The XXIII Conference of the International Organization of Citrus Virologists.

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Abstracts

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THE INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS - IOCV

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INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

XXIII International Organisation of CITRUS VIROLOGISTS CONFERENCE MILDURA, AUSTRALIA 2025

2025 IOCV XXIII Abstracts

Abstracts presented at the 23nd Conference of the International Organization of Citrus Virologists (IOCV), in Mildura, Australia, March 16-20, 2025 are now available on line at the **Journal of Citrus Pathology (JCP)** (http://journalofcitruspathology.com/).

The abstracts are arranged in the order they were presented at the conference. The conference session each abstract was presented at is also noted (see example below). The conference program can be found at: https://iocv.ucr.edu/conferences/xxiii-iocv-conference

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Davis R.I. 2025. Diagnostic challenges for plant health surveillance in extremely remote locations. The part that huanglongbing has played through 35 years of northern Australia quarantine strategy plant health surveys [abstract]. *Journal of Citrus Pathology*, 12(2):3. https://doi.org/10.5070/C4.61717.

Session 1 -

Huanglongbing I

DIAGNOSTIC CHALLENGES FOR PLANT HEALTH SURVEILLANCE IN EXTREMELY REMOTE LOCATIONS. THE PART THAT HUANGLONGBING HAS PLAYED THROUGH 35 YEARS OF NORTHERN AUSTRALIA QUARANTINE STRATEGY PLANT HEALTH SURVEYS.

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Abstract

The Northern Australia Quarantine Strategy (NAQS) is a biosecurity initiative operated by the Australian federal government's Department of Agriculture, Fisheries and Forestry (DAFF). It has been operating for over 35 years now and remains unique throughout the world, though many other island nations face similar challenges. The need for NAQS lies with the fact that the north of Australia is vast, sparsely populated, and close to neighbouring countries with very different plant and animal health status. Incursion threats, both natural and human mediated, are very real. The heart of the NAQS strategy is physical surveillance for early detection of exotic pests and diseases should they arrive from northern neighbouring countries and establish on Australian soil. Such early detection, followed by suitable interventions, will protect plant and animal-based industries, mostly heavily concentrated to the south, from devastation.

One pillar to the success of NAQS has been its interaction with First Nations communities. The cooperation and goodwill of Aboriginal and Torres Strait Islander Traditional Owners and ranger groups has been integral to NAQS boots on the ground surveillance work. Today, the DAFF funded Indigenous Ranger Biosecurity Program engages over 60 ranger groups in fee for service activities to support biosecurity surveillance. The second key pillar of the NAQS story has been its ability to keep 'a finger on the pulse' of the plant and animal health status of those neighbouring nations to the north. This 'over the horizon' insight into immediate threats, has been achieved via a long history of collaborative international plant health surveys, working side by side with the biosecurity scientists of those countries. This relationship has been rich and synergetic. NAQS staff built long-term, hands-on experience with key target organisms whilst counterpart agencies overseas benefitted from close collegiate relationships and ready access to Australian and international diagnostic networks when needed.

Australia's citrus industry has always rated highly in the formulation and targeting of NAQS plant health surveillance. Amongst the list of NAQS targets, huanglongbing disease of citrus has been especially significant. The history of NAQS surveillance for this disease is long and intriguing. The main challenge has been difficulties in field triage and implementing effective diagnostic testing when trees are located in extremely difficult to reach places. This story began in the early 1990s when adoption of molecular tools to achieve meaningful diagnostics was in its infancy. As the NAQS plant pathologists learned to address these challenges over the next 20-30 years, a very unusual HLB epidemiological story played out on the island of New Guinea. The details of this journey will be explained in the context of meeting the challenges of diagnostics on multi target, multi host plant health surveys in Australia's extreme north and adjacent island nations.

HUANGLONGBING PROGRESS, CONTROL PRACTICES, AND CURRENT SITUATION IN CALIFORNIA

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Abstract

The Asian citrus psyllid (ACP, *Diaphorina citri* Kuwayama), the vector of huanglongbing (HLB), was first detected in California in San Diego County in 2008. By 2017, ACP had been detected across all citrus-growing regions of the state. The first HLB-positive tree was confirmed in 2012 in Los Angeles County. By 2024, over 650,000 trees and over 330,000 ACP samples were tested, identifying and eradicating 9,336 HLB-positive trees from residential areas in six southern California counties. HLB quarantine zones now cover over 2,400 square miles, with restrictions on the movement of citrus nursery stock and bulk citrus in and out of these areas. The ACP-HLB program, with a budget of over $47 million, is managed by the California Department of Food and Agriculture (CDFA) in collaboration with the United States Department of Agriculture (USDA) and the Citrus Pest and Disease Prevention Committee (CPDPC), includes extensive detection and quarantine efforts. Inspection activities are risk-based, focusing on areas with past HLB detections and incorporating data on human activities, such as travel in HLB-infested regions. In 2022, the program adjusted to a multi-pest survey approach, which led to the identification of citrus yellow vein clearing virus (CYVCV) in Tulare County. Biological control of ACP is ongoing, with over 31 million *Tamarixia radiata* released since 2011. This beneficial wasp is now established in southern California, and releases are concentrated around HLB quarantine zones, trade corridors, and newly infested areas. Native species such as hoverflies (*Allograpta obliqua*) have also been identified as natural enemies of ACP in California. Quarantine measures for ACP were redesigned in 2017 to create regional zones that limit the long-distance transport of bulk citrus, reducing the risk of ACP spread from southern HLB-infested areas to central California. The state is now divided into seven zones, allowing fruit packing closer to production sites. Similar restrictions apply to citrus nursery stock, which has been produced under the mandatory Citrus Nursery Stock Pest Cleanliness Program since 2010. Nurseries in high-risk areas must grow trees under protective structures and undergo regular inspections and HLB testing to ship trees to lower-risk zones. Public outreach and education are key components of the program. CDFA works with pest control districts and grower liaisons to encourage homeowners to remove backyard citrus, coordinate area-wide treatments, and address neglected or abandoned groves. Efforts are underway to expand HLB diagnostic capacity by incorporating private and industry-run high-throughput laboratories. HLB and ACP research continues with tens of millions of dollars in funding from industry and government agencies.

PROPOSED INTEGRATED HUANGLONGBING (HLB) MANAGEMENT OF CITRUS IN FLORIDA

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Abstract

The century-old huanglongbing disease (HLB), putatively caused by *Candidatus* Liberibacter asiaticus (CLas) and primarily transmitted by the insect vector Asian citrus psyllid (ACP; *Diaphorina citri*), has defied most management strategies and continues to spread in Florida and other citrus-producing areas worldwide. Through extensive collaborations and support from federal and state agencies in the US, our research team has been working since 2016 on several projects to develop practical solutions for citrus growers to combat HLB. In the absence of a cure, we have developed strategies utilizing several 'tools' effective in controlling HLB that growers in Florida are increasingly adopting. These strategies consist of the use of individual protective covers (IPCs) at planting, trunk injection of oxytetracycline (OTC) to alleviate the disease severity and symptoms, and application of brassinosteroids (Brs) and systemic acquired resistance inducers (SARs) to protect new shoots from reinfection. Additional tools, including HLB-tolerant interstocks and an automated trunk injection system, are being developed and evaluated. In several field studies using these strategies, we demonstrated the efficacy of IPCs in preventing CLas infection, OTC in the reduction of CLas titers and fruit-drop, and Brs and SARs in increasing tree productivity and protecting new shoots from ACP-mediated CLas reinfections, and citrus canker. Our results indicate that these IPM tools, collectively, can effectively control HLB in commercial citrus orchards and provide a path for sustainable citrus production under endemic conditions. The results of several ongoing field trials to integrate these tools into IPM programs will be discussed, and an IPM plan for effective HLB management in Florida orchards will be proposed.

Non-technical summary

Huanglongbing (HLB), a.k.a. citrus greening, is a disease of citrus that has proven to be incurable. We developed several practical tools for growers to use as part of the integrated management of HLB in Florida orchards. Growers who adopted our proposed IPM are more successful in managing the HLB in their orchards.

CITRUS ORCHARDS FOR PROFITABILITY UNDER HLB AND NATURAL HARDSHIPS - THE TEXAS EXPERIENCE

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Abstract

The Florida citrus industry peaked in 1971 at 849,000 acres; however, by completing my Ph.D. in Plant Pathology (1984), that number had dropped to 508,000 acres due to environmental challenges such as freezes and hurricanes, resulting in a 40% decline in Florida citrus acreage. Upon joining the Texas A&M System in 1988, I encountered a distinctly different landscape: only 29,000 acres of citrus, primarily grapefruit, and a few oranges were cultivated across three Texas counties. In Texas, the citrus industry was characterized by vertically integrated family-owned businesses that included orchards and nurseries, but the state had no "clean citrus nursery" practices. With the support of various people, including some IOCV members, a state-mandated clean citrus program was initiated in 1997, successfully distributing five million clean citrus buds to growers. By 2012, stringent state and federal regulations emerged in response to the threats posed by citrus canker, Huanglongbing (HLB), Blackspot, and Sweet Orange Scab diseases, leading to the decline of outdoor nurseries and the establishment of certified nurseries under protected insect covers. In the ensuing decade, skyrocketing property values forced many small family growers out of the market. Today, the thriving Texas citrus industry predominantly operates under corporate or cooperative models, enhancing fruit marketing capabilities. In this presentation, I will share insights from my transition from academia to entrepreneurship, navigating the complexities of "Acts of God" and government regulatory interventions, and developing a New Outlook for Citrus Production focused on profitability. I aim to equip and mentor the next generation of citrus scientists with practical knowledge to support growers and consumers better while maintaining their academic responsibilities.

THE FORGOTTEN DISEASE: WHY CTV RESISTANCE IS CRITICAL IN THE BATTLE AGAINST HLB

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Rootstock research has recently attracted wider interest, stimulated by new evidence suggesting that wild Citrus species native to Oceania show promising resistance to Huanglongbing (HLB). Scientists in various countries are using these wild species to produce hybrid rootstocks they hope may solve HLB. Unfortunately, these current efforts largely ignore the fact that it is not a new idea, it is not easy, and these wild species are extremely sensitive to Citrus Tristeza Virus (CTV). Through a historical review of disease, genetics, breeding and pathology we will show that scientists have been experimenting with these wild Oceania species for more than 130 years and yet have consistently failed to generate anything of commercial significance. Such consistent failure points to a single and fundamental limitation; they are extremely sensitive to CTV. We were fortunate to discover this limitation early in our breeding program and in 2008 recognised that "the horticultural value of these species cannot be assessed until this limitation is addressed". During the intervening period we have used conventional breeding to transfer the CTV resistance gene into a broad range of Oceanian species, frequently requiring the use of bridging species to overcome sexual compatibility barriers. Field trials are now showing robust performance from a broad range of CTV-resistant hybrids with pedigrees incorporating *C. australasica, C. australis, C. garrawayi, C. glauca, C. inodora, C. wakonai, C. warburgiana,* and *C. wintersii*. Whether any of these have useful resistance to HLB has yet to be determined and will require testing outside of Australia, which remains free of this devastating disease. Regardless of the HLB potential of Oceania species, the fundamental limitation of CTV must not be ignored in the rush to use them as rootstocks. CTV may be an old and unfashionable disease but it can not be forgotten in the battle against HLB.

Session 2 -

Huanglongbing II

STRATEGIES TO DEVELOP GENETIC SOLUTIONS TO CITRUS HUANGLONGBING DISEASE.

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Abstract

Huanglongbing (HLB) is considered the greatest challenge to citrus cultivation in regions where the disease is prevalent. Associated with an unculturable bacterial pathogen and spread by a prolific psyllid vector, HLB has no cure at present. The disease has severely impacted citrus production in Florida (USA), Brazil, and many other regions in the Western Hemisphere. The availability of HLB-resistant citrus cultivars will provide a sustainable, genetic solution for long-term citrus cultivation in the presence of HLB. We have identified sources of HLB resistance in wild Australian limes (*Citrus glauca*, *C. australasica*, *C. australis* and *C. inodora*), and developed a breeding program designed to introgress the resistance traits into citrus. HLB disease response in novel hybrids was evaluated in greenhouse and field trials through graft and psyllid challenges. Resistance was observed in the novel hybrids of the first generation (F_1), which also exhibited various undesirable fruit traits. Further breeding resulted in advanced hybrids with improved citrus-like fruit qualities. Selection of the breeding population was based on response to the HLB pathogen, organoleptic properties of fruit juice, and the presence of genomic fragments correlated with HLB resistance. We have sequenced the genomes of four wild Australian limes and three mandarins used in breeding. The *de-novo* assembled, phased genomes were used to align the sequences from selected hybrids for genetic analyses. Other strategies in developing genetic resistance include construction of a project-based pangenome (with breeding parents, and selected phenotyped progeny), identification of resistance-related genes, extensive genotyping, gene expression analysis in resistant and susceptible progeny, and metabolomic analysis of fruit juice (to select hybrids with organoleptically acceptable profiles).

Non-technical Summary

The development of HLB-resistant varieties using resistant/tolerant Australian wild limes through genome-assisted breeding is in progress and will provide sustainable solutions to many citrus industries.

CITRUS-RELATIVE GENOTYPES AS POTENTIAL SOURCES OF RESISTANCE TO HUANGLONGBING (HLB)

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Abstract

The global citrus industry faces significant challenges from Huanglongbing (HLB), also known as citrus greening. This disease is caused by phloem-restricted bacteria of the *Candidatus Liberibacter* genus, with *Candidatus* Liberibacter asiaticus (CLas) being the most widespread and destructive species. HLB is transmitted by the Asian citrus psyllid (*Diaphorina citri*), and since there is no cure for it, the development of resistant varieties is crucial. However, no resistance has been identified within the *Citrus* genus. Phylogenetically closer to *Citrus*, the Oceanian citrus *Eremocitrus glauca* and certain species from the genera *Microcitrus* and their hybrids have been suggested in field observations as potentially resistant to HLB. Based on this, we selected 25 genotypes of Fundecitrus germplasm to challenge through CLas-grafting under greenhouse-controlled conditions. First, we clonally propagated them onto the susceptible 'Rangpur lime' rootstock and evaluated them for two years. Accessions that remained free of CLas despite the susceptible rootstocks being colonized were classified as resistant. However, CLas was detected in their stem bark above the scion-rootstock graft union. To better understand the full-resistance phenotype, new experiments were conducted, evaluating: (1) CLas acquisition by *D. citri* fed on these genotypes; (2) CLas infection in sweet orange plants grafted with bark or budwood from these genotypes; (3) CLas infection in sweet orange plants top-grafted onto them; (4) CLas infection in new shoots from rooted plants; and (5) CLas infection in new shoots after drastic back-pruning. The results showed that insects feeding on Oceanian citrus genotypes, new flushes, and rooted cuttings, tested qPCR-negative for CLas. Furthermore, budwood from these genotypes failed to infect sweet orange through grafting. *Eremocitrus glauca* and *Microcitrus* are compatible with *Citrus* for grafting and pollination, presenting opportunities to assess them as potential *Citrus* rootstocks/interstocks, crossbreed them with *Citrus* varieties to generate new cultivars, and map specific loci involved in CLas resistance or lack of susceptibility. In contrast, more distantly related genera such as *Murraya paniculata* and *Bergera koenigii* have been described as partially resistant and immune to the HLB bacterium, respectively, and are more attractive to the insect vector than *Citrus*. Since they are incompatible with *Citrus* for both grafting and pollination, their use in breeding programs is better suited to bioinformatics and biotechnological approaches. To explore these genera,

bioassay designs for tissue collection, over which transcript profiles will be monitored, are critical and should be based on plant-insect-pathogen interaction observations. Therefore, we first identified the time course of CLas multiplication and whole-plant colonization immediately following inoculation by infected psyllids feeding for 2 days on new shoots (NS) of *Citrus* × *sinensis* (susceptible), *Murraya paniculata* (partially resistant), and *Bergera koenigii* (fully resistant). Following this, we proposed an RNA-seq comparative study at the previously identified key time points of early CLas colonization. Transcriptomic analyses were performed on new flushes from these species at 0, 10, 20, 30, and 60 days after a 48-hour exposure to CLas-positive or negative psyllids. Gene expression was significantly reprogrammed during flush growth, with thousands of differentially expressed genes identified. However, the transcriptomes of flushes exhibited minimal changes in response to CLas infection. Gene Set Enrichment Analysis highlighted physiological differences in CLas-infected flushes, including attenuation of photosynthesis-related processes in *M. paniculata* at t0, t10, t30, and t60, and in *B. koenigii* at t0 and t10. Additionally, sulfur amino acid pathways were downregulated in *B. koenigii* at t0 and t10. Notably, no plant immunity or bacterial defense responses were detected at any time point in either species. Orthologous gene expression analysis across the three species revealed activation of various biochemical pathways related to primary metabolism and other basic cellular functions, which could be associated with limiting CLas multiplication. In summary, our findings suggest that metabolic differences and physiological changes likely restrict bacterial proliferation. Understanding these interactions provides valuable insights into potential disease management strategies and resistance breeding. Further exploration of these mechanisms is essential for developing sustainable solutions against HLB.

NATIONAL EXPERIMENTAL PROGRAM FOR HLB TOLERANT ROOTSTOCK EVALUATION IN AUSTRALIA AND INDONESIA

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Abstract

Huanglongbing (HLB), also known as citrus greening, is one of the most destructive diseases of citrus worldwide. Infection leads to reduced productivity and fruit quality, tree decline, and death. There is no cure for HLB, but there are a range of strategies to potentially reduce its impact including the use of HLB-tolerant rootstock varieties. HLB and its associated vectors are not present in Australia, but industry and government are proactively preparing for an incursion. One preparedness activity was the importation of several purportedly HLB-tolerant rootstock varieties from breeding and evaluation programs in the United States for evaluation in field trials under Australian conditions. The National Citrus Rootstock Evaluation program is funded by Horticulture Innovation and is run by the New South Wales Department of Primary Industries and Regional Development (NSW DPIRD). HLB-tolerant rootstock varieties will also be trialled in Indonesia where HLB and associated vectors are present, through a project led by NSW DPIRD in collaboration with project partners in Indonesia and China and funded by the Australian Centre for International Agricultural Research and Horticulture Innovation. Twenty rootstocks, including the UFR series, B11 series, and N40 series, were imported from the University of Florida, Lake Alfred. Seven rootstocks, US-802, US-812, US-897, US-942, US-1283, US-1284, and US-1516, were imported from the United States Department of Agriculture, Fort Pierce. All rootstock varieties will be evaluated with a range of scion varieties. An update on the progress of the collaborative program will be presented.

EFFICACY OF OXYTETRACYCLINE (OTC) TRUNK INJECTION FOR HUANGLONGBING (HLB) MANAGEMENT OF RIO RED GRAPEFRUIT TREES IN TEXAS

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Abstract

Citrus Huanglongbing (HLB), also known as citrus greening disease, is associated with *Candidatus* Liberibacter asiaticus (CLas), a phloem limited bacterium transmitted by an insect vector, Asian citrus psyllid (ACP). Currently, there are no commercially viable treatment options available for managing HLB. Among various potential therapies, trunk injection of Oxytetracycline (OTC) is an emerging treatment. To understand its efficacy (Rectify, A.I: 95% Oxytetracycline Hydrochloride) in mitigating HLB, a completely randomized block design trial was conducted in two 'Rio Red' grapefruit orchards for two consecutive years (2023 and 2024). Treatment evaluations consisted of annual OTC truck injections at two different concentrations, 1.1g and 0.55g per tree. Trees were evaluated for various attributes including periodic bacterial titer in leaf and root tissues, morphometrics (canopy color, canopy density), yield (fruit size and weight), fruit internal quality (brix, titratable acidity) and residual of OTC in fruit (UHPLC-MS-TOF). Results indicate that OTC treatment had a positive effect on tree health. No OTC residue was detected in citrus juice after six months post-treatment. No significant yield increase was observed. Ongoing assessments intended to aid productivity in the second-year of the trial. Preliminary results from this comprehensive field trial will be presented. This is the first report on the efficacy of OTC in 'Rio Red' grapefruit for HLB management in South Texas.

Session 3 –

Mid-conference tour

Tours of the Dareton Primary Industries Institute and Auscitrus propagation scheme

Session 4 -

Huanglongbing and other bacterial diseases

CVC AND HLB IN BRAZIL – HISTORY, CURRENT SITUATION AND EFFECTIVENESS OF MANAGEMENT PRACTICES

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Citrus variegated chlorosis (CVC) and huanglongbing (HLB, citrus greening) are incurable vascular diseases of citrus transmitted by prolific insects that are difficult to control. The diseases result in blockage of sap flow, progress quickly within trees and orchards and cause devastating economic losses. CVC is caused by *Xylella fastidiosa* (*Xf*), a bacterium limited to the xylem and classified in distinct subspecies also affecting other important crops such as grapes, coffee, plum, and olive trees. The subspecies of *Xf* that causes CVC is transmitted by 13 species of xylem-feeding cicadellid sharpshooters. Symptoms include yellow spots on leaves, wilting, leaf drop and premature fruit maturation. The fruits do not fall, are sweeter, and the juice does not lose flavor, thus, the fruits can be consumed freshly or be processed by the juice industry. HLB is associated with three putative species of Liberibacter, '*Candidatus* Liberibacter americanus' (*C*Lam), '*Ca.* L. asiaticus' (*C*Las) and '*Ca.* L. africanus' (*C*Laf), which are associated with the American, Asiatic and African forms of the disease. *C*Las tolerates higher temperatures than *C*Lam and *C*Laf and reaches higher titers in diseased trees. It also has higher dissemination rates and a wider geographic distribution than *C*Lam and *C*Laf. The liberibacters are transmitted by phloem-feeding psyllids. Because of their geographic distributions, *C*Las is transmitted mostly by *Diaphorina citri* and *C*Laf mostly by *Trioza erytreae,* although both *C*Las and *C*Laf can be transmitted by both insect vectors. *C*Lam is known to be transmitted only by *D. citri*. In contrast to CVC, which affects only sweet oranges, HLB affects all commercially cultivated citrus species and hybrids causing leaf mottling, root death, fruit deformation, and leaf and fruit drop. Juice from affected fruits is more acidic, lower in sugar content and with a bitter taste, thus, the fruits are usually not consumed freshly or processed by the juice industry.

Comparative analysis of the insects that transmit the pathogens shows that sharpshooters prefer the older stages of new flush growth to feed and reproduce, possess longer life cycles, reach lower populations, migrate to shorter distances in citrus orchards, and are less efficient in transmitting *Xf* than the psyllids in transmitting *C*Las. These are probably the main characteristics that help to explain why it has been easier to control CVC than HLB using the same management practices to protect trees from infection, namely, (i) the use of certified pathogen-free trees produced in insect-proof screen houses for planting, (ii) removal of diseased trees to reduce sources of inoculum, and (iii) applications of systemic insecticides to young trees, and frequent sprays of contact insecticides to mature trees. In addition to citrus, sharpshooters feed and reproduce also on weeds, wild shrubs and coffee trees, and the *D. citri* on the ornamental, and citrus relative, orange jasmine, *Murraya paniculata*. However, none of these plants are susceptible to the *Xf* that causes CVC or to *C*Las, thus, not serving as important sources of inoculum to citrus trees. In the case of CVC, because *Xf* moves at speed apparently slower than *C*Las within orange trees, it is possible to prune trees to remove the symptomatic branch and restore the tree health. However, pruning against CVC will be effective only if the tree is mature (over ca. 3 years of age), the symptoms are premature and present in only a few leaves of a single branch, and if the cut to remove the symptomatic branch is made near the main trunk. Also, because CVC affects only orange trees and the other citrus are resistant to *Xf* infections, it is possible to reuse rootstocks of highly affected

diseased trees and, by grafting, substitute the diseased to new healthy scions, which grow faster and start production earlier than trees coming from regular plantings. Unfortunately, these two practices, or any other supposedly curative actions, were ineffective against the Asiatic HLB, given the susceptibility of commercial citrus species and hybrids to the disease, and the fast movement of *C*Las through the phloem, reaching roots long before the appearance of symptoms on leaves.

In Brazil, both CVC and HLB were first detected in the main citrus belt of the country, namely, São Paulo (SPS) and Minas Gerais (MGS) states, which comprises over 200 million trees growing on ca. 400 thousand hectares. The first trees with CVC were detected in the north of SPS in 1987. Soon afterwards, the disease was found in other regions of the state, and later in other states of the country and various countries in the South and Central Americas. The first trees with American and Asiatic HLB were detected in central SPS in 2004. As for CVC, shortly afterwards, the Asiatic and American HLB were found in other regions of SPS and, today, two decades later, only the Asian HLB is found in six Brazilian states, and several other southern, central, and northern American countries, causing great damage.

In the major SPS/MGS citrus belt, the incidence of CVC increased to a maximum of 43% in 2009, but progressively declined to 7% in 2015, 1% in 2020 and 0.45% in 2024. The incidence of HLB reached averages of 18% in 2018, 24% in 2022, 38% in 2023, and 44% in 2024. The progress of both diseases has been affected by the environment, with the hotter and drier regions mostly favoring CVC but disfavoring HLB. During a survey conducted in 2024, the incidence of CVC varied from 0.0% in the north to 2% in the center, and the incidence of Asiatic HLB varied from averages of 0.11% in the north to 79% in the center-east. The overall sharp decline in CVC incidence after 2009 has been attributed mainly to the removal of large areas affected by the disease, and to the more frequent applications of systemic insecticides and shorter spray-intervals of contact insecticides to control *D. citri*, which may have had probably greater impacts on sharpshooter populations. On the other hand, the overall abrupt increase of Asiatic HLB incidence in the last two years has been attributed mainly to an increase in inoculum sources inside and outside the orchards plus a high increase in psyllid populations, demonstrated through analyses of the Fundecitrus Psyllid Alert System data, which, since 2011, has monitored populations of *D. citri* fortnightly with the use of over 30 thousand yellow sticky traps distributed throughout the major citrus belt. The increase in inoculum sources of HLB resulted from a reluctance of citrus growers and homeowners to remove many diseased trees present in commercial plantings and backyards, and the increase in psyllid populations resulted from insufficient or poor quality insecticide applications, and the prevalence of insecticide-resistant insects, caused by a continued use of active ingredients with the same mode of action. Atypical climates observed in recent years in addition to an increase in the practice of irrigation and top pruning of trees may also have influenced the rates of *C*Las dissemination, as a result of changes in citrus flushing patterns, insect reproduction rates, and *C*Las multiplication in diseased trees.

The much higher incidence and more severe damage caused by Asiatic HLB than CVC, or any other citrus disease, make HLB a priority in research activities, at Fundecitrus and other collaborative national and international institutions, aimed at developing more effective and sustainable practices to manage the disease. Research has focused on (i) identifying genes that make some Oceanian species resistant to *C*Las so the genes they carry can be

incorporated into commercial citrus cultivars, (ii) making citrus trees genetically unattractive to psyllids, and *Bergera koenigii* toxic to *D. citri*, in this case to be planted around the borders of citrus groves to attract and kill the vector, and (iii) finding molecules that may minimize HLB damage, to be applied in the currently large geographic area of SPS affected by the disease. Meanwhile, with technical support from Fundecitrus, growers are taking additional actions to better protect their trees from *C*Las infections, including (i) keeping mature trees, through top and lateral pruning, at heights and width that favor adequate insecticide coverage, (ii) use of kaolin, mainly in the border of young groves, to repel the vector, and (iii) removal of all diseased citrus trees and orange jasmine in a 5-km radius surrounding the farm to reduce inoculum and psyllid sources. Fundecitrus helps growers by monitoring new flushes and *D. citri* populations to trigger alerts of critical moments to act against psyllid and testing old and new chemicals against the psyllid and the pathogen. To escape HLB pressure and take advantage of the high prices currently paid for the orange fruits, many growers are moving and starting new plantings in less risky but more distant areas that still remain free or have very low disease incidence. With all these actions it is predicted that, in the years to come, overall production and productivity of citrus orchards in Brazil will not be greatly impacted by HLB, and the country will continue providing most of the orange juice consumed in the world.

EFFECTOR *C*Las0185 TARGETS METHIONINE SULPHOXIDE REDUCTASE B1 OF *CITRUS SINENSIS* TO PROMOTE MULTIPLICATION OF '*CANDIDATUS* LIBERIBACTER ASIATICUS' VIA ENHANCING ENZYMATIC ACTIVITY OF ASCORBATE PEROXIDASE 1

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Abstract

Citrus Huanglongbing (HLB) has been causing enormous damage to worldwide citrus industries. As the main causal agent, '*Candidatus* Liberibacter asiaticus' (*C*Las) delivers a set of effectors to modulate host responses, while the modes of action adopted remain largely unclear. Here, we demonstrated that CLIBASIA_00185 (*C*Las0185) could attenuate reactive oxygen species (ROS)-mediated cell death in *Nicotiana benthamiana*. Transgenic expression of *C*Las0185 in Wanjincheng (*Citrus sinensis*) enhanced plant susceptibility to *C*Las. We found that methionine sulfoxide reductase B1 (CsMsrB1) was targeted by the effector, and its abundance was elevated in *C*Las0185-transgenic in citrus plants. Their interaction promoted *C*Las proliferation. We then determined that CsMsrB1 sustained redox state and enzymatic activity of ascorbate peroxidase 1 (CsAPX1) under oxidative stress. The latter reduced H_2O_2 accumulation and was associated with host susceptibility to *C*Las infection. Consistently, citrus plants expressing *C*Las0185 and CsMsrB1 conferred enhanced APX activity and decreased H_2O_2 content. Taken together, these findings revealed the tactic taken by *C*Las0185 to benefit *C*Las colonization via targeting CsMsrB1, which facilitated the antioxidant activity and subtracted ROS during pathogen infection.

Non-technical summary

Our study unveils the virulence strategy utilized by *C*Las0185 to depress plant innate immunity. This comprehensive investigation provides a detailed examination into the molecular events associated with pathogen-host interactions, thereby offering valuable insights for establishing control strategies against HLB.

ENHANCING qPCR DETECTION OF '*CANDIDATUS* LIBERIBACTER ASIATICUS' WITH A NOVEL SYNTHETIC INTERNAL STANDARD

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Abstract

'*Candidatus* Liberibacter asiaticus' (CLas), the bacterium associated with Huanglongbing (HLB), is typically detected in citrus and psyllid hosts using quantitative polymerase chain reaction (qPCR) assays with hydrolysis probes. Internal standards, usually host household genes, are included to mitigate false negatives caused by qPCR inhibitors. When the internal standard is detected while CLas is not, it is generally assumed that the pathogen is absent from the sample. Our study demonstrates that this assumption may be incorrect, as trace levels of CLas can go undetected if the internal standard is either too abundant or insufficiently similar to the CLas target sequence. To overcome these limitations, we developed a synthetic internal standard (IS) tailored to the CLas target. This IS uses the same primer-binding sites as the CLas sequence, with a modified internal region derived from smooth hammerhead shark (*Sphyrna zygaena*) DNA (IS-SHK). The IS-SHK standard matches the G/C content and melting temperature of the CLas target, ensuring compatibility with the qPCR reaction while avoiding interference from other nucleic acids present in citrus samples. To minimize competition between the IS-SHK standard and the CLas target, an average of 21 IS-SHK molecules are added per qPCR reaction. When the IS-SHK standard is detected at expected levels, but CLas is not, the absence of CLas can be confirmed. Conversely, the absence of both IS-SHK and CLas indicates the presence of qPCR inhibitors, prompting retesting of the sample. This optimized internal standard significantly enhances the reliability of qPCR assays, ensuring accurate detection of CLas for diagnosing HLB in citrus plants.

Non-Technical Summary

The bacteria associated with huanglongbing (HLB), a devastating citrus disease, are detected in citrus or psyllid samples using a laboratory test called qPCR. To ensure reliable results, the test includes a standard as a control. We developed a new type of standard, based on a shark DNA sequence, that ensures more reliable detection of the disease-associated bacteria. This improvement helps citrus growers get accurate disease diagnoses, reducing the risk of undetected infections.

CHARACTERIZATION OF A NEW RNA VIRUS ISOLATE OF CITRUS, NMV-M/CFL AND EXPLORATION OF ITS POTENTIAL FOR HLB CONTROL

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Abstract

The citrus industry in Florida, United States is fighting for survival against a devastating bacterial disease, huanglongbing (HLB). In search of HLB-resistant/tolerant bud-sport mutants, we discovered the NMV-M-like virus via high-throughput sequencing of grapefruit bud-sport selections that showed improved HLB resistance/tolerance. The virus is a non-enveloped, isometric virus with a single-stranded, positive-sense RNA genome of 6,731 nucleotides. It encodes a single polyprotein of 2,055 amino acid residues, and shares sequence identities from 76% to 96% with the known NMV isolates. This is the first report of a virus closely related to NMV-M infecting citrus and suppressing HLB, and it is designated as NMV-M/CFL. Our transmission studies indicated that this virus was transmitted by Asian citrus psyllids (*Diaphorina citri*). However, the virus did not cause any obvious symptoms (adverse effects) on any tested citrus varieties, including sweet orange and grapefruit. Based on the genome sequences, we developed a sensitive and specific RT-qPCR detection method, and using this method, we determined that the virus was systematically distributed in citrus plants, including roots. It is worth noting that plants infected with the NMV-M/CFL showed improved HLB resistance/tolerance by suppressing HLB symptoms without reduction of titer of the HLB bacterium, '*Candidatus* Liberibacter asiaticus'. The symptom suppression effects conferred by NMV-M/CFL infection were associated with the altered expression of H_2O_2 and ATP accumulation-related genes. The NMV-M/CFL infectious clone and its expression system for potential management of HLB and other applications in citrus genetics will be discussed.

Non-technical summary

We discovered a new RNA virus, NMV-M/CFL in citrus that does not cause disease but appears to suppress symptoms of HLB in Florida, United States. The virus, its infectious clone and expression system are being explored for use in management of citrus HLB.

EVALUATION OF THE SPATIAL AND TEMPORAL DISTRIBUTION OF '*CANDIDATUS* LIBERIBACTER AFRICANUS' IN CITRUS HOST PLANTS

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Abstract

Huanglongbing (HLB), also known as citrus greening, is one of the most devastating diseases affecting citrus orchards globally. It causes significant yield losses, compromises fruit quality, and poses a severe threat to the sustainability of the citrus industry and the economies of citrus-producing countries. HLB is caused by three closely related and phloem-restricted bacterial species: *'Candidatus* Liberibacter asiaticus' (CLas), *'Ca.* L. americanus' (CLam), and *'Ca.* L. africanus*'* (CLaf). The term "African Greening" specifically refers to the milder form of the disease caused by CLaf, which is vectored by the African citrus psyllid (*Trioza erytreae*). African Greening has been endemic to South Africa for over a century, during which growers and researchers have implemented various strategies and protocols to mitigate its impact on citrus production. However, compared to its more aggressive counterpart, CLas, CLaf remains relatively understudied, and many aspects of its biology and epidemiology are not clearly understood.

To address this knowledge gap, we conducted a comprehensive survey of a commercial citrus orchard to investigate the occurrence and progression of African Greening symptoms on foliage and fruit. Disease symptom incidence and severity were monitored across the orchard over the course of a growing season. Seven symptomatic trees were selected to be examined in more depth. DNA was extracted from bark samples collected at various set points along the trunks and branches, as well as from corresponding leaf samples over the growing season. These samples were analysed using a quantitative PCR assay to determine the presence and concentration of CLaf at different positions within the trees over the growing season.

Our preliminary findings, as expected, show a heterogeneous distribution of CLaf within individual trees, raising important questions about the pathogen's movement and colonization patterns. Orchard wide the distribution pattern is typical of a vector-borne pathogen with symptoms being more severe during the cooler times of the season.

Understanding the spatial and temporal distribution of CLaf is a critical step toward developing more effective disease management strategies.

CITRUS TRISTEZA VIRUS (CTV) VECTORS AS AN EPIGENETIC TOOL TO IDENTIFY THERAPEUTICS TO MITIGATE HUANGLONGBING (HLB)

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Abstract

Huanglongbing (HLB), also known as citrus greening, is an extremely devastating disease of commercial citrus. It is associated with the gram-negative intracellular bacterium *Candidatus* Liberibacter asiaticus (Clas). Almost all elite commercial citrus scion lines lack tolerance to HLB. In Florida, nearly 100% of productive trees are infected, leading to a >90% reduction in yield and more than 50% decrease in acreage from peak production in 2003-2004. The disease is spreading in two other major citrus producing states in the USA, Texas and California. Clas, which is vectored by the Asian citrus psyllid (ACP), is not in pure culture, making it impossible to screen therapeutics against the bacteria *in vitro*. Clas, as well as CTV, reside within the phloem tissue of citrus where the ACP feeds. CTV isolates have a range of phenotypes from pathogenic but nonvirulent to extremely virulent, which prevent the profitable production of citrus in certain geographical locations. In Florida, the T36 genotype is mild on all scion rootstock combinations except that of sour orange rootstock where it causes quick decline. The Florida CTV-T36 genotype has an infectious clone that proved stable as an over expression and RNA interference (RNAi) vector for many years using reporter and phenotype revealing genes. Thus, to identify therapeutics that would mitigate the effect of HLB on the citrus industry, we are using the CTV-T36 strain vectors as a phloem bio delivery tool. Our focus is to target the bacteria directly, identify citrus genes that induce resistance in the plant upon silencing or overexpression that would be later modified using CRISPR technology and amend the ACP citrus phloem diet. Using this approach, we identified antimicrobial peptides (AMPs) as well as citrus gene targets that induce a tolerant phenotype upon overexpression and silencing respectively. Due to their small size (<80 amino acids) and stability within the CTV vector, efficacious CTV-delivered AMPs will be used in budwood sources as a remedy until a permanent solution for HLB is available. Further, the ACP insect vector reproduction was severely affected by the overexpression of a *Bacillus thuringiensis* pesticidal protein or down regulation by RNAi of multiple ACP genes. The drawback for this approach is that the pesticidal protein is not highly stable, and RNAi targeting of ACP genes works about 50% of the time. There are other uses for the CTV overexpression and RNAi vectors. In short, the CTV vectors carry enormous potential for the fast identification of therapeutics that would mitigate HLB.

Non-technical summary:

To identify therapeutics that mitigate HLB, a pathogenic but nonvirulent CTV-T36 based expression/RNAi vectors are being used to deliver potential therapeutics to the phloem tissue of citrus where the bacteria causing citrus greening, *Candidatus* Liberibacter asiaticus, resides and the Asian citrus psyllid insect vector, *Diaphorina citri*, feeds.

Session 5
- Viruses I

LOOKING FOR RESISTANCE TO TRISTEZA DECLINE IN GENETICALLY MODIFIED SOUR ORANGE THROUGH RNA INTERFERENCE AGAINST THE THREE VIRAL SILENCING SUPPRESSORS

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Abstract

After the spreading of *Phytophthora spp*. worldwide, only citrus scions grafted onto sour orange remained healthy. Since then, bud grafting of scion varieties onto sour orange became a universal practice. Decades later, trees grafted onto sour orange started to decline because of `tristeza´, caused by the citrus tristeza virus (CTV). The virus infects naturally phloem-associated tissues of species of the genera *Citrus* and *Fortunella* within the family Rutaceae, and it is transmissible by several aphid species or by grafting. CTV caused a disaster due to the almost exclusive use of sour orange as rootstock, since all varieties grafted onto it, except lemons, decayed and ended up dying a short time after the viral infection due to vascular necrosis at the graft union.

Since then, different *Citrus* genotypes and relatives have been used as rootstocks and breeding programs were initiated to select rootstocks resistant to CTV. However, none of these rootstocks have the excellent attributes of sour orange in terms of tree performance and rusticity. The CTV genome is a plus-sense, single-stranded (ss) RNA molecule of ~20 kb organized into 12 open reading frames (ORFs) and two 5′- and 3′-untranslated regions (UTRs). The ten 3′ proximal ORFs include genes for the minor and major coat proteins (CPs), p27 and p25, respectively, and proteins p33, p6, p65, p61, p18, p13, p20 and p23. The p23 protein is a pathogenicity determinant, which facilitates CTV escaping from the phloem in sweet and sour orange and increases virus accumulation in the latter host. Proteins p23, p20 and p25 act as RNA silencing suppressors. Most strategies for engineering protection to CTV have been based on pathogen-derived resistance, namely by incorporating genes or sequences derived from the viral genome into different citrus genotypes. However, most of them only yielded partial resistance or delayed symptom appearance upon graft or aphid inoculation with CTV. The best results have been obtained so far with Mexican lime using an RNA interference (RNAi) strategy targeting three important determinants of virus pathogenicity (p23, p20 and p25). Resistance was achieved for 100% of the transgenic plants inoculated with the virus by means of bark grafting, either of the rootstock (non-transgenic) or directly on the transgenic scion. Here, we have incorporated the same construct directed to block p23, p20 and p25 into sour orange to attain transgenic resistance against CTV-induced decline (or tristeza syndrome) in this highly desirable rootstock. We tested resistance to tristeza through the inverted graft test, which permits assessing the graft incompatibility reaction in the sour orange transgenic lines grafted on CTV-infected citrus rootstocks. Results from these experiments will allow us to anticipate what may happen when sweet orange trees (or other citrus scions) are grafted on transgenic sour oranges in long-term field trials.

TESTING FOR POTENTIAL OF CITRUS TRISTEZA VIRUS (CTV) HYBRID T36-VT GENOTYPE EMERGENCE IN MIXED INFECTIONS USING ENGINEERED INFECTIOUS CLONES

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Abstract

Initially, field isolates of CTV were classified based on their phenotypes in different citrus genotypes. With the emergence of RNA seq technology, sequencing revealed that most field isolates are composed of different CTV genotypes/strains with different proportionalities. Sequencing also revealed that some CTV genotypes are recombinants between the 6 major CTV strains. There are three major CTV genotypes in Florida, namely T30, T36 and VT, that exist mostly in mixed infections. To evaluate the potential for recombinants to emerge among the CTV genotypes present in Florida, we simplified the system and engineered clones between T36 and VT genotypes using unique restriction sites focusing on different domains enabling engineering of the full-length infectious VT clone. We divided the 5' end responsible for replication into 5 genetic regions that included three hybrids within the two leader protease domains, 5'UTR till end of methyl transferase domain, the interdomain region and the helicase/replicase domain. The 3' half of the genome was divided into two domains one that included the quintuple gene block responsible for virion formation and small movement protein, whereas the other domain included the two strongest silencing suppressors, p20 and p23, the p13 gene and part of the p18 ORF. Additionally, the clones assembled sequentially from the 5' end to create the full-length VT infectious clone. Clones were tested for ability to initiate infection in *N. benthamiana* and analyzed for replication level by Northern blots and dilution ELISA. We were able to obtain a range of replicating hybrids. Some T36-VT infectious hybrids replicated better than either the parental T36 or VT full length infectious clone whereas others did not replicate at all. Hybrid clones that infected *N. benthamiana* were able to infect *Citrus macrophylla* albeit with different replication profiles based on dilution ELISA. In short, to create viable hybrids between the CTV T36 and VT strains, it is necessary to have the large IDR and the replicase/helicase domain of the same genotype.

A T30 GENOTYPE OF CTV CAUSES QUICK DECLINE OF CITRUS ON SOUR ORANGE ROOTSTOCKS IN CALIFORNIA

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Abstract

Citrus tristeza virus (CTV) induced Quick Decline (QD) of citrus has been the foremost challenge for citrus production on sour orange rootstock in the early to mid-20th century. In the 1930s-50s in southern California, the CTV-QD epidemic forced the industry to move to Central California and switch to CTV-tolerant rootstocks such as trifoliate and its hybrid rootstocks. T36 is a well-studied genotype of CTV, and it has been reported to induce QD in Florida. However, despite several decades of CTV surveys in California, T36 was never detected in QD trees. In recent years, next-generation sequencing (NGS) has streamlined deciphering full-length genome sequences. Over the past three years, 19 QD samples representing five properties in Tulare County were subjected to broad-spectrum and MCA13 ELISA and strain-specific RT-qPCR. Fourteen samples from QD trees were subjected to NGS. After removing host sequences, 16.8M reads were obtained, and *de-novo* assembly resulted in 13K contigs of 100bp or longer. With direct mapping of small RNAs against the representative sequences of the CTV genotypes, we obtained complete genome coverage for T30 (T385) alone, while the coverage for other genotypes was scattered across 3' genes but not enough coverage to reconstruct the complete genome. QD-sampled trees typically exhibited classic CTV-QD symptoms of budunion necrosis with some honeycomb pitting on the sour rootstock. Therefore, we conclude that our T30 strain (Accession PQ603092 not released yet) obtained from dying QD trees causes CTV-QD in California. Further examination of biological stresses along with molecular studies are needed to determine which gene sequences are involved in QD induction by generating hybrid infectious CTV clones with gene knockouts or substitutions and examining the phenotype of inoculated sweet orange on sour orange rootstock.

Non-technical summary

Quick Decline (QD) of citrus trees on sour orange rootstock has been a longstanding issue in California for over a century. While the T36 genotype of Citrus tristeza virus (CTV) was reported to induce QD in Florida, extensive surveys conducted in California over the past several decades have never detected T36 in QD-affected trees. Instead, samples from QD trees underwent Next-Generation Sequencing, which consistently identified the T30 genotype in all cases. This indicates that the T30 genotype of CTV is responsible for causing QD in citrus trees on sour orange rootstocks in California. Further research is needed to uncover the molecular signatures contributing to developing QD symptoms.

TRANSMISSION OF CITRUS YELLOW VEIN CLEARING VIRUS IN CALIFORNIA

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Abstract

Citrus yellow vein clearing virus (CYVCV) was detected in citrus and citrus relatives in urban landscapes in the cities of Tulare in central California in 2022 and Hacienda Heights in southern California in late 2023. These infections mark the first time the virus has been detected in the Western Hemisphere. CYVCV is reported to be transmitted mechanically and by aphid and whitefly vectors to citrus and non-citrus hosts. To determine the threat and spread potential of CYVCV to the citrus industry of California, we conducted greenhouse and growth chamber experiments on the transmission and host range of CYVCV. Using a whole genome sequenced isolate of CYVCV, the virus was graft inoculated to 22 different commercial propagations of citrus and citrus relatives representing orange, mandarin, tangelo, grapefruit, pummelo, lemon, lime, and kumquat cultivars. All inoculated cultivars became infected with CYVCV and maintained high virus titer. Strong symptoms were observed in Sour Orange and lemon cultivars. Mild symptoms of vein clearing, and slight leaf distortion were observed in new flush growth of sweet orange and mandarin cultivars but appeared to be somewhat ephemeral disappearing with warm greenhouse temperatures. Growth measurements of the trees one-year post inoculation indicate little effect on tree size. Mechanical transmission with purified CYVCV virions was achieved on inoculated leaves of mallow, Chenopodium, peas, Alyssum, mustard greens, and peppers. No vector transmission has been achieved from citrus to citrus to date, but these experiments are continuing with citrus and herbaceous hosts. The data being collected will provide critical insights on the spread and economic impact of CYVCV.

Non-technical summary

Experimental mechanical transmission of CYVCV was achieved using purified virions to a wide number of non-citrus host plants. Aphid and whitefly transmission of CYVCV has not yet been achieved but vector research has shifted to virus acquisition of purified virions via artificial feeding sachets and vector inoculation of herbaceous hosts as well as citrus. These data will be useful in developing grower strategies to mitigate the spread of CYVCV.

ClBeclin1 POSITIVELY REGULATES CITRUS DEFENSE AGAINST CITRUS YELLOW VEIN CLEARING VIRUS THROUGH MEDIATING AUTOPHAGY-DEPENDENT DEGRADATION OF ClAPX1

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Abstract

Autophagy is a highly conserved cellular-degradation mechanism implicated in antiviral defense in plants. Beclin1 is a homologue of ATG6 in plants and yeast, and involved in autophagy induction, and response to biotic and abiotic stresses. Previous study showed that the expression level of *ClBeclin1* was upregulated in Eureka lemon infected with citrus yellow vein clearing virus (CYVCV). However, the function of *ClBeclin* during CYVCV-infection is still unknown. In this study, we found that the titer of CYVCV was significantly reduced in Eureka lemon hairy roots over-expressed *ClBeclin,* and increased in hairy roots silenced *ClBeclin.* These results suggested that *ClBeclin* positively regulates Eureka lemon resistance to CYVCV. In addition, autophagy was induced by the interaction between ClBeclin and ascorbate peroxidase 1 (ClAPX1), which increased the accumulation of reactive oxygen species by suppressing the activity of ClAPX1. Further study showed that exogenous application of H_2O_2 reduced CYVCV content in plants. These results provide a new insight into host-CYVCV interaction.

Non-technical summary

During citrus yellow vein clearing virus (CYVCV) infection, ClBeclin1 targets ClAPX1 for degradation through autophagy pathway to promote reactive oxygen species production, enhancing CYVCV resistance in Eureka lemon.

CITRUS VEIN ENATION VIRUS ENCODES TWO DISTINCT SUPPRESSORS OF RNA SILENCING

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Abstract

Citrus vein enation virus (CVEV, genus *Enamovirus*, family *Solemoviridae*), employs two distinct viral suppressors of RNA silencing (VSRs), P0 and ORF3, to counteract host antiviral silencing. P0 acts as both a local and systemic VSR, while ORF3 (a putative coat protein) primarily acts as a systemic VSR. Host antiviral silencing suppression of these two proteins was demonstrated via *Agrobacterium*-mediated co-infiltration assays in *Nicotiana benthamiana* 16c plants. Mass spectrometry-based co-immunoprecipitation (co-IP) experiments revealed that CVEV-P0 interacts with S-phase kinase-associated protein 1 (SKP1) and cullin 1 (CUL1), via its F-box motif, forming a multi-protein SCF E3 ubiquitin ligase complex, further enhancing its suppression capabilities. Through co-IP and pull-down assays, we also found that P0 mediates autophagic degradation of Argonaute protein 1 (AGO1), a key component of the host RNA-induced silencing complex (RISC). Targeted deletion mutagenesis identified amino acid residues 36-83 and 176-245 as critical for localized silencing suppression by P0 and for inducing AGO1 degradation. In contrast, CVEV-ORF3 exerted systemic RNA silencing suppression without directly interacting with any proteins involved in host RNA silencing. ORF3 functionality likely involves indirect mechanisms that need further investigation. Deletion mutagenesis identified amino acid residues 68-100 as critical for the systemic silencing suppression activity of ORF3. We also found that neither P0 nor ORF3 binds directly to any RNA species, distinguishing their modes of action from other known VSRs. Incorporation of CVEV-P0 or -ORF3 into a potato virus X (PVX) infectious vector enhanced viral RNA accumulation and symptom severity in *N. benthamiana*, confirming their role as CVEV pathogenicity determinants. ORF3, although less efficient than P0, also significantly suppressed systemic RNA silencing and enhanced PVX virulence. This study provided novel insights into the distinct mechanisms used by CVEV to subvert host antiviral systems. Studies like this enhance our current knowledge of such quarantine-significant viruses, furthering ongoing efforts to develop targeted strategies to manage viruses in commercial citrus.

Non-technical summary

Citrus vein enation virus uses two proteins, P0 and ORF3 to suppress natural plant antiviral defenses, allowing the virus to spread more effectively. P0 degrades key components of the defense machinery, while ORF3 works indirectly. These findings help us understand how such plant viruses cause disease and inform strategies for protecting citriculture.

Session 6 -

Viruses II

INVESTIGATING THE BIOLOGY OF CITRUS YELLOW VEIN-ASSOCIATED VIRUS (CYVaV): INSIGHTS FROM A CALIFORNIA FIELD TRIAL

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Abstract

Yellow vein disease (YVD) of citrus was first observed in a small number of limequat trees in California in the late 1950s, and it was recently associated with an umbra-like viral RNA named citrus yellow vein-associated virus (CYVaV). Early studies by Prof. L.G. Weathers established YVD as a graft-transmissible disease with a broad host range under greenhouse conditions and no evidence of natural spread through pollen, insect vectors, or mechanical means. Despite its discovery decades ago, YVD remained understudied until recent advances in sequencing technologies enabled the complete characterization of the CYVaV genome. To evaluate CYVaV's potential quarantine significance to citriculture, a replicated field trial was set up at University of California, Riverside in 2020. The objective of this trial was to further examine CYVaV biology, natural transmissibility, and CYVaV-driven impacts on citrus health and productivity under field conditions. For the trial, 12 scion/rootstock combinations of commercially popular citrus varieties were graft-inoculated with a CYVaV isolate and monitored over three years. CYVaV infection was confirmed in 32% of graft-inoculated trees, after three rounds of graft inoculations, primarily in Lisbon lemon and mandarin varieties. Varieties such as Parent Washington navel, Cara Cara navel, and Miho Wase satsuma remained negative for CYVaV even after three graft-inoculation events. Visual symptoms were mild (mainly yellow veining on leaves) or absent, and molecular screening revealed systemic CYVaV presence in petioles, stems, roots, fruit, and pollen sampled from infected field trees. Two in-field pollen transmission bioassays showed no evidence of CYVaV transfer from infected to healthy trees via pollen. Furthermore, despite the presence of local aphid populations feeding on young flushes, no CYVaV spread was detected among the field trees. Tree growth metrics collected during the trial and two seasons' harvest data demonstrated no significant effects of CYVaV infection on tree health, fruit yield or quality. This study provided critical insights into CYVaV biology and transmissibility under field conditions, generated data for quarantine risk assessments, and emphasized the importance of proactive monitoring of emerging pathogens. While our findings suggest that CYVaV does not currently pose significant risks to commercial citrus, understanding its infection potential and transmission dynamics was essential to ensure that existing quarantine measures remain effective in preventing unexpected outbreaks or novel pathogen interactions.

Non-technical summary

Citrus yellow vein-associated virus (CYVaV) was studied in a replicated field trial to evaluate its natural transmissibility and potential impact on commercial citrus health and productivity. This study confirmed that CYVaV establishes systemic infections in citrus and can be detected in pollen, with no evidence of pollen- or aphid-mediated transmission, nor any significant negative effects on tree health, fruit yield or quality, under field conditions. While CYVaV poses no immediate threat to commercial citrus, this research highlights the importance of ongoing monitoring to ensure efficacy of existing quarantine measures and preparedness for potential future risks from emergent pathogens.

UNLOCKING VIRAL SYNERGISM: INTERACTIONS BETWEEN CITRUS VEIN ENATION VIRUS AND CITRUS YELLOW VEIN-ASSOCIATED VIRUS.

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Abstract

Mixed viral pathogens can interact synergistically, antagonistically, or both, significantly influencing virus-virus and virus-host interactions. Citrus vein enation virus (CVEV) and citrus yellow vein-associated virus (CYVaV), both endemic to California, are typically considered mild citrus pathogens when present individually. However, co-infection results in dramatic changes in disease outcomes, including plant death in sensitive citrus species. CYVaV, an umbra-like viral RNA, lacks a coat protein or movement protein, is graft-transmissible, and is asymptomatic in most citrus cultivars, with no apparent natural transmission mechanism. Such subviral entities have the potential to engage in helper-dependence relationships with unrelated viruses for full viral functionality. On the other hand, CVEV is an aphid-transmissible virus from the genus *Enamovirus* (family *Solemoviridae*), a family of viruses known for acting as a helper-virus in these disease complexes. To investigate the interaction mechanisms between CVEV and CYVaV, disease bank trees from the Citrus Clonal Protection Program (Riverside, California, USA) were used for single and co-pathogen graft inoculations on sensitive Mexican lime seedlings. Viral titer, negative-strand titer, disease severity index (DSI), and host parameters were compared between single and co-infected plants under standard greenhouse conditions. Co-infection led to faster and more severe CYVaV symptom development, increased DSI, and elevated overall titer and negative-strand accumulation (measured via RT-qPCR) of CYVaV compared to single infections. Interestingly, during co-infection, CVEV viral titer and negative-strand accumulation were also elevated; however, there was a decrease in CVEV's DSI and in the overall replication efficiency of CVEV's coat protein, a mild silencing suppressor, compared to single infections. These findings suggest a potential helper-dependence relationship between CVEV and CYVaV, as such interactions often enhance the symptoms and accumulation of at least one virus in the relationship. Furthermore, the decrease in replication efficiency of CVEV's silencing suppressor indicates additional, more complex virus-virus or virus-host interactions between CYVaV and CVEV. Understanding these interaction mechanisms in mixed infections is essential for elucidating differences in pathogenesis, host responses, and the potential transmission mechanisms of CYVaV.

Non-technical summary

Citrus vein enation virus (CVEV) and citrus yellow vein-associated virus (CYVaV) cause more severe disease and higher pathogen titer when in mixed infection than in single infections. Both viruses have characteristics suggesting they may be involved in a helper-dependent relationship, which may help elucidate the transmission mechanism of CYVaV.

DETECTION OF *BREVIPALPUS* TRANSMITTED VIRUSES IN MULTIPLE HOSTS IN CALIFORNIA, FLORIDA AND HAWAII ENHANCE THE POSSIBILITY OF CITRUS LEPROSIS DISEASE REEMERGENCE IN UNITED STATES

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Abstract

Citrus leprosis disease (CiLD) is associated with two unrelated taxa of single stranded RNA viruses; one is positive sense kitavirids (*Cilevirus* and *Higrevirus*) and the other is a negative sense *Dichorhavirus*. The relationship between *Brevipalpus* spp. and viruses associated with CiLD transmissions is often very strict as multiple false spider species (*B. yothersi*, *B. azores*, *B. californicus s.l., B. papayensis*, and *B. phoenicis s.s*) are involved. Even though CiLD was first reported from Florida, there is no record of CiLD since the mid-1960s. Currently, seven virus species [three kitavirids; citrus leprosis virus C (CiLV-C), citrus leprosis virus C2, hibiscus green spot virus 2 and four dichorhavirus; orchid fleck virus (OFV), citrus leprosis virus-N, citrus chlorotic spot virus, and citrus bright spot virus] and multiple strains of CiLV-C, CiLV-C2 and OFV are associated with CiLD syndrome. Hibiscus strain of CiLV-C2 (CiLV-C2H) is the only *Cilevirus* so far reported from the USA. The known natural host range of CiLV-C2H has been expanded when it was detected in passion fruit in Hawaii. OFV infects citrus, includes two strains (OFV-Orc and OFV-Cit) and each strain has two variants (OFV-Orc1, OFV-Orc2, OFV-Cit1 and OFV-Cit2). In 2021, *Brevipalpus* transmitted OFV was detected in rough lemon and mandarin orange in Hawaii and identified as OFV-Orc2 variant. Both the variants of OFV-Orc were detected either in single or in mixed infection in monkey grass (*Liriope* spp.), greenbrier (*Smilax auriculata*), lilyturfs (*Ophiopogon* spp.), pandan grass (*Pandanus amaryllifolius*), and cast-iron (*Aspidistra elatior*) plants in Florida and in orchids (*Cymbidium* sp., *Dendrobium* sp., and *Dendrochilum magnum*) in California utilizing OFV strain/variant specific RT-qPCR assays. Furthermore, high throughput sequencing followed by bioinformatic analysis was utilized to confirm the association of OFV-strain/variant in infected citrus, orchids and ornamentals. Interestingly, RNA1 and RNA2 contigs from three ornamentals (Liriope, Ophiopogon, and Aspidistra) shared 90-91% and 97% nt identities to OFV-Orc1 variant and generic OFV-Orc strain sequences, respectively. Phylogenetic analysis of RNA1 nt sequences created a separate clade in the dendrogram and confirmed the existence of a new OFV strain, which is more closely related to OFV-Orc1 followed by OFV-Cit2 than the OFV-Orc2. Taxonomic identification of local flat mites associated with the new OFV strain and CiLV-C2H, followed by transmission to orchids, citrus and ornamentals, will lead to knowing the possibility of re-emergence of citrus leprosis in Florida and other citrus growing states.

THE ROLE OF CYSTEINE-RICH PROTEIN IN ENHANCING MANDARIVIRUS INFECTIVITY AND PATHOGENICITY

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Abstract

Mandariviruses represent a major threat to the citrus industry due to their wide-ranging transmission routes and high pathogenicity. Despite their economic importance, the molecular mechanisms underlying mandarivirus pathogenicity remain largely elusive, particularly regarding the roles of the virus-encoded cysteine-rich proteins (CRPs) during infection. In this study, we employed a potato virus X (PVX) vector to ectopically express two CRPs originating from citrus yellow vein clearing virus (CYVCV) and citrus yellow mottle-associated virus (CiYMaV) in *Nicotiana benthamiana*. Both CRPs significantly exacerbated disease symptoms and enhanced viral accumulation. Notably, the CRP from CiYMaV triggered cell death in *N. benthamiana* leaves, and a zinc finger motif was identified as the key structural element responsible for this hypersensitive response. The CYVCV CRP served as a suppressor of local RNA silencing specifically induced by single-stranded GFP, but not by double-stranded GFP. Furthermore, mutational analyses of infectious clones for both CiYMaV and CYVCV in citrus plants revealed that these CRPs are indispensable for symptom development and viral accumulation. Collectively, these findings establish CiYMaV and CYVCV CRPs as pathogenicity determinants, thereby expanding our understanding of the functional repertoire of the mandarivirus proteome and providing a promising target for developing citrus resistance strategies against mandariviruses.

Non-technical summary

Our study presents compelling evidence that CiYMaV and CYVCV CRPs, nonstructural proteins, act as pathogenicity determinants with multiple functions. This comprehensive investigation provides a broad understanding of the function of mandarivirus CRPs, thereby offering valuable insights for establishing effective prevention and control strategies against mandariviruses.

CHARACTERIZATION OF TWO DISTINCT VIRAL SUPPRESSORS OF RNA SILENCING ENCODED BY CITRUS TATTER LEAF VIRUS

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Abstract

Citrus tatter leaf virus (CTLV), a strain of the apple stem grooving virus (ASGV), is a single-stranded positive-sense RNA virus in the family *Betaflexiviridae*. It is associated with bud union crease and tree decline in trifoliate and trifoliate hybrid rootstocks that are widely used in commercial citrus production. This study identified two viral suppressors of RNA silencing (VSRs) encoded by CTLV: the coat protein (CP) and the movement protein (MP). Using *Agrobacterium*-mediated co-infiltration assays in *Nicotiana benthamiana* 16c plants, the CTLV MP was shown to function as a local silencing suppressor, while the CP exhibited systemic silencing suppression activity. The CP was found to enhance viral systemic infection and accumulation, while the MP primarily influenced local silencing suppression and initial infection sites. Deletion mutagenesis in the context of a potato virus X (PVX) infectious vector identified regions within the CP (amino acids 36-70) and MP (amino acids 112-143) critical for their silencing suppression activity. Mutants lacking these regions failed to suppress silencing or enhance PVX infectivity in *N. benthamiana*. RNA immunoprecipitation and RNA-protein pull-down assays revealed that the MP binds to double-stranded RNA (dsRNA), suggesting a potential role in shielding viral RNAs from host silencing mechanisms. However, mass spectrometry-based immunoprecipitation proteomics demonstrated that neither the CTLV CP nor the MP interact directly with cellular components involved in host RNA silencing pathways. Both CTLV VSRs significantly increased the infectivity and symptom severity of the PVX infectious vector when introduced into *N. benthamiana*. These findings provided critical insights into the molecular interactions between CTLV and its hosts, demonstrating the virus's reliance on two distinct VSRs to establish and maintain infection. These results not only advance our understanding of the role of VSRs in viral pathogenesis but also underscore their potential as targets for developing innovative management strategies against CTLV and related viruses.

Non-technical summary

Citrus tatter leaf virus (CTLV) suppresses plant antiviral defenses by using two proteins that enhance viral infection and symptom severity: the coat protein (CP) and the movement protein (MP). This study provides valuable insights into the mechanisms by which these two proteins help CTLV overcome host defenses and may inform new strategies in the future to protect commercial citrus from CTLV and related pathogens.

Session 7 -
What's in a name?

BINOMIAL NOMENCLATURE FOR VIRUS AND VIROID SPECIES

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Abstract

The International Committee on Taxonomy of Viruses (ICTV), created in 1966, is responsible for the classification and taxonomic nomenclature of all viruses, viroids and some satellite viruses infecting animals, plants, fungi, bacteria and archaea. Historically, virus nomenclature diverged from the universal binomial system used for other organisms. However, the ICTV has recently adopted binomial nomenclature for virus and viroid species, following the standard procedure used in scientific names, consisting of a genus name followed by a species epithet. This system provides consistent and accurate species identification across languages and geographic regions. By standardizing names, it eliminates ambiguity, facilitates global scientific collaboration, and ensures precise identification of closely related species. Furthermore, by clearly differentiating scientific and common names, it makes it easier to distinguish between viruses, the entities that infect living organisms, and virus species, taxonomic categories defined by shared properties (Zerbini et al., 2022). Moreover, only the species names have been changed, not the common names. For example, the causal agent of tristeza disease is still called citrus tristeza virus (the common name) but is now classified as a member of the species *Closterovirus tristezae*. Likewise, citrus leprosis disease is still caused by citrus leprosis virus C, which now belongs to the species *Cilevirus leprosis* and citrus exocortis is still caused by citrus exocortis viroid, of the species *Pospiviroid exocortiscitri*. Although these new species names may initially seem challenging to memorize, sooner or later we will get used to them. And I am sure we will all learn to like them!

Citations

Zerbini, F.M. et al. Differentiating between viruses and virus species by writing their names correctly. Arch Virol 167, 1231–1234 (2022). https://doi.org/10.1007/s00705-021-05323-4

Session 8 -

Programs

SAFEGUARDING CALIFORNIA CITRUS FOR OVER 65 YEARS: INNOVATIONS AND ACHIEVEMENTS OF THE CITRUS CLONAL PROTECTION PROGRAM

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Abstract
Ensuring the availability of pathogen-tested, true-to-type citrus propagative materials is essential for the sustainability of citrus production and research. The Citrus Clonal Protection Program (CCPP), part of the National Clean Plant Network (NCPN) at the University of California, Riverside, has been safeguarding California's citrus industry since its inception in the 1930s through pioneering citrus virus research. In partnership with the United States Department of Agriculture, the California Department of Food and Agriculture, and key industry stakeholders such as the Citrus Research Board and the California Citrus Nursery Board, the CCPP provides a secure pathway for introducing and distributing citrus accessions into California and across the U.S. from citrus-producing regions worldwide. The CCPP utilizes advanced technologies in pathogen detection, bioindexing, tissue culture, and controlled environment agriculture, integrating circular economy principles and laboratory information management systems. Real-time electronic monitoring ensures the program's pivotal contributions to the competitiveness of citrus nursery stock and fruit producers, as well as its role in supporting research on critical challenges such as Huanglongbing (HLB). Since 2019, the CCPP has processed 365 citrus introductions, conducted 7,716 pre-index laboratory tests, and intercepted citrus pathogens in 95 introductions. It has completed 4,306 shoot-tip grafts (STGs) for 325 introductions and 7,500 therapy success tests for 419 STGs of 276 introductions. The program has performed 5,918 laboratory and 10,199 biological tests to release 217 accessions (107 for commercial and 110 for research purposes) from 13 countries. It has distributed 405,007 buds of 424 accessions to 3,761 nurseries, growers, scientists, and citrus enthusiasts, reflecting over a 100 percent increase in budwood distribution compared to previous years. Economic analyses estimate average per-acre benefits of up to $6,000 for clean citrus cultivars, underscoring the program’s vital contributions to the citrus industry and research communities.

Non-technical summary
The Citrus Clonal Protection Program (CCPP) ensures the availability of pathogen-tested, true-to-type citrus propagative materials, vital for sustaining citrus production and research. Using advanced technologies and global partnerships, the program has introduced and distributed hundreds of disease-free citrus varieties while supporting efforts to tackle critical challenges like Huanglongbing (HLB). Its contributions significantly enhance citrus industry productivity and economic benefits.

THE JOURNEY OF POST ENTRY QUARANTINE OF CITRUS IN SOUTH AFRICA

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Abstract

The disease management unit at the Agricultural Research Council's Tropical and Subtropical Crops, Mbombela campus (ARC-TSC) is a role player in the mandate of the Citrus Improvement Scheme (CIS) to ensure the supply of pathogen-free propagation material to the South African citrus industry. The Post Entry Quarantine (PEQ) function, assigned by the Department of Agriculture (DoA) to the ARC-TSC is critical to ensure that no foreign pathogens are introduced with imported budwood. Budwood imports are subjected to shoot tip grafting (STG), to eradicate graft transmissible pathogens, *i.e.* viruses, viroids and bacteria. Once STG is completed, the material is indexed on biological indicators and molecular tests to detect various pathogens. Seed imports are subjected to grow out tests and subsequent molecular diagnosis for pathogens as per the quarantine requirements. An historical summary of citrus introductions will be discussed in this presentation and current cultivar importation trends and statistics over time will be shared.

Non-technical summary

The Post Entry Quarantine of citrus is critical for maintaining high biosecurity standards in a country and policy makers and the citrus industry rely on the service to ensure disease-free citrus production.

DYNAMICS OF PAKISTAN'S CITRUS SECTOR: THREATS AND CHALLENGES

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Abstract

The Pakistan citrus sector is facing significant challenges that threaten its survival. These challenges extend beyond production and transcend to fruit quality, putting the very existence of citrus orchards at risk. Both biotic and abiotic factors are adversely affecting the production of various citrus varieties, particularly Kinnow mandarin, under current management practices in Sargodha, Punjab - the primary citrus-growing region of Pakistan. Over the past four years, climate change manifested in erratic rainfall, hailstorms, extreme summer heat, severe winter cold, and prolonged smog and fog has exacerbated the situation. These conditions have led to increased infestations of insect pests, such as sucking and chewing insects, as well as the spread of fungal and viral diseases. As a result, citrus exports have been declining each year, with poor fruit quality further diminishing the sector's competitiveness in international markets. This season (2024-25), the yield of Kinnow mandarin has dropped by 35-40% compared to last season, primarily due to the combined effects of climate change, pest attacks, and diseases. The 2024-25 harvesting season has also been delayed by 15 days because of prolonged summer and the late onset of cold weather, further shortening the fruit availability window. At the downstream end of the fruit value chain, poor post-harvest handling, inadequate storage facilities, and inadequate modern processing technologies have further compromised fruit quality, making it difficult to meet the stringent standards required for export. Key pests affecting Pakistani Kinnow mandarin include citrus psylla, leaf miners, citrus thrips, mealybugs, whiteflies, red mites, red scales, and cottony cushion scales. Major pre-harvest diseases include citrus melanose, citrus scab, citrus canker, citrus phytophthora, and citrus tristeza virus. Last year, the sector suffered significant commercial losses due to nearly 45 days of smog and fog, with the devastating effects of citrus melanose. A recent survey report warns that if timely and scientifically sound measures are not implemented to mitigate the impact of climate change and control fungal and viral diseases, Kinnow exports could become unsustainable within five to six years. Already, half of the 250 Kinnow processing and packing houses have shut down due to heavy financial losses. The decline in fruit quality has led to reduced demand in key export markets, further straining the industry. This crisis threatens the livelihoods of 300,000 people and risks an investment of Rs. 300 billion in the industry. Urgent action is needed to improve fruit quality, adopt modern agricultural practices, and enhance post-harvest management to safeguard the future of Pakistan's citrus sector and restore its position in the global market. A value chain approach of whole chain collaboration between the chain actors and the development partners is being considered as a potential pathway to tackle the wicked problems faced by the Pakistani Kinnow industry.

HISTORY AND EVOLUTION OF THE AUSCITRUS BUDWOOD SCHEME

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Abstract

At an Australian fruit industry conference in 1927, a collective decision was made to establish a controlling body for the buying and selling of selected citrus budwood in New South Wales. This led to the formation of the Cooperative Bud Selection Society in 1928 with the help of government start-up funding of £1500. The Society evolved over the next 77 years into what we know today as Auscitrus; the only Australian supplier of citrus propagation material from health-tested sources to industry.

In the early days, buds were selected from 'healthy' trees for propagation, with increasing rigour added to the process over time, largely influenced by pathogen discoveries. The industry changed to *Citrus trifoliata* in the 1940's after extensive orchard loss due to Phytophthora, but then those rootstocks were ravaged by citrus exocortis viroid (CEVd) until disease-free sources were identified. In the 1990's, the discovery of orange stem pitting strains of citrus tristeza virus (CTV) in the Central Burnett region of Queensland led to their arm of the national network ceasing budwood supply.

The Auscitrus scheme evolved with financial and technical support from the New South Wales Department of Primary Industries and Regional Development (NSW DPIRD). Auscitrus now operates autonomously on their own land, but NSW DPIRD provides an independent pathogen elimination and testing service.

In the modern-day scheme, imported and Australian selections of new varieties are tested and cleared of graft-transmissible pathogens (endemic and exotic) before release to industry. Foundation trees of these new varieties are then placed in the biosecure environment of the National Citrus Repository. Budwood and rootstock seed supply trees are propagated from high health foundation trees, and these daughter trees are used to supply propagation material to industry. The National Citrus Repository house at NSW DPIRD's Elizabeth Macarthur Agricultural Institute also serves as an offshore plant quarantine facility for New Zealand.

Industry and government programs work collectively to support and strengthen Auscitrus and Australian citrus biosecurity in the areas of diagnostics and surveillance, germplasm management, public education, policy and strategy. This includes a research program developing new or validating published diagnostic assays to ensure we are using the best methods available. The use of health-tested propagation material is not mandatory in Australia but there is agreement that this should be achieved prior to an incursion of the devastating citrus disease, huanglongbing.

Non-technical summary

Australian industry foresight combined with long-term government support enabled the evolution of Auscitrus into an independent and self-funded industry organisation. Supporting activities funded by industry and government continue to strengthen Auscitrus operations against citrus biosecurity threats.

Session 9 -

Viruses and Viroids

ROOTSTOCK SENSITIVITY TO CITRUS VIROIDS - VALENCIA FIELD AND GLASSHOUSE TRIALS

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Abstract

Citrus viroids are mechanically transmitted and can unintentionally be introduced to, and spread in nurseries and orchards by practices such as budding, pruning, girdling and harvesting. Citrus dwarfing viroid (CDVd) is associated with tree stunting on sensitive trifoliate or trifoliate-hybrid rootstocks and is often detected in older, commercial orchards, frequently in combination with hop stunt viroid (HSVd) in South Africa. Adjacent, newly planted orchards are exposed to viroid infection if cutting tools are not sanitised between use in orchards. Additionally, where existing cultivars are not performing, farmers are more frequently opting to top-work new scion cultivars to existing orchard rootstocks, rather than orchard renewal by replacement with nursery trees. There is therefore the danger of top-working to a viroid infected orchard. A further source of infection is the use of field-cut budwood as a cost-cutting measure, rather than purchasing pathogen free budwood from the Citrus Improvement Scheme. A change in orchard establishment practices in South Africa, necessitated research to investigate the risks pertaining to these changes.

We have limited experience regarding the effect of viroids on hybrid rootstocks introduced in the past two decades, including new selections from the USA. A field trial is underway to test the sensitivity of newer, commercial or potentially commercial rootstocks to citrus dwarfing viroid (CDVd) and the non-cachexia variant of hop stunt viroid (HSVd). A Valencia trial was planted in 2019 in Mpumalanga Province to investigate the effect of CDVd as a single infection and as a combined infection with HSVd. Smaller canopy volumes were associated with viroid treatments with some rootstocks and a reduction in yield mirrored canopy size reduction with these treatments.

We are further testing the impact of CDVd on growth of rootstocks in a glasshouse trial and comparing viroid concentrations. An initial trial with five rootstocks is in progress. Rootstocks Carrizo Citrange, Furr C-57, Carpenter C-54, Swingle Citrumelo and Rough Lemon were graft-inoculated with CDVd as a single infection and control plants were uninoculated. One shoot was allowed to grow and side shoots were removed. Growth was determined by stem-length measurements of various growth periods after stems were cut-back over a total period of 23 months. Absolute quantification was done for inoculated rootstocks to compare viroid concentrations. Growth of five rootstocks was not affected by CDVd infection over a cumulative 23-month period. Rough Lemon showed lower CDVd copies compared to the trifoliate-hybrid rootstocks. Results suggest that CDVd-induced tree stunting is likely impacted by the scion-rootstock combination and not solely the rootstock.

DETERMINING CITRUS VIROID PREVALENCE IN AUSTRALIA WITH MULTIPLEX RTqPCR ASSAYS

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Abstract

Citrus production is a significant economic activity found in disparate growing regions across mainland Australia, with total production valued at $1.11 billion (Hort Innovation). Viroids are small, circular RNA molecules that are spread mechanically via pruning and hedging tools or through grafting infected propagules. Symptoms associated with viroid infection can range from severe to mild and are determined by the viroid or viroids present, and the scion and rootstock combination. Citrus samples collected between 2020 and 2024 from across Australia were tested for viroids using two RT-qPCR assays. The first assay detected citrus exocortis viroid (CEVd, *Posipiviroid exocortiscitri*), hop stunt viroid (HSVd, *Hostuviroid impedihumuli*), and citrus bark cracking viroid (CBCVd, *Cocadviroid rimocitri*) (Osman *et al.* 2017), while the second TaqMan RT-qPCR assay we designed and developed to detect all citrus-infecting apscaviroids include citrus bent leaf viroid (CBLVd, *Apscaviroid curvifoliumcitri*), citrus dwarfing viroid (CDVd, *Apscaviroid nanocitri*), citrus viroid V (CVd-V, *Apscaviroid epsiloncitri*), citrus viroid VI (CVd-VI, *Apscaviroid zetacitri*), and citrus viroid VII (CVd-VII, *Apscaviroid etacitri*).

Over 700 samples, collected from all production areas of Australia, were tested using the two assays, and viroids were detected in approximately 20% of samples as individual or mixed infections. CDVd was the most prevalent viroid and was detected in 14% of samples; both CEVd and HSVd were found in ~5% of samples. CBCVd was not detected in this survey and the remaining citrus-infecting apscaviroids (CBLVd, CVd-V, CVd-VI, CVd-VII), were found in less than 1% of samples. A number of *Murraya paniculata* plants were also sampled as part of the survey, given this species is a potential transient host of HLB, with CBLVd and CDVd detected. Development of the new pentaplex assay will allow the Auscitrus budwood scheme to more efficiently test budwood and rootstock seed source trees to ensure high health status propagation material is supplied to the Australian citrus industry.

Citations

Osman F, Dang T, Bodaghi S, Vidalakis G. 2017. One-step multiplex RT-qPCR detects three citrus viroids from different genera in a wide range of hosts. Journal of Virological Methods 245:40–52

UNVEILING THE ROLE OF THE TERMINAL RIGHT DOMAIN IN MODULATING ACCUMULATION AND PATHOGENICITY IN CITRUS EXOCORTIS VIROID

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Abstract

Citrus exocortis viroid (CEVd), a pathogenic noncoding RNA with five functional domains, can infect most citrus varieties especially trifoliate orange and cause the exocortis and bark-scaling disorder diseases. The relationship between pathogenicity and the functional domains of CEVd remains unclear. The terminal right (TR) domain is essential for the accumulation and pathogenicity of certain viroids. An artificial chimera of CEVd (CEVd-CB) was constructed through TR domain exchange with the closely related citrus bark cracking viroid (CBCVd) to assess changes in infection. CEVd-CB caused attenuated symptoms and accumulated lower levels in tomato, citron, and tobacco than the original CEVd, due to the reduced secondary structural stability and nuclear import ability. Furthermore, the artificial chimera of CBCVd (CBCVd-CE), created by exchanging the TR domain of CEVd, demonstrated enhanced capabilities in nuclear entry and accumulation of CBCVd. These results demonstrate the crucial role of the TR domain in modulating viroid accumulation and pathogenicity, thereby deepening our understanding of the domain function of pathogenic noncoding RNAs in citrus plants.

Non-technical summary

Citrus exocortis disease poses significant challenges in the production of virus-free seedlings. The pathogenic mechanisms underlying this disease remain poorly understood. In this study, we employed a chimeric viroid to elucidate the role of the terminal right domain in both pathogenicity and viroid accumulation by influencing secondary structure stability and nuclear import capabilities. Our findings enhance the understanding of the relationship between functional domains and viroid pathogenicity in woody plants. This insight will facilitate the development of more effective strategies for combating viroid diseases in fruit trees.

CURRENT AND FUTURE RESEARCH INTO VIRAL AND OTHER GRAFT TRANSMISSIBLE DISEASES OF CITRUS IN PAKISTAN

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Abstract

Pakistan's citrus industry is highly susceptible to various graft-transmissible pathogens, including citrus tristeza virus (CTV), citrus viroids, and *'Candidatus* Liberibacter asiaticus' (CLas), the causative agent of huanglongbing (HLB). This paper provides a comprehensive overview of current research on these pathogens in Pakistan, highlighting advances in molecular characterization, phylogenetic analysis, and disease management.

Recent studies have revealed the distribution and molecular diversity of CTV isolates in Pakistan, utilizing techniques such as restriction fragment length polymorphism (RFLP) and biological indexing. Genetic studies have identified new CTV variants with potential for cross-protection, while viroid research has focused on citrus viroid V and I-LSS variants, tracing their geographic origins and evolutionary patterns. Additionally, transcriptome analyses have identified novel viroid variants, such as citrus bark cracking viroid (CBCVd), and provided insights into host-pathogen interactions in citrus.

The discovery of citrus yellow vein clearing virus (CYVCV) and a newly identified mandarivirus associated with leaf yellow mottle disease marks a significant milestone in citrus virology in Pakistan. Furthermore, genome sequencing of CLas strains from Pakistan has led to the identification of unique prophages, offering clues about the pathogen's evolution and potential management strategies. These findings underscore the importance of continuous surveillance, molecular diagnostics, and the exploration of mild strains for cross-protection.

Future research should focus on expanding the genetic resources available for these pathogens, improving disease resistance through breeding programs, and developing sustainable management practices to safeguard Pakistan's citrus industry.

VIRUSES AND VIROIDS IN CITRUS PLANTS: INSIGHTS FROM HISTORIC LATIN AMERICAN HERBARIUM SAMPLES

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Abstract
Citrus production is a key activity for the global agricultural sector, playing a substantial role in economic development, sustainability, and public health. While China leads in lemon and lime production, Brazil is the main producer of sweet oranges (*Citrus sinensis*). Due to their widespread cultivation, citrus plants are vulnerable to various pathogens, including viruses and viroids. This study investigated the diversity of viruses and virus-like pathogens affecting sweet orange plants since the establishment of commercial and non-commercial orchards across several Latin American countries. To achieve this, high-throughput sequencing was carried out on eight herbarium samples collected between 1932 and 1975 from trees that originally exhibited necrosis, chlorosis, and mosaic symptoms. These samples were preserved at the Herbarium of the Instituto Biológico in São Paulo, Brazil. Bioinformatics analyses revealed the genomes of viruses belonging to the genus *Cilevirus* (family *Kitaviridae*) causing citrus leprosis (CL). The genome of seven isolates of citrus leprosis virus C (CiLV-C; *Cilevirus leprosis*) were recovered from Argentina, Brazil, and Paraguay samples collected in the period 1932-1975, and one isolate of citrus leprosis virus C2 (CiLV-C2; *Cilevirus colombiaense*) sampled in Venezuela, in 1937. Particularly, the Paraguay isolate of CiLV-C (1932, named ASU) characterized a novel lineage in the CiLV-C population. CiLV-C ASU shares ~85% nucleotide sequence identity with viruses of the known CiLV-C lineages, i.e. CRD, found throughout the Americas and present in Brazil at least since 1932, and SJP, identified in 2015 in commercial orchards in the Brazilian citrus belt. Moreover, we identified the complete genome of the oldest known isolate of citrus tristeza virus (CTV; *Closterovirus tristezae*) from Brazil (1975), along with the full genomes of viroids from three species within the family *Pospiviroidae* in four samples from Brazil and Paraguay (1932–1941). Lastly, from samples of Brazilian citrus plants affected by zonate chlorosis disease (ZCD) (1933 and 1965), only the genomes of four isolates of the kitavirus hibiscus green spot virus 2 (HGSV2, *Higrevirus waimanalo*) were recovered, which strongly implicates the HGSV2 as the ZCD causal agent. Previously, HGSV2 was associated with a leprosis-like disease in Volkamer lemons in Hawaii, USA, 2012. This study improves our understanding of viral and viroid diversity in citrus plants since the citrus industry´s onset in the Americas, providing valuable insights into disease epidemiology and virus evolution, and demonstrating the potential of herbarium samples for plant archaeovirology research.

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GROWTH EVALUATION OF CITRUS ROOTSTOCK SEEDLINGS AND GRAFT-TRANSMISSION OF CITRUS VIRUSES AND VIROIDS IN DIFFERENT ROOTSTOCKS

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Abstract
Virus and viroid infections are graft-transmissible, and their infections may affect growth and production of citrus trees. Citrus is predominantly grown on sour orange (*Citrus aurantium*) rootstock in the Lower Rio Grande Valley (LRGV) of South Texas. Citrus tristeza virus (CTV), citrus tatter leaf virus (CTLV), citrus exocortis viroid (CEVd), and hop stunt viroid (HSVd) have been reported in the LRGV. Moreover, we recently reported several CTV strains in the Upper Gulf Coast area. Although the insect vector, the brown citrus aphid, has not been reported, CTV-positive trees were occasionally found in the LRGV. Citrus grown on sour orange rootstock is highly susceptible to CTV. There is a need for the evaluation of alternate rootstocks in Texas that are tolerant to citrus viruses and viroids and perform well in highly calcareous South Texas soils. Seedlings of sour orange, trifoliate orange (*Poncirus trifoliata*), US-942 (*C. reticulata* 'Sunki' x *P. trifoliata*), Bitters C-22 citrandarin (x *Citroncirus sp.*), C146 (*Citrus* x *aurantium* 'Sunki' x *Citrus trifoliata* 'Swingle'), X639 (*C. reshni* 'Cleopatra' x *Poncirus trifoliata*), and Alemow (*C. macrophylla)* were established and kept under insect resistant screenhouse conditions. Growth parameters of seven rootstock seedlings were measured at three and six months after planting and before graft-inoculation. Rootstocks C146 and X639 had a faster rate of growth and more leaves. Rootstocks alemow and sour orange had a larger canopy radius and therefore are more vigorous. This study evaluated the response of rootstocks against CTV, CTLV, HSVd, and CEVd + HSVd by graft-inoculation. CTV, CTLV, HSVd, and CEVd were successfully transmitted by grafting infected buds onto rootstock plants. All rootstocks used in this study were susceptible to virus and viroid infection. Viruses and viroids were detected in the roots of all rootstocks except for CTLV in US-942. Symptoms of leaf curl, chlorosis, and distorted leaves were observed in one C22 rootstock plant graft-inoculated with CTLV. Corky veins were observed in one sour orange rootstock plant graft-inoculated with CTV. In addition, three sour orange plants showed a leaf cupping symptom.

Session 10 –

Diagnostics I

OPTIMIZING RNA EXTRACTION PROTOCOLS FOR RELIABLE DETECTION OF CITRUS VIRUSES AND VIROIDS USING RT-qPCR

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Abstract

Accurate detection of plant pathogens is fundamental to effective disease management and the success of citrus certification schemes, which aim to ensure the propagation of healthy, pathogen-free plant material. The efficiency of detection assays is greatly influenced by the quality of RNA used, making the RNA extraction protocol a critical step in the diagnostic process. This study evaluated four RNA extraction protocols, differing in maceration buffer and precipitation method (CTAB:LiCl (CL), CTAB:Ethanol (CE), Trizol:LiCl (TL) and SDS:Isopropanol (SI)), to determine their performance in detecting citrus tristeza virus (CTV), citrus virus A (CiVA), and three citrus viroids (CEVd, CDVd, HSVd) in infected plant material using RT-qPCR.

Significant variation was observed in the detection efficiency of viruses and viroids depending on the RNA extraction method. For CTV, the CL method produced the highest copy number indicating its suitability for positive sense RNA virus detection. CiVA was most efficiently detected using the SI protocol, which also demonstrated improved sensitivity across replicates. Viroid detection showed notable variability among the protocols. CE and CL provided the most reliable results for CEVd and CDVd detection, while HSVd detection was optimal using CE, but was also adequately detected using SI and CL protocols. The TL method, while performing sufficiently for CDVd and CiVA, was less consistent across all targets, highlighting the importance of method selection for reliable pathogen detection.

Overall, the CE protocol showed consistent results for viroid detection across diverse tissue types. In contrast, the CL protocol, while excelling in CTV detection, displayed moderate variability for viroids. The SI protocol emerged as the most versatile for mixed pathogen detection. Inconsistencies in viroid detection, observed across all protocols, can be attributed to the uneven distribution of viroids in plant tissues and sampling fluctuations, emphasizing the need for RNA extraction methods that maximize recovery and ensure representative sampling.

This study highlights the importance of optimizing RNA extraction protocols for RT-qPCR-based diagnostics in citrus certification schemes. By identifying effective RNA extraction methods, we enhance the reliability of pathogen detection, support the production of disease-free propagation material, and contribute to sustainable citrus production systems. These findings provide a strong foundation for improving diagnostic workflows in citrus pathology.

INNOVATIVE ON-SITE SAMPLE PREPARATION AND DETECTION METHODS FOR CITRUS PATHOGENS USING MICRO-HOMOGENIZERS AND RT-LAMP ASSAYS

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Abstract

Rapid and accurate detection of citrus pathogens is critical for effective disease management, particularly in field conditions where laboratory resources are unavailable. This study presents two innovative approaches combining portable sample preparation techniques and downstream diagnostic assays for detecting multiple citrus pathogens, including citrus tristeza virus (CTV). The first method utilizes a micro-homogenizer for chemical and mechanical lysis, enabling efficient nucleic acid extraction. Simple serial dilution or chromatography paper-based purification rendered crude lysates suitable for quantitative polymerase chain reaction (qPCR) and reverse transcription-quantitative PCR (RT-qPCR). These methods achieved up to 76.5% DNA and 63.3% RNA yields compared to conventional mortar and pestle grinding. Nucleic acids stored on paper disks demonstrated long-term stability under various conditions, supporting their use in on-site diagnostics or mail-in analysis. Various citrus pathogens representing both RNA and DNA genomes were analyzed using this method. The second approach integrates semi-automated sample preparation with reverse transcription-loop mediated isothermal amplification (RT-LAMP) for rapid, colorimetric detection of CTV. Using an OmniLyse micro-homogenizer and cellulose paper disks, total nucleic acids were extracted in under 15 minutes. Optimized RT-LAMP assays achieved detection limits of 43 copies/µL for CTV and 5 copies/µL for cytochrome oxidase (COX), with a total assay time of 40 minutes in laboratory settings. In-field testing with lyophilized RT-LAMP reaction mixes enabled accurate detection in greenhouse conditions within 35 minutes. Together, these approaches demonstrate portable, efficient, and reliable methods for pathogen detection, offering practical solutions for in-field diagnostics or decentralized testing. These strategies can be universally applied for diverse plant pathogens, empowering growers and first responders with accessible and accurate diagnostic tools.

Non-technical summary

We developed portable and rapid methods to detect citrus pathogens directly in the field, eliminating the need for complex laboratory equipment. Using a micro-homogenizer and simple tools like paper disks, we achieved efficient sample preparation and reliable detection of pathogens within minutes. These approaches make disease diagnosis more accessible, helping growers and first responders manage citrus diseases more effectively and prevent their spread.

DEVELOPMENT AND VALIDATION OF A MULTIPLEX REAL-TIME RT-PCR ASSAY FOR THE DETECTION OF THREE DICHORHAVIRUSES ASSOCIATED WITH CITRUS LEPROSIS DISEASE SYNDROME

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Abstract

Two taxonomically distinct groups of *Brevipalpus* transmitted viruses, belonging to the families *Kitaviridae* and *Rhabdoviridae* are associated with citrus leprosis disease (CiLD) complex. In total three kitavirids (citrus leprosis virus C, citrus leprosis virus C2 and hibiscus green spot virus 2) and four dichorhaviruses (orchid fleck virus; OFV, citrus leprosis virus-N; CiLV-N, citrus chlorotic spot virus; CiCSV, and citrus bright spot virus; CiBSV) were reported from multiple *Citrus* sp. The relationship between *Brevipalpus* spp. and dichorhavirus transmissions is often very strict as multiple false spider species are involved. All known dichorhavirus vectors have been reported from the United States (US), thus posing a risk of a reemergence of CiLD and its spread through the multibillion-dollar US citrus industry. To prevent the introduction of CiLV-N and CiCSV, and establishment of OFV in the US, a multiplex real-time RT-PCR assay was developed. Furthermore, to confirm the absence of CiLV-N, CiCSV and OFV in the *Brevipalpus* transmitted virus suspected diagnostic samples and check the quality of RNA, an internal control gene (*Nad5*) primer pair and TaqMan probe was also included. CiLV-N and CiCSV isolates from Brazil and OFV isolates from Mexico and the US (California, Florida, and Hawaii), were obtained to validate specificity of the assay. Citrus leprosis caused by kitavirids, CiBSV, dichorhaviruses infecting other crops, and other viruses infecting citrus species were included for specificity testing. The assays successfully detected the targets in common plant matrices like healthy sweet orange, and orchid tissues. The validated quadruplex TaqMan RT-PCR assay's work instruction was shared with the collaborators in Brazil for Tier 2 validation. Results obtained by the quadruplex real-time RT-PCR (cut off C_T value ≥37) were fully consistent with those obtained using the end-point PCR tests. No amplification was detected in assays using both healthy plants and non-template controls. Overall, this assay will be an effective screening tool for the detection of CiLV-N, CiCSV and OFV in known hosts and the discovery of unknown hosts that may exist in nature.

A REAL TIME PCR ARRAY FOR RAPID DETECTION OF MULTIPLE CITRUS PATHOGENS

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Abstract

Citrus is affected by many graft-transmissible viral and bacterial pathogens. The use of infected material can be devastating since citrus is largely a clonally propagated crop. Rapid testing methods are needed for safer germplasm exchanges between different citrus industries. A 96-well real time PCR array was developed for simultaneous detection of 24 citrus viral and bacterial pathogens. The array included three sets of 23 different duplex assays for detection of 20 pathogens, single-plex assays for four pathogens, and two assays for reference genes. In addition, a fourth set of wells also included synthetic gene blocks containing target sequences used as templates for positive controls. Starting with extracted RNA from the test plants, three samples can be assayed using a single 96-well array within 90 minutes. The assay includes a 15-minute reverse transcription reaction and a 35-minute PCR. The arrays stored at -20 °C have been stable for a minimum of 18 months.

Using the array, we tested 80 plants from the pathogen positive inventory in our germplasm facilities. Next-generation sequencing (NGS) libraries were also created to sequence the transcriptomes of these plants with a minimum of 30 million paired-end reads generated per sample. A bioinformatics pipeline was used to create *de novo* assemblies of non-host sequences to identify viral and bacterial pathogen sequences using blast search of an in-house pathogen database. The NGS analyses validated the results from assays using the array. The array developed in this study is modular with possibilities for either modification or addition of individual assays as needed.

Non-technical summary

Safer use of citrus budwood for propagation requires testing for freedom from multiple citrus pathogens. A technique was developed here to rapidly test a plant extract for 24 different viral and bacterial pathogens within about 90 minutes.

DEVELOPMENT AND VALIDATION OF A SUITE OF E-PROBES FOR ELECTRONIC DIAGNOSTIC NUCLEIC ACID ANALYSIS (EDNA) FOR 20 GRAFT-TRANSMISSIBLE PATHOGENS OF CITRUS USING MiFi® AND TESTING WITH NOVICE USERS

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Abstract
Graft-transmissible citrus pathogens threaten productivity. Citrus quarantine germplasm programs worldwide are responsible for producing and maintaining pathogen-tested tree sources to supply propagative materials for the commercial citrus industry, as well as for non-commercial uses such as research, landscaping, and public distribution. The success of these programs relies on the availability of reliable pathogen detection assays. We developed and validated a suite of electronic probes (e-probes) for Electronic Diagnostic Nucleic Acid Analysis (EDNA) to detect 20 citrus pathogens, including viroids, viruses, and bacteria, from raw high-throughput sequencing (HTS) data. These e-probes were optimized using the MiFi® bioinformatics platform for specificity, sensitivity, and detection limits through both *in silico* and *in planta* testing. Validation included comparisons with qPCR and extensive ring tests across multiple users to ensure robustness and reproducibility. EDNA consistently matched the diagnostic capabilities of conventional molecular methods, accurately identifying pathogens with high precision and enabling extensive multiplex testing without compromising sensitivity (all tests can be performed simultaneously on one sample HTS data). This innovative approach offers a scalable, efficient, and cost-effective pathogen detection tool to enhance citrus disease prevention and management and safeguard citrus germplasm at a global scale.

POINT OF CARE DETECTION OF CTV AND HLB IN CITRUS: APPLYING WORK UNDERTAKEN IN OTHER CROPS

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Global citrus production has reached 158.5 million tonnes, with 13.9 million tonnes of citrus fruits being exported. However, economic losses in citrus production have increased globally in recent years due to diseases like huanglongbing (HLB) and citrus tristeza virus (CTV). HLB and CTV pose a particularly significant threat to the world's leading citrus producers, including Brazil, China, India, Mexico, and the United States. One of the main bottlenecks for management and eradication of diseases is early detection of pathogens in-field before large scale symptoms appear. Advances in molecular diagnostics, such as PCR and isothermal amplification techniques, have improved speed and accuracy but require specialized equipment and laboratory infrastructure, making them unsuitable for field applications. Point-of-care diagnostic devices have emerged as transformative tools for on-site pathogen detection; these devices must be real-time connected, easy to use, affordable, highly sensitive and specific, user-friendly, rapid in delivering results, independent of complex equipment, and suitable for deployment in diverse agricultural settings. We previously developed a field detection kit for point-of-care detection of Papaya Meleira Virus (PMeV). Using the lateral-flow device dipstick method we developed, Skybury Papaya was able to select disease-free clones ensuring their farm was free from the disease. This knowledge was applied to develop a detection kit for HLB and CTV using Surface Enhanced Raman Spectroscopy (SERS) based bionanosensors. The technique was sensitive enough to detect 10^{-20}gm (10 zeptogram) of the HLB and CTV DNA in the laboratory. We are currently building onto this to convert the lab-based method to a robust point-of-care detection kit that farmers can routinely use in the field.

Session 11 –

Diagnostics II

Discussion session

Session 12 -
Other citrus pathogens

UNRAVELLING BLACK CORE ROT IN AUSTRALIAN CITRUS

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Abstract

Black core rot is an emerging citrus disease caused by *Alternaria* species that impacts Australian fruit quality and yields. The disease is characterised by pre-harvest fruit drop and internal fruit rot which can go undetected until fruit reaches consumers or juicing plants. Affected fruit often have little to no external symptoms and disease incidence is erratic among citrus varieties, major growing regions and seasons, which has resulted in a varied impact across the industry. *Alternaria alternata* is the reported causal agent of black core rot, however, this fungus is also known to cause Emperor brown spot in citrus. The symptoms and geographical distribution of these two diseases are apparently distinct, yet the differences between the pathogen identities and disease cycles remains a significant gap in knowledge. This has left the industry with little capacity to effectively manage black core rot. Multi-locus phylogenetic analyses of *Alternaria* isolates collected in this study obtained from Black core rot affected citrus fruit were placed within the *Alternaria alternata* clade, which is polyphyletic and does not resolve the pathogen identities of both diseases. Detached fruit inoculation assays have been established to assess and compare the ability of isolates of these two *Alternaria* diseases and *Alternaria* isolates from non-diseased citrus material to cause black core rot symptoms. To elucidate the black core rot disease cycle further and evaluate potential disease control options, a multi-year field trial with a time-staggered fungicide application and three chemical products (azoxystrobin, cuprous oxide, copper gluconate) is in its third year. An additional field trial using artificial inoculations of black core rot *Alternaria* isolates in situ to identify the timing of infection is in progress in the Riverina, NSW. These field trials, combined with ongoing sampling of citrus foliage and fruit at different physiological growth stages may pinpoint the sources of inoculum of black core rot in Australian orchards. New knowledge on the pathogen identity, pathogenicity and the disease cycle from this study may aid the development of a cost effective and sustainable integrated disease management program.

ALTERING VOLATILES TO CONTROL CITRUS PESTS AND DISEASES

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Abstract

Plant volatile organic compounds (VOCs) are secondary metabolites involved in plant-to-plant communication and in the interactions of plants with other organisms, such as pollinators or herbivores whose host selection is based not only on visual, tactile or gustatory cues, but also by olfactory stimuli. These organisms can be classified as generalists, if their requirement is to find resources among a wide variety of plants, or as specialists, if they identify specific hosts by their characteristic profile of VOCs (among other cues) emitted by concrete plant species. Plant VOCs also influence microbial endophyte populations, modify visiting insects' sexual behaviour, or confer allelopathic effects on other surrounding plants. In addition, VOCs can play a role in plant defence as deterrents against phytophagous organisms, by attracting natural enemies of herbivores or by acting as alarm signals to neighbouring plants. Altogether, knowledge of the field indicates that manipulating plant volatile emission can alter the relationship with both specialist and generalist pests and pathogens. Thus, modifying plant VOCs seems to be an ecological cost-effective manner to control crop losses and, in addition, the advantage of preventing herbivores/pests developing resistance to such changes. This strategy has been examined in different herbaceous plants, with modified, transgenic lines showing an improvement in their defences, in both a direct (repelling herbivores) and an indirect (attracting herbivore natural enemies) manner. Therefore, altering citrus VOC emission profiles provides a feasible and sustainable strategy to fight important pests, insect vectors and diseases. The volatile profile of citrus species contains a complex mixture of volatiles with a predominance of monoterpenes. During the last two decades our laboratory has generated modified orange lines in which their natural aroma has been altered by genetic modification, either by repression of the monoterpene biosynthetic D-limonene synthase gene in the fruit peel, by over-expression of a linalool synthase in green tissues (in collaboration with the NIFTS from Japan) or by overexpression of the sesquiterpene biosynthetic gene β-caryophyllene synthase, in young shoots (in collaboration with Fundecitrus from Brazil). The response against insects (such as the pest *Ceratitis capitata*, or *Diaphorina citri*, insect vector of '*Candidatus* Liberibacter asiaticus' that causes huanglongbing), pathogenic fungi (*Penicillium digitatum*, causing green mold, or *Phylloctista citricarpa*, causing citrus black spot), and the bacterium *Xanthomonas citri* ssp. *citri*, (causing citrus canker), as well as the behaviour of different herbivores (birds and mammals) to such altered aromas will be shared and discussed.

EFFECTS OF TEMPERATURE AND WATER CONTENT FOR STORAGE OF CITRUS ROOTSTOCK SEEDS

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Abstract

Seed germination has traditionally been the primary method for propagating citrus rootstock seedlings. The availability of high-quality seeds, for vigorous germination and the growth of strong rootstock seedlings, is a critical factor for citrus nursery production. In citrus germplasm programs, rootstock seedlings are used for shoot tip grafting (STG) therapy under sterile conditions. Storage of viable seeds is crucial to ensure continuous production of rootstocks year-round. The lifespan of seeds during storage depends on several factors, including moisture content, temperature, and the presence of pathogens, which have not been extensively studied for most citrus species or varieties. Rapid loss of viability is one of the major challenges for uniform and consistent propagation of rootstock seedlings throughout the year. This study evaluated the effect of Carrizo citrange seed moisture content and storage temperatures to develop optimal citrus seed storage protocols. Seeds were collected from mature fruit and treated for mucilage removal before air drying under 19°C and 44% relative humidity. Seed water content from 40 to 10% was evaluated. Seeds were stored in sealed plastic containers under 8 and 2 °C. Germination and vigor tests were conducted on fresh seeds and dry seeds at the beginning, and then every 5 months until completing 15 months of storage. Germination trials were performed in laboratory settings using surface sterilized seeds following the standard *in vitro* seed germination protocol to ensure and confirm the production of sterile seedlings for their safe use for STG. Germination data were collected daily for 21 days to generate reverse survival curves or germination curves. Seeds vigor was determined by the number and length of roots and shoots growing in culture tubes containing 25 ml of seed germination medium. Our results indicated that desiccation lower than 30% water content significantly reduces germination percentage and seed vigor. Stored seeds under both temperatures with water content higher than 30%, were able to maintain high (>95%) germination percentages over time, with 40% water content as the optimal treatment as faster germination and seedling development were observed with this treatment. Scientific information obtained from this project will be critical to developing short- and long-term storage protocols for viable seeds and can be potentially implemented as part of citrus germplasm programs and nurseries protocols for seed storage and rootstock production.

Non-technical summary

Citrus seed viability and longevity depend on factors like moisture content, temperature, and pathogens. Desiccation sensitivity significantly impacts seed storage success. Identifying optimal seed moisture levels and storage temperatures for each variety is essential to maintain seed viability and vigor.

Session 13 -

Diagnostics III

GLOBAL SANITARY DIAGNOSIS OF ALULA CITRUS ORCHARDS

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Abstract

The establishment of a high value-added citrus production chain is the goal of High Value Citrus for AlUla (HVCA), a research and sustainable development project for the region of AlUla (Saudi Arabia), supported by the French Agency for AlUla Development in collaboration with the Royal Commission for AlUla.

During the first phase of this project, a sanitary diagnosis was carried out for orchards present in 6 areas and 2 culture types of AlUla. 80 sampling pools representing 259 citrus trees and consisting of either 2 asymptomatic or 5 symptomatic trees (with or without symptoms potentially attributable to a disease of biotic origin) were sent to the ANSES Quarantine Unit. Two local nurseries were also sampled. In parallel to this, budwood of 11 trees constituting initial mother plant candidates (IMPc) chosen among the local diversity of varieties (sweet orange acidless, orange, clementine, Mexican lime, lemon, sweet lemon, citron hybrid and pumelo) were introduced into the French quarantine station for cultivation and sanitary evaluation. Using targeted tests, all these sample categories were tested against up to 20 pests, part of the most impacting pests on citrus production, including European Union regulated quarantine and non-quarantine pests. The chosen pests include 8 viruses (belonging to the genera *Badnavirus*, *Capillovirus*, *Cilevirus*, *Closterovirus*, *Dichorhavirus*, *Ophiovirus*, *Sadwavirus*), 3 viroids (genera *Cocadviroid*, *Hostuviroid*, *Pospiviroid*), 1 phytoplasma (*Ca.* Phytoplasma aurantifolia), 7 bacteria (*Ca.* Liberibacter spp., *Spiroplasma citri*, *Xanthomonas citri* pv. *citri* and *aurantifolii*, *Xylella fastidiosa*) and also 1 fungus (*Plenodomus tracheiphilus*). High-Throughput Sequencing (HTS) analyses were also carried out to detect diverse viruses and viroids in order to complete the sanitary picture of current citrus plants in this territory.

All plants analysed were found to be free from the European Union regulated quarantine pests. Various viroids were detected in this region, including in asymptomatic trees and nurseries. Their management will be improved through the generalization of good practices in the incoming AlUla nurseries and orchards. We recorded a few cases of detection of citrus psorosis virus (CPsV, species *Ophiovirus citri*) in the microclimates of Al Hijr and North Core. In addition, 2 symptomatic pools tested positive for *Spiroplasma citri* bacteria in the microclimates of Al Hijr and Fursan. Our presentation will document (1) these detections in more detail, (2) the complementary detections obtained by HTS, and (3) the use of the knowledge acquired from this first project phase to initiate phase 2 (2025 – 2027).

Non-technical summary

Overview of the activities carried out by the ANSES Quarantine Unit as part of an international collaborative project. Our Unit hosts France's official multi-species quarantine station and notably holds the national reference mandate for citrus virus detection.

IMPROVED SHOOT TIP GRAFTING (STG) TECHNIQUE FOR PATHOGEN ELIMINATION OF CITRUS GERMPLASM

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Abstract

In vitro shoot tip grafting (STG) is an effective technique for eliminating graft-transmissible pathogens from citrus germplasm and other crops. This method has facilitated the establishment and introduction of thousands of pathogen-free cultivars into the global citrus industry. Since 1976, the Citrus Clonal Protection Program (CCPP), National Clean Plant Network, at the University of California, Riverside, has routinely used STG to eliminate disease-causing organisms from citrus varieties introduced into California. STG involves grafting a small shoot tip (0.1–0.2 mm) onto an *in vitro* rootstock seedling under aseptic conditions. Optimizing the STG procedure requires standardizing protocols for *in vitro* citrus seed germination to produce rootstock seedlings and culturing budwood for shoot production. The success of STG heavily depends on the quality of rootstock seedlings, which is influenced by factors such as seed quality, culture medium, sterilization methods, and seed pre-treatments. Four culture media were tested for their effectiveness in promoting germination and seedling development of Carrizo Citrange. High germination rates were observed in all treatments (>82%), but significantly faster and higher germination (93%) was achieved with a medium containing ¼-strength Murashige and Skoog (MS) medium. This medium also produced the highest number of seedlings with greater length. Budwood culture protocols have also been significantly improved over previous standards used by the CCPP, enabling indefinite maintenance of budwood cultures until successful STG is achieved. Budwood culture stabilization media were evaluated to promote consistent shoot growth across multiple subcultures. Driver and Kuniyaki (DKW) medium proved more effective than MS medium, particularly when supplemented with plant growth regulators. DKW medium with vitamins, 30 g/L sucrose, 1.25–2.5 µM N6-benzyladenine (BA), and 0.5 µM α-naphthaleneacetic acid (NAA) yielded high rates of shoot proliferation across diverse citrus varieties. After successful STG, shoots reaching 10 mm in length are grafted onto rough lemon seedlings to ensure acclimatization. This study established an efficient and reliable STG protocol for obtaining pathogen-free citrus varieties, enhancing the success and sustainability of *in vitro* citrus propagation.

Non-technical summary

The CCPP employs *in vitro* shoot tip grafting to eliminate pathogens from citrus varieties introduced into California. Tiny shoot tips from budwood cultures are grafted onto *in vitro* rootstock seedlings under sterile conditions. Optimizing this process involves standardizing protocols for citrus seed germination and budwood culture to ensure consistent seedling and shoot production.

TOWARDS POINT-OF-CARE TESTING WITH CRISPR/CAS-BASED ASSAYS IN THE FIELD TO DETECT EXOTIC PATHOGENS IN CITRUS AND IMPROVE PREPAREDNESS

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Abstract

Rapid, efficient, sensitive, and easy-to-use portable diagnostic tests for plant pathogens are essential for identifying potentially threatening diseases in surveillance, inspections, evidence of area freedom, containment, asset protection, and managing responses to post-border incursions. With a view toward field-deploying CRISPR/Cas-based diagnostic assays for the simultaneous detection of multiple pathogens per plant commodity, we have started developing individual CRISPR/Cas assays based on literature and Australia's National Priority Plant Pests List, targeting citrus exotic pathogens as a proof of concept.

The technology employs the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated nucleases, such as Cas12 (targets dsDNA) and Cas13 (targets RNA), in combination with reverse transcription and isothermal nucleic acid amplification systems, such as Recombinase Polymerase Amplification (RPA). These systems can specifically detect exceptionally low levels of pathogen DNA or RNA. The resulting collateral activities of CRISPR/Cas systems can be visualised in the laboratory using fluorescence readers and in the field with portable platforms.

Here, I will present an update on the development of CRISPR/Cas12a diagnostic assays for detecting multiple bacterial pathogens (causal agents of huanglongbing, Pierce's disease, and citrus canker) and viruses (citrus tristeza virus, citrus vein enation virus, and hop stunt viroid), as well as CRISPR/Cas13 diagnostic assays for detecting multiple citrus tristeza virus strains in citrus. In a one-pot reaction (i.e., a single-tube process), infected plant material can be identified, with demonstrated sensitivity to synthetic DNA molecules down to attomole concentrations.

The results, future challenges, and benefits of CRISPR/Cas technology will be discussed in the context of maximum strain capture and field deployment.

Non-technical summary

This research develops portable CRISPR/Cas-based tests to quickly and accurately detect multiple citrus plant pathogens. The tests can identify even tiny amounts of pathogen bacteria or viruses, offering a powerful tool for early disease detection and plant protection in the field.

LIGHT MANIPULATION UNDER CONTROLLED ENVIRONMENT AGRICULTURE CONDITIONS AFFECTS VIRAL SYMPTOM EXPRESSION IN BIOLOGICAL INDEXING

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Abstract

The Citrus Clonal Protection Program (CCPP), National Clean Plant Network, at the University of California, Riverside, is responsible for the introduction of citrus accessions into California. These accessions must undergo therapy and rigorous laboratory and bioindexing pathogen testing under quarantine to prevent the introduction and spread of citrus pathogens. While bioindexing is an invaluable biosecurity tool, it creates a bottleneck in the introduction process, as symptoms of graft-transmissible pathogens take months to develop on slow-growing citrus plant indicators, compared to days for laboratory tests. Alternative agriculture techniques applying next-generation indoor vertical farming and controlled environment agriculture (CEA) were used to optimize the current bioindexing protocols carried out at the CCPP. Four different wavelength ratios of red (R) and blue (B) light spectra from adjustable LEDs and one full-spectrum LED light (red dominant) conditions were used to test the effects on viral symptom onset and expression in sensitive citrus indicator seedlings compared to standard greenhouse conditions. High percentages of red light (95R:5B and 85R:15B) were found to induce more rapid viral symptom expression, increase the disease severity index (DSI%), and elevate viral titer (measured via RT-qPCR) in several regulatory-significant viral pathogens. By incorporating these alternative agricultural techniques, the bioindexing procedures at the CCPP can be improved by expediting and increasing observable symptoms in plant indicators and thus reducing false-negatives in plants of unknown pathogen status, as well as shortening the overall time required for citrus accession introduction under quarantine.

Non-technical summary

New indoor vertical farming techniques with LED lights were used to improve the required bioindexing procedures used in citrus quarantine in California. High levels of red light were able to accelerate symptom expression and increase disease severity in several viral pathogens.

Session 14 -
Vectors

MANAGING THE PRIMARY PSYLLID VECTOR OF THE HUANGLONGBING PATHOGEN

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Abstract

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is an economically important insect pest of citrus worldwide, mainly due to its role as a primary vector of "*Candidatus* liberibacter asiaticus", which causes huanglongbing (HLB) or citrus greening disease. ACP-HLB is one of the world's most serious vector-disease complexes, devastating citrus industries worldwide. ACP is reported from 57 countries and HLB from 50 countries. In Florida, this vector and disease were reported in 1998 and 2005, respectively, and became established.

ACP management is critical to reducing the spread and severity of HLB. In the last two decades, we evaluated cultural, biological, and chemical methods of pest control for managing ACP in citrus produced for processed and fresh fruit markets. Biological control played a significant role in suppressing citrus pests for decades before the upsurge of ACP, which continued with several first responders, such as ladybeetles, lacewings, and entomopathogens, causing mortality in its populations averaging 80% or more. The species-specific parasitoid, *Tamarixia radiata* (Waterston), was introduced from China, Pakistan, and Vietnam, and mass-rearing facilities were developed which provide parasitoids to stakeholders for release in commercial and urban environments. Parasitism rates improved 3-4 times with augmentation from 10% or less observed with a limited release made soon after ACP was discovered. Chemical control increased after HLB was found and contributed significantly to ACP suppression with strategies developed for dormant and growing season populations. ACP management continues to evolve, and in the last decade, we investigated novel Citrus Under Protective Screen (CUPS) and Individual Protective Covers (IPCs) systems for citrus production and protection against ACP and HLB. Overall, CUPS and IPCs were effective in bringing young trees into production with better health and higher yields than open production systems and without ACP-HLB. Some non-target pests, small predators, and parasitoids were observed in these novel environments. The occurrence and functioning of beneficial organisms suggest opportunities for strengthening biological control in these systems. Findings from the research and implementation of ACP-HLB management in traditional open and protected environments will be discussed.

Non-technical summary

Tools and tactics developed for managing ACP-HLB complex using cultural, biological, and chemical methods of pest control provide opportunities for stakeholders to protect and produce citrus crops in traditional open and protected environments and to make advances in the management of pests and diseases.

STUDYING THE BIODIVERSITY OF AUSTRALIAN NATIVE INSECT SPECIES AND ASSOCIATED MICROBIOMES AROUND CITRUS

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Abstract

Australia's citrus industry is threatened by potential incursions of plant pathogens vectored by some Hemipteran insects such as aphids spreading citrus tristeza virus (CTV), and psyllids spreading the *'Candidatus* Liberibacter*'* bacteria that causes huanglongbing (HLB) disease, which has greatly impacted citrus worldwide and caused billions in losses. The main insect vectors of HLB are the exotic African citrus psyllid (*Trioza erytreae*) and the Asian citrus psyllid, ACP (*Diaphorina citri*). Fortunately, no psyllids are known to feed on citrus in Australia, but ACP which can vector HLB is present in neighbouring countries, and Australian biosecurity has intercepted it at the border in the past.

Australia is a global hotspot of psyllid biodiversity but there is limited knowledge about what native Psylloidea and their associated microbial communities are already present. Using psyllids as a vector model, we explore their biodiversity, and their associated microbiome around citrus orchards. This will improve the understanding of Australian native diversity so that potential incursions of exotic species can be easier to identify.

Trapping methods were compared across three states and four major citrus growing regions, and species were then identified using an integrative taxonomy approach combining traditional morphology and molecular techniques.

As a result, over 200 psyllid species across 40 genera were identified, and more than 500 DNA sequences were generated. Trapping systems enabling capture of the most comprehensive insect diversity were determined and the taxa present across most citrus growing regions revealed. Information obtained on the insects' bacterial communities will allow comparison of the microbiome of Australian native species with those of exotic psyllid pests. An improved genetic database for insect and bacterial identification will streamline processes for identifying potential pests of the Australian citrus industry.

Non-technical summary

A number of exotic insects are important vectors of plant pathogens harming citrus production overseas, but they are not present in Australia. We used psyllids and their associated bacteria as a model to show the importance of studying what insect species and microbes are already present in Australia so that we can better prepare Australian biosecurity for exotic insect incursions.

MURRAYA SPP. AS THE ALTERNATIVE HOST OF *CANDIDATUS* LIBERIBACTER ASIATICUS AND THE INSECT VECTOR, *DIAPHORINA CITRI*

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Abstract

Huanglongbing caused by *Candidatus* Liberibacter asiaticus transmitted by the psyllid vector *Diaphorina citri* has been infecting *Citrus* spp. and the hybrids, and the relative species. *Murraya* spp in Indonesia included *M. paniculata* or orange jasmine, *M. sumatrana, M. lucida*, and *M. omphalocarpa*. CLas were repeatedly detected by PCR and qPCR as a natural infection on *M. paniculata* and *M sumatrana* in Java, on the other hand no natural infection were found on the other two species. The colonization of *D. citri* was very commonly found on *M. paniculata* and sometimes on *M. sumatrana* and were not found on the other two species. The WGS of chloroplast confirmed that those 4 species were separated in different clades; however, *M. lucida* is located in the sister clade of *M. sumatrana* whereas *M. omphalocarpa* in the sister clade of *M. paniculata.*

Poster Session 1

MIQE GUIDELINES: A FRAMEWORK FOR ENSURING ACCURACY AND RELIABILITY IN qPCR-BASED CITRUS PATHOGEN DETECTION

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Abstract

The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines provide a standardized framework for reporting quantitative real-time polymerase chain reaction (qPCR) experiments. These guidelines promote transparency, reproducibility, and accuracy in qPCR research by outlining essential information to include when reporting qPCR experiments. This includes experimental design, sample characteristics, nucleic acid extraction and quality control, primer and probe sequences, qPCR conditions, data analysis, and validation procedures. In the context of plant pathology, the detection of citrus pathogens such as *'Candidatus* Liberibacter asiaticus' (CLas), the agent associated with the citrus greening disease, requires reliable and standardized qPCR protocols. The implementation of MIQE guidelines ensures the transparency, reproducibility, and accuracy of qPCR results, which is critical for the development of effective management strategies for citrus greening disease. The validation process follows criteria set by the MIQE guidelines and the Diagnostic Assay Validation Network (DAVN), a platform designed to promote validated methods by sharing standard diagnostic method development, ensuring easy access to statistical tools, registries of methods, and controls. For example, when detecting '*Candidatus* Liberibacter' species, *S. citri*, and viroids in citrus plants, the process of assay design, validation, and implementation is a multistage collaborative effort that needs to adhere to MIQE guidelines for quantitative and qualitative qPCR. The MIQE guidelines stipulate essential criteria such as sensitivity, specificity, robustness, reproducibility, and transferability and recommend reporting key experimental details, including primer and probe sequences, qPCR conditions, and data analysis parameters, such as threshold settings and baseline corrections. Implementing MIQE guidelines is crucial for ensuring the quality and reliability of qPCR results in plant pathology research. By following these guidelines, researchers can ensure that their qPCR results are transparent, reproducible, and accurate, which is essential for developing effective management strategies for citrus greening disease.

Non-technical summary

Researchers have developed guidelines called MIQE to help ensure that diagnostic experiments using qPCR are done correctly and reported clearly. This is especially important in plant pathology, where qPCR is used to detect pathogens of diseases like HLB. By following MIQE guidelines, researchers can ensure their qPCR results are accurate, reliable, and useful, ultimately contributing to developing effective management strategies for citrus diseases.

DEVELOPMENT OF A POINT-OF-CARE FIELD DETECTION KIT FOR HUANGLONGBING (HLB) USING APTAMER-BASED TECHNOLOGY

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Abstract

Huanglongbing (HLB), caused by *Candidatus* Liberibacter species and transmitted by psyllid vectors, is one of the most destructive diseases affecting citrus crops worldwide. Since there is currently no effective cure, early and accurate pathogen detection is essential to control its spread and minimize crop losses. Our approach focuses on developing a point-of-care diagnostic kit using aptamers with high binding affinity for HLB-specific effector molecules. By identifying the most suitable aptamer pairs, we have integrated them into a dipstick-based platform for in-field detection. Previously, we successfully achieved DNA detection sensitivity to 10^{-18} g using advanced techniques. Building on this, the new portable system utilizes a handheld spectrometer for rapid and quantitative analysis in real time. Designed for field use, this method is not only highly sensitive and specific but also cost-effective, with an estimated cost of test < $5 per sample. The proposed diagnostic tool combines affordability, portability, and precision, enabling citrus growers to conduct early detection of HLB on-site. This early diagnostic capability can play a critical role in the timely implementation of control measures, thereby helping to mitigate the devastating impact of HLB on citrus production globally.

IMPROVING PLANT INDICATOR GROWTH FOR BIOINDEXING IN CITRUS GERMPLASM PROGRAMS

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Abstract

Bioindexing remains a cornerstone of citrus quarantine programs, providing a critical safety net for detecting unknown or laboratory-undetectable pathogens. To modernize this process, the Citrus Clonal Protection Program (CCPP), National Clean Plant Network, at the University of California, Riverside, initiated a project to optimize plant indicator growth using recirculating ebb-and-flow hydroponics and automated fertigation systems. Traditional bioindexing methods face challenges in today's fast-paced citrus introduction landscape, including resource-intensive operations, inconsistent nutrient delivery, and the risk of nutrient deficiencies that can mask disease symptoms. Transitioning to smaller containers and adopting hydroponics offer the potential to increase throughput, reduce greenhouse space requirements, and improve nutrient delivery efficiency, but these approaches require optimization to reach their full potential. In a pilot study, non-inoculated Mexican lime indicators were grown in hydroponic systems with coconut coir as a sustainable substrate and fertilized with a multipurpose blend. The frequent and dramatic fluctuations in nutrient solution pH, combined with plant health metrics (i.e., leaf nutrient analysis, visual observations of nutritional deficiencies, and photosynthetic markers), highlighted the need for further optimization of vegetative growth to ensure reliable symptom expression. Maintaining the pH of the nutrient solution within an acceptable range is essential for efficient nutrient absorption and optimal vegetative growth. Preliminary screenings with Dweet tangor indicators and alternative fertilizers were conducted to identify formulations more resistant to pH fluctuations and to explore species-specific responses and nutrient requirements. Findings from these trials will be presented and discussed. Future efforts will expand trials to additional indicator species, incorporate automated dosing systems, and utilize real-time nutrient monitoring tools. These innovations aim to develop a robust standard operating procedure for high-throughput bioindexing, ensuring consistent symptom development and diagnostic reliability.

Non-technical summary

Modern automated systems for plant indicator growth are critical for sustainable, efficient, and scalable bioindexing processes that exclude graft-transmissible pathogens from citrus germplasm, safeguarding citrus nursery production and citriculture.

DETECTION OF CITRUS-ASSOCIATED RHABDOVIRUS IN AUSTRALIA USING HIGH THROUGHPUT SEQUENCING

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Abstract

As part of a nationwide surveillance program targeting huanglongbing and citrus variegated chlorosis, a sample was received from the Northern Territory of Australia. The budwood sample was from an asymptomatic kumquat plant (*Citrus japonica* Thunb.). Both DNA and RNA were extracted from green bark tissue using a MagMAX Plant RNA extraction kit (ThermoFisher Scientific, United States) according to the manufacturer's instructions, except with the omission of the DNaseI digestion step. Extracts were tested using molecular methods for '*Candidatus* Liberibacter spp.' and *Xylella fastidiosa* subsp. p*auca* which are not reported in Australia and a range of graft-transmissible viruses and viroids known to be endemic in Australia. Following the detection of citrus leaf blotch virus (CLBV; citrivirus, Betaflexiviridae), a daughter tree was propagated from this budwood onto Carrizo citrange (*Citrus* ×*insitorum* Mabb.) rootstock and grown in a controlled environment.

To investigate the genomic diversity of CLBV in Australia, total RNA was extracted and sent for high throughput sequencing. Following sequencing, quality control and de novo assembly, eight contigs with 95-99% identity to CLBV (NC_003877) were detected with a local NCBI ref_viruses_rep_genomes database and a further three contigs were generated that had 78-83% identity to *Cytorhabdovirus caricae* (papaya virus E strain) (NC_055504). Mapping analysis of reads to *C. caricae* (citrus-associated rhabdovirus strain) (MT302546), determined that the consensus sequence was 92.8% identical to citrus-associated rhabdovirus (CiaRV) isolates from China that infect citrus trees.

Testing of other Australian citrus samples using RT-PCR found four other plants with the virus present. Sequence analysis of the amplified fragment of the nucleoprotein gene showed a high sequence similarity between samples. Mechanical inoculation of the virus to passionfruit (*Passiflora edulis* Sims) plants was attempted and was not successful after 4 weeks. This is the first report of CiaRV outside of China and most likely will not be the last.

FIRST REPORT OF CITRUS VEIN ENATION VIRUS IN LEMON TREES IN COMMERCIAL ORCHARDS OF TUCUMÁN, ARGENTINA

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Abstract

Citrus vein enation virus (CVEV) is a phloem-limited virus and the causal agent of citrus vein enation disease, which is graft- and aphid-transmissible, affecting sensitive citrus species and causing serious losses to the citrus industry. This study reports the first detection and identification of CVEV in commercial citrus orchards in two different locations of Tucumán Province, Argentina. In 2019, several Lisbon lemon trees (*Citrus limon* (L) Burm f.) grafted onto Swingle citrumelo (*Poncirus trifoliata* x *Citrus paradisi*) rootstock were observed exhibiting symptoms of prominent woody galls on branches and enations on secondary midvein leaves. This was the first documented occurrence of these symptoms in the region. Buds from these trees were collected, grafted onto *C. volkameriana*, and bark samples were inoculated in sour orange (*C. aurantium* L.) and Mexican lime (*C. aurantifolia*) seedlings in a greenhouse maintained at temperatures between 18-27ºC. Six months later, galls developed around the inoculation sites on the indicator plants and enations on midvein leaves confirmed graft transmission suggesting the presence of CVEV. In 2024, similar field symptoms were observed on Lisbon lemon trees grafted onto P.T. Flying Dragon rootstock in another commercial orchard. In both instances, RNA was extracted from the bark tissues of the field tree samples for molecular analysis. Reverse transcription polymerase chain reaction (RT-PCR) targeting conserved motifs in the RNA-dependent RNA polymerase domain of ORF 2 yielded positive results in both cases. Amplicons were sequenced, and subsequent BLAST analysis revealed 99% nucleotide identity with CVEV GenBank reference sequences (MN187035.1; LC433635.1; LC089851.1; NC_021564.1). Further studies are needed to evaluate the virus epidemiology and potential impact on lemon production in Tucumán.

Non-technical summary

Citrus vein enation virus (CVEV) was detected for the first time in Argentina, causing galls on stems and vein enations on leaves of Lisbon lemon trees in different commercial orchards from Tucuman province. This study confirmed the presence of the virus using biological and molecular techniques and underscores the need for further research to assess its impact on lemon production and guide disease management strategies.

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Poster Session 2

DEVELOPMENT OF qPCR MARKERS FOR ASSESSING RESISTANCE AND SUSCEPTIBILITY TO *PHYTOPHTHORA SPP.* IN NEW *CITRUS* GERMPLASM

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Abstract

Phytophthora spp., despite being well-known pathogens causing soil and fruit diseases on citrus since the 19th century, remain a major problem in most world citrus countries. In the Mediterranean basin, *Phytophthora citrophthora* causes both gummosis and root rot; it also attacks aerial parts of the trunk and major limbs and causes significant fruit losses in Clementines (*Citrus clementina* Hort. Ex Tan), being hard to cure with common fungicide treatments. In tropical countries, *P. nicotianae* is the most common *Phytophthora* species, causing foot rot and root rot though usually it does not infect far above the ground. Despite the use of resistant or tolerant rootstocks and the application of antifungals, genetic resistance within citrus is limited to the *Poncirus trifoliata* genus and some of its hybrids, as well as very few species of the *Citrus* genus, which restricts the use of most *Citrus* genotypes as rootstocks. In our laboratory, we are working at incorporating resistance to the main *Phytophthora* species into *Citrus* germplasm using biotechnology. Currently, the only method available to evaluate the presence of *Phytophthora* in trees is through isolation from soil or root tissue and identification by microscopy or PCR. However, this does not allow us to determine whether the pathogen is attempting to infect host cells and whether those host cells are responding to *Phytophthora* challenge inoculation. This restricts the possibilities to evaluate resistance in new germplasm generated through breeding or biotechnology. In this study, we have developed two qPCR molecular markers based on: 1) *ITS2* which indicates the presence of *Phytophthora* in plant tissue (i.e.: roots), and 2) an effector belonging to a family of copper-dependent lytic polysaccharide monooxygenases (*LPMO*) which assess on host infection by the oomycete.

Additionally, the expression of an osmotin-like pathogenesis-related (PR-5) gene has been also characterised as a marker whose expression is upregulated in *Citrus* hosts responding to *Phytophthora* infection. All three markers were first validated under laboratory conditions in two different genotypes: Swingle citrumelo (*Citrus x paradisi* Macfad. × *Poncirus trifoliata* L. Raf), described as resistant to *Phytophthora spp.*, and rough lemon (*Citrus jambhiri* Lush), considered as susceptible. Then, these markers were validated in Pera sweet orange trees grafted on the susceptible Sunki mandarin rootstock under field conditions in Sao Paulo (Brazil), where the predominant *Phytophthora* species is *P. nicotianae*.

EXPLORING THE BOUNDARIES OF ENGINEERING CITRUS YELLOW VEIN CLEARING VIRUS GENOME BY INSERTING AN EXOTIC GENE CASSETTE

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Abstract

Citrus yellow vein clearing virus (CYVCV) poses a significant threat to California's citrus industry. Infected lemon trees exhibit characteristic symptoms such as yellow vein clearing, water-soaked leaf appearance, and leaf deformities. However, our understanding of CYVCV pathogenesis is limited, including its host range and the specific host cells it colonizes. To develop fundamental knowledge on CYVCV, an infectious clone of a California isolate was constructed. *Agrobacterium*-mediated infiltration revealed that the wild CYVCV-type CYVCV infectious clone could infect a broad range of citrus cultivars. However, our infectious clone only induced local infection in *Nicotiana benthamiana* with no systemic spread observed. Various approaches to modify the CYVCV infectious clone were performed with limited success. For example, modified vectors incorporating TGB3-GFP, NB-GFP, or free GFP between TGB3 and CP failed to produce infections. A vector with GFP inserted at the 3' end resulted in mild GFP expression in *N. benthamiana* but did not establish infection in citrus plants. Exploring further vector construction based on the CYVCV genome by inserting foreign genes is crucial for studying CYVCV's tissue tropism, specifically identifying the host cells it colonizes. This research will contribute to understanding virus transmission dynamics among different hosts.

Non-technical summary

An infectious clone of a California isolate of CYVCV was constructed which was able to systemically infect a broad range of citrus cultivars. However, the infectious clone only induced local infection in *Nicotiana benthamiana* with no systemic spread observed. A vector with GFP inserted at the 3' end resulted in mild GFP expression in *N. benthamiana* but did not establish infection in citrus plants. Further research is ongoing to modify the CYVCV infectious clone to express GFP in citrus.

STRATEGIES TO IDENTIFY OR ENGINEER MILD CROSS-PROTECTING STRAINS OF CITRUS TRISTEZA VIRUS TO SAFEGUARD THE AUSTRALIAN CITRUS INDUSTRY

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Abstract

Citrus tristeza virus (CTV) is a significant citrus pathogen, estimated to have caused the loss of over 100 million trees worldwide. While the quick decline disease phenotype associated with CTV is now largely controlled through the use of resistant rootstocks, the stem pitting phenotype remains a major issue, reducing yield and shortening the productive lifespan of citrus trees. CTV is a member of the complex *Closteroviridae* family, with a large ~19.3 kB single-stranded RNA genome. As a phloem-limited virus, CTV is transmitted in the field by several aphid vectors and during citrus propagation via grafting infected budwood.

The virus has a complex genome, and multiple genotypes of CTV can accumulate in citrus over time. This complexity makes it challenging to link specific pathotypes to individual genotypes, and the sequence signatures of disease-causing variants remain unknown. One proven strategy to mitigate the effects of severe CTV strains is cross-protection, where mild strains are intentionally introduced to pre-infect citrus trees, thereby preventing or delaying infection by more virulent strains. This strategy has been commercially successful in Australia, where it is used to protect grapefruit from severe stem pitting disease, but the underlying mechanisms of cross-protection are only beginning to be understood.

We aim to advance the understanding of viral cross-protection, focusing on developing or identifying mild CTV strains to protect citrus from the potentially devastating orange stem pitting disease, which is currently confined to Queensland in Australia. To achieve this, we will employ high-throughput sequencing to catalog the diversity of CTV genotypes present in Australia and assemble infectious CTV clones for testing in the model plant *Nicotiana benthamiana*. This experimental host provides a rapid platform for engineering mild, cross-protective CTV strains tailored for citrus.

CITRUS EXOCORTIS VIROID SYMPTOM MITIGATION USING BIOCHAR AND BOKASHI FERMENTED CITRUS FRUIT WASTE AS POTTING MEDIA AMENDMENTS

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Abstract

Citrus exocortis viroid (CEVd), the causal agent of exocortis disease in citrus, is also pathogenic to tomatoes, causing stunting, chlorosis, leaf curling, and eventually plant death. In this study, we tested the hypothesis that tomato plants grown in potting media amended with two types of agricultural waste -bokashi-fermented citrus fruit (BOK) and almond shell biochar (BC)- would exhibit increased immune responses to CEVd infection. BOK is reported to contain beneficial microbes and an optimal carbon-to-nitrogen ratio, both of which are associated with enhanced plant growth and health. BC is known for its porous structure, which provides a habitat for beneficial microbes and improves water-holding capacity. Together, these agricultural waste amendments are believed to enhance crop growth and health while potentially mitigating disease symptoms. To investigate the impact of BOK and BC on CEVd disease expression, 'Rutgers' tomatoes -frequently used as a model system for CEVd studies- were grown under standard greenhouse conditions in four potting media mixes: control (equal parts of perlite, peat moss, and coconut coir), 12.5% (by volume) BOK, 10% BC, and 12.5% BOK + 10% BC. At three weeks of age, the tomatoes were inoculated with CEVd by rubbing the leaves of recipient plants with those of CEVd-infected source plants, simulating a natural viroid transmission event. CEVd-infected tomatoes grown in BOK- and BC-amended potting media exhibited greater growth, delayed symptom development, lower viroid RNA titers, faster fruit ripening, and more uniform fruit production compared to those grown in the control potting media. These results suggest that BOK and BC indirectly suppress pathogen activity and enhance the plant's systemic resistance, despite the spatial separation between the inoculation site and the soil mix containing the amendments. These findings highlight the potential of agricultural waste as a resource for disease mitigation strategies and provide a foundation for future citrus studies, offering insights into the mechanisms of plant immune responses to CEVd.

Non-technical summary

Tomato plants infected with the exocortis viroid showed improved growth, delayed symptoms, and faster fruit production when grown in soil enriched with bokashi-fermented citrus fruit and almond shell biochar. These natural soil additives appear to enhance the plants' immune response and suppress the viroid's activity.

EFFECT OF ROOTSTOCKS ON THE DEVELOPMENT OF HUANGLONGBING DISEASE ON LEMON

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Abstract

Citrus is one of the most important fruit species around the world. In many countries, it suffers from huanglongbing (HLB), a serious disease of the crop. In Indonesia, Japansche citroen (*Citrus* ×otaitensis (Risso & Poit.) Risso, syn. *C. limonia* Osbeck) (JC) is still the dominant rootstock used in citrus nurseries. However, some citrus nurseries use 'Salam' (*C. japonica* Thunb., syn. *Fortunella japonica* (Thunb.) Swingle) for this purpose. This study aimed to observe the response of these two rootstocks and Volkameriana lemon (*C.* ×*otaitensis*), Troyer citrange (*C.* ×*insitorum* Mabb.) and Carrizo citrange (*C.* ×*insitorum*) on the development of HLB in scions of the lemon variety 'California'. Inoculation of rootstocks with HLB was achieved by two methods: by grafting budwood of an infected lemon (in June 2023); and by grafting the bark of bud sticks from an infected lemon (in November 2023). Controls were grafted using budwood or bark from HLB-free lemon plants. The diameters of scions and rootstocks, plant heights, disease intensity, and number of fruits were evaluated every 3 months after inoculation. Other morphological and biochemical parameters investigated were: the number of stomata, wet and dry biomass, chlorophyll contents, production of compounds associated with resistance (total phenolic content, phenylalanine ammonia-lyase, tyrosine ammonia lyase) and metabolic profiles. Scion diameters and plant heights were influenced by the rootstock type and method of inoculation. The rootstocks inoculated using bark had larger diameters than those inoculated using budwood, especially for JC and Carizzo. Plant height was influenced by HLB disease status with HLB-infected plants being shorter than healthy ones. Initial fruit production by the healthy (mock inoculated) lemon plants occurred 17 months after inoculation, especially for plants grafted onto JC and Troyer citrange. In contrast, lemons grafted with infected bud wood did not produce fruit at the same time. Lemon grafted onto Troyer and Carrizo citrange and inoculated using bark had fewer fruits than the healthy plants. The rootstocks influenced resistance of the lemon to HLB disease symptoms. Troyer and Carrizo showed high symptom expression if the rootstocks were grafted with infected budwood. Plants grafted onto Carrizo showed more severe symptoms than those on other rootstocks when inoculated using bark. Huanglongbing infection affected chlorophyll contents, wet and dry biomass, production of resistance enzymes, and metabolic profiles. The selection of HLB tolerant rootstocks influences the success of lemon cultivation, so further research is needed regarding the suitability of rootstocks for each citrus variety.