they are, at least in theory, effective. This mainly stems from the complexity of taking into account the biological processes and the human interventions that both impact disease spread. A promising approach to optimize disease management consists in modeling both the epidemic processes and control measures. In this way, the most influential parameters can be identified, and alternative control strategies can be proposed and tested *in silico* in order to assess their potential efficiency.

MATERIALS and METHODS

We developed a spatially-realistic stochastic SEIR (susceptible – exposed – infectious – removed) model simulating disease dynamics (introduction, latent period and dispersal function) and management (surveillance, removal and replanting conditions). Then, we defined epidemiological and economic criteria that were used in global sensitivity analyses and to optimize the model parameters.

RESULTS

First, we carried out generic sensitivity analyses with parameter ranges large enough to encompass values that are typical of many perennial plant diseases and of their management. These analyses revealed the importance of the latent period duration, and highlighted the key contributors to disease management impact. Then, we specifically scrutinized the main processes affecting sharka epidemics, caused by *Plum pox virus*, a quarantine pathogen of prunus trees (especially apricot, peach and plum) in many areas of the world (Rimbaud et al., 2015). Using realistic parameter ranges given the present knowledge of sharka epidemiology, the introduction of infectious plants and the removal procedure appeared to be the main levers for management optimization. A last sensitivity analysis on the most promising control parameters enabled the theoretical economic optimization of sharka management strategy.

CONCLUSIONS

We are now expanding this framework to study the potential impact of disease management based on landscape optimization. The identified optimal control strategies are discussed with the organizations responsible for sharka control in order to help the design of durable and cost-effective strategies.

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GOING VIRAL: USING SOCIAL NETWORK THEORY TO EXPLORE THE SOCIAL DYNAMICS OF THE CASSAVA MOSAIC VIRUS PANDEMICS

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BACKGROUND and OBJECTIVES

Many crop diseases are propagated mechanically through infected stem cuttings that move along farmers' informal seed exchange networks. In Africa, the Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD) are major threats to regional food security and cause vast economic losses to staple crops. CMD and CBSD both originated from East Africa but have been steadily expanding westwards towards Central and West Africa (Legg *et al.*, 2011). Successful control of crop pandemics requires a better understanding of the social factors that can enhance the spread of viruses through open and unrestricted seed exchanges or, on the contrary, slow down the adoption of pathogen-resistant cultivars through tighter social control of local seed systems.

MATERIAL and METHODS

Using a phylodynamic approach, we investigated how the spread of plant pathogens is influenced by local seed systems, by analysing spatial patterns of genetic variation in the African cassava mosaic virus (ACMV) in Gabon, central Africa. Viral diversity was characterized through DNA sequencing of the replication associated protein (Rep) open reading frame on the ACMV genome. Because genetic relationships between haplotypes not solely represent evolutionary ties but reflect also the movements of viral strains through regional networks of seed exchange, we analysed regional patterns of ACMV diversity not only from the point of view of population genetics but also from the point of view of social network analysis.

RESULTS

In small-scale farming communities, circulation of crop landraces is often channelled by social structures which determine the connectivity of farmer populations and, *in extenso*, favour or limit germplasm exchanges among communities (Delêtre *et al.*, 2011). We show that social structures leave distinctive molecular signals on intra- and inter-population levels of DNA polymorphism in virus metapopulations, which reflect in the topology and statistical properties of local and regional haplotype networks of viral DNA.

CONCLUSIONS

Knowledge on the role played by informal seed systems in the diffusion of crop diseases is essential for implementing efficient control strategies to curb crop disease pandemics. The comparison of anthropological, genetic, and plant epidemiological data provides a unique opportunity to explore how the interplay of social structures and local networks of seed exchange influences regional dynamics of plant viruses.

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COMMUNITY PHYTOSANITATION AND THE EPIDEMIOLOGY OF CASSAVA VIRUSES

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BACKGROUND and OBJECTIVES

The major constraints to cassava production in Africa are the virus diseases: cassava mosaic disease (CMD) caused by cassava mosaic geminiviruses (CMGs) (*Geminiviridae*; *Begomovirus*) and cassava brown streak disease (CBSD) caused by cassava brown streak viruses (CBSVs) (*Potyviridae*; *Ipomovirus*). Recent epidemics of CBSD have affected large parts of the Great Lakes region of East and Central Africa. Phytosanitary measures are frequently used to control plant virus diseases, but this has rarely been done at community-wide scale. Recent evidence on the transmission and epidemiological characteristics of CBSVs has suggested that they are semi-persistently transmitted, not retained for long periods, and spread over relatively short distances. This encouraged the piloting of a community phytosanitation approach for the management of CBSD in East Africa.

MATERIALS and METHODS

Two communities in Tanzania participated fully in the community phytosanitation programme; one (Mkuranga) in the Coast Zone, and one in the north-western Lake Zone (Chato). The aim was to start in a small isolated portion of each community by removing all virus-diseased cassava plants of local varieties, and replace these with virus-tested planting material of a CMD-resistant improved variety – *Mkombozi*. This first portion of the community to receive the material was referred to as the Primary Recipient Group (PRG). A community taskforce worked with PRG farmers to ensure that any symptomatic plants were removed during the course of the growing season. After the first season, the same procedure was extended to a Secondary Recipient Group (SRG) and in the third year to a Tertiary Recipient Group (TRG). Ten farms in each of the PRG, SRG and TRG were selected at random and monitored at regular intervals through each growing season. Records were taken of incidence and severity of CMD and CBSD, as well as the abundance of the whitefly vector, *Bemisia tabaci*. Surrounding cassava crops were assessed to determine the inoculum pressure for each monitored field. Ten Control Recipient Group (CRG) fields were planted with virus-tested *Mkombozi* in adjacent 'control' communities in which none of the phytosanitary measures were implemented.

RESULTS

Sustained low levels of infection in PRG, SRG and TRG fields through the three-year observation period have confirmed that phytosanitary measures can be effective in managing CBSD at community level. Lower incidences of CBSD in treated versus control areas demonstrated that significant benefits are obtained from practicing phytosanitation when compared to simply introducing virus-tested material. The epidemiological characteristics of CBSD spread through community phytosanitation treated and control villages will be presented.

CONCLUSIONS

Community phytosanitation is effective in managing CBSD in areas severely affected by the disease, but its potential for scaling out is constrained by the high cost and complexity of social factors that have to be addressed during implementation.

POSTERS

OCCURRENCE AND DIVERSITY OF CASSAVA BROWN STREAK DISEASE-ASSOCIATED VIRUSES (*Potyviridae: ipomovirus*) IN UGANDA

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BACKGROUND and OBJECTIVES

Cassava brown streak disease (CBSD), caused by two different *potyvirus* species, *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV) is an important constraint to cassava production in Uganda (Mbanzibwa *et al*., 2011). In Uganda, CBSD has been reported to be widely distributed throughout the country (Alicai *et al*., 2007), with the highest incidence in Central and Southern regions. However, there is limited information on the distribution of the causal viruses. In this study, comprehensive surveys were undertaken in December 2014 and 2015 in 6 agro-ecological zones of Uganda to determine the geographical distribution and genetic diversity of CBSD-associated viruses in Uganda. Here, we report the findings.

MATERIAL and METHODS

102 and 120 cassava leaf samples were collected from farmers' fields in December 2014 and 2015 respectively. Total RNA was extracted and CBSD-associated viruses detected and discriminated as described by (Mbanzibwa *et al*., 2011). Partial genome sequences of about 1600bp of PCR amplicons of the partial HAM1-like protein, complete coat protein and partial 3'UTR region of 78 distinct and geographically diverse CBSD-associated virus isolates were generated.

RESULTS

CBSD symptoms were observed in at least one farmers' field in 51 of the 54 districts of Uganda surveyed in 2014. CBSV and UCBSV were detected occurring either singly or dually in 85.3% of the cassava leaf samples analyzed, no virus was detected in 14.7% of the samples. Of the samples that tested positive for CBSD-associated viruses by RT-PCR, 28.7%, 58.6%, and 12.6% were infected with CBSV, UCBSV and both CBSV and UCBSV respectively. For 2015, 120 CBSD symptomatic samples were analyzed, of which 97.5% and 2.5% detected positive and negative for CBSD-associated viruses respectively. Of the positive samples 26.5%, 55.6% and 17.9% were infected with CBSV, UBSV and both CBSV and UCBSV respectively. A phylogenetic analyses of the partial HAM1-like protein and coat protein sequences partitioned the CBSD-associated virus isolates into two major clusters, comprising of CBSV and UCBSV isolates previously sequenced. The two clusters share 55.4 - 98.3% and 77.8 – 99.4% nucleotide identities among the CBSV and UCBSV isolates respectively

CONCLUSIONS

UCBSV predominates the current CBSD epidemic in Uganda. However there is an overall increase in the percentage of cassava plants dually infected by both UCBSV and CBSV. In Uganda, the different species of CBSD-associated viruses are not geographically restricted although no single infection of CBSV was not encountered in two surveys in West Nile region. Next generation sequencing is recommended for CBSD symptomatic samples that fail to amplify with RT-PCR.

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EXPLORING PLANT-LUTEOVIRID INTERACTIONS: A PROTEOMIC APPROACH

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BACKGROUND AND OBJECTIVES

Viruses in the family *Luteoviridae*, collectively referred to as luteovirids, cause economically important diseases on crops worldwide. In nature, luteovirids are transmitted exclusively by aphids in a persistent, circulative manner. Using less than ten viral proteins, luteovirids regulate many factors in both plant hosts and insect vectors, including tissue tropism, movement of virions, and immunity evasion. Due to the virus' requirement for a living host to attract a vector, and a living vector to infect a new host, these multi-trophic interactions must be delicately balanced; yet, are largely uncharacterized. Using Protein Interaction Reporter (PIR) technology, a mass spectrometry-compatible cross-linker, we probed plant host-virus protein-protein interactions in the luteovirid *Potato leafroll virus* (PLRV).

MATERIALS AND METHODS

PIR technology utilizes a chemical cross-linker which is compatible with mass spectrometry, facilitating the elucidation of direct protein-protein interactions, as well as providing information which can be used for structural modeling of the interaction topologies. Here, we expand on previous studies of PLRV – *Nicotiana benthamiana* interactions (DeBlasio et al 2015) using PIR technology on PLRV virions partially purified from infected plant tissue.

RESULTS

By significantly increasing the quantity of purified virus used and improving our previous protocol, we were able to identify novel interactions between plant and viral proteins, and confirm some interactions found previously. We will outline work in progress to further characterize host-virus molecular interactions and their functional roles. New work using mutants of *Arabidopsis thaliana* and a related luteovirid, *Turnip yellows virus* (TuYV) will be described.

CONCLUSIONS

By integrating cutting-edge proteomic techniques with classical *Arabidopsis* genetics, we will unravel the complex molecular interactions which are essential for luteovirids' infectivity. These new data not only shed light on the life cycle of luteovirids, but may inform new control strategies in the future.

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FIELD DETECTION OF TOMATO LEAF CURL NEW DELHI VIRUS WITH IMMUNOSTRIP®

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BACKGROUND and OBJECTIVES

The Tomato leaf curl New Delhi virus (ToLCNDV) is a begomovirus whitefly-transmitted. It has been described first in India in 1995 on tomatoes. Initially reported on different solanaceae, this virus is also responsible of many damages on cucurbits (cucumber, melon, watermelon, and zucchini). Symptoms caused by ToLCNDV were observed on zucchini in 2013 in Spain in Almeria province. Since the disease is spreading in south of Spain, and was also detected recently in Tunisia, Morocco, Algeria, Turkey, Italy and Greece. The virus listed on the EPPO Alert List, has a wide host range including important crops for Mediterranean countries as tomato, potato, cucurbits.
To help in the monitoring of the disease in the fields and greenhouses, Agdia Inc. has recently developed a specific lateral flow device (ImmunoStrip®) for the detection of the virus in less than 30 minutes. Data for cross reaction with other begomoviruses will also be presented. Currently the strip can be run in Squash, Cucumber, Melon, Tomato, Potato, and Pepper plant tissue.

MATERIALS and METHODS

Material: Agdia ImmunoStrip® for ToLCNDV, commercially available ELISA kits for ToLCNDV
Method: Comparison of the new Agdia ImmunoStrip® for ToLCNDV to commercially available ELISA kits to demonstrate comparable sensitivity on plant tissue.

RESULTS

The ToLCNDV immunostrip was tested with fresh samples collected in the fields and greenhouses in Murcia and Almeria regions in Spain. The immunostrip reacted very strongly with all symptomatic samples.
Dilution studies showed that the ToLCNDV immunostrips have the same sensitivity of the commercial available ELISA tests.
We are currently investigating the crossreactivities of the immunostrips with other viruses belonging to the begomovirus group.

CONCLUSIONS

The new Agdia ImmunoStrip® for ToLCNDV is a comparable test in sensitivity to commercially available ELISA tests and will represent a diagnostic tool to help the Mediterranean vegetable industry to fight against this damaging virus.

MOLECULAR CHARACTERIZATION OF A MOSAIC DISEASE OF VERNONIA AMYGDALINA INDUCED BY A POTYVIRUS IN NIGERIA

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BACKGROUND AND OBJECTIVES

Vernonia is a genus of about 1000 species of forbs and shrubs in the family Asteraceae. Several species of *Vernonia*, including *V. calvoana*, *V. amygdalina*, and *V. colorata*, are eaten as leaf vegetables. Common names for these species include bitterleaf, ewuro, ndole and onugbu. They are common in most West African and Central African countries where they are of great economic value. Bitter leaf (*Vernonia amygdalina*) plants with severe mosaic, vein banding and yellowing were observed in both experimental and commercial fields in southwest, Nigeria. The aim of the present study is to identify and characterise the causal virus causing vein-banding disease of *V. amygdalina*

MATERIALS AND METHODS

Host range test was carried out using some leguminous and non leguminous plant species. The total RNA was extracted from both symptomatic and asymptomatic leaf samples. RT-PCR was carried out using total RNA isolated from infected leaf samples and a pair of potyvirus-specific primers. Amplicon obtained were purified and sequenced. The phylogeny analysis was done using mega6 soft ware.

RESULTS

In host range studies, only species in the families Solanaceae and Chenopodiaceae were susceptible, and neither of *Vigna unguiculata*, *Capsicum annum, Capsicum frutescence* nor *Glycine max* (L.) became infected. The expected size (~700bp) amplicon was detected from all symptomatic samples but not from samples of healthy plants, indicating the presence of a potyvirus infection. The basic local alignment search tool analysis revealing the newly derived sequence as having the highest identities (68–67%) with Jasmine ringspot virus (JRSV) isolates deposited in the GenBank database. The phylogenetic analysis of the virus sequences with available identical potyvirus sequences and those with reported serological relationship with a previously suggested Vernonia green vein-banding virus (VGVBV) also revealed the closest relationship with JRSV with evolutionary divergence value of 0.42. The sequence had identity with CP genes of other potyviruses with evolutionary divergence values between 0.46 and 2.22.

CONCLUSIONS

Based on sequence identity at nucleic acid and protein levels as well as phylogeny and evolutionary divergence matrix analyses, the isolate in this present study was considered not an isolate of Jasmine ringspot virus but corroborates the earlier suggestion that VGVBV was a new uncharacterized potyvirus. To the best of our knowledge, we hereby report the first molecular characterization of VGVBV causing severe vein banding disease on *Vernonia* in Nigeria.

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INFECTION OF CHRYSANTHEMUM BY TMV & USE OF A TMV-BASED TRANSIENT EXPRESSION VECTOR IN CHRYSANTHEMUM

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BACKGROUND and OBJECTIVES

Chrysanthemus (*Dendrathema morifolium*) are susceptible to a number of viruses and two viroids. Tobacco mosaic virus (TMV) has been reported to infect chrysanthemum, but no details were given. Therefore, we examined the infection of chrysanthemum by TMV and also determined whether a TMV expression vector could infect chrysanthemum systemically.

MATERIAL and METHODS

Florists' chrysanthemum (cv. Vivid Scarlet) and was inoculated with TMV (strain U1) and with TMV expressing the green fluorescent protein (TMV-GFP) and incubated under ambient conditions to determine the pathogenicity of these viruses in chrysanthemum. Plants also were assessed for the replication and movement of TMV and TMV-GFP using RT-PCR, and for GFP expression under UV light.

RESULTS

Infection of chrysanthemum by TMV produced stunted plants with aborted leaf development and abnormal flower formation resulting in tubular flowers. Infection by TMV-GFP produced symptomless plants which contained TMV sequences and the GFP gene in upper leaves, as determined by RT-PCR, but did not show green fluorescence under UV light. Only the inoculated leaves showed florescence, but limited to the major veins. This is in contrast to *Nicotiana benthamiana* infected by the same TMV-GFP constructs, which showed a strong green fluorescence and sytemic symptoms.

CONCLUSIONS

TMV infection of chrysanthemum resulted in severe symptoms on the plants, while the expression vector TMV-GFP resulted in a symptomless infection. Thus, TMV-based vectors can infect chrysanthemum.

VIRUS-INDUCED CHANGES IN PHOTOSYNTHETIC APPARATUS AND ANTIOXIDANT ENZYME ACTIVITIES IN TOMATO LEAVES

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BACKGROUND and OBJECTIVES

Tomaboviruses are more widely distributed viruses in the world, causing dangerous diseases in tomato crops and significantly affecting plant productivity. Virus infections affect especially protein balance or manifest itself as oxidative stress leading to the intensive synthesis of reactive oxygen species. The aim of this study was to clarify the effects of virus infection on photosynthetic characteristics, thylakoid membrane proteins and antioxidant enzyme activities of tomato leaves.

MATERIAL and METHODS

Tomato plant samples with characteristic symptoms for tomaboviruses were collected from various gardens and greenhouses of Absheron penisulla during phytopathological monitorings. The collected leaf samples were tested for viruses by immunochromatography-ELISA and ELISA using polyclonal antibodies to ToMV, TMV, CMV, TSWV, PMMoV (Bioreba AG, Sweden). The activities of the enzymes were assessed spectrophotometrically and polypeptide analyses of thylakoid membrane samples were performed electrophoretically.

RESULTS

Serological tests showed that symptomatic tomato samples collected from Absheron penisula were infected by *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus* (TMV) and *Pepper mild mottle virus* (PMMoV). Immunostrip-test bands and the results of ELISA completely coincided. Analyses of thylakoid polypeptides of virus-infected leaves indicated a decrease in the content of 123, 55, 47, 33, 28–24, 17 and 15 kD polypeptides in comparison with the healthy controls. Photosystem II efficiency and the content of photosynthetic pigments also decreased in all infected samples. Viral infection caused an increase in the amount of malondialdehyde, alterations in activities of antioxidant enzymes and quantitative/qualitative changes in their molecular isoforms.

CONCLUSIONS

In conclusion, we determined that viral infection caused a decrease in the synthesis rate of thylakoid membrane proteins and induced rapid senescence of the infected leaves. The enhanced activity and expression of new peroxidase isozyme bands may be related to tobamovirus infections and could be used as a selection marker in breeding programs.

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ANALYSIS OF FITNESS TRADE-OFFS LIMITING HOST RANGE EXPANSION IN PEPPER-INFECTING TOBAMOVIRUSES

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The acquisition of new hosts, or host range expansion, will provide a virus with more opportunities for transmission and survival, but may be limited by across-host fitness trade-offs. A major cause of across-host trade-offs in viruses is antagonistic pleiotropy. A relevant case of host range expansion is resistance-breaking (RB), in which viruses acquire the capacity to infect otherwise resistant plant genotypes. Under the gene-for-gene (GFG) model of host-pathogen interactions, resistance breaking should be associated to fitness costs in non-resistant hosts. RB-associataed costs have been reported for tobamovirus pathotypes that overcome *L*-gene resistance in pepper (Fraile et al. 2014).

To explore the causes of RB-associated costs, full-length infectious cDNA clones were obtained from two field isolates of Pepper mild mottle virus (PMMoV) that overcome L^2 resistance. Then, all reported coat-protein mutations determining RB of alleles L^3 and L^4 were introduced, and the parental and mutant genotypes was assayed in the susceptible pepper genotypes L^+/L^+, L^1/L^1, L^2/L^2 and L^3/L^3. Virus accumulation was quantified as a proxy to fitness, and virulence was estimated as the decrease of plant biomass due to infection.

Results show that virus fitness depended on the interaction virus genotype (G) x Environment (E), host genotype being the environment, indicating pleiotropic effects of RB mutations. When these effects were quantified, it was found that pleiotropy was antagonistic or positive depending on the specific RB mutation. Similarly, the fitness of the non-RB P_0 pathotype isolates depended on the host plant. Last, RB-mutations also affected virulence, but fitness and virulence were not correlated.
These results are significant as they show that selection for RB does depend not only on the resistance allele deployed but on the genotype of susceptible hosts. Thus, results stress the complexity of the mechanisms underlying host range expansion in viruses, and the difficulty of predicting the evolution of RB and, hence, of resistance durability. Also, these results are significant in that they will provide the bases for future model analyses of the evolution of resistance breaking under realistic scenarios.

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DIVERSITY OF VIRUSES INFECTING OPEN-FIELD PEPPER CROPS IN COTE D'IVOIRE

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BACKGROUND and OBJECTIVES

Pepper (*Capsicum* spp.) is a major vegetable crop, grown in all regions of Côte d'Ivoire, throughout the year. It is usually grown in open fields and, given the diversity of agro-ecological conditions in Côte d'Ivoire, is exposed to a large panel of pathogens, including viruses.

MATERIAL and METHODS

To get an exhaustive and accurate view of the major viral constraints to pepper production, we performed a survey of pepper fields in several agro-ecological zones (AEZ) of Côte d'Ivoire in 2014 and 2015. Leaf samples were collected, kept desiccated on calcium chloride and tested by enzyme-linked immunosorbent assay (ELISA) for infection by *Pepper veinal mottle virus* (PepVMV),*Cucumber mosaic virus*(CMV), *Potato virus Y* (PVY),*Tobacco mosaic virus* (TMV) and *Pepper mild mottle virus*(PMMoV) with polyclonal antisera.Poleroviruses were looked for by ELISA on a sample subset, using *Cucurbit aphid-borne yellows virus* (CABYV) polyclonal antiserium. Then, the most prevalent viruses and poleroviruses were characterized molecularly by RT-PCR and sequencing.

RESULTS

Among 525 plant samples corresponding to 110 farms and 43 localities, 142 (27.0%) presented virus infections. PepVMV was the most frequent virus and was detected in 92 samples (21.2%). It was followed by CMV (11%), PMMoV (2.5%) and TMV (1.3%). No PVY-infected sample was observed and some of the samples reacted positively with CABYV antibodies.Virus prevalence varied greatly between AEZ. Among AEZ where at least 29 samples were analysed, virus prevalence varied from 11.8% (AEZ VI, Sudanese savannah in North and North-West Côte d'Ivoire) to 52.2% (AEZ II, humid forestin South-West Côte d'Ivoire).PepVMV and CMV were most frequently detected in all AEZ, except in AEZ VI, where no PepVMV was detected. RT-PCR products corresponding to parts of the VPg and coat protein (CP) coding sequences of PepVMV isolates were sequenced directly. After withdrawal of sequences corresponding to mixed infections, phylogenetic trees were obtained with 43 VPg and 26 CP-coding sequences and allowed identifying four and three major clades, respectively. A high amino acid diversity was observed in the central region (positions 115 to 128) of the VPg, shown to be critical for adaptation to resistance genes in the case of PVY. Indeed 10 different haplotypes, could be distinguished using the amino acid sequence in this region. Concerning the detected poleroviruses, partial sequencing revealed that they belonged to the species *Pepper vein yellows virus* (PeVYV), and clustered with PeVYV isolates from Mali in the phylogenetic tree. This is the first report of PeVYV in Côte d'Ivoire.

CONCLUSIONS

The most frequent virus in open-field pepper crops throughout Côte d'Ivoire is PepVMV, except in the northern part of the country (AEZ VI). Pepper cultivars carrying a resistance to PepVMV are being released in Africa. Resistance is based on the combination of two recessive genes encoding eukaryotic translation initiation factors 4E that interact with the virus VPg. The high amino acid diversity in the central part of the VPg of PepVMV isolates from Côte d'Ivoire raises concerns about the efficiency and durability of this resistance.

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MOLECULAR CHARACTERIZATION OF THE GENOME SEQUENCES OF *CUCUMBER MOSAIC VIRUS* ISOLATES IN POLAND

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BACKGROUND and OBJECTIVES

Cucumber mosaic virus (CMV) is responsible for important agronomic losses in many crops worldwide and has one of the broadest host range among plant viruses (over 1300 different plant species in 500 genera of more than 100 families). CMV shows a high degree of diversity, as revealed by a large number of isolates differing in both biological and molecular properties. CMV is a type member of the genus *Cucumovirus* in the family *Bromoviridae*. The genome is composed of three single-stranded positive-sense RNAs (3300, 3000 and 2200 nt). Moreover, the genomic RNA of virus belonging to Cucumovirus genus can be often associated with shorter RNA particles as satellites. These particles can modulate the symptoms induce on infected plants (Simon and Roossinck 2004). The total length of each RNA can differ slightly according to the strain or strain grouping, but each ORF of different strains has a similar size, except for ORF 2a and ORF 2b from subgroup II, which are smaller than their counterparts in subgroup I. Based on serological and sequence data, CMV strains have been categorized into three subgroups, IA, IB, and II. The virus is mechanically transmissible by plant sap and spread innature by more than 80 species of aphids, in a non-persistent manner (Bashir et al. 2006).

MATERIAL and METHODS

Recently, the increased presence of CMV isolates has been observed in zucchini crops in Poland. In this study, the molecular characterization of a new, necrotic isolates of CMV (namely: CMV 7 and CMV 21), was performed. The isolates induced very severe, necrotic symptoms on zucchini plants. The nucleotide sequence of the coding regions of both isolates were obtained and compared with others deposited at GenBank. The single nucleotide polymorphism was analyzed and phylogenetic trees were constructed. The sequences were also analyzed in the term of genetic determinants of necrotic symptoms.

RESULTS

In our study the isolates differ in the length of two genes, 2a and 2b respectively. In both isolates R461 in the protein 1a and L55 in the protein 2b were noticed. These two mutations have been previously described as determinants of necrotic symptoms. The phylogenetic analyzes showed that CMV 7 is a genetic reassortant; RNA 1 of CMV 7 shows similarity to phylogenetic subgroup I while the RNA 2 and RNA 3 shows similarity to phylogenetic subgroup II. The CMV 21 isolate was assigned to subgroup I.

CONCLUSIONS

It confirms the genetic diversity of Polish isolates of CMV.

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MODELING THE RELATIVE IMPORTANCE OF VECTOR LIFE HISTORY AND BEHAVIOR IN DRIVING PATHOGEN SPREAD

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BACKGROUND AND OBJECTIVES

Plant viruses that are spread by arthropod vectors impact ecosystems worldwide. While it is well known that vector movement influences pathogen spread, we currently lack a comprehensive understanding of the role that vector movement plays in the spread of plant viruses. We aim to develop a general conceptual model for the relative contributions of vector life history and behavior relevant to host preference on the spread of vector-borne plant viruses. In particular, we consider the case where vector life history (growth rate, carrying capacity) and behavior (departure and settlement rates) parameters are conditional on whether the plant host is infected or healthy and whether the vector is viruliferous or not. A recent empirical study showed that viruliferous vectors prefer noninfected hosts, while nonviruliferous vectors prefer infected hosts (Ingwell et al. 2012), and modeling indicated that conditional vector preference influences disease spread (Roosien et al. 2013).

MATERIAL AND METHODS

We ran simulations under a wide range of parameter combinations and quantified the fraction of hosts infected over time. We ran one version of the model parameterized to *Barley yellow dwarf virus*, a persistently transmitted virus (PT) case study, and one version parameterized to *Potato virus Y*, a non-persistently transmitted virus (NPT) case study. We explored the relative importance of condition-dependence for each vector behavior and life history parameter by running simulations where only one vector parameter type at a time was condition-dependent (different for infected and healthy hosts and/or vectors), while other vector parameters were not dependent on vector or host status.

RESULTS

We found two general types of mechanisms in our model that increased the rate of pathogen spread. First, increasing factors such as vector intrinsic growth rate, carrying capacity, and departure rate from hosts (independent of whether these factors were condition-dependent) led to more vectors moving between hosts, which increased pathogen spread. Second, changing condition-dependent factors such as a vector's preference for settling on a host with a different infection status than itself, and vector tendency to leave a host of the same infection status, led to increased contact between hosts and vectors with different infection statuses, which increased pathogen spread.

CONCLUSION

Our findings will contribute to the knowledge base in plant virus epidemiology and to potential strategies to more effectively manage plant virus diseases.

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Q-BANK AND VIRUSCOLLECT: A COMMON REFERENCE COLLECTION OF PLANT VIRUSES AND VIROIDS

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The availability and accessibility of suitably characterised plant virus and viroid reference isolates is vital for research and diagnostic laboratories. The maintenance of such collections has come under severe pressure due to a reduction in the number of scientists (in particular virologists) and associated budgets. As a result there is a need for international collaboration.
The Euphresco project VirusCollect aimed to establish a common reference collection of viruses and viroids by linking collections maintained by individual institutions via Q-bank.
Q-bank, the comprehensive databases on plant pests and diseases, offers an excellent platform to share data on plant virus collections (http://www.q-bank.eu/Virus). Over a thousand virus species are included and relevant information for each species is provided. The inclusion of additional data and corresponding nucleotide sequences will allow provisional identification of 'unknown' virus isolates using the 'search' and 'BLAST' functions of Q-bank. Moreover, information on the availability of over 500 virus isolates from international laboratories can be obtained.
Within the VirusCollect project, standard operating procedures (SOP's) were developed and implemented by participating laboratories to guarantee the quality of isolates and data. In addition, more than 60 virus isolates of phytosanitary and/or economic importance were characterised and the corresponding data included in Q-bank. Characterised isolates were adequately stored to maintain their viability and guarantee the availability for future reference and use.
VirusCollect has enabled the first step in collaboration between curators and standardisation of maintenance of virus collections. Fundamental standards laid the basis for improving the quality of individual collections and the layout of Q-bank as a platform to share data and information. The achievements towards a common reference collection were appreciated and resulted in a follow-up project, Euphresco VirusCollect II, in which eight countries have expressed an interest to join.

INCIDENCE OF PLRV AND TRANSMISSION EFFICIENCY BY FOUR POTATO APHIDS IN NORTH-EASTERN TUNISIA

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BACKGROUND and OBJECTIVES

PVY and PLRV are the most damaging viruses on potato inducing yield loss and downgrading seeds quality. Epidemiology of PVY was studied during spring 2007 until 2012 in Tunisia. PVY^{NTN} was the major virus transmitted by 15 aphid species abundant in yellow traps. Among them *M. persicae*, *A. gossypii* and *A. spiraecola* were the most efficient vectors (Boukhris-Bouhachem, 2011). For a better understanding of PLRV epidemiology, the incidence, the updating of aphids colonizing potatoes and their transmission efficiency are estimated.

MATERIAL and METHODS

Aphids were collected in the Cap Bon region, with yellow water trap in seed potato field from beginning of February to late May from 2013 to 2015. Captured aphids were identified with Remaudière & Seco (1990) key. Furthermore, one hundred potato leaves were collected every two weeks to identify colonizing aphids. PVY and PLRV incidences were determined based on 204-potato leaves var. "Spunta" tested by DAS-ELISA. Transmission of PLRV by the four aphid species was applied on *Physalis floridana*. The transmission capacities were studied.

RESULTS

Field monitoring revealed the presence of PLRV (20.5%) compared to PVY (85%). More than 170 aphids were captured in yellow water trap. Fifteen species were abundant: *Aphis gossypii, Macrosiphum euphorbiae, Myzus persicae, Aphis fabae, Aulacorthum solani, Aploneura lentisci, Brachycaudus helichrysi, B. cardui, Hyalopterus pruni, Hyperomyzus lactucae, Acyrthosiphon pisum, Rhopalosiphum padi, R. maidis, Aphis spiraecola,* and *Lipaphis erysimi*. These latest were previously proved to be efficient to transmit PVY.
On potato leaves, four species are identified: *M. persicae, M. euphorbiae, A. fabae,* and *A. gossypii.* All these colonizing species were efficient to transmit PLRV with different capacities, 90%, 80%, 50% and 30% respectively. *M. persicae* and *M. euphorbiae,* are the best vectors of PLRV as reported by Robert (1971). Conversely, the PVY transmission efficiencies of *M. euphorbiae* (3%) was the poorest one compared to *M. persicae* 95% while *A. gossypii (68%)* and *A. fabae (43%)* have moderate efficiencies.

CONCLUSIONS

These results will lead to a better understanding of PVY and PLRV epidemiology for successful management of key aphid vectors in order to improve seed potato sanitary conditions.

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IMPACT OF WHEAT STREAK MOSAIC VIRUS ON WHEAT PRODUCTION IN THE NORTHERN GREAT PLAINS REGION OF THE UNITED STATES: A REVIEW

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BACKGROUND and OBJECTIVES

The Great Plains region of the United States produces more than half of the total U.S. wheat. This region is characterized by prairie and grassland and receives relatively low rainfall (510 mm) making this region favorable for wheat viruses, principally *Wheat streak mosaic virus* (WSMV). WSMV is devastating to wheat, because WSMV is endemic in most of this region and can lead to complete loss of the wheat crop, depending on the cultivar and time of infection. WSMV is transmitted by wheat curl mites (WCMs, *Aceria tosichella* Keifer). WCMs also transmit *Triticum mosaic virus* (TriMV) and *Wheat mosaic virus* (formerly High Plains virus), but these are not as widely spread as WSMV. This review aims at summarizing the impacts of WSMV epidemics on wheat in the Great Plains region and to highlight research efforts in managing WSMV.

MATERIALS and METHODS

Published literature on WSMV in the Great Plains region was accessed and summarized. Information on impact of WSMV was also obtained from extension and outreach-type publications.

RESULTS

Yield loss studies reported in literature used mechanical inoculation to quantify yield loss. Greatest yield loss occurred in winter wheat infected in fall compared to spring inoculation. Grain yield loss ranged from 14% to 98% across wheat cultivars and environments. Yield loss was more pronounced with WSMV co-infecting with other viruses such as TriMV. In a few instances, WSMV infection led to total loss of the wheat crop in producer fields. The most common method of WSMV management reported was use of tolerant or resistant cultivars.

CONCLUSIONS

Wheat streak mosaic virus caused severe yield losses in both spring and winter wheat and the extent of yield loss depended on time of infection and if infection was by WSMV alone or with other viruses transmitted by WCM. Cultivar resistance was the most effective management practice in the WSMV management.

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SEQUENCE IDENTIFICATION OF A NOVEL BEGOMOVIRUS AFFECTING BEAN CROPS IN SOUTHWEST COLOMBIA

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BACKGROUND & OBJECTIVES

Geminiviruses belong to a fast growing family of plant pathogens. In bean crops, a series of begomoviruses have been reported to affect productivity in Latin America. The whitefly-transmitted Bean leaf crumple disease (BLCD) was first described in 2002 affecting green beans in the department of Valle del Cauca, Colombia (1,2). Diseased plants showed severe leaf deformation with mild variegation (incidence of 80%). Since then, in the department of Valle del Cauca, there has been a drastic decrease (60-70%) of the cultivated area of green beans due to recurrent and sporadic outbreaks of begomovirus-associated symptoms. We organized surveys to monitor the reported recent outbreaks of BLCD, identified the begomovirus species responsible for the symptoms and performed phylogenetic analysis for its proper classification.

MATERIAL & METHODS

Bean samples collected between 2008 and 2015 in Valle del Cauca were tested for Geminiviruses by PCR using generic primers or for circular DNAs by rolling circle amplification (RCA) using random hexamer primers.

RESULTS

RCA allowed us to obtain the whole viral bipartite genome. Blast analysis of the replication-associated protein sequence (Rep) (DNA-A) showed 86-88% nucleotide identity with Calopogonium golden mosaic virus, a virus reported from Costa Rica, associated to different symptoms, in 1992 (3). For the movement protein (MP) on DNA-B, the sequence comparisons showed 93% nucleotide identity with *Sida micrantha mosaic* virus. The phylogenetic analysis of both segments revealed that all isolates clustered separately from any previously reported begomovirus infecting beans in the Latin America.

CONCLUSIONS

RCA is an easy, fast and unbiased detection method for all infecting circular DNA components. Here we successfully implemented RCA to detect and identify a novel group of begomovirus severely affecting bean crops in southwestern Colombia. Sequence identity values, different symptoms and distinct geographic distribution suggest a new begomovirus infecting beans for which we suggest the name “Bean leaf crumple virus” (BLCrV).

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BIOLOGICAL CHARACTERIZATION, DISTRIBUTION AND SYNERGISTIC INTERACTIONS OF A TORRADOVIRUS WITH A POLEROVIRUS INFECTING CASSAVA IN THE AMERICAS

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BACKGROUND & OBJECTIVES

Field experiments in Colombia using clean cassava planting material showed that Cassava torrado-like virus (CsTLV) (family *Secoviridae*) and Cassava polero-like virus (CsPLV, family *Luteoviridae*), two novel viruses found in mixed infections in cassava affected by severe root symptoms (1) are the first to accumulate during growing season in 2014. To characterize CsTLV and CsPLV and further understand their role in pathogenicity, full genome sequence, biological studies and sequence diversity were performed.

MATERIAL & METHODS

We characterized isolates of CsTLV and CsPLV and studied its co-infection using grafting and sap-inoculations in cassava and a series of herbaceous hosts. Plants were monitored for leaf and root symptoms development as well as average plant height, number of commercial stakes and root weight per plant was determined. Deep sequencing using small interfering RNAs (siRNAs) and RT-PCR from a CsTLV isolate Sec13 were used to complete CsTLV genome.

RESULTS

CsTLV causes chlorotic spot symptoms in young cassava leaves while CsPLV did not induce any visible symptoms in leaves. None of them could be mechanically transmitted neither were they through botanical seed. Full genome analysis of CsTLV revealed the presence of a Maf/HAM domain. Only two other plant viruses have been reported to contain a Maf/HAM domain: *Cassava brown streak virus* and *Euphorbia ringspot virus* (family *Potyviridae*) and all have been isolated from species of the family Euphorbiaceae (2, 3). Field surveys show the presence of CsTLV in three surveyed regions but it was more prevalent in province of Sucre (62%) where the percentage of root diseases was higher. Phylogenetic analyses of CsTLV isolates revealed wide sequence diversity and suggest the presence of at least three different strains that share between 80-85% a.a. similarity in the RdRp. When CsTLV was tested in co-infection with CsPLV, root yield of cassava showed up to 90% decrease. The protein function of CsTLV Maf/HAM is under study.

CONCLUSIONS

The high incidence and the early infection of CsTLV and CsPLV contribute to the rapid accumulation of mixed viral infections in cassava leading to great yield losses.

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DEVELOPMENT OF SENSITIVE NESTED-RT-PCR METHOD FOR DETECTION OF *CITRUS PSOROSIS VIRUS*

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BACKGROUND and OBJECTİVES

Psorosis disease caused by *Citrus psorosis virus* (CPsV) is one of the most important viral diseases of citrus in many citrus growing region of the world including Turkey. Even though the existence of CPsV in Turkey has been known for a long time, the virus was detected only in the Eastern Mediterranean region. CPsV induces different symptoms ranging from chlorotic flecks on the leaf, scaling of the bark to decline of citrus trees. In recent years, a significant number of studies have been conducted about serological and molecular detection of CPsV in the world. Although a number of serological and molecular methods have been used for detection CPsV, the sensitivity of the methods is generally low and detection is some time limited to certain period. The virus is most efficiently detected from flower tissue which is available only for a limited period of time. Therefore, a more sensitive method is need for detection of CPsV in readily available leaf tissue. The objectives of this study were detection of CPsV in Antalya province and development of a more sensitive RT-PCR method for detection of CPsV in leaf tissue.

MATERIALS and METHODS

Field surveys conducted in different citrus growing areas of Antalya province located in western Mediterranean Region of Turkey. Samples were first tested two serological methods, direct tissue blot immunoassay (DTBIA) and enzyme linked immunosorbent assay (ELISA) using commercial kit with monoclonal antibody (Agritest). Then total RNA was isolated from all leaf samples by one step RNA solution (BioBasic) and the coat protein (CP) gene of CPsV were amplified by a two-step reverse transcription-polymerase chain reaction (RT-PCR) method using degenerate primers. Finlay, a nested-PCR was performed to all samples using PCR products of degenerate primers as template for second PCR reaction conducted with internal primers specific to the CP gene. The results of serological and PCR methods were all compared to determine the most efficient method of detection.

RESULTS and CONCLUSIONS

Leaf samples were collected from 51 orange trees showing typical symptoms of psorosis disease in different citrus orchards in different areas of Antalya Province. A total of 6 found to be infected with CPsV by both serological tests. When all samples were tested with RT-PCR, 1320 bp DNA fragment corresponding to the CP gene of CPsV was amplified from nine of the 51 tested samples. Further testing with nested PCR showed that 350 bp portion of CPsV CP gene was amplified from 45 of the 51 tested samples indicated that majority of trees were actually infected with CPsV. Comparison of the results of the serological and RT-PCR tests demonstrated the nested-PCR was the most sensitive detection method for CPsV from the leaf samples. The finding of this study not only showed the presence of CPsV in western Mediterranean Region and but also revealed that nested-PCR is a sensitive method that can improve the detection of CPsV in different citrus growing regions of the world.

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MONITORING *TOMATO CHLOROSIS VIRUS* INFECTION PROCESS IN TOMATO BY REAL-TIME RT-PCR ASSAY

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BACKGROUND and OBJECTİVES

Tomato chlorosis virus (ToCV) causes severe intervenal chlorosis in tomato and recently spreading in tomato production regions of the world especially in the Mediterranean Basin including Turkey. The virus is transmitted by different whitefly species and spread rapidly especially in the greenhouses. In the absence of natural resistance to ToCV, the virus becoming an important pathogen of tomato the disease development process and has not been studied in great detail. To understand the infection and disease development processes a graft inoculation method was developed for monitoring ToCV inoculation in tomato plant in controlled environmental conditions.

MATERIALS and METHODS

At least 10 seed-grown tomato seedlings were graft inoculated with phloem tissue containing stem segments from a ToCV-infected tomato plants to monitor ToCV infection. Another group of tomato plants were grafted with similar stem segments from healthy tomato plants and used as control muck inoculation. The CP gene of ToCV was amplified and cloned into pGEM-Teasy vector under the control T7 promoter and RNA was synthesized by in vitro transcription using T7 RNA polymerase and RNA was quantified by spectrophotometer and serially diluted to obtain standards containing 10^1 to 10^6 copy of the virus used for absolute quantification of ToCV in inoculated samples. Total RNA was isolated from leaf samples collected from ToCV inoculated and muck inoculated control plants before the inoculation and 1, 2, 4, 7, 10, 14, 20, 25, 30, 35, 40, 45 and 60 days post inoculation (dpi). The presence and the quantity of ToCV were determined from all ToCV inoculated or muck inoculated control plants by qRT-PCR methods using TaqMan probes specific to the coat protein gene.

RESULTS and CONCLUSIONS

The results showed that ToCV was detected as early as 15 dpi only in small portion (20-30%) of inoculated plants. The infection rate was increased progressively as the inoculation period advanced and reached to 70-80% by 60 dpi. The copy number of ToCV in inoculated plants were determined as using RNA standards, the virus titer was at the detectable level at 15 dpi and increased and reached to maximum level by 40 dpi and then started to decrease. The results showed that the ToCV was readily transmitted by patch grafting in tomato and grafting can be used as experimental system studying ToCV-tomato interaction. The results revealed that graft inoculation of ToCV infection require at least 15 days and the virus titer was reached to the maximum level at 40 dpi suggesting that ToCV infection process should be conducted between 15 and 40 dpi.
This study was supported by a grant from TUBİTAK Project No 111O646

MOLECULAR CHARACTERIZATION OF GREEK VEGETABLE AND LEGUME ISOLATES OF CUCUMBER MOSAIC VIRUS AND ITS SATELLITE RNAs

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BACKGROUND and OBJECTIVES

Cucumber mosaic virus (CMV, genus *Cucumovirus*, family: *Bromoviridae*) has probably the broadest host range in nature. A variety of plant diseases are caused by the three CMV isolate subgroups (IA, IB and II) and the associated satellite RNAs (satRNAs) (Jacquemond, 2012). In Greece, field surveys showed that CMV is the major seed-borne virus encountered in legumes and also in several other vegetable crops. The aim of this study was to analyze the molecular variability of the coat protein (CP) of the Greek CMV isolates and the presence and variability of their satRNAs.

MATERIAL and METHODS

In total 42 CMV isolates collected throughout the country were analyzed: 15 isolates originated from tomato, 15 from legumes (12 common bean, three lentil), ten from cucurbits (seven melon, two cucumber, one zucchini) and two from pepper. Primers designed and used were targeting the CP gene of CMV and the full sequence of its satRNAs. Amplicons were sequenced and the respective phylogenetic analysis was performed.

RESULTS

All Greek legume and vegetable CMV isolates originating from different areas were clustered together with isolates from subgroup I, according to their CP sequences. None of the isolates was classified in subgroup II. The genetic diversity of the Greek CMV isolates was limited when compared with the respective ones from the worldwide database. SatRNAs were detected in four tomato and one pepper isolate. Among the legume isolates, seven common bean and three lentil isolates were associated with satRNAs, while among the cucurbit isolates, one cucumber and three melon isolates contained satRNAs. In phylogenetic analysis of the tomato and legume satRNAs, all isolates clustered within the necrogenic subgroup. However, their presence was associated with necrotic symptoms only in tomato. Greek CMV satRNAs showed broad genetic diversity when they were compared with those from the worldwide database. The analysis of the cucurbit and pepper satRNAs is in progress.

CONCLUSIONS

In this study the analysis of the CP gene of several legume and vegetable CMV isolates showed a dominance of subgroup I. Necrogenic satRNAs are widespread within Greek CMV population, but they are only associated with necrotic symptoms in tomatoes. The presence of necrogenic satRNAs in hosts with mild symptoms may represent their "surviving ecological niches".

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INCIDENCE AND MOLECULAR CHARACTERIZATION OF THE MAJOR APHID-BORNE VIRUSES OF LEGUME CROPS IN GREECE

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BACKGROUND and OBJECTIVES

In Greece, a plethora of local legume landraces are cultivated under low input conditions exploiting variable soils and environments. Aphid-transmitted viral diseases represent major constraints for legume production (Makkouk et al., 2012; 2014), while local landraces kept «*in situ*» by farmers can be severely affected by the seed-borne viruses. This study aims to investigate the incidence and the molecular variability of the major aphid-transmitted legume viruses in Greece.

MATERIAL and METHODS

Field surveys were performed from 2009 to 2015 in bean (*Phaseolus* sp and *Vigna* sp), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.) and Cyprus-vetch (*Lathyrus ochrus* L.) crops, during their flowering-podding stage. Leaf samples with virus-like symptoms were analyzed by ELISA for the presence of *Alfalfa mosaic virus* (AMV), *Bean yellow mosaic virus* (BYMV), *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Bean leafroll virus* (BLRV), *Cucumber mosaic virus* (CMV), and *Pea enation mosaic virus-1* (PEMV-1). Subsequently, the molecular variability of BCMV was studied by using primers targeting the nuclear inclusion protease (NIa-pro) and the RNA helicase (Cl) genes. For BLRV the coat protein (CP) gene and the 3' end region were targeted, whereas for PEMV-1 primers amplifying the P3-P5 read-through structural protein (CP-RTD primers) and the read-through domain (RTD) were used. In all cases the amplicons were subsequently sequenced and subjected to phylogenetic analysis.

RESULTS

CMV (64.3%) and BCMV (57.1%) prevailed in bean crops. Although AMV and BYMV were recorded in all legume crops, their incidence was erratic. BCMNV was reported for the first time in a limited number of bean samples. BLRV and PEMV-1 were the major viruses encountered in lentil (36.1% and 35% of the symptomatic plants, respectively) and pea (48% and 50%) crops. BLRV also prevailed in faba bean (37%) and PEMV-1 in Cyprus-vetch (53.8%) crops. Twelve BCMV isolates were classified in two distinct phylogenetic branches when their NIa-pro and Cl sequences were analyzed. Five lentil, eight faba bean and three pea isolates of BLRV shared 95% identity for the CP gene and 99% for the 3' end region. Two pea and three lentil PEMV-1 isolates clustered together with an isolate from Germany according to their CP-RTD region, while the analysis of the Cyprus-vetch isolates is underway.

CONCLUSIONS

Winter and spring Greek legumes are threatened by different aphid-transmitted viruses. Bean crops are mainly affected by the non-persistently, seed-borne CMV and BCMV. On the other hand, the persistently transmitted BLRV and PEMV-1 represent threats for pea, lentil, faba bean and Cyprus-vetch crops. Low levels of molecular variability were detected in BCMV, BLRV and PEMV-1 isolates suggesting a narrow genetic base for these viral populations in the country.

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MOLECULAR EPIDEMIOLOGY OF CASSAVA BEGOMOVIRUSES ASSOCIATED WITH CASSAVA MOSAIC DISEASE IN ZAMBIA

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Cassava is the most important root crop in Zambia, however, productivity is affected by a number of biotic stresses of which cassava mosaic disease (CMD) is the most important. The study carried out in a country wide survey between April and September 2015 identified three new cassava viruses in Zambia. Using universal and specific primers, the viruses identified include; *East African cassava mosaic Cameroon virus* (EACMCV), *South African cassava mosaic virus* (SACMV) and *East African cassava mosaic Zanzibar virus* (EACMZV). Distinct geographical distributions were observed for the three viruses. EACMZV was more prevalent in Northern, Luapula, Lusaka, Central and Eastern provinces while SACMV and EACMCV were prevalent in Eastern and Lusaka provinces. Polymerase chain reaction (PCR) diagnosis revealed that mixed infection (up to four co-infected viruses) occurred in 0.9% (6/634) of the samples and were associated with higher (3.39) symptom severity scores. Mixed infections of ACMV+EACMZV occurred in 7.1% (45/634) of the positive samples. Single infections of ACMV were found in 66.4% (421/634) of the positive samples. Mean whitefly population was high in Western (11.3) and Lusaka (7.6), and low in Eastern (0.9) and Luapula (0.3) provinces. This study has established new and westward spread of cassava viruses in Zambia. In order to reduce the threat posed by the viruses, it will be essential to identify, develop and deploy effective strategies for CMD management.

WIND-MEDIATED CONTACT TRANSMISSION OF *PEA SEED-BORNE MOSAIC VIRUS* IN FIELD PEA

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BACKGROUND AND OBJECTIVES

Pea seed-borne mosaic virus (PSbMV) infection causes serious losses in yield and seed quality in semi-leafless field pea (*Pisum sativum*) crops (Coutts et al 2008, 2009). It is non-persistently aphid-borne in the field, but the possible role of contact transmission as a secondary means of spread has not been investigated.

MATERIALS AND METHODS

Five glasshouse experiments were done to assess the stability of PSbMV infectivity in sap incubated at room temperature for different time-periods (5min to 30h). The sap was rubbed onto leaves of healthy faba bean (*Vicia faba*) plants without use of abrasive. PSbMV exhibited moderately-high stability, remaining infective in sap for at least 30 hours. To assess whether transmission would occur due to passive contact or leaf-rubbing, eight experiments were done. Each experiment consisted of two sets of ten pots, one set containing a healthy and a PSbMV-infected pea plant growing in each pot such that the plants were able to intertwine (passive contact). In the second identical set of pots, the leaves of ten healthy pea plants were rubbed with a PSbMV-infected pea leaf. To simulate wind-mediated contact transmission in the field, eight glasshouse experiments were done. Oscillating fans were used to blow rows of healthy pea plants which each included one PSbMV-infected plant, 6-12 times for 1-2 hours at a time.

RESULTS

Following leaf-rubbing, PSbMV was transmitted from infected to healthy pea plants at a rate of 20-40% but no passive contact transmission occurred. Wind-mediated contact transmission occurred in each of 3 experiments when maximum glasshouse temperatures were between 14-20°C and PSbMV concentration in the infector plants was high. However, PSbMV transmission only occurred in 1/5 experiments when maximum glasshouse temperatures were 20-30°C, and virus concentration in the infector plants was low. This could be explained by PSbMV being a temperate climate-adapted virus reaching higher concentrations under cooler temperatures.

CONCLUSION

These results suggest that wind-mediated PSbMV contact transmission may play an important role in its spread in field pea crops by enlarging PSbMV infection foci in young crops for aphid transmission later in the season, accelerating PSbMV spread. This information is important in improving understanding of PSbMV epidemiology and can be implemented into a forecasting model that predicts PSbMV epidemics in field pea.

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AMPLICON NEXT GENERATION SEQUENCING AS A TOOL TO STUDY THE DIVERSITY OF ILARVIRUSES IN *PRUNUS* TREES

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BACKGROUND and OBJECTIVES

PCR amplicon next generation sequencing (NGS) offers an alternative and sensitive approach to study virus diversity within a sample and between samples compared metagenomics NGS [1]. It is possible to sequence amplicons from multiple samples in a single run. In this study amplicon NGS was used to determine the distribution and diversity of *Ilarvirus* species populations amongst *Prunus* species in Australia.

MATERIAL and METHODS

Forty seven *Prunus* trees tested positive using an *Ilarvirus* genus-specific nested RT-PCR [2] and the 371bp amplicons were used for NGS library preparation. The amplicon libraries were quantified, pooled and sequenced using the Illumina Miseq, with a paired read length of 301bp. The sequence reads were quality trimmed, paired and subject to a BLASTn search of GenBank. The BLASTn output was analysed using MEGAN [3].

RESULTS

Metagenomic analysis revealed sequence matches to 15 *Ilarvirus* species, with varying number of reads, across the 47 samples. Mixed infections of *Ilarvirus* species and populations of quasi-species occurred within individual samples. A high number of the total reads in each sample were attributed to a selected few ilarviruses and the remaining reads represented ilarviruses occurring with low frequency. *Prunus necrotic ringspot virus* (PNRSV) was detected in 37 of the 47 samples. In each of the 47 samples a few sequence reads were attributed to a virus of unknown origin. These reads had a 70-80% identity to *Ilarvirus* type species *Tobacco streak virus,* suggesting the presence of new ilarviruses in the samples.

CONCLUSIONS

Amplicon NGS provided a highly sensitive tool to assess the frequency and diversity of *Ilarvirus* species within an individual plant sample and between multiple plant samples in a single NGS run. High read numbers for an individual virus gives confidence that it is present in a sample. Low read numbers could indicate a low-level infection beyond the limit of conventional detection methods. PNRSV was the most frequently detected *Ilarvirus* species amongst all the samples that were analysed. The results also suggested the presence of novel Ilarviruses in some *Prunus* samples. The presence of known and novel Ilarviruses associated to a low number of reads could be a potential biosecurity concern and metagenomics NGS will be used to confirm their existence in the samples.

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EPIDEMIOLOGY OF GRAPEVINE RED BLOTCH AND LEAFROLL VIRUSES IN OREGON VINEYARDS

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BACKGROUND and OBJECTIVES

We initiated this study focusing on the grapevine leafroll-associated virus strain 3 (GLRaV-3) and its vector the grape mealybug (*Pseudococcus maritimus*) across Oregon, U.S.A. In 2012 a grapevine virus later named grapevine red blotch-associated virus (GRBaV) was identified in grapevines exhibiting leafroll-like symptoms (Sudarshana et al. 2015). Studies began in 2013 to investigate the epidemiology of GRBaV in Oregon vineyards. The objectives of the studies were to determine: 1) baseline incidence of GRBaV in Oregon viticultural regions; 2) whether GRBaV is spreading in study sites; 3) potential vectors of GRBaV in Oregon and their prevalence in study sites; 4) whether GLRaV-3 and GRBaV coexist in grapevines.

MATERIALS and METHODS

Manual surveys and pheromone-baited sticky traps were employed in 6-8 vineyards in Oregon from 2010-2012 to determine phenology and incidence of *P. maritimus*. To assess potential insect vectors of GRBaV, the baited sticky traps from 2010-2012 were revisited, and in 2014 and 2015 passive sticky traps were placed in the remaining vineyard sites plus additional study sites. Sweep net samples were taken from blocks with known GRBaV infection. Leaf collections were made in fall, nucleic acid extracts were obtained, and qRT-PCR and qPCR tests were conducted to detect GLRaV-3 (fall 2012-2014) and GRBaV (2013-2015), respectively. Insect and virus distribution were assessed using the statistical platform, Spatial Analysis by Distance Indices and plotted using the mapping software Surfer.

RESULTS

From the studies conducted in 2010-2012, it was determined that *P. maritimus* was the main vector species for GLRaV-3 in Oregon (Walton et al. 2013). Virus symptoms occurred in most study sites, yet PCR analyses for GLRaV-3 did not match the occurrence of symptoms in the field. Subsequent PCR tests showed presence and significant increases in the occurrence of GRBaV at many locations from 2013-2015. More than 20 species of leafhoppers were present in sticky card and sweep net samples.

CONCLUSIONS

GRBaV is present in most Oregon viticultural regions, and appears to be spreading. Spatial analysis of GRBaV and GLRaV-3 indicates that the two viruses are independent of one another and likely have separate insect vector species.

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METAGENOMICS-BASED EXPLORATION OF THE GENETIC DIVERSITY OF SCYLV IN THE SUGARCANE COLLECTION OF GUADELOUPE.

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BACKGROUND and OBJECTIVES

Sugarcane yellow leaf virus (SCYLV) is a phloem-restricted RNA virus species in the genus *Polerovirus* of the family *Luteoviridae*, which is either transmitted by aphids or propagated through plantation of sugarcane infected cuttings. Yellow leaf disease had emerged two decades ago in Guadeloupe where it was firmly identified for the first time in 1996. Since its introduction in Guadeloupe, the virus incidence has continuously increased throughout the island and has reached in 2010 levels of 20% in the commercial fields and 50 % in the sugarcane variety collection. While the genotype (REU) was initially over-dominant at the commercial field level (2005, 98%) two other genotypes (BRA-PER and CUB) were subsequently identified throughout the island. Interestingly, the incidence of the genotype CUB, which is the most aggressive SCYLV genotype, has tremendously increased the last decade (>85% in 2010). Additionally genotypes mixed infections were frequently observed at the variety level. However, intra-plant SCLYV population structure remains widely unknown, which hampers straightforward analyses of SCYLV microevolution.

MATERIAL and METHODS

We here use the virion-associated nucleic acids (VANA) metagenomics-based approach for estimating the genetic diversity of SCYLV in the sugarcane variety collection context in 2012. Leaf samples were randomly collected from 300 sugarcane varieties (1). The VANA-based 454 pyrosequencing approach was used to analyse individually the virome of each of the 300 sugarcane samples.

RESULTS

SCYLV-related reads were identified from 134 samples out of the 300 processed plant samples, which suggest an overall SCYLV prevalence of 45%, which is in full agreement with recent SCYLV prevalence studies. We focus on thirty-three sugarcane samples for which more than 400 SCYLV-related reads were obtained. For the thirty-three samples, based on the reads mapping into ORF1 and ORF3, we show that 12 varieties are infected by the SCYLV genotype REU, 11 varieties by genotype BRA-PER and 4 varieties by the genotype CUB. We also find that 4 varieties are co-infected by two genotypes (CUB/REU) and, unexpectedly, that 2 varieties, namely ROC7 and PR1059 are infected by SCYLV chimeric variants that are assigned to CUB (ORF1) / REU (ORF3) genotypes and BRA-PER (ORF1) / REU (ORF3) genotypes, respectively. This result suggests the occurrence of recombination events between SCYLV genotypes. We further show that the chimeric variant obtained from the variety ROC7 results from a recombinant event, which may involve the transfer of the CP-RDT zone from a SCYLV REU isolate (minor parent) to a SCYLV CUB genome (major parent). We could also assembled scaffolds covering at least 80% of publicly available SCYLV genomes from 21 of the 33 varieties. Those 21 varieties are infected by a single genotype (3 CUB; 11 REU; 7 BRA-PER) and phylogenetic analyses reveal that all 21 SCYLV isolates unambiguously group within the SCYLV REU, BRA-PER and CUB phylogenetic clusters. Interestingly, the 3 CUB isolates are forming a subgroup within the CUB phylogenetic cluster.

CONCLUSIONS

Our results suggest that SCYLV has been locally evolving the last two decades, suggesting that the virus virulence can quickly evolve enabling SCYLV adaptation to local varieties and vector complex.

(1): Accessions supplied by the "Tropical Plants Biological Resources Center, INRA-CIRAD, French Antilles"

OCCURRENCE AND VARIABILITY OF LITTLE CHERRY VIRUS 1 AND 2 IN BELGIUM

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BACKGROUND and OBJECTIVES

Little Cherry disease (LChD) can be caused by two distinct viruses (*Little cherry virus* 1 and 2), and is having a large impact on yield and quality in commercial sweet (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.) production (Rott and Jelkmann, 2001). Several other *Prunus* spp. can be infected - yet often latently - by both viruses, including the popular oriental flowering cherry (*P. serrulata* L.). For LChV-1, no vector has been identified so far, but at least two distinct species of mealybugs (Hemiptera, Coccoidea, Pseudococcidae) are known to transmit LChV-2, namely the apple mealybug (*Phenacoccus aceris* Signoret) and grape mealybug (*Pseudococcus maritimus* Ehrhorn) (Slykhuis *et al*., 1980; Mekuria *et al*. 2013).

MATERIALS and METHODS

During 2014 and 2015, an intensive survey has been conducted to monitor the incidence and spread of LChV-1 and -2 in symptomatic and non-symptomatic host trees, eventually leading to re-evaluate the status of LChV infections in Belgium. Leaf symptoms such as premature reddening or bronzing, as well as the development of small fruits, uneven ripening and an insipid taste were observed in many orchards. A total of 306 trees were sampled and tested by RT-PCR for the 2 viruses. LChV-1 or -2 -specific PCR products spanning different open reading frames, were subsequently sequenced and used as markers for characterization.

RESULTS

Both viruses were widely detected in individual or mixed infections, with a slightly higher incidence for LChV-2 in samples from sweet and sour cherries. The disease was found to be prevalent in many cherry production areas in nearly all places where cherries are grown. Additionally, both viruses were also found in ornamental *Prunus* spp. in private gardens and in lane trees. Along with published homologous genomic data from other isolates, the genetic diversity of Belgian *Little cherry virus* (LChV-1 & 2) isolates originating from different hosts and geographic locations was assessed, based on sequences corresponding to the partial RNA-dependent RNA polymerase (RdRp), Heat-Shock Protein homologue (HSP70h) and Coat Protein (CP) genes.

CONCLUSIONS

Preliminary phylogenetic analysis revealed a low genetic variability for the Belgian LChV-1 and LChV-2 isolates, yet suggests a long-term establishment for both viruses in our region.

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CHARACTERIZATION OF BEGOMOVIRUSES INFECTING NONE-CULTIVATED CROPS IN SOUTH WESTERN NIGERIA

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BACKGROUND and OBJECTIVES

Whitefly transmitted Begomoviruses (family Geminivirdae) are a threat to crop production around the world and a threat to food security in West Africa due to the diseases that they cause in cassava, a major staple food in West Africa. Continues emergence of new Begomoviruses species/strains, which is driven by mutation, genetic drift and recombination, is a concern and a challenge to the global efforts towards the management of *Begomovirus* diseases. Thus proper understanding of the role of none cultivated plants in *Begomovirus* epidemiology remains crucial.

MATERIAL and METHODS

During field surveys conducted in 2012, 22% of 50 none cultivated plants collected within and around cassava farms in South Western Nigeria tested positive to *Begomovirus* infection by PCR using a degenerate *Begomovirus* coat protein primer pair. The coat protein genes from two of the viruses were cloned and sequenced.

RESULTS

One of the sequenced isolate had 97-99% nucleotide (nt) and 97-100 % amino acid (aa) identity with most *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) coat protein sequences available in the Genebank, while the second cloned isolate had 99% nt and 98% aa identity to the coat protein of *Soybean mild mottle virus* (SbMMV). The coat protein sequence of this second was also homologous, in varying degrees, to several previously characterized Begomoviruses from vegetables and weeds.

CONCLUSIONS

Knowledge of the alternative hosts involved in cassava mosaic disease epidemiology is crucial to the management of these diseases. The role of the begomovirus isolates characterized in this study in cassava virus disease dynamics need to be understood therefore the potential of these viruses, singly and in mixed infection, to induce disease in cassava is will be investigated.

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PRE-ACQUISITION STARVATION DOES NOT INCREASE THE TRANSMISSION RATE OF *CUCUMBER MOSAIC VIRUS* (CMV) BY *APHIS GOSSYPII*

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BACKGROUND AND OBJECTIVES

Most plant viruses are transmitted in a nonpersistent (NP) manner by aphids. NP transmission occurs during very superficial probes of less than five minutes with subsequent transmissibility being lost within a few hours. Previous studies have shown that a pre-acquisition starvation period increases the transmission rate of NP viruses by altering their probing behaviour to make them more likely to acquire virions from epidermal cells. The main objective of this work was to study the effect of pre-acquisition starvation on the transmission rate of CMV and on the probing behaviour of *Aphis gossypii*.

MATERIAL AND METHODS

A series of transmission tests were carried out to compare the transmission rate of several CMV isolates (M6, Fny, V698, Ls, Val 24 and B20) by groups of five starved or nonstarved *A. gossypii* adults subjected to an acquisition access period (AAP) of 5 min on zucchini plants. Moreover, the probing behavior of single starved and nonstarved *A. gossypii* on a CMV-Fny infected plant and its associated virus transmission rate was compared using the Electrical Penetration Graphs (EPG) technique. Nonviruliferous aphids with a gold wire attached to their dorsum were divided in two different batches: some aphids were suspended in the air using a gold wire and thus not allowed to feed (starved) and others were placed directly on a healthy melon leaf and allowed to feed (nonstarved). After one hour, both starved and nonstarved aphids were placed on a CMV-Fny infected source zucchini plant until they produced a single intracellular stylet puncture (pd). Then, aphids were removed and individually transferred to a healthy zucchini plant for a 24 h inoculation access period (IAP) to assess the CMV-Fny transmission rate.

RESULTS

No significant differences were observed on the transmission rates of most CMV isolates between starved and nonstarved aphids. Only for the V698 isolate did starved aphids transmit significantly better than nonstarved aphids. CMV- Fny was the isolate with the highest transmission rate by *A. gossypii* after an AAP of 5 min. Pre-acquisition starvation did show a significant effect on the probing behavior of both *A. gossypii.* Starved aphids began probing faster and made longer intracellular punctures than nonstarved aphids. However, there were no significant differences on the transmission rate of CMV-Fny isolate between starved and nonstarved *A. gossypii*.

CONCLUSIONS

Pre-acquisition starvation of *A. gossypii* does not increase the transmission rate of most of the CMV isolates tested. However, starved *A. gossypii* behaved in a way that is known to enhance the transmission of other NP viruses such as Potyviruses, but such changes in probing behaviour did not increase the transmission rate of CMV.

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APHID TRANSMISSION OF MULTIPLE STRAINS OF *POTATO VIRUS Y* ACQUIRED EITHER SEQUENTIALLY OR FROM MIXED INFECTIONS

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BACKGROUND and OBJECTIVES:

Aphid transmission may be a contributing factor to a recent shift in the predominant strains of *Potato virus Y* (PVY) affecting the U.S. potato crop. Surveys of U.S. seed potato fields over the past decade indicate the ordinary strain of PVY, PVY^{O}, is being replaced by recombinant strains and that a majority of seed fields harbor multiple PVY strains. Previous studies have suggested that the recombinant strains may be more efficiently transmitted and that the virus strains may differ in their ability to bind to or be released from the aphid acrostyle. Our objectives were to compare transmission efficiency when aphids were allowed either sequential acquisition access to two PVY strains or acquisition from plants infected with two strains.

MATERIALS and METHODS:

Individual green peach aphids (*Myzus persicae*, Sulzer) were allowed a 2-3 min acquisition access period on potato leaves infected with different combinations of the three strains, or a 2-3 min acquisition access period on a leaf infected with PVY^{O}, PVY^{NWi} or PVY^{NTN}, followed by another 2-3 min acquisition access period on a second potato leaf infected with a different PVY strain, Single aphids were then transferred to healthy potato seedlings for a 24 hr inoculation access period. All possible combinations of the three strains were tested. Strain-specific infection of the recipient plants was determined by DAS-ELISA and IC-RT-PCR 28 days post-inoculation.

RESULTS:

When aphids acquired PVY^{NTN} and PVY^{O} in either sequence, PVY^{NTN} was transmitted to a majority of the plants. PVY^{NWi} acquisition either prior to or after acquisition access on PVY^{O} or PVY^{NTN}, led to the transmission of PVY^{NWi} to more than 80% of the plants. Interestingly, when aphids acquired two strains of PVY from mixed infected plants, PVY^{O} was found to be the infecting virus in a majority of the recipient plants regardless of whether PVY^{NTN} or PVY^{NWi} was the other strain in the mixed infection.

CONCLUSIONS:

The data from the sequential transmission assays suggest that PVY^{NWi} and PVY^{NTN} may preferentially bind to the aphid acrostyle over PVY^{O} or they may be preferentially released during inoculation. Preferential transmission of PVY^{NTN} or PVY^{NWi} was dependent upon the isolate of PVY^{NTN} found in the mixed infection. Transmission from mixed infected plants indicates that PVY^{O} outcompetes the other strains if the viruses are acquired simultaneously and suggests that different mechanisms are at play depending on how viruses are acquired or inoculated. Based on all of our data, the contribution of aphid transmission to the shift in predominant PVY strains affecting the potato crop remains uncertain and it is unknown if differential transmission of PVY strains in these experiments was due to aphid-virus interactions in the stylet or virus strain competitions in the plant following aphid inoculation.

POTATO CULTIVAR AND VIRUS ISOLATE AFFECT THE DISEASE DYNAMICS OF MIXED-STRAIN INFECTIONS OF POTATO VIRUS Y

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BACKGROUND and OBJECTIVES

Infections of more than one strain of Potato virus Y are not uncommon in potato grown in the U.S. The disease dynamics in a mixed infection overtime may influence the virus available for aphids to acquire and spread to other plants and it may affect the number of daughter tubers infected with either or both strains. Our objective was to follow the spatial and temporal distribution of two PVY strains in systemically infected potato plants when both viruses are inoculated at the same time.

MATERIALS and METHODS

Two isolates of PVY^{O} (Oz, NY31) and two isolates of PVY^{NTN} (ME4, NY29) were mechanically inoculated in all possible mixtures to three potato cultivars, Pike, Goldrush and CalWhite. Relative virus titer was monitored at various leaf positions over a four week period and then again just prior to harvest to determine how quickly both viruses moved from the inoculated tissue and if they were detected at similar levels in various positions on the plant over time. Additionally, tubers were harvested from the plants. After suitable dormancy they were planted and the resulted stems tested to determine relative titers of both strains. Immuno-fluorescence confocal microscopy was used to monitor the distribution of both virus strains in leaf tissues and individual cells.

RESULTS

PVY infection of CalWhite plants resulted in very mild foliar symptoms regardless of infecting strain, but all four isolates reached similar titers when inoculated alone. Mixed infections of NY31 and ME4 resulted in both viruses reaching similar titers in all tissues, whereas NY31 and Oz completely dominated NY29. Titer of ME4 was more than twice that of Oz. Virus found in the daughter tubers reflected what was in the leaves of the mother plants. PVY^{O} infection of Goldrush resulted in severe mosaic symptoms; PVY^{NTN} infection induced noticeable mosaic. Both isolates of PVY^{O} were completely dominated by both isolates of PVY^{NTN} in all leaf tissues up to four weeks post inoculation. However by harvest, PVY^{O} titers were similar to PVY^{NTN} except in the Oz and NY29 mixture. Virus found in the daughter tubers reflected the relative virus titers in the leaves at harvest except from plants infected with NY31 and NY29. Virus titer in the leaves was similar for both strains at harvest, but NY31 was found alone in most of the tubers. PVY^{O} infection of Pike usually results in a systemic hypersensitive response manifested as vein necrosis and leaf drop. This was true for the Oz isolate, but not NY31, which induced mosaic symptoms as did infection by the two PVY^{NTN} isolates. Both NY29 and ME4 dominated the mixed infections with Oz, whereas relative titers of both NY29 and ME4 were similar to NY31 in the mixed infections. Virus found in daughter tubers reflected relative titers in leaves at harvest, except, similar to Goldrush, NY31 was found alone in tubers from plants infected with both NY31 and NY29. Immuno-fluorescence studies indicate that both PVY^{O} and PVY^{NTN} can co-infect cells in potato and tobacco and mixed infected cells dominate most of the infected cells in mature leaf tissues.

CONCLUSIONS

These studies indicate that potato cultivars as well as isolates within a virus strain can vary considerably in infection dynamics when two virus strains are competing. In this study both viruses were inoculated at the same time; it is likely that staggered inoculation times would have a more profound impact on virus titer and distribution in the plant and thereby influence virus acquisition by aphid vectors and also virus transmitted vertically to daughter tubers.

EVIDENCE FOR MAIN APPLE VIRUSES IN OLD EXTENSIVE ORCHARDS, GARDENS AND IN THE WILD GROWING TREES

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BACKGROUND AND OBJECTIVES

The extensively managed orchards and gardens with traditional apple cultivars sustained in their original regions and wild growing trees under natural selection should be less likely to suffer from significant pathogen infestations. Nevertheless, problems can still occur. The aim of the present study was to uncover the state of health of old apple orchards, gardens and of wildly growing trees, situated on the outskirts of the capital Prague, in the Czech Republic. Of the many viruses affecting apple trees, *Apple mosaic virus* (ApMV), *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV), and *Apple chlorotic leaf spot virus* (ACLSV) have been chosen for the purpose of our survey.

MATERIAL AND METHODS

Fully expanded leaves were randomly collected from 100 apple trees growing in two extensively managed gardens, ten orchards and from 51 wild-growing apple trees in 2015. The age of the trees was estimated approximately from 60 to 90 years. The virus presence was assessed out by qRT-PCR. Additionally, full/partial CP-coding gene sequences of tested viruses originating from the wild or cultivated apple populations were determined.

RESULTS

Altogether 151 apple trees were individually tested. In the case of orchards and gardens, 80% samples were infected with at least one monitored virus. ACLSV was detected in the highest number of tested trees (70%), followed by ASPV (62%), ASGV (47%) and ApMV (13%). Mixed infections were found in 69% tested trees. Of the total 51 wildly growing seedlings, 9.8% were infected with at least one monitored virus. ASPV was confirmed in the 7.8% trees, ASGV in 5.9% and ApMV in 2%. No ACLSV infection was detected. Mixed infections were found in 3.9% tested trees.

CONCLUSIONS

Acquired data clearly showed that the presence of monitored viruses is common in the region and are in accordance with previous studies devoted to the virus surveys in intensively kept orchards and gardens throughout the Czech Republic.
Since vegetative propagation of infected planting material is considered the main and mostly the only transmission pathway of all tested viruses, the evidence of ApMV, ASGV, ASPV and ACLSV in old trees could indicate their common and frequent presence in our region before the Second World War, thus in the time, when the orchards and gardens were established. However, since no information is available concerning the natural spread of apple viruses in the field without human endeavor, the question is how ApMV, ASGV and ASPV reached the seedlings. The most probable explanation of the phenomena could be an unidentified way of their transmission.

OCCURRENCE AND DISTRIBUTION OF IRIS YELLOW SPOT VIRUS ON ALLIUM SPECIES IN ZIMBABWE

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BACKGROUND and OBJECTIVES

Iris yellow spot virus, IYSV, (genus *Tospovirus*, family *Bunyaviridae*), an emerging pathogen of alliaceous crops, has been reported in many countries worldwide (Bag *et al*., 2015). The pathogen has recently been reported infecting garlic, leek and onion crops in Zimbabwe (Karavina *et al*., 2016a, b). This study was carried to determine the incidence, severity and distribution of IYSV disease in Zimbabwe.

MATERIALS and METHODS

Disease surveys were carried out in 18 districts representative of the country's five agro-ecological zones between May 2014 and November 2015. Symptomatic alliaceous leaf samples were tested for IYSV presence by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using a commercial kit supplied by Loewe® Biochemica GmBh (Sauerlach, Germany). The presence of IYSV was further confirmed by host indexing, transmission electron microscopy, and molecular analysis followed by partial sequencing of the nucleocapsid protein (N) gene.

RESULTS

The pathogen was detected in garlic (*Allium sativum*), leek (*A. ampeloprasum*), onion (*A. cepa*) and shallot (*A. aggregatum*) in all districts. IYSV disease incidences ranged from 15-85%, and alliaceous fields with high disease incidences also had high disease severities. Two types of IYSV-induced symptoms were observed namely, the irregularly-shaped, straw-colored necrotic lesions, and the diamond-shaped, necrotic lesions with chlorotic islands. Onion seedlings that were mechanically inoculated with sap from diseased plants produced typical IYSV symptoms. Electron microscopy studies revealed spherical enveloped particles, 80-120nm in diameter. Partial N gene sequence analysis of the Zimbabwean IYSV isolates showed they clustered together with isolates from other African countries, Spain, Italy, Australia, India and the USA. However, they clustered differently with isolates from The Netherlands, The UK, Iran, Serbia and Slovenia. Zimbabwean IYSV isolates were at least 94% identical to each other.

CONCLUSIONS

The study revealed the high IYSV incidence and severity in *Allium sp.* throughout Zimbabwe. Further studies to ascertain IYSV's economic impact and alternative hosts need to be carried out. This study lays the foundation to develop effective management strategies for IYSV in Zimbabwe.

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VIRUSES INVADING THE NEW ZEALAND FLORA

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BACKGROUND AND OBJECTIVES

Some of the 220 plant viruses introduced into New Zealand[1] (NZ) over the last 200 years have invaded the native flora. Half of the NZ flora is represented by two families: the Asteraceae and the Poaceae. Very little work has been done on the Asteraceae but, because of their economic importance, the grasses have been investigated. Plant viruses are not usually considered biological invaders in the same way as weeds, insects and vertebrate pests; we aim to investigate the characteristics of plant virus invaders.

MATERIAL AND METHODS

Known examples of viruses invading the NZ flora were analysed in an effort to understand invasion risk factors in an effort to avoid or mitigate further invasions of the flora.

RESULTS

Luteoviruses have invaded ≥12 native grass species in NZ[1]. They are common in nine introduced grasses and cereals. PAV viruses are having a significant effect on the growth and survival of some species. There is a correlation between the presence of weed grasses and infection of the natives, indicating that weed control could help protect from these incursions.
Cucumber mosaic virus (CMV) has been recorded in 93 hosts in NZ[1], five of which are natives. CMV infects NZ's only native cucurbit *Sicyos australis* and may be contributing to the decline of this rare and endangered species. CMV causes severe symptoms and decreases growth by over 60% in this herbaceous climber. CMV causes a mild mottle in the large woody vine *Clematis paniculata.* Infection is associated with a sudden decline in this usually long lived species. CMV infection has been reported from three other natives showing conspicuous symptoms but little else is known.
Cauliflower mosaic virus (CaMV) , *Turnip mosaic virus* (TuMV) and *Turnip yellows virus* (TuYV) cause yellowing and decline in NZ's rare and endangered coastal cresses (*Lepidium* spp).TuMV infects 26 species in NZ[1] including six native alpine and coastal species. TuYV infects 36 species including the coastal cresses and although CaMV has a narrow host range (10 brassicas) 36% of the *Lepidium* plants are infected.

CONCLUSION

Viruses with dispersive vectors, with many potential hosts in a single family, or with broad host ranges are causing problems in the native flora. All of the above viruses are transmitted by aphid species. The use of insecticides in conservation areas is undesirable however the expanded use of aphid parasitoids in agricultural systems will help reduce the spread and build-up of vectors in native species. Most of the outlined pathosystems include an association with one or more weed hosts, therefore removal of these introduced reservoirs is a potential management tool.

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DEFECTIVE RNAs DERIVED FROM THE GENOME OF *TOMATO BLACK RING VIRUS* INTERFERE WITH ITS REPLICATION

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BACKGROUND and OBJECTIVES

Tomato black ring virus (TBRV) causes diseases of economic importance in a wide range of cultivated annual, perennial and woody plants. The genome of TBRV consists of two single stranded RNAs of positive polarity. RNA1 is responsible for viral replication and polyproteins' processing and RNA2 for encapsidation and movement in plant (Le Gall et al. 1995). The TBRV infection can be accompanied by subviral particles such as satellite or defective RNAs. D-RNAs do not encode any proteins and their replication, encapsidation and spread depend on the parental virus. D-RNAs may interfere with helper virus accumulation and affect the symptoms produced on infected plants (defective interfering RNAs – DI-RNAs) (Graves et al. 1996). The goal of this study was to check whether additional, small RNA particles which arose from TBRV genome during prolonged passaging in *Chenopodium quinoa* plants, interfere with replication of parental virus.

MATERIAL and METHODS

In this study, two isolates of TBRV from tomato [with (TBRV+D-RNA) and without additional RNA particles (TBRV-D-RNA)] were analyzed. *Ch. quinoa* plants were infected with the equal amount of viral RNA and the symptoms and viral accumulation were monitored 7, 14, 21 and 28 dpi. The accumulation level of TBRV was measured by real time quantitative PCR using LightCycler® 96 (Roche) and statistical analyses were performed. Relative quantity of viral RNA in each sample was estimated by interpolating individual *Ct* values in the standard curve from three independent qPCR assays. The experiments were repeated twice.

RESULTS

The preliminary data showed that viral RNA replication and accumulation in systemically infected plants decreased in the presence of D-RNAs. Moreover, the plants infected with TBRV+D-RNAs showed only chlorotic spots whereas those infected with TBRV-D-RNA displayed severe chlorosis symptoms, leaves malformation and growth reduction.

CONCLUSIONS

Interference of D-RNAs with the replication of TBRV delays the progress of infection, reduces the virus load and symptoms.

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EXPLORING PLANT TOLERANCE TO VIRUSES AS A SUSTAINABLE MEANS OF DISEASE CONTROL

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BACKGROUND and OBJECTIVES

The use of resistant plant cultivars is an efficient, cost-effective and environmentally-friendly method of disease control, particularly against viral pathogens. However, resistance is subject to breakdown, through the often rapid adaptation of viral populations to newly deployed resistant genotypes.
As it exerts a weaker selection pressure on parasite populations, plant tolerance to parasites - the mechanisms that reduce the negative impact of infection on host fitness or yield, despite relatively elevated parasite concentrations - appears as an interesting alternative to resistance *sensu stricto* - mechanisms that reduce pathogen accumulation (1). However, the durability of tolerance has seldom been experimentally tested, and counter-examples exist (2). Moreover, little is known about the genetic determinants controlling tolerance to pathogens.
We have chosen the interaction between pepper and *Cucumber mosaic virus* (CMV) as a model to study plant tolerance to viral pathogens and evaluate the durability of tolerance.

MATERIAL and METHODS

We will exploit available pepper mapping populations in order to identify doubled haploid (DH) lines with contrasted levels of tolerance and resistance *s.s.* to a highly aggressive CMV isolate. Both virus titre and plant health will simultaneously be evaluated for each DH line. Virus accumulation will be quantified using serological methods (DAS-ELISA, Double Antibody Sandwich Enzyme Linked Immunosorbent Assay). The impact of infection on plant health will be measured using different methods, including the reduction in fresh weight of infected plants compared to mock-inoculated plants, and the AUDPC (Area Under the Disease Progress Curve) index, which combines time of symptom emergence and symptom intensity.
QTLs (Quantitative trait loci) governing symptom expression *versus* QTLs controlling virus accumulation will be mapped.
Tolerant DH lines will be exploited in experimental evolution assays, through serial virus inoculations, in order to evaluate the durability of this defense mechanism in controlled conditions.

RESULTS and CONCLUSIONS

We have recently initiated a screen using a pepper DH mapping population. Our preliminary screening efforts have allowed the identification of lines displaying different levels of resistance and tolerance to CMV. Screening of additional DH lines will allow us to map QTLs controlling tolerance or resistance *s.s*., and to compare the genetic architecture of these two defense mechanisms.
Further efforts will aim at confirming tolerance and resistance levels of a small subset of DH lines with contrasted levels of resistance and tolerance to CMV, and testing their response to a set of isolates representative of CMV diversity.
Experimental evolution assays should indicate whether tolerance is evolutionarily more stable than resistance *s.s.* and whether breeding tolerant crops may contribute to sustainable control of plant viruses.

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BANANA BRACT MOSAIC VIRUS AND CANNA YELLOW MOTTLE VIRUS FROM FLOWERING GINGER IN HAWAII

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BACKGROUND AND OBJECTIVES

Flowering ginger (*Alpinia purpurata*) is an important component of the diversified agriculture in Hawaii. Typical symptoms of viral infections were found on flowering ginger in Hawaii in 2010. The purposes of this study were to detect and to characterize the viruses from the symptomatic ginger plants in Hawaii.

MATERIAL AND METHOD

Double-stranded RNAs were isolated from symptomatic leaves and used in deep sequencing analyses. Viruses were characterized in bioassays and molecular analyses. Sensitive assays were developed for rapid and reliable detections of the viruses.

RESULTS

Banana bract mosaic virus (BBrMV) was detected from leaf samples of symptomatic ginger plants. Our preliminary study demonstrated that BBrMV could be transmitted from flowering ginger to its natural host banana, raising a serious concern for risk to the rapidly growing banana industry in Hawaii. To quickly monitor this virus in the field, we have developed a robust immunocapture reverse-transcription loop-mediated isothermal amplification (IC-RT-LAMP) assay. Deep sequencing of the BBrMV isolate from flowering ginger revealed the presence of a potyvirus with a single-stranded RNA genome of 9,713 nt potentially encoding a polyprotein of 3,124 aa, and another predicted protein (PIPO) in the +2 reading-frame shift. Deep sequencing also revealed *Canna yellow mottle virus* (CaYMV) infecting some symptomatic ginger plants. We have sequenced the CaYMV isolate using a combination of deep-sequencing and traditional Sanger sequencing techniques. The complete genome is 7,120 bp with an organization typical of *Badnavirus* species. A specific PCR assay was developed to detect CaYMV and to study its distributions in Hawaii.

CONCLUSIONS

BBrMV and CaYMV were detected from the symptomatic ginger plants. Many but not all the symptomatic plants were infected by both of these viruses. Complete genomes of BBrMV and CaYMV were determined using deep sequencing technology. BBrMV could be a potential problem for the banana industry in Hawaii. Phylogenetic analysis supports CaYMV as a distinct species within the genus *Badnavirus*, family *Caulimoviridae.* Sensitive LAMP and PCR assays were developed for detection of BBrMV and CaYMV, respectively.

MOLECULAR ANALYSIS OF THRIPS DIVERSITY AND THRIPS-BORNE *IRIS YELLOW SPOT VIRUS* (*TOSPOVIRUS*) FROM PAKISTAN

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BACKGROUND AND OBJECTIVES

Although thrips are globally important crop pests and vectors of destructive plant viruses, species identification is difficult because of their small size and inconspicuous morphological differences. Sequence variation in the mitochondrial COI-5' (DNA barcode) has proven effective for the identification of species in animals and groups of insect-pests. Thrips-transmitted *Iris yellow spot virus* (IYSV) is an economically important viral pathogen that causes severe losses of *Allium* crops worldwide. A survey to determine the incidence of IYSV was carried out in onion-growing regions of Pakistan.

RESULTS

We analyzed barcode sequence variation among 496 thrips from various plant hosts in north-central Pakistan. The Barcode Index Number (BIN) system assigned the 471 sequences to 55 BINs. The recursive partitions by Automatic Barcode Gap Discovery revealed the presence of 55 groups. Sequence analysis revealed that the intraspecific and interspecific distances ranged from 0.0% to 7.5% and 2.3% to 22.3%, respectively. The Neighbor-Joining clustering and Bayesian inference supported the presence of 56 monophyletic lineages while three major pest and vector species (*Haplothrips reuteri* (Black flower thrips), *Thrips palmi* (Melon thrips), *Thrips tabaci* (Onion thrips), and one predatory thrips (*Aeolothrips intermedius,* (Banded thrips) showed deep intraspecific divergences, strongly suggesting that each is a cryptic species complex. A comparison of our species with those previously reported from this region showed that one species (*Apterygothrips pellucidus* Ananthakrishnan) from Tubulifera and seven species (*Chaetanaphothrips orchidii* Moulton, *Chirothrips meridionalis* Bagnall, *Megalurothrips distalis* Karny, *M. usitatus* Bagnall, *Neohydatothrips samayunkur* Kudo, *Taeniothrips major, Thrips trehernei* Priesner) from Terebrantia and four genera (*Apterygothrips, Chaetanaphothrips*, *Neohydatothrips*, *Taeniothrips*) were the first reports from Pakistan. Plants with symptoms suggestive of IYSV infection were collected and tested for the presence of the virus by ELISA and RT-PCR. Sequence analysis of RT-PCR amplified nucleocapsid (N) gene confirmed IYSV infection of onion in Pakistan. Based on the *in silico* RFLP analysis of all known IYSV N gene sequences, global IYSV population could be grouped into two genotypes, $IYSV_{BR}$ and $IYSV_{NL}$ and the Pakistani IYSV isolate turned out to be the $IYSV_{NL}$ genotype.

CONCLUSION

The study compiles the first barcode reference library for the thrips of Pakistan and examined the global haplotype diversity in four important pest and virus-vector thrips species. RFLP approach was found to be a useful and relatively rapid method to determine the IYSV genotypes and for studying the movement and evolution of this important virus of onion.

A VIRULENT NEW *TURNIP MOSAIC VIRUS* (TuMV) STRAIN THAT BREAKS ALL TuMV RESISTANCES IN *BRASSICA NAPUS*

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BACKGROUND & OBJECTIVES

In 2012, a virulent new resistance-breaking strain of *Turnip mosaic virus* (TuMV) was detected causing symptoms of severe mosaic, leaf deformation and plant stunting in *Brassica napus* (canola) crops growing in the Liverpool Plains region of Northern New South Wales, Australia. In 2013, a survey of *B. napus* crops in this region found high incidences of this virulent strain. No resistance was found when plants of diverse *B. napus* germplasm were inoculated with it (1). However, TuMV resistance gene *TuRBJ01* withstood it in plants of *B. juncea* (Indian mustard) (3). It has not yet been pathotyped using standard *B. napus* differentials.

MATERIAL & METHODS

Virulent isolates 12.2 and 12.5, and Australian TuMV isolates in pathotypes 1 (NSW-2), 7 (NSW-1) and 8 (WA Ap1) were sap inoculated to plants 18 *B. napus* cultivars, and isolates 12.2, 12.5 and WA-Ap1 to plants of nine other *Brassicaceae* species. TuMV isolates 12.2, 12.5 and WA-Ap1 were sequenced by Illumina HighSeq 500, and their reads subjected to de novo assembly using a CLC genomics workbench to obtain complete genomes. The genomes obtained were compared to others from Genbank.

RESULTS

At least one of isolates NSW-1, NSW-2 and WA Ap-1 caused one or other of four different resistance phenotypes in each of the 18 *B. napus* cultivars inoculated. Isolates 12.2 and 12.5 always overcame these four resistances causing a severe systemic mosaic in the 18 cultivars. In contrast, isolates 12.2, 12.5 and WA-Ap-1 all induced resistance phenotypes in 4/9 other *Brassicaceae* species (*B. oleracea*, *Camelina sativa*, *Eruca sativa, Raphanus sativus*). The 12.1 and 12.5 isolate complete sequences were close to previously sequenced isolates NSW-1 to NSW-6 (2). Isolate WA-Ap-1 was closest to isolate WA-Ap.

CONCLUSIONS

All of the 18 *B. napus* cultivars carried at least one of four distinct strain specific TuMV resistances. Isolates 12.2 and 12.5 overcame all of them, but their resistance-breaking properties were ineffective against TuMV resistances present in *B. oleracea*, *C. sativa*, *E. sativa* and *R. sativus*. Phylogenetic analysis revealed that their sequences were similar to those of other TuMV isolates from the same region. There is widespread concern that this virulent TuMV strain will spread to *B. napus* crops in other Australian regions.

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FURTHER INFORMATION ON THE GENETIC STRUCTURE OF *PLUM POX VIRUS* STRAIN M

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BACKGROUND AND OBJECTIVES

Plum pox virus (PPV) shows high genetic diversity, as nine viral strains, M, D, EA, C, Rec, T, W, AM, CR, have been identified so far (3). PPV strain M (PPV-M) has been characterised as a threat to the Mediterranean stone fruit industry (1). A previous study on the genetic diversity of PPV-M described the existence of two phylogenetic clades formed by isolates of similar geographical origin, from either the Mediterranean countries or the Central and Eastern European countries, respectively (2). A large scale survey conducted during 2013-2015 indicated that PPV-M is the prevalent viral strain in Greece and also widespread throughout the country. The purpose of this study was to shed light on the phylogenetic relationships of the Greek PPV-M isolates with those originating from other European countries.

MATERIALS AND METHODS

Phylogenetic analysis was performed using a 5'-terminal fragment of the CP gene from 14 Greek isolates, which were sequenced in the course of this study, as well as 27 PPV-M isolates of European origin and isolates from C, D, EA, T and W strains, retrieved from the databases.

RESULTS

Two geographical groups (Ma, Mb) were formed, as previously described by Dallot et al (2011). The majority of Greek isolates clustered with PPV-M isolates of Mediterranean origin (Ma group). One appears to follow an intermediate evolutionary route, between the two geographical groups, while another isolate grouped with Central and Eastern European isolates (Mb group). The former Greek isolate also shares two out of the three point mutations in the N-terminal of the CP gene, described as conserved amongst Mb isolates by Dallot et al (2011).

CONCLUSIONS

This is only the second molecular study on the intrastrain genetic diversity of PPV-M. Sequencing of more isolates from Greece and abroad is needed to establish geographical differentiation as well as to trace the strain's evolutionary history.

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DEVELOPING INTEGRATED APPROACHES FOR PEST AND DISEASE CONTROL IN HORTICULTURAL FIELD CROPS (IAPAD)

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BACKGROUND and OBJECTIVES

Turnip yellows virus (TuYV) causes up to 180 million GB pounds worth of losses in arable and vegetable brassica crops in the UK each year. It was previously known as Beet western yellows virus (BWYV) and is spread by the peach-potato aphid (*Myzus persicae*). It is a phloem-limited, single stranded, +sense RNA virus. In white cabbage (*Brassica oleracea* L. var. *capitata*) TuYV infection results in an internal disorder known as tipburn[1] during storage, causing collapse or discoloration of tissue at the leaf margins within the head. The occurrence of tipburn often results in cabbage consignments being rejected by processors and going to waste. A range of control strategies for TuYV identified from earlier research are now being evaluated in the field with the aim of developing an integrated strategy for reducing TuYV infection, insecticide inputs and yield losses in vegetable brassicas.

MATERIALS and METHODS

Two multifactorial field experiments, located in Lincolnshire and Warwickshire, UK, were carried out during 2015. The field experiment in Lincolnshire relied on natural TuYV infection from neighbouring commercial crops and weeds, whereas the experiment in Warwickshire had sources of *M. persicae* and TuYV inoculum introduced to ensure high vector and virus pressure. The effectiveness of a range of control strategies to reduce TuYV infection was evaluated individually and synergistically. The different control strategies tested were, insecticide seed treatment, insecticide sprays applied following detection of TuYV in *M. persicae* caught in traps adjacent to experiments, delayed transplanting date and partial plant resistance. Plant infection with TuYV was monitored during the growing season and at harvest. Cabbage head yield was determined and tipburn will be assessed following storage. We also monitored the numbers of *M. persicae* caught in water traps adjacent to the crops and Rothamsted Insect Survey suction traps throughout the season and whether the trapped aphids were carrying TuYV. The insecticide-resistance status of trapped *M. persicae* was also determined.

RESULTS

Numbers of *M. persicae* caught in both water and suction traps between mid-June and mid-July were very high. A large proportion of *M. persicae* were carrying TuYV. At the mid-May transplanting date, low numbers of *M. persicae* were caught in traps, this coincided with yield differences relative to the later transplanted cabbage. Yield and virus titre differences were seen between the partially-resistant cultivar and the more susceptible cultivar. Insecticide seed treatment also had an effect on virus titre and cabbage head weight yield. Cabbage heads are currently stored and will be assessed for tipburn symptoms later this year.

CONCLUSIONS

We quantified the effectiveness of individual and combined components of an integrated control strategy against TuYV infection in field-grown white cabbage and established synergy between some treatments. In the longer term we are working on more extreme sources of resistance to TuYV in a range of *B. oleracea* types.

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PROSPECTS OF FUNGAL ENDOPHYTES FOR MANAGEMENT OF MAIZE LETHAL NECROSIS

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BACKGROUND AND OBJECTIVES

Maize lethal necrosis (MLN) is caused by a co-infection of maize by *Maize chlorotic mottle virus* (MCMV) and any of the cereal potyviruses such as *Sugarcane mosaic virus* (SCMV), or *Maize dwarf mosaic virus* (MDMV). With reported yield losses of upto 100%, MLN is a dire threat to food security in eastern Africa. Since MLN is new to Africa, there are no known effective management strategies against the disease. Therefore, the present study examined the potential of 10 selected fungal isolates to colonize maize plants and induce resistance against viruses (e.g. MCMV and SCMV) causing MLN.

MATERIAL AND METHODS

Maize seeds were inoculated with fungal endophytes by soaking the seeds in fungal inoculum for 5h. Inoculated seeds were sown in pots and evaluated for endophyte colonization at three phenological stages: leaf formation; stem elongation; and flowering stages. Maize plants colonized with the effective endophyte strains were further challenged by SCMV and MCMV at 21 days post-inoculation to assess their effects on disease incidence, and severity. The virus titers in the maize plants were assessed over time with ELISA.

RESULTS

Three isolates, *Trichoderma harzianum* (F2L4), *Trichoderma atroviridae* (F5S21) and *Hypocrea lixii* (F3ST1) colonized different sections of maize plants at different phenological stages. Confirmatory tests proved successful colonization of maize plants prior to challenge with MLN-inducing viruses. All plants inoculated with either MCMV or SCMV tested positive by PCR. Fungal endophytes in the inoculated maize plants reduced disease severity and titers of SCMV upto 1.36 and 2.72 folds respectively, compared to the controls. However, the fungal endophytes did not have an effect on disease incidence and severity in MCMV-infected plants.

CONCLUSIONS

This study demonstrates that seed treatment with fungal endophytes can induce resistance in maize plants against SCMV which can be deployed in integrated management of MLN. Further screening for endophyte strains that could be effective against MCMV is needed.

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VIRUS DISEASES OF CUCURBITS IN COTE D'IVOIRE IN LIGHT OF CLIMATE CHANGE

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BACKGROUND AND OBJECTIVES

Cucurbits crops have a high significance for farmers in Côte d'Ivoire because of their nutritional value and as a source of income. These crops are threatened by virus diseases which present a limiting factor for their production. Indeed, virus infected plants not only show severe symptoms on leaves but more so discoloration and distortion of fruits rendering vegetables unmarketable and as a consequence, farmers abandon the culture.
In West Africa despite a high prevalence of plant diseases, there is in general a lack of information about the causal agents and hence this survey study was initiated to identify viruses in cucurbit crops.

MATERIAL AND METHODS

Cross-sectional surveys in different regions of the country were conducted to estimate virus incidence and prevalence. Samples collection was conducted during the two seasons to evaluate virus prevalence
Using ELISA tests covering a range of the known cucurbit viruses were performed to detect three viruses. Identity of the viruses was confirmed by RT-PCR and sequencing.
Cucumber mosaic virus (CMV) and *zucchini yellow mosaic virus* (ZYMV) inoculation on young cucumber plants was conducted in temperature controlled chambers at 20°C, 25°C and 30°C, to simulate climate effects on virus infections, the development of symptoms and plant growth. Scoring based on symptoms developing was done following the disease severity formula used by, Hassan and Al-masri (1991).

RESULTS

A total of 757 cucurbits samples and weeds were collected. CMV, ZYMV and PRSV (*papaya ringspot virus)* were detected. In dry season the prevalence of CMV was 32% followed by ZYMV (27%) and PRSV (10%). In the rainy season, ZYMV was more prevalent (34%) followed by CMV (23%) and PRSV (3%). *Pepo aphid-borne yellows virus* was reported for the first time.
CMV-inoculated plants at 20°C had a severity score of 3 with mosaic symptoms and slow plant growth. CMV symptoms were milder at 25°C while at 30°C no apparent symptoms were found although virus infections were detected by ELISA. In contrast to CMV, ZYMV-infected plants kept at 20°C showed very severe symptoms with stunted plants and leaf blistering and distortion and reduction of the surface (score = 4). At 25°C only vein banding symptoms were observed on otherwise vigorously growing plants. In contrast, at 30 °C leaves of infected plants showed a marked reduction of leaf size and severe leaf distortion hence the most dramatic effects of virus infections.

CONCLUSIONS

The prevalence of the viruses was determined in dry and rainy seasons in Cote d'Ivoire.
Virus incidence was determined through an experimental study of temperature on CMV and ZYMV behavior.

IDENTIFICATION OF *GRAPEVINE RUPESTIRS STEM PITTING-ASSOCIATED VIRUS FROM* IRAN

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BACKGROUND and OBJECTIVES:

Vein necrosis (VN) is a latent virus-like disease in all European grapevine cultivars and also in most American rootstock species and hybrids. The necrotic reactions develop first in leaves at the base of shoots, then, as shoots grow, on the younger and upper leaves. Necrotic spots also appear at the adaxial side of leaf blades. Severe forms of VN induce necrosis of tendrils and dieback of green shoots, followed by an almost complete cessation of growth and, sometimes, death of the host plant. *Grapevine rupestris stem pitting-associated virus* (*GRSPaV)* was found to be consistently present in VN-infected vines and there is co-evolution between *GRSPaV* and its hosts. *GRSPaV* is a member of family *Betaflexiviridae*. The genome of *GRSPaV* consists of 8,725 nucleotides excluding a poly(A) tail. It contains six open reading frames (ORFs) in which ORF1 potentially encodes a polypeptide with a methyltransferase domain, a papain-like proteinase domain, a helicase domain, and a RNA-dependent RNA polymerase domain. Other coding regions are a triple gene block (ORF2-4), coat protein (ORF5) and a protein with unknown function (ORF6). In this study, we report an isolate of GRSPaV which is associated with grapevine vein necrosis from Iran and characterize phylogenetic relation of this isolate with other reported isolates.

MATERIAL and METHODS:

Samples of grapevine showing vein necrosis symptoms were collected from Zanjan, Iran. Total RNAs were extracted using a method described by Rouhani et al (1993). Then cDNA samples were synthesized using 1 microgram of the RNA samples and random hexamer primers. These cDNA samples were used as template to amplify a part of coat protein gene of GRSPaV using specific primers, GRSP-F and GRSP-R. The amplified products were sequenced by Macrogen (Korea). The obtained sequences were aligned with other sequences from Genbank and a phylogenetic tree was prepared using MEGA6 program.

RESULTS:

Vein necrosis symptoms were observed in grapevines in the northwest of Iran. These symptoms were similar to the symptoms reported in grapevines infected with *GRSPaV*. Using RT-PCR with *GRSPaV* specific primers, a DNA fragment (approximately 320 bp) was amplified from these samples. Analysis of the obtained sequences revealed that these plants were infected with GRSPaV. Phylogenetic analysis showed that this isolate is similar to a GRSPaV isolate from Spain, called E120-RSP.

CONCLUSION:

This is the first report of GRSPaV form grapevines showing vein necrosis from Iran. This isolate has the highest similarity (72 %) with a GRSPaV isolate from Spain.

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IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF PRUNUS NECROTIC RING SPOT VIRUS ISOLATED FROM NORTH WEST OF IRAN

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BACKGROUND and OBJECTIVES

Stone fruit trees are the most important crops grown in the Northwest of Iran. *Prunus necrotic ring spot virus* (PNRSV) is a member of *Bromoviridae* family in the *Ilarvirus* genus. The genome of this virus encompass three single strand RNAs with positive polarity. The aim of this study was to identify the PNRSV-infection in the stone fruit orchards in Northwest of Iran and analysis the amplified gene by phylogenetic programs**.**

MATERIAL and METHODS

Plant Samples were collected from stone fruit orchards in the Northwest of Iran. Extracted RNAs were subjected to reverse transcription polymerase chain reaction (RT-PCR) with a pair of universal primers to *Ilarvirus* genus. Then, RNAs of positive samples in the previous test were used to amplify the complete coat protein (CP) gene by a pair of specific primers. The obtained sequences from new isolates were compared with previously reported isolates around the world in GenBank sequence database. In addition, phylogenetic tree was generated with Maximum Parsimony (MP) method using MEGA6 program.

RESULT

Major symptoms such as leaf necrotic lesions were observed in the studied orchards. PNRSV-infection was revealed by amplification of a fragment of PNRSV-CP using RT-PCR. The full length of CP gene was amplified from 21 out of 65 tested samples. Comparing the new sequences in the GenBank showed a high similarity between new PNRSV isolates and some isolates from Iran, Poland and India. Phylogenetic tree showed that these new isolates were grouped together with PNRSV isolates from diverse geographical regions including Iran, Poland, Uruguay, China, Chile, Montenegro and USA.

CONCLUSION

To our knowledge this is the first report of PNRSV-infection in the main stone fruit growing region of Iran. There are genetic diversity between new PNRSV isolates and other reported isolates from around the world based on the CP sequence.

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PHYLOGENETIC ANALYSIS OF THE NEW ISOLATES OF *TOMATO SPOTTED WILT VIRUS* BASED ON THE NUCLEOCAPSID GENE FROM IRAN

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BACKGROUND and OBJECTIVES

Tomato spotted wilt virus (TSWV) is the type member of *Tospovirus* genus in the family *Bunyaviridae* that is transmitted by thrips. The genome consists of three linear single-strand RNA molecules denoted as small (S) RNA, medium (M) RNA and large (L) RNA. Our aim was to identify the TSWV isolates from tomato fields and phylogenetic analysis of the isolates.

MATERIAL and METHODS

Tomato leaf and fruit samples were collected from some tomato fields from Iran. Reverse Transcription-Polymerase Chain Reaction was carried out using a pair of specific primers (TSWVF/TSWVR) corresponding the complete N gene of TSWV. Amplified DNA fragments were sequenced and the new sequences were aligned with the sequences in the GenBank. A maximum parsimony phylogenetic tree was generated by MEGA6 program.

RESULTS

Necrotic spots were observed at young stages of tomato plants on the leaves and yellow ring spots on the fruits. A fragment with about 777 bp in length corresponding to the S genomic region was amplified from 7 out of 17 samples (41%). By optimization procedure, the best result was obtained when the annealing temperature was set to 52 °C. Result showed that the newly generated sequences correspond to Nucleocapsid gene of TSWV. The identities between the Iranian and other reported isolates were 95%-98%. Also, alignment of deduced amino acid sequences showed that the identities between the Iranian isolates and the other isolates were 98%-99%. In addition, the highest similarity was observed between Iranian isolates and two isolates from Italy (P202/WT) and Turkey (SC13).

CONCLUSIONS

TSWV is a widespread viral disease in tomato fields in North West and West regions in Iran. This is the first report of TSWV infection in tomato plants in the studied regions. Genetic variation between detected isolates and other isolates around the world based on CP gene was observed. The sequences of two new isolates have been submitted to the GenBank nucleotide sequence database and have been assigned the accession numbers KT899947 and KT899948 which are belonged to TOS 101 and TOS102.

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HOST RANGE AND GENETIC VARIATION OF *MAIZE CHLOROTIC MOTTLE VIRUS* AND *SUGARCANE MOSAIC VIRUS* IN KENYA

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BACKGROUND AND OBJECTIVES

In eastern Africa, maize lethal necrosis (MLN) is caused by a co-infection of maize with *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV) (Wangai et al. 2012).Maize lethal necrosis was first reported in 2011 in Bomet County, Kenya and has since spread to other parts of Kenya and eastern Africa (Wangai et al. 2012; Lukanda et al. 2014). In Kenya, reports indicate that the disease is caused by a dual infection of maize with MCMV and SCMV. Since the disease is new to Africa, there is little information on its epidemiology. Therefore, we aimed at determining the host range of SCMV and MCMV in Kenya.

MATERIALS AND METHODS

Leaf samples of cereals and grasses were collected from maize fields in Nyamira, Bomet, Vihiga, Makueni and Machakos counties. MCMV and SCMV were detected from the samples by DAS-ELISA and confirmed by RT-PCR and sequencing. The PCR products were sequenced in both directions, consensus sequences generated and compared with similar sequences from the GenBank.

RESULTS

A total of 16 sequences, accession numbers KT630791-KT630806 were submitted to the GenBank. Six grass species tested positive for MCMV and SCMV namely; velvet crabgrass (*Digitaria velutina*), couch grass (*Digitaria abyssinica*), star grass (*Cynodon dactylon*), kikuyu grass (*Pennisetum clandestinum*) and signal grass (*Brachiaria brizantha*). Nut grass (*Cyperus rotundus*) and Napier grass (*Pennisetum purpureum*) tested positive for MCMV alone. Sugarcane (*Saccharum officinarum L.*), finger millet (*Eleusine coracana*) and sorghum (*Sorghum bicolor*) tested positive for both MCMV and SCMV. The nucleotide sequences of MCMV isolates were 98-100% similar based on replicase and P7 genes and mostly related to the Kenya and Rwanda isolates (99-100%). SCMV isolates were 91.70 – 99.84% similar based on the CP gene and 3'UTR and were most related to the Rwanda isolate (95-99%). Based on the 3' UTR region, a deletion of 20 nucleotides was observed in the SCMV isolates as compared to isolates from USA and Germany.

CONCLUSIONS

Our results indicate that there is a wide host range of MCMV and SCMV that could be important in the epidemiology and management of MLN. The information on genetic variation of the two viruses is important in breeding and screening for resistance against MLN.

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MODELLING DIFFERENTIAL TRANSMISSION CHARACTERISTICS OF WHITEFLY-TRANSMITTED CASSAVA VIRUSES

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BACKGROUND and OBJECTIVES

The whitefly, *Bemisia tabaci*, is one of the most economically important insect vectors of plant viruses. It transmits over 200 viruses through either semi-persistent or persistent mechanisms. Cassava mosaic geminiviruses (CMGs) and cassava brown streak viruses (CBSVs) have been associated with region-wide spread of a dual pandemic of cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Although both diseases are spreading through East and Central Africa, their epidemiological characteristics differ greatly. We compared local transmission characteristics of both viruses using modelling techniques, to derive novel insights into factors driving these epidemics. Since CMGs are persistent viruses and CBSVs are semi-persistent, modelling results may be relevant to other crop systems where virus groups with similarly contrasting transmission characteristics occur.

MATERIALS and METHODS

Field data for model fitting were obtained from experimental trials planted with CMD-susceptible and CMD-resistant cassava varieties (both susceptible for CBSD) in Ukerere Island in north-western Tanzania and Namulonge, in south-central Uganda. Initially disease-free planting material of each variety was monitored for two periods of one year per site, with incidences of CBSD and CMD, and *B. tabaci* abundance recorded monthly.
A system of ordinary differential equations was formulated with mechanisms to illustrate differences between transmission effects of CMGs and CBSVs on cassava plants at field level. Plants were divided into healthy, latent and infectious compartments. Whitefly populations were assumed to feed only on cassava plants and were classified based on their infectious status: non-viruliferous, and viruliferous. Model parameter value ranges were estimated from literature. The field data sets were fitted against dynamic models using a standard parameter identifying scheme (nonlinear least-square regression) which minimizes the error between the model solution and the data.

RESULTS

Our simulations show that these models not only fit the field data well, with biologically meaningful parameter estimates, but additionally they also capture the differences between the two types of viral infections: CMGs and CBSVs. These data-validated models may be employed to simulate infection dynamics subject to various starting plant infection levels and whitefly abundance values.

CONCLUSIONS

The models generated here will be building blocks for models with larger spatial features at the regional level with multiple fields, which will ultimately serve as tools aiding management decisions.

THE ROLE OF RNA-DEPENDENT RNA POLYMERASE 1 GENES IN ANTIVIRAL DEFENSE IN *CUCUMIS*.

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BACKGROUND and OBJECTIVES

RNA-dependent RNA polymerase (RDR) is a gene family involved in development and defense against viruses. RDR1 and RDR6 play crucial roles as defense enzymes against viruses, by amplification of viral-dsRNA in the gene silencing pathway. RDR1 is regulated by a range of phytohormones such as salicylic acid. The resistance based on gene silencing can be effective against viruses correlated with increased RDR1 expression and the production of endogenous siRNA [1].

MATERIAL and METHODS

Cucumber (*Cucumis sativius*) and melon (*C. melo*) RDR1 genes were cloned and sequenced using Cucurbit Genomics Database (http://www.icugi.org/) and Melonomics (https://melonomics.net). Kinetic analysis of RDR1 gene expression and virus accumulation was determined by qPCR. Silencing of *RDR1c* was performed with ZYMV as VIGS vector.

RESULTS

In cucumber we identified four unique functional RDR1 genes; RDR1a, RDR1b, RDR1c1 and RDR1c2. RDR1a and RDR1b are expressed to high levels compared to a very low level of RDR1c1 and RDR1c2 expression in healthy plants. However, the gene expression levels of *RDR1*(a-c) were induced in cucumber plants infected with different viruses. The expression level of *RDR1b* increased 10- to 20-fold in a broadly virus resistant cucumber transgenic line that accumulates high levels of transgene small RNAs [2] and in several virus-resistant cultivars. The association of virus resistance and RDR1b gene expression is independent of salicylic acid levels. *RDR1c* was highly induced (25- to 1300-fold) in susceptible cultivars infected with RNA or DNA viruses, but only weakly by infection with *Pseudomonas syringae* or powdery mildew. In addition, silencing of RDR1c leads to increased virus accumulation which may indicate the antiviral function of these genes. However, in melon only RDR1a is expressed, while both RDR1b and RDR1c genes are truncated. The expression levels of RDR1a, RDR2 and RDR6 in melon do not increase following virus infection.

CONCLUSIONS

We showed that the cucumber RDR1 genes have different levels of inducible expression and may have different or overlapping functions, while in melon, susceptible to more viruses than cucumber, only one RDR1 gene was induced by virus infection and functioned to provide a lower level of protection. We assume that the RDR1b gene is a new virus resistance gene in cucumber. Understanding RDR1 gene regulation and antiviral functions may help with the development of broad-virus resistant plants.

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CASSAVA MOSAIC DISEASE IN MADAGASCAR: COMPLEX EPIDEMIOLOGY AND EVOLUTIONARY DYNAMICS OF CASSAVA MOSAIC GEMINIVIRUSES

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BACKGROUND and OBJECTIVES

Cassava is the staple food for hundreds of millions of people in Africa but its cultivation is seriously constrained by cassava mosaic disease (CMD) in Madagascar and in Africa in general. We have undertaken (1) to identify the cassava mosaic geminiviruses (CMGs) involved in CMD in Madagascar and their epidemiological characteristics from country-wide surveys, and (2) to study the genetic and spatial structure of Malagasy CMGs populations.

MATERIAL and METHODS

Altogether 708 cassava leaf samples were collected with and without CMD symptoms from the main cassava-growing areas of Madagascar during 2009 to 2011. Prevalence and symptom severity of CMD, origin of infection (whitefly or cutting), and abundance of whiteflies were assessed.

RESULTS

Molecular diagnosis of CMGs in Madagascar revealed an unprecedented diversity and co-occurrence of six CMGs. Distinct geographical distributions were observed for the six viruses. While EACMCV and SACMV were widespread, ACMV was more prevalent in the central highlands, and EACMV and EACMKV were prevalent in lowlands and coastal regions. PCR diagnosis revealed that mixed infection (up to four co-infected viruses) occurred in 21% of the samples and were associated with higher symptom severity scores. Pairwise comparisons of virus associations showed that EACMCV was found in mixed infections more often than expected while ACMV and SACMV were mostly found in single infections. Even if the whitefly vector *Bemisia tabaci* was more abundant in lowland and coastal areas, infected cuttings remain the primary source of CMD propagation (95%) in Madagascar.
SACMV and ACMV, the two most prevalent viruses, displayed low degrees of genetic diversity and have most likely been introduced to the island only once. By contrast, EACMV-like CMG populations (EACMV, EAMCKV, EACMCV and complex recombinants of these) were more diverse, more spatially structured, and displayed evidence of at least three independent introductions from mainland Africa.

CONCLUSIONS

Our study highlights both the complexity of CMD in Madagascar, and the distinct evolutionary and spatial dynamics of the different viral species that collectively are associated with this disease.

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A COMPARISON OF COMPLETE CUCURBIT POTYVIRUS GENOMES FROM EAST TIMOR AND NORTHERN AUSTRALIA

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BACKGROUND AND OBJECTIVES

Viromics were used to investigate connectivity between virus populations infecting crops in northern Australia and East Timor, and test the hypothesis that viruses are arriving in northern Australia via wind-borne insect vectors coming from neighbouring countries.

MATERIAL AND METHODS

In 2015, samples from East Timorese cucurbit plants with virus-like symptoms were blotted onto FTA cards and sent to Australia. Similar samples from cucurbit plants in Broome in north-west Australia were air freighted to Perth. Four cucurbit virus isolates were obtained from Australia's Northern Territory (NT). With Australian samples, virus-like symptoms associated with sampled plants and virus symptoms in indicator hosts inoculated with their sap were recorded and ELISA used for virus identification. East Timorese samples extracts from FTA cards, and extracts from Australian samples were subjected to RT-PCR. RNA extracts were used to prepare libraries with Ribozero™ Plant chemistry. These were sequenced by Illumina HiSeq 2500. The RNA-seq reads obtained were subjected to de novo assembly using a CLC Genomics Workbench.

RESULTS

Indicator host, ELISA and RT-PCR tests found *Zucchini yellow mosaic* (ZYMV) and *Papaya ringspot virus* (PRSV) infecting crops in East Timor and Broome. The complete genomes obtained were: three (ZYMV) and five (PRSV) for East Timor, seven of each for Broome, and two of each for NT. These constitute the first complete cucurbit potyvirus genomes from East Timor and Australia. For south-east Asia, the only complete cucurbit potyvirus genome available was one of ZYMV from Singapore. On phylogenetic analysis, ZYMV genomes from East Timor, Broome and NT all grouped separately, but those from within each location grouped together. The Singapore genome grouped with the East Timorese genomes. The PRSV genomes from East Timor grouped separately, but those from Broome and NT grouped together.

CONCLUSIONS

When previously obtained coat protein (CP) sequences (1) were compared with new CP sequences from this study, the findings with ZYMV and PRSV agreed with those for whole genomes. In addition, new ZYMV CP sequences from NT grouped with others from NT, while East Timorese CP sequences were closest to previous sequences from Kununurra in north-west Australia.

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ARTICHOKE YELLOW RINGSPOT VIRUS IS ASSOCIATED WITH A NEW LETTUCE DISEASE IN SOUTHERN GREECE

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BACKGROUND and OBJECTIVES

In 2015, lettuce plants showing yellow ringspots and line patterns were observed in Northeastern Peloponnese. The symptoms resembled those caused by nepoviruses and especially by *Artichoke yellow ringspot virus* (AYRSV) that is known to infect artichoke and onion crops, in this area. Testing some symptomatic samples by a specific RT-PCR (Maliogka et al., 2006) confirmed the presence of AYRSV. The aim of this work was to further characterize the AYRSV isolate identified and investigate its association with the observed disease.

MATERIAL and METHODS

Mechanical inoculations were initially performed onto a number of test plants using leaf extracts from diseased lettuce samples. In order to retrieve the sequence of AYRSV and to verify whether other viruses were also present in the diseased plants, one lettuce sample showing characteristic symptoms was analyzed using next-generation sequencing (NGS) of siRNAs. Additionally, a large number of symptomatic and asymptomatic lettuce samples collected from the same fields were analyzed by RT-PCR for the presence of AYRSV. Finally, nearby crops and weeds were also surveyed for AYRSV.

RESULTS

The test plants showed symptoms similar to those caused by AYRSV and its presence was confirmed by RT-PCR. Also, NGS analysis of siRNAs extracted from a symptomatic lettuce revealed infection only by AYRSV. Large fragments from both RNAs of the virus were obtained and sequence comparisons of the RdRp gene showed highest similarity with the AYRSV isolate from onion (98%) but lower with the isolates from artichoke and *Vicia faba* (91%). AYRSV was detected in all symptomatic lettuce samples, but not in the apparently healthy ones. Finally, the virus was also detected in a number of cultivated plant species and weeds.

CONCLUSIONS

In this study a new isolate of AYRSV was associated for the first time with a lettuce disease, thus increasing the known natural host range of the virus. Additionally, our findings indicate that AYRSV has become endemic in this area. AYRSV presence in a number of cultivated and wild plant species strongly indicates the involvement of a vector, a question demanding future research.

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BEGOMOVIRUS DISEASE COMPLEX: EMERGING THREAT TO ORNAMENTAL PRODUCTION SYSTEMS OF INDIA

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BACKGROUND and OBJECTIVES

Ornamental crops play a major role in the livelihoods of the rural poor in India. Among major constraints to ornamental production are diseases caused by a group of viruses belonging to the genus *Begomovirus*, family *Geminiviridae*, which are transmitted by the whitefly vector *Bemisia tabaci*. In this study *Begomoviruses* diversity infecting ornamental crops in India was checked with the aim of developing RNAi mediated resistance against potential *Begomoviruses*.

MATERIAL and METHODS

During 2010 – 2015, leaves showing typical *Begomovirus* symptoms were collected from various parts of India, which was subsequently identified, cloned, sequenced and Southern Hybridized. Infectivity assay was carried out through, *Agrobacterium* mediated gene transfer (in *Lycopersicon esculentum* and *Nicotiana benthamiana*), Seed transmission test and White fly transmission studies. RNAi mediated studies was carried out against number of *Begomoviruses*, e.g. for the plant expression plasmid containing ihpRNA targeted to βC1 gene (HQ631430) was constructed and cloned in sense and anti-sense orientation with short intron. To monitor silencing, down-regulation of GUS protein expression was followed using binary pBin121-GUS construct. This plasmid was also used to make pBin121GUS-βC1 construct. For this, βC1 gene ligated downstream to GUS gene in the *SacI–PstI* site of pBin121-GUS [1].

RESULTS

76 *Begomoviruses* and its associated satellites were found infecting number of Ornamental crops such as *Alternanthera sessilis*, *Bougainvillea peruviana*, *Calendulla officinalis*, *Chrysanthemum indicum*, *Clerodendrum inerme*, *Duranta erecta*, *Hibiscus rosa-sinensis*, *Jasminum sambac*, *Justicia adhatoda*, *Lantana camara*, *Melia azedarach*, *Millingtonia hortensis*, *Mimosa pudica*, *Ocimum sanctum Tagetes patula*, *Rosa sp.*, *Tecoma stans*, *Vinca alba*, *Vinca minor* and *Vinca rosea*. The *Begomoviruses* revealed diversity among themselves and showed identity with *Begomoviruses* reported from South East Asia, Central Asia and East Africa. For the silencing effect, the *N. benthamiana*, leaves were first agroinfilterated with pCAMBIARNAiβC1 harboring *Agrobacterium* and incubated at 25 ^{o}C for 76 h followed by infiltration of the pBin121-GUS-βC1. The results showed the complete shutdown of GUS expression after the 24 h infiltration due to its fusion to the βC1, confirmed by infiltrating pCAMBIARNAiβC1 followed by pBin121-GUS. GUS expression was also observed in infiltrated leaf. However, GUS-expression was not detected when infiltrated alone with pCAMBIARNAiβC1.

CONCLUSIONS

The diversity of *Begomovirus* across the sub-continent is higher than previously realized and is higher than for any other ornamental plant species so far analyzed. Ornamental plants serve as alternate hosts for these viruses in the absence of the main crops. There is a need for a more comprehensive study to identify possible further *Begomoviruses* infection in the country. Producing siRNA in *N. benthamiana* plants may be significantly important for producing antiviral resistance. Results of these techniques effectively applied for disease management, crop protection and development of quarantine strategies at state and national level in India. This will form the basis of our future investigations.

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DEVELOPMENT AND VALIDATION OF A MULTIPLEX IC-PCR PROTOCOL FOR THE SIMULTANEOUS DETECTION OF FOUR BANANA STREAK VIRUS SPECIES

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BACKGROUND and OBJECTIVES

Banana Streak virus (BSV) is currently considered a major constraint to banana improvement and poses a threat to *Musa* production worldwide. On *M. balbisiana* (including plantain) the epidemiology of BSV is correlated with the presence of BSV sequences integrated into banana genome. It is necessary to develop and use BSV detection strategies that are both reliable and sensitive. Four specific IC-PCR protocols are presently used in official French analyses, one for each BSV species, with an antiserum only available by research (Le Provost, Iskra-Caruana et al. 2006).

MATERIALS and METHODS

In this study, we first compared 8 different antisera (commercial and research). We then optimized an Australian IC PCR multiplex protocol (Geering) with commercial antisera capable of detecting four BSV species simultaneously at a time. Secondly, we validated this protocol using a range of the four BSV species comprising different varieties and geographic locations in different countries. We used 39 infected samples, 18 non-infected samples and 8 infected samples at 5 levels of dilution. The method was assessed following the European standard EN ISO 16140 and the EPPO standard PM7/98. We thus characterized the analytical specificity (inclusivity and exclusivity), the analytical sensitivity and the repeatability, and compared them with the simplex-specific IC-PCR.

RESULTS

We obtained the best result with a commercial serum used for the validation. We found the same level of performance with either multiplex or simplex IC-PCR; analytical specificity and repeatability were maximal. Applying a multiplex protocol did not change the analytical sensitivity (1/100). This technique reduces the cost of the analyses four-fold and also the time for producing an official analysis report.

CONCLUSIONS

In conclusion, our results show that multiplex IC-PCR remains a highly reliable method for routine detection of BSV and is both faster and cheaper. It will be delegated to approved laboratories once published as French official method.

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DISCOVERY OF VIRUSES IN THE SOYBEAN APHID BY DEEP SEQUENCING

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BACKGROUND and OBJECTIVES

Soybean aphid (*Aphis glycines*) is one of the most economically important insect pests of soybean. Unknown in the USA before 2000, populations subsequently exploded across the Midwest of the USA. The arrival of the soybean aphid led to widespread application of chemical insecticides ranging from seed treatment to aerial crop dusting, to the potential detriment of honey bees (Dolezal *et al.*, 2016). Moreover, soybean aphid is the main vector of most soybean viruses, placing crops at risk of disease. As no viral pathogens of soybean aphid have been described, we sought to identify viruses that infect or associate with this pest, with potential for use as non-chemical, biological control agents. Viral sequences derived from soybean aphid were identified using Illumina deep sequencing (Liu *et al.,* 2011).

MATERIALS and METHODS

Three live colonies established with soybean aphids collected from Michigan, Ohio and Iowa (USA) were maintained on soybean plants in growth chambers at Iowa State University, prior to virus extraction. Dead aphids collected in China and sent to Iowa at ambient temperature were also analyzed. Virions were partially purified by sucrose gradient ultracentrifugation, RNA extracted and amplified without poly(A) selection. Sequencing (100 bp paired-end) was conducted on an Illumina HiSeq 2000. Sequences were trimmed and assembled into contigs using CLC Genomics Workbench 6.0. After iterations of querying the NCBI database by BLASTn and BLASTx, and contig assembly selecting for viral sequences, the largest overlapping contigs were assembled into near full-length viral genomes. Genomes were aligned and phylogenetic trees generated using MEGA 6.0.

RESULTS

Low numbers of reads of fragments derived from a wide range of plant, insect, and vertebrate virus genomes were found. Large numbers of reads derived from six viruses were obtained, allowing for assembly of near-complete viral genomes. The highest number of reads (hundreds of thousands to millions) corresponded to Aphid lethal paralysis-like viruses (Liu *et al.,* 2014) and *Rhopalosiphum padi* virus, both of which are dicistroviruses known to infect other aphid species. We also discovered (i) a tetravirus-like virus in the Michigan, Ohio and Iowa aphids, (ii) a dicistrovirus similar to partial genome sequences associated with honey bees that were called Big Sioux River virus, (iii) a virus distantly related to ciliviruses of plants and Negeviruses of insects, and (iv) Cotton leafroll dwarf virus (CLRDV), a polerovirus found only in the aphids from China.

CONCLUSIONS

Dicistroviruses related to ALPV and RhPV are abundant and likely replicating in soybean aphid based on the high number of reads. Also abundant is a new tetravirus-like virus that we find in all living soybean aphid collections. The cilivirus-like virus is highly divergent from known viruses and whether it infects plants or insects remains to be determined. The presence of CLRDV sequences may indicate that soybean aphid is a vector for this polerovirus. However soybean aphid is not known to colonize cotton, nor is CLRDV known to infect soybean. CLRDV has not been reported previously in China (M. Sharman, personal communication). While deep sequencing is a rapid and powerful method for virus sequence discovery, confirmation of the presence of infectious virions and determination of host for viruses identified is required. .

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THE RESISTANCE TO VIRUS TRIGGERED BY APHID INOCULATION IN *VAT* MELON IS NOT SYSTEMIC.

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BACKGROUND and OBJECTIVES

Host plant resistance is an essential mean of controlling virus epidemics in crops. Resistance to viruses in plant belongs to three major families, either recessive or dominant resistance genes, as well as the antiviral defense system based on RNA silencing. Most NBS-LRR antiviral resistances are triggered by the recognition of the NBS-LRR protein and a virus protein, playing the role of avirulence factor. The *Vat* resistance gene in melon is unique among the dominant resistance to virus genes. It is a CC-NBS-LRR gene, it is triggered by the recognition between an aphid avirulence factor, delivered in plant cells by *Aphis gossypii* puncturing, and the CC-NBS-LRR protein produced by Vat plants. This resistance is efficient against unrelated viruses transmitted on the non persistent mode (Boualem et al., 2016). We investigated if the resistance to virus triggered by *A. gossypii* in *Vat* plants is systemic.

MATERIAL and METHODS

Two batches of *Vat* plants were prepared, one pre-inoculated with CMV (*Cucumber Mosaic Virus)* by *A. gossypii* NM1 clone, the other one without pre-inoculation. The *A. gossypii* clone NM1, is known to be highly efficient in triggering virus resistance on *Vat* plants (Boissot et al., 2016). 1h30, 12h, 24h after the pre-inoculation with NM1, the two batches were inoculated with CMV either mechanically or using *Myzus persicae* as vector. Pre-inoculated plants were inoculated on the same leaf than for the pre-inoculation with NM1. Plantlets with and without symptoms were recorded 2 weeks later.

RESULTS

All plantlets only pre-inoculated with CMV by NM1 aphids were symptomless. All plantlets mechanically inoculated with CMV, both pre-inoculated and not, exhibited symptoms. More than 80% of the plantlets inoculated using *M. persicae* as vector exhibited symptoms without significant difference between pre-inoculated or not pre-inoculated plantlets. The results showed that a pre-inoculation with CMV inoculated by *A. gossypii* NM1 clone did not protect the *Vat* plants against viruses inoculated 1h30, 12h, 24h later, mechanically or using *M. persicae*.

CONCLUSIONS

The resistance to virus triggered by *A. gossypii* inoculation in *Vat* melon is not systemic at the leaf level. This results reinforced the hypothesis that *Vat* plant responses triggered by *A. gossypii* aphids puncturing, block the viruses in the inoculated cell or the neighboring cells (Sarria Villada et al., 2009).

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MOLECULAR CHARACTERIZATION OF BANANA BUNCHY TOP VIRUS REVEALS WIDESPREAD LOW GENETIC VARIATION AMONG ISOLATES IN DEMOCRATIC REPUBLIC OF CONGO

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BACKGROUND and OBJECTIVES

Banana bunchy top virus (BBTV), is the most devastating and widespread banana virus. Banana and plantain (*Musa* spp.) are major crops in terms of household income and food security in Democratic republic of Congo (DRC) (1). Despite the large territory under banana and plantain cultivation in the country, the genetic characterization of BBTV isolates has been undertaken for two provinces. The main objectives of this work were to understand the genetic variability of DRC BBTV isolates and to determine their origin.

MATERIALS and METHODS

In this study, genetic variation in BBTV was assessed from 52 BBTV isolates collected in five out of 11 provinces in DRC (Bandundu, Bas-Congo, Katanga, Kinshasa and Kasai Oriental). 52 full sequences of DNA-R and 30 full sequences of all BBTV components were analysed.

RESULTS

Full genome sequencing of DNA-R components was performed revealing a low genetic variation (98-100% nucleotide identity) amongst the BBTV isolates detected in seven of 11 DRC provinces. The phylogenetic tree showed that all DRC isolates gather a unique clade in the South Pacific group of BBTV. Based on the coding region for replication initiator protein, haplotype diversity was estimated to be 0.944±0.013, with 30 different haplotypes from 68 isolates in DRC. In addition, fives isolates were selected from each province for total genome sequencing, confirming low genetic variation among isolates from seven provinces (97-100% nucleotide identity).

CONCLUSION

This study strengthens the hypothesis of a single BBTV introduction some time ago, followed by the spread of the virus in the country. Analysis of the geographical dispersion reveals specific haplotypes groups according to the different agro-environmental conditions.

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STUDY ON THE ORIGIN OF CACAO SWOLLEN SHOOT VIRUS AND ITS DISPERSAL ON CACAO TREES IN WEST AFRICA.

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BACKGROUND and OBJECTIVES

Cacao swollen shoot virus (CSSV) is a member of the family Caulimoviridae, genus Badnavirus naturally transmitted to Theobroma cacao by several mealybug species. Typical symptoms of the disease on cocoa trees are red vein banding of young leaves, mosaic on older leaves and swelling of the orthotropic shoots. The virus, restricted to West Africa whereas the cacao tree originates from the Western Hemisphere, could therefore most probably have an indigenous origin on the West African subcontinent. The disease has caused enormous economic damage in Ghana since the1930s but was only restricted to small areas in Togo and Côte d'Ivoire until recently. Now, renewed outbreaks in the main producing areas in Côte d'Ivoire, Ghana and Togo cause serious problems. The knowledge of the viral biodiversity in the different outbreaks will in turn help to provide a better understanding of the development of the epidemics, and of the evolution of viral populations and may permit to retrace the emergence and dispersal of CSSV.

MATERIAL and METHODS

Prospections were made over several successive years in Ivory Coast, Togo and Ghana. Virus variability was studied by PCR amplification with CSSV primers, direct sequencing, sequence alignment and phylogenetic studies.

RESULTS

CSSV diversity is genetically structured in twelve groups according to the diversity in the first part of ORF3 and the 20% threshold of nucleotide divergence. However, according to ICTV recommendations which consider the nucleotide diversity in the RTAse region, we could describe at least seven different species.

CONCLUSIONS

The high variability observed within CSSV populations compared to its very short evolutionary history on cocoa trees, suggests the existence of many emergences from native hosts to cacao trees in the various countries of West Africa. Moreover, based on the geographical dispersal of the different species, we could propose the existence at different times of parallel emergences in each of the West African countries.

NEXT GENERATION SEQUENCING REVEALS OCCURRENCE OF SEVERAL VIRUSES IN COMMON BEAN PLANTS GROWN BY THE SMALL SCALE FARMERS IN TANZANIA.

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BACKGROUND AND OBJECTIVES

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume in developing countries. In Africa and America, for example, it is estimated that common beans provide about 15% of total daily calories and 36% of total daily protein (Todorović *et al*., 2008). It is therefore not only grown for cash but also plays a nutritional role. Common beans may be intercropped or grown in rotation with non-leguminous crops such as maize and potato thereby playing role in improvement of soil fertility. Tanzania is among the top ten major producers in sub-Saharan Africa but the average yields of common beans in smallholders' fields there is generally low (<500 kg/ha). Apart from abiotic and improper agronomic practices, low yields are caused by fungal, bacterial and viral diseases. Diseases of common beans caused by viruses are poorly studied in Tanzania mainly due to lack of diagnostic and detection tools. Thus a survey to document symptoms and collect samples for detection of viruses was carried out in 2015 in four agro-ecological zones.

MATERIALS AND METHODS

Samples were collected from four agro-ecological zones in Tanzania. Total RNA was extracted using the CTAB method with modifications. We then employed next generation (deep sequencing of small RNAs) and Sanger sequencing techniques to detect and characterize viruses, which infect common bean in Tanzania. The *denovo* assembly of small RNAs were achieved using a velvet assembler programs.

RESULTS

In a survey we observed and recorded symptoms including mosaic, vein banding, rugosity, leaf curl and leaf malformation. NGS has revealed that viruses infecting common beans in Tanzania belong to at least four families, namely *Potyviridae*, *Bromoviridae*, *Tombusviridae* and *Endornaviridae*. We note further that at molecular level, isolates of *Bean common mosaic virus* (BCMV) are diverse while a single strain of BCMNV is detected in all agro-ecological zones.

CONCLUSION

We have found that viruses are distributed in all zones where they cause devastative disease symptoms such as leaf curl, mosaic, rugosity and malformation. Viruses from many families are infecting beans in each zone and this has implication on breeding programs for management of viral diseases in Tanzania. Further challenge on breeding for resistance may be posed by the genetic diversity of BCMV.

We acknowledge financial support from the Bill and Melinda Gates Foundation.

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DETECTION OF TOSPOVIRUS IN SOME PLANTS AND ITS IMPLICATION IN HOST RESPONSE

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BACKGROUND AND OBJECTIVE

Viral diseases in plants have become a serious concern across the globe and curtailing these diseases through biological means is of paramount importance.An attempt has been made to identify viral agent causing diseases in some vegetable crops and response of plants to its infection.

MATERIAL AND METHODS

- Plant species screened for Tospovirus infection are *Solanum melongena*, *Solanum lycopersicum*, *Vigna unguiculata*, *Vigna radiate* and *Vigna mungo* from the local agricultural fields.
- Isolated viruses were mechanically infected and maintained in *Vigna unguiculata.*
- Virus identification was performed with tospovirus specific TAS ELISA Kit (AS-0118-0226/1) – DSMZ, Germany with PC-1109 (*Nicotiana benthamiana)* as positive control.
- The protocols developed by Rio *et al.* (2010) and Renukadevi *et al.* (2016) were used for RNA extraction and PCR amplification of L gene of Tospovirus and sequenced with standard protocol.
- Antioxidant assay (Szabo *et al*. 2007), Total phenolic estimation (Singleton *et al.* 1965) and Protein Estimation (Bradford 1976).

RESULTS

Five virus infected plants were collected from the agricultural field and screed for presence of tospoviruses using the TAS ELISA technique. *S. melongena* showed the maximum presence of nuclear viral capsid proteins (178.4%) followed by *V. unguiculata* (110.4%) V. mungo (106.4%) *V. radiata* (32%), when compared to the positive DSMZ control. The presence of tospovirus was further confirmed by PCR amplification using primers specific for the viral capsid gene. All the infected plants showed the presence of tospovirus. Additionally, the biochemical response of the tospovirus infected plants was evaluated by measuring the antioxidant activity, total phenolic and total protein contents. The infected plants showed increased concentration of total phenolic compounds however the highest increase (215%) had occurred in *S. melongena*. Similarly, there was an increase in protein content in all the plants analyzed however relatively higher (48%) total protein content was observed in *S. lycopersicum* when compared to other plant species. In contrast, there was a decrease in the content of chlorophyll and antioxidant activity in all the virus infected plants. The maximum (30.72%) and minimum (14.65%) decrease in chlorophyll content occurred in *S. melongena* and *S. lycopersicum* respectively. All the infected plants showed decrease in antioxidant activity between 8% to 10%. The results obtained will be presented and discussed in detail during the conference.

CONCLUSION

The presence of tospovirus in vegetable and crop plants has been ascertained with ELISA and virus specific gene amplification. Changes in biochemical response of the plants in terms of total phenolic content, total protein and antioxidant activity has been observed.

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QUANTIFYING RESISTANCE, TOLERANCE AND SUSCEPTIBILITY FOLLOWING DUO INFECTION OF CASSAVA BROWN STREAK VIRUSES

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BACKGROUND and OBJECTIVES

Cassava brown streak disease (CBSD) caused by *Cassava brown streak virus* (CBSV) and the *Ugandan cassava brown streak virus* (UCBSV) is among the threats to global food security. In Sub-Saharan Africa, the disease persistently reduces the overall root quality resulting in up to 100% yield losses in susceptible varieties. The disease also reduces stem quality limiting the seed systems. Complexities observed in CBSD symptom expression, has showed that symptom expression alone is not reliable in evaluating CBSD resistance, necessitating the need to develop more robust and durable methods of controlling these viruses.

MATERIALS and METHODS

This study quantifies the response of 41 cassava varieties according to CBSD symptom expression and relative CBSV and UCBSV load in roots and foliar under field trial in Uganda. Symptom expression scores and sampling was done at three, six, nine and twelve months after planting. Relative viral load was measured using quantitative RT-PCR using COX as an internal housekeeping gene.

RESULTS

A complicated situation was revealed with indications of variability in symptom expression. Six response categories were defined. Symptom expression was not always positively correlated with virus load. Considerably, many genotypes depicted a difference in the type and level of virus. This suggested difference in mechanisms of resistance, either resistance to one virus species or the other, or some form of interaction between virus species.

CONCLUSIONS

An extensive amount of research still needs to be undertaken to fully understand the genetic bases of resistance. This information will be useful in informing breeding strategies and restricting virus spread.

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SAMPLE COLLECTION METHODS FOR POTATO VIRUS Y (PVY) EPIDEMIOLOGY SURVEYS IN KENYA

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BACKGROUND AND OBJECTIVES:

Potato virus Y (PVY) causes major yield and crop quality losses in the potato growing regions in Kenya. Use of resistant cultivars to the main PVY strains is considered the most sustainable and long term means of managing the disease. Starting cultivar resistance screening for PVY requires field surveys in the potato growing areas to understand occurrence, distribution and relative importance of the different PVY strains. Unfortunately, distance and remoteness of the potato growing regions often compromise quality of plant tissue samples for reliable serological and molecular detection especially if samples do not reach the laboratories in time. This work aims to identify sustainable and reliable alternatives for sample collection for PVY epidemiological surveys.

MATERIALS AND METHODS:

Tobacco leaf samples infected with each of the four PVY strains (PVY^{N}, PVY^{O}, PVY^{NTN} and PVY^{Wi}) were applied to two types of filter paper cards (Whatman's FTA® and ordinary filter paper-OFP). Two mm discs punched from the cards were incubated overnight in SEB and dilutions of the eluate were tested for PVY in DAS-ELISA, dot blot and in RT-PCR in comparison with equal amount of leaf sap and also tested for infectivity by bioassay on *N. tabacum* cultivar White Burley.

RESULTS:

The four PVY strains were successfully detected on PVY infected samples collected using FTA® and OFP paper in DAS-ELISA and RT-PCR but not in dot blot. In contrast, dot blot and DAS-ELISA gave comparable results when using leaf sap. Infection of healthy tobacco plants using sap of the four PVY strains eluted from both FTA® and OFP was successful and PVY was detected using DAS-ELISA at two weeks after inoculation.

CONCLUSIONS:

From this work, it was clear that PVY samples obtained from remote potato growing areas can either be stored in FTA® or OFP cards awaiting further serological and molecular analysis and subsequent use in PVY resistance screening. Though OFP appeared to be a cheaper alternative to FTA®, its ability to retain virus samples over a longer period of time still needs to be investigated and compared to FTA® cards.

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CASSAVA VIRUS DISEASE DIAGNOSTIC SURVEY IN GHANA

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Cassava is an important staple crop not only in Ghana but in most of the tropics. The productivity of the crop is beset with pests and diseases attacks. Outbreak of viral diseases of cassava poses a threat to productivity of the crop in Ghana. Cassava mosaic disease has been reported in almost all the cassava growing ecologies in the country. However, with the emergence of virulent strains of the African cassava mosaic virus and the incidence of Cassava brown streak virus in Eastern parts of Africa, there is the need to undertake regular surveys and monitoring to ascertain the prevalence of any of these viruses in the country.
The objective of this work was to assess the current status of cassava viral disease incidence and severity as well as the prevalence of whitefly vectors in the various cassava growing ecologies of Ghana.
Field surveys were conducted in the summer of 2015. Coordinates of all cassava fields where samples were taken were captured with the GPS (Garmin™). Number of Cassava farms within any two locations along the routes where samples were taken were also counted and recorded. In each location visited diagonal transect walks were made and 30 plants within the diagonal transects were assessed for viral disease incidence and severity. Severity was assessed based on a scale of 1-5 with 1 representing no infection and 5 very, very severe infection whilst disease incidence was assessed as a percentage of number of plants infected per field/location. Samples of diseased plants showing varying symptoms and severity that were collected were carefully labeled and kept in herbarium pressers for laboratory diagnostics using PCR techniques. Whiteflies were collected from at least, 5 plants per location using the aspirator and then kept on 70% ethanol, labeled and then sent to the laboratory for analysis. Two hundred and fifteen (215) different fields/locations were visited and about 900 leaf samples collected in addition 190 collections of whitefly vectors.
Field data collected is being analyzed to draw disease maps that will guide breeders in developing disease-free planting materials and their deployment to interested stakeholders. Preliminary, diagnostic analyses of some of the samples collected have identified African cassava mosaic virus. No Cassava brown streak virus has been detected in any of the samples analyzed.

EPIDEMIOLOGY AND GENETIC DIVERSITY OF CUCURBIT CHLOROTIC YELLOWS VIRUS IN GREECE

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BACKGROUND and OBJECTIVES

Cucurbit chlorotic yellows virus (CCYV) (genus *Crinivirus*, family Closteroviridae) is implicated in Cucurbit Yellows Disease (CYD) and is transmitted semi-persistently by *Bemisia tabaci* biotypes B and Q (Okuda et al., 2010). It was recently reported in Greece in cucurbit fields in Rhodes and Crete islands (Orfanidou et al., 2014), but its presence in other regions of Greece is unknown, whereas knowledge on its epidemiology and genetic diversity is rather limited. The aim of this work was to study the host range, transmission parameters by the biotype Q, as well as to molecularly characterize CCYV isolates from different plant species.

MATERIAL and METHODS

During 2011-2015, 509 cucurbit samples (melon, watermelon, cucumber, zucchini) showing yellowing symptoms were collected from different regions of Greece while 342 weed samples (40 different species, 23 families) were also collected from the island of Rhodes, where CCYV prevails. Virus testing was done using total RNA extraction and RT-PCR targeting the HSP70h gene (Orfanidou et al., 2014). For the molecular characterization of CCYV, the coat protein (CP) gene of 6 isolates was sequenced and used in Maximum Likelihood (ML) phylogenetic analysis along with homologous published sequences from other isolates. Moreover, a colony of *B. tabaci* biotype Q was used in order to evaluate transmission efficiency of CCYV in cucumber. Acquisition access period (AAP) of 24h and 48h as well as the number of adult whiteflies (5, 10, 15, 25, 40, 60) were tested.

RESULTS

Results revealed the presence of CCYV in cucumber, melon and watermelon in Rhodes and Crete but not in the other areas surveyed. Moreover, 13 different weed species belonging to 10 families were identified here for the first time as alternative hosts of CCYV. Sequencing analysis of the 6 Greek isolates showed limited genetic diversity in the CP gene. The ML phylogenetic tree clustered the CCYV isolates in two groups. All Greek isolates were classified in Group I while Group II consisted only of isolates from Iran. Finally, transmission experiments revealed statistically significant differences ($P<0.05$) regarding 24h and 48h of AAP. Also, the number of whiteflies was positively correlated with the transmission efficiency, which, however, did not reach 100% in any treatment.

CONCLUSIONS

CCYV is a recently reported virus in Greece which seems to be restricted so far in the Eastern-Southern islands. Its wide host range in combination with its efficient transmission by *B. tabaci* render it a serious pathogen of cucurbit crops and therefore further studies are needed to address the mechanisms underlying its epidemiology in our country.

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SINGLE NUCLEOTIDE POLYMORPHISMS IN THE COAT PROTEIN OF PEPMV ARE RESPONSIBLE FOR YELLOWING PATHOTYPES IN TOMATO CROPS

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BACKGROUND and OBJECTIVES

Pepino mosaic virus is one of the most important viral diseases in tomato production. The symptoms in infected plants are highly variable. In the recent years, a new pathotype consisting of severe yellowing of the leaves and severe deformation of the fruits has been described. Two different phenotypes were observed: intervenal yellowing and severe mosaic patterns. Several single nucleotide polymorphisms were identified. Studies to reveal the impact of these mutations on symptomatology have been performed and will be presented in this paper.

MATERIAL and METHODS

Samples showing different types of yellowing symptoms were collected in commercial tomato crops in Poland, Belgium, The Netherlands and France. Mechanical transmission of symptoms was verified through inoculation on test plants. Total RNA was isolated and the nucleotide sequences of the coat protein (CP) gene were determined. The sequences were aligned to identify single nucleotide polymorphisms. Relevant point mutations were introduced individually into previously constructed full- length infectious clones of a mild PepMV isolate using PCR-based mutagenesis (Hasiów-Jaroszewska et al. 2009). The circularized PCR products were used for transformations of competent cells. After isolation of the plasmid DNA, the plasmids were cut with restriction enzymes and purified. The purified linear plasmids were used for transcription. The obtained RNA was mechanically inoculated onto *Nicotiana benthamiana* and subsequently onto tomato plants. Plants were monitored for development of symptoms.

RESULTS

Nucleotide sequence alignments of yellowing and mild isolates identified several single nucleotide polymorphisms in the CP gene. Initially, mutations in positions 155 and 166 were shown to be responsible for the yellowing symptoms (Hasiów- Jaroszewska et al. 2013). The ratio of mutant and wild-type sequences determined the severity of symptoms. Recently, new samples showing severe yellowing symptoms were collected. None of the previously described mutations were found in these samples. However, a third point mutation was identified. Site-directed mutagenesis and subsequent bio-assays confirmed that this mutation is also responsible for the yellowing symptoms.

CONCLUSIONS

Different point mutations have been identified and confirmed as responsible of severe yellowing symptoms on tomato leaves. Our results provide a better understanding of single nucleotide polymorphisms in the PepMV genome and their impact on viral symptomatology and virulence.

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MOLECULAR EPIDEMIOLOGY OF EMERGING BEGOMOVIRUS DISEASES ON VEGETABLE CROPS IN BURKINA FASO

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BACKGROUND and OBJECTIVES

Begomoviruses (family *Geminiviridea*) are transmitted by *Bemisia tabaci* and are responsible for serious diseases in the world. Except cassava-infecting bipartite begomoviruses (DNA-A and DNA-B components), all African begomoviruses described on crops are monopartite (DNA-A component). In recent years, a complex of more than 10 monopartite begomoviruses responsible for tomato (yellow) leaf curl diseases (TYLCD-ToLCD) has been described on tomato in sub-Saharan Africa [1]. Faced with the upsurge of vegetable virus diseases in Burkina Faso, we have undertaken (1) to identify the causal agents and their molecular diversity, and (2) to investigate their main epidemiological parameters in the field and under controlled conditions.

MATERIAL and METHODS

Thirty-nine localities from the main vegetable-growing areas of Burkina Faso were surveyed during 2013 to 2015. Altogether 939 leaf samples from cultivated and uncultivated crops were collected. Samples were subjected to begomovirus diagnosis by PCR, RCA, cloning and sequencing. Agroinfectious clones were constructed in order to assess the pathogenicity of some characterized viruses.

RESULTS

We have cloned and sequenced 143 DNA sequences (109 DNA-A and 34 DNA-B). Whereas, nucleotide similarity analyses confirmed the presence of at least four known African monopartite begomoviruses (CLCuGV, PepYVMV, ToLMLV and ToLCGHV), it also revealed for the first time in West Africa the mastrevirus CpCDV on tomato and a new species named “tomato leaf curl Burkina Faso virus”. Unexpectedly, we characterized a DNA-B molecule on cultivated and uncultivated plants, always associated with the DNA-A of PepYVMV, which is distantly related to the known DNA-B. Our agroinoculation tests demonstrate that the sole PepYVMV DNA-A doesn't induce TYLCD-ToLCD symptoms on tomato in experimental conditions. However, in mixed infection the DNA-B is an activator of the virulence of the cognate PepYVMV DNA-A.

CONCLUSIONS

A complex of four known begomoviruses (CLCuGV, PepYVMV, ToLMLV and ToLCGHV) and a new begomovirus species on tomato (ToLCBFV) were described on diseased vegetable crops in Burkina Faso. Interestingly, a mastrevirus (CpCDV) was described for the first time in West Africa on tomato. Finally, we demonstrate experimentally that the newly characterized DNA-B component, naturally associated with PepYVMV DNA-A component in the field, represents a major pathogenicity activator. Our first investigations may suggest that this DNA-B component recruited by PepYVMV DNA-A could be the main epidemiological factor for the emergence of PepYVMV as the most prevalent and severe plant virus disease on tomato and pepper in Burkina Faso.

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STUDY OF PATHOGENICITY DETERMINANTS IN CASSAVA BROWN STREAK VIRUS (CBSV)

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BACKGROUND and OBJECTIVES

Cassava brown streak virus (CBSV) is a positive sense ssRNA *Potyviridae*, genus *Ipomovirus*. Along with *Ugandan cassava brown streak virus* (UCBSV), CBSV is one of the main causes of yield reduction and economic losses of cassava in Africa. It is transmitted by whitefly (*Bemisia tabaci*) and distributed across the East coast of Africa.
CBSV encodes a polyprotein of 2912-2916aa, cleaved into 10 mature proteins. Functions have not been attributed to most of them, the rest have been predicted by homology comparison with proteins in other Potyviruses. CBSV lacks the Helper component proteinase (HC-Pro) involved in silencing suppression, viral amplification, long distance movement, protease activity, virus-vector interaction and symptom expression. CBSV encodes the Ham1-like protein, a novel protein in *Ipomovirus* that only appears in *Euphorbia ringspot virus*.
The present research aims to investigate the functions of CBSV proteins that can induce synergism with other viruses, then understand which elements of this virus act as pathogenicity determinants, silencing suppressors or movement proteins, and identify the function of Ham1-like protein during infection.

MATERIAL and METHODS

Transgenic lines, expressing individual CBSV encoded proteins were generated in *Nicothiana tabacum*. These plants were tested for synergistic interactions, infecting them with *Tobacco mosaic virus* (TMV) and *Potato virus Y* (PVY). Interaction between transgenes and the virus were analysed, measuring viral titre by ELISA assays and comparing symptom development of transgenic plants and wild type *N. tabacum*.
All CBSV encoded proteins were analysed for silencing suppressor activity, performing co-Agro-infiltration assays into *Nicothiana benthamiana* 16c line.

RESULTS

Synergistic interactions between showed that CBSV encoded proteins Ham1, P3 and NIb enhanced titre levels of TMV. The presence of the protein CI, NIb and 6K2 enhanced symptomatology, showing intense bleaching on veins and stems. PVY infection was delayed in presence of Ham1 and NIb.
The first Potyviral protein (P1) was confirmed as a silencing suppressor in CBSV, enhancing overexpression of the green fluorescent protein (GFP) in the 16c line, in a similar manner as displayed for P1 from UCBSV and HC-Pro from PVY[1].

CONCLUSIONS

P1 acts as the only silencing suppressor in CBSV, while P3, Ham1 and NIb interact in synergism. Therefore, they can be implicated in silencing suppression, movement or as pathogenicity factors[2], further studies are being performed to determine if other functions can be attributed to these proteins in CBSV.

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GLOBAL ANALYSIS OF THE MIGRATION PATTERN AND EVOLUTIONARY LINEAGES OF POTATO VIRUS Y

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BACKGROUND AND OBJECTIVES

Potato virus Y (PVY) is considered one of the most important plant viruses globally and affects worldwide potato production. PVY displays biological, serological, and molecular diversity as it exists as several distinct strains that differ in their ability to cause a wide range of phenotypes on both potato plant and potato tuber. New strains continue to emerge making control difficult. Despite its global incidence and importance, the origin, evolution and pathways of spread of PVY were not studied in detail.

MATERIAL AND METHODS

Using the complete genomic sequences of 149 PVY isolates reported worldwide, we studied the migration and evolutionary pattern of PVY using Bayesian phylogenetic approaches to assess the influence of host (potato) and geography on both virus evolution and spread. To estimate the evolutionary rate and timescale of PVY genomes, Bayesian phylogenetic analyses in BEAST v2.1.3 was performed. The published sampling time of the PVY isolates or sequences reported were used for calibrations of the molecular clock. We used Bayes factors to determine the best-fitting molecular-clock model and coalescent prior for the tree topology and node times. Both strict and relaxed (uncorrelated exponential and uncorrelated lognormal) molecular clocks, respectively were compared and to infer the demographic history we compared five demographic models - constant population size, expansion growth, exponential growth, logistic growth, and the Bayesian skyline plot.

RESULTS

Recombination has been a hallmark of PVY evolution, and some strains had a different evolutionary history than others. Some strains had accumulated a higher number of recombination events compared to others. Molecular clock analyses showed that the viral genes evolved at rates between 1.44 and 2.39×10^{-4} substitutions/site/year, similar to those with an RNA genome. The haplotype network of PVY isolates (149 samples with 116 haplotypes) suggested that PVY haplotypes could be divided into clearly two major groups. One was composed of N and NTN, while the other was of haplotypes from O and O5. Two smaller groups having haplotypes belonging to N:O and Wi/Wilga strains were also observed. All haplotype groups have accumulated their own unique set of mutations. The unique strains isolated from non-potato hosts, such as C, MN and NC57, were found to evolve separately with unknown median vectors i.e., missing haplotypes. The network was found to be spread out rather than star-shaped indicating that there were multiple recombination events. All the haplotype groups had such structures and thus might have accumulated unique mutations in each genomic region.

CONCLUSIONS

Bayesian phylogenetic analysis of the origin and global spread of PVY showed strong Bayes factor (BF) support for CP that the virus had spread not only intra-continental in Europe but also outside to different parts of the world. The origin of majority of PVY recombinants can be traced to Europe from where they were likely re-introduced with returning Spanish explorers. PVY probably spread from a single population from Europe to other parts of the world with subsequent frequent gene flow between populations in Europe and the USA.

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GRAPEVINE RED BLOTCH-ASSOCIATED VIRUS IN FREE-LIVING *VITIS* SP. PROXIMAL TO CULTIVATED GRAPEVINES

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BACKGROUND AND OBJECTIVES

Red blotch is an emerging disease of grape caused by grapevine red blotch-associated virus (GRBaV; Sudarshana et al. 2015). The only known hosts for this virus are Vitis sp., and no vector of epidemiological significance has been conclusively identified. GRBaV has a single-stranded, circular DNA genome and is a proposed member of the family *Geminiviridae*. GRBaV has been detected in all major grape-growing regions of the United States and Canada, likely as a result of the dissemination of infected propagation and planting material (Krenz et al. 2014). In 2012, a vineyard in California showed an unusual apparent clustering of vines with severe red blotch. The number of symptomatic vines was greatest along one edge of the block proximal to an uncultivated riparian habitat that included free-living vines (*Vitis* sp.). Our objectives were three-fold: 1) to assess the genetic diversity of GRBaV isolates from vines within a single block of the same cultivar and clonal line, 2) to determine if GRBaV was present in free-living vines proximal to a vineyard with GRBaV-infected vines, and 3) to determine the relatedness of any GRBaV isolates from free-living vines and those present in an adjacent vineyard.

MATERIAL AND METHODS

GRBaV was monitored in a vineyard in Napa Valley, California based on visual observations, virus detection by multiplex PCR, and the sequencing of virus genomes (Krenz et al. 2014).

RESULTS

No genetic heterogeneity was observed in a 587 nt region of the GRBaV genome in a population of 44 Cabernet franc clone 214 isolates. By contrast, genetic differences were observed in isolates from other cultivars and clones in adjacent blocks. GRBaV was confirmed infecting four free-living vines, two of which were shown to be *V. californica* x *V. vinifera* hybrids. The genomes of three free-living GRBaV vine isolates and seven from *V. vinifera* cultivars were compared; free-living vine isolates were shown to be more similar to each other and a Merlot isolate than to the other cultivated vine isolates.

CONCLUSIONS

The finding that GRBaV is present in free-living *Vitis* sp. indicates the virus can be spread by natural (non-human-mediated) means, and we hypothesize in-field spread of GRBaV is occurring. Free-living *Vitis* sp. can function as reservoirs for the virus and need to be considered in the management of this virus.

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ANALYSING THE INFLUENCE OF LANDSCAPE CHARACTERISTICS ON DISEASE SPREAD AND MANAGEMENT STRATEGIES

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BACKGROUND and OBJECTIVES

Using modelling, many studies have tried to understand disease dynamics to predict epidemics and improve management strategies (Keeling *et al*., 2008). Spatially explicit models generally represent disease dispersal using epidemiological and management parameters. They are mostly used in a fixed landscape and rarely account for landscapes characteristics. However, the landscape can influence epidemic dynamics; thus, the impact of management strategies is not necessarily transposable from one landscape to another. Here, we present a generic *in silico* approach which predicts the influence of landscape characteristics on the direct and indirect costs associated with an epidemic. We apply this approach to sharka, the most damaging disease of *Prunus* trees, caused by *Plum pox virus* (PPV, family *Potyviridae*).

MATERIALS and METHODS

PPV epidemics were simulated using a spatiotemporal stochastic model based on an SEIR (susceptible – exposed – infectious – removed) architecture (Rimbaud *et al.,* 2015). This model uses epidemiological and management parameters as inputs, and outputs the number of fully productive trees and the net present value (i.e. an economic criterion balancing the cost of the control measures and the benefit generated by healthy trees). We simulated various landscapes, differing in plot density and aggregation, by modifying real landscapes and using a T-tessellation algorithm. Then, simulations of PPV dispersal were carried out for the different landscapes, and model outputs were compared. Sensitivity analyses were undertaken to assess the relative influence of landscape, epidemiological and management parameters.

RESULTS

A range of landscapes was created for various levels of plot density and aggregation. The use of these landscapes in the model enabled to show their impact. Simulations highlighted that plot density and aggregation influence the economic criterion, and sensitivity analyses revealed how the influence of management and epidemiological parameters changes depending on the landscape characteristics.

CONCLUSIONS

This study shows how useful it is to take landscape characteristics into account to predict epidemics. This approach, which is transposable to many epidemics, could thus be used to improve management strategies. In addition to plot density and aggregation, other landscape characteristics may be tested, like the spatial allocation of resistant varieties.

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EVIDENCE FOR APHID IMMUNE SYSTEM MODULATION BY PLANTS INFECTED WITH CIRCULATIVE VIRUSES BUT NOT STYLET BORNE VIRUSES

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BACKGROUND and OBJECTIVES

Viruses in the Luteoviridae (Luteovirids) are transmitted exclusively by aphids in a persistent, circulative manner. These viruses are sequestered in membrane-bound vesicles and do not replicate in aphid tissues. How they manage to avoid replication is not known. We hypothesized that aphids activate their siRNA pathway to minimize or avoid widespread replication in their tissues. We used next generation small RNA (sRNA) sequencing to test the whether sRNAs targeting *Potato leafroll virus* (PLRV) are produced in viruliferous *Myzus persicae*.

MATERIAL AND METHODS

Aphids were fed to potato plants infected with PLRV, PVY or mock inoculated (healthy) or to artificial diets containing purified PLRV or diet. In all treatments where aphids were fed to a potato plant, after a 48h acquisition access period, aphids were transferred to turnip, a non-host of PLRV, for three days to clear their guts of sRNAs generated in potato. Deep sequencing was performed using an Illumina sequencer and clean reads were aligned to the potato and *M. persicae* reference genomes as well as all viral sequences deposited in NCBI. *M. persicae Densovirus* (MpDNV) was quantified in aphids by qPCR.

RESULTS

Aphids did not produce significant number of sRNA reads against PLRV, showing no luteovirid replication in the aphid vector. However, aphids did produce sRNAs that aligned to the aphid virus MpDNV. Interestingly, an abundance of unusual long sRNA (MpDNV-derived), ranging from 33-39nt, were observed only in aphids fed to PLRV-infected plants, which indicates that a circulative virus (PLRV), but not a stylet-borne virus (PVY), influences the antiviral defense of its aphid vector. Additionally, aphids fed to PLRV-infected plants produced significantly less 22 nt long sRNA reads, which is the most common size of siRNA processed by Dicer-2 (Dcr-2). These data suggest that in aphids fed to PLRV-infected plant, the siRNA pathway is altered or that an alternative enzyme or antiviral pathway is activated. Alignment of the long siRNA generated in these aphids shows that these long sRNAs may be immature siRNAs that were not processed into the functional 22 nt form or they may be a product of a different enzyme in another pathway. Regardless, the antiviral pathway in aphids exposed to PLRV-infected plants, it is not as efficient as the fully active Dcr-2 siRNA pathway, since the titer of MpDNV in PLRV-infected aphids tends to be higher than in aphids fed to plants or diets lacking PLRV, as measured by qPCR.

CONCLUSIONS

The mechanism used by aphids to avoid Luteovirus replication is not a siRNA-based mechanism. A circulative transmitted virus, PLRV, but not a stylet-borne virus, PVY, influences the immune response of aphids to replicative viruses, such as *Myzus persicae Densovirus*..

THE ROLE OF IPM IN THE SUSTAINABLE MANAGEMENT OF PLANT VIRUSES

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BACKGROUND and OBJECTIVES

Integrated Pest Management (IPM) is considered the central paradigm of insect pest management and is often characterized as a comprehensive use of multiple control tactics to reduce pest status while minimizing economic and environmental costs. As the principal precursor of IPM, the integrated control concept formulated the economic theory behind pest management decisions and specified an applied methodology for carrying out pest control. Sampling, economic thresholds and selective insecticides were three of the critical elements of that methodology that are now considered indispensable to the goals of IPM. While IPM is principally about integrating control tactics into an effective and sustainable approach to pest control, this overarching goal can only be achieved through well-trained practitioners, knowledgeable of the tenets conceived in the integrated control concept that ultimately yield informed pest management.

MATERIAL and METHODS

Insect vectors of plant viruses are often overlooked when it comes to appraising the destructive role of insects in crop losses. Basic knowledge about epidemics of vectored viruses is often lacking to the point where the component parts are seldom fully elucidated. However, at least two vector species that are cosmopolitan in distribution, *Myzus persicae* and *Bemisia tabaci*, are as well known for their overall pest status as they are for their potential to transmit scores of plant viruses to commercially valuable crops. A great number of management-based studies incorporating various control methods have been performed on these two species. These include vector exclusion methods that block contact or interfere with vector landing and feeding on plants, breeding of virus-resistant cultivars, biological control methods that aim to reduce vector numbers, and insecticidal methods for reducing vector numbers and disrupting transmission by altering normal feeding behaviours.

RESULTS

Managing plant viral diseases in an economically effective manner can be an enormous challenge if vector pressure is persistently high throughout crop development. Even in situations of relatively modest vector numbers, high inoculum potential in a region can result in a rapid spread of virus that can be difficult to combat. Well-timed insecticide applications can be relatively inexpensive while affording strong protection, but overdependence can lead to resistance problems and be disruptive of sustainable management. Both *M. persicae* and *B. tabaci* have been documented as being among the most resistance-prone insect pests that threaten crops through direct feeding damage and as virus vectors.

CONCLUSIONS

The key to successful management of virus vectors is understanding their intrinsic potential for increase in various environments while also knowing the capacity of countermeasures to suppress population growth and/or disrupt virus transmission. Although complications of resistance threaten sustainability of insecticide use, diversification of insecticidal modes of action have greatly improved the choices available for management of virus diseases in crops.

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BARLEY YELLOW DWARF INCIDENCE AND BIRD CHERRY-OAT APHID PREFERENCE, IN FOUR U.S.A. WHEAT VARIETIES

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BACKGROUND and OBJECTIVES

Barley yellow dwarf-associated viruses (B/CYDV) are transmitted by several species of cereal aphids. The bird cherry-oat aphid (BCOA), *Rhopalosiphum padi* (L.) is known to be an efficient vector of BYDV (Power et al. 1991), which also causes direct feeding damage in cereals (Leather et al. 1989). In recent years, outbreaks of the PAV species of BYDV have been challenging winter wheat production in Idaho, U.S.A. (Marshall and Rashed 2014). These outbreaks have been attributed, at least in part, to the expansion of corn acreage in the region. Identifying and planting wheat varieties with less relative susceptibility to aphid infestation and BYDV may help to limit virus spread at a regional scale, especially when summer hosts begin to mature. This study was conducted to: *i* compare BCOA preference in colonizing four Idaho winter wheat varieties, and *ii* quantify BYDV titer, and BYDV incidence in the same four varieties. In addition, relationships between root and foliar biomasses, and BCOA pressure were quantified in the field.

MATERIAL and METHODS

BYDV-positive BCOAs were presented with the choice to colonize seedlings of winter wheat varieties SY Ovation, Brundage, Stephens, and WB-Junction. The number of aphids on each variety was used to assess BCOA feeding preference. Using qPCR, BYDV titer in each of the four genotypes is currently being quantified. In a two-year field study, BYDV incidence was compared among the varieties. Each year consisted of 10 cage replicates per variety. Each cage was infested with 40 BYDV-positive BCOAs. ELISA was used to detect BYDV within BCOA-infested cages.

RESULTS

Although Stephens and SY Ovations consistently harbored relatively less aphid numbers than WB-Junction and Brundage, overall differences in aphid preference for the evaluated wheat varieties were not statistically significant. BYDV titer in foliar tissue of the evaluated varieties is currently being quantified with qPCR. BYD incidence in field cages was not statistically different among the four varieties, however SY Ovation had the lowest BYD incidence in both years.

CONCLUSIONS

Consistent trends in relative varietal attractiveness to BCOA and BYD incidence were observed in two varieties. Studies with additional cultivars may reveal detectable differences in susceptibility to BYDV, and assist with selecting varieties that may limit virus spread. Such studies would also help to better understand disease epidemiology at the regional scale.

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PREVALENCE OF TWO BEGOMOVIRUS, TYVSV AND ToLDeV, IN TOMATO CROP IN THE NORTH OF CHILE

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BACKGROUND AND OBJECTIVES

Arica and Parinacota is the northern region of Chile. It is characterized by favorable soil and climate conditions to grow vegetables throughout the year, being the Azapa Valley the main provider of tomato for fresh consumption for central Chile during winter season. In 2007, tomato crops from this area were affected by viral diseases causing yield losses between 30- 70%. Surveys conducted in the period 2009-2010 showed that the major viruses present corresponded to Pepino mosaic virus (PepMV), Peru tomato mosaic virus (PTV)) and Begomoviruses. The aim of this work was to determine the prevalence of the two begomovirus identified in the region: *Tomato yellow vein streak virus* (TYVSV) and *Tomato leaf deformation virus* (ToLDeV) affecting tomato crops, weeds and native plants present in the productive valleys of the region of Arica and Parinacota.

MATERIALS AND METHODS

During 2012-2015 years a total of 713 leaf samples from tomato crops, weeds and native plants, were collected in three vegetable producing valleys of Arica and Parinacota region (Azapa, Lluta and Chaca). Total DNA of each sample was extracted using the protocols of Dellaporta *et. al* (1983). Viral DNA was detected by dot blotting, using specific digoxigenin (DIG)-labelled DNA-probes for TYVSV and ToLDeV.

RESULTS

The results indicated that 43% of total samples analyzed were positive for TYVSV, while 16% were positive for ToLDeV. The prevalence for TYVSV and ToLDeV in Azapa valleys was 49.88% and 16.47%, while Lluta was 34.85% and 11.7% and finally Chaca valley was observed 43.25% and 20.75% respectively. In addition, 8.3% of the samples showed mixed infection.

CONCLUSIONS

This study showed evidence that TYVSV and ToLDeV were widespread distributed in the three productive valleys of Arica and Parinacota region, in the north of Chile. TYSV showed a higher prevalence than ToLDeV. So far, there is no report of the presence of these viruses or its whitefly vector in other tomato growing areas of the country. Begomoviruses are an emerging threat worldwide and in Latin America because its causes severe damage to major crops. Therefore, effective management practices should be put in place to prevent their spread into virus free locations in the country.

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A NEW STRAIN OF *Euphorbia mosaic Venezuela virus* DISPLAYS SEVERE SYMPTOMS IN TOMATO PLANTS

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BACKGROUND and OBJECTIVES

Begomoviruses are one of the major constraint for crop production in tropical and subtropical regions. *Euphorbia mosaic Venezuela virus* (EuMVV) is a begomovirus first reported infecting milkweed (*Euphorbia heterophylla*) and tomato (*Solanum lycopersicum*) in Venezuela and displaying mild symptoms of yellowing and curling of leaves (1). In this study seven tomato plants exhibiting begomovirus-like symptoms were collected in a field of Zulia, Venezuela. The samples were analysed by PCR using universal primers for begomoviruses (2). Begomovirus incidence was confirmed for all samples and sequencing of the expected viral DNA fragments revealed the presence of EuMVV in three samples. One of the EuMVV isolates was highly divergent when compared with previous reported isolates (1). In order to characterize this EuMMV isolate (hereafter, Zulia 216) further assays were carried out to perform full-genome amplification and production of an infectious clone.

MATERIAL and METHODS

Amplification products obtained by TempliPhi kit (GE Healthcare, Munich) were digested and inserted into pBluescript II SK+ (Stratagene, La Jolla) to transform *Escherichia coli* DH10B cells. Tomato plants var. Rio Grande were biolistically inoculated with viral DNA components. The symptoms were observed after 15 days post inoculation and confirmed by PCR using EuMVV specific primers (3).

RESULTS

In a BLAST search the complete sequence of DNA-A (2.618 bp) and DNA-B (2599 bp) components showed 93% and 87% nucleotide identity with DNA-A (JN368145) and DNA-B (JN368146) of EuMVV, respectively. In accordance with demarcation criteria of International Committee of Virus Taxonomy (ICTV), the isolate Zulia 216 is a new strain of EuMVV. In contrast to the previous study (1), plants of tomato var. Rio Grande displayed severe leaf curl when inoculated with isolate Zulia 216. Additional sap inoculations showed the mechanical transmission of this isolate onto tomato, *Nicotiana benthamiana*, *Nicotiana glutinosa* and *Nicotiana tabacum*.

CONCLUSIONS

Overall, our results show a new strain of EuMVV which is able to develop severe symptoms in tomato plants raising the question about its capacity to emerge as a risk for tomato production in Venezuela.

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RESISTANCE SOURCES AGAINST A NEW AN OLD WORLD BEGOMOVIRUSES ASSOCIATED WITH EPIDEMIC DISEASES IN CUCURBIT CROPS

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BACKGROUND and OBJECTIVES

The cucurbits include several important vegetables to human consumption which are threatened for epidemic diseases such as those caused by begomoviruses. The Old World begomovirus *Tomato leaf curl New Delhi virus* (ToLCNDV) was first described in India and is currently spreading in some Mediterranean countries (1, 2). Meanwhile, the New World begomovirus *Melon chlorotic mosaic virus* (MeCMV) has been only described in Venezuela (3). This work aims to evaluate melon lines in order to look for resistance sources against these viruses.

MATERIAL and METHODS

Thirty melon accessions were selected from a germplasm collection at National Institute for Agricultural Research (Avignon, France). The majority of the selected accessions have been previously found to be resistant or tolerant to at least one plant pathogen. An infectious clone of MeCMV, previously developed (2), and another of ToLCNDV, developed in this work, were biolistically inoculated onto plantlets of melon cv. Védrantais. Infectivity of clones was confirmed by symptom observation and PCR with specific primers at 30 days post inoculation (dpi). Subsequently, sap inoculation assays for both begomoviruses were performed onto the 30 melon accessions. In addition, the accession IC-274014 was selected for further inheritance studies of resistance factors against both begomovirus.

RESULTS

Infectivity of the clones was confirmed in susceptible melon cv. Védrantais. The majority of the 30 melon accessions were susceptible to both begomoviruses. Accessions IC-274014, PI 124112, PI 282448, WM7 and WM9 did not exhibit clear symptoms of either ToLCNDV or MeCMV. Accession IC-274014 was selected for inheritance studies of resistance factors. Thus a F1 generation obtained from parents IC-274014 and Védrantais was challenged to viral sap inoculations. The F1 generation exhibited intermediate resistance involving systemic chlorotic spots, however, the size of plants was similar to the resistant parent at 45 dpi.

CONCLUSIONS

In this work five potential sources of tolerant/resistance to MeCMV and ToLCNDV were identified, of which IC-274014 accession showed the best performance. Furthermore, intermediated resistance displays by F1 generation of IC-274014 and Védrantais suggests at least to one recessive gene according to the absence or presence of viruses evaluated in this work. Overall, our results provide evidences of an interesting resistance source against two genetically distant begomoviruses.

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OCURRENCE AND MOLECULAR CHARACTERIZATION OF VIRUSES PRESENT IN THE POTATO SEED TUBER PRODUCING AREA OF CHILE

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BACKGROUND AND OBJECTIVES

Annually about 50,000 ha with potatoes are grown in Chile. The production is destined almost entirely to the domestic market, with an annual per capita consumption of 35-45 kg. This crop is present in several regions along the country, including native potato varieties originating in the Chiloé archipelago. In recent seasons, there has been an increase in viral diseases affecting potato crop in southern Chile, which has caused significant losses in yield and quality as well as an increase in the rejection of seed tuber for certification. The main objective of this work was to update our knowledge about the occurrence and distribution of viruses threatening potato tuber seed production in Chile.

MATERIALS AND METHODS

A survey was conducted in three regions of the south of Chile (La Araucanía, Los Ríos and Los Lagos) to study the occurrence and distribution of the three viruses included in the local potato seed certification program (PVX, PVY and PLRV) plus PVS and TSWV. A total of 434 fields were sampled for asymptomatic tubers, which would be used as seed in the next season. ELISA test was used to asses the presence of the following viruses: PVS, PVY, PLRV and TWSV. Then, 112 PVY positives samples were characterized by strains using a multiplex RT-PCR assay described in Lorenzen et al. (2006). A total of 28 PVS isolates were characterized by sequencing of the coat protein gene, followed by phylogenetic and pairwise identity analysis. Additionally, a metagenomic approach was used to search for previously non-described viruses infecting native potatoes in Chiloe.

RESULTS

ELISA tests results showed that a 71.4% of the sampled field were positive for one or more viruses. PVS had the highest prevalence with a 66.1% of fields positive for this virus, followed by PLRV (28.8%), PVX (21%) and PVY (20.5%). There were no samples positives for TSWV. The PVY-strain characterization showed that a 61,6% of the isolates were classified as either PVY^{NTN}, PVY^{N}, PVY^{Na-N} ,PVY^{Na-NTN} or $PVY^{N:O}$, while 17.9% were PVY^{O}. In the case of PVS, 26 of the samples grouped with the PVS^{O} strain group and 2 genotypes with the PVS^{A} strains. Lastly, deep sequencing followed by assembly of viral small RNAs (vsRNAs) to a reference virus genome, revealed that *Potato mop-top virus* (PMTV) was infecting native potatoes in Chiloe Archipelago, in the south of Chile.

CONCLUSIONS

This study showed evidence of the high virus prevalence in the tuber-seed production area in Chile. PVS is widespread (66%) and there should be effort to reduce its frequency in the field, and even consider its re-inclusion in the tuber-seed certification program. As it has been described in another coutries, high prevalence of necrotic and tuber necrotic inducing strains of PVY are described in the area. Finally, the metagenomic approach allowed the identification of a quarantine virus pathogen for Chile, PMTV. Thus, these results provide important information to be considered in programs of seed tubers and plant quarantine management of potato pathogens in Chile.

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GENETIC DRIFT AS A TOOL TO INCREASE THE DURABILITY OF PLANT QUALITATIVE RESISTANCE TO VIRUSES

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BACKGROUND and OBJECTIVES

Plant qualitative (i.e. total) resistance to viruses is an efficient way of protecting crops, but it is often broken down by the emergence of adapted virus variants, able to infect those resistant plants. This evolutionary phenomenon can occur particularly quickly in monocultures deployed in vast areas. Different strategies have been studied, aiming at increasing the durability of resistant crops, such as the use of multiline cultivars or cultivar mixtures in space and time. Here we study a complementary strategy that can yield interesting results in terms of yield increase and resistance durability. It exploits the evolutionary principles governing virus populations for the management of plant resistance, particularly regarding the effect of genetic drift on the evolution of plant viruses.

MATERIAL and METHODS

We have developed a stochastic plant epidemic model at the field scale, coupling epidemiology and population genetics. It allowed to study the effect of genetic drift controlled by quantitative (i.e. partial) resistances on qualitative resistance durability. Our model yields predictions of the added value of pyramiding quantitative resistances controlling virus effective population sizes, and thus the intensity of genetic drift, on crop damage reduction. We investigated the role of field features - such as the intensity of epidemics or the proportion of resistant plants -, and of population genetics features - such as virus bottlenecks sizes and their evolutionary equilibrium within hosts - on yield benefit.

RESULTS

The model provided insightful guidelines for the management of cultivars pyramiding qualitative and quantitative resistances controlling virus effective population sizes. Notably, those quantitative resistances should be particularly helpful when the qualitative resistance gene is not highly efficient, or when the adapted variants are not already present at high frequency in the landscape. Two additional points emerged from the simulations in our framework, (i) it is often preferable to deploy a large proportion of resistant cultivars and (ii) when helpful, it is better to choose quantitative resistances leading to the smallest virus effective population sizes, hence to the strongest genetic drift.

CONCLUSIONS

This model is, to our knowledge, the first one to depict the impact of genetic drift on plant disease resistance durability. We decided to build it as simple as possible, to be able to discriminate which underlying processes enhance durability. It provided promising qualitative results, encouraging further finer research on this subject. In all, we were able to prove the interest of introducing quantitative resistances controlling virus effective population sizes in breeding strategies to increase the durability of plant qualitative resistances.

CHARACTERIZATION AND DETECTION OF CARROT TORRADOVIRUS, TENTATIVE NEW MEMBER OF THE TORRADOVIRUS GENUS

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BACKGROUND AND OBJECTIVES

Carrot torradovirus is a tentative new member of the Torradovirus genus placed in 2007 to describe two new viruses affecting tomato (Verbeek et al., 2007), *Tomato torrado virus* (ToTV) and *Tomato marchitez virus* (ToMarV). It was found in 2013 with Carrot Closterovirus 1 (CtCV1), when the causes of internal necrosis in carrots were being investigated (Adams et al., 2014). However, no association was found between the presence of the new viruses and the development of symptoms in the infected plants.

MATERIAL AND METHODS

New sets of primers were designed and Reverse-transcriptase PCR (RT-PCR) and real-time RT-(q)PCR molecular assays were developed for the detection of CaTV in samples collected from the field. Sensitivity and specificity of the designed assays were checked and RT-qPCR assay validated according to the requirements established by the European Plant Protection Organization (EPPO). Transmission experiments were also carried out with *Nicotiana benthamiana*, Chervil and Carrots with two different aphis species, *Myzus persicae* and *Cavariella aegopodii*.

RESULTS

Surveys done in 2014 and 2015 across the UK indicated high percentage of virus infection in carrot crops and revealed the incidence of the new viruses. RT-PCR and RT-qPCR methods successfully amplified CaTV from infected samples and not from healthy and negative controls. Genome annotation of CaTV showed the closest related member was Motherwort yellow mottle virus (MYMoV). Also, virus transmission experiments carried out in the glasshouse indicated that CaTV could be, unlike other tomato-infecting Torradoviruses, naturally transmitted by *Myzus persicae*.

CONCLUSIONS

CaTV was found to be present in carrot fields in UK but no association has been found with the development of disease due to high prevalence of other carrot viruses. Further work will be performed by NGS in order to try to establish a relation between virus presence and development of symptoms.

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EVALUATION OF SELECTED CASSAVA CLONES UNDER DIFFERENT AGRO-ECOLOGICAL ZONES IN TANZANIA

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BACKGROUND and OBJECTIVES

Cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) are the most important constraints to cassava production in Africa. The viruses causing both are transmitted by the whitefly, *Bemisia tabaci* and through vegetative propagation of infected planting material. Recent outbreaks of CBSD have forced farmers in north-western Tanzania to abandon cassava cultivation leading to food shortages. CBSD is caused by at least two species of cassava brown streak viruses (CBSVs) (genus *Ipomovirus*, family Potyviridae). CMD is caused by cassava mosaic geminiviruses (CMGs) (family Geminiviridae, genus *Begomovirus*). CMD has been controlled through distribution of improved varieties but prevalence of severe CMGs threatens the sustainability of cassava production in Tanzania. Strategic management of these virus diseases requires the availability of clean planting material and disease resistant varieties that are adapted to the different agro-ecologies. Most of the CMD-resistant varieties that are currently available are susceptible to CBSD, highlighting the urgent need to breed for varieties with dual-resistance. A study was therefore designed to evaluate a set of at least 25 elite cassava clones at eight sites with contrasting agro-ecological characteristics.

MATERIALS and METHODS

Plantlets of at least five tissue-cultured and virus-indexed varieties were obtained from each of Kenya, Malawi, Mozambique, Uganda and Tanzania. Following hardening-off and field multiplication, at least 25 clones per site were planted at each of the eight sites in Tanzania using an alpha design. These sites included: Misungwi, Bunda, Bukoba and Chato in northwestern, Dodoma in central, Namtumbo and Mtwara in southeastern and Bagamoyo and Zanzibar in coastal Tanzania. Trials will be run for 12 months. Vector abundance and CMD and CBSD disease scoring assessments will be conducted at two, four, six and twelve months after planting (MAP). Leaf samples will be collected for virus testing, characterization and quantification during each assessment, and inoculum pressure will be determined at 2MAP. At harvest, roots will be assessed for necrotic rot and samples taken for virus testing and quantification.

RESULTS

Preliminary data on virus inoculum pressure, virus diversity and early patterns of infection for each of the eight evaluation sites will be presented.

CONCLUSIONS

Results of this study are expected to be useful in selecting varieties to be used as sources of dual-resistance and provide information on how different cassava genotypes interact with CBSVs and CMGs under different environmental conditions.

TOLERANCE OF PLANTS TO VIRUS INFECTION: MECHANISMS AND ROLE IN PLANT-VIRUS CO-EVOLUTION

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BACKGROUND and OBJECTIVES

Tolerance, the ability of a host to diminish the negative impact of infection on its fitness, is a major defence of plants to pathogens. Plant tolerance to virus infection remains underexplored, both in its underlying mechanisms and regarding its role in plant-virus co-evolution. Tolerance analysis of *Arabidopsis thaliana* to *Cucumber mosaic virus* (CMV) showed that tolerant genotypes reduced the impact of infection on seed production by reprogramming their development and reallocating proportionally more resources to reproduction than to growth, as compared to non-infected plants. Accordingly, tolerant genotypes delayed flowering upon infection (Pagán et al. 2008).suggesting that tolerance is attained though the regulation of flowering genes upon infection. However, as flowering responds to many environmental cues, it could be that tolerance did not evolve as a defence to CMV infection but be a by-product of adaptation to the abiotic environment.

MATERIAL and METHODS

To test the first hypothesis we analysed two Arabidopsis genotypes genotypes that co-exist in wild Arabidopsis populations: the short-lived, non-tolerant *Landsberg erecta* (Ler) and the long-lived, tolerant *Llagostera* (Ll-0).The time-course of flowering genes expression was quantified after CMV infection along with genetic analysis involving different lines in which Ll-0 alleles of two major flowering genes (*FRI* and *FLC*) had been introgressed into the Ler and Col-0 backgrounds. To test the second hypothesis, 77 wild Arabidopsis genotypes from different habitats in the Iberian Peninsula were analysed for their adaptation to the environment and for tolerance to CMV.

RESULTS

Results indicate that the expression of tolerance requires functional alleles of *FRI* and *FLC*, and that upon CMV infection flowering repressor genes are stimulated, while flowering promoting genes, such as *FT* and SOC, are repressed. Thus, tolerance is due at least in part by changes in the expression of the genes that regulate flowering time. On the other hand, while architecture and flowering time of mock-inoculated plants of 77 Iberian genotypes correlated with variables of the abiotic environment, tolerance did not.

CONCLUSIONS

These results allow concluding that CMV infection modulates the expression of flowering genes early after infection, which will alter the plant's developmental schedule and result in resource reallocation and tolerance. Results also show that tolerance is not a by-product of adaptation of flowering time to the abiotic environment, but a specific response to CMV infection. Hence, results suggest that tolerance is a co-evolved defence reaction involving developmental pathways not previously associated to virus defence responses.

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MACRO- AND MICRO-LEVEL CHARACTERIZATION OF INTERACTIONS BETWEEN A *TOSPOVIRUS* AND ITS THRIPS VECTOR

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BACKGROUND AND OBJECTIVES

Tospovirus is the only plant-infecting genus in the family *Bunyaviridae*. Thrips transmit tospoviruses in a persistent and propagative manner. We attempted to characterize interactions between *Tomato spotted wilt virus,* a *Tospovirus,* and its thrips vector, tobacco thrips, *Frankliniella fusca* (Hinds) (Order Thysanoptera: Family Thripidae). Macro-level studies were conducted using microcosms (Plexiglass cages). Results indicated that TSWV infection increased thrips oviposition, but suppressed thrips feeding, reduced thrips fecundity, and delayed development (Shrestha et al. 2012). The positive and negative effects of TSWV infection on its vector are in line with a number of studies conducted in other *Tospovirus* pathosystems. However, studies examining molecular bases for such interactions are relatively rare. Few studies conducted so far have focused solely on one vector, Western Flower Thrips, *Frankliniella occidentalis* (Pergande).

MATERIALS AND METHODS

With Next Generation Sequencing, using a Hiseq Illumina Platform, we constructed transcriptomes of larvae, pupae, and adults of *F. fusca* with and without TSWV infection, and conducted differential expression analyses. The results were validated for a subset of contigs using quantitative real time PCR.

RESULTS

Results revealed that more contigs were differentially expressed in adults (562) than in larvae (395) and pupae (204) following TSWV infection. Numerous contigs associated with virus entry and movement such as cell receptor aminopeptidase N, clathrin and adaptor were upregulated up to 10 folds in larvae and adults of viruliferous thrips than non-viruliferous thrips. Homologous contigs associated with virus replication such as 40S ribosomal protein 15, 60S ribosomal protein po, and host cell factor 1 protein among others were also upregulated following TSWV infection. In addition, contigs associated with virus transport, lipid and carbohydrate metabolic processes were upregulated following virus infection. Furthermore, contigs related to immune pathways including proteolysis, apoptosis and RNAi were upregulated in viruliferous thrips than in non-viruliferous thrips; contigs that negatively regulate immune pathways were also upregulated in viruliferous thrips than non-viruliferous thrips. A closer examination of contigs associated with thrips fitness revealed an upregulation of contigs associated with egg production, and embryo development in TSWV-infected thrips than in non-infected thrips. At the same time, there was a strong downregulation of contigs associated with growth and development in viruliferous thrips than non-viruliferous thrips. All associations described are based on *in silico* interpretations

CONCLUSIONS

Differential expression analyses also suggested effects on thrips fitness similar to macro-level experiments. This study also identified several targets for RNA-induced silencing experiments; experiments are currently underway to examine their effects on thrips fitness.

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DIAGNOSTIC OF TOBACCO RINGSPOT VIRUS IN RUSSIAN FEDERATION

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BACKGROUND and OBJECTIVES

One of the main problems for horticulture is improving product quality and reducing crop losses and also to obtain healthy planting material.
Testing of the plants for viruses can allow stopping their spread. Asymptomatic infected plant material in a mixed consignment and absence of phytosanitary control can lead to serious losses in the future in case of recontamination sensitive plants.
In 2015 in the quarantine lists of Russia was extended. One of the most important viruses that were added then is a member of the genus Nepovirus: *Tobacco ringspot virus* (TRSV) that has wide variety of pathways. The virus is spread by nematodes of the genus *Xiphinema*, some insects, as well as vegetative and by seeds. This is a cause of potential risk for a wide and rapid spread of TRSV.
TRSV was first discovered and described in 1927. The virus has a wide range of plant hosts, which includes field crops, ornamentals and weeds, belong to annual and perennial crops.

MATERIALS and METHODS.

In the virology laboratory of All-Russian Plant Quarantine Centre during the work by PCR and ELISA kits were testing plant material of ornamental and vegetable crops Russian and foreign origin.
More than 1000 plants were tested. For screening test was used different ELISA kits: Agdia (USA), Adgen (UK), DSMZ (Germany) and others. Some species have been surveyed: Streptocarpus, Impatiens, Pelargonium, Iris, Fuchsia, Petunia, *Ruellia*, Lilium, Tulip, Eggplant and other vegetables, and ornamental plants. Were tested vegetative parts of the plant, as well as bulbs and rhizomes of ornamental plants and vegetable seeds.
Were used different kits for RNA extraction: "Proba-NK" (Agrodiagnostika, Russia) and the "Phyto-Sorb" (Syntol, Russia). For the RT reaction was used MMLV-RT-kit (Agrodiagnostika Russia) and for amplification 5X Mas^{CFE}TaqMIX-2025 (Dialat, Russia), including a "hot start" SmarTaq-polymerase.

RESULTS

On 4 Impatiens plants and seedlings of eggplant (Russian origin), as well as plants of the Iris (the Netherlands origin) was detected TRSV by ELISA. All positive samples, as well as positive controls Bioreba (Switzerland) and DSMZ TRSV PC-0234 (Germany) were tested by PCR with specific primers TRSV F / R (Jossey S, Babadoost N, 2006).
All PCR products were sequenced. The sequences were processed and analyzed by program BlastN (NCBI). By sequences all results were confirmed the presence of TRSV in the samples.
Genetic differences between the samples ranged from 3 to 5%. The sequences of the virus isolates from Impatiens and Iris were identical, that confirms the foreign origin of planting material of Impatiens that was introduced in previous years.
All consignments with infected plants were destroyed.

CONCLUSIONS

Control of import and movements of plant material for the presence TRSV can prevent introduce and further spread this virus in the territory of Russia.

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EVOLUTIONARY ADAPTATION OF *ZUCCHINI YELLOW MOSAIC VIRUS* P3 PROTEIN IN CUCURBITS GROWING IN ITALY

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BACKGROUND and OBJECTIVES

Zucchini yellow mosaic virus (ZYMV) is one of the most prevalent and devastating viral pathogens in the cucurbit-growing regions. Transmitted by aphid in stylet-borne fashion, its control in field is difficult and genetic resistance is still the most cost-effective and reliable approach to protect crops. As typical member of the genus *Potyvirus*, ZYMV genome consists in a positive single-stranded monopartite RNA molecule which contains one long open reading frame (ORF) encoding 11 viral proteins. The function of the non-structural P3 protein is still poorly understood though a single mutation in the P3 coding region of ZYMV that produced an amino acid alteration was sufficient to induce severe symptoms in tolerant zucchini squash (Desbiez et al., 2003). The P3 coding region is also reported as a highly variable region of potyviruses (Urcuqui et al., 2001) so that other specific codon could show a positive selection pressure favouring a new virus pathogenic behavior towards resistant cultivars. Therefore, it seems useful to collect more data to study the variability of ZYMV-P3 coding region, and the P3N-PIPO coding region that overlaps with its half-amino terminal, in particular where commercial resistant cultivars are commonly used and the selection pressure is higher.

MATERIAL and METHODS

From CREA-PAV virus collection, 28 Italian isolates of ZYMV were selected over a period of more 20 years. ZYMV infection in cucurbits was identified by either serological (in the past) or molecular assays. Total RNAs were purified from leaf and fruit samples and two primer pairs were designed to amplify the complete P3 coding region.

RESULTS

Amino acid sequence alignments and phylogenetic tree reconstruction (MEGA6) in the P3 coding region with the Italian ZYMV sequences and those of worldwide isolates available in GenBank were performed and all isolates of the present study grouped in a non-Asiatic group but were distributed within different clades where, in general, the year of collection was not a demarcation criterion. In Italy, the single point mutation at amino acid position 917 (aggressive variant) was first reported in 2001 by Desbiez et al. (2003) even if according to our samples it was detected from 2007. Interestingly, year 2012 demarcates the appearance of an emergent but well established ZYMV population showing a new single point mutation at position 903 which was not revealed in any other P3 published sequences.

CONCLUSIONS

More studies are needed to correlate this last mutation in P3 coding region with virus adaptation to current commercial varieties that, indeed, are genetically uniform for virus resistance and could be responsible for the rapid genetic diversity of ZYMV interacting with the host plant.

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MOLECULAR VARIABILITY OF *ZUCCHINI YELLOW MOSAIC VIRUS (*ZYMV) ISOLATES FROM TURKEY

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BACKGROUND and OBJECTIVES

Cucurbits are widely grown in different provinces of Turkey. However their production is restricted by plant viruses. ZYMV is one of the most economically important viruses of cucurbit crops worldwide, including in Turkey. Several strains have been defined based on their biological, serological and molecular variability – particularly in the coat protein (CP) coding region (Lecoq & Desbiez, 2012). One molecular cluster (A1) appears highly prevalent worldwide, but other clusters (A4, A5) have been observed recently in several European and Mediterranean countries, probably in relation to recent introductions (Lecoq& Desbiez, 2012). However the situation in Turkey remains unknown. The aim of this research was thus to characterize the molecular variability of ZYMV in Turkey and look for the presence of emerging strains.

MATERIAL and METHODS

During the years 2010-2012, 230 cucurbits samples were collected from different provinces of Turkey and tested by ELISA for the presence of ZYMV. 22 ZYMV-positive isolates from Adana, Antalya, Muğla, Antakya and Anamur in 1994 were also included in the analysis. After RNA extraction and RT-PCR, the partial CP sequences of ZYMV isolates from Ankara, Antalya, Konya and Karaman provinces of Turkey were obtained and compared to the worldwide diversity of the virus using MEGA6 software.

RESULTS

According to ELISA results, 37% of the samples were infected with ZYMV. Full CP sequences were optained for 43 isolates collected from different provinces of Turkey in 2010-2012. The CP coding regions of Turkish ZYMV were 837 nt long and encoded 279 amino acids (aa). Forty isolates of different region were classified in the subgroup A1 and these isolates showed 99 % sequence identity with Fars, Iraq and Germany isolates. Three isolates collected from Antalya region in 2011 were classified in subgroup A4 and showed 99 % sequence identity isolates from the same subgroup that have emerged recently in France. Based on partial CP sequences, the 22 isolates from 1994 belonged to subgroup A1.

CONCLUSIONS

The CP coding region is the most frequently used for studies of genetic diversty in Potyviruses. In this study, sequence analysis of ZYMV isolates of Turkey showed that most isolates belonged to a major molecular group (A1), the most widespread worldwide. However three isolates from Antalya region belonged to a different molecular group (A4) that has been observed in several countries including China and Poland and seems to be now emerging in south-eastern France. Further surveys are required to determine if group A4 is now emerging in Turkey.

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MOLECULAR CHARACTERIZATION OF CUCURBIT APHID-BORNE YELLOWS VIRUS AFFECTING CUCURBITS IN TURKEY

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BACKGROUND and OBJECTIVES

Cucurbits are economically important vegetable in Turkey. Cucurbit crops are seriously affected by many viruses including Cucurbit aphid-borne yellows virus (CABYV). CABYV, a widespread polerovirus causing yellowing symptoms on older leaves that were previously associated to nutritional or physiological disorders. CABYV was first identified in 1988 in France, now, CABYV is widely spread in the whole Mediterranean region (Lecoq and Desbiez 2012). In Turkey, CABY reported firstly by Yardımcı and Özgönen (2007) as serologicaly. The purpose of this study was to characterize the molecular variability of CABYV in Turkey, through determining partial coat protein (CP) nucleotide sequences of (CABYV) isolates of Turkey.

MATERIAL and METHODS

To characterize the molecular variability of CABYV in Turkey, cucurbits samples were collected from cucurbit crops in the cucurbit plantation areas of Turkey in 2012-2013. After sample collection, RNA exraction and reverse transcription–polymerase chain reaction (RT-PCR), Tthe nucleotide sequences of the partial CP sequences of CABYV isolates from different province of Turkey were characterized and compared using MEGA5.

RESULTS

Phylogenetic analyses revealed that Turkey isolates belong to two molecular groups. İsolates collected in 1994 and 5 isolates from 2012-2013 clustered in the “European- African” group whereas 3 isolates from 2012-2013 clustered in the “Asian” group. And one isolate presented a mixed infection with the two groups

CONCLUSIONS

In conclusion a phylogenetic analysis showed that at least two genetic groups coexist in the field in Turkey. To our knowledge this is the first study of the molecular variability of CABYV in Turkey. Furter work is needed to understand better the epidemiology and evolution of CABYV in Turkey.

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RECOMBINANT STRAINS OF POTATO VIRUS Y OVERCOME MATURE PLANT RESISTANCE IN POTATO (*SOLANUM TUBEROSUM*)

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BACKGROUND AND OBJECTIVES

Potato virus Y (PVY) is vectored in a non-persistent manner by aphids and vector control measures such as the application of insecticides have limited effect on virus spread. Moreover, different strains of PVY (including ordinary, necrotic and recombinants such as NTN and N-Wi) are increasing in incidence, making PVY the most economically important virus in many potato production systems worldwide. PVY can be controlled by natural resistance genes but these are lacking in many commercial cultivars. Mature plant resistance (MPR) is a poorly understood phenomenon where plants become more resistant to infection as they age. MPR could be a useful component for virus control, however, a better understanding of the mechanism MPR is needed to allow it to be effectively incorporated in epidemiological models for integrated pest management.

MATERIALS AND METHODS

MPR against four PVY strains (PVY^O, PVY^N, PVY^{NTN} and $PVY^{N\text{-}Wi}$) was studied in four cultivars of potato: Maris Piper, Atlantic, Desiree and Shepody. Plants were challenged by manual inoculation at four stages of development (6 leaf, stolon, tuber bulking and flowering). PVY infection was detected by DAS-ELISA. PVY movement into phloem was investigated in inoculated leaf tissue at different stages by live cell imaging of the of the GFP expression from a PVY^N infectious cDNA clone and solute transport from source leaves to sink tissues was studied using a phloem mobile fluorescent probe carboxyfluoresceine diacetate (CFDA) to monitor changes in solute transport (a proxy for virus movement).

RESULTS

PVY infection of non-inoculated leaf tissues was significantly affected by PVY strain, host genotype and plant growth stage. MPR was effective against PVY^O and resistance in all cvs increased with developmental stage. So that when inoculated at flowering, PVY^O was not detected in the upper non-inoculated leaves and did not spread to progeny tubers. MPR was less apparent in cvs inoculated with the other strains. Foliar infection decreased markedly at the flowering stage for all cultivars except Shepody (which did not display MPR) and $PVY^{N\text{-}Wi}$ and PVY^N were not readily detectable in foliar tissue of most plants, however, PVY^{NTN} was detected in upper leaves of approx. 25% plants. Despite decreased foliar infections, PVY^{NTN} and $PVY^{N\text{-}Wi}$ were detected in all progeny tubers of plants inoculated at the flowering stage. CFDA translocation studies showed unloading was not detected in developing leaves at the top of the stem and there was a reduction in solute unloading in tubers at flowering stage. However, GFP-tagged PVY^N was detected in phloem tissue of inoculated leaves at all growth stages. Therefore, PVY is not prevented from entering the phloem in inoculated leaves during MPR.

CONCLUSIONS

These results show that MPR can be a useful measure to control PVY^O and PVY^N in some cvs but recombinant PVY strains, despite decreased foliar infection, were able to infect progeny tubers at all developmental stages. Studies are ongoing to investigate the mechanism of resistance and understand the difference in pathogenicity of recombinant virus strains but change in solute transport and inhibition of phloem entry does not appear to be associated with MPR.

IMPACT OF *BARLEY YELLOW DWARF VIRUS* INFECTION ON GRAIN YIELD AND QUALITY IN WHEAT AND BARLEY

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BACKGROUND and OBJECTIVES

Barely yellow dwarf virus (BYDV) causes an economically important disease which affects cereal crops such as wheat, barley and oats (Lister and Rochow, 1979), often resulting in significant losses of grain yield and quality. BYDV is transmitted by several aphid species and the most common vector in Australia is the bird cherry-oat aphid, *Rhopalosiphum padi* (Trebicki et al, 2015). Epidemiology of BYDV is not well understood and up to date information on the impacts of BYDV infection on yield in Australia is scarce. In this study, a field trial was conducted in Horsham, Australia to assess the effects of BYDV on plant growth, grain yield and grain quality in wheat and barley.

MATERIAL and METHODS

The field trial consisted of three treatments: early BYDV infection (inoculated at two-leaf stage), later BYDV infection (inoculated at early tillering stage) and a non-inoculated control. BYDV was transmitted to randomised replicated plots of wheat and barley using viruliferous *R. padi* contained in 90cmx180cm cages that covered plants within the plot. Plants were tested by tissue blot immunoassay (TBIA) (Freeman et al, 2013) to confirm virus presence. Plants were harvested and assessments included plant height, biomass, grain yield and grain count and 1,000 grain weight and harvest index were calculated.

RESULTS

Grain yield was significantly reduced due to BYDV infection in both wheat (80%) and barley (64%) but there was no significant difference in grain yield between the two different BYDV inoculation times in barley. Differences in grain count as a result of BYDV infection were also highly significant in both wheat and barley. 1,000 grain weight was not significantly affected by BYDV infection in wheat but there was a small but significant difference in barley. Additionally, BYDV infection significantly reduced plant biomass and other parameters measured in wheat and barley.

CONCLUSIONS

Despite its importance and high level of infection in recent years in Victoria, Australia, losses due to BYDV infection are poorly understood. Implications of BYDV infection on grain yield and quality for the grain industry will be summarised and discussed.

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STRAIN DIVERSITY OF PLUM POX VIRUS IN DIFFERENT REGIONS OF UKRAINE

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Plum pox virus (PPV) is the pathogen causing dangerous disease of stone fruit crops, which is widespread throughout the world. As the quarantine object, PPV attracts significant attention of researchers and causes substantial economic losses. PPV has a great evolutionary potential, with both mutation and recombination playing major roles in genetic diversification and pathogenicity. PPV has seven distinct strains differing in their pathogenicity, host range, serological and molecular characteristics, and geographical distribution (García & Cambra, 2007). Although these divergent strains of PPV represent an important threat, there is only limited information available on their prevalence on cultivated and wild hosts, variability, biological properties and epidemiological impact (Šubr & Glasa, 2013). The disease caused by PPV was first described in Ukraine in 1966. PPV is widespread in almost all regions and is a serious threat to horticulture of our country, but the variety of strains is not thoroughly studied (Budzanivska et all., 2011).

The aim of our research was to investigate the diversity of PPV strains. Samples were selected from the central and northern regions of Ukraine by visual symptoms. Enzyme-linked immunosorbent assay (ELISA) was used for initial detection of PPV in collected samples of peaches, cherries, apricots, and plums. Polymerase chain reaction (PCR) was proved as a promising and accurate method for detecting viral infections. The advantages of PCR are based on its even higher sensitivity and ability to differentiate the strains of PPV. ELISA-positive samples were also analyzed by RT-PCR with further sequencing of obtained partial amplicons of PPV genome. According to molecular data, D strain (found in samples of apricot, peach, and plum) prevailed in studied regions of Ukraine. Rarely M strain (found in apricot and peach samples) was detected, whereas several samples were infected by more than one strain of PPV. Phylogenetic analysis of sequenced parts of PPV genome confirmed the identity of the strains.

Depending on the strain of the virus, different kinds and varieties of plants can be damaged and losses of crop can significantly vary. Therefore, it is important to determine the diversity of PPV strains and their similarities to other isolates. Establishing the phylogenetic relationships among Ukrainian isolates, as well as their comparison with known strains and isolates of PPV, allows the monitoring of the development of virus spread to/from different regions of Ukraine and the neighboring countries, establishing the origin(s) of the disease, and predicting the occurrence of possible epidemics caused by more aggressive strains.

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‘CHERNOBYL’ ISOLATE OF TMV IN LUPINE: IS THIS THE EFFECT OF RADIOACTIVITY ?

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As communicated earlier, novel strain of *Tobacco mosaic virus* (TMV) has been recovered from lupine plant (*Lupinus perennis*) when monitoring viral infections of wild flora in 30 kilometer estrangement zone of Chernobyl Nuclear Power Plant. It was suggested that alteration in the properties of ‘classical’ TMV could be attributed to mutational effects of chronic radioactivity (1).

We have partially cloned and sequenced genes corresponding to polymerase, coat and movement proteins of ‘Chernobyl’ isolate of TMV and conducted the phylogenetic analysis of these sequences with those available in the Genbank database (TMV isolates sampled from different geographical regions and hosts) (2).

Phylogenetic comparison of all three partially sequenced genes of the ‘Chernobyl’ isolate of TMV demonstrated that this isolate was homologous to those recovered from ‘atypical’ host plants belonging to *Balsaminaceae* and *Oleaceae* families. The ‘Chernobyl’ isolate of TMV was most closely related to IM strain of TMV isolated from *Impatiens* plant and to SXFQ strain, suggesting possible divergence of these isolates from a single common parent form of the virus or convergence resulting from analogous mutations in different phylogenetic lines.Using BEAST 2 software package (3) we showed that tentative timescale of divergence of these three isolates/strains of TMV (‘Chernobyl’, IM, and SXFQ) from a common parent form fluctuated in the range of 50-100 years.

This data may invalidate the hypothesis of the intense radioactivity (which developed after the Chernobyl disaster only 30 years ago) being the driver of virus divergence. However, the statistical confidence of the BEAST 2 results lies within a range of 50 years, suggesting that full-genome analysis of the ‘Chernobyl’ isolate of TMV is essential for making solid conclusions about the role of chronic radioactivity in virus microevolution.

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VIRUSES INFECTING CUCURBITS IN UKRAINE AND THEIR PHYLOGENETIC ANALYSIS

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BACKGROUND and OBJECTIVES

Viral infections on cucurbits may lead to substantial losses. Approximately 60 viruses were reported to infect plants from *Cucurbitaceae* family [1]. The information about genetic diversity of these viruses is limited in Ukraine. Thus, the purpose of this study was to detect viruses infecting cucurbits in Ukraine and evaluate phylogenetic relationships of all obtained isolates.

MATERIALS and METODS

Symptomatic plant samples were collected during 2009-2015 years in different regions of Ukraine. Detection of viral antigens was performed by DAS-ELISA using commercial test systems. Further, total RNA was extracted from plant samples using RNeasy Plant Mini kit (Qiagen, UK). RT-PCR was accomplished using specific primers to coat protein (CP) gene of CMV, nuclear inclusion-coat protein (NIb-CP) genome region of WMV-2 and ZYMV (expected product size – 500 bp, 800 bp, 600 bp respectively). Then obtained amplicons were purified and sequenced using Applied Biosystems 3730x1 DNA Analyzer with Big Dye terminators, version 3.1 (Applied Biosystems, USA). Phylogenetic analysis was conducted using Neighbor-Joining method in MEGA 6.

RESULTS

ZYMV, WMV-2 and CMV were detected in plant samples by DAS-ELISA. Infected plants shared variable symptoms. ZYMV caused yellow mosaics, leaf blade deformation, knobs and malformations of fruits. Using C-terminal part of NIb and N-terminal part of CP for analysis, five ZYMV isolates was defined as members of group A. They were clustered with isolates from Slovenia, Hungary, Czech Republic, Austria and France within subgroup AI. Obtained isolates were characterized with high homology (98-100%).

The symptoms of WMV-2 included dark green mosaic, vein banding and dark mottle on leafs, deformation of fruits and stunting. According to the topology of Neighbor-Joining tree based on sequences of NIb-CP genome region, Ukrainian isolates of WMV-2 belonged to group G1. Obtained isolates were highly homologous (94-99%).

CMV-infected plants developed leaf yellow mosaics, dark green spots, distortion, vein banding, and malformations of fruits. Seven sequences of partial CP gene of CMV were obtained and aligned with other CMV strains from different locations and phylogenetic subgroups. Isolates CMV-514, CMV-1213, and CMV-2114 showed the highest homology (96-99%) with strains from subgroup IA. Homology between isolates CMV-1409, CMV-8, CMV-Ukr-tom2, CMV-Ukr-sq13 and members of subgroup IB was more than 95%. The topology of Neighbor-Joining tree confirmed subgroup attribution. The homology within both subgroups was surprisingly high: >99%.

CONCLUSIONS

Viruses infecting cucurbits in Ukraine belongs to the most frequent phylogenetic groups, which are common for other European countries: CMV-IA, WMV-G1 and ZYMV-A. Moreover, CMV isolates of subgroup IB, which had been originated from East Asia, were detected. The data obtained in this study provides a useful basis for developing effective control strategies for Ukrainian farmers.

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AN INTEGRATED BADNAVIRUS INFECTS BLACKBERRY

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BACKGROUND and OBJECTIVES

A new badnavirus, tentatively named as blackberry virus F (BVF) was discovered in plants displaying blackberry yellow vein disease (BYVD) symptoms. BVF was characterized at the molecular level and several epidemiological attributes including genome integration, distribution, host range, population structure and association with disease symptoms were assessed. This discovery changes the standards in the production of virus-free propagation material as there are not methods developed to eliminate the integrated genomes of badnavirus. A sensitive detection protocol was developed that could assist in the elimination of BVF-infected material from the propagation pipeline.

MATERIALS and METHODS

The isolate used for virus characterization was obtained from a plant from Mississippi showing BYVD symptoms. Phylogenetic and recombination analysis was performed essentially as described in Thekke-Veetil et al. (2013). Twenty herbaceous plant species were tested as alternative hosts for the virus whereas more than 400 BYVD plants, collected from six US States, were assessed for the presence of the virus. The population structure was determined based on 23 isolates. Virus integration was examined as described in Laney et al. (2012).

RESULTS

The dsDNA BVF genome is 7663 base pairs and phylogenetic analysis revealed close relationships with several badnaviruses, indicating that the virus is a new member of the genus. A recombination signal was identified by the majority of the programs used in the analysis. Fourteen plant species can sustain virus replication. BVF in not closely associated with BYVD, but can integrate in the host genome. A RT-PCR protocol, developed based on the studied, fairly homogeneous virus population, is able to detect all BVF isolates studied and may be used for efficient virus screening in research and regulatory settings.

CONCLUSIONS

BVF is a putative new member of the genus *Badnavirus.* The population structure of the virus is homogeneous and recombination may be the driving evolutionary force for the virus. As BVF can integrate in the host genome it can affect the propagation pipeline. Blackberry virus diseases are often caused by virus complexes and infected material with a virus which may never be eliminate from the germline because of its integration in the host genome may serve as the trigger for epidemics as field plants are being infected by additional viruses.

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ELDERBERRY (SAMBUCUS SPP.) AS A MAJOR CONTRIBUTOR OF CARLAVIRUS EVOLUTION

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BACKGROUND and OBJECTIVES

Five new carlaviruses infecting elderberry were characterized at the molecular level. The new viruses form two distinct phylogenetic groups indicative of two speciation events in the host. A universal detection assay that potentially detects all carlaviruses was developed. The implications of the new findings in elderberry propagation and production will be discussed.

MATERIALS and METHODS

Material was collected from the NCGR elderberry germplasm collection in Corvallis, Oregon. Virus contigs were assembled using VirFind (Ho and Tzanetakis, 2014). Sequence gaps were obtained using virus-specific PCR reactions and termini were obtained using 5' and 3' RACE as described previously (Thekke-Veetil et. al., 2013). A universal test able to detect all members of the genus was developed based on sequence data of 37 carlavirus genomes. Phylogenetic analyses were performed on the five new elderberry carlaviruses and all ICTV-recognized carlaviruses and analysis of recombination patterns was performed using RDP4 with default parameters (Martin et al., 2015).

RESULTS

The genomes of these five carlaviruses ranged between 8540-8628 nt excluding the polyadenylated tails. An RT-PCR protocol able to detect all carlaviruses was developed and successfully tested against all carlaviruses infecting elderberry. Elderberry carlaviruses originated from two distinct ancestors with three evolving from one and the remaining from another. The genomes share 42-66% nt identity among themselves and 40-58% to other carlavirus species. One of the viruses was found to be a recombinant whereas another one or its ancestor was predicted to be a parent virus for *Cowpea mild mottle virus*.

CONCLUSIONS

Elderberry has a complex virome. The two elderberry carlavirus groups appear to have emerged from distinct lineages, containing closely related viruses that infect the same host, indicative of sympatric speciation. This, in addition to the recombination analysis, imply that elderberry, is a major contributor to carlavirus evolution. Mixed infections did not allow for the assessment of the role of individual viruses in pathogenicity. Given that the effect of the new viruses in single or mixed infections is unknown and the biological characterization is on-going (Postman, Martin and Tzanetakis, unpublished), current efforts focus on eliminating those viruses from plants used in nursery propagation. The universal detection protocol developed will be of great assistance for the nursery production of elderberry plants as it provides reliable, unbiased, detection which is not affected by the population structure of individual viruses having been developed from the consensus of 37 carlavirus genomes.

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A RECOMBINANT TOMATO YELLOW LEAF CURL VIRUS HAS REPLACED ITS PARENTAL VIRUSES IN SOUTHERN MOROCCO

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BACKGROUND and OBJECTIVES

Tomato yellow leaf curl virus (TYLCV) and Tomato yellow leaf curl Sardinia virus (TYLCSV) are two begomoviruses (Geminiviridae) which cause Tomato yellow leaf curl disease (Tylc) in the Mediterranean countries. The disease has been successfully managed from the end of the 90s with the use of cultivars bearing the tolerance gene Ty-1. In 2010, a new TYLCV/TYLCSV recombinant virus (IS76) was detected on tolerant plants exhibiting the typical Tylc symptoms in Souss, the region with the most intensive tomato production in southern Morocco. The prevalence of IS76 was determined with an intensive survey and its origin was inferred with Bayesian analysis.

MATERIAL & METHODS

As IS76 was not detectable with the tools previously reported to detect TYLCV/TYLCSV recombinants, a multiplex PCR test was designed to determine the infection status of each individual plant. A total of 800 tomato plant samples collected between 1998 and 2014 in the Souss and other tomato producing areas of Morocco were tested for the presence of the Mediterranean Tylc-associated viruses (TYLCV-IL, TYLCV-Mld, TYLCSV-ES) and potentially all TYLCV/TYLCSV recombinants. The date of IS76 recombination event was inferred with BEAST using Genbank sequences of TYLCV and sequences of TYLCV and IS76 generated in this study.

RESULTS

With a 76 nt TYLCSV inherited fragment, IS76 was identified to be different from the canonical TYLCV/TYLCSV recombinant previously reported [(1 & 2)]. It has virtually replaced its parental viruses, (TYLCV-IL, TYLCSV-ES) in the Souss between 2003 and 2012 and has spread northwards up to the Mediterranean coast of Morocco where it was detected in co-infection with parental viruses and canonical recombinants[(3)]. Its detection was not necessarily associated with Tylc symptoms, which was consistent with the fact that Ty-1 tolerant tomato plants remained symptomless following their infection with an infectious clone of IS76. According to phylogeny and Beast inference, the date of the recombination event leading to IS76 was most probably at the end of the 1990s and it emerged in the Souss region.

CONCLUSIONS

This is the first report of a TYLCV/TYLCSV recombinant virus that entirely displaced its parental viruses. As the population shift coincided with the increasing use of tolerant Ty-1 bearing cultivars in the Souss, it was suggested that these plants have positively selected the new recombinant. Drift was not the favored hypothesis because of year round tomato production. Studies to confirm the selective hypothesis IS76 and to explain why its peculiar recombination pattern has not been reported elsewhere are presented in the sister presentation of Z. Belabess.

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EFFECTS OF PEPINO MOSAIC VIRUS AND CUCUMBER GREEN MOTTLE MOSAIC VIRUS ON DROUGHT TOLERANCE IN NICOTIANA BENTHAMIANA

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BACKGROUND and OBJECTIVES

Viruses are often seen as pathogens which cause disease and limit the growth of the host. However, it may be time to rethink that paradigm. Earlier it was observed that a virus infection can induce drought tolerance in a plant and increase its survival (Xu *et al.*, 2008). We wanted to know if a virus infection of a symptomatic (*Pepino mosaic virus*, PepMV) and a non-symptomatic virus (*Cucumber green mottle mosaic virus*, CGMMV) can induce drought tolerance in *Nicotiana benthamiana*, and possibly affect its drought survival.

MATERIALS and METHODS

N. benthamiana plants were inoculated with CGMMV or a mild PepMV strain in Tomato. Negative controls were inoculated with water. Once systemic infection was confirmed by DAS-ELISA, watering was halted for two weeks. Wilting symptoms were scored visually and the daily weight loss of the pot was measured to calculate the transpiration rate per plant. Whole plant thermal pictures were taken to analyse the leaf temperature as an indication of the aperture of stomata. Proline contents were determined from systemically infected leaves 7 and 14 days after the on-set of water starvation, and used as an indicator of water stress.

RESULTS

Drought symptoms were delayed in PepMV-infected plants compared to CGMMV- and non-infected plants. PepMV infection caused mild symptoms, a smaller size, a higher leaf temperature and a lower transpiration rate compared to non-infected plants. The CGMMV-infected plants did not show symptoms and the transpiration rate was similar as non-infected plants. Proline contents were not significantly different between the treatments. PepMV-infected plants had a higher survival rate upon extended drought before re-watering in comparison to non-infected plants.

CONCLUSIONS

PepMV, but not CGMMV, induced an improved drought tolerance in *N. benthamiana*. PepMV infection caused a delay in the appearance of drought symptoms and a clear extension of the period in which the plants could recover from and survive extended drought ('tipping point'). Results, including thermal pictures, indicate that the extended drought tolerance conferred by PepMV is likely based on a lower rate of transpiration and a more efficient use of water.

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FULL GENOME CHARACTERISATION OF 10 TOSPOVIRUSES BY NEXT GENERATION SEQUENCING

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BACKGROUND and OBJECTIVES

The genus *Tospovirus* currently contains 11 officially recognized species (ICTV, 2015) however, more tentative species have been described. For most tospoviruses no specific antisera are available and only partial sequence data have been described. This hampers reliable detection of these agronomical important viruses. To facilitate the development of diagnostics and ensure the future availability of reference isolates the full RNA genomes of 10 different tospoviruses were determined, their sequences and biological data included in Q-bank and the individual isolates included in the physical plant virus collection at Wageningen UR.

MATERIALS and METHODS

Isolates of the different tospoviruses were obtained from plant virus collections from the Dutch NPPO and Wageningen UR and inoculated on indicator plants. Total RNA extracts were used for Illumina RiboZero 125 base paired-ends library preparations with individual MIDs and subsequently run in batchon a HiSeq 2500. After MID splitting individual datasets were fed into custom designed workflows within CLC Genomics Workbench (Qiagen, Denmark). These workflows comprised '*De novo*' and reference-assemblies with and without substraction of plant-related reads. Resulting contigs were analysed by BlastN and BlastX against the NCBI database to identify tospovirus related sequences. Primer walking and 5'- and 3'-RACE and Sanger sequencing was used to determine complete tospovirus RNA sequences.

RESULTS

Most assemblies of the individual tospovirus datasets resulted in near full length RNA segments (L, M and S). A few RNAs were split in two contigs most likely by poor assembly of the hairpin regions. Primer walking and conventional Sanger sequencing resulted in linking contigs enabling assemblies of full length RNA sequences. Full sequences and additional information on the tospoviruses will be made publically available through Q-bank (www.q-bank.eu/virus).

CONCLUSIONS

NGS proofed to be a quick and relatively easy method to determine the complete sequences of a significant number of tospoviruses simultaneously. These sequences can now be used for the development of diagnostics and the identification of viral sequences obtained from plant samples through the virus ID function in Q-bank/virus.

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STRAWBERRY LATENT RINGSPOT VIRUS IN LILY IS SEED TRANSMITTED AND LOCALISED IN THE EMBRYO

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BACKGROUND and OBJECTIVES

Strawberry latent ringspot virus (SLRSV, family *Secoviridae*, not assigned to a genus) has a very broad host range in both dicotyledons and monocotyledons. One of its hosts is Lily (*Lilium spp.*) in which SLRSV does not cause symptoms. However, SLRSV causes economic losses in Lily as infected bulbs cannot be exported to certain countries due to phytosanitary legislations.
Early work on SLRSV in vegetable crops revealed the nematodes *Xiphinema diversicaudatum* and *X. coxi* as vectors (Lister, 1964; Murant, 1974). Also seed transmission was reported in the crops Raspberry, Celery and Mentha, and in weeds such as Henbit dead-nettle (*Lamium amplexicaule*), Chickweed (*Stellaria media*) and Goosefoot (*Chenopodium quinoa*) (Murant, 1974).
The objective of our research was to determine whether SLRSV is seed-transmitted in cultivated Lily.

MATERIALS and METHODS

Experiments were set-up with two SLRSV-infected Lily cultivars which are known as 'Garden Lilies' and are normally propagated by seed. Seed pods were harvested from SLRSV-infected plants, and from a non-infected control plant, dried and kept at 4°C for several months in order to break dormancy. Individual seeds were tested in DAS-ELISA and RT-PCR for the presence of SLRSV. Seeds were also sown in sterilised soil for growing-out tests. In order to localise the virus in the seeds, seeds were dissected. Seed coat, endosperm and embryo were tested for SLRSV separately with RT-PCR.

RESULTS

In a number of individual seeds SLRSV was detected using DAS-ELISA. These results were confirmed by RT-PCR using specific primers for the SLRSV isolate present in the research material.
In the sowing-out test, up to 30% of the young seedlings turned out to be infected.
Localisation of the virus within the seed proved that SLRSV is present in the embryo.

CONCLUSIONS

From our experiments it can be concluded that SLRSV is seed transmitted in Garden Lilly. The virus could be detected within the embryo of the infected seed, and therefore disinfection methods cannot be applied. To what extent this seed transmission contributes to the epidemiology of SLRSV in Garden Lily remains to be established.

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EMERGENCE OF TWO NEW VARIANTS OF THE TORRADOVIRUS, TOMATO NECROTIC DWARF VIRUS, IN CALIFORNIA

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BACKGROUND AND OBJECTIVES

Tomato necrotic dwarf virus (ToNDV) is a whitefly-transmitted virus in the genus *Torradovirus*, family *Secoviridae* that caused significant losses for tomato production in the Imperial Valley of southern California during the 1980s, but was not fully characterized until recently. The virus produces icosahedral virions approximately 30 nm in diameter, and can be transmitted by three whitefly species; *Bemisia tabaci*, *Trialeurodes abutilonea*, and *T. vaporariorum*, as well as mechanically and by grafting. Whitefly transmission was efficient and comparable with both *T. abutilonea* and *B. tabaci* MEAM1, whereas transmission by *T. vaporariorum* was less efficient in laboratory studies. The ToNDV genome is composed of two RNA molecules of 7.2 and 4.9 kb. RNA1 contains a large ORF encoding a 2150 aa polyprotein, whereas RNA2 encodes two ORFs of 189 and 1190 aa, respectively, with the latter also expressed as a polyprotein. These original isolates of ToNDV are most closely related to *Tomato marchitez virus* at 80% and 91% identity for the RNA1 and RNA2 polyproteins, respectively.

MATERIALS AND METHODS

In the fall of 2015, RNA was isolated from nine tomato plants exhibiting typical ToNDV symptoms of stunting, foliar necrosis, and leaf miniaturization from a late season planting in Kern County in the San Joaquin Valley of California, resulting in identification of ToNDV by RT-PCR with confirmation by sequencing. Limited surveys to determine if ToNDV was present elsewhere in California identified a second isolate present in *Datura stramonium* plants growing in an undisturbed area in Imperial Valley near agricultural land. Both isolates were sequenced and compared with the 1980s isolates and to other torradoviruses. Host range analysis and transmission studies are continuing.

RESULTS

Preliminary sequencing of approximately 75% of the genome of the Kern isolated determined the new isolate shared 91-94% identity with the 1980s Imperial Valley isolates (RNA1: NC_027926, RNA2: NC_027927). The datura isolate is much more distantly related to the original isolates, sharing only 80-81% nucleotide identity with the original isolates and producing stunting and yellowing, but less necrosis than is typical of tomato isolates. Tomato plants infected with the Kern isolate were also infected with *Tobacco mosaic virus* based on immunostrip tests. No other viruses were identified from plants infected with the datura isolate.

CONCLUSIONS

The two new ToNDV isolates are geographically distant from one another in distinct production regions. Although ToNDV was a serious problem for tomato production in the Imperial Valley during the 1980s, it had not been seen in fields for nearly 30 years; however, there is currently very little tomato produced in the region. Whitefly populations in the San Joaquin Valley develop near the end of the tomato season, therefore infection of tomato by whitefly-transmitted viruses is not common. Further sequencing as well as biological characterization of both isolates is in progress and may clarify genetic diversity of this California torradovirus.

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PHYLOGENETIC ANALYSIS OF WATERMELON MOSAIC VIRUS IN GREECE

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BACKGROUND and OBJECTIVES

Watermelon mosaic virus (WMV, genus *Potyvirus*, family *Potyviridae*) is a serious pathogen of cucurbit crops with a worldwide distribution. It has a wide host range (>170 species) and it is transmitted non-persistently by several aphid species (3). Previous molecular analyses based on full-length WMV sequences indicated the presence of 3 major groups, namely G1, G2 & G3. Until 2000 in the Mediterranean basin only G1 and G2 groups were observed, with many G1-G2 recombinants. The third group (G3 or ''EM'' for emerging) probably originating from Asia was first observed in south-eastern France in 2000 (2). Despite the occurrence of WMV in Greece at least since 1983, no information was available on its genetic variability.

MATERIAL and METHODS

In 2013-2015, a survey was conducted in 28 geographic areas in Greece for the collection of WMV isolates. Three regions of the virus genome (P1, P3-CI and Nib-CP) were sequenced and used in phylogenetic analysis. Homologous regions from 14 isolates collected in Greece in 1994 and stored as dried leaf material were also included in the analysis (1).

RESULTS

Phylogenetic analyses revealed the presence of G1, G2 (or G2-G1 recombinants) and EM isolates in Greece in 2013-2015. Interestingly, several isolates collected during all three years did not belong to any known molecular group. In contrast, all WMV isolates from 1994 that were analyzed in this study belonged to G1 (or G2-G1) only.

CONCLUSIONS

Our analyses suggest the presence of high diversity and frequent intra-specific recombination in WMV populations in Greece. The identification of EM-group isolates only in our survey and not in samples collected 20 years ago indicates that they have probably been introduced recently in Greece as observed in other countries in the Mediterranean Basin (2). Of special interest are also the isolates identified herein as forming separate molecular groups from those already known. Whether these are endemic or introduced from an unknown origin remains to be determined. Further studies are currently conducted in order to understand the mechanisms of appearance and evolution of variability in WMV populations, as well as its agronomic consequences.

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DETECTION AND DIFFERENTIATION OF *POTATO VIRUS Y* INFECTING POTATO IN THE MSINGA AREA IN KWAZULU-NATAL, SOUTH AFRICA

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BACKGROUND & OBJECTIVES

Accurate identification of plant pathogens is crucial towards initiating control strategies to ensure safe and sustainable agriculture. The aim of this study was to detect and differentiate *Potato virus Y* (PVY) isolates infecting potato (*Solanum tuberosum L*.) in Msinga area in the Province of KwaZulu-Natal, South Africa. Farming plays a huge role in this poverty-stricken community thus keeping hunger at bay. The aim of this study was to detect the different strains of PVY and identify its major strains in several locations of Msinga as well as to analyse full genome sequences of four randomly selected isolates of PVY infecting potatoes in Msinga with hope that this will help in determining if there is any recombination patterns amongst the isolates thus assisting in understanding PVY evolution in Msinga area.

MATERIAL & METHODS

Initial detection of PVY in the leaf samples, collected in different locations of Msinga, was achieved through triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA). PVY-infected samples were further tested to determine their serotypes using antibodies specific to the serotypes O and N. Reverse transcription-polymerase chain reaction (RT-PCR), using primers specific to the coat protein of all PVY, were performed on total RNA of four PVY infected sample randomly selected as a confirmation test. *Nicotiana tabacum* cv Samsun were individually inoculated mechanically with all PVY-infected samples detected with ELISA. RT-PCR confirmation test yielded the expected 512 bp amplicon. Total RNA extracted from PVY-infected tobacco leaves was used as a template for next generation sequencing (NGS). NGS was run on Illumina Hiseq using paired-end chemistry 125x125bp reads De novo was performed according to default settings and the resulting contigs were blasted to the GenBank database and those matching PVY were selected for phylogenetic analysis which was conducted using 39 closely related PVY sequences.

RESULTS

Symptoms on inoculated tobacco were observed from seven days post inoculation and consisted of vein clearing, faint mosaic patterns, yellowing, chlorotic spots and vein necrosis. The veinal necrosis symptom, characteristic of *PVY*^{N}, *PVY*^{N}Wilga and *PVY*^{NTN} strains, was also observed on N*. tabacum* cv Samsun. After virus purification, samples were viewed under an electron microscope and flexuous rod particles that were approximately 730nm long and 12nm wide were observed. Such particles are typical of PVY. The NGS results showed that PVYN Wilga and PVYNTN strains co-exist in this farming region

CONCLUSIONS

Screening techniques confirmed that PVY exists in potatoes grown in Msinga area. It also confirmed that PVY^{N} is the most prevalent strain in Msinga with over 96% existence in the area. NGS proved that the detection of PVY^{NTN} strain dominating in Msinga area is a huge concern for farmers as they rely heavily on farming for their livelihood.

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FIRST REPORT OF CHRYSANTHEMUM STEM NECROSIS VIRUS IN CHRYSANTHEMUMS IN KOREA

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MATERIAL and METHODS

CSNV-Kr was initially isolated from a *Chrysanthemum* plant (cv. 'Jinba') in Gyeongnam province and various diagnostic tools (such as RT-PCR using N gene-specific primers) confirmed the single infection of CSNV-Kr in the plant. Total RNA was extracted from the infected leaf and stem tissues of the chrysanthemum plant infected with CSNV-Kr. Using Illumina HiSeq™ 2500 platform, we obtained the complete genome sequences of CSNV-Kr from analyses of CLC Genomics Workbench 7.0, BLASTn searches and other bioinformatic tools. The complete nucleotide sequences of the L, M and S segments of CSNV-Kr have been deposited to the GenBank (Accession No. LC126116, LC126117 and LC126118, respectively).

RESULTS

The nucleocapsid protein (N) gene of CSNV-Kr was amplified successfully using RT-PCR from a chrysanthemum plant. Simultaneously, the N genes of *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV) were not detected from the chrysanthemum plant. RT-PCR analysis using virus-specific primers for chrysanthemum-infecting viruses reported previously further confirmed that the chrysanthemum plant is singly infected with CSNV-Kr. To determine the complete sequences of CSNV-Kr genomic RNAs, total RNA was subjected to NGS (Illumina HiSeq™ 2500), according to manufacturer's instructions. Our NGS results showed that the complete sequence of CSNV-Kr L RNA is 8959 bp in length, encoding RNA-dependent RNA polymerase (RdRp). And the complete sequence of ambisense M RNA is 4835 bp in length, encoding a non-structural protein (Nsm) in the sense and GcGn glycoprotein precursor in the minus sense. The complete sequence of S RNA is 2936 bp in length, consisting of two ORFs, a non-structural protein (NSs) gene and N gene, in an opposite orientation. The N gene and the translated product N protein of SCNV-Kr share nucleotide (nt) and amino acid (aa) sequence identities of 98.0-98.5 % and 99.2-100.0 % with the corresponding gene and its protein of other CSNV isolates, and lower nt and aa sequence identities of 41.9-77.1 % and 26.1-79.3 % with other tospoviruses respectively, as reported previously (1). In the phylogenic analysis of N proteins of tospoviruses, CSNV-Kr was grouped into other CSNV isolates and classified in the same genetic group as TSWV and INSV (2).

CONCLUSIONS

Biological and molecular properties of CSNV-Kr isolated from a chrysanthemum in Gyeongnam province, Korea, 2013 was characterized in the present study. The sequences of CSNV-Kr RNA were completely determined using NGS and analyzed for phylogenic relationships with those of other tospoviruses. To our knowledge, this study is the first report of incidence and sequence determination of CSNV in Korea.

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TRANSMISSION OF THE FIG MOSAIC DISEASE AGENTS BY *Ceroplastes rusci* L. and *Aceria ficus* Cotte.

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BACKGROUND and OBJECTIVES

Fig mosaic disease is common in different fig growing orchards of Turkey. Up to now, fig (*Ficus carica*) is infected by many different viruses; *Fig leaf mottle-associated virus* 1-2 (FLMaV 1-2), *Fig mosaic virus* (FMV), *Fig mosaic associated virus* 1-2 (FMaV 1-2), *Fig latent virus*-1 (FLV-1), *Fig mild mottle associated virus* (FMMaV), *Fig cryptic virus* (FCV), *Fig badnavirus*-1 (FBV-1), *Arkansas fig closterovirus* 1-2 (AFCV 1-2), *Fig fleck-associated virus (*FFkaV) in different countries of world. It is known that Fig mosaic disease is spread by graft and an eriophyid mite, but not by seed in nature. FMV was determined to be transmitted by *Aceria ficus* Cotte. from fig to fig and *Catharanthus roseus*. We have little knowledge of the Fig mosaic disease agents transmission by other vector. *C. rusci* is a potential vector for many virus transmission in other plants. For this reason, it is investigated that transmission of Fig mosaic disease agents by *C. rusci* and *A. ficus* in this study.

MATERIAL and METHODS

C. rusci and *A. ficus* were observed on donor fig leaves which showing typical fig mosaic symptoms. These leaves were examined under a stereo microscope and first nymphs of the *C. rusci* and *A.ficus* adults were transferred separately on the young leaves of healthy fig seedlings. Donor plants leaves which showing virus symptoms and transmitted plants leaves were analyzed with RT-PCR using 12 fig virus specific primers. All transmission experiment perform with two years/ two replication.

RESULTS

Leaf deformation were observed in transmitted plants after 20 days and 7 weeks whereas no symptoms were observed in control plants for six months. FLMaV-1, FMV and FMaV-1 were determined on the donor plant. While FMV and FMaV-1 were detected on the *A.ficus* transmitted plants, FLMaV-1 was detected with RT-PCR on the *C.rusci* transmitted plants and amplicon has a 352bp size.

CONCLUSIONS

These results are taken as evidence that *C. rusci* is able to transmit FLMaV-1 from fig to fig under experimental conditions. It is first result for transmission of FLMaV-1 by *C. rusci*. In addition to it is determinated once again FMV were transmitted by *A. ficus.*

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EXPRESSION OF SYNTHETIC tasiRNAs: AN ANTIVIRAL STRATEGY IN PLANT BIOTECNOLOGY

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BACKGROUND and OBJECTIVES

MIGS (miRNA-induced gene silencing) technology, based on syn-tasiRNAs [1], has been recently added to the repertoire of RNA silencing-related strategies to suppress gene expression, which also includes more classical approaches based on sense or inverted repeat RNAs and amiRNAs [2]. MiR173-triggered production of tasiRNAs can be settled in heterologous plants, such as *Nicotiana benthamiana*, and has been used to engineer single or multiple copies of synthetic tasiRNAs (syn-tasiRNAs), which are able to silence endogenous genes.
In this study we wanted to gain insight into general and particular features of syn-siRNAs generated by MIGS and to explore the antiviral potential of this technology, using as experimental system the infection of *Plum pox virus*, a positive strand RNA virus of the genus *Potyvirus.*

MATERIAL and METHODS

We engineered the TAS1c locus to impair Plum pox virus (PPV) infection by replacing the five native tasiRNAs with two 210-bp fragments from the CP and the 3´NCR regions of the PPV genome. We used a transient agro-infiltration assay in *Nicotiana benthamiana* to assess the small RNA species produced by both constructs *in planta* by deep sequencing analyses. The antiviral effect of both constructs was also evaluated in the presence or absence of miR173.

RESULTS

We found that phased processing of the syn-tasiRNAs is construct-specific. While in syn-tasiR-CP construct the processing was as predicted 21-nt phased in register with miR173-guided cleavage, the processing of syn-tasiR-3NCR is far from what was expected. A 22-nt species from the miR173-guided cleavage was a guide of two series of phased small RNAs, one of them in an exact 21-nt register, and the other one in a mixed of 21-/22-nt frame. In addition, both constructs produced abundant PPV-derived small RNAs in the absence of miR173 as a consequence of a strong sense post-transcriptional gene silencing induction. The antiviral effect of both constructs showed that the impairment of PPV infection was not significantly higher when miR173 was present.

CONCLUSIONS

The results show that syn-tasiRNAs processing depends on construct-specific factors that should be further studied before the so-called MIGS technology can be used reliably.

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