16th ISPVE

International Symposium of Plant Virus Epidemiology

June 30th to July 3rd, 2025

Cásper Líbero | São Paulo - BR

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Fundecitrus Ciência e Sustentabilidade para a Citricultura

ISBN AND PUBLICATION INDEX

16th International Symposium of Plant Virus Epidemiology
June 30th to July 3rd, 2025 | São Paulo, SP, Brazil

Technical Edition
Marcelo Eiras & Juliana Freitas-Astúa

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PRESENTATION

The city of São Paulo, Brazil will host, in June 2025, the 16th edition of the International Symposium of Plant Virus Epidemiology – 16th ISPVE-São Paulo 2025. The theme of the symposium will be: "Epidemiology and management of plant viruses and their vectors: a challenge for agriculture in the 21st century", focusing on ecology, epidemiology, and pathogen-host-vector interactions.

The event in São Paulo (Brazil) will bring together specialists on plant virus epidemiology and other vector-transmitted plant pathogens for four days and will be an excellent opportunity to present and discuss scientific advances in this area. It will also be a chance to promote and stimulate debates and reflections on the global social and economic consequences of plant virus epidemics and the challenges for the 21st century to solve problems in an intelligent, efficient, and sustainable way.

The event will feature oral and poster presentations, involving the topics listed above. The activities will begin, each day, with the presentation of a topic by a prominent researcher in the area, followed by oral presentations, previously selected from all abstracts submitted for this topic. Each day, there will be time allocations for personal discussions to encourage collaboration among the Symposium participants. In addition, on the last day of the event, participants will have the opportunity to visit research institutions and cultivation areas to learn about research and agriculture in Brazil.

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COMMITTEES

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PROGRAMME

June 30th

Schedule	Activities
07:30 - 08:30	REGISTRATION/WELCOME RECEPTION
08:30 - 09:00	OPENING CEREMONY
09:00 - 10:00	**OPENING LECTURE: Behavioural aspects influencing plant pathogen transmission by insect vectors** *Speaker: DR. ALBERTO FERERES - ICA-CSIC, Spain*
10:00 - 10:45	COFFEE BREAK AND POSTER EXHIBIT
10:45 - 12:15	**Session 1:** GENERAL EPIDEMIOLOGY
10:45 - 11:15	**KEYNOTE LECTURE 1 : Epidemiology-based strategies for important vector-borne citrus diseases in Brazil: Leprosis and Huanglonbing** *Speaker: DR. RENATO BEOZZO BASSANEZI - Fundecitrus, Brazil*
11:15 - 12:15	**ORAL PRESENTATIONS**
12:15 - 14:00	**LUNCH AND POSTER EXHIBIT (LOCAL)**
14:00 - 19:00	**Session 2:** PATHOGEN-VECTOR-INTERACTIONS
14:00 - 14:30	**KEYNOTE LECTURE 2 : From vectors to victims: how pathogen acquisition changes vector biology** *Speaker: DR. KERRY E. MAUCK - University of California, USA*
14:30 - 16:00	**ORAL PRESENTATIONS**
16:00 - 16:45	**COFFEE BREAK AND POSTER EXHIBIT**
16:45 - 17:45	**ORAL PRESENTATIONS**
17:45 - 19:00	**POSTER SESSION P1**
19:00 - 20:00	Welcome Cocktail

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July 1st

Schedule	Activity
08:30 - 12:15	**Session 3:** VIRUS DISCOVERY AND ROLE OF METAGENOMICS IN EPIDEMIOLOGY
08:30 - 09:00	**KEYNOTE LECTURE 3: Data mining in the new era of virus discovery** *Speaker: DR. NICOLÁS E. BEJERMAN - CONICET-INTA, Argentina*
09:00 - 10:00	**ORAL PRESENTATIONS**
10:00 - 10:45	**COFFEE BREAK AND POSTER EXHIBIT**
10:45 - 12:15	**ORAL PRESENTATIONS**
12:15 - 14:00	**LUNCH AND POSTER EXHIBIT**
14:00 - 16:00	**Session 4:** DISEASE MANAGEMENT AND RESISTANCE
14:00 - 14:30	**KEYNOTE LECTURE 4: Advancing Sustainable Virus Disease Management in Sub-Saharan Africa: Insights from Yam Mosaic Virus Control** *Speaker: DR. LAVA KUMAR - IITA, Nigeria*
14:30 - 16:00	**ORAL PRESENTATIONS**
16:00 - 16:45	**COFFEE BREAK AND POSTER EXHIBIT**
16:45 - 20:00	**MASP VISIT TOUR**

July 2nd

Schedule	Activity
08:30 - 12:00	**Session 5:** VIRUS ECOLOGY AND EVOLUTION
08:30 – 09:00	**KEYNOTE LECTURE 5: Changing environments and variations in the rates of plant virus evolution** *Speaker: DR. SANTIAGO ELENA – CSIC-UV, Spain*
09:00 – 10:00	**ORAL PRESENTATIONS**
10:00 – 10:45	**COFFEE BREAK AND POSTER EXHIBIT**
10:45 – 12:00	**ORAL PRESENTATIONS**
12:00 – 13:30	**LUNCH AND POSTER EXHIBIT**
13:30 – 15:30	**Session 6:** OTHER VECTOR-BORNE DISEASES

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Schedule	Activity
13:30 – 14:00	**KEYNOTE LECTURE 6: Biological and multi-omics interrogation of psyllid-Liberibacter interactions** *Speaker: DR. JUDITH K. BROWN – University of Arizona, USA*
14:00 – 15:30	**ORAL PRESENTATIONS**
15:30 – 16:00	**COFFEE BREAK AND POSTER EXHIBIT**
16:00 – 17:00	**Session 7:** CLIMATE AND EPIDEMICS
16:00 – 16:30	**KEYNOTE LECTURE 7: Challenges with insect-transmitted viruses infecting crop plants in a changing climate** *Speaker: Dr. ANDERS KVARNHEDEN – Swedish University of Agricultural Sciences, Sweden*
16:30 – 17:00	**ORAL PRESENTATIONS**
17:00 – 18:30	BUSINESS MEETING
19:00 – 23:00	**CLOSING DINNER:** **Tickets available (not included at ISPVE Registration)

July 3rd

Schedule	Activity
07:30 - 19:00	**Field trip: Sakata Seed Experimental Station – Vegetable diseases**
07:30 – 19:30	**Field trip: Citrus Research Center "Sylvio Moreira" (CCSM) and citrus diseases in commercial orchards**

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LECTURES

OPENING LECTURE: Behavioural aspects influencing plant pathogen transmission by insect vectors
Speaker: DR. ALBERTO FERERES – ICA-CSIC, Spain

Behavioural aspects influencing plant pathogen transmission by insect vectors

Alberto Fereres
ICA-CSIC, Madrid, Spain.

Major insect vectors of plant viruses include aphids, whiteflies and mealybugs (Hemiptera: Sternorryncha) together with thrips (Thysanoptera). On the other hand, psyllids (Hemiptera: Psyllidae) together with spittlebugs and sharpshooters (Auchenorryncha: Cicadomorpha) are the major vectors of devastating diseases caused by plant pathogenic bacteria such as Candidatus *Liberibacter spp. or* Xylella fastidiosa*. Host plant recognition by insect vectors requires a series of steps that are linked to plant pathogen transmission, including host searching or pre-alighting behaviour, probing on superficial plant tissues, settlement and stylet penetration to the target feeding tissues and salivation or egestion followed by continuous sap ingestion from the preferred feeding sites (mesophyll, phloem or xylem tissues). My presentation will focus on how vector behaviour modulates the transmission and spread of plant viruses depending on the mode of transmission (non-persistent versus persistent). The role of aphids and whiteflies as vectors of plants viruses and their particular probing and feeding behavioural processes leading to the transmission of cuticula-borne and circulative viruses will be discussed. The transmission mechanisms of xylem-limited and phloem-limited bacteria will be also described. Plant-mediated indirect and direct effects of plant viruses on vector behaviour such as changes in the attractiveness, settlement or feeding preference together with changes on vector fitness will be discussed. My presentation will also focus on which are the most likely retention sites within the aphid's mouthparts of noncirculative-transmitted plant viruses and novel ways to interfere with the transmission process.*

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KEYNOTE LECTURE 1 : Epidemiology-based strategies for important vector-borne citrus diseases in Brazil: Leprosis and Huanglonbing
Speaker: DR. RENATO BEOZZO BASSANEZI - Fundecitrus, Brazil

Epidemiology-based management strategies for important vector-borne citrus diseases in Brazil: Leprosis and Huanglongbing

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Citrus leprosis, caused by citrus leprosis virus C (CiLV-C), and Huanglongbing (HLB), caused by the phloem-restricted bacterium Candidatus *Liberibacter asiaticus (CLas), are the main vector-borne citrus diseases in Brazilian citriculture. Aspects of leprosis pathosystem make feasible the disease control measures applied only within the citrus orchards to prevent secondary infections. CiLV-C is transmitted only by the active phases of the flat mite* Brevipalpus yothersi *and the virus has very few alternative hosts besides citrus. The virus-vector relationship is persistent circulative, and the virus can persist in the vector for 12 days. CiLV-C is not systemic, and the virus can be acquired only on the lesioned tissues. Therefore, the disease spatial-temporal progress is totally dependent on the presence of inoculum and the mite population inside the orchard. The disease incubation period is relatively short (2 to 5 weeks). High lesioned fruits and leaves fall prematurely, and the inoculum accumulates from one season to the next mainly on affected branches. Thus, the harvest of all the fruits and pruning of symptomatic branches are efficient to decrease the disease progress rate and reestablish the health of diseased tree. Mite cycle varies from 15 to 35 days, and its population increases in dryer and warmer periods during fruit growth. The mite prefers fruits for feeding and rearing and can be distributed internally and externally throughout the tree. Active mite dispersal is limited to the closest neighbor trees, with a spatial dependence of 30 m among symptomatic tree and infective mites. These characteristics are useful to design protocols for the mite monitoring and the aggregated spatial distribution pattern of leprosis can be used for a rational control with acaricides. On the other hand, aspects of HLB epidemiology make its control very difficult. CLas is transmitted by the Asian psyllid citrus* Diaphorina citri *and is systemic. The bacteria rapidly spread to actively growing tissues such as new shoots, developing roots, and fruits, rendering canopy pruning or thermotherapy ineffective for curing the tree. The disease latent period (~14 days) much shorter than the incubation period (≥4 months) allows infected trees to serve as an inoculum source months before they are detected what makes the disease eradication almost impossible. Inoculum sources outside commercial orchards and the long-distance dispersal of the vector makes the disease primary spread as or more important than the secondary spread for the epidemic progress, resulting in almost no effect of removing diseased trees only within commercial orchards on reducing the disease incidence. Psyllids prefer new shoots to feed on, which requires a greater frequency of insecticide sprays to keep a protective barrier to prevent the bacteria transmission during the development of new leaf tissues. Secondary infections are almost fully controlled with fortnightly insecticide sprays that break the development of psyllid life cycle on infected trees and kill adult psyllids that acquired the bacteria on diseased trees before the latency period of the bacteria in the vector was concluded. In turn, primary infections by infective psyllids that were raised in diseased trees and moved to commercial orchards are partially controlled even with weekly insecticide sprays during the vegetative flushing period (poor coverage, rain-wash, growth of unprotected new tissues). The decreasing gradient of the psyllid and diseased trees populations from the orchard edge to the center has been directed the inspections for the vector and diseased trees, as well as the application of measures that reduce the primary dispersion to the interior of the property, such as: planting density and direction, planting of varieties with different vigor, trap-crop, more intensive applications of insecticides and repellent kaolin.*

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The seasonality of the psyllid population and the period of greater disease symptom expression have directed the periods of highest frequency of insecticide application and actions to detect symptomatic trees, respectively. However, all mentioned measures can partially control the primary infections, and their efficacy is dependent on the amount of primary inoculum. Thus, for the effective HLB control, citrus growers must control the vector and reduce inoculum sources both inside and outside the orchards in a coordinated and joint way, which still difficult to achieve in practice due to their diverse profile. Good disease control was achieved where regional and integrated management was carried out, and this is the way until more effective and sustainable measures, such as resistant citrus cultivars, are available.

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KEYNOTE LECTURE 2 : From vectors to victims: how pathogen acquisition changes vector biology
Speaker: DR. KERRY E. MAUCK - University of California, USA

From vectors to victims: how pathogen acquisition changes vector biology

Kerry E. Mauck
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Hemipteran insects are ideal vectors for plant viruses, which rely on the presence of intact cells for invasion and proliferation within hosts. During the process of infection, plant viruses can drastically alter the very same chemical and nutritional aspects of the host that mediate interactions with phloem-feeding insects. Thus, by virtue of their tremendous capacity to serve as efficient virus transporters, hemipteran vectors are frequently subjected to rapidly shifting plant suitability and palatability following host selection and virus transmission. Recognition of this challenge has led researchers to study how virus-induced changes in host-plant phenotypes influence subsequent numerical and behavioral responses by vectors, and thereby, virus fitness. As a result of this body of work, we now have evidence that plant viruses can manipulate specific host traits in ways that enhance their own transmission. This work is providing new insights into virus evolution and hemipteran feeding and plasticity, but we still lack information about the robustness of virus effects across pathosystems. Using several model systems under study in my laboratory, I will discuss the ways in which virus effects on host chemistry and vector behavior vary according to virus transmission mode, relate this to the evolution of manipulative traits, and explore the constancy of virus-induced signals over time and environmental variation.

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KEYNOTE LECTURE 3: Data mining in the new era of virus discovery
Speaker: DR. NICOLÁS E. BEJERMAN - CONICET-INTA, Argentina

Data mining in the new era of virus discovery

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Data mining of publicly available databases is an invaluable tool for virus discovery. Novel resources, such as Serratus, are crucial to speed up the process of virus hunting, illustrating the key importance of openscience practices to gain knowledge on the unexplored virosphere. I will present our analysis of publically available data on neglected plant virus families, as well as on underexplored hosts, such as gymnosperms, in order to fill gaps in the global virus "dark matter" linked to plants. I will describe our efforts oriented to uncovering the plant virosphere hidden diversity illustrating the significance of the analysis of NCBI-SRA public data as a valued tool not only to fasten the discovery of novel viruses but, also, to increase our understanding into their evolution and to improve virus taxonomy. Some examples of our results on data mining analysis will be presented. Moreover, I will present evidence that the increased number of virus sequences deposited in publicly available databases not only will aid to develop new tools to ease the discovery of distant viruses, but also will allow to deepen our understanding on virus emergence and spread. Finally I will discuss the current challenges and opportunities associated to data mining as the new standard for virus discovery.

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KEYNOTE LECTURE 4: Advancing Sustainable Virus Disease Management in Sub-Saharan Africa: Insights from Yam Mosaic Virus Control
Speaker: DR. LAVA KUMAR - IITA, Nigeria

Advancing Sustainable Virus Disease Management in Sub-Saharan Africa: Insights from Yam Mosaic Virus Control

Lava Kumar PULLIKANTI1, Aighewi Beatrice2, Balogun Morufat1, Kolade Olufisayo1, Djana Mignouna3, Asrat Amele2
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Successful virus disease management in sub-Saharan Africa (SSA) has largely relied on resistant crop varieties, as demonstrated by the control of cassava mosaic begomoviruses in cassava and maize streak mastrevirus in maize. However, managing many other viral diseases remains challenging due to limited grower awareness, insufficient research funding, and inadequate scaling of solutions. This presentation focuses a decade long effort to device effective management for the control of yam mosaic virus (YMV, genus Potyvirus*), a major threat to yam (*Dioscorea *spp.) production in West Africa. In the absence of YMV-resistant varieties, YMV management efforts rely heavily on clean seed systems to mitigate yield losses. However, rapid YMV reinfection of clean yam seedlings in the field undermines the effectiveness of the clean seed-system based approach. Here, we report on how understanding YMV epidemiology and the introduction of rapid clean seed propagation technologies promise to prevent yield losses due to YMV. We also discuss reinfection rates, YMV-induced seed degeneration, and integrated approaches to reducing field-level infections. Lastly, we present key lessons for developing sustainable virus disease management strategies in SSA.*

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KEYNOTE LECTURE 5: Changing environments and variations in the rates of plant virus evolution
Speaker: DR. SANTIAGO ELENA - CSIC-UV, Spain

Changing hosts and variations in the rates of plant virus evolution

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It is assumed that host genetic variability for susceptibility to infection will necessarily condition the rates of virus evolution, either by driving them to diversification into strains that track the different host defense alleles (e.g., antigenic diversity), or by canalization to infect only the most susceptible genotypes. Associated to these processes, virulence may or may not increase. To tackle these questions, we performed evolution experiments with turnip mosaic virus (TuMV) in genotypes of Arabidopsis thaliana differing in their susceptibility to infection. TuMV lineages evolved in A. thaliana mutants in genes involved in resistance pathways and in genes whose products are essential for potyviruses infection. Plant genotypes classified into three categories: hypersusceptible (severer symptoms), equal to wildtype and hyposusceptible (weaker or no symptoms). We found that evolution proceeded faster in hyposusceptible hosts than in hypersusceptible ones. Most of the phenotypic differences shown by the ancestral virus across host genotypes were removed after evolution, suggesting the combined action of selection and drift. When all evolved viral lineages were tested in all plant genotypes used in the experiments, we found compelling evidences that the most restrictive plant genotypes selected for more generalist viruses, while more permissive genotypes selected for more specialist viruses. Sequencing the genomes of the evolved viral lineages, we found that selection targeted the multifunctional protein VPg in most host genotypes.

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KEYNOTE LECTURE 6: Biological and multi-omics interrogation of psyllid-Liberibacter interactions

Speaker: DR. JUDITH K. BROWN - University of Arizona, USA

Biological and functional genomics interrogation of psyllid vector-"Ca" Liberibacter interactions in divergent coevolved pathosystems

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*"*Candidatus *("Ca.") Liberibacter solanacearum" (CLso) is the causal agent of zebra chip and vein-greening diseases of potato and tomato, respectively, and is transmitted by the potato psyllid (PoP)* Bactericera cockerelli *(Sulc.) in a circulative, propagative manner. Both similar and distinct biological characteristics are shared by the citrus greening disease pathosystem, "Ca." Liberibacter asiaticus (CLas) and its' vector the Asian citrus psyllid (ACP). Previous studies have revealed that "Ca. Liberibacter"-psyllid pathogenesis involves gut invasion and propagation, and salivary glands-mediated acquisition processes implicated perturbation of physiological processes in the ACP late-nymphal and teneral adult stages that are biologicallyassociated with pre- and/or post-acquisition of CLas. Although PoP also supports a circulative, propagative transmission mode, both immature and adult life stages may be capable of acquiring and transmitting CLso, suggesting the gut-blood-SGs barriers and basis for pathogen-vector specificity may be distinct for the two pathosystems. To elucidate organspecific mechanisms involved in "Ca." Liberibacter spp. invasion of the psyllid host gut and in salivary glands-acquisition, comparative transcriptomic, proteomic, yeast di-hybrid, and/or TEM analyses have been undertaken. A major goal of functional genomics efforts has been to identify pathogenesisrelated genes and utilize Gene Ontology and KEGG pathway-prediction tools to develop a model that characterizes gut invasion and salivary glandsacquisition. Results indicated that "Ca"Liberibacter utilizes psyllidhost/vector endo-exocytotic pathways and enlist cytoskeletal remodeling to invade, colonize, and exit the psyllid gut in both pathosystems. Gut and salivary glands analyses revealed over- and under-expression of genes in immature and/or adult stages, associated with responses of various physiological processes to "Ca"Liberibacter-infection. Intracellular stationary and motile lifestyles appear to be essential for "Ca Liberibacter" navigation psyllid host organs and tissues. Potentially, differences in defense and immunity genes/pathway responsiveness to infection contribute to the extent of early or late-instar stage processes that lead to systemic infection of the ACP compared to PoP hosts. New knowledge about molecular and cellular interactions involved in Liberibacter-psyllid vector circulative invasion and acquisition processes that culminate in transmission will further the understanding of fundamental aspects, processes, and mechanisms governing transmission of fastidious bacteria that co-evolve with their homopteran insect vectors. Also, identification of psyllid genes required for Liberibacter-gut and salivary glands invasion can guide the identification of gene targets by dsRNA-mediated knockdown (reverse genetics) to validate predicted gene functions, and potentially advance the development of RNAinterference biopesticide technology.*

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KEYNOTE LECTURE 7: Challenges with insect-transmitted viruses infecting crop plants in a changing climate
Speaker: Dr. ANDERS KVARNHEDEN - Swedish University of Agricultural Sciences, Sweden

Challenges with insect-transmitted viruses infecting crop plants in a changing climate

Anders Kvarnheden
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Consequences of climate change are now visible throughout the world with extreme weather events of long dry periods or heavy rainfall. Climate change has severe consequences for agricultural production, including the cultivation of crops. When it comes to outbreaks of virus diseases of crops, they are often associated with changes in growing conditions or agricultural practices. Most plant-infecting viruses have insect vectors, such as aphids, whiteflies, leafhoppers, planthoppers or thrips. The epidemiology of the insect-transmitted viruses is to a large extent determined by the activities and biology of the vectors that are strongly affected by the weather conditions. As a consequence of climate change, the vegetative period in northern Europe has been prolonged, which means increased possibilities for cultivation of new crops on a commercial scale, such as maize and grapevine. However, warmer and longer autumns have also resulted in higher incidence of infection with turnip yellows virus in oilseed rape and regular outbreaks of yellow dwarf viruses in winter cereals. In addition, if mild winters allow the aphids to overwinter as adults, it will increase the risk for early infections of different spring crops. For predicting future virus disease outbreaks caused by climate change and to mitigate the potential effects of them, it is important that we generate a basic understanding of the presently occurring viruses with identification of their different hosts and vectors as well as other important factors determining their spread. This knowledge is currently often lacking.

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ORAL PRESENTATIONS

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Session 1: GENERAL EPIDEMIOLOGY

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COMPLEXITY OF CITRUS LIBERIBACTER DIAGNOSTICS AND PSYLLOID VECTOR SURVEILLANCE IN AFRICA

Glynnis Cook1, Rochelle de Bruyn1, Muriel Rikhotso1, Rachelle Bester2,4, Hans J. Maree2,4, Aruna Manrakhan1,3, Evans Mauda1

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ABSTRACT:

Citrus Greening or Huanglongbing (HLB) in Africa is associated with three related bacteria; *Candidatus* Liberibacter asiaticus (CLas), *Ca.* L. africanus (CLaf) and *Ca.* L. africanus subsp. clausenae (CLafCl). CLaf is present in central, eastern and southern African countries. Wherever CLaf is present in Africa, it is spread by the indigenous psylloid - African citrus triozid (ACT), *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae). CLas was first reported on the African continent, in Ethiopia, in 2010. Five years later, there was a report of CLas in Uganda followed by a report from Tanzania. In 2017, a South African-led survey in East Africa investigated these reports and found that CLafCl was misidentified as CLas at the time. Subsequently, CLafCl, was detected widely in Uganda, Ethiopia and Kenya. The presence of CLas in Ethiopia was confirmed. The report of CLas in Kenya is currently being investigated. CLas is naturally vectored by *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), commonly known as Asian Citrus Psyllid (ACP) and to date was detected in African countries spanning both East and West Africa. Efforts to detect ACP incursions and to monitor populations of ACT as well as associated Liberibacters, in citrus environments, were initiated in southern Africa in 2021. Trapping and active collections indicated the presence of a range of triozid species, morphologically similar to ACT. Morphological characterisation and molecular phylogeny supported the distinction of seven triozid species including ACT. CLaf was only detected in ACT, and CLas was not detected. Our results also suggest the presence of other triozid species in other African countries which were misidentified for ACT. We developed tools to accurately identify ACT, a citrus pest with vectoring capacity for both CLaf and CLas, to distinguish it from closely related non-pest, indigenous species. Similarly, we detected undescribed, indigenous *Diaphorina* spp., morphologically similar to ACP, some of which harbour Liberibacter spp. detected by diagnostic assays for CLas and CLaf. Trapping and testing of psylloids for Liberibacters is complex in the African context, given the natural diversity of psylloids and their rutaceous hosts which carry related Liberibacters. Given that assays used to detect HLB pathogens differ in their ability to differentiate the citrus pathogens from other, related bacteria present in psylloids, an integrative assay approach is currently being followed.

KEYWORDS: Huanglongbing; *Candidatus* Liberibacter; psylloid vectors

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CARRIER (SC) TREES: THE EFFECT ON PRODUCTION AND GENETIC DIFFERENCES BETWEEN ASBVD SC RELATED STRAINS

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ABSTRACT:
Avocado sunblotch viroid (ASBVd) is an infectious RNA molecule that induces avocado sunblotch disease (ASBD) in avocado (*Persea americana* Mill.). Symptomless carrier (SC) trees play a significant role in the epidemiology of ASBVd, serving as primary sources of infection by spreading the disease through budding and grafting practices. In this study, two separate experiments were conducted to (i) investigate the impact of ASBVd-infected SC trees on tree morphology, fruit maturity, yield, and quality of 'Haas' avocado, and (ii) detect molecular differences between ASBVd variants within a clonal population of symptomless 'Fuerte' avocado plants. It was discovered that flower overbearing with the shedding of leaves and lower yields can be used as indicators of ASBvd infection in 'Haas' orchards but molecular diagnostics is required. Yield counts indicated that medium and highly infected trees produced between 83 and 96% lower yields compared to healthy trees. Whole genome sequences of ASBVd variants were obtained through conventional RT-PCR and 42 variants were identified. The sequence lengths of the obtained variants varied between 248 and 253 nucleotides (nt). The sequences of these variants were deposited into the NCBI GeneBank database and assigned accession numbers ON135462 to ON135503. Phylogenetic analysis of the variants revealed a high sequence identity of 97% with reference ASBVd variants obtained from the GeneBank database. These findings from this study can be incorporated into a ASBVd management strategy in 'Haas' orchards and the sequences are crucial for accurate detection of ASBVd.
KEYWORDS: Avocado sunblotch disease/viroid; Avocado; ASBVd variants

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DYNAMICS OF SINGLE AND MIXED YELLOW DWARF VIRUS INFECTIONS IN CEREAL AND GRASS HOSTS IN AUSTRALIA

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ABSTRACT:
Yellow dwarf viruses (YDVs) are significant pathogens that reduce cereal yields, with mixed infections often increasing disease severity. This study examined the prevalence of single and mixed YDV infections in symptomatic cereal and grass plants in Victoria, Australia, over three years. Symptomatic plants from cereal fields and surrounding areas were tested using tissue-blot immunoassay for multiple YDV species and related viruses. Results showed that 34% of virus-positive plants had mixed infections, with wheat (47%) and wild oat (36%) exhibiting higher proportions than barley (8%) and brome grass (17%). The overall prevalence of mixed infections was nearly four times higher than reported in a similar study from 1985. While the proportion of mixed infections remained consistent across years, host species significantly influenced infection rates. These findings highlight the complex epidemiology of YDVs and the challenges in breeding virus-resistant cereals, emphasizing the need for ongoing surveillance to inform effective disease management strategies.
KEYWORDS: Yellow dwarf viruses; mixed infections; surveillance

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EMERGENCE AND RE-EMERGENCE OF VIRAL PATHOGENS IN COTTON: AN ENIGMATIC ISSUE FOR COTTON PRODUCERS AND RESEARCHERS IN GEORGIA, USA

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ABSTRACT:
Since 2018, cotton growers belt wide have experienced a complex disease characterized by diverse symptoms often associated with biotic and abiotic factors. A key focus was the association of the symptoms with a pathogen eventually identified as cotton leafroll dwarf virus (CLRDV) causing “cotton blue disease” in South America. Over the next two years, intensive research was conducted on its genomic, epidemiological and potential impact on yield and quality in Georgia and other cotton-growing regions in the USA, in an effort to develop management studies. Following a period during which the disease symptoms diminished, it was challenging to detect the virus in GA. The disease reemerged in 2024 with intensified severity in late planted cotton in many commercial fields in South Georgia, causing renewed concern among stakeholders. The symptoms are widespread across numerous counties, closely resembling those of previously known bronze wilt, a physiological issue without any known pathogen. The detection of CLRDV in these symptomatic leaves and plant tissues since 2018 has led to a renewed designation as "CLRDV-induced bronze wilt." Additionally, in early 2023, stakeholders reported terminal abortion and stem bifurcation in some cotton plants from the belt wide, with the detection of caulimoviridae-like sequences in symptomatic plants. These caulimoviridae-like sequences were also detected and confirmed in Georgia. These emerging and reemerging virus diseases highlight the enigmatic nature of the cotton diseases affecting Georgia and the wider cotton belt.
KEYWORDS: Cotton leafroll dwarf virus; Cotton blue disease; Enigmatic

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Session 2: PATHOGEN-VECTOR-INTERACTIONS

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IMPACT OF THE ENTOMOPATHOGENIC FUNGUS *METHARIZHIUM BRUNNEUM* ON THE FEEDING BEHAVIOR OF *PHILAENUS SPUMARIUS* ON OLIVE PLANTS: IMPLICATIONS FOR *XYLELLA FASTIDIOSA* MANAGEMENT

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ABSTRACT:

The meadow spittlebug, *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae), is the primary vector of *Xylella fastidiosa* in Europe, posing a serious threat to several economically important crops such as olives, grapevines and almonds. Given the limitations of chemical control measures, alternative strategies are needed for sustainable vector management. Entomopathogenic fungi (EF), have emerged as promising biological control agents due to their ability to infect insects both directly and through endophytic colonization of host plants. This study evaluates the endophytic colonization of *Metharizhium brunneum* strain EAMa 01/58-Su in olives, and the feeding behavior and survival of *P. spumarius* when exposed to EF-treated olive plants. The Electrical Penetration Graphs (EPG) technique was used to characterize changes in spittlebug probing and feeding behavior after foliar and root-drenched application of *M. brunneum* on olive plants. Our results demonstrate that direct foliar application significantly altered *P. spumarius* feeding, leading to an increase in the number of total probes, number of unsuccessful probes and number of non-probing (np) events, an increased np duration and a reduced xylem contact (Xc) duration. Additionally, the time required to perform a successful probe (with xylem ingestion) was significantly longer on EF-treated leaves. However, no significant differences in insect mortality were observed after short-term exposure. This study highlights the potential of *M. brunneum* as a biocontrol agent and suggests its application in integrated pest management (IPM) programs targeting *P. spumarius*.

KEYWORDS: Biological Control; Integrated Pest Management (IPM); plant pathogenic bacterium

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POPULATION DYNAMICS OF WHITEFLIES AND TRANSMITTED VIRUSES IN SÃO PAULO, BRAZIL

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ABSTRACT:
In Brazil, the whitefly *Bemisia tabaci* is considered one of the most important pests and vectors of significant viruses affecting tomatoes, peppers, soybeans, and beans. Brazil has a long history with whiteflies, first detected in Bahia in 1928. Native whiteflies were not regarded as important virus vectors, with only sporadic problems associated with viruses infecting beans during the 1970s. The dynamics of the insect population changed dramatically following the introduction of the Middle East-Asia Minor 1 (MEAM1), formerly known as the "B biotype" and *B. argentifolii* Bellows & Perring, as well as the Mediterranean (MED), formerly the "Q biotype" and considered the *B. tabaci* sensu stricto. MEAM1 and MED are the most invasive within this complex and are widespread globally. Both are efficient plant virus vectors, contributing to the spread, establishment, and epidemics of many destructive viruses. MEAM1 was responsible for the first outbreak of begomovirus infecting tomatoes in Brazil. Ten years have passed since MED was first reported in São Paulo State, associated with ornamental plants. Two years later, outbreaks of the insect were often observed in pepper greenhouses, bringing significant problems to this crop. Its migration to soybean open field conditions was reported in 2021. Since then, we have conducted studies regarding virus transmission by four different populations of MED and one of MEAM1. Tomato plants infected by tomato severe rugose virus (ToSRV), tomato rugose mosaic virus (ToRMV), the crinivirus tomato chlorosis virus (ToCV) and beans infected by the cowpea mild mottle virus (CPMMV) and bean golden mosaic virus (BGMV) were used as inoculum sources for the transmission tests. Nonviruliferous adults of MEAM1 and MED were separately placed into insect-proof cages containing the infected source plants for a virus acquisition access period (AAP) of 24h. After the AAP, 10 adult insects per seeding were transfered to cages containing healthy tomatoes and beans plants for an inoculation access period (IAP) of 24h. After the IAP, the seedlings were sprayed with acetamiprid and pyriproxyfen. Our data reveals that MED is not a good vector of begomoviruses, but it is an excellent vector of ToCV and CPMMV. Our studies highlight the importance of understanding the differential transmission of viruses by whitefly species and suggest that the prevalence of *B. tabaci* MED may alter the epidemiological scenario of viruses in different crops.
KEYWORDS: *Bemisia tabaci*; begomovirus; carlavirus

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TWO WHITEFLY (*BEMISIA TABACI*) PROTEINS INTERACT WITH MONOPARTITE AND BIPARTITE BEGOMOVIRUS CAPSID PROTEINS AND REGULATE VIRUS RETENTION AND TRANSMISSION

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ABSTRACT:
A few sweetpotato whitefly (*Bemisia tabaci*) proteins have been known to interact with single stranded begomoviruses. Two additional proteins in our laboratory have been identified and validated to interact with capsid proteins (CPs) of monopartite (old world) and bipartite (new world) begomoviruses. The viruses evaluated include tomato yellow leaf curl virus (TYLCV), cucurbit leaf crumple virus (CuLCrV), and sida golden mosaic virus (SiGMV). The two proteins include a C2H2 zinc finger like protein (vesicular endothelial zinc finger like - vezf) and cyclic adenosine monophosphate (cAMP) dependent phosphodiesterase (PDE4). The protein-protein interactions were identified using an in vitro yeast two hybrid screen (Y2H) against cloned cDNA whitefly library with cloned capsids as baits. The interactions were further validated using a series of other in vitro approaches such as pull-down assays with glutathione-S-transferase (GST) tagged CPs and in vivo approaches including co-immunoprecipitation and confocal microscopy. vezf belongs to a most abundant class of transcription factors in insects. When a virus-induced gene silencing (VIGS) approach was used with a tobacco rattle virus (TRV) vector, Vezf silencing in the whitefly resulted in increased retention of targeted begomoviruses, but did not affect their transmission (Ghosh et al. 2023). cAMP is a secondary messenger that is involved in a series of signaling cascades. Adenosine triphosphate (ATP) conversion to cAMP by adenylyl cyclase upstream and PDE4 hydrolyzation of cAMP to AMP downstream regulate intracellular cAMP levels. dsRNA-based silencing and chemical inhibition of PDE4 (Rolipram) resulted in enhanced cAMP levels with higher virus retention. Conversely, dsRNA-based silencing and chemical inhibition of adenylyl cyclase (SQ22536) led to reduced cAMP levels and reduced virus retention. TRV-based silencing of cAMP and PDE4 also indicated the same. cAMP inhibition resulted in reduced transmission of targeted begomoviruses, whereas PDE4 inhibition resulted in increased cAMP and increased transmission of begomoviruses (Ghosh et al. 2025). Results indicate that both vezF and cAMP-specific PDE4 interact with begomovirus capsids, and that their manipulation could modulate virus retention and transmission. These proteins could have implications for virus management and options are currently being explored.
KEYWORDS: *Bemisia tabaci* proteins; Capsid proteins; interaction

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A SYSTEMIC COINFECTION INVOLVING A MONOPARTITE BEGOMOVIRUS AND A BIPARTITE BEGOMOVIRUS DNA-A SEGMENT IS MAINTAINED OVER MULTIPLE CYCLES OF WHITEFLY TRANSMISSION

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ABSTRACT:
Begomoviruses are whitefly-transmitted, single-stranded DNA viruses that occur frequently in coinfections that can influence disease severity and genome evolution. They include monopartite viruses with a single genome segment encoding all viral proteins, and bipartite viruses with two genome segments (DNA-A and DNA-B) that differ in their sequences and are packaged in separate virions. Proteins responsible for replication and most other functions are encoded on DNA-A, the movement proteins are encoded on DNA-B. Both segments are required to establish a systemic, single-virus infection. In this presentation, we document whitefly (*Bemisia tabaci* MEAM1) transmission of systemic partial co-infections (PCI) involving the monopartite Tomato yellow leaf curl virus (TYLCV) and the DNA-A of bipartite Tomato mottle virus (ToMoV), following virus acquisition from coinfected tomato plants (TYLCV + ToMoV DNA-A and DNA-B). PCIs in tomato were maintained over 3 rounds of sequential whitefly transmission. The probability of whiteflies transmitting a PCI from a coinfected plant was significantly less than transmitting a full coinfection or single infections of TYLCV or ToMoV. ToMoV DNA-A accumulated more slowly in PCI than single or coinfected tomato plants. It did not clear from midrib vasculature over time in PCI or coinfected plants as it does in ToMoV infected plants and exhibited a phloem-limited distribution characteristic of TYLCV rather than the phloem-associated distribution of ToMoV. Symptoms of PCI plants were intermediate between single infections of either virus, and coinfections. These findings show that whitefly transmission of a DNA-A segment and a monopartite genome segment of different viruses can produce an infection resulting in more severe disease symptoms than either virus alone. They also show that the dependence of a DNA-A segment of a bipartite begomovirus on its cognate DNA-B for the movement function, which is necessary to establish systemic infections, can be uncoupled in a coinfection with a monopartite begomovirus.
KEYWORDS: Begomovirus; Coinfections; Whitefly

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TRANSMISSION AND EPIDEMIOLOGY OF THE FIRST WHITEFLY-TRANSMITTED POLEROVIRUS IN PEPPER

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ABSTRACT:
Poleroviruses (*Luteoviridae*) are phloem-restricted, positive-sense RNA plant viruses that are exclusively transmitted by aphids. We previously reported the first known whitefly-transmitted polerovirus infecting bell pepper (*Capsicum annuum*), which, similar to other aphid-vectored poleroviruses infecting pepper, induces characteristic symptoms such as vein yellowing, leaf rolling, and fruit discoloration. This newly identified virus, which we have named Pepper whitefly-borne vein yellows virus (PeWBVYV), is transmitted specifically by the whitefly *Bemisia tabaci* Middle East Asia Minor 1 (MEAM1) but not by the Mediterranean (MED) species. Genomic analysis of PeWBVYV revealed a unique recombination pattern, with its 5′ half exhibiting over 95% sequence identity to the aphid-transmitted Pepper vein yellows virus (PeVYV) from Israel, whereas its 3′ half shares homology with African eggplant yellows virus (AeYV). The virus is persistently transmitted by *B. tabaci* MEAM1 and has demonstrated a competitive advantage over PeVYV in host plants. In recent years, PeWBVYV has entirely displaced PeVYV in the Jordan Valley, where it was first identified, but remains geographically restricted and has not yet established dominance in other major pepper-growing regions of Israel. To better understand the transmission dynamics, disease progression, and the relationship between virus infection and fruit discoloration, we conducted extensive field surveys alongside controlled laboratory experiments. Our findings indicate that multiple factors contribute to the observed disease incidence and severity in the field, including environmental conditions, pepper variety, plant physiological state, whitefly population density, and the prevalence of virus inoculum. While PeWBVYV exhibits high transmission efficiency and disease incidence under field conditions, laboratory-based transmission assays have shown relatively low efficiency, making it challenging to isolate individual factors and assess their specific contributions to disease dynamics. These findings highlight the complexity of PeWBVYV epidemiology and underscore the need for further research to better understand its interactions with host plants, vectors, and environmental conditions.
KEYWORDS: *Bemisia tabaci*; Transmission; Vector

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MATCHING KEYS TO LOCKS: THE ROLE OF DIFFERENT STYLINS IN THE TRANSMISSION OF CAULIFLOWER MOSAIC VIRUS AND TURNIP MOSAIC VIRUS

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ABSTRACT:
Non-circulative stylet-borne viruses are primarily transmitted by aphids. The acrostyle, a specialized cuticular structure located at the tip of their maxillary stylets, plays a key role in the retention and transmission of such viruses, including the cauliflower mosaic virus (CaMV). The acrostyle is lined with cuticular proteins, known as stylins, which feature exposed domains at the virus-vector interface. Beyond virus retention, the acrostyle also interacts with aphid salivary effectors, emphasizing its multifunctional role in both feeding behavior and virus transmission. Among stylins, Stylin-01 was previously identified as a receptor candidate for CaMV through various approaches. To confirm its function, we used CRISPR-Cas9 knockout aphid lines in which stylin-01 gene was completely disrupted. Here, we will present the characterization of the phenotypes of these mutants. Our results showed that the absence of Stylin-01 prevents CaMV retention and transmission, definitely demonstrating Stylin-01 as the main receptor for CaMV. Interestingly, our results also suggest a partial functional compensation by another stylin, which was overexpressed in the KO mutant line. Building on these findings, we expanded our investigation to receptors of potyviruses, a group of aphid-transmitted viruses of agronomic importance. We used turnip mosaic virus (TuMV) as a model in our study. We are currently exploring the role of stylins in TuMV transmission. The latest findings will be presented and discussed.
KEYWORDS: Aphid stylins; Cauliflower mosaic virus; Turnip mosaic virus

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IMPATIENS NECROTIC SPOT VIRUS EXHIBITS TISSUE-SPECIFIC ACCUMULATION IN RESISTANT LETTUCE VARIETIES AND MAY INFLUENCE VIRUS SPREAD

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ABSTRACT:
Impatiens necrotic spot virus (INSV; *Orthotospovirus impatiensnecromaculae, Orthotospovirus*, *Tospoviridae*), has emerged as one of the most economically important pathogens of lettuce in the United States (U.S.). The virus causes tissue necrosis and wilting in lettuce, and has resulted in complete crop loss in coastal California where over 75 percent of U.S. lettuce is grown (Hasegawa and Del Pozo-Valdivia, 2023). In California, INSV is transmitted primarily by the western flower thrips (*Frankliniella occidentalis*). Recent studies have demonstrated that INSV commonly infects several weeds that are abundant in lettuce production regions and that these hosts likely contribute to the disease dynamics in the region. Recently, new varieties of lettuce have been released that exhibit reduced INSV infection and disease severity. Field evaluations demonstrated that INSV exhibits tissue tropism in these lettuce varieties, such that lettuce varieties that are considered moderately resistant had virus titers that were concentrated in crown and root tissue whereas susceptible varieties exhibited virus titers that were more uniformly distributed throughout the plant. To further characterize this tissue tropism, four moderately resistant romaine varieties and the susceptible variety, Parris Island, were inoculated with INSV using viruliferous western flower thrips. Six weeks post-inoculation, tissue samples were collected from leaves, crown, and roots of plants, and tested by ELISA with INSV-specific antiserum. Results demonstrated relatively uniform infection in susceptible plants; tolerant varieties exhibited varying degrees of tropism, with greatest accumulation in crown tissue. To determine how this may influence plant-to-plant spread, 15 first instar thrips were given a 48-hour acquisition access period on leaf tissue collected from susceptible and resistant romaine plants and then reared to maturity on green beans for three weeks. Adult thrips were collected from green beans and used in transmission assays to susceptible romaine plants or subjected to RT-qPCR to compare INSV titers between thrips that acquired virus from resistant or susceptible lettuce. Results will clarify the potential of new moderately resistant lettuce varieties to reduce vector acquisition of INSV and determine their impact on plant-to-plant spread within fields. These studies contribute to an industry-wide effort to reduce incidence and spread of INSV throughout the region.
KEYWORDS: virus titer; tospovirus; virus transmission

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EVALUATION OF DIAGNOSTIC METHODS FOR IMPATIENS NECROTIC SPOT VIRUS AND TOMATO SPOTTED WILT VIRUS IN HOST PLANTS AND INSECT VECTORS

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ABSTRACT:
Ornamental crops used for indoor and outdoor gardening are affected by many diseases, including viruses. In international trade, these plants can be a source of latent infection. One of the most dangerous genera of viruses is *Orthotospovirus*. This genus currently includes 33 species, many of which are polyphagous. Impatiens necrotic spot virus (INSV) and Tomato spotted wilt virus (TSWV) are widespread in many countries and affect more than 1300 plant species (EPPO, 2020). Orthotospoviruses are not seed-transmitted, but bulbs, rootstocks, seedlings and plants of ornamental crops are the sources of latent infection and the introduction of viruses into new areas (Shneyder et al., 2010). After introduction into new areas, INSV and TSWV are transmitted by thrips, *Frankliniella occidentalis, F. intonsa, F. fusca* and *F. schultzei*, as well as other vectors known for TSWV, *F. bispinosa, F. cephalica, F. gemina, Thrips setosus* and *T. tabaci* (EPPO, 2020). INSV and TSWV are regulated as quarantine organisms in many countries. For accurate diagnosis, including testing of asymptomatic plants, highly sensitive and specific diagnostic methods are needed to detect the virus in time to eliminate potential risks. It is also necessary to control insect vectors - thrips, which spread the infection, increasing the speed of long-distance movement of the virus. Trapping insects with glue or pheromone traps and then testing thrips can speed up the understanding of virus spread. As part of this study, ELISA kits from different manufacturers were tested and the feasibility of using primers for real-time RT-PCR for INSV for detection in host plants and thrips, as well as primers for conventional RT-PCR, was investigated. For TSWV diagnosis, primers for real-time RT-PCR and conventional RT-PCR were evaluated (Zhivaeva et al., 2021). The best primers and ELISA kits were selected and recommended for use in quarantine laboratories.
KEYWORDS: INSV; TSWV; Orthotospovirus

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TRANSMISSION DYNAMICS OF RESISTANCE-BREAKING TOMATO SPOTTED WILT VIRUS STRAINS BY WESTERN FLOWER THRIPS

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ABSTRACT:
Novel resistance-breaking (RB) strains of tomato spotted wilt orthotospovirus (TSWV) that overcome single-gene resistance in tomato (Sw-5b) and pepper (Tsw) have been reported worldwide. Thrips, as supervectors of TSWV, transmit these strains across a wide range of specialty and staple crops. However, the transmission biology of RB strains remains largely unexplored. We investigated key transmission parameters-including inoculation efficiency, sex-specific differences in transmission, virus accumulation, and source-sink relationships-using six novel TSWV strains (Tom-BL1, Tom-BL2, Tom-CA, Tom-MX, Pep-BL, and Non-RB), transmitted by western flower thrips (WFT) over four consecutive 24-hour inoculation access periods (IAPs). Most strains were inoculated across all IAPs, but at varying rates. Overall, WFT exhibited the highest inoculation efficiency in the first IAP and the lowest in the second. Female thrips carried higher virus titers, yet males were more efficient at inoculating TSWV. No significant positive correlations were observed in virus titers among acquisition tissues, thrips, and thrips-inoculated leaf discs. Males inoculated RB strains with 87% efficiency and the Non-RB strain at 80%, whereas females inoculated RB and Non-RB strains at 77% and 75% efficiency, respectively. This study provides new insights into the transmission biology of TSWV RB strains, particularly regarding inoculation efficiency and thrips sex-specific transmission dynamics, establishing a foundation for future molecular studies on emerging TSWV variants.
KEYWORDS: Vector-virus interactions; Cross-kingdom viruses; New viral strains

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PHYTOPLASMAS IN PIPER: PRELIMINARY STUDY OF A NEW ASSOCIATION PRESENT IN NATIVE FORESTS OF COLOMBIA

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ABSTRACT:
Wild plant populations play an important role in enabling interactions within the ecosystem and regulating natural processes. Currently, in wild populations of *Piper grande* in Teruel (Huila, Colombia), which marks the structure and function of the tropical forest, symptoms of phyllody (leaf-like structures in floral organs) have been observed; however, the cause has not been determined, nor the degree of affection in biotic interactions in wild ecosystems. Phyllody is associated with infection by phytoplasmas, obligate phytopathogenic bacteria without cell walls. Phytoplasmas have the ability to alter the metabolism and physiology of their plant hosts, causing symptoms such as witches' broom, phyllody, small leaves, epicormic shoots, among others. Although it infects at least 80 plant families, it has been studied in plants of commercial interest and no phytoplasma-associated symptoms have been reported in wild communities. Therefore, we evaluated whether phyllody symptomatology in P. *grande* was associated with phytoplasma infection and determined the species of the pathogen. A study of symptomatology prevalence, morphological and structural characterization of symptomatic and asymptomatic organs was carried out, where changes in the size and shape of bracts and anthers were observed, and it was determined that the prevalence of symptomatology in the study site was 33%. Nested PCR of the 16S rRNA gene was performed with universal primers for phytoplasmas from DNA extracts of stems, leaves and flowers of seven symptomatic and asymptomatic plants. The amplicons obtained (1250 bp) were evaluated by RFLP (Restriction Fragment Length Polymorphism) and sequencing. PCR results suggest the presence of phytoplasmas in all structures evaluated in symptomatic and asymptomatic plants. So far, RFLPs patterns seem to be inconclusive about the group of phytoplasmas present in *P. grande*, the sequences obtained have a high percentage of similarity with 'Candidatus Phytoplasmas Asteris', result that will be validated by specific qPCR for this species of phytoplasmas. These results allow the generation of new knowledge on the characterization of diseases associated with phytoplasmas in Colombian native forest populations, which opens a wide spectrum of questions about the possible effect of phytoplasmas in wild ecosystems.
KEYWORDS: Phyllody; Wild plant populations; Molecular detection

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Session 3: VIRUS DISCOVERY AND ROLE OF METAGENOMICS IN EPIDEMIOLOGY

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A NOVEL NANOPORE-BASED METHOD ENABLING IN-DEPTH PROFILING OF DNA VIRUS POPULATIONS BY UNAMBIGUOUS IDENTIFICATION OF LOW ABUNDANT VARIANTS AND PARTIAL GENOMIC COMPONENTS

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ABSTRACT:
The emergence of hyper-virulent strains of single-stranded DNA viruses poses significant threats to global food security. Current mitigation strategies often rely on genetic resistance, which can be rapidly overcome by evolving virus populations. To address this challenge, we developed a novel pipeline1 leveraging Oxford Nanopore long-read sequencing technology and the Genome Detective analysis tool2 to accurately identify and characterize full-length sequence variants of monopartite and multipartite circular viral DNA genomes. This approach enables the detection of virus variants with single nucleotide differences and relative abundances as low as 1%. Our pipeline successfully profiled the populations of emerging Tomato leaf curl New Dehli virus (ToLCNDV) and Banana bunchy top virus (BBTV), including the unambiguous detection of previously unreported partial genomic sequences. The use of duplex reads significantly enhanced analysis accuracy, allowing for reliable identification of true virus variants. This pipeline offers a powerful tool for monitoring the emergence of new virus variants and studying their adaptation to host plant responses and environmental conditions, with potential applications in cross-protection strategies against severe viral isolates. Our work highlights the utility of long-read sequencing technologies in overcoming the limitations of short-read sequencing for in-depth analysis of plant virus populations.
KEYWORDS: full length viral genome; nanopore; partial genomic components

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A QUARANTINE SPECIES HIDING IN PLAIN SIGHT FOR DECADES?

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ABSTRACT:
This study reports the first official finding of American plum line pattern virus (APLPV) in two reference collections of ornamental *Prunus* spp. in the Netherlands in 2024. APLPV is a regulated quarantine pest under EU Regulation 2019/2072. Globally APLPV is rarely reported and is included in certification testing schemes. At the first location, two infected trees were initially sampled in 2022 as part of a high-throughput sequencing (HTS)-based categorization project for the National Plant Protection Organization (NPPO) reference collection. Due to its clonal link to the first site, the second location was subsequently investigated, leading to the identification of additional infections. The Prunus trees infected with APLPV were also infected with other viral species, including close relatives, for which they were originally maintained in collection. Sequence analysis of all sampled Prunus trees revealed three distinct APLPV genome sequence types, suggesting multiple introductions. While the exact source remains unclear, historical Prunus accessions indicate that the virus may have persisted in some accessions or clonal lines for decades. Real-time RT-PCR and sequence analyses suggest limited local spread, restricted to neighboring trees, possibly facilitated by natural root grafts. To prevent further spread, all infected trees were destroyed, while uninfected neighboring trees remained under measures and will be retested in 2025. To preserve valuable viral isolates for research, living material has been maintained in quarantine greenhouse facilities. This report provides important insights into the rare occurrence of the quarantine organism APLPV in living Prunus accessions and highlights the importance of using HTS for investigating important reference collections. HTS is a powerful tool for virus detection, identification and epidemiological studies, often leading to unexpected yet significant findings.
KEYWORDS: Quarantine finding; Living reference collections; Untargeted screening with HTS

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VIROMIC EXPLORATION FOR DIVERSITY AND EPIDEMIC RISKS OF UNKNOWN CITRUS VIRUSES

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ABSTRACT:
We have established containment system for citrus viral epidemics in China mainly through nationwide deployment of certified virus-free seedlings. However, viral epidemics often emerge from obscure sources, highlighting the critical need for proactive monitoring of these hidden risks. From 2018 to 2024, we discovered nine new viruses from both cultivated citrus in agroecosystem and wild citrus in natural ecosystem (1). Among them, citrus-associated rhabdovirus was found to be defective in the G gene while the strains infecting other geographically adjacent plant hosts had intact genomes, implying the transmission of citrus strains by human activities like grafting instead of some hemipterans (2). This case exemplifies how plant viruses spill over to new hosts and reshape their lifestyles likely via accumulative neutral mutations. Some of the new viruses may have evolved through interspecies or intergenus genomic recombination (3,4), i.e. citrus leaf blotch virus-2 (CLBV-2) and citrus yellow spot virus, both were homologous in polymerase to another known citrus betaflexvirid (CLBV). In addition, three new closterovirids that were identified by virome analysis from wild citrus in Yunnan, the citrus origin center in China, exhibited not only the interstrain and cross-kingdom genetic exchanges, but also the alteration of gene encoding strategy, representing special evolutionary trajectories (5). This is the first discovery of new closterovirids since we have known citrus tristeza virus (CTV) for the past decades. The endemic status of the three viruses, as indicated by their frequent mix infections with CTV in both cultivated and wild citrus, and their potential pathogenicity, suggested their possible natural transmissions by some mobile but ecologically restrained viral vectors. However, there is yet lack of information on the definite biological vectors, despite we became vigilant for the hidden epidemic risks. In conclusion, epidemics of citrus viruses could depend on their flexible transmissions whereby viruses evolved in an intrinsic manner that intracellular nucleic acids act, to modify genetics in new environments and prepared for their next travel. More recently, we discovered at least seven plant-associated viruses from wild citrus, continually expanding the knowledge on diversity and origin of citrus viruses. Now, our efforts will focus on biological property and epidemic potential of the viruses newly identified from citrus since 2018 in China.
KEYWORDS: Virus disease; High-throughput sequencing; Virus ecology

SUPPORT

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CHARACTERIZING A NOVEL NEPOVIRUS USING A COMBINATION OF EXPERIMENTATION, DATA MINING, AND DATA SHARING TO DETERMINE RISK

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ABSTRACT:
Samples of a *Tilia* sp. from the Yorkshire Arboretum were collected from a tree showing virus like symptoms of leaf deformation/twisting, reduced leaflet size and vein clearing, and from a second adjacent tree which was asymptomatic. High Throughput Sequencing indicated the presence of novel nepovirus, tentatively named "Tilia nepovirus 1" (TiNV1) from the asymptomatic tree. The virus had a high identity with a partial sequence on NCBI GenBank originating from a bee virus study (Pascall et al, 2021). Real-time RT-PCR primers were designed to the RNA1 and RNA2 of the virus, and these indicated virus presence in both the symptomatic and asymptomatic tree samples. Separately, a phytosanitary inspection at a botanic garden in Scotland, found the presence of a virus infecting *Lobelia*. Biological characterization indicated the virus was mechanically transmissible to *Nicotiana benthamiana* and *N. occidentalis*; with *N. benthamiana* developing leaf rugosity and necrosis, and *N. occidentalis* developing ringspots. Nanopore sequencing of the inoculated plants indicated the presence of TiNV1, and it was subsequently confirmed in the original *Lobelia* by real-time RT-PCR. A complete viral genome sequence with significant similarity to the two described isolates was later deposited on NCBI under the name "Coral plant nepovirus". This was derived from Sequence Read Archive (SRA) data of *Berberidopsis corallina*, as part of a plant taxonomy study (Sidharthan et al, 2024). Further investigation revealed that the *Berberidopsis* plant had originated from the same botanic garden in Scotland as the *Lobelia*. Honeybee pollen sacs, from a bee foraging study, were also tested using real-time RT-PCR to potentially identify distribution of the virus, showing the virus was present in foraging bees from across South-East Scotland. A site specific survey at the Yorkshire Arboretum indicated the virus was present in 9 of 26 *Tilia* spp. sampled. Additional national survey work has been conducted by citizen science volunteers through the Euphresco VirNotree project. The potential for integrating data gathered through metagenomic sequencing, specific diagnostics, SRA searches, biological assays, with local and national survey work to support and epidemiology investigations of a novel virus discovery, will be discussed.
KEYWORDS: nepovirus; risk assessment; sequence read archive

SUPPORT

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PLANT VIRUSES ON THE LAND-WATER INTERFACE

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ABSTRACT:
Several plant viruses are environmentally stable and can persist outside their hosts for extended periods. We previously demonstrated that metagenomic approaches can reveal a high diversity of plant viruses in environmental waters (Maksimovic et al., 2024) and that infectious plant viruses can enter ecosystems via urban sewage, even after wastewater treatment (Bacnik et al., 2020). To further investigate plant virus epidemiology at the land-water interface, we pursued multiple research directions. First, we explored the presence and diversity of plant viruses in aquatic plants, key hosts at this interface. Although ecologically significant, aquatic plants (macrophytes) remain largely unexplored for viral infections beyond a few crop species. To address this gap, we conducted a literature review, mined existing high-throughput sequencing (HTS) datasets, and generated new HTS data for selected globally traded ornamental aquatic plants. Our findings reveal associations with diverse plant viruses, including known crop pathogens (e.g., polerovirus, cucumovirus and crinivirus), as well as numerous putative novel viruses. Notably, we identified novel begomoviruses and a potentially widespread potyvirus. Begomoviruses, detected in two globally traded ornamental aquatic plant species, may contribute to observed plant ornamental traits. The novel potyvirus was found in multiple *Sagittaria* species, with follow-up surveys confirming its presence in samples from online stores in Slovenia and in plants intercepted during importation into the UK. Second, to investigate plant virus movement across ecosystem components, we established a research site at the land-water interface, including a wastewater treatment plant effluent, a recipient river, and multiple upstream and downstream sampling points. Over two growing seasons, we collected 61 water samples and 1,386 plant samples from 245 species. HTS-based virome analysis is ongoing, aiming to elucidate plant virus fluxes within the studied ecosystem. Preliminary results indicate a high diversity of predominantly unknown plant viruses and suggest overlaps between different sample types. These findings provide foundation to understand plant virus epidemiology at the land-water interface and reveal the diversity of known and novel viruses in aquatic plants. Further studies will assess the ecological and agricultural implications of the findings.
KEYWORDS: plant viromes; ecosystem; water

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SOLVING THE FROGSKIN MYSTERY: SINGLE INFECTIONS BY A TORRADOVIRUS EXPLAIN CASSAVA FROGSKIN DISEASE IN THE AMERICAS

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ABSTRACT:
Cassava is an important food security crop in the tropics and cassava frogskin disease (CFSD) was first identified in Colombia in 1971 and has been a challenge to solve due to the frequent occurrence of mixed infections in the vegetatively propagated cassava crop. In this study, we employed a sentinel approach to capture single-pathogen infections from affected fields. After eight months of exposure to a high CFSD inoculum, 6.9% of the susceptible sentinel plants exhibited characteristic root symptoms of CFSD. Sentinel plants were then multiplied and transferred to a screenhouse for a second infection cycle and storage root development in the absence of further infection. Notably, (RT)-PCR diagnostics found no evidence linking CFSD to phytoplasma or reovirids, pathogens historically associated with the disease. Instead, single infections by torradoviruses were sufficient to account for CFSD root symptoms. Further analysis by high-throughput sequencing confirmed the absence of other pathogens but torradoviruses in symptomatic roots, unveiled a second torradovirus species in these plants. Field surveys in the north coast of Colombia confirmed the association of torradoviruses with CFSD in farmers' fields. These findings have significant implications for cassava seed certification, early detection of infected planting material, and breeding programs aimed at identifying sources of resistance to CFSD. Ultimately, this new diagnostic for CFSD will play a crucial role in mitigating the impact of the disease on cassava production.
KEYWORDS: high throughput sequencing; emergent disease; virus evolution

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EPIDEMIOLOGY OF GRAPEVINE VIRUSES IN CANADIAN VINEYARDS: RELATIVE INCIDENCE, GENETIC DIVERSITY, AND VIROME COMPOSITION

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ABSTRACT:

Grapevine viruses pose a significant threat to the sustainability and productivity of vineyards in Canada. This study summarizes three key studies investigating the epidemiology of grapevine viruses in Canadian vineyards. The research highlights the spread of Grapevine Pinot gris virus (GPGV) and Grapevine red blotch virus (GRBV), the phylogenetic relationships of GPGV isolates across Canada, and a metagenomic survey of grapevine viruses in Canadian wine-producing provinces. In the first study, forty virus-free sentinel vines were introduced into two heavily infected vineyards with GRBV and GPGV. High-throughput sequencing (HTS) and PCR analysis 15 months post-introduction revealed widespread GPGV infection (13/18 vines in the organic vineyard vs. 1/19 in the conventional), while GRBV was not detected in any sentinel vines. Wild grapevines (*Vitis* spp.) were identified as potential reservoirs for GPGV. A phylogenetic study of 25 Canadian GPGV isolates (from British Columbia, Ontario, Nova Scotia, and Quebec) compared with 43 global isolates revealed distinct clustering of North American GPGV strains, separate from European and Asian lineages. Analysis of the MP and CP gene regions from 169 global isolates identified two major clades: Clade 1 (predominantly asymptomatic infections) and Clade 2 (predominantly symptomatic). This study provides the first genetic characterization of GPGV in Canada, suggesting multiple introductions and adaptive evolution. A metagenomic survey of 310 grapevine samples from four Canadian provinces identified 37 viruses and three viroids. Key pathogens included Grapevine rupestris stem pitting-associated virus (GRSPaV), Grapevine leafroll-associated viruses, GRBV, and GPGV. 13 viruses were listed in grapevine certification programs, and nine were newly reported in Canada. The study revealed significant regional and varietal differences in virus prevalence, emphasizing the need for improved management strategies and clean plant programs to mitigate virus spread. The findings underscore the complex epidemiology of grapevine viruses in Canada, with GPGV showing higher transmission potential than GRBV. The genetic diversity of GPGV suggests multiple introduction events, while the national virome survey highlights the presence of certain viral species across cultivars and regions. Strengthening phytosanitary measures and implementing clean plant programs are critical steps toward reducing virus impact on the Canadian grapevine industry.

KEYWORDS: Grapevine viruses; Canada; high-throughput sequencing

SUPPORT

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SEVERELY SYMPTOMATIC ZUCCHINI VIROMES FROM CROATIA REVEAL DIVERSITY AND HIGH PREVALENCE OF POTYVIRUSES

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ABSTRACT:
Zucchini (*Cucurbita pepo* L. 'Naxos F1') fruits and/or leaves showing severe virus-like symptoms were collected from several localities in the vicinity of Pitomaca (Eastern Croatia) in 2021. Symptoms included leaf deformation, mottling, mosaic, and vein yellowing. Also, zucchini fruits were unmarketable due to severe deformations, including blistering and lumpiness. Virome analyses were performed on two out of six representative fruit samples using Illumina and Oxford Nanopore Technologies (ONT). Both approaches predominantly detected potyviruses, including watermelon mosaic virus (WMV), zucchini yellow mosaic virus (ZYMV), and Moroccan watermelon mosaic virus (MWMV). Nanopore sequencing of the sample T3 yielded 80,200 reads, covering 2.94% of the viral genomes with 238 reads assembled to the MWMV reference, allowing for its partial polyprotein gene sequencing (GenBank acc. no. OQ729739). Illumina sequencing of the sample T5 generated 10,559,780 reads, covering 15.45% of the viral genomes, with 2,049,672 reads mapping to MWMV, leading to the complete genome assembly (GenBank acc. no. OQ729737). A Kraken algorithm-based metagenome analysis revealed the prevalence of genus *Potyvirus* reads with a proportion of 42.85% and 50.5% of T3 and T5 samples, respectively. RT-PCR and Sanger sequencing confirmed that all six severely symptomatic zucchini plants were co-infected with MWMV and WMV, while ZYMV was detected in only one sample. The comparative analyses suggest MWMV is the primary cause of the observed symptoms, with WMV and ZYMV contributing to a lesser extent. Phylogenetic analyses revealed that Croatian MWMV isolates clustered within the Mediterranean group, with NIb/CP sequence forming a distinct highly supported (99% bootstrap) subclade. Based on the CP region of WMV, Croatian isolates were assigned to the Group 3, subgroup EM3 (emerging isolates). The NIb/CP sequence of one identified ZYMV zucchini isolate from Croatia clustered in the Group A, subgroup A1, with the majority of European isolates representing non-emerging strains. This study represents the first record of MWMV in Croatia and provides insights into potyviral diversity and epidemiology affecting this important cucurbit crop.
KEYWORDS: MWMV; WMV; ZYMV

SUPPORT

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APPLICATION OF HIGH-THROUGHPUT SEQUENCING FOR MONITORING INSECT-TRANSMITTED VIRUSES IN ZUCCHINI CROPS AND THE ROLE OF WEEDS AS RESERVOIRS

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ABSTRACT:
High-throughput sequencing (HTS) is a powerful tool for detecting plant viruses, facilitating the prediction of potential epidemics in crops. In this study HTS was applied for monitoring insect-transmitted viruses in zucchini crops and for investigating the role of weeds as potential reservoirs of known and/or new viruses. Sequential samplings were conducted in the Vassilika region of Thessaloniki (Macedonia) during 2023 and 2024. The first sampling, targeting both zucchini and weeds, occurred in November 2023. Additional samplings were performed in March 2024 (before crop establishment), July 2024 (during the growing season), and November 2024 (in the subsequent growing season). A total of six pooled samples from wild plant species and six pooled samples from zucchini plants showing virus-like symptoms were collected to assess the contribution of weeds in sustaining viral loads across growing seasons. Total RNA was extracted from each pooled sample, followed by cDNA library construction, and sequencing on the Illumina NovaSeq platform. Bioinformatics analysis included raw read quality assessment, trimming and host genome sequence removal. Cleaned reads were de novo assembled and analyzed using BLASTn/x against viral genome databases. HTS revealed the presence of well-known viruses, such as cucumber mosaic virus (CMV), watermelon mosaic virus (WMV), and cucurbit aphid-borne yellows virus (CABYV), in both cultivated zucchini plants and weeds, exhibiting high genetic similarity (>97%). Cucurbit yellow stunting disorder virus (CYSDV) and zucchini yellow mosaic virus (ZYMV) were also detected in zucchini plants. Conventional RT-PCR assays confirmed the presence of CMV, WMV, and CABYV in both pooled and individual samples. Notably, contigs of putative novel poleroviruses were identified in both weeds and zucchini plants, sharing a high percentage of sequence identity (>98%). Validation of these sequences is currently ongoing to establish whether they represent distinct polerovirus species. These findings contribute to a better understanding of the epidemiology of insect-transmitted viruses in zucchini crops and highlight the importance of weed management in disease control strategies.
KEYWORDS: weeds; virome; HTS

SUPPORT

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INSIGHT INTO THE *BREVIPALPUS* MITE VIROME: NOVEL VIRUSES AND GEOGRAPHICALLY DETERMINED PROFILES.

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ABSTRACT:

The genus *Brevipalpus* Donnadieu (Tenuipalpidae) comprises phytophagous mites predominantly found in tropical and subtropical regions. These mites infest numerous economically important crops and ornamental plants, with fewer than a dozen of the nearly 300 known species identified as vectors for plant-infecting viruses belonging to the *Kitaviridae* and *Rhabdoviridae* families. Notably, some of these viruses cause citrus leprosis in several countries, which is considered the most significant viral disease affecting Brazilian citrus orchards. However, the diversity of viruses beyond kitaviruses and rhabdoviruses within the *Brevipalpus* virome remains entirely unexplored. In this study, we collected *Brevipalpus* mites representing nine species from field-infested plants or laboratory-reared populations across five continents. Total RNA extracts were obtained from 100-200 individuals per sample, and high-throughput sequencing (HTS) libraries were prepared following ribosomal RNA depletion. Viral diversity at the family level was assessed using Kaiju software to analyze the 45 generated libraries. Preliminary results indicate that the *Brevipalpus* virome is primarily structured by geographic origin rather than by host plant or mite species. Hierarchical clustering analyses further revealed distinct virome compositions between laboratory-reared and field-collected populations of the same mite species. Comparisons among males, females, and nymphs of *B. chilensis* collected from the same plant showed that while the virome composition was largely shared across these groups, certain viruses were detected exclusively in one or two subpopulations. Of particular significance, we identified two novel plant-infecting viruses-a cilevirus (*Kitaviridae*) and a dichorhavirus (*Rhabdoviridae*)-currently under characterization, from two *B. chilensis* populations. Globally, however, reads corresponding to plant viruses other than cileviruses and dichorhaviruses were rare or present at low abundance, suggesting specific vectorial activity by these mites. Additionally, we recovered complete genomes of novel viruses from other taxonomic groups commonly detected in arthropods, such as nege-kita-like viruses, which appear to form part of the Brevipalpus-specific virome. Our findings highlight a complex viral community within Brevipalpus mites, providing insights into its evolutionary dynamics, virus-virus interactions, and potential applications for mite biocontrol strategies.

KEYWORDS: kitaviruses; high-throughput sequencing; dichorhaviruses

SUPPORT

The Brevipalpus virome research consortium comprises researchers from over a dozen laboratories worldwide.

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Session 4: DISEASE MANAGEMENT AND RESISTANCE

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OPTIMAL PLANTING WINDOW: EVALUATING THE RESPONSE OF CBSD-TOLERANT AND CBSD-SUSCEPTIBLE CASSAVA VARIETIES IN COASTAL TANZANIA

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ABSTRACT:
Planting time significantly impacts crop yield due to the necessity of aligning crop growth with favourable growing conditions and ensuring freedom from pests and diseases. Cassava has been regarded as a resilient crop that can withstand a wide range of environmental conditions while producing optimally. We evaluated the root production of three cassava varieties: a cassava brown streak virus disease (CBSD)-tolerant variety *Kiroba* and two CBSD-susceptible varieties *Albert* and *Mkombozi* over an extended planting window at Chambezi, Bagamoyo in Coastal Tanzania and Ukiriguru in the Lake Victoria zone of north-western Tanzania. Plantings were spread between October and May flanking the two main planting seasons in Tanzania. Chambezi is a high-disease pressure hotspot while Ukiriguru is a low-disease pressure site. *Kiroba* was planted at both sites while *Albert* was planted only in Chambezi and *Mkombozi* only at Ukiriguru. Yields were generally higher in Chambezi (28.1 t/ha) than at Ukiriguru (9.0 t/ha). While soil moisture conditions were adequate for effective growth and root production for most of the planting dates tested at Chambezi, yields at Ukiriguru were primarily determined by soil moisture, with a very narrow planting date period providing adequate moisture for effective growth and root production. At Chambezi, fresh root yields were similar for both varieties for all planting dates. However, at Chambezi, conditions were optimal for CBSD spread for planting dates from October to December but not from March to April. Most of the fresh root yield for planting dates October to December was therefore unusable (due to CBSD-induced root necrosis), while in April most of the root yield was usable. In Chambezi, planting date is critical for varieties that are sensitive to CBSD but less so for CBSD-tolerant varieties. In Ukiriguru, the key determinant is soil moisture rather than CBSD. These results highlight the benefit of extending the planting window for cassava while emphasizing the importance of isolating farmer-preferred CBSD-sensitive cassava varieties from virus inoculum and promoting the use of clean planting material.
KEYWORDS: Planting date; CBSD; Root yield

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CHARACTERIZING THE RNA SILENCING PATHWAY IN CASSAVA MOSAIC VIRUS RESISTANCE

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ABSTRACT:
Cassava mosaic disease (CMD) is a major constraint to cassava (*Manihot esculenta*) cultivation posing a significant threat wherever cassava is grown. Resistance to cassava-infecting begomoviruses remains the only effective strategy for disease management. Three types of resistance were identified; the CMD1 resistance, derived from *Manihot glaziovii* Muell.-Arg confers a recovery phenotype characterized by reduced viral load and attenuated symptoms; the CMD2 resistance, from an African landrace TME 3, provides near complete protection against the begomoviruses so far known to infect cassava while CMD3 resistance (CMD1 x CMD2 cross) confers near-immunity. These resistant sources have been extensively utilized in breeding programs. Despite being a dominant trait, the molecular mechanisms underlying CMD resistance remain unclear. This study examined East African cassava mosaic virus (EACMV) infections in CMD1- and CMD2-resistant, as well as susceptible, cassava genotypes. Using RNAscope® ISH and qPCR, we localized the virus and analyzed the expression of Xyloglucan endotransglycosylase (XET)-implicated in defense responses-alongside RNA silencing genes, Dicer-like (DCL), Argonaute (Ago), and RNA-dependent RNA polymerase (RdRP), which are key to antiviral defense. EACMV was introduced via graft inoculation, and virus movement was tracked in leaf and stem tissues. Viral transport from the graft site occurred in all resistant plants, indicating phloem movement however, with only minimal viral accumulation in leaf tissues of resistant plants. EACMV primarily localized in the phloem but high-resolution RNAscope® ISH further demonstrated its presence in mesophyll cells of infected leaves. CMD resistance is characterized by a significantly reduced viral replication and a delay in systemic movement. Notably, CMD2- and CMD3-resistant cassava lines exhibited elevated XET expression, particularly in stem tissues. However, this upregulation was not specific to EACMV infection, suggesting a broader role in plant defense responses. In a time-series analysis of meristematic tissues from TMS 30572 (CMD1), we observed a pronounced upregulation of RNA silencing genes during viral invasion, strongly implicating the RNA silencing machinery in cassava's defense against begomoviruses. These findings provide novel insights into CMD resistance mechanisms and underscore the critical role of RNA silencing pathways in antiviral defense in cassava.
KEYWORDS: plant response; tissue localization; silencing genes

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INTER AND INTRA PATHOGEN INTERACTIONS ARISING FROM COINFECTION WITH DIFFERENT FUNGAL AND VIRAL STRAINS IN CANOLA CULTIVARS WITH DIFFERING HOST RESISTANCES

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ABSTRACT:
Few recent investigations examine coinfection interactions between fungal and viral plant pathogens. Here, we investigated coinfections between *Leptosphaeria maculans* and turnip mosaic virus (TuMV) in canola (*Brassica napus*). Different combinations of non-resistance breaking and resistance breaking *L. maculans* isolates and a resistance breaking TuMV isolate were inoculated to three canola cultivars with differing pathogen resistances/susceptibilities. They were inoculated first to entire or half cotyledons 10-12 days after emergence, and second to opposite entire or half cotyledons on the same day (day 0) or 3 or 7 days afterwards. The parameters measured were *L. maculans* cotyledon disease index (%CDI), and TuMV systemically infected leaf symptom intensity (SI) and virus concentration (VC). Except when both day 0 inoculations were with the resistance breaking *L. maculans* isolate, %CDI values were suppressed strongly or only weakly when either *L. maculans* isolate were inoculated to plants of cultivars with *L. maculans* single gene resistance (SGR) or polygenic resistance (PGR), respectively. However, except when the non-resistance breaking *L. maculans* isolate P11 was inoculated first and its resistance breaking isolate second, these values declined after inoculation day 0 in plants of the cultivars without SGR. TuMV infection suppressed %CDI values, although this decrease was usually smaller following day 0 half cotyledon than entire cotyledon inoculations. When TuMV temperature sensitive systemic invasion resistance was present in a cultivar and both inoculations were with TuMV, SI and VC values diminished greatly. However, the extent of this decrease was reduced when second inoculations were with *L. maculans*. SI and VC values were also smaller when SGR was present and second inoculations were with *L. maculans*. When either SGR or PGR *L. maculans* resistance was lacking in a cultivar, SI and VC values were smaller when second inoculations to entire cotyledons were with *L. maculans* rather than TuMV. This also occurred after second half cotyledon inoculations with the non-resistance breaking *L. maculans* isolate but not with its resistance breaking isolate. Therefore, diverse inter or intra pathogen interactions developed depending upon host resistance, isolate combination, cotyledon inoculation approach and second inoculation timing. This study provides a foundational step for further research on effects of pathogen mixtures on host resistance effectiveness .
KEYWORDS: pathogen interactions; host resistance; resistance breaking strains

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INVESTIGATING THE IMPACTS OF SUGARCANE YELLOW LEAF VIRUS ON LOUISIANA SUGARCANE AND ITS IMPLICATIONS REGARDING VIRUS MANAGEMENT

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ABSTRACT:

Sugarcane yellow leaf virus (SCYLV; *Polerovirus SCYLV*; *Polerovirus*; *Solemoviridae*), the causal agent of yellow leaf disease, is found in all sugarcane growing regions around the world. Globally, yield losses of 15-50% have been reported in asymptomatic infections, such as those observed in Louisiana. In the state, if SCYLV incidence exceeds 10% in propagative material (seed cane) fields they cannot be certified, reducing its value to seed cane farmers and companies. Despite this regulation, little is known regarding the impact of SCYLV on common sugarcane cultivars grown in Louisiana. This study aims to quantify virus titer to ensure the proper tissue is being collected for detection and evaluate SCYLV-sugarcane interactions that may impact yield to justify current regulations. In 2024, tissue was collected from the cultivar LCP 85-384 monthly (April-August) from three canopy layers. A SYBR-based, reverse-transcription, quantitative polymerase chain reaction assay determined no difference in SCYLV titer, regardless of canopy layer and month of collection (p=0.217). These data indicated detection was feasible at any point during the growing season from all canopy levels. Infected sugarcane exhibits reduced chlorophyll concentrations and increased starch accumulation in bundle sheath cells; both of which are known to negatively impact sucrose biosynthesis. In both infected and non-infected plants, chlorophyll a (ChA), b (ChB), and total chlorophyll (ChT) were extracted using methanol and quantified with spectrophotometry. Early in the growing season, there was no observed difference in ChA, ChB, and ChT concentrations (p=0.112, 0.089, 0.181, respectively), but mid-season, higher ChB and ChT concentrations were observed in healthy tissue compared to infected (p=0.013 and 0.006, respectively). This work will be repeated in the popular cultivar, L-01-299. Preliminary data shows similar chlorophyll patterns late in the growing season. Microscopy of cross sections following Lugol´s iodine staining will be paired with computational analysis to quantify starch present in bundle sheath cells in healthy and infected leaf tissues. These data will be used to assess SCYLV-sugarcane interactions between cultivars, determine the appropriate time and tissue for optimal detection, and set the stage for future yield assessments. This work is paramount to establishing proper certification protocols and investigating the risk SCYLV poses to the Louisiana sugarcane industry.

KEYWORDS: disease management; detection; sugarcane

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IMPACT OF DIAGNOSTICS AND PHYTOSANITARY REGULATIONS ON BIOSECURITY AGAINST TRANSBOUNDARY PLANT VIRUSES

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ABSTRACT:
The global trade of agricultural commodities and exchange of germplasm have the potential to introduce new viruses which may pose potential risk to the agriculture of importing country. The National Plant Protection Organizations have the responsibility to protect their crops from exotic pests including plant viruses. The combination of regulatory and technical approaches would ensure biosecurity of crops against plant viruses for a country/region. The Directorate of Plant Protection, Quarantine and Storage under the Ministry of Agriculture and Farmers' Welfare is responsible for quarantine and for inspection/ disinfestation of agricultural commodities for commercial purpose in India. The imported germplasm including transgenics are quarantined at ICAR-National Bureau of Plant Genetic Resources. The Plant Quarantine (Regulation of Import into India) Order, 2003 (PQ Order), requires Additional Declarations to be included in the Phytosanitary Certificate by the exporting country for seeds and other planting material as free from pests. As per the PQ Order, 264 viruses are regulated pests which are of quarantine significance for India. Early, sensitive and accurate detection of viruses is necessary in quarantine. ICAR-NBPGR has developed multiplex RT-PCR protocols to detect exotic viruses affecting soybean and maize during quarantine. Adopting Standard Operating Procedure of post-entry quarantine growing/inspection followed by EM/ELISA/RT-PCR, 51 viruses of quarantine significance for India were intercepted in imported germplasm including transgenics in the last 25 years. The interceptions include 25 viruses not yet reported from India and several viruses not known to occur on particular host(s) in India. However, their potential vectors and favourable conditions exist to multiply and spread exotic viruses. India need to establish a network of accredited laboratories to quickly identify new viruses/strains. To enhance preparedness, there is a need for "National Plant Pests Diagnostic Network". Plant viruses don't stop at nation's borders and miles of oceans don't prevent their spread. Hence, strictly implementing quarantine regulations and using sensitive techniques for detection of viruses would ensure crop biosecurity against transboundary plant viruses, virus-free exchange of germplasm and trade.
KEYWORDS: Plant quarantine; diagnostics; transboundary plant viruses

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WHEN IS YOUR VIRUS "DEAD"? A TEST TO DISTINGUISH INFECTIOUS FROM NON-INFECTIOUS VIRUS

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ABSTRACT:
The recent worldwide outbreak of tomato brown rugose fruit virus (ToBRFV) has again showed that the spread of plant viruses may quickly go unnoticed and can have significant agricultural and economic consequences. Diagnostic testing to ensure virus-free seed material and applying strict hygienic measures are crucial in trying to prevent and contain virus outbreaks. Especially tobamoviruses are of particular concern in this respect as they are extremely robust and often occur in very high concentrations. Virus particles may remain infectious for months on surfaces in greenhouses, tools or on crates and other materials to transport or store tomatoes. Various disinfection strategies may be deployed to de-contaminate affected surfaces and materials. Often DAS-ELISA and RT-qPCR assays are used to test for the presence of but neither of these methods can distinguish between the mere presence of viral coat protein, degraded viral RNA and truly infectious virus particles. Within the EU Horizon 2020 funded VIRTIGATION project we have developed a method based on the Luminex x TAG platform which is characterized by the true multiplex detection of multiple targets in a single assay. Our assay detects long RT-PCR fragments, each with multiple target regions covering the near full genome, which is indicative of intact viral RNA and thus infectious virus. Test results obtained with our developed method on different dilutions of tomato mosaic virus (indicative of a typical tobamovirus) plant sap treated for 20 minutes at various temperatures between room temperature and 95oC showed a clear decline in intact virus and a 100% correlation with a bioassay of the heat-treated dilutions on *Nicotiana glutinosa*, a test plant very sensitive for tobamovirus infections. In contrast to our Luminex x TAG assay, DAS-ELISA and TaqMan test were not able to distinguish between infectious and non-infectious virus resulting from the different heat treatments. These results indicate that our method is equally effective as a bioassay in distinguishing "life" and "dead" virus while only requiring 1,5 working days to achieve results. This method can easily be adapted to other viruses which make it a broadly applicable method and alternative to the bioassay.
KEYWORDS: Tobamovirus; virus detection; bioassay

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Session 5: VIRUS ECOLOGY AND EVOLUTION

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PHYLODYNAMICS, RECOMBINATION, AND MOLECULAR CLOCK ANALYSIS OF COTTON LEAFROLL DWARF VIRUS GENOMES FROM US-COTTON BELT STATES

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ABSTRACT:
Following the initial 2017 outbreak of the aphid-transmitted, cotton leafroll dwarf virus (CLRDV) (*Solemoviridae, Polerovirus*) in Alabama (USA), the results of surveillance efforts reported widespread CLRDV infection throughout the southeastern U.S. cotton belt. Total RNA was isolated from leaves collected from cotton plants established in sentinel plots located in U.S. cotton belt states. RNAseq libraries were prepared and enriched for CLRDV by target enrichment followed by Illumina sequencing and assembly of near full-length CLRDV genomes (n=103). Initial identification of CLRDV-like isolates was established by comparison to reference genomes available in the GenBank database. Near full-length genomes were subjected to phylogenetic, recombination, and molecular clock analyses. The phylogenetic tree (Bayesian) grouped CLRDV isolates representing the U.S. genomes and reference CLRDV genomes from South America and Asia (GenBank database) into three major clades with a basis in extant geographical origin: North America, South America, and Asia, respectively. The network tree structure was similar to that of the Bayesian tree and predicted extensive recombination in the majority of the U.S. CLRDV genomes. Among molecular-clock tree priors combinations tested the recombination-aware Coalescent Bayesian Skyline model provided the most robust support, reinforcing an underlying role of genomic admixture in CLRDV evolutionary trajectories. The Bayesian tree structure strongly supported multiple inter- and intra-continental hybridization events. Molecular clock analysis predicted that the CLRDV species emerged during the 1940?s, possibly in Asia, followed by South America in the 1980?s and the first U.S. introduction in the 1990?s. The initial invasion of CLRDV may possibly have been linked to unusual symptoms first reported in cotton in the Midsouthern and Southeastern U.S., referred to as “bronze wilt”, during June-July of 1995-1996, and 1998 when temperatures were unusually high. A new strain of Agrobacterium tumefaciens was identified as the possible causal agent. Bronze-wilt susceptible varieties were subsequently eliminated from U.S. varietal germplasm, suggesting that CLRDV susceptibility was selected against despite an unconfirmed etiology. In 2017-2018, cotton plants in Alabama and Georgia, albeit rarely, developed bronze wilt-like symptoms, including copper-colored leaves and maroon stems, potentially due to gene remnants in cotton gene pool admixtures.
KEYWORDS: Aphid-transmitted polerovirus; Emergent cotton virus; Plant virus phylogenomics

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DIVERSITY AND EVOLUTION OF PLANT ORTHOTOSPOVIRUSES INFECTING IMPORTANT CROPS IN ALABAMA

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ABSTRACT:
Plant viruses in the genus *Orthotospovirus*, including the tomato spotted wilt virus (*Orthotospovirus tomatomaculae*, TSWV) and soybean vein necrosis virus (*Orthotospovirus glycininecrovenae*, SVNV), pose significant threats to legume crops such as peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.), which are valued at $54 billion in the USA in 2023. These viruses possess an ambisense, single-stranded RNA genome composed of three segments that encode five open reading frames (ORFs): NSs (putative non-structural silencing suppressor), N (nucleocapsid), NSm (putative non-structural movement), GN and GC (glycoproteins), and L (RNA-dependent RNA polymerase). To examine the genetic diversity of TSWV and SVNV in Alabama, 172 peanut and five tomato samples were collected for TSWV across 2021-2023, while 33 soybean samples were collected for SVNV in 2023. The N, NSs, and NSm genes were sequenced and compared to previous sequences in GenBank. TSWV exhibited three amino acid changes in N from peanut and tomato, no changes in NSs, and one change in NSm only seen in tomato. In contrast, SVNV showed greater variability, with nine amino acid changes in N, six in NSs, and three in NSm. A phylogenetic analysis of these genes revealed that N is the most diverse in both viruses, suggesting its potential role in adaptation. Further investigation into the impact of these mutations on viral protein expression showed that they altered protein localization within host cells, indicating possible functional consequences. These findings provide new insights into the genetic evolution of TSWV and SVNV, highlighting specific mutations that may influence viral- host interactions. Identifying these genetic changes can help monitor virus strains that may overcome existing resistance and guide the development of breeding programs focused on enhancing resistance in peanut and soybean cultivars.
KEYWORDS: Tomato spotted wilt virus, soybean vein necrosis virus; nucleocapsid protein (N), non-structural silencing protein (NSs), non-structural movement protein (N; Sanger sequencing, amino acid mutations, and protein localization.

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EXPERIMENTAL EVOLUTION OF THE HOST RANGE FOR TWO ISOLATES OF POTATO VIRUS Y

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ABSTRACT:

Potato virus Y (PVY) remains one of the most economically damaging plant viruses, characterized by high genetic diversity and remarkable adaptability to different hosts. Despite its importance, the role of host species in driving PVY evolutionary trajectories, and eventually resulting in host range expansion, remains poorly understood. Here, we investigated the evolutionary dynamics of two PVY isolates (PVYNb and PVYSt) subjected to serial passages in three solanaceous hosts: *Nicotiana benthamiana*, *Solanum lycopersicum*, and *Solanum tuberosum*. Over ten passages, we monitored changes in infection efficiency, virus accumulation, and host-specific adaptation patterns, combining infectivity assays with high-throughput sequencing of evolved viral populations. Our results revealed isolate-specific and host-dependent evolutionary responses. PVYNb exhibited high adaptability, with robust infection efficiency and sustained viral replication in *N. benthamiana*, which acted as a source host facilitating viral diversification. In contrast, PVYSt displayed restricted infectivity, with tomato acting as a sink host, limiting viral accumulation and reducing the potential for sustained transmission. Serial passage experiments highlighted complex dynamics, with significant interactions between viral genotype, host species, and passage number shaping viral population size and stability. Infectivity assays of evolved lineages demonstrated that PVYNb populations passaged in *N. benthamiana* showed enhanced infectivity across all hosts, suggesting the potential for host generalism driven by a permissive hosts. Conversely, PVYSt populations and lineages evolved in tomato or alternating hosts exhibited reduced fitness, indicative of evolutionary constraints. Genome sequencing revealed higher intra-population diversity and host-specific fixed mutations in PVYNb lineages, while PVYSt populations remained more genetically stable, with few fixed changes across hosts. These findings underscore the critical role of host species as selective environments influencing viral adaptation, host range evolution, and, ultimately, virus emergence potential. This study highlights the key role of host species in shaping PVY evolution and adaptation, with implications for understanding virus evolution and developing effective management strategies for PVY in diverse agricultural systems.

KEYWORDS: source-sink dynamics; *Potyvirus*; virus evolution

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NATURAL REASSORTMENT IN CITRUS LEPROSIS VIRUS C MODULATES VIRAL LOAD DYNAMICS: EPIDEMIOLOGICAL IMPLICATIONS FOR CITRUS LEPROSIS DISEASE

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ABSTRACT:
Citrus leprosis virus C (CiLV-C, *Cilevirus leprosis*) is the main causal agent of citrus leprosis (CL) disease in Brazil, responsible for estimated annual economic losses exceeding US$ 54 million. CiLV-C population comprises three lineages: CRD, SJP, and ASU, whose members share approximately 85% nucleotide identity (nt id). CL is characterized by the local symptoms caused by the virus, that remains restricted to the feeding site from its *Brevipalpus* mite vector. Mixed infections involving SJP and CRD lineages have been documented in single lesions on citrus trees within the Brazilian citrus belt. A natural reassortant CiLV-C isolate, Crd08, was identified in 2020 on a sweet orange tree (*Citrus x sinensis* (L.) Osbeck) in Cordeirópolis, SP. This isolate carries a CRD-derived RNA1 (98.8% nt id with Crd01) and SJP-derived RNA2 (99% nt id with SJP01), initially noticed through RT-PCR with strain specific primers and later confirmed through high-throughput sequencing (HTS) analysis. To investigate the biological implications of the reassortment event, we assessed viral load dynamics in sweet orange leaves infected with Crd08 and its parental strains and verified the transmission by *B. yothersi* to *Arabidopsis thaliana*. Non-viruliferous adult *B. yothersi* mites were allowed to feed on infected sweet orange leaves and subsequently transferred into groups of five to healthy Arabidopsis plants. RT-PCR confirmed the presence of Crd08 in all 14 Arabidopsis leaves across six plants. Replication dynamics analyzed using RT-qPCR revealed altered RNA1/RNA2 ratios in Crd08, characterized by a higher accumulation of RNA2 compared to RNA1 (R1/R2: 0.96 ± 0.09; R2/R1: 1.05 ± 0.12), an inverse trend relative to CRD strain (R1/R2: 1.02 ± 0.03; R2/R1: 0.98 ± 0.03) and SJP (R1/R2: 1.13 ± 0.18; R2/R1: 0.91 ± 0.15). Absolute quantification of one of the genomic segments, i.e. gene RdRp (RNA1), showed that Crd08 exhibited intermediate viral loads (8.82E+05 ± 1.15E+05), surpassing CRD (1.26E+05 ± 2.80E+04) but remaining lower than SJP (2.80E+06 ± 4.12E+05). Ongoing digital PCR studies aim to precisely quantify genomic and subgenomic RNA segments, improving our understanding of the replication dynamics of the reassortant isolate. This study elucidates how segment reassortment contributes to CiLV-C diversification, altering replication dynamics and potential viral fitness - insights critical for improving surveillance and targeted control strategies in citrus production.
KEYWORDS: *Cilevirus*; *Brevipalpus yothersi*; RT-qPCR

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ANALYSIS OF 40 NEW ISOLATES OF BEAN YELLOW MOSAIC VIRUS REVEAL IMPORTANT NEW INSIGHTS INTO ITS PHYLOGENY, EVOLUTIONARY DYNAMICS AND BIOLOGY

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ABSTRACT:
Bean yellow mosaic virus (BYMV) has a very extensive natural host range including both dicots and monocots. The diseases it causes pose a significant threat to many cultivated plants, including legume crop and pasture species. In Australia, faba bean (*Vicia faba*) and lupin (*Lupinus* spp.) are important legume crops whose production potential is greatly hampered by BYMV infection. This study conducted a comprehensive analysis of 40 new Australian BYMV genomes from diverse crop, pasture and other hosts. These included both historical and recent BYMV isolates collected over a 73-year period. In addition, some isolates were inoculated to legume hosts to study their virulence. Genomes from different BYMV isolates exhibited >20% nucleotide (nt) sequence divergence. Phylogenetic analysis revealed the existence of ten distinct phylogroups including a new phylogroup (designated X) dominated by red clover (*Trifolium pratense*) isolates from Australia, Japan and Europe. Mechanical inoculation of faba bean plants with new isolates from phylogroup X, revealed unusually severe foliage symptoms including severe mosaic, and leaf and systemic tip necrosis followed by plant death. Moreover, the isolates within phylogroup X exhibited the highest evolutionary divergence 0.22 nt difference. BYMV's coat protein (CP) gene was the most highly conserved genome component having the highest number of nt polymorphisms (SNPs). Furthermore, our study identified episodic positive selection primarily in the P3 and CP genes. At the codon level, our analysis found distinct patterns of codon usage bias and selection pressures within the BYMV genome, with varying levels of genetic diversity and evolutionary constraints among the CP and P3 genes. Recombination analysis revealed more than 50 breakpoints providing new information relevant to the role of recombination in the genetic diversity and evolutionary dynamics of BYMV. Our findings provide valuable insights into the genetic mechanisms driving BYMV evolution, adaptation and spread with implications for understanding its virulence, host-virus interactions and improved management.
KEYWORDS: Bean yellow mosaic virus; Phylogeny; Evolutionary dynamics

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CONTRIBUTION OF WILD POACEAE SPECIES TO THE MAINTENANCE OF THE WHEAT DWARF DISEASE

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ABSTRACT:
Harvest represents a challenge for the persistence of insect-borne viral diseases in agroecosystems. To overcome this challenge, some viruses infect non-crop plants (hereinafter spillover) as reservoirs for future introduction of viruses to newly sown fields (hereinafter spillback). The wheat dwarf disease (WDD), one of the most important viral diseases on cereals, is caused by the wheat dwarf virus (WDV) and is transmitted by the leafhopper *Psammotettix alienus*. While the epidemic process of this disease has been studied in crop areas, very few data is available on factors influencing spillover/spillback events. To better understand the contribution of non-crop species in the epidemiology of WDD, plant-virus (i.e. infection rate) and plant-vector (i.e. survival and fecundity) interactions were monitored on 20 non-crop Poaceae (NCP) species. Results showed that i) host range of WDV is wider than expected and ii) NCP species can be clustered according to host quality for WDV and/or *P. alienus*. *Bromus hordeaceus* and *Phalaris arundinaceae* (two species with contrasted host quality for WDV and *P. alienus*) were included in multi-host arena experiments to assess the impact of a heterogeneous plant environment on leafhopper preferences and WDV infection. Collected data showed that *P. alienus* prefers *B. hordeaceus* (a poorly efficient host for WDV) for oviposition. This could lead to a dilution of viruliferous vectors in environments containing *B. hordeaceus* plants as hosts for WDV. Altogether, results of this work indicate that host quality and the composition of plant populations are important for WDD maintenance in non-cultivated areas.
KEYWORDS: Virus epidemiology; Virus and vector flux between crop and wild plants; *Geminiviridae*

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VIRUS EVOLUTION IN THE NATURAL ENVIRONMENT: A 12-YEAR STUDY OF A BEGOMOVIRUS COMMUNITY IN A NON-CULTIVATED PLANT

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ABSTRACT:
Begomoviruses (whitefly-transmitted, ssDNA viruses) have notoriously high rates of mutation and recombination, which together contribute to their high adaptive capacity. Begomoviruses are major threats to agriculture, especially in tropical and subtropical regions of the world. The emergence of new diseases often results from the proximity of wild (non-cultivated) hosts to domestic hosts. Wild plants constitute reservoirs of viral diversity and contribute to spillover events which are a pre-condition to virus emergence in crops. Studies exploring the ecological and evolutionary dynamics of viruses in non-cultivated plants are still in their infancy. In this work, we took advantage of the presence of a perennial population of the non-cultivated plant *Sida acuta* in a small area with little human intervention, in close proximity to our lab, and carried out a 12-year study of the begomovirus community infecting this host. The results indicate that *Sida acuta* is a highly permissive begomovirus host. The begomovirus community in *Sida acuta* is composed of viruses classified in at least five species (>91% nucleotide sequence identity), some of them subdivided into strains (>94% identity) and variants (>98% identity). In the first years of collection, Oxalis yellow vein virus (OxYVV) was the most prevalent virus in the area. The OxYVV population was composed of two strains, with one of the strains further subdivided into five variants. A major shift in the composition of the community occurred in 2016, when OxYVV was outnumbered by Sida yellow leaf curl virus (SiYLCV). The shifts in species composition within the community could be the result of random events that reduced the effective size of the OxYVV population, allowing the establishment of SiYLCV, or of migration of viruses from other areas. The low variability of the SiYLCV population and the maintenance of the same recombination event by all sequences suggest a common evolutionary origin for these isolates. The hypothesis of differential fitness among OxYVV variants was tested with infectious clones of the OxYVV-S1a and OxYVV-S1b variants. OxYVV-S1a showed greater accumulation in the early stages of infection, but viral load decreased over time, eventually equaling that of OxYVV-S1b. Transmission assays indicated no major differences in transmission rates between the two variants. The results are consistent with the hypothesis of fitness differences at the level of replication between OxYVV variants.
KEYWORDS: geminivirus; differential fitness; recombination

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STUDY OF THE GENETIC COMPOSITION AND EPIDEMIOLOGY OF POLEROVIRUSES RELATED TO PEPPER YELLOWS DISEASE IN GREECE

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ABSTRACT:
Pepper yellows disease (PYD) is widespread worldwide and a group of phylogenetically related poleroviruses (PeVYVs) is associated with it. To study the variation in the genetic composition of PeVYVs populations in Greece over the years, samples were collected from pepper plants displaying PYD symptoms from greenhouses of Crete from 2020 to 2023. Subsequently, samples from individual plants or groups of plants were subjected to high throughput sequencing (HTS) and the results showed the presence of PeVYV-6, which has been endemic in Crete for more than 15 years, and other genetically differentiated isolates (PeVYV-u). The PeVYV-u isolates exhibit similarity to PeVYV-6 up to the N-terminal end of P5 but they also show significant differences at the C-terminal end (4 resemble PoPeVYV by 86% in nt. and 81% in aa). However, one isolate (PeKuB31) from the PeVYV-u group exhibits higher similarity in each individual ORF with different PeVYV species. The RDP program detected recombination in ORF5 among a PeVYV-6 and/or PeVYV-5 and a PeVYV-2 ancestor. Remarkably, the change in the genetic composition of the population coincided with a significant reduction in the incidence of PeVYVs in pepper in 2022 and 2023. Specifically, PeVYV-6 was detected in 2020, along with PeVYV-u in 2021. In 2022, only PeVYV-u were detected, and in 2023 no PeVYV was found. This is possibly due to two separate introductions of poleroviruses (PeVYV-2, PoPeVYV-like), which although managed to displace PeVYV-6, have not yet established their presence in the field. Furthermore, the transmission of PeVYV-6 by aphids and its host range were investigated as factors affecting its prevalence in the region. Four different pepper colonizing aphid species were studied for their vectoring ability: *Aphis gossypii, Myzus persicae, Macrosiphum euphorbiae* and *Aphis nasturtii*. Results showed that *A. gossypii* was the most efficient vector, even though differences were observed among its different clones. Regarding the host range of PeVYV-6, many species of the Solanaceae family and most plants of the *Capsicum* genus can be infected by the virus under field or laboratory conditions. PeVYV-6 seems to have a broader host range compared to other PeVYVs.
KEYWORDS: PeVYV-6; genetic diversity; epidemiology

SUPPORT

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SEARCH FOR FACTORS INVOLVED IN THE EMERGENCE OF TOMATO LEAF CURL NEW DELHI VIRUS IN FRANCE

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ABSTRACT:
Tomato leaf curl New Delhi virus (ToLCNDV, *Begomovirus solanumdelhiense*) is currently emerging in cucurbit crops throughout the Mediterranean basin. Mediterranean ToLCNDV presents a high genetic uniformity, distinguishing it from its Asian relatives and suggesting a monophyletic origin. In France, ToLCNDV was first detected in autumn 2020 in the South-east(1). Although not observed in 2021, it has been detected again in the same areas every year since 2022, suggesting a local maintenance or repeated introductions. Complete sequencing of French ToLCNDV isolates by Sanger or Cider-Seq indicated that all isolates belonged unambiguously to the Mediterranean clade of ToLCNDV. However, the French isolates clustered in two molecular subgroups for DNA-A and four subgroups for DNA-B, with two A+B combinations being present since 2020, and other combinations detected since 2022. Surveys of weeds close to infected cucurbit crops indicated that the wild perennial cucurbits bryony (*Bryonia dioica*) and squirting cucumber (*Ecballium elaterium*) could constitute reservoirs allowing the local overwintering of ToLCNDV. These wild hosts often presented complex intraplant populations with infections by several molecular subgroups. Surprisingly, some French ToLCNDV isolates, observed since 2020, presented an atypical "recover" phenotype, where symptom severity in susceptible cultivated cucurbits decreased in the course of infection and virus accumulation was reduced compared to severe isolates. Experiments in controlled conditions did not reveal differences in host range or transmission that could counterbalance the apparent low fitness of the "recover" isolates. However, in mixed infection of ToLCNDV with watermelon mosaic virus (WMV, potyvirus), a very common virus in southeastern France, symptoms were more severe than in single infections; the recover phenotype was no more observed and ToLCNDV accumulation was increased without effect on WMV accumulation. The impact of mixed infections on ToLCNDV whitefly transmission remains to be determined. These results suggest that the interactions with alternative hosts and with other viruses play a key role for ToLCNDV maintenance and evolution in southeastern France.
KEYWORDS: Begomovirus; Emergence; Cucurbit

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Session 6: OTHER VECTOR-BORNE DISEASES

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OLIVE RESISTANT CULTIVARS SIGNIFICANTLY REDUCE TRANSMISSION EFFICIENCY OF *XYLELLA FASTIDIOSA* ST53 BY THE MAIN EUROPEAN VECTOR, *PHILAENUS SPUMARIUS* (HEMIPTERA: APHROPHORIDAE)

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ABSTRACT:

The epidemic of Olive Quick Decline Syndrome (OQDS) caused by *Xylella fastidiosa* subsp. pauca ST53 in South Italy olive groves has a dramatic impact at the economic and social levels. The combination of a landscape dominated by susceptible olive cultivars and high density populations of the insect vector *Philaenus spumarius* (Hemiptera: Aphrophoridae) led to the loss of millions of trees in about ten years[1]. In the framework of the containment measures, planting species immune or resistant to the bacterium is one of the strategies adopted for landscape and olive industry restoration. While replanting susceptible olive cultivars is forbidden by the phytosanitary legislation, replanting olive groves with the resistant/tolerant olive cultivars is currently promoted and financially supported. In this study we investigated the transmission efficiency of *X. fastidiosa* ST53 by *P. spumarius* from and to resistant plant sources (Leccino and FS17 olive cultivars). Several acquisition and inoculation trials were performed in different seasons during three years in both field and controlled conditions in the infected area in Apulia region of Italy, comparing resistant olive cultivars with the most common susceptible ones grown in the infected area. The experiments demonstrated that i) both resistant olive varieties used in our study were inefficient sources of the bacterium for the vector (with very low subsequent inoculation rates by insect vector), ii) when used as recipient plants, both resistant and susceptible olive cultivars were infected at a similar rate, iii) the inoculation rate on both susceptible and resistant plants was positively related to the number of infectious insects that fed on test plants. These results highlight how the cultivation of resistant olive cultivars can contribute - at an epidemiological level - to reduce the rate of spread and the incidence of the infections, by suppressing the bacterium inoculum and therefore the infectivity of the vector populations. However, as resistant varieties are not immune to the infections, control of the vector populations - coupled with the reduction of the sources of inoculum - at the crop and landscape levels remain of paramount importance[2]. Our results provide useful data for modeling the future evolution of epidemic in Italy and implementing the use of resistant olive varieties, in order to cope with OQDS in infected areas and neighboring regions at risk of *X. fastidiosa* introduction.

KEYWORDS: vector-borne disease; Olive Quick Decline Syndrome; non circulative-persistent transmission

SUPPORT

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CSSAMT OVEREXPRESSION ALTERS VOLATILE PROFILES AND REDUCES *DIAPHORINA CITRI* PREFERENCE IN *CITRUS SINENSIS*

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ABSTRACT:
Diaphorina citri is the vector of *Candidatus* Liberibacter asiaticus (CLas), the causal agent of Huanglongbing (HLB), a devastating citrus disease. The insect acquires CLas by feeding on infected plants and transmits it to healthy ones. Disease management relies on broad-spectrum insecticides, leading to insect resistance and environmental concerns. Plants produce volatile organic compounds that can attract or repel insects, influencing its behavior. Methyl salicylate (MeSA) is a key volatile compound released by plants in response to pathogens. Plant cells accumulate salicylic acid (SA), which is converted into MeSA via SA-dependent methyltransferase (SAMT). Previous studies have shown that transgenic plants overexpressing *CsSAMT* gene in *Citrus sinensis* increases MeSA production, enhancing resistance against Citrus Canker and HLB. However, although MeSA plays a crucial role in plant defense, it also seems to attract psyllids, even at low concentrations. Therefore, to assess the response of transgenic plants to *D. citri,* we evaluated the insect's attractiveness, multiple-choice preference, and oviposition behavior toward MeSA-emitting plants overexpressing *CsSAMT* (Hamlin and Valencia cultivars) compared to non-transgenic (WT) plants. Experiments were conducted over three consecutive days using H-shaped and X-shaped cages, with both healthy and CLas-infected plants. The results indicated that *CsSAMT* overexpression did not significantly influence the attractiveness of *D. citri*. Interestingly, WT plants were preferred for oviposition, suggesting that the increased presence of MeSA may have a repellent effect. Given these unexpected findings, we investigated whether MeSA-emitting plants also modified the profile of other volatiles that could influence insect preferences. Therefore, we analyzed the volatile composition of essential oils extracted from the leaves of CsSAMT lines and WT controls using gas chromatography. GC-MS analysis revealed that *CsSAMT* overexpression significantly affected the concentrations of several compounds, including sabinene, limonene, linalool, β-caryophyllene E, and β-sinensal. Additionally, we identified unique compounds, such as geranyl linalool, which were present in CsSAMT lines but absent in WT samples. In conclusion, this study demonstrates that CsSAMT overexpression in sweet orange alters the plant's volatile profile, not affecting *D. citri* attractiveness but influencing its feeding preferences.
KEYWORDS: Insect attraction; airborne defense; Volatile organic compounds

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TARGETED MYC2-PROTEIN STABILIZATION CONFERS CITRUS HUANGLONGBING RESISTANCE

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ABSTRACT:
Citrus is one of the most widely cultivated fruit crops globally with the highest fruit yield annually. Huanglongbing (HLB), also known as citrus greening, is currently the most devastating citrus disease, causing the most serious economic losses in citrus industry. HLB is primarily caused by the phloem-colonizing bacterium *Candidatus* Liberibacter asiaticus (CLas) and is mainly transmitted in the field by the Asian citrus psyllid (*Diaphorina citri*). Deploying Resistance (R) genes during breeding is the most effective and sustainable approach for combating crop diseases. However, no R-gene or cure currently exists for this disease and all commercial citrus varieties remain susceptible to HLB. Therefore, in this study, we identified new susceptibility gene and developed new approaches to inactivate these S genes for citrus resistance breeding. We report an HLB-resistant regulatory circuit in Citrus composed of the PUB21 gene, encoding an E3 ubiquitin ligase PUB21 and its substrate MYC2 transcription factor, the latter regulates defensive phytohormone jasmonate (JA)-mediated responses. MYC2 is degraded by PUB21 and diminished in HLB-sensitive Citrus species due to a helitron transposition within the PUB21 promoter. The helitron insertion acquires multiple MYC2 binding cis-elements and creates a regulatory circuit linking the PUB21 promoter-PUB21-MYC2 to demolish MYC2. Ectopic expression of inactive PUB21C39A, a natural dominant-negative variant discovered in Citrus distant relatives, stabilizes MYC2 and confers immunity against HLB. This study provides new insights into developing strategies to address unculturable pathogenic diseases through targeted immune protein stabilization.
KEYWORDS: Candidatus Liberibacter asiaticus; PUB21-MYC2 regulatory circuit; helitron transposon

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TOWARDS RNAi-BASED CONTROL OF *SCAPHOIDEUS TITANUS*, VECTOR OF FLAVESCENCE DORÉE PHYTOPLASMAS

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ABSTRACT:
RNA interference (RNAi) is a mechanism to regulate gene expression in eukaryotes and can be exploited for crop protection by exogenous applications of double-stranded RNAs (dsRNAs). Two vector species of Flavescence dorée phytoplasma (FDp), *Scaphoideus titanus* (natural vector) and *Euscelidius variegatus* (laboratory vector) (Hemiptera: Cicadellidae), show efficient responses to dsRNAs. Flavescence dorée (FD), a serious threat to European viticulture, controlled by planting of healthy material, roguing of infected plants and insecticide treatments to limit disease spread in the vineyards, with obvious environmental and health consequences. We explored a set of essential genes, already proved to negatively affect survival of other insect species, for their ability to control the natural FDp vector *S. titanus*. To maximize specificity of RNAi triggering molecules, candidate target mRNAs of both vector species were aligned with correspondent transcripts of the off target *Apis mellifera*, to identify portions with the lowest percentage of homology. Due to the high degree of homology shared by the vector species at the selected genes, preliminary tests were conducted on the laboratory vector E. variegatus, easiest to rear. Microinjection was selected as the most efficient delivery strategy of dsRNAs to newly emerged E. variegatus adults, and target silencing was assessed at 7, 14 and 21 days post injection, together with mortality rate for each treatment. One of the best performing target was associated to reduced progeny, and gender-cross experiments confirmed that this was mainly due to gene silencing of females. Based on results obtained on E. variegatus, one candidate target was tested on the natural FD vector, *S. titanus*. Silencing resulted in significant decreased survival compared to the control insects, and in compromised ovary phenotype, also in this species. Silencing of genes with a lethal phenotype, coupled with those involved in different phases of vector body colonization by the FD phytoplasma may represent a valid strategy to cope with insecticide bans in the EU, provided an efficient method of dsRNA delivery to these vineyard phloem-feeder insects can be identified.
KEYWORDS: *Candidatus* Phytoplasma vitis; *Vitis vinifera; Euscelidius variegatus*

SUPPORT

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EVIDENCE OF ADAPTATION TO TEMPERATURE IN A VECTOR-BORNE PLANT PATHOGEN

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ABSTRACT:
There is a pressing need to understand range shifts of diseases under a changing climate in order to safeguard biodiversity and food security. Disease models generally use current species distributions to infer future geographic distributions under different climate scenarios. However, pathogens may also adapt to novel conditions, such as temperature extremes, increasing the risk of unexpected disease expansions. We used a set of 188 *Xylella fastidiosa* genomes obtained from diseased grapevines across a ~1,000-kilometer latitudinal gradient in California, USA, to study the potential for pathogen adaptation to a temperature gradient. Gene-environment association models accounting for population structure were used to identify 213 significant SNPs; the vast majority of SNPs were associated with summer (17 distinct genes and intergenic regions, mostly in 3 loci) rather than winter (1 locus) temperatures. Surprisingly, the vast majority of climate-associated variants were of recombinant origin. This work shows that risk assessments should consider pathogen adaptation if feasible.
KEYWORDS: Climate; adaptation; *Xylella fastidiosa*

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DOES WATER DEFICIT PROMOTE 'CANDIDATUS LIBERIBACTER SOLANACEARUM' SPREAD?

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ABSTRACT:
The bacterial pathogen '*Candidatus* Liberibacter solanacearum' (Lso) was first reported in the early 2000s infecting solanaceous plants and vectored by the potato psyllid, *Bactericera cockerelli*. After its discovery, this pathogen was reported associated with several plant families and different psyllid vectors worldwide. Anecdotal accounts point to a role of water deficit in the percentage of infectious potato psyllids in the field. To evaluate the role of water deficit in the epidemiology of the diseases caused by this pathogen, we previously investigated the effect of water deficit on potato psyllid fitness and discovered that psyllids developing on drought-stressed plants had improved fitness (1). Here, we report our findings evaluating the role of water deficit on the disease caused by two different Lso haplotypes, A and B. Four-week-old tomato plants were subjected to two weeks of water deficit or control conditions. They were then infested for one week with psyllids carrying LsoA, LsoB or without Lso. After insect removal, the plants were maintained for at least 8 weeks, period of time after which LsoB-infected plants usually die. Plant height, symptom development, and Lso presence in different leaves of the plant were evaluated bi-weekly. Our results suggest that water deficit did not affect LsoB infection. However, water deficit improved tomato tolerance to LsoA infection. We also discovered an effect of water deficit on Lso accumulation and distribution within the plant. The consequences of this accumulation difference in bacterial acquisition by the vector have not been investigated yet.
KEYWORDS: psyllid; drought; tomato

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PROPOSED INTEGRATED HUANGLONGBING (HLB) MANAGEMENT FOR CITRUS IN FLORIDA

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ABSTRACT:
Huanglongbing (HLB), also known as citrus greening, is a devastating disease primarily linked to *Candidatus* Liberibacter asiaticus (*C*Las) and spread by the Asian citrus psyllid (ACP; *Diaphorina citri*). Despite extensive management efforts, the disease continues to threaten citrus production in Florida and other regions worldwide. Since 2016, our research team, in collaboration with federal and state agencies, has been developing practical solutions to help citrus growers combat HLB. While no cure exists, we have introduced several management strategies that growers in Florida are increasingly adopting. These include the use of individual protective covers (IPCs) during planting to prevent infection, trunk injections of oxytetracycline (OTC) to mitigate disease symptoms, and the application of brassinosteroids (Brs) and systemic acquired resistance inducers (SARs) to shield new growth from reinfection. Additionally, we are assessing new tools such as HLB-tolerant inter-stocks and an automated trunk injection system. Field studies evaluating these approaches have demonstrated promising results. IPCs effectively block *C*Las infection, OTC reduces bacterial titers and fruit drop, while Brs and SARs enhance tree productivity and protect new shoots from ACP-mediated reinfections and citrus canker. Collectively, these integrated pest management (IPM) tools offer a viable strategy for controlling HLB in commercial citrus orchards, supporting sustainable citrus production despite the disease´s presence. Ongoing field trials aim to refine and integrate these tools into comprehensive IPM programs, with a proposed management plan tailored for Florida orchards.
KEYWORDS: citrus greening; trunk injection; therapeutics

SUPPORT

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Session 7: CLIMATE AND EPIDEMICS

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WEATHER-BASED APHID FLIGHT FORECASTING: ESTIMATING VIRUS RISK AND ENHANCING MANAGEMENT

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ABSTRACT:
In France, open-field melon crops are frequently infected by viruses, among which cucurbit aphid-borne yellows virus (CABYV, *Polerovirus*). This virus is becoming a serious threat in an increasing number of cucurbit producing countries. It is transmitted in a persistent, circulative, non-propagative manner by a few aphid species (*Aphis gossypii*, *Macrosiphum euphorbiae* and *Myzus persicae*). In the absence of curative methods, virus control relies exclusively on measures limiting virus introduction and spread. Whatever the control method under consideration (chemical, genetic, biological), its efficiency can be expected to be enhanced with an accurate knowledge of epidemic drivers, in particular those linked with aphid vectors. An eight-year field experiment database was set up and used to investigate the relationship between CABYV epidemics and aphid vector abundances. A systematic search for correlations between virus variables (estimated parameters of logistic models describing epidemic progress curves) and aphid variables computed by aggregating abundances on periods relative to the planting date was undertaken. The abundance of *A. gossypii* during the first two weeks after planting was found to be a good predictor of CABYV dynamics suggesting that an early control of this aphid species could mitigate the onset and progress of CABYV epidemics in melon crops. In a further step, a model-based statistical analysis was performed aiming at forecasting *A. gossypii* flight patterns as a function of local weather conditions. Significant relationships were established between aphid abundances and climatic variables concerning mainly temperature and wind direction. Although promising, these relationships fail to correctly predict some flight peaks, suggesting that local weather conditions cannot appropriately explain the arrival of aphids from more distant sources. This hypothesis was explored by calculating air-mass trajectories during the arrival periods of aphids in the fields, via the Tropolink application (1) relying on the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT, 2). New data sets including the possible long-distance origins of aphids were used to improve the weather-based covariates in our predictive models.
KEYWORDS: aphid vectors; CABYV; climatic variables

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CLIMATE CHANGE EFFECTS: ELEVATED CO_2 MODIFIES WHEAT CANOPY TEMPERATURE, WATER USE AND VIRUS PLANT INTERACTIONS

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ABSTRACT:
Climate change, driven by rising atmospheric carbon dioxide (CO_2) levels and increasing temperatures, poses significant challenges to agricultural production. Understanding how these factors influence plant-virus interactions is essential for predicting future crop performance. The Barley and Cereal yellow dwarf virus (B/CYDV) complex is a major viral disease impacting cereal crops globally, with transmission primarily facilitated by aphids, particularly *Rhopalosiphum padi*. While elevated CO_2 (eCO_2) can enhance plant biomass and yield, it also induces physiological changes that may alter disease dynamics and severity. This study examines the direct effects of eCO_2 on plant canopy temperature and water use efficiency in virus free and BYDV-PAV infected wheat. Using thermal imaging technology, we monitored temperature variations and plant responses under controlled conditions. Our findings reveal that eCO_2 significantly increases plant canopy temperature by reducing water use. Additionally, virus-infected plants under eCO_2 conditions exhibit even higher canopy temperatures compared to virus-free plants, suggesting an amplified physiological response to infection. These changes could have far-reaching consequences for plant health, vector populations and viral transmission under future climate scenarios. Our results suggest that rising CO_2 levels may reshape virus interactions in agricultural ecosystems, potentially impacting crop productivity and sustainability. This study underscores the urgent need for further research to develop adaptive strategies for managing plant viruses in a changing climate.
KEYWORDS: climate change; plant virus interaction; plant canopy temperature

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Session 1: GENERAL EPIDEMIOLOGY

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COWPEA MILD MOTTLE VIRUS: HIGH INCIDENCE IN SOYBEAN AREAS OF SÃO PAULO AND NATURAL TRANSMISSION THROUGH SEEDS.

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ABSTRACT:
Brazil is the leading producer and exporter of soybean (*Glycine max*). However, during the 2023/2024 soybean season, the prolonged El Niño phenomenon resulted in high temperatures and reduced rainfall. This climate event contributed to an increase in the whitefly (*Bemisia tabaci*) (Hemiptera: Aleyrodidae) population in the fields, as higher temperatures accelerate its life cycle. Whiteflies cause physiological disturbances in soybean plants through feeding and virus transmission. This pest is considered a virus supervector, capable of transmitting numerous viruses to crops, such as the cowpea mild mottle virus (CPMMV). CPMMV belongs to the *Betaflexiviridae* family and the *Carlavirus* genus, primarily infecting Fabaceae crops, including soybeans and common beans. To identify the possible source of inoculum of CPMMV in the soybean fields, this study aimed to understand the extent to which soybean seeds could transmit CPMMV. We collected 100 leaf samples from ten different soybean genotypes in commercial fields. Total RNA was extracted (Bertheau et al., 1998[1]) and subjected to RT-PCR to confirm CPMMV infection. All the evaluated genotypes tested positive for CPMMV, with infection ranging between 74% and 100%. At the end of the soybean cycle, at least 1000 seeds from these genotypes were collected, planted in trials, and kept in a greenhouse. When the first trifoliate leaf emerged, total RNA was extracted and tested for CPMMV via RT-PCR. Out of the ten evaluated soybean genotypes, seven exhibited CPMMV transmission through seeds, with transmission rates ranging between 0.2% and 1.4%. Symptoms observed included mottling, yellow mosaic, blistering, and leaf curling, while some plants showed no symptoms. Based on the results of this study, further research is needed to develop seed care management strategies to prevent CPMMV transmission and minimize the source of inoculum of the virus in soybean fields.
KEYWORDS: seed infected; carlavirus; soybean breed.

SUPPORT

São Paulo State University (UNESP), School of Agricultural Sciences, Brazil.

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A DECADE LONG STUDY REVEALS DISTINCT EPIDEMIOLOGICAL TRAJECTORIES OF GRAPEVINE RED BLOTCH VIRUS AND ZONAL ROGUING AS A DISEASE MANAGEMENT STRATEGY

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ABSTRACT:

Red blotch disease, a serious threat to grape production in the United States, is caused by grapevine red blotch virus (GRBV, *Grablovirus vitis, Grablovirus, Geminiviridae*). GRBV isolates group into two distinct phylogenetic clades, both of which are implicated in disease etiology. The dissemination of infected planting material has resulted in the widespread presence of GRBV across the United States. Secondary spread has been observed in Northern California and Southern Oregon. The three-cornered alfalfa hopper, *Spissistilus festinus*, transmits GRBV in the greenhouse and in vineyards in Northern California. The transmission is circulative, non-propagative. Epidemiological studies over the course of a decade in a diseased ?Cabernet franc? vineyard experiencing secondary spread showed an increase in disease incidence from 3.9% in 2014 to 36.4% in 2023. Interestingly, increases in disease incidence were observed three years following a growing season with less rainfall (r=-0.68), suggesting a more active *S. festinus* presence in vineyards, potentially due to a reduced availability of preferred feeding and reproductive hosts in natural and riparian areas outside the vineyard. An increase of newly symptomatic vines three years following periods of little rainfall is congruent with an extended latency period of GRBV. Further, five subsections of the study vineyard, with varying proximity to a disease focus, experienced distinct spread dynamics with virus transmission primarily occurring from diseased vines to neighboring vines, as determined by Spatial Analysis by Distance Indices. Testing every vine within the five subsections for GRBV revealed the presence of asymptomatic infections of isolates from both phylogenetic clades in each subsection. Current recommendation strategies suggest roguing of individual diseased vines when incidence is less than 30%, and removal of the entire vineyard when greater than 30%. Considering the presence of asymptomatic infections, join-count analysis suggested zonal roguing based on incidence and level of aggregation is more appropriate to reduce virus incidence. Together, this study determined spread dynamics shaped by virus incidence, aggregation patterns, vector dispersal and weather conditions. This information was paramount for refining red blotch disease management guidelines and proposing zonal roguing in response to a viral disease in a perennial fruit crop.

KEYWORDS: grapevine; geminivirus; management

SUPPORT

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BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF PEPPER NECROTIC SPOT VIRUS: AN EMERGING ORTHOTOSPOVIRUS WITH POTENTIAL SEED TRANSMISSION ABILITY

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ABSTRACT:

The emergence of new viruses or viral variants in agriculture represents a significant threat to food security, leading to substantial economic losses. Agricultural intensification characterized by reduced genetic diversity has facilitated the emergence of viral diseases. In South-America, pepper necrotic spot virus (PNSV) has emerged as a phytosanitary-relevant orthotospovirus, primarily affecting solanaceous crops. Viruses of this genus are transmitted by thrips, possess a single-stranded tripartite RNA genome (L, M, and S), and exhibit a high genetic and phenotypic variability, complicating their management. However, unlike other orthotospoviruses, which are transmitted exclusively by insect vectors, the potential seed transmission of PNSV suggests an unconventional epidemiological scenario within the genus. To date, research on PNSV has been limited to the partial sequencing of the S-segment from two Peruvian isolates (HE584763.1 and HE584762) infecting tomato and pepper, with biological characterization restricted to symptomatology in a narrow host range. To expand our understanding of this emerging pathogen, we conducted a comprehensive molecular and biological characterization of the Chilean PNSV isolates, evaluating their epidemiology. For this purpose, we sequenced the nucleocapsid (N) and RNA silencing suppressor (NSs) genes from local isolates, finding 96% amino acid sequence identity with Peruvian isolates, suggesting a close phylogenetic relationship and the potential presence of the same isolates in Chile. Pathogenicity assays revealed infection in three plant families (Asteraceae, Brassicaceae, and Solanaceae), with significant differences in disease severity progression. Among the observed symptoms were necrotic rings, chlorosis, leaf curling, and floral/foliar abortion. Notably, seed transmission assays using RT-PCR detected PNSV in symptomatic pepper seeds, as well as in cotyledons, true leaves, and roots of germinated seedlings, suggesting a potential vertical transmission. These findings highlight the need to further investigate the genetic variability and transmission mechanisms of Orthotospoviruses, considering that species such as soybean vein necrosis (SVNV) and groundnut ringspot virus (GRSV) have shown differences in their dispersal strategies. The confirmation of PNSV seed transmission could redefine the epidemiological paradigm of the genus, with direct implications for phytosanitary management of horticultural crops.

KEYWORDS: Vertical transmission; Orthotospoviruses; emergence diseases

SUPPORT

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DEVELOPMENT OF A DODDER-BASED APPROACH FOR EFFICIENT INFECTIOUS CLONE TRANSFER TO WOODY HOSTS

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ABSTRACT:
Infectious clones provide a fully characterized single virus source that can be used to study virus biology and its interactions with hosts or vectors. Agrobacterium-mediated transformation is commonly used to deliver these clones to targeted hosts. However, this method can be inefficient, particularly when working with woody hosts, which are often recalcitrant to genetic transformation. Dodder species (*Cuscuta* spp.) have long been utilized in plant virology to facilitate virus transmission between unrelated plant species by forming connections with the vascular tissue of the parasitized plants. Here, we introduce a novel technique that addresses the challenges posed by woody hosts by using dodder as an intermediary to facilitate the transfer of infectious clones from agroinoculated plants to those woody hosts. To test this method, we used infectious clones from two viruses: a systemic virus, blackberry chlorotic ringspot virus (BCRV), and a phloem-limited virus, blackberry yellow vein associated virus (BYVaV). The dodder-based approach demonstrated superior efficiency compared to direct agroinoculation methods in facilitating virus transmission in *Rubus* spp. Specifically, transmission efficiency increased from 9% to 73% for BCRV and from 0% to 46% for BYVaV. This novel dodder-based approach expands the potential for virus research across a broader range of plant species, overcoming the limitations of traditional agroinoculation methods that have often hindered work with woody plants.
KEYWORDS: transmission; dodder; infectious clone

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EXPLORING THE VIROME OF CITRUS SPP. IN GREECE

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ABSTRACT:

Citrus is recognized as one of the most significant crops globally. In prior research, we investigated the role of reservoir weed hosts in the transmission of citrus viruses and viroids within citrus orchards. The current study aims to enhance our knowledge of the virome associated with citrus trees in Greece. Thirty-six citrus samples, representing seven species, were collected from 18 orchards, previously surveyed for weed hosts. These samples were analyzed by RT-PCR using primers specific for five viroids and three viruses (1). The findings revealed a high prevalence of HSVd (91%) and CDVd (86%), followed by CBCVd (80%), CEVd (72%) and CBLVd (52%), detected in all different citrus hosts, in accordance with a previous epidemiology study in Greece (1). Among viruses, CPsV was the most prevalent (47%), followed by CTV (19%), while CLBV was not detected. The virome of four pooled samples (C1-C4), each containing 3-6 sub-samples, was explored by high-throughput sequencing (HTS) on the Illumina platform. HTS confirmed the presence of the citrus viroids and viruses previously detected by RT-PCR. Moreover, CiVA was identified in all four pooled samples, and the complete genome of CCDaV (3,641 nt) was recovered as a single contig from sample C1. Notably, analysis of the sequencing reads allowed to reconstruct a second shorter form of CCDaV differing by a single large 113 nt indel. Given that the Rep protein of other Geminiviridae members is often expressed following a splicing event, this indel might represent the excision of an intron. PCR analysis confirmed that four out of the six samples pooled in C1 were infected with CCDaV. By designing specific primers flanking the indel region, we conducted RT-PCR and amplicon sequencing confirming the presence of an intron in CCDaV genome. An ongoing analysis of all individual samples aims to confirm and further elucidate the presence of CiVA and CCDaV in Greek citrus plants. The detection of CCDaV represents its first identification in the European Union, following reports from China, Thailand, and Turkey (2).

KEYWORDS: citrus; viroime; RNA splicing

SUPPORT

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UNDERSTANDING AFRICAN GREENING: A CASE STUDY ON THE SPATIAL AND TEMPORAL DISTRIBUTION OF CANDIDATUS LIBERIBACTER AFRICANUS IN SOUTH AFRICA

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ABSTRACT:
Huanglongbing (HLB) is currently the most significant biological threat to the global citrus industry. In its severe form, it causes catastrophic economic losses by reducing yield, compromising fruit quality, and ultimately leading to tree decline. HLB is caused by three closely related bacterial species: *Candidatus* Liberibacter asiaticus (CLas), *Ca*. L. americanus (CLam), and *Ca*. L. africanus (CLaf). While CLas has caused devastating outbreaks in various citrus-growing regions, African Greening, the form of the disease caused by CLaf, is a mild form of HLB. Commercial citrus production can be sustained in areas where African Greening is endemic, by adjusting citriculture practices. However, it remains an ongoing challenge. *Trioza erytreae*, the vector responsible for transmitting CLaf in South Africa, plays a key role in the persistence and spread of the disease in affected orchards, and is the focus for controlling disease spread. Despite being present in South Africa for over 100 years, aspects of CLaf pathogenesis remain to be elucidated, particularly its movement within the host, long-term effects on tree health, and epidemiological patterns in commercial orchards. The aim of this case study is to advance our understanding of African Greening and its vector by examining the spatial and temporal distribution of CLaf within trees, orchards and the whole production region.The study focused on a commercial orchard where diseased trees were visually assessed, tree-by-tree, and the development of foliar disease symptoms tracked over time. To investigate pathogen distribution within trees, CLaf concentrations were quantified by collecting and analysing bark and leaf samples from multiple locations along the trunks and branches, tracking liberibacter concentrations during the season. In addition, yellow sticky traps were placed throughout the production region to monitor *T. erytreae* populations. Captured specimens were screened for liberibacter to estimate the percentage of infected vectors, providing insight into disease pressure in the region.These insights will contribute to the development of improved diagnostic tools and protocols, better disease management strategies, and a more comprehensive understanding of CLaf epidemiology, ensuring the long-term sustainability of citrus production in the region.
KEYWORDS: Citrus; *Trioza erytreae*; Huanglongbing

SUPPORT

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THE CURRENT STATUS OF COTTON LEAFROLL DWARF VIRUS IN THE UNITED STATES

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ABSTRACT:
Cotton leaf roll dwarf virus (CLRDV) has been reported to infect *Gossypium hirsutum* in Asia, South America, and the United States. CLRDV is spread to cotton by *Aphis gossypii* in a persistent and non-propagative manner. Research over the past five years has been conducted to better understand the epidemiology of this pathosystem. Studies examining weekly seasonal dynamics of virus spread by the vector showed that the vector is active mid- and late-season, and virus spread occurs 3-15 weeks during the cotton growing season, depending on the geography. Data from on-farm studies characterizing abundance of *A. gossypii* and CLRDV over a two-year period at 64 locations characterized the causal relationships between aphid abundance, CLRDV incidence, weed host presence and landscape features. Additionally, aphid gut content analyses were conducted to determine alternate hosts of the aphids prior to infesting cotton in the Southeastern United States Cotton Belt. A summary of the major findings from these recent studies as they relate to vector-virus-host relationships responsible for CLRDV spread will be presented and discussed.
KEYWORDS: Polerovirus; Aphididae; Cotton

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TOMATO LEAF CURL NEW DELHI VIRUS: AN EMERGING PATHOGEN AFFECTING GREEK CUCURBITS

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ABSTRACT:
The whitefly-transmitted tomato leaf curl New Delhi virus (ToLCNDV, genus *Begomovirus*, species *Begomovirus solanumdelhiense*) is one of the most devastating viruses around the world. In Europe, ToLCNDV can occur in tomato but is prevalent in cucurbits in the Mediterranean basin (1). It was first reported from Greece in 2018 (2) and this study reports its epidemiological (natural host range, incidence, vectors) and genetic (molecular variability of isolates) profile. Field surveys were conducted from 2022 onwards in major vegetable cultivation areas. Open field and protected cucurbit crops were randomly sampled and tested for the presence of ToLCNDV by TAS-ELISA (DSMZ AS-1109). To investigate the contribution of ToLCNDV to important cucurbit diseases, field plants with conspicuous virus symptoms were also tested for common aphid-borne viruses. When ToLCNDV was confirmed, cultivated plants and weeds in the vicinity of the positive plant were sampled and tested for ToLCNDV. To date, more than 2200 samples have been analysed. ToLCNDV was widespread in open field zucchini, cucumber, melon, and snake melon crops, in Northern Greece (Attica, Viotia, Messinia, Lasithi, Evia, Ilia, Argolida) and became the predominant virus during the late summer-autumn period. Especially severe symptoms were recorded on zucchinis when ToLCNDV appeared in mixed infections with cucurbit aphid-born yellows virus or cucumber mosaic virus. Among the 55 weed species tested, ToLCNDV was confirmed only in the perennial *Ecballium elaterium* that may function as virus reservoir. To investigate the molecular variability of the virus, DNA extracted from selected ToLCNDV positive samples was amplified by PCR and RCA followed by genome sequencing. A HTS analysis of two samples was performed using total RNA extracts omitting DNase treatment. The molecular analysis of virus isolates from different hosts and geographical regions confirmed the close genetic relationship with ToLCNDV-ES strain described from Spain. *Bemisia tabaci* populations are also being sampled across Greece for biotype identification using COI gene DNA sequencing, and analysis of the cryptic species within *B. tabaci* is ongoing. This study demonstrated that isolates from the ToLCNDV-ES cluster are significantly affecting cucurbit crops in Greece. However, to fully understand their epidemiology, further work is needed to identify infection sources, virus reservoirs, and the whitefly populations responsible for virus transmission.
KEYWORDS: *Begomovirus*; whiteflies; HTS

SUPPORT

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UNDERSTANDING THE INTERPLAY BETWEEN *IMPATIENS NECROTIC SPOT VIRUS* AND PYTHIUM WILT IN LETTUCE

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ABSTRACT:
Lettuce (*Lactuca sativa*) is produced year-round in the United States, with the state of California leading production for domestic consumption in the U.S. and for export markets. Monterey County in California is the largest county for lettuce production, however, it has recently been threatened by *Impatiens necrotic spot virus* (INSV), a tospovirus transmitted by the Western flower thrips, *Frankliniella occidentalis*, and was first reported in 2008 (1). Pythium Wilt, a soilborne disease caused by the oomycete *Pythium uncinulatum* was first reported in 1995 (2), but recently, severe outbreaks of both pathogens have occurred in lettuce fields and resulted in over 150 million dollars lost in 2022. Furthermore, field evaluations documented a high frequency of coinfection by both pathogens. Here, we sought to characterize the potential interactions between INSV and Pythium Wilt using methods that represent natural infections. *P. uncinulatum* was propagated on millet and mixed with autoclaved potting soil in a ratio of 1:7. Lettuce seedlings var. Parris Island were then transplanted into the inoculated soil and INSV transmission assays using thrips were performed at three different time points of plant growth. A total of four treatments were setup to assess disease symptoms and quantify the titer of each pathogen in lettuce roots. Lettuce treatments included: 1) *P. uncinulatum* inoculation only, 2) INSV inoculated only, 3) *P. uncinulatum* + INSV inoculation, 4) Uninoculated control. All treatments were maintained in a greenhouse for four weeks, evaluated for disease symptom development, and harvested to extract DNA and RNA from the roots. Samples were evaluated using a multiplex RT-qPCR assay that quantified INSV and *P. uncinulatum* from a single sample. Plants that were inoculated with INSV became infected regardless of the growth stage at which they were inoculated. Interestingly, plants that were co-infected with both pathogens showed more severe symptoms than plants that were singly infected. Additional replications are in progress. This work is a first step towards understanding the role of INSV in the re-emergence of Pythium Wilt in California lettuce. Understanding the interactions between INSV and Pythium Wilt provides new knowledge that will improve the selection of appropriate disease management strategies.
KEYWORDS: Tospovirus; Western flower thrips; *Pythium uncinulatum*

SUPPORT

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SPATIOTEMPORAL SPREAD OF GRAPEVINE LEAFROLL-ASSOCIATED AMPELOVIRUSES IN COMMERCIAL VINEYARDS OF NORTHEN GREECE

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ABSTRACT:
Grapevine leafroll disease (GLD) is one of the most economically important diseases affecting grapevine worldwide. Five viruses of the *Closteroviridae* family (grapevine leafroll-associated virus 1, -2, -3, -4, -7 - GLRaV-1, -2, -3, -4, -7) have been associated worldwide with grapevine leafroll disease (GLD). GLRaV-1, GLRaV-3 and GLRaV-4 are widely spread in Greek vineyards. Based on this knowledge, a study was carried out to investigate their rate of spread within vineyards over time. For this purpose, 194 grapevine samples of Malagouzia cultivar (8 years old in 2021) from Epanomi Thessaloniki and 72 and 70 grapevine samples from two young vineyards (one year old in 2021) of the cultivars Xinomavro and Muscat De Hambourg, respectively, from Goumenissa Kilkis were collected. The three vineyards are near to older vineyards showing typical GLD symptoms. The same number of marked vines from each vineyard were sampled at the end of each growing season for three consecutive years (2021-2023). The collected samples were subjected to total RNA extraction and tested for the presence of GLRaV-1 and GLRaV-3, using real-time RT-PCR methods, and for GLRaV-4 through conventional RT-PCR. The three viruses were detected in all three vineyards during the first sampling (2021) with GLRaV-3 and GLRaV-4 being the most common viruses in the Malagouzia vineyard. GLRaV-1 and GLRaV-4 prevailed in the Muscat De Hambourg vineyard and GLRaV-3 in the Xinomavro vineyard. During the following two years' surveys, there was an increase in the number of infected vines, with GLRaV-4 showing the highest rate of spread. Overall, our findings suggest a possible implication of vectors in the spread of the three viruses in vineyards of Northern Greece.
KEYWORDS: Spatiotemporal spread; ampeloviruses; Grapevine leafroll disease

SUPPORT

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MONITORING THE SANITARY STATUS OF IMPORTANT INDIGENOUS GRAPEVINE VARIETIES CULTIVATED IN GREECE

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ABSTRACT:
Grapevine is an economically important and widespread crop in Greece, and it is infected by numerous viruses and viroids. Within the framework of the research program "NATIVINE" a total of 20 indigenous cultivars (101 grapevine samples) originating from seven viticultural areas of Greece were tested for the presence of specific viruses. More specifically the varieties included Black Muscat from the area of Thessaly, Mavrodaphni, Malagousia and Xinomavro from Macedonia, Aidani, Mavrotragano, Athiri and Asyrtiko from Santorini Island, Asprouda of Serres, Pamidi, Zoumiatiko and Kiniariko from Macedonia, Kara Pappas from Komotini, Vidiano, Thrapsathiri, Tsardana and Kotsifali from Crete and Fokiano, Muscat of Samos and Ritino from Samos Island. Phloem tissue was collected from mature canes of every sample and subjected to total RNA extraction. Subsequently, the samples were tested for the presence of grapevine leafroll-associated virus 1 (GLRaV-1), grapevine leafroll-associated virus 3 (GLRaV-3), arabis mosaic virus (ArMV) and grapevine fanleaf virus (GFLV) according to the Commission implementing directive (EU) 2020/177 using molecular detection methods. The samples were also examined for the presence of grapevine virus A (GVA) and grapevine virus B (GVB) as they are widely spread in Greek vineyards. Most of the vines were heavily infected by more than one virus. The results revealed the presence of mixed infections in the same vine in all tested cultivars, with GLRaV-3 and GVA coexisting in most of them. Only 7 vines belonging to the varieties Mavrotragano, Asyrtiko, Zoumiatiko and Fokiano were found to be free from the viruses of the legislation but were infected with GVA and/or GVB. Our results unveiled the impaired sanitary status of important grapevine varieties cultivated in Greek vineyards and highlight the need for the production and use of high-quality, certified propagative material.
KEYWORDS: Greek varieties; viruses; propagative material

SUPPORT

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MANAGEMENT OF CORN STUNT COMPLEX REQUIRES CONSTANT MONITORING OF THE LEAFHOPPER VECTOR AND ITS NATURAL INFECTION WITH THE MAIZE PATHOGENS

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ABSTRACT:
Corn stunt complex (CSC) consists of bacterial pathogens belonging to the Class Mollicutes, which are the maize bushy stunt phytoplasma (MBSP) and the corn stunt spiroplasma (CSS), and the maize rayado fino virus (MRFV). These pathogens are persistently transmitted by the corn leafhopper, *Dalbulus maidis* (Hemiptera: Cicadellidae). In the past, CSC diseases outbreaks were sporadic and localized. Within a decade, serious outbreaks of the CSC diseases have been reported across the country. A significant outbreak occurred in the southern state of Santa Catarina (SC), where producers and technical personnel were initially unfamiliar with the pathosystem (Oliveira and Frizzas, 2022). We conducted an epidemiological study on the incidence of the corn leafhopper and its natural infection with the CSC pathogens in SC, within a proposal for providing information to the production sector ("Programa Monitora Milho SC"). The monitoring was carried out over a period of forty weeks, during the 2021/22 and 2022/23 growing seasons. Approximately 20 commercial maize fields were selected in the state for monitoring during the early planting season, and 10 fields were selected for the late planting season. At the edge of each field crop, supports for yellow sticky traps were installed. Weekly, field technicians replaced the traps. Captured *D. maidis* were counted. A portion of them was assessed using molecular procedures to detect infection with the CSC pathogens, using seven samples in triplicates per location. Approximately 3000 traps were examined in each growing season. The fluctuation of the corn leafhopper was divergent in the two agricultural years. The number of *D. maidis* captured in 2022/23 was 37.26% higher than in the previous crop season. The highest incidence of *D. maidis* occurred in the late season for both agricultural years. Approximately 3000 samples were submitted for pathogens detection for each agricultural year. CSS was the most predominant over MBSP (2021/22: CSS 29%; MBSP 18%; 2022/23: CSS 23%; MBSP 10%). MRFV was predominant over bacteria (2022/23: 54%). Natural infection of *D. maidis* with CSC pathogens fluctuates over the weeks and can be intermittent or clustered, at each municipality. The monitoring program has been informing farmers regarding CSC status in SC. The dynamics of *D. maidis* and its infection with CSC pathogens differed for each season, suggesting that the complex requires constant monitoring aiming the management of the insect vector.
KEYWORDS: Re-emergent diseases; Insect vector monitoring; Translational epidemiology

SUPPORT

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SURVEY OF MAIZE RAYADO FINO VIRUS IN MAIZE (ZEA MAYS L.) IN THE STATE OF PARANÁ, BRAZIL

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ABSTRACT:

Maize (*Zea mays* L.) is one of the main cereal crops produced in Brazil. A major challenge to maize production is its susceptibility to pathogens associated with the corn stunt disease complex (CSDC). Among these pathogens are the mollicutes *Spiroplasma kunkelii* (family Spiroplasmataceae) and '*Candidatus* Phytoplasma asteris' (family Acholeplasmataceae), and the Maize rayado fino virus (MRFV) (*Marafivirus maidis*, family *Tymoviridae*). These CSDC pathogens are transmitted by the insect vector *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae). Symptomatic maize plants present chlorotic punctuations, pale and reddish spots on leaves, dwarfism, shortened internodes, and poorly developed ears. Diseased plants can have significantly reduced productivity, with substantial economic losses for the growers. This study aimed to identify and monitor the presence of MRFV associated with CSDC in the state of Paraná, Brazil. A total of 392 maize samples from 89 municipalities were analyzed. The samples were collected from commercial maize fields in different regions of Paraná between the 2022 summer and 2024 winter crops. Analyses were carried out at the Plant Virology Laboratory of the Paraná Rural Development Institute - IAPAR-Emater (IDR-Parana) in Londrina, PR, Brazil. The symptomatic maize leaves were washed with running water, dried, and processed with liquid nitrogen. Total RNA was extracted using Trizol® reagent (Invitrogen) and virus detection was performed by Reverse Transcript Polymerase Chain Reaction (RT-PCR), using the primers MRFV 09 and MRFV 10. Among the 392 analyzed samples, 179 tested positive for the virus. The regions with the highest incidence of MRFV-infected plants were central north (53.25%), west (52.63%), and northwestern (31.86%) of the state of Paraná. These areas are characterized by extended maize sowing periods, warmer temperatures, and lower altitudes, which provide favorable conditions for the corn leafhopper (*D. maidis*), the MRFV insect vector. Therefore, the presence of the pathogen MRFV was confirmed in all corn producing regions of the state of Paraná. The fields in the central-north region of the state had the major incidence of MRFV. However, monitoring studies of insect vectors and virus diversity are necessary to understand the pathosystem in the state.

KEYWORDS: MRFV; Disease; Virus

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INCIDENCE OF *DALBULUS MAIDIS* AND ITS NATURAL INFECTION WITH THE CORN STUNT COMPLEX PATHOGENS OVER EARLY AND LATE PLANTINGS OF THE 2023/24 AGRICULTURAL YEAR

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ABSTRACT:
Maize is a key global crop, with Brazil as the third-largest producer. Despite its economic importance, maize faces major phytosanitary threats, including the severe corn stunt disease complex. This complex is caused by pathogens of the Mollicutes class, represented by the maize bushy stunt phytoplasma (MBSP), and the corn stunt spiroplasma (CSS). Additionally, two viruses, maize rayado fino virus (MRFV) and maize striate mosaic virus (MSMV), have been associated with the disease. The corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott, 1923) (Hemiptera: Cicadellidae), serves as a persistent vector for these pathogens, facilitating their spread in crops. A comprehensive epidemiological investigation was conducted to determine the occurrence of *D. maidis* and natural infection in Santa Catarina, Brazil, attending the "Programa Monitora Milho SC". Monitoring was conducted for approximately 38 weeks from July to April during the 2023/24 crop year in 10 crops across the State. Yellow sticky traps were placed at each field edge, and captured *D. maidis* specimens were counted, and analyzed for infectivity individually or in batches (3 replicates of up to 5 insects). The population fluctuation of *D. maidis* varied throughout the weeks. The presence of the *D. maidis* was more intense between weeks 10 and 30, with a population peak between weeks 26 and 29 (January and February). The insect population increased progressively from the early maize growth stages (VE-V2), reaching its peak in the reproductive stages (VT-R1-R3/R4-R6) for both early and late plantings. After harvest, a sharp decline in the insect population was observed. Natural infection of *D. maidis* with MBSP, CSS, MRFV, and MSMV varied throughout the maize development cycle (Positive samples over the assessment: 42.29, 6.72, 31.97 and 42.41% respectively). During the off-season, MSMV exhibited the highest infection rates (64%). Infection with MBSP progressively increased, peaking in the reproductive stages (VT-R1-R3/R4-R6), coinciding with an increase of CSS and MRFV. After harvest, MBSP and CSS remained detectable in the *D. maidis* population. The monitoring program provides weekly reports that help farmers track *D. maidis* incidence and infection status. Continuous monitoring and integrated management strategies are essential for reducing pathogen spread and mitigating yield losses.
KEYWORDS: Vector-borne phytopathogens; Epidemiological monitoring; Vector population fluctuation

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UNDERSTANDING PATHOGEN INTERACTIONS CAUSING CASSAVA FROGSKIN DISEASE IN SOUTH AMERICAN CASSAVA AND EXPLORING RESISTANCE IN AFRICAN VARIETIES

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ABSTRACT:
A severe disease syndrome, Cassava Frogskin Disease (CFSD), significantly impacts cassava cultivation in South America and is expanding its geographical range, posing a potential threat to cassava-growing regions worldwide. The disease manifests predominantly in tuberous roots of the crop after several months of growth, making early detection and management difficult to achieve. CFSD is an infectious disease with a highly complex biology that often leads to total crop loss. Although novel viruses, torrado- ampelo,- poleroviruses and phytoplasmas have been associated with CFSD, definitive evidence of their roles is lacking, complicating accurate detection. Recently, evidence was provided that a torradovirus (CsTLV2) 2 was the primary virus linked to CFSD.To further resolve the conundrum and provide biological evidence, we have isolated single viruses from CFSD-infected plants collected in Brazil and transferred to different cassava lines by grafting to assess symptoms associated with each virus and reproduce the disease. To support diagnosis, we developed a sensitive detection pipeline for "all" so far known viruses and pathogens found in CFSD-infected tissues. Our bio-assays showed that most South American cassava genotypes were susceptible and became infected with visible foliar symptoms, while several African cassava varieties showed tolerance/ resistance, with no foliar symptoms expressed. To better understand virus-host interactions, we tracked viruses in situ using a highly sensitive "RNAscope® in situ hybridization" method. Bioassays are critical for elucidating CFSD's etiology and further complex biological studies are necessary to fully reconstruct pathogenesis of the disease. Additionally, the development of sensitive diagnostic assays is crucial for detecting the full spectrum of pathogens involved in CFSD, ensuring disease-free cassava crops and preventing further spread to cassava-growing regions worldwide. While studies are not finalized, our findings suggest that African cassava varieties can be valuable sources for further virus resistance exploration, offering a foundation for resistance breeding. The outcome of this study will guide pre-breeding and preemptive breeding efforts while providing information about resistance phenotypes and potential host resistance.
KEYWORDS: Cassava FrogSkin; Resitance-breeding; RNAscope®

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FACILITATING PLANT VIRUS SURVEILLANCE: INTEGRATED DATA MANAGEMENT AND ANALYSIS FOR EPIDEMIC STUDIES

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ABSTRACT:

In recent years, the tracking of emerging viral pathogens has greatly benefited from high throughput sequencing data. This technology enables early detection of variants of interest and concern while also helping to identify factors influencing viral spread. However, leveraging sequencing data for broader research presents several challenges, including centralized data management, standardized epidemiological annotations, controlled access within organizations or consortia, and user-friendly bioinformatics tools for analysis. Genome Detective (www.genomedetective.com) is a web-based platform that provides such an integrated solution for managing sequencing data. It was successfully employed to trace virus outbreaks during the recent SARS-CoV-2 epidemics. We will demonstrate how it has been successfully utilized in the Horizon 2020 project VIRTIGATION (www.virtigation.eu) to study ongoing ToBRFV and ToLCNDV epidemics. The platform allows members to submit their data to a centralized yet private database, ensuring secure storage and controlled access. Additionally, it employs robust algorithms to analyze multiple samples from different consortium members, generating standardized and comparable reports across institutions. Researchers can also define and store per-sample epidemiological metadata alongside sequencing data, enabling epidemiology analyses. Genome Detective also supports virus-specific analyses when required. Leveraging expert knowledge, we developed and integrated a ToBRFV subtyping tool within the platform, enabling the identification of specific variants. This flexibility allows tailored solutions to be implemented efficiently, ensuring that consortium members can access advanced analyses without the need to set up their own bioinformatics infrastructure. In conclusion, Genome Detective offers a comprehensive and scalable solution for managing and analyzing sequencing data, facilitating collaboration and enhancing our understanding of plant virus epidemics.

KEYWORDS: Bioinformatics; Data management; Software

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EPIDEMIOLOGY OF CACTUS VIRUSES

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ABSTRACT:
The American continent is considered the center of origin of cacti plants but they are currently present worldwide. A recent literature survey found 31 distinct viruses, naturally infecting more than 70 cacti species around the world. Most of them were detected in species used for ornamental purposes or economic exploitation. Only on rare occasions, viral infection of cacti resulted in external symptoms. There are only two registered cases of viruses' detection in cacti growing in their natural habitat: (1) the saguaro cactus (*Carnegiea gigantea*) in the desert of Arizona, USA, infected by the alphacarmovirus Saguaro cactus virus; (2) some *Opuntia* species collected in Utah, Nevada and Arizona, USA, were infected by potexviruses cactus virus X and zygocactus virus X, and the tobamovirus Sammon's Opuntia virus. Most potexviruses, tobamoviruses, carlaviruses, and the sole alphacarmovirus found infecting cactaceous plants seem to be specific to cacti. They have not been detected infecting non-cacti plants in nature yet. Viruses from the families *Geminiviridae, Genomoviridae,* and *Caulimoviridae*, besides viroids, recently identified by metagenomics, still lack information regarding their biology. On the other hand, the three orthotospoviruses and the polerovirus beet western yellows virus detected in some cacti, are possibly cases of accidental infection, being carried by vectors from non-cacti sources. It is unknown yet how cacti viruses are dispersed in the natural habitat since members of the genera *Potexvirus, Tobamovirus*, and *Alphacarmovirus* do not have known vectors. The most likely explanation should be the dissemination by pollen or seed. A large number of cases of worldwide virus-infection in ornamental and cultivated cacti (dragon fruit, prickly pear) undoubtedly are due to human activity. Firstly, by dispersing a wide variety of cacti species around the world, and secondly, by disseminating viruses through pruning and grafting procedures in nurseries and collections, or through cultural traits in the field, since most known cacti viruses are easily transmitted by mechanical means.
KEYWORDS: Cactaceae; *Potexvirus*; *Tobamovirus*

SUPPORT

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AN INFECTIOUS CLONE OF MAIZE STRIATE MOSAIC VIRUS FOR INVESTIGATING ITS PATHOGENICITY

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ABSTRACT:
Geminiviruses are plant viruses that possess circular single-stranded DNA (ssDNA) genomes encapsulated in twined icosahedral particles. These viruses cause devastating diseases in various crops worldwide. Among the 14 recognized genera, *Mastrevirus* members exhibit monopartite genomes and are efficiently transmitted by leafhoppers. Maize streak disease (MSD), caused by the maize streak virus (MSV; genus *Mastrevirus*), results in significant yield losses in Africa (Bosque-Pérez, 2000). Recently, maize striate mosaic virus (MSMV; genus *Mastrevirus*) was reported in maize and sugarcane in the Americas (Fontenele et al., 2018; Batista et al., 2021; Ruiz-Posse et al., 2023). MSMV is transmitted by the leafhopper *Dalbulus maidis* in a persistent, nonpropagative manner (Vilanova et al., 2022). In this study, we constructed an infectious clone of MSMV to investigate its pathogenicity and detection. Based on the genome sequences of MSMV isolates (NCBI accession numbers MF167297-MF167307), we synthesized a construct containing a 1.66× tandem repeat of the viral genome, including duplicated long intergenic regions (LIRs). This construct was cloned into the binary vector pCB301 and transformed into *Agrobacterium tumefaciens* strain C58C1. The transformed bacteria were used to agroinoculate B73 maize seedlings. At 20 days post-inoculation, 27.5% (11/40) of inoculated plants tested positive for full-length MSMV genomes via PCR using a pair of abutting primers. Although no visible symptoms were initially observed, mild chlorotic streaks became apparent in infected plants at 5-6 weeks post-inoculation.Additionally, we validated a pair of primers targeting the *coat protein* gene (MSMV-QF: CAGCTACCTTCCAGGCTTCC; MSMV-QR: TACAGAGCTCCGCACGTAAC) for specific quantification of MSMV genomic DNA using quantitative PCR. These primers showed no cross-reactivity with total DNA from healthy B73 maize leaves. This infectious clone provides a critical tool for elucidating MSMV-host interactions, transmission mechanisms, and developing disease management strategies.
KEYWORDS: Maize striate mosaic virus; Infectious clone; Detection

SUPPORT

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STUDY OF TWO OLPIDIUM-TRANSMITTED OPHIOVIRUSES INFECTING PEPPER CROPS IN GREECE

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ABSTRACT:
In March 2021, two viruses of the genus Ophiovirus were detected in pepper greenhouse crops for the first time in Greece (Rhodes), using high-throughput sequencing (HTS). These are ranunculus white mottle virus (RWMV) and lettuce ring necrosis virus (LRNV). Ophioviruses have a divided, negative polarity RNA genome and their transmission occurs through zoospores of fungi that belong to the genus Olpidium. The aim of this study was to further characterize these viruses, to improve their detection methods and to study their transmission to various plant species. The sequences from isolates of both viruses were determined by HTS and their analysis showed high rates of genetic variability. Phylogenetic analysis indicated that RWMV has greater intraspecific variation than LRNV. Based on the sequences retrieved and those available at GenBank, primers were designed and used in RT-PCR assays to reliably detect RWMV and LRNV. These methods were applied to test plant material from peppers collected during 2021-2023 from Rhodes. RWMV and LRNV were detected in two out of four greenhouses from which samples were collected in 2021, and in 2023 only RWMV was detected in one of them. *Olpidium virulentus* was also identified both microscopically and molecularly through PCR, in the soil of the greenhouses, where ophioviruses had been detected. Virus transmission tests of both viruses were carried out under lab conditions on pepper and tomato plants with soil coming from the greenhouses where the viruses and *O. virulentus* were detected. The results of the experiments highlighted that both ophioviruses can be effectively transmitted under lab conditions to pepper and tomato plants. In fact, it was observed that virus detection rates were higher three months after inoculation, compared to two months and were also higher in roots than in the foliage. Resting spores of *O. virulentus* were observed in the root system of the inoculated plants by light microscopy and the identity of *O. virulentus* was confirmed by PCR. Overall, our work advances our understanding of the poorly studied pathosystem of Olpidium-transmitted ophioviruses.
KEYWORDS: Ophiovirus; pepper; Olpidium

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WILD RADISH: A KEY WEED IN THE EPIDEMIOLOGY OF VIRUSES INFECTING CULTIVATED BRASSICAS IN THE NEOTROPICAL REGION

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ABSTRACT:
To understand epidemiological aspects of weedy Brassicaceae in the aphid fauna composition and their influence on the dispersion of viruses transmitted in a non-circulative manner, two fields of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) and broccoli (*Brassica oleracea* var. *italica*), located at latitudes 23°34'22" and 23°39'21" S in the green belt of São Paulo (the largest vegetable producer in the Neotropical region), were monitored for three years. Symptomatic and asymptomatic weedy Brassicaceae were collected and submitted to ELISA to confirm the occurrence of the main viruses infecting brassicas in Brazil: cauliflower mosaic virus (CaMV), cole latent virus (CoLV), cole mild mosaic virus (CoMMV) and turnip mosaic virus (TuMV). To evaluate their potential as reservoirs, these plants were mechanically inoculated with previously characterized viral isolates. Aphids were collected from colonies established on weedy Brassicaceae at the edges of production plots and identified based on dichotomous keys. The influence of rainfall and temperature was assessed by the Pearson index and species clustering was performed using the PAST program (Jaccard coefficient). The probability of transmission by a single aphid associated with the vector pressure index was also evaluated. Of the five species of weedy brassicas reported in the areas, wild radish (*Raphanus sativus*) was susceptible to TuMV in both single and co-infections with CaMV, CoLV and CoMMV, playing a key role in maintaining colonies of *Myzus persicae* (polyphagous) and *Brevicoryne brassicae* (oligophagous). Wild mustard (*Brassica rapa*) plants, although asymptomatic, harbored aphid colonies also found on wild radish. However, wild mustard seedlings, when challenged by mechanical inoculation, showed hypersensitivity reaction to TuMV and immunity to other viruses. No aphid colonies or symptoms were observed on shepherd's purse (*Capsella bursa-pastoris*), bittercress (*Cardamine bonariensis*) and Virginia pepperweed (*Lepidium virginicum*). Nevertheless, mechanical inoculations revealed that these species could act as asymptomatic reservoirs. In the cluster analyses, aphid species identified in wild radish and wild mustard were most frequent in spring, when rainfall and temperature positively influenced colony formation. *Myzus persicae* was the most efficient in terms of vector pressure both in the non-persistent transmission of CaMV, CoLV, CoMV and TuMV, and in the semi-persistent transmission of CaMV.
KEYWORDS: Brassicaceae; Aphid; *Raphanus sativus*

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CITRUS SUDDEN DEATH-ASSOCIATED VIRUS: WHAT CAN A SINGLE VIRUS TEACH US ABOUT VIRUS-HOST INTERACTIONS?

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ABSTRACT:
More than 25 years after citrus sudden death (CSD) disease was first reported in Brazil, its causal agent remains undetermined. The Citrus sudden death-associated virus (CSDaV) is strongly associated with CSD-symptomatic plants; however, its low titer, consistent co-infection with Citrus tristeza virus, and unknown biological vector have made it difficult to establish its direct role in disease development. To overcome these challenges, we developed a CSDaV infectious clone that efficiently infects the model plant Nicotiana benthamiana. Locally infected leaves exhibit severe necrosis (HR-like symptoms), whereas systemically infected leaves remain asymptomatic. In local infections, the HR marker gene HIN1 is upregulated, while reactive oxygen species (ROS) accumulation is suppressed and a ROS-scavenging gene (Cat1) is downregulated. Notably, CSDaV virions accumulate around chloroplasts, which show structural changes and clumping upon infection. Whether these strong local plant responses are mediated by chloroplast function remains unknown, but prXQ and bas1, two chloroplastic ROS scavenging genes, are also downregulated in infected tissues. Additionally, CSDaV encodes a proline/serine-rich protein (p16) that localizes to chloroplasts when is transiently expressed as a GFP fusion. Mass spectrometry and phosphoproteomics analyses suggest that p16 interacts with chloroplastic proteins, acts as a phospho-substrate, and potentially interferes with phosphorylation of plant chloroplastic proteins. These findings suggest that p16 may be a key factor in CSDaV-chloroplast interactions, potentially modulating chloroplast-mediated plant defense responses to facilitate infection.
KEYWORDS: Citrus sudden death-associated virus; Virus-chloroplast interaction; Viral protein function

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CURRENT STATUS OF *SPONGOSPORA SUBTERRANEA* AS A VECTOR OF POTATO MOP TOP VIRUS (PMTV) IN CHILE: A MULTIPLEX REAL-TIME PCR APPROACH FOR DETECTING POWDERY AND COMMON SCAB IN SYMPTOMATIC TUBERS

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ABSTRACT:

Spongospora subterranea f. sp. *subterranea* (Sss) is an obligate biotrophic protozoan and the causal agent of potato powdery scab. It also acts as a vector for potato mop top virus (PMTV). The symptoms of powdery scab can be confused with common scab, a disease caused by Gram-positive bacteria of the genus Streptomyces, particularly *Streptomyces scabiei*. Accurate identification of both pathogens is essential, as their tolerance limits for certified seed potato tuber seed can vary significantly.In this study, a multiplex real-time PCR methodology was developed for the simultaneous detection of *S. subterranea* and *S. scabiei*. A qPCR strategy using SYBR Green was designed with reference sequences from the ITS region of *S. subterranea* (AY604171-AY604172) and the *nec1* pathogenicity marker gene of *S. scabiei* (MN393487). This approach allows for the individual or simultaneous detection of both pathogens and was validated on lesion samples from minitubers in seed potato production. The protocol demonstrates high efficiency and sensitivity, making it a valuable tool for managing powdery and common scab in seed potato certification.Based on the 2017 report by the Servicio Agrícola y Ganadero (SAG), which documented the distribution of *S. subterranea* and the presence of PMTV in the main potato-producing regions of southern Chile, and using samples from a spore collection gathered between 2004 and 2024, this study expanded the analysis to include untested spore samples, powdery scab lesions, and fresh potato galls from the central-northern region of the country. RT-PCR was applied using two previously described primer sets targeting the coat protein (RNA-CP) region and RNA2 of PMTV.While previous reports had confirmed the presence of PMTV in critical areas such as Chiloe, along with the widespread distribution of powdery scab in the Los Lagos and Los Rios regions, this study provides new insights by detecting both the vector and PMTV in powdery scab lesions and root galls from the Coquimbo region, which had not been previously assessed for these pathogens. Given the potential impact on local agriculture, urgent measures must be implemented to prevent the further spread of PMTV.

KEYWORDS: Powdery scab; PMTV; Common scab

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OCCURRENCE AND DISTRIBUTION OF THE GEMINIVIRUS BRAZILIAN CURLY TOP VIRUS IN TOMATO PLANTS IN BRAZIL

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ABSTRACT:
Among the viral pathogens affecting the tomato crop in Brazil, the Brazilian curly top virus (BraCTV), belonging to the family *Geminiviridae* and the genus *Topilevirus*, has been identified in open-field cultivations for fresh consumption. BraCTV is persistently transmitted by leafhoppers. After infection, symptoms manifest as chlorosis, reduced vegetative growth, leaf curling and deformation, as well as purplish coloration along the leaf margins, significantly compromising the plant's physiological performance and, consequently, productivity. BraCTV is associated with curly top disease (CTD). The disease's incidence in Brazil declined from the 1950s onward. Since then, no records of its occurrence were observed in tomatoes until the detection of similar geminiviruses in cleome and also in tomato plants. The present study aimed to investigate the presence of BraCTV in major tomato-producing regions of Brazil, including the Federal District, Pernambuco, Mato Grosso, Santa Catarina, and São Paulo states. To achieve this, techniques, such as RCA, PCR, and sequencing, were employed to detect the presence of BraCTV in the collected tomato samples. In the Federal District, out of 100 plant samples from symptomatic or asymptomatic plants, 20% tested positive for BraCTV. Additionally, the presence of other viruses, such as ToSRV, was detected in the area. In Santa Catarina state, 20.9% of the 43 evaluated samples tested positive for systemic BraCTV infection. In São Paulo, 79.2% of the 24 samples were positive. The plants that tested positive for BraCTV displayed characteristic symptoms of viral infection, primarily severe leaf curling, a purplish coloration along the leaf margins, and stunting. BraCTV isolates were also detected in samples collected in Mato Grosso and Pernambuco states, but not in samples from Goiás and Paraná states. These results confirm the presence of BraCTV in different tomato-producing regions in Brazil and highlight the potential losses this virus may be causing for growers. Efforts to monitor the disease and seek management methods are urgently needed.
KEYWORDS: Geminivirus; Viral Epidemiology; Curly Top Disease

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THE EMERGENCE OF WATERMELON CHLOROTIC STUNT VIRUS HAS ALTERED VIRUS INFECTION PATTERNS IN SOUTHWESTERN U.S. MELON AND WATERMELON PRODUCTION

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ABSTRACT:
Cucurbit production in the southern and western United States (U.S.) is severely impacted by whitefly (*Bemisia tabaci*)-transmitted viruses (WTVs), and many of these viruses occur together in mixed infections (Mondal et al., 2023). During the 2023 fall melon season in the southwestern U.S. desert production region, watermelon and melon plants with leaves exhibiting yellow mosaic symptoms or chlorotic spotting, respectively, along with foliar distortion, predominantly on newer growth, were observed in commercial fields. Symptoms were particularly severe on watermelon. Plants were determined to be infected with common WTVs and most notably with watermelon chlorotic stunt virus (WmCSV; *Begomovirus citrulli*, *Begomovirus,* Geminiviridae); this was the first report of WmCSV infecting cucurbit crops in the U.S. (Wintermantel et al., 2024). Molecular assays for the detection of WmCSV, as well as a multiplex assay that detects WmCSV along with other common WTVs, were developed. Surveys were conducted during the summer and fall of 2024 to determine virus prevalence in cucurbit crops as well as weeds common throughout the production area, and results confirmed that WmCSV is now one of the most prevalent viruses infecting melon and watermelon in the southwestern U.S. Results demonstrated that WmCSV infects many common weeds as well as some non-cucurbit crops in the region, including many perennial plants, and evaluation of these plants as virus reservoir hosts from which whiteflies can acquire and transmit WmCSV to cucurbit crops is in progress. The emergence of WmCSV has also altered seasonal infection patterns among whitefly transmitted viruses in the southwestern U.S. Previous studies demonstrated that the crinivirus, cucurbit chlorotic yellows virus, was the dominant virus causing yellowing in spring and summer melons, whereas a different crinivirus, cucurbit yellow stunting disorder virus (CYSDV), dominated fall infections (Mondal et al., 2023). With the establishment of WmCSV in the region, these seasonal differences have been greatly reduced and co-infections of WmCSV particularly with CSYDV have become common. This suggests the possibility of a selective advantage to mixed CYSDV-WmCSV infections, and this is under evaluation.
KEYWORDS: mixed infection; whitefly; virus competitiveness

SUPPORT

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STUDY OF THE SPREADING OF POTATO VIRUS A IN THE RUSSIAN FEDERATION

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ABSTRACT:
Potatoes are used as a food, animal feed and technical crop. The Russian Federation is one of thecountries with high levels of potato production and consumption. Currently, viral diseases areseverely affecting potato production (1). In recent years, many potato viruses, in addition tobeing included in certification schemes, have been controlled as quarantine pests or regulatednon-quarantine organisms, prohibiting the importation of infected material from countries ofspread. For example, Potato virus A (PVA, Potyvirus atuberosi) is considered one of the mostimportant viruses infecting crops in the Solanaceae family. Losses due to the virus can reach 30-40% on unresistant potato varieties. PVA infection of host plants causes symptoms of mosaic,spotting with slight crinkling of plants. On resistant potato varieties, the virus infection may beasymptomatic and thus sensitive and specific diagnostic methods are important. Seven aphidspecies are considered vectors of the virus: *Aphis frangulae, A. nasturtii, Brachycaudushelichrysi, Macrosiphum euphorbiae, M. persicae, Neomyzus circumflexum* and *Rhopalosiphum padi* (2). Potato virus A is included in the quarantine lists of 10 countries, of which 8 countrieshave included it in the last 10 years, including BRICS countries: Brazil (2018), China (2021) (3).For the Russian Federation, PVA is a non-quarantine organism but it is regulated by seedcertification schemes.In this study, samples of plant material of seed and food potatoes were collected in 15 regions ofthe Russian Federation. More than 300 potato samples were tested by ELISA and real-time RT-PCR methods. To detect the virus by ELISA method, a diagnostic kit manufactured by Bioreba(Switzerland) was used. RNA isolation of the microorganism was carried out using the reagentkit "FitoSorb" (Syntol, Russia). For PVA identification by real-time RT-PCR, commercial kit of Syntol (Russia) with combined reverse transcription (real-time RT-PCR) and AgroDiagnostica (Russia) together with a reverse transcription kit of the same manufacture (2-step format) wereused.As a result of the tests, the presence of Potato virus A was detected in 1.6% of food potatosamples from Amur Region (1 sample), Irkutsk Region (1 sample) and Sverdlovsk Region (3 samples). It should be noted that seed potatoes were free from PVA, which indicates high qualityof planting material.
KEYWORDS: ELISA; Real-time RT-PCR; PVA

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Session 2: PATHOGEN-VECTOR-INTERACTIONS

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VIRUS, CHLOROPLAST, AND INSECT VECTOR: HOW DO THEY INFLUENCE EACH OTHER?

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ABSTRACT:
Sugarbeet farming is threatened by aphid-transmitted yellowing virus infections. Banning of neonicotinoids, commonly used to kill aphids, leads to increased spread of the viruses, which now requires managing this problem in a more sustainable way. Previous research indicated that chloroplast degradation caused by yellowing virus infections greatly impacts aphid longevity and fecundity (Schop, 2024), but how virus-chloroplast interactions influence vectors is not well understood. This project specifically investigates how beet yellows virus (BYV) and beet chlorosis virus infections influence sugarbeet chloroplast structure and function, and how these influence virus transmission by *Myzus persicae* aphids, the insect vector. Chloroplast structural changes in various virus-infected sugar beet plants tissue types will be checked under light and electron microscopy, and compared to non-infected plants. Changes in the chloroplasts photosynthetic and defense functions in virus-infected sugarbeet plants will be assessed by several means, including analysis of chlorophyll content, chloroplastic fluorescence, gene expression, transcriptome-proteome dynamics, and will be compared to non-infected plants. Ultimately, the influence of these interactions on vector competency will be checked by analysing aphid behaviour and the efficiency of virus transmission in various viruses-influenced chloroplast conditions. Currently, experiments are ongoing to determine time point of the first systemic BYV detection and the initial onset of yellowing as a base for selecting the sampling time point to investigate BYV-influenced chloroplast structural changes.
KEYWORDS: Yellowing virus; Sugarbeet; Aphid

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LIMITED ROLE OF *BEMISIA TABACI* MED POPULATIONS IN THE TRANSMISSION OF BRAZILIAN BEGOMOVIRUSES IN SÃO PAULO STATE

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ABSTRACT:
Whiteflies from the cryptic species complex *Bemisia tabaci* cause significant damage to agriculture due to their polyphagous feeding habits and their role as vectors for various viruses, particularly those from the *Begomovirus* genus. In Brazil, the predominant species of *B. tabaci* is Middle East-Asia Minor 1 (MEAM1), which has been the most common since 1990. The cryptic species *B. tabaci* Mediterranean (MED) was detected in the country in 2014, and after ten years of this invasion, many questions arose regarding the epidemiology of diseases caused by viruses transmitted by whiteflies. The main objectives of this study were to evaluate the transmission efficiency of the begomoviruses tomato severe rugose virus (ToSRV) and tomato rugose mosaic virus (ToRMV) to tomato plants, as well as the bean golden mosaic virus (BGMV) to bean plants, by *B. tabaci* MEAM1 and four populations of *B. tabaci* MED. The results indicated that *B. tabaci* MEAM1 is an excellent vector of begomoviruses, efficiently transmitting ToSRV and ToRMV to tomato plants and BGMV to bean plants. In contrast, *B. tabaci* MED was unable to transmit ToSRV and ToRMV to tomato plants. Only one population of *B. tabaci* MED was capable of transmitting BGMV to a bean plant, demonstrating that this species has low efficiency in virus transmission. When assessing the acquisition of viruses by the insect, it was found that both *B. tabaci* MEAM1 and *B. tabaci* MED can acquire ToSRV, ToRMV, and BGMV. These findings suggest that *B. tabaci* MED plays a limited role in the transmission of begomoviruses, which may impact the distribution of these viruses in the fields.
KEYWORDS: *Begomovirus*; *Bemisia tabaci*; Virus transmission

SUPPORT

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COMPARATIVE TRANSCRIPTOMICS OF THE APHID MELANAPHIS SORGHI FEEDING ON SORGHUM INFECTED WITH PERSISTENTLY AND NON-PERSISTENTLY TRANSMITTED VIRUSES

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ABSTRACT:
The sorghum aphid (*Melanaphis sorghi*) is a major pest of sorghum (*Sorghum bicolor*), capable of reducing crop yields by up to 70%. This species was recently implicated in a mass aphid outbreak in the Americas, previously attributed to the sugarcane aphid (*Melanaphis sacchari*). M. sorghi also vectors the persistently transmitted sugarcane yellow leaf virus (SCYLV; *Polerovirus SCYLV, Solemoviridae)* and the non-persistently transmitted sugarcane mosaic virus (SCMV; Po*tyvirus sacchari, Potyviridae*). However, the impact of plant viral infections on aphid biology and host interactions remains poorly understood. To address these gaps, we assessed the impact of virus acquisition on *S. bicolor* infected with SCYLV (persistent, circulative), SCMV (non-persistent), or both viruses simultaneously on the *M. sorghi* transcriptome. We infected sorghum seedlings with these viruses, confirmed the infections by RT-PCR detection, allowed aphids feeding in infected and healthy controls and sampled groups of five aphids after 48 hours for further analysis. RNA was extracted, and RNA sequencing was performed for four samples per treatment. Quality control and viral read scanning with fastv enabled the assembly of a novel SCMV isolate genome (RIB-3) using SPAdes. Phylogenetic analysis revealed its similarity to other sugarcane isolates. Transcriptome assembly was performed using a genome-guided approach on Trinity, and annotation was performed with the dammit pipeline. Gene expression was quantified via Salmon, and differential expression analysis was conducted with the DESeq2 R package. These analyses identified highly specific differentially expressed genes across treatments and enrichment of key biological pathways. Aphids feeding on SCMV-infected plants exhibited changes in reproduction and carbohydrate metabolism, while those on SCYLV-infected plants showed enrichment in cellular oxidant detoxification and protein processing. Aphids feeding on co-infected plants displayed changes in transcription antitermination and peptidoglycan transport. Future work will focus on constructing a weighted gene coexpression network to identify additional metabolic pathways and regulatory hubs involved in responses, alongside RT-qPCR validation.
KEYWORDS: Polerovirus; Potyvirus; virus aphid interactions

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VARIATION IN THE ABILITY OF *BEMISIA TABACI* TO TRANSMIT CASSAVA VIRUSES

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ABSTRACT:
Cassava brown streak virus (CBSV) is a significant pathogen affecting cassava crops, primarily transmitted by whiteflies of the *Bemisia tabaci* species complex in a semi-persistent manner. CBSV is predominantly found in East Africa but of great concern for food security is its spread towards inland areas due to the movement of infected plant material and vectors. The transmission efficiency of CBSV by insects is influenced by several factors, including the species of whitefly, their bacterial endosymbionts and environmental conditions. Grasping these factors is essential for understanding CBSV transmission dynamics, which is vital for devising effective management strategies to control and mitigate the impact of CBSV.We assessed the capacity of three *B. tabaci* populations in East Africa to transmit two viruses responsible for the disease both in the field and in control conditions, highlighting variations in vector competence. We unravelled that all *B. tabaci* regardless of their vector abilities were able to acquire and retain the virus. Transcriptomic analysis of insects varying in their vector competence provided some insights into the molecular mechanisms associated with successful transmission. We discuss the potential impact of these results for the management, modelling and prevention of CBSV spread with a particular emphasis on the risk of cassava brown streak disease to cassava production in West Africa.
KEYWORDS: CBSV; semi-persistent transmission; epidemiology

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INCIDENCE OF TURNIP YELLOWS VIRUS IN CANOLA CROPS AND APHIDS IN VICTORIA, AUSTRALIA

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ABSTRACT:
Canola is an important grain crop in Australia and is infected by several viruses including turnip yellows virus (TuYV). *Myzus persicae* is considered the most efficient vector of this virus. In the pre-sowing period of 2024, a high incidence of TuYV was observed in "green bridge" hosts and a surveillance program was implemented in Victoria to monitor disease incidence. Four virus surveys were conducted in Victoria: (i) pre-season green bridge survey from the Wimmera and South-west regions (ii) the monitoring of five experimental canola crops from the Wimmera region; (iii) a survey of canola from the south-west region at stem elongation; and (iv) the monitoring of four canola crops in the drier Mallee region of Victoria. At each sampling point, 50 random canola plants were collected per crop and the presence and incidence of TuYV was determined by tissue blot immunoassay (TBIA). In the green bridge survey, all eight self-sown canola paddocks were infected by TuYV with an incidence range of 18-96%. In the five experimental canola crops from the Wimmera region, the incidence of TuYV was zero in August, 0-3% in September, while TuYV was detected in all 5 crops in October with an incidence ranging from 3-45%. In the South-west region, 5/9 canola crops were TuYV-infected, and the virus incidence ranged from 2-14%, while the TuYV incidence in a forage turnip crop was 82%. In the Mallee, all four canola crops were found to be TuYV-infected in both September and October with an incidence range of 6-74% and 60-100%, respectively. At the five experimental canola crops, fortnightly aphid inspections were also conducted within the crop and yellow sticky traps (YST) were deployed and collected fortnightly to better understand the movement of TuYV into the crops. Aphid colonies first appeared in October and the predominant aphid species was *Brevicoryne brassicae*, but *Lipaphis pseudobrassicae* and *M. persicae* were both present in low numbers. Seventeen aphid species were morphologically identified on the YSTs across the season. Additionally, RNA was extracted from 420 pools, each consisting of up to 10 aphids, which were tested for the presence of TuYV using qPCR, and 48% of the pooled aphid samples tested positive. Virus incidence, while driven primarily by differences in aphid populations, also varied with crop type, region and environmental conditions. The intricate relationships between these factors will be discussed.
KEYWORDS: Turnip yellows virus; Canola; Aphids

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IMPROVING THE KNOWLEDGE AND MANAGEMENT OF A RE-EMERGENT THRIPS-TRANSMITTED ORTHOTOSPOVIRUS AFFECTING LETTUCE IN CALIFORNIA

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ABSTRACT:
California produces over 75% of all lettuce (*Lactuca sativa*) in the United States and is valued annually at over 3 billion USD. Over the last five years, Impatiens necrotic spot virus (INSV; Order *Bunyavirales*, Family *Tospoviridae*) has emerged as a primary concern for the industry, resulting in hundreds of millions of dollars in losses. While INSV resistant lettuce cultivars remain largely unavailable, chemical management of the thrips vector, Frankliniella occidentalis has been ineffective in preventing disease outbreaks. As a first step towards understanding the patterns of recent INSV epidemics, we applied Spatial Analysis by Distance IndicEs (SADIE) to characterize the distribution and progression of INSV incidence and thrips infestations in commercial lettuce fields in California. Over the course of 7 weeks, INSV rapidly increased from ~15% to ~80% incidence in two fields and aggregated at specific field edges as early as 3 weeks after planting (Ia = 1.63, Pa = 0.0100, and Ia = 1.53, Pa = 0.0300). In contrast, thrips were inconsistently abundant in areas that experienced the most INSV, highlighting fundamental challenges that are associated with developing vector and virus management programs (1). We also sought to understand the role of weeds and other non-crops as secondary hosts for INSV. Over 4,000 plants comprising 70 species were sampled and tested for INSV using a combination of ELISA and RT-PCR. INSV was consistently detected from several common weed species, including, *Malva parviflora, Sonchus oleraceus, Chenopodium murale, Capsella bursa-pastoris,* and *Chenopodium album*. Studies are ongoing to characterize the potential for thrips to acquire INSV from each of the weed species and their competency to transmit back to lettuce crops. We'll discuss the significance of these findings and highlight the industry's synergistic response to improve the management of INSV in California lettuce.
KEYWORDS: Thrips; Tospovirus; Lettuce

SUPPORT

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MIXED INFECTION OF SUGARCANE MOSAIC VIRUS (SCMV) AND MAIZE STRIATE MOSAIC VIRUS (MSMV) INCREASES MSMV TRANSMISSION BY THE CORN LEAFHOPPER BUT NOT SCMV TRANSMISSION BY THE CORN LEAF APHID

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ABSTRACT:
Mixed infections of plant viruses can result in synergistic, neutral or antagonistic interactions, influencing disease severity and transmission by insect vectors. Surveys in Brazil have shown that sugarcane mosaic virus (SCMV; *Potyvirus*) and maize striate mosaic virus (MSMV; *Mastrevirus*) frequently co-infect maize. However, the impact of these infections on virus transmission by their vectors, *Rhopalosiphum maidis* (SCMV) and *Dalbulus maidis* (MSMV), remains unknown. Here, we assessed the effect of SCMV+MSMV mixed infections in both source and test plants on the transmission efficiency of MSMV by *D. maidis* and of SCMV by *R. maidis*, in independent experiments. To obtain source plants with single or mixed infections, groups of 20 viruliferous leafhoppers were confined per plant for 48 h for MSMV inoculation, while SCMV inoculation was performed mechanically 48 h before MSMV inoculation (mixed infection). For MSMV transmission, aviruliferous leafhoppers were confined on the source plants for a 96-h acquisition access period (AAP), and then groups of 20 individuals were transferred per healthy or previously SCMV-infected test plant for a 48-h inoculation access period (IAP). For SCMV transmission, aviruliferous aphids were confined on detached leaves of source plants placed in Petri dishes with agar (1%) for a 20-min AAP, then groups of 10 individuals were transferred per healthy or previously MSMV-infected test plant for a 24-h IAP. Previous infection with SCMV in both source and test plants increased the efficiency of MSMV transmission by *D. maidis*. The overall transmission rate of MSMV from plants with single or mixed infections was approximately 38 and 57% across three to four replicates of the experiment, respectively, with no significant difference ($P > 0.05$). However, MSMV transmission from single-infected source plants to test plants previously infected with SCMV was more than twice as high (85%) compared to transmission to healthy test plants (38%), showing a significant difference ($P < 0.05$). Mixed infection with MSMV did not affect the efficiency of SCMV transmission by *R. maidis* ($P > 0.05$), with overall transmission rates of approximately 85% (single infection), 80% (mixed infection) and 79% (from a single-infected source plant to a test plant infected with MSMV) across two experimental replicates. These findings contribute to the understanding of interactions between viruses in mixed infections and their epidemiological implications on maize crops.
KEYWORDS: maize viruses; co-infection effects; vector transmission

SUPPORT

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EFFICIENCY OF TOCV TRANSMISSION TO POTATO PLANTS BY *B. TABACI* MEAM1 AND MED AND RATE OF DETECTION IN TUBERS AND VERTICAL TRANSMISSION OF THE VIRUS TO THE NEXT GENERATION

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ABSTRACT:
Potato is a staple food worldwide, but viral diseases threaten its production. Tomato chlorosis virus (ToCV, *Crinivirus tomatichlorosis*) primarily affects solanaceous crops, especially tomatoes, but its impact on potatoes remains understudied in Brazil. This study evaluated the transmission efficiency of ToCV by *Bemisia tabaci* MEAM1 and MED in potatoes and assessed vertical transmission via tubers. Transmission assays were conducted using plants of cultivars Agata and Asterix. The insects were allowed a 24-hour virus acquisition access period (AAP). Subsequently, the insects were transferred (n = 10) to a healthy potato plant from the respective cultivars. The insects were provided a 24-hour inoculation access period (IAP) to transmit the virus. For each cryptic species, 10 potato plants from each cultivar were inoculated. ToCV transmission was confirmed 30 days after inoculation by RT-PCR. The experiment was conducted three times. To assess the detection rate of ToCV and its vertical transmission, tubers produced by plants of the Agata and Asterix cvs. infected with the virus were analyzed. Half of the tubers produced by each plant was used to evaluate the ToCV detection rate. This involved extracting total RNA from the tubers followed by RT-PCR analysis. The remaining half of the tubers were used for vertical transmission analysis. These tubers were planted in pots containing substrate, and 20 days after plant emergence, total RNA was extracted from the leaf tissue of the plants. RT-PCR was then performed to confirm the presence of ToCV. MED transmitted ToCV to potato plants of cv. Agata with higher efficiency (36.6%) than MEAM1 (10%). For the Asterix cv., the transmission efficiency for MEAM1 was 30% (9/30), while for MED was 36.6% (11/30). The evaluated Agata plants produced 124 tubers. Total RNA was extracted from 64 tubers, and ToCV was detected in 44 of them (68.75%) via RT-PCR. The remaining 60 tubers were planted to evaluate the vertical transmission rate of the crinivirus to the next generation. ToCV was detected in 76.67% of the evaluated plants (46/60). For the Asterix cv., ToCV was detected in 35 out of the 48 tubers analyzed (72.91%) by RT-PCR. The vertical transmission rate of the virus to the next generation was 88.1% (37/42). These findings highlight the potential risk of ToCV spread through tubers and whitefly transmission, emphasizing the need for continuous monitoring of potato fields and vector populations.
KEYWORDS: *Crinivirus*; *Solanum tuberosum*; whitefly

SUPPORT

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ACQUISITION OF CANDIDATUS LIBERIBACTER ASIATICUS BY NON-DIAPHORINA CITRI PSYLLIDS OCCASIONALLY LANDING ON CITRUS AND THEIR POTENTIAL TRANSMISSION RISK

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ABSTRACT:
Candidatus Liberibacter asiaticus (*C*Las), the prevalent etiological agent in the Americas of the Huanglongbing (HLB), threatens global citrus production. This phloem-limited bacterium is transmitted by *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). However, other psyllid species occasionally land on citrus, mainly during dispersal periods. Therefore, this study investigated: (*i*) Which psyllids, besides *D. citri*, land on citrus? (*ii*) What are their survival rates during the acquisition access period (AAP) on CLas-infected citrus, followed by a latency period (LP) on breeding hosts, and a post-latency period (PLP) on healthy citrus? (*iii*) Can they acquire *C*Las and retain it for a minimum of 20 days (*iv*) Does *C*Las reach the head-mesothorax, where the salivary glands are located, after feeding on infected citrus? Based on a field sampling (August 2017-March 2020, São Paulo, Brazil) using sweep net and suction devices, three non-*D. citri* species, *Isogonoceraia divergipennis*, *Heteropsylla cubana*, and *Triozoida limbata*, were collected on sweet orange [*Citrus sinensis* (L.) Osbeck] trees in São Paulo State, Brazil. Laboratory-reared adults (n=100, one-weekpost-emergence) of each psyllid species were confined on *C*Las-infected citrus (AAP, 3 days), then transferred to breeding hosts (LP, 14 days), and finally to healthy citrus (PLP, 3 days). Acquisition ability was assessed by qPCR at the PLP's end. Survival rates were calculated, and potential transmission was inferred by molecular analysis of dissected individuals into 'head-mesothorax' and 'metathorax-abdomen' sections. Survival rates were significantly lower for *H. cubana* (24.6%), *T. limbata* (26.3%), and *I. divergipennis* (34.1%), compared to *D. citri*. Despite their lower survival, all three species acquired *C*Las. The highest aquistion rate was observed for *D. citri* (90.2% females, 80.8% males, followed by *I. divergipennis* (9.8% females, 5.7% males), *T. limbata* (2.44%) and *H. cubana* (1.07%). Spearman's correlation analysis revealed a significant positive correlation between EF-Ts gene copy numbers in 'head-mesothorax' and 'metathorax-abdomen' across species. Notably, *T. limbata* was the only species, aside from *D. citri*, in which *C*Las detected in the 'head-mesothorax'. *H. cubana* and *I. divergipennis* showed significantly lower bacterial titer, with *C*Las detection restricted to the 'metathorax-abdomen'. This study provides the first evidence that non-*D.citri* psyllids can land on citrus, survive, and acquire *C*Las.
KEYWORDS: Huanglongbing; non-colonizing psyllids; potential vectors

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VECTOR RANGE AND TRANSMISSION BIOLOGY OF MAIZE STRIATE MOSAIC VIRUS (MSMV, *MASTREVIRUS*) BY THE CORN LEAFHOPPER

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ABSTRACT:
Maize striate mosaic virus (MSMV) is a new mastrevirus recently reported in maize in Brazil, transmitted by the corn leafhopper *Dalbulus maidis*. However, the transmission characteristics by *D. maidis* and transmission specificity have not yet been investigated. Here, we characterized the transmission of MSMV by *D. maidis* in terms of persistence, latency period (LP), and transmission efficiency in relation to access to acquisition (AAP) and inoculation (IAP) periods. Additionally, we determined the range of MSMV vectors. Transmission persistence was assessed in successive 48-h IAPs following a 48-h AAP. The LP was evaluated after 2-h AAP, with successive 2-6 h IAPs by insect cohort. Transmission efficiency was tested by varying the AAP, followed by a constant IAP (96 h), or by using a constant AAP (96 h) followed by a variable IAP. The variable AAP and IAP values used were 0.25, 0.5, 1, 2, 4, 8, 24, 48 and 96 h. In the LP and IAPs assays, only adults were tested. The leafhoppers transmitted the virus until the fourth (nymphs) or fifth (adults) successive IAP of 48-h, with transmission efficiency decreasing over time after the acquisition. The "half-life" of the virus in the insect was estimated at 7.1 days for nymphs and 9.1 days for adults. The virus was transmitted after an LP of 4 h from the start of acquisition, with higher transmission efficiency after an LP of 9 h. Similarly, the frequency of virus detection by PCR in the insect head increased with time after the acquisition, peaking at 11 h. Nymphs were more efficient at acquiring the virus, with 30 and 100% of samples from two individuals testing positive by PCR after AAPs of 0.25 and 0.5 h, respectively, compared to 13 and 40% of samples from adults. Transmission efficiency was positively correlated with both AAP and IAP durations. Collectively, these results suggest a persistent but not propagative mode of transmission of MSMV by *D. maidis*, as expected for a mastrevirus. Among the species tested as vectors of MSMV, only *Planicephalus flavicosta* and *Agallia albidula* were able to transmit the virus. Although the species *Exitianus obscurinervis*, *Balclutha frontalis*, *Peregrinus maidis* and *Rhopalosiphum maidis* failed to transmit the virus, virus acquisition was detected in individuals of all these species by PCR. This study provides valuable insights into the transmission of a new mastrevirus by leafhoppers in maize, which is crucial for understanding the epidemiology and management of the disease.
KEYWORDS: Transmission characteristics; *Dalbulus maidis*; Transmission specificity

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REEMERGENCE OF BRAZILIAN CURLY TOP VIRUS: EVIDENCE OF TRANSMISSION BY LEAFHOPPERS

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ABSTRACT:

The disappearance of Brazilian curly top disease (BraCTD) from tomato fields in Brazil has been an intriguing mystery for decades. First reported around 70 years ago, the disease caused distinctive symptoms, including stunting, leaf curling, vein swelling, and purpling of tomato plants. Although the causal agent remained unidentified at the time, it was shown to be transmissible by the leafhopper *Agallia albidula* and distinct from the curtovirus beet curly top virus. Since the 1990s, however, begomoviruses have dominated tomato cultivation, largely due to the spread of the whitefly *Bemisia tabaci*. Recently, tomato plants exhibiting BraCTD-like symptoms have reappeared in Brazil. Molecular analysis revealed the presence of a distinct geminivirus closely related to tomato-associated geminivirus 2, named BraCTV, a member of the genus *Topilevirus* within the family *Geminiviridae*, whose vector remains unknown. To investigate the transmissibility of GV-CTD-MT (genomic DNA of BraCTV), we tested *A. albidula*, the reported vector of the original BraCTD virus, and *Planicecephallus flavicosta*, a common leafhopper in São Paulo agricultural regions. Using infected tomato and *Datura stramonium* plants as sources, both leafhopper species successfully transmitted the virus after a 48-hour acquisition and inoculation access periods to tomato and *D. stramonium* plants. The similarities in host range and A. albidula transmission strongly suggest that GV-CTD-MT is an isolate of the original BraCTD virus. Ongoing studies aim to determine its distribution and potential impact on tomato production in Brazil.

KEYWORDS: Geminvirus; Tomato; leafhopper transmission

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ATTRACTIVENESS OF *APHIS GOSSYPII* AND *MYZUS PERSICAE* TO PASSION FRUIT PLANTS INFECTED WITH COWPEA APHID-BORNE MOSAIC VIRUS

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ABSTRACT:

In Brazil, passion fruit woodiness is the most critical disease affecting passion fruit crops. It is caused by the potyvirus cowpea aphid-borne mosaic virus (CABMV). To effectively delay CABMV spread, systematic roguing of diseased plants is recommended within the first five months or until 20% of plants become infected after transplantation. However, recent studies indicate that removing diseased plants does not significantly hinder its spread once the virus is introduced into the crop. This study aimed to evaluate the attractiveness of *Aphis gossypii* and *Myzus persicae* to CABMV-infected (asymptomatic and symptomatic) passion fruit plants and identify possible volatile organic compounds (VOCs) involved. Healthy passion fruit plants were used as controls. Aphid preference tests followed Safari et al. (1), using 50 aphids per test, with three replicates. Aphids were placed in a Petri dish covered with aluminum foil to eliminate visual cues. Each test compared infected asymptomatic vs. healthy plants and infected symptomatic vs. healthy plants. Attractivity was evaluated at 2, 7, and 24 h by counting the cumulative number of aphids that moved at least 1 cm toward the plants. Tests were repeated 10 times. VOC capture was performed using dynamic headspace sampling. Quantitative analysis of the molecules was conducted using TD-GC-MS (thermal desorption coupled to gas chromatography with mass spectrometry detection), as described by Volpe et al. (2). Healthy passion fruit plants (8), asymptomatic plants infected with CABMV (8), symptomatic infected plants (8), and an empty bag were compared. An evaluation was conducted. On average, after 72 hours, 631 *A. gossypii* individuals were attracted to infected asymptomatic plants, compared to 243 for healthy plants. The numbers for symptomatic infected plants versus healthy plants were 585 and 259, respectively. For *M. persicae*, 515 individuals were attracted to infected asymptomatic plants compared to 303 for healthy plants. Comparing symptomatic infected plants vs. healthy plants, the numbers were 518 and 225, respectively. Two VOCs, (E)-4,8-Dimethyl-1,3,7-nonatriene (DMNT) and (3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), were identified exclusively in infected plants (asymptomatic and symptomatic). Ongoing studies aim to confirm whether these VOCs contribute to aphid attraction in CABMV-infected passion fruit plants.

KEYWORDS: Passifloraceae; Potyvirus; Disease epidemiology

SUPPORT

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NEAR-CHROMOSOMAL AND IMPROVED DRAFT GENOME ASSEMBLIES OF DIVERSE BEGOMOVIRUS-VECTORS WITHIN THE *BEMISIA TABACI* SPECIES COMPLEX

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ABSTRACT:
Whiteflies belonging to the *Bemisia tabaci* species complex are among the world's most devastating agricultural pests, causing direct feeding damage on a wide range of plants, as well as further crop losses through vectoring several hundred viruses. To date, eight draft genomes of B. tabaci species have been published with reported genome lengths of 615-658Mb and N50 of 0.18-10.84Mb. The small size of B. tabaci whiteflies combined with their high genome heterozygosity has, however, meant that these draft genomes have been generated from 1000s of individuals, varying from field-collected populations to full-sib inbred populations (F5-F7). Such methods generate challenges for interpretation of duplicated genes and repeat sequences. We have generated improved genomic resources for the *B. tabaci* species complex by generating high quality draft genomes from single whiteflies using PCR-free methods. Near chromosomal level assemblies (N50 = 45-65Mb) of three species SSA1-SG1 (2 different populations), MED-ASL and AsiaII-1 have been achieved by scaffolding the single whitefly assemblies using Dovetail-HiRise TM assembly methodology. These genomes provide improved reference genomes for studies on differential virus transmission within the species complex, with our focus to date having been on cassava viruses.
KEYWORDS: *Bemisia tabaci*; whitefly vector; genome assembly

SUPPORT

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CITRUS HOST EFFECTS ON *BREVIPALPUS YOTHERSI*: IMPLICATIONS FOR CILV-C TRANSMISSION AND DETOXIFICATION GENE EXPRESSION

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ABSTRACT:
Brevipalpus mites are important vectors with high plasticity for adaptation to different hosts and strong ability to transmit plant viruses, known as BTVs (*Brevipalpus*-transmitted viruses). One of the most notable associated disease is citrus leprosis, with major impact on orchards, caused by *Cilevirus leprosis* (CiLV-C, *Cilevirus*: *Kitaviridae*). Most *Citrus* spp. are described as susceptible to the pathogen, with some considered tolerant and/or resistant. Our research has identified differentially expressed genes (DEGs) in *Brevipalpus yothersi* in response to CiLV-C infection and in association with various host plants. Special interest was given to the cytochrome P450 gene family, which may play a key role in the mites' adaptive responses to plant hosts and xenobiotics. The virus-vector interaction can be quite complex and, in the case of citrus leprosis, remains poorly understood. Here, we evaluated the functional response in the mite-host interaction by comparing the expression of P450 family genes in *B. yothersi* when colonizing sweet orange (*Citrus sinensis*) and Tahiti lime (*C. latifolia*) as well as by challenging the latter as a host of CiLV-C. In the functional analysis of mites maintained on Tahiti lime, we observed that the *CYP1* gene (P450 family) was significantly downregulated. In controlled inoculation experiments, there was no transmission of CiLV-C to Tahiti lime, supporting its classification as resistant and/or tolerant to the disease. Viruliferous mites, when maintained on Tahiti lime fruits, tested negative for CiLV-C by RT-PCR after 60 days, suggesting no viral replication. These findings suggest a distinct functional response of the mites regarding detoxification in different citrus hosts, and that CiLV-C does not replicate in Tahiti lime, preventing viral persistence and reinfection in vector populations. Further experiments are being conducted to validate this hypothesis.
KEYWORDS: Citrus leprosis, P450 gene family, virus-vector interaction, *Citrus latifolia*, host resistance

SUPPORT

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Session 3: VIRUS DISCOVERY AND ROLE OF METAGENOMICS IN EPIDEMIOLOGY

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THE GENETIC DIVERSITY OF *ORTHOTOSPOVIRUS IMPATIENSNECROMACULAE* (IMPATIENS NECROTIC SPOT VIRUS; INSV) IN AUSTRALIA

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ABSTRACT:

Impatiens necrotic spot virus (species *Orthotospovirus impatiensnecromaculae*, genus *Orthotospovirus,* INSV) is an economically important pest that has a wide range of ornamental, vegetable and weed hosts. In Australia, INSV was first found in New South Wales (NSW) in 2010 in *Begonia* sp. and reported to be successfully eradicated. In 2018 and 2019 INSV was detected again in NSW in *Lactuca sativa* (lettuce), approximately 130km south of the 2010 detection. Between 2021-2025, during surveillance for orthotospoviruses and thrips in southeastern Australia, INSV was detected in a range of ornamental plant species, including *Eustoma* sp. (lisianthus) and *Hoya* spp., in four different ornamental nurseries and in *Ocimum basilicum* (basil) in a production facility in Victoria. It was also found in lisianthus in one cut flower production facility in NSW, near the location where INSV was first reported in 2010. The presence of INSV poses a high risk to the Australian nursery, cut flower and vegetable industries, as its vectors, *Frankliniella occidentalis* (western flower thrips) and *Thrips tabaci* (onion thrips) and host plants are prevalent and could lead to a wider spreading of the virus nationally. To investigate the source and the diversity of the INSV in Australia, genomes of Australian isolates were generated by a combination of metagenomic high throughput and Sanger sequencing. Phylogenetic analyses were used to compare the S, M, and L genome segment sequences between the Australian INSV isolates and with isolates from overseas. For each genome segment, three distinct clusters of Australian isolates were formed that were associated with hosts and locations and might also suggest at least three incursions of INSV into Australia. Metagenomic sequencing also detected Hoya tobamovirus 2 in *Hoya* spp. plants for the first time in Australia.

KEYWORDS: Orthotospoviruses; Metagenomic; INSV

SUPPORT

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HIGH THROUGHPUT SEQUENCING UNVEILS THE COMPLEX VIROME OF GREEK VINEYARDS

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ABSTRACT:
Grapevine is among the most important crops in Greece, and it is cultivated in several areas both in the mainland and the islands of the country. A big number of local varieties are currently widely grown and used to produce high-quality wines. Nevertheless, the viral status of these varieties is largely unknown. Therefore, the target of this study was to unveil their virome using a high-throughput sequencing (HTS) approach. For this purpose, 25 samples originating from different Greek grapevine varieties were collected during 2023-2024 from the main viticultural areas of Greece. All samples were subjected to total RNA extraction, which was afterwards analyzed through HTS. Bioinformatic analysis of the obtained data was performed using a pipeline that included quality check, trimming of the reads and de novo assembly of contigs followed by BLASTn/BLASTx analysis. The results revealed the presence of twenty-five viruses and four viroids in the samples. Specifically, the presence of seventeen viruses and all viroids was already known in Greek vineyards, including viruses associated with grapevine leafroll disease, rugose wood complex, leaf degeneration, as well as grapevine fleck virus and grapevine Syrah virus-1 and the viroids Australian grapevine viroid, grapevine yellow speckle viroid 1, grapevine yellow speckle viroid 2 and hop stunt viroid. Eight viruses were detected for the first time in Greek vineyards, namely grapevine associated jivivirus 1, grapevine associated narnavirus-1, grapevine asteroid mosaic associated virus, grapevine Kizil Sapak virus (GKSV), grapevine-associated mitovirus 9, grapevine satellite virus, grapevine virus T (GVT) and a putative novel satellite virus. The presence of GKSV and GVT has already been confirmed in the samples initially analyzed by HTS and the study of the remaining viruses, some of which might not originally have grapevine as a host, is in progress.
KEYWORDS: grapevine; virome; high throughput sequencing

SUPPORT

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IMPACT OF VIRAL INFECTIONS ON MICRORNA EXPRESSION IN GRAPEVINE (*VITIS VINIFERA*)

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ABSTRACT:
Grapevine (*Vitis vinifera* L.) is among the most widely cultivated fruit crops. However, it is susceptible to numerous pathogens, including viruses. To date, over 100 viruses have been identified in grapevines, some of which cause severe disease symptoms, impacting both the quantity and the quality of the yield (1). Viral infections can also influence the expression of microRNAs (miRNAs) in plants (2). miRNAs are a class of small non-coding RNAs that regulate target gene expression at the post-transcriptional level (3). In this study, we analyzed the impact of viral infections on miRNA expression in the grapevine cultivars Refosk and Zeleni Sauvignon. Young leaves were sampled from virus-free and virus-infected grapevine plants. For miRNA expression analysis, small RNAs were extracted, and libraries were prepared for sequencing. Sequencing was conducted using the Ion Proton system. The VirusDetect tool was used to identify viruses present in the samples based on sequencing data. Expression of miRNA was analyzed using three miRNA prediction tools: miRador, ShortStack and miRDeep2. The results from all three tools were combined and used for differential expression analysis of miRNAs between infected and virus-free samples of each cultivar, which was performed using the R program and the DESeq2 package. Potential targets of differentially expressed miRNAs in the grapevine genome were predicted using the psRNATarget tool. Gene ontology (GO) analysis was performed to determine the biological functions of the target genes. By sequencing of small RNAs we obtained between 6,344,090 and 25,041,733 reads per library. In virus-free samples, the absence of viruses was confirmed, while infected samples contained the expected viruses GPGV, GRSPaV, and GRVFV. Differential expression analysis identified 21 differentially expressed miRNAs in cultivar Zeleni Sauvignon and 26 in cultivar Refosk. 17 miRNAs were differentially expressed in both cultivars. Potential targets for all differentially expressed miRNAs were predicted in the grapevine genome using psRNATarget, resulting in 111 potential targets in Refosk and 79 in Zeleni Sauvignon. Gene ontology (GO) enrichment analysis revealed that predicted target genes were involved in a broad range of biological processes and molecular functions. The identified differentially expressed miRNAs and their predicted targets will be further validated through qPCR, degradome sequencing, and a modified VIGS method (VbMS).
KEYWORDS: *Vitis vinifera*; miRNA; plant-pathogen interactions

SUPPORT

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THE EXPANDING CITRUS VIROME: UNCOVERING VIRUSES, ONE BY ONE

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ABSTRACT:
The advent of high-throughput sequencing (HTS) technologies, coupled with bioinformatics, has revolutionized plant virus research, enabling the rapid and unbiased discovery of viral sequences. This surge in genomic exploration has led to an unprecedented expansion of the plant virome, with novel viruses being identified at an accelerating pace. While this progress enhances our understanding of plant viral diversity, it also raises critical questions regarding the biological relevance of these newly detected sequences. The mere presence of viral sequences in HTS datasets does not confirm pathogenicity, necessitating rigorous biological characterization to determine their role in citrus health. To fully characterize a virus, experimental validation is required, including the fulfilment of Koch´s postulates, elucidation of transmission mechanisms, identification of potential vectors, determination of host range, and comprehensive symptomatology studies. These follow-up investigations are essential to differentiate between incidental viral sequences and biologically significant pathogens with potential agricultural impact. Additionally, understanding virus-host interactions and potential synergistic effects with other pathogens is crucial for assessing disease outcomes and economic consequences. Despite the excitement surrounding viral discoveries, scrutiny is necessary to assess their implications for citrus production and disease management. Some newly detected viruses may be latent or have negligible effects on plant health, while others could represent emerging threats requiring rapid response and intervention. The integration of HTS-based viral discovery with robust biological validation is therefore essential to provide deeper insights into the ecology, evolution, and epidemiology of citrus-infecting viruses. Furthermore, standardized criteria for virus classification, along with international collaboration in data sharing and validation studies, will enhance the reliability of new discoveries. By striking a balance between exploration and functional characterization, the scientific community can ensure that newly identified viral sequences contribute meaningfully to plant pathology, biosecurity, and sustainable citrus production. This approach will not only expand our understanding of the citrus virome but also support the development of effective diagnostic tools and management strategies for the long-term health of citrus industries worldwide.
KEYWORDS: High-throughput sequencing; HTS; Plant virus characterization

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GENOME SEQUENCING OF A PLUM POX VIRUS ISOLATE INFECTING PRUNUS DOMESTICA CV D'AGEN IN ARGENTINA USING THE ONT PLATFORM

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ABSTRACT:
Plum pox virus (PPV), the causal agent of Sharka disease in Prunus fruit trees, was detected in Argentina in San Juan (2006) and Mendoza (2007) province in Japanese plum, European plum, and apricot varieties. In acute infections, this virus causes a drastic reduction in production and affects fruit quality, rendering it commercially unviable for consumption, industry, and/or export. PPV presents different races or variants, which are usually specific to the host, region, and vector (when transmitted by aphids) and cause varying degrees of plant damage, with some affecting up to 80% of susceptible cultivars. In Argentina, it is a quarantine disease restricted to the Cuyo region. Given its significance, official measures have been adopted for its management and control. To identify a PPV isolate present in Cuadro Benegas production farms in San Rafael, whole viral genome amplification was carried out using the ONT platform. We started with symptomatic leaves of European plum cv D'Agen (H3 P7-Samper), extracted total RNA from two plants using the RNA kit (Sigma-Aldrich), and quantified it by spectrometry (Nanodrop-1000) and fluorometry (Quantus). The extracted RNA was used for library construction with PCR-cDNA Barcoding (SQK-PCB109). Sequencing was performed on a MinION device with a flow cell R9.4.1 on the ONT platform at CIAP, INTA. Raw sequences were filtered for quality and size (-q >10, size > 250 bp), and the resulting reads were assembled de novo and polished using Medaka version 1.12.0 and Racon v1.4.1. A BLASTn database was generated from 14,819 viral genome sequences (NCBI, RefSeq). With the curated assemblies, a BLASTn search was performed against the database at https://usegalaxy.eu/ (e-value 0.001, one hit per contig). The presence of a 9,286 bp viral contig corresponding to PPV was identified. This sequence was compared with a specific database of viral genomes of the PPV species in the NCBI (NCBI: txid12211), showing homologies higher than 97% with PPV strain D, including 99% identity with isolate 3.30RB/GF-IVIA, 98.89% with isolate PEEN 5, 98.95% with isolate SK3, and 98.85% with isolate Ou1 for the complete genome. This is the first result of obtaining a complete genome sequence of a PPV isolate from Argentina using the ONT platform.
KEYWORDS: sharka disease; Prunus domestica; PPV ONT sequencing

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A VIROME AND RISK ANALYSIS OF MULTIPLE "NEW" VIRUSES IDENTIFIED FROM MASHUA (*TROPAEOLUM TUBEROSUM*)

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ABSTRACT:
Vegetatively propagated crops often contain viruses which in many cases may remain unnoticed. A recent Euphreso project (PRONC) identified numerous "new"viruses in tuberous plant species originating from the South American Andean region. Many of these plant species are often grown in home gardens and the tubers are freely traded through international webshops. However, the presence of the "new" viruses may pose yet unknown risks to European agriculture. In this project we investigated the virome of Mashua (*Tropaeolum tuberosum*) and the possibly associated risks for Dutch agriculture. Multiple tubers from 5 different Mashua cultivars were obtained from international webshops and planted in a Q-greenhouse with most plants remaining symptomless. Total RNA samples were extracted from individual plant leaves and subjected to Illumina HTS. Resulting reads were analyzed using the CLC Genomics Workbench (Qiagen_V20). Contigs resulting from De novo assembly were analyzed for possible viral origin using a "long-read mapping" module against a list of plant virus reference sequences as well as subjected to Blastn analyses against a from NCBI downloaded virus RefSeq database.These analyses identified a significant number of new viruses of which most were present in multiple cultivars. Viruses represent species from the genera potyvirus, potexvirus, carlavirus, polerovirus, umbravirus, cytorhabdovirus and alphanucleorhabdovirus.To investigate whether these viruses could constitute a potential phytosanitary risk a selection of viruses was tested for pathogenicity through mechanical inoculation on tobacco indicator plants as well as on potato, tomato and sweet pepper.
KEYWORDS: Virome analyses; virus risk analysis; bioassay

SUPPORT

The project received financial support from Topsector Tuinbouw & Uitgangsmaterialen (TKI-TU18095 Fytosanitair Belangrijk voor Nederland BV), in which industry, research institutions and the Dutch government collaborate on innovations in the sustainable production of healthy and safe food, and the development of a healthy and green living environment.

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HIGH-THROUGHPUT SEQUENCING DISCLOSES THE VIROME OF COMPANION PLANTS IN OPEN-FIELD TOMATO CROPS

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ABSTRACT:

Companion or banker plants are increasingly used in integrated pest management (IPM) to support beneficial insects, but their potential role as reservoirs of plant viruses remains largely unexplored. In this study, we employed high-throughput sequencing (HTS)-based approaches to investigate the presence of viral pathogens in two companion plant species, *Phacelia tanacetifolia* and buckwheat (*Fagopyrum esculentum*), grown adjacent to an open-field tomato crop during the summer of 2024.Total RNA was extracted from pooled samples, each consisting of six leaves per plant species, and subjected to HTS. The raw sequencing reads were quality-checked, trimmed, and assembled into contigs using de novo assembly approaches. The resulting contigs were screened against viral reference databases using BLAST to identify potential viral sequences. Initial bioinformatic analyses revealed the presence of cucumber mosaic virus (CMV) and broad bean wilt virus 2 (BBWV2) in both species. In *P. tanacetifolia*, three different umbravirus species were detected, along with beet western yellows virus, turnip yellows virus (TuYV), turnip mosaic virus (TuMV) and beet mosaic virus. In *F. esculentum*, an umbravirus and a novel cytorhabdovirus were identified, expanding the known viral diversity in companion or banker plant systems. RT-PCR confirmed the presence of CMV, BBWV2, TuYV and TuMV in the companion plants, while confirmation of the remaining viral sequences presence is ongoing. To further assess the epidemiological implications of our findings, tomato plants from the adjacent crop were also sampled, and their virome will be analysed once banker plant viral profiles are fully characterized. These findings highlight the complex viral landscape associated with companion plants. Understanding whether these plants serve as viral reservoirs and contribute to virus transmission in the crops is critical for optimizing their use in sustainable agricultural practices.

KEYWORDS: companion plants; virome; HTS

SUPPORT

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INSIGHTS INTO GENOME REGULATION AND FUNCTIONAL COMPLEMENTATION IN MULTIPARTITE VIRUSES

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ABSTRACT:
Multipartite viruses rely on coordinated interactions among genome segments for successful replication and infectivity, yet their organization remains challenging to fully understand [1]. Advances in metagenomics have facilitated the characterization of these viruses, providing insights into their genome composition and evolutionary dynamics [2]. In this study, we investigated the genomic architecture of milk vetch dwarf virus (MDV), focusing on segment composition, relative genome abundance, and reassortment between nanoviruses. Using genome formula analysis, we identified segment distribution trends in MDV and examined their role in viral replication and genome stability. These findings contribute to a broader understanding of multipartite virus genome organization and maintenance. Reassortment plays a fundamental role in the evolution of multipartite viruses, facilitating the exchange of genome segments among co-infecting species [3]. We also explored functional complementation and segment reassortment between nanoviruses to assess segment compatibility and potential cross-species interactions. This study underscores the importance of quantitative genomic approaches in deciphering genome organization, reassortment, and segment interactions, particularly in nanoviruses.
KEYWORDS: Multipartite Viruses; Milk vetch dwarf virus; Reassortment

SUPPORT

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FIRST REPORT OF *TOMATO YELLOW LEAF CURLY VIRUS* (TYLCV) INFECTING TOMATO PLANTS IN BRAZIL

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ABSTRACT:
Tomato yellow leaf curly virus (TYLCV) (genus *Begomovirus,* family *Geminiviridae*) has occurred epidemically and caused major losses to tomato (*Solanum lycopersicum*) crops in several regions around the world. Typical symptoms associated with TYLCV infection in tomato plants involve chlorosis, upward curling of leaflet margins, reduction in leaf area, yellowing of leaflets, and stunting of the plant. In December 2024, tomato plants were found in commercial crops in the municipality of Faxinal, state of Paraná, Brazil, with symptoms similar to those associated with TYLCV infection. It is worth noting that the presence of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae), a potential vector of viruses belonging to the genus *Begomovirus*, had also been reported in large amount on tomato plants. Furthermore, tomatoes are produced in Faxinal basically under semi-protected environment, with an estimated area of 360 ha of screenhouses. Symptomatic tomato samples were brought to the Virology laboratory of the Instituto de Desenvolvimento Rural do Paraná/IAPAR-Emater – IDR Paraná for analysis. Total DNA was extracted from the samples and subjected to polymerase chain reactions (PCR), using the primers pairs Pal1v1978/PAR1C496 and AV494/AC1048. Subsequently, the samples that presented positive results in the PCR reactions were submitted to genomic sequencing (Applied Biosystems ABI 3730XL DNA Analyzer, Foster City, CA, USA). In the PCR reactions, DNA fragments of 1,100 bp and 700 bp were amplified with the primers pairs Pal1v1978/PAR1C496 andAV494/AC1048, respectively, from tomato samples collected in commercial crops in the municipality of Faxinal. The genomic sequencing results revealed 97.79% similarity with the TYLCV-CJ-cgh isolate of TYLCV obtained from tomato in the city of Cheju, South Korea. Although the presence of the virus may have been suspected previously, this is the first confirming report of the occurrence of TYLCV infecting tomatoes in Brazil. Additional studies are needed to determine the occurrence of TYLCV in other tomato-producing regions in Brazil.
KEYWORDS: *Begomovirus*; *Solanum lycopersicum*; TYLCV

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NEW EMARAVIRUS FOUND IN CACAO PLANTS (THEOBROMA CACAO L.) IN AMAZONAS, PERU

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ABSTRACT:

Cacao crop is the engine of the chocolate industry. Peru is the world's second-largest producer of fine-aroma cacao and shares with Ecuador and Colombia the Amazonian area where the crop originated. To preserve Peruvian cacao germplasm is necessary to identify propagation accessions free of pathogens such as viruses, which currently constitute a threat to the crop, mainly in West Africa. In this study, we aimed to obtain the virome of cacao from Amazonas. A survey was carried out in the native cacao collection bank of the Universidad Nacional Toribio Rodriguez de Mendoza de Amazonas, Peru. Ten leaf samples were collected from plants with symptoms associated with the presence of viruses, such as mosaics, yellowing, or deformation. Total RNA was extracted from each of the samples and sequenced using Next-Generation Sequencing (NGS). The raw sequence reads were processed using CLC Genomics Workbench. In two samples we found four RNA segments matching the typical genomic structure of the Emaravirus genus: RNA1 (7133 nt encoding a viral replicase P1), RNA2 (2234 nt, encoding a glycoprotein P2), RNA3 (1270 nt encoding a nucleocapsid protein P3) and RNA4 (1286 nt encoding a movement protein P4). BLASTx analysis indicated that the four genomic RNAs have between 32.6% and 45.9% amino acid identity with the closest viral species, the European mountain ash ringspot-associated Emaravirus. These data indicate that it is a new Emaravirus, belonging to the family Fimoviridae, whose tentative name is Theobroma cacao emaravirus A (ThCEV-A). A phylogenetic tree with the amino acid sequence of P1 - P4, corresponding to the new emaravirus and 24 emaravirus available in the genbank, was constructed, finding that ThCEV-A forms a new phylogenetic clade. Additionally, specific primers were designed and 60 samples of native cacao accessions from Amazonas, were analyzing. Eleven accessions were positive for all the 4 RNAs. The symptoms associated with the presence of the virus correspond to leaf deformations and yellow spots. This is the first emaravirus described in cacao in the world. Future surveys are in progress in order to detect the possible presence of ThCEV-A in wild cacao plants and identify potential vectors.

KEYWORDS: Bunyavirales; Fimoviridae; cacao plants virome

SUPPORT

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INDEXING CASSAVA GERMPLASM FOR VIRUSES AND PATHOGENS REVEALS HIGH VIRAL DIVERSITY, JUSTIFYING STRICT MEASURES TO PREVENT INTRODUCTION OF HARMFUL ORGANISMS

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ABSTRACT:
The genetic improvement of crops through breeding requires genetic resources providing novel traits to enhance yield, quality and resilience. Since the centers of diversity of major crops, from tomato to cassava, are in far distant geographies and ecosystems and the internationalization of breeding efforts, crop improvement means, the global exchange of germplasm and improved breeding lines. An intensive international exchange of breeding and raw materials from a global division of production is associated with the introduction of unwanted organisms that can pose a risk to agriculture and environment. Plant viruses are of particular concern when introduced into new areas with plant propagules and the recent outbreaks of Sri Lankan cassava mosaic virus (SLCMV) in South East Asia, maize lethal necrosis (MLN) in Eastern Africa and tomato brown rugose fruit virus (ToBRFV) in Europe and the world provide ample evidence for the impact caused. The DSMZ Plant Virus Department serves as a leading transit quarantine center for cassava and other root crops, ensuring the safe transcontinental movement of genetic resources. To mitigate viral transmission, rigorous growing-on tests combined with high-throughput sequencing (HTS) are employed to screen *in vitro* plants for viruses and virus-like organisms. HTS indexing of cassava germplasm revealed a vast diversity of known and novel viral sequences, including isolates and variants that would remain undetected using conventional molecular assays. Some newly identified viruses exhibited mild or transient symptoms, or no clear disease association in their native environments, highlighting the superior sensitivity of HTS in plant health assessment. In vegetatively propagated crops like cassava, geographically distinct viral isolates can coexist within a single plant. HTS also provided insights into historical virus introductions, tracing early germplasm exchanges across continents. Transmission experiments and mixed-infection studies allowed risk assessments of novel viruses, guiding exclusion measures to prevent their establishment in new regions. To safeguard cassava germplasm, we established a robust transit-quarantine workflow integrating HTS at the tissue culture stage with confirmatory glasshouse screening. This approach ensures comprehensive detection of RNA and DNA viruses, as well as phytoplasmas, reinforcing global biosecurity in cassava breeding and conservation.
KEYWORDS: genetic resources; transcontinental exchange; plant health

SUPPORT

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FIRST REPORT OF CUCUMBER MOSAIC VIRUS INFECTING *PETROSELINUM CRISPUM* IN SERBIA

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ABSTRACT:
The Apiaceae family, a large and complex family with about 3820 species distributed throughout the world, includes important leaf and root vegetables such as carrot, parsley, celery, and parsnip. A number of species are cultivated as aromatic herbs and spices, while certain species have long been used in medicine. It is well-known that this family has been threatened by numerous pathogens, of which more than 30 widespread viruses have been described. Cucumber mosaic virus (CMV; *Cucumovirus CMV*, *Cucumovirus*, *Bromoviridae*) is one of the most widespread viruses, causing significant agronomic losses in many crops, including over 1,300 plant species from more than 500 genera and 100 families, with a high potential for rapid spread to new hosts. In autumn 2023, during a survey to determine the presence of Apiaceous viruses in Serbia, symptomatic parsley plants with chlorotic spots and line patterns on the leaf surface were observed in the Medvedja locality (district of Rasina). The collected samples were analyzed by double-antibody sandwich (DAS)-ELISA and CMV was detected in 7 out of 9 tested samples. The presence of CMV in parsley was also confirmed by RT-PCR with specific primers. The amplified product of isolate 4/23 was sequenced using Sanger sequencing method and analyzed by MEGAX software. BLAST search result revealed a 99,52% identity with Polish CMV isolate from host *Lonicera caprifolium* (EU 191027). The constructed phylogenetic tree showed that the Serbian parsley isolate belongs to CMV subgroup II. In Yugoslavia's former geographical region, CMV's presence was suspected based on symptoms observed on celery plants in the fields. To our knowledge, this is the first report of CMV on parsley in Serbia.
KEYWORDS: parsley viruses; RT-PCR; phylogeny analyses

SUPPORT

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VIROME PROFILING OF IMPORTED SEEDS IN KOREA REVEALS RISK OF VERTICAL VIRUS TRANSMISSION

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ABSTRACT:
Plant viruses remain a major threat to crop production worldwide, causing substantial yield losses. While many viruses are transmitted horizontally via vectors or mechanical contact, certain species are also capable of vertical transmission through seeds. In recent years, the reliance of domestic seed companies on overseas breeding and production facilities has increased, along with the import of commercial seed lots from foreign sources. This practice raises the risk of introducing foreign viruses, including quarantine-regulated or previously unreported species, into Korean agriculture. Although current quarantine systems rely on PCR or RT-PCR methods to screen for known viruses, these approaches are limited by their dependence on prior sequence information and may fail to detect novel or mutated variants. To address this gap, we conducted a virome analysis using high-throughput sequencing (HTS) on pooled RNA samples extracted from commercially available seeds of various origins sold in Korea. The HTS results revealed the presence of 15 distinct plant viruses, including previously uncharacterized members of the genera *Betapartitivirus* and *Allexivirus*. To evaluate their transmission potential, germination tests were conducted and viral presence in the sprouted seedlings was confirmed. Notably, all 15 viruses detected in the original seed lots were also found in the corresponding seedlings, demonstrating their ability to be transmitted vertically through seed. These findings highlight the potential for international seed trade to serve as a route for the introduction of both known and novel plant viruses. The study underscores the need for more comprehensive virus surveillance tools, such as HTS, in seed quarantine and certification systems to ensure agricultural biosecurity.
KEYWORDS: Seed transmission; Imported seeds; Virome analysis

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VIROME ANALYSIS OF *CNIDIUM OFFICINALE* IN KOREA: COMPLEX CO-INFECTION PATTERNS OF NOVEL AND ESTABLISHED VIRUSES

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ABSTRACT:
High-throughput sequencing (HTS) has become a powerful tool in plant virology, enabling the simultaneous detection of known and previously uncharacterized viruses, as well as the assessment of complex viral infections in diverse plant hosts. In this study, we investigated the virome of *Cnidium officinale* Makino, a medicinal crop grown in the mountainous regions of Korea, including Gyeongsangbuk-do, Gangwon-do, Chungcheongbuk-do, and Jeollabuk-do.Total RNA was extracted from samples collected across nine regions and subjected to HTS. *De novo* transcriptome assembly using CLC Genomics Workbench (v22.0.4) facilitated the reconstruction of viral genomes, and BLAST analysis enabled identification of both known and novel viral sequences. We observed substantial variation in the number of viral reads among regions, with Bonghwa (Socheon-myeon) exhibiting the highest read count (74,074,044) and Jecheon the lowest (8,009,876).A total of 15 viruses were identified, including cnidium vein yellowing virus-1 (CnVYV-1), cnidium virus X (CnVX), and cucumber mosaic virus (CMV), along with several novel viral candidates. Full-length genomes of CnVYV-1, CnVX, and CMV were successfully assembled, while partial sequences were recovered for others. Comparative analysis revealed host-specific features in viruses such as apple stem grooving virus (ASGV) and CMV, which are typically associated with fruit trees or solanaceous and cucurbit hosts, respectively. Despite geographical variation in host cultivation, viral isolates showed high sequence similarity across regions.These findings shed light on the complex viral communities and co-infection dynamics affecting *C. officinale* in Korea and underscore the utility of HTS in monitoring virus populations in medicinal crops.
KEYWORDS: *Cnidium officinale*; Co-infection; Virome analysis

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COMPARATIVE ASSESSMENT OF THE VIROME ASSOCIATED WITH HOP AND CITRUS IN BRAZIL AND ITALY

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ABSTRACT:
Citrus cultivation holds significant economic importance in both Brazil and Italy. Recently, hop cultivation has also seen notable growth in these countries, driven by the rise of craft breweries. In the European Union, viroids pose the most pressing challenge for hop production. Initially, outbreaks were caused by hop stunt viroid (HSVd), but epidemics of citrus bark cracking viroid (CBCVd) have become a major concern. Originally identified in citrus, CBCVd has now crossed over to hop through an as-yet-unknown transmission pathway. In Brazil, CBCVd has recently been reported in hop but not in citrus, raising concerns about viroid transmission between these two crops. Conversely, in Italy, CBCVd has been identified in citrus but not in hop. The overall presence and distribution of viruses and viroids affecting hop crops in both countries remain poorly understood. As part of a collaborative project between CNR (Italy) and FAPESP (Brazil), our objective is to characterize the virome of hop, citrus, and associated weeds in both countries by metatranscriptomics analysis. Total RNA from leaf samples (citrus, hop and associated weeds) collected from different Brazilian regions (States of Sao Paulo, Rio de Janeiro, Minas Gerais, Paraná and Santa Catarina) and Southern Italy (Campania, Calabria and Lazio provinces) were pooled and analysed by RNAseq (Illumina). Preliminary bioinformatic analysis of Brazilian hop libraries revealed the absence of CBCVd in the tested samples, whereas hop latent viroid (HLVd) was found to be widespread. Additionally, various known and potentially novel viral species, along with a viroid-like agent, were identified in Brazilian samples. In Italy, CBCVd was found in citrus from Campania and Calabria, and a variability study of the Italian isolates of this viroid has been conducted. These findings, including the identification of new viral and subviral agents, phylogenetic analyses, and epidemiological insights, will be presented.
KEYWORDS: citrus bark cracking viroid; Metatranscriptomics; hop

SUPPORT

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Session 4: DISEASE MANAGEMENT AND RESISTANCE

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REGULATION OF RIBOSOME-ASSOCIATED PROTEIN QUALITY CONTROL BY VIRAL INFECTION IN PLANTS

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ABSTRACT:
The ribosome-associated protein quality control (RQC) pathway resolves unproductive translation events caused by ribosomes that fail to reach a stop codon and terminate translation. The RQC complex, conserved across eukaryotes, is responsible for recycling 60S ribosomal subunits and targeting incomplete polypeptides for degradation. In plants, RQC components were recently characterized in Oryza sativa for their role in stabilizing the thermo-sensitive genic male sterility phenotype in two-line hybrid rice systems. Since viral infections can generate truncated mRNA species as byproducts of mRNA degradation via the small interfering RNA (siRNA) response, and viral transcripts commonly contain secondary structures, we hypothesized that viral infection could provide substrates for the RQC pathway, potentially linking these genes to virus disease resistance. Our first objective was to characterize *AtLTN1*, a key component of the RQC pathway. *AtLTN1* (AT5G5840) codes for a homolog of the E3 ligase of the RQC that ubiquitinates incomplete polypeptide chains attached to the 60S ribosomal subunit, marking them for degradation via the proteasome. We first performed a phenotypic characterization of two lineages of *Arabidopsis thaliana AtLTN1* SALK T-DNA insertional mutants collection (*atltn1.1, atltn1.2*). These plants exhibited an approximately 30% reduction in biomass compared to wild-type plants and mild defects in root development. To investigate the involvement of LTN1 homologs in the response to viral infections, we analyzed the expression of these genes by Q-PCR in *Arabidopsis thaliana* and *Nicotiana benthamiana* infiltrated with an infectious clone of Potato virus X (PVX). A two-fold increase in *AtLTN1* and *NbLTN1* expression was observed in infiltrated plants compared to non-infiltrated controls. To further investigate the impact of *AtLTN1* loss-of-function on viral infection, ongoing experiments are being conducted using Turnip mosaic virus (TuMV), which naturally infects *A. thaliana*. These findings suggest that the RQC pathway plays a role in plant development and in the response to viral infection.
KEYWORDS: RQC pathway; LTN1; viral infection

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GROUNDNUT BREEDING FOR ROSETTE RESISTANCE IN MALAWI

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ABSTRACT:
Groundnuts (*Arachis hypogaea* L.) greatly aid food security and economic stability, especially in sub-Saharan Africa. However, biotic stressors, environmental variability, and restricted access to better varieties limit its output. This study used multi-environment trials in nine locales to find stable, disease-resistant, and high-yielding groundnut cultivars. Pod yield, kernel yield, and 100-seed weight were among the important agronomic parameters for which genotype-by-environment (G×E) interactions were evaluated. The findings showed that genetic and environmental factors significantly differed in yield and seed quality characteristics. Positive correlations between pod and kernel yield and seed size emphasized the importance of selecting varieties with desirable market traits. Conversely, early leaf spot and groundnut rosette disease negatively impacted yield, reinforcing the need for disease-resistant varieties. GGE biplot analysis identified superior genotypes such as CG 9, CG 13, and CG 15, demonstrating broad adaptability. These findings emphasize the importance of multi-environment trials and advanced statistical tools in selecting resilient groundnut varieties. Targeted breeding strategies incorporating genetic and environmental factors can enhance productivity and sustainability, contributing to food security in groundnut-growing regions.
KEYWORDS: Peanut; Groundnut Rosette Disease (GRD); GXE

SUPPORT

Peanut Innovation Lab

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PROOF OF LIFE: USING MOLECULAR DIAGNOSTICS TO DEMONSTRATE VIRUS VIABILITY

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ABSTRACT:
Accurate, reliable, and rapid diagnostic tools for determining the causal agents of these diseases are important for surveillance and outbreak management. Molecular methods (such as PCR and high throughput sequencing) are established as detection methods in plant health laboratories, however, these methods cannot be used directly to infer the biological relevance of a detection. Tomato brown rugose fruit virus (ToBRFV) is a tobamovirus. Since emerging in Jordan in 2014 it now affects tomato production around the globe and is a focus for regulatory phytosanitary controls. ToBRFV is highly stable, readily transmissible by contact and through seed-borne-transmission. Viral RNA is still detectable by RT-qPCR from seed samples and contaminated surfaces even after heat or chemical treatments have inactivated the virus (Davino et al., 2020; Skelton et al., 2023). Currently, bioassay using a local lesion indicator host plant is the standard method to verify the viability of the virus (ISF, 2024). This method lacks reliable sensitivity, requires skill, and takes up to 21 days to obtain a result making it unsuitable as a routine test. In this study, targeted whole genome sequencing by combining long range PCR and tiled amplicon multiplex PCR with high throughput sequencing using Flongle (Oxford Nanopore Technologies) are developed to detect whole ToBRFV genome. This technique has been compared to RT-PCR, real-time RT-PCR, ELISA and bioassay to allow result quantification. The working dilution for source of viral inoculum has been determined for all methods to ensure these are performing within their relative limits of detection. Following virus deactivation, using methods such as heat and chemical treatment, the detection methods will be used to investigate the difference between detection of treated (non-viable) and untreated (viable) virus samples. The methods developed in this study and the current standard diagnostic methods will be compared to understand the correlation of the test results with virus viability. This study will assess tiled amplicon sequencing as a proxy diagnostic tool in indicating the virus viability in diagnostic samples. The latest results and potential applications of this approach will be discussed.
KEYWORDS: tomato brown rugose fruit virus; tiled amplicon sequencing; biological relevance

SUPPORT

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A NOVEL GEMINIVIRUS-BASED VIRAL VECTOR SYSTEM FOR RNAI DELIVERY IN *NICOTIANA BENTHAMIANA* AND GRAPEVINE

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ABSTRACT:
Plant viral diseases pose a significant threat to global agriculture, often leading to severe yield losses with limited options for management. Since curative treatments for viral infections are unavailable, disease control primarily relies on prevention, including vector management, resistant crop varieties, and biotechnological approaches such as RNA interference (RNAi). Viral vectors have emerged as powerful tools for gene silencing and foreign gene expression, offering potential applications in disease control. However, their effectiveness depends on the characteristics of the virus and its ability to replicate and move within host plants. In this study, we developed grapevine geminivirus A (GGVA)-based viral vectors from two different isolates to induce RNAi in *Nicotiana benthamiana* and grapevine plants. We constructed an infectious defective viral genome of GGVA (Def-GGVA), whose replication and movement are facilitated by the wild-type GGVA (WT-GGVA) in infected plants. Target sequences were cloned into Def-GGVA for RNA silencing or protein expression, allowing multiple Def-GGVA clones with different target sequences to be introduced simultaneously alongside WT-GGVA. Using this system, we successfully targeted *phytoene desaturase* (PDS) and *ChlH* transcripts in *N. benthamiana* and another grapevine virus in grapevine plants. Our results demonstrated that GGVA-based viral vectors can achieve low-level protein expression and induce strong silencing effects specifically in phloem-associated tissues. Furthermore, we observed that co-infiltrating two Def-GGVA clones, one carrying the sense and the other the antisense sequence of the target gene, enhanced the silencing effect. These findings highlight the potential of GGVA viral vectors not only as efficient gene silencing tools but also as a promising strategy for disease control in grapevine.
KEYWORDS: Grapevine geminivirus A (GGVA); viral vector; RNA interference

SUPPORT

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DOES GREEN CROP PROTECTION INFLUENCES VIRUS DETECTION IN PLANTS?

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ABSTRACT:
The Flower Bulb Inspection Service (BKD) has been tasked by the Dutch Ministry of Agriculture, Fisheries, Food security and Nature with inspecting the quality of all flower bulb crops in the Netherlands on both quality defects and quarantine pathogens. Therefore, samples of flower bulb crops are tested using ELISA and/or PCR tests that were designed and optimized for these samples. The opinion on the use of chemical pesticides has undergone significant transformation in recent years. Growing concerns about environmental impact, human and animal health risks, and the long-term sustainability of chemical pesticides have led to a shift in social opinion. In response to these concerns, there has been a noticeable increase in the use and development of biological pest control methods, such as microorganisms and other eco-friendly agents. As these agents are substantially different from the existing chemical agents, we investigated whether the use of the new biological agents influences the performance and results of our ELISA and PCR tests.
KEYWORDS: ELISA; PCR; Biological pest controle methods

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WHY 'MUNGER' BLACK RASPBERRY IS THE PREFERRED INDICATOR FOR RUBUS VIRUSES: INSIGHTS FROM THE BLACKBERRY CHLOROTIC RINGSPOT VIRUS ACCUMULATION

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ABSTRACT:
Virus indicator plants are those that exhibit heightened susceptibility to viral infections and are commonly used for diagnostic purposes. In the case of Rubus viruses, 'Munger' black raspberry (*Rubus occidentalis*) is the preferred indicator. Here, we aim to determine whether the observed elevated susceptibility in 'Munger' is linked to its ability to sustain higher virus titer accumulation. To address this, we compared how virus expression differs amongst two Rubus species: *R. occidentalis* ('Munger') and *Rubus* L. subgenus *Rubus* Watson (blackberry, 'Natchez'). An infectious clone of blackberry chlorotic ringspot virus (BCRV) was used to obtain single infected BCRV plants for both 'Munger' and 'Natchez', providing a uniform source of inoculum. Plants were sampled over a period of six months, and RT-qPCR was used for virus detection. For viral quantification, the cycle threshold (CT) values were normalized using 18S rRNA as an internal control. Subsequent statistical analyses showed that 'Munger' could sustain higher virus accumulation than 'Natchez', over 47,300 times higher. The above enhances our understanding of the interaction between BCRV and two Rubus genotypes, moving us closer to resolving the long-standing question of why 'Munger' is the preferred indicator species for virus detection in *Rubus* spp.
KEYWORDS: replication dynamics; restistance; Rubus

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MOLECULAR DIAGNOSTIC TESTS TO STRENGTHEN SEED SYSTEMS OF ROOT AND TUBER CROPS

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ABSTRACT:
Yam (*Dioscorea* spp.) productivity is severely compromised by viruses and the lack and high cost of clean planting material. Propagation through seed yam tubers encourages the recycling of infected planting materials, increasing virus incidence and yield losses, a common issue for vegetatively propagated crops. In Ghana, yam farmers often use low-quality, virus-infected planting material from their own or neighbouring farms. The only effective control method is to use virus-free seed yam. The Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) developed an aeroponics and hydroponics system to enhance seed yam production. This system has a high multiplication rate, generating thousands of plantlets from a single plant. However, virus titres in the plantlets are reduced but not eliminated, complicating a reliable virus detection and making it difficult to ensure that planting material is virus-free. This could lead to false virus-negative certification of planting materials, spreading infected materials and threatening crop production and food security. We have developed and evaluated isothermal amplification- and high throughput sequencing (HTS)-based diagnostic tests for virus detection in various crops. The yam tests have been transferred to CSIR-CRI, improving their capacity in diagnostics and seed certification and contributing towards the production and sustainable supply of high-quality seed yams. We will discuss the potential wider application of these tests and the factors influencing their future deployment in support of seed systems of root and tuber crops.
KEYWORDS: Loop mediated amplification (LAMP), on-site diagnostics, virus detection; High Throughput Sequencing (HTS); MinION sequencing

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IMPACT OF HOST RESISTANCE AGAINST A BEGOMOVIRUS ON HOST-VECTOR-VIRUS INTERACTIONS, VIRUS TRANSMISSION, AND VIRUS POPULATION GENETICS

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ABSTRACT:
The sweetpotato whitefly (*Bemisia tabaci*) transmitted single stranded DNA virus, tomato yellow leaf curl virus (TYLCV), is a serious pathogen in tomato production worldwide. Host resistance plays a significant role in managing the virus and mitigating losses. Host resistance to TYLCV in tomato is conferred by six (Ty1-6) semidominant genes. The functionality of the genes varies from RNA-dependent RNA polymerase (RDRP), leucine rich repeat (LRR), to mRNA surveillance factor (pelota), thereby insinuating that resistance mechanisms could vary with genes. Transcriptomes (genome guided) from tomato with six genes (individually) on the same genetic background are currently being synthesized and annotated. Details will be discussed during the presentation. Semi-dominant genes-conferred resistance is characterized by mild symptoms and reduced accumulation of TYLCV in infected plants. Experiments were conducted to assess if TYLCV infection in resistant genotypes would enable them to function as inoculum sources. Results indicated that TYLCV infection induced a subdued phenotype in resistant genotypes when compared with the susceptible genotype, and fewer non-viruliferous adults were attracted to resistant TYLCV-infected genotypes in comparison with the resistant genotype. Also, TYLCV resistant genotypes accumulated less TYLCV loads than the susceptible genotype over different intervals. Overall, results suggested that TYLCV resistant genotypes would serve as less effective inoculum sources when compared with the susceptible genotype. TYLCV whole genomes were sequenced from resistant and susceptible genotypes from field collected samples. Phylogenetic analyses did not distinguish the genomes collected from susceptible and resistant genotypes. In addition, in the greenhouse, one susceptible and one resistant genotype were challenged with TYLCV for ten passages. Whole genomes were sequenced after one, five, and ten passages from resistant and susceptible genotypes. After ten passages, results indicated that there were more substitutions in resistant genotype genomes than the susceptible genotype genomes. However, there was no evidence of positive selection against *Ty* genes. Results reiterate that TYLCV resistant genotypes with semi-dominant genes might not breakdown due to repeated planting, and that they will remain vital for TYLCV management.
KEYWORDS: Tomato yellow leaf curl virus; *Bemisia tabaci*; Management

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IDENTIFICATION OF TOMATO SPOTTED WILT VIRUS RESISTANT BREAKING (TSWV-RB) ISOLATES FROM PEPPER IN GREECE

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ABSTRACT:
The control of tomato spotted wilt virus (TSWV, *Orthotospovirus tomatomaculae*), one of the most destructive plant viruses, has relied on the use of resistant hybrids in tomato (*Sw-5*) and pepper (*Tsw*). Their extensive cultivation, however, has led to the emergence of resistant-breaking virus isolates (TSWV-RB) in various growing areas worldwide (1, 2). Over the last few years, most TSWV outbreaks in Greece were recorded in pepper cultivation. In the present study, four pepper isolates were characterised biologically and molecularly regarding their potential to overcome *Tsw* resistance. Indeed, an isolate, Veria 2024, derived from a resistant pepper crop in Northern Greece, broke the resistance of pepper resistant hybrids Unigold F1 and Chi7 (*Tsw*). Following mechanical inoculation with Veria 2024, apical leaves of Yolo wonder, a standard sensitive variety, and those of the resistant hybrids exhibited similar symptoms and produced comparable OD_{405} readings in ELISA when tested at 21 dpi. Subsequently, the full genome sequences of L, M and S RNAs of Veria 2024 were obtained by HTS (Illumina Novaseq 6000). The nucleotide sequence of the *NSs* gene, which encodes an RNA silencing suppressor protein responsible for breaking the *Tsw* resistance in pepper plants (3), was further confirmed via Sanger sequencing. BLASTp analysis of the predicted protein showed the highest amino acid identity (99.4%) with a non-RB isolate, p170, obtained from pepper filed samples in Italy (GenBank Acc. No. ABD92813). A multiple alignment of NSs proteins from isolates with known non-RB and RB traits revealed three candidate amino acid substitutions in the NSs of Veria 2024 TSWV isolate, which could be related to the *Tsw* resistance breaking. This is the first report of a TSWV-RB pepper isolate in Greece, which, at the same time, was unable to break the resistance of a reference tomato-resistant cultivar (Mospomor *Sw-5*). Furthermore, our current results support the absence of tomato TSWV-RB isolates in the country.
KEYWORDS: *Tsw* resistance breaking; *Capsicum annuum*; NSs protein

SUPPORT

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RESISTANCE TO WHITEFLY-TRANSMITTED VIRUSES IN *CUCURBITA PEPO* L. (SQUASH)

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ABSTRACT:
Criniviruses (Cucurbit chlorotic yellows virus (CCYV) and cucurbit yellow stunting disorder virus (CYSDV)) and the begomovirus Cucurbit leaf crumple virus (CuLCrV), all whitefly-transmitted, pose severe threats to cucurbit production, particularly in yellow squash. Given the lack of commercially resistant cultivars, identifying novel resistance sources is critical. Greenhouse screenings using viruliferous whiteflies revealed *Cucurbita* several accessions, notably PI 420328 and PI 458731 as tolerant to CCYV, displaying reduced symptom severity and lower viral titers. PI 420328 also exhibited tolerance to CYSDV, attributed to an enhanced RNA silencing response characterized by increased 21- and 22-nt viral small RNAs and distinct viral genome targeting. To overcome the limitations of whitefly-mediated resistance screening, an infectious partial tandem repeat construct of CuLCrV was developed. To address the challenges of agroinfection posed by the dense mesophyll tissue in cucurbit leaves, which restricts stomatal air spaces, an efficient agroinoculation method was established. This method, utilizing microneedle-induced leaf injuries, reliably induces systemic CuLCrV infection in squash. These findings collectively contribute to the development of sustainable disease management strategies for cucurbit crops.
KEYWORDS: Cucurbit chlorotic yellows virus; Cucurbit leaf crumple virus; Infectious clones

SUPPORT

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GRAPEVINE PINOT GRIS VIRUS IN SLOVENIA: MONITORING AND MANAGEMENT STRATEGIES

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ABSTRACT:
Grapevine Pinot gris virus (GPGV) was first identified in Italy in 2012 (1). GPGV is a member of the *Trichovirus* genus and *Betaflexiviridae* family. The virus causes chlorotic mottling, leaf deformations, puckering, low vigor, shortened internodes, stunting, inner necrosis of berries, but may also be latent. This work aimed to confirm the GPGV presence in traditional Slovenian grapevines (Cipro, Pokalca, Malvazija, Volovnik, Rebula, Poljsakica, Laski rizling, Zeleni Sauvignon, Refosk) using high-throughput sequencing (HTS) of virus-derived small RNAs. HTS data revealed that GPGV was the most prevalent virus in our study, detected in all 16 analyzed libraries with 95.3-100% genome coverage. Individual testing (RT-PCR) further confirmed the presence of GPGV in over 90% of the analyzed samples. It was found in mixed infections with several other viruses and viroids: grapevine leafroll-associated virus 1 (GLRaV-1), grapevine leafroll-associated virus 2 (GLRaV-2), grapevine leafroll-associated virus 3 (GLRaV-3), grapevine rupestris stem pitting-associated virus (GRSPaV), grapevine fanleaf virus (GFLV) and its satellite RNA (satGFLV), grapevine fleck virus (GFkV), grapevine rupestris vein feathering virus (GRVFV), grapevine satellite virus (GV-Sat), grapevine Syrah virus-1 (GSyV-1), grapevine red globe virus (GRGV), raspberry bushy dwarf virus (RBDV), hop stunt viroid (HSVd), and grapevine yellow speckle viroid 1 (GYSVd-1). A polymorphism showing C/T variation introducing a premature termination codon was observed in the movement protein (MP). Phylogenetic analysis based on partial MP and CP sequences clustered our GPGV isolates with those from neighboring countries, suggesting that infections likely spread through infected propagation material. Twenty-eight samples were randomly selected for the elimination of GPGV and viruses/viroids found in coinfection, combining *in vivo* thermotherapy (36-38 °C) with *in vitro* meristem tip (0.1-0.2 mm) micrografting onto hypocotyls of Vialla (*Vitis labrusca* x *Vitis riparia*). Using the described approach all viruses were successfully eliminated, however, viroid elimination was less effective, with success rates of 39.2% for HSVd and 42.6% for GYSVd-1.
KEYWORDS: virus-derived small RNAs; thermotherapy; micrografting

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TRANSCRIPTIONAL RESPONSE OF COMMON BEAN LINES WITH DIFFERENT MECHANISMS OF RESISTANCE TO CPMMV

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ABSTRACT:
Cowpea mild mottle virus (CPMMV) has become more prevalent in common bean (*Phaseolus vulgaris*)-producing areas in recent years, causing significant yield losses. A common bean cultivar with natural tolerance to CPMMV was developed (BRS FC420 RMD), presenting none or few symptoms of the disease[1], although the virus can still replicate in the plants. More recently, variation in resistance to CPMMV was identified in the IPR Celeiro cultivar. After four cycles of selection by mechanical inoculation of CPMMV, an individual line (10.12.1) was selected within the IPR Celeiro cultivar, showing no viral symptoms and reduced viral load. We hypothesize that the mechanisms of tolerance and resistance in these two lines are different. To test this hypothesis, a transcriptomics study was conducted by performing RNA-seq of three common bean genotypes: one susceptible (IPR Celeiro susceptible), one tolerant (BRS FC 420 RMD), and one resistant (10.12.1), mechanically inoculated with CPMMV or mock-inoculated (with buffer only), totaling six treatments, analyzed in triplicate. Total RNA samples were extracted and sequenced using the Illumina NGS platform. In total, 18 libraries were generated and sequenced to obtain comprehensive data on the transcriptional response of the lines. The analysis revealed approximately 55,000 transcripts, representing around 30,000 genes, of which approximately 1,500 were identified as differentially expressed genes (DEGs), highlighting significant variations in the genotypes' response to CPMMV. Interestingly, among the genes with significantly higher expression in CPMMV-inoculated plants (compared to mock-inoculated) of the susceptible IPR Celeiro and the tolerant BRS FC420, two were common: Phvul.011G026700, which encodes proline-rich protein 4, involved in cell wall reinforcement and stress response, suggesting its upregulation may contribute to mitigating viral damage; and Phvul.006G202700 (not annotated), in addition to CPMMV genes. A different set of genes was upregulated in the resistant line 10.12.1, including those involved in plant defense, such as the pathogenesis-related protein Bet v I from the PR-10 family, which may help restrict CPMMV replication and support an active resistance mechanism. Furthermore, CPMMV genes were not expressed in the inoculated resistant plants. These results may improve breeding strategies like marker-assisted selection and genome editing, advancing research and improving bean varieties.
KEYWORDS: Carlavirus; *Phaseolus vulgaris*; plant defense

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EVALUATION OF PEPPER HYBRIDS' SUSCEPTIBILITY AND PLANT DEFENSE-INDUCING AGENTS FOR THE CONTROL OF PEPPER VEIN YELLOWS VIRUS-6

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ABSTRACT:
Pepper vein yellows virus-6 (PeVYV-6) belongs to the genus *Polerovirus* and is persistently transmitted by aphids. Its control relies, among other methods, on the use of insecticides, which have adverse environmental impacts and lead to the development of resistance in the aphid vectors. Therefore, the implementation of more environmentally friendly preventive measures, such as the use of resistant/tolerant hybrids and agents (chemical, biological) that stimulate plant defense mechanisms, are proposed. In this study, seven commercial pepper hybrids were evaluated to determine their susceptibility to PeVYV-6. Furthermore, four biological or chemical agents: Acibenzolar-S-methyl, *Bacillus amyloliquefaciens* strain MBI600, *Trichoderma harzianum* strain T-22 and *Pseudomonas chlororaphis* Toza7 were tested in Raiko hybrid as plant defense activators. The agents were evaluated according to the following experimental design: Three applications were performed within ten days and the plants were infected with PeVYV-6 two days after the second application of the agent. To assess the susceptibility of the hybrids and the effectiveness of the activators, PeVYV-6 was transmitted using aphids (*Aphis gossypii*), followed by recording the viral titer in the plants with absolute quantification with the RT-qPCR method constructing a standard curve with seven decimal dilutions (seven points). The measurement of the viral copies in both experiments was performed 30 days post inoculation with PeVYV-6. Differences were observed among the seven pepper hybrids regarding their susceptibility to the virus, according to the quantification of the viral titer. Nevertheless, further evaluation should be conducted under field conditions. In addition, a reduction in viral copies was recorded during the applications of acibenzolar-S-methyl. Studies are in progress to develop an integrated effective virus management strategy based on the use of tolerant hybrids and plant defense activators.
KEYWORDS: PeVYV-6; pepper hybrids; plant defence activators

SUPPORT

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TOWARDS CASSAVA BROWN STREAK DISEASE RESISTANCE: GENETIC AND MOLECULAR APPROACHES TO A MECHANISTIC EXPLANATION

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ABSTRACT:
The scope of our research focuses on cassava diseases worldwide, aiming to understand disease agents, develop control strategies, and mitigate their impact. We base our work on our expanding collection of viruses and virus-like agents originating from South America, Africa, and Asia and the worldwide collaboration among cassava researchers from international and local research institutions.A key element of our virus work focuses on combating the severe Cassava Brown Streak Disease (CBSD), a critical threat to food security in Sub-Saharan Africa. We identified and characterized resistant cassava lines from South America, the center of origin of cassava, defined lines with differential virus resistance, organ-specific resistance, and immunity,y and deployed these valuable genetic resources to epicenters of the disease in Eastern and Central Africa and, to regions at risk for pre-emptive breeding.To understand the virus resistance responses of cassava, cDNA clones of CBSVs and modified virus genomes were used to infect cassava and Nicotiana benthamiana to unravel viral and host genes associated with resistance. Notably, the Ham-1 gene was identified as a pivotal player for cassava infections implicated in virus replication, movement, symptom expression, and resistance in cassava. To further understand plant resistance, we conducted Genome-Wide Association Studies (GWAS), mapping by sequencing, and small RNA analysis on resistant and susceptible plants. Weak signals from GWAS and GBS on chromosome 11 implicated NLRR genes as potential contributors to CBSV resistance. Additional signals on chromosome 3 were associated with UCBSV resistance, warranting further verification. Small RNA sequencing further identified novel miRNAs implicating lipid transfer genes as potential players in plant immunity. Preliminary proteomic data provided evidence for a lipid-binding protein as a candidate for resistance, while transcriptomic data highlighted heat shock proteins as important candidates in early stages of cassava infection. To investigate the genetic relationships between South American cassava lines and those from other regions, we constructed a phylogenetic tree, which revealed distinct clusters. Additionally, weighted gene co-expression network analysis (WGCNA) using public RNA-seq data indicated co-expression of gene modules that warrant further investigation. These findings contribute to our understanding of resistance to viruses infecting cassava
KEYWORDS: virus-resistance; CBSD; cassava

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DIFFERENTIAL ANTI-VIRAL ACTION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPRS) IN TOMATO PLANTS

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ABSTRACT:
Viruses pose a significant threat to global agriculture, leading to substantial economic losses. The use of Plant Growth-Promoting Rhizobacteria (PGPRs) is considered as an innovative and environmentally friendly approach to manage plant diseases, while an increasing number of studies highlight their potential against plant viruses. In a previous study (1), we reported the antiviral mode of action of *Bacillus amyloliquefaciens* strain MBI600 (Serifel®, BASF) in tomato plants (*Solanum lycopersicum* Belladona F1 hybrid) against tomato spotted wilt virus (TSWV). In this study, we evaluated the efficacy of (i) *Bacillus velezensis* K165 (2) and Serifel® against cucumber mosaic virus (CMV), tomato yellow leaf curl virus (TYLCV), tomato brown rugose fruit virus (ToBRFV) and potato spindle tuber viroid (PSTVd) and (ii) *Pseudomonas putida* Z13 (3) and *Paraburkholderia eburnea* EP3 (4) against CMV and TSWV. Our findings revealed differential plant-host responses to the various virus/PGPR combinations, with notable inhibition recorded against TYLCV and PSTVd infection by K165 and Serifel®. Specifically, attenuated symptoms and reduced virus titre were recorded during TYLCV infection, regardless its mode of transmission (whitefly vector, *Bemisia tabaci* MED or agro-inoculation with an infectious clone). In the case of PSTVd, infection was delayed by at least 10 days. None of the PGPRs tested had an effect on CMV onset. Our future research will focus on the identification of gene markers that could predict the outcome of viral infection in the presence of specific PGPRs. The latter will facilitate the selection of the most appropriate PGPR for field application and will contribute to the development of more effective and environmentally friendly biocontrol strategies against viral pathogens in crops.
KEYWORDS: Virus control; Plant growth-promoting rhizobacteria (PGPRs); Induced resistance

SUPPORT

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EXPLORING THE VIRUS-CONTROLLING POTENTIAL OF ROOT-PROMOTING MICROBIAL AGENTS IN SOLANACEOUS HOSTS

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ABSTRACT:
Biological control agents, particularly microbial-based products, are increasingly utilized in agriculture to manage plant viral diseases. This study evaluated the antiviral effects of a soil microbial agent, a formulation containing *Trichoderma harzianum* and *Bacillus velezensis*, on two economically important viruses: tomato yellow leaf curl virus (TYLCV) and tomato spotted wilt orthotospovirus (TSWV). Both viruses are major pathogens in solanaceous crops, including tomato and *Nicotiana benthamiana*, and are associated with substantial yield losses. To assess the virus-suppressing potential of the microbial agent, two different concentrations were applied to nutrient-free soil prior to virus inoculation. Plants were inoculated with TYLCV or TSWV and evaluated at multiple time points for viral accumulation and symptom development. Real-time PCR was used to quantify virus levels, while plant growth parameters-including plant height, leaf number, and shoot dry weight-were measured alongside symptom scoring. Microbial population changes in the soil and rhizosphere were monitored through dilution plating and qPCR analysis. The higher concentration of the microbial agent resulted in reduced viral titers and milder symptoms compared to untreated controls. These findings suggest that soil-applied microbial agents formulated for root growth promotion may also contribute to the suppression of plant viruses, supporting their potential role in integrated virus disease management strategies.
KEYWORDS: Root-promoting microbial agents; Tomato-infecting virus; *Trichoderma*

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ANTIVIRAL EFFECTS OF A STROBILURIN-BASED FUNGICIDE ON TYLCV IN TOMATO PLANTS

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ABSTRACT:
Strobilurin-based fungicides are commonly used to control fungal diseases in crops, but recent studies have suggested that they may also exhibit inhibitory effects on plant viruses. This study aimed to evaluate the virus-suppressing potential of commercially available strobilurin-containing fungicides in Korea against tomato yellow leaf curl virus (TYLCV). Since no formulation containing strobilurin alone is currently marketed by Korean companies, we selected a mixed fungicide formulation composed of fluxapyroxad (a carboxamide fungicide) and pyraclostrobin (a strobilurin fungicide). The fungicide was applied once to the soil at the time of seedling transplantation. TYLCV was introduced into tomato and *Nicotiana benthamiana* plants using agroinfiltration. The experiment consisted of three biological replicates with five plants per treatment group in each replicate. Leaf samples were collected from the uppermost fully expanded leaves at 12 different time points between 2 and 30 days post-inoculation (dpi). Viral accumulation was quantified by real-time PCR using EF1α as the reference gene. Compared with the untreated control group, plants treated with the fungicide formulation showed consistently lower levels of TYLCV accumulation, suggesting that the tested strobilurin-based fungicide may contribute to the suppression of virus replication. These findings provide preliminary evidence that strobilurin-containing fungicides, while developed for fungal control, may hold potential as part of an integrated strategy for managing viral diseases in tomato crops.
KEYWORDS: TYLCV; Strobilurin; Fungicide

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ADVANCING SUSTAINABLE VIRUS DISEASE MANAGEMENT IN SUB-SAHARAN AFRICA: INSIGHTS FROM YAM MOSAIC VIRUS CONTROL

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ABSTRACT:
Successful virus disease management in sub-Saharan Africa (SSA) has largely relied on resistant crop varieties, as demonstrated by the control of cassava mosaic begomoviruses in cassava and maize streak mastrevirus in maize. However, managing many other viral diseases remains challenging due to limited grower awareness, insufficient research funding, and inadequate scaling of solutions. This presentation focuses a decade long effort to device effective management for the control of yam mosaic virus (YMV, genus *Potyvirus*), a major threat to yam (*Dioscorea* spp.) production in West Africa. In the absence of YMV-resistant varieties, YMV management efforts rely heavily on clean seed systems to mitigate yield losses. However, rapid YMV reinfection of clean yam seedlings in the field undermines the effectiveness of the clean seed-system based approach. Here, we report on how understanding YMV epidemiology and the introduction of rapid clean seed propagation technologies promise to prevent yield losses due to YMV. We also discuss reinfection rates, YMV-induced seed degeneration, and integrated approaches to reducing field-level infections. Lastly, we present key lessons for developing sustainable virus disease management strategies in SSA.
KEYWORDS: disease management; epidemiology; reinfection control

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IN PLANTA SYSTEM-MEDIATED DOUBLE-STRANDED RNA DELIVERY TARGETING MELANIZATION GENES CAUSES DEVELOPMENTAL DELAY AND MORTALITY IN *DIAPHORINA CITRI*

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ABSTRACT:
Currently, *Diaphorina citri* is responsible for the transmission of phytopathogenic bacteria of the genus *Candidatus* Liberibacter, associated with one of the most devastating diseases of citrus, Huanglongbing (HLB). As an alternative form of HLB management, gene silencing by interference RNA (RNAi) is one of the biotechnological promises used to control pests from specific target genes. In this study, we focused on knockdown genes related to cuticle melanization of *D. citri*, lac2 and tyr3-monooxygenase. Firstly, we evaluate the expression level of lac2 and tyr3-monooxygenase in life stages of *D. citri*. Secondly, we delivered the dsRNAs in plant (*Murraya paniculata*) system to *D. citri* and we evaluated the knockdown of target genes at 24, 48 and 96 hours after ingestion of dsRNA. Moreover, we analyzed the viability and development of nymphs, and daily the mortality from 24 to 144 hours. The results showed the knockdown for both genes over time, the nymphs viability was reduced, the nymphs development was delayed and the increase of the mortality of *D. citri* over time. Thus, RNAi is an important tool for the technological advances to control *D. citri*, and consequently, the HLB disease in citrus.
KEYWORDS: RNA interference; Asian citrus psyllid; citrus

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A HIGH-RESOLUTION REMOTE SENSING BANANA CROP MAPPING TOOL FOR TARGETED SURVEILLANCE OF BANANA BUNCHY TOP VIRUS IN SUB-SAHARAN AFRICA

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ABSTRACT:
Bananas and plantain (*Musa* spp.), widely cultivated as mixed crops by smallholder farmers across sub-Saharan Africa (SSA), are increasingly threatened by the invasive banana bunchy top virus (BBTV, genus *Babuvirus*). Since BBTV was introduced in the Benin Republic in 2010, it has continued to spread, reaching Nigeria in 2011 and Togo in 2018. Surveillance for BBTV is challenging due to the small, fragmented nature of banana production, making traditional field surveys time-consuming and costly. To address this, we have developed remote sensing-based Artificial Intelligence (AI) tools to identify banana fields for targeted BBTV surveillance. Using synthetic aperture radar (SAR) data from the Copernicus Sentinel-1 satellite of the European Space Agency (ESA), we applied AI algorithms-including random forest (RF) and convolutional neural networks (CNN)-to map banana-growing areas in Nigeria, Benin, Togo, and Ghana. Our models were trained and validated using Unmanned Aerial Vehicle (UAV) RGB images and GoPro action camera photos. The results demonstrated over 85% accuracy in predicting banana fields and supported effective BBTV detection, mapping, and containment strategies across SSA.
KEYWORDS: BBTV surveillance; machine learning; Remote sensing

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UNRAVELLING THE ROLE OF GRAFTING IN TOMATO PLANTS UPON VIROID INFECTION

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ABSTRACT:

Tomato is one of the most important solanaceous crops due to its economic and nutritional relevance. In addition to their organoleptic properties, local tomato ecotypes serve as a valuable source of resistance/tolerance to pests. Indeed, tomato hosts a wide range of plant pathogens, including potato spindle tuber viroid (PSTVd; family *Pospiviroidae*), that elicits stunting and leaf curling symptoms. Grafting is an agricultural method employed as a sustainable approach to manage both biotic and abiotic stresses. Besides to boosting the performance of various horticultural crops, grafting has the potential to increase tomato plants' tolerance to viral infections. In the frame of the project DiVInGraft, we are investigating the effect of grafting in response to viroid infection in tomato plants. To this end, we used high-throughput imaging phenotyping to study the responses of grafted and non-grafted tomato plants to PSTVd infection. Two tomato varieties, Manduria (Ma) and UC82 (UC), tolerant and susceptible to viral infections, respectively, were tested. Non-grafted, self-grafted UC (UC/UC) and UC grafted onto Ma (UC/Ma) were mechanically inoculated with PSTVd or mock-inoculated. Quantitative data of morphological parameters were acquired at 9 time points up to 36 days post-inoculation (dpi). PCA analysis showed that grafting has a global effect on PSTVd infection, with a slightly positive effect of UC/Ma respect self-grafted tomato plants. Simultaneously, using the same experimental design, a parallel set of plants was prepared to extract RNA from leaf tissue at 15 dpi to be submitted for high-throughput sequencing (HTS) of small RNAs and messenger RNAs. The transcriptomic responses upon PSTVd infection, of the two tomato varieties and of the grafted and non-grafted plants were compared.Finally, the susceptibility of three Campanian tomato ecotypes to PSTVd infection was evaluated. Symptom observation and quantification of viroid accumulation showed that all tested ecotypes were susceptible to PSTVd. Specifically, San Marzano showed mild symptoms and reduced accumulation of PSTVd, unveiling a certain level of tolerance. In contrast, the ecotype Giallo determinato da serbo was particularly susceptible to PSTVd. These preliminary results demonstrate that phenotyping is a promising tool to evaluate tomato responses upon viroid infections and grafting. These responses may be further explored at molecular level by HTS data.

KEYWORDS: potato spindle tuber viroid; tolerance; tomato

SUPPORT

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YELLOW MOSAIC IN GRAPEVINE: AN OVERVIEW OF THE MOLECULAR AND CYTOLOGICAL CHANGES ELICITED DURING THE SYNDROME THROUGH RNA-SEQ AND ELECTRON MICROSCOPY INVESTIGATIONS

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ABSTRACT:
Yellow mosaic (YM) in grapevine is associated to systemic infection of grapevine fanleaf virus (GFLV; species *Nepovirus foliumflabelli*). The syndrome is characterized by a persistent yellow discoloration of the leaves that occurs during spring and summer on multiple branches of the vine, while others may remain normally pigmented. Such yellowing reverts to a normal green by late summer, and it could be accompanied by typical leaf deformities and degeneration caused by GFLV. Although various grapevine cultivars consistently exhibit the same phenotype over the years, serological and molecular techniques are unable to differentiate between different GFLV isolates or variants. In this context, three YM-affected vines were selected in Apulia vineyards (Italy) and sampled in both green and yellow leaf tissues. Total RNAs were submitted for sequencing (mRNA and smallRNA). Quality-checked reads were assembled with rnaSPAdes, then annotated using BLAST. The virome analysis detected the presence of viral agents commonly affecting grapevine, without showing a discrimination pattern between yellow and green leaf tissues. GFLV showed a higher genome coverage than the other viruses, with the isolate sequence correlated with the source plant accession, not with the symptom. In addition, RT-qPCR of GFLV demonstrated that its titer was similar in both leaf tissues. More than 3500 statistically significant differentially expressed genes (DEGs) were identified, with about 1400 genes overexpressed in the yellow leaf tissues, and over than 2100 underexpressed in the same tissues. GO enrichment of the related DEGs revealed a higher transcriptional activity, ethylene-activated signaling pathways and chloroplast/photosynthesis-related proteins in green tissues, whilst yellow leaf tissues were characterized by an increased metabolism of carbohydrates. About 30 DEGs were selected for validation by RT-qPCR. Genes showing the highest fold-change values in both leaf tissues were also used in a monthly analysis, from May to September, conducted on the same vines. Differentially expressed miRNAs were also consistently validated. Finally, a noteworthy result was the chloroplast analysis in EM. In YM leaf tissues, chloroplasts showed a strong alteration of the thylakoids' membrane organization. These alterations range from a complete lack of thylakoids to vesiculations of the thylakoid's membrane, compatible with their evolution into gerontoplasts.
KEYWORDS: yellow mosaic; grapevine; grapevine fanleaf virus

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MONITORING AND TACKLING MAIZE LETHAL NECROSIS (MLN) IN EASTERN AND SOUTHERN AFRICA FROM 2014 TO 2024

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ABSTRACT:
Maize (*Zea mays* L.) is a critical staple crop and economic resource in sub-Saharan Africa (SSA), cultivated on over 35 million hectares and yielding more than 70 million metric tons (MMT) annually, primarily within smallholder farming systems. Maize Lethal Necrosis (MLN), first reported in Kenya in 2011, has since emerged as a major threat to maize production in eastern Africa. MLN results from the synergistic interaction between Maize Chlorotic Mottle Virus (MCMV) and Sugarcane Mosaic Virus (SCMV), both prevalent in the region. Most commercial maize hybrids are highly susceptible to MLN, which spreads rapidly through insect vectors and contaminated seeds, threatening food security and livelihoods.Over the past decade, CIMMYT, in collaboration with global and national partners, has implemented effective plant health strategies to combat MLN. Surveillance data from 2013-2023 indicate that MCMV incidence reached nearly 20% in eastern Africa in 2023, raising concerns over its continued spread. More than 18,735 surveillance data points have been collected and published on the MLN web portal, covering Kenya, Ethiopia, Uganda, Rwanda, Tanzania, Malawi, Zambia, and Zimbabwe. Rwanda contributed the highest number of entries (6,733), while 6,904 and 11,831 samples were recorded from southern and eastern Africa, respectively. A robust network of collaborators has been trained in MLN diagnostics using immunostrip and ELISA-based assays, facilitating cost-effective, rapid, and reliable disease detection in field conditions. The collected data was systematically recorded using ODK tools and uploaded to the MLN web portal, ensuring accessibility and transparency for stakeholders. This surveillance initiative demonstrates the effectiveness of leveraging digital tools, standardized diagnostic protocols, and strong multi-institutional partnerships in monitoring and managing viral maize diseases. By strengthening disease surveillance and response strategies, these efforts contribute significantly to sustaining maize production and improving food security in SSA.
KEYWORDS: Maize; Surveillance; MLN

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Session 5: VIRUS ECOLOGY AND EVOLUTION

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DIVERSITY AND SPREAD OF CASSAVA BROWN STREAK VIRUSES, CBSV AND UCBSV, IN EASTERN AND CENTRAL AFRICA

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ABSTRACT:
In Africa, two ipomoviruses, the cassava brown streak virus (CBSV) and the Ugandan cassava brown streak virus (UCBSV) cause the severe cassava brown streak disease (CBSD). This disease has a devastating impact on cassava production often leading to the destruction of the edible tuberous roots. Understanding the diversity and composition of virus populations is crucial for the effective deployment of resistant cassava varieties, particularly when resistance is strain-specific.To this end, we monitored disease outbreaks, analyzed the genetic diversity of virus isolates, and investigated plant responses. Cassava ipomoviruses are unique in possessing a Ham1 gene upstream of the coat protein that functions as a pathogenicity factor. The P1 gene plays a key role in suppressing the plant's RNA-silencing defense mechanism and this gene is most diverse among the virus isolates, separating a further CBSV lineage from the predominant CBSV clade. UCBSV isolates exhibit further genetic diversification, with isolates from Zambia and Malawi clustering separately from those in Kenya and Rwanda.We reconstructed the spatial and temporal evolution of virus genomes to examine their spread patterns. The southernmost extent of CBSD virus expansion is currently in Zambia and Mozambique, while westward spread has been detected in the central region of the Democratic Republic of Congo, particularly in North and South Kivu. Although CBSV and UCBSV often co-occur, there are regions, including Zambia, Malawi, and North Kivu, where only UCBSV is currently established. This presents an opportunity for deploying UCBSV-resistant cassava varieties. Our analysis indicates that the spread of U/CBSV into Zambia, Malawi, and eastern Congo is a result of recurrent introductions of diverse virus strains from Tanzania, Uganda, and Rwanda. While Bemisia tabaci whiteflies contribute to virus transmission, human-mediated movement of infected planting materials plays a more significant role in disease dissemination at both field and regional levels. This may explain why UCBSV is more widespread than CBSV, as infected planting materials used for vegetative propagation often appear asymptomatic. To mitigate the impact of CBSD, effective management strategies should focus on the development of virus-resistant cassava varieties, the provision of virus-free planting material, and the implementation of phytosanitary measures to prevent further spread into new regions.
KEYWORDS: virus distribution; cassava brown streak disease; genome diversity

SUPPORT

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EXPLORING REPLICATION INITIATOR PROTEIN DYNAMICS AND EVOLUTIONARY PATTERNS IN SSDNA VIRUSES OF THE PHYLUM CRESSDNAVIRICOTA

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ABSTRACT:
The phylum Cressdnaviricota encompasses a wide array of circular single-stranded DNA (ssDNA) viruses infecting plants, animals, and protists, many of which display striking genomic and ecological diversity [1]. Despite this variability, a defining feature across the phylum is the replication initiator protein (Rep), a multifunctional enzyme essential for rolling-circle replication and a key molecular marker in phylogenetic and taxonomic studies [2]. Emerging evidence from comparative genomics and metagenomic analyses suggests that the Rep gene exhibits considerable sequence variation and may undergo recombination or exchange across taxonomically distinct viral lineages [3]. Such genetic plasticity raises intriguing questions about the evolutionary dynamics of Rep and its role in the diversification and speciation of ssDNA viruses. This study aims to investigate the potential for natural reassortment of the Rep gene within Cressdnaviricota by examining conserved sequence motifs, domain architectures, and phylogenetic relationships. Through these analyses, we seek to identify signatures of inter-lineage gene exchange and evaluate their impact on genome structure and evolutionary divergence. While the investigation is currently in an exploratory phase, the findings are expected to provide important insights into the role of replication-associated proteins as potential drivers of adaptive evolution and lineage emergence in ssDNA viruses.
KEYWORDS: Cressdnaviricota; Replication initiator protein (Rep); Viral evolution

SUPPORT

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ECOLOGY AND TRANSMISSION OF CITRUS YELLOW VEIN CLEARING VIRUS (CYVCV) IN CALIFORNIA

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ABSTRACT:
Citrus yellow vein clearing virus (CYVCV) is an emerging citrus pathogen in California, with the potential to significantly impact the citrus industry. CYVCV is a single-stranded, positive-sense RNA virus in the subgenus *Mandarivirus* (family *Alphaflexiviridae*) and has been reported in multiple regions worldwide. This virus has a broad host range, including herbaceous crops, weeds, and citrus, and exhibits a complex epidemiology involving multiple hosts and insect vectors. This virus is suspected to be mechanically transmitted, insect-vectored, and spread through grafting of infected budwood, though its precise transmission pathways remain uncertain. To date, CYVCV infection has been confirmed in a few citrus trees from different counties in California, exhibiting vein clearing and chlorosis. To better understand CYVCV ecology and vector specificity, we conducted extensive sampling across Southern California, targeting citrus, weeds, and potential vectors such as whiteflies and aphids. We also tested mechanical transmission from infected to healthy plants. Under laboratory conditions, we successfully inoculated lemon plants, which developed symptoms within two months. Additionally, both aphids and whiteflies in certain locations tested positive for CYVCV, suggesting their potential role in virus transmission. We have also amplified and sequenced the whole genome of this virus isolated from these insects. Together, this study will provide crucial insights into CYVCV's ecology and transmission dynamics.
KEYWORDS: Citrus; Mandarivirus; emerging pathogen

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EVALUATION OF THE PATHOGENICITY OF MAIZE YELLOW MOSAIC VIRUS (MAYMV) ISOLATES IN MAIZE

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ABSTRACT:
Maize yellow mosaic virus (MaYMV; *Polerovirus MaYMV*, *Solemoviridae*) is a newly reported virus in maize (*Zea mays* L.), first identified in China and later detected in other countries, including Brazil. It is transmitted by the corn leaf aphid *Rhopalosiphum maidis* and other aphids. Despite MaYMV being a potentially emerging virus, its symptomatology and the extent of maize damage have not yet been fully investigated. Here, we assessed the pathogenicity of seven MaYMV isolates from São Paulo (SP) and Minas Gerais (MG) states, Brazil, based on infection rates via *R. maidis* transmission, symptom severity on leaves, and plant height (hybrid LP-2020). Aviruliferous aphids were confined on RT-PCR-MaYMV-positive maize plants for an acquisition access period (AAP) of 48 h, and then transferred to healthy maize seedlings (V2 stadium, 10 aphids/test plant) for an inoculation access period (IAP) of 96 h. Control plants were either insect-free or exposed to non-viruliferous aphids (mock). The experiment was repeated two to three times per isolate, with inoculation of 4-15 test plants per isolate in each replicate. Overall, the rate of MaYMV infection confirmed by RT-PCR was 21%. The infection rate was low for all isolates: <20% for isolates named SP-CP1, SP-CP2, and SP-M2, 20-25% for the isolates SP-M1, SP-CB, and MG-U, and 30% for the isolate SP-P. Among the infected plants, mild symptoms were observed in 42% of them, characterized by small chlorotic spots on the upper third of younger leaves, mainly at 15 days post inoculation (dpi). The rate of symptomatic plants was higher in plants infected by SP-M2 (75%) and SP-P (60%), and null for MG-U. There was a reduction in plant height of up to 23% (SP-M1), 13% (SP-P), and 11% (MG-U) compared to the mock. When comparing the height of infected plants among isolates, a difference was observed only at 15 dpi, with SP-M1 and SP-M2 differing statistically from SP-CB but not from the other isolates. In a complementary experiment with a different hybrid (BY-2023), we evaluated the impact of infection by the SP-P isolate on yield components. The rate of infection in two experimental replicates was 44%, with most plants being asymptomatic or showing mild symptoms, and no significant reduction in plant height or grain yield was observed compared to the mock treatment. Our results suggest that symptom severity varies among MaYMV isolates from null to mild, with no detectable effects on grain yield for the genotype and isolate studied.
KEYWORDS: *Polerovirus*; Corn leaf aphid; Infection rate

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GLOBAL SPATIO-TEMPORAL DYNAMICS OF YAM MILD MOSAIC VIRUS INTO BRAZIL: EVIDENCE OF DISTINCT, HOST-RELATED DISPERSION ROUTES

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ABSTRACT:

Plant virus epidemics pose a major threat to global food security. Yams (genus *Dioscorea*) are a tuber crop of significant importance, particularly in tropical regions, where they serve as staple food and play a crucial role for nutritional assurance and local economic returns. Virus infections can lead to significant yield losses in yam. Among them, occurrence of yam mild mosaic virus (YMMV; genus Potyvirus) has been reported over a broad geographic range including Africa, Asia, Oceania, the Caribbean, and South America. In this study, we have conducted genetic and phylogeographic analyses to infer YMMV global evolution and dissemination history, with close attention to the dynamics of YMMV into Brazil. Bayesian analysis of the whole set of YMMV coat protein (CP) sequences available in NCBI GenBank, plus a newly-assembled sequence from Paraíba State (Brazil), placed the Brazilian YMMV sequences within a new phylogroup, supporting distinct evolution of YMMV from the South-American continent. Evolutionary relationships among YMMVs were explored using BEAST and Nextstrain analyses, providing consistent results and estimation of the appearance of a most recent common ancestor presumably between 743-550 years ago in China. Of interest, gene flow and genetic diversification analyses supported the findings obtained with BEAST and Nextstrain, and identified distinct transmission routes for YMMV in *D. alata* compared to *D. trifida*. Specifically, our investigations support that YMMV found in *D. alata* likely originated directly from China, while YMMV from *D. trifida* followed a route through China, Africa, French Guiana, and eventually to Brazil. Our study further reinforces the value of sequence data of plant viruses to investigate their global spread patterns and infer spatio-temporal dynamics of plant virus introductions and dissemination.

KEYWORDS: phylogeography; introductions; dissemination

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DIFFERENTIAL ACCUMULATION OF CITRUS LEPROSIS VIRUS C AMONG LINEAGES, CITRUS VARIETIES, AND TISSUES: EPIDEMIOLOGICAL IMPLICATIONS ON DISEASE.

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ABSTRACT:
Citrus leprosis (CL) is the most significant viral disease affecting the Brazilian citriculture, primarily caused by the citrus leprosis virus C (CiLV-C, *Cilevirus leprosis*). CiLV-C has two positive single-stranded RNA molecules with short bacilliform particles, is persistently transmitted by *Brevipalpus yothersi*, and causes chlorotic or necrotic lesions in sweet orange (*Citrus* × *sinensis*) fruits, stems, and leaves. Although three lineages of CiLV-C have been identified either in herbarium samples or the field, only SJP and CRD occur in the Brazilian Citrus Belt (BCB). The SJP lineage, first detected in São José do Rio Preto (SP) in 2016, predominates within the BCB but is absent in major citrus-producing regions outside this area. Conversely, the CRD lineage is widely distributed across Latin America but occurs at lower frequencies in the BCB, where it is primarily found in mixed infections with SJP. To elucidate the molecular epidemiology of CiLV-C lineages and their host-pathogen interactions, we developed a lineage-specific RT-qPCR assay to quantify the viral load of CiLV-C. This method enabled the assessment of lineage-specific viral accumulation across diverse sweet orange tissues, the accumulation of the SJP lineage among scion varieties, and the impact of contrasting edaphoclimatic conditions in the States of São Paulo and Bahia on CRD lineage prevalence. The RT-qPCR assay was performed on 194 fruit samples (121 infected by the SJP and 73 by the CRD lineages) and 85 independent CL leaves, of which the SJP infected 62 and 23 by the CRD lineages. We found that CiLV-C_SJP viral loads were up to four times higher than those of CiLV-C_CRD. Furthermore, regardless of the lineage, viral concentrations in fruits were three times higher than in leaves. CiLV-C_SJP showed the highest accumulation in the fruits of 'Natal' and the lowest in 'Valência Americana' varieties. These findings provide insights into the potential adaptive advantages of the SJP lineage and its epidemiological impact, with implications for genetic improvement programs and CL management strategies.
KEYWORDS: *Cilevirus*; *Kitaviridae*; *Brevipalpus*-transmitted viruses

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EXPERIMENTAL HOST RANGE AND PATHOGENICITY OF A POTENTIALLY EMERGING MASTREVIRUS IN MAIZE TRANSMITTED BY THE CORN LEAFHOPPER

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ABSTRACT:
Maize striate mosaic virus (MSMV) is a new mastrevirus (*Mastrevirus*) first reported in maize (*Zea mays*) transmitted by the corn leafhopper *Dalbulus maidis*, and later was detected in sugarcane (*Saccharum officinarum*). MSMV symptoms in maize include mottling with mild chlorotic streaks, but the extent of its damage and host range remains unstudied. Here, we assessed the susceptibility of 16 plant species to MSMV by evaluating symptom expression and detecting the virus via PCR after inoculating 10 to 30 test plants per species using viruliferous *D. maidis* (20 leafhoppers per plant) during a 2-day inoculation access period (IAP). Plants exposed to non-viruliferous leafhoppers served as negative controls. Maize was included in all experiments as a positive control for virus detection and experimental conditions. In a separate experiment, we evaluated MSMV pathogenicity in maize test plants of two experimental hybrids. Plants were inoculated by confining groups of approximately 20 viruliferous leafhoppers for a 3-day IAP. After inoculation, plants were sprayed with insecticides and grown in pots in a vector-free greenhouse to assess symptoms, phenotypic traits, flowering, and yield components. MSMV was detected by PCR in four inoculated grass species: *Urochloa brizantha* [brachiaria grass, cv. Marandu (70.0%) and cv. Xaraés (25.0%)], *Andropogon gayanus* [gamba grass (33.3%)], *S. officinarum* [cv. CTC-9001 (7.7%) and cv. CTC-9005 (21.4%)], and *Pennisetum glaucum* [pearl millet (13.3%)]. Only *S. officinarum* (cv. CTC-9005) exhibited symptoms, characterized by mild chlorotic streaks. In maize, the virus was detected in 51.8% of tested plants (n = 164), of which 46% showed symptoms characteristic of the virus and 54% were asymptomatic. In the pathogenicity assessment, infection type (symptomatic vs. asymptomatic) significantly influenced damage to various phenotypic traits and yield parameters. Regardless of hybrid (LP-2019 or BY-2023), asymptomatic infection did not impact plant development but significantly reduced grain yield in LP-2019. In plants with symptomatic infection in both hybrids, severe damage was observed across all evaluated parameters, including severe dwarfism, stunted roots, and delayed flowering, with grain yield losses ranging from 93% to 96%. This study is the first to report MSMV host range and its impact on maize, highlighting the need for further research to understand the role of other grasses as virus reservoirs for maize and other crops.
KEYWORDS: maize striate mosaic virus; *Dalbulus maidis*; Damage

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GENETIC CHARACTERISTICS OF TOMATO BROWN RUGOSE FRUIT VIRUS (TOBRFV) ISOLATES IN SOME COUNTRIES

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ABSTRACT:
The tomato brown rugose fruit virus (*Tobamovirus fructirugosum*, ToBRFV), belonging to the genus Tobamovirus, is a quarantine pathogen regulated by the Eurasian Economic Union, the European and Mediterranean Plant Protection Organization, and numerous countries worldwide. Tobamovirus virions are highly stable and can survive outside a host on inert and biological surfaces, as well as in nutrient solutions, water, and soil for months without losing virulence. Identifying the sources of phytopathogenic virus infections is a critical step in limiting their spread. This can be achieved by comparing the nucleotide sequences of detected viral isolates with isolates of diverse geographical origins. The study objectives were ToBRFV isolates identified in tomato plants from the Russian Federation, the Republic of Kazakhstan, and the Republic of Uzbekistan. The resulting amplicons were sequenced using a modified Sanger method. For phylogenetic analysis, the identified isolates were compared with several ToBRFV isolates deposited in the NCBI GenBank. The tomato mosaic tobamovirus (MW197140, Iran) was included as a phylogenetic outgroup. Sequencing of amplification products yielded sequences for 15 ToBRFV isolates from the Russian Federation, 10 isolates from Kazakhstan, and 5 isolates from Uzbekistan. Sequence analysis confirmed that all isolates belonged to *Tobamovirus fructirugosum*. Russian Federation: 12 ToBRFV isolates showed high genetic similarity, while the remaining 3 differed significantly. Kazakhstan: All isolates were genetically closely related to each other and to reference isolates from the Netherlands. Uzbekistan: Six ToBRFV isolates exhibited 99.27-100% nucleotide identity with reference isolates. A 100% identity was observed in the 126 kDa replicase gene fragment with an Italian isolate. Phylogenetic analysis revealed: 1. Three isolates from Russia and two from Kazakhstan formed a cluster with isolates from Israel and Jordan. 2. Four isolates from Kazakhstan and one from Uzbekistan grouped with isolates from Turkey and China. 3. Russian isolates formed two distinct clusters. The study demonstrates high genetic heterogeneity among ToBRFV isolates circulating in the Russian Federation, suggesting multiple independent introductions of the virus. Isolates from Kazakhstan showed close genetic affinity to Dutch isolates, while one Uzbek isolate was highly identical to an Italian strain.
KEYWORDS: molecular diagnostics; sequencing; phylogenetic analysis

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FIRST DETECTION OF HIGH PLAINS WHEAT MOSAIC EMARAVIRUS (HPWMOV) IN THE RUSSIAN FEDERATION

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ABSTRACT:
Wheat is the most important crop in the Russian Federation and is grown on more than 28.5 million hectares annually. One of the most harmful virus of wheat is high plains wheat mosaic emaravirus, HPWMoV (species *Emaravirus tritici*), which belongs to the genus *Emaravirus* of the family *Fimoviridae*. In April-May 2024, we conducted surveys of winter wheat crops in 8 regions of the Russian Federation. Samples with symptoms of virus-like pathologies were taken from the crops and tested by DAS-ELISA for the presence of a complex of viruses infecting wheat plants, including HPWMoV. This virus was detected in 3 regions of the Russian Federation in 3.8% of the 237 samples tested. The presence of HPWMoV in seropositive samples was confirmed by RT-PCR tests with primers WmoV-F2/WmoV-R2 (1), WMoV-F/WMoV-RR (1), H1/H4 (2), HPV-F/HPV-R (3), designed to RNA-2, RNA-3 and RNA-7 of this virus, and with primers HPWMoV-RNA7-pF9/HPWMoV-RNA7-pR9 and HPWMoV-RNA8-pF5/HPWMoV-RNA8-pR5 designed by the authors of this article. HPWMoV was predominantly found in monoinfection, but individual samples with coinfection of this virus with wheat streak mosaic virus, WSMV (species *Tritimovirus tritici*), wheat spindle streak mosaic virus, WSSMV (*Bymovirus tritici*) and Barley yellow dwarf virus PAV, BYDV-PAV (*Luteovirus pavhordei*) were detected. We found that based on the nucleotide sequence at the RNA-7 site of 513-522 nucleotides in length, the identified HPWMoV isolates were most highly identical (88.1-88.5%) to isolate K1 (KT988892, USA) and slightly less identical (87.6-88.2%) to isolates W1 (KT970504, USA), 20RH2 (OR900952, USA) and HPVWMoV NW2 (MN250351, USA). This represents the first detection of HPWMoV in the Russian Federation and only the second detection in Europe after Ukraine (4).
KEYWORDS: High Plains wheat mosaic emaravirus; PCR; DAS-ELISA

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TRACING THE EVOLUTIONARY ORIGINS OF BAMBOO MOSAIC VIRUS: CHLOROPLAST-DEPENDENT REPLICATION ACROSS PLANT LINEAGES

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ABSTRACT:
Bamboo mosaic virus (BaMV) is a positive-sense single-stranded RNA virus in the *Alphaflexiviridae* family, genus *Potexvirus*. While BaMV is known to infect bamboo species, it also successfully infects *Nicotiana benthamiana*, a commonly used experimental host. To better understand its infection strategy, we investigate the involvement of host factors in viral entry and replication. Upon cell entry, BaMV RNA associates with chloroplast phosphoglycerate kinase (PGK), which binds the 3′ untranslated region (UTR) and facilitates chloroplast targeting. Within chloroplasts, BaMV exploits host enzymes—including carbonic anhydrase (CA), ferredoxin- $NADP^+$ reductase (FNR), and the oxygen-evolving complex protein NbPsbO1—to promote viral genome replication and subgenomic RNA synthesis. Given BaMV's dependence on chloroplast-associated processes and its ability to infects both monocot (e.g., bamboo, barley), and dicot (*N. benthamiana*) hosts, we hypothesized that any chloroplasts-containing plant could support BaMV replication. To test this, we examined evolutionarily basal plants, including liverwort *Marchantia polymorpha* (a bryophyte) and a green alga from *Chlorophyta.* Both species supported BaMV replication, suggesting that the virus's evolutionary origins may trace back to early chloroplast-bearing eukaryotes. These findings provide evidence that BaMV may have co-evolved with photosynthetic lineages from ancestral algae to modern flowing plants, offering novel insights into the adaptation, and cross-species transmission of RNA viruses in the plant kingdom.

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'*CANDIDATUS* PHYTOPLASMA SOLANI' GRAPEVINE-VECTOR-WEED PATHOSYSTEM COMPLEXITY INVESTIGATIONS IN CROATIA

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ABSTRACT:
'*Candidatus* Phytoplasma solani' (CPs) is an etiological agent of the grapevine 'bois noir' (BN) disease as well as similar diseases affecting many other cultivated plants and causing significant economic losses. CPs is endemic in the Euro-Mediterranean zone, but can be sporadically detected worldwide. It circulates in different pathosystem consisting of cultivated plants, polyphagous cixiid planthopper vectors and reservoir plants, often weeds, in nature. Involvement of different CPs strains in BN disease and their different epidemiological cycles can be revealed through multilocus sequence typing (MLST). Genes *tuf*, encoding the translation elongation factor Tu, *secY*, coding for a translocation protein, and two for variable surface proteins *vmp1* and *stamp* are frequently used to asses BN genetic diversity and thus better inform the BN disease control strategies. In this study, CPs genetic diversity research in over a decade is presented. Two new *stamp* (ST59) and *vmp1* (V28) CPs genotypes were revealed in the grapevine and totally 28 different MLST genotypes with the prevalence of CPsSqt21 (S6/ST6/V-18/tuf-b2) type linked to *Hyalesthes obsoletus* vector and *Urtica dioica* reservoir plant. Other two important genotypes for the grapevine are CPsSqt28 (S39/ST46/V3/tuf-a) and CPsSqtT2 (S1/ST9/V4/tuf-b1) associated respectively with *U. dioica* and *Convolvulus arvensis*. *Cixius wagneri* vector harbors yet different *vmp1* CPs genotypes all over the county. CPs was also detected in *Dicthyophara europaea* insect and two new potential reservoirs (*Ailanthus altissima*, *Robinia pseudoacacia*). With such high level of CPs genetic variability, it is evident that several independent cycles have to be considered regarding the BN epidemiology in Croatia, a country with unique geographical characteristics covering different eco-climatic zones within south-east Europe.
KEYWORDS: bois noir; MLST; molecular variability

SUPPORT

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RNAI-MEDIATED SILENCING OF ESSENTIAL GENES IN THE SPITTLEBUG PHILAENUS SPUMARIUS, VECTOR OF XYLELLA FASTIDIOSA, INCREASES INSECT MORTALITY AND REDUCES BACTERIAL LOAD

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ABSTRACT:

RNA interference (RNAi) is a post-transcriptional gene silencing mechanism that can be triggered by administering double-stranded RNA (dsRNA) molecules to a target organism. Exogenous application of dsRNAs to crops has emerged as a powerful tool in agriculture to control insect pests. We aimed at exploring RNAi mechanisms/effects on the spittlebug *Philaenus spumarius*, the main vector of *Xylella fastidiosa* (Xf) ST53 to olive in the Apulia Region of Italy. An extensive bibliographic search to select possible target genes causing lethality and/or sterility effects was done. Among the seventeen genes identified, six sequences of potential target genes involved in glucose metabolism, protein homeostasis maintenance and ions channels transports were retrieved, corresponding dsRNAs were produced and delivered to the insects either by microinjection or feeding on plant petiole dipped in a dsRNA solution. The existence of an active RNAi mechanism in *P. spumarius* was confirmed by in-silico identification of the main genes involved in RNAi core machinery, downregulation of the two pathway genes and by the presence of specific small RNAs. Two targets, once silenced, determined a significant increase in mortality rate. Finally, to investigate if transmission competence of *P. spumarius* adults was affected by gene silencing, dsRNA molecules targeting two genes, whose silencing caused different levels of mortality, were separately delivered to *P. spumarius* adults in an experiment based on the following steps: dsRNA microinjection, Xf pauca ST53 acquisition by feeding on infected plants (AAP), two inoculation periods (IAP1, IAP2) by feeding Xf-exposed insects on healthy periwinkles. RNAi did not impact directly the acquisition, neither inoculation rates, that were rather correlated with differences in spittlebugs infectivity (number of Xf-positive insects per recipient plant). However, the bacterial load within the foregut of treated insects was up to eight times lower than in dsGFP controls. In conclusion, several lines of evidence confirmed the existence of an active RNAi mechanism in *P. spumarius*, that can be exploited to increase insect mortality and possibly decrease Xf transmission efficiency. Although the delivery of dsRNA to sap-sucking insects is still very challenging, the perspective of coupling mortality and transmission interference effects is intriguing for future RNAi applications to control the vector as well as *X. fastidiosa* spread.

KEYWORDS: RNA interference; spittlebug; *Xylella fastidiosa* vector

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BEHAVIOR PREFERENCES OF *RUSSELLIANA CAPSICI* BURCKHARDT (HEMIPTERA: PSYLLIDAE) ON DIFFERENT CULTIVATED AND WILD PLANTS

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ABSTRACT:

Several psyllid species are vectors of '*Candidatus* Liberibacter spp.', a genus of bacteria associated with diseases of economically important crops. Recently, a new bacterium of the group was discovered in the psyllid *Russelliana capsici* Burckhardt, '*Candidatus* Liberibacter capsica' (1). This insect is considered highly host-specific until now. It is still unknown whether the bacterium is phytopathogenic and no details are known of the pathosystem. More studies on the insect, the new bacterium and the possibility of dispersal to other host plants are therefore indispensable. The goal of this essay was to start studies about the behaviour of the insect in other plants, to support future bacterial dispersion research. For that was carried out the evaluate settling and oviposition preferences of *R. capsici* in choice tests among vegetables (*Daucus carota*, *Solanum tuberosum*, *S. lycopersicum*, *Capsicum annuum* and *C. chinense*) and common weeds (*Amaranthus retroflexus*, *Nicandra physalodes*, *Parthenium hysterophorus*, *Solanum americanum* and *Bidens pilosa*). One plant of each species (vegetables and weeds were tested separately) was placed around the center of observation cages (n=6), in which 80 unsexed adults of *R. capsici* were released on a flight platform located 10 cm above the top of the plants. The number of adults per plant was counted at 0.5, 1, 3, 6, 24 and 48 h after the release, and the number of eggs laid per plant was counted after 48 h. *Russelliana capsici* preferred to settle, with statistically equal probabilities, in *C. chinense* (chilli pepper named "biquinho" in Brazil), *C. annum* (green pepper) and *S. tuberosum*, but the highest statistically values for oviposition occurred on chilli pepper and green pepper and, to a lesser statistical degree, on *S. tuberosum* and *S. lycopersicum*. Regarding the wild plants, the highest statistically numbers were found on *N. physalodes* and *S. americanum*, both for settle and oviposition variables. These results indicate that this insect may not be as specific to host plants as previously thought. Further studies, including tests using the Electrical Penetration Graph (EPG) technique, are being conducted by us to assist in designing possible insect or bacterium dispersal management strategies, if necessary, in the future.

KEYWORDS: Psyllid; '*Candidatus* Liberibacter spp.'; Host plant

SUPPORT

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SIMPLIFYING PHYTOPLASMA GENOME SEQUENCING: A CASE STUDY OF FLAVESCENCE DORÉE PHYTOPLASMA IN GRAPEVINE

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ABSTRACT:
Phytoplasmas are non-culturable obligate intracellular bacteria that cause considerable damage in agriculture. Genome sequencing is an important tool to understand their biology, host dependence and interactions with plant and insect hosts. However, genome studies on phytoplasmas are hampered by their low titres in several naturally infected host plants and the high proportion of host DNA in the infected samples. Therefore, the propagation of phytoplasmas in test plants is often required. This is a time-consuming process that requires specialized greenhouses, especially when working with quarantine phytoplasmas such as the phytoplasma that causes Flavescence dorée (FD), a serious threat to grapevine in the EU.To overcome these challenges, we have developed a protocol to enrich phytoplasma DNA directly from field samples using sequencing approaches from Illumina and Oxford Nanopore Technologies. In this study, we investigated six sample preparation protocols to determine their impact on the output of HTS in terms of phytoplasma genome coverage and assembly quality. We started with a liquid nitrogen homogenised grapevine sample containing a low phytoplasma titre and gradually added phytoplasma enrichment steps. For five of the protocols, we determined the sequence of the enriched nucleic acids using Illumina sequencing, and only for the protocol with the best performance we additionally used nanopore sequencing.Of the protocols tested, the one that included differential centrifugation, CTAB extraction and enrichment with the NEBNext Microbiome Kit proved to be the most effective, as it resulted in a significant increase in phytoplasma reads compared to protocols without it.*De novo* assemblies of the obtained datasets confirmed that the combination of pre- and post-extraction enrichment steps is crucial for successful phytoplasma genome assembly. The Illumina-based protocol produced the most contiguous assemblies, while hybrid assemblies improved certain assembly metrics. The final hybrid *de novo* assembled genome of the Slovenian FD isolate achieved 96% coverage of the reference genome, with good contiguity and low error rates compared to the available reference genome.This easily accessible protocol, which does not require specialised facilities and equipment, enables high quality genome sequencing of naturally infected grapevines, facilitating broader phytoplasma research and potentially enabling sequencing of other naturally infected phytoplasma hosts.
KEYWORDS: phytoplasma; genome; enrichment

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SCAPHOIDEUS TITANUS AND VITIS VINIFERA INTERPLAY IN THE CONTEXT OF GRAPEVINE RESISTANCE TO FLAVESCENCE DORÉE PHYTOPLASMA

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ABSTRACT:

Flavescence dorée (FD) is an economically important disease of grapevine caused by a phytoplasma (FDp). FDp is transmitted in a persistent propagative manner by the leafhopper *Scaphoideus titanus* and it is a regulated pest in the EU. Control of the disease relies on planting of healthy material, roguing of infected plants and compulsory insecticide treatments against the vector, these latter having raised concerns about non-target effects and human health. Grapevine genotypes show different susceptibility to FD in the field, but complete resistance has not been confirmed under controlled conditions. Nevertheless, in the process of building up knowledge for future breeding programs, several *Vitis* cultivars (cvs) were ranked from highly susceptible to tolerant to FD infection. Some *Vitis* accessions, including some rootstocks and *V. vinifera* subsp. *sylvestris*, resulted partially resistant/tolerant to FD in preliminary studies. Among these, Brachetto and Moscato bianco, widely grown in north-western Italy, also proved to be less suitable for the vector *Scaphoideus titanus* compared to the highly susceptible Barbera, as confirmed by reduced vector fitness when feeding on these tolerant cultivars. Two selection criteria were explored in an attempt to identify accessions that disjunctively exhibit pathogen resistance and vector repellence: i) a close parental relationship with a resistant cultivar and ii) a high-frequency introgression of genomic elements belonging to a resistant genotype. Two grapevine cultivars, each meeting one of the two listed criteria and not yet studied for their susceptibility to FD, were analyzed in this work. Vector feeding behaviour on the selected cultivars was assessed by electropenetrography (EPG) to disentangle resistance due to the plant's response to phytoplasma rather than to repellence/antibiosis toward the vector. Our results showed that high-frequency introgression of the *V. vinifera sylvestris* genome was not sufficient to confer FD resistance to a susceptible *Vitis* cultivar. On the other hand, the cultivar distantly-related to the resistant Moscato bianco showed FD resistance in a context of vector repellence.

KEYWORDS: Scaphoideus titanus; Susceptibility; electropenetrography

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CONTRIBUTIONS TO THE KNOWLEDGE OF THE PLANT HOSTS OF CICADELLIDAE PHYTOPLASMA-INSECT VECTORS IN CUNDINAMARCA, COLOMBIA

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ABSTRACT:
Phytoplasmas are plant pathogenic bacteria of the class Mollicutes, which lack cell walls, are phloem-obligated phloem and are fastidious to culture. They are mainly transmitted by hemipteran insect vectors of Cicadellidae and Psylloidea species but also by vegetative propagation and contaminated plant material. '*Candidatus* Phytoplasma asteris' (group 16SrI) and '*Candidatus* Phytoplasma fraxini' (group 16SrVII) are known pathogens of urban trees, potato and strawberry crops, grass and weeds in Cundinamarca, central Colombia. The phytoplasmas' host ranges depend on their insect vector´s feeding habits. There is little biological information about the Cicadellidae and Psylloidea groups in Colombia. Using standard entomologic methods (nets, aspirators and yellow sticky traps), we collected specimens of Cicadellidae and Psylloidea on trees, grass, potato crops and weed species. The taxonomic identification was performed using morphological characters and DNA barcoding. Our results show that at least 30 species of Cicadellidae thrive on the vegetation. At least 17 species of Cicadellidae inhabit the grass *Cenchrus clandestinus*, and 11 of those reproduced on this plant host, including the known phytoplasma insect vectors *Amplicephalus funzaensis*, *Exitianus atratus* and *Baldulus* sp., a species that transmits phytoplasmas in incrimination assays. Some species were collected in more than one plant host, for instance, *A. funzaensis* and *E. atratus* were collected on grass, potato plants and *Quercus humboldtii* (Fagaceae) trees; *Baldulus* sp. on grass, *Q. humboldtii* and maize; and a species of Idiocerinae on grass, and *Magnolia grandiflora* (Magnoliaceae) and *Fraxinus uhdei* trees (Oleaceae). Other species were mainly associated with one plant host. *Synoza cornutiventris* (Psylloidea) was found on *Ficus americana* (Moraceae) and *C. clandestinus*. Our results show the high biodiversity of Cicadellidae and the existence of polyphagous insect species that may be involved in the transmission of phytoplasmas in this ecosystem. The epidemiological consequences of these findings are discussed.
KEYWORDS: '*Candidatus* Phytoplasma asteris' vectors; '*Candidatus* Phytoplasma fraxini' vectors; polyphagous insect vectors

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DETECTION OF PHYTOPLASMAS IN WILD ANDEAN OAK (*QUERCUS HUMBOLDTII* BONPLAND) FORESTS IN COLOMBIA

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ABSTRACT:
Andean oak forests are wild ecosystems in the Colombian Andes, growing from 1,000 to 3,200 m of altitude. *Quercus humboldtii* (Fagaceae) is an endemic umbrella tree species for these ecosystems. About 25 years ago, Andean oak trees were planted in Bogotá as urban trees. In Bogotá, '*Candidatus* Phytoplasma asteris' and '*Candidatus* Phytoplasma fraxini' have been reported to infect at least 11 urban tree species, including Andean oaks. Infected trees display symptoms such as witches' brooms, yellowing, tufted foliage, proliferation of axillary shoots, and general canopy deformation, affecting their ecosystem services. Phytoplasmas are plant-pathogenic bacteria of the class Mollicutes that infect the phloem of plants and the hemolymph of insect vectors. They lack cell walls, are obligate parasites, and are difficult to culture. In this project, we estimated the risk of phytoplasma infection in wild *Q. humboldtii* and other plant species growing in Andean oak forests. Symptom observations and sample collections were conducted in forests of the departments of Boyacá, Cauca, Cundinamarca, and Santander. Deviations from normal morphology were observed in Andean oaks and other plants growing in the forests. When possible, samples of Andean oaks and other symptomatic and asymptomatic plants were collected. Leafhoppers were also collected from vegetation growing inside or at the edges of the forests. Plant and insect DNA extracts were tested by nested PCR of the 16S rRNA gene, and the amplicons were analyzed by RFLP to determine their group affiliation. To predict the risk of phytoplasma infection, a regression model was built that considers epidemiological variables of the disease. In total, we visited eight forests, and in seven of them, at least one tree showed symptoms suggestive of phytoplasma infection. In five forests, we recorded the presence of Melastomataceae and Oleaceae plants with phytoplasma symptoms. Phytoplasmas from the 16SrI and 16SrVII groups were detected in 24 plant samples using molecular methods. A few leafhopper specimens were collected inside the forests. However, leafhoppers were abundant in herbaceous areas or grasslands inside or around the forests. Specimens of two confirmed insect vectors of phytoplasmas were found in grasslands inside or around forests. The multiple regression model provided a useful estimate of disease risk. This work provides new information about phytoplasma diseases in wild ecosystems in Colombia.
KEYWORDS: 16SrI group; 16SrVII group; risk assesment

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SHORTER INSECTICIDE SPRAY INTERVALS REDUCE '*CANDIDATUS* LIBERIBACTER ASIATICUS' TRANSMISSION DURING SHOOT DEVELOPMENT IN SWEET ORANGE TREES

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ABSTRACT:
The Asian citrus psyllid (ACP), *Diaphorina citri*, is a phloem-feeding insect that vectors the bacterium '*Candidatus* Liberibacter asiaticus' (CLas) and result in the most devastating citrus disease: the huanglongbing (HLB). Frequent foliar application of insecticides at 7 to 14-day intervals is recommended to prevent ACP from completing its life cycle from egg to adult and, consequently, for HLB management. However, there is limited information on the effectiveness of these spraying intervals in reducing CLas transmission under conditions of continuous influx of ACP carrying CLas (ACP_{CLas+}) originating from outside the orchard (primary dispersal) and during the flushing period of sweet orange trees. To investigate this, experiments were conducted using 'Valencia' sweet orange seedlings, initially with shoots at the V2 stage. The seedlings received foliar application of the insecticides thiamethoxam or spinetoram, with treatments varying intervals: spray every 14 (S14), 7 (S7), and 3 (S3) days. Non-sprayed seedlings were used as control. To simulate continuous ACP primary dispersal, seventy ACP_{CLas+} were released every two days into a screened greenhouse containing seedlings from all treatments and control. Vegetative stage, percentage of seedlings with at least one ACP (occupancy), and number of ACPs per seedling (abundance) were assessed over the experiment every two days, ending on the 13th day after the first spraying, when all live ACPs were removed. Six months after this ACP removal, seedlings were tested for CLas by qPCR to assess HLB incidence. The effect of each insecticide was evaluated separately in independent experiments, each conducted twice. Results showed that reducing the interval between insecticide applications decreased both ACP occupancy and abundance. Compared to control, HLB incidence was similarly reduced in S7 and S14 treatments by 55.6% and 66.7% for both insecticides. In S3 treatment, HLB incidence reduced by 87.5% for thiamethoxam and 94.4% for spinetoram, also compared to control. These findings suggest that the shorter the interval between insecticide sprays, the greater the ACP control and the lower CLas transmission, during shoot growth and continuous ACP primary dispersal. However, complete prevention of CLas transmission was not achieved. Our findings reinforce the importance of eliminating HLB-infected plants through regional eradication and the necessity for integrated insecticide sprays with other ACP control strategies.
KEYWORDS: Greening; Chemical control; Vector-borne disease control

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TRANSMISSION EFFICIENCY OF GENETICALLY AND BIOLOGICALLY DISTINCT *XYLELLA FASTIDIOSA* STRAINS BY *MACUGONALIA LEUCOMELAS*

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ABSTRACT:
Xylella fastidiosa is a phytopathogenic bacterium with a wide range of host plants, responsible for economically important diseases such as Citrus Variegated Chlorosis (CVC), Plum Leaf Scald (PLS) and Olive Quick Decline (OQD). Due to the wide genetic and phenotypic variability of the species, this phytopathogen is divided into subspecies and subdivided into strains, which are grouped into sequence types (ST) and associated with different host plants. In order to understand the influence of the variability of the species on the interaction with the insect vector, the transmission efficiency of different strains by the sharpshooter *Macugonalia leucomelas* Walker was evaluated based on two factors: aggregation level and pathogenicity of genetically close strains associated with CVC, using strains u24d and 9a5c, both classified within ST13 and 11399 and j1a12, belonging to ST12, and genetic distance, in which strains 9a5c from citrus (ST13), 327 from plum (ST67) and 361 from olive (ST84) were evaluated. Each bacterial strain was cultivated in Xfm culture medium and then resuspended in artificial diet (L-glutamine, 0.7 mM; L-asparagine, 0.1 mM; sodium citrate, 1 mM; pH 6.4), which was fed to the insects through an in vitro acquisition system using parafilm membrane sachets, for an acquisition access period of 6 hours. The insects were then confined in groups of three on healthy *Catharanthus roseus* L. plants for 72 hours. With regard to genetically close strains, insects that fed on the artificial diet containing cells of strain j1a12 had a higher acquisition rate (100%) than insects that fed on the diet containing strains u24d, 9a5c and 11399, which were acquired with efficiencies of 58.7%, 73.6% and 81.5%, respectively ($X^2 = 9.08$; d.f. = 3; $P = 0.02$) and there was no significant difference in inoculation rates between the strains ($X^2 = 1.11$; d.f. = 3; $P = 0.77$). Regarding the genetically distant strains, no significant difference was found between the acquisition ($X^2 = 4.11$; d.f. = 2; $P = 0.13$) and inoculation ($X^2 = 3.12$; d.f. = 2; $P = 0.21$) rates. The results suggest that the genetic basis of the bacterium influences to some extent its transmission efficiency, making it relevant for understanding the epidemiology and implementing management strategies.
KEYWORDS: xylem-limited bacterium; strain variability; transmission rates

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BALDULUS SP. (HEMIPTERA: CICADELLIDAE): EVIDENCE OF NEW PHYTOPLASMA INSECT VECTOR FOR COLOMBIA USING INCRIMINATION ESSAYS

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ABSTRACT:
Phytoplasmas are pathogenic bacteria that lack cell walls, are limited to the phloem, unculturable and transmitted mainly by insect vectors. In Cundinamarca, phytoplasmas are associated with diseases in trees and crops. *Baldulus* sp. (Deltocephalinae subfamily), is a non-described species of this genera, which thrives in phytoplasma infected grassalands. We tested if *Baldulus* sp. carried phytoplasmas and its ability to transmit them to artificial feeding media. DNA was extracted from 68 *Baldulus* sp. individuals and tested by nested PCR, using universal primers for the 16S rRNA gene. Tests were conducted on DNA extracted from the heads of eleven individuals and phytoplasmas were detected in five of them. Of thirteen DNA extracts obtained from complete specimens, 11 were positive for phytoplasmas. RFLP analysis of PCR amplicons showed the presence of group 16SrI in eleven individuals, mixed infections of groups 16SrI and 16SrVII in four individuals, and 16SrVII in one individual. The fact that *Baldulus* sp. carry phytoplasmas in their heads suggest that their salivary glands may be infected and threfore are potential phytoplasma vectors. Transmission essays to artificial media were conducted for 31 adults. Of these, four survived feeding on the artificial medium more than 24h and were positive for phytoplasmas. Nested PCR tests performed on DNA extracts of the artificial feeding media were positive for phytoplasmas in all the cases, suggesting that they secreted their saliva with phytoplasmas. Therefore, incrimination tests suggest that *Baldulus* sp. maybe a phytoplasma vector. Confirmation tests to plants are needed. Additionally, DNA barcodes were generated for this species by amplification of the *COI* mithocondrial gene. Project INV-CIAS-3916.
KEYWORDS: Artificial feeding medium; '*Candidatus* Phytoplasma asteris'; '*Candidatus* Phytoplasma fraxini'

SUPPORT

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PROSPECTION OF ANTAGONISTIC BACTERIA FOR THE BIOCONTROL OF CANDIDATUS *LIBERIBACTER ASIATICUS*

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ABSTRACT:
Huanglongbing (HLB), caused by the bacterium *Candidatus* Liberibacter asiaticus (C*Las*), is currently the most devastating and difficult to control citrus disease. Studying this pathogen is challenging since CLas cannot yet be cultured in vitro. Given this limitation, the search for microorganisms with antagonistic potential against CLas has emerged as a promising strategy for developing new control approaches. The aim of this study is to prospect bacteria with antimicrobial activity against CLas, using *Sinorhizobium meliloti* as a model organism for *in vitro* assays and validating the most promising candidates in infected plants.To achieve this, we selected isolates from our citrus rhizobacteria collection belonging to genera previously reported in the literature as antagonists of plant pathogens. So far, we have identified 11 bacterial strains with antimicrobial potential, demonstrated by *in vitro* antagonism tests against *S. meliloti*. These strains belong to the genera *Bacillus, Burkholderia, Chryseobacterium, Lelliottia, Methylobacterium, Serratia*, and *Pseudomonas*. Following the initial screening, an additional 194 isolates from these genera are currently being evaluated. The strains with the best *in vitro* performance will be further tested in *in planta* assays to confirm their efficacy in controlling CLas, using qPCR to quantify bacterial load.
KEYWORDS: Huanglongbing; *Sinorhizobium meliloti*; Biocontrol

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IMPACT OF DIFFUSIBLE SIGNAL FACTOR OVERPRODUCTION IN TRANSGENIC PLANTS ON VECTOR FEEDING BEHAVIOR AND *XYLELLA FASTIDIOSA* ACQUISITION

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ABSTRACT:

Xylella fastidiosa is a bacterium responsible for several economically significant plant diseases, including citrus variegated chlorosis (CVC). Its transmission relies on the colonization of both the host plant xylem and the foregut of insect vectors, a process regulated by the quorum sensing (QS) system. Our research group developed a transgenic sweet orange (*Citrus sinensis* cv. 'Hamlin') overexpressing the *rpfF* gene, which encodes the diffusible signal factor (DSF), a fatty acid involved in QS regulation. These transgenic plants exhibited increased tolerance to CVC by enhancing bacterial adhesion and restricting its movement within the plant. However, the influence of DSF overproduction on the interaction between the bacterium and its insect vector in these plants remains unknown. This study investigated whether DSF overproduction in transgenic plants influences insect feeding behavior and bacterial acquisition. The Electrical Penetration Graphs (EPG) technique was used to monitor the feeding behavior of *Macugonalia leucomelas* sharpshooters on four plant treatments: (i) transgenic without the bacterium (TSXF), (ii) transgenic with the bacterium (TCXF), (iii) wild-type without the bacterium (WTSXF), and (iv) wild-type with the bacterium (WTCXF). A total of 20 sharpshooters per treatment were analyzed over an eight-hour recording period. Insects spent significantly less time ingesting xylem sap on TSXF (169.1 min) plants compared to WTSXF (270.7 min) plants and exhibited increased resting behavior on transgenic plants, with an average of 134.6 min (TSXF) and 94.7 min (TCXF), compared to wild-type plants, which showed 51.2 min (WTSXF) and 44.9 min (WTCXF). Furthermore, PCR analysis revealed that 68.5% of insects acquired the bacterium from transgenic plants, whereas 81.5% became infective when feeding on wild-type plants. These findings suggest that DSF overproduction in transgenic plants may reduce bacterial transmission efficiency in the field by decreasing the duration of insect xylem sap ingestion, potentially impairing bacterial acquisition.

KEYWORDS: vector sharpshooters; insect-plant-pathogen interaction; Electrical Penetration Graphs

SUPPORT

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INORGANIC ELICITATION ROLE IN THE DEFENSE OFALSTROEMERIA PLANTS AGAINST THE WESTERN FLOWER THRIPS

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ABSTRACT:
The western flower thrips complex presents a major threat to genus Alstroemeria,damaging leaves, flowers, and transmitting plant diseases such as tomato spotted wilt virus and other tospoviruses, leading to significant losses inthe floriculture industry. Traditional pest control methods heavily rely on chemicalpesticides, which have negative environmental impacts. As a sustainable alternative, plantelicitation using inorganic elicitors has gained interest. This study investigates theeffectiveness of two inorganic elicitors, potassium phosphite and calcium carbonate, inenhancing plant defenses against thrips infestation. Both elicitors were applied as foliarsprays at concentrations of 150 and 300 ppm to the red variety of Alstroemeria,susceptible to thrips. Flowers were collected at various time points post-application, andplant defense was assessed by analyzing changes in the phytochemical profile throughliquid chromatography, thrips feeding behavior (consumption area), and the activity ofthree key detoxifying enzymes. Results demonstrated increased levels of defense-relatedcompounds, reduced feeding by thrips, and elevated enzymatic activity, indicating thatboth elicitors effectively enhance Alstroemeria's defense against thrips. This approachoffers a promising, eco-friendly alternative to chemical control in floriculture. Thisresearch was funded by the Vice-Rector for Research at the Universidad Militar Nueva Granada through the IMP-CIAS-3739 research project, valid from 2023 to 2025.
KEYWORDS: Pest Control; Phytochemical profile; Thrips feeding behavior

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RE-EMERGENCE OF VIRUSES CAUSING YELLOWS DISEASE OF SUGAR BEET IN CZECH REPUBLIC

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ABSTRACT:

The virus yellows disease (VY), caused by a group of aphid-transmitted viruses, poses a significant threat to sugar beet production, both in terms of yield and quality. The main viruses involved in VY disease include beet yellows virus (BYV), beet mild yellowing virus (BMYV), beet chlorosis virus (BChV) and beet western yellows virus (BWYV). The viruses have occurred on a significant extent in Europe in recent years, most likely due to the restriction of foliar or seed treatment with neonicotinoids under the EU Directive (No. 485/2013). As a result, vector populations have increased significantly over the last decade in the Czech Republic and elsewhere. We have monitored the occurrence of viruses in sugar beet as well as in weeds throughout the Czech Republic. The BYV, BChV and TuYV viruses in particular were detected most frequently and occurred either as single or mixed infections. In the case of BChV, this is the first detection in the Czech Republic. We have developed a multiple RTX-PCR based on a one-step system for the detection of viruses. All detections were additionally confirmed by sequencing and RT-qPCR. A mixed infected field sample was subjected to high-throughput sequencing (HTS), which yielded full-length sequences of BChV and BYV. The BChV of the Czech isolate BChV-CZ has a genome sequence of 5,744 bp. Phylogenetic analysis showed that the BChV-CZ isolate is clustered with other European isolates and has a sequence similarity of ?94% for nucleotides (nt) and ?91% for amino acids (aa) (overall similarity with all full-length BChV isolates is 94.8-99.9% nt and 89.1-99.7% aa). The BYV of the Czech isolate BYV-CZ has a genome sequence of 15,480 bp. Phylogenetic analysis showed that the BYV-CZ isolate is clustered with the Californian isolate (BYV-4) and has a sequence similarity of ?99.9% nucleotides (nt) and ?99.8% amino acids (aa) (overall similarity with all complete sequences of BYV isolates is in the range of 97.3-99.9% nt and 93.3-99.9% aa). We also analysed the level of resistance of commonly grown sugar beet genotypes to BChV and BYV. The genotypes tested showed different degrees of susceptibility to the both viruses, with the exception of the cultivar Laser, which showed moderate resistance revealing mild virus symptoms as well as low virus titres.

KEYWORDS: Sugar beet; yellows disease; virus detection, RTX-PCR, RT-qPCR, BYV, BChV, TuYV, diversity.

SUPPORT

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THE TOBRFV EPIDEMIC IN MOROCCO: AGRONOMIC AND SOCIO-ECONOMIC IMPACT

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ABSTRACT:
Tomato (*Solanum lycopersicon* L.) is one of the most important vegetable crops in Morocco, with an estimated production of 1,444,678 tons in 2023. The major threats to tomato intensive cultivation are viral diseases, which are responsible for significant yield and fruit quality losses. *Tobamovirus fructirugosum* (ToBRFV) is an emerging tobamovirus that affects tomato, capsicum and chili. ToBRFV is easily transmitted by mechanical means, via seeds, bumblebees, and via contaminated water and soil, enabling its spread locally and over long distances. In Morocco, the virus was first detected in October 2021 in the Souss-Massa region and later in the Dakhla region in March 2022 (EPPO Reporting Service: 2023/2025). Still, little is known about the current situation of ToBRFV including its agronomic and socio-economic impact in the country. In this work, we conducted surveys through a questionnaire shared online and through structured interviews with farmers in the Souss Massa Region, data was generated and analyzed. Losses caused by ToBRFV constituted an average of 30% of crop loss production per hectare. Moreover, the ToBRFV epidemic caused an increase in production costs related to the prophylactic measures exceeding 1,000 USD per hectare. The epidemic also impacted the supply chain of tomatoes destined for export by promoting a shift to less sensitive crops, reduction in tomato cultivating areas, and decreased farm workforce. The ToBRFV epidemic has an important impact on tomato production, implying the use of efficient management strategies to mitigate its impacts.
KEYWORDS: *Tobamovirus fructirugosum* (ToBRFV); Epidemic; Socio-economic impact

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THE NORTH AMERICAN SUNFLOWER VIRUS PROJECT: DEVELOPING A PHYLOGENETICALLY-ROBUST PREDICTIVE FRAMEWORK FOR STUDYING EPIDEMIOLOGY ACROSS CROPS AND THEIR RELATIVES

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ABSTRACT:
Phylogenetic relatedness across host taxa may be a predictive factor in determining virus epidemics in native and cultivated plants, particularly for taxa that have diverged recently and in the presence of viral symbionts. This study will examine how phylogenetic relationships between members of genus *Helianthus* shape virus community diversity, specificity and disease symptoms. In addition to describing the virome of native Helianthus species across natural North America prairies and grasslands, this project will also explore implications for sunflower crop disease and protection. By elucidating the host phylogenetic and evolutionary mechanisms governing plant-virus interactions, we will develop predictive frameworks for monitoring virus spillover in future climate scenarios and identify potential genetic resources for breeding resilient cultivated sunflower varieties. Those interested in crop wild relatives, phylogenetic relatedness between hosts and North American sunflower development are encouraged to stop by to engage with researchers as we build this multi-institutional project.
KEYWORDS: Sunflowers; Spatial phylogenetics; Predictive modeling

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INTERPLAY OF VIRUS INFECTION AND WATER STRESS IN SUGAR BEET: EFFECTS ON PLANT GROWTH, SUGAR CONTENT AND APHID FEEDING BEHAVIOR

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ABSTRACT:
Current climate change conditions have led crops to undergo more frequent and severe drought periods. Drought affects crops directly by impacting plant development and indirectly by affecting the activity of their insect pests and the transmitted pathogens. Here, we first studied how severe drought in association with infection by beet yellows virus (BYV, *Closterovirus*) affects plant development and sugar yield of sugar beet (*Beta vulgaris* L.) in comparison to virus-free droughted plants. Additionally, we studied the effect of water stressed and well-watered sugar beets on the feeding behaviour and further transmission of BYV by viruliferous *Myzus pesicae* (Sulzer) aphids, the main vector of BYV. The interplay between virus infection and drought significantly reduced several sugar beet traits in comparison to the healthy and droughted plants, such as fresh and dry weight of leaves and roots. More importantly, BYV-infection and drought led to a significant reduction of the amount of sugar concentration in comparison to the non-infected and droughted plants. Therefore, the interplay between virus and drought negatively impacted most of the traits studied for these plants in comparison to the only-drought scenario. Also, preliminary results from the EPG experiments also suggest an impact of drought on the feeding behaviour of viruliferous aphids. Aphids took longer to produce a phloem-pd ´stylet activity associated to BYV transmission´ under water stress than when feeding on well-watered plants, suggesting that virus transmission events will require longer when plants are grown under water stress. Our results point out a significant effect of virus infection and drought conditions on sugar beet. Results from the EPG studies will be discussed on the basis of the further analysis of the results obtained. This study again remarks the importance of efficient pest control strategies to disrupt virus spread and further transmission to crops especially under the more often drought scenario on agriculture.
KEYWORDS: Climate change; Drought; Electrical penetration graphs

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